CHAPTER II

EXPERIMENTAL

2.1 Materials

2.1.1 Method of Sampling. Food and food products were purchased monthly at different well-known supermarkets and grocery stores in downtown areas in Bangkok. Eighty-nine samples were collected during December 1976 through January 1977. These preserved food were:- fish, meat, cereal, milk, milk powder and milk products which were packed in can or air-tighted sealed plastic bag. Samples were selected in such a way that they would represent both collecting from contaminated and non-contaminated area. A list of types of samples relating to the countries distributed was shown in Table 1.

2.1.2 Sample preparation.

Sample were prepared relating to the types of analysis that was :

2.1.2.1 For gamma-counting: Individual sample was weighed and blended with an automatic glass blender. This was done in order to control a homogeneity of sample. Then, it was transferred into a 1000 ml. Marinelli beaker (Figure 1) for gamma counting.

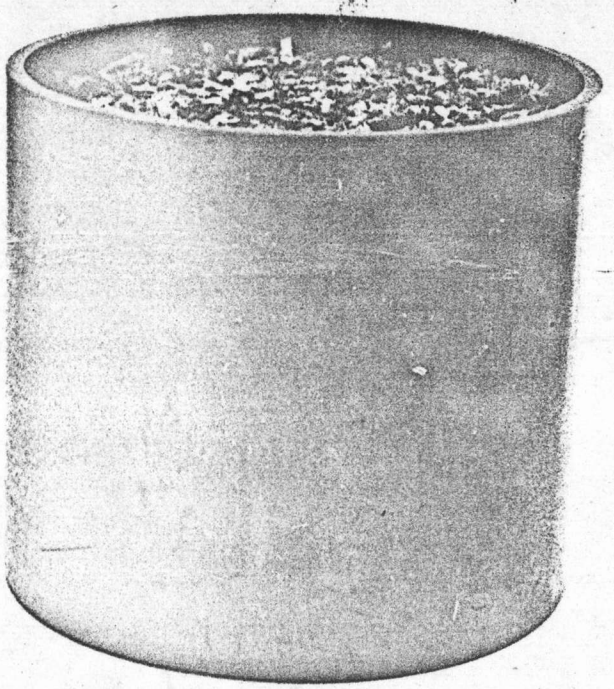

FIG. I MARINELLI BEAKER.

Table 1

Types of Sample & country distributed

Sample No.	Type of sample	Countries distribute
	Marine fish and Shellfish	
1.	Sardine	Norway, Thailand, Japan, Denmark, U.S.A., Portugal, Southwest Africa, Morocco
2.	Salmon	Japan, Canada
3.	Tuna	Thailand
4.	Herring fellet	U.S.A.
5.	Mackerel	Japan
6.	Threadfin	Thailand
7.	Clam	Japan
8.	Oyster	Japan, Korea
9.	Pilchard	England
	Meat	
10.	Corned beef	Australia, France
	Cereals	
11.	Corn flake, barley, Oat	Australia, U.S.A., Switzerland, West Germany, Malasia, Thailand
	Milk	
12.	Milk powdered	Japan
13.	Condensed milk	Thailand
	Food products	
14.	Butter	Australia
15.	Baby food	Thailand

2.1.2.2 For beta-counting: After gamma-activity measurement, sample was transferred into porcelain dishes and dry-ashed. The ashing technique was done in 3 steps. Firstly, sample was dried in an oven at temperature 120-200°C for about 6 hours until it became char. Secondly, the dishes were placed in a muffle furnace. The sample was preliminary ashed at temperature around 120-300°C, depending on the type of sample, for a period of 8 hours (Table 2) and finally at 500°C for 12 hours. Thirdly, allow the dished to cool down and put them in the oven again for 6 hours. After cooling in the desiccator for 1 hour, the ash sample was pulverized, weighed and kept in a clean container.

