CHAPTER II

EXPERIMENT

2.1 Chemicals

All chemicals used were of reagent grade, no further purification was attempted unless otherwise stated. Hygroscopic substances were kept in the desiccator containing anhydrous silica gel as a drying agent. Triply distilled mercury (distilled from the BDH Analar grade Hg) which had been tested for purity by differential pulse polarographic (DPP) analysis was used throughout this study. Purified nitrogen gas used for deaerating the test solutions was prepared by passing the nitrogen gas (obtained from the Royal Thai Army Chemical Department) through a vanadous chloride solution and then through the double deionized water before entering the cell (57).

All solutions were prepared with the double deionized water.

2.2 Apparatus

Polarograms were obtained with a PAR (Princeton Applied Research, Inc.) Model 174 A Polarographic Analyzer, equipped with a PAR Model 174/70 drop timer, and a

Hewlett - Packard 7040 A X-Y Recorder.

The cell employed in all DPP analyses is a conventional H-type with two compartments (see Figure 4): one compartment served for the saturated calomel electrode (SCE) and the other served for the test solution. The two compartments are separated by a cross member filled with 4 % agar-saturated potassium chloride gel, which is held in position by a sintered glass disc. The three electrodes used are a dropping mercury electrode (DME) as the working electrode, the SCE as the reference electrode and a carbon rod counter electrode.

All potentials reported herein were measured against SCE.

The pH values of 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° C or were digested in the 300 cm^3 Kjeldahl flasks by an electrothermal heating mantle.

All glassware used was soaked with detergent, cleaned with tap water and ca. 3 M HNO_3, and rinsed several times with the double deionized water.

All measurements were carried out at room temperature.

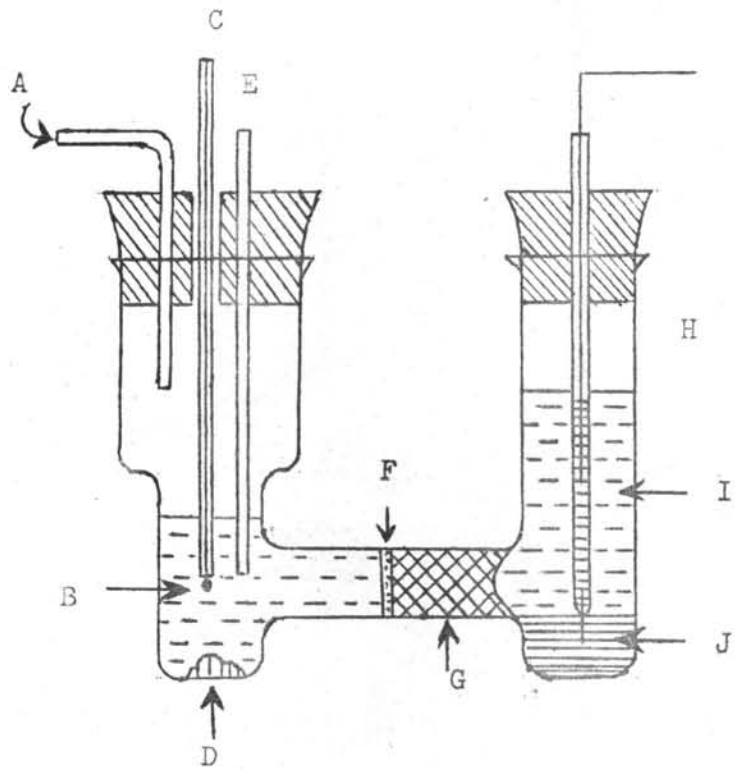

Figure 4. The H cell used in DPP analysis. The labelling letters are A- purified N_2, B- solution of the sample, C- the DME working electrode, D- Hg, E- carbon rod counter electrode, F- sintered glass disc, G - agar plug, H- the SCE reference electrode, I- saturated KCl and Hg_2Cl_2 solution, and J- solid KCl, calomel paste and Hg.

2.3 Procedure

2.3.1 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.

