CHAPTER 2

EXPERIMENTAL

2.1 Chemical stand materials

2.1.1 Standard chemicals

- Stock solution (1,000 mg L^{-1}) of lead (SCP Science, USA)

2.1.2 General chemicals and solvents

- Cadmium nitrate-4- hydrate (MERCK, Germany)
- Mercury nitrate (MERCK, Germany)
- Copper nitrate (MERCK, Germany)
- Lead nitrate (MERCK, Germany)
- Xanthone 97.0 % (Fluka, Switzerland)
- Xanthene 98.0 % (Fluka, Switzerland)
- Acridone 98.0 % (Fluka, Switzerland)
- Thioxanthone 97.0 % (Fluka, Switzerland)
- Ammonium acetate 97% (APS, Australia)
- Sodium acetate (APS, Australia)
- Potassium nitrate (APS, Australia)
- Sodium nitrate (BDH, England)
- Tris(hydroxymethyl)aminomethane (MERCK, Germany)
- Nitric acid 69-70% (w /v), AR grade (J.T. Baker, USA.)
- Sulfuric acid 96% (w /v), AR grade (LAB-SCAN, Ireland)
- Perchloric acid 70% (w /v), AR grade (Panreac, Spain)
- Ammonium hydroxide 28.0-30.0%, A.C.S reagent (J.T. Baker, USA.)
- 8-Hydroxyquinoline 99.99 % (Fluka, Switzerland)
- Acetonitrile (LAB-SCAN, Ireland)
- Tetrabutylammoniumhexafluorophosphate: TBAP (Fluka, Switzerland)
- Graphite powder, < 20 Micron (Aldrich, Germany)

- Liquid paraffin (BDH, England)
- de-ionized water

2.1.3 Samples

Canned fish samples (mackerel in tomato sauce) of ten brands were purchased from Lotus supermarkets (sampling date 5 July 2007).

2.2 Instruments and apparatus

2.2.1 AUTOLAB PGSTAT 100 (Metrohm, Switzerland)

- AUTOLAB PGSTAT 100 combined the general purpose electrochemical system (GPES) software is a computer controlled electrochemical measurement system. It consists of a data acquisition system and a potentiostat/galvanostat shown in Figure 2-1.

2.2.2 Electrochemical cell and electrodes

- Glassy carbon electrode (2 millimeter diameter) served as the working electrode for study electrochemical behavior (Metrohm, Switzerland)
- A hanging mercury drop electrode (HMDE) from the multi-mode electrode (MME, Metrohm, Switzerland) as the working electrode.
- Platinum wire served as the auxiliary electrode (Metrohm, Switzerland)
- Ag/AgCl (3 M KCl) served as the reference electrode (Metrohm, Switzerland)
- Stirrer (Metrohm, Switzerland)
- 10 mL of electrochemical cell (Metrohm, Switzerland)

2.2.3 Apparatus

- pH meter Model 15 (Denver Instrument, USA)
- Hot plate (Fisher Scientific, USA)
- Microlitre pipette model: P20, P100 and P 1,000 µg L^{-1} (Gilson, France)
- General glassware such as volumetric flask and beaker
- Polyethylene bottles
- Polyethylene bags

2.2.4 Carbon paste electrode (CPE)

- Teflon rod (0.8 and 2 centimeter of diameter)
- Copper wire (2 millimeter of diameter)
- Filter paper (Whatman, No. 42)
- Transparent paper

Figure 2-1 AUTOLAB PGSTAT 100 (Metrohm, Switzerland)