Table 2:

Dry-ashing temperature relating to the type of sample

Type of sample	Oven-dried temperature °C	Preliminary-ashing temperature °C	Final ashing temperature °C
Fish	120	burning	500
Meat	120	burning	500
Milk(dry)	200	-	500
Milk(wet)	120	120-300	500
Grains	200	200-300	500

2.1.2.3 For chemical seperation: The third operation was to put the ashed sample into solution. A portion of each ashed sample was weighed and digested with concentrated HNO_3 for half an hour. The solution was filtered and the clear solution was stored in a 125 ml. polyethylene boltle and kept for the analysis of Sr-90 and stable calcium. The volume of the clear solution must be accurately measured.

2.2 Experimental procedures.

2.2.1 Gamma-emissions searching.

Each of fresh sample, as described under 2.1.2.1, in 1000 ml Marinelli beaker was counted for gamma emission for 18 hours using 128-channel pulse height analyzer attached to 3" x 3" NaI(Tl) crystal detector.(Figure 2, Figure 3). The long-lived radionuclides identified were potasium-40(K-40) and cesium-137 (Cs-137). The area under the photopeak of 1.46 MeV. and 0.66 MeV. of K-40 and Cs-137 respectively were used for calculation by Covell's method. The background radiation was counted identically as the sample but with only empty Marinelli beaker. The counting rates obtained of sample were also corrected for background.

The activity concentrations of K-40 and Cs-137 in the sample was computed with the standard solution of KCl and Cs-137 and reported as pCi/g wet weight. according to the formula:

