5-1-1977

The Differentiation of Mercury Species in Submicrogram Quantities in Aqueous Solution

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THE DIFFERENTIATION OF MERCURY SPECIES IN SUBMICROGRAM QUANTITIES IN AQUEOUS SOLUTION

by

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Bachelor of Arts, University of North Dakota, 1972

A Dissertation

Submitted to the Faculty

of the

University of North Dakota

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Grand Forks, North Dakota

May
1977

This Dissertation submitted by David A. Hildebrand, in partial fulfillment of the requirements for the Degree of Doctor of Philosophy from the University of North Dakota is hereby approved by the Faculty Advisory Committee under whom the work has been done.

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ACKNOWLEDGEMENTS

I wish to acknowledge my advisor, Dr. Richard J. Baltisberger, for his assistance, the University of North Dakota for the use of their facilities and for financial assistance through teaching assistanceships, and for financial support by funds provided by the United States Department of the Interior, Office of Water Resource and Technology, as authorized under the Water Resources Research Act of 1964, Public Law 88-379, through the Office of Water Resources Institute, Fargo, North Dakota, annual allotment Grant A-050-NDAK and a matching Grant B-020-NDAK.

I would also like to thank Dr. Thomas A. Ballentine for his technical assistance and many helpful discussions during the course of this study.

The author also wishes to thank his parents for their support and encouragement.

I dedicate this dissertation to my wife, Bonnie, without whose patience, understanding and dedication, this dissertation would not have been completed.

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ABSTRACT

Although trace analysis procedures for the analysis of total mercury have been available for a number of years, a major shortcoming of the procedures has been the inability to differentiate between the exact chemical forms of mercury species in solution. This is needed due to the varing toxicity of the different species. An investigation was made of the possible analytical procedures which could be used for a separation and quantitation of mercury (0), mercury(I), mercury(II), methyl- and phenylmercury(II). The method of flameless atomic absorption spectroscopy (FAA) was used to detect mercury vapor produced by reduction of all mercury species or disproportionation of mercury(I) in an aeration cell. To increase the sensitivity of this method a new design of aeration cell was tested. The parameters that affected the instrument response were investigated and fixed to give a reproducible instrument response. These parameters were sample volume, carrier gas flow rate, aeration solution volume, and concentration of the sample analyzed.

The stability of various mercury solutions was investigated to have available standard solutions to be used for calibration of the FAA spectrophotometer.

Mercury(II) solutions in the ppb range could not be stored for more than one working day without the formation of mercury(I) in the solution. This was suspected to have been caused by the oxidation of water by mercury(II). Solutions containing mercury(0) were found to be air oxidized to mercury(I). The preparation of solutions containing mercury(0) under oxygen free conditions eliminated the oxidation of mercury(0). Solutions of mercury(I) could be quantitatively prepared by the reduction of mercury(II) by mercury(0) under oxygen free conditions.

An ion exchange liquid chromatographic procedure for the separation of the mercury species was studied on both Bio Rad AG 2 X8 and ECTEOLA cellulose ion exchange materials. Methyl- and phenylmercury(II) chloride were quantitatively separated on the Bio Rad AG 2 X8 resin but the inorganic mercury species were irreversibly absorbed. On the ECTEOLA cellulose polymer mercury(I) and mercury(II) were retained under the same solvent conditions with mercury(I) disproportionating on the column. These ion exchange procedures were of little use for the separation of the inorganic forms of mercury.

Because of the failure of the ion exchange procedures in giving a separation of the inorganic mercury species, an analysis scheme based on selective chemical reactions was studied. This was done in order to develop a means of analyzing a mercury mixture <u>in situ</u>. Mercury(0) could be vaporized from an acid media in the absence of a

reducing agent and detected in a UV detection cell. When mercury(I) was also present in the sample, the chloride concentration was adjusted to 0.01M to prevent the disproportionation of mercury(I) from forming additional mercury(0). When care was taken to exclude chloride from a sample of mercury(0) and mercury(I), the mercury(I) was found to quantitatively disproportionate to mercury(0) and mercury(II). The mercury(0) observed was equal to the sum of the quantity of soluble mercury(0) and the quantity of mercury(0) formed by disproportionation. A non-reducing analysis performed in basic media was unsatisfactory for the analysis of inorganic mercury species because of a partial or total reduction of the mercury sample. The inorganic mercury concentration of a sample was determined in a hydrochloric acid-tin(II) media. The total mercury concentration of a sample (including organo mercury) was determined in a basic reducing mixture of tin(II)- and cadmium(II) chloride. The quantity of organic mercury was obtained by subtraction of the analyses in acidic and basic reducing solutions.

The prevention of the disproportionation of mercury (I) by chloride was studied in detail. It was found that a chloride concentration greater than 10^{-7}M retarded the disproportionation reaction and a concentration of 0.01M chloride prevented disproportionation during aeration for several hours. The reason for the stability of mercury(I) chloride towards disproportionation was postulated to have

been caused by the aging of a colloidal type precipitate.

A mixture of mercury(II) and mercury(I) was analyzed by the addition of a drop of elemental mercury and chloride ions under nitrogen . The increase in concentration of the sample due to the formation of mercury(I), from the reduction of mercury(II) by mercury(0) liquid, was equal to the quantity of mercury(II) in the original sample. The mercury(I) in the sample was quantitated by subtraction of the concentration of mercury(II) from an initial total mercury analysis of the sample.

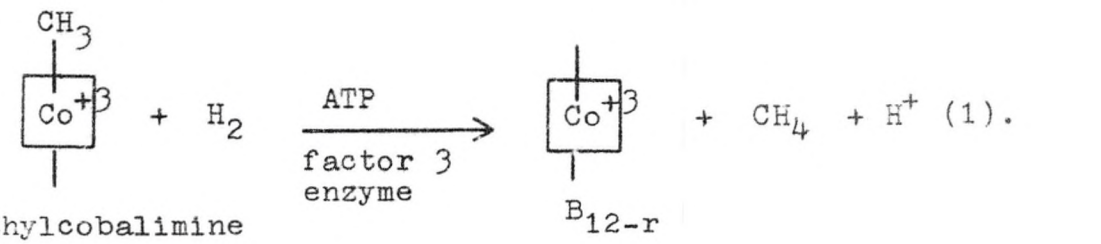
INTRODUCTION

The subject of this study will be to develop analytical procedures by which one ionic form of mercury can be distinguished from another in aqueous solution of trace levels of 10^{-7} to 10^{-9}M.[1] Before discussing the inorganic and analytical chemistry of mercury compounds, an overview of some of the problems created by the indiscriminate use of mercury and its compounds will be presented. This discussion will show the need for analytical procedures capable of differentiating the various ionic forms of mercury in aqueous solution.

Environmental problems Mercury contamination in aqueous or soil samples can be a serious problem but there is normally a natural background concentration of mercury in our environment due to its release by weathering of rocks and mineral deposits.[1] In a biomagnification process, this mercury is first absorbed by the lower order plants and animals and is ultimately concentrated at the top of the food chain. In a second process, bioaccumulation, mercury builds up in the predators body with age.[2] These processes have occured for thousands of years and have only become a problem when man introduced higher concentrations through indiscriminate use and disposal of mercury.[3,4] An industrial

incident occured in Minamata Bay, Japan in the early fifties in which forty one people died and many others became severely ill from the discharge of mercury into the bay.[5] This mercury, used as a catalyst for the industrial production of acetic acid, was concentrated in the fish and shellfish which were the primary diet of the people affected. An agricultural example of this type of problem happened in Sweden in the early sixties.[6] Certain bird populations decreased drastically due to mercury poisoning from the extensive use of mercury compounds as fungacide in the agriculture industry. Very large mercury concentrations were also found in the agricultural products. Analysis of fish samples from areas of Sweden, which have been contaminated with industrial waste of phenylmercury(II) and mercury(II) cations, have a very high mercury content.[3] This high mercury content is present entirely as the methyl mercury(II) cation.

This latter data indicates that there is some type of mechanism for the conversion of the phenyl and inorganic forms of mercury to the methyl mercury cation. This mechanism was found to be an adaptation of the last step in the natural bacterial degradation of organic matter to methane, given below in reaction,[7,8]



Wood found that when low levels of mercury(II) were present no methane was formed but the production of B_{12-r} was uninhibited.[7] Methyl and dimethylmercury(II) were found to be the reaction products. A very rapid reaction rate at high mercury concentrations lead Wood to suspect a nonenzymatic pathway for the reaction. It was found that mild reducing conditions (Zn^{o}) in acid solution (10% HNO_3) gave the desired reaction of a nonenzymatic pathway. At high mercury concentrations only the monomethyl form was produced indicating that dimethylmercury(II) is the ultimate product of the reaction when an adequate supply of bacteria is present. When these conditions were investigated, only the dimethyl form was found in the product analysis.[8] The dimethyl form isn't often found in the analysis of environmental samples because of its highly reactive nature.[7] Dimethylmercury(II) reacts with HCl as in reaction,

$$(CH_3)_2Hg + HCl \longrightarrow CH_4 + CH_3HgCl. \qquad (2)$$

The reaction that is more likely to be encountered under environmental conditions is reaction,[8]

$$(CH_3)_2Hg + HgCl_2 \longrightarrow 2CH_3HgCl \ . \qquad (3)$$

Not only is methylmercury(II) chloride the form of mercury expected to be found in nature but it is the extremely toxic form.[5,6,9] One reason for the high toxicity of methylmercury(II) chloride is that it is absorbed to a greater extent by plants than the inorganic forms and concentrated in the fruit of the plant by a

factor of 33 to 3 times that of the inorganic forms.[2,10] This trend is followed in the animal life.[11,12] The methylmercury(II) cation is concentrated faster and retained six times longer than the inorganic forms of mercury. It was found by radio isotope labeling that 25% of the inorganic mercury was in the particulate matter in streams eventually settling out into the silt while only 4% of the methyl form was found on the particulate matter. The methyl form required five times longer to be carried down stream relative to the inorganic forms. Evidence of slow cycling in the human body is pointed out be a woman that was treated with a mercury containing ointment for a year.[13] She was exposed to four grams of mercury per month in the ointment. Three years after her last treatment she was still excreting 330ppb mercury in her urine and her liver contained 94.5ppb which is two hundred times the normal level of mercury. The form of mercury found in this case was probably methylmercury but it was not determined in the analysis. The body converts the mercury absorbed to the methyl form by the process discussed earlier. In the body methylmercury (II) chloride collects in the central nervous system and in particular the nerves associated with vision.[14] The first symptons of alkylmercury poisoning are impaired vision or blindness. The biological chemistry is not really understood but a good explanation for the accumulation and extremely slow release rate for mercury in living tissue could be the almost irreversible bond formed between mercury

compounds and the RSH groups on the amino acids and proteins present in all living tissues.[7,9,15-18] Due to the large difference between the inorganic and organic forms in toxicity and retention in biological systems, there is a definate need to be able to differentiate between the different forms of mercury in aqueous samples. Such a differentiation method would give investigators a better profile of the toxicity level of mercury pollution in environmental samples.

Analytical procedures for differentiation of mercury species

The major methods of differentiation of organic and inorganic mercury at trace levels can be grouped into four general classes. Trace level concentrations in environmental samples generally are on the order of 1 to 100ppb (10^{-9} to 10^{-7}M) for aqueous samples while animal or plant tissues are of the order of 0.1 to 10ppm.

The first general type of differentiation is done by using gas chromatography (glpc).[8] The inorganic and several organic forms of mercury are separated as their chloride forms using a number of possible column packings and detected by an electron capture detector which is extremely sensitive for the analysis of halogen containing compounds.[19] A mass spectrograph can also be coupled to a glpc and mass spectrum of each peak can be taken to conclusively identify the compounds. This is needed because the retention time of a peak is not conclusive proof as

to the identity of a compound. A variation on this method uses a flame ionization detector with a reducing flame in place of the electron capture detector.[20] The reducing flame converts all the mercury forms to mercury (0). This flame ionization detector is coupled with a flameless atomic absorption instrument (FAA) in order to increase the sensitivity. The flame detector destroys organic compounds which are a major source of interference in the FAA method. The FAA method first described by Hatch and Ott is based on the ease of forming mercury atoms at room temperature which can be measured by atomic absorption spectrophotometric methods.[21] In the analysis the mercury compounds are reduced to the metallic form which is vaporized from aqueous solution into an inert gas stream. The mercury vapor is carried into a 20-30cm gas cell in which the quantity of mercury present in the gas stream is measured by UV absorbance measurement of the 254nm light emitted from a mercury lamp source. The most severe disadvantage of using glpc is the sample has to be extracted into an organic solvent prior to its injection.

The second general type of analysis is based on the possible separation by liquid chromatography (LC) of the inorganic and organic forms prior to measurement of the mercury. One of these methods is a cation exchange separation using the isothiocyanatopentaaquochromium(III) complexes of mercury(I), mercury(II) and methylmercury(II).[22] After separation on a cation exchange column the organic fractions

are oxidized to mercury(II) using hydrogen peroxide and perchloric acid. The determination was done by FAA. In another method the dithiazone complexes of organo and inorganic mercury are isolated by thin layer or column chromatography using alumina as the absorbent. The detection in this method is made by the colorimetric measurement of the dithiazone complexes.[23]

The third general type is based on the approach of using an analysis method that only detects inorganic mercury. The sample to be tested was divided into two parts. One sample is analyzed for inorganic mercury by placing the sample in acid-tin(II) media in which the inorganic mercury is reduced; organomercury compounds are very slowly reduced in acidic tin (II) media with essentially no mercury(0) produced from these compounds during the time of analysis. The resulting mercury vapor is analyzed by FAA. A second sample is oxidized to convert all forms of mercury to mercury(II). This sample is then analyzed as in the first step. The difference in quantity between the two analyses gives the organomercury concentration, while the first analysis gives the inorganic mercury concentration.[24] The only difference in most of the methods of this type is the oxidizing agent used. Examples of common classical wet methods of oxidation used are $K_2Cr_2O_7$, $K_2S_2O_8$, $KMnO_4$, H_2O_2, $HClO_4$, O_2 bond, and Cl_2 gas.[25-30] The organomercury species can also be decomposed by irradiation with an intense UV light source.[31] These methods are the most widely used, it should be pointed out

that other methods can be used. A solution of $HgCl_3^-$ and $HgCl_4^{-2}$ has a wavelength maximum at 230nm which has been used to detect mercury(II) in the presence of the methyl form. This can be accomplished because of the much smaller molar absorptivity of methylmercury(II) chloride at that wave length.[32] The analysis was performed by measuring the solution absorbance to determine the mercury(II) concentration. This sample was then irradicated with UV radiation to decompose the organic forms to mercury(II) and the absorbance is remeasured. The increased absorbance is then due to the concentration of the organic forms in the sample.

The fourth general class of methods uses a special reducing agent for the FAA method. This reducing agent will reduce the organomercury species without a preoxidation step to obtain the total mercury content. There are two variations of this technique in use at present time. In both techniques the inorganic mercury concentration of a mixture is measured by FAA using a tin(II) chloride acid mixture to generate mercury(0). In a following step a basic tin(II) chloride media is used to reduce a second sample so that the mercury total is measured by FAA. In one case copper(II) is added as a catalyst and in a second case cadmium(II) is used. The organomercury content is then calculated from the difference of the total and inorganic analysis.[33,34]

As evident from the above discussion of analytical

techniques the emphasis has been placed on the analysis and differentiation of organomercury from inorganic mercury forms. In the subsequent discussion of the chemistry it will be evident that inorganic mercury can exist in solution as mercury(0), mercury(II), and mercury(I). No methods have been proposed which will allow one to measure the individual amounts of the species at the ppb level. This thesis will address itself to this particular problem.

Inorganic chemistry of mercury Inorganic mercury exists in three distinct valence forms, mercury(0), mercury(II), mercury(I), in aqueous solution.[35] These three forms are related by the equilibrium reaction,

$$Hg_2^{+2} = Hg^{+2} + Hg^{o}_{(aq)} \cdot \qquad (4)$$

The disproportionation constant written for reaction 4 is,

$$K_{eq} = \left[Hg^{+2}\right] \left[Hg^{o}_{(aq)}\right] \left[Hg_2^{+2}\right]^{-1} \cdot \qquad (5)$$

The most commonly quoted value in the literature for the constant is 5.5×10^{-9} moles per liter at 25°, reported by Moser and Voight.[36] Experimentally, the value was measured by adding radioisotopic labeled mercury(I) chloride to water and determining the amount of labeled mercury(0) at equilibrium. The mercury(0) concentration has a limiting effect on the equilibrium expression due to its low solubility in water. This solubility has been very well documented under a variety of conditions. One analysis in 1962 obtained a value of 63.1ppb in pure water by neutron

activation.[37] In 1974 the solubility was redetermined by FAA in water and sea water to be 63.1 and 54.9ppb, respectively. The lower value was explained by the salting out effect that an electrolyte has on a nonelectrolyte.[38] In another study the solubility was determined by measurement of the UV absorbance of mercury(I) generated by the reaction of the mercury(0) with an excess quantity of mercury(II). Neither mercury(II) or mercury(0) absorb at 236.5nm, the wavelength of maximum absorbance of mercury(I) dimer.[39] The molar absorptivity of the dimer was reported to be $2.8 \times 10^{5} m^{-1} cm^{-1}$ which permitted sufficient sensitivity for the determination of the solubility to be 61ppb. The loss of mercury(0) from a solution of mercury(I) will shift the disproportionation reaction in the direction to produce more mercury(0). These type of losses have been observed from mercury(I) solutions.[40,41] This loss was used for a determination of the solubility of mercury(0) in air of 2.16×10^{-8} gm/ml.[36] In the same study the mercury(0) solubility in water was found to be 60ppb at 25°C by the use of radioisotopic labeling. A value for the proportion constant for the extraction of mercury(0) from water to air was calculated to be 0.36. This constant was redetermined in 1976 to be 0.4 from acid solution by FAA.[42] This constant was found to increase with temperature as did the solubility of mercury(0).[37,42] When mercury(0) is present well above the solubility value, liquid mercury(0) will form as the aqueous concentration of mercury(0) remains at the constant value of its solubility.

The equilibrium reaction can be rewritten as,

$$Hg_2^{+2} = Hg^{+2} + Hg(liq.). \quad (6)$$

$$R = \frac{[Hg^{+2}]}{[Hg_2^{+2}]} \quad (7)$$

This disproportionation ratio was determined by Sillen to to be 0.0077 by potentiometric titration with chloride and followed by using mercury electrodes.[43,44] The disproportionation constant and ratio are related through the solubility reaction,

$$Hg(liq) = Hg(aq) \quad (8)$$

to give

$$K = R\,[Hg^{o}(aq)] \quad . \quad (9)$$

A value of K calculated from Sillen's data and the mercury(0) solubility is 2.3×10^{-9}M compares well to the Moser and Voigt value of 5.5×10^{-9}M. Both the disproportionation constant and the ratio indicate that mercury(I) is the most stable form of mercury in aqueous solution and should not disproportionate. To shift this reaction towards disproportionation, mercury(II) would have to be stabilized relative to mercury(I) by using complexing ligands or one of the reaction products would have to be removed from the reaction mixture.

The reduction potentials at 25°C of mercury(I) and mercury(II) to form metallic mercury are,

$$Hg^{+2} + 2e^- = Hg^{o}(liq) \quad E^{o} = 0.854V \quad (10)$$

$$Hg_2^{+2} + 2e^- = 2Hg^{o}(liq) \quad E^{o} = 0.789V \quad (11)$$

as reported by Latimer.[45] The value of the disproportionation ratio calculated from these potentials is 0.00625 which agrees well with the number of 0.0077 reported by Sillen. In the absence of complexing agents, the values of the two potentials are so close together that no oxidizing or reducing agent can be added to the mixture which will completely reduce or oxidize one species without reacting with the other.

