TRACE MERCURY ANALYSIS

TRACE ANALYSIS FOR MERCURY
IN
BIOLOGICAL MATERIAL

By

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SCOPE AND CONTENTS:

The need for accurate trace analysis for mercury due to its very toxic nature and widespread distribution is discussed. Critical investigations of the two currently most widely used analytical techniques, neutron activation analysis and cold vapour atomic absorption, are reported in detail. Many problems with the methods of analysis for mercury are delineated, and improvements are outlined. Procedures for obtaining best results are recommended. An instrumental improvement involving a photon-counting readout for the atomic absorption method is reported. A comparison of the analytical techniques is given.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 GENERAL INTRODUCTION

1.1 Brief Outline of the Aims of This Work *

It is now well established that mercury is an element which is highly toxic to human and many other life-forms. Alkyl-mercurials (such as methyl-mercury compounds) have been found to be especially potent. Maximum acceptable daily intake of mercury has been estimated to be in the order of 100 micrograms per day for inorganic forms, or 30 micrograms per day for alkyl-mercurials.

Mercury can be absorbed through the lungs by breathing contaminated air, or through the skin from contact, but for the general populace, ingestion in food and drink appears to be the main source. If three kilograms is used as an estimate of daily intake of food and drink, the average level of mercury contamination should be below thirty-three parts per billion for inorganic mercurials, or less than ten parts per billion for alkyl-mercurials. So far, evidence

* For the sake of brevity and clarity, no references are given in this section, and details are kept to a minimum. Full details and references are presented in Section 1.3.

suggests that mercury in food occurs largely in the latter form.

Despite its highly toxic nature, the unique properties of this element have led to its widespread use in industrial processes such as chlor-alkali plants, plastic production (as a catalyst), pulp and paper production (as a slimicide), mining (amalgamation-collection of silver and gold), and electrical instruments and apparatus manufacture; in agriculture as a seed dressing and fungicide for fruit and turf growing; and in science for thermometers, manometers, mercury diffusion pumps, electrodes, and many other laboratory uses. Losses from various processes invariably occur and lead to pollution of the environment. Furthermore, the agricultural uses result in direct application of mercurials to the growth of foodstuffs. In addition, the smelting of ores and burning or other treatment of fossil fuels is now recognized as a major source of mercury pollution.

The problem, then, is basically the investigation of man-made pollution: its sources, its distribution, and its effect on the environment and on man himself. In this regard, it would also be very useful to establish normal levels. This problem has had a great deal of attention focussed on it in the past decade as the result of increasing awareness of widespread mercury pollution in the environment. To solve the problem, an analytical method is required which can give <u>accurate</u> results down to a concentration of ten parts per billion. The expe-

riences of many researchers have proven that trace analysis for mercury is an extremely difficult task due to its volatility and the problem of contamination at these very low levels of concentration.

This thesis presents the results of a critical investigation into the two main analytical techniques currently employed in the trace analysis of mercury: neutron activation and cold vapour atomic absorption. Many problems encountered with techniques are outlined and discussed, and an instrumental improvement for the atomic absorption method is presented. Recommended procedures are delineated, and a comparison of the two methods is given.

1.2 Organization of the Thesis

The thesis has been organized in the following manner: Chapter 1 is a general introduction to the problems of trace analysis for mercury, including a survey of the vast literature on this subject. Chapter 2 considers the volatility of mercury. The results which have been obtained were so surprising that it was felt that this topic warranted a chapter of its own to emphasize its importance. Chapter 3 describes a detailed critical investigation of the neutron activation analysis technique. Chapter 4 gives a detailed critical investigation of the cold vapour atomic absorption technique. Each of these last two chapters is complete unto itself, as it was felt that this would be the most convenient presentation. Chapter 5 presents a general discussion and a comparison of the two methods which have been investigated.

1.3 General Aspects of Trace Mercury Analysis

1.3.1 Introduction

Many excellent monographs on the larger problems of mercury pollution exist and should be consulted for a more detailed account. These include: "The Environmental Mercury Problem", by D'Itri;[1] "Mercury Pollution Control", by Jones;[2] and "Environmental Mercury Contamination", by Hartung and Dinman.[3] Grant[4] gives an account of "Mercury In Man" with many references to its toxic effects observed in Japan, Sweden, Canada and the United States. The excellent Swedish research on mercury pollution has been outlined by Ljunggren et al[5] and Ackefors,[6] among others. A number of accounts are also available in the more popular literature, such as "The Toxic Metals", by Tucker;[7] and "Mercury, Man's Deadly Servant", by Putman and Madden.[8] These lend some additional perspective to the situation.

1.3.2 Toxicity of Mercury

The extremely toxic nature of mercury and its compounds has been brought dramatically to the public's attention in the last two decades due to several serious epi-

demics of poisoning from food containing mercurials. The tragedy that occurred at Minimata, Japan (1 - 4, 7, 8, 9) between 1953-1961 has led to the dubious honour of diagnosing the symptoms of mercurial poisoning as "Minimata Disease." Another outbreak of mercury poisoning occurred at Niigata, Japan (10) in 1964-1965. The source of the poisoning was found to be methyl-mercury compounds ingested in fish. Other wide-spread tragedies have occurred in Iraq (8, 11) through the direct use for food of seed-grains dressed with organo-mercurials. A similar case has been documented in the United States. (12) Many other incidents of pollution of the environment and poisoning of wild-life have been reported. (1 - 8, 13, 14) From these experiences, and also from industrial experience in handling inorganic forms of mercury, (15 - 18) a tentative maximum acceptable daily intake of mercury was first estimated as 100 micrograms. (7, 19, 20) Grant (4) and Tucker (7) make very interesting comments about the difficulty and uncertainty in establishing values for acceptable daily intake of mercury. A tentative limit of five parts per billion mercury in drinking water has been proposed. (21)

It has been found that organic forms of mercury compounds, especially alkyl-mercurials, are the most highly toxic of all mercurials. (1 - 4, 7, 22 - 25) Furthermore, it now appears that mercury occurs in food largely in the form of methyl-mercury compounds. (10, 12, 20, 25 - 31)

Because of their more toxic nature, a lower acceptable daily intake for alkyl mercurials has been proposed. (23, 25, 32) The suggested maximum level is 30 micrograms per day.

The above considerations have only involved an estimation of acute and chronic poisoning. Several authors have raised the question of what effect doses at levels lower than the amount which causes clinical manifestations might have.

It is possible that sub-clinical effects occur with exposure which influence mental fitness and ageing over the long term. The possibility of genetic effects has also been suggested. (20, 25, 33)

1.3.3 Sources and Distribution of Mercury Pollution

The unique properties of mercury make it extremely useful in a wide variety of applications. Because of this, expecially in highly industrialized countries, intake of mercury is high and has been increasing. It has become apparent that significant amounts of the mercury used have been lost to the environment, thus causing elevated levels in certain areas. The pollution tends to spread from the source, often via rivers and streams, and may accumulate in lakes some distance away. From this point, mercury enters the food chain in a surprising variety of ways and naturally tends to concentrate in animals at the top of the food chains. Fish and other aquatic

organisms are now well known to be "collectors" of mercury contamination.[34, 35] The possibility of mass transport (via bubble bursting processes) from the hydrosphere into the atmosphere, and thence back to the land, has been outlined.[36, 37] Transport of mercury from sediment to plants to cattle, as well as other food-chain-concentration mechanisms, have also been detailed.[38, 39] Other estimations of the degree and mechanism of mercury pollution are given in many of the previously quoted references, especially those of Section 1.3.1.

It is clear that certain areas of the North American environment have become polluted,[40] and there are indications of wide-spread problems in Canada.[1 - 4, 41 - 43] Possible sources of the Canadian contamination have been pinpointed by Fimreite.[44] Losses from various industrial processes, direct application to the environment as seed dressings and fungicides, and smelting of ores and burning or other treatment of fossil fuels are major sources of mercury pollution. The wide-spread burning of fossil fuels has been estimated to release a quantity of mercury comparable to that emitted as waste from industrial processes.[45] The uses of mercury compounds in agriculture have been reviewed by Smart.[46]

Plants are known to take up mercury when high concentrations are present in the soil.[35, 47, 48] Furthermore, it appears that application of mercurial fungicides

to the leaves of a plant leads to even greater translocation to the fruit and other parts of the plant.[49]

Although data on the amount of mercury present in the average North American diet are scarce, reports by Jervis et al[43, 50] and Rayudu et al[51] indicate alarmingly high levels. Other researchers have not substantiated these results,[52, 53] so an element of doubt as to the exact situation exists.

1.3.4 The Analytical Problem

It is obvious that an accurate and reliable analytical method is absolutely essential to the investigation of the problems of mercury pollution. The modern-day analyst is fortunate in that a large number of techniques are available for application to such an investigation, including colorimetry, emission spectroscopy, mass spectrometry, x-ray fluorescence, atomic (emission, absorption and fluorescence) spectrometry, fluorimetry, neutron activation analysis and other radiochemical techniques, gas chromatography, dithizone titration and anodic stripping voltammetry. The possibilities will be narrowed down by considering the practical restrictions imposed by the specific problem, namely trace analysis for mercury in biological materials.

From Sections 1.1 and 1.3.2, it is seen that the level of critical interest is somewhere in the low part per billion range. In general, this indicates that for an

analytical technique to be chosen, it must provide a detection limit at least two orders of magnitude lower than this, and preferably three or more, in order to achieve high accuracy. Although the sample size taken directly affects the relative detection limit for most techniques, and preconcentration steps are possible, there is always a practical limit to which these measures may be taken. In the present situation, a minimum of sample treatment is desirable because of the possibility of loss of mercury, and also of contamination. The latter problem certainly limits the amount of reagents which can be used, and so in turn limits the practical sample size.

In practice, the methods mainly employed have been those of: dithizone extraction, usually followed by a colorimetric determination; cold vapour atomic absorption; and neutron activation analysis. The large published literature does not instil confidence in the analyses obtained by these methods, as many difficulties have been reported, and the results of interlab comparisons on portions of the same sample have often been very poor. A sampling of the literature follows to illustrate this.

Difficulties, even when determining relatively high concentrations, have been outlined by Strafford and Wyatt.[54] A report by Monk et al[55] on a colorimetric method indicates incomplete recoveries of added mercurials and concludes that the method requires experienced workers and carefully screened reagents, as the reagent blanks were

often very high. A comparison of activation analysis with a photometric dithizone method by Ruzicka et al[56] showed very low results of the latter relative to the former. Comparison of neutron activation with a cold vapour atomic absorption method by Ulfvarson[57] gave poor agreement, and very erratic results for the latter below 10 nanogram sample contents. A North American check of results on fish tissue analysis by the methods of neutron activation and cold vapour atomic absorption reported by Uthe et al[58] showed good agreement between mean values, but the results of each technique showed a disturbing range of values: two-fold for a 1.4 ppm sample, and over ten-fold for a 0.1 ppm sample. Similar poor agreement and/or disturbingly large ranges of results have been reported by Jones et al,[59] Rottschafer et al,[60] Nadkarni and Ehmann,[61] Munns and Holland,[62] Lee and Laufmann,[63] and Pillay et al.[64] A report by Heinonen and Suschny[65] on results obtained for IAEA flour samples analyzed by neutron activation analysis again is not encouraging, and these authors conclude that reliability of data in the parts per billion range is unsatisfactory.

More recently, there appears to have been a note of optimism about the situation of trace mercury analysis, although this was hotly contested by others.[66] Such optimism must be labelled as premature in the light of recent results.[67] Rains had some interesting comments on the difficulties encountered by the National Bureau of Stan-

dards in certifying orchard leaves as a Standard Reference Material for trace mercury analysis.[68] In a 1973 conference he also commented on round-robin analysis results on coal and fly ash samples, for which the values for mercury content ranged from 0.02 to 95.0 ppm! Van Loon[69] commented on the validity of analytical data obtained for trace analyses for mercury under the rather appropriate title of "Pick a Number, Any Number." Recent results reported by Kadish et al[70] again show poor agreement, even though aliquots analyzed by each method were from the same sample after it had been ashed!

1.3.5 Selection From Available Techniques

Many of the available techniques for trace analysis may be ruled out as unfavourable because of insufficient sensitivity for application to this problem. These include: x-ray flourescence[71] with a detection limit in the order of 2 parts per million; atomic fluorescence[72 - 76] with a detection limit around 0.1 parts per million and problems with matrix effects of real samples;[77] and emission spectroscopy, with a detection limit of about 10 parts per million.[78, 79]

Several other techniques have sufficient sensitivity but appear to suffer from matrix effects, and so would require considerable development work for application to biological samples. These include: spark source mass spec-

trometry,[80] which provides the additional possibility of multielement analysis; anodic stripping voltammetry;[81] and helium plasma emission spectroscopy.[82, 83]

Three techniques have been widely applied to this problem. They are: dithizone colorimetry, cold vapour atomic absorption, and neutron activation analysis.

Dithizone colorimetry has been used for many years for trace mercury analysis, and is still utilized, as a sampling of the literature shows.[84 - 96] The method suffers several disadvantages, however. These include the necessity of considerable chemical processing, thus risking contamination from reagents and loss of mercury due to adsorption and volatilization; serious interference from a number of elements; and, most important of all, a detection limit in the order of 0.5 micrograms. The latter drawback necessitates the use of inordinately large sample sizes. Sandell[97] discusses the method, and Smart[46] reviews the application of dithizone colorimetry to trace analysis for mercury. Because of these disadvantages, dithizone colorimetry is being more and more replaced by neutron activation analysis and cold vapour atomic absorption.

The standard flame atomic absorption technique does not have a low enough detection limit to be applied to this problem,[98, 99] unless a complex modification such as that of Lindstrom[100] is used. Currently popular atomic absorption methods such as the carbon rod or graphite furnace

atomizers cannot be applied, because it is necessary to char the organic matrix before analysis. During the charring step, the mercury content is volatilized. The cold vapour technique has been found to be very suitable, and its application has been reviewed by Manning (101) and D'Itri. (1)

Neutron activation analysis has also been found to be very suitable for this application. (102, 103, 104) A number of simple radiochemical procedures for analysis by isotope exchange or dilution have been suggested as well. (105, 106, 107)

While these are simpler methods than neutron activation, they do not have the advantage of freedom from contamination, loss, and other interferences that neutron activation has.

One disadvantage that these methods have in common is that they determine only total mercury in a sample. It is desirable to know what portion of the total is made up by alkyl-mercurials because of their greater toxicity. Although a method for differentiation has been suggested for neutron activation, (108) and for atomic absorption, (109) neither appear practical. Pre-analysis separation steps have been suggested in special cases. (110,111,112) The application of chromatography would appear to be appropriate for determination of organo-mercurials. Thin layer chromatography (113) and gas chromatography (26-29) have been used. Use of a gas chromatograph followed by an atomic absorption detector shows considerable promise. (114,115)

The two major techniques currently in use are cold vapour atomic absorption, and neutron activation analysis, for the reasons discussed above. Section 1.3.4 has pointed out that the performance obtained by these methods is somewhat less than desirable, however. Since any investigation of the problem of mercury pollution must be based on sound analytical data, the importance of the question of the ability of these techniques to give good results can hardly be overemphasized! Accordingly, this thesis concerns itself primarily with a critical evaluation of neutron activation analysis and cold vapour atomic absorption and goes on to compare the two techniques.

2 THE VOLATILITY OF MERCURY

2.1 Introduction

In the literature on trace analysis of mercury, the extreme volatility of this element and its compounds has often been emphasized. During the course of the investigations reported in this thesis, considerable experience has been obtained with the problems associated with handling trace quantities of mercury compounds. The extreme volatility of mercury compounds under certain conditions has very important implications for any trace analysis procedure. Therefore, this chapter has been devoted to some observations on, and discussion of, the problem of the volatility of mercury.

2.2 *In Vivo* ^{203}Hg Fish Labelling Experiment

2.2.1 Introduction

One difficulty in checking out any given procedure is that in order to follow the mercury at any particular step, it is necessary to analyze for it, (generally by the method that is to be checked out) and so it may be that errors go unnoticed because of the method employed. Furthermore, the addition of simple mercury compounds to a sample in order to check recovery or suitability of a procedure must also be rejected as "proving" that a method is reliable, because there is considerable evidence to indicate that mercury occurs in biological samples in a different form which is more difficult to analyze.

Use of standard reference materials such as Bowen's kale, or NBS standard orchard leaves, is certainly very desirable, but objections must still be raised as to their utilization to "prove" a method. The reason for this is that these standards have been treated extensively by drying, mechanical grinding and sieving. These operations very likely alter the mercury content and the form in which it is present. It is probably coincidence, but it is interesting to note that both standards mentioned have gone

through similar treatment, and both now have the same mercury content.

Use of a radiotracer is an extremely useful and elegant method but again all to no avail if the tracer mercury is present in a different form from that of a real sample.

It was therefore decided to undertake an experiment to label suitable samples *in vivo* with long-lived ^{203}Hg ($T_{\frac{1}{2}}$ = 47 days) in order to obtain a tracer in a natural form. Fish were chosen as suitable specimens, because it has been demonstrated that they concentrate mercury[34] and that it appears to be present mainly in a methylated form.[25, 26, 27, 30] Furthermore, the analysis of fish is currently of primary concern.

2.2.2 Experimental Procedures and Results

A five-gallon tank was set up with several species of small tropical fish. After a period of a week, about 300 micrograms of $HgCl_2$, which had been irradiated 30 days in the McMaster Nuclear Reactor, were added to the water. The radioactive mercury content of the water was observed to decrease slowly, by checking the activity of one millilitre aliquots. At the end of the experiment, more than 90% of the starting radio-mercury was associated with the sediment in the tank. The fish concentrated some of the activity as expected. After a period of one month in this environment,

several fish were sacrificed and used in experiments designed to study the behaviour of the radiotracer mercury which had been incorporated. Table 2.1 outlines a few of the experiments performed and their results. In no case was significant loss of the tracer mercury observed -- not on standing at room temperature for long periods, nor on freeze drying, nor even on heating in a drying oven overnight at temperatures as high as 115°C. These results were so unexpected that it was felt that the behaviour of the incorporated mercury tracer might depend strongly on the form in which it was administered. The addition in an inorganic form possibly led to the surprising stability. The experiment was therefore begun again with a new set of fish. This time, the mercury tracer was administered in the form of methyl-mercury chloride (purchased from ICN, 26201 Miles Road, Cleveland, Ohio, 44124.) The fish were left in this environment for longer than one month in order to allow time for natural incorporation of the methyl-mercury tracer. Several plants were also grown in the tank and were observed to take up some of the mercury tracer. The experiments were repeated, with almost the identical results as found with the inorganic tracer! Data are shown in Table 2.2 for the fish and Table 2.3 for the plants. Only minor differences were observed in the two experiments. In the methyl-mercury treated fish, the tracer seemed to be more evenly distributed over the body of the fish, whereas in the inorganic-tracer

experiment, the tracer activity was concentrated in the head and gut area, with only small amounts in the tail section. Also, some volatilization of the methyl-mercury labelled fish occurred at a temperature of 104°C, (Table 2.2) somewhat lower than for the other fish. The extent of the uptake was estimated to be about 60 ppb in the fish, based on activity measurements.

The remaining fish were used for further experiments to study the effectiveness of various ashing techniques. These experiments are described in the chapter on atomic absorption, in Section 4.7.

Table 2.1

Drying of $^{203}Hg(+2)$ - *In Vivo* Labelled Fish

1) Whole fish (Red Wag), initial weight = 743 mg

Treatment	Weight After Treatment	Change in Tracer ^{203}Hg Activity *
Standing at room temperature for 1½ days	679 mg	+ 0.1 %
Freeze-drying for 3 hours	279 mg	+ 1.3 %
Freeze-drying for 3½ hours more	184 mg	+ 1.3 %
Standing at room temperature for an additional week	---	+ 1.2 %

2) Portion of a fish

Dried in an oven at 115 °C for 16 hours	- 2.6 %

* Change relative to initial activity. Slight increase is typically found due to better geometry and decreased gamma-ray attenuation as the sample dries.

Table 2.2

Drying of $CH_3{}^{203}HgCl$ *In Vivo* Labelled Fish

1) Blue Moon - Lyophilization (initial weight = 752 mg)

2) Goldfish - Oven Drying (initial weight = 202 mg)

3) Goldfish - Oven Drying (initial weight = 226 mg)

Treatment	Weight	Change In Tracer ^{203}Hg Activity
1) Freeze-drying for 3 hours.	159 mg	+ 2.8 %
2) Drying 1 hour at 78 °C	138 mg	+ 0.7 %
Drying 2 hours at 89 °C	33.5 mg	+ 1.3 %
Drying 18 hours at 104 °C	32 mg	- 7.5 %
3) Drying 2 hours at 89 °C	40 mg	+ 1.0 %

Table 2.3

Drying $CH_3{}^{203}HgCl$ *In Vivo* Labelled Plants

	Treatment	Weight	Change In Activity
1)		128 mg (initial)	
	½ hour drying at 92 °C		+ 0.9 %
	24 hours at 92 °C	7.5 mg	- 1.1 %
2)		149 mg (initial)	
	Standing at room temperature for 29 days	7.3 mg	+ 1.1 %

2.3 Loss of Mercury During Cooking

One interesting and important question concerning the volatility of mercury from food should be considered. Food appears to be the main source of mercury ingestion for most people. The previous section has shown that mercury is retained in various samples at temperatures exceeding 100°C. Many foodstuffs such as meat or fish are cooked at a considerably higher temperature before they are eaten, however, and so some volatilization of the mercury might be expected. An experiment was carried out to estimate mercury losses during cooking. Samples of fresh raw meat were taken. The remainder of the meat was then cooked in the normal manner and another sample was taken. The still remaining meat was eaten for supper.

Portions of the samples were analyzed for mercury content by the proposed neutron activation analysis technique, Section 3.20. Duplicate samples were used to determine water content and fat content (the latter by the method of Folch,[116] as modified by Tietz.[117]) These were used to calculate the weight loss on cooking. This value was verified experimentally in the case of bacon (75% weight loss.) Data from the experiment are given in Table 3.17. Lean meat (chicken liver and chicken breast) was found to take up "fat" during cooking. Each meat lost considerable weight, but the mercury content was not volatilized appreciably.

Table 2.4

Mercury Loss On Cooking

Sample	Water Content	Mercury Content (wet weight) ✓	Weight Loss on Cooking *
Chicken Liver (raw)	75%	16.0 ppb	
" " (fried)	60%	19.4 ppb	22%
Chicken Breast (raw)	75%	5.0 ppb	
" " (baked)	63%	7.6 ppb	27%
Ground Chuck (raw)	64%	1.4 ppb	
" " (fried)	49%	1.3 ppb	33%
Bacon (raw)	40%	1.8 ppb	
" (fried crisp)	10%	2.0 ppb	75%

✓ by neutron activation analysis.

* includes water and fat.

2.4 Discussion

Loss of mercury from samples has been reported many times in the literature. Similar observations have been made during this research, especially in evaporating dilute solutions of mercury radiotracer to smaller volumes. Acidic solutions lost a considerable fraction of the mercury, and alkaline solutions were even worse. Any solution taken to dryness invariably lost all traces of radioactive mercury. The worst experiences, however, were had with methyl-mercury chloride labelled with ^{203}Hg. It was found to be extremely difficult to handle, and it also had a very strong tendency to become adsorbed to plastic.

The behaviour of the mercury in real samples, as presented in the previous sections, has been found to be in strong contrast to that of the above simple mercurials. Some support of these surprising observations may be garnered from the literature. For example, several researchers have reported that in processing fish for the protein content, the mercury present in the fish all ends up in the fish protein concentrate.[118, 119] Also, since people have been poisoned by eating food containing mercury, and the food usually can be assumed to have been cooked, it may be concluded that the mercury content could not have been appreciably

volatilized. For example, the case of a family eating contaminated pork has been very well documented.[8, 12] Results recently reported by LaFleur[120] show similar evidence to that found here in freeze-drying experiments on portions of guinea pigs and rats which had been labelled *in vivo* by treating their food with various radioactively labelled mercurials. It should be noted that at the same time he reported serious losses on freeze-drying aqueous solutions of simple mercurials.

The evidence suggests that the mercury contained in a biological matrix is much more stable than has been generally suspected. This leads to the speculation that the problems with the analysis occur mainly during and after steps which are designed to extract the mercury from its matrix, or to destroy the matrix completely. Also, the evidence indicates that just as much care should be lavished on preparation of the standards as is given to the preparation of samples.

Despite the observations reported here, volatilization losses must still be considered as a serious threat to sample integrity. Lyophilization (freeze-drying) of the sample is considered in Section 3.17. Bacterial production of methyl-mercury from inorganic mercury has been observed.[121, 122]

Several workers have found volatilization of mercury from samples, apparently caused by bacterial degradation.[123, 124] Kovalevskii[125] has reported losses of

mercury from samples on standing at room temperature, and also very serious losses when soil samples were ground up.

Even relatively simple matrices such as water samples and biological fluids appear to offer formidable difficulties. Losses due to adsorption on container walls have been observed by many researchers.[87, 97, 126, 127] The latter reference also describes association with particulate phases. Prevention of such losses by acidification[127, 128, 129] and addition of an oxidizing agent[130] has been suggested. One mechanism of loss by volatilization, and its prevention by addition of permanganate has been detailed by Toribara et al.[131]

Further discussion of sampling problems peculiar to mercury may be found in reports by Pillay et al,[64] Sandell,[97] Linch et al,[112] Jervis,[132] and in Chapters 3 and 4 of this thesis. A possible example of contamination by vehicles used when obtaining a sample has been suggested by Dickson.[133] Maintaining sample integrity is a difficult problem at best.

Since mercury has proven itself to be such an extremely difficult element to determine accurately, all of the skill of the analyst must be brought to bear. This suggests strongly that the analyst should collect his own samples fresh, if at all possible, and follow them with care throughout the analysis. Samples collected in remote areas

and requiring storage for some period before they can be analyzed present a difficult problem. Sealing in quartz capsules is strongly recommended, if it is at all feasible.

This discussion has been an estimation of the sample integrity during sampling and storage, up to the time that processing for the chosen analytical procedure is begun. Losses (and gains via contamination) during extraction of the mercury from the matrix, or destruction of the matrix, and subsequent processing of the trace mercury content, present even more difficult problems! The subject of ashing technique is broached in Section 4.12. Contamination problems are considered in Section 5.2

3. NEUTRON ACTIVATION ANALYSIS

THEORY

3.1 Introduction

In general, a sample placed in a neutron flux will undergo various neutron-induced nuclear reactions. Many of the products will be radioactive, and these species may be measured conveniently some time after the end of the irradiation period. Specific emissions, in particular of gamma-radiation, are characteristic of a given product isotope, and so the presence of its parent in the original sample may be inferred from the detection of the radioisotope. Furthermore, its emission intensity is directly proportional to the amount of the parent isotope present in the sample, so that if a standard of the same element is co-irradiated with the sample, qualitative and quantitative analysis of the element may be achieved by comparison.

The following equation describes the buildup of radioactivity for a given neutron-induced nuclear reaction, followed by its decay after the end of irradiation:

$$A = \sigma\phi N (1-e^{-\lambda t}) \cdot (e^{-\lambda t'}) \qquad (3.1)$$

where: A is the activity in disintegrations per second

σ is the cross section for the nuclear reaction

ϕ is the neutron flux in the sample

N is the number of atoms of the given target isotope

t is the time of irradiation

t' is the time from the end of irradiation

λ is the constant decay of the produced radio isotope

$\lambda = \frac{\ln(2)}{T_{\frac{1}{2}}}$, where $T_{\frac{1}{2}}$ is the half-life.

Consider an analysis in which a sample (subscript s) and a standard (subscript st) of some element to be determined are irradiated together for the same length of time. Since the same isotope is being considered in both instances, reaction cross section and half-life are identical:

$$\sigma_s = \sigma_{st} \qquad (3.2)$$

$$\lambda_s = \lambda_{st} \qquad (3.3)$$

If both sample and standard are put into the neutron flux and removed from it at essentially the same time, the buildup factors will also be the same:

$$(1 - e^{-\lambda t_s}) = (1 - e^{-\lambda t_{st}}) \qquad (3.4)$$

If the sample and standard are physically situated close to each other during irradiation, the neutron flux received by each will be the same, provided that no large flux gradients are present. The assumption of equal fluxes may cause some error in certain cases, and is discussed in detail for mercury in biological samples in Section 3.2.3.

Assuming, then, that $\phi_s = \phi_{st}$, the ratio of the expressions for activity of sample and standard, after substitution of equations (3.2), (3.3), and (3.4) into (3.1) becomes:

$$\frac{A_{sample}}{A_{standard}} = \frac{N_s}{N_{st}} \cdot \frac{e^{-\lambda t's}}{e^{-\lambda t'st}} \qquad (3.5)$$

The factor $e^{-\lambda t'}$ gives the decay of the activity, and must be used to correct for the time which elapses between determination of the activity of the sample and determination of the activity of the standard. Taking this into account, the activity ratio of the radioactive isotope formed is equal to the ratio of the number of atoms of the parent isotope present in each. This equation is the basis of quantitative analysis by neutron activation.

The burn-up factor, $e^{-\sigma\phi t}$ is almost always very small, and so any error introduced by it (due to $\phi_s \neq \phi_{st}$) is negligible.

3.2 Nuclear Properties Used for Neutron Activation of Mercury, and Associated Errors.

3.2.1 Nuclear Data for Mercury

The pertinent information for neutron activation involves the isotopes of mass numbers 196 and 202. Table 3.1 summarizes the data.

The high neutron-capture cross section of $_{80}Hg^{196}$ (134, 135) helps to compensate for the low natural abundance, as is seen from the abundance-corrected cross section. (The cross section for this isotope given in (299) has been used in many places but appears to be low.)

3.2.2 Decay Scheme

The decay schemes for ^{197m}Hg and ^{197}Hg have been discussed by Helmer.(136) ^{197m}Hg is strongly converted,(137) undergoing internal transition to ^{197}Hg in 97% of decays. Because of this, and also its low effective cross section relative to that of ^{197}Hg formation, this isotope is of little use for analytical purposes. One point should be considered, however, with regard to interfering effects of the ^{197m}Hg decay on measurement of the activities of both ^{197}Hg and ^{203}Hg:

(1) $^{197m}Hg \xrightarrow{I.T.\ 97\%} {}^{197}Hg$

At the end of irradiation, ^{197m}Hg will add to the number of atoms of ^{197}Hg present. This will cause an increase in the activity over that expected by normal

Table 3.1 Pertinent Nuclear Data for Mercury

Stable Isotope	^{196}Hg	^{197}Hg	^{202}Hg
Natural Abundance	0.00146	0.00146	0.298
Product of (n,γ)	^{197m}Hg	^{197}Hg	^{203}Hg
Cross Section (σ) *	107 barns	3092 barns	5.0 barns
Abundance-Corrected Cross Section **	0.16 barns	4.5 barns	1.5 barns
Half-Life	23.8 hours	64.1 hours	46.6 days

* from Kim and Adams.[135] 1 barn = 10^{-24} cm^2.