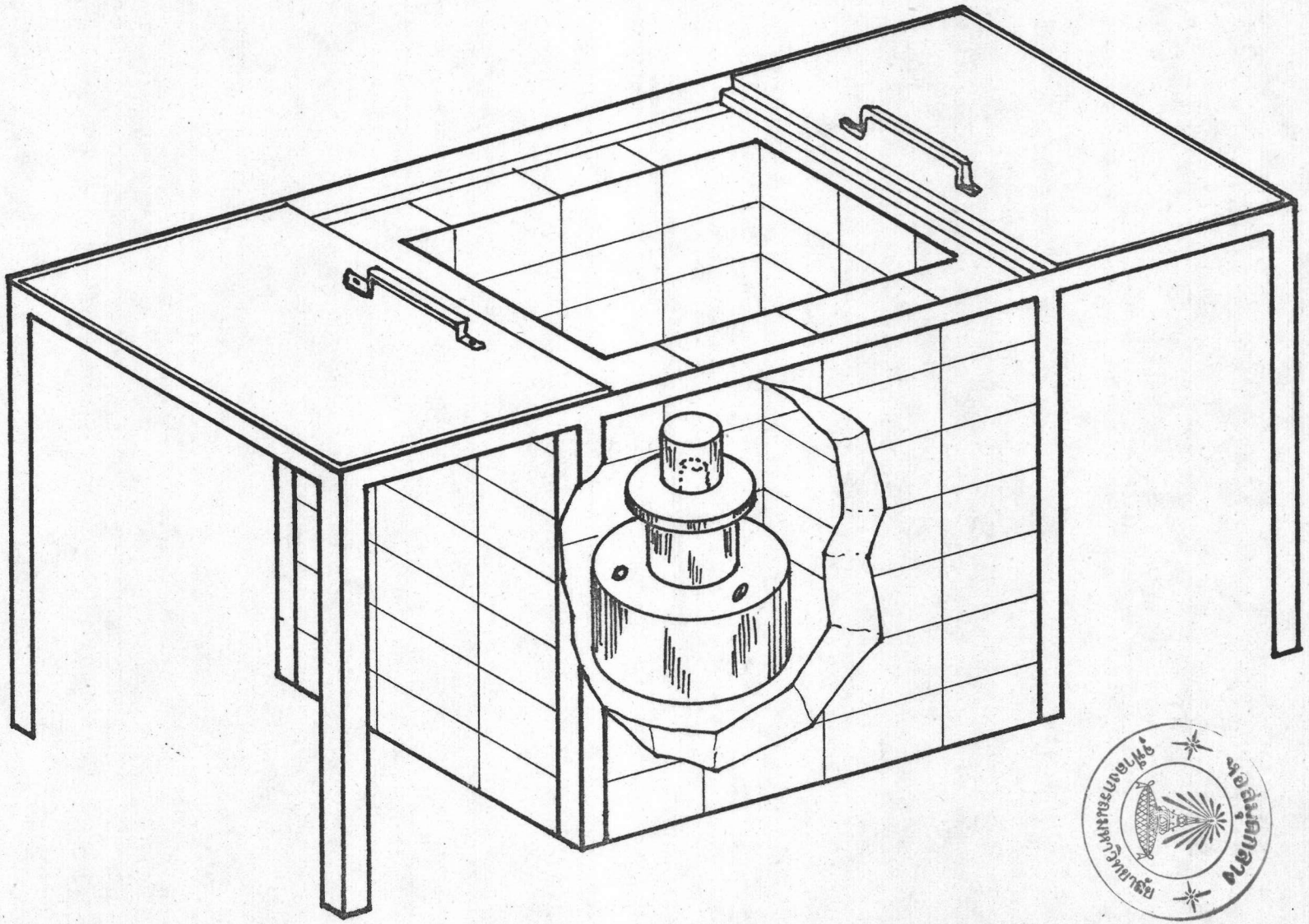

FIG. 2 MARINELLI BEAKER IN THE LEAD CAVE.

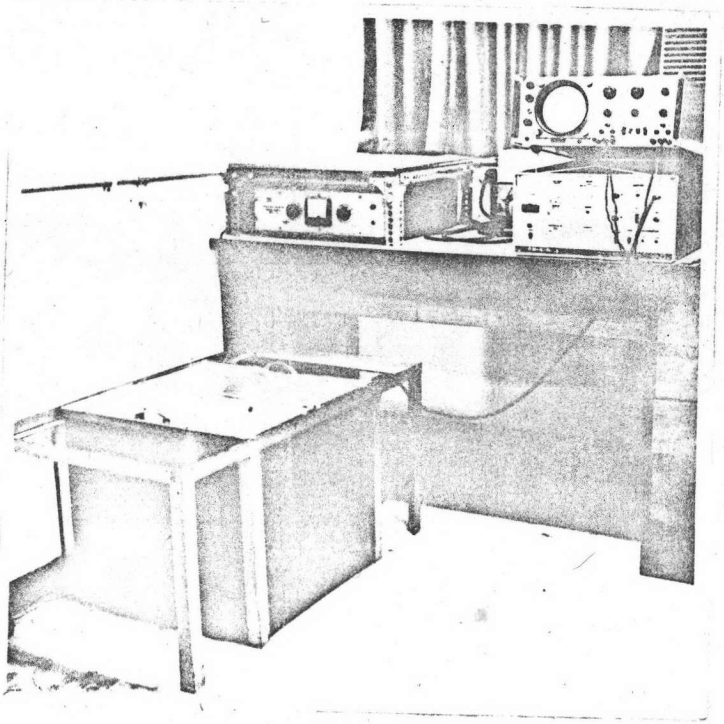

FIG.3 THE 128 CHANNEL ANALYZER CONNECTED WITH THE NaI (TI) DETECTOR.

$$\text{K-40 (pCi/g wet wt.)} = \frac{\text{corrected cpm}}{\text{efficiency} \times 2.22 \times \text{fresh wt.(g)}}$$

$$= \frac{\text{corrected ppm}}{0.02 \times 2.22 \times \text{fresh wt. (g)}}$$

$$= \frac{\text{corrected cpm}}{0.0444 \times \text{fresh wt. (g)}}$$

$$\text{and Cs-137 (pCi/g wet.wt)} = \frac{\text{corrected cpm}}{\text{efficiency} \times 2.22 \times \text{fresh wt.(g)}}$$

$$= \frac{\text{corrected cpm}}{0.03 \times 2.22 \times \text{fresh wt. (g)}}$$

$$= \frac{\text{corrected cpm}}{0.0666 \times \text{fresh wt. (g)}}$$

2.2.2 Gross beta activity measurement

Ashed sample was individually weighed in an aluminium planchet of 3 centrimetres in diameter and counted in the low background anti-coincidence G-M counter for 1000 seconds. The counting rates obtained of sample were also corrected for background radiation which was counted identically as sample but with using aluminium planchet alone.