Ideally one would like to add a complexing agent which would stabilize one species relative to the other, such that complete formation of either mercury(I) or mercury(II) is possible. The desired reaction would be to add a complexing agent, X, which would stabilize mercury(II) forcing all of the mercury(I) species in solution to disproportionate to form mercury(II) and mercury(0). The measurement of the mercury(0) produced would then be equal to the concentration of mercury(I) originally in the sample. No analytical procedure at trace levels based on this type of reaction have been reported, but in the qualitative analysis scheme this type of reaction is reported with the addition of ammonia to mercury(I) chloride. An alternative analytical procedure for trace analysis would be to add a complexing agent, Y, to a mixture which would stabilize mercury(I). In this case mercury(0) would be added to

react with mercury(II) to produce a solution entirely in the mercury(I) form. The amount of mercury(II) would then be equal to the quantity of mercury(0) oxidized to mercury(I). No analytical procedure at the trace level of this type has been reported.

In the equilibrium expression mercury(I) is written as a dimer, Hg_2^{+2}, because to date all studies have indicated the monomer, Hg^+, does not exist in solution.[35] Estimations of the constant for the dissociation of the dimer to form the monomer are of the order of 10^{-18} to 10^{-30} from thermodynamic data.[36] Higginson found that Beer's law plots of the UV spectra of mercury(I) perchlorate solutions become nonlinear near 10^{-7} molar.[46] In this study the solution spectra was measured by reading the maximum of mercury(I) perchlorate at 236.3nm where mercury(0) and mercury(II) have no absorbance. Higginson explained this nonlinearity by the formation of mercury(I) monomers and estimated a dissociation constant of 10^{-6} to 10^{-8} for the reaction,

$$Hg_2^{+2} = 2Hg^+ . \qquad (12)$$

Moser and Voigt in their solubility study of mercury(0) in mercury(I) solutions worked at the concentration range of 10^{-7}M.[36] They observed no evidence of mercury(I) monomers and indicated that the constant would have to be below 10^{-7}M. Onat in his study of the UV solution spectra of mercury(I) perchlorate solutions also observed no deviation from linearity down to 10^{-7}M.[39] These studies indicate that if there is any dissociation, it occurs

below 10^{-7}M. This is not very reassuring considering that most environmental samples are also below 10^{-7}M. Aqueous mercury(I) solutions are diamagnetic which indicates that the ionic form would be paramagnetic.[35] The Raman spectrum has a strong line in an aqueous solution of mercury(I) nitrate attributed to the stretching of the mercury-mercury bond. The force constant obtained for the bond was 2.5 mdyne/Å.[35] There is no real evidence of the existence of the monomer in aqueous solutions of mercury(I).

All experimental evidence for the kinetic stability of mercury(I) has been consistent with a rapid disproportionation of the dimer to mercury(II) and mercury(0). The exchange rate,

$$Hg_2^{+2*} + Hg^{+2} = Hg_2^{+2} + Hg^{+2*} \qquad (13)$$

was found to be complete within the time of mixing (one minute) by King.[47] The extent of the exchange was determined by precipitation of radioactive labeled mercury(I) and subsequent counting of the specific activity. If the labeled mercury was added prior to the precipitation, exchange was always complete within mixing time. If the radioactive label was added to the precipitate, exchange was found to be incomplete. The results were interpreted to mean that the exchange reaction is rapid in solution, but that the rate of recrystallization of some mercury(I) precipitates was slow.[47] A second study performed by Wolfgang and Dobson

obtained the similar results using the same method of selective precipitation.[48] They also found that the addition of cyanide ion slowed the exchange rate of mercury(I) and mercury(II) to that of the rate of exchange of mercury(II) with cyanide. This lead them to exclude the monomer as an intermediate in the exchange reaction.

The two reasonable mechanisms by which the rapid exchange can occur are by the rapid disproportionation of mercury(I),

$$Hg_2^{+2} \overset{rapid}{=} Hg^{+2} + Hg^{o} \qquad (14)$$

or by the formation of a trimer intermediate such as,

$$Hg_2^{+2} + Hg^{+2} = Hg_3^{+4} \; . \qquad (15)$$

The following kinetic data is consistent with the first mechanism being the pathway.

In kinetic studies, the oxidation of mercury(I) by two equivalent oxidizing agents was studied. One study used thallium(III) as the oxidizing agent and followed the reaction by the consumption of mercury(I) measured at 236.5nm.[49] The stoichiometry of the reaction is,

$$Hg_2^{+2} + Tl(III) \longrightarrow Tl(I) + 2Hg^{+2} \; . \qquad (16)$$

In this work, Halpern found a definite inverse dependence of the reaction rate on mercury(II). If the reaction proceeded by two one electron reactions the rate expression would not have this inverse dependence on mercury(II). The following mechanism was proposed in which the fast

disproportionation reaction of mercury(I) was followed by the rate determining reaction of thalium(III) with mercury(0) to produce mercury(II) and thalium(I). The proposed mechanism was,

$$Hg_2^{+2} \overset{fast}{=} Hg^{+2} + Hg^{o} \quad \text{and} \quad (17)$$

$$Hg^{o} + Tl^{+3} \xrightarrow{slow} Tl^{+1} + Hg^{+2} . \quad (18)$$

If the trimer, Hg_3^{+4}, were the reactive species a first order dependence on Hg(II) would be observed. A second study was made in the same manner except bromate ion (BrO_3^-) was used as the oxidizing agent.[50] In this study Sykes found the same inverse dependence on mercury(II). These authors concluded that a similar mechanism was followed as shown in reactions 17 and 18. One possible pathway that the studies didn't discuss was the breakup of the dimer to the monomer. The monomer would go on to react with the bromate ion and would give the rate law a square root dependence on mercury(I). This square root dependence was not observed in either study eliminating this type of mechanism as a possible pathway.

The disproportionation constant given in reaction 4 is for mercury(I) in a noncomplexing media. The presence of complexing ligands will shift the equilibrium position depending on the relative stability of the mercury(I) to mercury(II) complexes. Compounds that can not be made with mercury(I) are oxide, sulfide, and cyanide. These complexes with mercury(II) are stabilized to such a great extent that the formation of mercury(I) is no longer

favored.[35] This causes the mercury(I) to disproportionate to mercury(0) and mercury(II). The monohydroxide species does exist in acidic aqueous solution because of the highly acidic properties of mercury(I).[51,52] When the pH is raised to produce the dihydroxo form, mercury(I) disproportionates. In the study of mercury(I) complexes using the ligands PO_4^{-3}, $P_2O_7^{-4}$, $P_3O_{10}^{-5}$, and $P_4O_{13}^{-6}$ as well as some dicarboxylate anions, $C_2O_4^{-4}$, $(CH_3)_2C(CO_2)_2^{-2}$ and $(CH_2)_2(CO_2)_2^{-2}$ an important point was discovered.[53,54,55] In general the complexes of mercury(I) with ligands that formed strong covalent bonds to mercury(I), such as soft bases, destabilized mercury(I) to form mercury(II). Those complexes that formed by ionic bonding of the ligands, such as hard bases, formed stable mercury(I) complexes. In work done by Potts, another series of mercury(I) complexes were prepared with a series of ligands where the stability was investigated with respect to disproportionation.[56-64] No stable complexes could be prepared with ligands with greater stability than water in the spectrochemical series of $CN^- > NO_3^- > NH_4^+ > NCS^- > H_2O > (COO)_2^{-2} > OH^- > (NH_2)_2CO > (C_2H_5)_2NCS_2^- > F^- > (C_2H_5O)_2PS_2 > N_3^- > SCH^- > Cl^- > Br^- > I^-$. The first stable soluble complexes of mercury (I) with nitrogen donor atom ligands were made by Wirth.[65] Since then an entire series of substituted pyridine and quinoline compounds of mercury(I) have been made.[66] Their stability towards disproportionation seems to be highly dependent on the pK_b of the nitrogen ligand. In general the more basic ligands that had a pK_b below 8.5 caused the

mercury(I) to disproportionate. This disproportionation was suspected to have been caused by the high affinity of the strongly basic nitrogen donor atom for the more acidic mercury(II) than the mercury(I).[52,67-69]

In summary, mercury(I) has been shown to be stable with respect to disproportionation and that the exchange between mercury(I) and mercury(II) is very rapid. Mercury(I) can also be forced to disproportionate by the choice of ligand added to the solution. Because of this instability it took until the 1950's for stable complexes with many types of ligands to be found.[70] The fact that these ligands can be isolated from solution indicates that by the choice of the correct ligand mercury(I) could be stable enough to be separated from mercury(0) and mercury(II) in solution.

Storage problem A serious problem in the analysis of environmental mercury samples is the loss of mercury during storage of the samples. This problem occurs both for natural samples and prepared standards of low concentration. One reason for the problem is the high volatility of many of the mercury compounds especially mercury(0).[71-73] A second reason is the reaction of mercury compounds with trace reducing agents in the solution producing mercury(I) and mercury(0). The mercury(I) will disproportionate to mercury (0) which is lost. The third explanation is the absorption of mercury compounds on suspended particles that settle out of natural water samples.[11,12,71,72] This causes problems

of loss and nonhomogeneity of the sample. A final explanation attributes the loss to adsorption onto the walls of the containers.[71] Losses can be as high as 90% in ten days. Chemical attempts to preserve these solutions involve the addition of acid to the reagents to protonate the ion exchange sites on the glass surface and stop the absorption. To remove the problem of reduction and loss by volatility, oxidizing agents are added to convert all of the mercury forms to mercury(II). For example, when nitric acid and potassium dichromate were added to the solution there was no loss of mercury concentration during the first ten days of storage. An experiment conducted with radioisotopic tracers determined that without the preservatives, 77% of the mercury was adsorbed onto the walls of the container and 18% was lost by volatility in 21 days. When nitric acid-potassium dichromate mixture was used the losses were 2% onto the walls and no loss by volatility.[72] The major disadvantage of these chemical methods of storage is their destruction of forms of mercury in the mixture by conversion to mercury(II). When it is desired to determine the exact chemical form of the mercury species in solution, these methods are not usable. A satisfactory method for the storage of solutions containing organic and inorganic mercury has been found which uses hydrochloric acid (pH=1.5) to store mercury(II) in Pyrex bottles with no loss of concentration.[74] At that chloride concentration the mercury(II) will all be in the anionic form which would have no absorption onto the

anionic exchange sites on the surface of the glass.[75-79] Having the mercury(II) in the anionic chloro complex also seems to stop the reduction and loss by volatilization. It is not known what affect the formation of chloro complexes will have on the disproportionation equilibrium. This method of storage was the method of preference for this study.

Mercury(0) has been shown to diffuse through the walls of polyethylene bottles when used in the hydrochloric acid method of storage.[74] Problems of even higher losses have also been found using other methods of storage.[71] These types of containers are not recommended for storage.

Statement of the problem Although trace analysis procedures for the analysis of total mercury have been available for a number of years, a major shortcoming of the procedures has been the inability to differentiate between the exact chemical forms of mercury species in solution. This is needed due to the varying toxicity of the different species. Mercury exists in aqueous solution as mercury(0), mercury(I), mercury (II) and organomercury(II) where the organic group is methyl or phenyl for example. The objective of this project will be to develop analytical procedures which will quantitate the individual concentration of these types of mercury species. The research will be divided into four parts. First, the method of flameless atomic absorption spectroscopy will be used to detect mercury vapor produced by reduction or dismutation of a mercury(I) species. The parameters

affecting the instrument response will be investigated and controlled so that a reliable analytical monitoring scheme will be available. Second, the stability of various mercury solutions will be investigated in order to have available standard solutions to be used for the calibration of the flameless atomic absorption spectrophotometer. Third, a liquid chromatographic procedure for the separation of the mercury species will be studied. The resulting isolated species will be analyzed for mercury content by the appropriate method. Fourth, an analysis scheme based on selective chemical reactions will be studied in order to develop a means of analyzing a mercury mixture in situ. The major problem in this portion of the work will be to establish the stoichiometry of the disproportionation of mercury(I) ion in various aqueous media. The solution parameters that need to be controlled for a rapid, quantitative disproportionation of mercury(I) ion as well as its general chemical properties in dilute solution will be investigated.

EXPERIMENTAL

Reagents All reagents used in this study were analytical grade except where stated and all water was triply distilled. The second distillation was made from alkaline permanganate solution in a glass still. The third distillation was also done in a glass still and the collected water stored in glass containers with ground stoppers.

Mercury(II) Solutions The method of analysis of trace level mercury(II) solutions used in this study was flameless atomic absorption (FAA).[21] The method requires almost daily calibration of the instrument using a standard mercury(II) solution. Early in the study it was determined that mercury (II) solutions tend to lose strength through various chemical and physical methods when stored for several days. It was, therefore, necessary to make standard mercury solutions a few hours before use. In order to make these solutions rapidly, it was necessary to have available a salt of mercury(II) that could serve as a primary standard so that such solutions could be prepared by weight. The alternative to this approach would have been to standardize freshly made solutions by titration which would have become very time consuming.

In order to determine if the salt, mercury(II) acetate, obtained from Fisher Scientific Co. could be used as a primary standard, the following experiments were carried out. Mercury(II) acetate solutions were prepared by dissolving a weighed amount of the salt in 5 ml of concentrated perchloric acid which was diluted to 500 ml with water to give a final concentration of 0.0501M used for titration experiments or 1.04×10^{-3}M used for FAA analysis. Mercury(II) acetate stock solutions were standardized by titration with sodium thiocyanate which had been previously standardized by titration with primary standard silver nitrate. Iron(II) nitrate was used as an indicator in both sets of titrations.[80] The molarity of the mercury(II) acetate solution found by titration was 0.05014M which was within $\pm$.2% of the value of 0.05010M calculated from the weight of the original salt used to prepare the solution. This error was less than the standard deviation of $\pm$3% in FAA analysis and therefore the preparation by weight was suitable. The above results indicate that mercury(II) acetate salt can be used as a primary standard. All mercury(II) solutions for FAA analysis solutions were prepared by diluting aliquots of this stock solution. Solutions of all other mercury species were standardized by comparison of their FAA response to those of mercury(II) standard solutions of approximately the same concentration. Unless otherwise stated all mercury(II) standard solutions were stored at 0°C in 0.1M perchloric acid to minimize

the loss of mercury(II) by production of mercury(0) which was lost by vaporization from solution or by absorption of mercury(II) on the glass surface.[81]

Mercury(0) Solutions Liquid mercury(0) used in the preparation of solutions was obtained by three different methods with no observable difference caused by the source. The first source of mercury(0) was triply distilled mercury (0) obtained from Bethlehem Apparatus Co. which was used without further purification. The second source of mercury(0) was from the distillation of mercury(0) from mercury(II) oxide. The third source was from dirty laboratory mercury(0) which was pinholed, cleaned with 1M nitric acid, aerated for 14 hours and washed with distilled water. The mercury(0) was then distilled under vacuum with only the middle fraction being retained for use.

Mercury(0) solutions were prepared by two methods. Solutions prepared by the first method were used to measure the solubility of mercury(0) in water in the presence of ionic mercury. The source of ionic mercury in these solutions was the air oxidation of the mercury(0) because oxygen was not excluded from the solution in this method of preparation. In this preparation, two grams of mercury (0) were added to a solution of desired volume which was stirred for five days. Mercury solutions were always stirred by means of glass covered magnetic stirring bars

because teflon and plastic coated bars absorb mercury. In most mercury(0) solutions perchloric or nitric acid was added to prevent hydrolysis of any ionic mercury produced. Chloride ions prevent the disproportionation of mercury(I) present in the samples and therefore must be absent from the final solution. Perchloric acid contains enough chloride to interfere with the disproportionation (10^{-6} to 10^{-7}M) and should be distilled several times saving only the middle fraction for preparing the acid solutions.

The second method was used to prepare oxygen free mercury(0) solutions. Solutions were prepared in both distilled water and 0.1M perchloric acid. These solutions were used to measure the mercury(0) solubility in the absence of other mercury species. A round bottom flask of the desired volume was stoppered by means of a 24/40 joint with an attached three way stopcock. A glass covered stirring bar was added to the flask and the air purged from the flask by flowing nitrogen admitted by a 12 inch syringe needle through the stopcock. After purging five minutes, distilled water or a perchloric acid solution was added by syringe through the stopcock, stirred and purged with nitrogen for an additional 30 minutes. At this time two grams of liquid mercury were added to the flask and the mixture was purged for another 30 minutes. The stopcock was closed with a positive nitrogen pressure in the flask while the solution was equilibrated for five

days with stirring. When samples were withdrawn by syringe, a nitrogen flow was maintained through the stopcock to prevent oxygen from diffusing into the solution.

Mercury(I) Solutions Mercury(I) perchlorate solutions were prepared by two methods. The first method of preparation was used for mercury(I) solutions of general use in the investigation of the disproportionation where it was not required that the mercury(I) concentration remain constant for more than 3 to 4 hours. Due to the presence of mercury(0) and oxygen in these solutions, there was a continual increase of the mercury(I) concentration from air oxidation of the mercury(0). These solutions were standardized just prior to their use. These solutions were prepared by dissolving a weighed quantity of mercury (I) nitrate dihydrate or mercury(II) oxide in 5 ml of concentrated perchloric acid and diluted to volume to give a final concentration of 10^{-5}M mercury in 0.1M perchloric acid. In some cases sulfuric or nitric acid was used to prepare the stock solution. Approximately two grams of liquid mercury were added and the solution was equilibrated for 5 days with stirring. Mercury(0) was added to reduce all mercury(II) to mercury(I), except for about 2% mercury(II) present at equilibrium.[36,39]

The second method of preparation was used in solutions when it was necessary to have oxygen free mercury(I) solutions. Such solutions were needed whenever a slow

reaction of mercury(I) was investigated or when a study was continued for several days that would require the mercury(I) concentration to remain unchanged. These solutions were prepared in a similar manner as the oxygen free preparation of mercury(0) solutions. In this preparation a mercury(II) solution containing 0.01M sodium chloride, 0.1M perchloric acid, or 0.01M sulfuric acid, was syringed through the stopcock and aerated with nitrogen for 30 minutes. The presence of chloride or acid in the solution prevented the loss through volatilization of mercury(0) that otherwise would occur in its absence. A mercury(0) solution free of ionic mercury was specially prepared by purging, with nitrogen, 50 ml of distilled water containing 2 grams of mercury(0) liquid for 30 minutes. In this process the ionic mercury dissolved in the solution and drop of mercury was eventually all converted to mercury(0) and was lost by volatilization from solution. This ionic free solution of mercury(0) or only the mercury (0) liquid was added to the mercury(II) chloride solution by syringe and the mixture aerated for another 30 minutes. Mercury(II) was reduced to mercury(I) by reaction with the mercury(0) and the presence of chloride complexed the mercury(I) preventing any disproportionation of this species. The stopcock was closed and the solution was stirred to equilibrium for 5 days. Because of the disproportionation reaction of mercury(I), the solution at equilibrium will contain about 2% mercury(II).[36,39]

Solutions of lower concentration were prepared by dilution of these concentrated stock solutions and were stored over mercury(0) liquid for at least 24 hours prior to use in order to reverse any disproportionation that had taken place during dilution.

Methylmercury(II) Solutions Solutions of methylmercury(II) chloride were prepared by weighing a known amount of the salt which was dissolved in 5 ml of concentrated perchloric acid. This solution was transferred to a 250 ml volumetric flask and diluted to volume to make a final concentration of 5×10^{-5}M methylmercury(II) in 0.1M perchloric acid. All solutions of lower concentration were made by dilution of this stock solution in 0.1M perchloric acid. Solutions were stored at 0°C in the dark to prevent loss of total mercury concentration or photodecomposition of organomercury.[82]

Phenylmercury(II) Solutions Solutions were prepared by weighing a quantity of phenylmercury(II) acetate which was dissolved in 5 ml of 1M sodium hydroxide. The solutions were transferred to a one liter flask and diluted to volume for a final hydroxide concentration of 0.005M and a phenylmercury(II) concentration of 20ppm. Phenylmercury (II) solutions of 1ppm or higher in concentration were stored in basic media because of solubility problems in acid. Solutions below a concentration of 1ppm were adjusted to 0.1M in acid by addition of a known volume of concentrated

reagent grade acid. Solutions were stored at 0°C in the dark to prevent loss of total mercury concentration or photodecomposition of organomercury.[82]

Mercury analysis by the impinger method The method of analysis used in this study was flameless atomic absorption (FAA).[22,28] Mercury(0) vapor produced in a reduction process was determined with a Laboratory Data Control system which consisted of a model 1235 UV monitor, model 330 recorder, and a mercury aeration vessel(impinger type). The analyzer system is depicted in figure 1. In the impinger system a gas flow through an immersed open ended tube aerates a reducing solution. Mercury vapor was produced in this reduction cell by injection of a known volume of a mercury solution.

