ANALYSIS FOR TRACE MERCURY CONCENTRATION

I. CRITICAL EVALUATION OF CURRENT PROCEDURES

II. A PROPOSED METHOD FOR DETERMINATION BY INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS

by

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ABSTRACT

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II. A PROPOSED METHOD FOR DETERMINATION BY INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS

ADVISER: EVAN T. WILLIAMS

Current methods of sample pretreatments, digestion, lyophilization and extraction, have been found to lead to considerable loss of mercury, at an initial mercury concentration of 1 $\mu g/g$, and less. Storage of solutions of mercury at concentrations of less than 1 $\mu g/ml$, in glass, Teflon and polyethylene containers, leads to losses by adsorption. Electrochemical reduction of mercury to the metal, and subsequent volatilization, is postulated as the mechanism of loss from the samples studied, during lyophilization. A method of instrumental neutron activation analysis, which obviates the above pretreatments, has been developed for mercury concentrations as low as 1 ng/ml.

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I. Introduction

The abundance of mercury in the earth's crust is $5 \times 10^{-5}\%$, a concentration of $0.5\ \mu g/g$ and it is present in seawater at a concentration of about 30 pg/ml.[1] The element is not found free in nature but occurs as the oxide or sulfide, both of which have been mined since before the first century, long before mercury and its compounds were recognized as environmental toxicants. The Greeks, notably Plinus Secundus[2], were among the first to recognize the potential of mercury compounds in the treatment of cuts and rashes and also its ability to separate noble metals from the earth through amalgamation. The first reported cases of mercury poisoning, as the result of ingestion and inhalation of mercury metal during the mining process[2], were reported(and successfully treated) at this time. However, it wasn't until the mid-1600's, about the time of the invention of the barometer, that it was realized that chronic mercurialism could result from exposure to trace concentrations. These cases of chronic mercurialism were caused by a "secret ingredient", mercuric nitrate, which was used as a carroting agent in the treatment of felt hats and furs[3]. The usage of this compound affected not only those people who made the furs and hats, but as indicated by Lewis Carrol in his character "The

Mad Hatter", from the book Alice in Wonderland, the condition of chronic mercurialism existed for those people who wore felt hats[4], as well. Since the time of the printing of that book (about 1836) mercurialism has often been documented as being caused by occupational exposure. More recently, the enormity of the Minimata[5], Iraq[6], and New Mexico[7] disasters have focused the attention of the world on this serious problem; the entrance of mercury into the environment, and the levels of mercury concentration in our air, water and foodstuffs.

The symptoms of acute mercury poisoning are; nausea, dizziness, metallic taste in the mouth and gastric upset. These effects are immediate and violent, except in the case of organomercury poisoning, in which case the symptoms may have as long as a two week latency period. Acute poisoning, however, is not a significant analytical problem, since it is easily diagnosed by its symptoms and the appearance of large quantities of mercury in the discharges, gastrointestinal fluid and the bloodstream.

In contrast to this, chronic mercuralism can exist over a period of several weeks to months, the symptoms (such as nephritus, necrosis of the liver and brain damage) don't become apparent until prolonged exposure has occurred and

it is usually caused by ingestion or inhalation of trace amounts of mercury in food or air. This type of exposure can be monitored, but it does present a special problem, i.e., the accurate determination of mercury in the concentration range of less than 1 $\mu g/g$.

There are three major entrances for mercury into the human food chain, namely industrial waste, crop spraying with mercurial pesticides, and treatment of seed grains with mercury fungicides. Mercury present in the air is usually the result of high-temperature incineration, coal burning, or boiler operation. Industrial wastes(primarily inorganic mercury) do not directly enter the food chain. The waste is disposed of by dumping into rivers or oceans, where the mercury precipitates and becomes incorporated into the sediment. It can subsequently be incorporated into plant life, or after methylation[6], through bacterial action, be incorporated into aquatic life. Even though this affects only the lower forms of marine organisms, it is passed on to their predators through bioamplification. Bioamplification is the process by which organisms of higher order, in a food chain, concentrate a particular substance. This occurs because predators will ingest many times their own weight during their

lifetime. It has been demonstrated that when abnormally high levels of mercury have existed in the bodies of certain test animals, less than 75% of the total body content of mercury is excreted per day[9]. Hence, a large concentration factor is possible.

The amount of organomercury compounds in the environment started to become significant in 1915[8], when they were first successfully used as pesticides. Since that time, they have also been used as seedidressing to kill fungi that can grow during storage. Despite their known toxicity, they have been continually used even after incidents such as occurred in New Mexico[7]. In this case hogs were fed grain which was treated with organomercury compounds and subsequently slaughtered for family consumption. The tragic result was the death of two family members and the crippling of the remainder of the family. The level of mercury found in the urine of one family member, two weeks after the incident was as high as 400 μg/l.

The toxicity of alkyl mercury compounds is at least an order of magnitude greater than their aryl counterparts, which are slightly more toxic than ionic mercury compounds. The action of these compounds as insecticides and fungicides is denaturation of proteins by mercury linkages with either sulfur or amino groups[10]. For this same reason mercury is a

deadly toxicant to humans. It has been found that alkyl mercury compounds are excreted at a level of only 3 to 7% of total body content per day,[9,10] consequently, accumulation of organic compounds is much faster than accumulation of inorganic compounds. The low solubility and mechanism of incorporation into cellular fluid are the main reasons for its slow rate of excretion. The organic portion of the molecule dissolves in the fatty portion of the cell wall, causing a separation in the wall, which leads to leakage and entrance of mercury.[10] The mercury can exchange with ions of calcium or magnesium, which form bridges with protein molecules. Mercury, being a larger ion, changes the distance between the molecules and thus alters the chemistry of the proteins within the cell.

On the basis of experimentation with test animals, tolerence limits of ionic mercury concentrations have been set as 0.1 mg/m^3 of air,[1] and 0.3 mg/l of urine;[1] the values established for organomercury compounds are one tenth of those for ionic mercury.

Classical Analysis

The several classical methods of mercury determination are based on gravimetry and photometry, and in both cases it

is necessary to use, large sample size(about 100 g), large quantities of reagents, and an initial sample digestion.

Among the best known of the gravimetric processes is the Holloway-Eschka method.[11] After initial sample digestion, the mercury is precipitated as the sulfide, along with other metals, and heated with iron filings in a crucible, covered with a gold or silver plate. The process causes reduction to elemental mercury, which then condenses on the gold or silver plate to which it amalgamates. The plate is weighed before and after analysis in order to determine the mercury content of the original sample.

A second method involves electrodeposition of mercury(from a digested sample) onto a copper wire for a period of 18 to 24 hours. Again the weight difference of the wire is used to determine the total mercury in the sample.[12] However, at some point an alternative to this procedure was proposed. The mercury deposited on the wire was distilled from the wire into a glass capillary tube, and the size of the mercury droplet measured with a microscope.[12] The amount of mercury was then computed on the basis of the drop size. Sensitivity by either of these methods was claimed to be between 0.2 and 0.002 μg, depending upon the source consulted.

One of the earliest methods of mercury determination was

based on light scattering.[13] The mercury was separated as the sulfide, dissolved in aqua regia, and reacted with strychnine. This product forms a colloid, which is placed in a high-intensity light beam, and the measured intensity, either at right angles(nephelometry) or at 180^0 to the incident light beam(turbidimetry), was subsequently compared to a series of standards.

The reaction of mercury metal with iodine to form the stable colored complex HgI_4^{2-}, is the basis for another type of photometric analysis. After the sample is digested the mercuric ion is reduced using stannous chloride. The mercury is then reacted with iodine and the color of the solution is visually compared to a prepared series of standards(this was prior to the widespread use of the spectrophotometer).

The best known and most widely used of the classical photometric mercury determinations is the dithizone method.[14] After sample digestion, the pH is adjusted to four, and the solution is shaken with a solution of dithizone in carbon tetrachloride to produce the highly colored mercury complex. The spectrophotometer which affords excellent resolution of the various spectral lines of the elements, led to wide-

spread use of this instrument for accurate determinations of low concentrations of mercury. This served as the major method of trace-mercury determination through the early 1950's.

Modern Methods

Atomic absorption and flame photometry have become very widely used methods of quantitative elemental analysis because they have greater precision and sensitivity than the conventional methods and because they can simultaneously determine several elements. In each of these methods the sample is first digested, using concentrated acids with, or without, strong oxidants. The sample may then be further concentrated by solvent extraction or ion exchange, or analyzed directly by either flame photometry, or a flame or flameless atomic absorption instrument. The appropriate wavelength for emission or absorption(for mercury these are 253.6 and 253.7 nm) is selected and the sample is compared to a series of prepared standards. It is possible to separate two elements whose wavelengths are as close as 0.05 nm, thus eliminating many interferences[15]. The sensitivity of an instrument can be as low as 1 ng/ml.

Chromatographic analysis can be used for specific compounds of mercury, as well as for ionic mercury. The sample

is extracted using an appropriate solvent with or without complexing agent, and the extracts subjected to either gas or thin layer chromatography, and compared to a series of known organomercury compounds of known concentration. The most commonly used complexing agent is dithizone, and the corresponding mercury dithizonates are purportedly measured in the chromatographic procedure. The sensitivity of this method[16] using a tritium or nickel-foil electron-capture detector permits measurements of concentrations less than 10^{-7}M.

A recent method of mercury analysis, anodic-stripping voltammetry utilizes the limiting current of the ion in solution to determine concentration. As in the other methods, absolute determination is not feasible, and therefore this method also requires comparison standards. Most recent literature reports of this method[17] claim sensitivity to 10^{-8}M. This method also shows potential for multielement analysis.

Mass-spectometric isotope-dilution analysis is a very sensitive and specific method[18] of analysis which can be used for a large number of elements. Isotope exchange is performed with the sample using a tracer which is either a stable or long-lived radioactive isotope of the element to be determined. Since a known amount of tracer is added, a quantitative

recovery of the original amount or tracer is nor required in the analysis. The method does not lend itself to multi-element analysis, but has the capability of detecting as little as 10^{-12}g of an element and is virtually interference free.

Activation analysis is a method of quantitative elemental analysis in which the characteristic radiations of the activated atoms, in a sample, are counted and compared to standards. Although it is regarded as a new analytical technique, its history dates back to the mid-1930's, when the first analyses were performed by bombardment with charged particles.[19] The development of the nuclear reactor in the forties made available high neutron fluxes, which yielded higher specific activities(due to large thermal neutron capture cross-sections) and consequently better sensitivity. Nondestructive sample analysis, both qualitatively and quantitatively, can be performed by identification of the radiation spectra. The limit of detection for mercury is about 0.1 ng, and the dynamic range extends up to a few parts per thousand. Beyond the upper limit, self-attenuation of the neutron flux occurs leading to poor linearity of standards. More recently, it has been shown that photonuclear-activation analysis, involving the activation of elements by bombardment with

high-energy gamma rays, has great utility[20].

The distinct advantage of the modern methods is that the physical measurement used for analytical determination is specific for the element in question. A weight change (or change in color or turbidity) can be due to any element, but a signal due to a distinct electronic, atomic, radioactive transition or decay is characteristic of that nuclide; the half life further characterizes the radionuclide. Generally, there is more than one transition which occurs for a given element which leads to additional positive confirmation of the presence of the element.

Modern methods(except chromatography), share a common link with the classical methods of analysis; the measurement of total mercury content rather than the chemical form of the mercury. However, at concentrations between a part per billion and a part per million, testing for specific mercury compounds is difficult. In addition, the organomercury compounds are easily reduced to elemental mercury[21], thus giving the impression of a lower organomercury concentration than is actually present.

Instrumental activation analysis is the one modern

method that affords the advantage of obviating sample pretreatment, since as this research has shown, pretreatments can lead to severe losses of mercury. Proton activation, one of the various methods of activation, has the disadvantage of essentially being limited to analysis of the surface of samples, which for environmental samples may not be sufficiently informative. Photon activation has good specificity but its sensitivity is not as great as needed. Neutron activation accomodates large sample size, affords good sensitivity and can be used for a large number of elements without sample pretreatments.

In order for neutron activation analysis to be effective, the accumulated counts from the decay of the radioisotope produced must be sufficient for reliable statistical analysis. This requires a cross section large enough so that upon short irradiation (1-2 hours maximum), the activity produced is sufficient for analytical determination. A short irradiation time for an environmental sample is advantageous because of the large background activity resulting from the decay of ^{24}Na. Longer irradiation times would lead to both a lower signal to noise ratio and the necessity of a longer period of decay before counting so that there is minimal dead time in the instrument.

The equation for radioactive build-up is

$$A = N\sigma\phi(1 - e^{-\lambda t})$$

where,

N = the number of atoms present
σ = cross section for thermal-neutron capture, in cm^2
ϕ = neutron flux in neutrons/(cm^2-sec)
λ = 0.693/(half life in hours)
t = time of irradiation in hours.

Theoretically, for a pure sample of mercury, the instrumental sensitivity for a two-hour irradiation at a thermal-neutron flux of 10^{14} n/cm^2-sec, would be about 10^{10} atoms(of ^{196}Hg) by counting the ^{197}Au x-rays from the decay of ^{197}Hg produced by the reaction,

$$^{196}_{80}Hg(n,\gamma)^{197}_{80}Hg \xrightarrow{T_{\frac{1}{2}} = 64 \text{ hrs.}} {}^{197}_{79}Au + \beta^+ + \gamma + \text{x-rays}$$

This is calculated on the basis of cross section, half life and natural abundance of the ^{196}Hg isotope as compared with the other naturally occurring mercury isotopes. The natural abundance of ^{196}Hg is small but its large cross section and shorter half life lead to a greater sensitivity during a short irradiation(see Table 1). The most prominent peaks in the gamma-ray spectrum of neutron-irradiated mercury are the x-rays(64, 68, 77, and 80 KeV) from the electron-capture decay of ^{197}Hg. These x-rays can be detected with high efficiency with a thin solid-state detector, while much of the higher-energy gamma rays pass through.

TABLE 1
Some Nuclear Properties of Mercury Isotopes

Isotope	Abundance	Cross Section barns	Highest Intensity Gamma Ray(KeV)	Conversion Coefficient	Half Life of (n,γ)Product
196	0.15%	3×10^{3}	77.4	4.0	64 hours
198	10.10	1.8×10^{-2} (to 199^{m})	158	---	43 minutes
199	16.90	2×10^{3}	none	---	-------
200	23.10	60(to 201^{m})	520, 220	---	94 seconds
201	13.20	60	none	---	-------
202	29.70	5	279	0.163	46 days
204	6.80	0.4	203	---	5 minutes

However, the problem due to high background, in this low-energy region, in environmental samples, is ever-present. This background is due mainly to $^{31}Si(T_{\frac{1}{2}} = 2.6$ hours), $^{56}Mn(T_{\frac{1}{2}} = 2.6$ hours), and $^{24}Na(T_{\frac{1}{2}} = 15.0$ hours), which are ubiquitous in environmental samples(silicon being especially troublesome because the sample tubes are made of quartz). The high activities produced in the neutron irradiation of these nuclides, also increases the analog to digital conversion dead time and causes many of the peaks in the gamma-ray spectrum to be unsymmetrical(the analyzer is unable to record so many events simultaneously)and hence interfere in an analytical determination. It is necessary, therefore, to wait at least one day prior to instrumental analysis in order for the activities of these short-lived isotopes to be reduced to a reasonable level. The waiting period and decreased sensitivity can be obviated by using radiochemical separation, but this also has its disadvantages. For example, radiochemical separation requires sample destruction, whereas instrumental analysis does not. Consequently, instrumental analysis also minimizes the volume of radioactive waste and radiation exposure to the analyst.

Although many of the other modern methods of mercury

analysis are at least as sensitive as neutron activation, they require sample preconcentration, digestion, or both, and subsequent determination by comparison with standards. It has been demonstrated that in the course of the necessary processing, loss of mercury(sometimes considerable), may occur when widely accepted procedures are followed.

Recent findings[22,23] that mercury at low concentrations is lost due to adsorption on various surfaces, are of significance because the end product of a sample digestion is a solution. It can be inferred from the results of Feldman[23] and Rook[22] that adsorption of mercury onto these surfaces may be due to reduction either to Hg^{+1} or Hg^{o}. In experiments by these authors, adsorption was minimized when mercury was kept in the +2 oxidation state by strong oxidants such as dichromate or tetrachloroaurate(III). Hence, storage of mercury solutions of low concentration, for even short periods of time, in non-oxidizing media may lead to significant adsorption.

Adsorption is of particular importance in the various wet digestion procedures[24,13,11] now used prior to analysis which typically will reduce the mercury concentration by a factor of 100. Therefore, a sample whose initial mercury concen-

tration will not be appreciably reduced by adsorption, will become a sample for which adsorption of a significant amount may occur.

Rains and Menis[25], have demonstrated that loss of mercury during wet digestion can be as high as 30%. They were able to eliminate this loss by packing the condensing column with Raschig rings. Another expedient is low-temperature wet ashing, using permanganate and persulfate, as suggested by Snell[13]. However, it was found that the most satisfactory procedure was the dry-combustion method of Rook[22] in which the sample is burned in a stream of oxygen, and the combustion products are collected in a liquid-nitrogen condenser.

Lyophilization is a common pretreatment which facilitates storage, by drying and concentrating the sample. Pillay[26] has observed losses of mercury during lyophilization of environmental samples, whereas La Fleur[27] and Friedman[28] have reported no loss of mercury in their samples during lyophilization. In addition, losses of mercury from aqueous solutions at atmospheric pressure have also been reported by MacKay[29], Magos[30] and Shields[31]. Heretofore, there has been no conclusive evidence which summarized the extent of loss in different samples at different mercury concentrations and at

different pressures.

The classical and modern methods of solvent extraction of mercury employ dithizone,[11,14] despite the fact that solutions of the complex have been reported to be unstable.[13] Solvent extraction(with complexing agents or pure solvents) is a common method of preconcentration. The efficiency of extraction and stability of the complexes formed have never been reported for environmental samples.

In view of the known toxicity of mercury and its compounds when present in trace quantities, the analytical determination of mercury at these low concentrations is a significant problem. The object of this research was a thorough investigation of all the abovementioned sample pretreatments, with special emphasis on nuclear methods for monitoring these procedures. It was also hoped that this investigation would help to develop the best method for mercury determination in environmental samples.

II. Standards Preparation and Adsorption

All modern methods which determine trace mercury require that the measured analytical signal from the sample, be compared to standards. A necessary requirement for any standard is that the concentration, or amount, of material to be determined, remain constant during preparation, storage and sample analysis. It is important to determine for standard preparation and storage whether or not adsorption of the species of interest occurs. Adsorption studies with mercury and iron showed that solutions of mercury are unique with respect to rapid adsorption onto the container walls.

Therefore, a critical evaluation of methods of trace mercury analysis must begin with a careful examination of standards preparation.

Experimental Procedures

Standards Preparation. A solution of $^{203}Hg(NO_3)_2$ (2 mCi, ICN Isotopes Division) to which was added 1.72 mg of mercuric nitrate, was diluted to 1 liter with 1 N nitric acid, to make a final mercury concentration of 1.5 μg/ml. This solution served as the stock solution for both the adsorption and lyophilization studies.

A solution of $^{59}FeCl_3$(1 mCi, ICN Isotopes Division) to

which was added 1.02 mg of iron wire, was diluted to 1 liter with 0.5 N HCl, to make a final iron concentration of 1.09μg/ml.