The gross beta activity was computed with using the KCl standard solution and reported as pCi/g ash wt. as formula:

$$\text{gross } \beta \text{ activity (pCi/g ash)} = \frac{\text{corrected cpm}}{\text{efficiency} \times 2.22 \times \text{ash wt(g)}}$$

2.2.3 Strontium-90 determination by TBP extraction

2.2.3.1 Glassware and Apparatus

a) 250 ml. peared-shape separatory funnel

b) 150 ml beaker

c) 50 ml. centrifuge tube

d) 1, 5 ml. pipette

e) Propipette

f) Glass stirrer rod

g) pH paper

h) 125 ml. polyethylene bottle

i) Glass fibered filtered paper 2.1 cm in diameter

j) Aluminium planchet

k) Complete set of Millipore apparatus (Figure 4)

l) Infrared lamp

m) Water bath

2.2.3.2 Reagents

a) Tributyl phosphate solution (TBP)

The solution used must be equilibrated with 14M HNO_3 by shaking equal volume of TBP and 14M HNO_3 for 1 minute, discarded aqueous phase.

b) Concentrated HNO_3

c) Concentrated HCl

d) 1:1 NH_4OH solution

the reproducibility of analyses was obtained by repeating two or more determinations of the same sample identically and considered for the agreement between those numerical values. The data which is precise does not indicate the accuracy. The results are therefore questionable unless the procedure was previously tested for the accuracy.

The precision of this study was made by analyzing the standard Sr-90 solution obtaining from the Radiochemical, Amersham and was diluted to concentration of 6.0 pCi. The results of four investigation was shown in Table 3.

The reliability of the chemical procedure was performed by analyzing a standard reference fresh water sample W-2 which was distributed by IAEA. The result was shown in Table 4.

2.4 Determination of stable Ca in food samples

The permanganate titration procedure was used to determine the calcium in food sample.

2.4.1 Principle of method

Calcium oxalate was precipitated from a portion of ashed sample which had been dissolved in a weakly acid solution. The precipitate was dissolved in dilute sulfuric acid and titrated with $KMnO_4$.

e) Sr-carrier (20 mg of Strontium per one ml. strontium nitrate solution)

f) Y-carrier (20 mg of yttrium per one ml. of yttrium nitrate solution)

g) Sr-90 solution from the Radiochemical Centre, Amersham. The solution was diluted to 6.0 pCi per ml corrected at the time of analysis

h) Reference Standard Solution W-2(fresh water) from International Atomic Energy Agency (IAEA)

i) Sr-85 solution from the Radiochemical Centre, Amersham. The concentration was 200 μCi per ml. and was diluted to 0.05 μCi per ml. for analysis of Sr-90

2.2.3.3 Electrical Aparatus

a) Shaker with shaking heads, for four seperatory funnels from Arthur H. Thomas Company (Figure 5)

b) 1/3 H.P. vacumm pump from General Electric

c) Centrifuges with 4 x 100 ml. horizontal head.