The mercury(0) produced in the reduction solution was purged from the solution and carried into the gas cell by circulating air. The mercury vapor was measured by absorption of the 254 nm light in the gas cell; the absorbance value of the gas was recorded on the strip chart recorder. The pen deflection was similar to that response obtained in a gas chromatograph. The peak slope was found to be extremely sharp with a long tail starting at half the peak height. The peak height and area of the tail were found to be dependent on the volume of solution in the aeration cell, the speed of injection, and the volume of the sample injection. Rather than attempt to

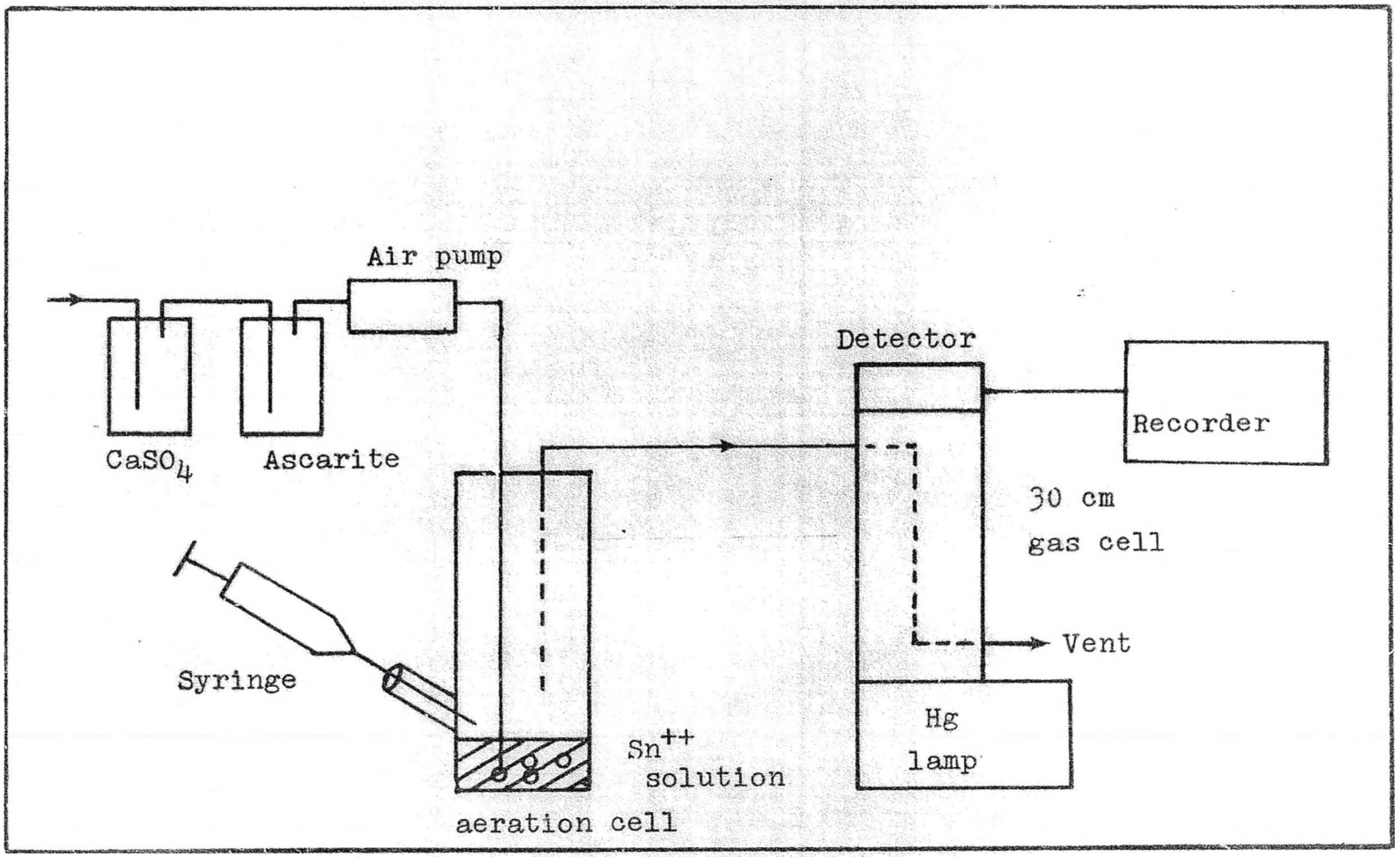


Figure 1 -- Mercury Analysis System

control these variables and solvent conditions so that the peak height could be used for analysis, the concentrations were determined by comparison of the areas under the peaks by cutting and weighing of the chart paper.

The impinger cell was connected to the detection cell using Chromatronix model T 125063 cheminert(1/16th inch i.d.) tubing. No difficulties were experienced with the water vapor background at the mercury levels used with this analysis system. The aeration vessel was cleaned periodically by allowing it to stand overnight in basic peroxide solution in order to obtain a stable baseline. Cleaning was necessary when solutions considerably less concentrated in mercury than the previous levels were to be analyzed.

A constant 920 ml/min air supply filtered through calcium sulfate and ascarite was obtained using a Masterflex model 7540 constant speed air pump fitted with a model 7015 head. Sample injections were made with Plasticpak 5602, 1 ccTB disposable syringes to which were glued 1 or 2 inch Hamilton, N 722, stainless steel needles. Scotch tape was used to cover the markings on the syringes to allow their repeated usage. The syringes were cleaned by repeated rinsing with 0.1M hydrochloric acid solution and distilled water. If the syringe was still contaminated with mercury or if the plunger became sticky, it was discarded. Different syringes were utilized for concentrations that varied by a factor of ten.

The sulfuric acid reducing solution used in the

impinger reaction cell reduces only inorganic mercury to mercury(0).[28] This acid reducing solution was prepared by dissolving ten grams of tin(II) sulfate in 80 ml of water. Eleven ml of concentrated sulfuric acid was added to the solution and the mixture was diluted to 100 ml. This solution was shook well before use due to the precipitation of tin(II) sulfate upon the addition of the sulfuric acid. Ten ml of this solution were added to the aeration vessel initially and the solution was replaced after 15 injections of 0.2 ml samples or an equivalent volume of sample. The reducing mixture in the aeration cell was ready to use as soon as a stable baseline was obtained on the UV absorbance readout.

The impinger analysis procedure described was difficult to use because peak heights and areas were not reproducible from injection to injection. Therefore the analysis train was modified in an attempt to obtain a more reproducible UV response.

An improved method of mercury analysis The newly designed system used in this study was the same as the impinger system except a different type of aeration vessel, a drying tube after the aeration vessel, a gas pressure regulator, and a gas flow regulator were added. Tank gas, nitrogen, was used in place of the circulating pump as the gas source. The new system is shown in figure 2. The carrier gas entered the aeration cell through an 18 mm medium porosity

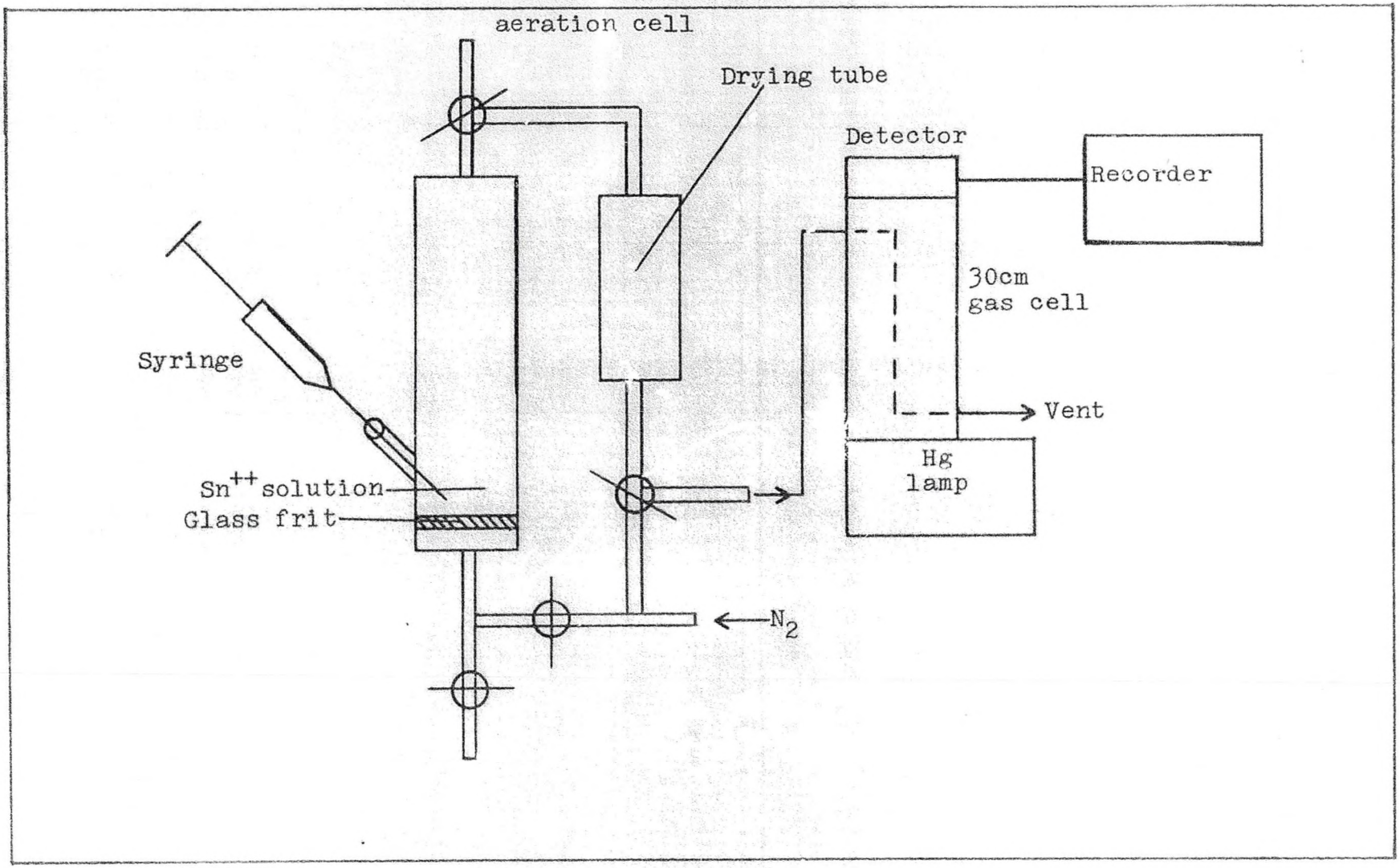


Figure 2 -- Improved Mercury Analysis System

glass frit through which the aeration produced an almost gaussian shaped UV response, increased sensitivity, and allowed the flow to be decreased to 200 ml/min. A typical response is shown in figure 3. The peak height for a sample was found to be insensitive to the speed of injection and to be constant above a flow of 85 ml/min. The peak height was much less sensitive to the volume in the aeration cell so that the peak height was found to be directly proportional to the concentration of mercury. The peak height was reproducible from day to day and allowed a decrease in the amount of reducing solution required (5 ml). Up to ten 0.2 ml samples of mercury were analyzed before replacing the reducing solution. Predried sodium hydroxide was used in the drying tube which also increased sensitivity by removing the background of water vapor which causes light scattering in the gas cell.

The carrier gas used was tank nitrogen at a flow of 200 ml/min. This flow was controlled with the tank regulator set at 30psi, a low pressure regulator set at about 5psi, and a needle valve to control the gas flow within ± 3 ml/min. Flow rates were measured by means of a soap bubble flow meter connected to the end of the analysis gas train.

A hydrochloric acid reducing solution which only reduced inorganic mercury in the sample was used in the new analysis system. The advantage of this solution over the sulfate reducing solution was that all of the tin(II) chloride was soluble while the tin(II) sulfate was not. This solution

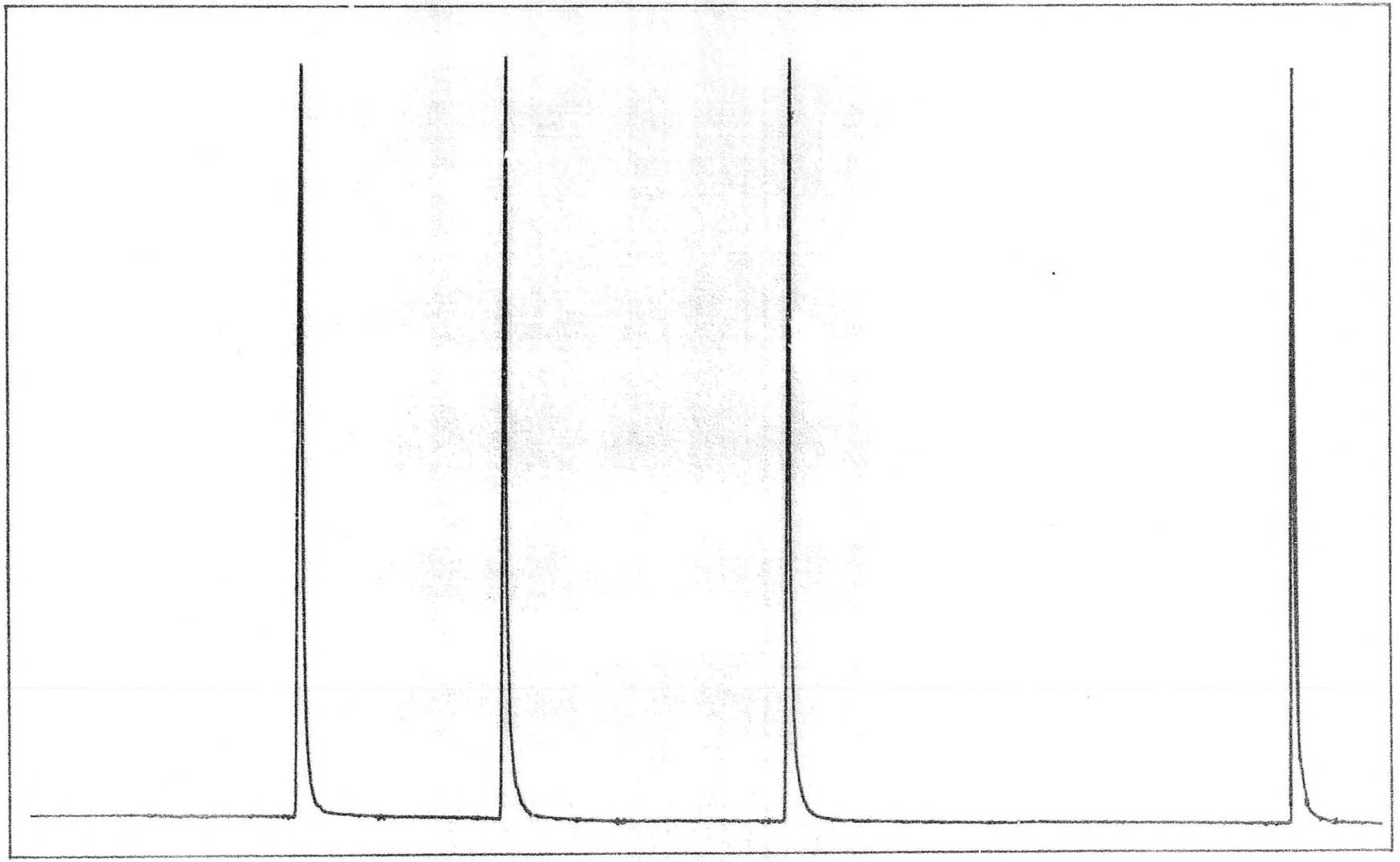


Figure 3 -- Typical UV responses for the reducing analysis of Hg^{+2}

was prepared by dissolving 8.8 grams of tin(II) chloride dihydrate and 2 ml of concentrated hydrochloric acid into water to give a final volume of 100 ml. Five ml of this solution was used for every 10 injections of 0.2 ml or an equivalent volume of sample. Injections of samples were made into this solution as soon as a stable baseline was observed for the absorbance value on the recorder.

Sample injections were made with 5, 2.5, 1, and 0.5 ml syringes. The size of the injections were varied with the concentration of the sample involved. The minimum detectable quantity of mercury was 2×10^{-9} grams and the maximum detectable quantity was 4×10^{-7} grams at which point the absorbance became nonlinear. Injections of highly concentrated mercury solutions were made with a 50 µl Hamilton gas tight syringe, model 1710N, to lower the quantity of mercury analyzed to fall within the linear detection range. The majority of the syringes used were of the polyethylene type described earlier with an occasional glass syringe used to check for possible interference problems from the polyethylene. Extreme care was taken not to contaminate the solution being analyzed by transfer of the reducing agent on the needle of the injection syringe. One syringe was used only for sampling of the unknown solution and delivering that sample into a clean test tube without touching the sides or bottom. A second syringe was then used to remove a known volume of the sample from the test tube for injection into the

aeration cell.

The drying tube in the new system was filled with predried sodium hydroxide, held by cotton plugs on each end. Cotton was used because it collected the water aerosol in the gas stream more efficiently than glass wool. The tube was washed, dried, and refilled every 4 hours during operation. The sodium hydroxide was prepared by drying at 115° for 2 days. The pellets were stirred every 15 minutes for the first four hours of drying to prevent formation of a single large lump. Before cooling, the pellets were stored in a tightly capped bottle to prevent readsorption of water vapor. The effectiveness of the sodium hydroxide was evaluated in the new analysis system by performing a set of analyses with and without the drying tube. The analyses were performed by injection of 0.2 ml of 20.8ppb mercury(II) acetate in 0.1M perchloric acid into 5 ml of the hydrochloric acid reducing solution. The UV response was determined by measurement of the area under each analysis peak by cutting and weighing. The results depicted in table 1 show that the drying tube gives a slight increase in sensitivity as well as making the response more reproducible.

The reaction cell was cleaned by first washing out the majority of the reducing solution with water. The glass frit was then cleaned by slowly filtering 10 ml of concentrated nitric acid which was followed by washing with distilled water to remove the remaining acid from the frit.

TABLE 1

EFFECT OF THE NaOH DRYING TUBE ON THE INSTRUMENT RESPONSE[a]

DRYING TUBE	GRAMS PAPER[b]	% RESPONSE[c]
without	0.2271	85.0
	0.2609	98.0
	0.2501	93.9
	0.2497	93.8
with	0.2720	102.0
	0.2662	100.0
	0.2663	100.0

[a] These values were obtained by injection of 0.2 ml of 21 ppb mercury(II) into the impinger aeration cell containing ten ml of the tin(II) chloride-hydrochloric acid reducing solution.

[b] These values were obtained by cutting out the peaks and weighing them.

[c] These values were obtained by defining 0.2662 grams as 100% for comparison purposes.

The aeration cell was then filled with 0.1M sodium hydroxide which was slowly filtered. Finally, the frit was washed with water to remove the remaining base. If after this cleaning, the aeration cell did not produce a stable baseline in the analysis procedure or if the frit was clogged, a concentrated solution of sodium hydroxide and hydrogen peroxide was used for additional washing. This solution was prepared by mixing 10 ml of 30% hydrogen peroxide, 10 ml of 14M sodium hydroxide, and 30 ml of water. This cleaning method was used sparingly because it will dissolve enough of the glass from the frit to cause it to crumble and fall out. This solution was washed out of the frit with distilled water as soon as all of it was filtered to minimize the dissolution of the glass frit. Gravity flow in all cases, except for water washes, was used to allow sufficient contact time for the cleaning process. Other methods of cleaning were investigated but were found to create a large amount of noise in the baseline.

Procedural parameters affecting the mercury analysis In an attempt to develop a reproducible method of analysis, those factors that effect the response of the system were investigated; such factors as the aeration cell volume, sample volume, gas flow rate, and mercury concentration were studied.

