CHAPTER III

EXPERIMENTS

3.1 Materials

3.1.1. Tracer Specification. The mercury-203 used in this experiment was obtained from the Radiochemical, Amersham, England. The specific activity of the mercury-203 was 0.64 curie/gram. The total volume of 5.9 millilitre of the original tracer was firstly diluted to 50 millilitre with diluted hydrochloric acid obtaining exactly 323.2 microgram of mercury per millilitre. An appropriate desired concentration can be prepared by further dilution with diluted hydrochloric acid.

3.1.2. Deionised Water. The deionised water was prepared by passing distilled water through a mixed resin bed of 50-100 mesh Dowex-HCR-W (hydrogen form) and 100-200 mesh Ionac (hydroxide form). Analysis for stable mercury revealed none.

3.1.3. Standard Reference Materials. Two of National Bureau of Standards Reference Mateials named as Orchard Leaves and Bovine Liver and a Standard Kale Sample prepared by Dr. H.J.M. Bowen of the University Whiteknights Park, Reading, Department of Chemistry, England and distributed by IAEA as an intercomparison sample were used to check the reliability of the developed procedure.

3.1.4. <u>Chemicals</u>. All chemicals used were of analytical reagent grade except mercuric oxide was spectroscopic pure grade. No purification was made otherwise stated.

3.1.4.1. Test solution

a) Stock solution. Dissolve 0.56 microgram of mercuric oxide in 500 millilitre diluted hydrochloric acid. This solution contains 100 microgram of mercury per millilitre.

b) Working solution. Prepare by successive dilution of the stock solution with diluted hydrochloric acid, and used as test solution for the method development of the present study.

3.1.4.2. Carbontetrachloride.

3.1.4.3. Dithizone stock solution. (0.01%) Dissolve 0.01 g. dithizone in 100 millilitre of carbontetrachloride. This solution must be kept in a brown glass-stoppered and stored in a refrigerator.

3.1.4.4. Hydrochloric acid 0.1 M. Purify by dithizone extraction.

3.1.4.5. Oxygen gas. It was locally product.

3.1.4.6. Sodium acetate. Dissolve 8.2 g. of sodium acetate in 100 millilitre of Hg-free water.

3.1.4.7. Zinc Chloride 0.1 M. Dissolve 1.36 g. of zinc chloride in 100 millilitre of diluted hydrochloric acid.

3.1.4.8. Ammonia solution.

3.1.4.9. Acetic acid 1M.

3.1.4.10 Zinc dithizonate stock solution (0.01 %). Dissolve 1 g. of ascorbic acid in 25 millilitre of Hg -free water in a 500 millilitre separatory funnel, add 75 millilitre of 1M. acetic acid and 75 millilitre of 1M. sodium acetate, and purify by dithizone extraction. Add 5 millilitre of 0.1M. zinc chloride solution, adjust pH to about 5 with ammonia, add 150 millilitre of dithizone stock solution and extract for about 2 minute. The separated filtered solution is zinc dithizonate. It must be kept in a dark container, cool place and away from light.

3.1.4.11. Zinc dithizonate working solution. Prepare daily by diluting the suitable portion of the stock zinc dithizonate solution (0.01 %) with carbontetrachloride to the desired concentration.

3.1.4.12. Buffer and masking solution. Dissolve 5 g. of citric acid in Hg-free water, add 25 millilitre of 1M. sodium hydroxide and purify by dithizone extraction. The solution of 13 millilitre, 0.1M. EDTA was added and dilute to 250 millilitre with deionised water.

3.1.4.13. Hydroxylamine hydrochloride.(20%) Dissolve 20 g. of hydroxylamine hydrochloride in 100 millilitre of deionised water and purify by dithizone extraction.