** The Abundance-Corrected Cross Section is the product of the Natural Abundance and the Cross Section.

decay with the half-life of ^{197}Hg. The extent of this effect may be calculated by the following equation, which may be found in Reference 142.

$$A_{197} = C \cdot \frac{(\lambda_{197m})(\lambda_{197})}{\lambda_{197} - \lambda_{197m}} \cdot N^{o}_{197} \cdot (e^{-\lambda_{197m} \cdot t} - e^{-\lambda_{197} \cdot t})$$

where A_{197} is the activity of ^{197}Hg due to decay of ^{197m}Hg.

C is the decay branching probability, 0.97.

λ_{197m} is the decay constant for ^{197m}Hg.

λ_{197} is the decay constant for ^{197}Hg.

N^{o}_{197m} is the starting number of ^{197m}Hg atoms at the end of irradiation.

Figure 3.1 shows the buildup of ^{197}Hg activity during irradiation from (n,γ) reaction and from decay of ^{197m}Hg. Note that the scales differ by two orders of magnitude. At the end of ten hours of irradiation (arrow) the decay of the ^{197}Hg already formed is shown, and the formation of ^{197}Hg by decay of ^{197m}Hg is also depicted. Again note that the scale for the latter is two orders of magnitude lower than that of the former. The relative activity shown is expressed as disintegrations per second per atom of mercury. A flux of 2×10^{13} neutrons/cm^2/sec. was assumed.

The observed activity is the sum of the two curves. Thus, errors may be made in half-life corrections. The extent of these errors will be small, however, as can be seen from Figure 3.1. The largest contribution of ^{197m}Hg

Figure 3.1

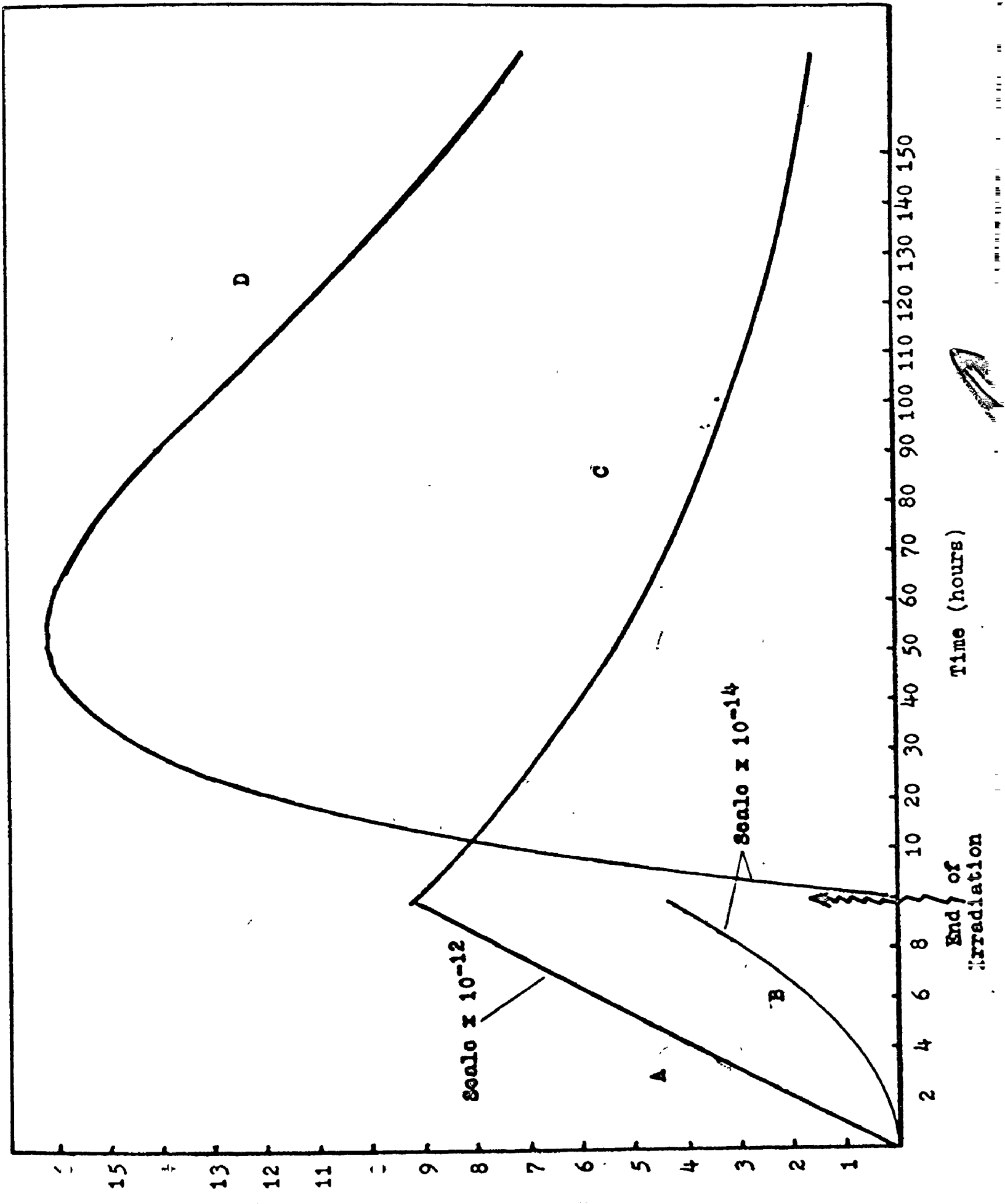
Contribution to ^{197}Hg from ^{197m}Hg:

A) Buildup of ^{197}Hg Activity During Irradiation from $^{196}Hg\ (n,\gamma)\ ^{197}Hg$.

B) Buildup of ^{197}Hg Activity During Irradiation from $^{196}Hg(n,\gamma)^{197m}Hg \xrightarrow{I.T.} {}^{197}Hg$.

C) Decay of Total ^{197}Hg Activity Present at the End of Irradiation.

D) Buildup and Decay of ^{197}Hg Activity from Decay of ^{197m}Hg Which is Present at the End of Irradiation.


Scale x 10^{-12}
Scale x 10^{-14}
A
B
C
D
End of irradiation
Time (hours)
2 4 6 8 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
1 2 3 4 5 6 7 8 9 10 11 12 13 15

to the activity occurs between the end of irradiation and just over two days later. This amounts to about 3% of the activity due to ^{197}Hg which has been formed by the end of the irradiation. In practice, several days of decay are generally allowed after the end of irradiation, and so errors from this source will be negligible. For example, a count made 70 hours after the end of irradiation and corrected to 30 hours later by using the ^{197}Hg half-life will be in error by -0.3% according to Figure 3.1.

(ii) 279 keV Gamma Radiation from ^{197m}Hg

The decay scheme[136] shows a decay path of 3% probability, giving a 279 keV gamma ray from ^{197m}Hg decay. For relatively short irradiations, the activity buildup factor for ^{197m}Hg will be much greater than that for ^{203}Hg. Despite the unfavourable effective cross section and decay path, at the end of short irradiations ^{197m}Hg contributes a significant amount to the activity observed. Table 3.2 gives calculated contribution data for different irradiation times based on data from Table 3.1 and gamma ray yields from reference.[138] Figure 3.2 shows the buildup of activity of the 279 keV gamma radiation during a ten hour irradiation at a flux of 2×10^{13} n/cm^2/sec., followed by the decay after the end of irradiation. Activity is expressed as gamma rays per second per atom of mercury. Note that the scales differ by a factor of ten. The observed activity will decrease more rapidly than might be expected from the ^{203}Hg half-life of 47 days for several days after

Table 3.2

^{197m}Hg Addition to 279 keV Emission

Irradiation Time	^{197m}Hg Activity Relative to ^{203}Hg at 279 keV *					
(hours)	(Decay Time After End of Irradiation)					
	0 hr.	12 hr.	24 hr.	48 hr.	72 hr.	100 hr.
0.17	18.3%	13.0%	9.2%	4.7%	2.3%	1.1%
1	18.1%	12.8%	9.1%	4.6%	2.3%	1.0%
5	17.1%	12.1%	8.6%	4.4%	2.2%	1.0%
10	16.0%	11.3%	8.1%	4.1%	2.0%	0.9%
20	14.0%	9.9%	7.1%	3.6%	1.8%	0.8%
40	11.0%	7.8%	5.5%	2.8%	1.4%	0.6%
80	7.3%	5.2%	3.7%	1.9%	0.9%	0.4%
100	6.1%	4.4%	3.1%	1.6%	0.8%	0.4%

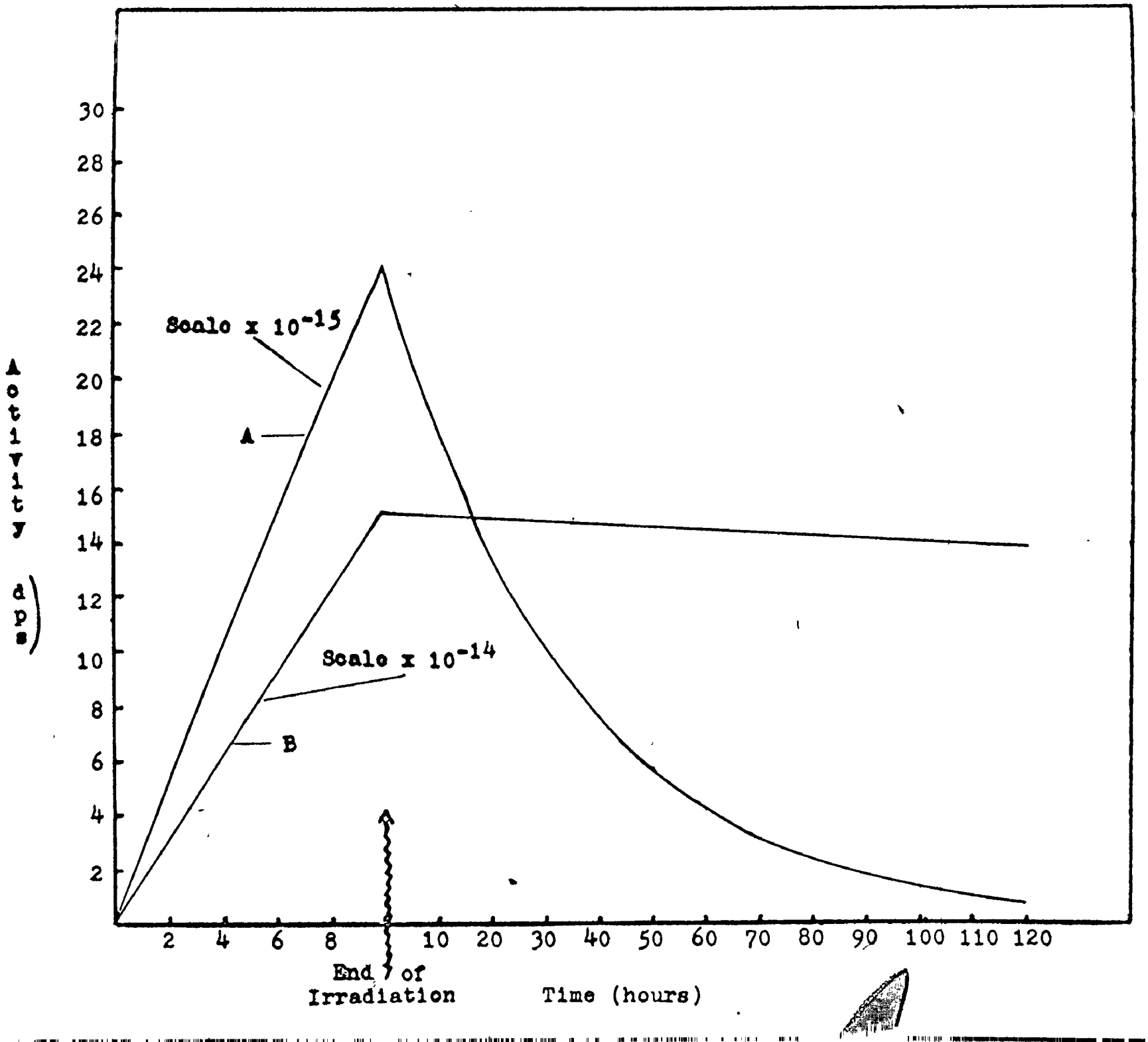
* Data calculated assuming a flux of 2×10^{13} n-cm^{-2}-sec^{-1} and using the data from Table 1. See Figure 3.2 for a plot of the case of irradiation time = 10 hours.

Figure 3.2

Contributions to 279 keV Gamma-Ray Emission by ^{197m}Hg and ^{203}Hg

A) Buildup (During Irradiation) and Decay of 279 keV Gamma-Ray Activity Due to ^{197m}Hg.

B) Buildup (During Irradiation) and Decay of 279 keV Gamma-Ray Activity Due to ^{203}Hg.


Activity (dps)
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
Scale x 10^{-15}
A
Scale x 10^{-14}
B
2 4 6 8 10 20 30 40 50 60 70 80 90 100 110 120
End of Irradiation
Time (hours)

the end of irradiation.

Both effects described above are relatively small and so would not normally have to be considered if sufficient time is allowed for decay of ^{197m}Hg. One case in which they are of importance, however, is that of very short irradiation times (in the order of minutes) when a rapid result is required.

The abundance-corrected cross section shows that ^{197}Hg is the most favourable isotope for analytical purposes. Taking into account that a reasonably short irradiation time is desirable from the point of view of cost and time elapsed before the result is available, the advantage over ^{203}Hg is magnified considerably. In a typical ten hour irradiation followed with three days of decay, the calculated advantage factor is 23 : 1, this being the ratio of gamma ray emission intensity of the 77.6 keV ^{197}Hg to the 279 keV ^{203}Hg emission. This value agrees well with what has been observed experimentally. If a large enough mercury concentration is present in a sample, obviously both isotopes can be used to give increased confidence to the analysis.

Besides the gamma rays emitted by the isotopes so far discussed, the production of a considerable number of different energies of x-rays is also encountered. These come from mercury, gold (to which ^{197}Hg decays), thallium (to which ^{203}Hg decays), and also possibly from the sample

support (for example platinum, if it were used for amalgamation collection of the Hg in a chemical separation) and from the lead shielding employed for most detectors. The latter are generally not very noticeable unless the sample gives off intense (B^-) emission.[139] Table 3.3 lists various x-ray energies in keV after Johnson and White.[140]

3.2.3 Neutron Flux Variations

The development of equation 3.5 depends on the assumption that the sample and standard receive identical neutron flux. Several things must be taken into consideration for this to be valid, and these will be outlined in the following paragraphs.

The elemental absorption cross section for mercury is 370 barns,[141] which is very large, and so self-shielding could be a significant problem. The number of atoms of mercury per unit volume is very important in such considerations, because the sample matrix may serve to dilute strongly absorbing species to such an extent that the problem becomes negligible. This is the case for biological samples with trace mercury content at or below the parts per million level, but is not necessarily true for a standard. The problem is considered in some detail by J. Roesmer,[103] and De Soete, Gijbels and Hoste,[142] with the resultant recommendation being that a standard should not exceed an

Table 3.3 Selected X-Ray Energies

Element	Energy (keV)			
	K_{B_2}	K_{B_1}	K_{a_1}	K_{a_2}
Pb	87.3	84.9	75.0	72.8
Tl	84.9	82.6	72.9	70.8
Hg	82.5	80.3	70.8	68.9
Au	80.2	78.0	68.8	67.0
Pt	77.9	75.7	66.8	65.1

amount of a few micrograms. If a larger standard is to be used, it should be diluted with some material which is not strongly absorbing, preferably as closely matched to the sample matrix as possible. Care must be exercised to ensure that the mercury present is in fact diluted, and is not concentrated in one spot.

Another problem may be that of flux depression. This is caused by the presence of a strong neutron absorber in the near vicinity of the sample and standard and results in a steep gradient in the flux with distance. Flux gradients in the order of 10% / cm have been observed but are generally somewhat less.[139, 142] In the McMaster Reactor, gradients are usually $\leq$ 2% / cm.[143]

Thermal flux enhancement caused by additional moderation of neutrons in aqueous samples can occur and may result in 5% increases or more depending on the amount of moderator present and also on how large the fast neutron flux is at the irradiation position relative to the thermalized flux.[144, 145, 146] Johnson[147] found a 2% enhancement for a sample in 10 ml of water and larger effects for larger volumes.

These problems may be overcome by using small amounts of dilute standards which have similar mercury content to that expected in the samples, and by irradiating samples and standards as closely together as possible. Since biological samples have a matrix similar to water as far as neutron-thermalizing effects are concerned (in fact, undried samples

typically contain roughly 75% water), flux enhancement for sample and standard will be very similar if standard solutions are employed. Also, flux enhancement will be small if small samples and standards are used. Thus, negligible error is expected from this source. In packing for irradiation, samples and standards are alternated so that a flux gradient will be detected.

3.2.4 Burn-Up

Since such a high cross section is involved in the production of ^{197}Hg, burn-up of ^{196}Hg may be expected to occur. An estimation of the burn-up may be calculated from the equation:

$$N = N_o e^{-\sigma \phi t}$$

Table 3.4 gives values of burn-up for various fluxes.

Table 3.4 Burn-Up of ^{196}Hg

Neutron Flux ($n/cm^2/sec.$)	Rate of Burn-Up (% per hour)
1×10^{12}	0.001
1×10^{13}	0.01
1×10^{14}	0.1

For long irradiations and/or high fluxes, the burn-up can be significant. Since samples and standards will undergo burn-up at the same rate, this will only be a problem if flux monitors are used instead of standards, or if standards are used over again, as might occur in non-destructive instrumental analysis.

3.2.5 Interfering Nuclear Reactions

The mercury isotopes of interest for analysis are essentially free from interfering nuclear reactions, (including second order reactions[148, 149]) as can be seen from a chart of the nuclides. The following are the possible exceptions:

^{206}Pb	(n,α)	^{203}Hg	
^{203}Tl	(n,p)	^{203}Hg	(cross section of 4×10^{-6} barns[143])
^{205}Tl	(γ,pn)	^{203}Hg	(gamma flux estimated to be 10^{9} cm^{-2} - sec^{-1})

Since the cross sections for the given reactions are many orders of magnitude lower than the (n,γ) reaction under consideration, large excesses of Pb and/or Tl must be present in the sample to cause interference. In biological samples these are trace elements, so interference will not occur. No problem is anticipated for the ^{196}Hg (n,γ) ^{197}Hg reaction.

3.2.6 Anomalous Isotopic Abundances

In equation (3.5), N is the number of atoms of a given isotope present in sample or standard. Since the number of atoms of a given element present is generally the quantity which interests the analytical chemist, and since many elements, including mercury, do not consist of a single stable isotope, a constant relative isotopic abundance must be assumed to calculate the elemental concentration.

Bronsted and Hevesy suggested the possibility of a partial separation of the isotopes of mercury and observed a slight shift in the atomic weight in experiments of evaporation at low pressure.[150, 151] No evidence of significant natural variation was observed, however.[152] A. O. Nier[153] published results of a careful redetermination of the relative abundances of the isotopes of mercury, in which he suggested that his previous results[154] should be recalculated, taking into account the square root of the mass ratios because of isotopic fractionation through distillation during the analysis. Haeffner[155] found that the isotopic composition of mercury could be changed by passing an electric current through a column of mercury. The extent of the effect was several per cent. V. H. Dibeler[156] found small but significant differences in relative isotopic abundances from the values of Nier in a specially prepared sample. His analysis was confirmed

by three other laboratories.

Thus it appears that small differences in the isotopic composition of mercury from different sources may occur. In particular, the lighter isotopes likely become enriched slightly during careful purification processes based on distillation at low pressure. It should be kept in mind that standards are generally made from reagent grade mercury metal, which has been triply distilled. Therefore, results by neutron activation based on the $^{196}Hg\ (n,\gamma)\ ^{197}Hg$ reaction may be low by several per cent because the standard is enriched to this extent in this isotope. Some compensation of this error may occur, depending on what processes the mercury in the sample has undergone. Since the average atomic weight of mercury is 200.6, the ^{202}Hg isotope can be expected to have undergone slight depletion if ^{196}Hg is enriched. Thus, analysis based on both isotopes would be desirable, if possible, in order to detect this effect and compensate for it. The error amounts to possibly a few per cent at most and is likely less, and therefore would not normally be considered to be serious as far as the trace analysis of mercury is concerned.

It is interesting to note that mass spectrometry, which could be expected to shed some light on the problem of anomalous isotopic abundances, suffers from the experimental difficulty that the vacuum in the instrument is

maintained by mercury diffusion pumps. Furthermore, the isotope of most interest in this case, ^{196}Hg, has an extremely low relative abundance of 0.15% and so gives a very small relative peak size. It is unlikely that a small difference in isotopic abundance would be detected because of this. The former problem might be surmounted by the method of Dibeler,[156] who measured dimethylmercury.

3.3 Measurement of Activity

3.3.1 Introduction

In order to obtain a quantitative analysis, the activity of a given isotope of interest must be determined and substituted in equation (3.5). The measurement that is actually made is related to the activity by the following equation:

$$R = KEA$$

where R is the measured activity (counts/sec.)

K is a geometry factor

E is the efficiency of detection

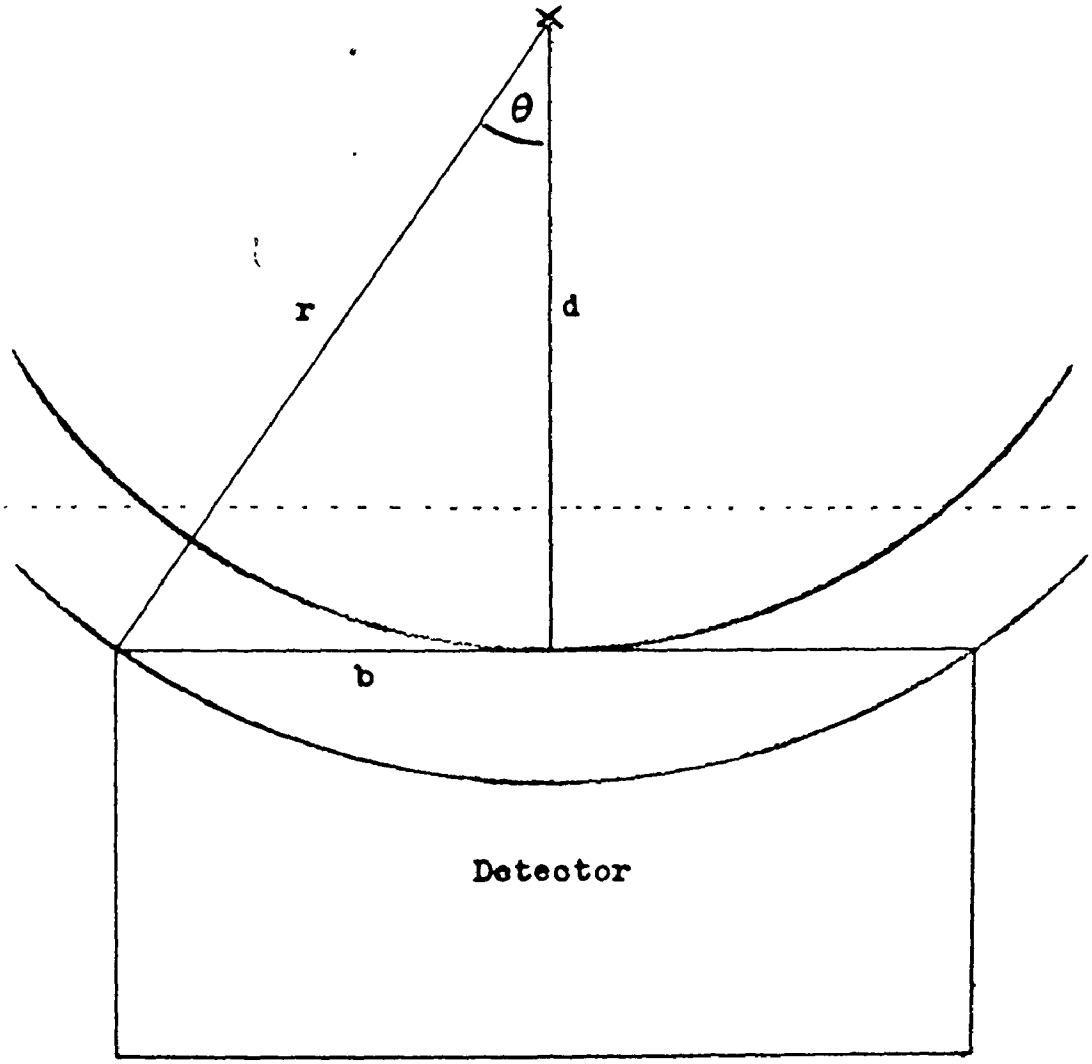
A is the absolute activity (gamma-rays/sec.)

The measured activity, R, is normally used in equation (3.5). This will not introduce any error, provided that K and E remain constant for sample and standard.

The geometry factor, K, depends on the solid angle which the detector subtends at the source. For a point source, K varies as $(1-\cos(\theta))$ where θ is the half-angle subtended by the detector at the sample. This is illustrated in Figure 3.3. The relationship is derived in Appendix I. Although the relative change in K may be minimized between samples by placing them a large distance from the detector, this is usually impractical, since the observed count rate will decrease correspondingly. Thus samples are often counted from a position very close to the detector, making it extremely important to be able to

Figure 3.3

Angle Subtended by the Detector at a Point Source.


θ
r
d
b
Detector

reproduce the geometry for each successive sample.

The detection efficiency, E, depends on the energy of the gamma radiation for a given detector. Since this is constant for a given isotope, the efficiency should be a constant factor. However, a gamma ray spectrometer is a complex instrument, and a number of subtle effects can occur which give apparent changes in efficiency. The most important of these are changes caused by high counting rates.

At high counting rates, the probability of random coincidence of the events increases dramatically because of the finite system response time. Chance summing results in events not being stored in the proper channels, and so the activity determined is too low. Furthermore, high rates oause other problems associated with the pulse shaping electronics which are too detailed to be included here.

3.3.2 Attenuation of Gamma Radiation

Gamma radiation undergoes attenuation on passing through matter according to the following exponential law:

$$I = Io\ e^{-\mu l}$$

Where Io is the initial intensity of the radiation

I is the intensity after passing a distance (l) into the attenuating material

μ is the linear absorption coefficient of the material

l is the distance the radiation travels through the attenuating material

The linear absorption coefficient, μ, depends on the atomic number of the material, its density and the energy of the radiation. The interaction of high energy photons with matter may occur in several distinct ways.[157] The dominant interaction depends on the energy of the photons. Pair production becomes increasingly important at energies in excess of that required to produce an electron-positron pair (1.022 MeV). The Compton effect (inelastic scattering) is another form of interaction. The photo-electric effect becomes increasingly important for photons of lower energy.

For mercury, gamma rays of 279 keV from ^{203}Hg, and 77.6 keV from ^{197}Hg are the principal emissions observed, as well as a composite x-ray peak at even lower energy for ^{197}Hg (see Table 3.3). These emissions are of relatively low energy and therefore will undergo significant attenuation passing through a given material. Consideration of this fact is important when determining the activity of a sample. The form in which the radioactive mercury from a sample is isolated will have a strong effect on the activity observed from that sample. Table 3.5 gives some absorption coefficients, with transmission factors calculated for a few examples. Note especially the predicted strong attenuation caused by mercury and mercuric sulphide.

Table 3.5

Gamma Ray Attenuation

Material	Absorption Coefficient (cm^{-1}) * 68 keV	78 keV	279 keV	Path Length	Transmission 68 keV	78 keV	279 keV
Water	0.18	0.17	0.12	5 mm	0.91	0.92	0.94
				6 mm	0.90	0.90	0.93
				10 mm	0.83	0.84	0.89
Aluminum	0.57	0.51	0.30	1.5 mm	0.92	0.93	0.89
Mercury **	48	35	(6.5)	1 mm	0.01	0.03	0.52
Mercuric Sulphide **	21	15	2.9	0.1 mm	0.81	0.86	0.97
				0.2 mm	0.66	0.74	0.94
				0.5 mm	0.35	0.46	0.87
				1.0 mm	0.12	0.21	0.75

* Data taken from Evans[158]

** Values estimated from x-ray mass absorption coefficient data on Pb, Au and Pt given in the Handbook of Chemistry and Physics[159] and from Reference[160]

EXPERIMENTAL PROCEDURES AND RESULTS

3.4 Experimental Equipment and Radiation Measurement Techniques

A high resolution Ge(Li) detector from Nuclear Diodes Inc. was employed with a cooled pre-amplifier. It had the following specifications:

Relative peak efficiency at 1.33 MeV 4.7%

Resolution at 1.33 MeV (FWHM) ..... 1.9 keV

Resolution at 122 keV (FWHM) ..... 0.9 keV

Peak-to-Compton ratio at 1.33 MeV 25/1

An Ortec model 410 linear amplifier was used for pulse amplification, and either a Canberra model 8100, or a Nuclear Data model 2200 multi-channel analyzer for pulse height analysis. The low energy portions of mercury spectra after various decay times obtained with this equipment are shown in Figure 3.4.

The detector was found to have an effective depth below the surface of the cap of 2.0 cm for a 60 keV (^{241}Am) source. For higher energy, it will appear to have greater depth because of greater penetration. Table 3.6 gives calculated geometry factors for variations in height of the sample above the detector cap for effective detector depths of 2.0 cm and 3.0 cm.

Figure 3.4

Low Energy Mercury Spectra:

A) Shortly After a One Minute Irradiation.

B) Same Sample Four Weeks Later.

C) ^{203}Hg Activity Only.