The first parameter investigated was the effect of

gas flow rate on the peak height of the absorbance for a given sample. This study was performed at flow rates of 50 to 100 ml/min by injection of a series of 0.2 ml sample aliquots of 21ppb mercury(II) acetate solution in 0.1M perchloric acid into the aeration cell containing 5 ml of the hydrochloric acid reducing solution. The peak height response of the UV monitor shown in figure 4 indicated that above a flow of 85 ml/min the response became constant for a constant quantity of mercury injected. A flow rate of 200 ml/min was used as the standard analysis condition because the amount of tail of the peak was reduced and the peak height not effected which allowed a shorter analysis time.

The hydrochloric acid and basic reducing solutions were used interchangeably for the analysis of inorganic mercury in this study because no difference was observed between the use of the two reducing solutions. The analysis of 100ppb mercury(II) solution in 0.01M sodium chloride in the basic reducing mixture gave an average value for ten injections of 55 ± 3 compared to 56 ± 2 for the analysis in the hydrochloric acid reducing solution for 5 injections. These analyses are the same value within the random experimental error of a single injection. The difference between the two reducing solutions was that the basic mixture reduces the organic forms of mercury where as the acid solution does not.

The basic reducing solution was prepared in two parts

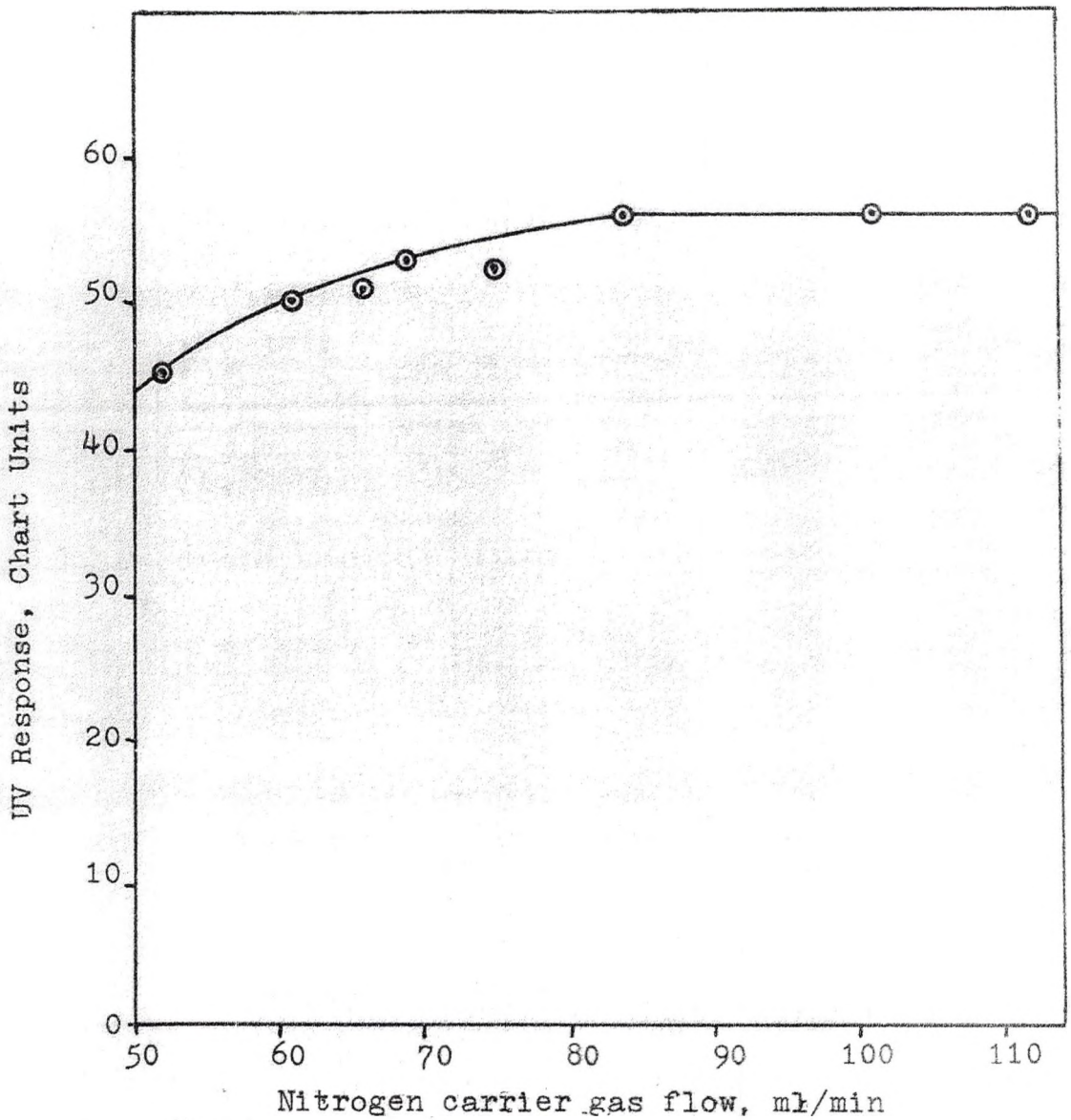


Figure 4 -- Effect of the carrier gas flow rate on the UV response

because metallic tin or cadmium precipitate out of solution when mixed for several hours prior to the analysis. The first solution was prepared by dissolving 8.8 grams of tin(II) chloride dihydrate in water and diluting to 100ml. The second solution was prepared by dissolving 5 grams of cadmium(II) chloride in a minimum amount of water. To this solution was added 56 grams of sodium hydroxide and sufficient water to make 100 ml volume. A great deal of cooling was needed during the dissolution of the sodium hydroxide before and during dilution to 100 ml volume. Cadmium(II) hydroxide precipitated on the addition of the sodium hydroxide requiring the solution be shook well before use. Equal portions of the two solutions were added to the aeration cell by syringe for the analysis. Both solutions were injected individually with a single syringe, which was cleaned with distilled water between injections to prevent the formation of precipitate in the needle. An 18 gauge needle was recommended because of the viscosity of the basic solution. Five ml total volume of this basic reducing mixture was used in the aeration cell when more than 6 analyses were made and only 2 ml when less than 6 analyses were performed.

The second parameter investigated was the effect of the volume of the solution in the aeration cell on the response of the UV peak. This study was performed by injection of a series of 0.2 ml sample aliquots of a 104ppb mercury(II) chloride solution in 0.01M sodium chloride

in o the aeration cell containing 2 ml of the basic reducing sol tion. After each injection of a mercury sample, one ml of w ater was added to the aeration cell to increase the volu e of solution in the aeration cell. The peak height respo se on the UV monitor as a function of volume in the aerat on cell is depicted in figure 5. As shown in figure 5, the peak height decreased with increasing volume in the ae ation cell indicating the need for replacing the reduci g solution after a total sample volume of 2 ml has been in roduced into the cell. Within this limit, the variati n of response is within the random error of ±3% for a s ngle injection at constant volume for this method. The ran om error will be discussed in the error analysis section

Th third parameter investigated was the effect of the sam le volume on the response of the analysis system. This st dy was performed by injecting 0.1 to 0.6 ml aliquots of a st ndard mercury(I) solution into 2 ml of a basic reducing solution in the aeration cell. The peak height respons e of the UV monitor is shown in figure 6. The solid line i the experimental value and the dashed line is the expect d response if no line broadening occured which would lower the peak height. As shown, the response begins to decre se when injections of larger than 0.4 ml are made. Samp es larger than 0.4 ml are not recommended for this type of continuous flow analysis.

The last parameter to be investigated was the response

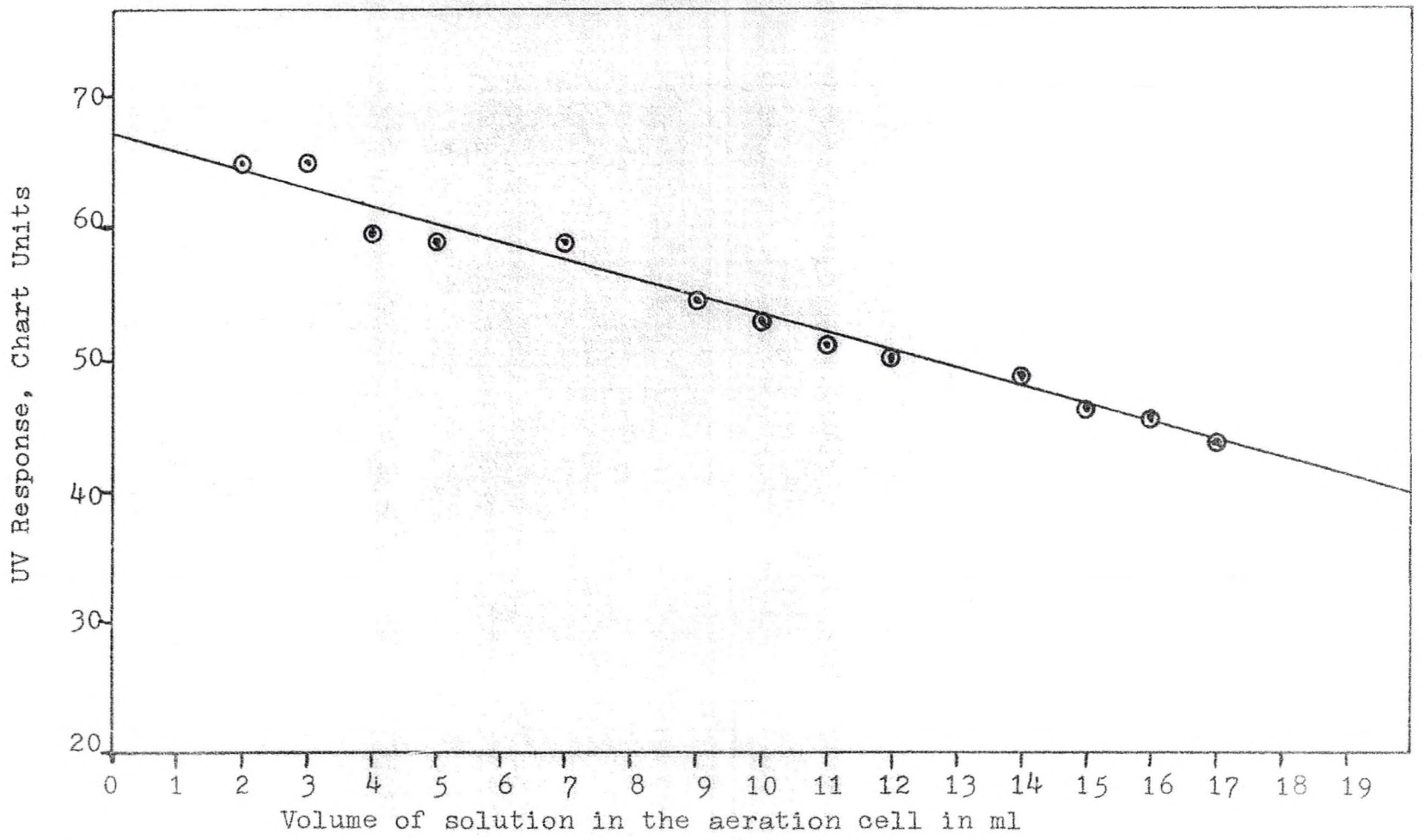


Figure 5 -- Effect of solution volume in the aeration cell on the peak height

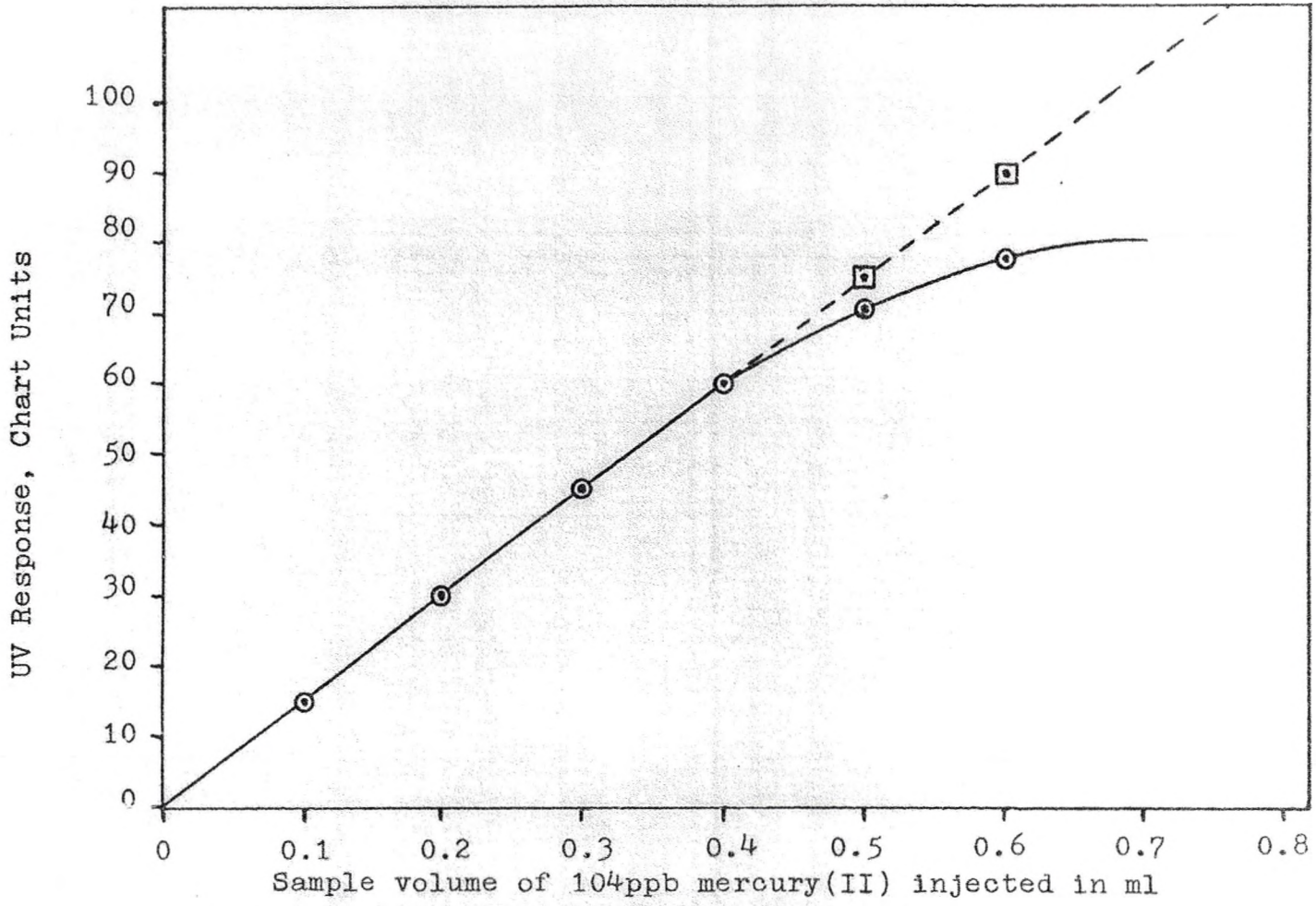


Figure 6 -- Effect of sample volume injected on the peak height

of the analysis system as a function of mercury concentration. In this study, 0.2 ml aliquots of mercury(II) samples of varing concentrations were injected into the aeration cell containing 5 ml of the hydrochloric acid reducing solution and the response recorded on the strip chart recorder. Figure 7 indicates that Beer's law was followed in the sample range from 1 to 7 micrograms of mercury. As indicated from the previous discussion, the flow rate was held constant at 200 ml/min and no cumulative addition of sample greater than 2 ml was allowed. In the continuous flow method, 0.5 microgram sample analysis is the limit of detectability (signal = 2 X noise). The upper limit of detection was determined by injection of a series of 0.2 ml mercury(II) samples of increasing concentration. This limit was found to be 40 micrograms above which the UV response no longer followed Beer's law.

The standard set of conditions selected for use for most analyses was 0.2 ml sample injection volume, 200 ml/min nitrogen flow rate, a 5 ml reducing media volume, no cumulative volume additions greater than 2 ml in the cell were allowed, and sample injections were limited to 0.5 to 40 micrograms of mercury per injection.

Mercury analysis by amalgamation The lower limit of detectability of 0.5 micrograms for the continuous method ofanalysis was not sufficiently sensitive for many environmental samples. One method of increasing the

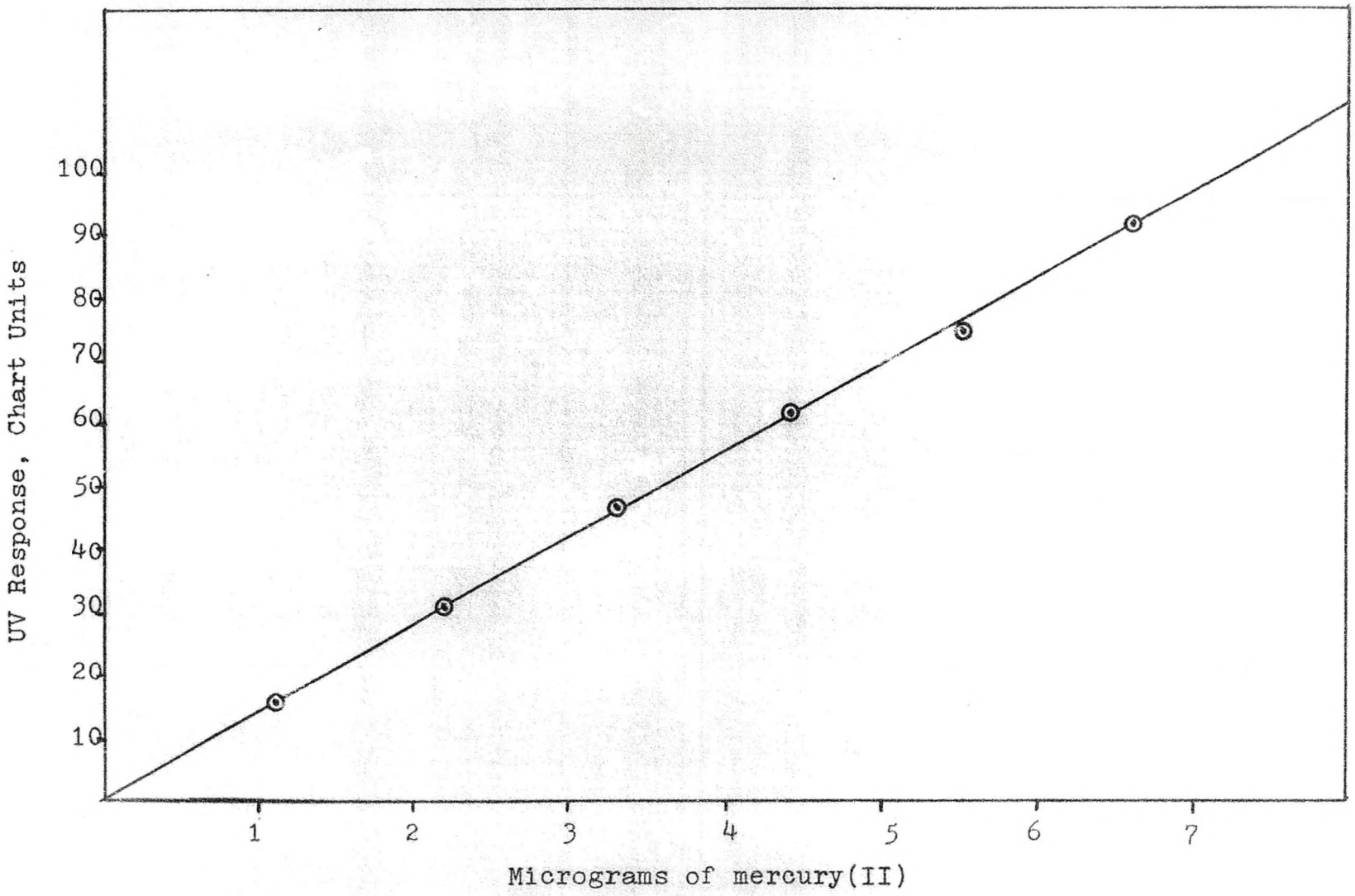


Figure 7 -- Beer's law plot for continuous flow method

sensitivity was to concentrate the sample prior to its detection.[83-89] This was accomplished by placing a tube containing gold foil between the aeration cell and the gas cell to collect the mercury(0) vapor by amalgamation from the gas stream. A flow of 200 ml/min was used in this method of study. The mercury(0) was first concentrated on the gold and then released by resitance heating for UV analysis. The tube was prepared with three grams of shredded gold placed in the middle of a 6mm by 24cm quartz tube wrapped with a nichrome resistance heater. During the heating process 28 volts were applied to the 1.8 ohm 22 gauge nichrome resistance wire. This heating vaporized the collected mercury in less than 30 seconds. The absorbance of the vaporized mercury was then measured in the 30cm gas detection cell. The gold in the tube was cleaned by repeated heating and cooling cycles until a blank UV absorbance value obtained on the heating cycle became constant. The tube required six minutes to cool after the heating cycle before collection of another sample was started. Low blank values were obtained by stopping the nitrogen flow over the gold during the cooling cycle. The flow was restored at the beginning of the collection of the sample and stopped when the heating was ended. In an analysis cycle, a sample or blank solution was injected into the aeration cell and the mercury(0) produced was collected for three minutes on the gold foil. The foil was then heated and the mercury absorbance of the mercury

vapor in the gas train was measured in the UV cell. The response cycle from baseline to peak to baseline was complete in two minutes. The data was analyzed by subtracting an average blank value obtained in a separate analysis from the peak height obtained from a sample containing mercury. This peak height was directly proportional to the amount of mercury injected into the aeration cell. The average blank value was the mean of the pre- and post analysis peak heights obtained when a sample containing no mercury was injected into the aeration cell. Table 2 indicates the blank response relative to a typical response for 4 micrograms of mercury. In most cases the pre- and post blank heights were the same value if enough time was allowed for all of the mercury to be reduced in the sample cycle. Table 2 shows that a three minute collection time was sufficient for vaporization of all the mercury. Long collection times were not desirable because the blank values increase with the collection time.