3.1.4.14. Interferences. Dissolve appropriate amounts of salts of cobalt (II), manganese (II), copper (II), silver (I), iron (III), cadmium (II), indium (III), lead (II), bismuth (III), nickel (II), tin (II), thallium (III) and thallium (I) in suitable solvents obtaining 10 milligram of element per millilitre and then further dilute to 200 microgram of element per millilitre

3.2. Apparatus.

3.2.1. Counting equipment. A Single Channel Analyzer, Model 1431 (Canberra) in conjunction with a 3 x 3 inches, thallium-activated, sodium iodide crystal with 3.5 inches thick, lead, copper and cadmium lined, gun barrel shielding (Fig 3-1) was used to measure gamma activity of mercury-203 throughout this study.

3.2.2. Mechanical shaker. A shaking apparatus from Arthur, H. Thomas Co. was used. (Fig 3-2).

3.2.3. Magnetic stirrer.

3.2.4. Combustion flask with platinum holder. The flask was flat bottomed, of 5-litre capacity, with center neck 29/32 and one angle side neck (15°) 14/23 standard taper with ground joint, DURAN 50/sovirel glass.(Fig 3-3)

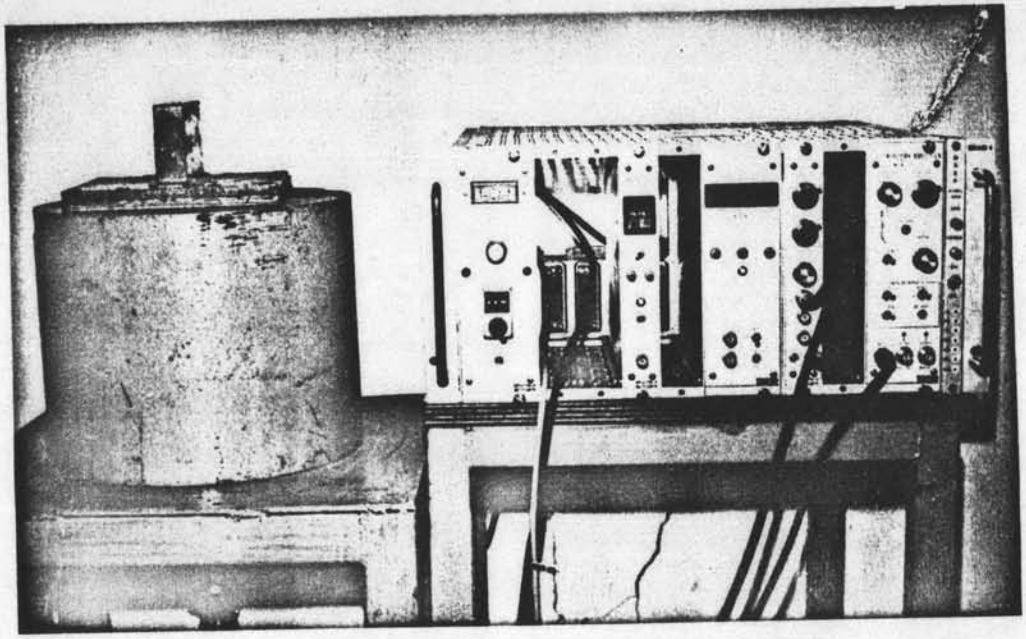

Figure 3-1 SINGLE CHANNEL ANALYZER

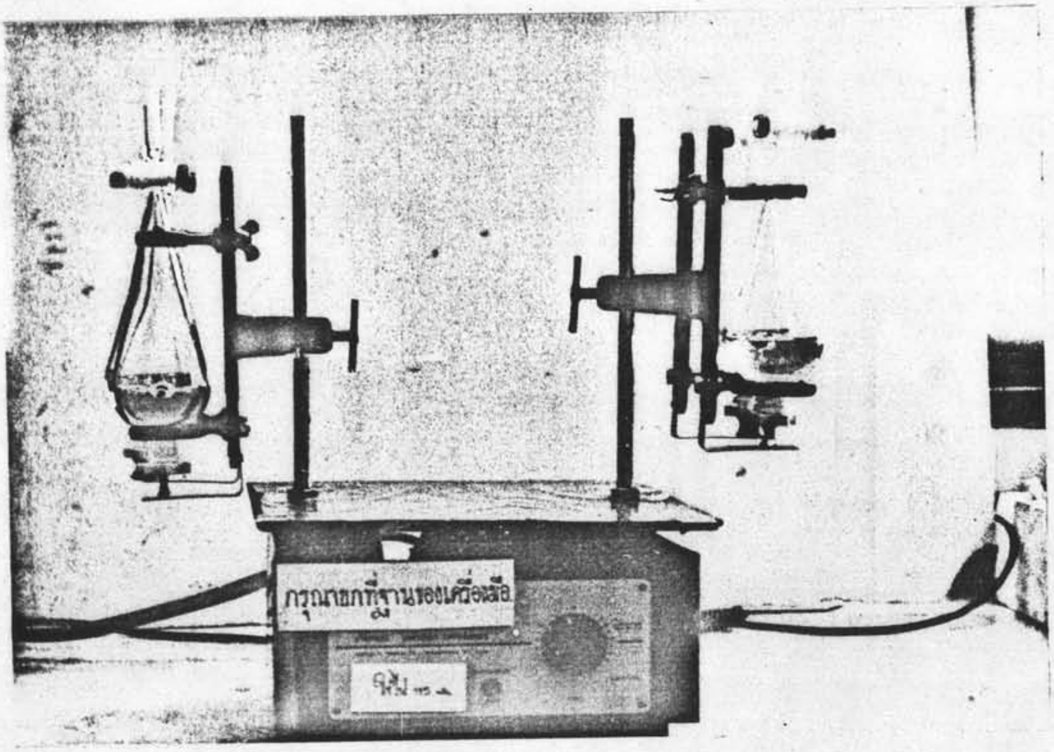

Figure 3-2 MECHANICAL SHAKER

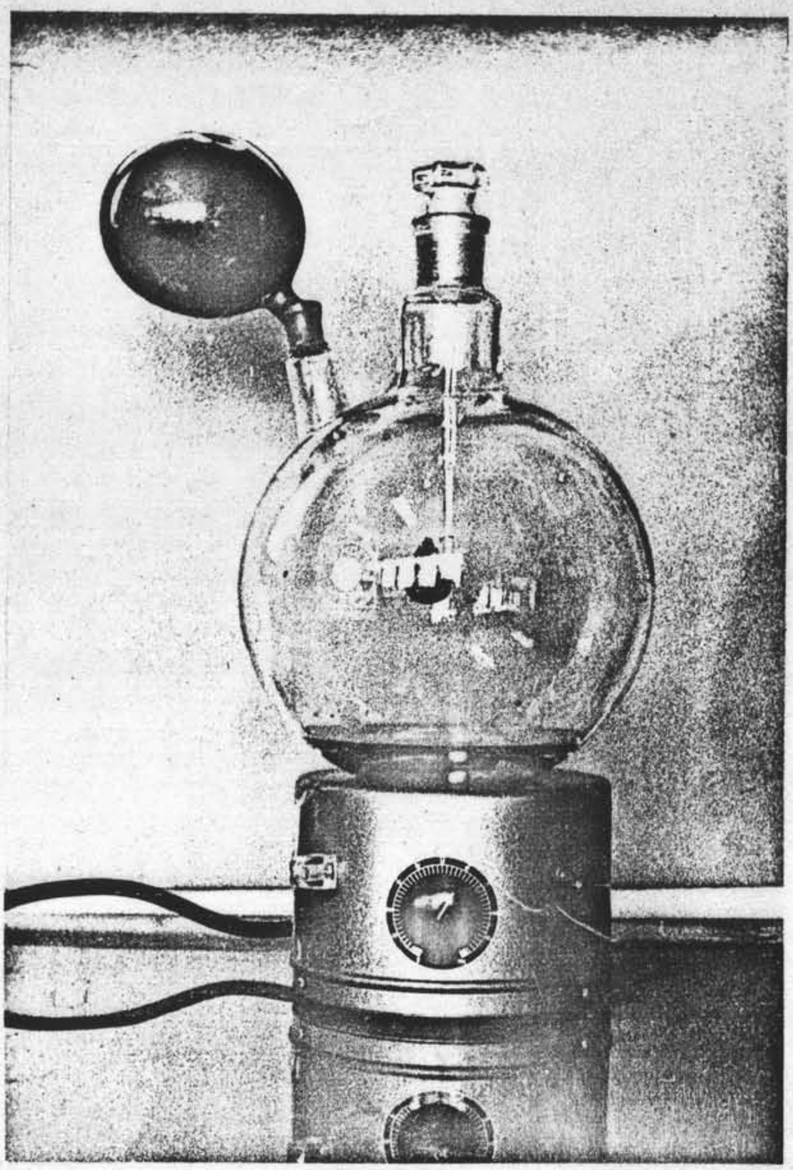

Figure 3-3 COMBUSTION FLASK WITH PLATINUM HOLDER.

The flask's stopper was connected with a glass rod fused with a platinum wire, which holding a platinum basket for placing a sample for combustion, at the other end.

3.3. Procedures.