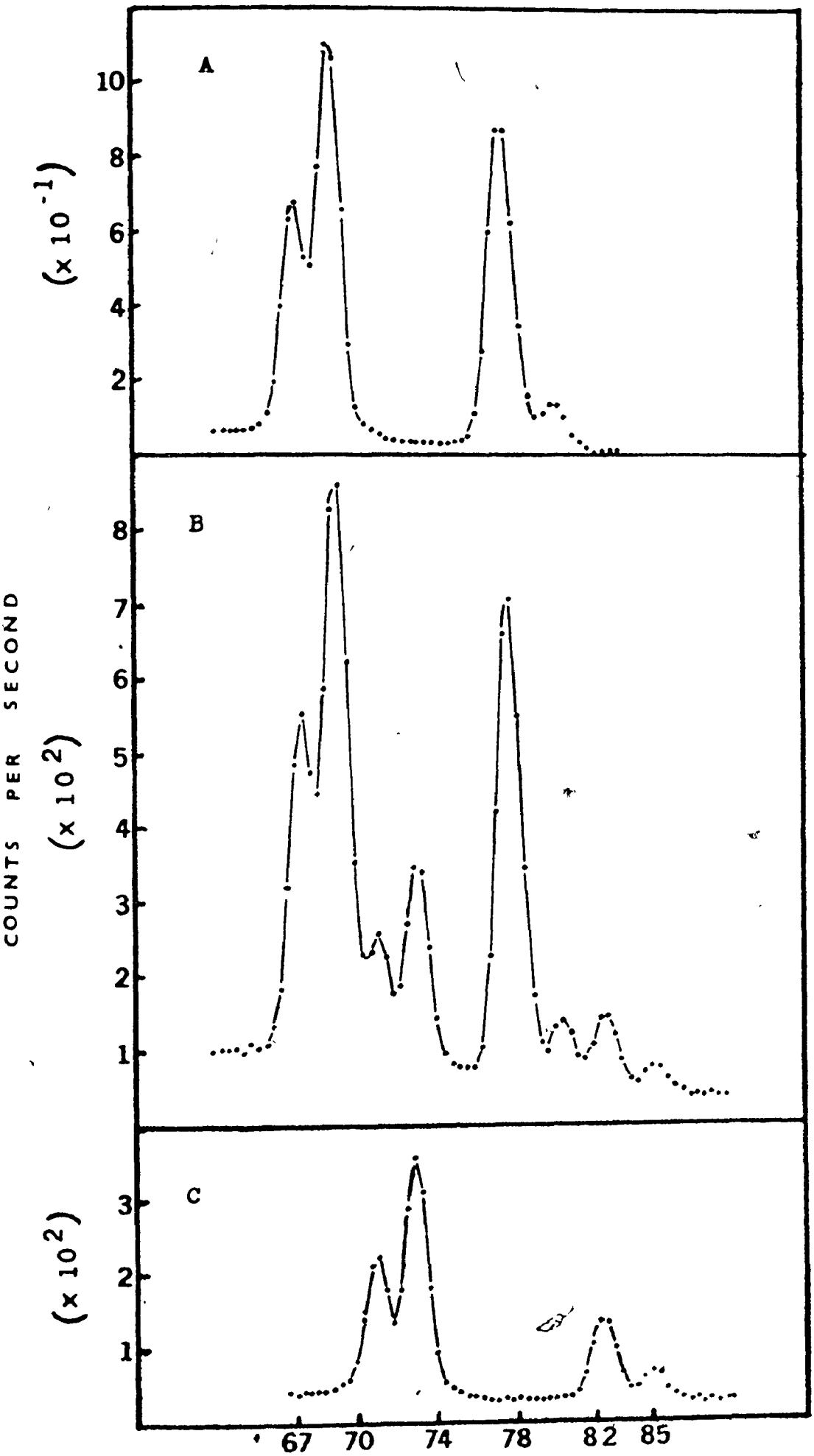

A
B
C
COUNTS PER SECOND
(x 10⁻¹)
(x 10²)
(x 10²)
10
8
6
4
2
8
7
6
5
4
3
2
1
3
2
1
67
70
74
78
82
85

Table 3.6

Geometry Factors

Height Above Cap	Relative Activity Observed For Given Depth Below Cap	
	2.0 cm	3.0 cm
0.0 cm	100.0%	100.0%
0.01 cm	99.4%	99.5%
0.02 cm	98.9%	99.0%
0.05 cm	97.2%	97.6%
0.1 cm	94.5%	95.2%
0.2 cm	89.3%	90.8%
0.5 cm	75.8%	79.0%
1.0 cm	58.7%	63.7%
2.0 cm	37.4%	----
3.0 cm	----	31.3%
10.0 cm	4.9%	7.2%

For tracer studies with radiochemically pure mercury, a well-type NaI scintillation detector was usually employed instead of the solid state detector described above. It had the following dimensions:

Diameter	7.56 cm
Length	7.56 cm
Well Diameter	1.7 cm
Well Depth	5.0 cm

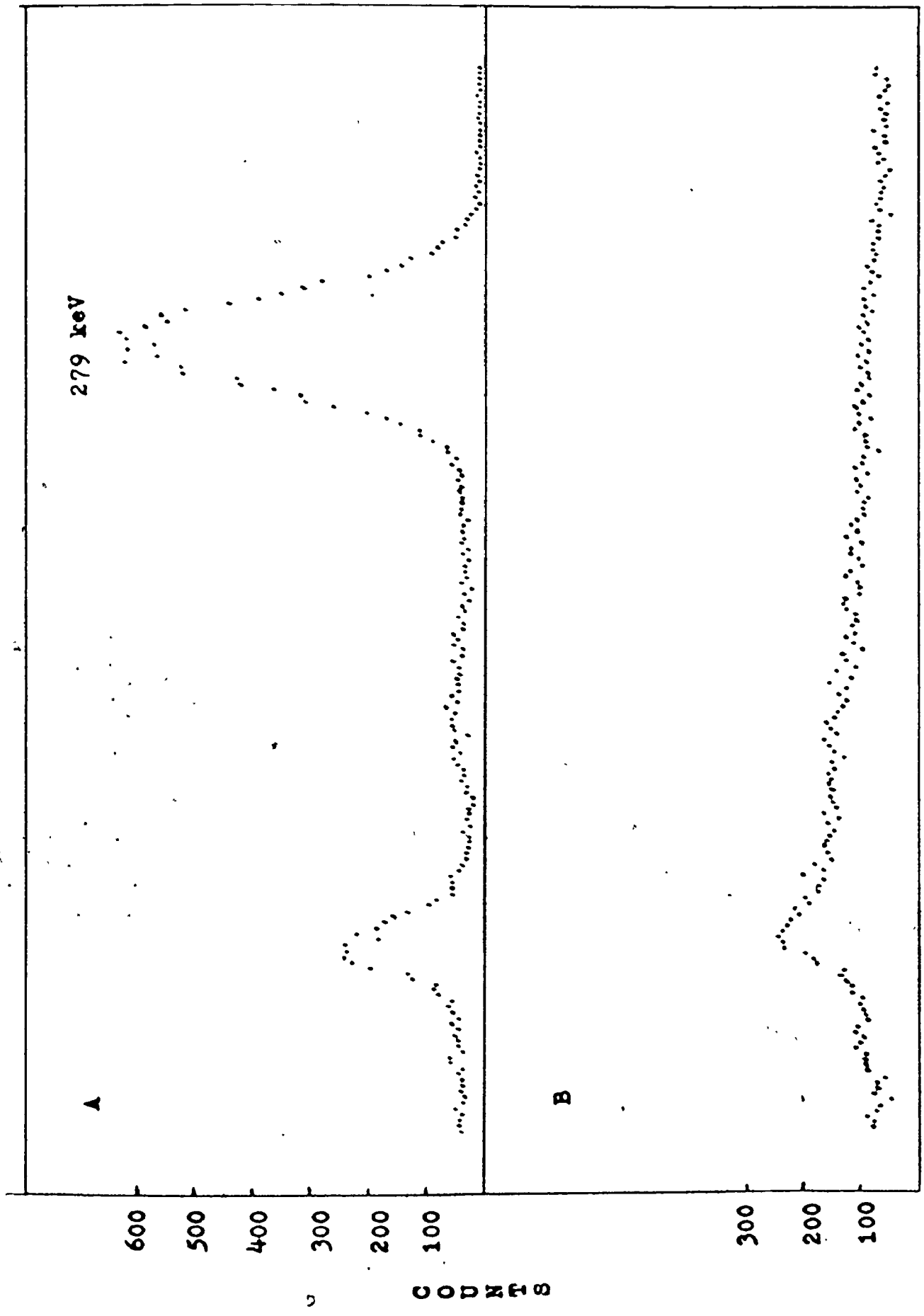
Figure 3.5 shows a spectrum of ^{203}Hg obtained by using this detector and the background. It is obvious that much poorer resolution is attained relative to the Ge(Li) detector. The low-energy peaks are unresolved. It is generally assumed, however, that the greater efficiency of the NaI detector will lead to a better detection limit, and so if a radio-chemically pure fraction can be obtained, use of this detector should be preferred. The background is also greater, and if a sufficiently high resolution Ge(Li) detector can be used, the detection limit can be superior to the NaI at low counting rates.(161)

For the equipment described, the well-type NaI detector gives a peak area for ^{197}Hg which is 5.3 times that given by the high-resolution Ge(Li). The background ratio is 5.1 : 1. This gives a relative signal-to-noise ratio of $5.3/\sqrt{5.1} = 2.35$ in favour of the well counter. Thus, the high resolution of the Ge(Li) detector can be made use of for positive identification with only a small sacrifice in detection limit. Longer counting times are necessary

Figure 3.5

A) ^{203}Hg Spectrum Using NaI.

B) Background.


279 keV
A
B
600
500
400
300
200
100
300
200
100
COUNTS
ENERGY

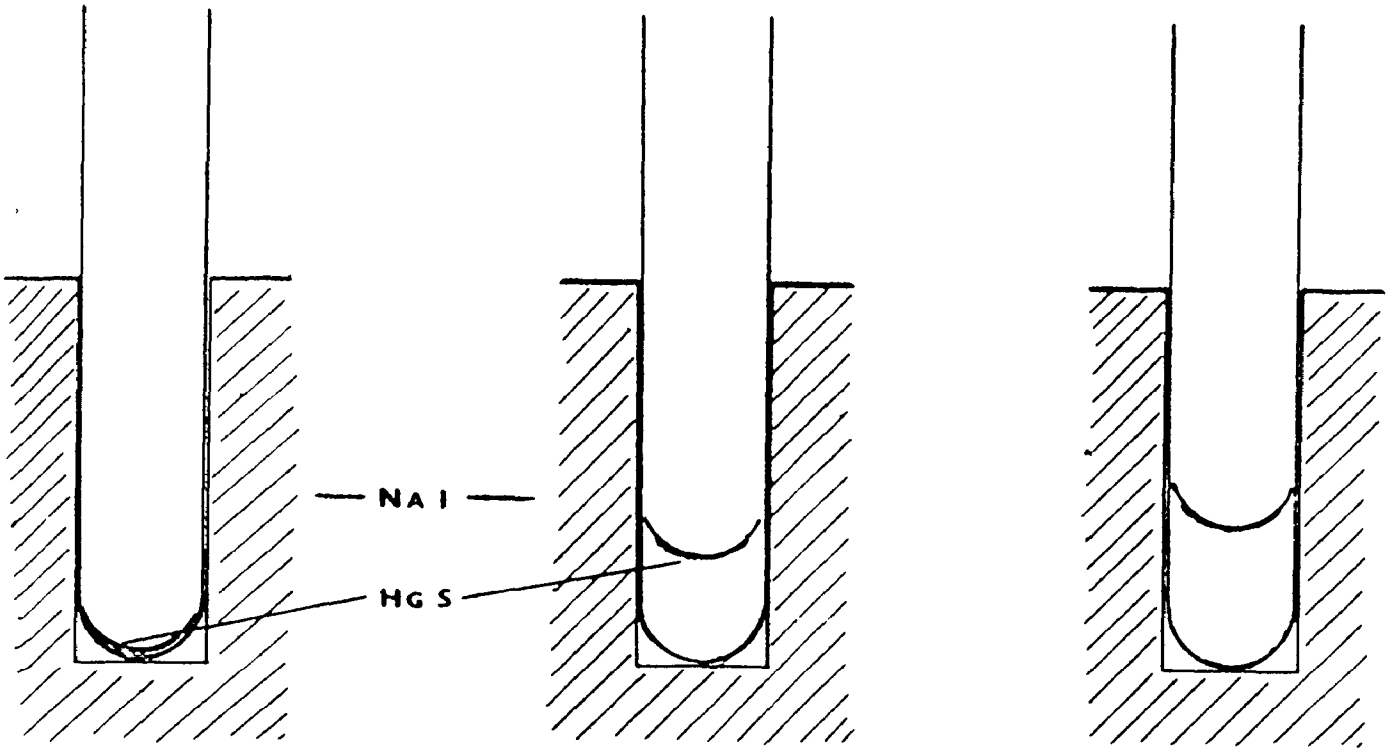
with the Ge(Li) detector in order to get similar counting statistics. This is particularly important with very weak sources.

A well-type detector is generally assumed to give very small geometry-related errors relative to a solid flat detector, because the sample is almost completely surrounded by the former.

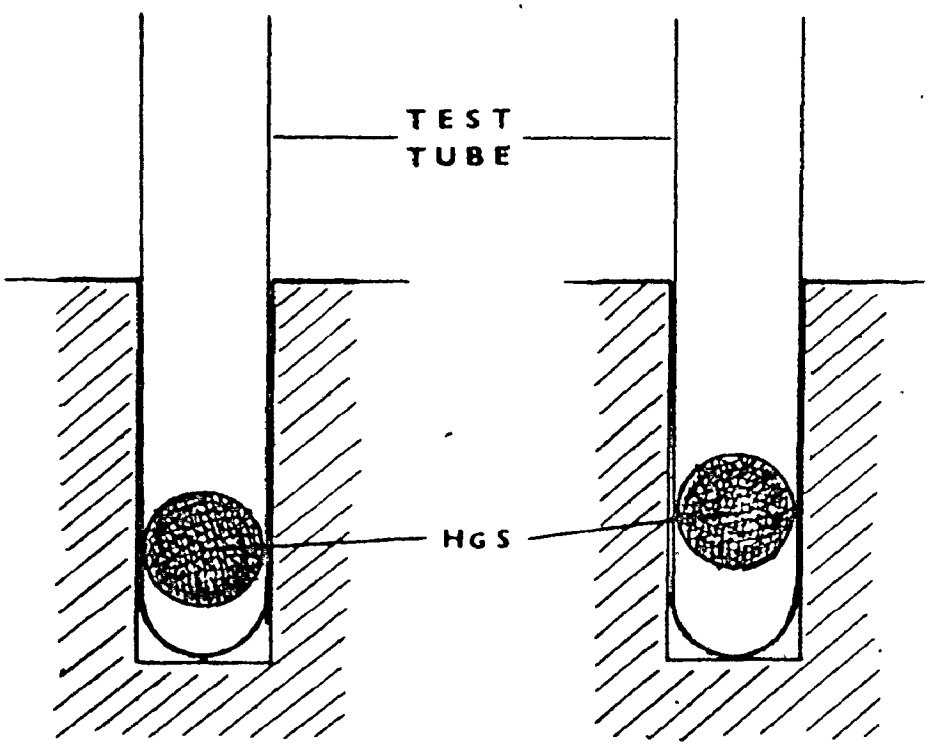
An experiment was carried out to determine exactly what geometry effects do occur for a sample counted in different positions in the well. A HgS precipitate weighing 65 mg and containing ^{203}Hg tracer was used. The precipitate was distributed in a circle 16 mm in diameter on a glass fibre filter disc of 25 mm diameter. A piece of tape held the precipitate in place. Figure 3.6 shows five positions in which the precipitate was counted in a plastic counting tube placed inside the well. Table 3.7 gives data for the geometry effects observed and also for dilution of a solution.

Figure 3.6

Geometry of Precipitates Counted in a NaI Well-Counter.



1 2 3



4 5

Table 3.7

NaI Geometry Effects

Position Number	Effect Relative to f
2	96.5%
3	94.0%
4	97.5%
5	95.3%

Amount of Solution	Activity Relative to 20 μl of Solution
1.0 ml	97%
2.0 ml	95%

^{203}Hg was tracer used. Data based on detection of 279 keV gamma ray.

3.5 Gamma-Ray Attenuation Data. (Variation of Absorption With Yield of Carrier.)

The possibility of attenuation of the low-energy gamma-ray and x-ray emission from ^{197}Hg has been outlined in Section 3.3.2, with calculations of the expected effect given in Table 3.5. These data were checked experimentally. Some results are given in Table 3.8. Of particular interest is the predicted strong absorption in a mercuric sulphide precipitate, which has been verified experimentally. The precipitate containing ^{197}Hg has the effect equivalent to a layer of mercuric sulphide approximately 0.2 mm thick, according to Table 3.5. This suggests the possibility that the attenuation due to the precipitate will vary strongly depending on the yield of the carrier. For example, if the same amount of ^{197}Hg were present in half as much mercuric sulphide, Table 3.5 predicts that the activity determined would be approximately 1.19 times larger, because the precipitate should be only half as thick and so would give less attenuation.

Experiments have been carried out to determine in practice just how large a variation in the attenuation will occur when the amount of carrier mercury recovered as mercuric sulphide varies. Different sized aliquots of carrier solution were taken. Equal amounts of radioactive tracer were then

Table 3.8

Gamma Ray Attenuation Data

Attenuating Material	Transmission ($1/1_0$ x 100%)	
	^{197}Hg	^{203}Hg
1.5 mm Aluminum	91%	97%
6.0 mm plexiglass	91%	97%
2.0 mm glass	90%	96%
10.0 mm plastic	87%	92%
addition of 1 ml water	96%	---
Precipitation with 50 mg carrier mercury as HgS	75% *	96%

* value depends on amount of carrier

added to each. The mercury was isolated as mercuric sulphide and the activity was determined in the well counter in position 4, as described in Section 3.4. Data for both ^{203}Hg tracer and ^{197}Hg tracer are given in Table 3.9.

Table 3.9

Variable Attenuation With Yield: ^{203}HgS

Amount of Carrier Mercury Added	Activity Found*
31.5 mg	294,886
38.4 mg	294,842
44.8 mg	292,394
51.1 mg	293,675
57.3 mg	294,206

* counts per 400 seconds.

Variable Attenuation With Yield: ^{197}HgS

Amount of Carrier Mercury Added	Activity Found Relative to Smallest Aliquot Added	
	#1	#2
12.8 mg	100 %	---
19.1 mg	98 %	100 %
31.5 mg	95 %	100 %
38.4 mg	---	96.9 %
44.8 mg	---	96.6 %
51.1 mg	---	95.2 %
57.3 mg	---	93.0 %
63.8 mg	90 %	93.0 %

3.6 Irradiation in Plastic Containers

Samples must be sealed for irradiation. The use of plastic containers was investigated for this purpose. Standards were prepared by pipetting small aliquots of dilute acidic solutions of mercuric chloride onto filter paper which was supported in a large beaker filled with hydrogen sulphide gas. The gas was blown gently over the filter paper while the solvent was evaporated by the use of a heat lamp. The spots were cut out carefully and sealed in small polythene bags, fashioned from polyethylene layflat tubing (0.1 mm thick), using a heat sealer (both items from Canus Equipment Ltd., 340 Gladstone Ave., Ottawa, Ontario.) The plastic had been washed carefully with aqua regia and then rinsed with distilled water before use. An irradiation time of two hours was used. After irradiation, the exteriors of the bags were washed, as activity due to ^{24}Na and ^{198}Au was often found.

The bags darkened and became brittle during irradiation and did not endure intact more than several hours in a flux of 10^{13} neutrons/cm^2/sec. Furthermore, they tended to stick together. These problems were alleviated somewhat by putting the samples in double bags and then wrapping them in aluminum foil. This kept the samples separate and also helped to keep them cool. Irradiation in plastic,

however, was eventually abandoned. It was judged not to be suitable for several reasons, despite the fact that it was a convenient procedure and allowed more samples to be irradiated at a time in one container than did sealing in quartz. The standards did not give consistently good calibration curves. This was likely due to the preparation procedure, although some volatility through the plastic was suspected. (The bags did not stand up well, even with double bags and wrapping in aluminum foil.) In many cases, irradiations of more than two hours were desirable. Large blank values were found (up to 10 nanograms): much of this was attributable to the plastic itself. Many other researchers have reported problems with samples sealed in plastic, especially due to apparent volatility through the walls and strong adsorption onto or into the walls.

3.7 Irradiation in Quartz Containers

Quartz capsules have been used for sample irradiation by many researchers. It is very unlikely that the mercury can escape from a properly sealed capsule. The low activity induced in high purity quartz (Suprasil quartz from Amersil Inc., 685 Ramsey Ave., Hillside, N. J., 07205) from contaminants makes this material ideal for instrumental activation analysis. After irradiation and a suitable cooling period to allow decay of ^{31}Si ($T_{\frac{1}{2}}$ = 2.6 hr.), only a slight amount of ^{153}Sm activity is present, and ^{198}Au is barely detectable by its 412 keV gamma ray. These are the most likely sources of specific spectral interference. Also, the overall activity was very low. The amount of mercury present in the quartz itself is below the detection limit, estimated in this case to be 0.01 parts per billion (10^{-11} g/g.)

Normal quartz exhibits an overall activity nine-fold greater than the high purity quartz, and in some samples a much higher level of contamination is present.

The possibility of mercury loss by recoil or diffusion into the walls of a quartz capsule during irradiation was investigated. A sample consisting of 8.44×10^{-9} g $HgCl_2$ in solution was sealed in Suprasil and irradiated for ten hours. After cooling, the quartz shards were found to con-

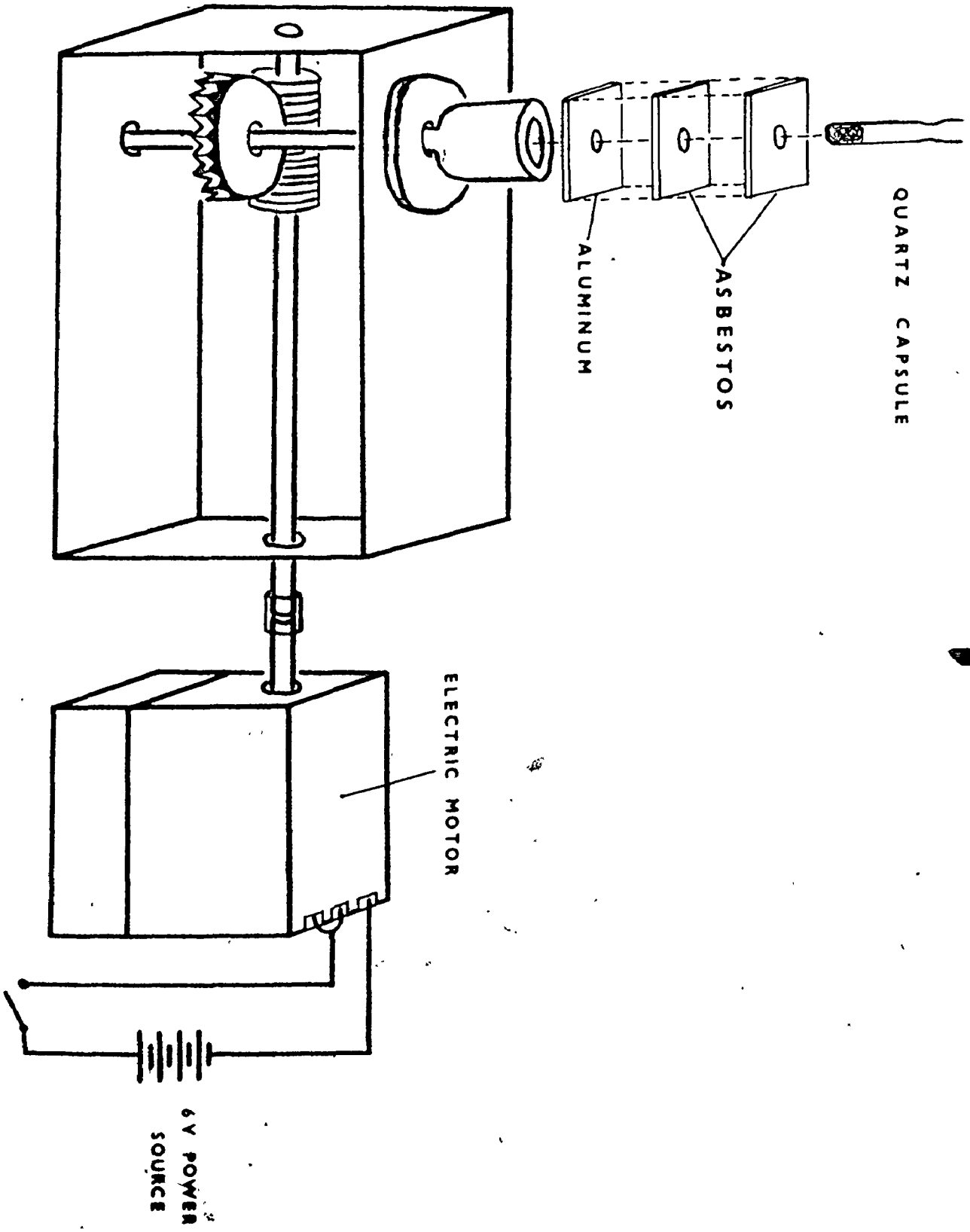
tain $\leq 5 \times 10^{-11}$ g Hg, thus showing negligible loss.

The most severe difficulty with this method comes in properly sealing the capsules. To do this, a very hot flame is necessary, and so the possibility of loss due to heating of the sample exists. The sealing must therefore be carried out as rapidly and carefully as possible. A quartz sealing device was built, as illustrated in Figure 3.7. A platform was rotated by an electric motor through a suitable set of gears to provide several revolutions per second. The platform held a container of the appropriate dimensions to surround the sample with coolant. An aluminum plate over the top had a hole drilled in it, of the right diameter to insert a quartz capsule to be sealed. Two layers of water-soaked asbestos pad were placed over the metal around the capsule to keep the flame from being deflected downwards. Samples may be frozen in liquid nitrogen, but keeping them cool in water was found to be very satisfactory. An oxy-acetylene torch with a fine tip was used to seal off the open end of the capsule. With some practice, it was possible to seal a capsule in about 30 seconds. The sample, which filled less than half the capsule, was kept cool and well shielded from the flame. Liquid samples must be well sealed (with thick end-walls) to withstand the pressure buildup from radiolysis during irradiation.

Methyl-mercury chloride was used to evaluate the capsule sealing technique. This is a very volatile mercury compound. The samples used were radioactively labelled with

Figure 3.7

Quartz Sealing Device.


ALUMINUM
ASBESTOS
QUARTZ CAPSULE
ELECTRIC MOTOR
6 V POWER SOURCE

^{203}Hg. Aliquots of a solution were placed in quartz capsules. The activity was measured, the capsules were sealed in the normal way, and the activity was determined again. No loss of mercury has been found. Table 3.10 shows data from one sealing experiment.

Table 3.10 Mercury Loss on Sealing Quartz Capsules

Estimated Amount of Hg as CH_3HgCl	Initial Count *	Count After Sealing *	Change
2×10^{-9} g	16817	16876	+ 0.4%
3×10^{-9} g	19795	19776	- 0.1%

*, each the mean of three counts for 200 seconds, with background of 390 subtracted.

3.8 Preparation of Standards

There is some evidence available to support the assertion that mercury in dilute solution, whether in inorganic or organic form, may be more volatile than the mercury present in a biological sample. While this is discussed in considerable detail in Section 2.4, it is mentioned here to emphasize that a great deal of care is required if accurate standards are to be made.

The simplest method of accurately preparing standards in the nanogram range is to dilute concentrated solutions in acidic media to the parts per million range and then pipet microliter quantities containing the desired amount. Table 3.11 gives data for a five (5) microliter automatic Eppendorf pipet (Brinkmann Instruments (Canada) Ltd., 50 Galaxy Blvd., Rexdale, Toronto, Ont.)

Water was pipetted into a freshly tared snap-cap poly-ethylene vial and weighed. All weighings were done on a microbalance, and the temperature changed from 23.5°C at the beginning to 24.5°C at the end. This results in a negligible change in density, amounting to 0.01% difference from the value at 24.0°C. This was calculated to be 4.987 mg for 5 µl from data given in the Handbook of Chemistry and Physics.[159] Some difficulty was encountered in transferring the water completely from the plastic tip to the plas-

Table 3.11

5µl Pipet: Precision and Accuracy Test

Tip	Weight (Milligrams)				
#1	5.00	4.95	4.92	4.91	4.80 *
#2	5.05	5.03	4.97	4.90	4.81 *
#3	4.99	4.57 *	4.88	4.83 *	4.91
#4	5.00	4.89	5.00	4.92	

* indicates values not considered in calculating the mean because droplets were left in the tip.

Mean (for n = 15): 4.955 mg

1σ = 0.055 (1.1%)

Accuracy = -0.64% (based on the expected weight of 5 ul of water at 24.0°C = 4.987 mg)

tic containers, and in the four instances indicated, tiny droplets were observed remaining in the tip. These data were not included in calculating the mean weight, which was found to be 4.96 mg (n = 15.) This is -0.64% relative to the expected value.

The pipet was then used to make up dilute standards by pipetting five microliter aliquots from solutions with appropriate concentrations(in the ppm range) into clean quartz capsules. The samples were frozen and sealed carefully shortly thereafter. Great care was taken, as this step was considered to be one of the most critical of the whole procedure. Standards were made up from independent stock solutions and irradiated with some of the above standards to check the reliability of this technique. Data are presented in Table 3.12. The expected value based on dilution of the stock solution was 114.5×10^{-9} g. Data obtained in the activation analysis gave a value of 114.6×10^{-9} g $\pm$ 2% (1σ) based on the induced activity of ^{197}Hg.

More dilute standards were made up from the first stock solution to give 22.9×10^{-9} g, and 4.58×10^{-9} g standards. A set of these standards was irradiated to determine their internal consistency. Data are given for this experiment in Table 3.13. Based on these experiments, the expected value from dilution was taken to be the accurate value. The remaining standards were used for various activation analysis experiments.