2.3.2 Standard arsenite solution

A stock solution containing 1.00 mg As/cm^3 was prepared by dissolving 0.13203 g of the analar grade As_2O_3 (BDH) in ca. 10 cm^3 of 10 % NaOH solution and diluting to the mark of the 100.0 cm^3 volumetric flask.

A series of standard solutions containing 0.10 - 1.00 μg As/cm^3 in 1 M HCl was prepared by successive dilution of the 1.00 mg As/cm^3 solution with concentrated HCl and the double deionized water. Other series of standard arsenite solutions in different electrolytes were also prepared in the same manner.

The percentage of As in the analar grade As_2O_3 (BDH) was determined by standardization against the standard KIO_3 solution in the presence of hydrochloric acid

and carbon tetrachloride (58). The extraction end point was very sharply marked by the disappearance of the last trace of violet color from carbon tetrachloride.

2.3.3 Salt bridge and reference electrode

2.3.3.1 Preparation of salt bridge (58)

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.

2.3.3.2 Preparation of the reference electrode (58)

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. A copper wire was dipped into a little portion of pure mercury contained in the glass tube. 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 bung with the glass tube associated with electrical connection was then inserted. The potential of the electrode prepared was measured against the PAR reference electrode Model 9331 (SCE) before being used.

2.3.4 Preparation of the cation exchange resins

In order to remove interfering cations which could be reduced at the potentials near the reduction potential of arsenite, cation exchange resins were added to the test solution. The resin employed was Amberlite IR - 120 (Na), 14-52 mesh, standard grade with the exchange capacity of 5.0 meq/g of dry resins or 1.9 meq/cm^3 of wet resins.

Preparation of the cation exchanger was performed by washing the resins with the double deionized water in a beaker until the color of the decanted water reached a minimum intensity and the fine particles were removed by decantation. Then the resins were soaked in a beaker of 2 M HCl solution about twice the volume of the resins for 60 minutes with occasional stirring. The

supernatant solution was decanted and the resins were washed by several aliquots of the double deionized water. Other portions of 2 M HCl were added, and the washing with the double deionized water was repeated until the supernatant solution was free of acid. After the resins were filtered off and dried in air, they were ready for the exchange of their hydrogen ions for cations presented in a given solution.

In the study of ion exchange, the process was carried out in the following manner. A 20-g of the prepared resins was placed in a required medium (e.g. 0.1 M HCl) in a beaker . A known amount of lead ion or arsenite ion solution was added to the resins and was maintained for 30 minutes with occasional stirring. Then the solution was filtered into a 100.0 cm^3 volumetric flask. The double deionized water and an adequate amount of the concentrated HCl were added so the final solution was in 1 M HCl. Then the solution was set for the DPP analysis.

Regenerations of the resins were performed with 2 M HCl, as the procedure mentioned above.

2.3.5 Preparation of sample

Only the edible parts of the vegetable 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 the following methods.

2.3.5.1 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 (59), and were treated with cation exchanger in the following manner.

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 and neutralized with 10 % NaOH. A 0.5 cm^3 of conc. HCl was added to the neutral solution and the dilution was made to 50.0 cm^3 with the double deionized water. Then 20 g of the cation exchange resins were added to the sample solution with occasional stirring. After 30 minutes, the solution was filtered into a 100.0 cm^3 volumetric flask. An addition of 8.4 cm^3 conc. HCl to the filtrate and the dilution with the double deionized water were performed to the mark of the volumetric flask. The resulted solution was in ca. 1 M HCl.

Reagent blank was performed in the same manner as the sample. To achieve recovery of the method, a known amount of standard arsenite solution was also treated by the same procedure.

2.3.5.2 Wet digestion process

Vegetable sample was digested by modifying the suggested method for plant materials by Reay (23), and by Aggett and Aspell (31).

