CHAPTER 2

EXPERIMENTAL

2.1 Chemicals

All chemicals used were of analytical grade, as follows:

1. Methyl-isobutyl ketone: $CH_3COCH_2CH(CH_3)_2$ (Baker analysed reagent, J. T. Baker, Holland)
2. Sodium diethyl-dithiocarbamate trihydrate: $C_5H_{10}NNaS_2 \cdot 3H_2O$ (Merck, Germany)
3. Sodium hydroxide: NaOH (Baker analysed reagent, J. T. Baker, Holland)
4. Nitric acid 65% (w/v): HNO_3 (Baker analysed reagent, J. T. Baker, Holland)
5. Methanol HPLC grade 99.9%: CH_3OH (Lab-Scan, Ireland)
6. Chromium potassium sulfate: $CrK(SO_4)_2 \cdot 12H_2O$ (Sigma-Aldrich, Germany)
7. Silver nitrate: $AgNO_3$ (Sigma-Aldrich, Germany)
8. Sodium peroxodisulfate: $Na_2S_2O_8$ (Sigma-Aldrich, Germany)
9. Metal stock solutions (1,000 mg l^{-1}): Cu, Pb, Cd, Ni and Zn (Merck, Germany)
10. Ethylenediaminetetraacetic acid: EDTA (Sigma-Aldrich, Germany)
11. Lead nitrate: $Pb(NO_3)$ (Merck, Germany)
12. Sr. Spec™ resin: 80-100 μm grain diameter (Eichrome, USA)
13. Ammonium ferrous sulfate: $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ (BDH, England)
14. Ion exchange resin, Dowex-50Wx8, 50-100 mesh (Fluka, Switzerland)
15. Hydrazinium chloride: $(N_2H_6)Cl_2$ (BDH, England)
16. Arsenic(III) oxide: As_2O_3 (Merck, Germany)

17. Sodium arsenate: $Na_2HAsO_4 \cdot 7H_2O$ (Fluka, Switzerland)

18. Potassium tetrachloroaurate(III): $KAuCl_4$ (Fluka, Switzerland)

19. Sulfuric acid: H_2SO_4 (BDH, England)

20. Hydrochloric acid 37% (w/v): HCl (Merck, Germany)

21. 1,10 –phenanthroline: $C_{12}H_8N_2 \cdot H_2O$ (Merck, Germany)

22. Ammonium ferric sulfate: $NH_4Fe(SO_4)_2 \cdot 12H_2O$ (BDH, England)

23. L(+)-ascorbic acid: $C_6H_8O_6$ (Merck, Germany)

24. Sodium nitrite: $NaNO_2$ (Merck, Germany)

25. 4-Acetamidophenol: $C_8H_9NO_2$ (Fluka, Switzerland)

26. Sodium acetate 3-hydrate: $CH_3COONa \cdot 3H_2O$ (Merck, Germany)

27. Acetic acid (glacial)100% (Merck, Germany)

2.2 Preparation of reagents and standard solutions

1. 1% w/v diethyl-dithiocarbamate (DDC)

1.0 g of DDC was dissolved with DI-water and the volume was made up to 100 ml.

2. Metal standard solutions (Cu(II), Pb(II), Ni(II), Cd(II), Zn(II) and Cr(VI) for the automated on-line solvent extraction system for some heavy metal determinations)

Standard solutions for each metal were prepared by diluting stock solutions 1,000 mg l^{-1}(Merck) of each metal with 0.1 M HNO_3.

3. Cr(III) stock solution (1,000 mg l^{-1})

Stock Cr(III) (1,000 mg l^{-1}) was prepared by dissolving 0.980 g of $CrK(SO_4)_2 \cdot 12H_2O$ in 0.1 M HNO_3 and the volume was made up to 100 ml. The standard solutions of Cr(III) for calibration were made by appropriate dilutions of the stock solution with 0.1 M HNO_3.

