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The cold vapor atomic absorption method for mercury determination was studied to improve the instrumentation and to develop procedures for chemical speciation. The major instrumental improvement was the construction of a continuous sample flow reduction vessel. A selective reduction scheme was developed to differentiate between sub-ppb levels of inorganic and organo-mercury.

Three different continuous sample flow reduction vessel designs were developed and optimized. The two most successful designs are based on delivery of tin chloride reductant and sample solutions with a peristaltic pump to a reduction vessel. Here the elemental mercury is volatilized out of solution with nitrogen and carried to an absorption cell where the atomic absorption at 254 nm is measured. The difference in absorbance between a blank and sample solution is proportional to the mercury concentration in solution. One reduction vessel design is based on stripping mercury from solution via bubble

aeration and provides a detection limit of 0.02 ppb and a response time (time for absorbance to stabilize between blank and sample solutions) of 1.3 minutes. The other design is based on stripping of the mercury from a thin stream of solution with a countercurrent flow of gas over the solution stream. With this design, the detection limit is 0.03 ppb and the response time is 0.9 min. These continuous sample reduction vessel cells are simpler to use and more amenable to remote operation than previous discrete sampling reduction cells in which sample and reductant solutions are added with syringes.

A selective reduction procedure was developed for use with a discrete sample reduction vessel. First tin chloride is used to reduce only the inorganic mercury in the sample. The resultant elemental mercury is swept into nitrogen and carried into an observation cell where the absorbance at 254 nm is measured. Next sodium borohydride is injected into the reduction cell and the organomercury is reduced, volatilized, and carried to the observation cell. The measured peak absorbances are proportional to the concentration of each type of mercury and the response produced by inorganic mercury is independent of organomercury and visa versa.

Calibration curves were established for Hg^{2+}, CH_3Hg^+, $CH_3CH_2Hg^+$, and $C_6H_5Hg^+$ with resulting detection limits of 0.003, 0.003, 0.003, and 0.005 ppb, respectively. Preservation studies revealed a breakdown of 20% of the methylmercury within one day in the presence of 1% (v/v) HNO_3 and 0.01% (w/v) $K_2Cr_2O_7$. Tuna, hair, urine, and tapwater samples were analyzed for inorganic and total mercury using this

speciation method. A KOH digestion was used with the biological samples with addition of 7.5% (v/v) HNO_3 (c) and dilution with a 1% (w/v) NaCl solution to prevent strong complexation of the Hg^{2+} by interferents.

Continuous Sample Flow and Mercury(II) Speciation Studies Utilizing the Cold Vapor Atomic Absorption Method for Mercury Determination

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INTRODUCTION

The cold vapor atomic absorption (CVAA) method for mercury determination, first utilized practically in 1968, had a limit of detection of 1 ppb for mercury(II) in solution. This method is based on the chemical reduction of mercuric ions to elemental mercury which is then swept into a gas stream where the atomic absorption of the mercury atoms is measured. Instrumentation developed in this laboratory in 1975 was able to detect 0.002 ppb of mercury(II) in a 1 mL sample with a relative standard deviation of about 2% for a concentration range of 0.05 to 10 ppb of mercury(II) utilizing a smaller reduction vessel and absorption cell path length of 60 cm. The reliability, accuracy, as well as the shortcomings of the CVAA technique are fairly well established. The existence of EPA approved methods and commercial devices devoted solely to mercury detection, that are based on the phenomenon of high volatility of elemental mercury at room temperatures testify to the acceptance of the CVAA method as well as to the extreme concern involved in closely monitoring this toxic substance.

With the principles of the method established, further improvement is sought in the form of simplifying the analysis procedure and automation of the system to handle large numbers of samples and to eliminate the need for constant devotion of an operator to the analyzer. The purpose of the first of two parts of this research was to design and optimize conditions for a reduction vessel based on continuous sample introduction. All previous CVAA instruments used a discrete sampling

approach in which each sample is injected into the instrument or brought to the instrument in a separate container, and is removed after the analysis.

The continuous sampling approach simplifies analyses and reduces the dependence on operator skill by eliminating the time-consuming manual use of syringes needed with discrete sample determinations. This method also allows automation in analysis for uninterrupted and/or remote monitoring of trace mercury levels and could also be used as an HPLC detector specific for mercury.

Three different reduction vessel designs for the continuous volatilization of mercury were proposed and were based on the release of mercury from a nebulizer-generated mist, by bubbling an inert gas through the solution, and by volatilization from a thin stream of solution. With the best reduction cell design (i.e. thin stream), automation is feasible with the appropriate hardware and a detection limit of 0.30 ppb was achieved. This is about a factor of ten above that achieved with the discrete sampling method and due in part to the inefficient volatilization of mercury. About three minutes are required per run.

The purpose of the second part of this research was to incorporate the different reducing strengths of $SnCl_2$ and $NaBH_4$ into a speciation procedure that would allow determination of inorganic and organic species of mercury(II) in a single run. Differentiation between inorganic and organic species of mercury is essential because of the difference in toxicity between the species. In the proposed procedure only total organomercury rather than specific organomercurials are

determined. However most organomercury in natural waters and biological systems is methylmercury so that as a first approximation, methylmercury is determined. Results of this study which employed a discrete sampling reduction vessel gave detection limits of 0.003 ppb for Hg^{2+}, CH_3Hg^+, and $CH_3CH_2Hg^+$ with relative standard deviations of 4-10% for concentrations between 3 and 0.03 ppb. The calibration sensitivity for phenylmercuric acetate was about 50% less with the resultant detection limit of 0.005 ppb. It was found that 1% (v/v) HNO_3 and 0.01% (w/v) $K_2Cr_2O_7$ preservatives, while aiding the retention of total amounts of mercury, contributed to about a 15% decomposition of methylmercury after one day. The developed speciation procedure was used to analyze canned tuna, hair, urine and tapwater samples. The biological samples were digested with KOH.

BACKGROUND

Introduction

The toxicological effects of the various organic and inorganic species of mercury, ranging from permanent neurological impairment and chromosomal abnormalities to physical deformations of afflicted fetuses, are well established (1-4). The tragic incident at Minamata, Japan, in 1952 where, over a period of nine years, 36 out of a total of 110 individuals with symptoms died of what was eventually diagnosed as methylmercury poisoning resulting from ingestion of fish contaminated by a plastics factory that was using mercury as a catalyst in the manufacture of polyvinyl chloride (5,6). This as well as various sobering incidents in, most notably, Sweden (6), again in Japan in 1965 (2), and the United States (7,8) served to emphasize the necessity of monitoring concentration levels of this toxic metal in food, water, and the environment, especially in areas of heavy industrialization.

The background levels of mercury in natural waters, although variable, are generally in the parts-per-trillion range (9,10). It has been estimated that the natural weathering process transfers about 5000 tons of mercury per year (10) to the oceans from continents via rivers, while man contributes about an equal amount each year. One of the major human sources of mercury pollution results as a by-product of coal burning power plants since coal contains volatile mercury that is eventually returned to the earth in rainwater (11). Crude oil has been shown to contain mercury in the ppm levels (8,12) which is also released to the atmosphere upon burning.

In addition, man has sought to isolate mercury for its various properties and proceeded to return it to the biosphere in concentrations and situations that become insidiously self-destructive. Unique properties of this liquid metal such as high electrical conductivity, uniform expansion over its liquid temperature range, high surface tension, and capability to readily amalgamate, make mercury an ideal choice for use in electrical apparatuses for the electrolytic production of chlorine and caustic soda, and for the industrial recovery of metals. Mercury compounds have been used as catalysts in industrial processes, such as those involving acetylene and plastics. The toxicity of mercury compounds is further underscored by their application as bactericides and fungicides in various industries as pharmaceuticals, pulp and paper processing, tanning, and in agriculture. Widely used as fungicides, farm crop seeds coated commercially with organomercurial compounds are one of the primary sources of mercury ingestion by wildlife (13). In 1968, 26% of about 20 million pounds of mercury consumed in the U.S. (6) was used for electrical apparatuses while the chloro-alkali industry accounted for 23% and agriculture for 4.6%.

Analytical Techniques for Inorganic Mercury

This manmade introduction of mercury into the environment poses the obvious question concerning the ultimate destination of the various unrecycled forms of mercury and how to monitor their presence. There are a number of review articles describing chemical and instrumental methods of total mercury analyses (14-17) and a partial tabulation of techniques and detection limits is shown in Table I.

Table I. Techniques for trace mercury determination

Technique	Detection Limit	Reference
Flame Emission	10 ppm	(18)
He Plasma Emission	0.02 ppb	(19)
Flame Atomic Fluorescence	2 ppb	(20)
Cold Vapor Atomic Fluorescence (CVAF)	0.005 ppb	(21)
Flame Atomic Absorption	500 ppb	(16)
Cold Vapor Atomic Absorption (CVAA)	0.002 ppb	(22)
Instrumental Neutron Activation Analysis (INAA)	1 ng	(20)
Colorimetric (dithizone)	10 ppb	(23)
Anodic Stripping Voltammetry (ASV)	0.005 ppb	(24)

As can be noted from Table I, ASV, INAA, plasma emission, CVAF and CVAA are the only methods that are presently directly capable of achieving sub-ppb detection limits comparable to background levels in natural waters. The inherent advantage of lowered detection limits is the elimination of preconcentration procedures such as lyophilization (25-27), ion exchange (28,29), or complexation (30,31) which enhances the probability of loss or contamination of mercury in the sample. Of the five analytical techniques, the combination of detection limit, sensitivity, freedom from interferences, simplicity, and financial considerations make CVAA the method of choice for routine analyses.

The techniques of mercury analysis by CVAA or CVAF are based on the volatility of elemental mercury and the solubility of mercury in water which can be described by the partition constant, K, where K =

concentration of mercury in air/concentration of mercury in solution = 0.4-0.7 at equilibrium (32) depending on the acid medium. Tong established a K value of 0.66 $\pm$ 0.04 in 2 M H_2SO_4 (33). Reduction of the oxidized forms of mercury to Hg(0) can be induced chemically by various agents, the most common being stannous chloride ($SnCl_2$), stannous sulfate ($SnSO_4$), and sodium borohydride ($NaBH_4$). The reduced mercury is swept out of solution with an inert gas into an observation cell where the atomic absorption or fluorescence of mercury at 235.7 nm is measured.

Development of the first practical CVAA system is generally attributed to Hatch and Ott (34) who, in 1968, achieved a detection limit of 1 ppb using a closed recirculating system to obtain a steady-state signal. Modifications of the method have since centered around the reduction vessel (38-41) and absorption cell (35) including conversion to an open system (36,37) in which the mercury vapor is swept once through the cell to produce a peak response. A significant advancement was contributed by Hawley and Ingle in 1975 (22) in which the mercury detection limit was reduced to 2 parts-per-trillion. A block diagram of the instrument is shown in Figure 1 (42). The absorption cell of large path length consisted of a 60 cm long, 2 mm i.d. Pyrex glass tube with inlet and outlet tubes fused to the wall, adjacent to quartz windows that faced the ends of the tube. In addition, the volume of the reduction vessel was significantly reduced compared to contemporary set-ups by introducing the volatilizing carrier gas through a fritted glass disc incorporated into the bottom of the reduction vessel (see Figure 2 (42)) as opposed to the immersion of

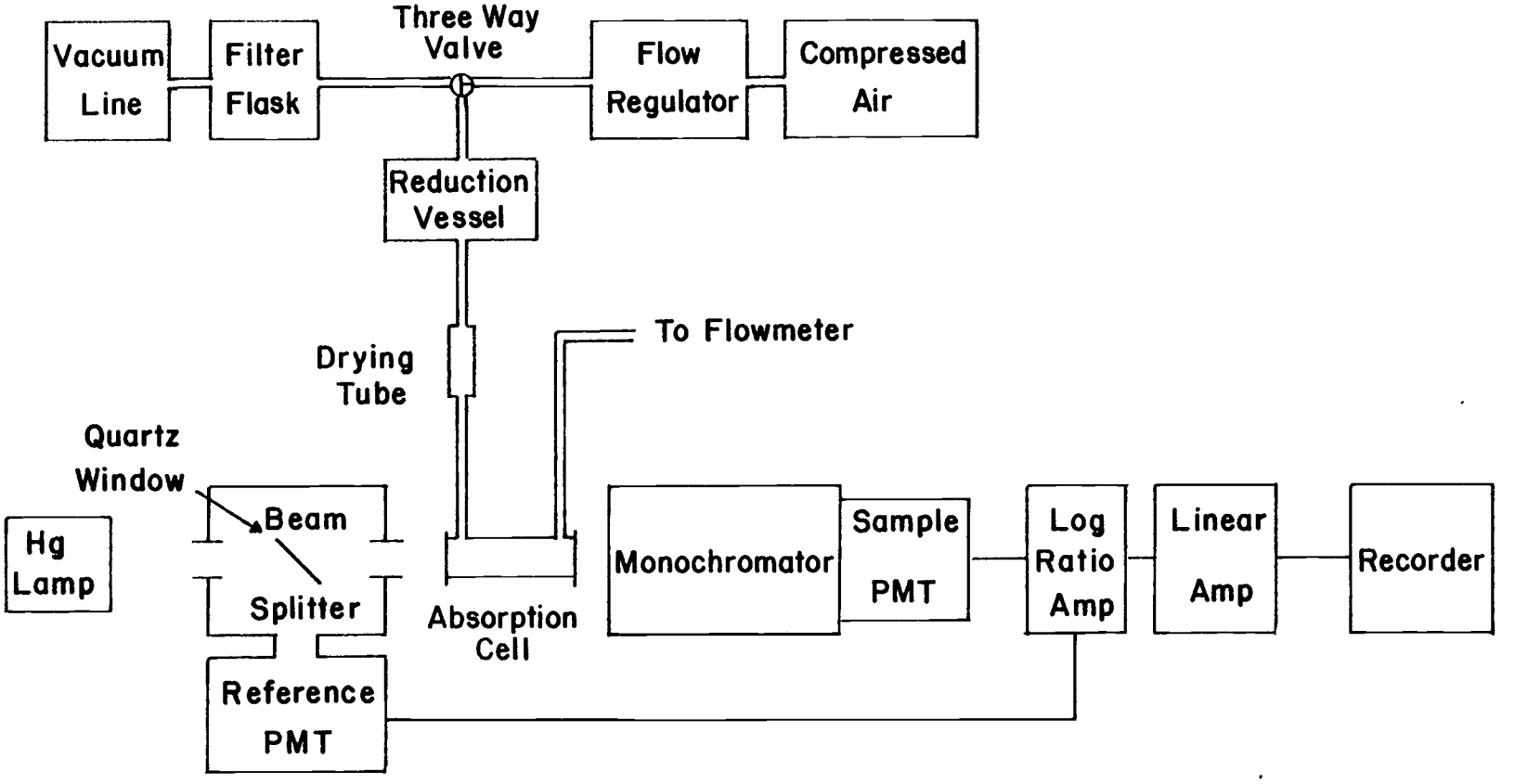


Figure 1. Double beam CVAA system used by Hawley.

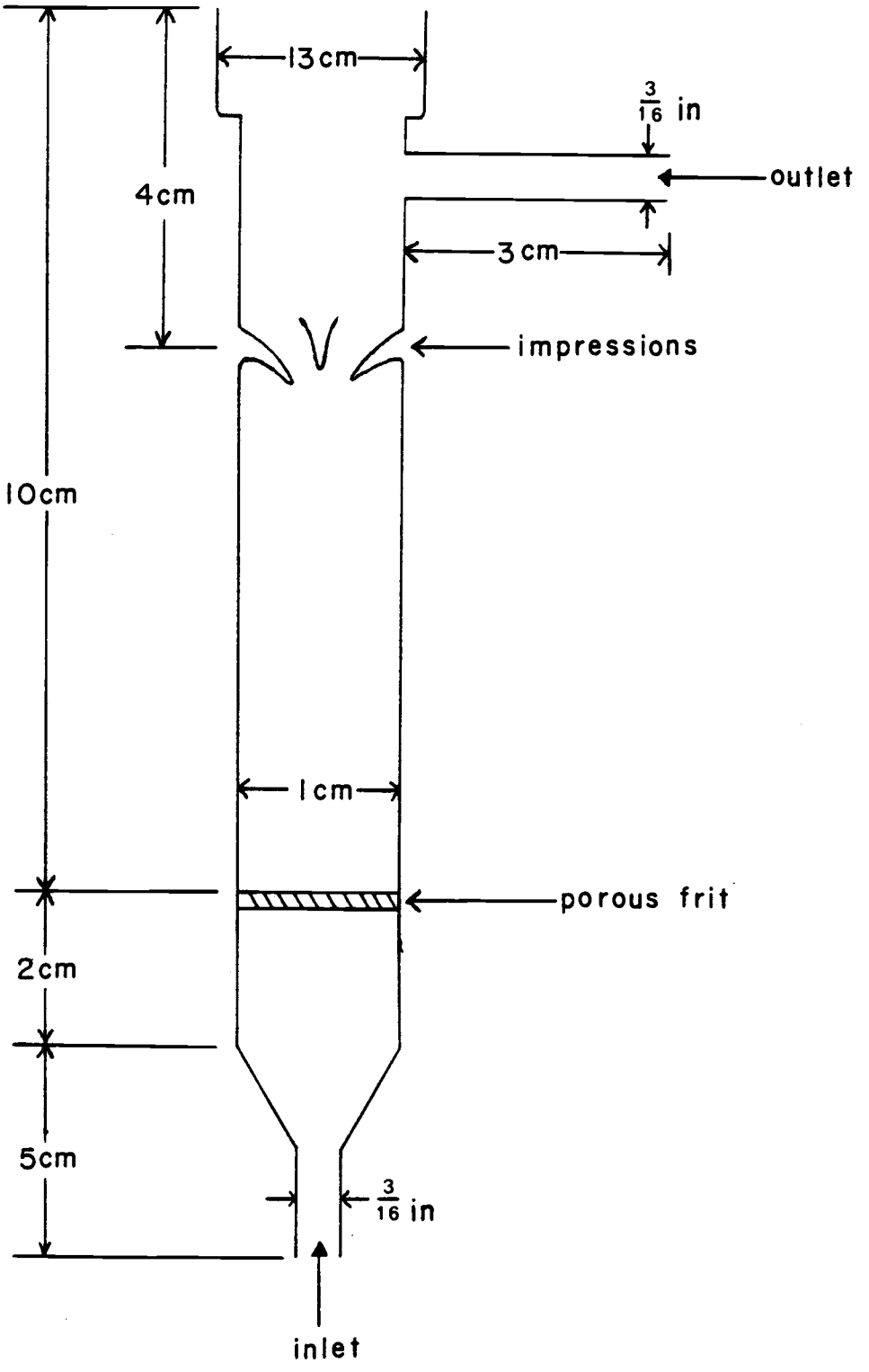


Figure 2. Discrete injection reduction vessel.

airstones in bottles which tended to require larger volumes. The efficient bubbling and small volume allows the Hg(0) to be swept out of the cell in a few milliliters of carrier gas to present a concentrated plug of Hg vapor to the absorption cell. The observation cell diameter was optimized so that the mercury plug fills the entire observation cell. A reference PMT monitors the Hg lamp intensity and this reference signal is used to correct for light source drift and flicker. The peak-to-peak baseline noise was about 0.0001 A.U. which allows small absorbance peaks to be measured.

During operation, 140 mL/min of N_2 flow upward through the reduction cell frit and 0.1 mL of 1% (w/v) of $SnCl_2$ is syringe injected into the reduction vessel through the rubber septum at the top of the cell. Next 1.0 mL of the sample or a standard solution is likewise injected into the reduction cell and the height of the resultant absorption peak is proportional to the mercury concentration. After about ten seconds, Hg has passed out of the reduction and observation cells and the reaction solution is evacuated through the reduction vessel frit into a vacuum flask to make the reduction cell ready for the next sample.

Addition of a pre-heating tube to this double beam atomic absorption system and electrical heating of the cell by Christmann and Ingle (43) eliminated noise and false absorbance peaks caused by water mist generated by the bubbling or by water vapor condensation on the windows. Previously a drying tube containing $Mg(ClO_4)_2$ as a dessicant was used and had to be replaced periodically.

Although highly specific for mercury, some interferences do exist such as erroneously high absorbances that result when some volatile

cyclic organic molecules, sulfur-containing molecules (15,16), or chlorine gas (44) are carried to the absorbance cell with the mercury. Attempts have been made to solve this by aeration of samples before reduction (37), extraction (45), precipitation of Hg as a sulfide (46), or background correction with a continuum source (47). The difference in the peak height before and after the above treatments or corrections is due to Hg. Water mist also posed a problem by causing scattering of the UV light, but has since been dispatched, as described above. Complexation such as bonding with sulfhydryl groups and especially amalgamation of elemental mercury onto the noble metals result in apparent losses. For example, Pt, Au, and Ag concentrations greater than about 1 μg/mL in solution decrease the mercury signal significantly with as much as 90% Hg loss by amalgamation when 5 ppm Au is present (48).

Preservation and Contamination

Preservation studies of inorganic and organomercury compounds, including a literature review (49), have been carried out to determine the best material for sample containment as well as chemical conditions suitable to minimize mercury loss in stored samples. Borosilicate glass and Teflon containers have been shown to confine mercury most effectively with polyethylene being least desirable with respect to analyte loss (27,50-52), although polyolefin bottles are nevertheless widely used for sample collection because of their low cost and high durability. Studies have been conducted on contamination and retention characteristics of various plastic containers (53-59). Several oxidizers such

as HNO_3 (53,65), $KMnO_4$ (64,65), H_2O_2 (54), $K_2Cr_2O_7$ (52), Au(III) (54), etc., as well as combinations (51,52) of these have been tested to prevent loss attributed mainly to adsorption onto container walls, volatilization of mercury compounds, or by reduction of Hg(II) to Hg(0) with subsequent volatilization or actual diffusion through vessel walls in the case of polyethylene (60). Toribara, Shields and Korval (61) have suggested that Hg(II) is easily reduced to Hg(I) followed by disproportionation of Hg(I) into Hg(II) and Hg(0) in the following manner:

$$Hg_2^{2+} \rightleftharpoons Hg^{2+} + Hg(0) \qquad E^o = -0.131\ V$$

$$\text{where } \left[Hg^{2+}\right] / \left[Hg_2^{2+}\right] = 6 \times 10^{-3} \text{ at equilibrium.}$$

However, the exact reduction mechanisms of the various species of Hg(II) are not completely known (27,63). Other possible methods that include adsorption on suspended colloids or particles, incorporation into stable complexes that may be difficult to decompose during analysis, or incorporation into stable amalgams after reduction, result in apparent mercury loss (i.e. become undetectable by the analytical procedure used). NaCl in excess of 3% has been shown to aid retention of Hg^{2+} (62) possibly by formation of Hg_2Cl_2 or $HgCl_4^{2-}$ complexes (50,56,61) and the sequestering amino acid cysteine has been shown to be an effective stabilizer (56). Christmann (43) and others (51) have shown that a very simple and effective preservation procedure for concentrations down to 0.1 ppb Hg^{+2} involves addition of 5% (v/v) HNO_3(c) and 0.01% (w/v) $K_2Cr_2O_7$, which induces complete retention for over 21 days. When a pH of about 0.5 is attained, HNO_3 apparently prevents adsorption

of mercury to the container walls while dichromate acts to inhibit Hg loss by reduction and subsequent volatilization (54). In the case of natural water, humic acid induces such volatilization (66).