Two sets of mercury standards were prepared; one by dilution of the stock solution with 1 N nitric acid and the other by dilution with deionized water. Aliquots of these dilutions were transferred to 10 ml volumetric flasks and sealed with snap caps and parafilm. These flasks were counted and then reserved for subsequent adsorption studies. Counting was performed by placing the 10 ml volumetric flasks into the well of the NaI(Tl) detector. Standards for neutron activation analysis were prepared in the same manner; after the dilutions were made, 0.1 ml aliquots were sealed into quartz vials.

Adsorption. I. The first adsorption study was accomplished by allowing solutions of mercuric nitrate, with tracer, 1 N in nitric acid, to remain in sealed volumetric flasks for various periods of time. Each flask was then weighed, vigorously shaken, counted, drained, reweighed and finally recounted.

II. The second method used to study adsorption(using Teflon, polyethylene and glass containers) employed large-volume flasks each of which contained 0.5 liters of mer-

curic nitrate solution 1 N in nitric acid. Small aliquots (5 ml) of each solution were removed periodically (following vigorous agitation of the container) during the next three weeks, and counted in a 10 ml volumetric flask as above.

The adsorption of iron, onto glass and polyethylene containers, from solutions of $^{59}FeCl_3$ and carrier 0.5 N in HCl, was monitored in the same way.

Reagents. Nitric acid, hydrochloric acid, mercuric nitrate and iron wire were Baker Analyzed Reagents. All containers were thoroughly rinsed with deionized water prior to storing solutions in them.

Neutron Activation. Samples were sealed in quartz tubes (50 mm x 3 mm ID). Possible loss of mercury in the sealing process(using an oxy-methane torch) was checked by addition of tracer solution to 70% capacity of the quartz tubes, the open end of which was sealed with parafilm, and the tubes then counted. The tubes were then sealed with a torch, without freezing the sample in liquid nitrogen, and recounted. Samples which were scorched during this procedure were not used in subsequent irradiations.

The polyethylene container, or rabbit, used in the reactor's pneumatic tube system had dimensions of 15 cm x

2 cm ID, which limited the number of samples to approximately twenty. This also meant that it was necessary to determine whether a flux depression due to so much quartz would be observed. Two sets of aqueous standards were prepared, carefully placed into the rabbit so that one set of standards occupied the outside position and the other the inside position, and irradiated.

Instrumentation. Irradiated samples were counted with both a 0.1% and a 7% efficient Ge(Li) detector and also with an intrinsic-germanium detector(for high resolution spectra) using a Northern Scientific Multichannel Analyzer model 2430. Tracer samples were counted with a 7.6 cm x 7.6 cm NaI(Tl) well detector and single channel analyzer. This well provided a constant geometry for counting the tracer samples in the 10 ml volumetric flasks.

Irradiations. All irradiations were performed in the reflector region of the Brookhaven National Laboratory High-Flux Beam Reactor in a thermal-neutron flux of $1 \times 10^{14} n/cm^2$-sec. Samples were allowed to decay one day prior to counting.

Statistics. Areas under the respective gamma-ray peaks were determined by the method of Covell[32]. For experiments using tracer solutions, the uncertainty of the total counts was

less than 1% (95% confidence level), much less than the statistical variation of replicate samples due to normal sampling errors.

Results and Discussion

Figure I shows good linearity of the standards solutions as prepared with the tracer, by dilution with both 1 N nitric acid and deionized water. Thus the linearity of the calibration curve is not affected by changing the pH between 1 and 4. However, if the mercury loss during the preparation of each of the successive concentrations was proportional to that concentration, the resultant curve could also yield a straight line, but displaced to reflect a lower specific activity. In order to show that this was not the case, the adsorption of mercury onto glass surfaces was studied as a function of time. Adsorption study I would show that the amount of mercury left in the flask after draining will be directly proportional to the amount of solution left in the flask after draining, if no adsorption occurs. No adsorption occurs for solutions of concentration from 0.375 to 0.0003 μg/ml of mercuric nitrate in the first few hours after preparation(Figure II). There is significant adsorp-

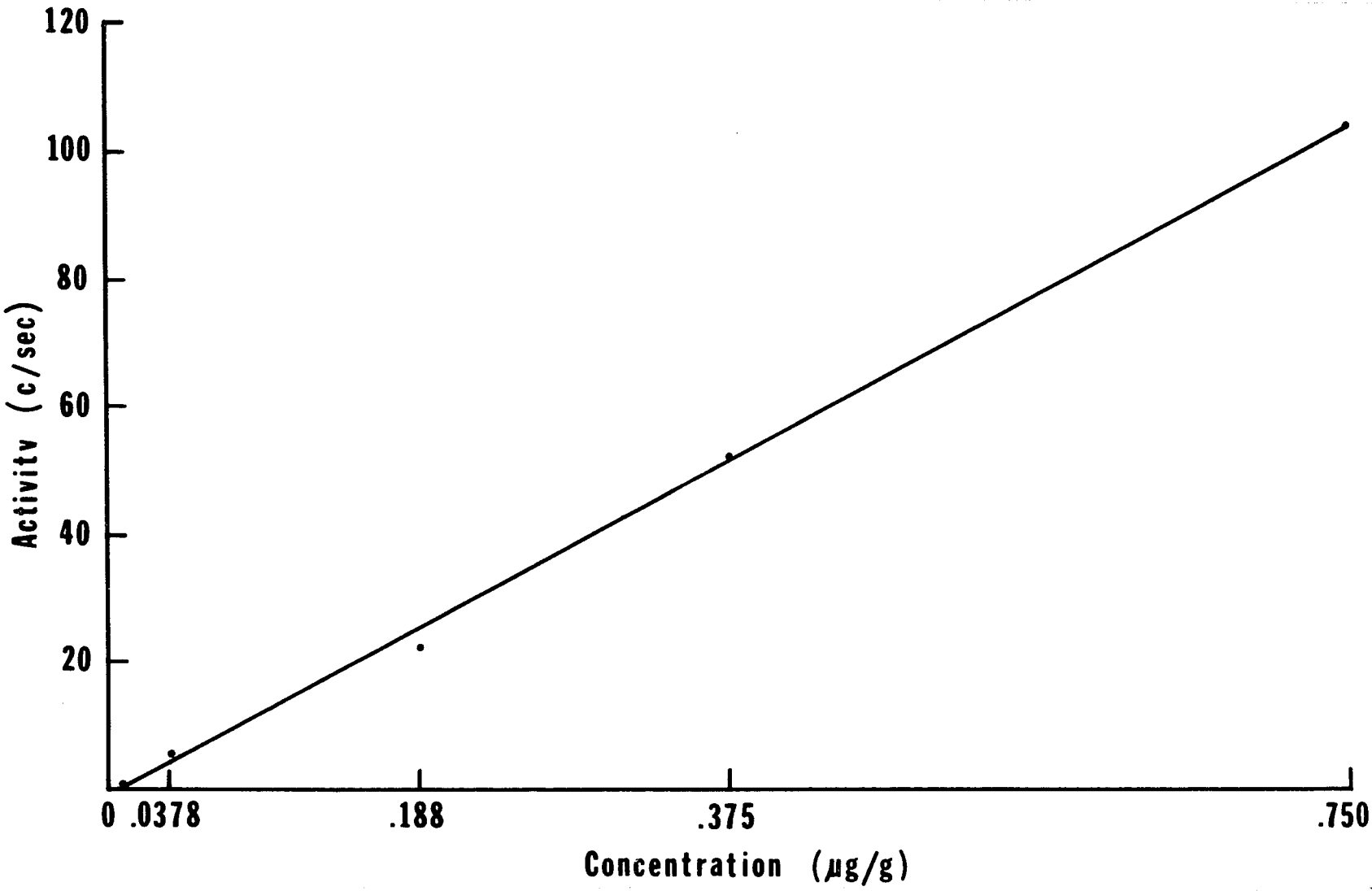

FIGURE I. Standards Preparation Curve for Mercury.

tion after a period of one week; the activity remaining in the drained flask is as much as 70 times greater than corresponds to the amount of solution left in the flask. Figure II shows that adsorption occurs at all concentrations but because the number of available adsorption sites are limited, the effect is not as noticeable at high concentrations. Therefore, the percentage of mercury lost, increases as the concentration decreases. It is also shown that negligible adsorption takes place within the first few hours of contact which is greater than the time necessary for preparation of standards. The former of these premises is further supported by adsorption study II.

Mercury solutions were stored for periods of 2-3 weeks in containers of Teflon, polyethylene and glass, respectively. The amount of mercury adsorbed onto the container walls reaches a maximum value as indicated by a constant tracer activity in the aliquots periodically withdrawn from the container. At concentrations of mercury above 1 μg/ml it was observed that the extent to which adsorption occurred was insignificant compared to the total amount of mercury in the solution. As can be seen in Figure III, the least amount of mercury is adsorbed by Teflon, a greater amount by glass, and the most by

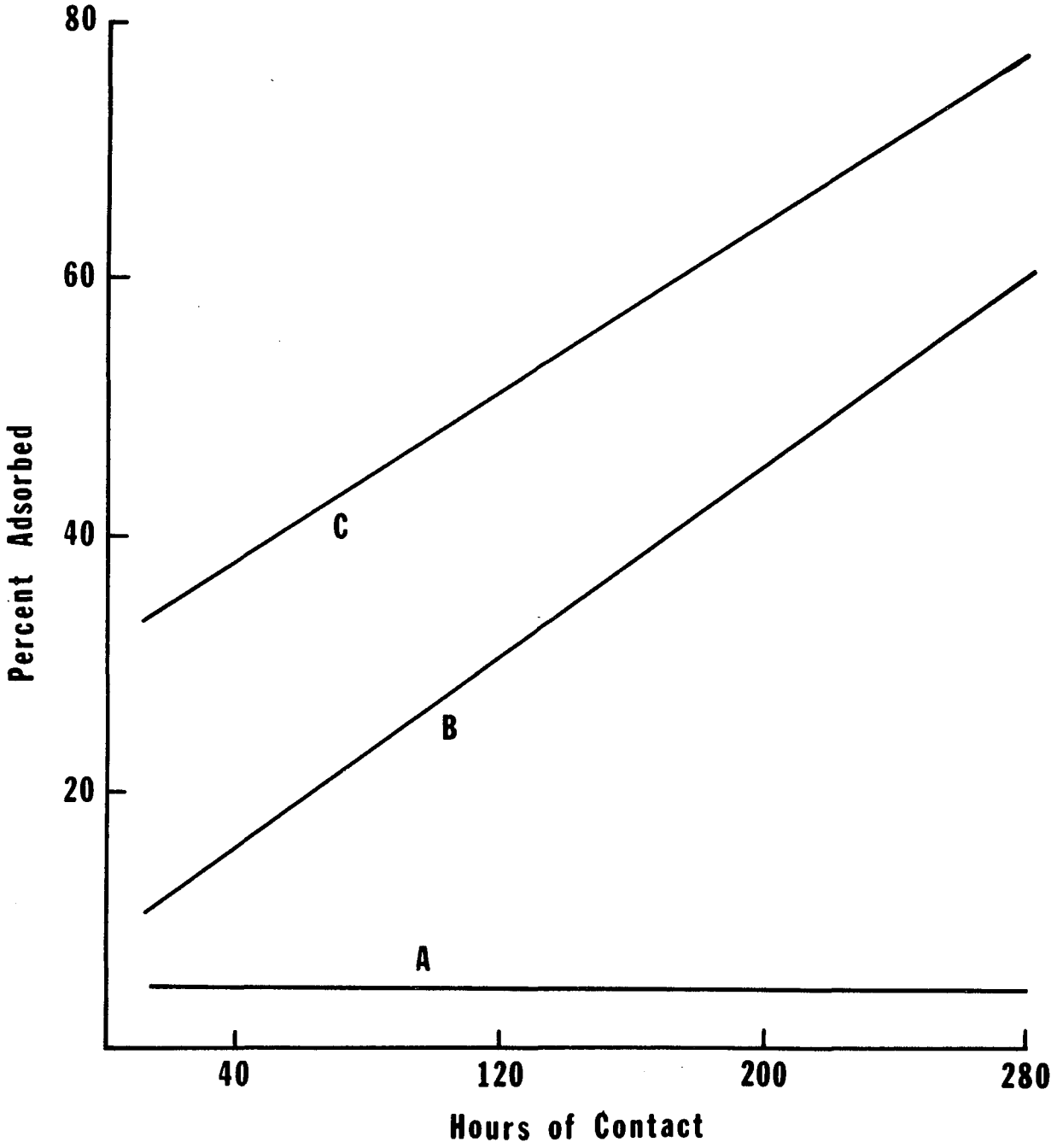

FIGURE II. Change in the Percent Mercury Adsorbed with Increasing Time of Contact. A)0.375μg/ml, Hg^{2+} B)0.015μg/ml, Hg^{2+} C)0.0003μg/ml, Hg^{2+}.

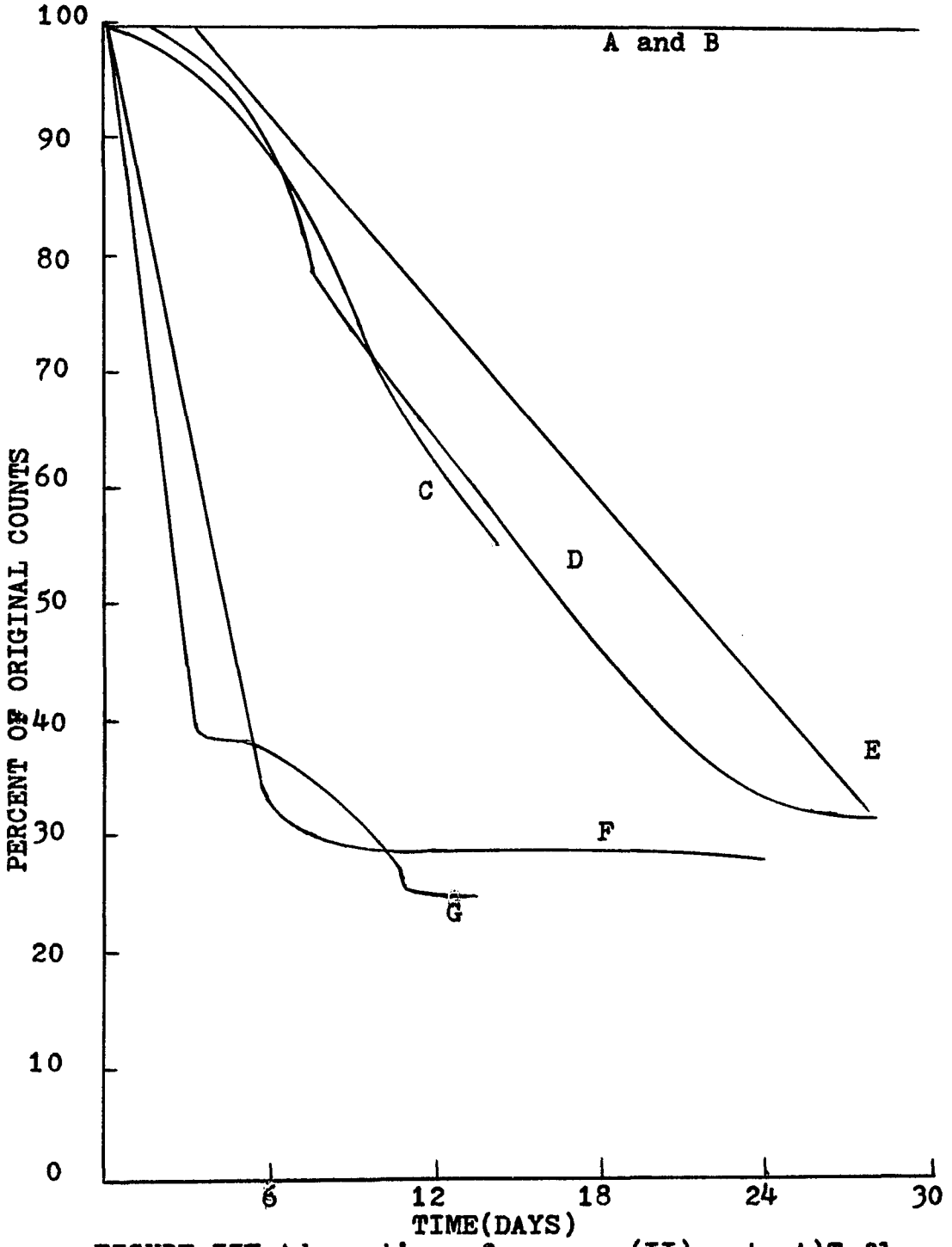

FIGURE III. Adsorption of mercury(II) onto A)Teflon 1.5 μg/ml B)Polyethylene, 3.41 μg/ml C)Teflon, 0.155 μg/ml D)Teflon, 0.015 μg/ml E)Teflon, 0.0015 μg/ml F)Polyethylene, 0.341 μg/ml G)Polyethylene 0.068 μg/ml. All solutions were 1 N in HNO_3. Total counts ranged from 10,000 to 800,000 total.

polyethylene. Adsorption on polyethylene can give significant loss of mercury in as little as four hours.

Reduction of the amount of mercury adsorbed onto glass surfaces was attempted by washing several new volumetric flasks with acid dichromate solution. However, no dichromate was added to the solutions to be studied for adsorption. It was observed that after one week there was a maximum of only 12% adsorption(using method I) at the lowest mercury concentration. It is known that chromium is strongly adsorbed by glass surfaces[32], from which it is inferred that chromium(either as the reduced chromic ion or as dichromate) occupied the available adsorption sites on the surfaces.

Solutions of $^{59}FeCl_3$ and carrier, in 0.5 N HCl which covered the concentration range from 1.09 to 0.001 μg/ml, showed no significant adsorption during a period of two weeks. The amount adsorbed was well below 1% of the total iron present, but of sufficient magnitude to be distinguished from background counts using the scaler.

During this study, it was found that only 60% of adsorbed mercury is removed after several washings with concentrated nitric acid. Several washings with acid-dichromate solution were necessary to completely remove the adsorbed

mercury. The behavior of adsorbed mercury appears to be unique. Similar studies with iron showed that adsorbed iron was easily removed by rinsing with deionized water.

Adsorption of mercury onto glass was also observed from solutions of mercury dithizonates and mercury complexes with tri-n-octylphosphineoxide in carbon tetrachloride, the amount adsorbed increasing with the time of contact. This will be discussed further in Chapter VI with regard to the stability of the complexes.

A possible loss of mercury can occur during the sealing of the quartz tubes in the preparation of standards for activation. The method that was used to determine if loss occurred, optimized conditions for mercury loss. The tubes were filled to 70% of their capacity and not frozen in liquid nitrogen, whereas samples are usually filled to only 50% of the tube's capacity and frozen. It was found that under these unfavorable conditions less than 1% of the initial activity from the mercury tracer is lost, even from the most dilute solutions.

The only other possible source of error in the standards preparation is that of diminished activity due to flux depression during irradiation. Since the Brookhaven reactor

does not have a rotating rack facility, there was no reason to assume that each sample would receive the same neutron flux in different positions of the rabbit. However, as can be seen in Figure IV, the curves for activity versus quantity of mercury for samples at the inside and outside positions, are identical, thus proving that in these irradiations there is no flux depression. This means that all possible sources of error for standards preparation and analysis(except counting geometry, which is discussed in Chapter VIII) have been examined and found to yield no significant loss when treated in the manner described here.

