CHAPTER 2

EXPERIMENTAL

2.1 Apparatus and chemicals

2.1.1 Apparatus

1) Atomic absorption spectrometer, model AAnalyst 800 (Perkin-Elmer, U.S.A)
2) THGA Graphite tube (Perkin-Elmer, U.S.A.)
3) Hollow Cathode Lamp (Perkin-Elmer, U.S.A.)
4) Micropipette: 20-100 µl, 30-200 µl, 200-1000 µl, P series (Gilson Medical Electronics, France)
5) Stirrer, model RH basic 1 and model C-MAG HS 7 with magnetic bar (Becthai Bangkok Equipment & Chemical Co., Ltd., Thailand)
6) Analytical balance, AB204-S series (Mettler Toledo, Switzerland)
10) Polyethylene (PE) bottles
11) A glass column (10 cm long and with 1 cm inner diameter)

2.1.2 Chemicals

1) Amberlite IRA-400 resin, Cl^- type, 16-50 mesh particle size, Sigma Aldrich, USA
2) Atomic absorption modifier solution 1000 mg/l, $Mg(NO_3)_2$ in 1% HNO_3, Perkin-Elmer, Norway
3) Atomic absorption modifier solution 1000 mg/l, $Pd(NO_3)_2$ in 15% HNO_3, Perkin-Elmer, Norway

4) Tin (IV) chloride pentahydrate ($SnCl_4.5H_2O$), 98%, M.W. 350.58, GR, Aldrich, Germany

5) Tin (II) chloride ($SnCl_2.2H_2O$), 97%, M.W. 225.63, AR, S.D. Fine Chem, India

6) Sodium chloride (NaCl), 99.5%, M.W. 58.443, RPE, Carlo Erba, Italy

7) Sodium hydroxide (NaOH), 98%, M.W. 39.997, RPE, Carlo Erba, Italy

8) Hydrochloric acid (HCl), 37.0% v/v, GR, Merck, Germany

9) Nitric acid (HNO_3), 65% v/v, GR, Merck, Germany

10) Tin (IV) standard solution 1000 ppm, AAS grade, Merck, Germany

11) Tin (II) standard solution 1000 ppm, AAS grade, BDH Chemicals, England

12) Lead (II) nitrate ($Pb(NO_3)_2$), 99.0%, M.W. 331.20, RPE, Carlo Erba, Italy

13) Nickel (II) nitrate ($Ni(NO_3)_2.6H_2O$), 99.0%, M.W. 290.81, RPE, Carlo Erba, Italy

14) Copper (II) nitrate ($Cu(NO_3)_2.3H_2O$), 99.5%, M.W. 241.60, RPE, Carlo Erba, Italy

15) Chromium (III) nitrate ($Cr(NO_3)_3.9H_2O$), 98%, M.W. 400.15, GR, Merck, Germany

16) Cadmium (II) nitrate ($Cd(NO_3)_2.4H_2O$), 99.0%, M.W. 308.47, Purum, Fluka, Germany

17) Ferrous sulfate ($FeSO_4.7H_2O$), 99.0%, M.W. 278.05, RPE, Carlo Erba, Italy

2.2 Preparation standard solutions and reagents

The ultrapure water from Milli-Q system (Millipore) was used for preparing all solutions. All labware used for handling solutions was cleaned with detergent solution, rinsed with tap water, soaked for at least 24 hours in 10% nitric acid solution, rinsed with ultrapure water and finally dried before use.

2.2.1 Hydrochloric acid solution

Stock solutions of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 M hydrochloric acid were prepared by adding 4.1, 8.2, 12.2, 16.3, 20.4, 24.5, 28.6, 32.7 and 36.8 ml of concentrated hydrochloric acid in ultrapure water and adjusting to 50 ml with ultrapure water in volumetric flask.

2.2.2 Sodium hydroxide solution

Stock solutions of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 M sodium hydroxide were prepared by dissolving 2.1, 4.1, 6.8, 8.2, 10.3, 12.4 and 14.4 g of sodium hydroxide in ultrapure water and adjusting to 50 ml with ultrapure water in volumetric flask.

2.2.3 Sodium chloride solution

Stock solution of 6.0 M sodium chloride was prepared by dissolving 35.24 g of sodium chloride in ultrapure water and adjusting to 100 ml with ultrapure water in volumetric flask.

2.2.4 Nitric acid solution

For the GFAAS determination, the 0.2% v/v nitric acid solution was prepared daily by adding 1.54 ml of concentrated nitric acid solution in ultrapure water and adjusting to 500 ml with ultrapure water in volumetric flask.

A 2% v/v nitric acid solution was prepared by adding 15.4 ml of concentrated nitric acid solution in ultrapure water and adjusting to 500 ml with ultrapure water in volumetric flask.