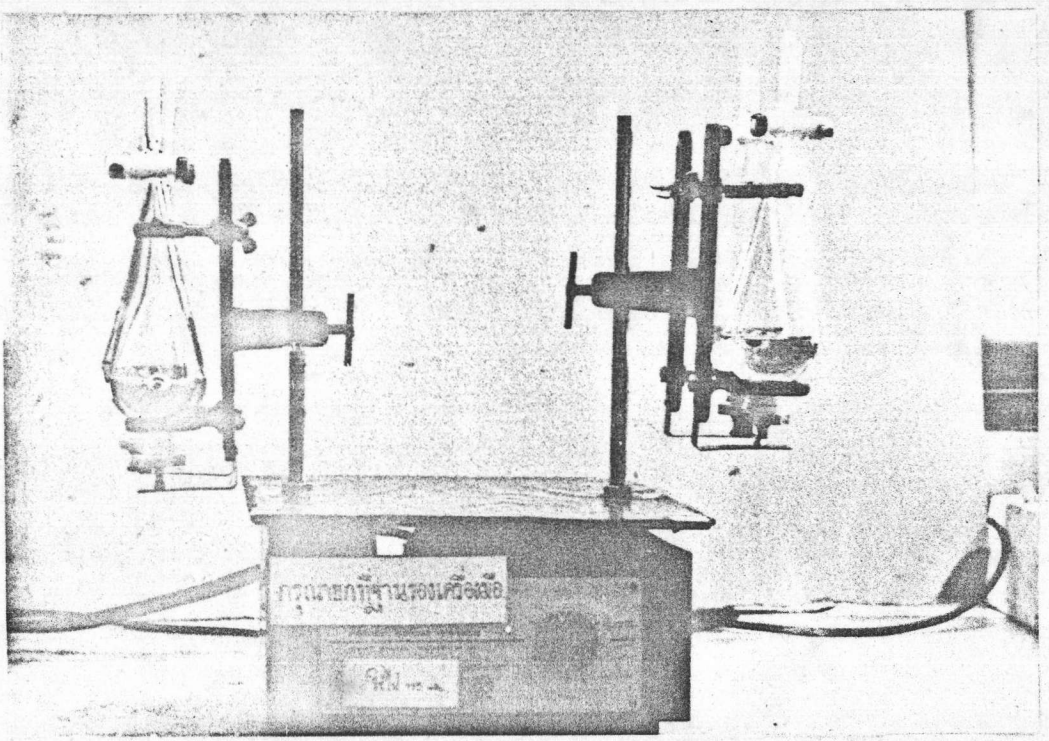

FIG. 5 MECHANICAL SHAKER.

2.2.3.4 Counting equipments.

a) Low background anti-coincidence G-M counter, Betaplan-50 (Panax) in connection with low background GM x 152 (19.8 mm in diameter) and guarded counter, M x 155. (Figure 6).

b) Single channel analyzer, Model 1431 (Canberra) in conjunction with a 3" x 3" NaI(Tl) crystal detector (Figure 7).

2.2.4 Method for strontium-90 determination by solvent extraction.

The tributyl phosphate extraction was used to determine the activity concentration of Sr-90 in food samples which had been dried, ashed and dissolved into a clear solution. The extraction into TBP provided the completeness of a pure yttrium separation.

2.2.4.1 Principle of method

About 4-5 grams of ashed sample was dissolved by 40 ml conc. HNO_3 for about half an hour, and the solution was filtered through glass fiber filtered paper. A clear solution obtained was transferred to polyethylene bottle, recorded the sample number and the volume.