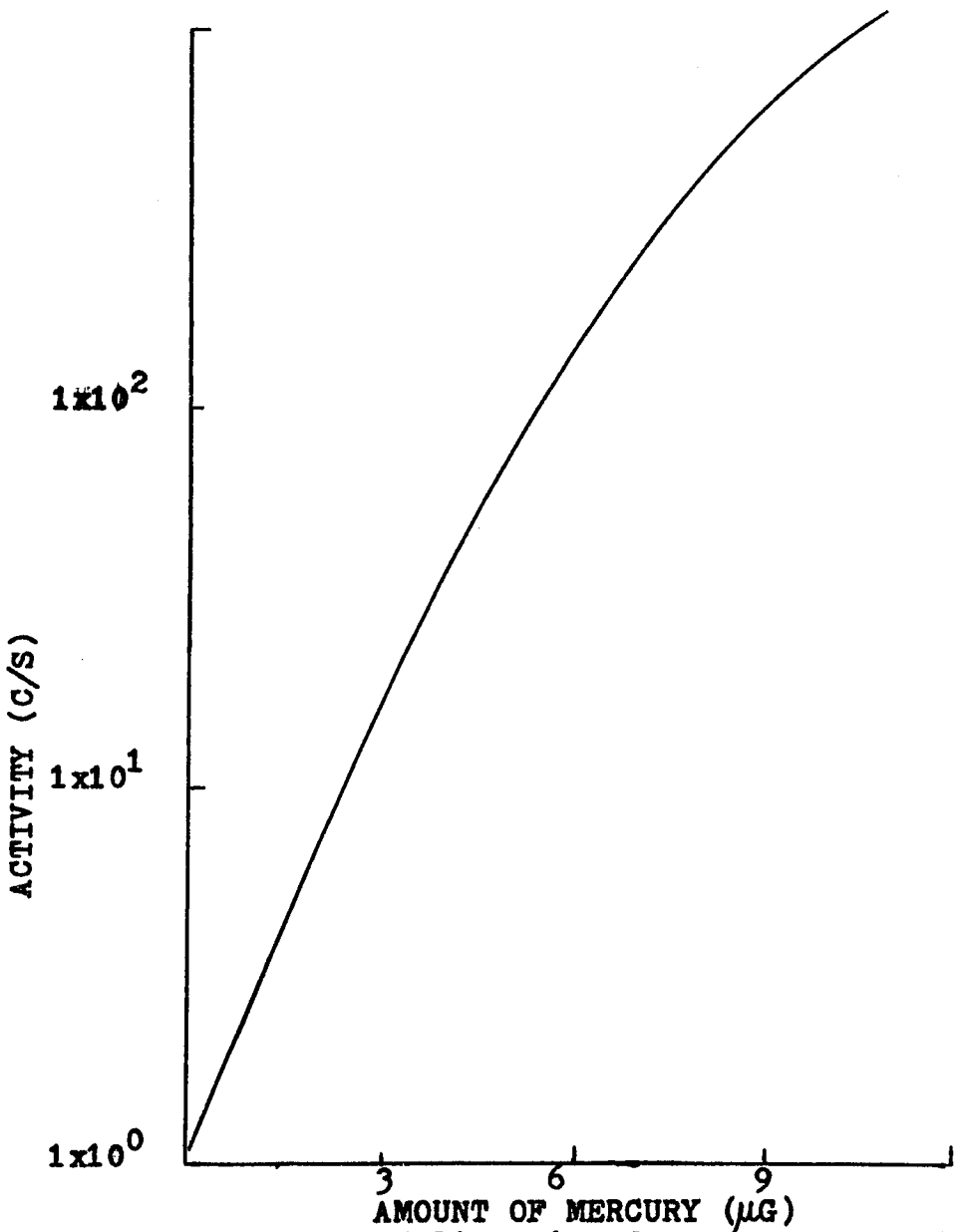

FIGURE IV. Calibration for mercury standards for inside and outside positions of the rabbit.

III. Wet Digestion and Dry Combustion

In most analyses it is necessary that the sample and standards be in the same physical form i.e., a solid, solution or vapor. The most convenient physical state for total mercury analysis is usually a solution. In order to achieve this, complete destruction of organic material present was accomplished by a standard method of wet digestion. However, analysis for mercury in an organic matrix presents problems due to its ease of electrochemical reduction and subsequent volatilization. The classical methods of wet digestion suffer from significant losses of mercury. The extent of these losses has been examined, and an alternate method of dry combustion has been explored.

Experimental Procedures

Wet digestion. Simulated samples for digestion studies consisted of 1 to 2 g samples of flour plus varying amounts of mercury tracer and carrier, corresponding to a total mercury content of 0.01 to 1μg. Concentrated nitric acid was added first, followed by slow addition of sulfuric acid to minimize the evolution of N_2O_4. A total of 20 ml of each acid was added and the final temperature was 60°C. The clear solution, after the digestion, was quantitatively transferred into a 100 ml volu-

metric flask and diluted to the mark with deionized water. This digestion procedure was also applied to two portions of flour doped with $^{59}FeCl_3$ and carrier, total iron content of each were 1.0 and 0.10 μg, respectively.

In a second digestion procedure, 5 to 10 ml of concentrated nitric acid was added to the simulated sample and the mixture stirred at 0^0C for five minutes before slow addition of 20 ml of concentrated sulfuric acid and 15 ml of concentrated nitric acid. The reaction temperature was not allowed to exceed 30^0C during the entire period of digestion.

A third procedure was performed on an environmental sample using the procedure of Snell[13] and in this case aliquots of the sample, initial and final digestion mixture were compared for mercury content by neutron activation analysis.

Dry Combustion. The dry-combustion procedure[22] was applied to laboratory standards, sediment samples(wet and lyophilized), and NBS SRMs following neutron irradiation; and also to tracer doped flour. Approximately 30 mg of mercuric oxide was added to the sample as carrier, prior to combustion. The combustion tube(Figure V) was flamed to red heat, in order to ensure that all the volatile oxidation products were flushed into the liquid-nitrogen condenser. These

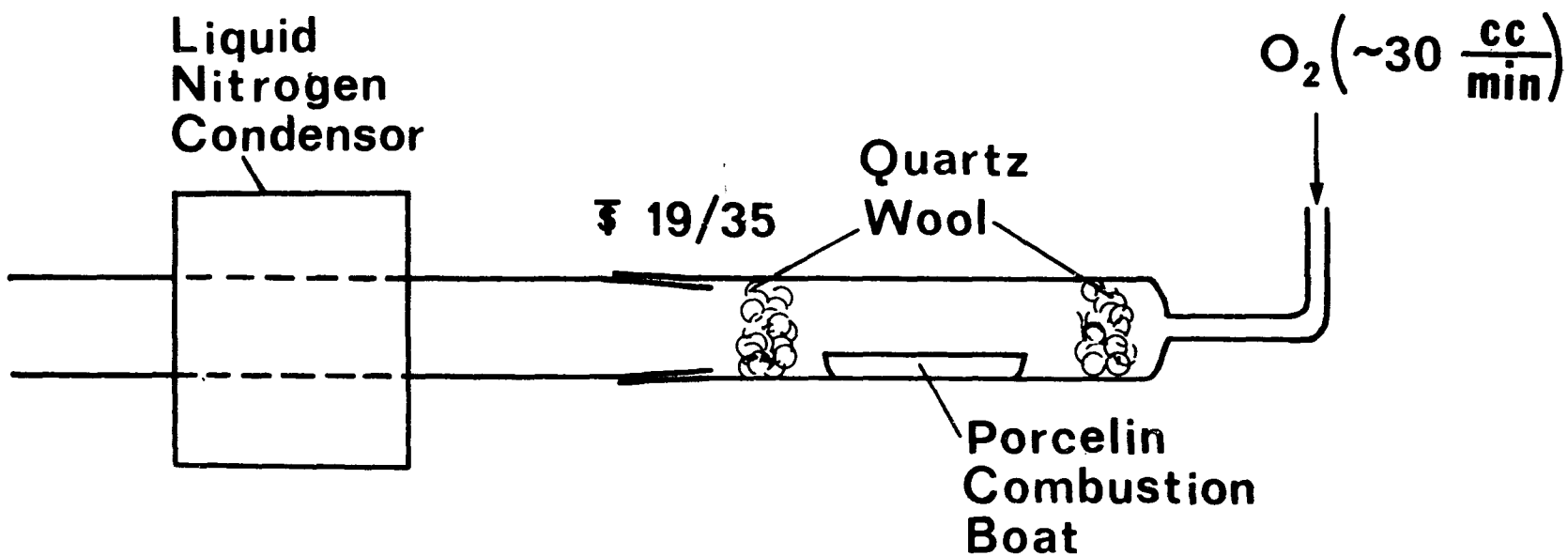

FIGURE V. Dry Combustion Apparatus

combustion products were then washed into a 50 ml flask (using concentrated nitric acid), and the flask warmed on a hot plate to be certain that all the mercury was dissolved. The solution was made basic with ammonium hydroxide, the mercury precipitated as the sulfide using thioacetamide, and collected on a Millipore filter.

Reagents Nitric and sulfuric acids, mercuric oxide, potassium permanganate, ammonium hydroxide, thioacetamide, and potassium persulfate were Baker Analyzed Reagents, National Bureau of Standards Standard Reference Material (NBS SRM's); 1571 (Bovine Liver), 1577 (Orchard Leaves) and 1630 (Coal) were used as obtained. Portions of each sample were lyophilized enabling subsequent sample-weight correction to dry-weight basis. Hecker's unbleached flour was used as available, as a representative matrix for digestion.

Results and Discussion

The classical methods of wet digestion suffer two inherent disadvantages; the concommittant dilution of the initial mercury concentration in the sample and the addition of large quantities of reagents which themselves contain trace mercury. Complete digestion of a 1 g sample typically requires 60 to 80 g of acid. If these acids contain an

average mercury concentration of 1 ng/g there is a reagent contribution of at least 60 ng of mercury. If the sample itself only contains 10 ng of mercury, the determination requires subtraction of a very large reagent blank, resulting in large uncertainties in the final value for the mercury concentration. Another factor which has been considered in the digestion procedure is possible loss of mercury by entrainment of water droplets in the water vapor. This loss cannot be due to volatility of the mercury salts in solution, which at this temperature is insignificant. The work of Menis [25] suggests the possibility of loss by entrainment, as does this work, the results of which are summarized in Table 2. Menis followed the classical method of Nitric Acid digestion but showed that by packing his condenser with Raschig rings he could obtain results that were 30% higher than without them, on the Standard Reference Materials; the higher of the two results agreeing with the NBS certified value for mercury. The low temperature (30°C) digestion using tracer, shows that at a lower temperature there were significantly smaller losses. A possible explanation is the less water volatilized, the less chance for entrainment of droplets containing mercury, (or as

TABLE 2
Loss of Mercury During Destruction of Organic Material

Method	Maximum Temperature	Mercury Concentration[1] (μg/ml)	Mercury Lost[2]
Wet Ashing[3]	60°C	0.0075	34.6%
Wet Ashing	60°C	0.075	27.4
Wet Ashing[4]	30°C	0.75	5.6
Wet Ashing	30°C	0.075	4.3
Wet Ashing	30°C	0.0037	1.1
Permanganate-Persulfate	70°C	1.00	34.0

[1]This column represents the mercury concentration which would be present in the digestate assuming no loss.

[2]Variance among duplicate samples was less than 5%.

[3]Wet ashing procedure of the Association of Official Analytical Chemists.

[4]Digestion flask was cooled in an ice bath during reagent addition otherwise same procedure as 3.

suggested by Menis [25], there is less chance for mercury metal, produced from reduction of mercuric ion, to be volatilized.)

The classical method of nitric acid digestion was also applied to flour samples doped with iron tracer plus carrier. There was no loss of iron observed in these experiments. This suggests that entrainment was not the mechanism by which mercury was lost, for if it was, there would have also been a loss of iron during the digestion.

This loss of mercury occurs not only during acid digestions but also during permanganate-persulfate digestion. The digestion of an actual environmental sample was monitored by analyzing aliquots of the digestion mixture at the beginning and at the end of the digestion, by activation analysis. Mercury in the reagents used was also monitored by instrumental activation analysis, and was found to contribute 1 μg of mercury to a sample containing 10 μg (initially). This sample lost 34% of its initial mercury content upon digestion, demonstrating that this method of digestion is also objectionable.

Analysis of Baker Analyzed sulfuric acid showed a mercury concentration of 0.050 μg/g, fifty times greater than the manufacturer's analysis listed on the bottle. This finding

indicates that all reagents must be checked for mercury content prior to each analysis ensuring that at least the reagent blank will be reliable.

The first experiments using the dry-combustion technique also indicated loss of mercury. However, the technique is very sensitive to poor heating of the combustion tube. That is, the entire tube must come to red heat, to ensure total decomposition of the mercuric oxide to mercury metal which is then flushed into the liquid nitrogen condenser. Furthermore, in the removal of the mercury from the condenser, the concentrated nitric acid removes all the combustion products from the condenser but does not immediately dissolve all the mercury (it should be kept in mind that mercury was present as carrier, so that there was a considerable amount present after the combustion). Therefore, it is necessary to warm the condenser washings until all the mercury metal is dissolved; the solution is then cooled and mercuric sulfide precipitated. When performed in this manner quantitative results are always obtained. Dry combustion of neutron irridated NBS SRM's 1571, 1577, and 1630, and subsequent x-ray analysis yielded the same values for the mercury concentration as the certified value.

IV. Lyophilization

Lyophilization of environmental samples is of great value in that it shrinks the size of the sample considerably, and in most cases obviates refrigeration. For example, as seen in Table 3, the percent water in those samples is upwards of 70%(except the sediment). This is true of most marine organisms as well as most food staples. Obviously, eliminating 70% of a sample's weight will facilitate its storage, and permit simultaneous collection of a greater number of samples.

Unfortunately, the trace mercury concentration of lyophilized samples is diminished during the process of freeze-drying. The extent of this loss in various environmental samples has been studied and a mechanism for this loss has been suggested.

Experimental Procedures

A VirTis lyophilization unit(model 10-100) was used exclusively. There was a liquid-nitrogen cold trap for each individual sample, in addition to the main trap of the unit which was maintained at -50°C(Figure VI). Samples in lyophilization bottles were initially frozen at dry-ice-isopropanol or liquid-nitrogen temperature, connected to the

TABLE 3
Lyophilization of Environmental and Laboratory Samples

Sample	Water Content Percent	Mercury Concentration[1] Before	After	Percent[2] Mercury Lost
$(CH_3)_2Hg$(on flour)	---	0.440 µg/g	0.202 µg/g	54
$Hg(NO_3)_2$(on flour)	---	0.0869	0.0470	45
Sediment #1	34	0.235	0.213	8
Sediment #2	20	0.126	0.0973	22
Sediment #3	44	0.615	0.424	31
Squid	78	8.8	2.5	71
Butterfish	74	7.8	2.3	70
Sea Cucumber	89	12.4	5.1	59

[1]Corrected to a dry weight basis.

[2]Uncertainty of duplicate samples is ± 7%, maximum.

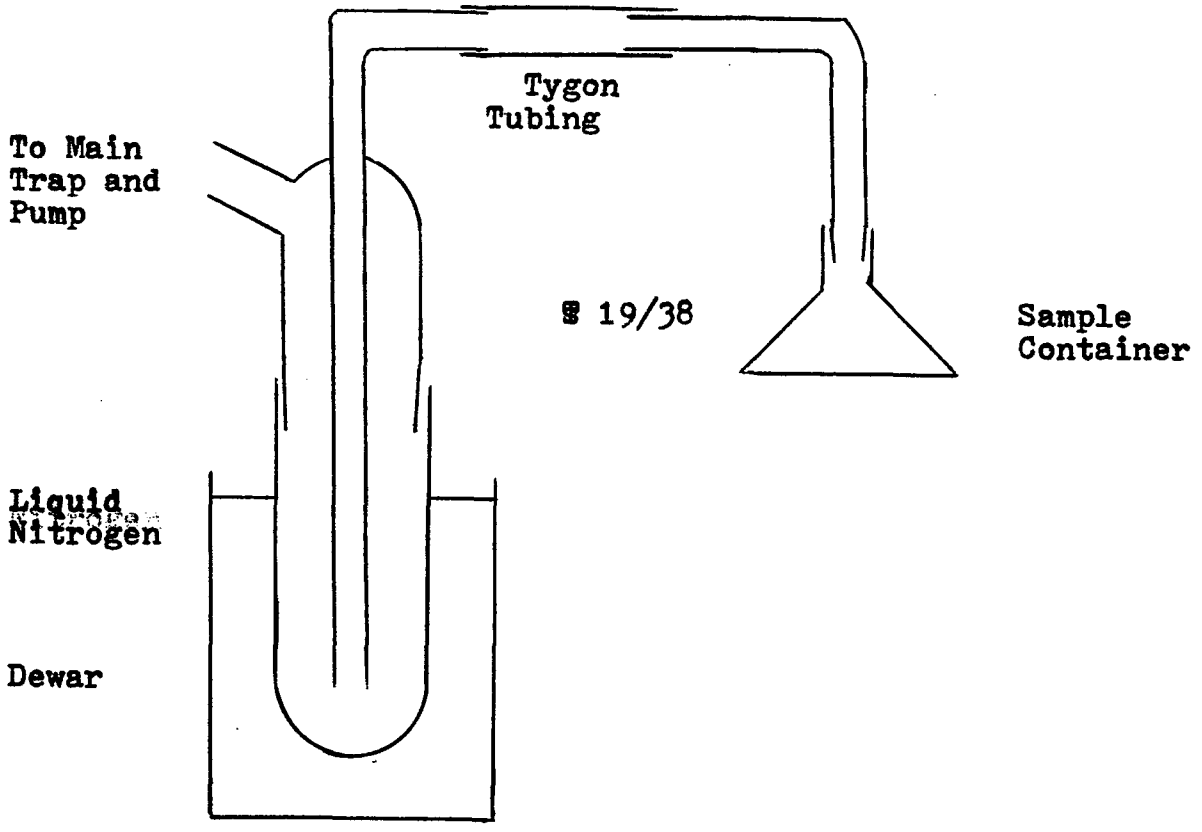

Figure VI. Lyophilization Unit

unit, the pressure reduced, and the samples allowed to warm to room temperature, over a period of between 6 and 24 hours. A pressure of 0.1 torr, as measured with a McLeod gauge, was established for samples which were subsequently irradiated. The gauge was disconnected after pressure measurement.

Liquid Samples. Solutions of $^{203}Hg(NO_3)_2$, with carrier, of concentration from 1.5 μg/ml to 4.5 ng/ml, were lyophilized at pressures ranging from 0.01 to 1.0 torr, in order to determine if pressure affected the amount of mercury lost during a 6-8 hour lyophilization.

A sample of synthetic seawater was prepared according to the concentrations reported by Horne[34] with sufficient mercury tracer and carrier added to bring the mercury concentration up to 0.015 μg/ml. This sample was lyophilized in the same manner as described above.

Solid Samples. Portions of flour were added to each of two roundbottom flasks, followed by addition of 50 ml of deionized water, in order to make a slurry. A freshly prepared solution of dimethylmercury(250 ml of a 1.46×10^{-1} ppm solution, total mercury = 36.5 μg) was added to one flask, and to the other was added mercuric nitrate(2.0 ml of a

9.13 ppm solution, total mercury = 18.26 μg) and 248 ml of deionized water. Both flasks were stoppered and stirred for four hours at room temperature to assure homogeneity. The samples were filtered and aliquots taken of the filtrate and wet flour prior to lyophilization, and of the dried flour and the material caught in the trap after lyophilization.