4. Acetate buffer (0.1 M)

7.70 g of CH_3COONH_4 was dissolved in 100 ml of water, 4 ml of acetic acid was added for adjusting pH to 4.5. The solution was diluted to 1,000 ml with DI-water.

5. Acetate buffer (0.5 M)

17.1 g of $CH_3COONa \cdot 3H_2O$ was dissolved with DI-water and acetic acid 7.1 ml was added in sodium acetate solution. The volume was made up to 250 ml.

6. 1% w/v sodium peroxodisulfate ($Na_2S_2O_8$)

1.0 g of $Na_2S_2O_8$ was dissolved with DI-water and volume was made up to 100 ml.

7. Pb(II) stock solution (1,000 mg l^{-1}) (for flow injection on-line preconcentration for lead determination by flame atomic absorption spectrometry)

Lead stock solution (1,000 mg l^{-1}) was prepared by dissolving 0.1582 g of $Pb(NO_3)$ in 0.1 M HNO_3 and volume was made up to 100 ml. The standard solutions of Pb(II) for calibration were prepared by appropriate dilutions of the stock solution.

8. Ethylenediaminetetraacetic acid solution (EDTA) (0.005 M)

1.9 g of EDTA was dissolved with DI-water and volume was made up to 1,000 ml. The pH was adjusted to 7 by NaOH solution.

9. Fe(II) stock solution (1,000 mg l^{-1})

Fe(II) stock solution (1,000 mg l^{-1}) was prepared by dissolving 0.7021 g of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ in 50 ml of DI-water and 1 ml of H_2SO_4 was added. The volume was made up to 100 ml with DI-water. The series of Fe(II) standard solutions were prepared by diluting the appropriate volume of Fe(II) stock solution with DI-water.

10. Fe(III) stock solution (1,000 mg l^{-1})

The Fe(III) stock solution was prepared by dissolving 0.8634 g of $NH_4Fe(SO_4)_2 \cdot 12H_2O$ in 50 ml of DI-water and added with 1 ml of H_2SO_4, then diluted to 100 ml with DI-water. The series of Fe(III) standard solutions were prepared by appropriate dilution of the Fe(III) stock solution.

11. Hydrazinium chloride (1,000 mg l^{-1})

0.1 g of $(N_2H_6)Cl_2$ was dissolved with DI-water and volume was made up to 100 ml.

12. As(III) stock solution (1,000 mg l^{-1})

As(III) stock solution (1,000 mg l^{-1}) was prepared by dissolving 0.132 g of As_2O_3 in the minimum amount of 5.0 M NaOH, adjusted to the pH about 3.5 with HCl and diluted to 100 ml with DI-water. A 5 mg l^{-1} of hydrazinium chloride was added to prevent oxidation of As(III) to As(V). Stock solution was stored at 4 ^{0}C and stable at least 1 month. As(III) working standard solutions were prepared by appropriate dilutions of the stock solution with 0.1 M HCl.

13. As(V) stock solution (1,000 mg l^{-1})

Arsenic(V) stock solution (1,000 mg l^{-1}) was prepared by dissolving 0.4162 g of $Na_2HAsO_4 \cdot 7H_2O$ in the minimum amount of 5.0 M NaOH, adjusted the pH about 7.0 with H_2SO_4 and diluted to 100 ml with DI-water. Stock solution was stored at 4 ^{0}C and stable at least 1 month. As(V) working standard solutions were prepared by appropriate dilutions of the stock solution with 0.1 M HCl.

14. Au(III) stock solution (500 mg l^{-1})

0.240 g of $KAuCl_4$ was dissolved and volume was made up to 250 ml with 1 M H_2SO_4.

