CHAPTER 2

Experimental

2.1 Chemicals, apparatus and instruments

2.1.1 Chemicals

All chemicals used in this research are analytical reagent grade. All solutions were prepared by dissolving in ultrapure water (Milli Q water, resistivity of 18.2 MΩ cm^{-1}). The chemicals used are listed below.

1. Acetic acid (99.7% purity): CH_3COOH (RCI Labscan, Thailand)
2. Bismuth nitrate pentahydrate: $Bi(NO_3)_3 \cdot 5H_2O$ (Carlo Erba, Italy)
3. Cadmium chloride dihydrate: $CdCl_2 \cdot 2H_2O$ (Fluka, Switzerland)
4. Hydrochloric acid (37% purity): HCl (RCI Labscan, Thailand)
5. Lead nitrate: $Pb(NO_3)_2$ (Merck, Germany)
6. Nitric acid (65% purity): HNO_3 (Carlo Erba, France)
7. Potassium hydroxide: KOH (Merck, Germany)
8. Sodium acetate trihydrate: $CH_3COONa \cdot 3H_2O$ (Carlo Erba, France)
9. Sodium hydroxide: NaOH (Merch, Germany)
10. Sulfuric acid: H_2SO_4 (96% purity), (RCI Labscan, Thailand)
11. Zinc sulfate heptahydrate: $ZnSO_4 \cdot 7H_2O$ (Ajex Finechem, Australia)

2.1.2 Materials and instruments

1. Micropipette 20, 100 and 1000 μL (Eppendorf, Germany)
2. pH meter model 744 (Metrohm, Switzerland)
3. Plasma cleaner (PDC-32G, Harrick, USA)
4. Voltammetric analyzer (VA-757, Metrohm, Switzerland)

2.2 Preparation of reagent

2.2.1 Acetate buffer solution, 0.1 M pH 4.5

$CH_3COONa{\cdot}3H_2O$ 2.50 g was dissolved in 400 mL Milli Q water and CH_3COOH 1.80 mL was added into the solution. The pH of solution was adjusted to pH 4.5 by adding 10 M KOH solution under pH monitoring and made up to a final volume of 500 mL with Milli Q water.

2.2.2 Acetic acid solution, 4% (v/v)

CH_3COOH 20.00 mL was diluted in 500 mL Milli Q water.

2.2.3 Acetic acid solution, 4% (v/v) pH 4.5

CH_3COOH 20.00 mL was diluted in 400 mL Milli Q water. The pH of solution was adjusted to pH 4.5 by adding 10 M KOH solution under pH monitoring and made up to a final volume of 500 mL with Milli Q water.

2.2.4 Metal standard solutions

Stock standard solution of cadmium(II) 1000 mg L^{-1} was prepared by dissolving 0.2032 g of $CdCl_2{\cdot}2H_2O$ in 0.1 M HCl and made up to a final volume of 100 mL with Milli Q water in a volumetric flask.

Stock standard solution of lead(II) 1000 mg L^{-1} was prepared by dissolving 0.1607 g of $Pb(NO_3)_2$ in 0.1 M HNO_3 and made up to a final volume of 100 mL with Milli Q water in a volumetric flask.

Stock standard solution of zinc(II) 1000 mg L^{-1} was prepared by dissolving 0.4444 g of $ZnSO_4{\cdot}7H_2O$ in 0.1 M H_2SO_4 and made up to a final volume of 100 mL with Milli Q water in a volumetric flask.

2.3 Preparation of working electrodes

2.3.1 Bismuth film electrode

Stock standard solution of bismuth(III) 1000 mg L^{-1} was prepared by dissolving 0.2368 g of $Bi(NO_3)_3{\cdot}5H_2O$ in 0.5 M HNO_3 and made up to a final volume of

100 mL with Milli Q water in a volumetric flask. The bismuth ions solution was *in situ* modified on carbon electrodes, it was directly added to the samples solution and electrochemically deposited simultaneously with the cadmium, lead and zinc ions on the electrode surface during the analysis.

2.3.2 Glassy carbon electrode

The commercially glassy carbon electrode (3 mm diameter, Metrohm, Switzerland) was polished with 1 μm alumina slurry on a polishing pad for about 10 min, then washed with deionized water to obtain a fresh surface.

2.3.3 Graphite electrode

The graphite electrode was made from graphite rod (3 mm diameter, Aldrich, USA). It was polished on paper until shiny surface was obtained or graphite does not slide on to the paper. Then, the graphite electrode was polished with 1 μm alumina slurry on a polishing pad for about 30 min, then washed with water to obtain a fresh surface.