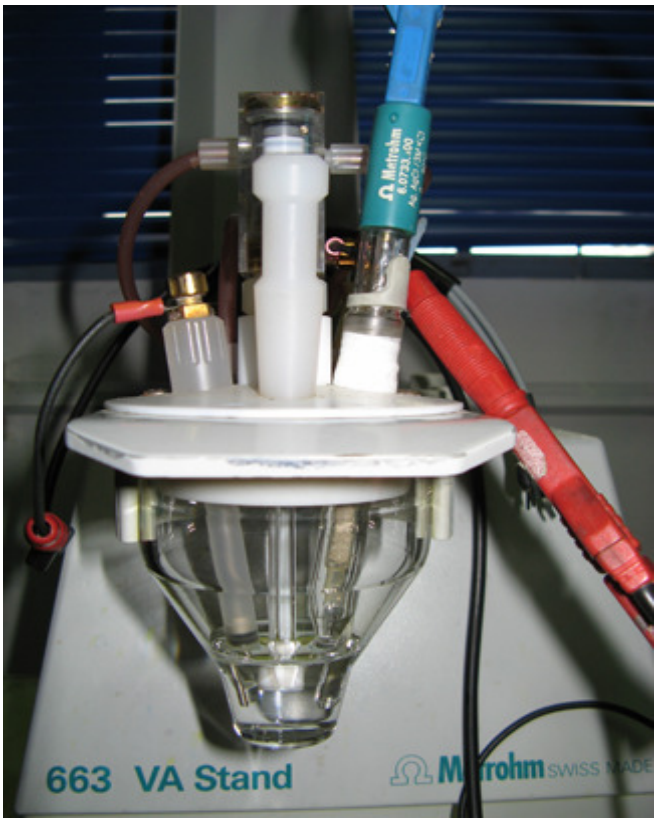

Figure 2-2 The cell with three electrode system

2.3 Methodology

2.3.1 Preparation of stock standard solution

Stock standard solution of 1,000 µg L^{-1} Pb(II) was prepared by using 1,000 mgL^{-1} Pb(II) standard solution and diluted for the corresponding stock solution.

Stock solution of 10^{-2} M 8-hydroxyquinoline was prepared in 0.2 M HCl and diluted with de-ionized water.

2.3.2 Cleaning of glassware and plastic ware

All glassware and plastic ware were soaked in 10% (v/v) nitric acid for 48 h followed by thorough rinsing with de-ionized water (Tarley *et al*., 2001).

2.3.3 Preparation of chemically modified carbon electrode

The configuration of the carbon paste electrode was obtained with a Teflon rod. The electrode body consists of two parts due to the face so that it can be compatible with the Metrohm system. The first part, Teflon, was cut to obtain a 5 centimetre piece and drilled to have the inside diameter 2 millimetre and drilled again at one end to expand the inside diameter to 4 millimetre (Mousavi *et al.,* 2001) with the depth of 5 millimetre. The copper with the diameter of 2 millimetre and 13 centimeter length was inserted into the other end through the centre of the rod to obtain a body of working electrode. The second part, the Teflon holder of working electrode was prepared as follows. Teflon rod was cut to obtained a 3 centimetre piece as Teflon holder and then drilled to have the inside diameter 2 millimetre and making its has shape trapeze cylindrical, one end has the outside diameter 1.6 centimetre and the other end has the outside diameter 0.8 millimetre. After that the second part was inserted in the first part to obtain the main probe (Rannurags, 2006).

The chemically modified carbon paste electrodes were prepared from three components: graphite power, modifier and binder. Modified carbon pastes of two different compositions were prepared by thoroughly hand-mixing 0.2775 g graphite powder and 0.0225 g group of xanthone compounds (referred as 7.5% modifier); and 0.2250 g graphite powder and 0.0450 g group of xanthone compounds (referred as 15% modifier); respectively, in a mortar and pestle. The liquid paraffin 0.1 mL was added and ground again until obtaining a homogeneous paste. (Mousavi *et al*., 2001). The pastes were packed into the hole of the electrode with spatula. The electrode surface was polished with filter paper follow transparent paper until shiny. The blank carbon electrode was prepared in the same way with out adding modifier. Figure 2-3 shows the complete chemically modified carbon paste electrode.