2.3 Instrument set ups

2.3.1 The set up of the automated on-line solvent extraction system for some heavy metal ions determination (Cu(II), Pb(II), Ni(II), Cd(II) and Zn(II)) and Cr(III)/Cr(VI) speciation

The set up of the system is shown in **Figure 2.1.** All components were controlled via a software program. Sample and DDC were delivered to the system by a peristaltic pump (P1; Ismatec, Model MS-CA 4/640, Switzerland). The sample was diverted to a mixing chamber or to waste via three-way solenoid valve (H). The mixing chamber was made from a 3 ml-plastic syringe. MIBK was aspirated into the system by a syringe pump (Org P; Model 351, Sage Instrument, USA). It was aspirated through a three-way solenoid valve (F). All the reagents and sample related to the extraction were transferred to the mixing chamber. In order to mix all reagents well and efficient, a small magnetic bar was used. After reagents and sample were mixed in the mixing chamber and then metal-complexes were extracted into an organic phase. After leaving for a while, organic and aqueous phases were separated from each other. The organic phase, which has lower density than water, being in an the upper layer was flowed to fill the sample loop by transferring water to the chamber with another peristaltic pump (P2; DESAGA, Struers sciencetific instruments, Denmark) via valve E, C, D to waste port. Then the valves E, C, D were switched simultaneously and metal-complexes in organic phase was transferred into the carrier stream of MIBK, which passed through the valve (B) to nebuliser of FAAS (Perkin Elmer, Model 5000, USA). After that methanol was sucked to clean the system by a vacuum (Heto type SUE3Q, Scandinavian). The rinsing procedure is necessary to avoid a carry over effect.

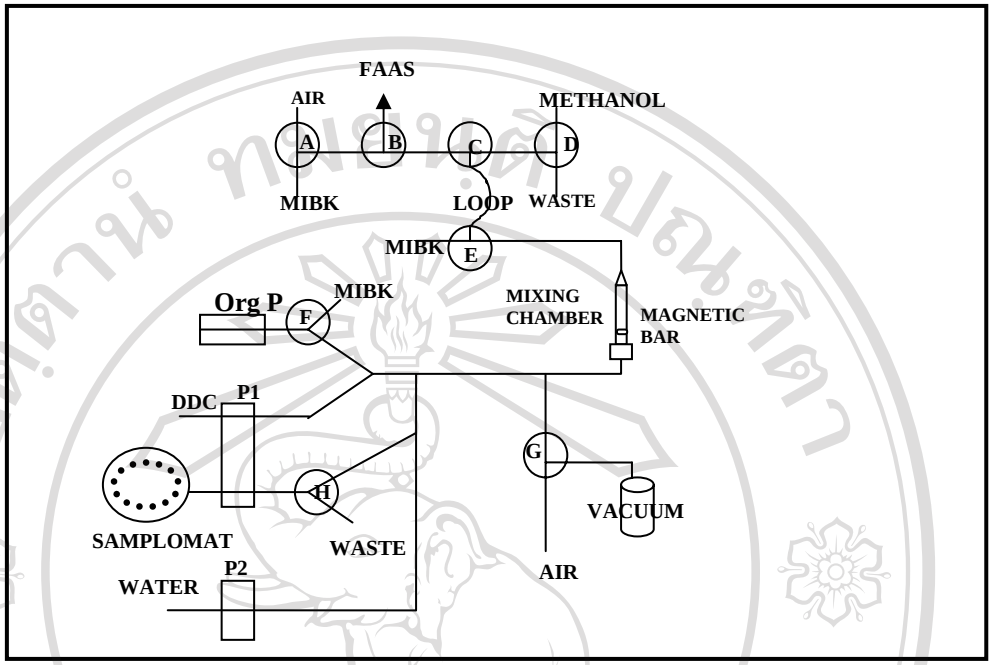


Figure 2.1 The set up of the automated on-line solvent extraction system for FAAS; P1= peristaltic pump1, P2 = peristaltic pump2, Org P= syringe pump for organic, A, B, C, D, E, F, G and H = three-way solenoid valves and FAAS = flame atomic absorption spectrophotometer

2.3.1.1 Operation procedure

The automated operation procedure was controlled by a computer. The procedure is described in each step below.

1. Pre-loading-sample/standard was aspirated to waste to replace previous sample/standard. This is to make sure that only current sample/standard was aspirated to the system.
2. Loading sample and reagents-sample/standard, DDC and MIBK were propelled to the system.