Cleaning of glassware and sample containers is also of concern at these mercury levels. Methods include use of concentrated mineral acids (52,56,62), chromic acid (52), permanganate (22) and mixtures (50) as well as dilute (~10%) HF (58) to leach the mercury from vessel surfaces. Subsequent heating of glassware to 500°C (51,62) effectively vaporizes any remaining mercury. While this treatment would not be feasible with polyethylene, Heiden and Aikens developed a chloroform and aqua regia vapor (CAR) pre-treatment (60). The loss of 1 ppb Hg(II) solutions stored in treated bottles was ~7-10% over a period of 15 days (without use of additional preservatives) which was more effective than addition of 5% HNO_3 without the CAR treatment for polyolefin bottles.

Chemical Speciation

Chemical speciation has also drawn considerable attention as an analytical problem where identifying and quantifying the chemical forms or oxidation states of a particular element may be as critical as and more difficult than the total elemental determination. It has been shown that the methylated form of mercury (CH_3Hg^+) is 10 to 100 times more toxic (1) than inorganic mercury. Here, inorganic mercury is considered as "mercury in the form of elemental mercury, mercurous salts, as well as those complexes in which mercuric ions can form reversible bonds to such ligands as thiol groups on proteins" (2).

It has been stated that elemental, liquid mercury "is not a poison; a person could swallow up to a pound or more of quicksilver with no significant adverse effects" (68). However, in the case of elemental mercury, toxicity even varies with the method of absorption. Only about 0.01% of the amount ingested by rats is actually absorbed (2) whereas 50-100% of inhaled vapor is retained. A small amount of this vapor readily diffuses across the blood-brain barrier into the brain and subsequently oxidizes to Hg(II) which induces cell damage (67). Calomel (Hg_2Cl_2) or mercurous chloride has been used as a diuretic, cathartic, and antiseptic, whereas 1-2 grams of mercuric chloride (10-30% absorption of $HgCl_2$), which tends to accumulate in the kidneys and liver, is fatal.

Organic mercury compounds, on the other hand, are those in which mercury is covalently bonded to at least one carbon atom, and can be further broken down into three major classes based on physical and pharmacological properties: arylmercury, alkoxyalkylmercury, and alkylmercury. Of the three, alkylmercurial compounds, and methylmercury in particular (>90% of methylmercury in food is absorbed by humans (6)), exhibit the highest neurotoxicity because of their ability to cross biological membranes and induce enzymatic disruption (69). Organomercury compounds have a high affinity for thiol and sulfhydryl groups in protein (such as hair where total mercury concentration is about 300 times higher than that of whole blood (70)) and enzyme systems, and since organic mercurials are more strongly complexed, they are also more completely absorbed than inorganic mercury. Compounds from the other categories such as methoxyethyl mercury and phenylmercury are

apparently quickly metabolized to inorganic mercury which poses somewhat less of a hazard. In fact, such chemicals as thiomersal ($C_9H_9HgNaO_2S$), phenylmercuric acetate, and nitrate are used as preservatives to prevent growth of microorganisms in eyedrops and vials for multidose injections. Although they have largely been replaced by nonmercurials that have been shown just as effective in many pharmaceuticals, organomercurials with such trade names as Merthiolate®, Mercurachrome®, and Mercresin®, are still widely used as antiseptics.

Rabenstein et al. (78) studied the various methylmercury species and complexes that exist at equilibrium in aqueous solutions. The occurrence of methylated forms of mercury in natural waters results not only from direct industrial or agricultural discharge but it has been shown that inorganic or phenylmercury salts are transformed into methyl- and dimethylmercury by microorganisms under aerobic conditions (71-74). Such processes occur in bottom sediments and to a limited extent in soils, so that methylmercury can be incorporated in the complex aquatic food web in which the biological half-life of mercury in some fish consumed by humans may be as much as 700-1000 days (67, 75) and the concentration factor may exceed 3000. The biological half-life in humans is about 70 days (67,75). There is evidence that methylation may also occur in birds, animals, and in various species of fish which have shown that 50-95% of the total mercury content appeared in the form of methylmercury (79,80).

One of the first attempts at mercury speciation involved dithizone extraction (81) to differentiate phenyl- and ethylmercuric compounds in the presence of Hg^{2+} ions (a study of the stability of the mercury-

mercury-dithizone complex done by Litman, Williams and Finston (82)), but was only useful to ppm levels. Another complexing reagent, isothiocyanatopentaaquochromium(III) was used by Baltisberger and Knudson (76b) in a cation exchange procedure that separated nanogram quantities of CH_3Hg^+, Hg_2^{2+}, and Hg^{2+}. Once the cations were separated flameless atomic absorption was employed to detect down to 20 ng of mercury in samples.

A more exact method of differentiating inorganic from organic forms involves determining each species directly utilizing gas chromatography (GC) which, at trace levels may require extraction or other method for selective preconcentration. The basic separation procedure based on the Westöö technique for determining methylmercury (84-86) consists of extraction with an organic solvent (such as benzene or toluene) of the methylmercury halide, back-extraction with a basic cysteine solution, re-extraction, then the GC analysis. GC was used to separate and quantify organo-mercury compounds using an electron capture detector (13,90) which has high sensitivity to halogenated compounds but relatively low sensitivity to dialkyl and diaryl compounds, and could detect 10^{-12} g of methylmercuric chloride (87). Dressman (88) and Longbottom (91) were able to analyze for dialkylmercury compounds to 0.1 ng without conversion to their respective chloride salts by temperature programming the GC column to achieve separation of the dimethyl, diethyl, dipropyl, and dibutyl mercury compounds and combusting each fraction in a flame ionization detector (FID) which released free Hg(0) to be analyzed by a cold vapor mercury apparatus. GC determination of inorganic mercury can be carried out by

initial conversion to an organomercurial such as methylmercuric chloride by reaction with tetramethyltin (i.e. $(CH_3)_4Sn + Hg^{2+} \xrightarrow{2Cl^-} CH_3HgCl + (CH_3)_3SnCl$) (89) or to a phenylmercury salt using sodium benzene sulphinate ($C_6H_5SO_2Na$) as was done by Jones and Nickless (90). The GC equipped with a microwave powered inert gas plasma detector used to detect sulfur- phosphorus- and halogen-containing compounds by emission spectrometry was developed by McCormack et al. (92) and subsequently modified and applied to methylmercuric salts by Bache and Lisk (93) and Talmi (94) as a more selective, sensitive and efficient replacement for electron capture detectors. Talmi reported detection limits of 0.5 and 2 pg for Ch_3HgX and $(CH_3)_2Hg$, respectively. Picogram detection limits have been achieved for methylmercuric chloride by a combined GC-mass spectrometer system (87).

Arah and McDuffie (95) made use of the difference in molecular absorbance of $HgCl_4^{2-}$, which absorbs strongly in the UV, and $CH_3HgCl_2^-$, which is only weakly absorbing, to detect at least 1% Hg^{2+} in the presence of methylmercury salts.

A second approach to speciation involves determination of the exact concentration of inorganic mercury and of the total inorganic plus organic by CVAA where the quantity of organomercury is calculated by difference. In the use of CVAA, $SnCl_2$ or $SnSO_4$ in acid media have been shown to be the reducing agents of choice for inorganic Hg^{2+}. They have negligible effect on the organomercury on the same time scale.

Several procedures have been applied to the organomercury decomposition process in order to produce the Hg^{2+} species that can be reduced by stannous chloride. Becknell, Marsh and Allie (28) decomposed

organomercury compounds in water samples with 30 minute treatments of Cl_2 generated from a $KClO_3$/HCl mixture and reported 100% mercury recovery at the ppb level while Farey, Nelson and Rolph (96) used a $KBrO_3$/KBr pre-treatment to achieve >95% recovery for six organomercury compounds in a 10-15 µg/L concentration range. Others have used permanganate-sulfuric acid mixtures (65,97), perchloric acid (98), and potassium persulfate (99,100), and other oxidizing agents (36, 99,101) to break down organic mercury while minimizing heating of the sample to prevent loss through volatilization. The approved U.S. Environmental Protection Agency (EPA) digestion procedure requires aqua regia and potassium permanganate as oxidants (102). Calder and Miller (103) compared several digestion procedures and found that a 2:5 mixture of perchloric and sulfuric acids was fastest (5-10 min) and could be performed at room temperature with a minimum of reagent interference. Matsunaga, Ishida and Oda (104) were able to reduce organic mercury from fish samples with stannous chloride in the presence of 1 M sodium hydroxide after homogenizing the samples and adding HCl and 0.5% cupric chloride. This mechanism of mercury release is apparently based on the preferential exchange of Cu^{2+} with organically bound mercury to set the mercuric ion free and requires about 20 minutes for total exchange. However, the need to perform individual analyses for total and inorganic mercury, the time required to prepare the samples, and the often hazardous reagents that may be required make these organomercury digestion approaches to speciation less feasible for routine analyses of large numbers of samples.

A 550 watt mercury arc lamp was used by Goulden and Afghan (105)

to irradiate samples in silica tubes and required 40 minutes to achieve total ultraviolet photochemical decomposition of methylmercuric bromide and 60 minutes for phenylmercuric acetate, while di-methyl, di-ethyl, and methyl mercury(II) compounds decomposed in 20 minutes. Kiemeneij and Kloosterboer (106) used low pressure Zn-Cd-Hg lamp irradiation times of about 20 minutes for organomercury decomposition, followed by CVAA to detect total mercury in the ppb range. Determination of samples with and without irradiation resulted in values for total and inorganic mercury, respectively. When compared to wet chemical decomposition which consisted of allowing the sample to stand for 20 hours with 2 or 4% $KMnO_4$ depending on the approximate mercury content, Kiemeneij's method agreed within 4% at 1 µg/L levels of mercury and resulted in lower blank values. Agemian and Chau (44) have shown that a 3 min UV digestion in the presence of sulfuric and nitric acids for total mercury determination resulted in recoveries of >90% for seven common organomercury compounds and determined mercury concentrations as low as 0.5 µg/L. In distilled water, methylmercuric chloride has a peak absorbance ($\varepsilon = 1.0 \times 10^4$ abs. $\underline{M}^{-1}$ cm^{-1}) at 189 nm (95). Use of these photochemical decomposition techniques greatly reduce the risk of contamination by added reagents, but still requires specialized silica irradiation cells and separate CVAA analyses for total and inorganic mercury.

Another approach to speciation involves use of reagents capable of selectively decomposing the organomercury compound while reducing the liberated Hg(II) in the same step. Magos (107) was able to determine inorganic mercury and total mercury in fish and rats by initially

digesting the solid sample in a cysteine, sodium chloride, and 45% sodium hydroxide solution, and subsequently reducing inorganic mercury in 1 - 20 mL of the diluted homogenate with 100 mg of stannous chloride and 20 mL of 45% NaOH to release the inorganic mercury. This was followed by 10 mL of 16 N H_2SO_4, and finally 1 mL of a 50% (w/v) stannous chloride - 10% (w/v) cadmium chloride reagent and 20 mL of 45% sodium hydroxide for organomercury. It was estimated that the homogenization procedure may have caused splitting of 5-8% of the methylmercury present. Magos also conducted studies of mercury in blood using the same basic procedure (108) and achieved a detection limit of 0.5 ppm in 1 mL of whole blood with an average recovery of 97%. Blank values for reagents ranged between 2.5 and 3 ng. It was also noted that the deflection for the 10 ng Hg internal standard used in whole blood was always less than that for a pure standard. Other reducing agents such as ascorbic acid in an alkaline medium and 40% hydrazine hydrate (109) have been used with lesser success.

Sodium borohydride, a well known reducing agent (110) has been employed in flame atomic absorption and emission spectrometry for determining various elements such as Sn, Sb, As, Se, etc. by the generation of their respective volatile covalent hydrides (111-114). $NaBH_4$ was used for mercury detection by Lyashenko and Stepanov (115) who found that for a 4×10^{-2} µg Hg/mL mercuric chloride standard, maximum absorbance was observed for pH values above 12.0 in a 5 mL total volume test solution and over a 0.01 - 3.0% range of reducing agent concentrations. Linearity was achieved over a 0.0005 - 0.14 µg range where the lower limit is the detection limit.

Toffaletti and Savory determined total mercury in urine and studied the effectiveness of $NaBH_4$ for reducing mercury(II) chloride, methylmercury(II) chloride, and phenylmercury(II) acetate using 1.5 mL of an aqueous 5% (w/v) $NaBH_4$ solution added to a buffered pH 6.5 system (116). Quantitative reduction of all three compounds was achieved for 0.5 M buffer solutions ranging in pH from 0.5 - 9.0 although more rapid rates of reduction were observed to occur at lower pH's. It was also noted that at room temperature the methylmercuric chloride peak area was one-third that of the phenylmercuric acetate peak while at absorption cell temperatures above 530°C (the absorption cell was constructed of quartz) the difference in responses was greatly reduced. The detection limit was 1-2 ng. Cu^{2+} at a concentration of 100 µg/mL resulted in only 70% recovery of 50 ng of mercuric chloride, although Cu^{2+} at lower concentrations accelerate the reduction of organomercury compounds.

Rooney studied interferences of the Hg^{2+} ion reduction by $NaBH_4$ (117) and found that, for mercury at a concentration of 100 ng/mL in acidified solutions of these various metals present at a 1 mg/mL level, Cu(II) reduced the mercury signal by 75%, bismuth(III) by about 60%, and silver by 50%, while Au(III), Pt(IV), Pd(II), Rh(III), and Ru(III) suppressed the mercury peak completely. Au(III) caused complete suppression of the peak down to levels of 100 µg/mL while having a negligible effect at 1 µg/mL. Lyashenko and Stepanov found that 30 µg Pt(IV), 10 µg Pd(II), and 0.4 mg Te(IV) interfered and 0.3 µg Ag^{+} depresses the result by about 50%. Most common anions did not interfere (115,116).

EXPERIMENTAL

Solution and Glassware Preparation

Only reagent grade chemicals were used in all solutions and all dilutions were made with deionized water from a Millipore Milli-Q system fed by house distilled water. The following solutions were prepared for the continuous sample flow section:

Reducing Solution:

1%(w/v) stannous chloride: 1.0 g $SnCl_2$ (Mallinckrodt, Inc.) + 1.0 mL HCl(c) (J. T. Baker Chemical Co.) diluted to 100 mL.

Mercury Stock Solution:

100 ppm inorganic mercury(II) stock solution: 0.1354 g $HgCl_2$ (Mallinckrodt, Inc.) + 50 mL HNO_3(c) (J. T. Baker Chemical Co.) + 10 mL 1% (w/v) $K_2Cr_2O_7$ (Mallinckrodt, Inc.) diluted to 1.0 L.

The stannous chloride should be dissolved completely in HCl before addition of water to prevent formation of tin hydroxide precipitates, and the solution was prepared daily.

The following solutions were prepared for speciation studies:

Reducing Solution:

1%(w/v) stannous chloride: prepared as described above.

1%(w/v) sodium borohydride: 1.0 g $NaBH_4$ (J. T. Baker Chemical Co.) + 1 mL 5 M KOH (J. T. Baker Chemical Co.) diluted to 100 mL.

Oxidizing Solutions:

20% (v/v) nitric acid, 1% (w/v) potassium dichromate:

20 mL HNO_3(c) + 1.0 g $K_2Cr_2O_7$ diluted to 100 mL.

Mercury Stock Solutions:

100 ppm Hg(II) inorganic stock solution: prepared as described above.

100 ppm Hg(II) methylmercuric chloride stock solution: 0.1252 g CH_3HgCl (Alfa Products Division, Ventron Corp.) dissolved in 25 mL acetone and diluted to 1.0 L.

50 ppm Hg(II) ethylmercuric acetate stock solution: 0.0720 g CH_3CH_2HgCl (Alfa Products Div., Ventron Corp.) dissolved in 25 mL acetone and diluted to 1.0 L.

10 ppm Hg(II) phenylmercuric chloride stock solution: 0.0156 g C_6H_5HgCl (Alfa Products Division, Ventron Corp.) dissolved in 25 mL acetone and diluted to 1.0 L.

The organomercury compounds did not go into aqueous solution readily and had to be dissolved initially in acetone, with the aid of an ultrasonic bath to disintegrate the crystals and speed dissolution. Phenylmercuric chloride was particularly difficult to solvate and the stock solution concentration was reduced to 10 ppm as a result. No preservatives were added to the organomercury stock solutions which were stored under refrigeration to reduce decomposition and loss. Solutions of 100 ppb Hg(II) concentration were made for each compound from Hg stock aliquots and subsequent dilutions were made from these solutions. Mercury standards prepared by dilutions of the 100 ppb solutions were preserved with 1% (w/v) HNO_3(c) and 0.01% (w/v) $K_2Cr_2O_7$ and

analyzed within twelve hours after preparation. Measurements of aliquots for standards were performed with Eppendorf fixed volume (error < ± 0.6% at 2.0-5.0 mL) autopipets. No dilutions greater than 1:1000 were made. The methylmercury aliquots for standards were added after initially diluting the HNO_3-$K_2Cr_2O_7$ preservatives to about 3/4 of the final volume to minimize decompositions of the organic species. Only weighings for mercury standards were carried out on an analytical balance (± 0.1 mg). All other weight measurements, of lesser criticality in terms of accuracy were performed on a top-loading balance (± 0.01 g). The $NaBH_4$ solution is useful for more than two weeks if refrigerated and kept under basic conditions (about pH 13) to prevent hydrolysis.

All glassware utilized in this work was borosilicate glass. Glassware was cleaned by soaking overnight in a 1% (w/v) $KMnO_4$-5% (v/v) HNO_3(c) solution followed by two HCl(c) washes to dissolve precipitated MnO_2 and a wash with HNO_3(c). Rinses with copious amounts of deionized-distilled water followed each cleaning step. Subsequent cleaning of treated glassware was done with HNO_3(c) and water washes. Periodic cleaning of the reduction vessel used in speciation studies was done by soaking in a 1:1 HNO_3-H_2SO_4 mixture.

Instrumentation

The double beam atomic absorption (AA) instrumentation was essentially identical to that developed by Hawley (21) and modified by Christmann (43). A few changes were made to this instrumentation. First was the addition of a constant current device constructed by

Riley Chan, student at OSU, to stabilize the DC pen lamp current. The mercury pen lamp was fired on the AC mode and allowed to warm for a minute before switching to the DC mode. The DC polarity was reversed periodically to increase lamp life. Second the monochromator was replaced by a Pomfret Research Optics Inc. 2537 $\pm$ 20 Å (120 $\pm$ 20 Å bandwidth, 18.5% transmittance at 2535 Å) optical interference filter attached in front of an aperture machined in an aluminum plate which allowed limited translation along three axes. The PMT housing was mounted to the aluminum plate on the opposite side of the aperture. Replacement of the monochromator by the filter caused no significant difference in baseline noise. Christmann (43) added a Variac controlled 13 mm i.d., 7 cm long pre-heating tube containing 1-mm diameter glass beads, completely wrapped with no. 28 nichrome wire (3 turns/cm), asbestos tape, and aluminum foil to replace Hawley's drying tube packed with magnesium perchlorate ($Mg(ClO_4)_2$). The pre-heating tube used here was reduced in volume by use of an identically wrapped 4 mm i.d. 5 cm long tube with glass wool as the filling material. Christmann also encased the absorption cell in nichrome wire heated with a Variac which prevented formation of water mist and condensation on the cell quartz windows. For this work, similar heated absorption cells were constructed and the windows were mounted with high-temperature epoxy or Varian Associates Torr Seal® Low Vapor Pressure Resin. Third the optical apparatus was permanently mounted on a 1/2 inch thick 2 ft x 4 ft aluminum plate with the help of John Archibald, O.S.U. Chemistry Department machinist, while the wooden pen lamp/beam splitter housing was reduced to about half its original size to economize on

space, and was painted a somewhat subdued shade of metallic blue in an attempt to promote aesthetic appeal.

A block diagram of the instrumentation (modification of diagram from Hawley) and pump set-up for continuous flow analysis are shown in Figures 3 and 4. The peristaltic pumps used in continuous sample flow systems were a calibrated Pharmacia Peristaltic Pump P-3 (0.6 - 410 mL/hr) for the inlet flow and a Cole-Parmer Masterflex Model 7020C peristaltic pump (up to 33 mL/min) for the cell outlet flow. The 1.17 mm (0.046 in) i.d. plastic "T" used to bring the reducing and mercury solutions together was from Value-Plastics Inc. Gas-Tight Hamilton syringes were used for discrete sampling analyses, and Roger Gilmont Instruments, Inc. flowmeters (size nos. 2 & 3) were used for measurement of pre-purified nitrogen carrier gas flow rates. The flowmeter calibration charts, calibrated for air or water flow did not require correction for measurement of pre-purified nitrogen flow (within stated accuracy of 2.0% of reading or one division, whichever was greater).

The final design of the continuous flow reduction vessel constructed by Mario Boschetto, O.S.U. Chemistry Department glassblower, consists of a 3.5 in long, 3/8 in i.d. Pyrex glass tube constricted at both ends to accept 0.25 in bore tygon tubing, with tapered inlet and outlet solution ports at opposite ends of the tube as depicted in Figure 5. The reduction vessel design used in the discrete sampling speciation studies is identical to that used by Hawley, i.e. modification of a 1.0 cm coarse frit sealing tube (21). PVC Tygon Tubing (0.25 in i.d.) was used for connection of all paths of nitrogen flow, while 1/8 in i.d. microbore tubing was used for interconnection between

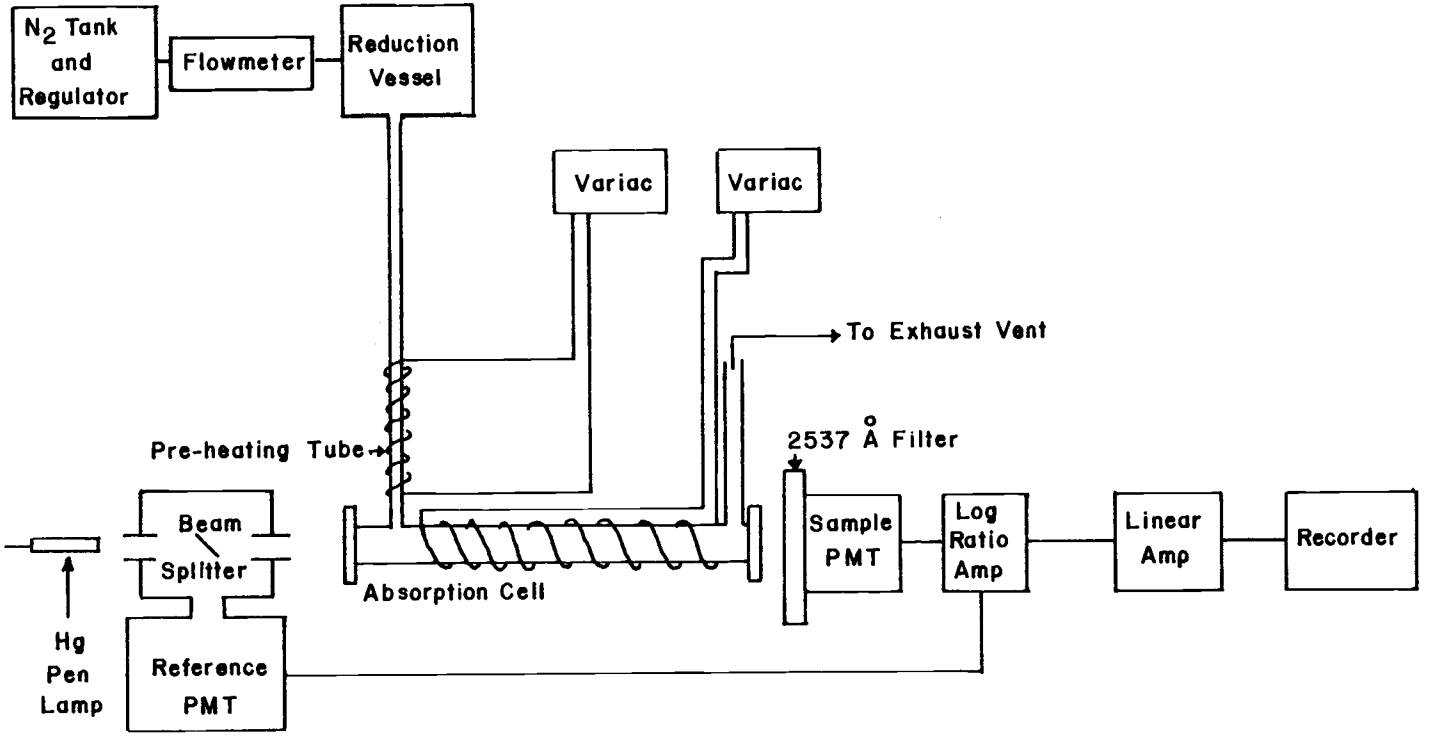


Figure 3. Block diagram of double beam CVAA system.