Samples. Samples of fish and invertebrates were collected from Lower New York Bay. Sediment samples were taken from the area of the Atlantic Ocean just south of Atlantic Beach(N.Y. Inner Continental Shelf).

Reagents. Dimethylmercury(DMM) was obtained from Eastman Organic Chemicals and used without further purification. Silver wool, mercuric nitrate and potassium dichromate were Baker Analyzed Reagents. A solution of $AuCl_4^{-2}$ (10 ng/ml) in 0.5 M HCl, was donated by H.L. Rook.

Results and Discussion

In many laboratories samples are routinely lyophilized prior to analysis in order to reduce their volume. The effects of lyophilization on the loss of mercury as reported[25-27] might appear to be contradictory. This investigation shows that loss may occur, depending upon the concentration and chemical form of the mercury, thus it cannot

be assumed that a variety of samples will behave identically even under the same conditions.

A comparison of the vapor pressure of water and DMM at -50°C shows that the vapor pressure of the latter is 23 times greater than that of water. Since DMM is known to form as a result of bacterial action in sediment of aquatic systems[8,35], and in the presence of certain coenzymes[36], one could expect the presence of this compound and other compounds of similar volatility, and consequently organic mercury compounds could be lost during lyophilization. Table 3 shows a definite loss of DMM during the lyophilization of the fabricated laboratory samples. It is of interest to note that there are also large losses of ionic mercury, the volatility of which is negligible compared with that of water.

It was found that the amount of mercury lost from solutions of mercuric nitrate in 1 N nitric acid was proportional to the concentration. Samples of higher mercury concentration lose a smaller percentage of the initial amount of mercury than do samples of lower concentration. Solutions containing 15 μg of mercury lost between 3.75 and 4.25 μg of mercury at a pressure of 1.5 torr; for solutions

containing 0.750 μg there was 0.525 μg lost. The results of lyophilization of solutions of different mercury concentrations are summarized in Figure VII. When the pressure is reduced these samples lose an even greater percentage of mercury as shown in Figure VIII. These observations, would appear to support loss of mercury by entrainment; similar loss was also observed in the wet digestions performed on tracer-doped flour. If entrainment were the reason for loss it would be expected that the amount of mercury lost would be proportional to the concentration. The results observed for both lyophilization and digestion, i.e., the disproportionate loss of mercury appears not to substantiate a mechanism of loss by entrainment. If entrainment is the mechanism for loss, then it should also be possible to observe similar losses for other ions in solution. In order to further test this hypothesis the same procedure that was used for the lyophilization of solutions of mercury was used for the lyophilization of solutions of $^{59}FeCl_3$ with carrier, in 0.5 N HCl. The result of the lyophilization of these solutions, at an iron concentration of 1.09 μg/ml, and a pressure of 0.25 torr, was quantitative retention of the iron <u>in the sample container</u>. This startling result indicates that the rsults obtained

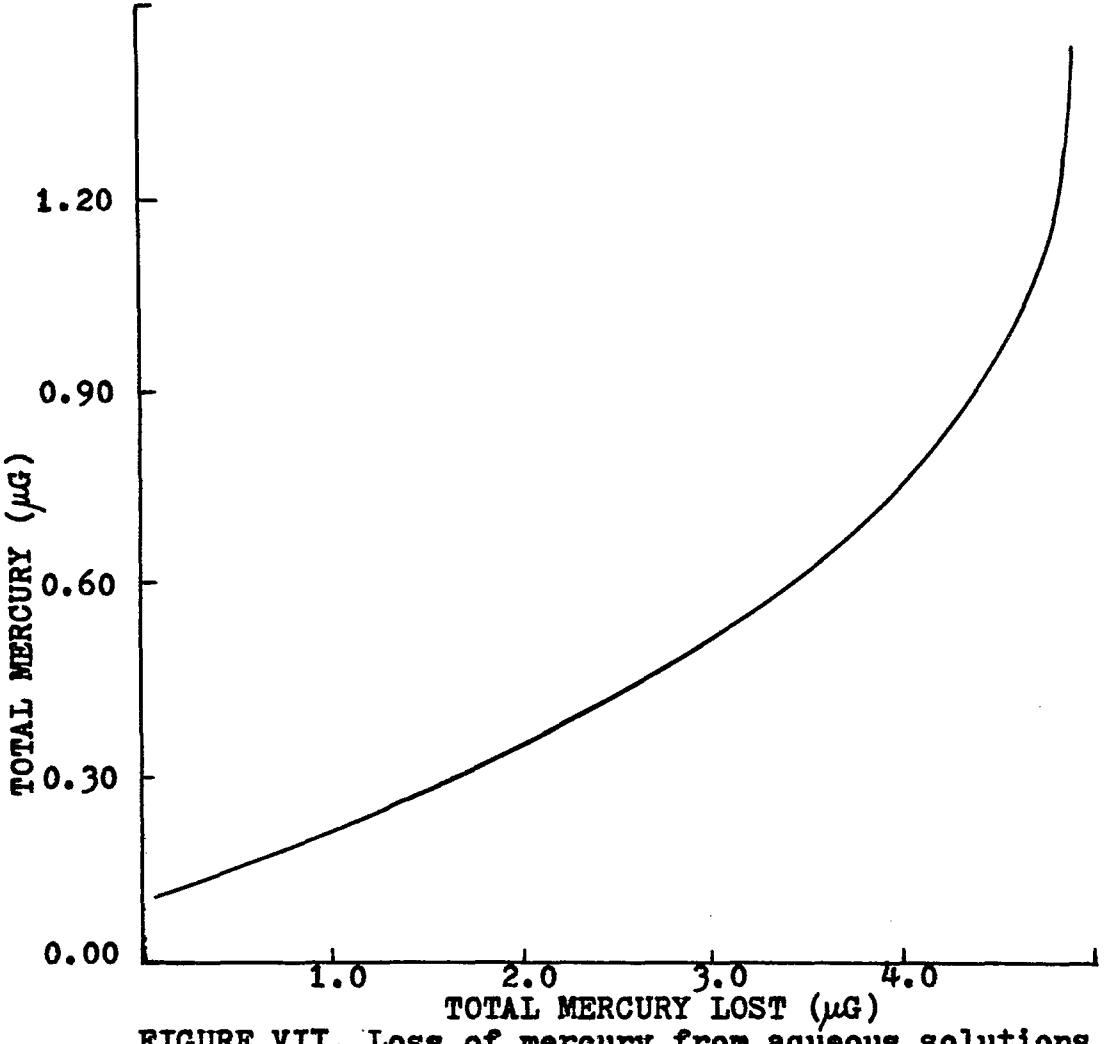

FIGURE VII. Loss of mercury from aqueous solutions as a function of the amount initially present during lyophilization. Volume of all solutions was 10 ml.

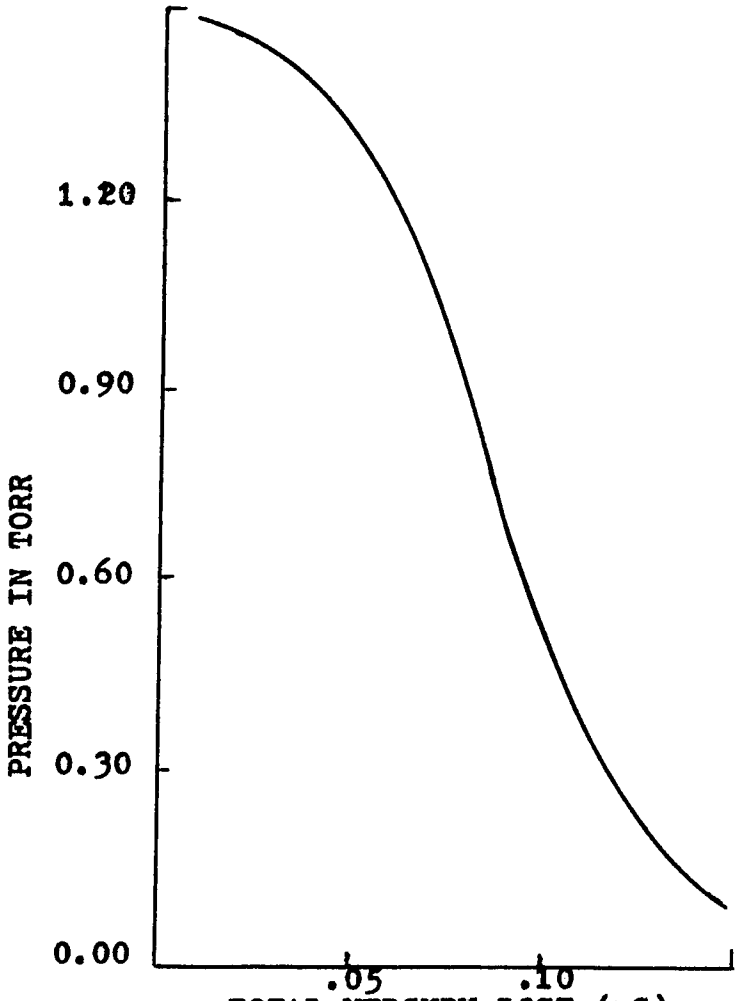

FIGURE VIII. Loss of mercury as a function of pressure, from aqueous solutions.

for mercury cannot be due to entrainment. It is possible that the mercuric ion is reduced to mercury metal; this is thermodynamically feasible according to the Nernst equation, substituting the activities of all the species present during the course of lyophilization. The nitric acid present is volatilized with the water during the lyophilization, as evidenced by the fact that the pH of the water caught in the primary trap was 1. Thus, the pH of the sample is increasing, making it more likely that the mercury can be spontaneously reduced(see appendix for Nernst equation calculations). Mercury metal, at this temperature and pressure, is a volatile constituent of the sample. If this is indeed the mechanism for mercury loss, the presence of a strong oxidant should keep mercury from being reduced. The two oxidizing agents, dichromate and tetrachloroaurate, were used to test this hypothesis in experiments identical to those already performed. These were the obvious oxidants to use because 1) they have previously been shown to prevent loss of mercury by adsorption[22,23] and 2) they are non-volatile.

Duplicate samples of mercury, 1 N in nitric acid, at a concentration of 1 ng/ml, were prepared, one of which con-

tained 10 ng/ml of $AuCl_4^-$. It was found that equal quantities of mercury were lost from both solutions(about 50%) upon lyophilization. Equal volumes of 1.5 μg/ml mercuric nitrate, 1 N in nitric acid, and 0.034 M in $K_2Cr_2O_7$ were lyophilized and also found to lose an amount of mercury equal to the amount lost from these same solutions without added dichromate. However, since nitric acid is being lost from solution, the dichromate cannot function as an oxidant because it needs 14 moles of H^+ per mole of dichromate in order to be effective. A solution of 100 ml of $^{203}Hg(NO_3)_2$ 1.5 μg/ml, 1 N in nitric acid and 0.55 ml of concentrated sulfuric acid was boiled down just to fumes of sulfuric acid. After cooling the volume was readjusted to 100 ml with deionized water; the sulfuric acid concentration was now 1 M. Three 10 ml portions of this solution(whose mercury concentration was now about 50% of its original value, see chapter on digestions) were taken, two of them made 0.017 M in dichromate, and then all three lyophilized. Since sulfuric acid shouldn't be lost, the dichromate should be able to fumction as an oxidant, and hence the mercury should remain as the ion. The pressure was decreased to 0.05 torr for 5 hours, after which most of the water was gone. The pressure then

rose to 0.3 torr for the next hour. Only the flask without the added dichromate had any sulfuric acid visible, the other two flasks appeared dry. However, this had no effect on the loss of mercury during the lyophilization. All samples lost approximately 60% of their original amount of mercury. Calculations based on the Nernst equation indicate that if the sulfuric acid was present in the solutions that contained dichromate then there certainly shouldn't have been any loss. It still appears that mercury is being reduced even in this strong oxidizing media, but it must be kept in mind that the Nernst equation is probably not applicable for an "aqueous solid solution". From the solutions containing only sulfuric acid, we would expect loss on the basis of the reduction potentials involved(see appendix).

Filby[37] has demonstrated that an increase in pH at constant mercury concentration decreases the amount of mercury lost during lyophilization. Lyophilization of a synthetic seawater sample(pH 7.4) with added mercury tracer, showed that less mercury was lost than at pH 1 at the same mercury concentration. This does not contradict the results of the previous experiments, because these solutions were

prepared prior to lyophilization, permitting the mercury present to form the hydrated oxide which is more difficult to reduce.

These results of the loss of mercury in these laboratory samples have significance. Homogenization of a sample often requires adding water and therefore the sample will approximate a solution. Prior to lyophilization all samples are frozen so that during the process they are all subject to the same type of chemical and physical changes, making possible losses similar to those observed in the aforementioned experiment.

These samples represented only the result of a simulated situation, and therefore it was necessary to determine that these losses could actually occur in an environmental sample. Table 3 shows a large loss of mercury due to lyophilization of biological samples. It is also seen in this same table, that sediment samples, whose mercury concentration was much less than that of the biological samples, lost much less mercury as well as a smaller percentage of the total initially present. The big difference between the two types of samples is the water content. We infer from these results that something more complicated than mere reduction and volatil-

ization.is taking place. Furthermore, the results obtained from lyophilization of the aqueous solutions, leads us to believe that mercury loss cannot be eliminated by maintaining an oxidizing medium during lyophilization.

It has been suggested[38] that baffles(such as inserting a glass wool plug in the line between the sample container and the trap) could reduce the amount of mercury carried into the trap, by providing a physical barrier and a site of adsorption, for the mercury atoms. This was investigated by lyophilization solutions of ionic mercury at a concentration of 1.5 μg/ml 1 N in nitric acid. The amount of mercury lost was the same as without the glass wool baffle, and there was no mercury found on the baffle.

In one reported study[39] mercury metal is collected from the atmosphere by passing large volumes of air over silver wool which traps mercury by amalgamation. Lyophilization of aqueous solutions was repeated with a silver wool baffle. Samples consisting of mercury tracer solutions(1.5μg/ml) were lyophilized with silver wool plugs placed in the arm of the sample container, in front of the Nalgon tubing(it was previously found that mercury was being deposited on the Nalgon tubing during lyophilization,Figure VI)which connects the sample container

with the cold trap. A third sample without any baffles, as before, served as a control. It was found that all three samples retained only 10% of the mercury initially present (pressure 0.06 torr). The samples which had the silver wool plugs, in contrast with the sample without the silver wool plug, had no activity in the traps or in the Nalgon Tubing. Although only about 60% of the mercury lost was accounted for by counting the silver wool, quantitative recovery is inferred(the efficiency for counting the silver wool was less than the efficiency for counting the tracer solution). The control sample had 9.4% of the original activity on the Nalgon tubing and 18% in the trap; The remaining 62% was not recovered. The mercury probably adheres to the Nalgon tubing because it is made of polyvinyl chloride, which possibly oxidizes the mercury metal to mercuric ion.

This shows conclusively that mercuric ion is being reduced to the metal during the lyophilization, and it is the metal that is volatilized. It is clear from this data, that loss of mercury from any sample is a possibility, and that the loss cannot be easily prevented, or accounted for. Furthermore, because this problem exists for mercury, it is possible that it will occur to some extent with other elements whose compounds in an

environmental sample are volatile, or which can be reduced to a volatile compound. Evidence for this was found in the analyses of the peaks of the gamma-ray spectra of the lyophilized and unlyophilized sediment samples. This was determined by counting the samples within a few hours of each other(two days after irradiation, to be certain that any elements whose half lives were shorter than ten hours had decayed) to minimize corrections due to decay, and the unlyophilized sample was always counted second. Although many of the peaks were not identified, it was found that some of them had activities that decreased as much as 50% by lyophilization. The activity due to bromine was found to be only 90% of its original activity before lyophilization.

V. Solvent Extraction

Concentration of trace mercury prior to analysis, has been achieved by various methods of solvent extraction, in particular, using the complexing agent dithizone. The mole ratio of the extracted complex has been assumed to be one mole of mercuric ion to two moles of dithizone anion[40] (i.e., dithizone minus H^+). This extraction has been considered very efficient[14] for concentrations of mercury in the part per million range.

Another solvent extraction system, using tri-n-octylphosphineoxide(TOPO) and/or thenoyltrifluoroacetylacetone (TTA) has been tested and a recommendation for the concentration of mercury with TOPO is presented.

Experimental Procedures

Extractions. Extractions of tracer solutions were performed manually as noted, otherwise in 25 ml capped glass burets (with Teflon stopcocks), mounted on a platform shaker. The solutions extracted were adjusted to pH 1 and pH 4 using nitric acid, aqueous ammonia or acetate buffer(0.1 M). Fresh solutions of dithizone were prepared regularly because of the compound's known instability in solution. For this same reason, shaking times for the extractions were limited

to a maximum of ½ hour and an excess of dithizone was always present. Solutions of TOPO and TTA(both 0.4 M in carbon tetrachloride) were diluted as necessary.

Tracer solutions of $^{203}Hg(NO_3)_2$ were prepared from a stock solution of 1.5 μg/ml in 1 N nitric acid to cover a concentration range from 1.5 to 0.015 μg/ml. The volume of aqueous and organic phases in the extraction were the same to obtain reproducible counting geometry. The salt concentration of the aqueous phase was either zero or made 1 M in NaCl or $NaNO_3$(by addition of these solids to the tracer solution), at constant mercury concentration, to determine if there is a salt effect.

Reagents. Diphenylthiocarbazone(dithizone), tri-n-octylphosphineoxide(TOPO), and thenoyltrifluoroacetylacetone(TTA) were obtaine from Eastman Organic Chemicals. Reagent grade carbon tetrachloride, methylisobutylketone(MIBK) and benzene were used for the extractions. All reagents were used as received without any further purification.

Counting. All solutions were counted in 10 ml volumetric flasks which fit snugly into the well of the NaI(Tl) detector. The same volume of solution was used in each instance and hence reproducible counting geometry was easily achieved.

Results and Discussion

Preconcentration of mercury for analysis of environmental samples has been achieved by either ion exchange[40], extraction[41] or lyophilization. Solvent extraction of mercury by the dithizone method has been extensively used because it can be made specific and it rapidly forms a highly colored complex. However, efficiency of the extraction at very low mercury concentrations, and stability of the complex formed, have never been adequately studied. The extraction efficiency was determined by calculating both the distribution coefficient[42], K, where,

$$K = \frac{(Hg^{2+})_{org}}{(Hg^{2+})_{aq}}, \qquad \text{(following extraction)} \quad \text{(I)}$$

and the percent extracted using,

$$\text{Percent Extracted} = \frac{(Hg^{2+})_{org} \times 100}{(Hg^{2+})_{\substack{\text{initially present} \\ \text{in aqueous phase}}}}. \qquad \text{(II)}$$

The classical method of expressing percent extracted uses the equation[41],

$$\text{Percent Extracted} = \frac{K \times 100}{K + (V_{aq}/V_{org})}. \qquad \text{(III)}$$

According to this equation, for K much greater than V_{aq}/V_{org},

the percent extracted will always be 100%. Neither expression I nor III are valid in this case because of the significant loss of mercury by adsorption. It will be shown that in this extraction procedure, quantitative recovery cannot be achieved and hence the second of these equations only, should be used.