3. Extraction-the magnetic bar was activated to mix sample/standard, DDC and MIBK together very well. Then metal-complexes were extracted into MIBK phase.
4. Pause-extracted organic phase was separated from aqueous phase owing to its lower density than water.
5. Filling up with water-water was drawn into the mixing chamber to transfer the upper layer extract (organic phase) to the sample loop.
6. Injection-the extract (organic phase) in the sample loop was injected to the FAAS. The signal was recorded on PC.
7. Sample and reagents discard-the solution left in the chamber was transferred to waste by vacuum system.
8. Rinsing with methanol-to avoid carry over effect, chamber was rinsed with methanol to wash out the previous solution.
9. Emptying methanol-after rinsing, methanol was sucked to waste by vacuum system.
10. Finish-the procedure was end and the results were displayed on PC.

2.3.1.2 Initial conditions

The initial conditions for the heavy metal ions determination are presented below. The initial operation procedure is expressed in **Table 2.1.**

Chemicals: 1.0% DDC as organic ligand, MIBK for organic phase and methanol for rinsing solution were employed.

Flow rates: MIBK 1.2 ml min^{-1}, standard/sample 2.5 ml min^{-1}, DDC 1.2 ml min^{-1}

Ratio of aqueous/organic volume: ≈ 3

Organic solvent uptake rate: 4.0 ml min^{-1}

Table 2.1 Initial operation procedure of the automated on-line solvent extraction

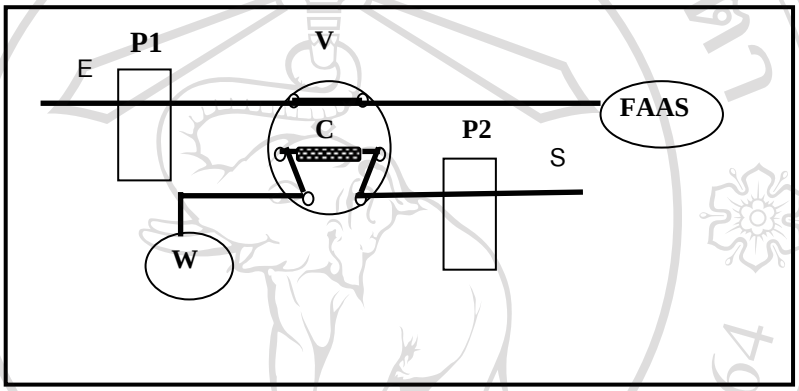
Procedure	Time (s)	Procedure	Time (s)
1. Pre-loading	10	6. Injection	10
2. Loading sample and reagents	40	7. Sample and reagents discard	20
3. Extraction	20	8. Rising with methanol	20
4. Pause	20	9. Emptying methanol	10
5. Filling up with water	12	10. Finish	5

2.3.2 The set up of FI on-line preconcentration for lead determination by FAAS

The manifold for FI in-valve mini-column for lead determination is illustrated in **Figure 2.2**. Perspex mini-column (C) with 3-mm i.d. and 20-mm length was incorporated within 6-port injection valve (V; Upchurch Scientific, Inc. Model V-451, USA) replacing one of sample loop. An immobilised crown ether resin (Sr.spec™) packed in the mini-column was used as an extractant. While the valve was at **inject position** (b), the eluent solution was aspirated through the column to admit the sample into the detector by using peristaltic pump1 (P1; Ismatec, Switzerland). EDTA was used as an eluent. When the injection valve was turned to **load position** (a), a sample solution in acidic medium was drawn through the mini-column by using another

peristaltic pump2 (P2; Eyela, MP-3, Japan). After a specific loading time, the valve was switched back to **inject position** and accumulated lead was eluted for continuously monitored at FAAS (Shimadzu, AA-670, Japan). Opposite flow direction of loading and eluting minimises problem due to column blocking.