Table 3.12 Verification of Standards

Hg Content of Standard	Specific Activity *	
	x-ray peak area	77.6 keV peak area
4×10^{-9} g	1476	809
4×10^{-9} g	1212	700
80×10^{-9} g	1393	786
80×10^{-9} g	1409	801
244×10^{-9} g	1426	799
370×10^{-9} g	1495	830
422×10^{-9} g	1491	823
	----	---
Mean	1415	793

Standard Set	Calculated Mercury Content	
1)	115.3×10^{-9} g	115.6×10^{-9} g
2)	110.9×10^{-9} g	110.9×10^{-9} g
3)	117.3×10^{-9} g	117.8×10^{-9} g
	----------	----------
Mean Value	114.6×10^{-9} g $\pm$ 2% (1σ)	

* given as counts per 1000 seconds per nanogram content of mercury, corrected for chemical yield, radioactive decay and background.

Table 3.13 Verification of Standards

Mercury Content Based on Dilution	Expected Relative Mercury Content	Observed Relative Mercury Content Based on ^{197}Hg Activity *
4.58×10^{-9} g	0.0400	$0.0395 \pm .0008$ (1σ)
22.9×10^{-9} g	0.200	$0.196 \pm .004$ (1σ)
114.5×10^{-9} g	1.00	---

* each value the average of three samples

3.9 Preparation of Samples

In order to take full advantage of the method, the minimum of sample handling possible was employed before irradiation. This consisted of placing a portion of the sample into a clean, tared quartz capsule, weighing and then carefully sealing the capsule, as described in Section 3.4.4. Some tools were employed as necessary to place the sample in the quartz capsule. A small plastic funnel was used for samples in a powder form (for example, Bowen's kale, or instant coffee.) It had been cleaned in aqua regia, rinsed with distilled water, and dried before use. A thin glass rod, cleaned in the same way, was used to push the samples down to the bottom of the capsule. Use of the funnel avoided getting sample on the capsule walls near the region to be sealed in the flame. This was difficult to avoid with very moist samples, such as fresh meat, but the loss on sealing was still very slight. A stainless steel knife was used for cutting if required. It was rinsed in distilled water and dried in the oven. A duplicate sample was taken and dried in an oven to determine moisture content in order to convert results to a dry weight basis.

Provided that care was taken in handling the sample, and rigorous cleaning of the capsule and tools was

maintained, contamination was unlikely. No evidence of contamination was observed. Blank capsules contained no detectable mercury, calibration curves were linear with no random very high values or non-zero intercepts, and results for biological standard reference material (Bowen's kale, Section 3.12) were in good agreement with the recommended value.

Evidence presented in Section 3.7 on sealing the capsules suggests that mercury losses at this stage were negligible. This is likewise supported by the above observations of consistent results.

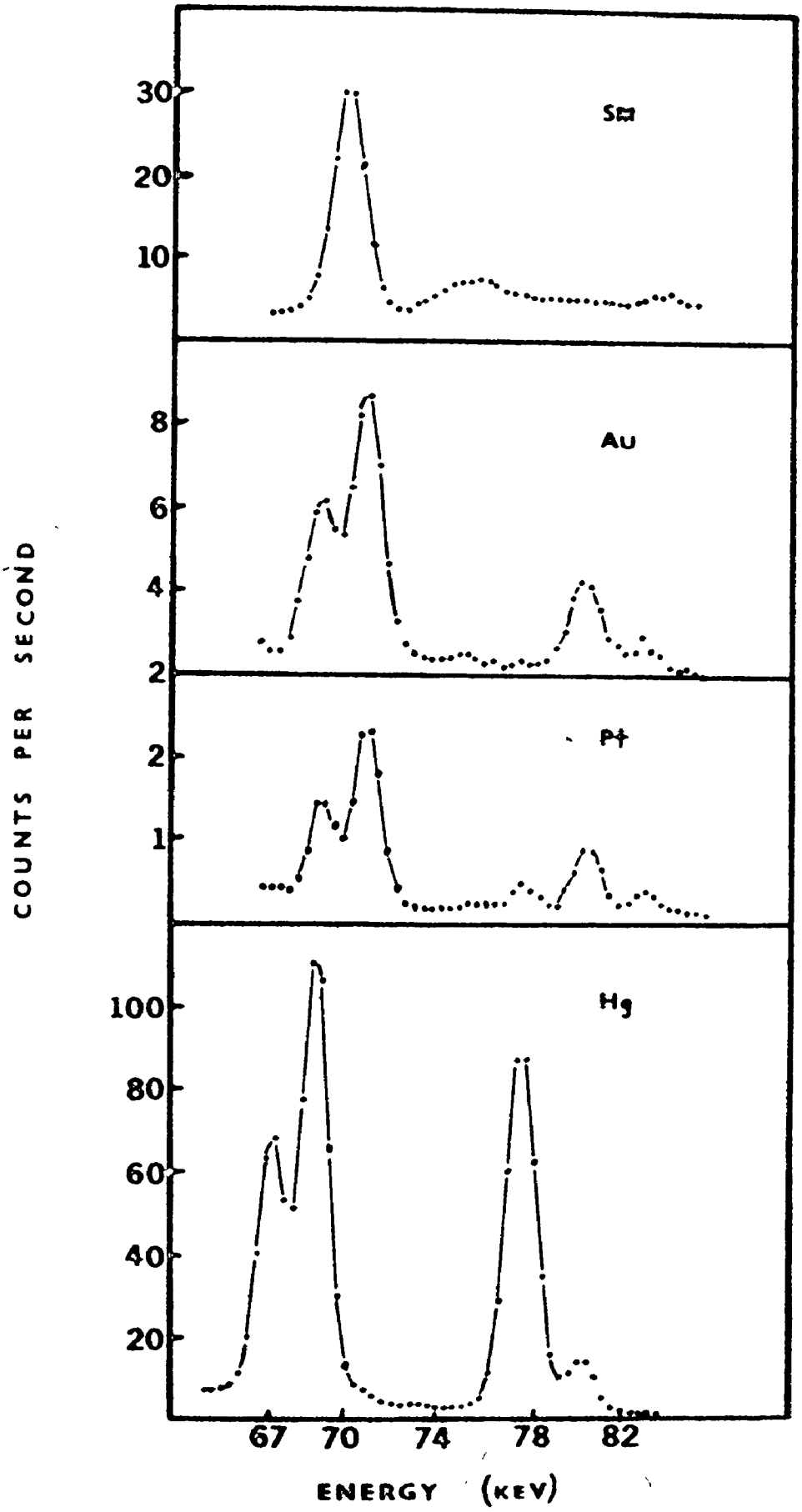
3.10 Instrumental Neutron Activation Analysis

Instrumental neutron activation analysis is certainly one of the simplest methods, because it requires essentially no processing of the sample after irradiation. Because of this, it is not subject to errors due to losses or contamination during sample handling. However, this technique has several significant drawbacks. The most serious of these is spectral interference from other radionuclides formed in the sample and in the container. If sealed quartz capsules are used to hold the sample, interference from rare earth elements giving low energy gamma rays, especially ^{153}Sm, can be severe. ^{198}Au is also often found to be present. The interference from the container can be minimized by using high purity quartz. It may be desirable to transfer the sample to another container for counting, but this will risk loss by volatilization and incomplete transfer.

Figure 3.8 gives spectra of several elements which may interfere, along with the spectrum of ^{197}Hg. These spectra were taken using the high resolution Ge(Li) detector described in Section 3.4.1. The quantities involved were: 437 ug of mercury, 500 ug of platinum, 43 ug of samarium, and 19 ug of gold. They were irradiated together

Figure 3.8

Spectral Interferences.


Sm
Au
Pt
Hg
30
20
10
8
6
4
2
2
1
100
80
60
40
20
67
70
74
78
82
COUNTS PER SECOND
ENERGY (KEV)

in separate packages for one minute in a neutron flux of 10^{13} neutrons/cm^2/sec. using the pneumatic tube (rabbit) system. All of these interfering elements give much more intense gamma ray emission at higher energy than the region under consideration here, and so their presence in a sample can be readily recognized and the extent of the interference taken into account and corrected for, if necessary.

From the spectra it can be seen that interference may be quite severe on the composite x-ray peak (here just resolved into a doublet) but that it is much less for the 77.6 keV peak region. Platinum shows the largest distinct peak at this energy, but for serious interference it would have to be present in at least ten-fold excess over the mercury. In biological samples this is unlikely, according to such compilations as Bowen's[162] and also in the author's experience. Some quantitative data on the interference from this and another experiment is given in Table 3.14. The samples were counted alone and in combination with mercury to estimate degree of spectral overlap.

Table 3.14

	Change in Peak Area From Mercury Alone	
Sample	X-ray Composite Peak	77.6 keV Peak
Hg + Au *	+ 2.5%	+ 0.1%
Hg + Sm *	+ 10.5%	+ 0.3%
Hg + Pt + Au **	+ 23.6%	+ 5.2%

* 437 ug Hg, 19 ug Au, 43 ug Sm.

** 290 ng Hg, 1.6 ug Pt, 30 ng Au.

Thus it is seen that a high resolution detector offers some relief to these problems of spectral interference.

The quantities of mercury involved are likely to be very small, and so give very weak activity which is often swamped by the much higher activities of other elements which are abundant in the sample, even though the gamma rays are far removed in energy. In biological material, ^{24}Na, ^{82}Br and ^{32}P generally exhibit intense emissions. The latter is a high-energy beta-ray emitter. This results in strong brehmsstrahlung. Also, intense beta-emitters result in lead x-ray emission from the shielding.

Because of these problems, it is generally necessary to allow a relatively long period of cooling so that at least some of the interfering activity will decay. Since the ^{197}Hg also decays, it is usually found to be better to use ^{203}Hg for the analysis. It is longer lived and gives a higher energy gamma ray. This, combined with the higher background, sacrifices several orders of magnitude in sensitivity relative to a chemical separation technique. ^{75}Se causes spectral interference with the 279 keV gamma ray. It may be corrected for with a factor derived from the 264.6 keV and 136.0 keV gamma rays of ^{75}Se, provided that the ratio of selenium to mercury is not too large.

Other problems with this technique include difficulties in reproducing the geometry between samples and standards, and the instrumental problems which may occur

when counting at high rates. Data in Table 3.6 shows that small changes in geometry can result in significant error when the sample is close to the detector. The shape and mean height above the detector cap may vary considerably from sample to sample, and from sample to standard. The difficulties of geometry and/or high counting rate may be circumvented by raising the samples to some height above the detector, but this will be at the expense of the counts received in the mercury peaks.

Another problem which can cause error is attenuation of the low-energy gamma rays by material between sample and detector. This includes different thicknesses of container walls and attenuation by the sample thickness itself, as well as attenuation by water in aqueous standards.

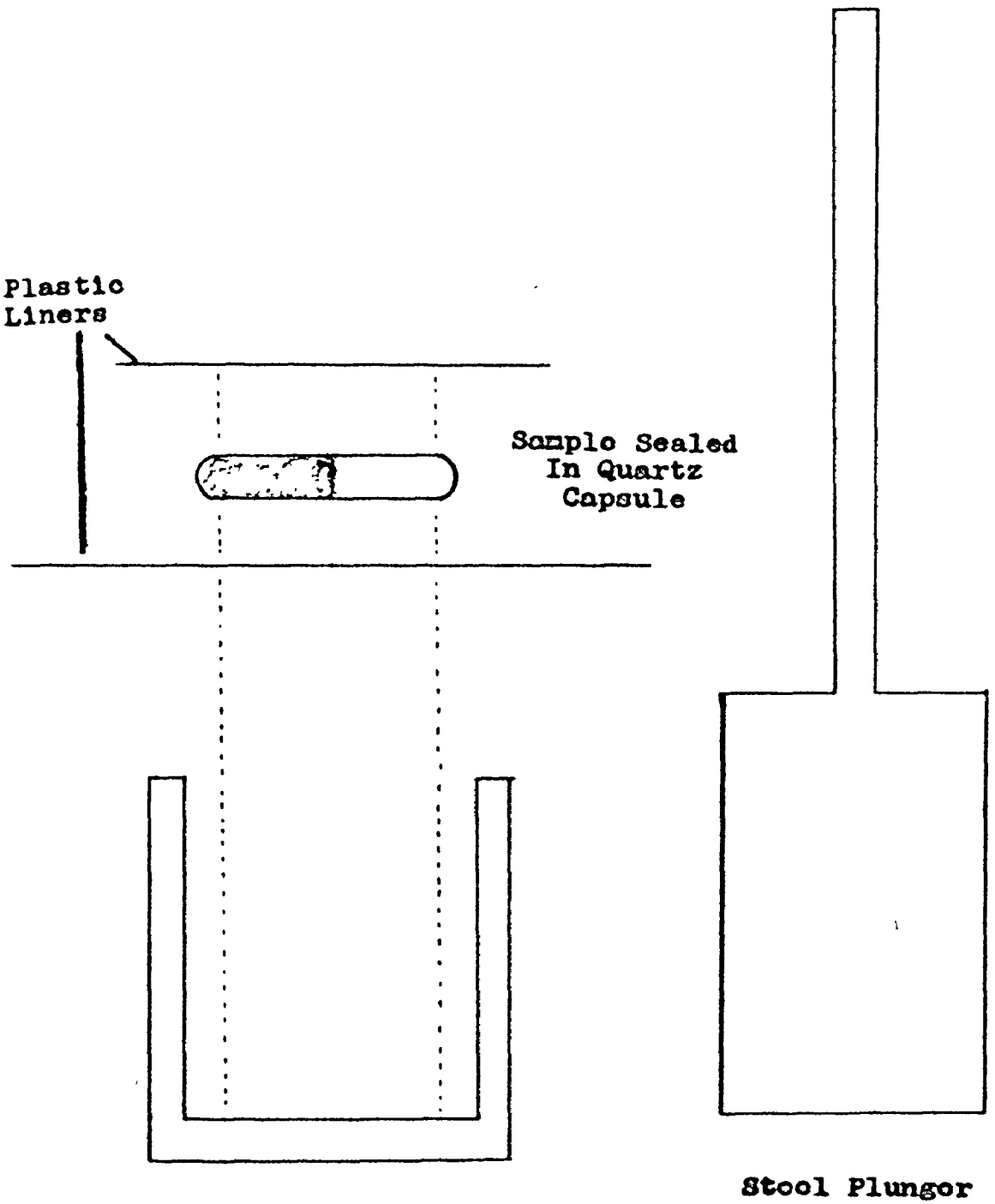
3.11 Radiochemical Separation Procedure For Mercury

The samples, sealed in quartz, were retrieved two or three days after the end of irradiation. This delay allowed short-lived radionuclides to decay and so made it easier to handle the samples from the point of view of radiation health hazards. It would be possible to process the samples so that the final separated fraction of HgS could be counted almost one half-life sooner, but this would be at considerable sacrifice of convenience due to the requirements of heavier shielding. Another alternative, if fast results are required, is to use a short irradiation in the pneumatic tube system (rabbit.) For a ten minute irradiation, a detection limit for mercury of about 10^{-9} g can be attained, and results could be available the same day.

The capsules were washed thoroughly in aqua regia, then rinsed in distilled water. ^{24}Na and ^{198}Au were often found on the outer surface from handling before irradiation. Individual capsules were frozen in liquid nitrogen and then broken open in a quartz smasher (Figure 3.9) between sheets of plastic. The sample, including plastic, was transferred completely to an ashing flask which was immediately connected to a reflux condenser. Concentrated sulphuric acid was used to wet all ground glass joints.

Figure 3.9

Quartz Capsule Smasher.


Plastic
Liners
Sample Sealed
In Quartz
Capsule
Stool Container
Stool Plunger

An aliquot of Hg(+2) carrier solution containing 50 mg mercury was added through the top of the condenser, and the sample was allowed to soak in it. Ten millilitres of concentrated sulphuric acid were then added and swirled with sample and carrier. Heat was applied to char the plastic, then three millilitres of fuming nitric acid were added and the mixture boiled vigorously. More nitric acid was added as required to ash the sample.

The solution was allowed to cool and 100 ml 1M hydrochloric acid was added. The solution was then filtered to remove the quartz shards and percolated through an anion exchange column (Dowex 1 x 8, 100 - 200 mesh). The column was washed thoroughly with dilute acid and water, and then the mercury was eluted with 100 ml of 0.1 M thiourea in 0.01 M hydrochloric acid.(163)

The mercury (+2) was precipitated with hydrogen sulphide and filtered with suction through a millipore filter apparatus onto a tared glass fibre filter disc. The mercuric sulphide precipitate was washed with water, ethanol, carbon disulphide, ethanol, and ether,(164, 165) and dried in an oven at 90°C. The precipitate was then weighed, taped front and back to hold the precipitate in place and counted on the high resolution Ge(Li) detector.

This procedure gives an essentially clean separation of the radioactive mercury from all other elements in the sample matrix, with the exception of gold. Generally, very

little, if any, gold is present, and so it does not interfere when the high resolution Ge(Li) detector is employed. If it is found to be necessary, gold can be added as carrier for the interfering activity and the mercuric sulphide precipitate redissolved and separated, but this has not been required as yet.

The ashing procedure has been investigated for losses due to volatility before complete exchange with the carrier mercury can be achieved. Aliquots of methyl mercury chloride, containing ^{203}Hg, were used in the procedure to check recovery. The activity recovered from three samples put through the ashing procedure was compared with two aliquots of tracer taken at the same time. These were counted, and then carrier mercury was added with potassium permanganate to break the methyl-mercury bond and promote exchange with the carrier. Mercuric sulphide was precipitated and counted for comparison with the samples. Table 3.15 gives data for this experiment. No significant loss due to volatilization during wet ashing has been detected. Further data are presented in Section 4.7.3 where wet ashing without addition of carrier mercury has been investigated for the atomic absorption method. No loss was found for tracer mercury, or for in vivo labelled fish.

Table 3.15

^{203}Hg Losses on Wet Ashing * - Carrier Addition

Order in Which Aliquot was Taken	Specific Activity **	Mean	90% Confidence Interval of Mean ≠
1) Standard	358	364.5	± 10
5) Standard	371		
2) Sample	349	356.3	± 8
3) Sample	346		
4) Sample	372		

* CH_3, ^{203}HgCl used.

** counts per 200 seconds per mg yield, background corrected.

≠ Calculation based on expected random errors, which are outlined in Section 3.21.1.

3.12 Procedural Checkout

From the experience gained in the experimental work described and consideration of the points outlined in the sections on theory, a recommended procedure has been derived. Table 3.16 gives a concise outline of this procedure as a flow chart in point form. For detailed information, the experimental investigations are referenced by page number. All important points are discussed, beginning at Section 3.13. Section 3.20 in particular should be referred to.

The proposed method of analysis by neutron activation has been tested by analyzing standard reference material. Preparation of Bowen's kale sample has been described in the literature.[166 - 168] Data on analyses from various labs are also reported by Bowen.[169, 170]

The most recent value for mercury in kale is 0.1663 ± 0.0235 ppb.[171] This is on a dry weight basis, and is the pooled result of 117 analyses reported by 20 labs, all using neutron activation analysis except for one. Results by the proposed method are given in Table 3.17. The mean value is 169 ± 5 ppb.

Table 3.16

Recommended Procedure Outline

1) Seal known amount of sample or standard in quartz with minimal pretreatment. Take duplicate sample for determination of water content. (Sections 3.7, 3.8 and 3.9)

2) 10 hour irradiation at 4×10^{13} $n\text{-}cm^{-2}\text{-}sec^{-1}$.

3) 24 - 36 hours cooling before beginning processing.

4) Transfer sample to wet-ashing flask. (Section 3.11)

5) Wet ash in presence of carrier (50 mg Hg). (")

6) Filter, adsorb on anion exchange resin (Dowex 1) and wash with dilute HCl. (Section 3.11)

7) Elute with 0.1 M thiourea which is 0.01 M in HCl. (")

8) Precipitate HgS. Wash, dry and weigh. (")

9) Determine activity.

Table 3.17

Bowen's Kale-Analysis for Mercury

Wet Weight	Mercury Content Found	Mercury Concentration
216.1 mg	34.7×10^{-9} g	161 ppb
155.3 mg	24.8×10^{-9} g	160 ppb
111.2 mg	17.7×10^{-9} g	159 ppb

Average Mercury Content = 160 ppb

Average Kale Water Content = 5.25%

Mercury Content (Dry Weight Basis) = 169 ppb ± 2.1 % *

* This is the 90 % confidence interval of the mean of 3 results (see Section 3.21.1).

Bowen's value based on 117 analyses from 20 different laboratories:

166.3 ± 23.5 ppb

DISCUSSION

3.13 Detection Equipment

The measurement of activity has been outlined in Sections 3.3 and 3.4. A high resolution Ge(Li) detector has been used throughout the experimental work reported here to determine the activity of irradiated samples and standards. The well-type NaI detector was used for tracer work, as a single radioisotope of mercury was known to be present. It was initially thought that the high resolution detector was required for "real" samples because of its greater freedom from spectral interference relative to the NaI detector. Use of the Ge(Li) detector sacrificed only a factor of 2.4 in detection limit compared to the well detector. Nevertheless, during this work it has been found that the high resolution is generally not required if a good chemical separation is employed. Gold is the only element which has been observed to interfere, and the error caused was usually negligible. A correction for the interference is easily made based on the 412 keV gamma-ray emission from this element. A NaI well detector has the additional advantage of requiring shorter counting times, and also gives a more easily reproducible geometry.

On the other hand, if an instrumental analysis is

to be attempted, the high resolution of the Ge(Li) detector is absolutely essential to the determination.

3.14 Attenuation of Gamma-Rays

Because of the low energy of the emissions from ^{197}Hg, attenuation is a serious problem. This must be kept in mind when doing tracer studies, as well as in the activation analysis. It has been found that the degree of attenuation varies with the chemical yield of carrier, as is to be expected. However, the observed effect is not as great as is indicated in Section 3.3.2. The reason for this appears to be that the HgS precipitate forms small clumps, which are distributed over the surface of the filter disc. Doubling the yield does not double the thickness of the precipitate, but rather produces about twice as many clumps, all roughly the same thickness, spread over the filter. The effect may be corrected for by an empirically determined correction curve, as indicated in Table 3.9. Care must be taken to ensure that the clumps do not pile up, as might occur if the filter were not perfectly horizontal during filtration. This would not only result in greater attenuation due to the greater thickness, but also in a different geometry, being higher and off-centre.

Use of a NaI-well detector helps reduce the possibility of geometry effects, and if the analysis can also be based on ^{203}Hg production, Table 3.9 shows that the attenuation of the higher energy gamma-ray is much less serious.

3.15 Irradiation In Plastic

Westermark and Sjostrand[139] showed that mercury can be very volatile under reactor irradiation conditions. Tracer studies utilizing various mercury compounds containing radioactive ^{203}Hg are described giving evaporation losses for various times at various temperatures. These studies showed that measurable evaporation occurred even at 50°C for organic-mercury compounds (but not for $HgCl_2$.) Careful wrapping in aluminum foil did not prevent losses under irradiation conditions. The D_2O moderator temperature was 35°C. The authors concluded that samples of interest for their mercury content must be completely sealed and used quartz tubes for this purpose.

Use of plastic containers for sealing samples has been made by many researchers. It is more convenient than quartz, because it is easier to seal and is thinner. These features allow more samples to be processed per unit time and per unit volume. The sealing temperature for plastic is much lower than that required for quartz, and so loss by accidental heating of the sample is minimized. Never the less, a considerable number of researchers have found erratic results which have been traced to apparent volatilization of mercury through the sealed plastic container. For example, Haller and co-workers[172] found that

results for mercury and other elements which exhibit volatility showed a larger standard deviation than those of "non-volatile" elements.

More specifically, L. C. Bate[173] found serious mercury losses from various sealed plastic containers relative to quartz-sealed standards and pointed out the consequences. The validity of these rather surprising results is backed up by the experiences of various researchers. Guinn and Kishore[174] noticed losses from plastic containers. McLain and Leddicotte[289] also confirmed Bate's work and now use quartz vials for sample irradiations in mercury analyses. Jervis and Tiefenbach[50] found severe losses from certain types of samples irradiated in plastic containers, especially fish, but no appreciable loss from many other biological tissues, such as meats, vegetation and hair. Jervis re-emphasized these results in a very interesting discussion on a paper on dental hazards[175] and in this same discussion, J. P. W. Houtman added that he found strong adsorption of mercury on the walls of plastic containers used in activation analysis, and so quartz was chosen instead. (The latter result had been observed by other researchers, especially for liquid samples.)[126,176]

A more general discussion on the volatility of mercury and losses by adsorption effects can be found in Section 2.4.

Bate[177] contributed some additional information to his other paper on the time-temperature dependence of

the mercury loss from sealed plastic containers. Weiss and Chew[178] found that acidity of a liquid sample played an important role in losses during irradiation in polyethylene containers. Unacidified samples "lost" significant amounts of mercury relative to their acidified counterparts. This loss was attributed to two factors. In a 60 minute irradiation, 12% was associated with the container walls and could not be readily recovered. Another 18% was lost by volatilization from the container.

McFarland[179] added considerable additional information to these results, including the possibility of cross contamination and leakage due to pressure buildup in some cases.

One possible solution to these problems has been suggested by Brune[146] and Brune and Landstrom.[180] Samples were kept frozen at -40°C during irradiation. The application of this technique was reported to offer the following advantages: Pressure buildup by water radiolysis is considerably reduced. Losses of activity due to vaporization effects as well as adsorption on container walls are strongly suppressed. The interference of nuclides from container walls is also avoided.

It becomes clear, then, that there may be a serious problem with losses of mercury from plastic containers, even if they are completely sealed. Although in specific cases it may be true that no loss occurs, it is not safe to generalize. Therefore, until convincing evidence to the contrary

is produced, it must be strongly recommended that plastic be avoided as an irradiation container. In this context, one must be wary of extrapolation of evidence from pure standard mercury compounds to the case of "real" samples. Similarly, evidence from relatively large quantities of mercury may not be applicable to samples which have orders-of-magnitude lower mercury content.

It has been suggested (181) that mercury could penetrate the walls of a quartz container to some extent. Even if it were only to a very slight depth, this might result in loss of that mercury, because it could not then be extracted again from the capsule. This possibility was examined in an experiment using high purity quartz to encapsulate a mercury sample, and it was concluded that loss of mercury to the quartz walls is not significant (Section 3.7).

3.16 Preparation of Standards

It has been the author's experience that small amounts of mercury contained in biological material are relatively stable (Chapter 2) but that in contrast to this, small amounts of simple inorganic (Hg(+2)) or organic mercury compounds (such as CH_3HgCl) in solution are extremely difficult to handle because of their volatility. The significance of this statement is that preparing accurate standards for activation analysis is much more difficult than might be expected, and may very well be the source of larger errors than the difficulties associated with sample preparation. In particular, evaporating solutions down to smaller volumes usually results in significant loss of tracer mercury, and taking solutions to dryness has invariably resulted in quantitative loss of the tracer mercury content. Even freeze drying will apparently result in serious loss, according to LaFleur.[120]

Mercury has a high elemental neutron cross section and so will exhibit strong self-shielding effects if more than a few micrograms are irradiated in a very small volume. Also, extrapolation from a standard exhibiting very high activity to a sample with orders-of-magnitude lower activity is an undesirable practice because of possible non-linearity. This may be caused, for example, by instrument

changes due to higher dead times and summing losses at high count rates. Thus, it is desirable to have standards with mercury content similar to that of the samples.

The stability of very dilute solutions of Hg(+2) has been discussed in Section 2.4. The more dilute the solution is, the harder it will be to handle. Acidic solutions (eg. 1M HCl) in the parts per million range have been found by the author to exhibit good stability.

Use of microlitre pipets has been favoured over using more dilute solutions and larger volumes. Larger pipets can give greater accuracy of delivery, but they are likely to cause other errors because the solutions are more dilute, and larger volumes are more difficult to handle. Furthermore, flux enhancement (Section 3.2.3) may become significant.

The precision obtained with Eppendorf microlitre pipets has been found to be 1.1% standard deviation (Section 3.8.) Some difficulty was experienced in achieving complete transfer from the plastic tips to the small plastic weighing vials used in this experiment. This is reflected in the finding that the accuracy of the mean delivery was -0.64%. This is significantly low, since the standard deviation of the mean is $\frac{1.1\%}{\sqrt{15}} = 0.28\%$.

However, complete transfer is more readily achieved into a glass or quartz container, and so this is not expected to have been a systematic bias.

3.17 Preparation of Samples

The volatility of mercury from biological samples is discussed in considerable detail in Section 2.4. Despite the surprising finding that the mercury content of fresh fish and meat is less volatile than expected, caution must be exercised, because during treatment of samples to extract the mercury content there is great danger of its loss.

One of the major features of the activation analysis technique is that minimal sample handling is required before irradiation. This aspect of the method should be used to full advantage in this case. Often the pre-treatment step of drying the sample is used. Removing the water allows more sample to be used in the same volume and reduces pressure buildup in the container from radiolysis during irradiation. The application of heat for drying purposes must be avoided. Westermark and Sjostrand[139] found organomercurials to be appreciably volatile, even at 50°C. Stein et al[182] found 95% losses on oven drying. Dry ashing, for example, with a low temperature oxygen asher, is reported to give essentially complete volatilization.[64, 280] Freeze-drying (lyophilization) appears to be the best drying technique. No losses were found in this study (Section 2.2) Stein et al[182] and Tanner et al[52] report similar results. On the other hand, Pillay et al[64] and Jervis and Tiefen-

Bach[50] found significant losses for certain types of samples. LaFleur[120] reports small losses from biological samples, and significant losses from aqueous solutions, especially of organo-mercurials. Besides volatilization, spattering of the sample can also be a problem.

In the author's experience, wet samples, or even aqueous samples, have been irradiated for at least ten hours at a neutron flux of 4×10^{13} n/cm^2/sec. with no problem, provided that the quartz capsules are properly sealed with a thick end wall. After irradiation, the samples are frozen in liquid nitrogen before breaking them open to avoid any possibility of explosion.

Because the pre-drying step appears to involve a high risk of loss and/or gain in mercury content, and it has been found not to be necessary, it is strongly recommended that it be avoided.

3.18 Instrumental Neutron Activation Analysis

The detection limit for instrumental activation analysis is much poorer than for a chemical separation technique. This is due to the spectral interference caused by the other activated isotopes present in the sample and the longer cooling periods required. Westermark and Sjostrand,[139] Ljunggren and Westermark,[183] and Weaver[184] found a detection limit of 0.03 micrograms using ^{197}Hg. Other workers have measured the activity of the ^{203}Hg isotope after a considerable period to allow decay and have found similar detection limits[174] or poorer.[186]

The errors involved in the analysis are also expected to be larger than for the separation technique. The counting statistics will be poorer because of the larger background and direct spectral interference by ^{75}Se.[187,188] Geometry and attenuation effects will also be larger, as it is more difficult to reproduce the exact position between sample and standard. Furthermore, instrumental errors may occur due to large differences in over-all counting rates between samples and standards. Therefore, the purely instrumental approach is considered unfavourable unless relatively large amounts of mercury are present in the sample.