A series of standard mercury(II) samples were analyzed by the gold amalgamation method to determine if Beer's law was followed in the concentration ranges studied. The analyses were performed by injection of 3 ml sample aliquots into the basic reducing aeration cell run under the standard conditions with 3 minute sample collection times. Due to the large sample volume, the reducing agent was replaced after each injection. Figure 8 shows that a linear response of the UV detector was observed for 3 ml injections of

TABLE 2

RESPONSE IN CHART UNITS FOR A 4 MICROGRAM MERCURY(II) SAMPLE FOR VARIOUS COLLECTION TIMES[a]

TIME	BLANK[b]	RESPONSE IN CHART UNITS	
		SAMPLE	(SAMPLE - BLANK)[c]
6 min.	22	66	44
3 min.	17	57	40
2 min.	8	50	42
1 min.	5	42	37
0.5 min.	3	9	6

[a] The aeration cell was run at 200 ml/min nitrogen flow by the gold amalgamation method. The mercury was removed from the gold by heating. The sample used was 2ppb mercury (II) in 0.01M NaCl with 0.2 ml being injected.

[b] The blank was a collection for the stated time with a blank sample injected.

[c] The values of 44, 40, and 42 are the same within the experimental error.

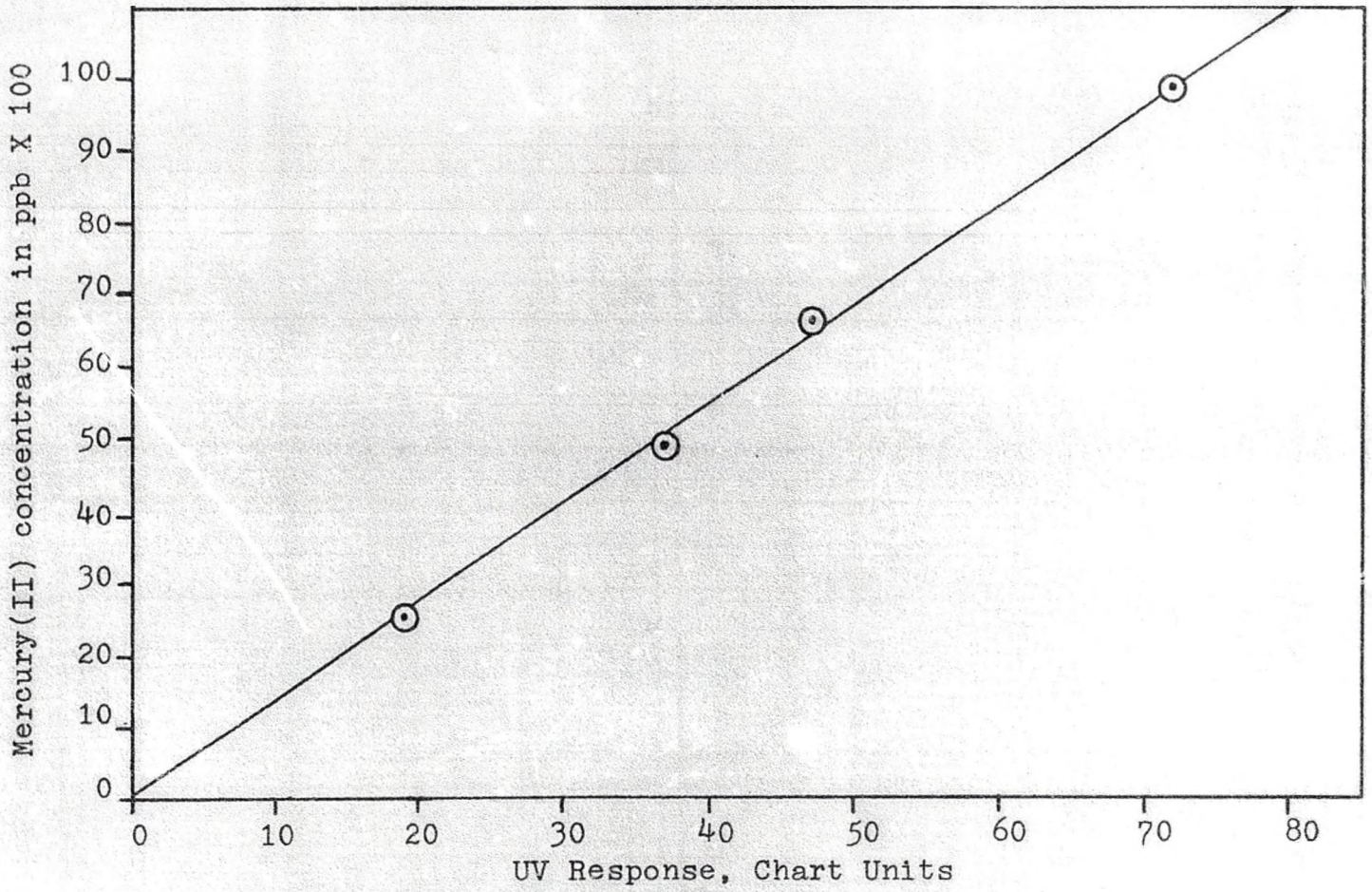


Figure 8 -- Beer's law plot for the gold amalgamation method

varing mercury(II) concentration in solutions from 0.2 up to 1ppb. The minimum amount of mercury analyzed by both the gold amalgamation and the continuous flow method was 0.5 micrograms of mercury. The difference between the two methods was that the gold allows larger injection volumes such as 3 ml compared to 0.2 ml for the other technique. Therefore, the gold method was over 25 times more sensitive for mercury analysis because a larger sample volume can be handled.

Error analysis The standard deviations were determined for both the continuous flow method and the gold amalgamation method of analysis. Both types of analyses were performed by injection of ten 0.2 ml sample aliquots of a freshly prepared 42ppb mercury(II) solution in 0.01M sodium chloride into the aeration cell containing 2 ml of the basic reducing solution. The aeration cell was run under the standard conditions, except for the reaction volume (2 ml), and a three minute collection time was used for the amalgamation method. The data shown in table 3 indicates the error at the 99.7% confidence level (3 sigma) for both methods was less than $\pm 3\%$. It should be noted that the peak height for the gold amalgamation analysis was 4 times that of the continuous flow analysis method for the same mercury(II) solution with similar sigma value.

Analysis of mercury(0) in the presence or absence of mercury(I)

TABLE 3

RESPONSE IN CHART UNITS FOR THE GOLD AMALGAMATION AND CONTINUOUS FLOW METHODS[a]

INSTRUMENT RESPONSE, CHART UNITS[b]	
CONTINUOUS FLOW	GOLD AMALGAMATION
76	76
75	74.2
74	74.2
75.3	73
76	74
75.3	73.7
76	74.4
75.2	73.1
72.9	73.8
76.3	73.2
sigma = 0.964	sigma = 0.814

[a] These values were determined by injection of 0.2 ml sample aliquots of a 42ppb mercury(II) solution in 0.01M NaCl into the aeration cell containing 2 ml of the basic reducing mixture.

[b] The readings were taken on two different attenuator settings on the recorder, the gold readings were 4 times those of the continuous flow method.

Mercury(0) was measured in the presence of mercury(I) and 0.01M sodium chloride by vaporization from an aeration cell containing 0.1M perchloric acid and no reducing agent. Mercury(I) disproportionates to form mercury(0) and mercury (II). Chloride ion prevents the disproportionation of mercury(I), thus no mercury(0) was produced from mercury (I). The analysis was performed by injection of 0.2 ml sample aliquots of a mixture of mercury(0) and mercury(I) into the aeration cell containing 5 ml of 0.1M perchloric acid in place of any reducing solution. The cell was run under standard flow conditions in which the mercury(0) present and that formed from disproportionation of mercury (I) were vaporized. Samples containing mercury(I) and mercury(0) were prepared both in the presence and absence of chloride to demonstrate that an analysis larger than the mercury(0) solubility was obtained when no chloride was used. Chloride slows the rate of the disproportionation reaction allowing the analysis of only the mercury(0) in the time frame of the analysis. Extreme care was taken not to contaminate the aeration cell with any type of reducing agent that would reduce the other forms of mercury present. In this regard the syringe in this analysis was never allowed to contact the reducing agents used in any of the other methods. The aeration cell was cleaned with distilled water in most cases. If the frit in the aeration cell became clogged or contaminated, the previously described basic peroxide solution was used for cleaning.

The water aeration cell was calibrated by comparison of the peak height response in the UV detector with that of the basic reducing aeration cell for the injection of 0.2 ml aliquots of 45ppb mercury(0) solution. The solution was prepared under oxygen free conditions and equilibrated for 10 days at 21°C. The aeration cells were run under standard flow conditions with the reducing cell containing 2 ml of the basic reducing mixture and the water aeration cell containing 4 ml of 0.1M perchloric acid. The response in chart units was 49 ±2 and 49 ±2 for six analyses performed by each method. Since the mercury(0) stock solution contained no ionic mercury, the results indicate the non-reducing cell can be used for mercury(0) analysis. This also indicates that the calibration curve used for the reducing media can be directly used for the response of the water aeration cell for the analysis of the mercury(0) concentration.

The comparison of peak heights was not an absolute comparison of the concentrations of the mercury(0) analysis in each analysis method because line broadening could occur causing a lowering of the peak height. To confirm that the comparison of the peak heights was valid for the two analysis methods for mercury(0), a series of analyses were performed by the gold method. The analyses were carried out by injection of a 0.2 ml sample of a saturated mercury (0) solution equilibrated at 25°C under oxygen free conditions into each type of aeration cell, non-reducing and the reducing cell. The vaporized mercury was collected

for 3 minutes on gold foil as in the amalgamation method previously described. The mercury was then analyzed in the gas cell as by the normal amalgamation method. The value obtained for the non-reducing analysis was 63 ±2ppb compared to 65 ±2ppb for the reducing analysis for an average of three injections into each aeration cell. These analyses values are the same within the experimental error and compare favorably to the literature value for the mercury(0) solubility of 63ppb.[37] The results show that the non-reducing cell vaporization of mercury(0) gives exactly the same peak response as does a reducing cell. Therefore, a calibration curve for the reducing method can be used for calibration of the non-reducing method.

The effect of oxygen was studied on mercury(0) standard solutions during the storage of these solutions. For this study two mercury(0) solutions were prepared in the presence of air and under nitrogen in 0.01M sodium chloride as described earlier. These solutions were analyzed by injection of 0.2 ml sample aliquots into the water and basic reducing aeration cells. The difference in the analyses was the amount of ionic mercury in the sample. The results shown in table 4 indicate that the solution prepared under the oxygen free method contained only mercury(0) but the solution prepared in air contained a high ionic mercury concentration as well as the mercury(0). The air oxidation of the mercury(0) in the sample was the source of ionic mercury. Due to these results, solutions that are to be

prepared to contain only mercury(0) should be prepared and stored under oxygen free conditions. The analysis values for the mercury(0) in table 4 and 5 are within the experimental error of ±3% of the literature value for the solubility of mercury(0) confirming the accuracy of the method of analysis.[37]

Table 4 indicates the uncertainty of the analysis of a series of injections of the same sample. Table 5 shows the analyses of a series of different samples of mercury(0) dissolved in water and prepared in air. This table indicates that even though different amounts of mercury(I) were present, the same solubility value of mercury(0) was measured by the non-reducing method. It is also clear from table 4 that chloride ion prevents the disproportionation of the mercury(I) in the solution prepared under air.

Differential analysis of organic and inorganic mercury

Organomercury cations such as methylmercury(II) and phenylmercury(II) were determined in the presence of inorganic mercury by two methods. Both methods depend on the fact that the organomercury forms are not reduced in acidic tin(II) media.[33,34] In one method, a preoxidation step was used to oxidize organomercury cations to mercury (II) in one portion of a sample. This was accomplished by adding 6 ml of 30% hydrogen peroxide and 1 ml of concentrated perchloric acid for every 100 ml of the sample containing organomercury compounds. The oxidation required 20 hours

TABLE 4

ANALYSIS OF SATURATED MERCURY(0) SOLUTIONS AT 25°C BY THE REDUCING AND NON-REDUCING CONDITIONS.[a]

	RESPONSE, CHART UNITS[b]		
SOLUTION PREPARATION	REDUCING CONDITION[c]	NON-REDUCING CONDITION[d]	Hg(0), ppb[e]
airless	66 ±2	64 ±4	62
air	191 ±2[f]	64 ±1	61

[a] These solutions were prepared in 0.01M NaCl.

[b] All values given in the table were based on not less than three analyses of each solution.

[c] These values were determined in 2 ml of basic reducing solution.

[d] These values were determined in 5 ml of 0.1M $HClO_4$.

[e] The literature value for the Hg(0) solubility is 63ppb at 25°C, reference 37.

[f] This value corresponds to 184ppb.

TABLE 5

ANALYSIS OF SATURATED MERCURY(0) SOLUTIONS PREPARED IN AIR AT 25°C BY THE USE OF REDUCING AND NON-REDUCING CONDITIONS.[a]

REDUCING ANALYSIS[b]		NON-REDUCING ANALYSIS[c]	
ppb	CHART UNITS	ppb[d]	CHART UNITS
217	224	61.2	63.5
184	191	61.2	63.5
93	96.6	59.0	61.3
105	109	55.6	55.7
145	150	60.0	62.3

[a] These solutions were prepared in air in 0.01M NaCl.

[b] These values were determined by injection of 0.2 ml sample aliquots of the mercury(0) solution into the aeration cell containing 2 ml of the basic reducing solution.

[c] These values were determined by injection of 0.2 ml sample aliquots into the aeration cell containing 5 ml of 0.1 ml of perchloric acid.

[d] The literature value for the mercury(0) solubility at 25°C is 63ppb, reference 37.

contact time at room temperature. The actual analysis of mercury in this solution was carried out in an aeration cell using a hydrochloric acid tin(II) chloride reducing solution. The step measured the total mercury content, so a second analysis of an unoxidized sample was performed in the same cell. The difference between the two quantities of mercury is equal to the organomercury concentration. For example, in a typical analysis a methylmercury(II) solution gave a response of 71 chart units when preoxidized compared to no detectable change in the UV baseline when analyzed without the preoxidation step.

The main disadvantage of the peroxide decomposition method was the 20 hours oxidation step. To eliminate the preoxidation time, a basic reducing solution was used which reduced all forms of mercury upon injection into the aeration cell. In this type of analysis and differentiation of the organic and inorganic mercury species, the sample was divided into two portions. The first portion was analyzed in the hydrochloric acid reducing solution to determine the inorganic mercury content. The second sample was analyzed by injection into a basic reducing mixture for measurement of the total mercury content. The difference in the quantities of these two analyses was equal to the organomercury content. By connecting in parallel two aeration cells to the nitrogen carrier gas supply, each cell can be used alternately for the two analyses allowing a total analysis time of less than three minutes.

To demonstrate that the basic reducing mixture gave the same analysis for a methylmercury(II) solution as the preoxidation-acid reducing method, analyses were performed by both methods on an identical methylmercury(II) solution. For these analyses, two 57ppb methylmercury(II) chloride solutions in 1M hydrochloric acid were prepared. One of these solutions was decomposed by the peroxide method and analyzed by injection of 0.2 ml sample aliquots into the aeration cell containing 5 ml of the hydrochloric acid reducing solution. The second methylmercury(II) solution was analyzed by direct injection of 0.2 ml sample aliquots into the aeration cell containing 5 ml of the basic reducing solution. Both analyses gave the same peak height response of 71 chart units demonstrating the ability of the basic reducing solution to reduce the organomercury forms without the need of the preoxidation step.

Ions interfering with the reduction methods In natural water samples there may be ions present that will complex mercury species which could slow down or prevent the reduction process in the analysis. To investigate this possibility a series of 104ppb mercury(II) solutions were prepared containing possible interfering anions. These solutions were analyzed in the acid and basic aeration cells run under standard conditions containing 5 ml of the reducing agents. The peak height response on the UV detector for a 0.2 ml injection of each of the solutions

was observed and the percent interference calculated. The percent interference was equal to the decrease in the peak height response of the solution containing the interfering ion divided by the peak height response for a solution containing no interfering ion. The data shown in table 6 points out that several ions do interfere with the reduction process with lower amount of interference seen in the basic reducing solution. The interference was thought to have been caused by the change in the reduction potentials upon complexation. Examples of the change in the reduction potential is shown in the reactions[45]

$$Hg^{+2} + 2e = Hg \qquad E^{\circ} = +0.854V, \qquad (19)$$

$$HgCl_4 + 2e = Hg + 4Cl^- \qquad +0.48, \qquad (20)$$

$$HgBr_4 + 2e = Hg + 4Br^- \qquad +0.21, \qquad (21)$$

$$HgI_4 + 2e = Hg + 4I^- \qquad -0.04, \text{ and} \qquad (22)$$

$$HgS + 2e = Hg + S^{=} \qquad -0.75 \qquad (23).$$

The trend followed by the reduction potentials is also followed by the formation constants as shown in the reactions[45]

$$HgCl_4^{=} = Hg^{+2} + 4Cl^- \qquad K = 8.3 \times 10^{-16}, \qquad (24)$$

$$HgBr_4^{=} = Hg^{+2} + 4Br^- \qquad 2.3 \times 10^{-22}, \qquad (25)$$

$$HgI_4^{=} = Hg^{+2} + 4I^- \qquad 5.3 \times 10^{-31}, \text{ and} \qquad (26)$$

TABLE 6

INTERFERENCES IN ACID AND BASIC REDUCING SOLUTIONS[a]

% INTERFERENCE[b]			
ACID[c]	BASIC[d]	COMPOUND	CONC.
—	27	Na_2S	0.0001M
100	10	$Na_2S_2O_3$	0.04M
100	30	Cysteine	0.04M
—	23	Cysteine	0.0001M
100	30	NaBr	0.1M
—	0	NaBr	0.0001M
100	0	NaI	0.16M
50	0	Na_2SO_3	0.04M
0	0	NaCl	4.0M

[a] These values were determined with a solution of 104ppb mercury(II) with the concentration of other ions given in the above table. Two-tenths of a ml were injected into the aeration cell containing 5 ml of the reducing solution.

[b] The percent interference was calculated from the decrease in response of a standard solution when the interfering anion was added.

[c] This solution is made up of $SnCl_2$ and HCl.