(a) Load position



(b) Inject position

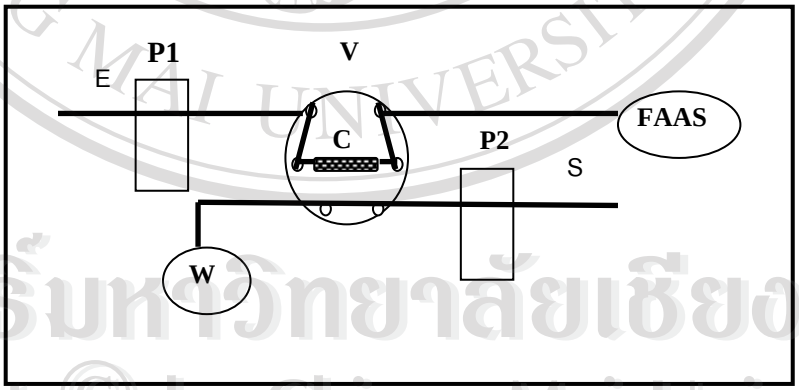


Figure 2.2 The manifold of FI in-valve mini-column for lead determination ((a) load position and (b) inject position), P1= peristaltic pump1, P2= peristaltic pump2, E= eluent, V= 6-port injection valve, S= sample/standard, C= mini-column packed with crown ether, W= waste and FAAS= flame atomic absorption spectrophotometer

Normally, calibration is made by varying sample concentration. However, in this work, single standard calibration was used instead of the normal one. Only one standard solution was used for constructing calibration graph by plotting between μg of lead sorbed on the resin and corresponding peak height. The μg of lead can be calculated from **equation 2.1**:

μg Pb = CxTxF ……equation 2.1

where C = sample/standard concentration, μg l^{-1}

T = loading time, min

F = flow rate of sample/standard, ml min^{-1}

Flow rate of standard and standard concentration were fixed, but loading time was varied. That means the **μg Pb** is propotional to the loading time. For sample determination, specific loading time was employed. By using single standard calibration, **μg Pb** will be obtained for sample. According to the **equation 2.1**, the concentration of Pb in sample can be calculated.

2.3.3 The set up of SI-column preconcentration for iron determination using FAAS

The set up of SI-column preconcentration for iron determination using FAAS(Shimadzu, AA-670, Japan) is shown in **Figure 2.3.** The SIA Analyser (Laboratory made, Center of Biotechnology, Turku University, Turku, Finland) was employed. In principle, Fe(II) can be retained on Dowex-50x8 resin which is a strong cation exchange resin. Then the sorbed Fe(II) was finally eluted by suitable eluent and

fed to detector. According to the operation procedure, water has to be aspirated to column for pre-conditioning step. Next, sample/standard of Fe(II) was passed through the column which placed between valve1 and valve2 for specific loading time. Then eluent was sent to strip accumulated Fe(II) on the column and detected with FAAS. Finally, column washing step was required to make sure sorbed Fe(II) can be released from column as much as possible. The sequence of the automatic procedure was performed via program as summarise in **Table 2.2**.