In order to ascertain the best conditions for the mercury extraction with dithizone, preliminary experiments of pH range, effect of zinc dithizonate chelating on mercury, time of reaching equilibrium, selectivity, effect of masking agent and effect of hydroxylamine hydrochloride were investigated using mercury-203 as tracer, as follows:

3.3.1. Determination of the effect of pH.

To a series of radiomercury solutions containing 0.6466 microgram of mercury, the appropriate amount of diluted hydrochloric acid or ammonia solution was added to reach the various desired pH. These solutions were simultaneously extracted with zinc dithizonate. The activity of two millilitre of individual organic phase was measured for mercury-203.

3.3.2. Determination of the effect of zinc dithizonate chelating on mercury. A series of 80 millilitre diluted hydrochloric acid solutions containing 0.6466 microgram of radiomercury was extracted with varying millilitre of 4×10^{-5} % zinc dithizonate. The activity of equal portion of organic phase was measured.

3.3.3. Determination of the effect of time for reaching equilibrium. A series of 0.6466 microgram of labelled mercury in 80 millilitre 0.1M hydrochloric acid solution was shaken with three millilitre of 4×10^{-5} % zinc dithizonate for varying period of time. The activity of two millilitre of each of organic layer was measured.

3.3.4. Determination of the selectivity of substoichiometric extraction of mercury. In order to investigate the effect of interfering substances with the extraction, the experiment was set, as follows: A series of solution containing 0.3233 microgram of radiomercury was substoichiometrically extracted with three millilitre of 2×10^{-5}% zinc dithizonate in the presence of 200 microgram of non-active cobalt (II), manganese (II), copper (II), silver (I), iron (III), cadmium (II), indium (III), lead (II), bismuth (III), nickel (II), tin (II), thallium (III) and thallium (I). The activity of mercury present in each of organic phase was measured.

An experiment was carried out exactly as above with 10 milligram of series of non-active elements.

3.3.5. Determination of the efect of masking agent on substoichiometric extraction of mercury. A series of solutions containing 0.3233 microgram of radiomercury, 200 microgram and 10 milligram of copper (II), 10 milligram of thallium (III) and thallium (I) respectively was shaken with three millilitre of $2 \times 10^{-5}\%$ zinc dithizonate with varying millilitre of EDTA. The individual equal portion of organic phase was counted.

3.3.6. Determination of experimental reproducibility of substoichiometric extraction of mercury. A series of solutions containing variable amounts of mercury labelled with 0.6466 microgram of radiomercury was extracted with three millilitre of $4 \times 10^{-5}\%$ zinc dithizonate for about 30 second. The activity of two millilitre of each of organic phase was counted.