The activity of ^{203}Hg is directly proportional to the amount of mercury present(as was demonstrated in Chapter II), and since equal volumes of organic and aqueous phases were used, the ratio of these activities in 10 ml aliquots is the means of determining the distribution coefficient and percent extracted. The distribution coefficient for a solution of mercuric nitrate(0.15 μg/ml, in 1 N nitric acid) shaken with dithizone in carbon tetrachloride for 1 to 10 minutes, would appear to be 181, but only 90% of the mercury initially present in the aqueous phase is extracted. The remaining mercury is not entirely found in the aqueous phase but also adsorbed on the glass surface of the extraction vessel. Dithizone in MIBK, under the same conditions yields an apparent distribution coefficient of 120 and an extraction efficiency of greater than or equal to 95%. Unfortunately, the dithizone is very short lived in MIBK(in carbon tetra-

chloride it is stable in a dark, cool place for about 1 week, but even with this precaution there is still some decomposition) within a few hours(at a concentration of 6 mg/l) turning completely orange. This is also what happens to dithizone in carbon tetrachloride after exposure to room light for a day or so. Decreasing the mercury concentration to 0.015 μg/ml lowers the apparent distribution coefficient and the percent extracted in carbon tetrachloride as shown in Tables 4 and 5. It can be seen that increased shaking time can decrease the percent extracted, and increase the amount adsorbed onto the glass surfaces.

The dithizone extractions were carried out in nitric acid media because samples for mercury analysis are either acidified or digested using nitric and/or sulfuric acid. The classical method[11] of extraction requires that the pH of the solution should be adjusted to four(with acetate buffer) in order to selectively remove mercury. Heating the solution of the sample to remove the nitrate, by decomposition to N_2O_4, would present problems such as those encountered during sample digestion and storage(see Chapter II and appendix). This means that even though nitrate a medium is poor for solvent extractions, there will be an appreciable nitrate concentration present. It is also found

TABLE 4

Percent Extraction of Mercury with Dithizone in CCl_4

Concentration / Time (minutes)	0.075 µg/ml		0.060 µg/ml		0.015 µg/ml	
	pH 4	pH 1	pH 4	pH 1	pH 4	pH 1
1	65%	94%	51%	83%	50%	64%
15	75	99	55	85	39	11
30	92	98	65	90	37	12

TABLE 5
Distribution Ratios of Mercury with Dithizone in CCl_4

Concentration	0.075 μg/ml		0.060 μg/ml		0.015 μg/ml	
	pH 4	pH 1	pH 4	pH 1	pH 4	pH 1
Time(minutes)						
1	160	170	180	180	190	180
15	180	180	160	160	90	70
30	180	180	160	160	90	70

that if acetate buffer is employed, we can attain 94% efficiency after one minute, whereas with nitric acid we needed 15-20 minutes to attain this efficiency. These extractions using acetate, did not contain any appreciable salt concentration(as would be found in an environmental sample) consequently these results do not accurately represent what can be expected after collection and digestion of an environmental sample. It must also be kept in mind that if the aqueous phase(total volume about 100 ml) was the result of a digestion of a one-gram sample, a mercury concentration from 0.15 to 0.015 μg/ml(before extraction) would correspond to a mercury concentration of between 15 and 1.5 μg/g(i.e., ppm) in the sample. Therefore for any real sample with a mercury concentration lower than this range, the procedure of digestion and extraction will lead to incomplete recovery of the mercury from solution.

Furthermore, it was observed that increasing the acid or salt concentration(by addition of concentrated acid or solid salt) decreases the amount of mercury extracted by approximately 10%. The concentration of chloride ion had a considerable effect, probably due to the formation of the complex $HgCl_4^{2-}$ in the aqueous phase, a type of salt-

ing-in effect. It has also been reported that successful extraction of mercury[11] can take place from solutions 6 M in sulfuric acid. At these low concentrations of mercury this is found not to be the case. At high acid concentration regardless of the acid species, the dithizone is converted into a yellow oxidation product, which is ineffective as an extracting agent. This was demonstrated by the following study. Four flour samples doped with mercury tracer were digested; two with concentrated nitric acid and two with 30 ml of concentrated sulfuric acid plus 20 ml of 8 M nitric acid. These digestates, whose final mercury concentrations were 10-20 ng/ml, were then extracted with a fresh dithizone solution. The extract of the nitric acid digestion was found to contain 55% of the original tracer activity in the digested sample, and the extract of the mixed-acid digestate was found to contain only 1% of its original activity. Increasing the time of extraction had no effect on these efficiencies.

The extraction procedure using dithizone also includes addition of EDTA as a holdback agent for zinc and copper, which are also extracted under these conditions. However, addition of EDTA also has the effect of decreasing the per-

cent mercury extracted by 10-20%.

These findings prompted an examination of the extraction of mercury by the solvents alone, in order to determine if high anion concentration affects the efficiency of extraction. The distribution coefficients for carbon tetrachloride, MIBK, and benzene were found to vary as a function of acid, anion and mercury concentration, as can be seen in Table 6. It is plausible that a mercury complex is formed, similar to the $HgCl_4^{2-}$ complex, which is not easily extracted by solvents or dithizone. The distribution coefficients for each of the solvents decreases by a factor of ten, upon addition of 1 M NaCl and by only 40% upon addition of the same concentration of $NaNO_3$. The decrease in the distribution coefficient due to nitrate can be explained in terms of the results of an experiment by Plane[43], in which he observed formation of a complex between mercury, nitrate and acetone. It is reasonable to assume that such a complex would also be formed with MIBK, hence explaining its large distribution coefficient, with respect to benzene and carbon tetrachloride, and also its small change upon addition of nitrate. Therefore, it might be expected from these observations, that dithizone(a thio-

TABLE 6

Distribution Ratios* for Pure Solvents at Various Acid and Salt Concentrations

Medium / Solvent	0.1 M HNO_3	0.1 M HCl	1.0 M HNO_3	1.0 M HCl	1.0 M Cl^- + 0.1 M HNO_3
Carbon Tetrachloride	0.109	0.02	0.02	0.02	0.004
MIBK	10.0	2.20	3.00	0.22	0.22
Benzene	0.040	0.04	0.004	0.004	0.002

*Mercury concentration in all cases was 0.15 μg/ml.

ketone) could also form this complex. Thus we can hypothesize a complex comprised of a mercury(II) ion, a molecule of dithizone and an anion. This is explored further in Chapter VI.

As a result of the shortcomings of the dithizone extraction, another extraction system was sought which might provide a more stable complex and a more consistent extraction efficiency. TTA was investigated as an extractant, and an efficiency of only 26% was achieved, independant of shaking time, from solutions of nitric acid at pH 1 and pH 4, and a mercury concentration of 0.075 μg/ml. It has been reported that TOPO also incompletely extracts mercury[44]. Using solutions of concentration from 0.4 to 0.01 M in carbon tetrachloride, an attempt was made to determine whether a synergic effect occurred with TOPO and TTA. The results of this study are shown in Table 7. TOPO, when used by itself, achieved 100% extraction after 1 minute(using a separatory funnel and shaking manually) for mercury concentrations in the range 2 to 0.02 μg/ml, at pH 1. This efficiency decreased with increased shaking time. When TOPO is used with TTA, the percent extracted increases with time to a maximum of 50%, which is evidence for a hindrance of extraction of TOPO by TTA at these mercury concentrations.

TABLE 7

Percent Mercury Extracted* by TTA and TOPO, 10 Minutes Shaking in a 25 ml Buret(pH 1/pH 4).

TTA \ TOPO	0.0 M	0.01 M	0.10 M	0.20 M	0.40 M
0.00 M		12/12	55/55	100/95	75/70
0.01 M	18/18				
0.10 M	26/26		70/61	84/84	
0.25 M	25/25			44/44	

*Mercury concentration in all cases was 0.075 μg/ml.

One of the shortcomings of using TOPO as an extractant is that it does not develop a color. An extraction using a mixture of dithizone and TOPO was tried, in order to see if the dithizone would work as an indicator for the TOPO, or if the TOPO would act as a stabilizing agent for the dithizone complex. However, the combination of dithizone and TOPO immediately turned yellow. In addition, solutions of mercury and TOPO are stripped back into the aqueous phase with only 50% efficiency.

The stabilities of the TOPO and dithizone complexes were compared by determining the amount of mercury adsorbed by glass surfaces from solutions of each complex at the same concentration. Several samples of mercury were extracted in the manner described, using an equimolar excess of TOPO or dithizone. The procedure used was that of adsorption method I(see Chapter II). Figure IX shows the percent mercury adsorbed from solutions of equal mercury complex concentration in the organic layer. The results obtained for the dithizone complex are not inconsistent with the known instability of dithizone in solution(the instability of the complex is examined more closely in the next chapter). Although an exact value for the reduction potential of dithizone has not been established, it is reasonable to assume that the

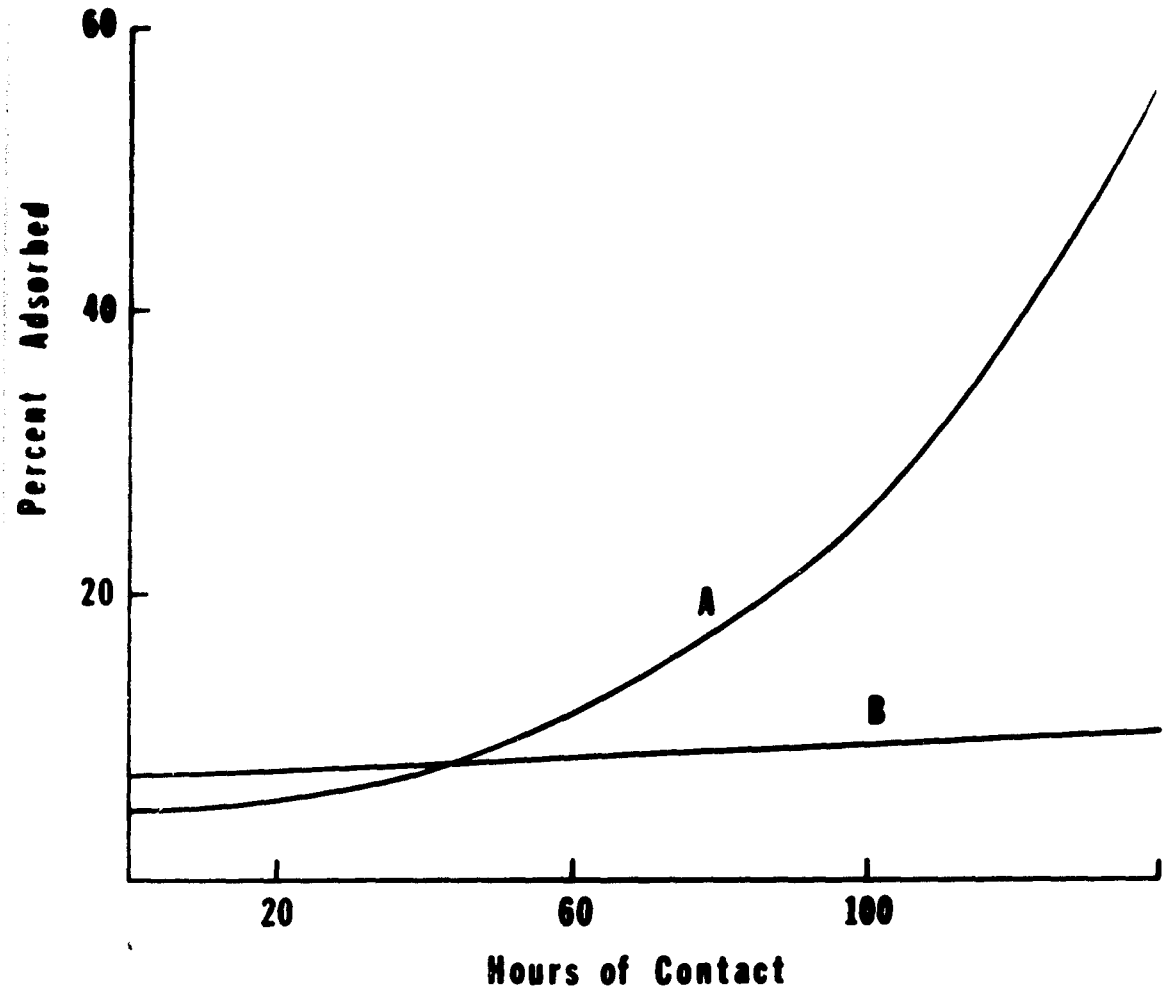

FIGURE IX. Percent Mercury Adsorbed with Increasing Time of Contact. A)0.075 μg/ml, Hg^{2+}(Dithizone in Carbon Tetrachloride) B)0.075 μg/ml, Hg^{2+}(Dithizone and TOPO in Carbon Tetrachloride).

mercury-dithizone complex is forming its own redox couple. Even if the complex formed a simple dissociation-association equilibrium, the Hg^{2+} in the absence of a strong oxidant would spontaneously be reduced to the metal. Thus, it is possible that the high percentage of mercury adsorbed from these solutions can be explained by reduction of the complex. TOPO is not oxidized under these conditions, and is not an oxidizing agent either. Therefore, it would also be expected that mercury would be reduced in this solution, but not as fast as in the dithizone solution, as shown in Figure IX.

VI. Spectrophotometric Determination of Mercury

The extraction of mercury using dithizone is not only used for isolation of mercury, but also for the determination of mercury concentration in a sample. The complex which is formed, has an absorption maximum in the visible region of the electromagnetic spectrum, and comparison of an unknown's absorbance to those absorbances from a series of standards will yield the unknown's concentration. However, it has been noted that dithizone and its mercury complex are unstable, and decompose when exposed to room light. The stability of ionic and organic mercury complexes with dithizone has been examined under a variety of conditions, and it has been observed that there is a considerable degree of instability of these complexes.

Experimental Procedures

Absorbance Spectra. The stability of both ionic and organic mercury dithizone complexes was monitored by recording their absorbance spectra from 520 to 380 nm, using a Cary 17 Spectrophotometer. The Cary 17 has an accuracy of approximately 0.002 absorbance units, between absorbances of 0.1 and 1.0, the region where all absorbances were measured. Ionic-mercury complexes were formed by manually shaking sol-

utions of $Hg(NO_3)_2$, of concentration 0.375 to 0.015 $\mu g/ml$ with a stock solution of 2.35×10^{-5}M dithizone (6 mg in 1 liter of carbon tetrachloride).

Solutions of dimethylmercury (12 mg in 100 ml of carbon tetrachloride) and diphenylmercury (18 mg in 100 ml of carbon tetrachloride) were freshly prepared; 1.5 ml aliquots of each of these solutions were separately added to 1.5 ml aliquots of the dithizone solution, in absorbance cells (no mixing is required, complexation is spontaneous). Duplicate samples of DMM and DPM in carbon tetrachloride with added dithizone were equilibrated with an aqueous phase maintained at either pH 1 or pH 4, and the organic phase then drained into the absorbance cell. The absorbance spectra of these solutions were recorded immediately following their preparation.

Solutions of methylmercuric chloride, of concentration from 11.3 to 1.13 $\mu g/ml$ of mercury, were prepared by dilution of a stock solution (13 mg in 100 ml of 0.1 N HCl). Aliquots of the final solutions were reacted with at least an equimolar amount of dithizone (using the stock solution of dithizone), at pH 1 (using HCl, HNO_3 or $HClO_4$) or pH 4 (using HNO_3 or an acetate buffer).

Complexes were formed for absorbance-spectra studies by manually shaking in a 60-ml separatory funnel(whose stem was drawn to a fine tip to exclude as much aqueous solution as possible) for 1 minute, after which the organic layer was immediately transferred into the absorbance cell and the spectra recorded(total time was about 3 minutes). The spectrum of the sample was recorded, after separation, at 3 to 7 minute intervals for 40 minutes. During these intervals samples were removed from the spectrometer and exposed to room light.

The effect of ionic strength of the aqueous phase on the extraction of the mercury-dithizone complex was studied by varying the salt concentration, at constant mercury concentration. The interval between separation and recording of spectra was maintained as constant as possible(to within 5 seconds). A solution of $Hg(ClO_4)_2$ was prepared by dissolving 2.39 mg of HgO in 1 liter of 0.1 M $HClO_4$(mercury concentration = 1.1×10^{-4} M). Portions of this solution were diluted to 1.1×10^{-6}M by addition of deionized water and solutions of NaCl and $NaNO_3$(final $[Cl^-] = 1 \times 10^{-7}$M to 1×10^{-5}M, final $[NO_3^-] = 1 \times 10^{-7}$M to 1×10^{-4}M). In this experiment the mercury was extracted by shaking with the dithizone stock solution in a separatory funnel for 1 minute.

Reagents. Methylmercuric chloride (MMC), dimethylmercury (DMM) and diphenylmercury (DPM) were obtained from Eastman Organic Chemicals and used without further purification. Perchloric acid, mercuric oxide, sodium nitrate and sodium chloride were Baker Analyzed Reagents.

Results and Discussion

Figures X, XI, and XII show the decrease of the absorbance of the dithizone complexes of both mercury and organomercury compounds when extracted from solutions of different pH and in the presence of different anions. It can be seen that the lower the mercury concentration, the greater the rate of the decomposition of the complex. Anticipating that an excess of dithizone would prevent such rapid decomposition, a solution of 40 mg/l of dithizone in CCl_4 was used to extract the mercury, and the absorbance spectrum again was measured at selected intervals. A decrease in both the absorption peak for the dithizone (present in excess) and the complex were observed, the dithizone peak disappearing at a faster rate initially (for 30 minutes) after which both peaks disappeared. It is postulated that as the complex decomposes, (see p.69), a fresh molecule of dithizone recomplexes with the mercury, the rate of which is faster than the decomposition of the com-

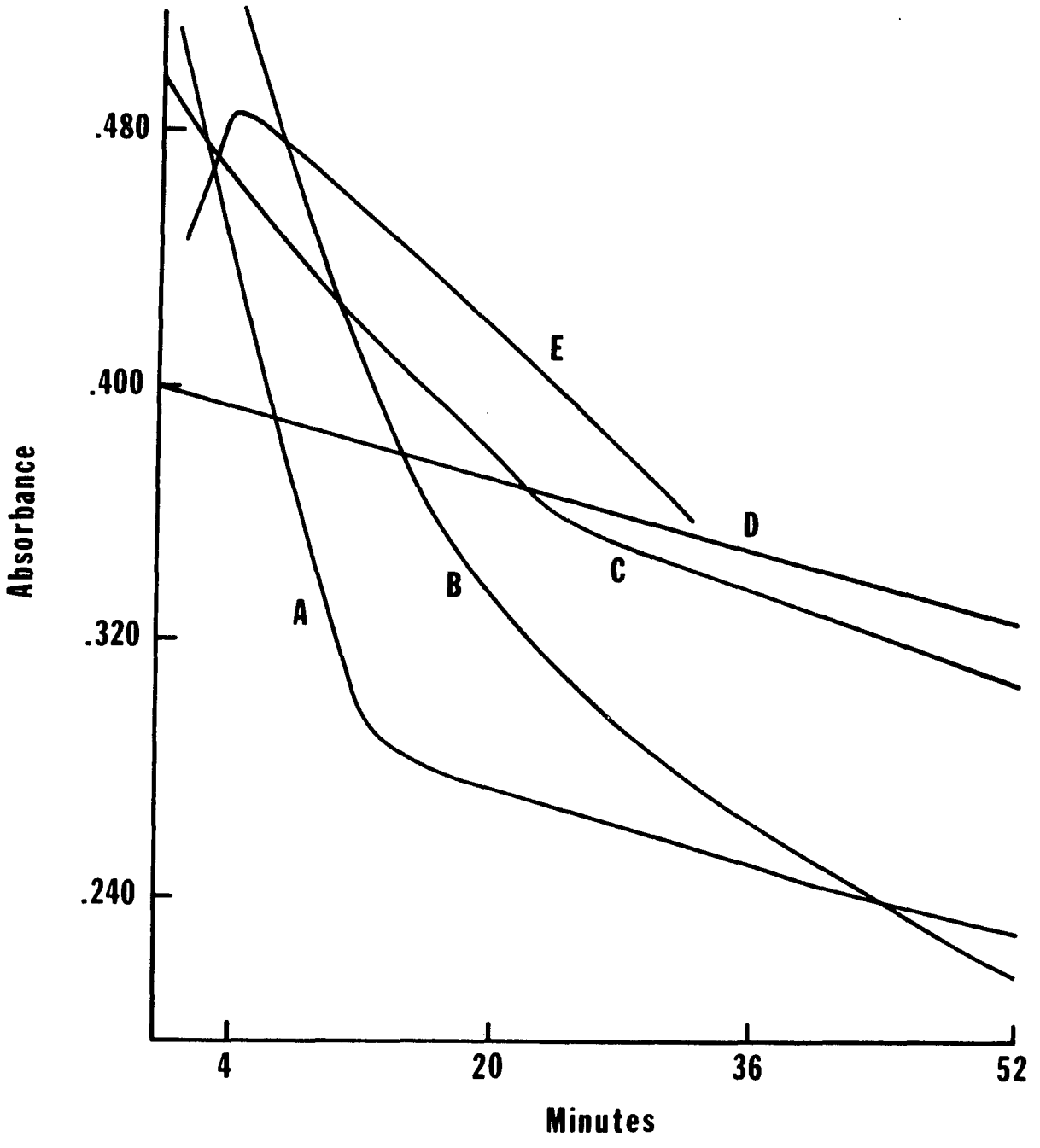

FIGURE X. Change in absorbance with time for mercury-dithizone complex extracted from 0.1 M nitric acid. A) 1μg/ml, Hg^{2+} B)1μg/ml, Hg^{2+}(with TOPO) C) 90μg/ml, DMM D) 10μg/ml, Hg^{2+} E) 270μg/ml, DPM.