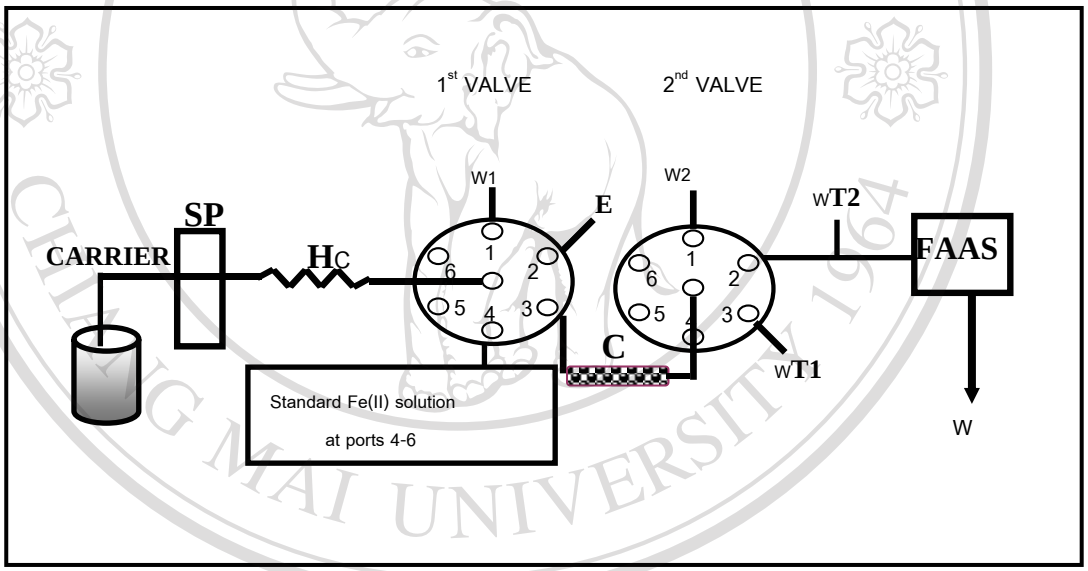


Figure 2.3 The set up of SI-column preconcentration for iron determination using FAAS: SP= syringe pump, HC= holding coil, W1 and W2= waste, FAAS = flame atomic absorption spectrometer, C= mini-column packed with Dowex-50x8, WT1 and WT2= DI-water and E= eluent (4 M HNO_3)

Table 2.2 Sequence of SIA operation for iron determination

Sequence	Volume, μl
1. Preconditioning column:	
1.1 Aspirate water (WT1) through column to HC	1,000
1.2 Aspirate water from reservoir to SP	2,500
1.3 Flow water forward from HC to W1	2,500
2. Fill line with standard	
2.1 Aspirate standard Fe(II) (port 4-6) to HC	500
2.2 Aspirate water from reservoir to SP	1,500
2.3 Send sample/standard to waste (W1)	1,500
3. Load sample on column	
3.1 Aspirate standard Fe(II) to HC	1,500
3.2 Aspirate water from reservoir to SP	1,000
3.3 Send standard Fe(II) through column to waste (W2)	2,500
4. Elute sample to detector	
4.1 Aspirate eluent (E) to HC	1,500
4.2 Aspirate water from reservoir to SP	1,000
4.3 Send E through column to strip Fe(II) to FAAS (During this step, WT2 was aspirated to FAAS by nebuliser)	2,500
5. Wash column with eluent	
5.1 Aspirate E to HC	1,500
5.2 Aspirate water from reservoir to SP	1,000
5.3 Send E through column to strip Fe to W2	2,500
5.4 Repeat 5.1-5.3 for 2 times	

2.3.4 The set up of SI-ASV for arsenic speciation

The set up of SI-ASV for arsenic speciation is depicted in **Figure 2.4**. It consists of SIA Analyser (Laboratory made, Center of Biotechnology, Turku University, Turku, Finland) and voltammograph with an electrochemical flow-cell (Bioanalytical System, 100B/W, USA). The operation procedure can be summarised in the **Table 2.3**.