3.3.7. Determination of the reliability of substoichiometric extraction of mercury. A series of mercury solutions containing 1.007 microgram of mercury labelled with standard solution containing 0.6466 microgram of radiomercury was extracted with three millilitre of $4 \times 10^{-5}\%$ zinc dithizonate. Two millilitre of each organic phase was measured for gamma activity of mercury-203.

3.4. Radiomercury measurement.

All mercury-203 analyses were made with an instrument described in 3.2.1. The gamma spectrum of 0.279 Mev. of mercury-203 was used for the determination. The counting rates obtained were corrected for background.

3.5. Mercury Determination.

3.5.1. Combustion technique. Weigh the sample on the cellophane and wrapp the cellophane around it, and again wrapp the filter paper around the cellophane. Place 40 millilitre of 0.1 M hydrochloric acid and one millilitre of standard radiomercury solution and a teflon covered magnetic stirrer in the combustion flask and a rubber balloon was firmly attached to the side-neck. The flask was then throughly flushed with oxygen. Place the package of sample in the wire basket and insert a fuse cut from filter paper into the sample, lit and immediately place in the flask. The stopper was held firmly in position until the combustion was completed, after which the flask was placed on the magnetic stirrer, which was operated fast enough to wash the inner surface of the flask with splashing solution. After 10-15 minute all visible vapours were condensed and all combustion products absorbed. The flask was removed from the stirrer, the wire basket, with ash, was carefully removed and the flask was closed again with the glass stopper. The flask was tilted in order to rinse its upper part, but the acid must not enter the balloon. The flask was opened again, the balloon was removed and the absorbing solution was poured through the side neck into 250 ml. separatory funnel. The flask was further rinsed twice with small portions of 0.1 M hydrochloric acid which were also added to the funnel.

Any oxidant occurring in the combustion product will cause trouble for the solvent extraction step. The experiment was therefore made to investigate the effect of the amount of hydroxylamine hydrochloride (strong reducing agent) on the substoichiometric extraction step, as follows:

A series of combustion product containing 0.6466 microgram of radiomercury containing variable amounts of hydroxylamine hydrochloride was extracted with three millilitre of 4 X 10^{-5}% zinc dithizonate. The gamma activity of mercury-203 in the organic phase was counted.

3.5.2. Substoichiometric isotope dilution technique.

To the combined acid solution in the separatory funnel, 10 ml. of buffer solution containing EDTA was added. Four millilitre of 20 % hydroxylamine hydrochloride was also added and the solution was shaken. Three millilitre of diluted zinc dithizonate (concentration corresponded to the amount of specific activity of radiomercury added) was added. The mixture was vigorously shaken for about 30 sec. and left for the phase to separate. Exactly two millilitre of organic extract obtained was measured for gamma activities of mercury-203 as described under section 3.4 (A_x)

Simultaneously with the sample, but only exactly same amounts of cellophane and filter paper, the steps of combustion and substoichiometric isotope dilution were treated identically as the sample. Again two millilitre of organic phase was counted to obtain the value of A.

From the equation (3), the mercury concentration in sample could be determined.

3.5.3 Determination of the reproducibility of the developed technique. Four various concentrations of mercury were separately dropped on the filter papers and treated as the unknown samples for reproducibility test. The individual dried filter paper was determined for mercury as described under section 3.5. The calculated concentration of mercury was compared to the corresponding known concentration.

3.5.4. Determination of the reliability of the developed technique.

Three Standard Reference Samples were used to check the reliability of the developed technique. The experiment was carried out as described in 3.5 and the content of the mercury in samples was evaluated from the activity measurement.