2.3.4 SPCE electrode

The SPCEs were made in laboratory by manual screen printing [Reanpang *et al.*, 2015]. The diameter of electrode was 3 mm. The first layer on the PVC substrate is made from carbon ink (Acheson, Singapore) and then drying in an oven for 30 min at 150 ^{o}C. Then, the carbon ink was used to print again as the second layer and drying in the oven as the same. Finally, an insulating ink was printed to cover the connections and define the working electrode area. Before used as a working electrode, the SPCE was cleaned in plasma cleaner chamber. The plasma cleaner was operated at pressure 0.4 torr for 60 s. Finally, the electrode was electrochemically cleaned from any remaining traces of metals at -1.6 V for 60 s in buffer solution under stirring at 2000 rpm.

2.4 Voltammetric system

Cyclic voltammetry (CV) and anodic stripping voltammetry (ASV) experiments were conducted with the electrochemical analyzer (VA-757, Metrohm, Switzerland) with three electrode electrochemical cell. Bismuth modified on carbon electrodes were used as a working electrode, a platinum wire as an auxiliary electrode and Ag/AgCl (3M KCl) as a reference electrode. These electrodes were immersed in the cell containing the electrolyte solution.

2.5 Study on sensitivity of different carbon electrodes

The glassy carbon, graphite and SPCE electrode were studied for the background current in 0.1 M acetate buffer solution (pH 4.5) and were compared sensitivity of these electrodes for measurement of 1.0 mg L^{-1} of bismuth *in situ* plated in 0.1 M acetate buffer solution (pH 4.5) containing 30 μg L^{-1} of cadmium, lead and zinc.

2.6 Optimization of ASV system

2.6.1 Study on effect of concentration of acetate buffer solution

The effect of concentration of acetate buffer was investigated by varying concentrations of acetate buffer solution at 0.01, 0.05, 0.10, 0.20 and 0.50 M. The optimal concentration was chosen by considering slope of calibration graphs of standard solutions of cadmium and lead in the range of 5 to 30 μg L^{-1} and zinc in the range of 10 to 40 μg L^{-1}. Conditions are as follow: a deposition potential of -1.2 V (vs Ag/AgCl), deposition time of 300 s, frequency of 50 Hz and voltage step of 5 mV, acetate buffer solution pH 4.5 as an electrolyte medium.

2.6.2 Effect of pH of acetate buffer solution

The effect of pH of acetate buffer was investigated by varying pH at 3.5, 4.0, 4.5, 5.0 and 5.5 while other conditions were the same as in part 2.6.1 and concentration of acetate buffer was 0.1 M.

2.6.3 Effect of bismuth concentration

The effect of bismuth concentration was investigated by varying concentrations of bismuth(III) ion at 0.0, 0.1, 0.4, 0.6, 0.8, 1.0, 1.5 and 2.0 μg L^{-1} and other conditions were the same as in part 2.6.2, with buffer pH 4.5. The optimal bismuth concentration was chosen by considering peak current of 30 μg L^{-1} for cadmium and lead and 60 μg L^{-1} for zinc.

2.6.4 Effect of deposition time

The effect of deposition time was investigated by varying time of deposit at 15, 30, 60, 90, 120, 180, 210, 240, 270, 300, 360 and 420 s and other conditions were the same as in part 2.6.3, and bismuth concentration of 0.4 μg L^{-1} was used.

2.6.5 Effect of deposition potential

The effect of deposition potential was investigated by varying potential of deposit at -0.9, -1.0, -1.1, -1.2, -1.3, -1.4 and -1.5 V and other conditions were the same as in part 2.6.4, with deposition time 240 s.

2.6.6 Effect of rotation speed of stirrer

The effect of rotation speed during deposition step was investigated by varying rotation speed of stirrer at 400, 600, 800, 1000, 2000 and 3000 rpm and other conditions were the same as in part 2.6.5, and the deposition potential was -1.3 V.

2.7 Analytical characteristics

2.7.1 Calibration curves

The analytical performance was investigated by simultaneous analysis of cadmium, lead and zinc. The square wave anodic stripping voltammograms were recorded for different concentrations of heavy metal ions.

