CHAPTER III

EXPERIMENT

3.1 Chemicals

All chemicals used were of reagent grade, no further purification was attempted unless otherwise stated. Hygroscopic compounds were kept in a desiccator over the anhydrous silica gel.

Purified nitrogen gas used for deaerating the test solutions in anodic stripping analyses was prepared by passing the oxygen free nitrogen gas (obtained from Thai Industrial Gas Co.) through a vanadous chloride solution and then through the double deionized water before entering the cell[59].

The percentage of the metal ion species in the analar grade reagent was determined. Cadmium nitrate tetrahydrate was potentiometrically titrated with EDTA and lead nitrate was amperometrically titrated with potassium dichromate.

All solutions were prepared with the double deionized water.

3.2 Apparatus

Voltammograms were obtained with a Princeton Applied Research (PAR) Model 174 A Polarographic Analyzer equipped with a PAR Model 315 A Automated Electroanalysis Controller and a Hewlett - Packard 7040 A X - Y Recorder.

The cell employed in all DPASV analyses is a conventional H - type with two compartments (see Figure 3): one compartment served for the reference electrode, saturated calomel electrode (SCE), and the other

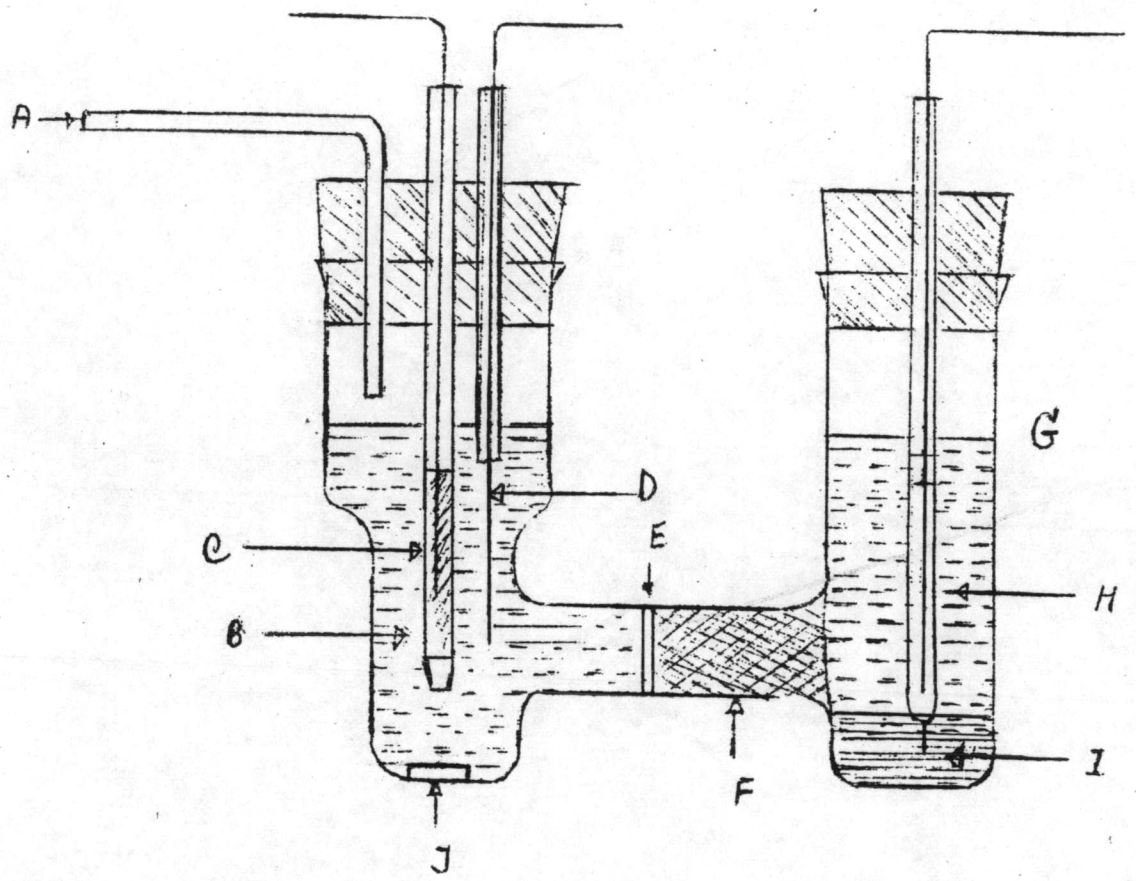

Figure 3. The H cell used in DPASV analysis. The labelling letters are A - purified N_2, B - solution of the sample, C - the GCE working electrode, D - platinum wire, E - sintered glass disc, F - agar plug, G - the SCE reference electrode, H - saturated KCl and Hg_2Cl_2 solution, I - solid KCl, calomel paste and Hg, and J - the magnetic stirring bar.