3.6. <u>Analytical scheme.</u> The scheme can be summarized as in Fig 3-4

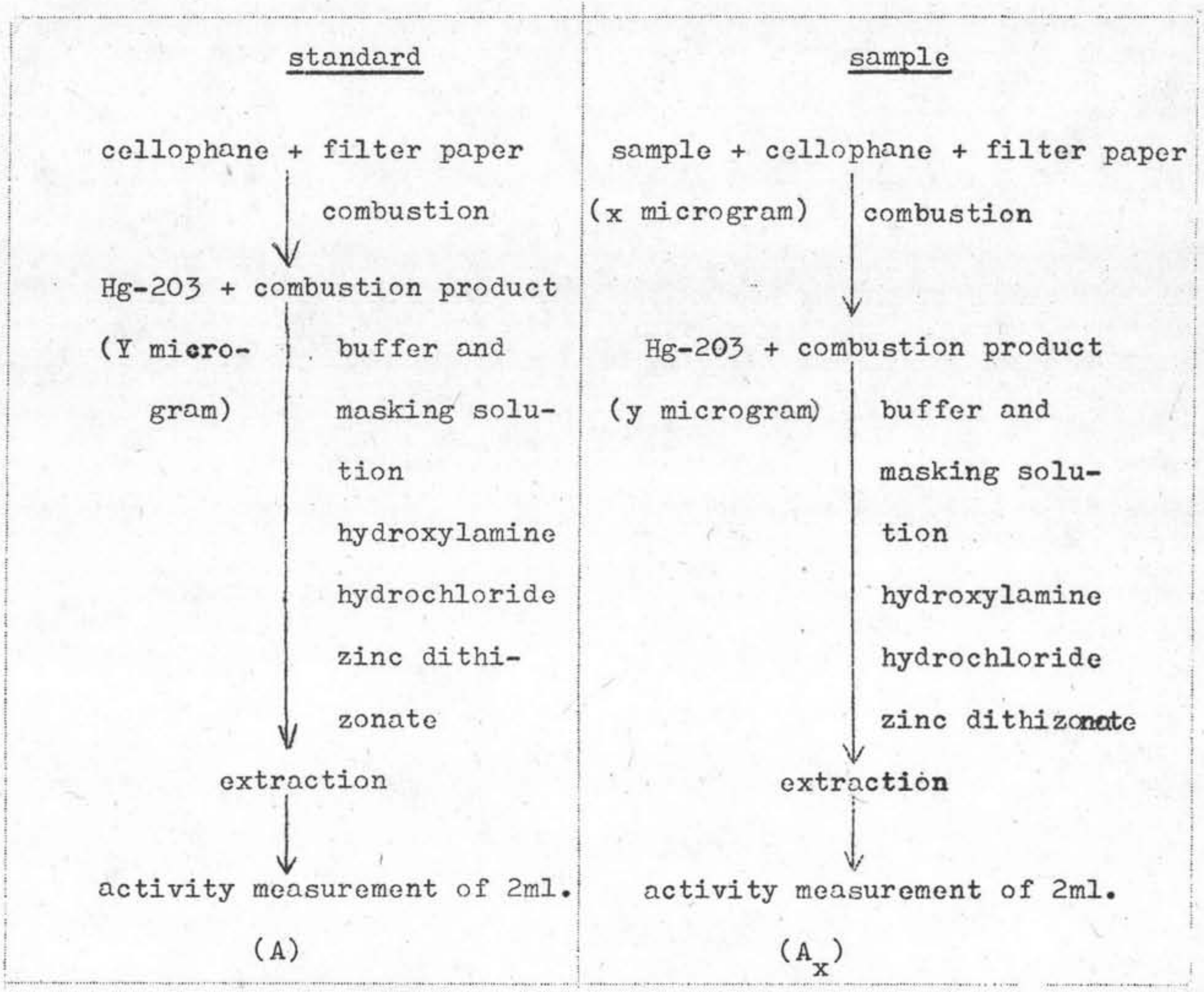

Fig 3-4 : ANALYTICAL SCHEME

The content of mercury in the sample (x microgram) was calculated from A_x and A by the equation

$$x = y\left(\frac{A}{A_x} - 1\right)$$