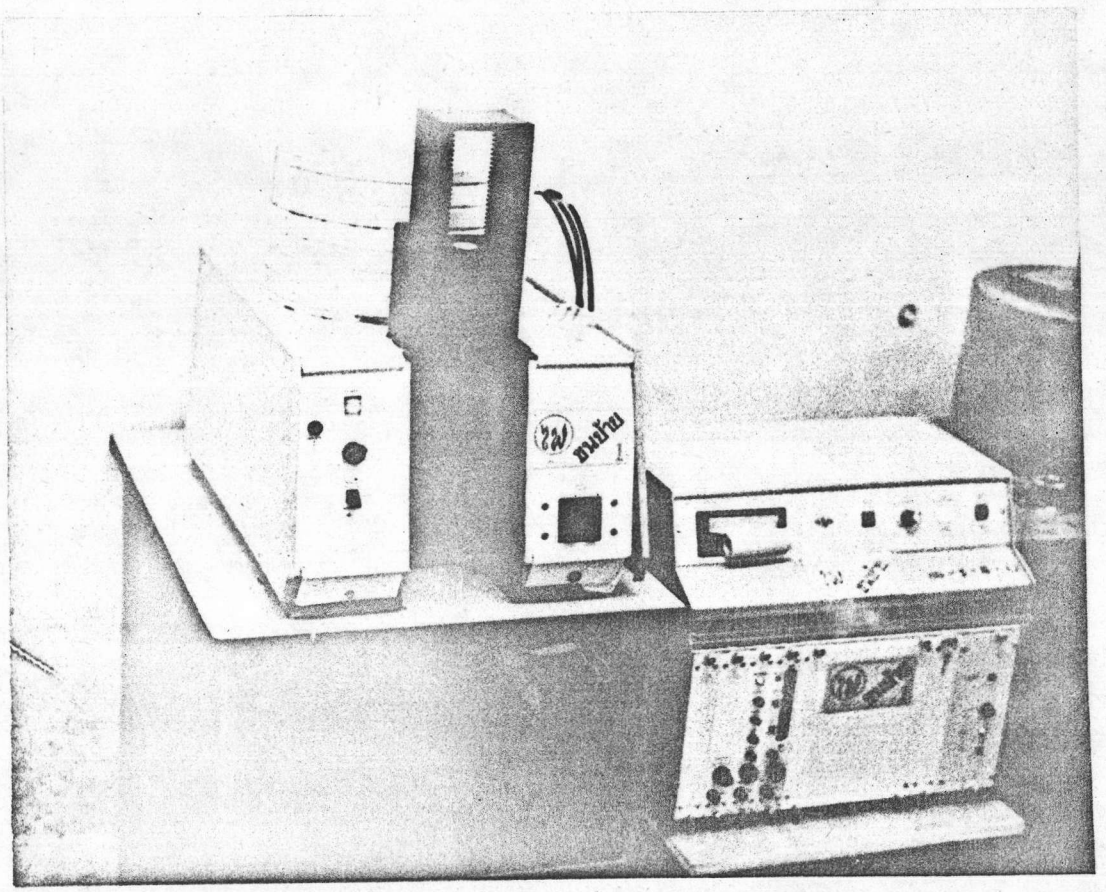

FIG. 6 LOW BACKGROUND ANTI-COINCIDENCE G-M COUNTER.

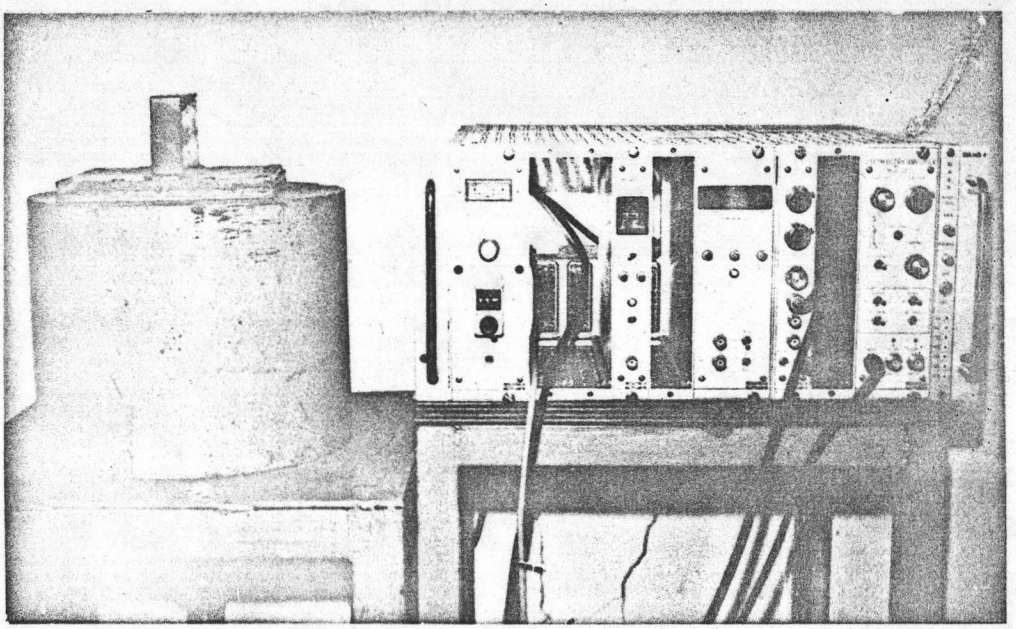

FIG.7 SINGLE CHANNEL ANALYZER

Yttrium in the solution was extracted into TBP, back extracted into distilled water and precipitated as yttrium oxalate. Since the extraction is strongly favoured by high concentrations of nitrate ion and of TBP. The extraction can be symbolized as:

$$Y^{+3}(aq)+2NO_3^-(aq)+3TBP(org) = Y(NO_3)_3 \cdot 3TBP(org)$$

Strontium 90 activity was computed from Yttrium-90 activity.