[d] This solution is made up of $SnCl_2$, $CdCl_2$ and NaOH.

$$HgS = Hg^{+2} + S^{-2} \quad K_{sp} = 16 \times 10^{-54} \quad (27).$$

The largest formation constant and the most negative reduction potential were with the sulfide ion. This ion should be expected to give the largest amount of interference which was seen in table 6. The lower amount of interference seen in the basic reducing mixture was due to the shift of the reducing potential of tin(II) in basic solution. As shown in reactions[45]

$$Sn^{+4} + 2e = Sn^{+2} \quad E^{o} = +0.15V \text{ and} \quad (28)$$

$$Sn(OH)_6^{+4} + 2e = HSnO_2^{-} + 3OH^{-} + H_2O \quad E_B^{o} = -0.93 \quad (29)$$

tin(II) has a larger oxidation potential in basic solution. This makes the reduction of the complexed mercury(II) more thermodynamically favorable in basic media than in acid. Table 6 indicates this point quite clearly. However, the reduction of mercury(II) in sulfide and sulfonyl containing solutions was still not quantitative. This may be due to a broadening of the peak and thus lowering the peak height response. One possible approach for the problem of sulfide ion in solution would be to prepare a set of standard solutions of mercury(II) for calibration that would contain the same concentration of sulfide ion as the samples. The concentration of sulfide ion in the samples could be determined by addition of a standard mercury(II) sample. The amount of the decrease in

the analysis response of the mercury(II) solution would be proportional to the sulfide ion concentration. This analysis method has not been investigated but should work.

Stability of methylmercury(II) chloride in hydrochloric acid The stability of methylmercury(II) in 1M hydrochloric acid was determined to see if chloride ion could be used in separation procedures involving this cation. For this determination a solution of 22ppb methylmercury(II) chloride in 1M hydrochloric acid was prepared and the inorganic mercury formation was measured as a function of time. This analysis was performed in the impinger analyzer run under standard conditions containing sulfuric acid reducing solution which only reduces inorganic mercury. The only source of inorganic mercury in the sample was from the decomposition of the methylmercury(II) sample. An identical methylmercury(II) sample prepared in 0.1M perchloric acid was decomposed by the peroxide method, and analyzed to define the total concentration of the sample. The samples were equilibrated at 50°C and a pH of 3.7 adjusted with perchloric acid. The peroxide decomposed sample gave a response of 0 units prior to decomposition and 63 chart units after oxidation. An aliquot of the methylmercury(II) solution in 1M hydrochloric acid which was not preoxidized with hydrogen peroxide gave a response of 4 chart units initially and 13 units after 6 days equilibration at 50°C. The amount of decomposition was about 2% per day. Therefore,

the amount of decomposition of a sample at 25°C would be unimportant because the time required for an ion exchange analysis was about 2 hours. Solutions of methylmercury (II) were prepared daily because of the slow decomposition. Other acid conditions were not studied because no problems with decomposition of the samples were experienced in the use of these solutions.

Mercury(II) standard solution storage Several methods of storing mercury(II) solutions were investigated, as well as several types of containers, to determine the solution conditions and the period of time for which a mercury(II) solution could be used as a standard. This study was carried out in several parts. In the first part of this study, four 170ppb mercury(II) solutions were prepared in 1, .1, .01, .001M sodium chloride at pH 2 adjusted with perchloric acid and stored at room temperature. Samples of these solutions were analyzed on consecutive days by injection into an aeration cell run under the standard conditions containing 2 ml of the basic reducing mixture. The results shown in figure 9 indicate that for 200 days there was no change of mercury(II) concentration within experimental error for the 0.1 and 0.01M sodium chloride solutions. The solutions of 1M and 0.001M sodium chloride increased rapidly in concentration for the first 50 days. The above data seems to indicate an optimum chloride concentration for the storage of mercury(II). Because

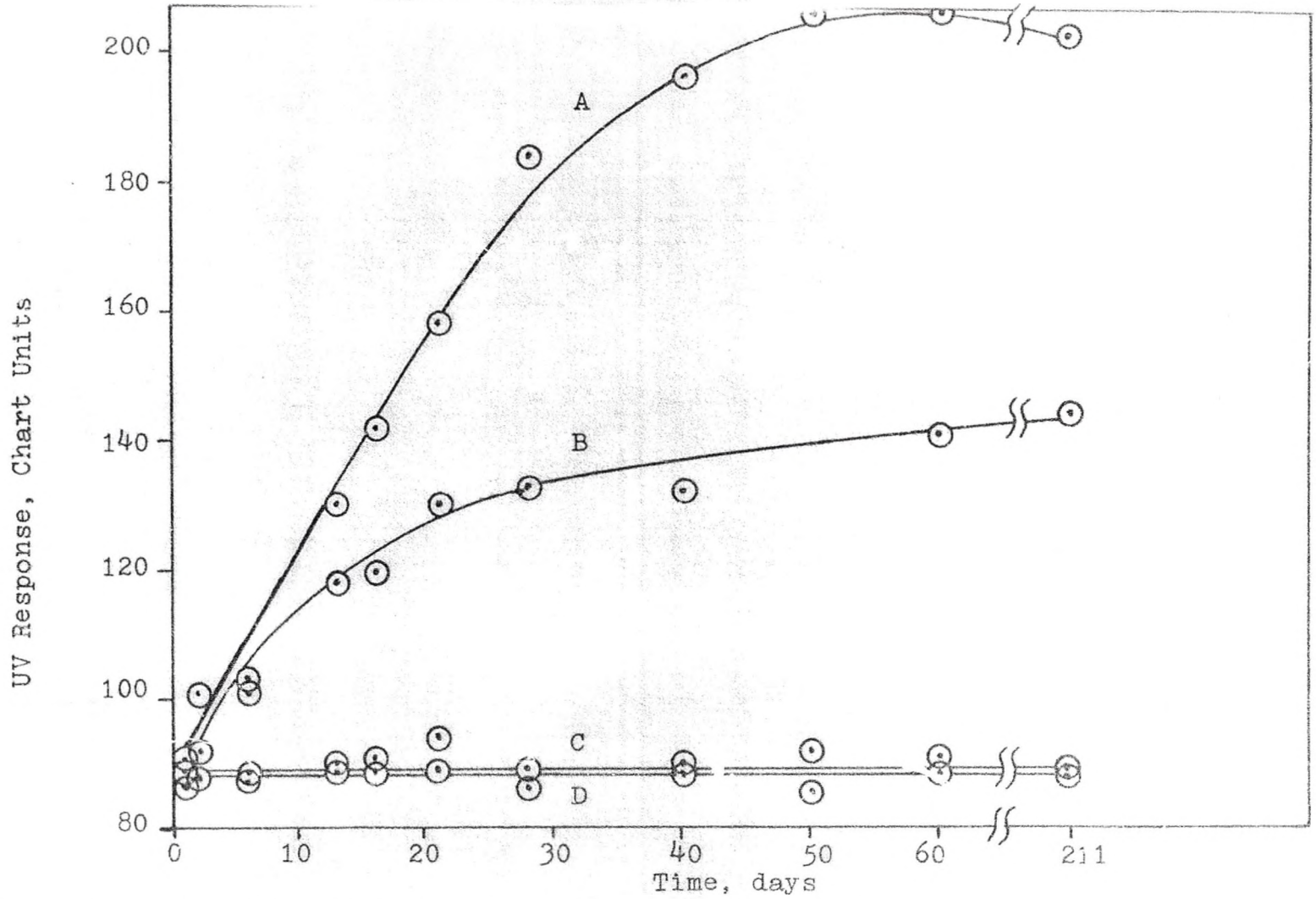


Figure 9 -- Variation of mercury(II) concentration during storage
(A) 0.001M NaCl pH 2, (B) 1M NaCl pH 2, (C) .1M NaCl pH 1.9, (D) 0.01M NaCl pH 2

of the above variation several more solutions were investigated in which the chloride, and hydrogen ion concentrations were studied. The solutions were analyzed as above with the results shown in figure 10. As seen in figure 10, very narrow limits of about 10^{-2}M chloride and 10^{-2}M hydrogen ion seem to be needed to store mercury(II) in solution without a change in concentration.

The mercury concentration in figures 9 and 10 showed no consistent pattern as the chloride concentration was varied. One possible source of this mercury is from the surface of the glass. To investigate this possibility a 0.04M sodium chloride solution was added to a mercury(II) contaminated flask which has previously been used to store 4ppm mercury(II) solution for several days. The mercury(II) concentration in the chloride solution was analyzed as a function of time for up to seven hours by injection of 0.2 ml sample aliquots into the aeration cell containing 5 ml of the sulfuric acid reducing solution. The results shown in figure 11 indicate the extremely fast rate of leaching of the mercury(II) from the surface of the glass into the 0.04M chloride solution. Due to these results, a washing procedure using dilute chloride solutions was used to remove mercury contamination from the surface of glassware by repeated soakings of fresh 0.001M sodium chloride solutions at pH 2.

Other types of containers were investigated to see if mercury(II) solutions could be stored more efficiently

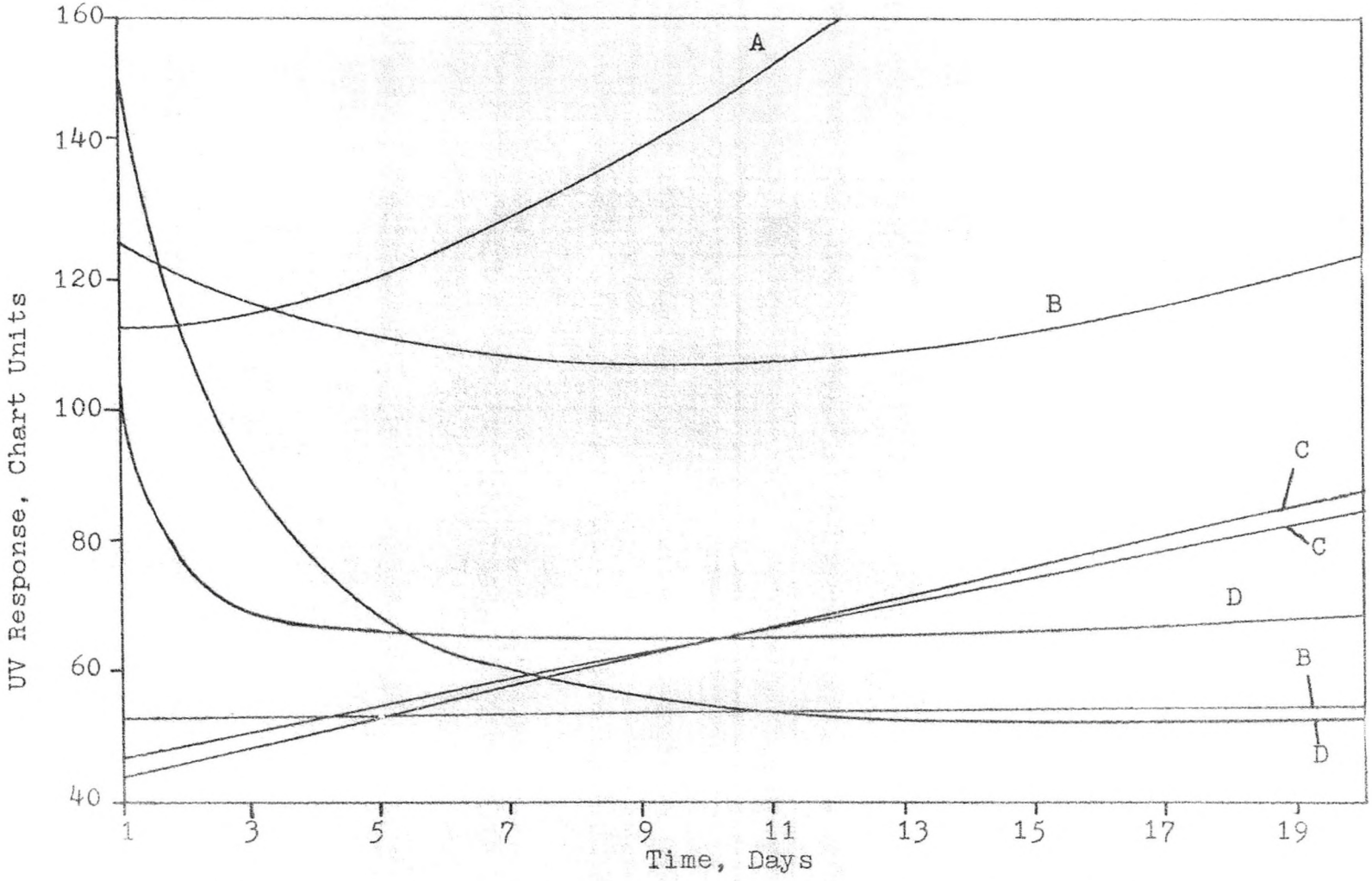


Figure 10 -- Variation of mercury(II) concentration during storage
(A) 0.001M NaCl pH 2, (B) 0.01M HCl, (C) 1M HCl, (D) 0.01M NaCl pH 5.5

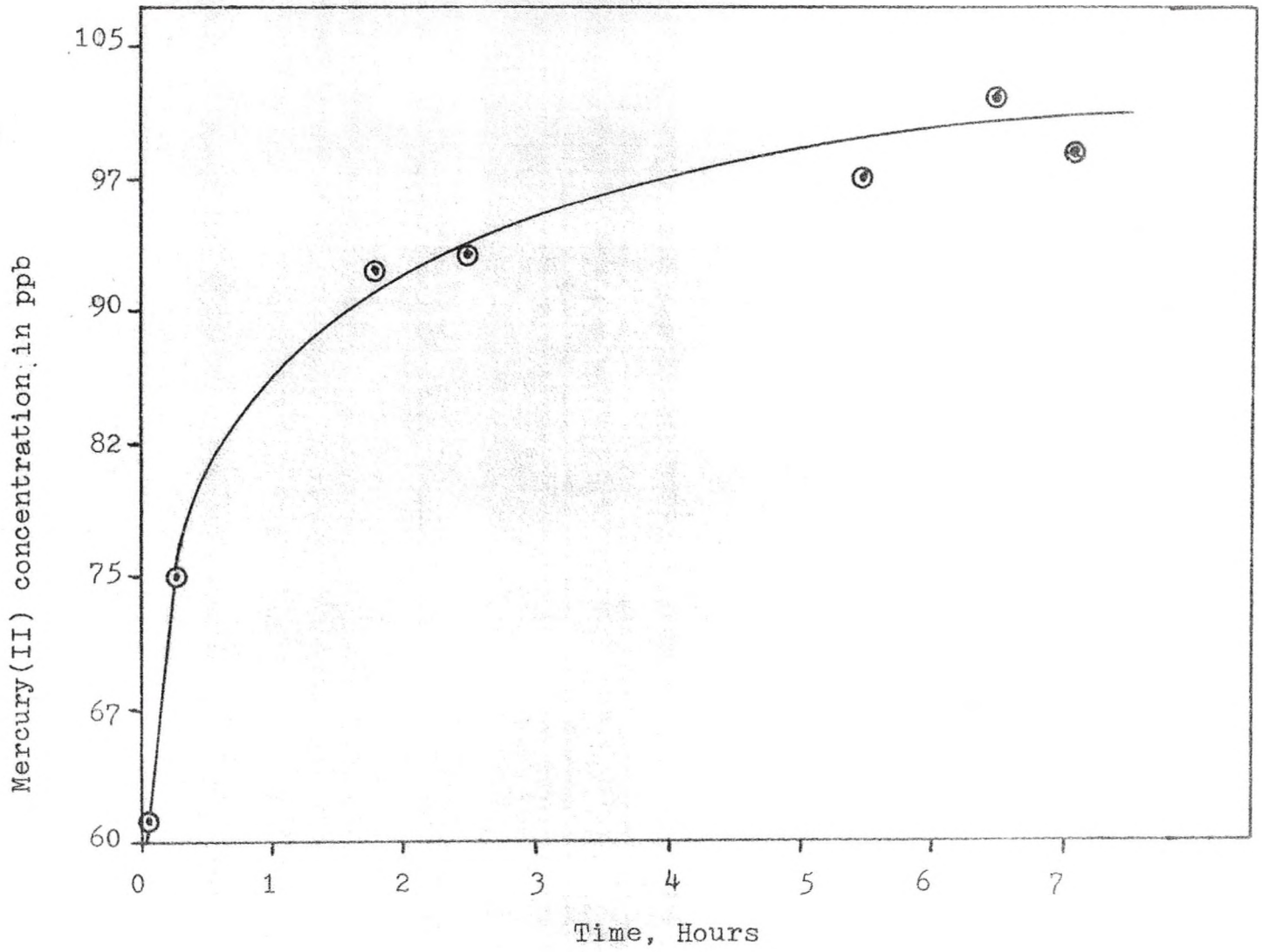


Figure 11 -- Mercury(II) concentration increase by leaching from glass surface

in wax, teflon, or glass. Mercury(II) solutions were prepared to be 21ppb in 0.1M perchloric acid. One hundred ml of this solution was placed in a teflon beaker, a wax coated flask and a Pyrex volumetric flask. Sample aliquots of 0.2 ml were withdrawn at time intervals and analyzed by injection into the impinger aeration cell containing 10 ml of the sulfuric acid reducing solution. The results graphed on figure 12 show that wax and teflon containers had a rapid absorption of mercury(II) in three hours. The Pyrex flask had no loss of concentration in 39 hours within the experimental error. Wax and teflon containers should not be used for storage of mercury(II) samples even for short time intervals. Teflon should also be avoided in the use of magnetic stirring bars due to the absorption of mercury. Pyrex glass seems to be the best type of container to use for the storage of mercury(II) solutions.

From the highly erratic behavior of the above solutions it is not recommended to store mercury(II) solutions of the ppb level for more than one working day. Mercury(II) was not only absorbed or desorbed on the container surfaces as shown above, but it also is slowly reduced to mercury(I) in aqueous solution. Grieble has shown that about 2-3% of mercury(II) was reduced to mercury(I) in 4800 minutes at 25°C.[90] If the only objective is for a total mercury analysis, for calibration of other solutions, it is possible to prepare ppb level solutions from 10^{-3} or 10^{-4}M stock

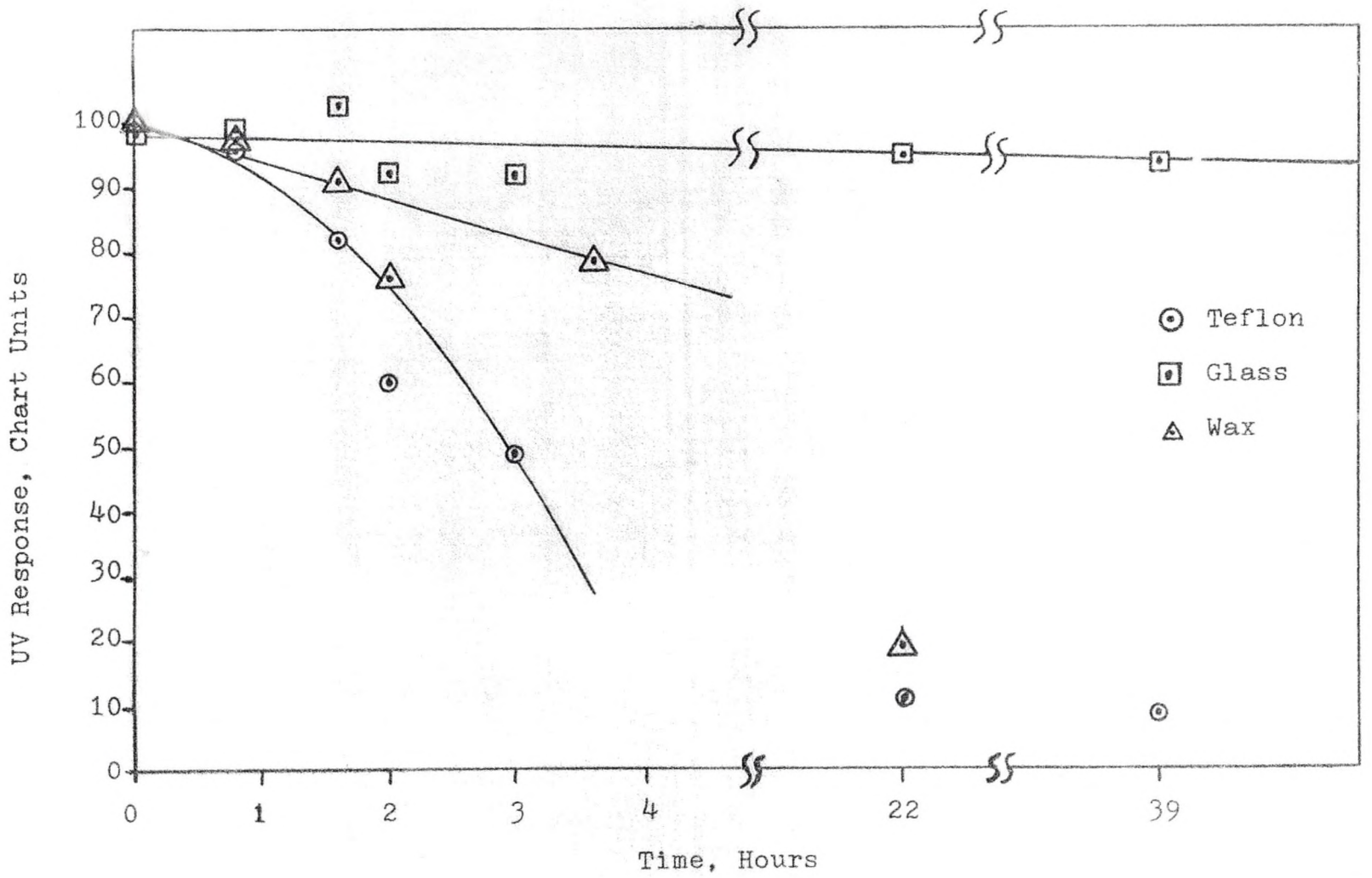


Figure 12 -- Mercury(II) concentrations lost by surface absorption in various containers

solutions of mercury(II) that are several weeks old. These stock solutions will contain mercury(I) but do not lose any appreciable concentration due to absorption on the glass. If the objective is to use the mercury(II) solution for a source of only mercury(II) ions, the solution must be prepared from the salt on the same working day they are used.