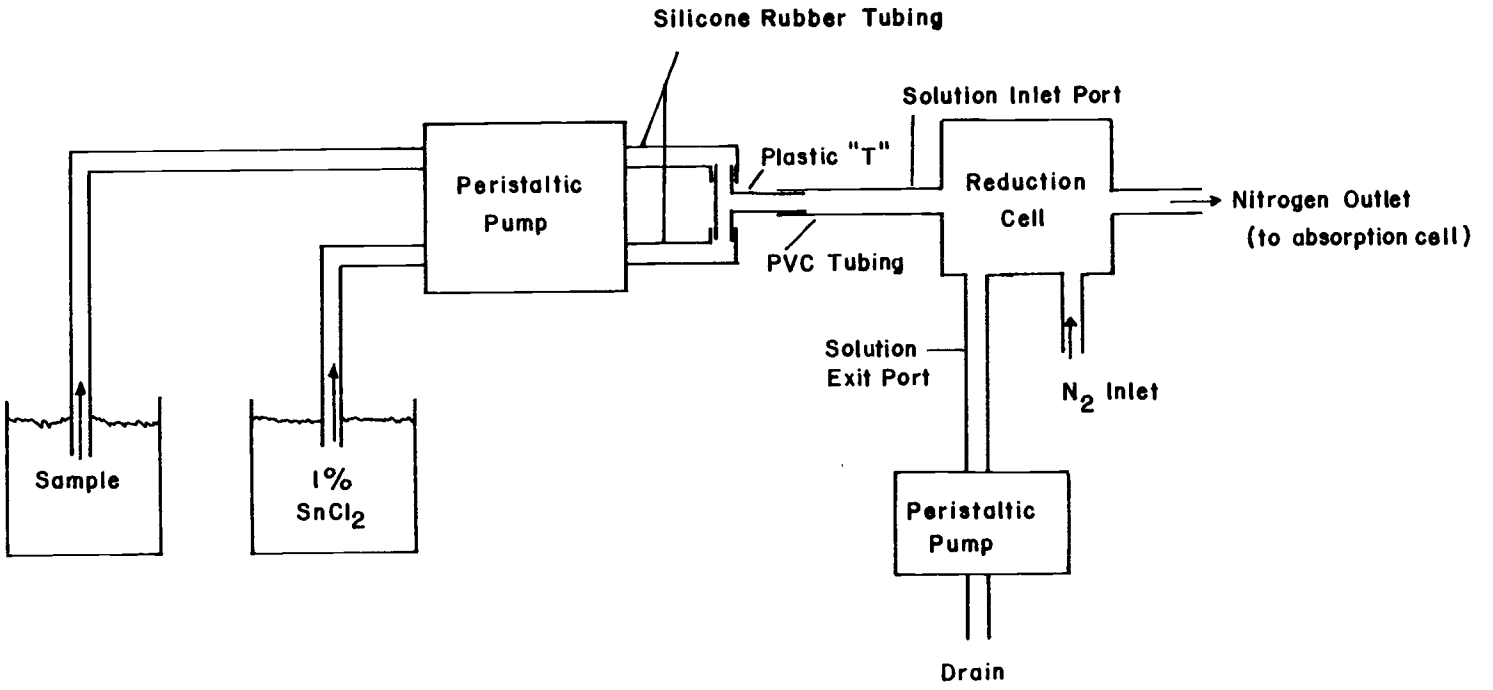


Figure 4. Block diagram of peristaltic pump set-up for the continuous sample flow cold vapor mercury analyzer.

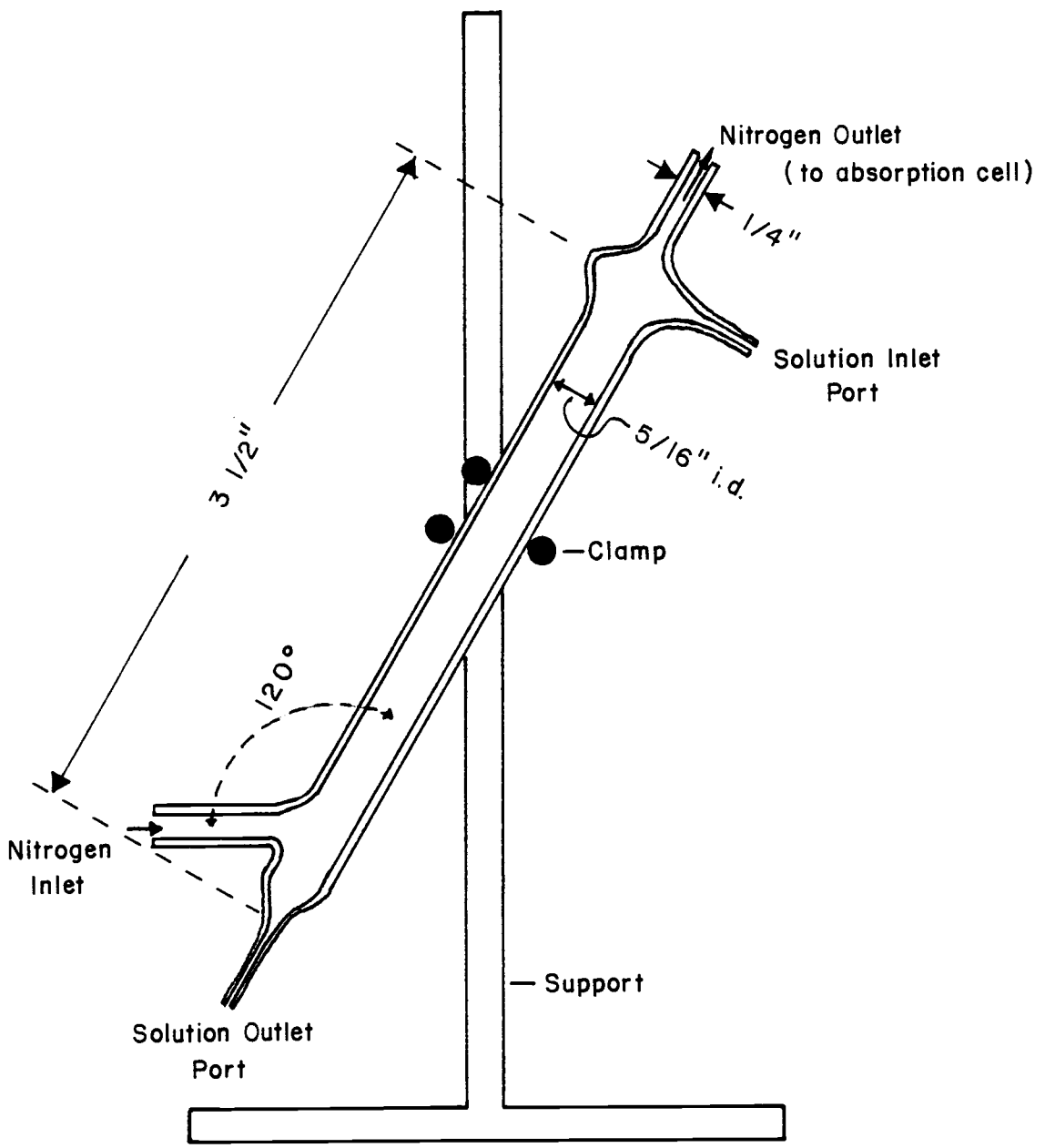


Figure 5. Thin stream reduction vessel.

peristaltic pump plumbing, with each pump requiring its own special internal tubing to function properly.

Procedure

All AA measurements were made with the basic double beam system used by Hawley (modifications discussed previously) where the transfer function of the logarithmic ratio amplifier is described by Equation 2.

$$E_o = k \log_{10}(i_s/i_r) \qquad (2)$$

where

E_o = output voltage, V

k = logarithmic slope, 1 V per decade

i_s = photoanodic current from sample PMT anode, A

i_r = photoanodic current from referenct PMT anode, A

Both the reference and sample photomultiplier power supply voltages were initially adjusted to produce a 10^{-5} A photoanodic current, and the sample PMT power supply was varied to adjust E_o to approximately zero with no Hg in the observation cell. The linear amplifier device was used in its current-to-voltage converter configuration (10^4-10^8 ohms feedback resistors) to set the initial reference and supply currents but assumed the function of a voltage amplifier (gains of 1-10^4) for all AA measurements. The mercury pen lamp was initially warmed for about 1 minute in the AC mode before being switched to DC. The lamp was previously determined to be most stable at ~7 mA of current. Lamp current polarity was reversed periodically to extend the life of the pen lamp. The absorption cell was optically aligned between the 0.2 in port in the pen lamp housing and the PMT to achieve maximum

throughput of radiation. The RC time constant for the voltage amplifier for continuous sampling was 1 s while for speciation studies using discrete injections a 0.32 s time constant was employed.

Continuous Sample Flow Methods

The optimal design of a continuous sample flow reduction vessel is the focus of this procedure and should provide the following desired conditions: 1) minimal solution and vapor dead volume to reduce peak response times, 2) efficient mixing of Hg(II) species and reducing solutions for maximum chemical interaction between the two species, 3) the vessel must be easily cleaned or self-flushing, preferably with non-wetting surfaces to reduce contamination and response time, and 4) maximization of the area of air-liquid interface to promote Hg volatilization into the inert gas stream. This last criterion was accomplished in three ways: by mist formation and subsequent diffusion of Hg^{o} out of the droplets, by vigorous bubbling of an inert gas through the solution mixture, or by passing a stream of gas over a thin film of solution.

The ideal chart recorder absorbance output from a continuously sampling flow system where the sample and blank are pumped alternately through the reduction vessel for equal lengths of analysis times, would closely follow that of a square wave (see Figure 6). The height which represents the difference is absorbance between the blank solution and the sample is proportional to the equilibrium concentration of mercury vapor passing through the cell (and consequently proportional to concentration

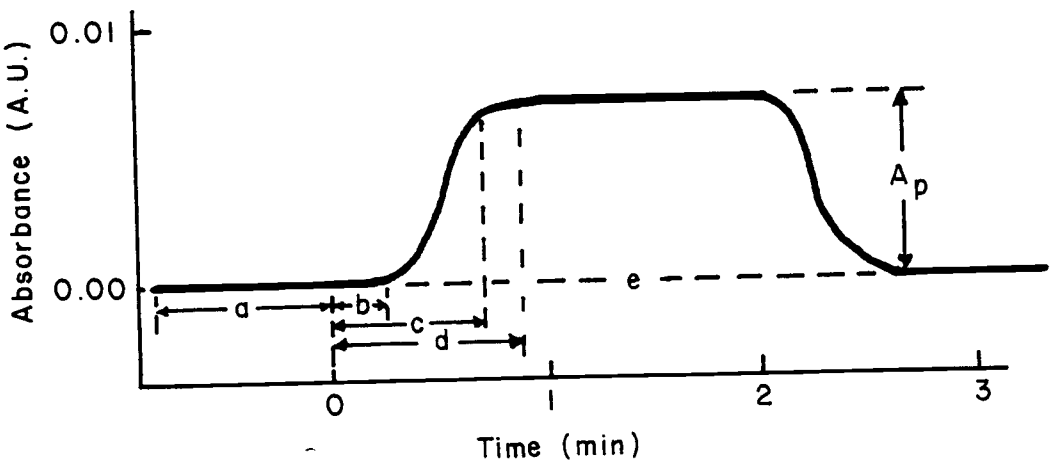


Figure 6. Typical observed absorbance plateau for a 1 ppb Hg^{2+} standard using a thin stream reduction vessel under optimum conditions.

a = baseline established by blank solution

b = time between solution entrance to reduction vessel and response

c = response time to achieve 90% A_p

d = response time to achieve 100% A_p

e = baseline interpolation

A_p = plateau absorbance

of mercury in the sample) and the breadth of the waveform is directly related to the time of sampling. However, practical considerations such as electronic, and more importantly, solution and carrier gas displacement lag times (collectively referred to as the response time) result in rounded corners and non-vertical sides. The measured plateau height is taken as the difference between a horizontal line connecting the blank baselines before and after the sample peak and a line best judged to represent the plateau of the peak. If baselines do not coincide exactly, an average is taken. The response time is determined to be the time elapsed after the mercury solution enters the cell ($\pm$ 2 s measurement error) and the point at which the tracing reaches a certain percentage of the absorption determined to be the top of the plateau. Response times to achieve 90% of the maximum absorbance (90% A_p) and the maximum absorbance (100% A_p) are presented and are used as figures of merit for comparison of reduction vessel efficiencies.

The following discussion is concerned with several reduction cell designs to maximize sensitivity while minimizing the response times and explores the concepts of mists, bubbles, and a thin stream as means of releasing elemental mercury from the aqueous sample.

Continuous sampling with mist formation: The production of water mist in an attempt to increase the water-to-gas surface area was achieved with the use of a total consumption burner-nebulizer (Beckman model no. 4030, medium bore oxygen-hydrogen burner) with the nozzle directed into the center of a 10 in long, 3/8 in i.d. Tygon tube section

(see Figure 7) that terminates at the absorption cell. A hollow stainless steel tube, sealed at one end with a hole notched into its side (similar to a basketball inflating needle) is inserted into the bottom of the bowed tubing and makes use of the carrier gas back-pressure to push the expended solution from the reduction vessel. This rate of drainage is controlled by an attached microbore PVC tube and screw clamp. A plastic "T" and 40/1000 in tubing brought the sample and $SnCl_2$ reducing solutions together into the nebulizer which required a minimum nitrogen flow rate of about 2 L/min to maintain aspiration of the solution (total uptake of approximately equal volumes of each solution was about 0.5-1.0 mL/min). The twenty-fold increase in the carrier gas flow rate compared to that necessary in discrete injections required changing from the 2 mm i.d. absorbance cell to a 5 mm i.d. (60 cm long) tube to reduce back-pressure and the 13 mm diameter preheating tube was replaced by a 5 in long, 1/4 in i.d. glass tube as mentioned earlier. The flowmeter (size no. 3 rather than the previously used size no. 2 was necessary to measure these large flow rates) was moved in front of the reduction vessel and remained at that position for the remainder of the continuous sample flow studies.

Continuous sampling with bubbling aeration: The reduction vessel to accommodate this method of aeration is a modification of that used by Hawley with the addition of solution entrance and drainage ports (see Figure 8). The sample and reducing solutions are brought together with a plastic "T" that leads directly to the reduction vessel and are carried separately before the "T" through silicone rubber tubes of the precisely controlled three-channel peristaltic pump that permits

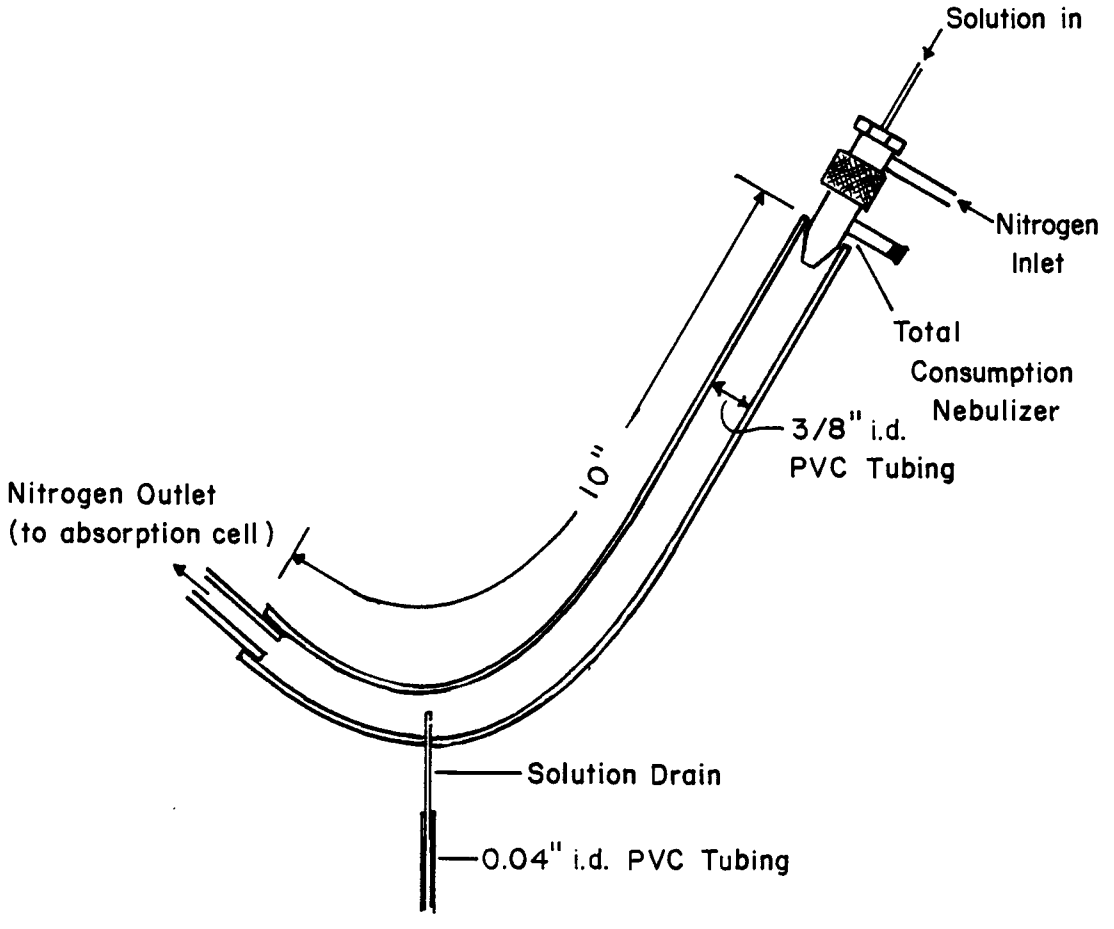


Figure 7. Mist formation mercury reduction technique.

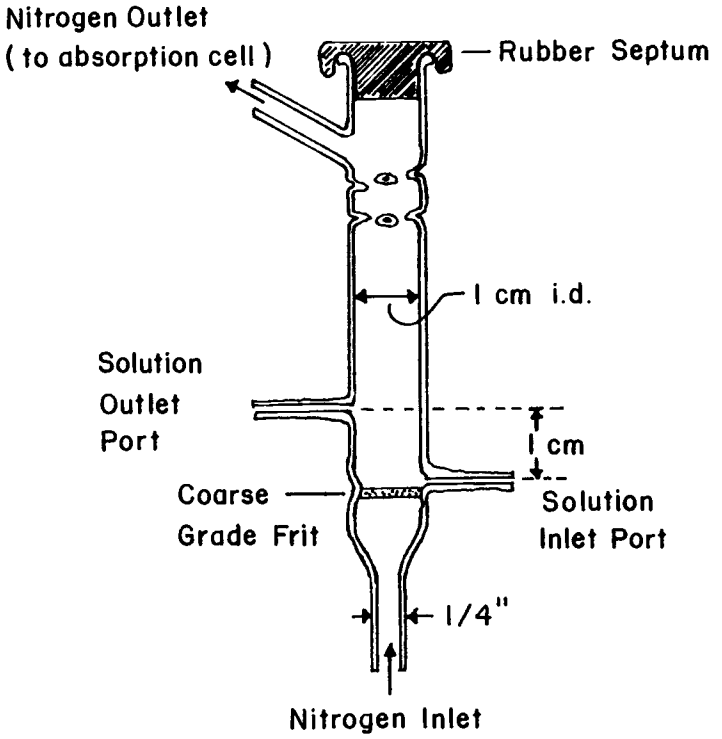


Figure 8. 1 cm i.d., 1-cm bubbling aeration reduction vessel (modification of discrete injection reduction vessel)

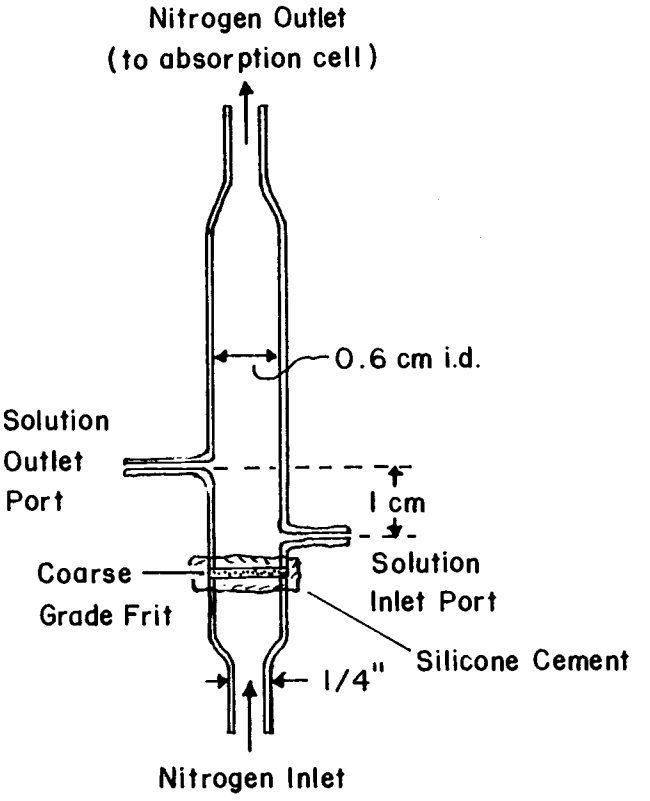


Figure 9. 0.6 cm i.d., 1-cm bubbling aeration reduction vessel.

constant flow (reproducibility about ± 1%). The sample moves through a special 3.1 mm i.d. silicone rubber pump tube (25-410 mL/hr) situated in the first channel of the rotating drum and, simultaneously, reducing solution flows into a 2.1 mm i.d. tube (10-160 mL/hr) seated in the second channel so that although the drum rotation rate is identical for both tubes, the smaller volume of reductant required may be controlled to a limited extent by the tube diameter.

The use of back pressure to expel the exhausted solutions was not effective because of different viscosities of air and solution. This caused fluctuations in gas flow (particularly noticable with lower flow rates) through the reduction and absorption vessels depending on whether liquid or gas from the bubbles was draining through the tube at any particular instant. This problem was overcome by using a peristaltic pump at the solution outlet port which provided a constant average total output flow despite the material viscosity passing through the exit tube. While the input pump flow rate was optimized the drainage pump (which itself was difficult to reproduce a speed exactly) was set visually to the minimum flow rate (about 15 mL/min) that would maintain a constant bubbling level in the vessel. Three reduction vessels with solution exit ports 0, 1, and 2 cm above the frit (the inlet port is situated as close to the frit as practically possible and the center of this port was about 0.2 cm above the frit surface) were examined to determine the effect of the volume of solution in the reduction vessel on absorbance and response time.

As will be discussed later the reduction vessel response time of about 45-60 s was slightly long for multiple analyses and one apparent

cause of this was the volume of solution which must be displaced in the vessel whenever a new sample is introduced. The increase in concentration of a given component in the reduction vessel follows an exponential function as a first approximation as the new solution enters the vessel, mixes with the solution already present, and the mixture exits. This characteristic implies that the greatest change in solution concentration occurs at the beginning of the dilution and, in this particular application, the duration necessary to achieve 90% of the final absorbance level of either blank or sample may be as long as the time to approach 99% of the final absorbance from 90% of the final absorbance.