2.2.5 Tin (II) chloride solution

Stock solution of 1000 mg/l tin (II) chloride was prepared by dissolving 0.1959 g of tin (II) chloride in ultrapure water and adjusting to 100 ml with ultrapure water in volumetric flask.

2.2.6 Tin (IV) chloride solution

Stock solution of 1000 mg/l tin (IV) chloride was prepared by dissolving 0.3013 g of tin (IV) chloride in ultrapure water and adjusting to 100 ml with ultrapure water in volumetric flask.

2.2.7 Interfering ion solution

Stock solution of 1000 mg/l interferring ions was prepared by dissolving 0.3820 g of $Cu(NO_3)_2$, 0.7852 g of $Cr(NO_3)_3$, 0.1615 g of $Pb(NO_3)_2$, 0.5005 g of $Ni(NO_3)_2$, 0.2744 g of $Cd(NO_3)_2$ and 0.0015 g of Zn powder in 2 % v/v HNO_3 and adjusting to 100 ml with 2 % v/v HNO_3 in volumetric flask.

2.2.8 Preparation of model solutions

2.2.8.1 Model solution for study on adsorption of Sn(II) and Sn(IV)

Model solution 200 µg/l of Sn(II) and Sn(IV) was prepared by transferring 20.00 ml of Sn(II) and Sn(IV) stock solution 1000 µg/l into 100 ml volumetric flask and adjusting with 0.2% v/v HNO_3.

2.2.8.2 Model solution for optimization of Sn(IV)

Model solution 200 µg/l of Sn(IV) was prepared by transferring 20.00 ml of Sn(IV) stock solution 1000 µg/l into 100 ml volumetric flask and adjusting with 0.2 % v/v HNO_3.

2.2.8.3 Model solution for study on interfering ions

Model solution of 0.1, 0.2, 1, 2, 3, 50, 250 and 500 mg/l interfering ions were prepared by transferring 20.00 ml of Sn(IV) stock solution 1000 µg/l and transferring 0.01, 0.02, 0.10, 0.20, 0.30, 5.00, 25.00 and 50.00 ml of interfering ion stock solution 1000 mg/l into 100 ml volumetric flask. Then, the solution was adjusted with 0.2 % v/v HNO_3.

2.3 Optimization of separation method

The several parameters that influence on the separation efficiency should be studied and optimized. There factors are eluent concentration, flow rate, amount of resin, elution volume, sample volume, time on adsorption of Sn(IV) and interfering ions. The separated solutions were analyzed by graphite furnace atomic absorption spectrometry. The optimum conditions in this study were obtained by the maximum of tin condition.

The procedure for optimization of Sn(IV) was tested by model solution, which contained 0.2 µg/ml of Sn(IV). A 400 mg of Amberlite IRA-400 resin was packed into a glass column. The column was passed through by model solution. Then, Sn(IV) was eluted by NaOH solution. The eluate was analyzed by graphite furnace atomic absorption spectrometry (GFAAS) for tin content.

2.3.1 Optimization of HCl concentration on adsorption of Sn(II) and Sn(IV)

The optimization of HCl concentration on adsorption of Sn(II) and Sn(IV) were studied by varying the HCl concentration in the range of 1.0-9.0 mol/l.

2.3.2 Optimization of NaOH concentration on desorption of Sn(IV)

The optimization of NaOH concentration on the recovery of Sn(IV) were studied by varying the NaOH concentration in the range of 1.0-7.0 mol/l.

2.3.3 Optimization of flow rate

The optimization of flow rate of model solution and eluent was investigated from 0.5 to 3.0 ml/min.

2.3.4 Optimization of resin amounts on desorption of Sn(IV)

The optimization of resin amounts was studied by varying the amounts of resin in the range of 200-700 mg.

2.3.5 Optimization of sample volume

The optimization of sample volumes on the recovery of Sn(IV) was investigated from 5 to 30 ml.

2.3.6 Optimization of eluent volume

The optimization of eluent volume was studied by varying the eluent volumes in the range of 1.0-6.0 ml.

2.3.7 Optimization of time on adsorption of Sn(IV)

The optimization of times on adsorption of Sn(IV) was varied from 0.5 to 6.0 hours.

2.3.8 Optimization of interfering ion concentration on separation of Sn(IV)

The optimization of interfering ions concentration was investigated in the range of 0.1-1000 mg/l of Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Fe^{2+} and Cr^{3+}.

2.4 Preparation of wastewater samples

The wastewater samples were filtered through a 0.45 μm pore size membrane filter to remove suspended particulate matter. The pH of the wastewater sample was measured. Then, this solution was transferred into PE bottle and kept in refrigerator for further use.