Anion exchange chromatography employing polystyrene matrices

The elution and separation of mercury(0), mercury(I), mercury(II), methylmercury(II), and phenylmercury(II) were studied utilizing carefully prepared columns containing Bio-Rad, AG2X8, 200-400 mesh resin. The exchange site in this case was a quaternary ammonium group bonded to a styrene-divinylbenzene copolymer. Prior to use fresh resin was soaked in distilled water for 24 hours, placed in a washing column, repeatedly backwashed with dilute acid, and allowed to settle which resulted in grading by particle size. The bottom 14% and the top 21% of the graded resin column was discarded. The middle 65% was stored and used in preparing columns that were used for mercury separations. The columns used in the study were prepared by the addition of 1cc of the prepared resin to a 6mm X 10cm glass column. The resin was retained with a small amount of glass wool. A fresh column was then washed with either 1M hydrochloric or 0.01M sodium chloride depending on the cation under study. Small samples (1-5 ml) containing various quantities

of mercury species were then loaded directly onto the column.

Once the sample was loaded, the eluent conditions were varied in an attempt to recover the cation in 100% yield in a relatively small retention volume. Chromatographs of the various carrier conditions were obtained from mercury analysis of one ml fractions of the eluent collected by a fraction collector. In order to determine whether quantitative yield of a mercury species was obtained, it was necessary to measure the mercury content background of the eluent solvent prior to the addition of a mercury sample to the exchange column and after passage of the mercury species through the column. In an alternate procedure, a sample blank was run through a column prior to use for a mercury sample.

In this study, mercury(II) was added directly to columns in 1M hydrochloric acid without special treatment of the sample. Mercury(0) and mercury(I) were added to the column in 0.01M sodium chloride. The mercury(0)-mercury(I) mixture had been prepared as described previously. Several columns were loaded with a solution containing only mercury(I) in 0.01M sodium chloride. In this case a mercury(I)-mercury(0) mixture was prepared by equilibration of mercury(II) over a drop of mercury(0). The solution was then removed from the drop of mercury(0) and aerated with nitrogen to remove the dissolved mercury(0). Mercury(I) does not disproportionate rapidly during this step which

will be discussed later. Methyl- and phenylmercury(II) were added to the resin in 1.0 or 0.1M hydrochloric acid. Each of the above species were retained quantitatively on the column in solvent mixture added except for mercury(0) which ran through the column. However, part of the mercury(0) (75%) was also retained on the styrene polymer.

Anion exchange chromatography employing cellulose matrices

The elution and separation of mercury(0), mercury(I), mercury(II), methylmercury(II), and phenylmercury(II) were studied utilizing carefully prepared columns containing Whatman ECTEOLA cellulose. The exchange site in this case was a tertiary ammonia group bonded to a cellulose polymer. Prior to addition to the column, the cellulose was soaked in distilled water for 24 hours. The columns used in the study were prepared by the addition of 1cc of the soaked cellulose to a 6mm X 10cm glass column. The cellulose was retained with a small amount of glass wool. The fresh column was washed with 0.1M hydrochloric acid to convert the active site to the quaternary ammonium chloride form. The column was then washed with sodium chloride solutions of varying concentrations and hydrogen ion concentrations depending on the cation under study. Small samples (1-5 ml) containing various quantities of mercury species were loaded directly onto the column.

Once the sample was loaded, the eluent conditions were varied in an attempt to recover the cation in 100%

yield in a relatively small retention volume. Chromatographs of the various carrier conditions were obtained from mercury analysis of 1 ml fractions of the eluent collected by a fraction collector. In order to determine whether quantitative yield of a mercury species was obtained, it was necessary to measure the mercury content background of the eluent solvent prior to the addition of a mercury sample to the exchange column and after passage of the mercury species through the column. In an alternate procedure a sample blank was run through a column prior to use for a mercury sample.

In this study, mercury(II) was added directly to the column in 0.01M sodium chloride at pH 5.8 without special treatment of the sample. Mercury(0) and mercury(I) were added to the column in 0.01M sodium chloride at pH 5.8. The mercury(0)-mercury(I) mixture had been prepared as described previously. Methyl- and phenylmercury(II) were added to the resin in various chloride concentrations. Each of the above species was recovered from the column in 100% yield with only the mercury(II) and mercury(I) being retained on the column longer than the dead volume. The mercury(I) sample when retained on the column disproportionated causing mercury(0) to bleed from the column.

RESULTS AND DISCUSSION

Elution characteristics of inorganic mercury from Bio Rad AG 2 X8 Due to the presence of chloride in almost all environmental samples and very high formation constants with all mercury species, anion exchange of the chloro mercury complexes was investigated as a possible separation technique for the mercury compounds. Mercury(II) was investigated on a Bio Rad AG 2 X8 strong base anion exchange resin. In this experiment a 5 ml sample of 60ppb mercury(II) in various chloride media was added directly to a 1 ml column of the Bio Rad AG 2 X8 exchange resin and was quantitatively retained. The sample was then washed with various solvents. The sample was retained on the column in all solvent mixtures as shown in table 7. Seymour and Fritz found that a mixture of 1M hydrochloric acid and 4.5M perchloric acid could be used to elute chloro complexes of mercury(II) from a macroreticular anion resin.[91] They postulated that neutral protonated chloro complex was formed in this mixture. Such an acid mixture was tried in this work and it was found that only eighty percent of the mercury (II) was recovered. The elution profile is shown in figure 13. The remaining twenty percent of the sample bled from the column at such low concentration that it

TABLE 7

ELUTION CHARACTERISTICS OF MERCURY(II) IN VARIOUS CHLORIDE MEDIA FOR BIO RAD AG 2 X8 RESIN.[a]

ELUENT SOLVENT	mls COLLECTED	UV RESPONSE, CHART UNITS[b]	% MERCURY RECOVERED
4M HCl	15	5	0
1M HCl	15	4	0
.1M HCl	20	3-4	0
.01M HCl	7	2-3	0
.01M NaCl (pH 5.5)	10	1-2	0
H_2O	10	1-2	0
ethanol	20	3-4	0

[a] A 5 ml sample of 60ppb mercury(II) in 1M hydrochloric acid was loaded on a 1 ml column with 1 ml fractions being collected.

[b] These values are an average analysis value for the fractions determined by injection of a 0.2 ml aliquot into the basic aeration cell containing 5 ml of the basic reducing mixture.

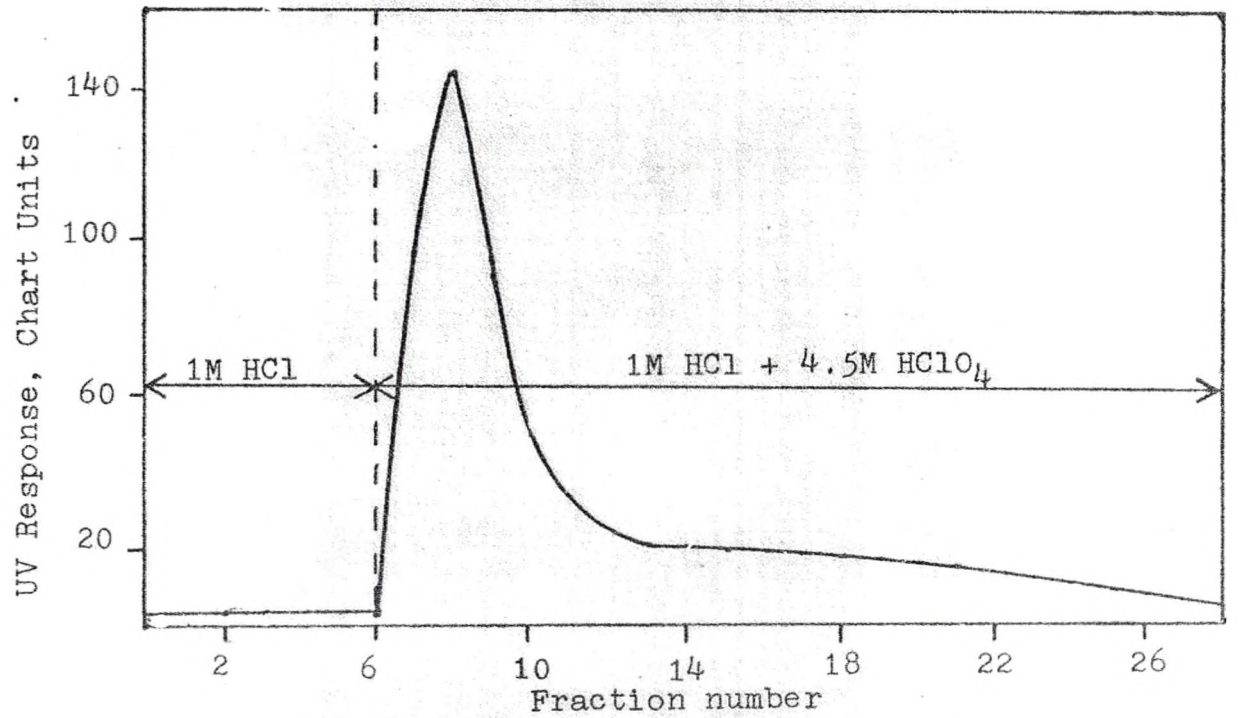


Figure 13 -- Elution of mercury(II) from Bio Rad AG2X8 resin.

could not be distinguished from the background or it may have remained on the column. The irreversible retention of mercury(II) on divinylbenzene type resin has been a common problem reported in the literature.[92] This irreversible absorption of mercury(II) is attributed to the mercuration of the meta position of the phenyl group of the polystyrene resin. Seymour and Fritz employing high pressure chromatographic techniques reported quantitative recovery of mercury(II) from macroreticular anion resin. They did note that in batch experiments nonquantitative recovery resulted due to the longer contact time in these experiments.

Mercury(0) was not expected to be retained on the anion exchange column because it is a non-ionic species. A 5 ml sample of 40ppb mercury(0) was loaded on the column in 0.01M sodium chloride at pH 2. The column was then washed with an additional 10 ml of the sodium chloride solution which eluted about twenty five percent of the mercury(0) sample in four 1 ml column volumes. The remaining seventy five percent of the sample was retained on the column during an additional wash with ethanol. The data is shown in table 8.

Mercury(I) was also investigated. A 5 ml sample of 91ppb mercury(I) in 0.01M sodium chloride at pH 2 was loaded onto the column and was completely retained. The column was then washed with a series of solvents with the sample quantitatively retained in all cases as shown

TABLE 8

ELUTION CHARACTERISTICS OF MERCURY(0) IN 0.01M SODIUM CHLORIDE AT pH 2.[a]

ELUENT SOLVENT	FRACTION NUMBER	UV RESPONSE, CHART UNITS[b]	% MERCURY RECOVERED
0.01M NaCl	1-5	0	0
	6	0	0
	7	1	0.4
	8	32	13.1
	9	23	9.4
	10	5	2
	11	0	0
ethanol	12-21	0	0

[a] A 5 ml of 40ppb mercury(0) in 0.01M sodium chloride was loaded on a 1 ml column, 1 ml fractions collected.

[b] The values were determined by injection of 0.2 ml samples into 5 ml of the basic reducing solution.

in table 9.

The inability to recover any of the inorganic mercury species quantitatively, eliminated this type of resin as a possible technique for a separation of mercury(0), mercury(I), and mercury(II).

Elution characteristics of organic mercury from Bio Rad AG 2 X8 In the investigation of the organic mercury species, it was found that phenylmercury(II) chloride was retained on a 1 ml column when a 5 ml sample of 60ppb was loaded in 1M hydrochloric acid. The column was then washed with a series of solvents, with the results shown in table 10. One molar sodium hydroxide partially eluted the sample with only nineteen percent recovery. This result would be expected if the retention of phenylmercury(II) chloride on the column was caused by the very low solubility of this species in acid media and its high solubility in basic media. A second experiment was performed using ethanol rather than water as the eluting solvent for the species. As shown in figure 14, quantitative recovery in 15 column volumes was obtained when ethanol was added to the column following the loading of the sample in 1M hydrochloric acid. This result seems to confirm that solubility was the mechanism of retention for phenylmercury(II) chloride. The shape of this elution profile was very similar to that obtained for mercury(II) (figure 13) with the elution width being about fifteen column

TABLE 9

RECOVERY OF MERCURY(I) FROM BIO RAD AG 2 X8 RESIN USING VARIOUS CHLORIDE CONCENTRATIONS.[a]

SOLVENT[b]	FRACTIONS	UV RESPONSE, CHART UNITS[c]	% MERCURY RECOVERED
0.01M NaCl[d]	1-2	1	0
0.01M NaCl ($91ppb\ Hg^{+2}$)	3-7	0.5	0
0.1M NaCl	8-21	0.5	0
.1M NaCl	22-29	0	0
1M NaCl	30-37	0	0
4M NaCl	38-48	1	0
4M HCl	49-52	2	0

[a] A 5 ml sample of 91ppb mercury(I) in pH 2 0.01M sodium chloride loaded on a 1 ml column with 1 ml fraction collected.

[b] Successive washing of column and sample with each of the following solutions.

[c] These analyses values were obtained by injection of 0.2 ml sample into an aeration cell containing 5 ml of the basic reducing mixture.

[d] Blank value for column (no mercury added).

TABLE 10

ELUTION CHARACTERISTICS OF PHENYLMERCURY(II) IN VARIOUS MEDIA FROM BIO RAD AG 2 X8 RESIN.[a]

ELUENT SOLVENT	FRACTION NUMBER	UV RESPONSE, CHART UNITS	% MERCURY RECOVERED
1M HCl[b]	1	6	0
1M HCl[c]	2	5	0
	3	2	0
	4	3	0
	5	3	0
	6	3	0
1M HCl[d]	6-18	3-4	0
.1M HCl	19-28	3-4	0
.01M HCl	29-32	3-4	0
4M HCl	33-50	4	0
	1-12	4	0
H_2O	13-17	5	0
0.01M NaOH	18-30	5	0
1M NaOH	31	44	7.6
	32	32	5.3
	33	19	2.9
	34	12	1.5
	35	9	.95
	36	8	.76
	37-39	4	0

[a] The analyses were made in the basic reducing aeration cell containing 5 ml of the basic reducing solution.

[b] Fraction just prior to addition of sample.

[c] A 5 ml sample of 60ppb phenylmercury(II) was added to the column followed by 1M HCl.

[d] Solvents varied in the order shown.

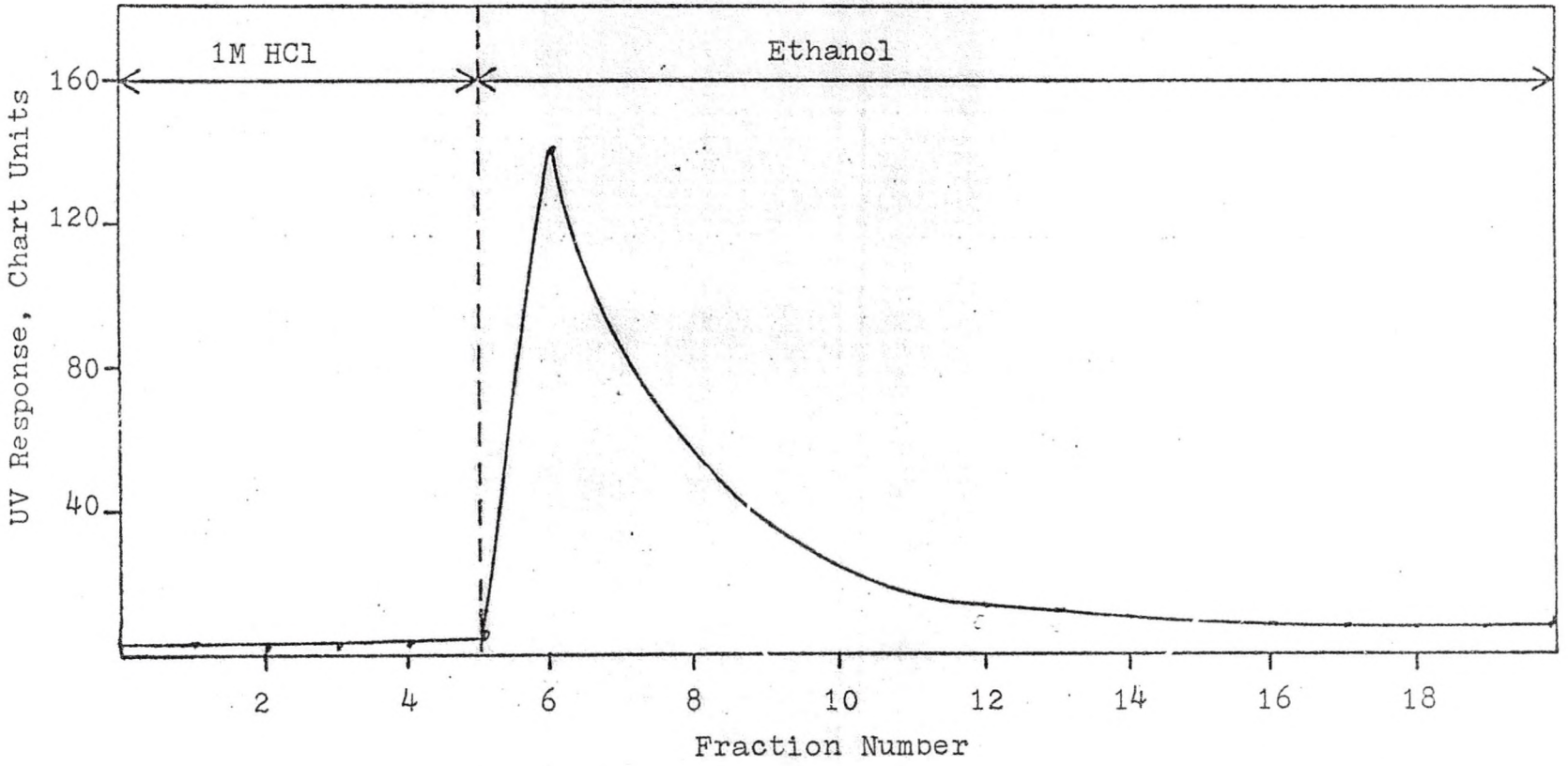


Figure 14 -- Elution of phenylmercury(II) chloride from Bio Rad AG 2 X8 resin.

volumes. The very large amount of tailing in these elution profiles indicates the amount of absorption of these species in the column. Because of the partial elution of the phenylmercury(II) in 1M sodium hydroxide, the possibility of using basic ethanol for elution was investigated. It was thought that the use of basic ethanol as the eluent would narrow the elution width of phenylmercury(II) chloride but the entire sample was retained in this solvent.