Nevertheless, this method is simpler than employing a chemical separation and, with a high resolution Ge(Li)

detector, may be conveniently employed for a concurrent multi-element analysis. (184, 186, 187, 189, 190, 191)

3.19 Chemical Separation

The detection limit achieved by instrumental analysis is generally not good enough for common biological samples. Chemical separation to isolate an essentially pure mercury fraction removes interfering activity from other radioisotopes and allows the activity to be determined sooner after the end of irradiation. This results in an improvement in the detection limit of three to four orders of magnitude and improves precision by decreasing possible variations due to attenuation, geometry, vastly differing overall count rates, and spectral interference.

Many chemical separation procedures have been outlined in the literature, including (59, 192 - 206), and many more have been reviewed by Koesmer.[103] These generally allow a multi-element determination to be carried out.

The critical step in a chemical separation procedure is ensuring exchange of the radioisotope with the carrier. After this point, the chemistry becomes that of an easily handled amount, rather than minute traces, and losses are corrected for by determining chemical yield. Contamination is unlikely, as it must be either in the form of radioactive mercury or else in significant amounts relative to the 50 mg of added carrier in order to cause a serious error.

The anion exchange procedure allows a clean separation

of the mercury from the other elements in the sample, with the exception of gold. Gold has not been found in any sample in quantities large enough to interfere with the mercury measurement. ^{199}Au from ^{198}Pt (n,γ,β^-) has never been observed in any of the samples analyzed. The ion exchange technique permits easy extension to a multielement analysis by simply varying the HCl concentration of the eluent solution.

Variable attenuation with yield has been observed and is discussed in Section 3.15. It must be corrected for with an empirically determined factor. Care should be taken to ensure an even deposit of the precipitate on the filter disc.

3.20 Quantitative Limit of Determination

A ten hour irradiation period has been used, followed by two to three days of ageing during which time short-lived radionuclides decay significantly. The separated mercury fractions (as HgS) are available for determination of their activity three to four days after the end of irradiation. At this time, the induced specific activity is typically found to be about 3200 counts in the 77.6 keV photopeak per 4000 second count per nanogram (10^{-9}g) mercury present. The background count over the same region for the same time is 400 counts. This allows an estimation of the "detection limit." An often used formula for this rather nebulous quantity is the signal which is equal to twice the standard deviation of the background.[207] In this case, the detection limit would be about 40 counts, which is equivalent to 1.3×10^{-11} g. However, this is an impractical estimate, and a much more useful estimate is that proposed by Currie,[207] as the "quantitative limit of determination." This is the amount of mercury which allows estimation of the result to within a rela-

tive standard deviation of ten per cent. Assuming that the counting statistics alone determine this limit, the value for the quantitative limit of determination is calculated to be 337 counts/ 4K sec., representing 1.1 x 10^{-10} g. Counting 40K sec. improves this value to 3 x 10^{-11} g.

The determination limit may be improved somewhat in two ways. A longer irradiation may be employed. For example, a three day irradiation will give a five-fold increase in the specific activity. Secondly, the sample could be processed about one half-life sooner. This would require considerable additional shielding because of the high activity involved. Also, larger sample sizes result in a lower relative determination limit. Sample sizes in the 0.05 g to 0.5 g range have typically been used in this work. A ten-fold increase would be feasible, although larger samples may result in larger errors from neutron flux variations.

Thus, pushing the technique to its practical limit in this way, and using a ten gram sample, could result in an impressive quantitative relative limit of determination of three hundred parts per quadrillion (3 x 10^{-13} g/g)!

If, on the other hand, a rapid result is required, a short irradiation in the pneumatic tube system may be used. A ten minute irradiation should give a quantitative determination limit of about 5 x 10^{-9} g, and results could be available the same day.

3.21 Errors

3.21.1 Random Errors

The possibilities of error in the activation process for mercury have been outlined in the sections of Theory. The most serious of these is neutron flux variation between sample and standard (Section 3.2.3.) The effect was minimized by using small volumes which have similar matrices and by irradiating samples and standards in close proximity. Note that the standards contained amounts of mercury similar to that expected to be in the sample. Typical flux gradients in the McMaster Reactor are in the order of 1.5% per centimetre. Experience indicates an overall error due to flux variations of about 0.5% standard deviation should be expected.

Errors in determining the activity come from variations in the geometry, variable attenuation and the statistical fluctuations inherent in radioactive decay. The geometry is extremely important. From Section 3.4, variations of 1 mm in height will result in 4% - 5% changes in measured activity. Reproducing the geometry to better than a millimetre between sample and standards has been possible, and so a standard deviation of 1% has been assigned to this error source. (Note that if a well-type detector is used, this error will become much smaller.)

Variable attenuation with yield of carrier is outlined in Section 3.5 and discussed in Section 3.15. It only affects the ^{197}Hg emissions significantly and is corrected for with an empirical factor. Nevertheless, there is some uncertainty in the correction, expected to amount to 0.5% (1σ), provided the mercuric sulphide precipitate is evenly distributed. The statistics of counting are readily calculated as the square root of the number of counts received (one standard deviation, neglecting background) and generally have amounted to about 1%, unless the source is extremely weak.

The remaining expected errors arise from the treatment of the sample and standards. Weighing errors will usually be negligible when compared with the others. Pipetting errors are expected in the order of 1% for the addition of carrier and 1% for the preparation of each standard, because of the use of micropipettes.

The expected total relative random error may be obtained by summing the individual relative variances and taking the square root:

$$\sigma \text{ total} = \sqrt{\sum_i \sigma_i^2}$$

Table 3.18 shows how the estimate of the total random error has been obtained. The error in a single result (sample compared to standard) is therefore:

$$1\sigma = \sqrt{(4\tfrac{1}{2} + 3\tfrac{1}{2}) \times 10^{-4}} = 2.8\%$$

Table 3.18 Random Errors

Standards

Error Source	Magnitude (1 σ)
flux variations	0.5 %
geometry	1 %
attenuation	0.5 %
counting statistics	1 %
pipetting standard	1 %
pipetting carrier	1 %

$$\sigma \text{ total} = \sqrt{(.25 + 1 + .25 + 1 + 1 + 1) \times 10^{-4}}$$
$$= \sqrt{4\tfrac{1}{2}} \times 10^{-2} = 2.1\%$$

Samples

Error Source	Magnitude (1 σ)
flux variations	0.5 %
geometry	1 %
attenuation	0.5 %
counting statistics	1 %
pipetting carrier	1 %

$$\sigma \text{ total} = \sqrt{(.25 + 1 + .25 + 1 + 1) \times 10^{-4}}$$
$$= \sqrt{3\tfrac{1}{2}} \times 10^{-2} = 1.9\%$$

If three standards are used, the error in a single result is computed as:

$$1\sigma = \sqrt{\left(\frac{4\frac{1}{2}}{3} + 3\frac{1}{2}\right) \times 10^{-4}} = 2.25\%$$

The standard deviations computed here as described may be used to set confidence intervals on data presented in this chapter. The interval quoted in appropriate tables has been that of 90% probability. This is found by taking $\pm$ 1.645 σ, as described by Dixon and Massey.(298)

Note that inhomogeneity of the mercury content at the sample size chosen will result in unexpectedly large random errors.

3.21.2 Systematic Errors

Systematic errors are usually very difficult to detect. Several possibilities have been given in the sections of Theory, including interfering nuclear reactions, (Section 3.2.5) anomalous isotopic abundance, (Section 3.2.6) and different neutron fluxes in sample and standard (Section 3.2.3.) The samples and standards were alternated so as to detect and correct for a flux gradient. Excellent linearity has been observed for the different standards. This also indicates that no serious self-shielding effects occurred. The same conclusion is reached for the Kale samples (Section 3.12) used to check the recommended method, as different sample sizes were taken. Furthermore, the good agreement shows that flux enhancement is not a serious effect

in this size of biological sample.

A systematic bias could occur if standards and samples were always treated the same way, but differently from each other. For example, different counting efficiencies can sometimes occur. The effect of carrier yield on attenuation of the low-energy gamma radiation of ^{197}Hg has been outlined in Section 3.5. Different dead time effects between samples and standards could occur, especially if one always has a higher activity than the other.

Other possibilities of systematic errors include consistent faulty chemical technique, introduction of contamination, incomplete exchange of the radioisotope with the carrier, and faulty preparation of the standards.

4. COLD VAPOUR ATOMIC ABSORPTION THEORY

4.1 Introduction

Atomic absorption spectrophotometry is now well established as a useful and popular analytical technique. The modern form of this method was suggested by Walsh in 1955,[208] and he and his co-workers have been responsible for many advances in the field since then. A theoretical treatment of the shape of analytical curves obtained in flame spectrophotometry has been presented by Zeegers et al[209], and De Galan and Samaey[300].

Mercury analysis by atomic absorption is a special case, however, because of the unique properties of this element. The application of this method to mercury predates 1955 considerably. In fact, Wood published a fascinating dissertation on the subject in 1910.[210,211] One result which is of interest to the analyst is that he found it necessary to open his windows to air out the laboratory before conducting experiments, because mercury vapour was present in the room to such an extent as to cause strong absorption! Based on Wood's research, and development by Hewlett, an instrument was built to detect mercury vapour. It was

described by Woodson in 1939.[212] The basis of the analysis for mercury in a sample is, therefore, to produce an atomic vapour from it and to pass this vapour through an absorption cell so that the instrument can measure the amount of absorption caused by the mercury vapour at a specific resonance wavelength (253.7 nm). The absorption by the mercury from the sample can then be related to the mercury concentration of the sample by establishing a calibration curve. Several early examples of the application of this technique may be found in references 213, 214, 215 and 245.

The method of production of the mercury vapour often employed is that of reduction in solution to the neutral atom from the Hg(+2) state by addition of reagent containing Sn(+2). The mercury atoms thus formed are swept from solution by bubbling a carrier gas through the solution. The carrier gas is then passed through an absorption cell positioned in the instrument.

Use of the reduction-aeration procedure had been made by many researchers as a concentration step during the analysis for mercury,[101] but Poluektov et al,[216, 217] followed by Hatch and Ott[218] were the first to make atomic absorption measurements on the evolved mercury vapour.

The absence of a high input of energy used to form the atomic vapour results in a reduction in possible interferences from other elements. Under these conditions, mercury is the only element which will form an atomic vapour.

Broad-band molecular absorption, especially from organic compounds, is a possibility, however.

The reduction-aeration method results in a transient signal as the mercury vapour flows into the cell and out again with the carrier gas. Each sample gives a "one-shot" run. This is in contrast to the conventional technique of flame atomic absorption, where the sample is aspirated into the flame at a steady rate, with the result that a constant signal is attained. Because of this, the normal instrument with digital read-out is not usable for making measurements on the mercury vapour. This has resulted in the application of a strip-chart recorder readout and calculation of concentration based on the peak height rather than the peak area. This rather dubious practice relies entirely on the release pattern being the same for sample and standard.

4.2 Reduction-Aeration With Flow-Through Cell

Assuming a Lambert-Beer Law relationship between the absorption caused by the mercury vapour and the number of atoms making up the vapour, the following equation obtains:

$$A(t) = K\,N(t)$$

where $A(t)$ is the absorbance due to the mercury vapour present in the absorption cell at time t. ($A \equiv -\log T$, T is the transmittance).

K is a constant which depends on the absorptivity and the cell dimensions.

$N(t)$ is the number mercury atoms present in the cell as a vapour at time t.

The total absorbance due to the mercury content of a sample passing through the absorption cell as an atomic vapour is:

$$A_{total} = \int A(t) = \int K\,N(t) = K\,N_{total}\,t_d$$

where N_{total} is the total number of mercury atoms which pass through the cell

t_d is the average dwell time of a mercury atom in the light beam

Since $t_d \propto \frac{\text{cell volume}}{\text{carrier gas flow rate}}$ we may write the relationship as:

$$A_{total} = (\text{constant}) \frac{N_{total}}{\text{gas flow rate}}$$

According to this equation, the product of the total absorbance and the carrier gas flow rate is the quantity which should be measured that is directly related to the total number of mercury atoms in the sample, provided that all of the mercury in the sample is passed through the absorption cell.

The relationship of the quantity A_{max} (the maximum absorbance, or "peak height") to N_{total} will now be considered. If an excess of reducing agent is added, we may assume that the reduction of the mercury to neutral atoms is rapid. The flow of mercury through the absorption cell then depends on its rate of release from the solution and its flow from the solution to the cell. The latter may include retention effects along the passage due to adsorption on the walls, and spreading by diffusion. The release from solution is expected to be a first order reaction, that is, it depends on the number of mercury atoms present:

$$\frac{dN}{dt} = - k N$$

$$\int \frac{dN}{N} = -\int k\,dt$$

$$\ln(N) = -kt + \text{constant}$$

$$\therefore \quad N = (e^{\text{constant}})\, e^{-kt}$$

at $t = 0$, $N = No$,

$$\therefore \quad N = No\, e^{-kt}$$

The "constant" k will depend on the temperature, the degree of saturation of the carrier gas with the mercury vapour, and the efficiency of the bubbling process. (The latter depends on such factors as the geometry of the solution and the efficiency of mixing.) Assuming that k is a constant under given conditions, and that the sample does not contain such a large amount of mercury as to result in saturation of the carrier gas, the flow pattern of mercury observed at the absorption cell, based solely on the above equation of release, can be predicted to be:

$$f(t) = \left[(1 - e^{-kt}) - (1 - e^{-kt^1})\right]$$

where $t = t^1 - \dfrac{\text{cell volume}}{\text{carrier gas flow rate}}$

The first exponential represents the expected flow of mercury vapour into the absorption cell. The second exponential represents the flow of the mercury vapour out of the other end of the cell at a later time equal to the average transit time of a mercury atom through the cell.

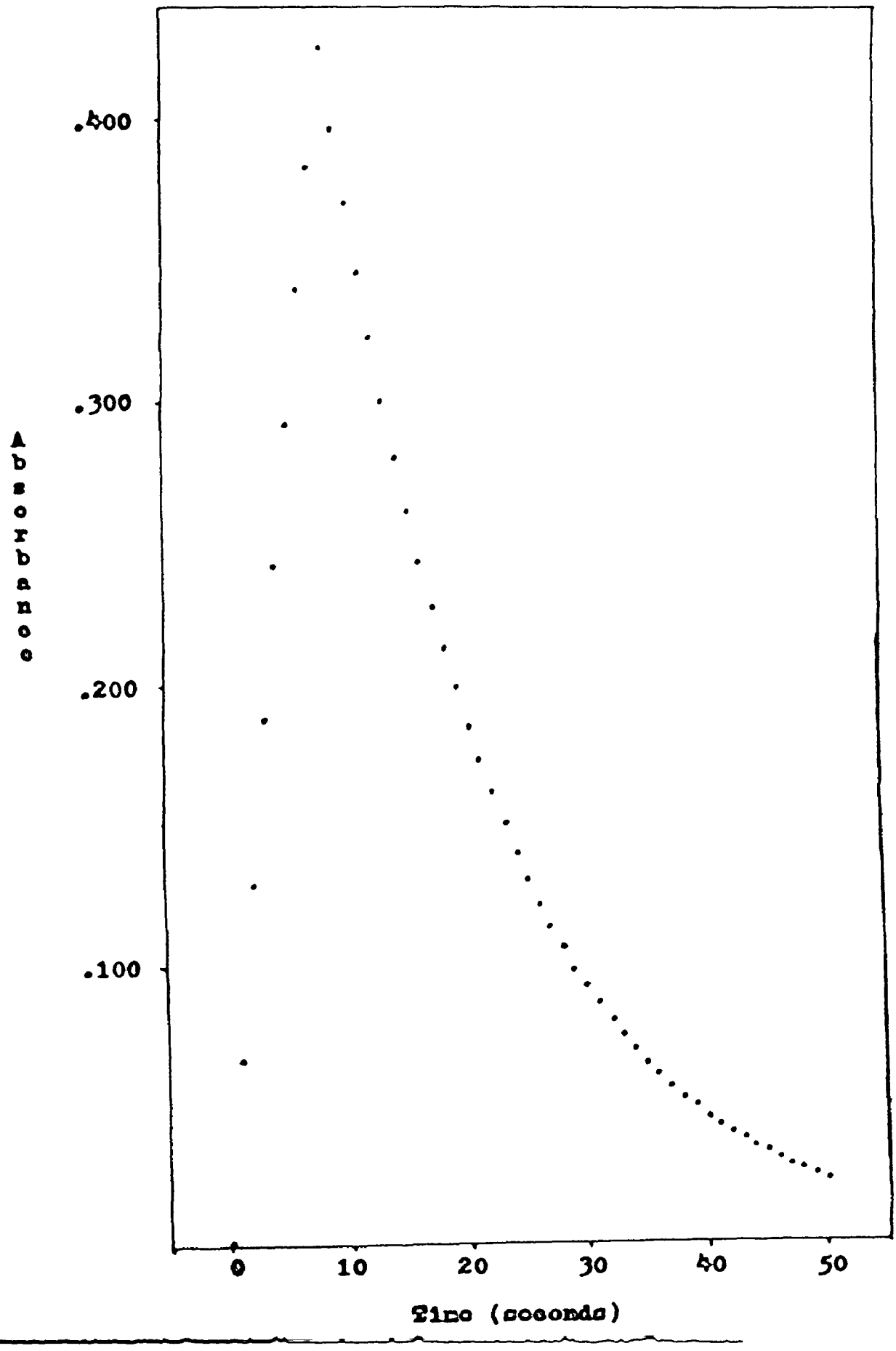
Figure 4.1 shows a plot of f(t) using k = 0.0693 and $t = t^1 - 8$ seconds (approximate observed values).

Clearly, $A(t) \propto f(t)$, and $A_{max} \propto f_{max}$.

A_{max} may be taken to represent N_o only if the same release pattern occurs for each sample and standard in a given analysis, thus giving a constant flow pattern function, f(t). This is the assumption in using peak height, rather than (peak area) x (gas flow rate) which truly reflects the amount of meroury present.

Figure 4.1

Theoretical Flow Pattern of Mercury Through the Absorption Cell.


.400
.300
.200
.100
Absorbance
0
10
20
30
40
50
Time (seconds)

4.3 PHOTON COUNTING READOUT

4.3.1 Introduction

It was desirable to improve the instrumental readout system in order to optimize it for the special application of cold vapour atomic absorption due to mercury, as outlined in the introduction. Furthermore, in order to check the theory presented in Section 4.2 and to develop it, it was absolutely necessary to have a readout which could conveniently and accurately follow the transient signal produced during the mercury analysis and allow determination of the total absorbance peak area. A photon-counting readout was therefore developed and adapted to this particular application.

A few researchers have suggested the application of photon counting to spectrophotometry, (219, 220, 221) and commercial equipment is now available. (222,284) The application is mainly regarded as a novelty, however, and only a few months ago has a report been submitted from this laboratory on the practical advantages that such a system offers for flame emission analysis. (223)

Photon counting is normally considered to be best suited to the measurement of low light levels, and not to

high levels such as are encountered in atomic absorption. Nevertheless, this readout mode has a number of advantages when applied to atomic absorption, which have been outlined by the author.[224] In particular, the very fast response time of this readout technique allows rapid signal fluctuations to be followed accurately. The system as adapted for the mercury analysis is described in the following sections.

4.3.2 Photoelectron-Pulse Amplifier-Discriminator

A block diagram of the photon amplifier-discriminator is shown in Figure 4.2. The photoelectric pulses arriving at the photomultiplier anode were several millivolts in magnitude and 5×10^{-9} seconds wide. These were amplified and then applied to a lower level discriminator to cut out noise pulses which were smaller than the photoelectron pulses. The actual circuit diagram is shown in Figure 4.3. At the discriminator output, the pulses were three volts in magnitude and 15×10^{-9} seconds wide. The recovery time of the circuit was observed to be 25×10^{-9} seconds, however, giving a maximum rate of 40 megahertz. In practice, the rate should be kept below about 400 kilohertz to avoid any significant dead time losses. The photon pulses were put through a scale-of-ten counter in order to give an output which was compatible with the somewhat slower circuitry used to record the count.

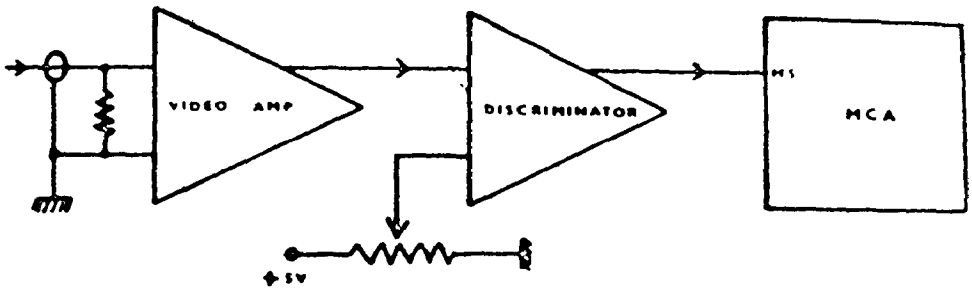
Figure 4.2

Photon Pulse Amplifier/Discriminator - Block Diagram.

A) Photon Count Accumulation by Application to Multi-Scaling (MS) Input of Multi-Channel Analyzer (MCA).

B) Photon Count Accumulation Synchronized With Light Chopping.

A


VIDEO AMP
DISCRIMINATOR
MS
MCA
+5V

B

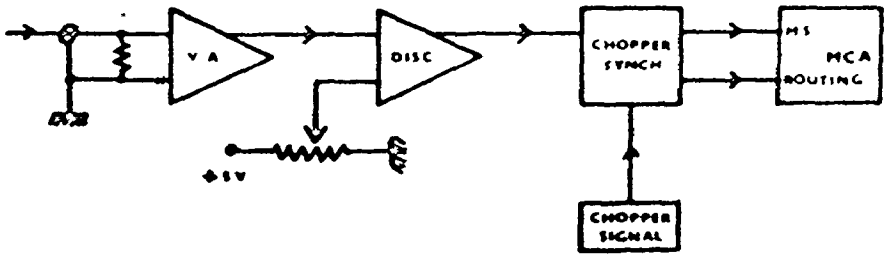
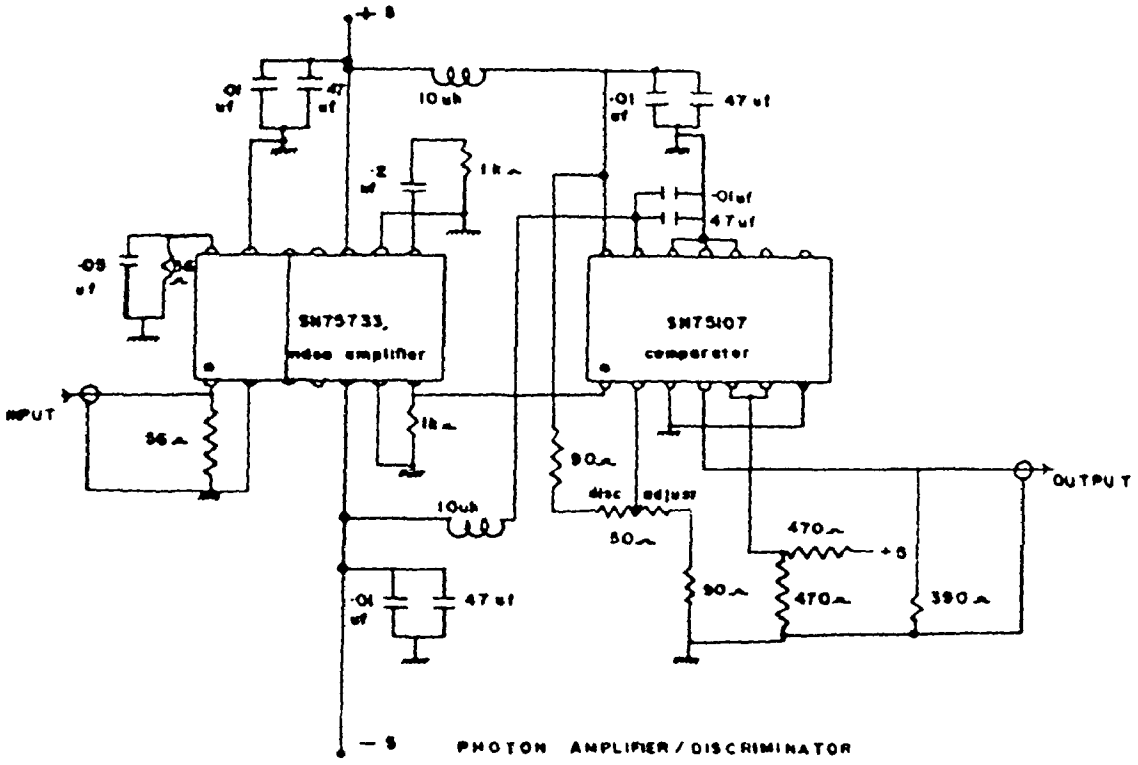

V A
DISC
CHOPPER SYNCH
MS
MCA ROUTING
+5V
CHOPPER SIGNAL

Figure 4.3

Photon Pulse Amplifier/Discriminator - Circuit Diagram.



PHOTON AMPLIFIER / DISCRIMINATOR

The circuit was constructed on a double-sided ground plane. A very short length of co-axial cable (50 ohm) was used to connect the phototube anode to the video amplifier input. The signal was terminated to ground with a 50 ohm resistor. Careful decoupling of the amplifier from the discriminator was absolutely essential, and the amplifier non-inverting output was terminated. Use of a very stable power supply in addition to the above precautions resulted in satisfactory stability.

In order to keep the counting rate at a moderate level, the mercury hollow cathode lamp was run at a lower current (3 mA), and the slits were kept narrower, than is normally recommended.[225,226,254] This had the advantage of helping avoid self-absorption and self-reversal in the lamp, and of resolving a nearby non-absorbing wavelength at 253.5 nm from the mercury wavelength at 253.7 nm. These would cause deviation from the Lambert-Beer Law.

4.3.3 Photon Count Accumulation

Each pulse coming from the circuitry described above in effect represented ten electrons ejected from the photocathode of the photomultiplier tube. Some of these do not represent photons, but rather (mainly) thermionic emission. This is the "dark count." It amounted to about 200 counts per second. This was negligible,

compared with the high rate due to the lamp. Since a transient signal was to be observed, it was necessary to record the photon count received in each successive small time interval, Δt, during the experiment. To do this, a counting register must add up the photon count for Δt, the count must be stored in a memory, the register cleared, and the count re-initiated. After the experiment, if the counts in the memory were displayed in the order they were taken, then the result was a digital display of photon count rate with time. Figure 4.4 shows such a readout. Skene et al (222) demonstrated how this could be done with a mini-computer. In the present experiment, the multi-scaling input of a multichannel analyzer (Canberra model 8100, or Nuclear Data model 2200) was used. The dwell time, Δt, was variable in integral increments from 1×10^{-6} seconds to 9 seconds per memory channel. The time required to store the count and clear the register was several microseconds, so this caused negligible loss of time unless very short dwell times were employed. The data were contained in magnetic core memory and could be observed on an oscilloscope. The data could also be read out, for example, on a teletype or paper tape.

Figure 4.5 shows the output of the photon count from the mercury 253.7 nm wavelength with the chopper on. The dwell time was one millisecond per channel. The chopper is seen to be in transit for a certain portion of the

Figure 4.4

Photon Count Accumulation: Mercury Vapour Passing Through the Absorption Cell.