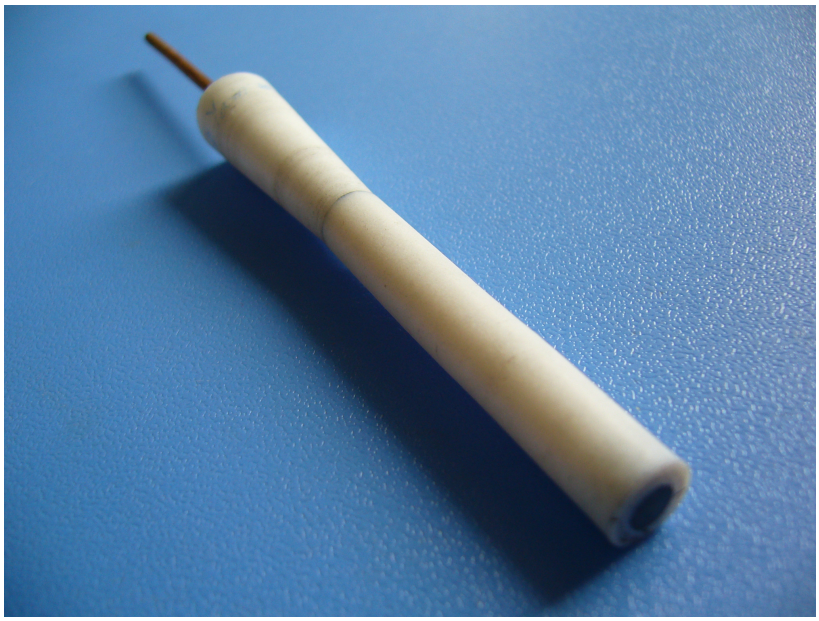

Figure 2-3 Show the complete chemically modified carbon paste electrode

2.3.4 Cyclic voltammetry procedures

2.3.4.1 Blank cyclic voltammetry experiments

All blank cyclic voltammetry experiments were carried out by purging nitrogen gas 3 min to eliminate interfering oxygen in 0.1 M Tetrabutylammoniumhexafluorophosphate (TBAP) and 50 mL CH_3CN solution at room temperature in a three electrode measuring cell. The working electrode as glassy carbon electrode was polished by alumina powder followed by cleaning with tissue paper (Kimberly Clark). The auxiliary electrode was a platinum wire and Ag/AgCl, 3M KCl electrode was used as the reference.

2.3.4.2 Cyclic voltammetry of metal ion in acetonitrile

Metals such as cadmium, copper, lead and mercury in 50 mL CH_3CN containing 0.1 M TBAP as electrolyte were added in electrochemical cell. The cyclic voltammograms were run after purging the solution for 3 min with nitrogen gas to eliminate interfering oxygen. Then cyclic voltammograms of each compounds solution were carried out at scan rate 100 mV s^{-1} and step potential 20 mV. Cyclic voltammogram was run compared with the blank solution.

2.3.4.3 Cyclic voltammetry of group of xanthone compounds

The group of xanthone compounds (xanthone, xanthene, thioxanthone and acridone) 1 mM in 50 mL CH_3CN containing 0.1 M TBAP as electrolyte was added in electrochemical cell. The cyclic voltammograms were run after purging the solution for 3 min with nitrogen gas to eliminate interfering oxygen. Then cyclic voltammogram of each compounds solution were carried out at scan rate 100 mV s^{-1} and step potential 20 mV. Cyclic voltammogram was run compared with the blank solution.

2.3.5 Stripping voltammetry of Cd(II), Cu(II), Hg(II) and Pb(II) by carbon paste electrode procedures

2.3.5.1 Effect of electrolyte for determination Cd(II), Cu(II), Hg(II) and Pb(II) by unmodified electrode

The solution of 10 mg L^{-1} Cd(II), Cu(II), Hg(II) and Pb(II) was prepared in different electrolyte , including 0.2 HNO_3, 0.2 M acetate buffer, 0.3 M CH_3COONH_4 and 0.2 M CH_3COONa. The experiments were performed at conditions as follows:

In the case of Cd(II), stripping voltammogram was run after purging the solution for 1 min with nitrogen gas. The accumulation potential of -1.10 V was applied to the carbon paste electrode under stirring. After a 2 min accumulation period and a 10 s equilibration time, the differential pulse stripping voltammogram was recorded form -1.10 to -0.70 V (Hu *et al*., 2003).

In the case of Cu(II), stripping voltammogram was run after purging the solution for 1 min with nitrogen gas. The accumulation potential was applied at -0.30 V for 2 min while stirring the solution. Following the accumulation period and after 10 s equilibration time, the stripping voltammogram was recorded by applying a positive going differential pulse scan from -0.30 to 0.10 V. (Alemu and Chandravanshi, 1998).