This problem of extended response times due to dilution may be alleviated by using a reduction vessel of smaller volume (i.e. smaller diameter) while maintaining the same solution flow rates. The lower size limit is determined by whether solution will be forced out of the reduction vessel and into the absorption cell by the velocity of the carrier gas. Since smaller diameter sealing tubes were not available a facsimile was fashioned by gluing, with silicone rubber cement (Dow Corning Silastic® 732 RTV), and a 1 cm glass frit between the facing ends of two 0.6 cm i.d. glass tubes, the upper segment having had solution tubes attached (see Figure 9). Nitrogen passed out of the vessel through the top rather than a side arm as was the case with the previous vessel. The actual analysis procedure is identical to that of the aforementioned modified Hawley continuous sample bubbling aeration flow cell.

Continuous sampling with a thin stream and countercurrent gas flow:

In this method, the carrier gas is passed over a thin stream or layer of solution containing the dissolved elemental mercury flowing in the opposite direction. The surface tension of aqueous solutions coupled with the desired non-wetting characteristic of the reduction vessel prevents the actual formation of an ideal thin film with large surface area. Instead a slow stream of solution is allowed to flow down the inside of the inclined 5/16 in i.d. glass tube with appropriate inlet and outlet gas and solution ports (see Figure 5). The combined sample and reducing solutions from the peristaltic pump enters from the upper port of the vessel while nitrogen flows into the 1/4 in tube near the bottom. Studies with water dyed with food coloring showed that slow solution movement from the pump resulted in laminar flow and very inefficient mixing as the combined solutions meet at the "T" and move to the reduction vessel. Unlike the bubbling aeration vessels where agitation and volatilization occur simultaneously in the reduction vessel, mixing must be achieved before the solutions enter the thin stream vessel. Various mixing chambers of minimal volume employing such devices as baffles (see Figure 10), constrictions, and obstructions were inserted in the stream to induce turbulence. The only effective modification made use of a magnetic stirrer and a 2 x 5 mm teflon coated stir bar. The entrance port of this 0.2 mL volume mixing chamber design was horizontal and the exit port had to be vertical to prevent entrapment of air bubbles (see Figure 11). However the effect of the additional stirring proved negligible for the effort expended. An ultrasonic bath was also used to induce mixing without success.

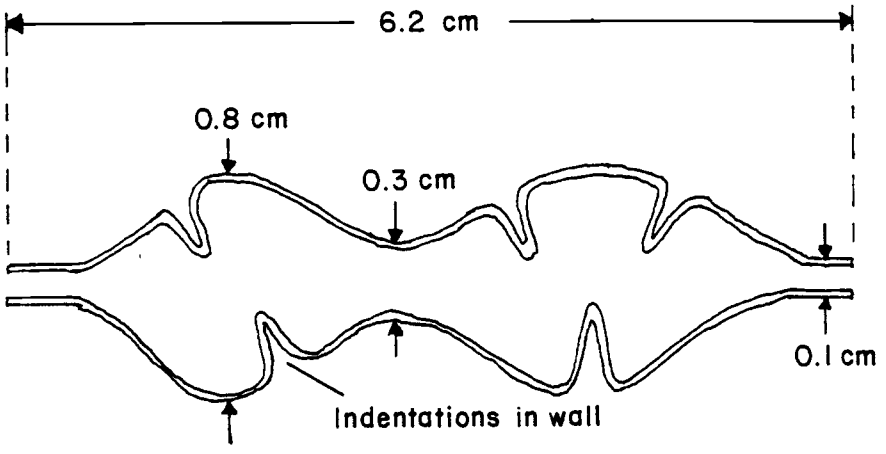


Figure 10. Baffles to induce solution turbulence.

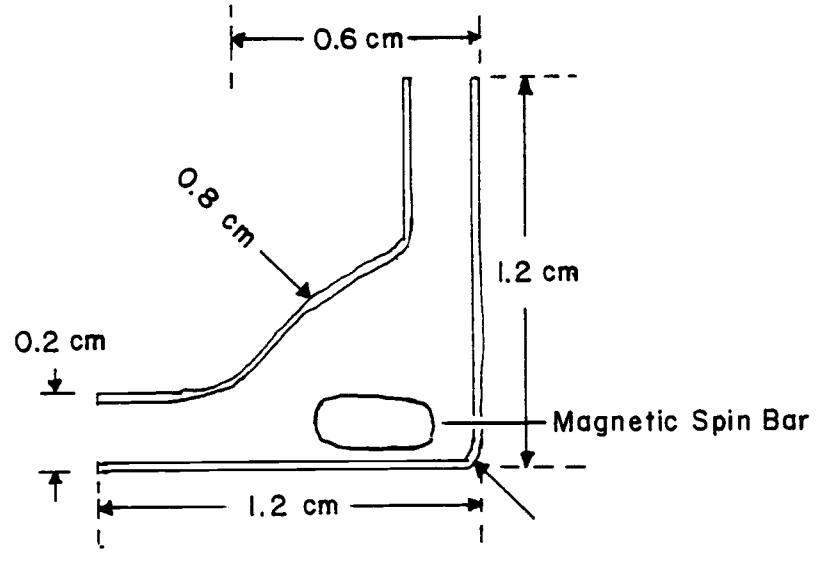


Figure 11. Micro-mixing chamber with 2 mm x 5 mm magnetic spin bar.

Optimization studies: As with any analytical instrumental technique, the analytical signal should be maximized for a given concentration (sensitivity) in the shortest length of time (response time) while maintaining acceptable limits of accuracy and precision. Unfortunately, one aspect is often compromised in order to compensate for another. In these continuous sampling systems the sample flow rate, nitrogen flow rate, reduction vessel volume, absorption cell temperature, and in the case of the thin stream reduction vessel, angle of inclination, length, and diameter of the reduction vessel were varied to optimize conditions for maximum sensitivity and signal-to-noise with minimum response time. A 1.0 ppb Hg^{2+} solution was used in all optimization studies. Sample flow rates were varied from 2.8 to 5.5 mL/min by changing the pump speed and the flow rate of reductant was changed between 1.1 and 2.2 mL/min. Manipulating the flow regulator or the micrometer adjustment on the gas flowmeter allowed changes of the carrier gas flow between 30 to 3000 mL/min. The temperatures of the nichrome wire wrapped absorption cell and pre-heating tube were varied between 58 to 178^{o}C by changing the voltage setting on the appropriate Variac. A chromel alumel junction thermocouple and voltage - temperature conversions from the Omega Engineering Inc. Temperature Measurement Book (118) were used to calibrate the Variac settings against pre-heating tube and absorption cell temperatures. At least 15 min were allowed for thermal equilibrium to be attained after changing a Variac setting.

Speciation Studies

The discrete injection procedure for inorganic Hg^{2+} used by Hawley consisted of injecting 0.1 mL of 1% $SnCl_2$ followed by 1.0 mL of the sample. For the speciation studies, the 2 mm i.d., 60 cm long absorption cell and Hawley's reduction vessel were used in the instrumental set-up diagrammed in Figure 3. Diffusion of Hg(0) out of solution resulted in a plug (the volume of carrier gas containing mercury atoms diffused from the solution) which exhibits a maximum peak height on the chart recorder when the front end of the plug reaches the end of the absorption cell, and tails off as all of the mercury diffuses out of solution and the mercury vapor concentration in the carrier gas approaches zero. The observed peak width is reduced if the volume of the mercury plug entering the cell or the cell volume is decreased (to the point that the cell volume is much smaller than the plug volume). The N_2 flow rate of about 85 mL/min was chosen as slow enough to produce maximum absorption as a result of minimum vapor dilution while maintaining reasonable response times and efficient agitation and bubble aeration using a coarse grade glass frit.

Sodium borohydride, a much stronger reducing agent than $SnCl_2$ is capable of reducing organically bound mercury(II) species where $SnCl_2$ fails. The following final sequence and volumes of injections is based on the selective reducing capability of the two reagents to differentiate and quantify inorganic Hg^{2+} and organomercury containing solutions:

1. 0.1 mL 1% (w/v) $SnCl_2$
2. 1.0 mL preserved sample or blank

3. 0.1 mL 1% (w/v) $K_2Cr_2O_7$ + 20% (v/v) HNO_3(c)

4. 0.1 mL 1% (w/v) $NaBH_4$ + 0.05 M KOH

To facilitate cleaning, a coarse frit reduction vessel was utilized in these speciation studies. With nitrogen flowing through the frit, the $SnCl_2$ is injected fairly quickly and the pen is allowed to stabilize to a baseline value before the sample or blank solution is injected. After the inorganic peak has come off, and baseline established again, the $K_2Cr_2O_7$-HNO_3 mixture and $NaBH_4$ are syringed in rapid succession which results in the organomercury peak. As before, the measured peak height is taken as the difference between the average of the horizontal lines representing the baselines before and after the peak (should the baselines not coincide) and the peak absorbance. A slight peak (<0.005 A.U.) may appear as solutions are being injected. This results from minutely varying gas flows as syringe needles are inserted and removed and solution is sprayed onto the frit, and is readily distinguished from a peak absorbance by the immediate response.

Maintenance of a consistent gas flow is critical in the precision of absorption measurements and the flowmeter reading was checked before each sample injection. Control of the nitrogen flow was carried out using the micrometer valve adjust of the Gilmont flowmeter although for the most part manipulating the low pressure flow regulator (applied pressure of 2-4 psi) or the three-way gas valve was more efficient and simpler. After the organomercury peak has returned to the baseline the reduction vessel is evacuated of its contents, the rubber septum removed and the walls are rinsed with copious amounts of deionized-distilled water. The vacuum is allowed to continue for a few seconds

to remove the remaining water (including water that may be trapped in the sidearm) and the septum is replaced preparing the vessel for another run. One-ppb concentrations relative to Hg(II) of inorganic and organomercury species were used to determine the reducing conditions and sequences.

Calibration curves using this procedure were run with combinations of mercuric chloride and each of three organomercuric compounds, methylmercuric chloride, ethylmercuric acetate, and phenylmercuric chloride. A study of the breakdown of CH_3Hg^+ under the various preservative conditions that would be employed for real samples was also conducted. Ratios of 0.03:1 and 1:0.03 ppb Hg^{2+} to CH_3Hg^+ were tested for recovery as a demonstration of selectivity. Finally, real samples were analyzed and the digestion procedure for organics was tested for interferences.

RESULTS AND DISCUSSION

Continuous Sampling Flow Methods

In this section, the analytical characteristics of the three basic designs employing mist formation, bubbling aeration, and a thin solution stream with countercurrent gas flow for a continuous sample flow reduction vessel described in the Experimental section will be presented. Their performance characteristics will be compared to ideal behavior and with one another. Optimization was based on maximization of sensitivity and signal-to-noise ratio with a minimum response time (preferably <1 minute).

As described in the Experimental section the recorder tracing reaches a plateau absorbance when the maximum equilibrium concentration of mercury atoms in the carrier gas is attained. A theoretical approximation of this maximum concentration present in the absorption cell for a given concentration of mercury in solution at specific solution and carrier gas flow rates can be calculated from equation 3

$$c_g = F_s \times c_s / F_g \qquad (3)$$

where

c_g = concentration of Hg(0) atoms in the carrier gas, mole/L

F_s = flow rate of solution into the reduction vessel, mL/min

c_s = concentration of Hg^{2+} ions in solution, mole/L

F_g = flow rate of carrier gas through the absorption cell, mL/min

This equation is based on the assumptions that all of the mercuric ions flowing into the cell are reduced and volatilized into the carrier gas, that all of the vapor is carried through the absorption cell

immediately after release, and that the amount of mercury vapor carried out through the solution drainage tube is negligible (<5% of the carrier gas volume). Application of Beer's Law (Equation 4) converts the Hg(0) concentration in the gas to absorbance units which may be measured instrumentally.

$$A = \varepsilon b c_g \qquad (4)$$

where

A = absorbance, A.U.

ε = molar absorptivity of mercury atoms at 254 nm, 4.1×10^6 $moles^{-1}$ L cm^{-1} (40)

b = cell path length, 60 cm

or

$$A = (4.1 \times 10^6 \text{ mole}^{-1} \text{ L cm}^{-1})(60 \text{ cm}) F_s c_s / F_g \qquad (5)$$

$$= (2.5 \times 10^8 \text{ mole}^{-1} \text{ L}) F_s c_s / F_g \qquad (6)$$

Equation 6 allows calculation of theoretical absorbances to which experimental data can be compared.

Continuous sampling using mist formation: The design based on mist formation was previously shown in Figure 7 in the Experimental section. The sample and reductant solutions flow into a "T" and are drawn by the vacuum imparted by a standard total consumption nebulizer. The mist is sprayed into a section of Tygon tubing fitted with a drain and the resulting mercury vapor passes through a pre-heating tube and into the absorption cell. In reaching this final design, the spraying of the mist into different diameter glass "T"'s or "Y"'s and different drainage systems was tested.

Several problems arose that resulted in abandoning this design as a viable approach to continuous mercury volatilization. 1) Memory effects and drift resulted from continuous formation of large droplets on the walls of the Tygon tubing immediately surrounding the nebulizer head. Attempts to vaporize the generated water mist would have been severely inadequate or physically impractical. 2) The sensitivity was insufficient, due in part to the dilution of the volatilized Hg in the more than 2 L/min of carrier gas required to induce efficient nebulization. The dilution factor would have resulted in a calibration sensitivity of about 6×10^{-4} A.U./ppb Hg^{2+} (see Equation 6) assuming all the mercury in solution was transferred to the carrier gas (which was probably not the case). This is already well below the reasonable working range of the CVAA set-up and was the major factor for dispensing with the method. 3) The baseline noise and drift were relatively large (typically 0.001-0.002 A.U. peak-to-peak) and probably due in part to mist entering the observation cell. The low calibration sensitivity and high baseline noise would have resulted in a high detection limit. 4) The uptake of the solution (0.25-0.5 mL/min of each solution) was based on a vacuum drawn by the nebulizer and did not necessarily insure consistent and equal flow through sample and reductant tubes leading to the "T". Slight differences in solution viscosity or particulate obstructions would cause considerable variability in results. 5) The large carrier gas volumes consumed would have required more frequent replenishing--a drawback when attempting to automate a system. This concept of mist formation was abandoned in favor of the following bubbling aeration method.

Continuous sampling using bubbling aeration: The bubbling aeration vessel shown in Figure 8 makes use of a coarse frit situated at the bottom of the vessel through which nitrogen passes. The sample continuously flows into and out of ports situated on opposite sides of the vessel and vigorous bubbling of the solution liberates Hg vapor which is carried to the absorption cell.

The continuous sampling bubbling aeration vessel with the exit port 2 cm above the inlet port (denoted 2-cm cell) was tested initially for feasibility of the method, employing a sample flow of ~4.1 mL/min, a reductant flow of ~1.9 mL/min and a nitrogen flow of ~75 mL/min. The optimization of absorption cell temperature, cell volume, carrier gas flow, and solution flow was then carried out once operation of the method was established. The flow rate of the peristaltic pump was calibrated and found to have maximum flow rates of 5.7 and 2.6 mL/min at the highest setting using 3.1 mm i.d. and 2.1 mm i.d. tubing, respectively. A small amount of pulsing was observed in the solution flow that could not be eliminated but did not pose a problem. The maximum flow for the Masterflex peristaltic pump used at the exit port was ~32 mL/min, and a flow of about 15 mL/min was set to maintain a constant solution level in the reduction vessel. The outlet pump had to operate at a higher rate than the solution inlet pump because gas from the bubbles as well as solution were drained to prevent overflow into the absorption cell. Although the occasional formation of large bubbles that rose past the baffles of the reduction vessel and forced solution into the absorption cell was a nuisance, no antifoam agent was really necessary or used for synthetic samples. A small tuft of

glass wool situated on the baffle just below the carrier gas exit tube facilitated the dispersion of bubbles that rose to that point.

As shown in Table II, the absorption cell temperature, controlled by a Variac connected to the nichrome heating wire coiled around the absorption cell did not significantly affect the measured absorbance. The absorbance for a 1 ppb Hg^{2+} solution was 0.015 $\pm$ 0.001 A.U. over the 100-150°C temperature range. Varying the pre-heating tube temperature over approximately the same range also did not affect the peak absorbance. Since there was no readily apparent advantage to using extremely high temperatures which broadens the absorption spectrum while reducing the amplitude (119), the absorption cell and the pre-heating tube were maintained at 135°C. This is hot enough to prevent condensation of water on the windows and expedite the evaporation of water that might blow over into the absorption cell without vaporizing the glue that holds the quartz windows. The use of improper or aging epoxy at these temperatures can result in apparent absorbances or positive drift due to volatilizing organics, as was encountered at one time. Other specialized high temperature cements than the one described in the experimental section are available (e.g. Techkits Adhesive Kit 100A or 120A, S-29 high temperature cement).

The optimization of carrier gas flow rate involves a compromise between plateau height (i.e. sensitivity) and response time. Lower flow rates resulting in increased experimental plateau absorbance (denoted A_e) but longer response times as shown in Table III and Figure 12. Typical recorder response curves are for a low and high flow rate as illustrated in Figure 13. This is behavior consistent

Table II. Effect of absorption cell temperature on sensitivity[a]

Variac voltage (V)	Temperature (^{o}C)	Absorbance (A.U.)
50	100	0.015
60	135	0.015
65	150	0.014

[a] $[Hg^{2+}]$ = 1.0 ppb, nitrogen flow = 75 mL/min, sample flow = 4.1 mL/min.

Table III. Results of testing the 2-cm reduction vessel[a]

N_2 flow rate, Fg (mL/min)	Absorbance (A.U. x 10^{-2})		% of theoretical absorbance	Fg x A_e	Response time (min)	
	A_e	A_t	A_e/A_t x 100	(A.U. mL/min)	for 90% A_e	for 100% A_e
26	4.0	20	20	1.0	2.6	8.2
70	1.7	7.3	23	1.2	1.2	3.1
120	1.2	4.3	28	1.4	0.67	2.0
164	0.89	3.1	29	1.5	0.44	1.6
204	0.70	2.5	28	1.4	0.42	1.2

[a]Vessel tested using 1 ppb Hg^{2+} solution. The sample flow rate was 4.1 mL/min.

A_e = experimental plateau absorbance.

A_t = theoretical plateau absorbance calculated from equation 6.

NOTE: For this and all succeeding tables and graphs, where exponents are indicated in the heading, the numbers listed are the mantissas of the blanketing exponent. e.g. In Table III above, the A_e value 4.0×10^{-2} is represented as 4.0 in the table.

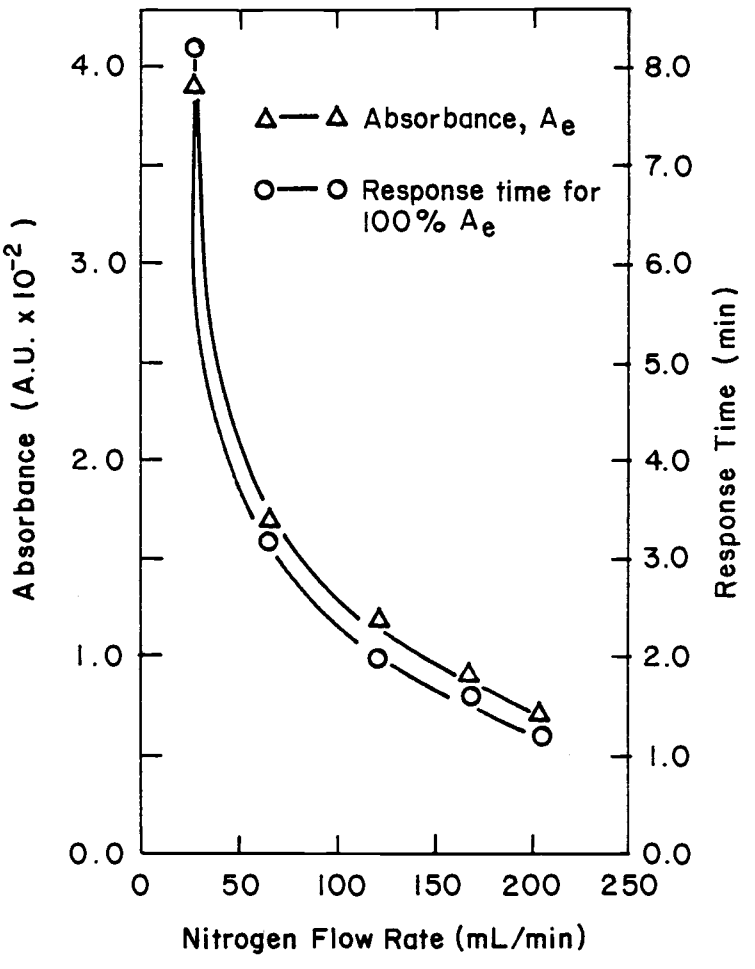


Figure 12. Effect of nitrogen flow rate on absorbance and response time for the 1 cm i.d., 2-cm bubbling aeration vessel with 1 ppb Hg^{2+}.

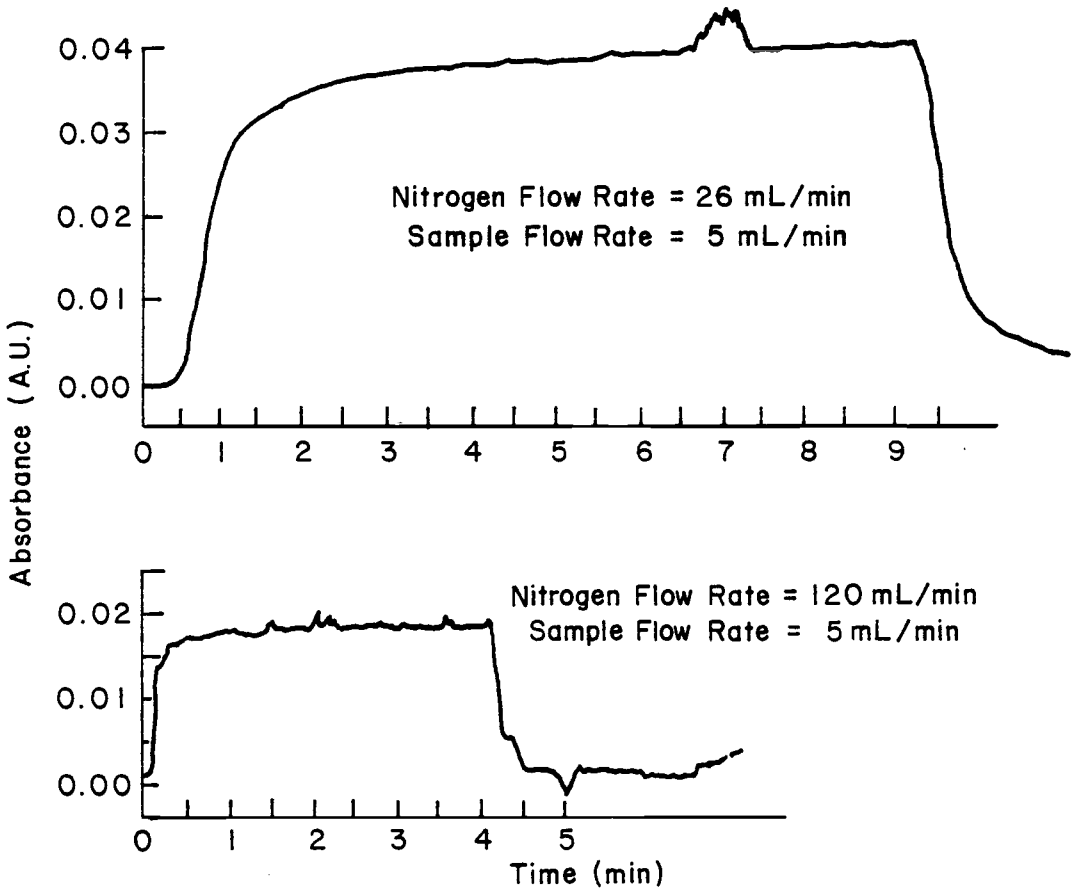


Figure 13. Comparison of typical response tracings of 1 ppb Hg^{2+} for low and high carrier gas flow rates using the 1 cm i.d., 1-cm bubbling aeration vessel.

with the concept described previously that higher flow rates of gas increase the dilution of mercury vapor with a consequent decrease in absorbance (see Equation 6). Higher flow rates also produce more efficient solution mixing and aeration of the gaseous dead volume with the effect of reducing the time necessary for the system to achieve equilibrium. Again, the response time was taken as the time interval between the point at which the mercury-containing solution entered the reduction vessel and that where an equilibrium concentration of mercury vapor was attained as defined by the top of the plateau. This point of solution entry was determined by following a bubble that was introduced to the stream when the sample tube was moved from the blank solution to the sample and vice versa. The time elapsed to reach 90% of the plateau absorbance (denoted 90% A_e) that is also listed can be compared to the 100% response time (100% A_e) to indicate the difference in relative rate of the initial rise in absorbance to the final rate of approach to the plateau absorbance.