2.7.2 Limits of detection

The limits of detection (LODs) were the lowest concentration of heavy metal ions distinguished from the peak current of blank. LODs were estimated from the three times standard deviation of y-intercept and slope of calibration graph which calculated from the equation 2.1 [Rojanarata, 2010].

$$\mathrm{LOD} = \frac{[(\bar{X} + 3\mathrm{S.D.})] - c}{m} \qquad 2.1$$

When $\bar{X}$ = mean of blank signal

S.D. = standard deviation of y-intercept from calibration graph

c = y-intercept of calibration graph from $y = mx + c$

m = slope of calibration graph from $y = mx + c$

2.7.3 Precision

The precision of the method was examined including of repeatability and reproducibility. The repeatability of the signal response of the Bi-SPCE was investigated by determining of heavy metal ions for 20 replicated measurements on the same electrode and the reproducibility was studied by determining of heavy metal ions for 2 replicated analyses each on 9 electrodes. The percentage of relative standard deviation (%RSD) value was used to evaluate the precision which calculated from the equation 2.2.

$$\%\mathrm{RSD} = \frac{\mathrm{S.D.}}{\bar{X}} \times 100 \qquad 2.2$$

When; S.D. = standard deviation

$\bar{X}$ = mean value

2.7.4 Accuracy

The accuracy was examined from the percentage of recovery. The recovery was performed by determining concentration of sample solution and concentration of spiking standard solution of heavy metals into samples solution via ASV. The recovery percentages were calculated from the equation 2.3.

$$\%\mathrm{Recovery} = \frac{(C\ \mathrm{sample{+}std} - C\ \mathrm{sample})}{C\ \mathrm{std}} \times 100 \qquad 2.3$$

When; $C_{\mathrm{sample+std}}$ = concentration of spiking standard solution into samples solution

C_{sample} = concentration of sample solution

C_{std} = concentration of spiking standard solution

2.8 Interferences

The interference study was performed by spiking various potential interfering metal cations including calcium(II), magnesium(II), ferrous(II) and copper(II) and anions including chloride, sulfate and carbonate in 100, 500 and 1000 µg L^{-1} into a standard solution containing of 30 µg L^{-1} cadmium, lead and 60 µg L^{-1} zinc. The results were reported as the percentage of difference and were calculated from the equation 2.4.

$$\%\text{Difference} = \frac{(S_{\text{interference}} - S_{\text{std}})}{S_{\text{std}}} \times 100 \qquad 2.4$$

When; S_{std} = peak current signal of standard solution

$S_{\text{interference}}$ = peak current signal of spiking interfering ion into the standard solution

2.9 Preparation of wastewater samples for analysis

2.9.1 Preparation of column resin for treating heavy metals

The Chelex 100 was used to treat heavy metals containing wastewater. The chemical properties of Chelex 100 as reported by the suppliers are given in the Table 2.1. The column method was used, ion exchange column was prepared by adding 5.0 g resin into column and was perfused through by solution of 1 M HCl and 1 M NaOH to remove possible impurities and adjusting pH of resin for acting as a cation exchanger as shown in Figure 1.6. Finally, resin column was washed with distilled water and the samples solution was passed through the column.

Table 2.1 Chemical properties of the chelating resins used

Properties	**Chelex 100**
Grade	Laboratory grade
Type	Styrene lattice with iminodiacetic acid sodium form
Particle size (mesh)	50 - 100
Capacity (meq mL^{-1})	0.4
Density (g mL^{-1})	0.65

2.9.2 Preparation of sample for analysis

All of 3 water samples being collected are surface natural water from around Chiang Mai University. Before being analyzed, samples were filtered through a filter paper (whatman No.1). The analytical application to real sample was investigated in 3 sections including (1) as collected water sample, (2) water sample as in (1) spiking with toxic metals (cadmium, lead and zinc) standard solution of 10 mg L^{-1}, and (3) water sample as in (2) after treatment by passing through the resin column. In case of water that treated by passing through the resin column, a constant flow rate 1 mL min^{-1} was used and 50 mL of effluent water were kept for each fraction, and fractions 2 and 4 were determined for heavy metal concentration. The percentage removal efficiency of the metal ion was calculated for each run from the equation 2.5.

$$\%\text{Removal efficiency} = \frac{C_{\text{initial}} - C_{\text{final}}}{C_{\text{initial}}} \times 100 \qquad 2.5$$

When; C_{initial} = initial concentration of metal ions

C_{final} = final concentration of metal ions

2.10 Preparation of ceramic samples for analysis

2.10.1 Effect of pH of supporting electrolyte

The effect of pH of supporting electrolyte was investigated by varying pH of 4% (v/v) acetic acid solution. The Bi-SPCE was used to determine heavy metals in extractant solution (pH 2.3) and pH adjusting extractant solution (pH 4.5).

2.10.2 Preparation of samples for analysis

Extraction of metals from glazed ceramic surface was carried out according to the standard method. First, ceramic sample was cleaned with detergent and dried in air. Then, it was filled with 4% (v/v) acetic acid and left for 24 h in a dark location. After 24 h, the extracted solution was collected in a plastic container for further voltammetric determination of the released metals. Before transferring to the container, the sample solution was adjusted to pH 4.5 by adding 10 M KOH solution under pH monitoring and made up with 4% acetic acid solution (pH 4.5) to a final volume of 100.0 mL.