In the case of Hg (II), stripping voltammogram was run after purging the solution for 1 min with nitrogen gas. The accumulation potential was applied at -0.30 V for 1 min while stirring the solution. Following the accumulation period and after 10 s equilibration time, the stripping voltammogram was recorded by applying a positive going differential pulse scan from -0.30 to 0.50 V (Guo and Guadalupe, 1999).

In the case of Pb (II), stripping voltammogram was run after purging the solution for 1 min with nitrogen gas. The accumulation potential of -0.70 V was applied to the carbon paste electrode under stirring. After a 1 min accumulation period and a 10 s equilibration time, the differential pulse stripping voltammogram was recorded form -0.70 to -0.30 V (Mousavi *et al*., 2001).

Differential pulse anodic voltammetry experiments were carried out with the modulation amplitude of 100 mV, a scan rate of 20 mV s^{-1} and a pulse interval of 0.25 s.

2.3.5.2 Stripping voltammetry of Cd(II), Cu(II), Hg(II) and Pb(II) by carbon paste electrode modified with group of xanthone compounds

The optimum electrolyte found in 2.3.5.1 was then used to study the stripping voltammetry of Cd(II), Cu(II), Hg(II) and Pb(II) by carbon paste electrode modified with group of xanthone compounds. Stripping voltammograms were obtained at freshly prepared unmodified and modified carbon paste electrodes. The standards solution were prepared at the concentration of 5 mg L^{-1} Cd(II) in CH_3COONH_4, 5 mg L^{-1} Cu(II) in 0.2 M acetate buffer, 10 mg L^{-1} Hg(II) in 0.2 M acetate buffer and 5 mg L^{-1} Pb(II) in 0.2 M HNO_3. Stripping voltammogram was run with the same condition in 2.3.5.1.

2.3.6 Optimization condition for determination Pb(II) by adsorptive stripping voltammetry

2.3.6.1 Adsorptive characteristics of the Pb-8-hydroxyquinoline complex

The solution of Pb(II) 1 mg L^{-1} in 0.01M ammonium acetate was prepared into 50 mL volumetric flask. Then, 0.1 mM 8-hydroxyquinoline was added, giving a final concentration of 0.1 M and the pH was adjusted to 8.0 by ammonium hydroxide. The solution was then transferred to a voltammetric cell. The solution was purged with nitrogen gas for 1 min. Cyclic voltammetry experiments were carried out with 1 min stirred adsorption at -0.4 V and 10 s equilibration time. Scan rate was 50 mV s^{-1} (Van Den Berg, 1986).

2.3.6.2 Comparison of square wave versus differential pulse

The aim of this experiment was to compare the pulse voltammetry from square wave versus differential pulse for determination Pb(II). The solution of Pb(II) 5, 10, 15, 20 μg L^{-1} was prepared in 0.01M ammonium acetate containing 10 μM 8-hydroxyquinoline and the pH was adjusted to 8.0 by ammonium hydroxide. The solution 10 mL was transferred to a voltammetric cell. The stirrer was switched on and the solution was purged with nitrogen gas for 1 min. The accumulation potential (-1.1 V) was applied to a fresh HMDE for 60 s while stirring the solution. Following the accumulation period, the stirrer was stopped, and after 10 s quiescence time, the voltammogram was recorded by applying a negative going scan from -0.3 to -0.8 V (Van Den Berg, 1986).

Differential pulse (DP) voltammetry experiments were carried out with a modulation amplitude 25 mV and a scan rate of 20 mV s^{-1} (Van Den Berg, 1986).

Square wave (SW) voltammetry experiments were carried out with amplitude 25 mV and a scan rate of 20 mV s^{-1} (Colombo and Van Den Berg, 1997).

2.3.6.3 Comparison of peak height versus peak area

The aim of this experiment was to compare the signal from of peak height and peak area. The solution of Pb(II) 5, 10, 15, 20 μg L^{-1} was prepared in 0.01M ammonium acetate containing 10 μM 8-hydroxyquinoline and the pH was adjusted to 8.0 by ammonium hydroxide. Stripping voltammogram was run in square wave mode with the same condition in 2.3.6.2.