Nitrogen flow rates much greater than 200 mL/min caused excessive bubbling and overflow. At very low flow rates and hence bubbling rates it is expected that the efficiency of mercury transferred to the carrier gas stream would be lower. This is confirmed by the ratio of experimental to theoretical absorbances on Table III where a constant ratio of 28 to 29% is actually observed for flow rates >120 mL/min. However, this ratio is smaller at much lower carrier gas flow rates. This behavior is probably due to poorer volatilization as the bubbling is visibly inefficient. From Equation 6, for a given mercury concentration and solution flow (i.e. constant $F_s \times c_s$), $F_g \times A_e$ should be constant

assuming that all or a constant fraction of total mercury in solution is volatilized. $F_g \times A_e$ values shown in Table III decrease with flow rate for gas flow rates less than 120 mL/min. This also demonstrates the decrease in volatilization efficiency at lower gas flow rates. Even for higher nitrogen flow rates, the efficiency of volatilization is not good and it was shown that about 10% of the original concentration was extracted when the reaction mixture was collected and passed through the reduction vessel a second time.

Compared to absorbances exhibited with no gas flowing through the absorption cell, nitrogen, at a rate of about 120 mL/min gave an apparent absorbance of about 0.001 A.U. which may be due to impurities in the gas (pure nitrogen does not absorb at 254 nm), contamination of the reduction vessel, absorption cell, or tube walls which release the interferent(s) at a constant rate. The baseline absorbance increased with F_g, however this did not affect measurements of mercury provided the nitrogen flow rate did not vary and the resulting background absorbance was constant. A nitrogen flow of about 120 mL/min was therefore chosen for further work as a compromise between sensitivity and response time. The response time of about 2 min is still rather long. It was decided to attempt to reduce the response time by reducing the volume of solution that is aerated before being drained, by changing the vertical distance between the inlet and exit ports.

Table IV shows that reducing this inlet-to-exit port separation to 1 cm or less reduces the response time by about 0.7 min (from 2.0 to 1.3 min). The response times are qualitatively consistent with expected results as the reduction in volume also means a reduction in the time

Table IV. Effect of solution volume on response time and sensitivity[a]

Vessel i.d. (cm)	Distance between exit port and frit (cm)	Calculated Volume[b] (mL)	Response time (min) for 90% A_e	Response time (min) for 100% A_e	Absorbance, A_e (A.U.)
1.0	0.2	0.16	0.31	1.3	1.4
1.0	1.2	0.94	0.45	1.3	1.4
1.0	2.2	1.7	0.67	2.0	1.2
0.6	1.2	0.34	0.16	1.2	0.88

[a] $[Hg^{2+}]$ = 1 ppb, N_2 flow = 120 mL/min, solution flow = 4.8 mL/min.

[b] Volume between frit and center of exit port.

NOTE: Distance between the center of the inlet port and the top of the frit is approximately 0.2 cm for each vessel. Distance between inlet and outlet ports were 0, 1, and 2 cm, denoted 0-cm, 1-cm, and 2-cm vessels, respectively, in the text.

necessary to attain equilibrium. The actual solution volumes involved and the dynamics of mixing are difficult to determine (the 0-cm cell appeared to have more than 0.16 mL in it at any instant) because of the vigorous bubbling. A crude model which can be used to estimate the general relative effects of varying the solution volume in the bubbling vessel is based on a stirred tank in which pure solution A pours into a vessel of pure solution B at a constant flow rate, F, while the mixed A + B solution exits from the bottom also at flow rate F. The fraction of solution that has been purged after a given length of time can be approximated by Equation 7 (120).

$$x = 1 - e^{-Vt/F} \qquad (7)$$

where

x = fraction of original solution B that has been removed

V = initial volume of solution B, mL

t = time after initial addition of solution A, min

F = inflow and discharge solution flow rates mL/min

According to this equation, about 15 min would have been the time necessary to achieve 90% solution A (and 30 min to achieve 99% A!) for the 1-cm vessel with stirring but no bubbling. In actuality the plateau is experimentally reached (a constant fraction of mercury vapor is volatilized and transferred into the absorption cell) in a fraction of the time calculated with the model. This indicates that the cell design may be more conducive to solution displacement than is predicted by the model and/or that the actual volume of solution in the cell that must be displaced is less than the calculated 0.94 cm^3.

This model may not exactly apply since it should not be necessary to totally displace all the solution previously in the cell to achieve the maximum concentration of mercury in the carrier gas. For instance, when the mercury solution starts to enter the cell, blank solution already in the cell should have no effect on the absorbance if all of the Hg^{2+} ions are reduced and volatilized immediately since it is the absolute amount of mercury present in the reduction vessel at any instant (for a constant input flow rate) that determines the absorbance. However, since only a fraction of the total mercury appears to be released even at equilibrium, the actual concentration of mercury (ions and atoms) in solution in the vessel may also have some bearing on the amount of mercury released before the solution equilibrium concentration of mercury is attained.

A semi-logarithmic plot of $\log_{10}$ of the percentage attained of the maximum plateau absorbance versus time shown in Figure 14 was constructed from data from a recorder tracing of the rising part of the absorbance signal. Time zero was taken at the point where the absorbance increased initially when Hg^{2+} was introduced into the vessel. This plot can be analyzed in two sections. The first non-linear part of the plot takes into account the very rapid initial increase in absorbance up to the point where 90% of the total plateau absorbance is reached, and appears to be based on the immediate release of mercury as the sample enters the vessel. The linear portion of the graph displaying greater than 90% of the plateau absorbance gives evidence of an exponential approach to the equilibrium concentration, and also indicates that smaller solution volumes should produce

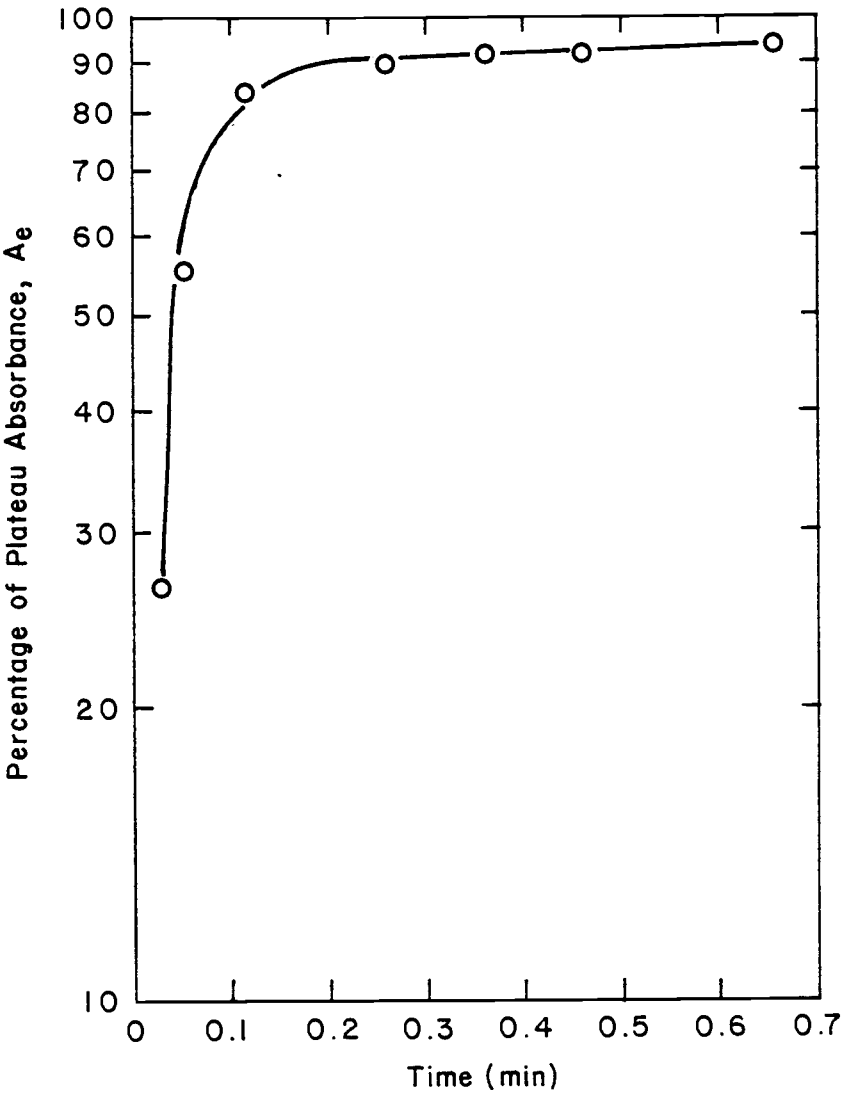


Figure 14. Plot of time versus $\log_{10}$ of the percentage of total absorbance using measurements extracted from a typical chart recorder output.

shorter response times.

The fact that the 2-cm cell exhibited a slightly smaller sensitivity than the other two designs was somewhat unexpected and could be attributed to the difficulty in exactly setting the gas flow rate consistently from day to day. Overall, the performances of the 0- and 1-cm vessels were quite comparable but the 1-cm vessel gave slightly less baseline noise due to the physical buffering effect on bubbling which is characteristic of a larger solution volume, and was selected as the optimum volume for further studies.

The plateau absorbance was measured using the 1-cm vessel as a function of sample flow rate as shown in Figure 15. Here a 1 ppb Hg^{2+} solution was continually fed into the reduction cell starting at a pump setting of 5 (2.4 mL/min). The setting was then incremented by one unit at a time to the maximum setting of 10 (5.8 mL/min) and the change in plateau absorbance at each increment was recorded. The procedure was repeated using a water blank in place of the 1 ppb mercury solution and the blank signals at corresponding solution flow rates were subtracted. As expected, at lower flow rates the plateau absorbance is linearly related to the flow rate as predicted by equation 6. This was consistent to the point (at about 4.5 mL/min) where apparently the fraction of mercury transferred to the carrier gas before the solution exits decreases due to a reduced residence time in the reduction vessel. Extrapolation of the plot to higher flow rates indicates that a plateau would be reached so that solution flow rates much higher than 5 mL/min results in wasted sample and reagents with non-commensurate increases in sensitivity. A trend toward shorter

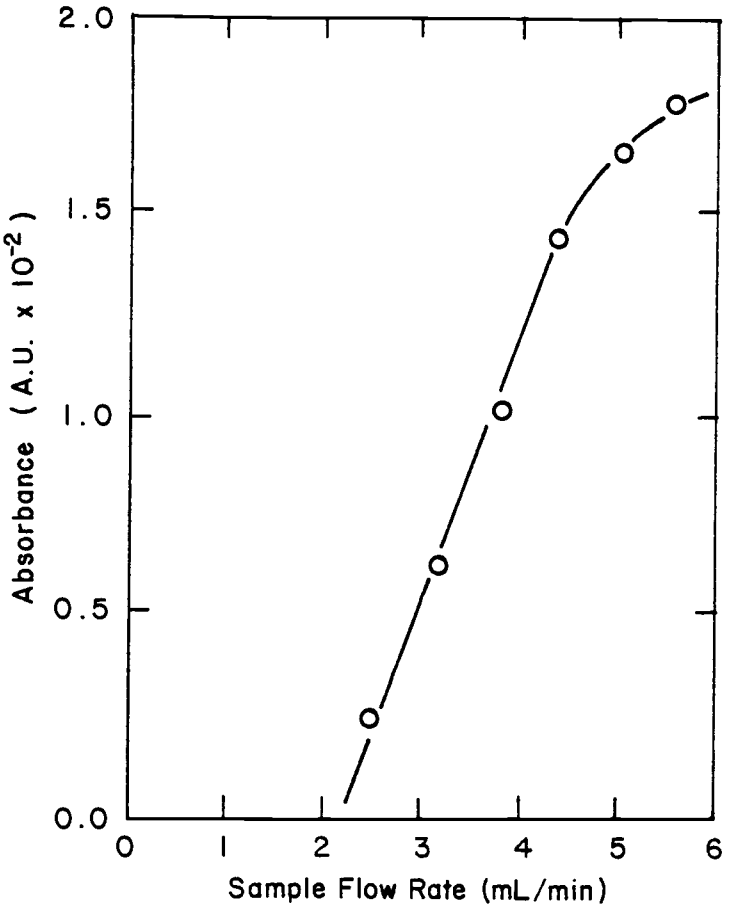


Figure 15. Effect of solution flow rate on absorbance for the 1 cm i.d., 1-cm bubbling aeration vessel with 1 ppb Hg^{2+}. N_2= 135 mL/min.

times at faster sample flow rates was exhibited. For example, in this study it took ~2.3 min to reach the plateau for a sample flow rate of 3 mL/min and ~1.2 min for a flow rate of 5 mL/min. A flow rate of about 4.8 mL/min was therefore chosen to be a reasonable working parameter for further studies.

The effect of changing nitrogen flow rates on noise was studied using the 1-cm vessel by pumping water through the sample tubes, increasing the amplification to 100, decreasing the time constant to 0.32 s, and calculating the standard deviation from 22 repetitive digital voltmeter readings taken at 5-second intervals for the various nitrogen flow rates. The absorption of the 254 nm radiation by non-analyte species such as water mist, volatile organics, or other spectral interferences being carried through the absorption cell could result in increased baseline noise. Any fluctuation in the gas flow which may result from the regulators, the gas flowmeter, movement of glass wool in the pre-heating tube, and mostly from the bubbling solution, will manifest itself as greater peak-to-peak baseline noise. The source flicker noise is minimized by the double-beam system while the amplifier-readout and dark current noises were negligible. The standard deviations of blank readings ranged from 7.4 to 10.5 x 10^{-4} A.U. which is not significant enough of a range to assert that varying the nitrogen carrier gas flow rate between 35 and 350 mL/min affected the baseline noise.

To reduce the volume and therefore the response time, the i.d. of the bubbling-aeration reduction vessel was decreased from 1 to 0.6 cm (about a factor of three reduction in volume), while maintaining the

coarse grade frit and 1 cm spacing between entrance and exit ports (see Figure 9 in the Experimental section). The characteristics of this reduction vessel are also reported in Table IV. The result was a slightly reduced response time which averaged 1.2 min to reach 100% A_e, with a range of 0.9 to 1.5 min for a 1 ppb Hg^{2+} solution and nitrogen flow rate of 120 mL/min. The absorbance for 1 ppb Hg^{2+} was 0.88 x 10^{-2} A.U. as compared to 1.4 x 10^{-2} A.U. obtained with the 1 cm diameter cell with the same solution and gas flow rate. Thus the fraction of mercury transferred to the gas stream is reduced by a factor of about one-third. The 90% A_e response time for 120 mL/min is about a factor of 3 better with the smaller diameter cell which indicates that the solution volume in the cell critically affects initial response times. The 100% A_e response time, however, is not much improved over the 1 cm i.d. vessel.

A slightly lower nitrogen flow rate of about 85 mL/min was actually chosen for most work with this design since at this flow rate the intensity of bubbling was about equivalent to a rate of 120 mL/min passing through the 1 cm diameter vessels. Solution carry-over often occurred for much greater carrier gas flows. A 1 ppb mercury solution tested at this reduced flow rate gave an absorbance of 1.2 x 10^{-2} A.U. and a 100% A_e response time of about 1.5 min. While providing somewhat of an improvement in generating more consistently usable data, this reaction vessel design is still plagued by slight fluctuations occurring on the plateau resulting from the aeration dynamics and the randomness of the formation, breaking, and sizes of the mercury vapor-containing bubbles. The complexity of rapidly occurring events that accompany

the bubbling make it difficult to identify the exact origin of much of the observed noise.

Table V lists the optimized conditions selected for the bubbling aeration design. Calibration data (Table VI) under these conditions gave a sensitivity of 0.014 A.U./ppb with significant negative deviation at 100 ppb. This slope compares to a calculated sensitivity of 0.050 A.U./ppb or an experimental recovery of 28% of the mercury when compared to the theoretical value. This plateau absorbance represents about half the 0.030 A.U./ppb sensitivity achieved by the discrete sampling method that will be discussed later.

A detection limit of 0.02 ppb was obtained and was calculated using equation 8.

$$c_\ell = 2s_b/m \qquad (8)$$

where

c_ℓ = concentration or absolute amount at the detection limit, ppb

s_b = experimental standard deviation in blank measurements, and is estimated as 1/5 of the peak-to-peak baseline noise, A.U.

m = slope of the calibration curve, A.U./ppb

In this case s_b was estimated to be 1.5×10^{-4} A.U.

The following is a summary of the effects of the various parameters on the bubbling aeration vessels' responses to mercury.

Distance between exit port and frit: As the distance between the two ports increases, the solution volume and therefore the response time also increases while the baseline noise decreases.

Table V. Optimized conditions for the bubbling aeration continuous sampling reduction vessel

Cell type:	1 cm diameter coarse frit with 1 cm between exit and inlet port
Nitrogen flow:	120 mL/min (12.5 reading on no. 2 size Gilmont flowmeter)
Sample solution flow:	4.8 mL/min (8.5 dial reading on Pharmacia P-3 peristaltic pump)
Absorption cell temperature:	135^{o} C

Table VI. Calibration data for 1-cm bubbling aeration reduction vessel[a]

Concentration Hg^{2+} (ppb)	Absorbance (A.U.)
0.01	not detected
0.1	0.0012
1.0	0.013
10.0	0.14
100.0	0.74

[a]Nitrogen flow = 120 mL/min, sample flow = 4.9 mL/min.

Diameter of the vessel: Increasing the vessel diameter also effectively increases solution volume with correspondingly slower response times but better sensitivity. Conversely, if the vessel diameter is very small, solution carry over into the absorption cell becomes a problem especially when a coarse frit is used which produces larger bubbles.

Carrier gas flow rate: At higher gas flow rates, both the response times and sensitivity are reduced. Depending on the vessel diameter such vigorous bubbling at higher flow rates can also induce a large amount of solution carry over.

Solution flow rate: Increasing the solution flow rate gives a corresponding increase in absorption up to the point where solution enters and exits too rapidly and a smaller fraction of the mercury in solution is extracted. Flow rates much greater than the optimum result in wasted reagents and sample without an increase in sensitivity.

Absorption cell temperature: Water mist-caused drift and false absorbances, and condensation on the quartz windows are problems that arise when the absorption cell (and preheating tube) temperatures are too low. Very high temperature results in lowered sensitivity, and, in some cases may cause volatilization of the glue that fixes the windows.

These bubbling aeration reduction vessels may be considered workable to a limited extent. However, the occasional solution carry overs and erratic appearance of spurious peaks and noise, especially at the top of the plateaus make it difficult to obtain highly reliable results, particularly in measurements of response times. The

semi-quantitative data presented were the average of acceptable runs in which the incidence of anomalies was low enough to allow extraction of reasonable data. This behavior coupled with the long response times made it necessary to seek a better design.

Continuous sampling utilizing a thin stream and countercurrent gas flow: As described previously the sample and blank solutions (with the stannous chloride reagent) are pumped one after the other without intermixing down the wall to the drain at the bottom of the inclined thin stream reduction vessel shown in Figure 5 in the Experimental section. The volatilizing carrier gas passes over the stream in the opposite direction. When the characteristics of this reduction vessel design are compared to the bubbling aeration reduction vessel some advantages become apparent. 1) Lower carrier gas flow rates are necessary for mercury volatilization due to a greater surface area-to-volume ratio, which means less dilution of the mercury vapor. 2) There is no bubbling and therefore less foaming of the sample which is normally a problem with bubbling aeration particularly when analyzing digested samples. 3) This design allows for faster response times since the sample and blank do not mix in the reduction vessel, thus avoiding the time-consuming exponential dilution problem.

Conversely, the passing of gas over a stream of solution may not be as efficient for complete volatilization of mercury as is vigorous bubbling because of the shortened residence time (<2s) of the solution in the vessel. Also the mixing of stannous chloride reagent with the sample is minimal since there is a lack of solution agitation. Mixing must therefore take place between the point that the solutions meet

in the "T" and their entrance into the reduction vessel. Flow patterns through the pump tubing and in the reduction vessel were examined by substituting the reductant and sample solutions with blue and yellow dyed solutions for visual analyses. These solutions clearly demonstrated that flow through the tubes was extremely laminar with a distinct boundary between the blue and yellow dyes as they passed into the reduction vessel.

Calculation of the Reynolds number, commonly used by chemical engineers, which normalizes density, viscosity, and flow rate of a liquid through a given tube diameter resulted in a value of about 12. A Reynold's number below 2300 is considered to indicate laminar flow while that above 4000 would indicate turbulent flow (121). To induce mixing, needles were inserted through the walls of the Tygon microbore tubing segment situated between the "T" and inlet port of the reduction vessel, and the set of baffles shown in Figure 10 of the Experimental section was also tested by placing it in-line between the "T" and reduction vessel inlet port. The microbore segment was immersed in an ultrasonic bath to induce agitation. However each of these attempts proved ineffective as the solutions merely separated and recombined virtually unchanged once they flowed past the needles or baffles, and the ultrasonic agitation did not have a significant effect in altering the flow patterns.

The 2 x 5 mm stir bar in the micro-mixing chamber inserted in the Tygon segment (see Figure 11 in the Experimental section) was mechanically practical and agitated the solutions very effectively. Since the chamber volume was minimized to reduce the response time, this

0.2 mL size also resulted in frequent stopping of the bar particularly when bubbles entered the chamber or occasionally even when it slipped to the side and struck the walls. The stirring chamber reduced the response times by about 0.1 min out of 0.9 min under optimized conditions and the absorbance signals were slightly less erratic, but these advantages did not justify the added hardware and constant vigilence required by the stirrer. The presence of the stirrer did not change the baseline absorbance but reduced the mercury signal about 20% for a reason that could not be explained.