Accurately weighed 10 g of sample (dry and ground) were transferred into a 300 cm^3 Kjeldahl flask. A solution of 150 cm^3 8 M HNO_3 was added, the mixture was swirled and gently warmed on a heating mantle until initial

reactions subsided. The solution was boiled at 120° C until it was clear and colorless, about 8 hours. If the solution was still brown or yellow, an addition of 20 cm^3 of nitric acid and further heating were carried out. Then the solution was heated to dryness and allowed to cool. After adding 10 cm^3 of the double deionized water, the solution was warmed to dissolve the residue. Then the solution was filtered and neutralized with 10 % NaOH solution. To the neutral solution, 0.5 cm^3 of conc. HCl was added and the dilution was made to 50.0 cm^3 with the double deionized water. Then 20 g cation exchange resins were added to the sample solution with occasional stirring. After 30 minutes, the solution was filtered into 100.0 cm^3 volumetric flask. An addition of 8.4 cm^3 conc. HCl was performed and the volume was made up to the mark with the double deionized water.

The same procedure was attempted for the reagent blank and the standard arsenite solution.

2.3.6 Differential pulse polarographic analysis

To obtain a polarogram of the test solution, the instrument (as shown in Figure 5) was set as followed:

potential scan rate	2 mV/sec
scan direction	" - "
potential range	3.0 V
initial potential	as needed

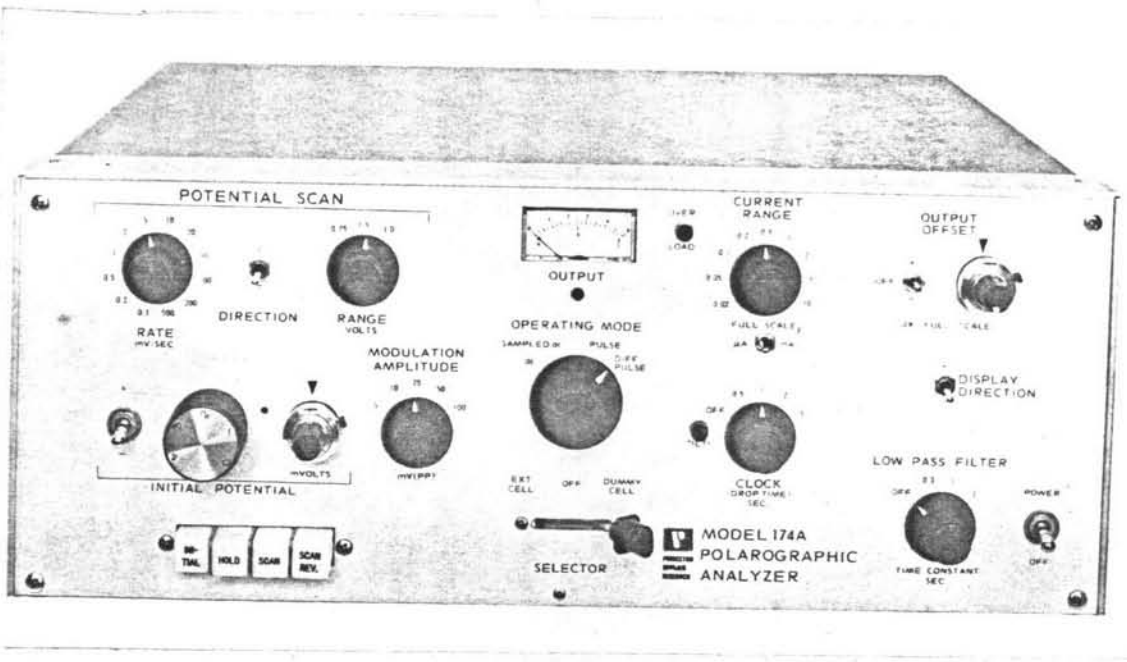

Figure 5. Model 174 A Polarographic Analyzer

modulation amplitude	50 mV
operation mode	diff. pulse
display direction	" + "
drop time	2 sec
current range	as needed

Before introducing any test solution into the cell, the test solution 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 counter electrode and the DME were placed. The polarogram was recorded. For the solution of vegetable sample, standard arsenite solution was subsequently added by means of pipet to the cell to approximately double the amount of arsenic presented. Again, the purified nitrogen gas was bubbled through the solution for 2 minutes and blanketed over the solution and the polarogram was recorded as before.