2.3.6.4 Effect of supporting electrolyte

The optimum pulse voltammetry of square wave found in the previous experiment was then used to study the effect of electrolyte. The solution of 20 μg L^{-1} Pb(II) was prepared in 0.01 M electrolyte, including CH_3COONH_4, CH_3COONa, Tris, KNO_3 and $NaNO_3$. Then, 8-hydroxyquinoline was added, giving a final concentration of 10 μM and the pH was adjusted to 8.0 by ammonium hydroxide. Stripping voltammogram was run in square wave mode with the same condition in 2.3.6.2.

2.3.6.5 Effect of supporting electrolyte concentration

The optimum electrolyte of CH_3COONH_4 found in 2.3.6.4 was then used to study the effect of electrolyte concentration. The solution of Pb(II) 20 µg L^{-1} was prepared in CH_3COONH_4 varied form 0.01-0.5 M. Then, 8-hydroxyquinoline was added, giving a final concentration of 10 µM and the pH was adjusted to 8.0 by ammonium hydroxide. Stripping voltammogram was run in square wave mode with the same condition in 2.3.6.2.

2.3.6.6 Effect of pH

The optimum electrolyte concentration found in 2.3.6.5 was then used to study the effect of pH. The solution of Pb(II) 20 µg L^{-1} was prepared in 0.1M CH_3COONH_4 containing 10 µM 8-hydroxyquinoline. The pH was varied from 6.0-10.0 and adjusted by using 0.1 M nitric acid and 0.1 M ammonium hydroxide. Stripping voltammogram was run in square wave mode with the same condition in 2.3.6.2.

2.3.6.7 Effect of 8-hydroxyquinoline concentration

The optimum electrolyte concentration and pH found in the previous experimentals were then used to study the effect of 8-hydroxyquinoline concentration. The solution of Pb(II) 20 µg L^{-1} was prepared in 0.1M CH_3COONH_4 containing 8-hydroxyquinoline was varied from 1-40 µM and the pH was adjusted to 7.5. Stripping voltammogram was run in square wave mode with the same condition in 2.3.6.2.

2.3.6.8 Effect of accumulation potential

The accumulation potential was studied in the range of -1.2 V to -0.2 V. The solution of Pb(II) 20 µg L^{-1} was prepared in 0.1M CH_3COONH_4 containing 15 µM 8-hydroxyquinoline and the pH was adjusted to 7.5.

2.3.6.9 Effect of accumulation time

The accumulation time was studied from 30 – 360 second with accumulation potential -0.7 V found in 2.3.6.8. The solution of Pb(II) 20 µg L^{-1} was prepared in 0.1M CH_3COONH_4 containing 15 µM 8-hydroxyquinoline and the pH was adjusted to 7.5.

2.3.6.10 Effect of scan rate and pulse amplitude

The solution of Pb(II) 20 μg L^{-1} was prepared in 0.1M CH_3COONH_4 containing 15 μM 8-hydroxyquinoline and the pH was adjusted to 7.5. The optimum scan rate and pulse amplitude were investigated by varied from 0.1 - 0.9 V/s and 10 - 50 mV respectively.

2.4 Analytical performances

2.4.1 Linear range

The standard stock solution of Pb(II) was diluted with de-ionized water to various concentrations in the range of 0.0 - 120.0 μg L^{-1}. The standard stock solutions of each concentration were analyzed by AdCSV at the optimum conditions from 2.3.6.1 - 2.3.6.10. The linear dynamic range obtained from plotting the current versus the concentration.

2.4.2 The limit of detection (LOD) and the limit of quantification (LOQ)

The limit of detection, C_L, is defined as the lowest concentration of analyte that can be measured at a specific confidence level. So, near limit of detection, the signal generated approaches that from a blank. The limit of detection is often defined as the concentration where the signal/noise ratio reaches an accepted value (typically between 2 and 4) (Mitra, 2003). Therefore, the smallest distinguishable signal, X_L, is

$$X_L = X_b + kS_b \tag{2.1}$$

Where X_b is the mean of blank measures and S_b the standard deviation of the blank measures and k is a numerical factor chosen according to the confidence level desired and the accepted value is at 3 confidence level of 99.6%.