Several of the variables optimized for the previous reduction vessels were also performed here. For these studies unless otherwise stated, the angle of the reduction vessel was 15^{o} from vertical. Table VII and Figure 16 show that there is a trade-off between calibration sensitivity and response times with nitrogen flow rate as observed previously with the bubbling reduction vessel (see Figure 12). Compared to the 1 cm i.d., 1-cm bubbling vessel, the absorbances at the same nitrogen flow rates were reduced by about 1/2 but the response time was also reduced by the same factor. A flow rate of about 80 mL/min was therefore chosen as an acceptable compromise between sensitivity (0.007 A.U./ppb) and response time (0.9 min to achieve 100% A_e). The noise on the plateau was also reduced compared to the bubbling aeration which is important for determining mercury levels near the detection limit. Peak-to-peak baseline noise from the recorder tracing was about $4\text{-}5 \times 10^{-4}$ A.U., about a factor of two reduction in noise compared to the bubbling aeration methods, and fairly independent of gas flow rate. This is probably due to the absence of significant

Table VII. Data for the thin stream reduction vessel[a]

N_2 flow rate, F_g (mL/min)	Absorbance (A.U. x 10^{-2}) A_e	A_t	% of theoretical absorbance A_e/A_t x 100	F_g x A_e (A.U. mL/min)	Response time (min) for 90% A_e	for 100% A_e
31	1.15	19.0	6.1	0.36	0.51	2.04
60	0.95	10.0	10.0	0.57	0.41	1.16
85	0.75	7.0	11.0	0.64	0.35	0.91
116	0.58	5.2	11.0	0.67	0.26	0.57
150	0.42	4.0	10.0	0.63	0.13	0.38

[a]Vessel tested using 1 ppb Hg^{2+} solution. The sample flow rate was 4.8 mL/min.

A_e = experimental plateau absorbance.

A_t = theoretical plateau absorbance calculated from equation 4.

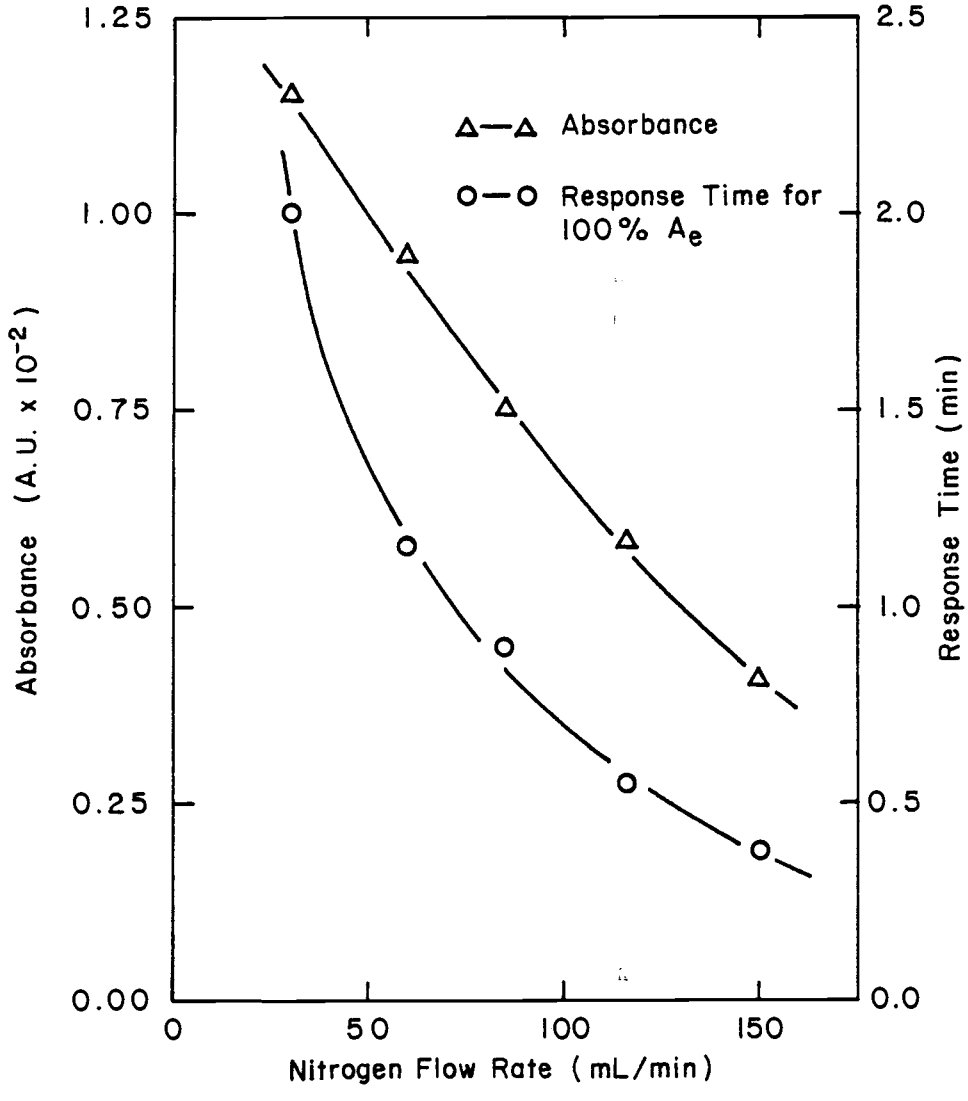


Figure 16. Effect of nitrogen flow rate on absorbance and response time for the thin stream vessel with 1 ppb Hg^{2+}.

water mist formation. However, occasional appearance of spurious peaks on the plateau has not been eliminated or explained. However, the frequency of appearance is much less than that observed with the bubbling aeration vessels.

Table VII is a summary of absorption and response time data for the thin stream reduction vessel. As can be seen, the volatilization efficiency is only 10-11% of the theoretical value, but this is not unexpected due to the lack of aeration of the solution flowing through the reduction vessel. The percentage released is constant for nitrogen flow rates above about 60-85 mL/min. The product $F_g \times A_e$ is also constant above about 85 mL/min. The velocity of nitrogen passing over the solution stream apparently has much to do with the volatilizing of mercury. Lower nitrogen flow rates result in slower diffusion of mercury out of solution since the concentration of elemental mercury immediately above the stream remains relatively higher.

The effect of solution flow on the plateau absorbance is shown in Figure 17. At lower flow rates, the smaller rate of delivery of mercury to the reduction vessel causes lower sensitivity even though the solution residence time is longer. At the highest flow rates, the decrease in residence time apparently reduces the fraction of mercury volatilized and causes the plot to plateau. This leveling off of sensitivity for an increase in flow rate occurred at about 4.8 mL/min and this value was therefore chosen as the optimum setting since higher settings mean wasted solution.

The effect of the angle of the reduction vessel on the plateau absorbance for 1 ppb Hg^{2+} is shown in Figure 18. The absorbance

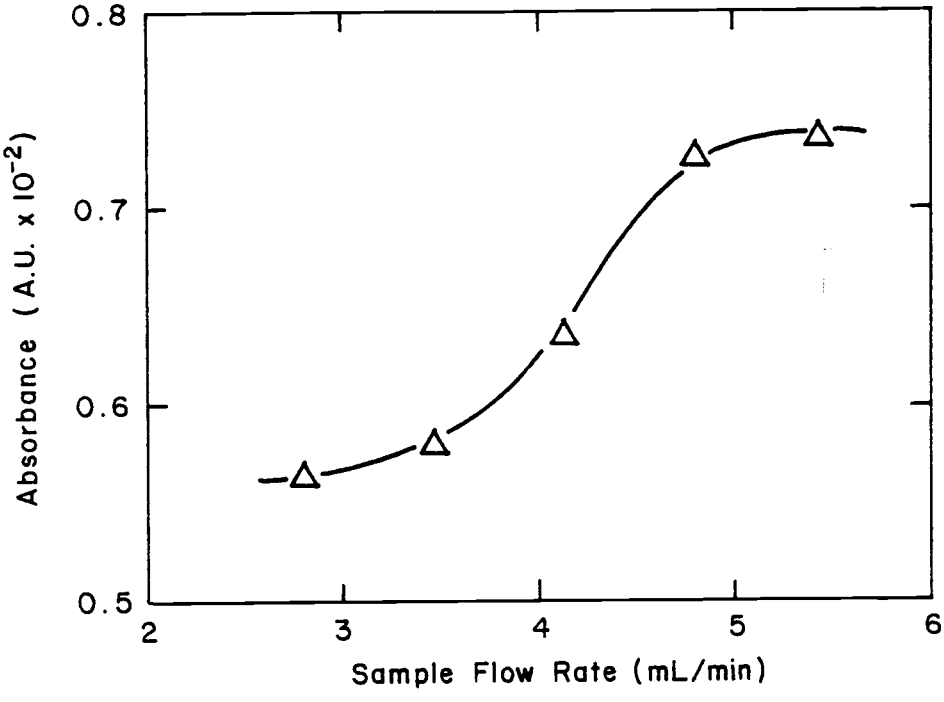


Figure 17. Effect of solution flow rate on absorbance.

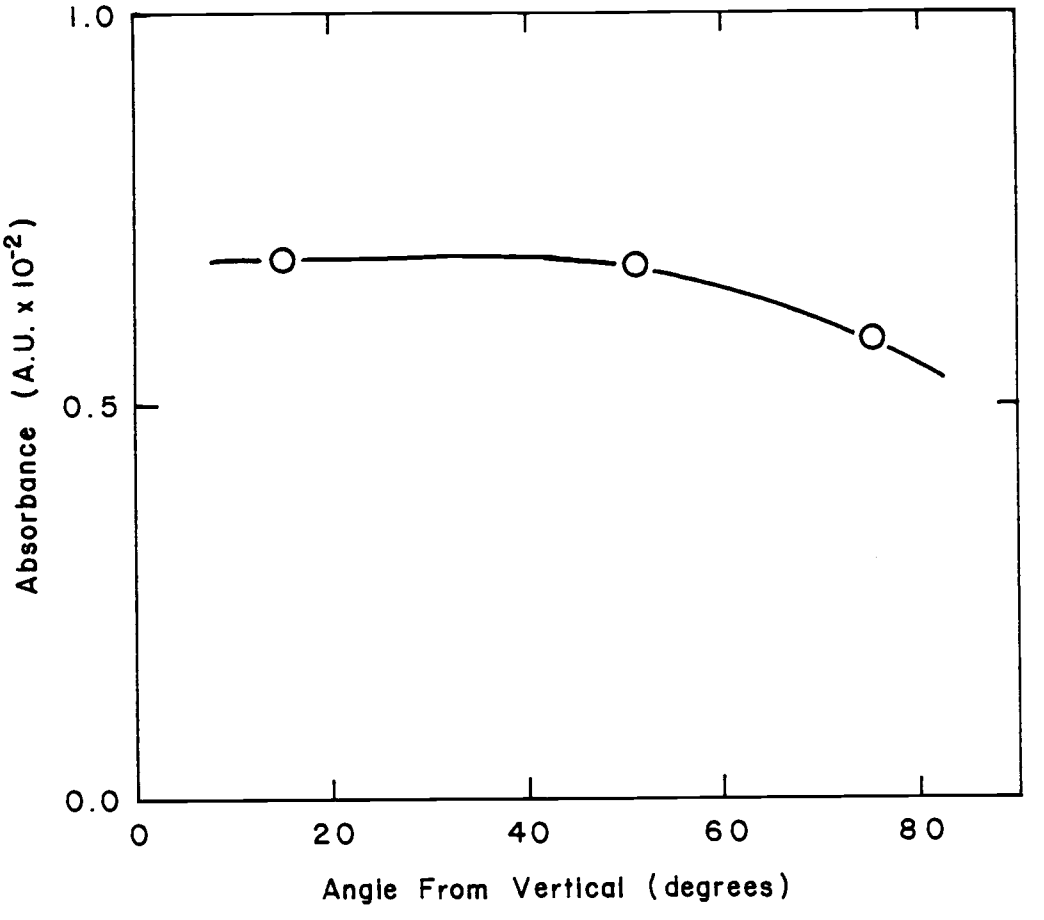


Figure 18. Effect of angle of reduction vessel on absorbance.

decreased slightly as the vessel was positioned more horizontally and may be due to poorer volatilization efficiency from the thicker stream present as the reduction vessel does become more horizontal.

A 10 mm diameter, 3.5 in long vessel was compared to the 5 mm diameter vessel of the same length to determine if the thin stream might be able to spread over a slightly larger surface area but there was no significant difference in absorbance while the response time of about 1.5 min was about 1/3 larger than that of the smaller diameter vessel. A 5.5 in long (5 mm i.d.) vessel achieved absorbances of about 0.001 A.U. (or about 13%) greater than the 3.5 in long vessel for 1 ppb Hg^{2+} (as with the bubbling aeration vessels, the length refers to the distance between solution entrance and exit ports) but the 3.5 in long vessel was retained for further studies for the sake of compactness.

A typical chart recorder tracing of an absorbance plateau is depicted in Figure 19. Compared to the peak shapes of the bubbling aeration vessels shown in Figure 13, the absorbance is about half that of the 1 cm i.d., 1-cm vessel but the noise is also considerably reduced. The time necessary to reach 90% and 100% A_e was generally at least 25% faster. A plot of log % A_e versus t of the rise to the plateau absorbance (similar to Figure 14) indicated the initial increase in absorbance was non-exponential.

The 5% HNO_3 and 0.01% $K_2Cr_2O_7$ used as preservatives had a corrosive effect on the pump's silicone rubber tubes when operated over a period of 2-3 months. The result was that the inner surface became less non-wetting and air bubbles introduced bewteen samples and blanks would adhere to and disengage from the walls at random, and show up as valleys

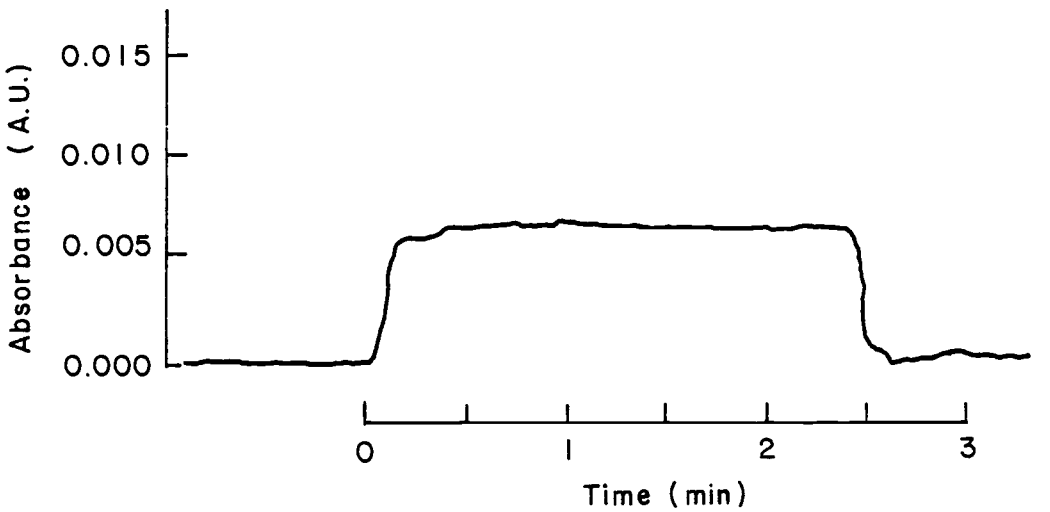


Figure 19. Typical chart recorder response tracing of 1 ppb Hg^{2+} for the thin stream vessel under optimum conditions.

in the middle of a peak tracing. The normally distinctly separated (by an air bubble) interfaces between succeeding solutions also became less distinct as one occasionally tailed into the other and led to a slightly longer (by about 0.1 min) response times. Replacement of this tubing after about 200 hours of operation was the only practical solution since a modified Tygon PVC tubing did not produce satisfactory results.

The optimized variables for the thin stream reduction vessel are summarized on Table VIII. A calibration curve shown in Figure 20 was run under these conditions and established a calibration sensitivity of 0.0065 A.U./ppb from a linear regression analysis of the slope (r^2 = 0.99995), and a detection limit of 0.03 ppb. The detection limit was calculated using Equation 8. The relative precision of this method is <5% for concentrations of 0.1 to 5 ppb.

Table VIII. Optimization of variables for thin-stream reduction vessel

Nitrogen flow rate:	80 mL/min
Sample solution flow rate:	4.8 mL/min
Diameter of reduction vessel:	0.5 cm
Length of reduction vessel:	3-1/2 in
Angle of reduction vessel:	15° from vertical

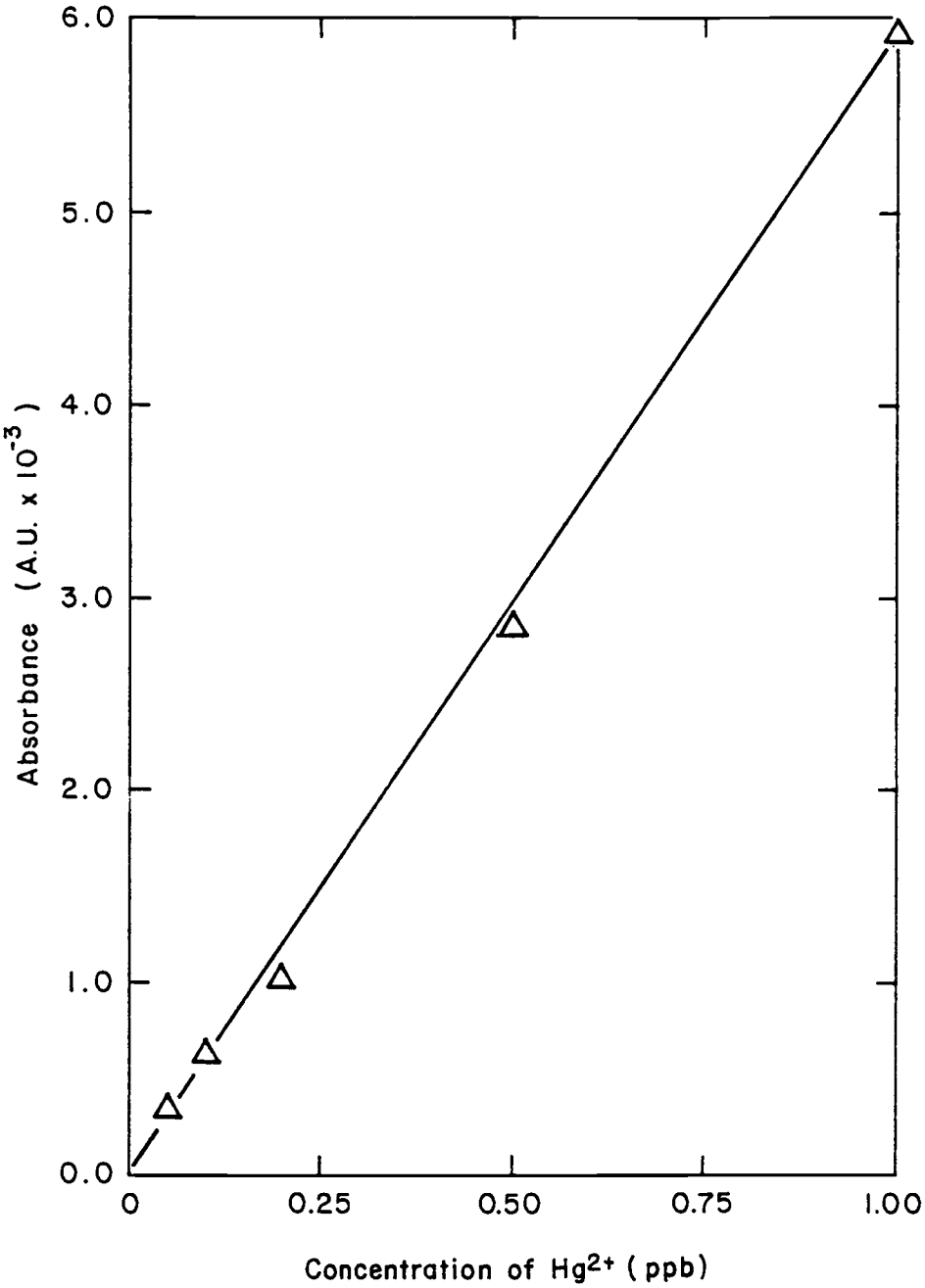


Figure 20. Calibration curve for Hg^{2+} using the thin stream reduction vessel.

Speciation Studies

Development of the method: The objective of this speciation study was to develop a simple and fast method by which inorganic and organomercurials at sub-ppb levels can be differentiated. Special attention was directed at highly toxic methylmercury since this is the most common organic form of mercury present in nature. Other alkylmercurials are less toxic while phenylmercurials have been shown to be of relatively low toxicity with chronic poisoning by phenylmercurials practically unknown.

As noted in the Experimental section, the reduction vessel and other instrumentation used previously (22) for the determination of inorganic mercury by the discrete injection technique were also utilized for speciation work because of the capability to achieve detection limits in the parts-per-trillion range. The final injection sequence consisted of 0.1 mL of 1% (w/v) $SnCl_2$ followed by 1.0 mL of sample or blank and 0.1 mL of a 20% (v/v) HNO_3 - 1% (w/v) $K_2Cr_2O_7$ mixture, and 0.1 mL of 1% (w/v) $NaBH_4$. Ideally all the inorganic mercury, but none of the organomercury, is reduced by the $SnCl_2$, whereas all the remaining organomercury is reduced by the $NaBH_4$. Also ideally no volatile species which absorb at 253.7 nm other than mercury will be produced during the injection of either reducing agent.

A small peak is often observed with the injection of $SnCl_2$ due to reduction and volatilization of residual mercuric ions in the reduction vessel by $SnCl_2$. In the injection sequence, the potassium dichromate and nitric acid mixture added to the sample between inorganic and

organomercury reductions serves two purposes. First, these reagents apparently catalyze the reduction process of the organomercury by borohydride, possibly by providing an oxidizing environment that may weaken the R-Hg bond. The exact effect of the dichromate on methylmercury is unknown except over an extended period of time has been observed to cause breakdown as will be shown later in the decomposition studies. Nitric acid serves to prepare the sample for borohydride reduction which occurs most effectively at pH's of 2 or less. Solutions and blanks were normally preserved with 1% (v/v) HNO_3 (c) and 0.01% (w/v) $K_2Cr_2O_7$ with a pH of less than 1. Omission of the dichromate and acid injection resulted in about a 40% reduction in the methylmercury peak height while total omission of even the preservatives gave a 90% reduction. Methylmercury solutions without preservation reagents produced normal peaks with the 20% HNO_3 and 1% $K_2Cr_2O_7$ injection.

The 20% concentration of HNO_3 was decided upon as a result of blank studies involving the interaction between $SnCl_2$ and $NaBH_4$. It was observed that when 0.1 mL aliquots of the 1% $NaBH_4$ and 1% $SnCl_2$ reagents were combined directly in the reduction vessel, an unknown yellow precipitate was produced. This precipitate turned black almost immediately (probably due to the formation of metallic tin) with the simultaneous evolution of a pungent yellowish vapor (assumed to be stannane, SnH_4) that absorbed strongly at 254 nm. The result was an intense peak (much greater than 0.2 A.U.) that required more than 2 minutes to return to the baseline if the reduction vessel was not evacuated immediately. Twenty percent HNO_3 was found to be the minimum concentration added to the $SnCl_2$ - containing solution that would prevent significant formation of the blank borohydride

peak. The acid may serve to speed conversion of the remaining easily oxidizable Sn^{2+} to SnO_2 which remains largely intact even with the introduction of borohydride.

The addition of borohydride has actually been applied to the detection of tin by the formation of volatile hydrides (112). Since gaseous SnH_4 and SnH_6 are thermally unstable (122) with appreciable breakdown occurring even at room temperature, this interference can be effectively reduced by an efficiently packed pre-heating tube which induces decomposition of the vapor into species that do not absorb at 254 nm. Even after addition of the 20% HNO_3 a poorly packed pre-heating tube resulted in blank absorbances of 0.001 to 0.004 A.U. while an efficiently packed tube reduced the interferent absorbance to 0.0 to 0.0005 A.U.

Preparation of the pre-heating tube to eliminate the apparent stannane peak proved to be most troublesome because unless the glass wool is arranged tightly enough, the stannane vapor is not completely decomposed and passes through the absorption cell to produce a blank absorbance peak. This behavior would worsen the detection limit which would then be determined by the ability to reproduce a blank peak rather than the baseline noise.