2.2.4.2 Calculation of Sr-90 activity.

$$\text{Sr-90 activity as pCi} = \frac{A}{B \times C \times D \times E} \quad ..(1)$$

A = net count rate of Y-90 (cpm)

B = correction factor $e^{-\lambda t}$ for Y-90 decay, where t is the time from separation of Y-90 to the time of β-counting

C = recovery of yttrium carrier

D = chemical yield of Sr-90 separation

E = efficiency of counting Y-90 as yttrium oxalate on 2.1 cm in diameter glass filtered(cpm/pCi)

2.2.4.3 Procedure

Five millilitre of dissolved sample solution was pipetted into a 150 ml beaker containing 1 ml each of strontium and yttrium carrier and Sr-85 tracer. The solution was well mixed and brought up to 30 ml volume with nitric acid and then warmed on a hot plate for 10 minutes while stirred continuously. Fifty millilitre

equilibrated TBP was added into a 250 ml Separatory funnel following with warmed sample solution. A small amount of nitric acid may be used for completely transferred of the sample solution. The funnel was then shaked vigorously for 15 minutes. After the phases were completely separated, the aqueous phase was drained off into another 250 ml separatory funnel containing 50 ml of equilibrated TBP. The funnel was again shaked for 15 minutes. The time and date of decantation was recorded as the end of Y-90 ingrowth and the beginning of its decay in the yttrium fraction.

When the phases were separated completely, the aqueous phase was transferred into a 125 ml polyethylene bottle and the remained organic phase was washed twice with 20 ml 14M HNO_3 by shaking the funnel for 10 minutes at a time. The washing solution was added into the same 125 ml polyethylene bottle. The organic phase was back-extracted twice with two successive 50 ml distilled water, and the aqueous solution was transferred into a 150 ml beaker and was evaporated to 10 ml. The solution was transferred to a 50 ml centrifuge tube with water and adjusted to pH 8 with 1:1 NH_4OH. The solution was allowed to cool to room temperature and centrifuged for 5 minutes and the supernatent was discarded.

The precipitate was dissolved with 1 ml conc. HCl and the solution was heated to 90°C and adjusted to 25 ml with distilled water. One ml of saturated $H_2C_2O_4$

was added and the pH was adjusted to 2 using NH_4OH. Yttrium oxalate was precipitated in the centrifuge tube while vigorous stirring. The precipitate was allowed to digest for 1 hour.

The precipitate was filtered through a 2.1 cm in diameter glass fiber filtered paper, which weight was accurately known, using a set of Millipore filtering apparatus (Figure 4). The oxalate precipitate was placed on the aluminium counting planchet and was dried under infrared lamp for about 1 hour. The precipitate was cooled to room temperature and weighed. The activity of yttrium oxalate was counted using low-background anti-coincidence G-M counter from Panax. (Figure 6). The time and date of counting was then recorded.

The volume of strontium phase was diluted to the neck of the polyethylene bottle and counted for gamma-activity of Sr-85 by a single-channel analyzer from Canberra (Figure 7).

The appropriate concentration of standard solution of Sr-90 was treated identically as the sample.

2.2.4.4 Calculation

The gross counting data obtained on the Y-90 precipitation was corrected to give the proper disintegration rate representing the Sr-90 in the sample (eq.1). The correction included those for counter background

and efficiency, strontium yield, the recovery of yttrium carrier as yttrium oxalate, yttrium-90 decay and expressed as picocurie.

The counter background was determined in each of counting tray with filter paper. The strontium yield was determined by measuring the recovery of Sr-85 tracer added to the sample. Since an aliquot of the original Sr-85 tracer solution was counted at the same time as the samples there was no need to know the disintegration rate of the tracer to apply for decay corrections.