served for the test solution. Glassy carbon electrode (GCE) and a platinum wire were used as working electrode and auxiliary electrode, respectively.

All potentials reported herein were measured against SCE.

The pH values of the solutions were measured with a pH meter (Radiometer Copenhagen type PHM 28).

Vegetable samples were ashed in an electric furnace with a temperature range of 0 - 1,400 ^{o}C.

All measurements were carried out at room temperature.

3.3 Procedure

3.3.1 Double deionized water

Double deionized water was obtained by passing the distilled water through a set of three columns. Each column had an inside diameter of 3.5 cm and a length of 55 cm. The first column was packed with anion exchange resins, Amberlite IR-45(OH). In the second column cation exchange resins, Amberlite IR - 120(H), was packed. The last column was half - filled with the anion exchangers and the other half with the cation exchangers.

3.3.2 Standard lead and cadmium solutions.

A 1.00 mg/cm^3 solution of Pb(II) ion or Cd(II) ion was prepared by dissolving the appropriate amount of its nitrate salt with the double deionized water.

Series of standard solutions of Pb(II) ion and Cd(II) ion in 1.0 M HCl were obtained by successive dilution of the 1.00 mg/cm^3 solution of each

metal ion species with concentrated HCl and the double deionized water. Other series of standard Pb(II) ion and Cd(II) ion solutions in different electrolytes were also prepared in the same manner.

3.3.3 Salt bridge, reference electrode and glassy carbon electrode

3.3.3.1 Preparation of salt bridge[60]

A 0.4 g of agar - agar was molten in ca. 10 cm^3 of saturated potassium chloride solution by gently warming the mixture on a hot plate. By clamping the clean and dry cell so that the cross member was vertical, the molten agar gel was transferred into the cross member by means of a dropper. The cell was allowed to stand undisturbed until the gel had solidified. No air bubble should be present in the KCl - agar salt bridge. When the H cell is not in used, the test solution compartment should be kept filled with the double deionized water to prevent the agar plug from drying out.

3.3.3.2 Preparation of the reference electrode[60].

The SCE was prepared in one compartment of the H cell. Electrical connection with the SCE was made by means of a platinum wire, sealed through a glass tube, the glass tube contains a little pure mercury into which an amalgamated copper wire dips. To set up the electrode, a saturated solution of KCl and Hg_2Cl_2 was first prepared. Pure mercury was placed in the bottom of the dry electrode compartment of the H cell for a depth of 0.5 cm. The mercury was then covered with a layer of calomel paste. The latter was prepared by rubbing pure Hg_2Cl_2, mercury and saturated KCl solution in a clean beaker with a clean dry glass rod.

The electrode compartment was filled with a saturated solution of KCl and Hg_2Cl_2 and the rubber bang with the glass tube associated with electrical connection was then inserted. The potential of the electrode prepared was measured against the Radiometer reference electrode (Copenhagen type PHM 28) before being used.

3.3.3.3 Preparation of the glassy carbon electrode[60]

A piece of 10- mm glassy carbon rod (vitreous carbon rod, 3mm.dia, from Ringsdorff - Werke GMBH) was glued in a 4 - mm (ID) soft glass tube with Araldit epoxy (Ciba - Geigy Limited, Basle, Switzerland). A fresh glassy carbon surface was always prepared for each run by polishing with a piece of silicon carbide paper, then washed with the double deionized water and gently rubbed with a piece of filter paper to get rid of dust and moisture.