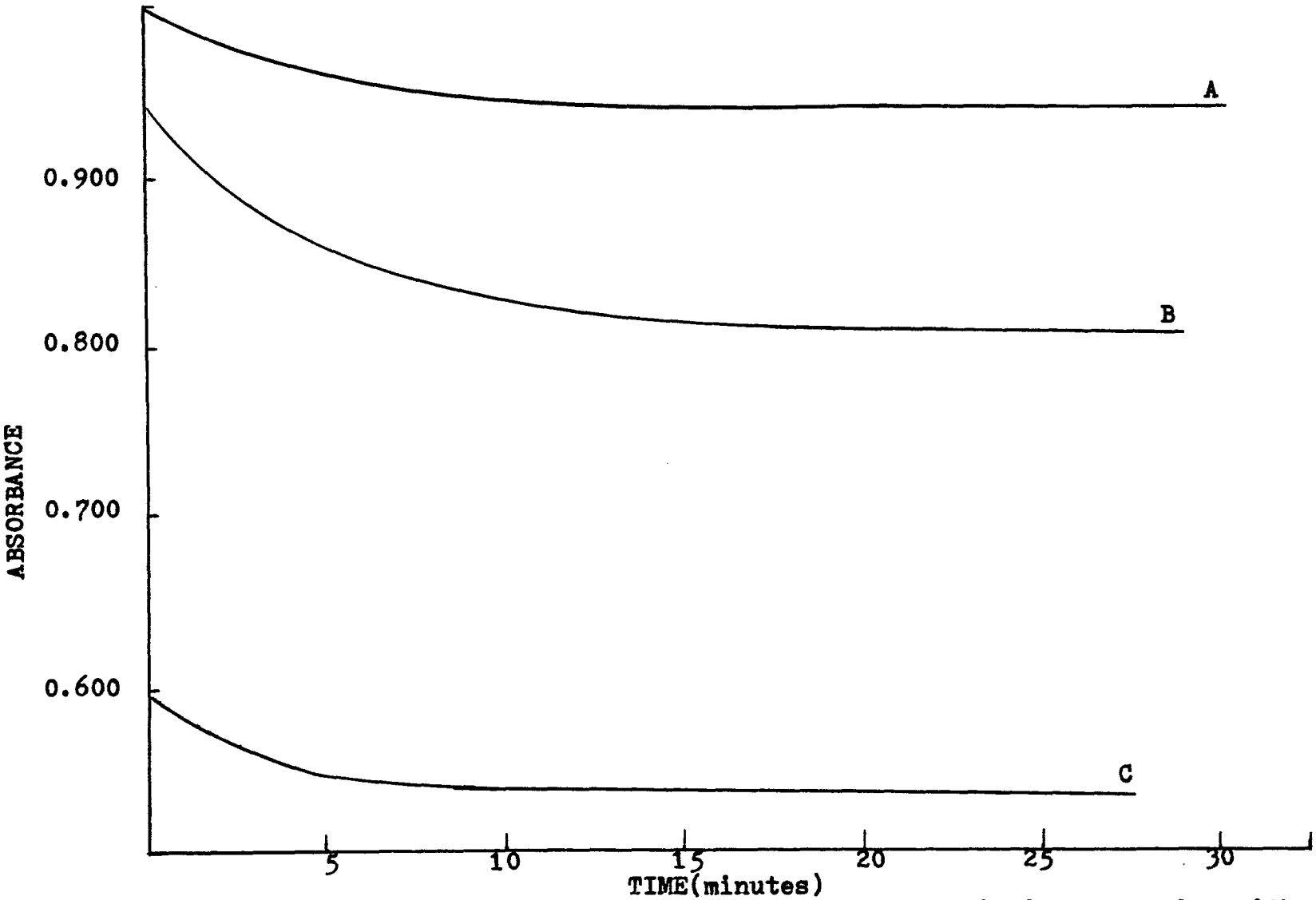

FIGURE XI. Change of absorbance of the methylmercury-dithizone complex with time, as extracted from 0.1 N HCl. A) 13 μg/ml B) 6.5 μg/ml C) 1.3 μg/ml.

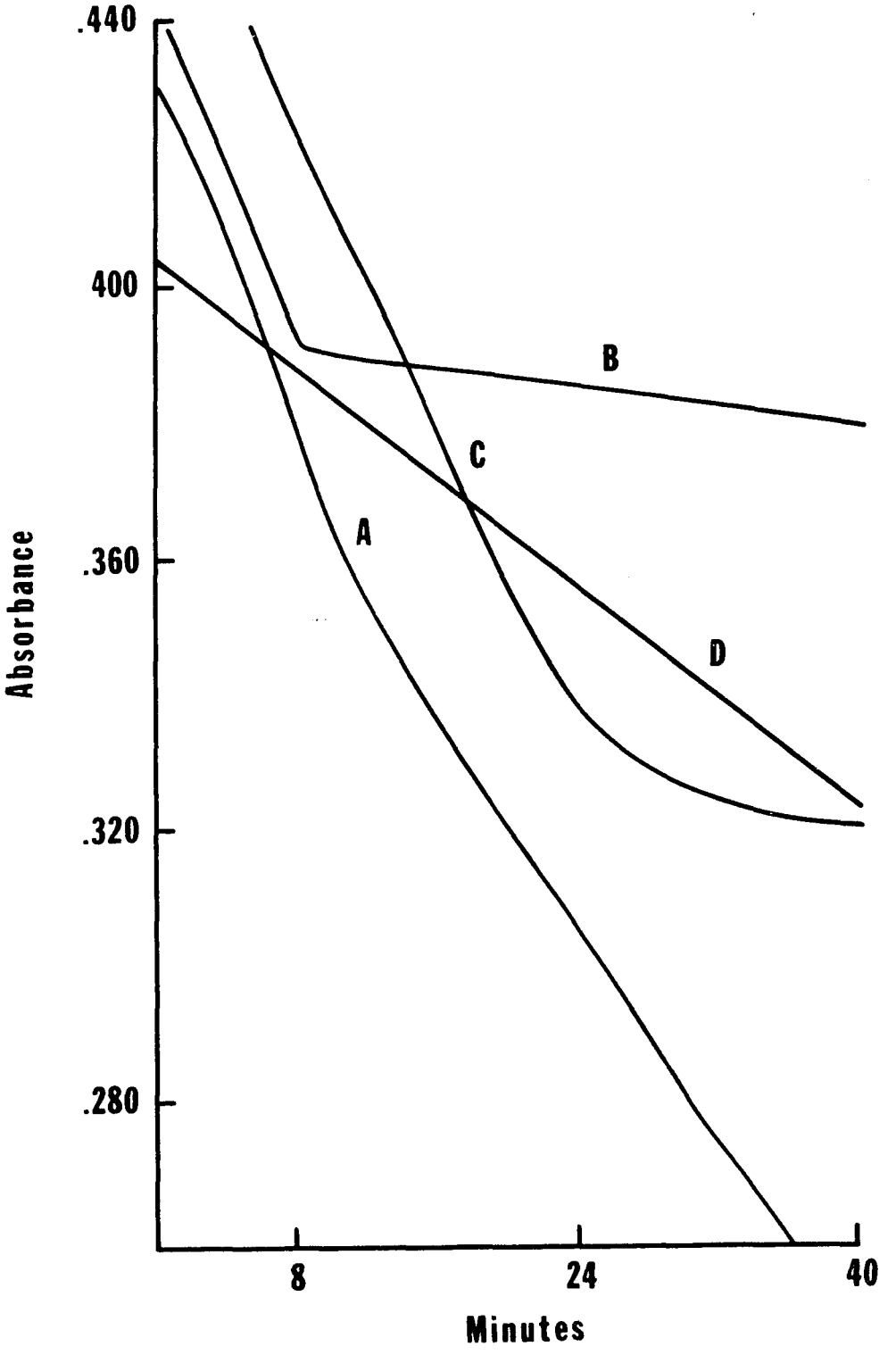

FIGURE XII. Change in Absorbance with Time for Mercury Dithizone Complex Extracted from $1x10^{-4}$ M Nitric Acid. A)10 μg/ml, Hg^{2+} B)15 μg/ml, Hg^{2+} C)140 μg/ml, DPM D)90 μg/ml, DMM.

plex, hence the faster decrease of the dithizone peak. Finally, the dithizone becomes depleted and the complex that remains undergoes self oxidation-reduction; the absorbance of both peaks decreasing to background during the next hour. Obviously this could be the cause of the high rate of adsorption of mercury from solutions of mercury dithizonates, onto glass surfaces.

Meriwether et al[45] have prepared mercury dithizonate (1 mole of mercury(II) and 2 moles of dithizonate anion) as a stable orange solid which is soluble in carbon tetrachloride. Solutions of this compound upon irradiation with UV-visible light changed color from orange (absorption maximum 490 nm), to royal blue (absorption maximum 605 nm) and returned to their original color upon standing in the dark. This compound was prepared by extraction of a high mercury concentration (at pH 4, Hg^{2+} concentration = 7.4×10^{-2} M) with only ½ the number of moles of dithizone (1.85×10^{-2} moles) present in the organic phase in order to effect total complexation. Prior to irradiation the solutions were sealed in glass under an inert atmosphere. This process, therefore, in no way duplicates the conditions for the processing of an environmental sample. Furthermore, the extraction of

mercury from an environmental sample(with dithizone solutions) yields an absorption spectrum with a maximum at 470 nm, and whose color irreversibly fades upon standing in room light. It was also observed that changing the anion to acetate from nitrate, shifts the absorption maximum to 490 nm, the color still fading irreversibly upon standing. Similar shifts were obtained using chloride and perchlorate, indicating that in these solutions the anion is taking part in the complexing reaction,

$$HgX_{2aq} \longrightarrow HgX_{2\ org} \quad (\text{where } X = Cl^-,\ ClO_4^-,\ NO_3^-,\ \text{or } Ac^-)$$

$$HgX_{2\ org} + HDz \longrightarrow HgXDz_{org} + (H^+ + X^-)_{aq}.$$

This mechanism is further supported by subsequent results of a salting-in effect.

The salting-in effect (that was observed in the extraction studies performed with tracer, see Chapter V) was studied by measuring the change in absorbance of the mercury-dithizone complex(or dithizone) as a function of salt concentration. If the absorbance of the dithizone peak increases with an increase in the salt concentration, then the amount of complex formed is decreasing. It can be seen in Figure XIII that this occurs at high and low ionic mercury concentrations, both at pH 1 and pH 4. The general shape of the

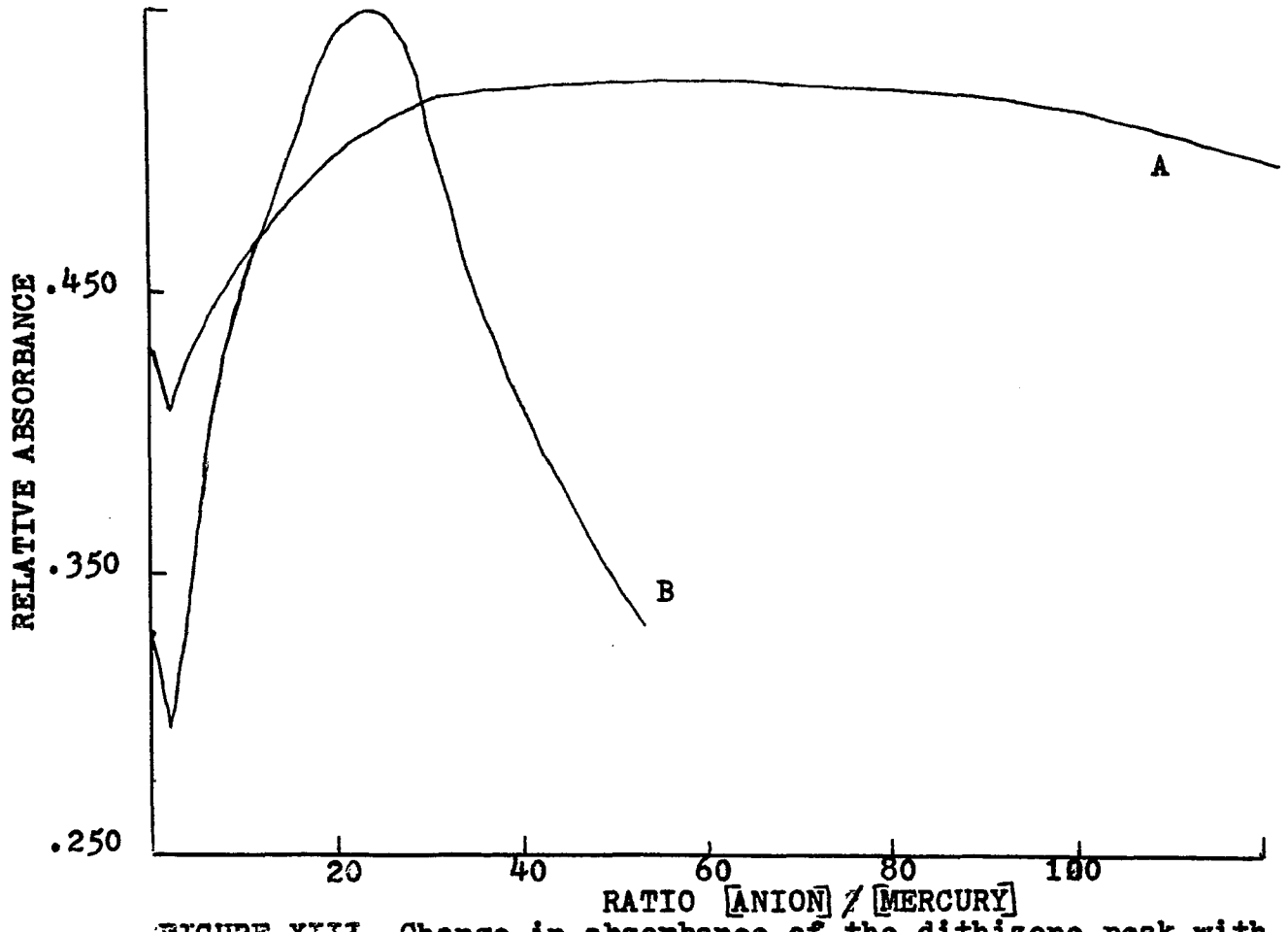

FIGURE XIII. Change in absorbance of the dithizone peak with anion concentration. A) Nitrate B) Chloride.

curve obtained is independant of the anion present, supporting the theory that the anion(chloride or nitrate) is involved in the extraction of the complex. The ratio of chloride or nitrate to mercury is plotted against absorbance and the **minimum** appears at about a 1:1 ratio. The change in absorbance with a change in the anion concentration is at most 0.1 absorbance units, which corresponds to a difference in concentrations(according to a set of calibration standards) of 30%. The change in absorbance of the complex going from no added salt to very high salt concentrations (approaching 1 M) is 12.5% which is in good agreement with the value obtained in the radiochemical determination of the change in percent extracted with high anion concentration.

These findings have significant bearing on the procedure for isolation of organomercury compounds from environmental samples as performed by Westoo[16], Tatton[46], and Bisogni[47]. They acidify a slurry of the macerated sample with 5 M HCl and extract with benzene(no dithizone present). The benzene phase is subsequently separated and concentrated to a small volume by distillation at reduced pressure. The benzene is then extracted with aqueous ammonia and sodium

sulfate and this aqueous extract is acidified with 6 M HCl which is back extracted into a fresh benzene solution. Our results indicate that the solvent of choice should be MIBK(see Chapter V) and not benzene. Furthermore, the concentrations of salt in these samples is formidable, and as has been shown this will decrease the extraction efficiency of inorganic mercury compounds. Similar results were obtained for DMM and DPM under identical conditions of high salt concentration. In addition, the instability of the dithizone complexes(which are prepared subsequent to the final benzene extraction in the above procedure) would prohibit an analytical determination of any mercury concentration in the sample.

VII. Gas Chromatographic Analysis

Determination of organomercury compounds is achieved by initial separation from the sample matrix by extraction, and subsequent analysis by either thin layer or gas chromatography. Analysis of organomercury compounds is hindered by the easy interconversion[36] of these compounds from dialkyl to monoalkyl to mercury, back to monoalkyl, etc. Furthermore it is observed that the dithizone complexes of these organomercury compounds are unstable during gas chromatographic analysis, making it impossible to give exact values for the concentration of these toxicants in an environmental sample.

Experimental Procedures

Gas Chromatography. Gas chromatographic analyses were performed using a Beckman GC-65 with a flame ionization detector. A glass column(3mm by 1 meter) was packed with 10% polyethylene glycol succinate on Chromsorb W(60/80 mesh, DMCS treated, acid washed), which was obtained from Hewlett-Packard.

Solutions of DMM(106 μg/ml in carbon tetrachloride), diethylmercury(DEM, 100 μg/ml in carbon tetrachloride), and DPM(200 μg/ml in carbon tetrachloride), and solutions of

their respective dithizonates (formed by shaking aqueous solutions saturated with these organic mercury compounds, with the stock solution of dithizone) were chromatographed at an oven temperature of 90°C and a nitrogen flow rate of 85 cc/min.