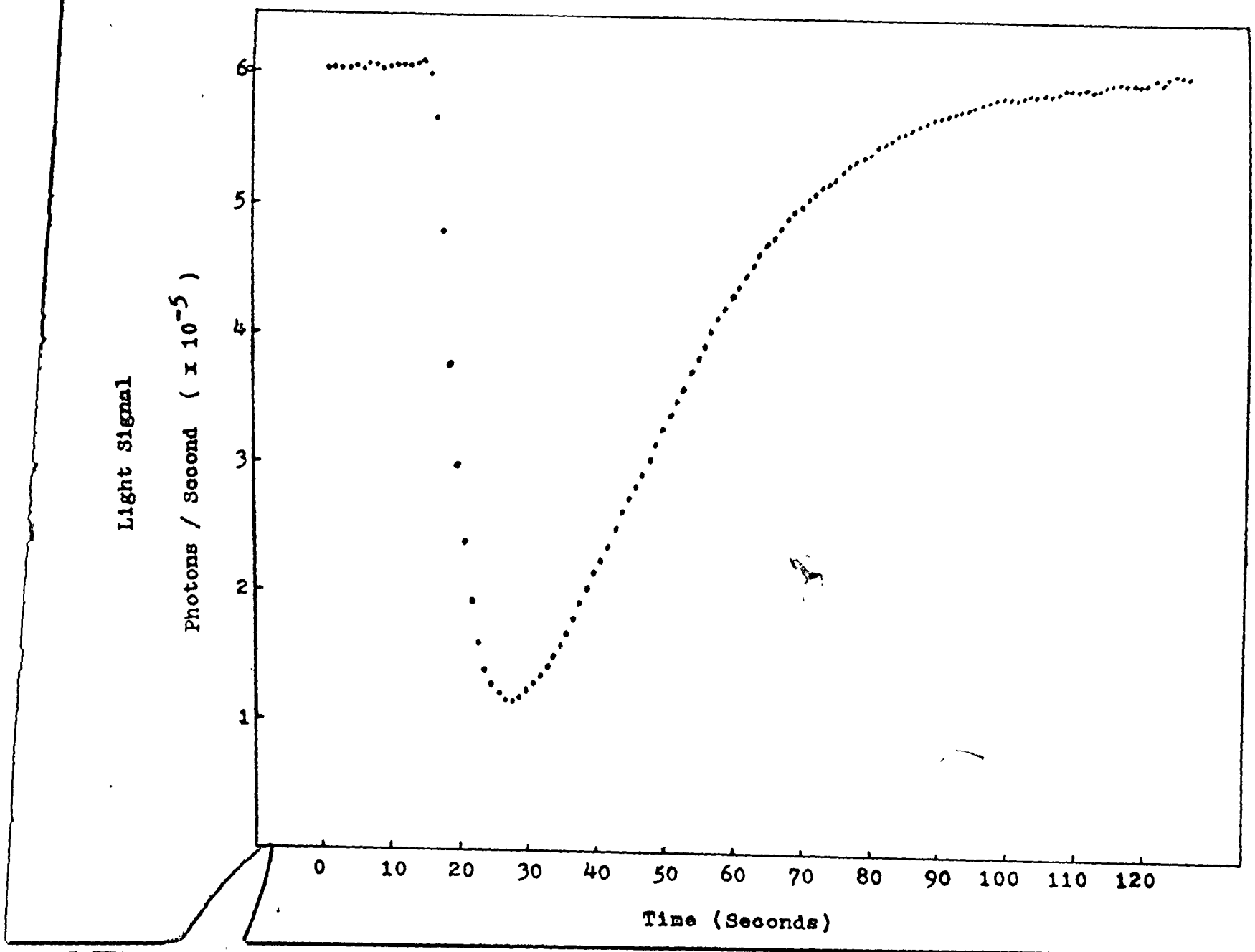
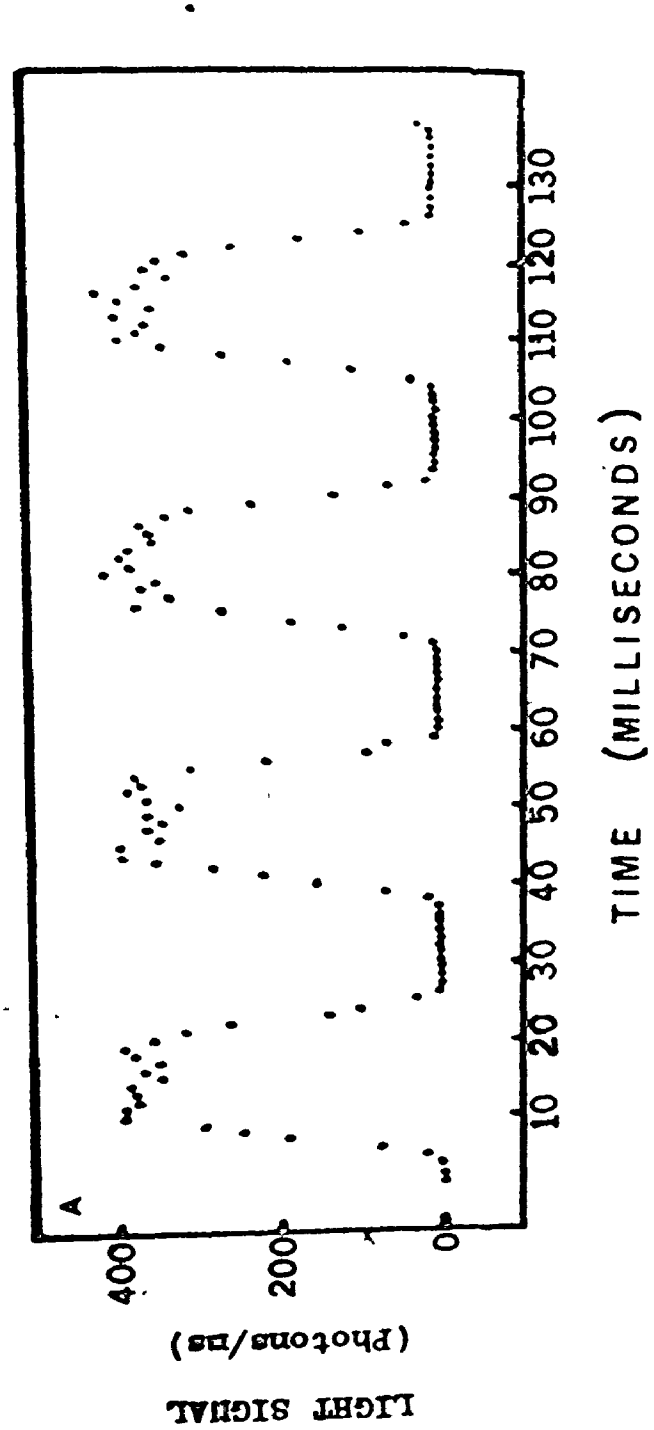

Light Signal
Photons / Second (x 10^{-5})
6
5
4
3
2
1
0
10
20
30
40
50
60
70
80
90
100
110
120
Time (Seconds)

Figure 4.5

Chopped Light Signal

(Chopping Rate = 30 Hz)


A
400
200
0
10 20 30 40 50 60 70 80 90 100 110 120 130
TIME (MILLISECONDS)
LIGHT SIGNAL
(photons/ms)

time. During these periods, meaningful data could not be collected, and so collection of the photon count had to be suppressed. This was simple to do in practice,[224] but resulted in a loss of time during which data was collected of about 33%. Furthermore, since the observation was on a "cold" vapour, no emission occurred, and any data collected when the lamp was cut off by the chopper just represented the dark count. This was essentially useless information in this case and resulted in a further 33% loss of time during which useful data could be collected. These losses were rather serious in this analysis, because the signal received was fleeting, and the normal atomic absorption mode in effect threw away two-thirds of it. Therefore, the chopping mode was unsuitable for this application and was not used.

4.4 The Reduction/Aeration Method

4.4.1 Introduction

The commonly employed flow-through cell technique was investigated. For this purpose, a Fisher model 82-731 mercury vapour atomic absorption accessory kit was purchased. Figure 4.6 shows the setup, and Figure 4.7 gives a flow diagram. Table 4.1 gives system volumes.

The apparatus was set up as recommended in the accompanying manual, and the recommended procedure [226] was evaluated, using inorganic mercury standards, both inactive and radioactive (as tracers.)

The instrument employed was a Heath model 703 atomic absorption/flame emission spectrophotometer with photon-counting readout as designed in this lab and described in the preceding section in detail. The absorption cell was attached to the top of the normal slot burner to allow accurately reproducible optical alignment in the light beam from the hollow cathode mercury lamp.

Table 4.2 indicates the saturation value of mercury vapour in the carrier gas based on the vapour pressure of mercury.

4.4.2 Drying Agents and Trapping of Mercury in the System

$CaCl_2$ was the drying agent strongly recommended in

Figure 4.6

Fisher Mercury Vapour Analyzer Accessory Kit.

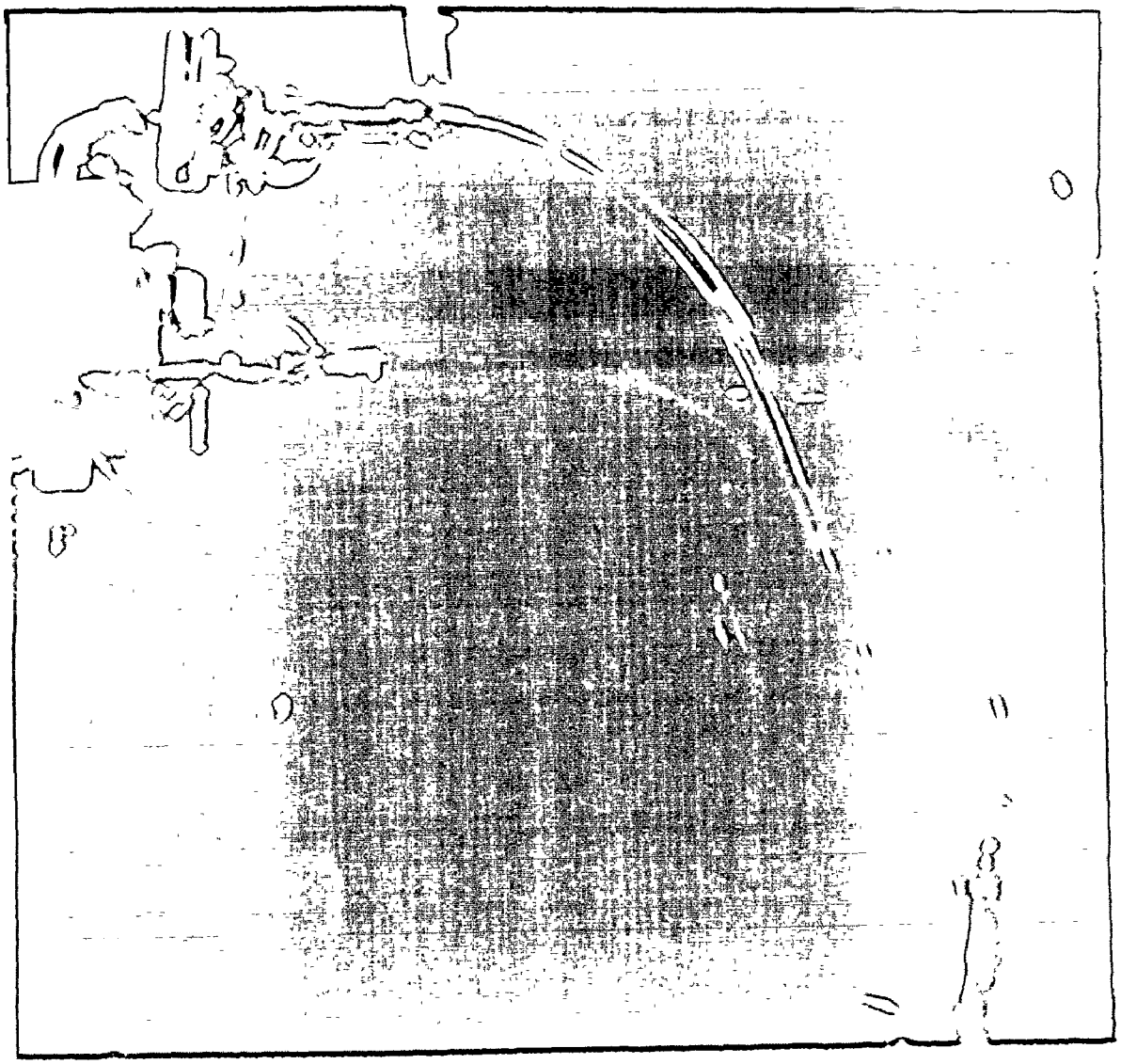


Figure 4.7

Flow Diagram of Aeration Apparatus.

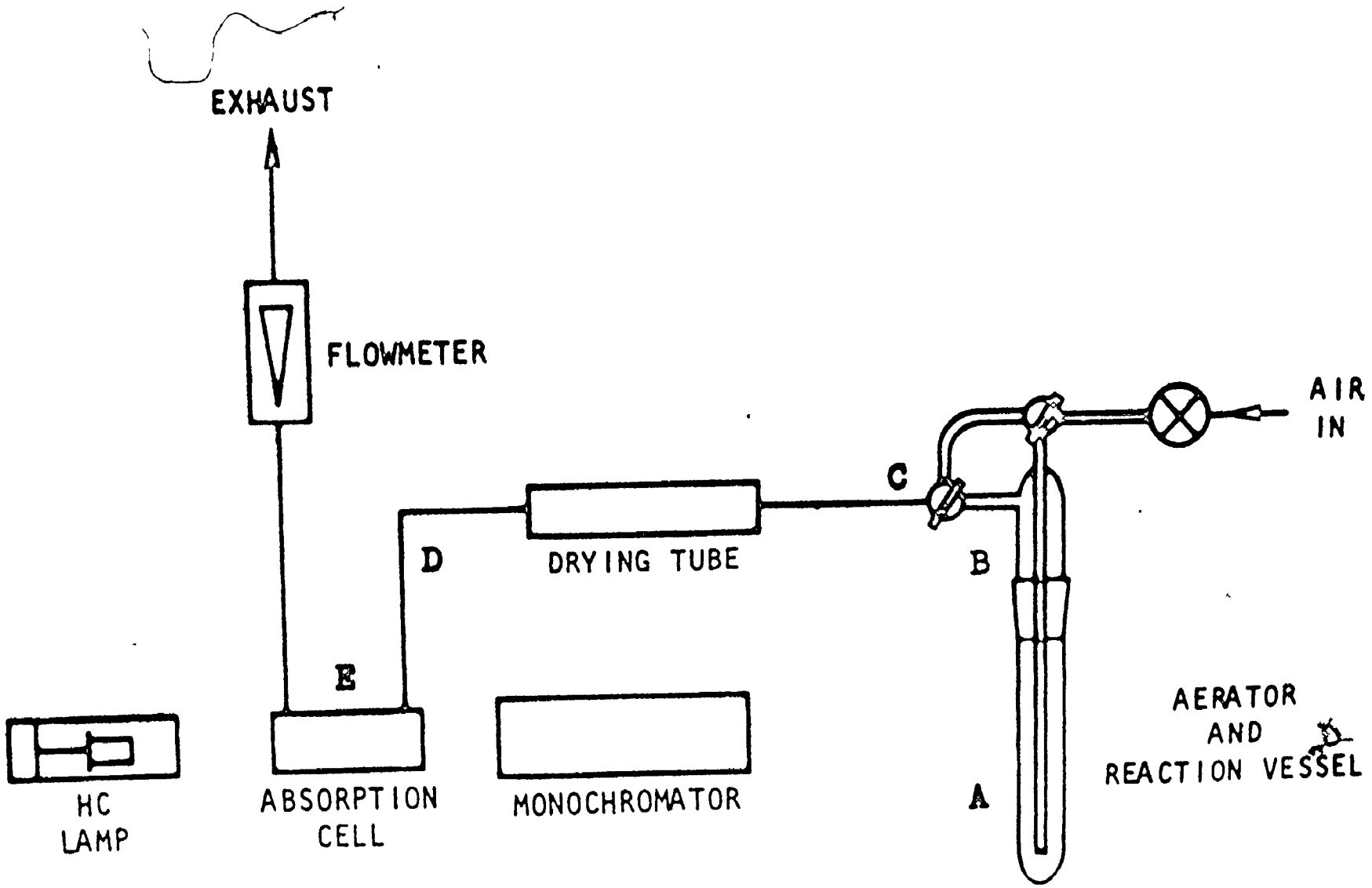

EXHAUST
FLOWMETER
AIR
IN
C
D
DRYING TUBE
B
E
HC
LAMP
ABSORPTION
CELL
MONOCHROMATOR
A
AERATOR
AND
REACTION VESSEL

Table 4.1

Volumes of Reduction/Aeration System

Figure 4.6 Label	Section	Volume
A	Reaction Tube	13.0 ml
B	Glass Tubing and Stopcock	6.5 ml
D	Plastic Tubing Connector	10.0 ml
E	Absorption Cell	29.0 ml

Table 4.2

Saturation Values of Mercury in Air

T(°C)	Hg per ml of * Saturated Carrier Gas	Maximum Amount of Hg Present in 29 ml Cell
0	2.18×10^{-9} g	63.2×10^{-9} g
10	5.57×10^{-9} g	161×10^{-9} g
18	11.1×10^{-9} g	322×10^{-9} g
20	13.2×10^{-9} g	383×10^{-9} g
22	15.5×10^{-9} g	449×10^{-9} g
24	18.3×10^{-9} g	531×10^{-9} g
26	21.5×10^{-9} g	623×10^{-9} g
30	29.5×10^{-9} g	855×10^{-9} g
40	62.4×10^{-9} g	1810×10^{-9} g
50	126×10^{-9} g	3654×10^{-9} g

* Calculated using ideal gas law based on mercury vapour pressure data from the Handbook of Chemistry and Physics (159)

the manual . Tests showed it to be extremely unsatisfactory. It was observed to absorb all the mercury from the sample, giving no absorption signal. This effect was later found to depend on how moist the $CaCl_2$ was. With reagent coming fresh from the drying oven, signals were observed which soon decreased in magnitude to negligible amplitude. Removal of the drying tube resulted in full restoration of the signal. Strong absorption of mercury by the $CaCl_2$ was confirmed in radiotracer studies and appeared to be due to the strongly acidic reaction of $CaCl_2$ on hydrolysis.

$Mg(ClO_4)_2$ was then used and found to be considerably better. Tracer mercury study again showed that the drying agent trapped several per cent of a 180×10^{-9}g standard, and that the glass wool plugs used trapped about 10%. This mercury is "exchangeable", as demonstrated by the fact that running an inactive standard through the system displaces the originally trapped radioactive mercury in part. The glass wool plugs were replaced by filter paper discs, and stuck in the glass tubing so that the air flow could not bypass them. This was found to be much more satisfactory, as the filter discs trapped only very small quantities ($\leq$ 1%).

During this experimentation, it became clear that a drying agent is not required, as water in the vapour phase was not observed to absorb at the mercury wavelength (253.7 nm). The problem was that bubbles coming to the top of a solution burst, causing the formation of an

aerosol.[37] This aerosol was carried along through the system. The larger droplets could be observed after a period of bubbling to be coating the inner walls of the tubing. These caused interference by acting as absorption sites to trap the mercury vapour. Even after they had dried out, the solid content was left. When the aerosol reached the absorption cell, absorption and scattering of the light beam resulted. Clearly, then, the aerosol must be prevented from travelling through the system. Two carefully inserted filter discs have been found to be effective for this purpose (point C, Figure 4.6.)

Using this improvement and a freshly cleaned system, an experiment was carried out to estimate mercury trapping in the system. Six radioactive mercury standards, each consisting of 29×10^{-9} g, were run. The system was then flushed with two inactive mercury standards of 103×10^{-9} g each. Data is given in Table 4.3. Approximately 15×10^{-9} g were found to be trapped in the system which could be displaced by successive samples. The experiment was repeated with 116×10^{-9} g standards, and about 10×10^{-9} g of displaceable mercury was found to be trapped in the system. This posed some question about the reliability of signals from very small amounts of mercury -- amounts less than the amount trapped in the system that can be displaced. Although the determination limit of this method is about 1×10^{-9} g as discussed in Section 4.14.2 the actual useful range for analysis may only be as low as the approximate

Table 4.3 Mercury Replaceably Trapped:

^{197}Hg Tracer Recovery, Using 29×10^{-9} g Standards

Sample	Absorbance	Activity Recovered After Absorption Cell
1)	0.051	90 %
2)	0.050	80 %
3)	0.059	88 %
4)	0.062	94 %
5)	0.062	98 %
6)	0.064	99 %

Amount missing based

on activity $\approx$ $.51 \times 29 \times 10^{-9} \approx 15 \times 10^{-9}$ g

Amount recovered by aeration of

two 103×10^{-9} g inactive mercury standards:

a) 48 % x 29 x 10^{-9} g

b) 2 % x 29 x 10^{-9} g

Total $\approx$ 15 x 10^{-9} g

level of mercury trapped! This amount may be minimized by minimizing the surface area exposed to the mercury vapour. Also, the material used is important. Based on present evidence, quartz is the best choice. Materials such as aluminum which form amalgams with mercury are obviously poor choices. Use of plastic should also be minimized. Whatever is used, it must be clean, and an experiment should be performed to estimate system trapping.

4.4.3 $SnCl_2$ Addition

An experiment was carried out to test the stability of standard solutions after addition of stannous chloride reagent. Radioactive tracer solutions were used containing 180×10^{-9} g mercury in a test tube. The test tube was placed in a 3" x 3" NaI well-type scintillation counter and the activity of the solution followed with time. Figure 4.8 shows typical experimental data. Addition of $SnCl_2$ results in an initial loss as the solution is stirred up by the addition. Slow but continuous loss follows. If the tube is shaken up, immediate losses of $\sim 10\%$ are typical. The addition must be made just before attachment to the aeration apparatus if losses are to be minimized.

4.4.4 Mercury Release from Solution, and Flow Through the Absorption Cell

The flow pattern of the mercury through the system, starting with the release from the test solution, has been

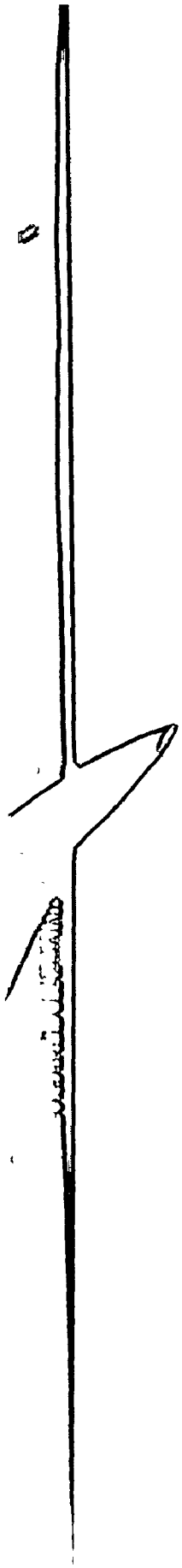
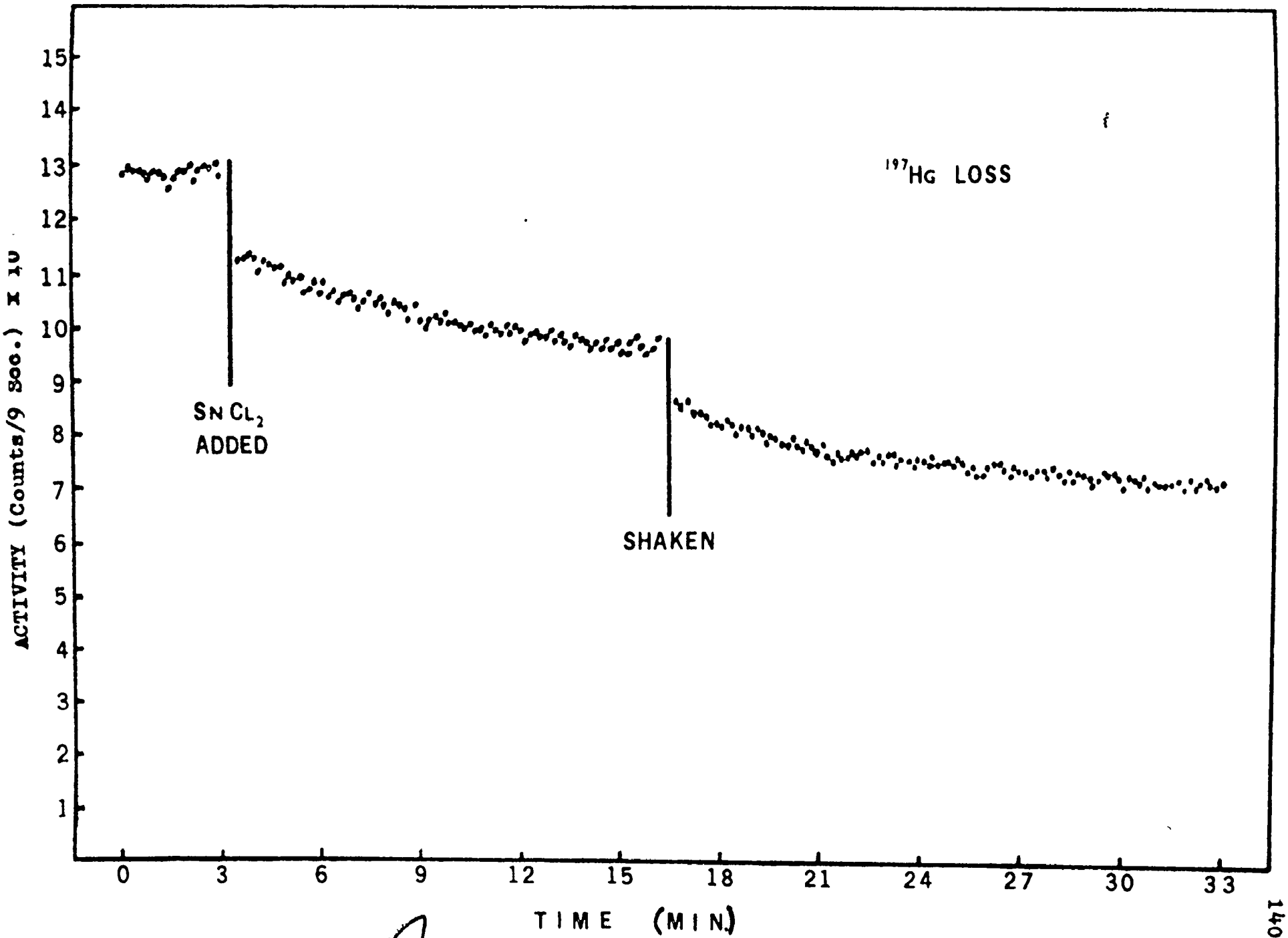


Figure 4.8

Loss of Mercury Upon Addition of $SnCl_2$.


^{197}Hg LOSS
$SnCl_2$ ADDED
SHAKEN
ACTIVITY (Counts/9 sec.) x 10
TIME (MIN.)
0
3
6
9
12
15
18
21
24
27
30
33
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

studied in order to better understand the process occurring and the possible interfering effects; especially to determine if the Hg^{o} vapour is held up in the flow-train, if its distribution is broadened, and the amount of mercury present in the cell at maximum absorbance. These measurements were facilitated by the photon counting readout technique. It made possible following the rapidly changing signal accurately and also precise measurement of timing of the flow.

Figure 4.9 (A) shows the typical release pattern from the solution. The sample was of radioactive mercury, and the reaction tube was in the NaI well counter. Loss of radioactivity from the sample was followed during reduction/aeration. From this data, Figure 4.9 (B) shows the expected mercury flow pattern through the system in terms of the amount of mercury having passed any given point plotted against time.

A typical photon counting readout (by Multi-Scaling) for one of these samples is shown in Figure 4.10 (A). Figure 4.10(B) is the integral of 4.10 (A), corrected for t_d, and gives the amount of mercury having passed a given point in the cell. From this, the amount in the cell at the point of maximum absorbance may be found. It is seen to be approximately 30% of the total amount which passes through the cell.

Figures 4.9 (B) and 4.10 (B), when scaled to reach

Figure 4.9

A) Experimental Release Pattern of Mercury.

B) Expected Mercury Flow.

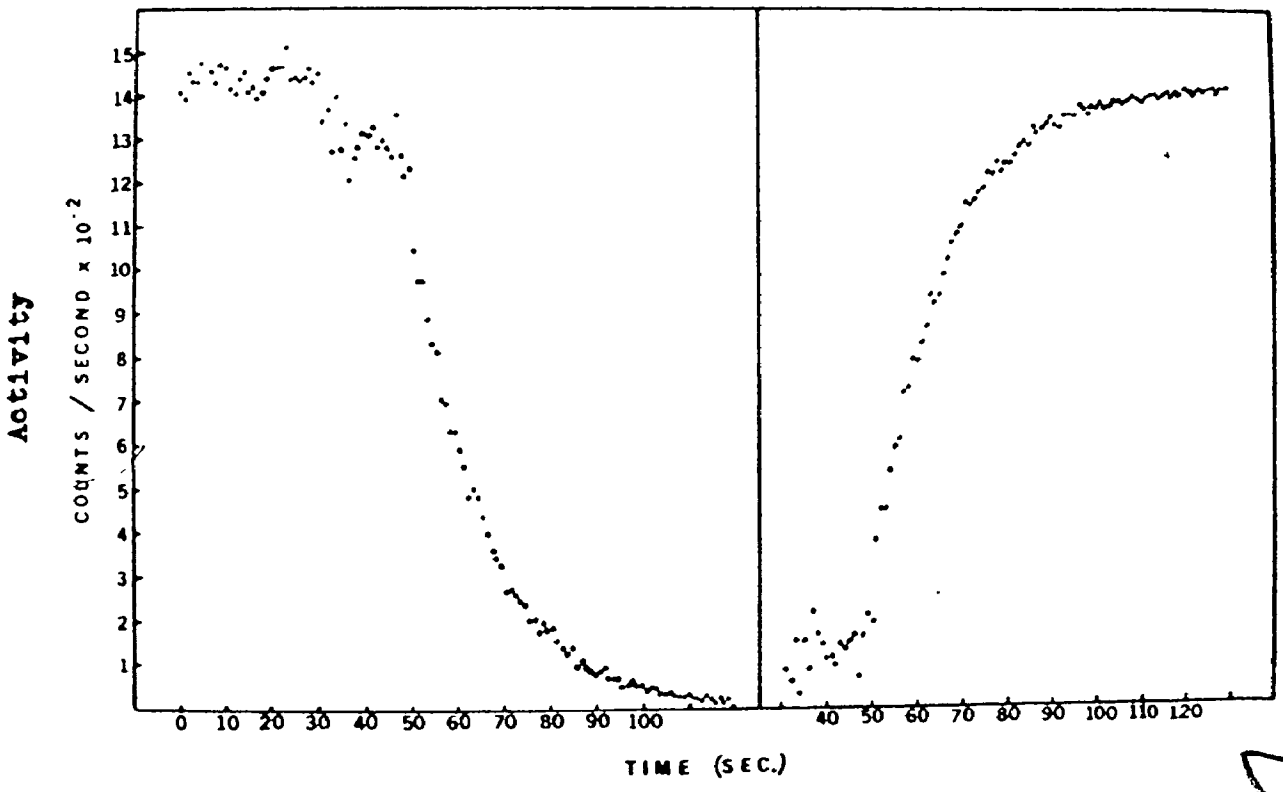
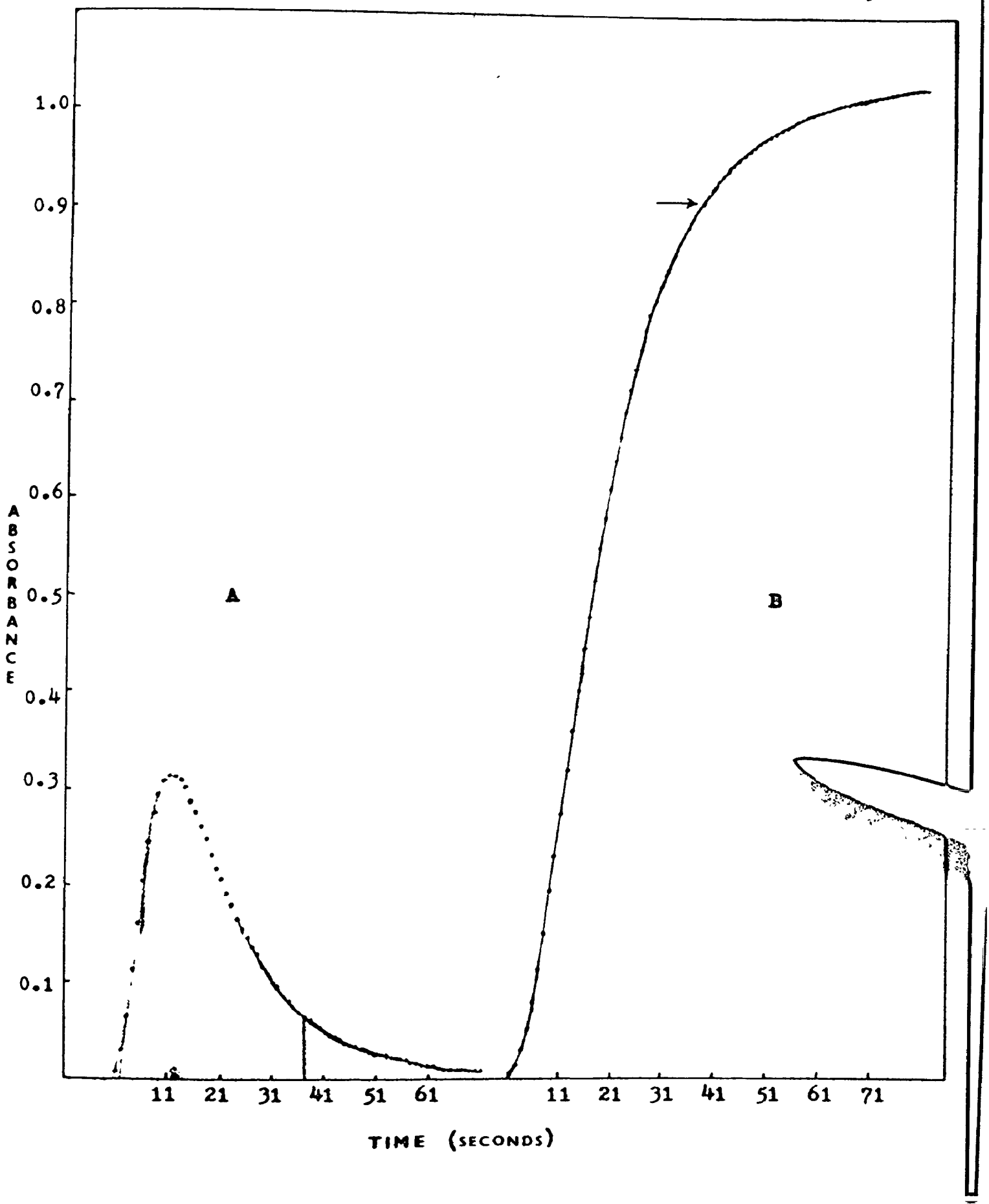

Activity
COUNTS / SECOND x 10^{-2}
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0 10 20 30 40 50 60 70 80 90 100
40 50 60 70 80 90 100 110 120
TIME (SEC.)