2.4.1 Determination of total tin in samples

A 10 ml concentrated nitric acid and 10 ml H_2O_2 was added to a 100 ml wastewater sample and heated at 100 °C for 6 hours to decompose the organic tin compounds. After that the wastewater samples were adjusted to pH 3.0 by adding ammonia and the volume was made up to 100 ml [22].

2.4.2 Procedures for the speciation of tin in wastewater samples

2.4.2.1 Resin preparation

In pretreatment of Amberlite IRA 400 resin (Cl^- type), it was purified by washing with saturated NaCl solution, deionized water, 1.5 mol/l HCl solution, deionized water, 1.5 mol/l NaOH solution, deionized water and saturated NaCl solution sequentially, in order to eliminate the contamination of trace metal ions, other inorganic and organic compounds. After that step, the resin was finally rinsed with deionized water and air-dried [6].

2.4.2.2 Procedures for the speciation of tin in wastewater samples

The 20 ml of wastewater sample was transferred into Amberlite IRA-400 resin column which passed through at a flow rate of 2.0 ml/min. Then, Sn(II) was eluted by 5.0 ml of 5.0 mol/l HCl at a flow rate of 2.0 ml/min. After 120 minutes, Sn(IV) was eluted by 3.0 ml of 2.0 mol/l NaOH at a flow rate of 2.0 ml/min. All effluents were collected into PE bottle and the analyte in eluted solution was detected by graphite furnace atomic absorption spectrometry (GFAAS) [6].

2.5 Preparation of the working standard solutions

Concentration of stock tin solution 1000 µg/l was prepared by transferring 25 µl of standard tin solution 1000 mg/l into 25 ml volumetric flask and adjusting with 0.2% v/v nitric acid. For the preparation of calibration curve at concentrations of 30, 60, 100, 120 and 150 µg/l were prepared by transferring portions of 300, 600, 1000, 1200 and 1500 µl of stock solution into 10 ml volumetric flask. Then, 25 µl of palladium modifier solution and 15 µl of magnesium modifier solution were added into volumetric flask. Finally, the solution was made up to 10 ml with 0.2% HNO_3 in volumetric flask.

2.6 Analytical characteristics of the method

1) Precision

The precision of the GFAAS instrument was studied by analyzing 100 µg/l standard solutions ten times. The relative standard deviation was calculated.

2) Detection limit

The detection limit of the method for the determination of tin was determined by analyzing blank ten times.

3) Accuracy

Accuracy was calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample.

The separated sample was prepared by spiking 80 µg/l of Sn(IV) standard solutions. The recovery was replicated three times and the results were calculated from the following equation.

$$\% \text{ Recovery} = \frac{spike\ sample\ result - sample\ result}{spike\ amount\ added} \text{ x } 100$$

2.7 Instrumentation

A Perkin Elmer AAnalyst 800 atomic absorption spectrometer equipped with THGA graphite furnace and Zeeman effect background corrector was used. A Sn Lumina hollow cathode lamp operated at 30 mA was used as the resonance line source. For graphite furnace measurements, argon (Ar) was used as inert gas. The operating parameters of instruments for tin were set according to the manufacturer recommendation. These conditions were given in **Table 2.1** and **Table 2.2**. Perkin-Elmer pyrolytic-coated graphite tubes with a platform were used. Sample solutions were injected into the furnace using Perkin Elmer AS-800 autosampler. The injected volume of the sample was 20 µl. The signals were measured as peak areas. The range of the calibration standards for tin was 30-150 µg/l.

Table 2.1 Instrument settings and analytical conditions

Parameters	Conditions
Wavelength (nm)	286.3
Slit width (nm)	0.7
Argon flow (ml/min)	250
Atomization site	Pyro/Platform
Reading time (s)	5
Pretreatment temperature (°C)	1400
Atomization temperature (°C)	2200

Table 2.2 Furnace heating program for tin determination

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Gas Flow (ml/min)
Dry 1	110	1	30	250
Dry 2	130	15	30	250
Pyrolysis	1400	10	20	250
Atomize	2200	0	5	0
Clean-out	2450	1	3	250

2.8 Wastewater samples

The wastewater samples were collected from various industry sites in Chiang Mai and Lamphun, Thailand. The No. 1, No. 2, No. 3 No. 4, No. 5 and No. 6 wastewater samples were collected in Chiang Mai and the No. 7 and No. 8 wastewater were collected in Lamphun. The wastewater samples from industry types were described in **Table 2.3.**

Table 2.3 The wastewater samples from various industry type

Sample	Industry type
No. 1	Wastewater treatment plant 1 (Chiang Mai University)
No. 2	Wastewater treatment plant 2 (Chiang Mai University)
No. 3	Sport equipment production
No. 4	Concrete production
No. 5	Electronic production
No. 6	Concrete production
No. 7	Northern Industrial Estate 1
No. 8	Northern Industrial Estate 2