3.3.4 Preparation of sample

The edible parts of the vegetable divided into two parts, leaf and stem, were analyzed. First, the vegetable sample was cleaned with tap water to remove all soil, dust and other residue presented. After rinsing with the double deionized water, the sample was left in open air about an hour or more for evaporating all water adsorbed on its surface. Then, it was weighed and dried in an electric oven at 100 - 110°C overnight and until a constant weight was achieved. After cooling down to room temperature, it was weighed again, therefore, the water content in plant tissue was known. Since the representative of the sample was necessary, the dry sample was ground and mixed thoroughly in a beaker with

a clean glass rod. The destruction of organic matter in the sample was proceeded by dry ashing process since it did not require constant attention and chemicals, as did wet oxidation.

3.3.4.1 Dry ashing process

In order to avoid the introduction of extraneous impurities, no ashing aid was utilized. Vegetable samples were ashed according to Doshi and Patel[61].

Accurately weighed 10 g of the dry and ground sample were transferred into a porcelain basin. The basin was placed in the 100°C muffle furnace. The furnace temperature was slowly raised up to 300°C and held until smoking ceased. The temperature then was increased to 450°C and at this temperature the sample was ashed for 8 hours or longer if carbon particles were still present. After cooling the ash down to room temperature in a desiccator, 5 cm^3 conc. HCl were added to the ash and followed by a few cm^3 of the double deionized water. The mixture was placed on a steam bath and evaporated to dryness. The residue was dissolved in ca. 10 cm^3 of the double deionized water, and the solution was filtered. An addition of8.7 cm^3 conc. HCl to the filtrate and the dilution with the double deionized water were performed to the mark of the 100 cm^3 volumetric flask. The resulted solution was in ca. 1M HCl.

3.3.5 Differential pulse anodic stripping voltammetric analysis

To obtain a voltammogram of the test solution, the PAR Model 174 A Polarographic Analyzer (as shown in Figure 4) was set as followed :

potential scan rate	5 mV/sec
scan direction	" + "
potential range	3.0 V
initial potential	0
modulation amplitude	25 mV
operation mode	diff. pulse
display direction	" - "
drop time	0.5 sec
current range	as needed
Low pass filter	off
output offset	off

The PAR Model 315 A Automated Electroanalysis Controller (as shown in Figure 5) was also set as followed :

conditioning potential	+ 0.00 V
deposition potential	- 1.00 V
final potential	+ 0.00 V
replications	as needed
deposition time	300 sec
purge	5 min
conditioning	3 min
equilibration	30 sec

overrides : purge, cell, cell check, stirrer and Recorder are all " auto "