Figure 4.10

A) Experimental Absorbance (Compare with Fig. 4.1).

B) Observed Mercury Flow.


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
ABSORBANCE
A
B
11
21
31
41
51
61
11
21
31
41
51
61
71
TIME (SECONDS)

the same final height, are found to be superimposable. This demonstrates that the shape of the absorbance curve is exactly as to be expected from data on the rate of release from solution. Therefore the flow path does not broaden the distribution of the mercury vapour appreciably. Nor does it hold the vapour up. The latter result is deduced from the carrier gas flow rate of approximately 4 ml/sec and the volumes of the flow path (Table 4.1), compared with the actual timing of the flow.

4.4.5 Peak Height and Peak Area

In Section 4.2, a theory has been presented which shows the relationships between peak area and the mercury content of a sample and also suggests how the peak height is related by the pattern of release from solution.

Table 4.4 shows some data from experiments investigating the relationship presented in the theory:

$$A_{total} = (\text{constant}) \frac{N_{total}}{\text{flow rate}}$$

The relationship is borne out very well.

The effect of a large number of variables on the release pattern has been investigated. Small variations in the volume and temperature of the test solution have no significant effect on A_{total} or $A_{maximum}$. The same is true for small variations in the concentration of the Sn(+2) reductant solution added. On the other hand,

Table 4.4

Effect of Flow Rate Variation

Flow Pate	A_{max}	A_{total}	A_{total} x Flow Rate
0.40 SCFH	0.187	6.17	2.47
0.43 "	0.181	5.63	2.42
0.46 "	0.211	5.46	2.51
0.46 "	0.196	5.31	2.45
0.50 "	0.180	5.14	2.57
0.55 "	0.193	4.76	2.62
0.60 "	0.200	4.31	2.58
	mean = 0.193		mean = 2.52
	1σ = 5.7%		1σ = 3.0%

Table 4.5

Effect of NH_2OH HCℓ

Amount Added of a 5g/10mℓ Solution	A_{max}	A_{total}	Number of Channels
None (N=3)	100%	100%	35
10 μℓ	97%	98%	34
20 μℓ	98%	98%	34
50 μℓ	85%	83%	32
100 μℓ	60%	65%	36
200 μℓ	34%	36%	35
300 μℓ	13%	11%	28

reagents, added for various purposes during the chemical processing (eg. ashing) of a sample usually have a very strong effect on the release pattern. These effects should always be investigated for a given procedure for each reagent added, and for the combination of all reagents. Several examples are shown in Tables 4.5, 4.6. 4.7. Table 4.5 shows the effect of addition of hydroxyl-ammine hydrochloride. Table 4.6 shows the effect of cysteine-hydrochloride. This compound is used in some mercury analysis procedures, and also may be taken to represent a possible component of a partially ashed sample. Table 4.7 shows the effect of addition of an anti-foaming agent such as 1-octanol. Data are for a constant carrier gas flow rate. The column "Number of Channels" represents the width of the absorbance peak (Figure 4.10 (A)) and so gives an estimate of how much spreading of the release pattern from the test solution is occurring. Figure 4.11 gives three typical experimental absorbance curves which illustrate what is happening. Figure 4.11 (C) shows a typical effect of a partially ashed biological sample. The effects of biological samples are reported in Section 4.7.

Table 4.6

Effect of Cysteine-Hydrochloride Addition

Amount of 1% Solution Added	A_{max}	A_{total}	Number of Channels
none	0.180	5.13	41
20 µl	0.186	5.32	40
50 µl	0.168	4.88	42
100 µl	0.120	5.32	62
200 µl	0.064	4.91	104

mean = 5.11
1σ = 4.2%

Table 4.7

Effect of 1-Octanol

Amount Added (and method)	A_{max}	A_{total}	Number of Channels
None (N=8)	100%	100%	37
5 µl	59%	96%	61
10 µl	60%	94%	58
"One drop" (as a ring)	52%	95%	62
"One drop" (as a ring - didn't reach the solution)	88%	100%	41

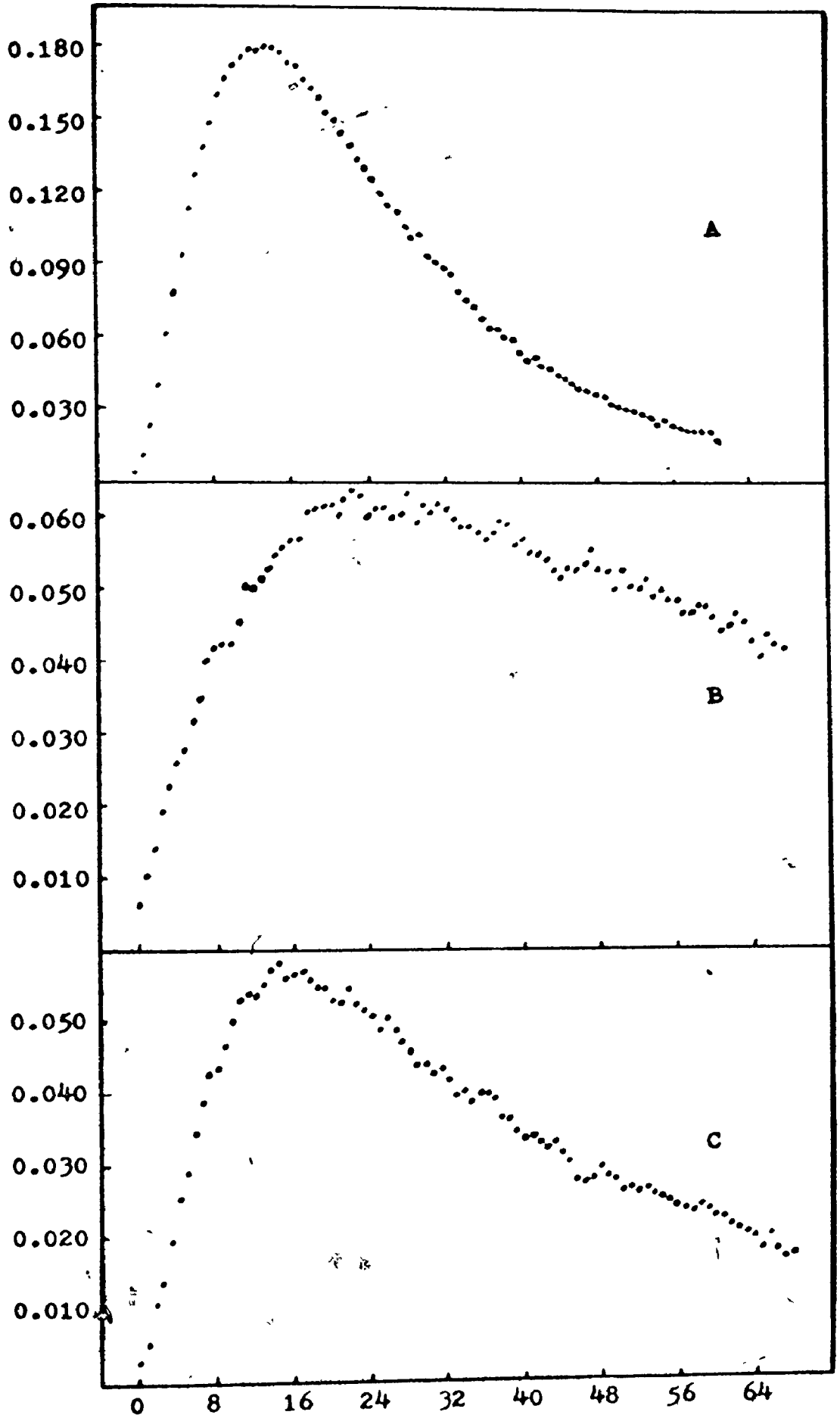
Figure 4.11

Absorbance Curves:

A) 76×10^{-9} g Hg^{+2} standard.

B) same as A), with 0.2 ml of 1% cysteine hydrochloride added. (as in Table 4.6).

C) fish sample by partial digestion method of Skare. (Section 4.7.2)


0.180
0.150
0.120
0.090
0.060
0.030
A
0.060
0.050
0.040
0.030
0.020
0.010
B
0.050
0.040
0.030
0.020
0.010
C
ABSORBANCE
0
8
16
24
32
40
48
56
64
TIME (Seconds)

4.5 Amalgamation Collection Followed By Volatilization By Heating

4.5.1 Introduction

Mercury is well known for its unique behaviour in readily forming amalgams with many elements. This property can be made use of in separating mercury from other elements. At first glance, this appears to be an ideal method for isolating traces of mercury from all other possibly-interfering components of a sample. The mercury can be released from the amalgam by applying heat to drive it out. This results in an atomic vapour, which can then be quantitated by measurement of atomic absorption. The sequence of steps involved should be specific for mercury. Several techniques of mercury separation by amalgamation have been investigated, and the results are presented in the following sections.

4.5.2. Collection on Copper Wire and on Silver-Plated Wire

An attractive method has been reported by Brander-berger and Bader.[227, 228, 229] They were able to collect mercury from solution on a coil of copper wire by applying a small negative voltage. The mercury could then be released into an absorption cell by passing a current through the wire. This method was for application to urine samples. For biological samples in general, it is necessary to release the

mercury from the matrix before it can be collected, and so some form of ashing is required. The technique of mercury collection by amalgamation was therefore studied by using mixtures which simulate diluted ashing solutions. Radioactive mercury tracer was used to evaluate various procedures.

Conditions of the experiment were varied as to the kind and amounts of various acids used to simulate the ashing mixture, electrolysis time, voltage and current density, size of the copper electrode (in the form of a coil of wire), temperature and dilution of the sample solution, and pH. Nitric, sulphuric and hydrochloric acids were used in many combinations. In a later series of experiments, biological samples were ashed with tracer ^{197}Hg added. Some representative data is given in Table 4.8. Neither quantitative nor consistent recoveries of the radioactive mercury tracer were ever attained. Studies were done using different amounts of Hg in the range of 2×10^{-9} g up to 1×10^{-6} g.

During this time, the high volatility of mercury in dilute solutions was observed. Even strongly acidic solutions were found to lose considerable amounts of mercury when they were gently evaporated to a smaller volume in open containers. Neutral or basic solutions were especially susceptible to loss. Any solutions going dry lost all of their tracer mercury.

Table 4.8 Recovery of Mercury by Electrolysis

Solution Conditions (total volume ~ 150 ml)	Electrolysis Time	Activity Recovery
10 ml HCl	40 min.	58 %
	1 hr. 40 min.	79 %
4 ml H_2SO_4	1½ hr.	29 %
5 ml H_2SO_4 + 5 ml HNO_3	1 3/4 hr.	88 %
5 ml H_2SO_4 + 4 ml HNO_3	10 hr.	89 %
5 ml each, H_2SO_4, HNO_3, HCl	35 min.	25 %
4 ml H_2SO_4 + 4 ml HNO_3 + 5 ml NH_4OH	4 hr.	70 %

In addition to these problems, it was found that the ^{197}Hg which had been collected on the copper wire could not be driven off quantitatively by ohmic heating. Some activity remained in every case. Several examples of retention found were 80%, 2%, 28% and 14% left after applying a 6 volt potential across the ends of the wire.

A silver plating was put on the copper wire in a further set of experiments. The silver plating technique is described in Reference (230). All of the ^{197}Hg collected in the electrolysis was found to be in the silver layer, but again both the collection and the release on heating were found to be non-quantitative and non-reproducible.

4.5.3 Copper Column Amalgamation Collection

At this point, the collection step on the copper wire was abandoned, since it obviously did not work satisfactorily. A new technique was initiated which involved passing the mercury-containing sample over a column made of copper powder. No mercury was found to pass through the column, and so collection was quantitative, providing that the column was carefully packed and that the flow rate was not too high. On a properly prepared column, all of the ^{197}Hg was found to be trapped at the top of the column.

The column was dried in an oven at 90°C. Typically 5 - 10% of the activity was lost. This problem was solved by adding an additional scoop of copper powder to the column after the mercury was trapped on it and running a small amount of dilute sulphuric acid through to clean it up.

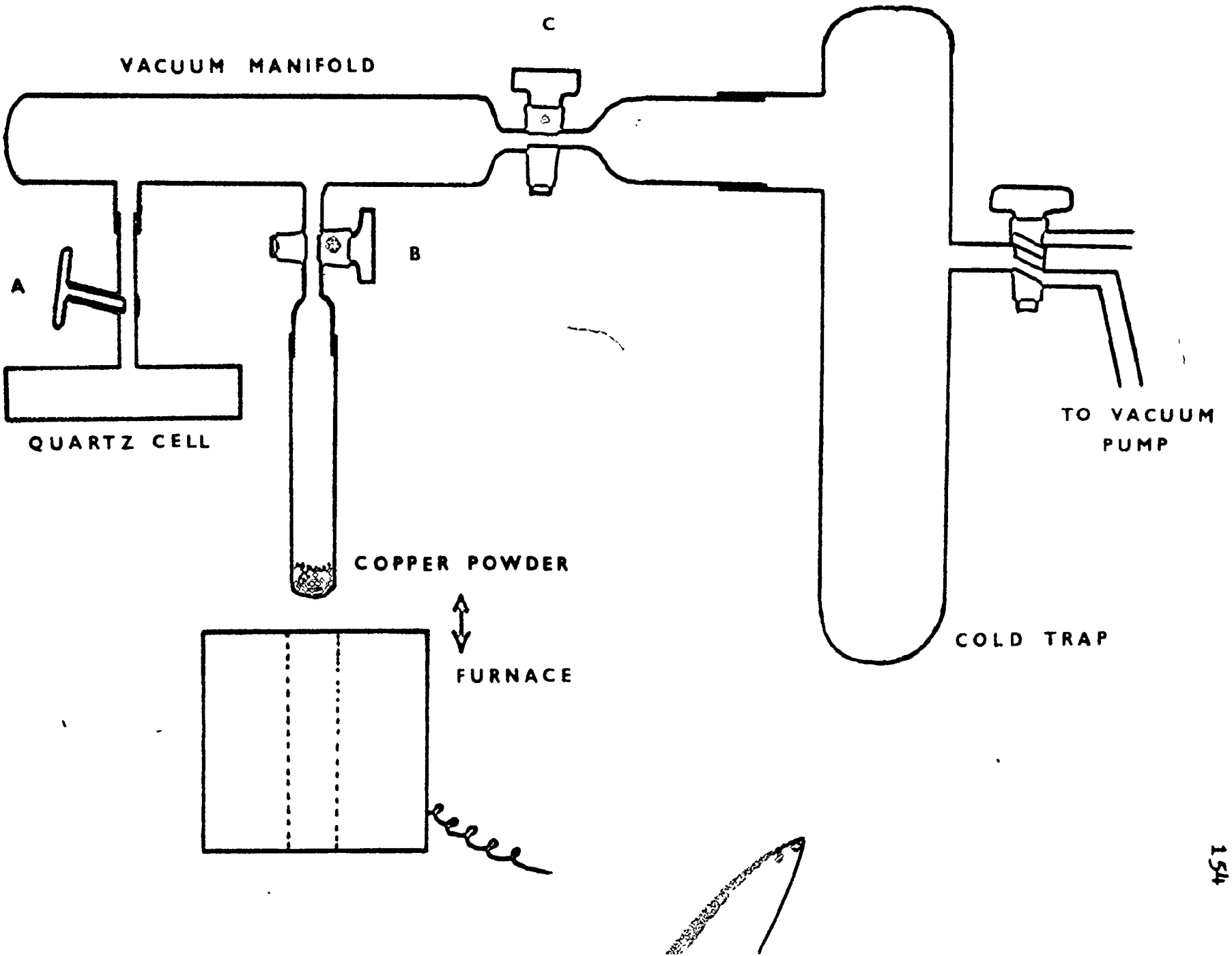
4.6 Static Vapour Absorption Measurement

Utilizing the method of mercury collection on copper columns described above, the mercury can then be released by heating, and collected in an absorption cell. The apparatus for this procedure is shown in Figure 4.12. A 10 cm long cylindrical Suprasil quartz cell with vacuum-tight fused windows (Hellma Ltd., 528 Huron Street, Toronto, Ont.) was fitted with a "vacuum" teflon needle valve (Fisher Scientific) and a ground glass joint for connection to a vacuum manifold. The volume of the cell and graded seal arm was 31.9 ml. From the vapour pressure of mercury, Table 4.2 it is readily calculated what amount of mercury as vapour can be contained in the cell at various temperatures. At room temperature only several hundred nanograms can exist in the vapour state in the cell, but as the temperature is raised somewhat, this becomes much greater.

The procedure for analysis is as follows. The copper column containing the sample's mercury is transferred into a test tube which is placed on the vacuum line with the quartz cell as shown. Both of these containers are evacuated. No loss of ^{197}Hg tracer was observed from the copper powder during evacuation. Stopcock C was then closed and the furnace (Brinkmann Canada Ltd.) was raised around the copper powder, which was brought to a red heat. A heating tape

Figure 4.12

Static Vapour Apparatus.


C
VACUUM MANIFOLD
A
B
QUARTZ CELL
TO VACUUM
PUMP
COPPER POWDER
FURNACE
COLD TRAP

surrounding the vacuum manifold warmed it to 100°C. The quartz cell was cooled by blowing vapour from liquid nitrogen over it in order to collect the mercury. Stopcock A was then closed and the furnace lowered. The cell was removed and its absorption measured on an atomic absorption instrument.

Unfortunately, standard curves gave very erratic results with this procedure. Several serious interferences were observed to occur:

(i) If any material was trapped on the copper column which was volatile at the temperature used to drive out the mercury, this was transferred to the cell and caused interference. Several times the copper columns were found to be damp, and water droplets condensed on the cell walls and windows. Organic material caught on the column would also cause interference.

(ii) It was found to be difficult to drive all of the mercury from the copper powder. In quite a few instances significant amounts of ^{197}Hg tracer remained. This effect was observed previously in the experiments with the copper wire collectors.

(iii) As the test tube containing the copper powder was evacuated, a very fine copper dust spread into the vacuum manifold. Great care must be taken to prevent this, as it will obviously trap the released mercury again.

(iv) During the absorption measurement, the quartz cell was heated by wrapping it in heating tape to ensure that all of the mercury would be in the vapour state as indicated by the data in Table 4.2. The absorption was observed to increase with increasing temperature and not to stabilize as expected!

4.7 Wet Ashing Techniques

4.7.1 Introduction

Section 4.4 has given a critical evaluation of the performance of the reduction-aeration technique, and Section 4.5 has presented an evaluation of amalgamation collection methods. These procedures presume the samples to consist of a solution in which the mercury is present in an inorganic form, as Hg(+2). Clearly, the mercury present in a biological matrix must be extricated from that matrix before the analysis can be carried out. Two general approaches, partial and complete digestion, will be considered.

4.7.2 Partial Digestion Methods

"Partial Digestion" techniques have become popular recently, although they have mainly been applied to biological fluids (blood and urine.) Since the matrix is not completely destroyed, this method relies on a complete extraction of the mercury from the matrix and breaking any carbon-mercury bonding to give Hg(+2) in solution. The advantage of this type of digestion is that it is not necessary to use high temperatures, and so loss of mercury by volatilization is less likely than for a complete ashing technique.

Various commonly employed partial digestion methods

have been evaluated by using fish samples labelled *in vivo* with radioactive mercury. The fish-labelling experiment is described in Chapter 2. The radiotracer was administered as methyl-mercury chloride. A period of at least one month was allowed for natural incorporation of the tracer. The amount taken up by the fish, based on the tracer activity, resulted in a concentration in the order of 60 parts per billion.

The methods used have been described in detail in the literature. They have been studied with regard to their ability to extract the mercury content from the sample matrix completely without loss. Initially, the possibility of loss due to volatilization of extracted mercury from open containers was investigated. Each method mentioned in this section was checked first by use of inorganic Hg(+2) radiotracer and then portions of *in vivo* labelled fish. Significant loss was noted only in one case -- that of pre-treatment of the inorganic tracer in alkaline solution. This treatment is normally recommended for homogenization of fish, and in the case of the fish, loss was not observed.

The methods studied are outlined briefly in Table 4.9. For full details, the publications should be consulted. The published procedures were applied to samples of *in vivo* labelled fish. After the prescribed treatment, the samples were filtered, and the amount of

Table 4.9

Partial Digestion Methods

A) Digest Solution : 150 mℓ 6% $KMnO_4$ + 20 mℓ conc. H_2SO_4
- to ≤ 1g sample, add 1.5 mℓ of digest solution, and allow to stand overnight
- add 0.3 mℓ 20% NH_2OH HCℓ

B) Same as A), but heat at 60°C overnight

C) - to a 1g sample, add 2.0 mℓ HCℓ + 100 mg $NaNO_3$, and allow to stand overnight
- add 10 mℓ H_2O and filter

D) Digest Solution : $HClO_4/HNO_3$ = 5/1
- to a 0.2g sample, add 1.5 mℓ digest solution and place in a 70°C → 75°C water bath overnight
- cool, add 1 mℓ H_2O, add 5 drops NH_2OH HCℓ

E) - to a 0.1 g sample, add 0.4 ml H_2SO_4. Heat to 50°C, cool and add cautiously 3 ml of 6% $KMnO_4$.
- heat again and allow to cool by standing. Repeat this step two more times.
- cool and add 20% NH_2OH HCl

A) typical of (231, 232, 233, 234, 235)
B) Uthe et al[236]
C) Toribara and Shields[237]
D) Skare[238]
E) Jacobs et al[239, 240]

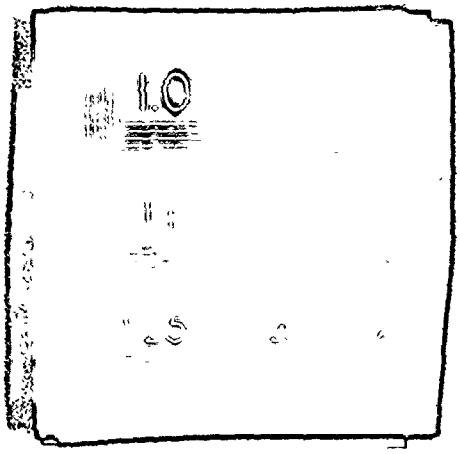
tracer mercury remaining in the residue was determined. The amount of tracer extracted into the filtrate was also measured. Results are given in Table 4.10.

In the case of Jacob's $KMnO_4/H_2SO_4$ [239, 240] digestion treatment and Skare's $HClO_4/HNO_3$ [238] digestion treatment, the filtrates from the above experiments were used to complete the method by reduction-aeration and cold vapour atomic absorption. In the former case, 43% and 37% of the tracer only could be aerated from two aliquots of the filtrate. The rest of the tracer remained in solution. In the latter method (Skare), 58% and 75% were aerated from two aliquots of filtrate.

This effect is the same as that observed for the addition of hydroxyl-ammine hydrochloride ($NH_2OH\ HCl$), as in Table 4.5. Note that many procedures call for its addition. This is not the cause of the hold-back effect in this experiment, however. Previous experiments had proven that if the amounts recommended in the published procedures were added, less than 5% of the mercury content of partially ashed samples could be aerated. Thus, in this experiment, minimal amounts were added - just enough to clear up the MnO_2 precipitate. The hold-back effect must, therefore, be attributed to a matrix interference from some component of the partially ashed biological sample.

3 3

OF/DE



In addition, sample absorption peaks were observed to be considerably drawn out in time relative to standards, even though the standards were prepared using the same reagents that were added to the samples. An example has been given in Figure 4.11 (C). The effect is similar to that observed for addition of cysteine hydrochloride (Table 4.6 and Figure 4.11 (B)). Again, this is an effect which must be attributed to interference caused by the partially ashed matrix. In this case, the interference may be satisfactorily corrected for by using peak area, rather than peak height.

4.7.3 Complete Digestion

The partial ashing experiments described in the previous section, although disappointing, revealed a trend. The performance of the method improved as the method gave more complete ashing of the sample (as observed visually.) This suggests that a complete ashing technique is mandatory. However, high temperatures must be applied to the sample,

Table 4.10

Partial Digestion of *In Vivo* Labelled Fish

Treatment	Tracer Left With Sample	Comments
A)	98.4%	
B)	90.3%	
C)	13%	- apparent 35% volatilization loss
D)	(15% in alkaline residue)	- 58%→75% aerated
E)	42%	- 43%,37% aerated

and this may result in loss of mercury by volatilization. During the course of this research, it has been discovered that an ashing mixture consisting of concentrated sulphuric acid which is present in at least two-fold excess over all other components possesses the ability to hold traces of mercury in solution, even at temperatures in excess of 270°C.

Considerable success has been found in employing the following method. A weighed portion of sample was placed in a 250 ml round-bottomed flask. The flask was connected to an efficient reflux condenser (Graham - type was used.) Ground glass joints were wet with a small amount of concentrated sulphuric acid. All reagents were added through the top of the condenser. Ten millilitres of concentrated sulphuric acid were added and the sample allowed to soak. Heat was then applied with a burner to begin charring the sample, and three millilitres of fuming nitric acid (90%) were added. Vigorous heating was used to completely ash the sample. Additional nitric acid was added if required. Considerable amounts of oxides of nitrogen were evolved and escaped through the top of the condenser. After complete ashing (clear solution) the digested sample was left to cool.

Volumes may be adjusted for amount of sample taken. The important factor appears to be that an excess of sul-

phuric acid over other components be maintained until after complete ashing and just before analysis. In this respect, it is important not to add water to the mixture before ashing.

Table 4.11 gives data for tracer experiments to investigate losses during this wet-ashing procedure using $CH_3{}^{203}HgCl$ samples of about 4×10^{-9} g. After ashing, carrier mercury was added and precipitated as mercuric sulphide, filtered and counted as described in Section 3.11. These samples were compared with the activity of two standard tracer aliquots taken at the same time. The latter were counted immediately, carrier was added with $KMnO_4$ to promote exchange, and precipitated as mercuric sulphide to compare with the wet ashing tracer to determine loss. No loss was observed.

A second similar experiment was carried out with in vivo labelled fish. The activities of these samples were determined before and after ashing. Table 4.12 gives the data for this experiment. The mercuric sulphide precipitate recovered is expected to give both attenuation of the gamma radiation and a counting geometry effect as described in Sections 3.5 and 3.4. From Table 3.7, the expected geometry effect of counting the precipitate in position 4 relative to the initial sample of fish counted in position 1 (Figure 3.6) is a reduction in observed activity of about 3%. Table 3.8 gives gamma ray attenuation data which shows that a further reduction of about 4% will be observed. Thus, when estimating the wet-ashing loss, approximately a 7% reduction in activity must be first accounted for by these

Table 4.11

^{203}Hg LOSSES ON WET ASHING - No Carrier Added

(Mercury added as C_6H_5HgCl)

Order Aliquot Taken	Specific Activity*	Mean
2) Standard	580.9	593
5) Standard	605.2	
1) Sample	579.4	600
3) Sample	599.7	
4) Sample	619.6	

* counts per 200 seconds per mg yield, background corrected

two effects. Taking this into consideration, it is clear that no significant loss has been observed during the suggested wet ashing procedure.

Table 4.12

^{203}Hg LOSSES ON WET ASHING IN VIVO LABELLED FISH

(CH_3HgCl used for labelling - see Section 2.2)

Sample	Weight	Activity Isolated After Wet Ashing * (as % of initial)
dried fish head	32 mg	92 %
fresh body	132 mg	93 %
fresh body	128 mg	93 %

* 7% reduction in measured activity is due to attenuation by precipitate, and different counting geometry.

4.8 Procedure Recommended, and Checkout

The procedure followed is outlined in point form in Table 4.13. The discussion should be consulted for details, especially Sections 4.12.3, 4.13 and 4.14. Section 4.7.3 should also be referred to.

Samples of the in vivo labelled fish (Section 2.2) were used in experiments to check the full procedure. Again no loss on ashing three fish samples was observed, based on counting a 1.0 ml aliquot of the ashed sample solution relative to the initial tracer activity.

During the reduction/aeration step, it was observed that employing a solution of 20% $SnCl_2$ in concentrated HCl in the reduction step resulted in incomplete aeration of the mercury. However, if a 20% solution of Sn^{+2} in H_2SO_4 diluted 1:4 with water was used, quantitative aeration was achieved.