Water mist or steam may also cause false peaks especially when injecting the reagents or samples. Injection peaks of about 0.001 A.U. occurred while adding sample or blank, or borohydride with a loosely packed pre-heating tube. This caused overlap between the injection and mercury peaks since the time between injection of the sample or $NaBH_4$ and appearance of mercury peaks was on the order of one to two seconds and before the injection peak returned to the baseline. The overlap

problem with a poorly packed pre-heating tube made it difficult to determine peak heights, especially at sub-ppb concentrations. Changes in baseline absorbance of up to 0.001 A.U. before and after injection of a sample into $SnCl_2$ were also observed and probably due to changing nitrogen flow rates which decreased with the presence of more solution above the frit. Closer packing of the pre-heating tube eliminated this effect by creating a back-pressure on either side of the frit that reduced the effect of greater pressure on the frit exerted by injection of the additional solution.

Although closer packing of the pre-heating tube gave more reliable and consistent data, there was also the problem that too much glass wool restricted gas flow and the increased nitrogen pressure necessary to maintain a reasonable flow through the absorption cell caused leaks in the system, especially through the rubber septum capping the reduction vessel. It was found that a well packed tube allowed a carrier gas flow rate of about 85 mL/min with an applied pressure of 6 psi and that this pressure could be handled by the system while maintaining a reasonable bubbling efficiency and eliminating the borohydride blank peak. This low flow rate resulted in about a six second delay between the sample or borohydride injection spikes and the resulting mercury peak.

Some previous work carried out by Debbie Zahnale, an undergraduate at Oregon State University, resulted in the selection of $NaBH_4$ as the most effective for organomercury reduction among other reducing agents such as ascorbic acid, hydrazine hydrate, hydroxylamine hydrochloride, hypophosphorous acid, hydroquinone, and formaldehyde. The

exact reaction mechanism of the borohydride reduction of organomercury compounds is unclear although rearrangement and deuterium studies indicate the formation of radical intermediates (126). The $CdCl_2$-$SnCl_2$ reagent used by Magos (104) for total mercury determination not only proved ineffective in this work but required such large concentrations of reagents that contamination became a problem if pre-purification was not undertaken.

Decomposition study: A study of the breakdown of a 1.0 ppb (with respect to mercury) CH_3HgCl solution caused by 1.0% (v/v) HNO_3 alone, 0.01% (w/v) $K_2Cr_2O_7$ alone, and a mixture of 1.0% (v/v)HNO_3 and 0.1% (w/v) $K_2Cr_2O_7$ which are normally used as preservatives for total mercury was carried out. Measurements of inorganic and methylmercury content using the speciation procedure developed were made within hours of preparation and after one, three, and eight days standing in 100 mL volumetric flasks at room temperature. The mercury concentrations were determined from the inorganic-methylmercury calibration curve that will be presented. The results were compared to those obtained with an unpreserved 1 ppb Ch_3HgCl solution and are shown in Figures 21-24.

It was previously found (43) that 0.01% (w/v) $K_2Cr_2O_7$ and 5.0% (v/v) HNO_3 were most effective in preventing loss in Hg^{2+} solutions at ppb concentrations. For these studies, the concentration of HNO_3 was reduced to 1.0% to minimize decomposition. However, even with the lowered acid concentration, about 20% of the methylmercury was observed to be converted to inorganic mercury (the form easily reducible by $SnCl_2$) under these conditions in slightly over a day (Figure 21). The

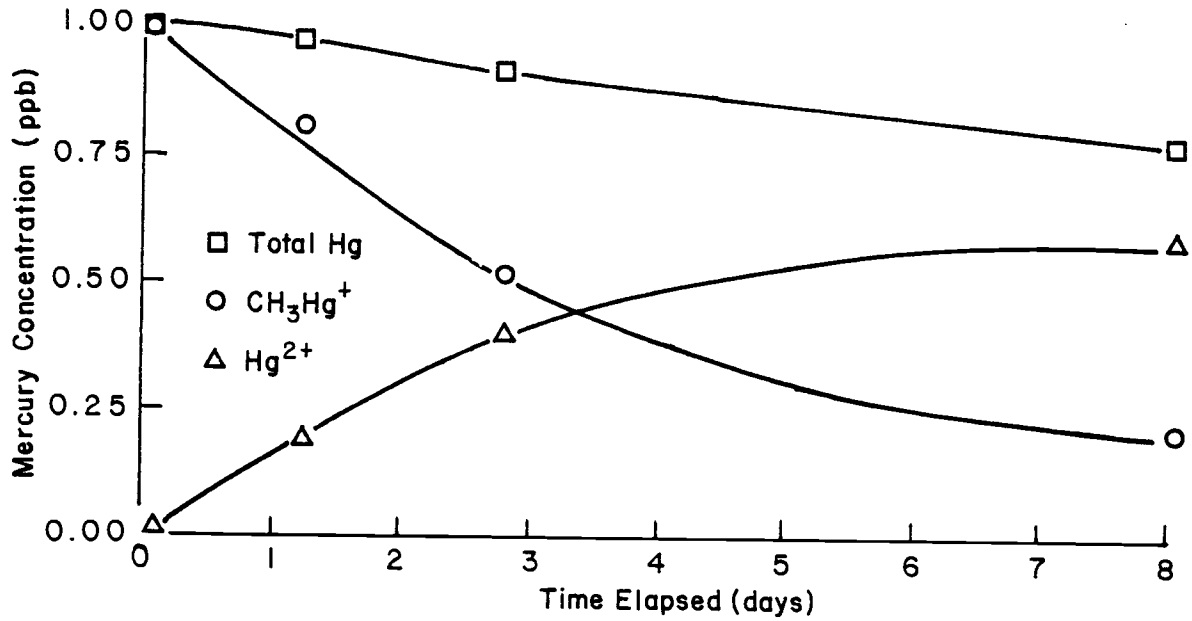


Figure 21. Preservation study of 1 ppb CH_3HgCl with 1.0% HNO_3 and 0.01% $K_2Cr_2O_7$.

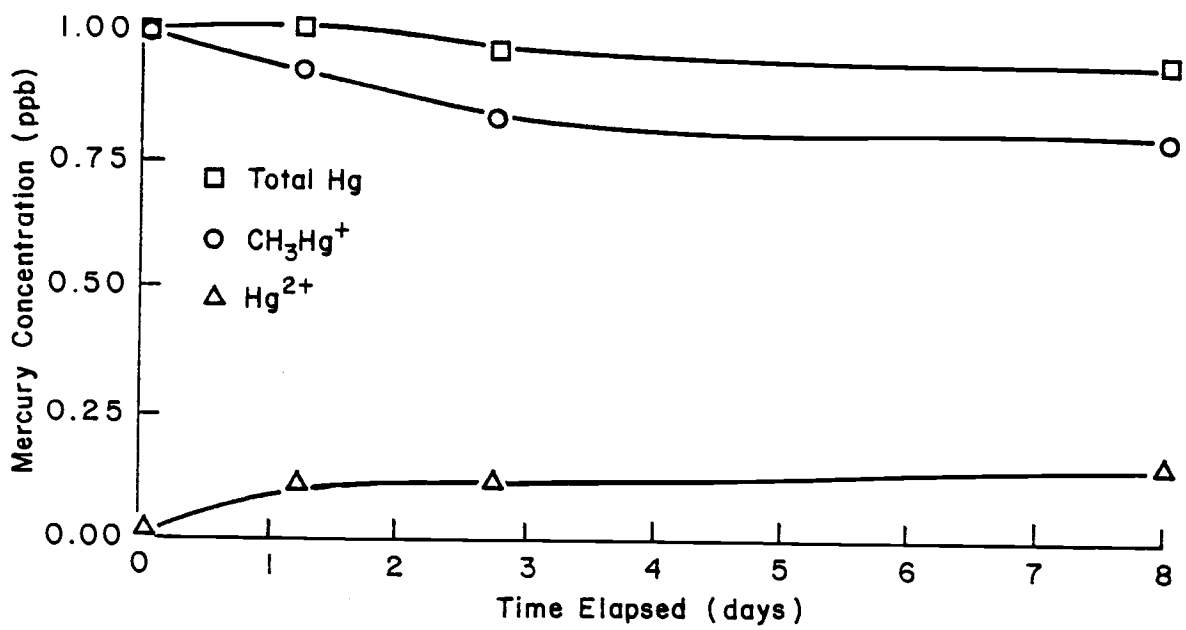


Figure 22. Preservation study of 1 ppb CH_3HgCl with 0.01% $K_2Cr_2O_7$ alone.

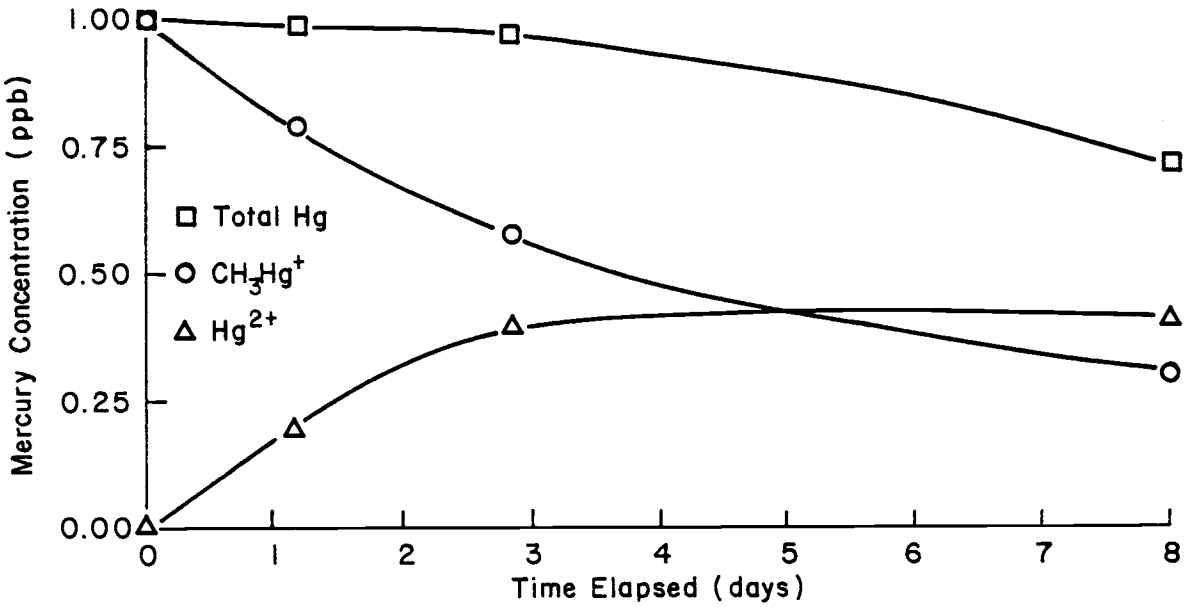


Figure 23. Preservation study of 1 ppb CH_3HgCl with 1.0% HNO_3 alone.

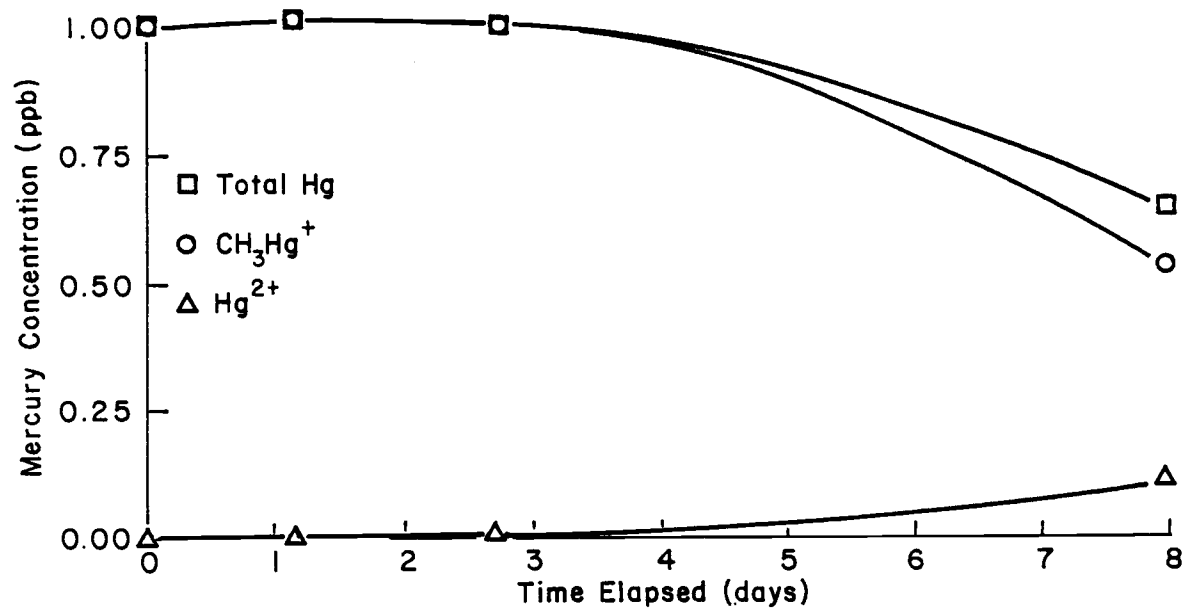


Figure 24. Preservation study of 1 ppb CH_3HgCl with no preserving reagents.

total amount of mercury (inorganic and organic) in solution remained fairly constant over a three day period with an approximate 25 ± 8% loss over a period of eight days. Comparison of decomposition induced by 0.01% $K_2Cr_2O_7$ alone (Figure 22) and 1.0% HNO_3 (Figure 23) alone to that caused by the combination of the two reagents indicates that the major factor appears to be the presence of HNO_3. Nitric acid alone converts almost half of the methylmercury to mercuric ions in just about three days and losses in terms of total mercury from the solution amounts to about 26 ± 5% over eight days. The $K_2Cr_2O_7$ is not nearly as destructive although about 15% of the CH_3Hg^+ is decomposed in three days while maintaining >90% effectiveness in retaining total mercury for more than a week.

It should be noted that in preparing any of the test solutions, the acid or dichromate was diluted to 50-75 mL with H_2O before addition of the CH_3HgCl solution so that the organomercury compound was never in direct contact with the concentrated preservation reagent. The unpreserved methylmercury (Figure 24) retained its concentration remarkably well over a three day period and as expected a minimum of methylmercury breakdown was observed over that time. However, losses of about 33 ± 12% of the total mercury concentration after eight days was noted, again as expected since no preservatives were present. The presence of inorganic mercury at the end of the test period may be partially attributed to photon induced decomposition since methylmercury is somewhat photosensitive (110),

This study seems to indicate that where speciation of mercury is the primary objective, the use of HNO_3 should be avoided to minimize

decomposition, or if added, the analysis must be run as soon as possible, preferably within hours of the addition. However, for extended periods of preservation, acid and dichromate should be used and a determination of total mercury can be obtained with a fair amount of accuracy since losses would be minimized. However, the original speciation information for the sample is no longer determinable unless analysis is carried out immediately after preservation.

Calibration curves: Once the technique for speciating inorganic and organomercury was established, the construction of calibration curves to obtain sensitivities and detection limits of the method for various compounds was carried out. Figures 25, 26, and 27 are calibration plots for equal concentration mixtures with respect to mercury of inorganic mercuric chloride and one of the organomercury compound. The calibration sensitivities (least squares slope of calibration slopes), and the respective detection limits for Hg^{2+}, CH_3Hg^+, $CH_3CH_2Hg^+$, and $C_6H_5Hg^+$ are shown in Table IX. All R^2 values were 0.998 or higher. Calculation of the detection limits (described previously) was based on the peak-to-peak baseline noise which ranged from about 0.2 - 1.0 X 10^{-3} A.U. over a one minute period when the system was performing well, and averaged 0.3 - 0.4 X 10^{-3} A.U. A peak-to-peak noise of 0.2 X 10^{-3} A.U. was used in calculating the detection limits to represent the system's capability under ideal conditions. The reported inorganic mercury calibration slope is the average of the three slopes that were determined with corresponding organomercury slopes. The methyl- and ethylmercury slopes are about 85-90% that of the inorganic Hg slope while the phenylmercuric chloride slope is about 50% of the

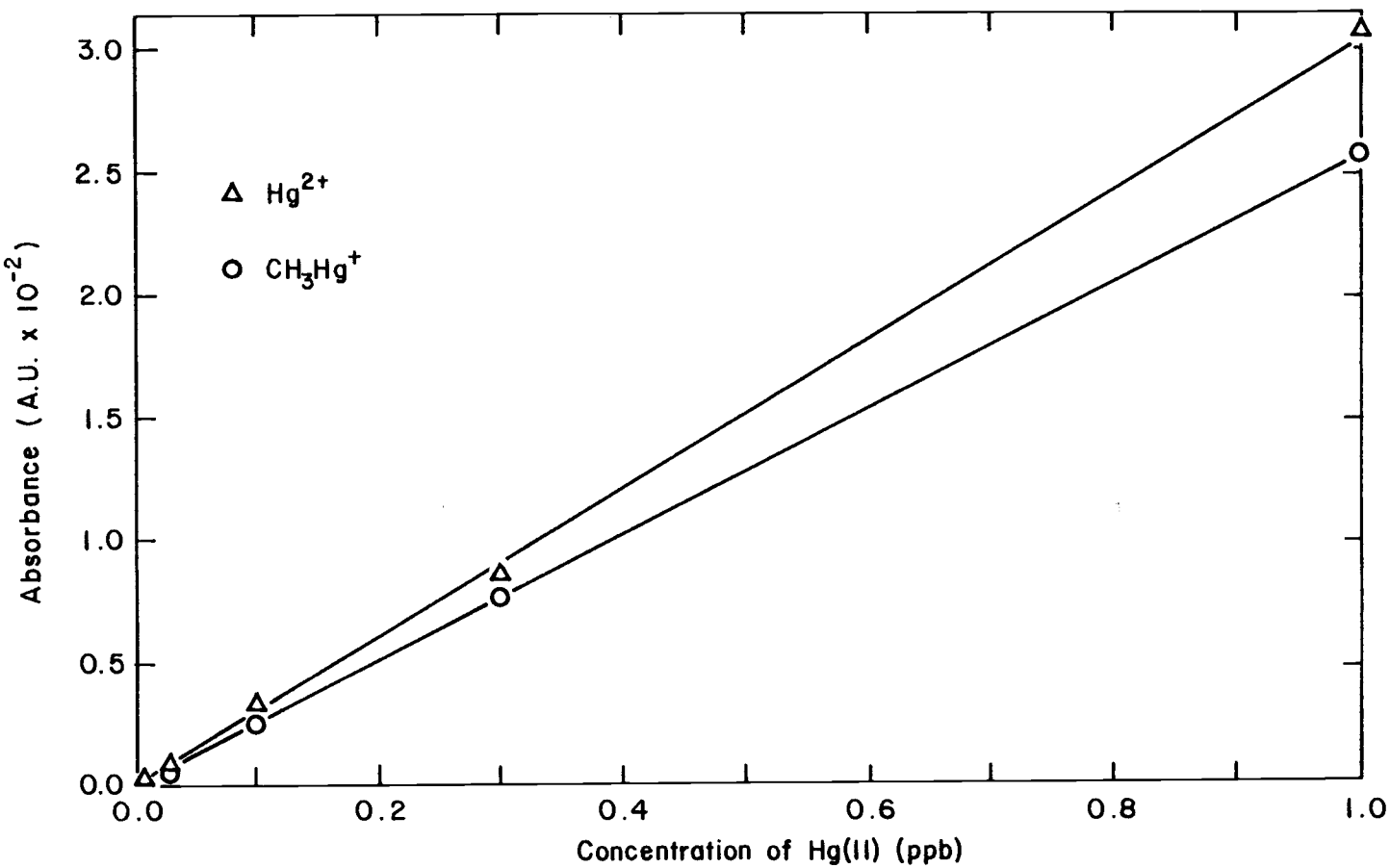


Figure 25. Calibration curve for equal Hg(II) concentration aqueous mixtures of $HgCl_2$ and CH_3HgCl.

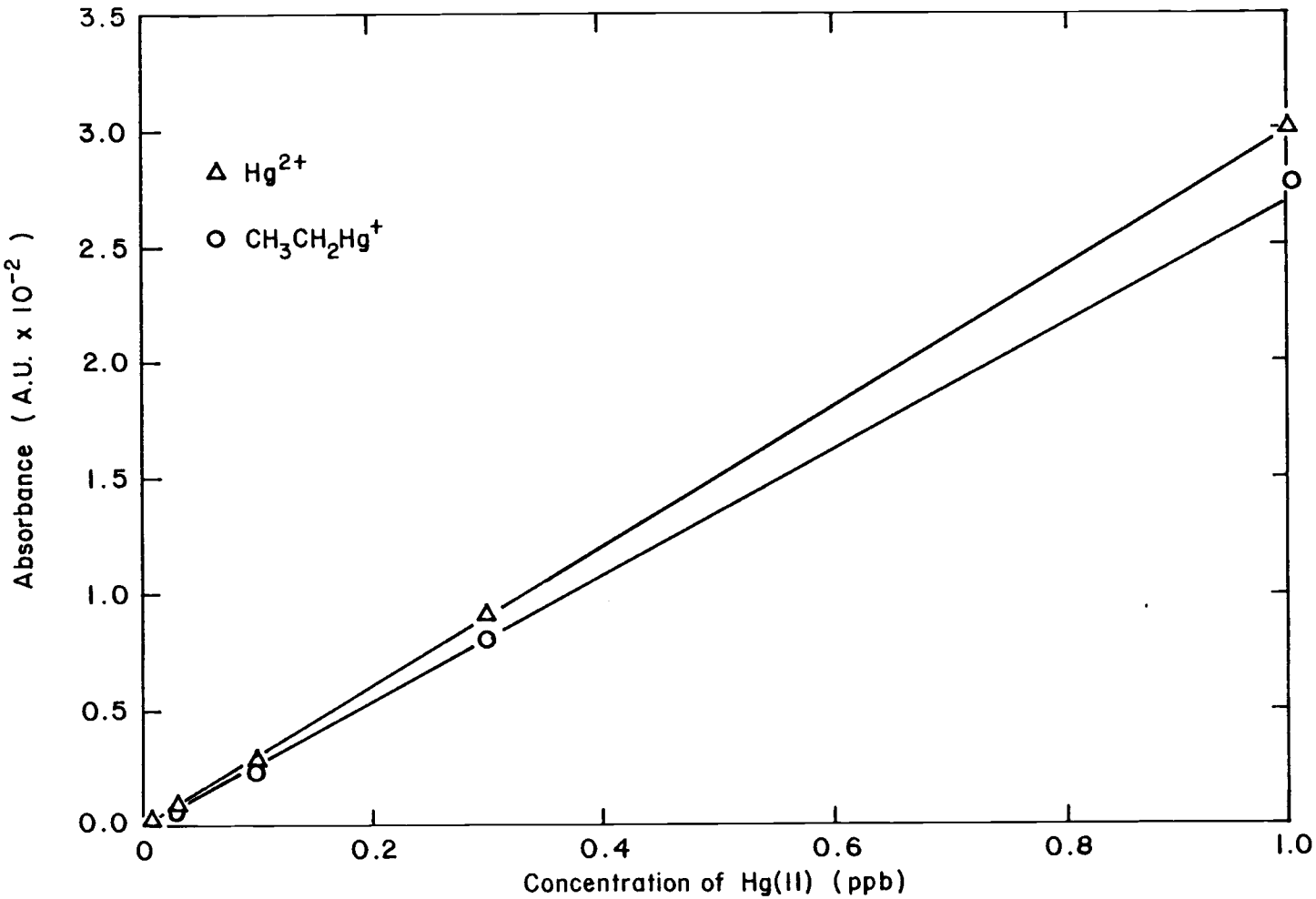


Figure 26. Calibration curve for equal Hg(II) concentration aqueous mixtures of $HgCl_2$ and $CH_3CH_2HgOOCCH_3$.