Results and Discussion

Westoo's isolation procedure for mercury(see Chapter V) is followed by both thin layer and gas chromatography. His gas chromatograms clearly show tailing(i.e., peaks which are not gaussian shaped, but broadened at longer retention times) of all the proposed dithizonate peaks. This is due not to poor manipulation of sample injection, but rather to decomposition of the complex before it gets to the detector.

This experiment was repeated using a shorter chromatographic column, so that if decomposition of the complex was actually occurring, the peak due to the yellow decomposition product of dithizone and peak broadening would be observed. Retention times of pure dithizone in carbon tetrachloride, and of DMM, DPM, and DEM(all in carbon tetrachloride) were compared to the retention times of the dithizonates of the organomercury compounds, and it was observed that the former had shorter retention times than the latter, as seen

in Figure XIV. The longer retention times for the dithizonates, double and broadened peaks, and the presence of a third peak(due to dithizone or its yellow decomposition product) indicates decomposition of the complex somewhere during the procedure, which has been substantiated by the work of Dressman[48]. Dressman observed that monoalkyl mercury salts were reduced to the corresponding dialkyl compounds in the injection port at temperatures of about 100°C. The peak due to dithizone might have been missed in Westoo's chromatograms because its retention time is very similar to that of DPM, leading to what might be an imperceptible broadening of the peak. The tailing of peaks at longer retention times indicates that some of the dithizonates are actually detected undecomposed(see Figure XIV), which is probably due to conditions of lower temperature in the column and injection port.

One shortcoming of chromatographic analysis is that it is insufficient, by itself, for determination of the presence of a compound. It shares the same disadvantage that the classical methods of mercury analysis suffered from, lack of specificity.

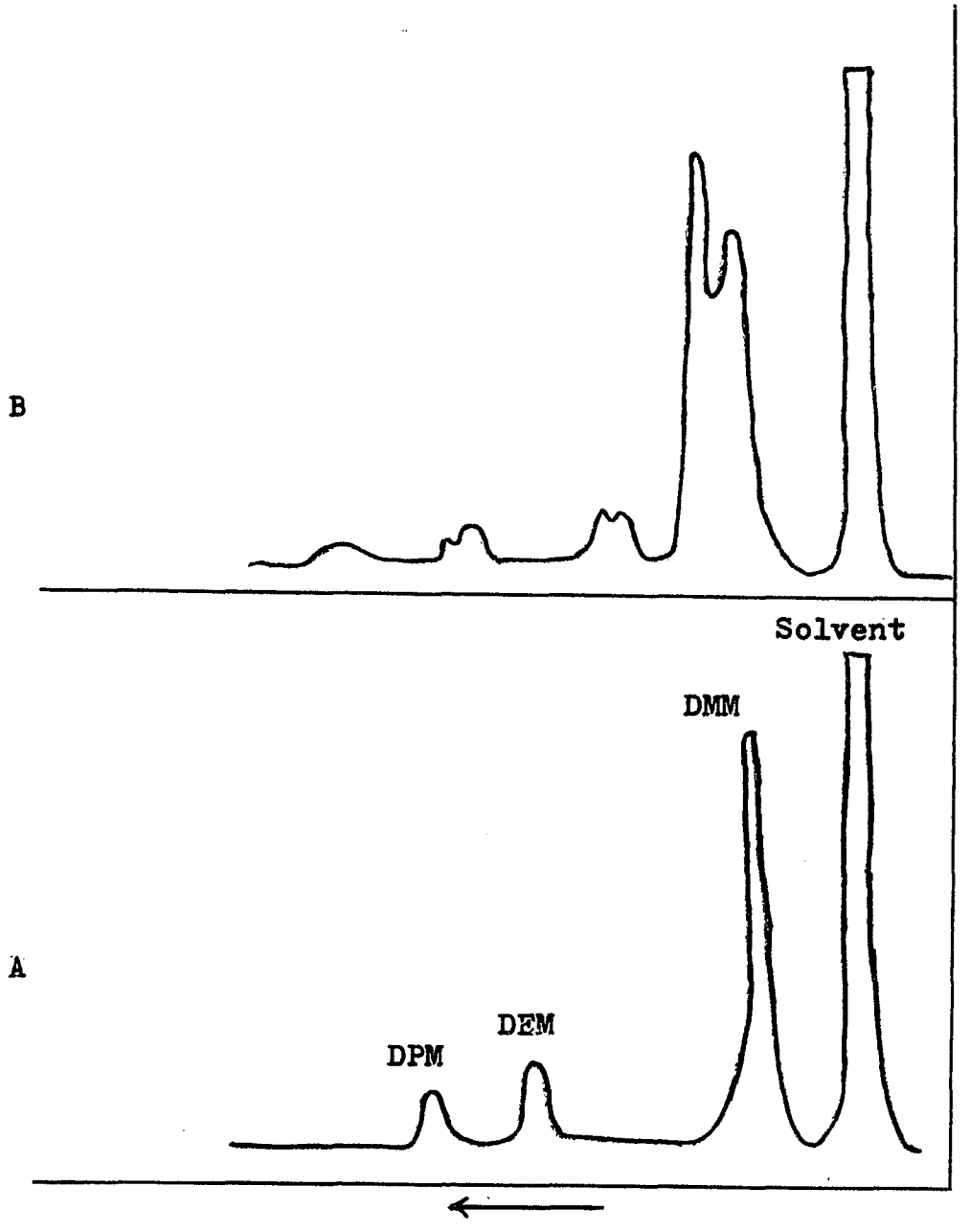

FIGURE XI. Gas chromatograms of A) Organomercury compounds in CCl_4 B) Organomercury dithizonates in CCl_4.

VIII. Neutron Activation Analysis

Instrumental neutron activation analysis (INAA) of a variety of samples is only possible if no matrix effect exists and if various positions of replicate samples, in the rabbit, yield the same specific activity. However, it is important to determine that there are no interfering gamma rays in the gamma-ray energy range that will be used for analytical determination. The 279-KeV gamma ray and the 68-KeV x-rays of mercury were used for analytical determination in the following analyses.

Experimental Procedures

Irradiations. Samples were irradiated for one hour in the reflector region of the High Flux Beam Reactor of Brookhaven National Laboratory. The samples were allowed to decay for one day before counting for analytical determinations.

Samples. NBS SRM 1596(tuna) was used as available. All other samples were used as described in Chapters III and IV.

Detectors. The following detectors were used for counting neutron-irradiated samples: A o.1% efficient Ge(Li), resolution of 2.6 KeV (FWHM) at 1.33 MeV. 2. A 7% efficient Ge(Li), resolution 2.1 KeV (FWHM) at 1.33 MeV. 3. A low-energy photon detector (LEPD) Ge(Li) resolution 280 eV at 68 KeV. 4. An

intrinsic germanium detector, resolution 400 eV at 68 KeV.

Results and Discussion

INAA has the distinct advantage of obviating sample pretreatments, except homogenization(which is necessary in most sample analyses). The x-rays from ^{197}Au are chosen for analysis because of the high $^{196}Hg(n,\gamma)$ cross-section, high conversion coefficient of the main gamma transition in ^{197}Hg and those reasons already discussed in Chapter I. Table 1 compares the different stable mercury isotopes in terms of these characteristics, and as can be seen ^{196}Hg is the isotope of choice. However, the x-rays produced as a result of the mercury decay may be obscured by radiations from other elements also activated during the irradiation. For example, the 280-KeV gamma-ray peak from selenium, a ubiquitous element, overlaps the 279-KeV gamma-ray peak of mercury, requiring spectrum stripping before analytical determination. The initial mercury determination in the NBS SRMs gave substantially higher values than those reported in the certificate of analysis. Upon careful examination of the higher-energy gamma rays in the spectrum, it was discovered that trace amounts of La, Sm, Eu, Pt, and Gd were present, which gave rise to gamma-ray transitions in the

energy range of the various mercury transitions(65-80 KeV). The resolution of the 0.1% efficient Ge(Li) detector that was used was 2.6 KeV(FWHM at 1.33 MeV) and was unable to resolve the low-energy gamma-rays from the x-rays.

In order to identify positively the interference, the samples were subjected to the dry-combustion procedure. The combustion residues as well as the evolved mercury, precipitated as mercuric sulfide, were counted and the subsequent spectra showed the ^{197}Au x-rays separated from interfering gamma rays. This result as well as the first determination and the NBS certified values are compared in Table 8. Sediment samples were also found to contain interfering activities following neutron irradiation, and were also subjected to the dry-combustion procedure. These samples were heated in the combustion tube for slightly longer period of time, because of the small amount of combustible material that was present(this insured the oxidation and subsequent decomposition of any mercury compounds present). The final mercuric sulfide precipitate(sealed in glassine paper) was counted with the 7% efficient Ge(Li) detector for analytical determination and on an intrinsic germanium detector to be sure that the interference had been removed. These two

TABLE 8
Various Methods of Determination of Mercury Concentration

	SRM 1571 Orchard Leaves	SRM 1577 Bovine Liver	SRM 1630 Coal
NBS	0.155 ± .006 μg/g	0.0145 ± .0034 μg/g	0.13 ± .01 μg/g
Instrumental	0.305 ± .012	0.200 ± .013	0.486 ± .060
Radiochemical Separation	0.161 ± .050	0.0180 ± .003	0.129 ± .01

detectors were not available in the original phase of the work.

Careful examination of the gamma-ray spectrum and half-life determination of the gamma-ray peak in the residue(at 68 KeV) indicates that the chief interference in the case of the SRMs was $^{153}Sm(T_{\frac{1}{2}}=46.5$ hrs.), produced by $^{152}Sm(n,\gamma)^{153}Sm$. Equal quantities of samarium and mercury upon activation would yield a ratio of activities of Sm/Hg = 32:1, on the basis of cross-section, natural abundance of the isotope, decay scheme, and half life of the isotope. The abundance of samarium in the earth's crust is almost an order of magnitude greater than that of mercury[1]; consequently, in an environmental sample not contaminated with mercury it might be possible to attain a ratio of activities of Sm/Hg = 320:1.

Figure XV shows the 68-Kev region of the gamma-ray spectrum of a prepared mercury standard and of SRM 1630, as counted with an intrinsic germanium detector, before and after separation of mercury. Even though the resolution of this detector is 400 eV at this energy, the 68-KeV x-ray from ^{197}Au can be seen only as a small shoulder on the large gamma-ray of ^{153}Sm. The higher-energy gamma ray(77 KeV)

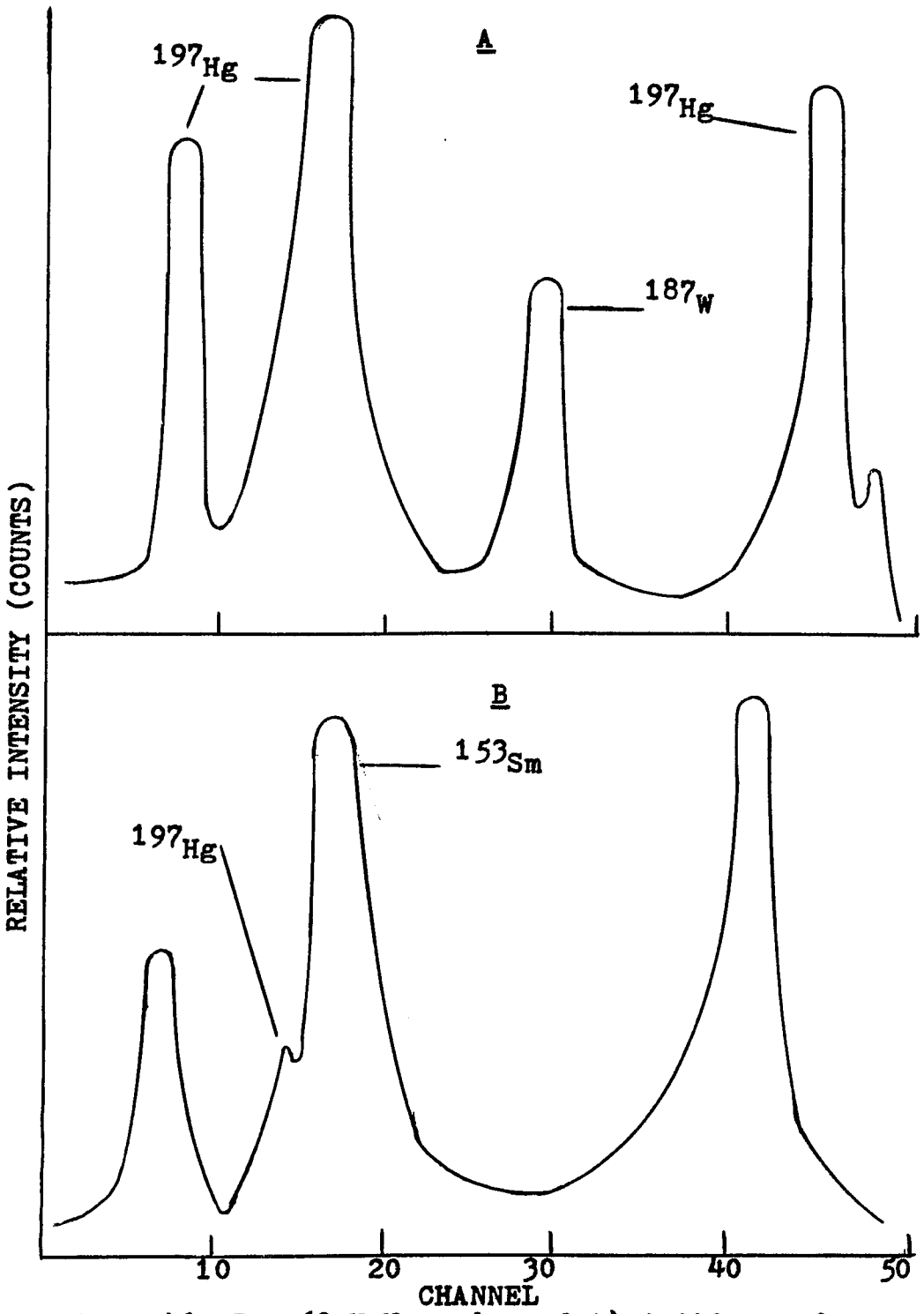

FIGURE XV. The 68-KeV region of A) 0.012 μg of mercury and B) SRM 1630, both as detected instrumentally by an intrinsic germanium crystal. The activities of both samples had decayed at least 1 day before these countings.

from a transition in ^{197}Au is also seen in the standard, but is subject to interference from the 79-KeV gamma rays of ^{169}Gd and ^{197}Pt. Figure XVI shows the same region after separation of mercury, as counted with a Ge(Li) detector. A comparison of the two spectra shows that the mercury has been completely separated from the samarium interference which remains in the residue in the combustion tube. The higher energy region of the gamma-ray spectrum revealed that bromine, selenium, and arsenic were also collected in the liquid-nitrogen condenser after combustion.

INAA of mercury has been applied to samples of diverse environmental origin. Since it was shown that the quartz tubes effected no flux depression on the aqueous samples, it was assumed that samples of matrices similar to quartz also will cause no flux depression. This assumption was experimentally verified by irradiating NBS SRM 1630(Coal and laboratory prepared aqueous standards, together. The areas under the 279-KeV gamma-ray peaks of the respective samples, counted under identical conditions with a 7% efficient Ge(Li) detector, gave the same specific activity. The contribution of the 280-KeV gamma-ray peak of selenium, which is significant in the gamma-ray spectrum of the coal sample, was subtracted

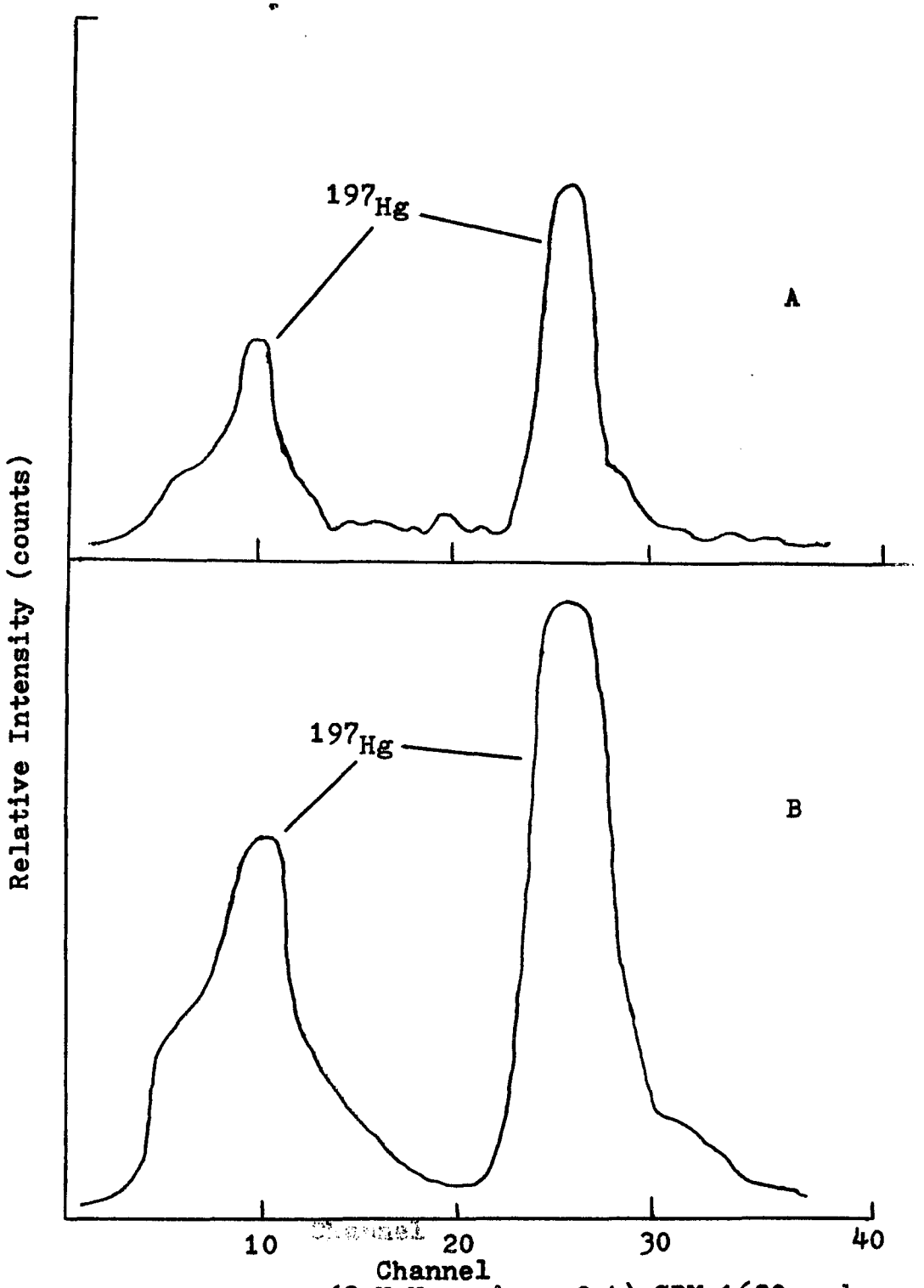

FIGURE XVI. The 68-KeV region of A) SRM 1630 and B) A prepared mercury standard, as counted with a Ge(Li) detector.

from the mercury gamma-ray peak by a standard method of spectrum stripping[32]. Therefore, the difference in the sample matrix in samples as diverse as water and coal, makes no difference in the analytical determination of mercury.