The procedure was then applied to samples of Bowen's kale to further test its reliability. Previous use of the kale had been made to check out the neutron activation procedure, as described in Section 3.12. In the present case, considerable difficulty was encountered. The kale has a 4% calcium content, and calcium sulphate is relatively insoluble. Consequently, at the end of the wet ashing procedure, a large amount of finely divided white precipitate formed. Dilution of the samples to a volume of 25 ml was insufficient to dissolve all of the precipitate, and it was clear from

Table 4.13

Outline Of Atomic Absorption Procedure

1) Weigh sample into ashing flask and connect to reflux condenser. Take a duplicate to determine water content.

2) Add concentrated sulphuric acid through the top of the condenser. The amount should be sufficient to ensure that twice as much H_2SO_4 as other components is present.

3) Ash the sample by heating vigorously and adding fuming nitric acid (90%) as required.

4) Allow the ashed sample to cool.

5) Rinse the sample into a 25 ml volumetric flask* with distilled water, and dilute to volume.

6) Take 5 ml aliquots* for determination of mercury.

7) Add 0.3 ml of reducing solution (made by dissolving 1 g $SnCl_2$ in 5 ml of H_2SO_4 : H_2O = 1 : 4 solution) and immediately attach to the aeration apparatus.

8) Determine the absorbance peak area.

* Use of larger volumes would be very desirable.
(see next page)

the results that the precipitate had trapped significant quantities of mercury. Results are given in Table 4.14. The remaining precipitate from sample 2) was collected and dissolved, and was found to contain 28 x 10^{-9} g of mercury.

With the equipment used, it was not feasible to dilute the samples further in order to dissolve all of the precipitate. The reason for this is that the aeration setup allowed a maximum sample size of 5 ml to be used. This limitation results in the mercury content of a 5 ml aliquot approaching undesirably close to the detection limit if the sample has been diluted to a great extent.

It was found that the precipitate would recrystallize if the last 5 ml of the volumetric were made up with 2 M HCl, after standing for several days. Results could also be obtained by analyzing the precipitate separately. The data given in Table 4.15 show that carefully done analyses by the above two approaches give results in excellent agreement with the value quoted by Bowen (accepted mean value = 166 parts per billion).

The problem of precipitation encountered here can be anticipated for certain types of samples, for example: minerals and metals, especially those containing lead or barium; and biological samples of high calcium content such as bone or teeth. In such cases it would be very desirable to have an apparatus which could accomodate larger samples.

Table 4.14 Kale Analysis

Weight (Wet) **	Mercury Content (Based on dry weight) **
1) 783 mg	128 ppb
	141 ppb *
2) 979 mg	99 ppb
	111 ppb *

* Aliquot was tested at a later date. Increase in concentration indicates recrystallization of precipitate to some extent, releasing some of the trapped mercury.

Table 4.15 Mercury In Kale

Weight (Wet) **	Mercury Content (Based on dry weight) **
783 mg	165 ppb
763 mg	168 ppb

** Water content of kale was 5.25%.

In addition, in some cases it would be necessary to use a different digestion solution; one which did not contain sulphuric acid.

DISCUSSION

4.9 Photon Counting and Normal Readout

The photon counting readout described in Section 4.3 has been utilized throughout the experimental work presented in this chapter. Its use has greatly facilitated the investigation of the reduction/aeration method with the flow-through absorption cell. It is clear from the data presented that the absorbance peak area must be used, rather than peak height. With a strip chart recorder, peak area is very difficult to estimate with sufficient accuracy, even though the recording pen should be able to follow the signal. Thomas et al (269) mention the use of a digital integrator in a pyrolytic method which may be applicable to this case.

Another possibility is to use a closed system carrier gas cycling apparatus similar to that of Hatch and Ott (218). The carrier gas is cycled through the absorption cell and then back through the sample in a continuous flow until the mercury is aerated and absorption has reached a maximum. The normal readout system is ideally suited to make measurements in this case, since a final signal level is slowly approached. This method would seem to require considerably more investigative work, however, in order to confirm its reliability. The amount of mercury actually measured relative to the total should be found, and sources of loss of mercury in the system must be studied.

4.10 Reduction/Aeration Method

The reduction/aeration method has been carefully investigated as to performance. Many interesting and pertinent aspects of this technique have been delineated through the use of tracer mercury and a photon counting readout.

Trapping of mercury in the system, and especially in drying agents, has been discovered. The mercury trapped in the system is displaced by successive samples, but not readily by simply flushing the system with carrier gas. This suggests that samples containing mercury in amounts less than that which is trapped in the system may give absorption signals which have larger random error than would be normally expected due to statistical fluctuations in displacement of the adsorbed mercury.

The use of drying agents (and glass wool!) must be minimized. The application of a simple filter plug to stop the aerosol (formed by bubbling gas through the sample) from spreading through the system is adequate.

Great care must be taken in adding Sn(+2) reagent to the sample. Section 4.4.3 demonstrates that immediate and continued volatilization of mercury occurs.

The flow pattern of mercury through the system is approximately as expected, based only on its release from the sample solution, as described in Section 4.2. The actual

flow pattern, Figure 4.10A has been modified slightly by immediate release of mercury from the solution upon addition of Sn(+2) reagent. This results in mercury vapour filling the air space above the sample in the short interval before aeration begins. The first part of the flow pattern is more linear than predicted because of this. Also, mixing and diffusion result in a more rounded absorbance peak. Since the release pattern from solution essentially determines the absorbance peak shape, anything affecting the release of the mercury will directly affect the absorbance peak height. Many things have been found to influence the release, including the ashed sample matrix. Consequently, the measurement of peak height must not be used, since it is not reproducibly related to the amount of mercury in the sample.

Interference in the analysis by holding some or all of the mercury in solution may occur. The effect of the presence of a complexing agent such as hydroxyl-ammine hydrochloride has been demonstrated (Table 4.5.) Other researchers have found similar interference for iodine. (57, 88, 241, 242) Any such complexing agent must be suspect.

Another possibility of interference is from the presence of an easily reduced species such as Cu, Ag, Au, Se, etc. Not only is there interference by reaction with the Sn(+2) reagent, but also by coprecipitation of mercury when a precipitate is formed. (88, 91, 243) The presence

of strong oxidants (eg. HNO_3) may also cause interference by attacking the Sn(+2) reagent.

Finally, compounds which can be aerated with the mercury and which cause broadband-absorption in the ultra violet region of the spectrum result in spurious absorption. This causes results to be too high. Molecular absorption has been observed for organic compounds,[101, 244, 245, 246, 247, 248, 249] and also for many other compounds, including oxides of nitrogen,[244, 250] hydrochloric acid,[251] and sulphur dioxide.[244] Furthermore, Saha and Lee[252] have observed such interference from fat remaining in the digest after wet oxidation of fish samples. A number of ingenious methods have been used to eliminate these interferences; in the latter case the fat was removed with a chloroform extraction. More generally, a correction can be applied. Ballard et al[245] took a second reading on a sample in a Beckman DU spectrophotometer; Teffus et al[253] used gold foil, and Windham[251] used $PdCl_2$, to collect the mercury vapour and so confirm that it was mercury causing the absorption. A more elegant method of background compensation has been proposed by various authors[246, 247, 248, 249, 254] by using a deuterium arc lamp. Manning[255] has found that if the beams are not adequately aligned, only partial compensation may be achieved. This problem can be circumvented with a true two-channel instrument by using a non-absorbing lamp emission line. The best and most elegant solution to this problem, however, is the use of a Zeeman-modulated source.[256]

4.11 Amalgamation Collection

Despite the promise of the amalgamation separation technique, it has not been found to give good performance with small traces of mercury. One publication describes the use of this method.(257) The data presented show very poor precision, as might be expected from the experience gained here. It is interesting to note that even electrolysis with larger quantities of mercury in solution has also been found not to give quantitative yields of the mercury.(64, 194)

Percolation of the sample solution through a small column packed with copper powder was discovered to be effective in collecting the mercury. The drawback of this method was that it was very difficult to wash all of the solution out of the column again in order to separate possible interferants. Collection of organic products of the matrix decomposition in particular can cause broad band absorption.(258, 259)

The efficiency of collection of an amalgamator may decrease in time. Surface effects such as oxidation or formation of a film of organics(259) and channelling so that the mercury can pass through without interaction are possibilities.

Furthermore, it was found to be very difficult to

drive off all of the mercury that had been amalgamated. Vaughn and McCarthy (185) found similar problems with platinum foil. "Ageing" effects may be important. Penetration into the interior by diffusion and formation of a surface layer (eg. by oxidation) would retard release. Penetration may be especially important for a thick amalgamator and lead to a memory effect if it is re-used. (260) Application of higher temperature ($\simeq 800^{\circ}C$) could improve the release, but it may lead to other problems. (259) If the mercury is driven off at very high temperature, oxidation is likely to occur, thus defeating the atomic absorption measurement. Amalgamators such as silver may distil to a cooler part of the apparatus and then trap the mercury vapour again. Spreading of a finely divided amalgamator (fine powder or mesh, or clippings) through the apparatus may occur, as was observed with the copper powder.

Finally, it should be noted that many of the above-mentioned difficulties will likely lead to a non-reproducible release pattern, so that again, as in the reduction-aeration method, peak height must not be used as a measure of the amount of mercury present.

Despite these findings, amalgamation-separations of mercury may be useful when properly applied. Examples of application of this technique may be found in References (176, 192, 204, 257 - 264), as well as the other publications mentioned in the Sections on amalgamation collection. Roesmer (103) and Long et al (264) give many additional references on this subject.

4.12 Ashing Techniques

4.12.1 Dry Ashing: Pyrolysis

Dry Ashing, even when carried out with a low temperature oxygen asher, [64, 265] has been found to result in essentially complete volatilization of the mercury in a sample. It has been suggested that this might be made use of as the basis of an analysis for mercury. Reed and Jovanovic [266] have applied a thermal release technique in neutron activation analysis of geological samples. A pyrolytic technique has been suggested for biological samples, followed by atomic absorption analysis of the mercury. [267 - 270]

The separation of mercury from interferants, especially organics, results in a very complicated apparatus, and the only separation which appears feasible is by an amalgamation collection of mercury. A double amalgamation technique has also been described. [45, 258, 271]

These methods are rather formidable in their complexity, and in the light of the experiments performed on simple amalgamation collection methods as presented in Section 4.5, and discussed in Section 4.11, it is very difficult to see how this technique could possibly give accurate results.

4.12.2 Partial Wet Ashing

This method was found to be unsuitable in experiments with fish samples. Incomplete extraction of the trace mercury from the matrix was observed. The degree of extraction correlated well with the degree of ashing (determined visually.) In this regard, a perchloric/nitric acids combination gave the best ashing mixture, and it is possible that it would be suitable for samples which are more readily ashed. Use of higher temperatures may be feasible to aid ashing, in which case this really becomes a complete ashing technique. The difficulties with this ashing mixture reported by Gorsuch[272] must be borne in mind, especially volatilization losses. Skare[238] noted some loss, even at lower temperature. It was also noted that after the partially ashed sample had been filtered off, the partial ashing solution retained significant amounts of the mercury from the sample after reduction-aeration. Furthermore, in certain cases, very large absorption signals were observed, even though very little mercury was present in the sample. This was probably due to incompletely ashed organics. Broad band absorption is an ever present possibility with partial ashing techniques. Finally, the mercury absorption signal that is observed was found to be drawn out in time relative to standards which were made up using the same reagents. This effect has also been noticed by Magos,[109] who suggested that since peak area measurement is impractical (!), internal

standards may be analyzed to give a correction factor. The use of peak area as described in this research has been found to give results far superior to the use of peak height, because the latter measurement cannot take the release pattern of mercury from the sample into account.

To conclude, then, partial ashing techniques have been found to be incapable of satisfactorily extracting the trace mercury content of fish samples, and suffer from strong matrix effects as well. Provided that a broad band compensation method is used, the experimental evidence indicates that results will be too low. Stein et al[182] observed similar effects, and suggested that the final atomic absorption measurement was made on less than seven per cent of the total mercury present. Stainton[273] also found that not all of the mercury present in a sample is available for measurement.

4.12.3 Complete Wet Ashing

The evidence presented in this thesis clearly indicates that complete wet ashing of the sample is absolutely essential to remove various strong matrix effects which occur. Several researchers have previously suggested that this might be the case,[182,272,274,275] even when analyzing urine samples.[276] However, many authors had reported losses of mercury due to volatilization during wet ashing at elevated temperatures.[84,86,96,231,272,277,278] Campbell and

Head[279] had reviewed published ashing techniques and suggested a procedure for the determination of mercury in urine.

In the present research, a method has been developed for biological samples which appears to give good results without loss of mercury by volatilization. One possible interference, which has been described in Section 4.8, is coprecipitation of mercury with an insoluble sulphate. In such an instance, a different ashing mixture may be required. Alternatives have been suggested,[280] including the oxygen bomb combustion method[92,281] and an acid-pressure decomposition method.[250,282,283] These approaches would, of course, require considerable investigation to determine their applicability in this particular case.

Even though most matrix effects appear to have been removed by wet ashing, peak area measurement rather than peak height is still necessary. Also, in the light of results reported by Saha and Lee,[252] some form of background compensation method should be employed (see Section 4.10), even when using a complete digestion technique.

4.13 Errors

4.13.1 Systematic Errors

Experience has shown that a great number of systematic errors can, and probably do, occur. The problem becomes one of minimizing them as much as possible.

Let us first assume that the sample integrity has been maintained during the sampling process, and also that volatilization losses and contamination additions do not occur significantly in the recommended procedure. Further, let us assume that broad band absorption interference, which has been discussed in Section 4.10, does not occur, or can be corrected for with the appropriate background corrector. The following operations are likely to produce systematic errors: Transfer from the ashing flask for accurate dilution is very difficult to achieve quantitatively, because only a small volume of rinsing solution could be used. Therefore, several percent may be left behind. During reduction-aeration great care is necessary in the addition of the Sn^{+2} reagent solution, since volatilization will begin immediately, and serious losses can occur. If the proper precautions are taken, such losses can be eliminated. It has been observed that in real samples complete aeration of the mercury is difficult to achieve, and the incomplete release often results in at least several percent of the mercury not being

measured. Trapping of the mercury by drying agents, glass wool, and other parts of the flow-through absorption measurement system has been described in Section 4.4. Such effects can be guarded against, if it is realized that they occur. The first several analyses in a new (clean) system will likely be low because of system trapping. If peak height measurement is used rather than peak area, the results will be significantly low, depending on the release pattern. Typically, the peak height gives a result which is 50% - 80% of that based on peak area.

Numerous chemical interferences may occur. Some of these have been outlined in Section 4.10. Further discussion of such interference may be found in References: 46, 217, 232, 282, 285 and 286.

The possibility of strong reversal of mercury resonance lines on commercial hollow cathode lamps has been suggested.[287] This effect will be particularly important at high lamp current and will cause deviation from the Lambert-Beer law.

Finally, sample storage before analysis presents a serious problem. This has been discussed in Section 2.4. Sealing in quartz capsules is recommended, if possible. A recent publication by Alberts et al[288] gives an indication that even this procedure can result in a problem of loss after four weeks' storage.

Note that all of the systematic errors considered which can not readily be corrected for, give low results.

4.13.2 Random Errors

Each of the systematic errors which have been outlined above will have a random component. The magnitude of these is very difficult to estimate. In addition to these, there is a random error associated with calculation of the absorbance peak area. This gives a standard deviation of about one per cent, provided that the peak is fairly large. If the peak is small, several things must be considered. The statistical fluctuations inherent in counting photons put a lower limit on the peak size that can be detected. The standard deviation is 0.1%, which leads to a lower limit of quantitative determination as defined by Currie[207] of total absorbance peak area equal to 0.04. This represents about 1×10^{-9} g of mercury.

However, the random error associated with small signals has generally been observed to be larger than expected from the above consideration of statistical fluctuations in photon counting. The other random error sources no doubt play a part, but the following source must also be considered. Mercury trapping in the system has been discussed in Section 4.4.2. Even when this was minimized as described, about 15×10^{-9} g of mercury was found to be adsorbed, and this was shown to be displaced in part by each sample which was aerated through the system. This suggests, therefore, that when samples containing an amount of mercury similar to or

less than the amount trapped in the system are aerated, statistical fluctuations in the displacement of mercury will occur, thus contributing significantly to the random error observed in the measurement. The net result is that the quantitative limit of determination is not as low as indicated above.

5. GENERAL DISCUSSION

5.1 Introduction

A critical evaluation of the two methods under consideration has been given. Chapter 3 presents a complete, self-contained investigation of the neutron activation analysis technique, and Chapter 4 presents the same for the cold vapour atomic absorption technique. Chapter 2 has been included to give an estimation of the very important problem of the volatility of mercury. From the experience gained in these investigations, it is now possible to compare the two methods on many points of interest to the analyst. This is done in Section 5.3.

5.2 Contamination Problems

During the course of this work, contamination has not been observed to be a serious problem. This is more likely due to previous experience in trace analysis leading to proper techniques, and careful screening of reagents and cleaning of equipment before use, than to the likelihood that contamination might not be a serious problem. Several cases of contamination have occurred, and reagent blanks should always be run with sample batches. The considerations involved in a well-run trace analytical laboratory have been outlined by Mitchell.(290) The laboratories used for this work had no known previous history of mercury spills.

Contamination from the air has been noted in several cases, including one of the first workers.(211) Trace mercury contents in materials and reagents used in analysis have been observed in many cases, for example by Monk et al,(55) Sandell(97) Toribara and Shields,(237) Byrne et al,(291) Weiss and Crozier,(292) and Christell et al.(102) Also, Reed and Jovanovic(266) studied contamination of samples standing in the open, especially with mercury present somewhere in the vicinity, and found that small amounts of mercury are picked up fairly readily, apparently adsorbed on the surface.

The integrity of the sample can be compromised by the technique used to collect the sample, and, in particular, pollution of the sample and its environment can occur at the time of sampling. Such a case has been suggested to have occurred by Dickson (133) and could be more common than is generally suspected. Nevertheless, it appears that contamination problems can be kept to a minimum, provided that the possibility is recognized, and that appropriate precautions are taken.

5.3 Comparison of Methods

5.3.1 Specificity, Sensitivity and Interferences

The most important question to be asked concerning an analytical method is, "Does it give the correct result?" No matter what other features it has, if the method gives the wrong answer, it is not of much use.

In terms of specificity, the neutron activation technique is superior to all others because it suffers from fewer interferences. In this case, there are no interfering nuclear reactions, and so based on specific chemical separation for mercury, the energy of gamma-ray emission, and the half-life of the radionuclide, there is no uncertainty of what is being determined. Also, the analysis can be based on more than one isotope. Minimal sample handling need be carried out before irradiation (although this is not always taken advantage of in practice, and so may be a source of error.) After irradiation, the mercury concentration is represented by the amount of activity induced in the various isotopes of mercury formed by neutron bombardment. Contamination at these times is therefore unlikely. A relatively large amount of inactive mercury is added to act as a carrier for the trace of radioactive mercury that has been formed. As soon as exchange of the mercury occurs, any later loss is also corrected for by a chemical yield of the carrier after the final

separation. This procedure has the very significant advantage that trace anounts of mercury therefore do not have to be dealt with during the chemical processing.

The atomic absorption method is plagued with numerous possibilities of errors and interference, chiefly because it requires chemical processing steps to be performed with the minute traces of mercury. Nevertheless, a procedure has been tentatively recommended which appears to give satisfactory performance.

For an estimation of the sensitivity of a technique, the quantitative limit of determination, as recommended by Currie,[207] is favoured. This is the level at which the measurement precision will be satisfactory for a practical quantitative determination, and has been approximated as being equivalent to ten times the standard deviation for a "well-known" blank. Under the described conditions, the values have been found to be: 3×10^{-11} g for the neutron activation analysis, and 1×10^{-9} g for cold vapour atomic absorption.

Despite the apparent advantages of the neutron activation method, results obtained do not always inspire confidence, as has been outlined in Section 1.3.4. There would seem to be two probable explanations for this. The first is the question of sample integrity as received by the analyst. This type of problem is very difficult to surmount. Initially homogeneous comparison samples should probably be sealed in

quartz for distribution. The second explanation is that analysts often do not make full use of the advantages that this technique offers. Considerable pre-irradiation processing, especially drying, is common. This operation carries with it a risk of loss, and loss is almost certain if standards are treated in this way. In addition, many researchers continue the questionable practice of irradiation in plastic containers. Often not enough attention is given to the preparation of standards.

To summarize, then, neutron activation analysis has been found to be superior to atomic absorption from the point of view of specificity, sensitivity, and freedom from interferences. Therefore, it has a much higher probability of giving accurate results. From the experience obtained, it may be predicted that the atomic absorption method will tend to give results that are low relative to the neutron activation method. This has been borne out in a number of reports. (64, 70, 289)

5.3.2 Availability and Applicability

Neutron activation analysis has often been summarily dismissed as being a technique for a few experts who have the facilities. In the past, this has been true. The average analyst had no possible opportunity to utilize this technique. There is also a shortage of trained personnel to carry out such analyses. However, both of these shortcomings diminish as reactor facilities become more common.

There is no doubt that the atomic absorption technique is presently more accessible for the average analyst. Unfortunately, a much greater degree of skill is required to obtain useful information by the cold vapour atomic absorption method than by neutron activation.

Both neutron activation and atomic absorption can handle most types of samples. Atomic absorption is more readily applicable to liquid samples than is neutron activation. On the other hand, activation analysis usually does not suffer from the problems which have cropped up in the atomic absorption technique, such as formation of a precipitate in the digest solution which traps the mercury, and various strong chemical interferences such as complexation (see Section 4.14).

5.3.3 Costs

The cost per analysis works out to be roughly equivalent for neutron activation and atomic absorption when done on a large scale.[289] If the analysis is to be done routinely, an automated system may be of great advantage to facilitate processing. De Goeij[293] has described an automated neutron activation analysis system. Lindstedt and Skare[234] have described an automated atomic absorption unit.

In order to utilize the cold vapour atomic absorption method, a reduction/aeration apparatus with an absorption

cell must be obtained. The cell can be attached to the normal atomic absorption instrument burner head to facilitate optical alignment. Kits such as the one used in this research are commercially available from almost all atomic absorption instrument manufacturers, (226, 294, 295) at a cost in the order of $400. to $500. It is recommended, however, that the researcher construct his own system in order to get exactly the system desired. Several helpful descriptions are available in the literature (296, 297) in addition to publications from various manufacturers. Some form of background correction device (for broad-band absorption) and a signal integrator are also necessary, as well as a mercury lamp.

For neutron activation analysis, a standard gamma-ray spectrometer setup is required with a NaI scintillation detector. The cost of this is comparable to the cost of an atomic absorption instrument.

SUMMARY

The importance of accurate analysis in the parts per billion range is briefly outlined, and evidence from the literature is presented to show that agreement of results between analysts and/or methods of analysis at this level of concentration is often poor.

A critical evaluation including radio-tracer studies, and analysis of standard reference material is given for each of the two analytical techniques currently most commonly employed in trace analysis for mercury : neutron activation analysis, and cold vapour atomic absorption.

The evaluation of neutron activation emphasizes the problems associated with such critical steps as : pre-irradiation treatment (eg. lyophilization), irradiation in plastic containers as opposed to quartz capsules, preparation of standards, and chemical separation procedures. A recommended procedure for neutron activation analysis is outlined in the light of the evaluation. A quantitative limit of determination of 3×10^{-11} g was found.

The evaluation of cold vapour atomic absorption presents a number of startling discoveries concerning this method, including trapping of mercury in drying agents (the use of which appears unnecessary), rapid loss of mercury from samples upon addition of $SnCl_2$ in the reduction-aeration method, poor performance of amalgamation-collection techniques and failure of partial digestion techniques to quantitatively

extract the mercury from biological samples. The application of a photon-counting readout technique which has been developed by the author for the purpose of accurately following the rapidly changing absorption signal is described. The results show clearly that peak area, and not peak height, must be used to represent the amount of mercury present. A theory is presented to describe the mercury flow through the absorption cell based on the rate of release from the test solution. Many factors are shown to influence this rate (including incompletely ashed biological samples and chemical reagents employed in sample treatment), thus affecting the shape of the absorption peak. In addition, incomplete aeration of mercury is shown to occur in a number of cases. A recommended procedure for cold vapour atomic absorption is outlined, which includes complete wet ashing of the sample without loss of its mercury content. A quantitative limit of determination of 1×10^{-9} g was found.

A chapter is devoted to the very important problem of the volatility of mercury, and includes a study of the effect on mercury content of food when it is cooked (no significant losses), and the description of <u>in vivo</u> labelling of fish and plants with $CH_3{}^{203}HgCl$ and $^{203}HgCl_2$ in order to study volatility, and to evaluate the performance of the methods of analysis.

Finally, the two methods of analysis are compared.

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APPENDIX: CALCULATION OF THE GEOMETRY FACTOR, K. (Section 3.3.1)

What solid angle does the detector subtend at a point source which is located on the central axis of the detector a distance d above it (Figure 3.3)? This problem is the same as calculating the solid angle of a cone of appropriate dimensions, as represented in Figure A.1. The solid angle is equal to: the surface area of a section of the sphere of radius r which is cut by the cone, divided by the square of the radius. This is shown in Figure A.2 as a "side-view", and in Figure A.3 as a "top-view". From this Figure, it is seen that,

$$m = r \sin \theta$$

$$\text{and } \Delta a = r \, \Delta\theta$$

Also, from Figure A.3, an increment of surface area of the sphere, ΔS, is seen to be:

$$\Delta S = (2\pi m)(\Delta a)$$

Thus, $dS = (2\pi r \sin \theta)(r \, d\theta)$,

and the total surface area of the sphere cut by the cone may be found by integrating:

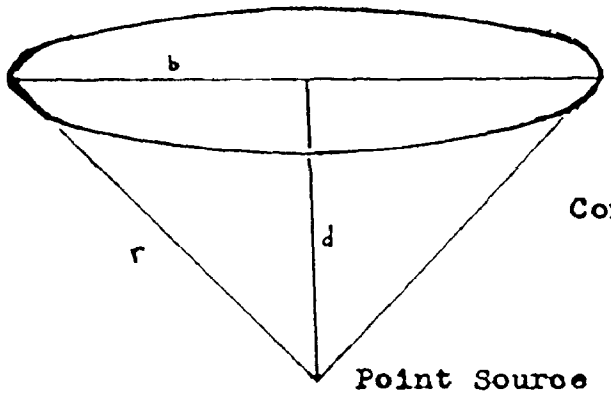
$$S = 2\pi r^2 \int_0^{\theta} \sin \theta \, d\theta$$

$$= 2\pi r^2 \, (-\cos \theta) \Big|_0^{\theta}$$

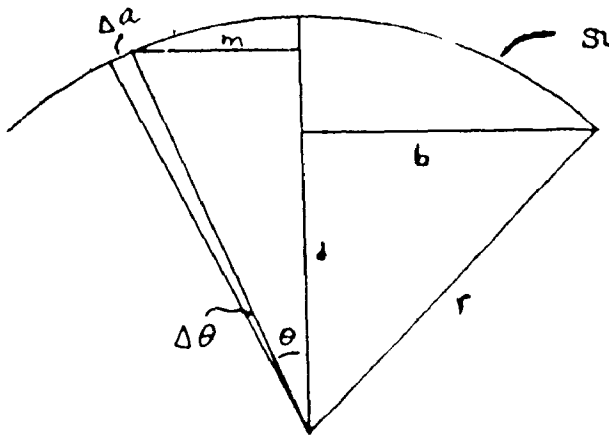
Figure A

Geometry Factor

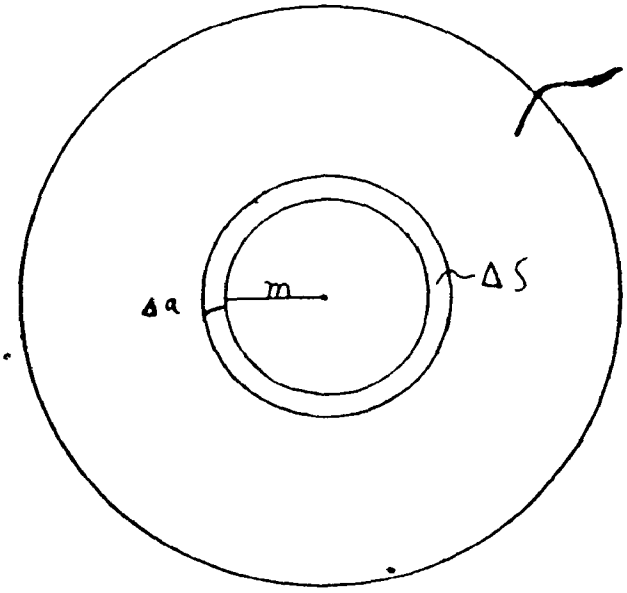
1



2



3



$$S = 2\pi r^2 (1 - \cos \theta)$$

The solid angle is $\frac{S}{r^2} = 2\pi (1 - \cos \theta)$.

Note that θ is the "half-angle". The amount of radiation falling on the detector depends on the solid angle, and will be given by the solid angle divided by 4π.

the geometry factor, $K = \frac{(1 - \cos \theta)}{2}$.

$$(\cos \theta = \frac{d}{r} = \frac{d}{(d^2 + b^2)^{\frac{1}{2}}})$$

Test of Derived Geometry Factor:

When $d \rightarrow 0$, $\theta = \frac{\pi}{2}$ radians, and $\cos \theta = 0$.

$$K \longrightarrow 50\%.$$

When d becomes large, the surface of the detector will approximate the surface of a sphere of radius $r = d$, and the inverse square law will hold ($d \gg b$). Table A.1 gives data calculated for the high resolution Ge(Li) detector, using $b = 2.15$ cm.

Several approximations are inherent in this formula when applied to the real case. The sample is not a point source, and this will give rise to some deviation as the distance d becomes small. Also, the gamma-rays penetrate some depth into the detector, making it necessary to use an "effective depth" for d, rather than the distance from the surface of the detector.

Table A.1 Geometry Factor, K

Distance From Detector (d)	$K = \frac{(1-\cos\theta)}{2}$	Ratio
0 cm	0.5	
0.1 cm	0.477	
1.0 cm	0.289	
10.0 cm	0.011	24.2
50 cm	4.62×10^{-4}	3.996
100 cm	2.90×10^{-4}	3.9990
200 cm	1.16×10^{-4}	3.9997
400 cm	7.25×10^{-5}	3.9999
800 cm	1.81×10^{-5}	