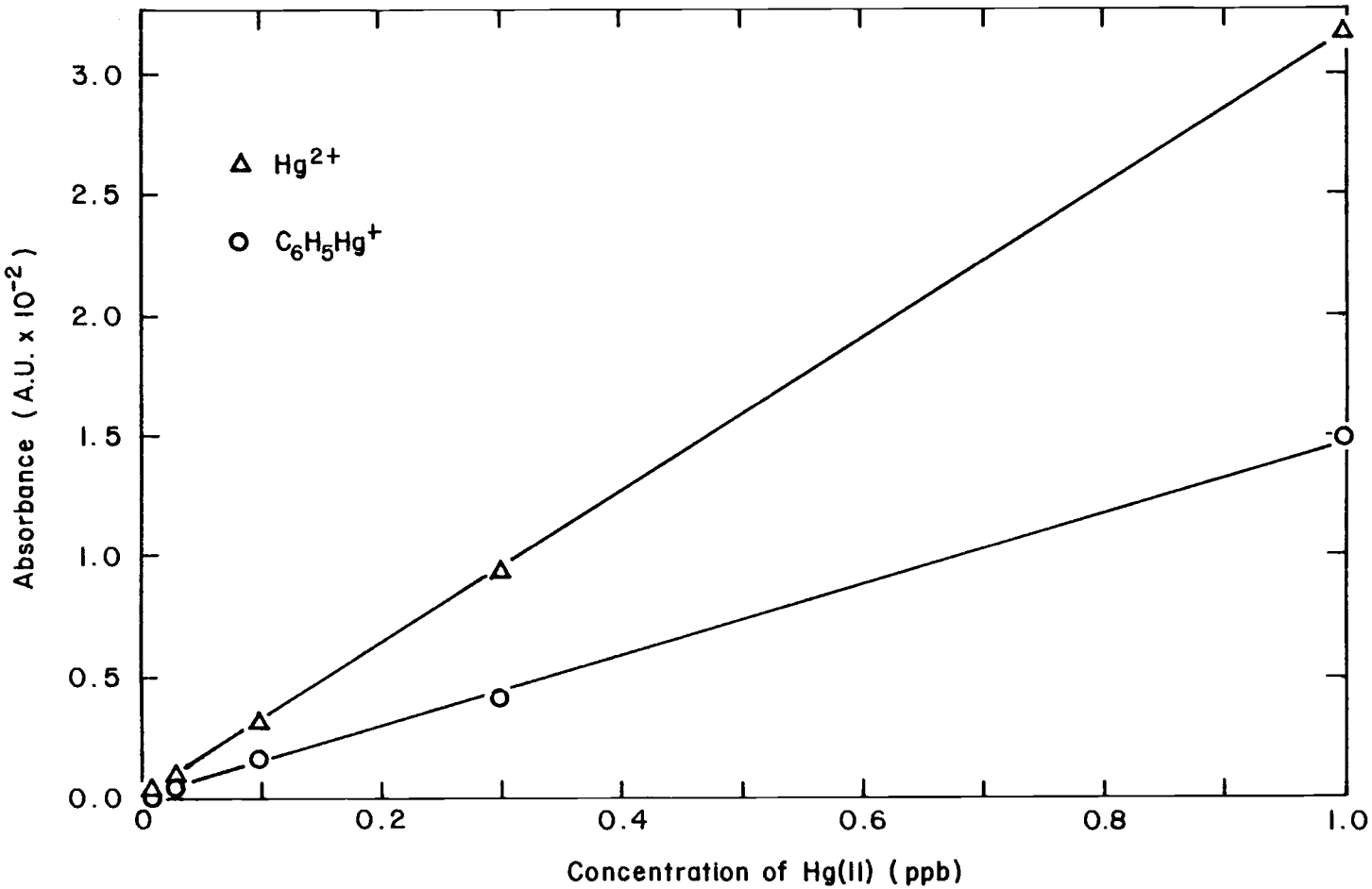


Figure 27. Calibration curve for equal Hg(II) concentration aqueous mixtures of $HgCl_2$ and C_6H_5HgCl.

Table IX. Calibration data for different mercury species

Species	Sensitivity (A.U./ppb)	Detection limit (pptr)
Hg^{2+}	0.031	3
CH_3Hg^+	0.026	3
$CH_3CH_2Hg^+$	0.026	3
$C_6H_5Hg^+$	0.015	5

inorganic mercury slope. A comparison of the peak shapes (see Figure 28) for inorganic and organomercury at 1.0 ppb Hg concentration indicate one possible reason for lower peak heights for methyl- and ethylmercury compounds. The organomercury peaks are slightly broader, indicating a slower reduction process and accompanying reduced rate of release of mercury into the nitrogen stream. Integration to obtain peak areas might reduce the differences in calibration sensitivity. However, the calibration curve established by the peak height measurements is linear from the detection limit to 3 ppb (i.e. at 3 ppb no significant deviations were obvious) and does not introduce appreciable error in real measurements of methyl- and ethylmercury compounds. For phenylmercury, the peaks are not much broader and the significant loss in calibration sensitivity appears to be due to the more tenacious phenyl C-Hg bond which was also found by Goulden and Afghan (105) to be much more difficult to decompose. Some phenylmercury probably remains in solution which could not be reduced even with increased amounts of borohydride or dichromate-nitric acid reagents. Recovery of inorganic mercury remained quantitative as no organomercury peak was observed when only Hg^{2+} was present.

Figure 28b depicts a typical recorder output for 0.030 ppb Hg^{2+} and CH_3Hg^+ and the associated noise. Figure 28c shows a water blank using the appropriate sequence of injections. A noticable feature of these two tracings is the presence of a large (0.0015 A.U.) negative peak that appears immediately after injection of the borohydride. This may be the result of the sudden release of hydrogen gas as borohydride begins to decompose and results in a dilution of the background contamination that may be present in the carrier gas flow. This

Figure 28. Typical recorder outputs for speciation analyses.

a. Mixture of 1 ppb (with respect to Hg) of Hg^{2+} and CH_3Hg^+.

A. Sample injection spike

B. Inorganic Hg^{2+} peak.

C. $NaBH_4$ injection spike.

D. Negative peak from H_2.

E. Organomercury peak.

b. Mixture of 0.030 ppb (with respect to Hg) of Hg^{2+} and CH_3Hg^+.

A. Sample injection spike.

B. Inorganic Hg^{2+} peak.

C. $NaBH_4$ injection spike.

D. Negative peak from H_2.

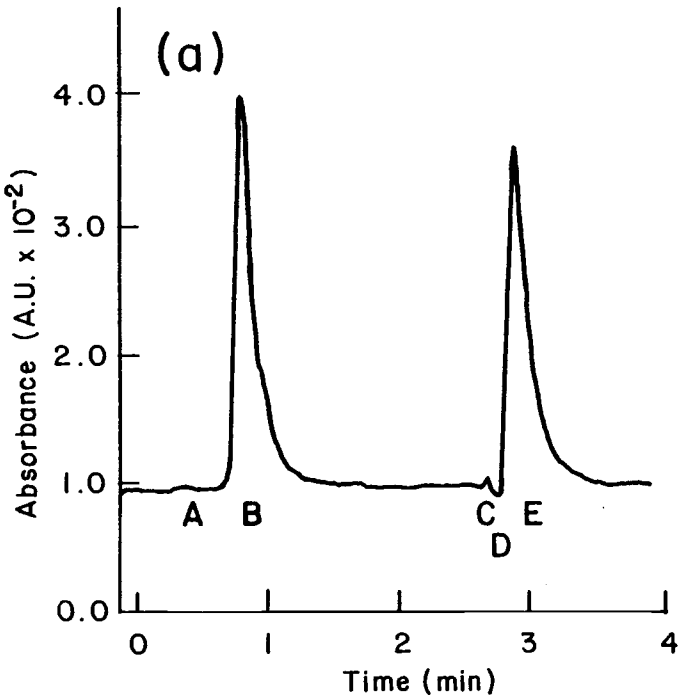
E. Organomercury peak.

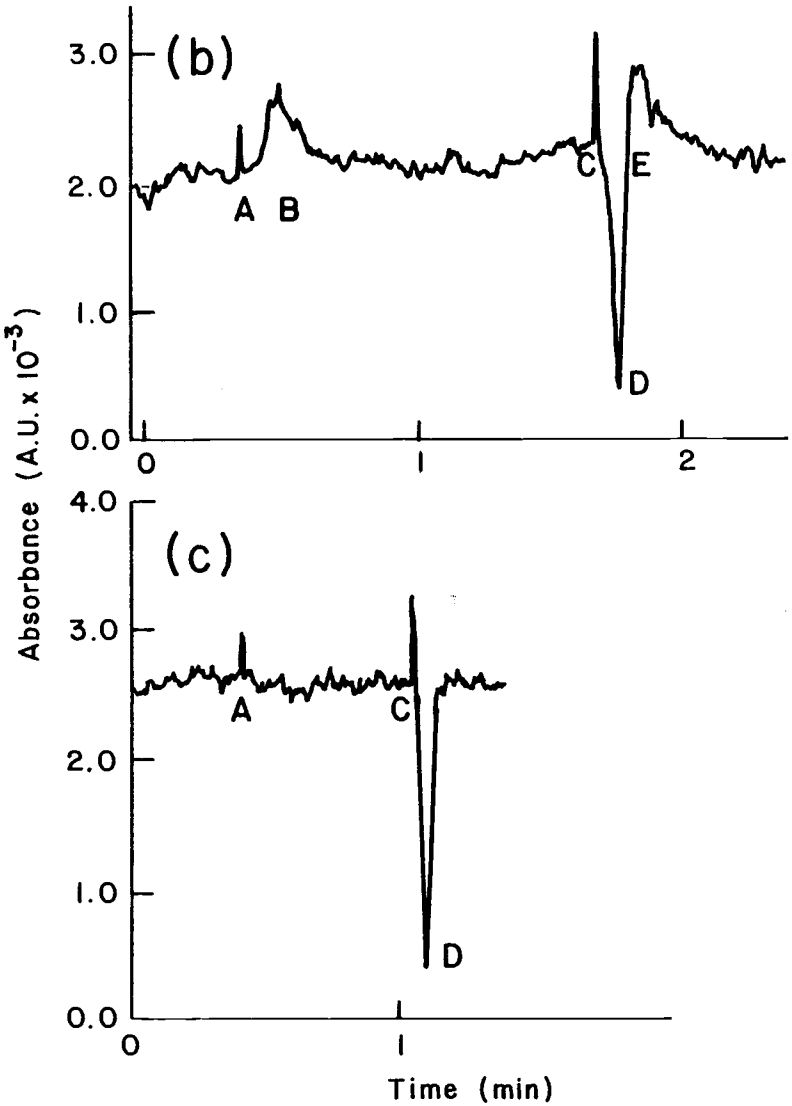
c. Water blank.

A. Blank injection spike.

C. $NaBH_4$ injection spike.

D. Negative peak from H_2.





Figures 28a, 28b, 28c.

peculiarity did not affect the mercury peak, however, since the delay time between injection of the borohydride and the appearance of the peak was greater than the time necessary for the negative peak to return to the baseline.

Real sample analysis: Spiked and unspiked samples of tapwater were tested with this speciation procedure as were digested samples of urine, hair, and tuna (hair and urine samples were donated by the researcher). The results are shown in Table X and each of these tabulations represents the average of three measurements. Two 1-cm segments were taken from the root end of a swatch of hair that had been removed 1 cm from the scalp and washed with laboratory glassware detergent and an ultrasonic cleaner to eliminate externally adsorbed mercury. The two samples of tuna were obtained from a single can of Bumblebee brand tuna after water had been drained.

The KOH digestion procedure employed was a modification of that used by Giovanoli-Jakubczak et al. (86) and consisted of heating 1 mL urine, 20-60 mg hair, or 0.1-0.3 g tuna in 2.5 mL 10 M KOH at 90° C in tightly capped two-dram vials for 15-30 min. Heating was accomplished by immersion of the vials in a beaker of boiling water. Potassium hydroxide blanks and 1.0 ppb mixtures of Hg^{2+} and CH_3Hg^+ were also processed under identical conditions for comparison and to verify complete recovery of each species while employing these digestion procedures. After digestion, the resultant solutions were centrifuged to separate remaining particulates and the supernatant was decanted into a 100 mL volumetric flask. The vials were washed three to four times with 1% (w/v) NaCl with centrifuging before each decant. When

Table X. Analysis of real samples

Sample	Inorganic Hg(II) (ppb)	Organic Hg(II) (ppb)	Total Hg(II) (ppb)
Urine	3.2	1.1	4.3
Urine	2.9	0.80	3.7
Hair	2.1×10^3	1.9×10^3	4.0×10^3
Hair	2.3×10^3	2.0×10^3	4.3×10^3
Tuna	35	4.1×10^2	4.5×10^2
Tuna	39	4.4×10^2	4.8×10^2
Test solution 1[a]	1.03	1.00	2.03
Tapwater	0.006	0.003	0.009
Tapwater	0.005	0.003	0.008
Tapwater, spiked[b]	0.035	0.034	0.069
Tapwater, spiked[b]	0.033	0.032	0.065
Test solution 2[c]	1.02	0.034	1.05
Test solution 3[d]	0.029	0.99	1.02

[a] 1.0 ppb $HgCl_2$ and CH_3HgCl carried through KOH digestion procedure.

[b] Spiked with 0.030 ppb $HgCl_2$ and 0.030 ppb CH_3HgCl.

[c] 1.0 ppb $HgCl_2$ and 0.030 ppb CH_3HgCl.

[d] 0.030 ppb $HgCl_2$ and 1.0 ppb CH_3HgCl.

the sample has been transferred, 7.5 mL HNO_3 (c) and 1 mL of 1.0% (w/v) $K_2Cr_2O_7$ were added and the remainder of the volume was diluted with 1.0% (w/v) NaCl.

Addition of these reagents is critical for quantitative recovery of inorganic mercury as shown by tests with the synthetic 1 ppb mixtures of HgCl, and CH_3HgCl. These tests showed that there was an apparent complexation of Hg^{2+} in the presence of either KOH or NaOH which acidification to pH 2 alone (addition of 2.5 mL of HNO_3 (c)) could not prevent or dissociate. The result was up to a 90% reduction of the inorganic peak since the $SnCl_2$ was not capable of reducing the complexed inorganic mercury which was in turn reduced by the borohydride to produce a larger than normal 1 ppb "organomercury" peak. Apparently the added 1% NaCl and 7.5 mL of HNO_3 (c) effectively competes with the complexing substance to form the $HgCl_4^{2-}$ complex which can be reduced as inorganic mercury. As shown by the test solution 1, in Table X there was no apparent breakdown of CH_3Hg^+ in the digestion procedure. Blank runs of KOH diluted with 1% NaCl gave absorbances of about 4×10^{-4} and 1×10^{-4} A.U. for the inorganic and organomercury reductions.

The actual analysis of more viscous solutions such as tuna required that Dow Corning® Antifoam A Spray (silicone base) be sprayed on the inside walls of the reduction vessel to prevent overflow and also to minimize broadening of peaks which occurred with excessive foaming. Because the hair and tuna solutions still contained some particulates and undigested organics, the tested solutions were evacuated through the top of the reduction vessel with a vacuum flask to prevent clogging of the frit. A 1:1 mixture of HNO_3(c)-H_2SO_4(c)

was used to wash the reduction vessel between runs to eliminate memory effects.

Total concentration of mercury in hair (relative standard deviations of 6-11%) was fairly consistent with existing data (123) which report mercury concentrations in hair ranging from 1-25 ppm for samples from rural to industrial areas. About half of the mercury found was in the organic form concentrated by the body with the other half probably in the form of externally adsorbed Hg^{2+}.

Tuna samples showed about 0.5 ppm total mercury contamination which is approximately normal for canned tuna (124). This is also the upper limit allowed by the USFDA. The organomercury-to-total mercury ratio is also in agreement with previous studies (78) with about 92% in the tuna appearing as organomercury (probably mainly methylmercury) and relative precisions of 18 and 7% for inorganic and organomercury, respectively.

The mercury in the urine sample was in the ppb range which is not unusual (1). The relative precision for the urine measurements were about 5 and 10% for inorganic and organomercury, respectively. Other researchers have been unable to numerically correlate what may be considered toxic concentrations of mercury in urine to that in the blood so that the author holds no undue concern. The inorganic form predominates as expected since most organomercury that is introduced to the body is absorbed or broken down before excretion.

The tapwater was tested, untreated, as it flowed out of the faucet which was left running for a few minutes before sampling. Parts-per-trillion levels of each species of mercury were evident. The upper

limit for mercury in water is 2 ppb as established by the Safe Drinking Water Act (125). The calculated concentrations of mercury in the tapwater are near or at the detection limit with relative standard deviations of 19 to 34 %. The 30 ppt spikes of inorganic-organomercury mixtures resulted in quantitative recovery of the mercury in tapwater.

Finally, the effect of a large excess (factor of 33) of one Hg species in solution with respect to the other was examined by running mixtures of 0.03 ppb Hg^{2+} and 1.0 ppb CH_3Hg^+, and 1.0 ppb Hg^{2+} and 0.03 ppb CH_3Hg^+. The gain of the linear amplifier was adjusted to be 10 for 1 ppb inorganic or organomercury and 100 for 0.03 ppb of either mercury species. This ensures a reasonable pen deflection for the lower Hg concentrations. Care was taken that the baseline was near 0.0 V when the amplification was increased ten-fold to insure that the peak would remain on scale. The results obtained with synthetic test samples 2 and 3 in Table X showed quantitative recovery within experimental error ($\pm$ 10%) of both species in each case. Relative standard deviations were 2 and 8% for 1 ppb Hg^{2+} and CH_3Hg^+, respectively and 11 and 16% for 0.030 ppb Hg^{2+} and CH_3Hg^+, respectively. This indicates that a 33-fold excess of one mercury species does not interfere with the determination of the other mercury species.

SUMMARY

Continuous Sampling Flow Methods

Modification of the discrete sampling reduction vessel which employed bubbling a carrier gas through the reaction mixture to release the elemental mercury as well as redesigning the vessel to employ the technique of volatilization from a thin stream allowed the determination of mercury in the continuous flow concept. Compared to the discrete sampling reduction vessel approach, some advantages were realized. The operation is simpler and less dependent on operator skill in that the sample tube need only be moved between sample and blank solutions as in a conventional flame AA spectrometer. This means the continuous sample scheme is easier to automate and more suitable for remote monitoring. The discrete sampling reduction cell could be automated with more difficulty with computer control of syringe injections of the sample, blank, and reagents and of the evacuation of the reaction mixture.

The primary disadvantages of the continuous sample reduction vessel approach compared to the discrete sampling approach are reduced calibration sensitivities, worse detection limits, and longer analysis times. The calibration sensitivities of 0.007 - 0.020 A.U./ppb and detection limits of 0.02 - 0.03 ppb are significantly worse than the respective values of 0.03 A.U./ppb and 0.003 ppb obtained with the discrete sampling cell. However, the detection limit is more than adequate to determine if mercury levels in water exceed the acceptable drinking water limit of 2 ppb. The reduced calibration sensitivities

are due in part to incomplete volatilization (10-29%) of mercury from the reaction solution. Also the baseline absorbance noise is a factor of 2-4 greater with continuous sample introduction even though a larger electronic time constant was employed. This increased noise is apparently due to pressure fluctuations and mist generated by the continuous flow reduction vessels. Response times of 0.9-1.3 min result in a typical analysis time about 2 minutes since both a blank and sample solution must be run. The sample analysis time with the discrete sampling reduction vessel is about 1 min because a blank run does not have to be made unless there is mercury in the reagent blank.

The thin film reduction vessel design is considered superior to the aeration design because the baseline noise is smaller and less spurious noise spikes are generated. In both cases there is a trade-off between calibration sensitivity and response time to consider when adjusting the nitrogen flow rate.

Further work could involve interfacing the continuous flow system to a high pressure liquid chromatograph as a mercury-specific detector. Interfacing to a microcomputer would enhance the capabilities of the system. An automatic switching valve could alternate the sample tube between sample and blank and allow the monitoring of the daily patterns of mercury in a stream with a minimum of attention. Speciation based on the selective reduction scheme developed in this thesis could be adapted to continuous flow cell. Preliminary studies revealed excessive formation of hydrogen bubbles in the tubing leading to the reduction vessel caused by the breakdown of $NaBH_4$. .This resulted in extremely erratic plateaus and imprecise data and carryover into the

absorption cell with the thin stream reduction vessel. However, separate ports leading to the reduction vessel that would transport reductant and sample could be employed so that mixing only occurs in the vessel and not in the delivery tubes.

Speciation

This selective reduction method of speciation developed takes advantage of the different reducing strengths of $SnCl_2$ and $NaBH_4$ to sequentially reduce inorganic mercury and organomercury compounds (primarily methylmercury), respectively. Calibration sensitivity and detection limits of about 0.03 A.U./ppb and 0.003 ppb, respectively, were obtained for Hg^{+2}, CH_3Hg^+, and $CH_3CH_2Hg^+$. For phenylmercuric chloride the calibration slope was about half the above value with a corresponding detection limit 0.005 ppb.

The significant advantage of this method over that employed by Magos is a factor of about 10^3 better detection limit and the need for fewer reagents of lower concentrations. This reduces the problem of contamination and eliminates an extra pre-purification step which was necessary for the 45% (w/v) NaOH and 16 N H_2SO_4 reagents that he used. Use of gas chromatography which has the advantage of differentiating the various organomercury compounds requires separate extraction and reaction steps to convert inorganic mercury to a detectable organomercury compounds. These steps are eliminated in the procedure presented in this thesis. HPLC, which has also been applied to mercury determination (126) at ppb levels effectively differentiated inorganic and various organomercury compounds but required between 5 and 14 minutes

to do so. In the method where total mercury is determined by either photochemical or chemical digestion of the sample to decompose organic forms of mercury, more reagent contamination and longer digestion times are required, especially where UV irradiation is employed. Also the organomercury is determined by difference between the inorganic concentration determined before digestion and the total mercury value. This technique is time consuming and subject to more error if most of the sample is inorganic mercury.

Investigation of methylmercury breakdown showed that 1% (v/v) HNO_3 (c) with 0.01% (w/v) $K_2Cr_2O_7$, together which are effective as a preservative for inorganic mercury, contributes to breakdown of methylmercury. Although the total amount of mercury in solution is maintained quantitatively over three days, breakdown of almost 50% of the methylmercury over the same period would invalidate attempts at speciation after this period of time. Of the two preservation reagents, HNO_3 has the most effect on decomposition. At this time, it is recommended that speciation analysis be carried out immediately after the water sample is acquired.

The applicability of the speciation procedure was demonstrated on several types of real samples. These samples revealed total mercury concentrations of 0.5 ppb with 92% organic mercury in canned tuna, 4 ppm with 48% organic mercury in hair, 4 ppb with 24% organic mercury in urine. In digestion procedures using KOH or NaOH, dilutions with 1% (w/v) NaCl and acidification with 7.5 mL HNO_3 (c) per 100 mL of solution and 0.01% (w/v) $K_2Cr_2O_7$ were necessary to obtain quantitative recovery of inorganic Hg^{2+} which is apparently normally complexed

without these reagents. Tapwater samples spiked with 0.030 ppb inorganic and organomercury resulted in quantitative recovery of each.

Further studies in this area could inlcude more detailed analyses on the complexation of the mercuric ion and possible employment of EDTA or cysteine to preferentially tie up the mercuric ion and release the mercury upon contact with $SnCl_2$. The simultaneous preservation of methylmercury without decomposition and inorganic mercury without loss requires further investigation. Computer integration of the peak areas might improve precision and accuracy especially with the broadened peaks encountered in viscous or poorly digested biological samples.

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