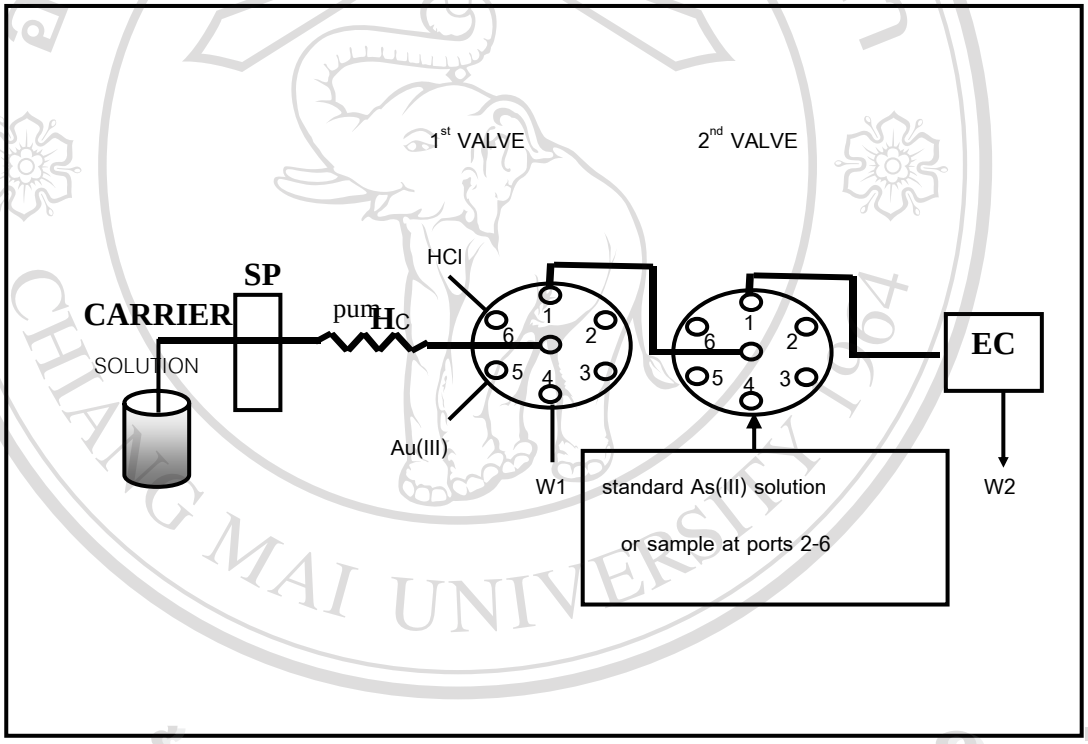


Figure 2.4 Schematic diagram of the SIA-ASV system for arsenic speciation determination: SP = syringe pump, HC = holding coil, W1 & W2 = waste and EC = electrochemical flow-cell

Table 2.3 Operation steps of SI-ASV

Step procedure	Time, s
1. Deaeration with pure nitrogen	300
2. Pre-plate gold film electrode	120
3. As(III) deposition	150
4. As(III) stripping and electrically detection	30
5. Cleaning EC with DI-water	25
6. Gold film stripping	30

2.3.4.1 Pre-plate gold film electrode

This was adapted from a report [90] for batch determination of As(III) and As(V). The glassy carbon electrode was used as substrate to plate gold film. Alumina slurry was used to polish electrode before plating. In order to plate gold film, Au(III) solution 500 mg l^{-1} was aspirated with the flow rate of 0.6 ml min^{-1} and apply the potential of -0.2 V vs. Ag/AgCl for 2 min. The gold solution was deposited on glassy carbon electrode (GCE) as a brown powder.

2.3.4.2 As(III) determination

As(III) in the range of 25-100 μg l^{-1} was determined. The solution was deaerated with pure nitrogen for 5 min. After the gold film electrode has been prepared by aforementioned process, the sample was flown through the flow cell described before while applying potential of working electrode at -0.3 V vs. Ag/AgCl. After deposition, stripping process was carried out in the differential pulse mode starting at -0.3 V with scan rate of 30 mV/s in positive direction to 0.5 V. Pulse width

of 50 ms and pulse height of 50 mV was applied. Duplicate determinations were performed.

2.3.4.3 Gold-film stripping

In order to minimise contamination from previous analysis, new gold film plating was required after at least 5 analysis cycles performed. The old gold film was removed by flowing HCl electrolyte through the electrochemical flow-cell while lying potential at 0.7 V vs. Ag/AgCl for a min.

2.3.4.4 Reduction of As(V) to As(III)

10 ml of 0.75 M ascorbic acid and 10 ml of 0.75 M KI were added into the standard/sample in a 100 ml-volumetric flask. The solution was left and shaken occasionally in order to completely convert As(V) to As(III). The reduction time was investigated. Then the solution was ready for total As determination.