The decay of Y-90 from the time of separation to the time of counting and also the recovery of 20 mg yttrium carrier as yttrium oxalate (one hundred percent recovery of 20 mg of yttrium carrier is 68 mg of yttrium oxalate) were corrected for.

The concentration activity of Sr-90 as pCi/g of ash weight in the sample was then calculated.

$$\frac{\text{amount of Sr-90 in the sample}}{\text{amount of Sr-90 in the standard solution}} = \frac{\text{total corrected count rate in the sample}}{\text{total corrected count rate in the standard solution}}$$

2.3 The Reliability test for Sr-90 determination by the TBP extraction.

The reliability in quantitative analysis is based on both precision and accuracy. The precision or

the reproducibility of analyses was obtained by repeating two or more determinations of the same sample identically and considered for the agreement between those numerical values. The data which is precise does not indicate the accuracy. The results are therefore questionable unless the procedure was previously tested for the accuracy.

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2.4.2 Reagents and glasswares

a) Ammonium hydroxide 15 M

b) Oxalic acid 2 M

c) Potasium permanganate 0.05 N

d) Sulfuric acid 2 M

e) Hydrochloric acid 6 M

f) Primary standard of sodium oxalate 0.05 N

g) 50 ml burette

h) 5 ml pipette

i) 250 ml Erlenmeyer flask

j) 50 ml volumetric flask

2.4.3 Experimental procedure

2.4.3.1 Preparation of 0.05 N Potasium ganate

1.6 gm of $KMnO_4$ was dissolved in 900 ml distilled water. The solution was heated to near boiling for 20-30 minutes and cooled overnight. The reagent was diluted to 1000 ml and filtered through Whatmam no.1 filter paper. The solution was standardized at least once each week against primary standard solution of sodium oxalate.

2.4.4 Standardize $KMnO_4$ with Primary Standard Solution $Na_2C_2O_4$.

Exactly 0.335 gm of $Na_2C_2O_4$ was dissolved in 25 ml 5% H_2SO_4 and the solution was diluted with distilled

water to 100 ml in volumetric flask. Five ml $Na_2C_2O_4$ was pipetted into 250 Erlenmeyer flask, added 1 ml H_2SO_4 and diluted to 10 ml with distilled water. The solution was heated to just below boiling, and titrated with 0.05 N $KMnO_4$ to the first faint pink endpoint which persisted for at least 30 seconds.

$$\text{Normality of } KMnO_4 = \frac{\text{mg. of } Na_2C_2O_4 \text{ standard} \times \frac{5}{100}}{(\text{ml } KMnO_4)(\text{meq.wt. } Na_2C_2O_4)}$$

$$\text{Ca (mg Ca/gm. ash)} = \frac{A \times B \times C}{E} \times \frac{100}{5} \quad \frac{D}{5}$$

A = vol. of $KMnO_4$ used for titration

B = morality of $KMnO_4$ (Meq./ml)

C = milli equivalent weight of Ca (mg/meq) = 20 mg

D = Total volume of dissolved ashed sample

E = wt. of ashed aample which was dissolved

2.4.5 Procedure

Five ml of dissolved ashed sample was pipetted into 150 ml beaker and evaporated to dryness. The residue was re-dissolved in 6 M HNO_3, and transferred to a 50 ml centrifuge tube using small amount of water and finally diluted to 30 ml with distilled water. The centrifuge tube was warmed and 5 ml of oxalic acid was again added. The solution was throughly mixed and adjusted to pH 3.5-4.0 with 15M NH_4OH. The solution was centrifuged and the supernatent was discarded. The

precipitate was washed twice with two successive of 40 ml hot distilled water and the filtrate was also discarded. The precipitate was dissolved in 2M H_2SO_4 and made up to 50 ml with distilled water in a 50 ml volumetric flash.

Exactly 5 ml of the solution was pipetted into a 250 ml erlenmeyer flask and 1 ml of H_2SO_4 was also added. The aliquot was adjusted to 10 ml and heated to 90°C on a hot plate. The solution was titrated while hot with standardized 0.05N $KMnO_4$ to a faint pink colour which persisted for at least 30 seconds.