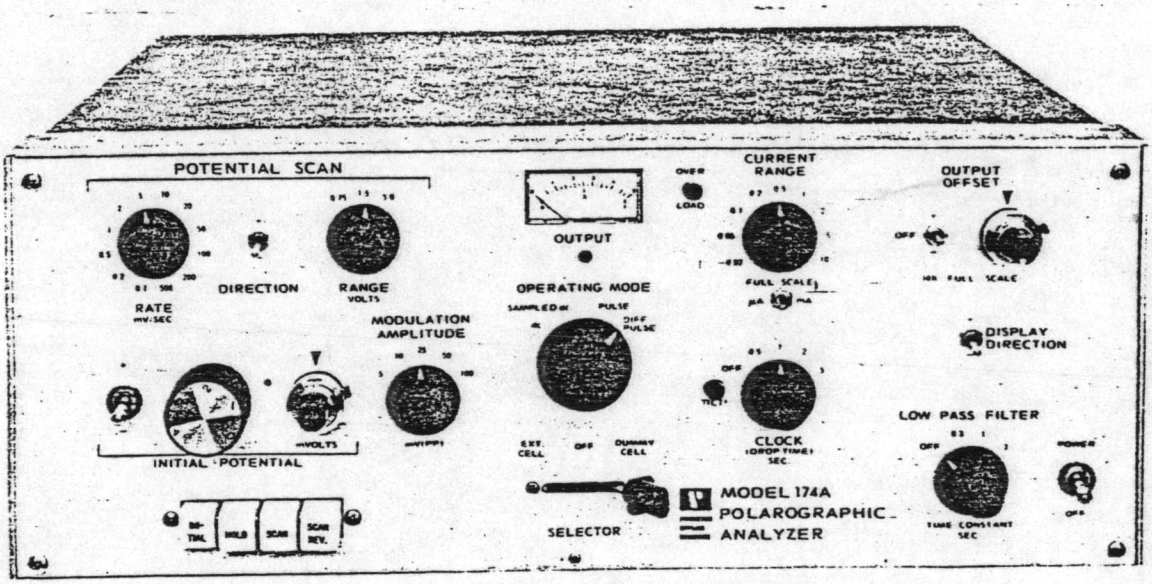

Figure 4 Model 174 A Polarographic Analyzer

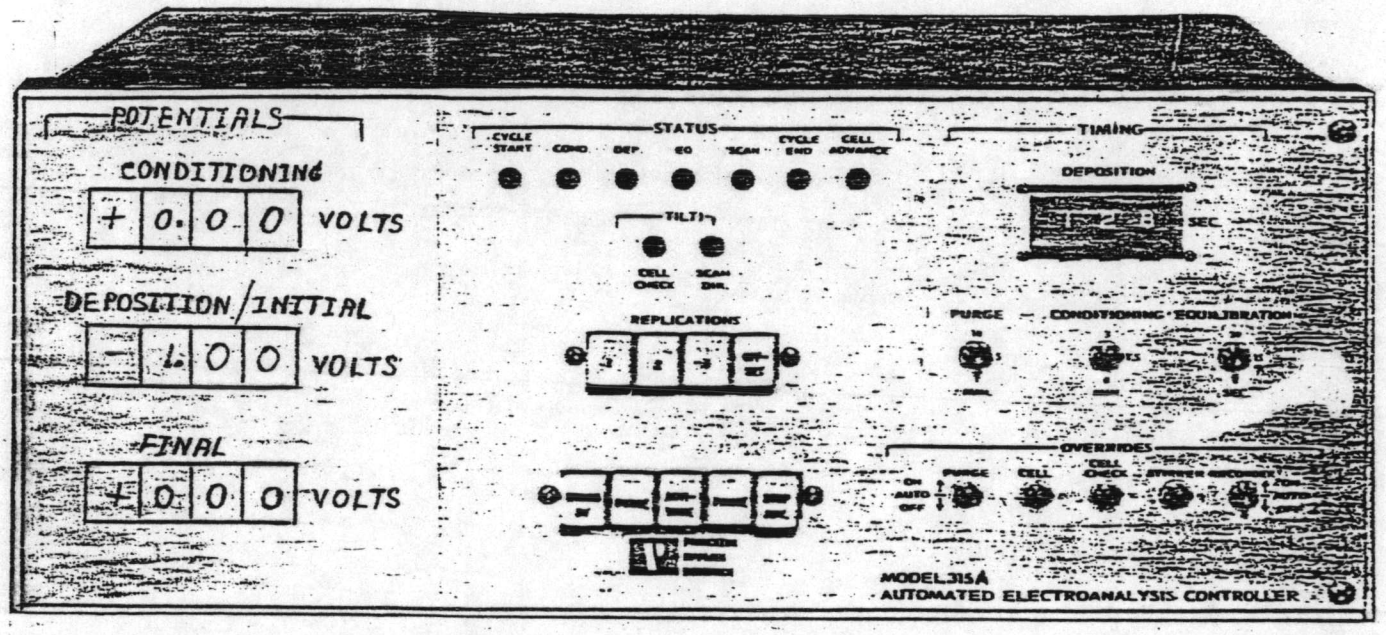

Figure 5 Model 315 A Automated Electroanalysis Controller

Before introducing any test solution into the cell for either voltammetric or stripping analysis, the test compartment was washed twice with ca. 3 M HNO_3, rinsed with the double deionized water for several times and finally with the test solution. A 15.0 cm^3 test solution was pipetted into the cell and the purified nitrogen gas was bubbled through the solution for 5 minutes and then was blanketed over the solution in which the auxiliary electrode and the GCE were placed. The voltammogram was recorded. For the solution of vegetable sample, standard lead and cadmium solutions were subsequently added by means of pipet to the cell to approximately double the amount of lead and cadmium present, Again, the purified nitrogen gas was bubbled through the solution for 3 minutes and blanketed over the solution and the voltammogram was recorded as before.

A typical differential pulse voltammogram is shown below.