A stringent requirement of INAA is reproducible sample-counting geometry. Ideally, any sample should be a point source with its position well defined and easily reproducible. This is not so important with a detector of large volume (e.g., a 30 cc Ge(Li) detector) as it is with a detector that has a small slit(e.g., an intrinsic germanium detector with a 3 mm window). This is because the solid angle subtended from a sample to a large detector will not be affected as much as will the solid angle subtended from a sample to a detector with a small window. However, at this point in the development of solid-state detectors, high resolution must be sacrificed for high efficiency, or vice-versa. Maximum sensitivity for any counting method is realized by proximity of the sample to the detector surface(i.e., if there is negligible dead time when the sample is this close to the detector surface). Unfortunately, at small distances, exactly reproducible geometry is difficult to achieve. Therefore, despite high sensitivity, efficiency and/or reso-

lution poor accuracy and precision can result.

The effect of not reproducing sample counting geometry was confirmed in an experiment performed with a sample of NBS SRM 1596(Tuna) and a laboratory prepared aqueous standard. Different volumes of each sample were sealed in quartz tubes. These samples were counted separately, approximately 2 inches from the surface of a Ge(Li) LEPD with the tubes placed horizontal to the detector surface. It was found that the mercury peak could be resolved from the interference present in the tuna with this detector. Counting the x-rays gave a mercury concentration in the tuna of 0.8352 μg/g; counting the 77-KeV gamma ray gave a value of 0.8309 μg/g, as compared with the laboratory prepared aqueous standard. However, after separation of the mercury by the dry-combustion technique, and recounting on a 7% Ge(Li) detector, the values for the mercury concentration as compared with the same standard(which was also subjected to the combustion) were both 1.01 μg/g, the value certified by the NBS. The low value obtained from the INAA of the sample must be due to failure to reproduce the sample counting geometry.

Prior to this experiment, all sample tubes were filled

to the same level to avoid this geometry effect. However, it still would be possible to obtain inconsistent results as a consequence of poor sample positioning or sample inhomogeneity. If this happened, then the results of all other INAA experiments could be in error. Fortunately, the prepared standards contained not only mercury but also arsenic. The value obtained for arsenic in the lyophilized and unlyophilized samples was found to be the same, 0.5 $\mu g/g$.

It has been shown that mercury metal may be lost in lyophilization as a result of electrochemical reduction, and subsequent volatilization of the metal, but we would not expect arsenic to be lost even if it was reduced in the same manner as mercury(this is because it is non-volatile under these conditions). Therefore consistent values for arsenic proves that sample geometry did not effect the mercury values in previous experiments. Furthermore, the values of arsenic in NBS SRMs 1577 and 1571 were found to agree with the NBS certified value, when counted in this same manner, thus verifying the reproduciblity of the sample geometry.

IX. Analysis for Other Trace Elements

Qualitative INAA has been applied to a wide scope of environmental samples for many diverse trace elements.[49,50] However, quantitative INAA has only recently been applied with success, to mercury,[28] but not to a significant number of other trace elements. Changes in trace element concentrations in the environment, during a period of months or years, can show the development of a trend or shift in the ecological balance. These changes may be either natural or induced, but in either case an accurate method of analysis that gives reliable results at less than the part per million level, must be developed. A set of aqueous standards for neutron irradiation and subsequent gamma-ray analysis, containing mercury, arsenic, zinc, and copper, were prepared with this purpose in mind.

Experimental Procedures

Arsenic Standards. A series of standards for arsenic were prepared from a stock solution (156.4 mg of $Na_2HAsO_4 \cdot 7H_2O$ in 1 liter of deionized water) containing an arsenic concentration of 37.58 μg/ml. Aliquots of the stock solution were diluted to make standards which covered the range of arsenic concentration from 1.01 to 0.010 μg/ml. The linearity

of the standards was examined by counting the 565 KeV gamma ray of ^{76}As($T_{\frac{1}{2}}$= 24 hours) after neutron irradiation.

Combined Standards. A multielement set of standards was prepared by dissolving 29.51 mg of mercuric nitrate, 158.6 mg of disodium arsenate, 11.85 g of cupric nitrate and 10.27 g of zinc acetate in 1 liter of 1 N nitric acid. Standards covering the following concentration ranges were prepared by dilution of the stock solution: mercury, 1.07 μg/ml to 2.33 ng/ml; arsenic, 2.37 μg/ml to 5.14 ng/ml; copper, 194 μg/ml to 0.421 μg/ml; zinc, 190.4 μg/ml to 0.413 μg/ml. The acid concentration was maintained at 1 N in nitric acid, by addition of concentrated nitric acid to the dilutions of the stock solution.

Irradiation. The standards and samples were irradiated in the Brookhaven National Laboratory High Flux Beam Reactor for one hour in a thermal neutron flux of 1×10^{14} n/cm^2-sec. The activities of the samples were allowed to decay for one day prior to counting on the 0.1% efficient Ge(Li) detector.

Results and Discussion

The arsenic standards yield a straight line for activity vs. micrograms as seen in Figure XVII. The lowest value attainable with these standards was 0.075 μg, below which the 559 KeV photopeak could not be distinguished from back-

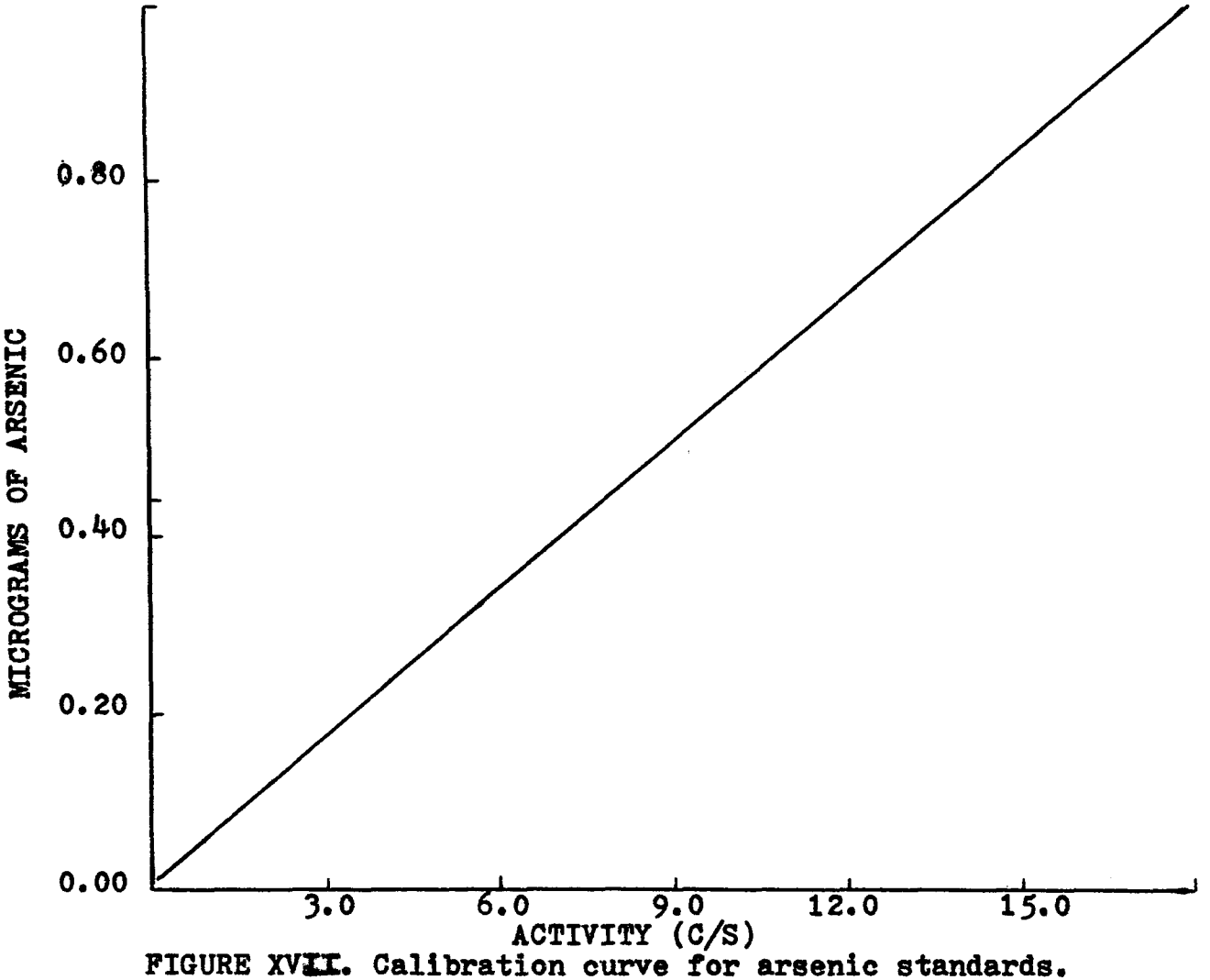

FIGURE XVII. Calibration curve for arsenic standards.

ground. It has been shown that solutions of arsenic, of concentration in the part per million range, are not subject to losses such as adsorption during storage[51], and therefore, it was decided that no studies of adsorption had to be performed(the redox potential does not favor reduction).

The quantity of arsenic in NBS SRMs was determined using these standards(only containing arsenic), and was found to be in agreement with the certified values, which were: Orchard leaves(1571), certified value 14.1 ± 1.0 μg/g, INAA value 13.5 ± 1.9 μg/g; Bovine Liver(1577),certified value 0.050 ± 0.006 μg/g, INAA value 0.050 ± 0.008 μg/g. The value for SRM Coal(1630) was also determined(21.2 ± 1.0 μg/g), but as of yet no certified value has been issued by the NBS.

INAA of the combined standards yielded linear calibration curves only for mercury and arsenic. The gamma-ray peaks from the zinc and copper standards were of insufficient intensity to use in quantitative analysis of samples(there appeared to be a contamination problem, because the standards with low concentration of zinc and copper had gamma-ray peaks which were as large as the high concentration standards).

Qualitative elemental analysis was performed upon sed-

iment samples and NBS SRMs, by determining the energy of the gamma rays present in the spectra of the irradiated samples, and then determining the half life for each particular photopeak by recounting(at constant geometry) several times over a period of one week. The intensities of the various gamma rays with the same half lives, were compared and the identity of the elements was confirmed by these (approximate) relative intensities and their respective energies.

Table 9 lists the various samples and their respective trace constituent elements. There were several photopeaks observed in the gamma-ray spectra which could not be accounted for by radioactive transitions of the trace elements identified. Some of these peaks are obviously due to pair production and subsequent escape from higher energy gamma rays(i.e., 573 KeV escape peak from the 1593 KeV gamma ray of ^{140}La, or the 452 KeV escape peak from the 1473 KeV gamma ray of ^{82}Br, etc.). Other peaks, however, were unresolvable.

The dry combustion procedure yielded an excellent separation of arsenic, bromine and selenium, as well as mercury, from the other trace elements. None of these elements were found in the combustion residue or in the filtrate from the

TABLE 9
Qualitative Analysis of Various Samples

Element / Sample	Hg	As	Br	Se	Sc	Eu	La	Sm	Pt	W	Os	Mo	Au	Sb	Cu	Zn
SRM 1630	x	x	x	x		x	x	x					x		x	
SRM 1571	x	x	x			x	x	x		x			x		x	x
SRM 1577	x	x	x			x							x		x	x
Sediment #1	x		x			x				x		x				
Sediment #2	x		x	x								x				
Sediment #3	x		x				x	x	x	x	x					
H_2SO_4(Baker)	x	x	x							x				x	x	

precipitated mercuric sulfide. Quantitative recovery was determined only for mercury, because the combustion of the samples was performed a week after the irradiation, and the arsenic had since decayed. Only an estimate of the quantiative recovery of the bromine and antimony could be made due to the lack of standards for these elements and the irreproducibility of counting geometry before and after combustion(the samples had different volumes before combustion because they were sealed in quartz vials).

X. Conclusions

This research has shown that trace mercury analysis, of environmental samples, presents serious difficulties in most routine analyses. These difficulties are adsorption, reduction to mercury metal and subsequent volatilization, and instability of mercury dithizone complexes. These losses occur at both high and low mercury concentrations but they are not so obvious(i.e., a smaller percentage is lost) at the higher concentrations.

These losses may be very reproducible in a set of standards or even for one type of sample. However, the concentration of mercury will govern the absolute quantity lost. Therefore, samples having different concentrations could appear to have the same concentration. Another very important consideration is the linear range of the standards. Simple extrapolation of a calibration curve to either high or low values, beyond the range of the standards, can lead to an appreciable error and has always been regarded as poor anlytical technique. This type of error was discovered in the very first irradiation of NBS SRM 1571, where the value, 2.4 μg/g(calculated from an experimental value of 0.11 μg of mercury from a 47 mg sample; the amount

of mercury actually present was 0.8 ng) was obtained by extrapolation of a standards curve, the smallest standard of which contained 0.5 μg of mercury. A second irradiation of a mercury standard which contained less than the expected amount of mercury in the sample yielded a value of 0.200 μg/g (which was still incorrect due to the interference from samarium, see Chapter IX). In other instances the precision of the results is good but accuracy may suffer. For example, in the lyophilization experiments, the losses observed were reproducible to within 5%, but if you didn't know that mercury was being lost it would be assumed that this was just a statistical variation of the method or of the sampling. Furthermore, the losses from samples of lower concentrations are smaller, which is probably due to the easier oxidation of mercury metal at lower concentrations (i.e., at lower mercury concentrations the potential for the overall oxidation equation is more positive).

In the case of the dithizone extraction, the difference in a few minutes shaking time will change the amount of mercury extracted, and different time intervals between absorbance measurements will yield various absorbance readings for the same sample. Both of these effects can lead to

errors in the final determination which are greater than statistical variation among replicate samples.

Mercury is very easily reduced in a medium without a strong oxidant, leading to loss of mercury during the dithizone extraction. Multiple extractions with dithizone have been attempted and they provide no additional efficiency probably because the mercury not extracted was reduced and/or adsorbed. The problem of adsorption in this case, and in any other method of analysis which requires pretreatment is of serious magnitude and is a problem for which there is no apparent solution. Therefore, mercury analysis should be performed with minimal sample treatments. INAA can obviate these treatments, but radiochemical separation must be used if an interference is present.

Qualitative INAA for other elements is easily performed, by relative gamma-ray intensities and half life determination. However, elements such as copper and zinc which have small thermal neutron capture cross sections require radiochemical separation in order to yield valid quantitative analysis. There are some elements, for example bromine and arsenic, which can yield good INAA results, but the level to which these elements, and other el-

ements can be determined instrumentally, may not be sufficient for the analytical determination of trace concentrations encountered in environmental samples.

APPENDIX

A concentration of 1 μg/ml of mercury corresponds to 5×10^{-6} M, and 1 ng/ml corresponds to 5×10^{-9} M. The reduction equations and standard potentials are[52]:

$$2e^- + Hg^{2+} \longrightarrow Hg \qquad E^o = +0.852 \text{ v}$$

$$2e^- + 4H^+ + 2NO_3^- \longrightarrow N_2O_4 + 2H_2O \qquad E^o = +0.810 \text{ v}$$

the net reaction is,

$$Hg^{2+} + N_2O_4 + 2H_2O \longrightarrow Hg + 2NO_3^- + 4H^+ \qquad E^o = +0.042 \text{ v}$$

At a concentration of 5×10^{-6} M, mercuric ion is sufficiently dilute so that its activity coefficient can be assumed to be unity, and hence its activity equals its molarity. The activity for nitric acid can be found by multiplying its concentration by the activity coefficients in the following table[52]:

Concentration	Activity Coefficient	Activity	Approximate pH
1.0 M	0.724	0.724	0
0.1	0.791	0.0791	1
0.01	0.830	0.0083	2

The Nerst equation for the reaction is:

$$E = +0.042 - \frac{.059}{2} \log \frac{[H^+]^4 [Hg] [NO_3^-]^2}{[Hg^{2+}] [N_2O_4]} \qquad (1)$$

If the value of E for this expression is **positive** then there will be spontaneous reduction of mercuric ion to mercury metal. Substituting the values of $[Hg^{2+}] = 5\times10^{-6}$ M, and

$N_2O_4 = 1.8 \times 10^{-4}$ M (based on an assumed molarity of 0.10 M, due to the decomposition of nitric acid, and a calculated activity coefficient of 1.8×10^{-2}), it is found that,

$$\text{at pH 0 } E = +0.042 - 0.03 \log \frac{(2.76 \times 10^{-1})(5.25 \times 10^{-1})}{(5 \times 10^{-6})(1.8 \times 10^{-4})}$$

$E = \pm 0.204$ v
at pH 1 $E = -0.091$ v
at pH 2 $E = +0.145$ v, therefore, at pH ≥ 2 we have a spontaneous reduction of mercuric ion to mercury metal. Similar calculations for mercuric ion concentration of 5×10^{-9} M yield,

at pH 0 $E = -0.264$ v
at pH 1 $E = -0.209$ v
at pH 2 $E = +0.055$ v, again at pH ≥ 2 spontaneous reduction to the metal. These calculations show that as the mercury concentration decreases (holding all else constant) the reduction becomes more unfavorable. This in part can explain why more mercury is lost through lyophilization at the higher concentrations than at the lower ones. It also implies less adsorption from solutions of small concentrations, which was also found. That is, there was a higher percentage of mercury lost from solutions of lower concentration, but a smaller absolute amount. The fact that nitric acid decomposes with time aids the reduction of mercury, due to an increase in N_2O_4 concentration.

In sulfuric acid solution a similar reaction can occur; the reduction equation and standard potential is:

$$SO_4^{2-} + 4H^+ + 2e^- \longrightarrow H_2O + H_2SO_3 \quad E^o = +0.20 \text{ v}$$

The overall reaction is:

$$Hg^{2+} + H_2O + H_2SO_3 \longrightarrow Hg + SO_4^{2-} + 4H^+ \quad E^o = +0.652 \text{ v}$$

The activity coefficient for the sulfuric acid species in solution is similar to that of nitric acid, but for convenience it will be assumed that it is one. Therefore, the Nernst equation for this reaction is,

$$E = +0.652 - \frac{.059}{2} \log \frac{[SO_4^{2-}][H^+]^4[Hg]}{[H_2SO_3][Hg^{2+}]} \qquad (2)$$

If the value of E is positive, then there will be spontaneous reduction of mercury. At a mercury concentration of 5×10^{-6} M, and assuming an H_2SO_3 concentration of 1×10^{-4} M it is found that,

at pH 0 E = +0.336 v.

At a mercury concentration of 5×10^{-9} M,

at pH 0 E = +0.245 v.

At pH's greater than zero, E becomes increasingly more positive and therefore leads to spontaneous reduction of mercury.

It is very important to note that in these calculations

we have assumed the concentration of the mercury metal in solution to be one. This is true for excess metal in equilibrium with a corresponding metal ion in solution. However, mercury metal has a solubility in water[29] of approximately 1×10^{-8} M . If this value were substituted into the Nernst equation, the logarithmic term would become increasingly more negative, leading to spontaneous reduction of mercuric ion at an even lower pH in both sulfuric and nitric acid solutions.

It seems reasonable to conclude from these calculations, that during the wet-digestion procedure it is possible that mercury is actually reduced. The concentration of N_2O_4 increases upon addition of sulfuric acid, and this increase leads to a more positive potential in equation (1) at constant concentration of all other species. Therefore, despite the high acid concentration during a wet digestion, mercuric ion can be reduced to the metal. The amount of mercury from a typical digestion would be no more than 1-2 parts per million(by weight), which is the solubility of mercury in water. Hence, the reduction would not be observable by the experimenter.

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