The limit of detection concentration, C_L, is obtained as a function of X_L and can be calculated as

$$C_L = (X_L - X_b)/m \tag{2.2}$$

Where m is the analytical sensitivity (slope of the calibration curves). Equation 2.3 is obtained by after substitution Equation 2.1 into 2.2

$$C_L = kS_b/m \quad (2.3)$$

The limit of quantification (LOQ) is the lowest concentration level at which a measurement is quantitatively meaningful is called. The limit of quantification is most often defined as 10 times the signal/noise ratio. If the noise is approximated as the standard deviation of the blank, the LOQ is ($10 \times s_{tbl}$) (Mitra, 2003).

In this study, the current of blank was measured and then the calibration curve of Pb was established by AdCSV at the optimum conditions from 2.3.6.1 - 2.3.6.10. Therefore the limit of detection and the limit of quantification in this investigation were evaluated by Equation (2.4-2.5).

$$\text{The limit of detection} = (3*SD)/m \quad (2.4)$$

$$\text{The limit of quantification} = (10*SD)/m \quad (2.5)$$

When, SD = standard deviation of blank

m = slope of calibration graph

2.4.3 Accuracy and precision

Accuracy

The accuracy of an analytical method is defined as the degree to which the determined value of analyte in a sample corresponds to the true value (Mitra, 2003). The accuracy of analytical method can be evaluated from percent recovery. The percent recovery can be evaluated by spiking 10.0, 20.0 and 30.0 μg L^{-1} of Pb(II) in canned fish sample. Pb(II) concentrations add and the percent recovery was calculated from standard addition graph.

Precision

Precision is a measure of reproducibility. The precision of an analytical procedure is usually expressed as the relative standard deviation (RSD). The calculation of RSD was evaluated by equation (2.6) (Mitra, 2003).

$$\text{RSD} = \frac{SD}{\overline{X}} \quad \text{or} \quad \%\,\text{RSD} = \frac{SD}{\overline{X}} \times 100 \quad \text{……..(2.6)}$$

When, RSD = the relative standard deviation

SD = the standard deviation

$\overline{X}$ = the mean or average value of the measurements

The precision of the AdCSV method was performed by analysis the standard solution of Pb(II) 1.0, 5.0 and 10.0 µg L^{-1} at the optimum conditions from 2.3.6.1 - 2.3.6.10.

2.5 Sample preparation and digestion

After opening each canned fish sample, fish and tomato sauce, was homogenized thoroughly in a food blender. Homogenized sample 1.5 g (wet weight) was placed into beaker and 15 mL of nitric acid : perchloric acid : sulphuric acid mixture (25 + 25 + 1 v:v:v) was added. The beaker was covered using watch glass and heated on the hot plate at 150 ^{o}C until the solution was clear. The clear solution was allowed to cool, transferred into a 25 mL volumetric flask and diluted to the mark with deionised distilled water (Ashraf *et al.,* 2006).

2.6 The comparison of the calibration and standard addition method for determination of Pb(II) in canned fish samples

The aim of this experiment was to study the determination method between calibration and standard addition method for analysis of Pb(II) in canned fish samples. The calibration was prepared by standard solution of Pb(II) with concentration 0, 2, 4 and 6 µg L^{-1}. The standard addition was studied by spiking known amount of Pb(II) standard solution at 0, 2, 4 and 6 µg L^{-1} into digestion sample. Canned fish samples were digested using hot plated digestion method (2.5) and measured Pb(II) by AdCSV at the previous optimum conditions that obtained from 2.3.6.1 - 2.3.6.10.

2.7 Interference studies

The effect of the interfering ions such as Fe^{2+}, Cr^{3+}, Mn^{2+}, Hg^{2+}, Sn^{2+}, Cd^{2+}, Zn^{2+}, Al^{3+}, Cu^{2+} and Ni^{2+} were evaluated by the addition of the interfering ion to a solution containing 20.0 µg L^{-1} of Pb(II) and carrying out the measurements at the optimized conditions (2.3.6.1 - 2.3.6.10).

2.8 Determination of Pb(II) in canned fish samples

The studied method was used for Pb(II) determination in canned fish samples. The 1.5 g of canned fish samples were digested using hot plate digestion (2.5) and determination Pb(II) by AdCSV at the previous optimum conditions that obtained from 2.3.6.1 - 2.3.6.10.