In contrast to phenylmercury(II), methylmercury(II) was eluted in a normal elution profile. A 5 ml sample of 62ppb methylmercury(II) in 1M hydrochloric acid was loaded onto a 1 ml column and retained for 8 column volumes of 1M hydrochloric acid wash. The column was then washed with 4M hydrochloric acid which quantitatively eluted the sample in a normal elution profile, shown in figure 15. The width of this elution was about 10 column volumes. Caution should be used when loading the sample in 1M hydrochloric acid because it will start to bleed through the column after about 8 column volumes of solvent have been collected. This was not a problem in this study because the sample was eluted from the column immediately after loading.

Based on the elution characteristics for methyl- and phenylmercury(II) chloride, a quantitative ion exchange separation was possible of these two species. The separation was performed by loading a 1 ml mixture of 50ppb

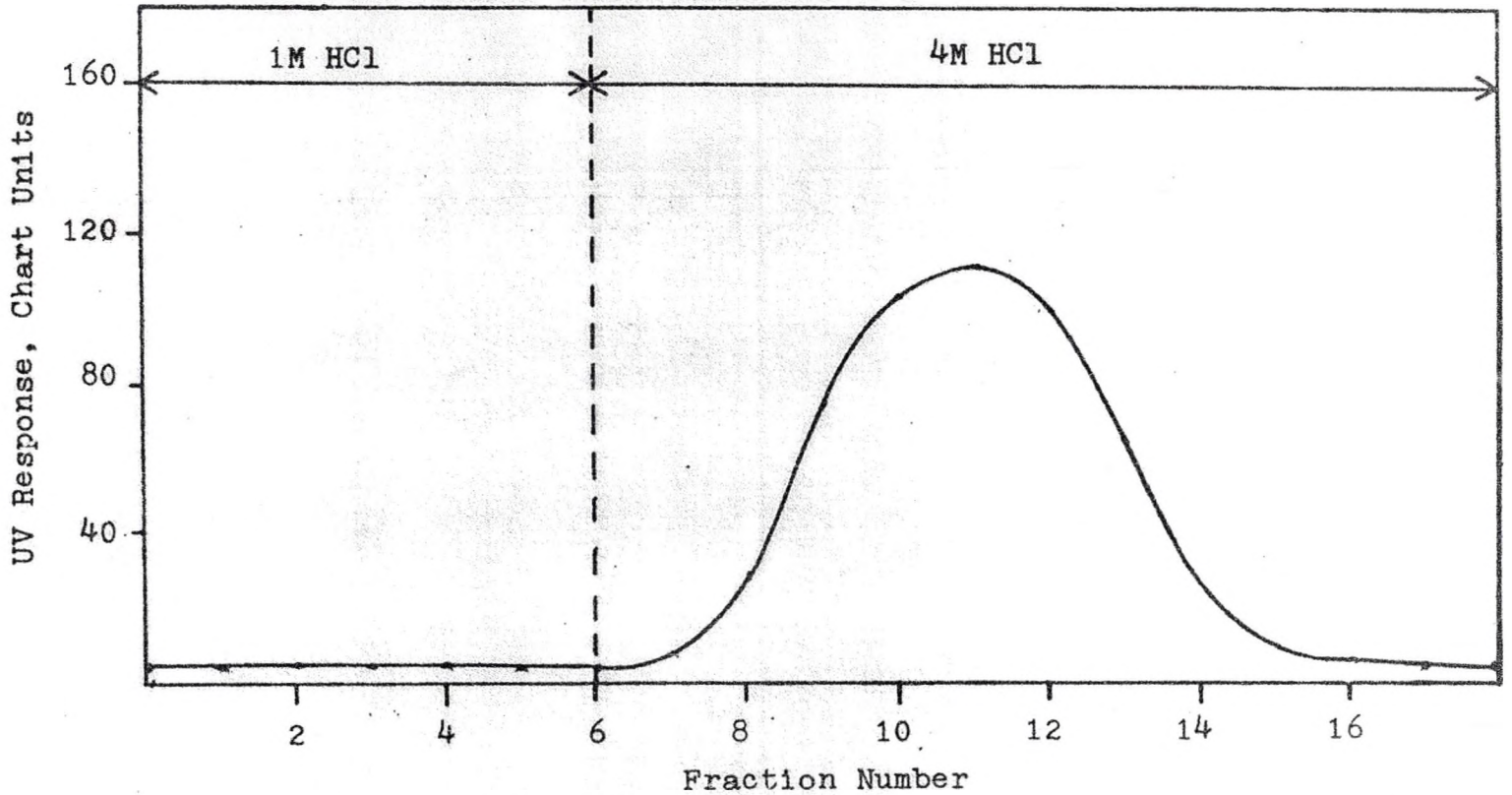


Figure 15 -- Elution of methylmercury(II) chloride from Bio Rad AG 2 X8 resin.

methylmercury(II) and 50ppb phenylmercury(II) chloride in 4M hydrochloric acid onto a 1 ml column. Under these conditions the methylmercury(II) was not retained on the column and was collected in the first ten column volumes following the dead volume of the column. After complete recovery of the methylmercury(II) chloride, the column was washed with ethanol to elute the phenylmercury(II) chloride in about 18 column volumes. The elution profile is shown in figure 16. A major problem with all of the ion exchange separations was that the sample was badly diluted relative to its initial volume.

Ion exchange properties of inorganic mercury on ECTEOLA cellulose matrix A second type of ion exchange material (ECTEOLA cellulose) was investigated in an attempt to achieve a quantitative separation of the inorganic mercury species. Mercury(II) was the first species to be investigated on the ECTEOLA cellulose ion exchange polymer. When a 1 ml sample of 104ppb mercury(II) in 1M hydrochloric acid was loaded on a 1 ml column, it was found to bleed through upon continued washing with 1M hydrochloric acid. The sample was recovered quantitatively in 17 column volumes. Several more experiments were performed to find a solvent condition in which mercury(II) would be retained on the column. In these experiments, the hydrochloric acid concentration was varied from 1M to 0.01M with only partial retention of the sample occuring at various

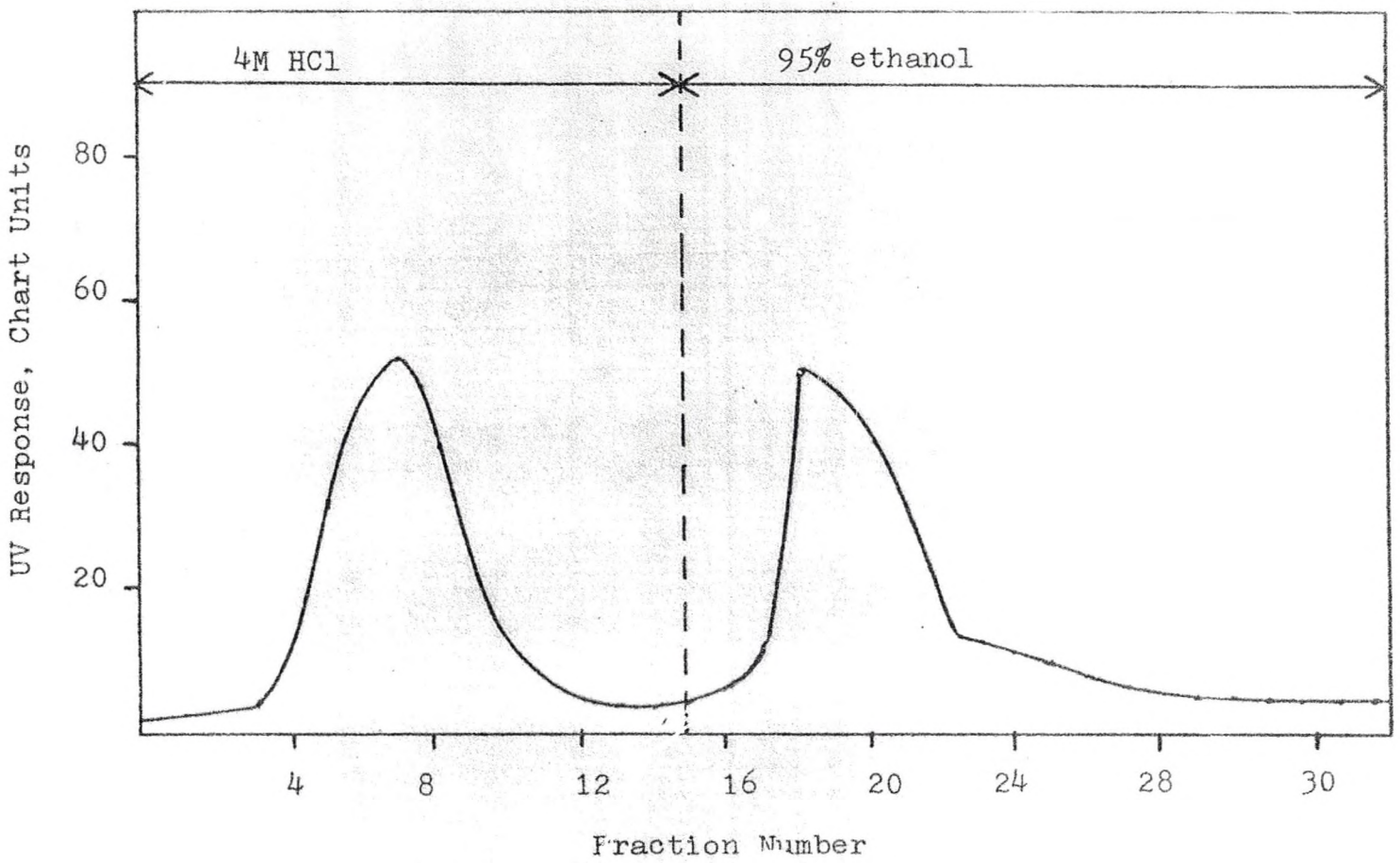


Figure 16 -- Elution of a methyl-and phenylmercury(II) chloride mixture.

concentrations as shown in table 11 and 12. Only at 0.01M or above .5M hydrochloric acid does the mercury(II) elute quantitatively in a narrow band. Between these concentrations the results are scattered and nonquantitative.

The next variable investigated was the chloride ion concentration range of 1M to 0.0005M at pH 5.8. This pH was used because retention of mercury(II) was achieved at that pH or greater. In these experiments the columns were preconditioned by washing with 20 ml of 0.1M hydrochloric acid followed by an additional 10 ml of sodium chloride solution at pH 5.8. The concentration and pH of this chloride solution were the same as that of the sample to be loaded on the column. A 1 ml mercury(II) sample in the stated chloride concentration and pH 5.8 was loaded on a 1 ml preconditioned column. The column was then washed with additional chloride solution. As seen in table 13 and 14, the entire mercury (II) sample was retained in 0.01M chloride for more than 15 column volumes. Upon change of solvent to 4M hydrochloric acid, the mercury(II) sample was quantitatively eluted from the column in 10 column volumes, as shown in figure 17. Also shown in figure 17 was a sample blank in which only 0.01M sodium chloride was added to the column in place of a mercury(II) sample. Quantitative elution for the mercury(II) sample was obtained by subtraction of the 4M hydrochloric acid blank values from those obtained during the mercury(II) elution. The blank

TABLE 11

ELUTION CHARACTERISTICS OF MERCURY(II) IN HYDROCHLORIC ACID CONCENTRATIONS FROM 4M HCl to 0.2M HCl FOR ECTEOLA CELLULOSE.[a]

FRACTION NUMBER	UV RESPONSE, CHART UNITS			
	4M HCl[b]	1M HCl	0.5M HCl	0.2M HCl
1	6	62	29	28
2	18	346	110	22
3	17	450	318	49
4	17	380	376	133
5	15	136	338	112
6	13	42	212	111
7	13	28	130	102
8	14	18	84	68
9	12	9	70	50
10	12	7	(116%)	37
11		3		37
12		(104%)[c]		20
13				(96%)

[a] A 5 ml sample of 60ppb mercury(II) in the stated hydrochloric acid concentration was loaded on a 1 ml column with 1 ml fractions collected. The analysis was performed by injection of 0.2 ml samples of each fraction into the aeration cell containing 5 ml of the basic reducing mixture.

[b] This experiment was performed as a blank using only 4M hydrochloric acid as a sample followed by an additional 4M hydrochloric acid.

[c] This percentage in parenthesis is the total mercury species removed from the column.

TABLE 12

ELUTION CHARACTERISTICS OF MERCURY(II) IN HYDROCHLORIC ACID CONCENTRATIONS FROM 0.16M HCl to 0.01M HCl FOR ECTEOLA CELLULOSE.[a]

FRACTION NUMBER	UV RESPONSE, CHART UNITS			
	0.16M HCl	0.1M HCl	0.05M HCl	0.01M HCl
1	8	4.5	7	3
2	9	13	18	26
3	36	28	123	690
4	90	143	266	447
5	53	105	156	105
6	48	86	58	14
7	33	91	44	(94%)
8	24	92	43	
9	17	86	40	
10	(46%)[b]	72	28	
11		(58%)	24	
12			(61%)	
13				

[a] A 5 ml sample of 60ppb mercury(II) in the stated hydrochloric acid concentration was loaded on a 1 ml column with 1 ml fractions collected. The analysis was performed by injection of 0.2 ml sample of each fraction into the aeration cell containing 5 ml of the basic reducing mixture.

[b] This percentage in parenthesis is the total mercury species removed from the column.

TABLE 13

ELUTION CHARACTERISTICS OF MERCURY(II) IN SODIUM CHLORIDE CONCENTRATIONS FROM 1 to 0.011M AT pH 5.8 FROM ECTEOLA CELLULOSE.[a]

FRACTION NUMBER	UV RESPONSE, CHART UNITS			
	1M NaCl[b]	0.1M NaCl	0.015M NaCl	0.011M NaCl
1	1	1	2	14
2	6	12	30	23
3	58	63	43	127
4	113	47	31	72
5	73	23	23	40
6	73	13	16	26
7	32	9	12	20
8	23	6	9	16
9	15	5	7	15
10	11	4	(24%)	(27%)
11	8	(93%)		
12	(100%)[c]			
13				
14				
15				

[a] A 1 ml column was prepared by washing with 20 ml of 0.1M hydrochloric acid followed by 10 ml of the stated chloride solution. A 1 ml sample of 104ppb mercury(II) in the stated chloride solution was loaded on the column with 1 ml fraction being collected.

[b] The analyses were preformed by an injection of 0.2ml sample aliquots into the basic reducing aeration cell containing 5 ml of the basic reducing agent.

[c] The percentage in parenthesis is the total mercury species removed from the column.

TABLE 14

ELUTION CHARACTERISTICS OF MERCURY(II) IN SODIUM CHLORIDE CONCENTRATIONS FROM 0.01 to 0.001M AT pH 5.8 FROM ECTEOLA CELLULOSE.[a]

FRACTION NUMBER	UV RESPONSE, CHART UNITS			
	0.01M NaCl[b]	0.0009M NaCl	0.0005M NaCl	0.001M NaCl
1	.5	10	3	3
2	1	12	24	45
3	.5	91	134	70
4	1	77	82	27
5	1	38	35	16
6	1	21	22	24
7	1	18	14	7
8	1	16	13	9
9	1	14	9	6
10	1.5	(25%)	(48%)	5
11	1.5			(35%)
12	1.5			
13	1.5			
14	1.5			
15	1.5			
	(0%)[c]			

[a] A 1 ml column was prepared by washing with 20 ml of 0.1M hydrochloric acid followed by 10 ml of the stated chloride solution. A 1 ml sample of 104ppb mercury(II) in the stated chloride solution was loaded on the column with 1 ml fraction being collected.

[b] The analyses were performed by an injection of 0.2ml sample aliquots into the basic reducing aeration cell containing 5 ml of the basic reducing agent.

[c] The percentage in parenthesis is the total mercury species removed from the column.

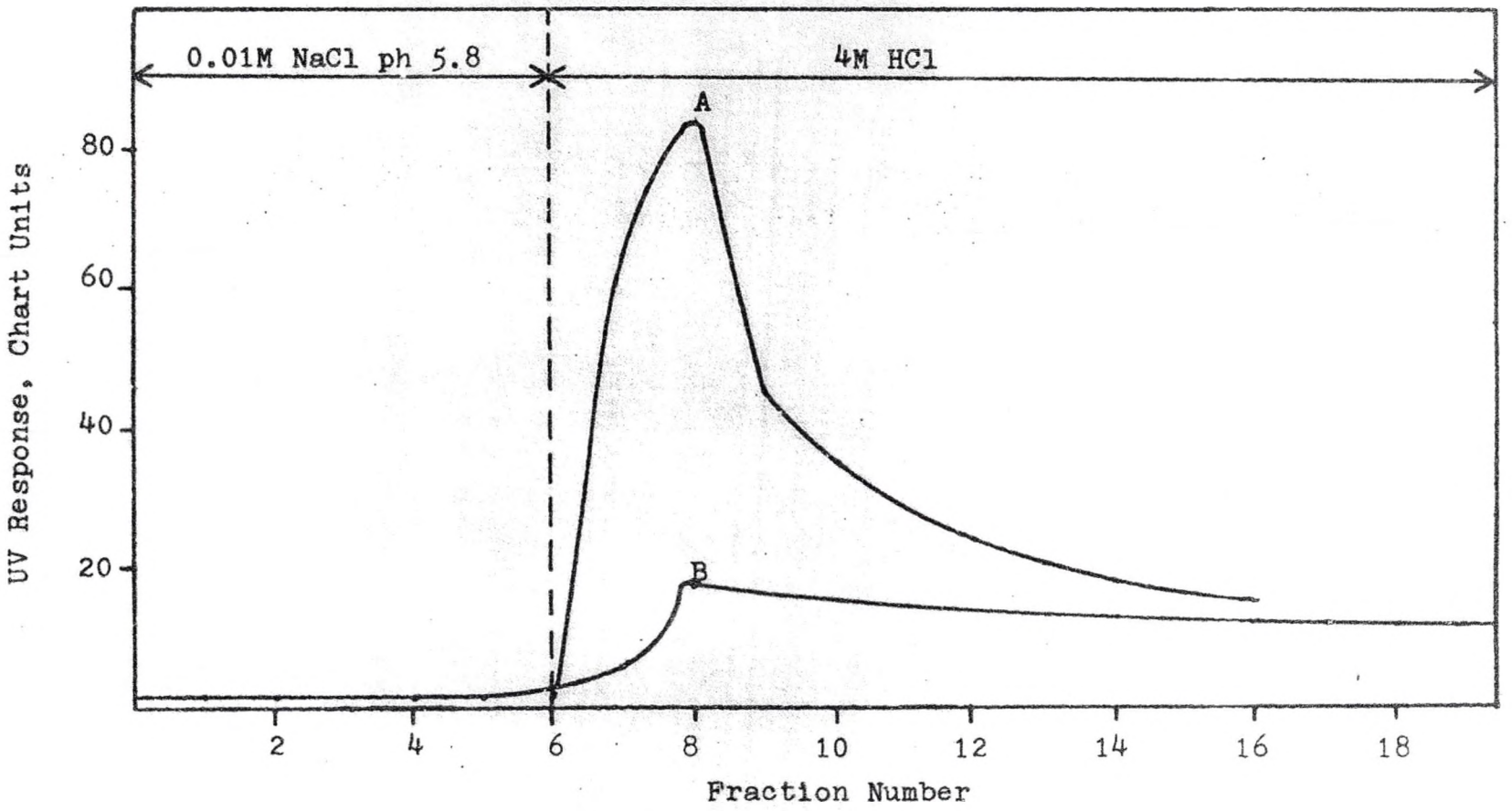


Figure 17 -- A - Elution of mercury(II) chloride from ECTEOLA cellulose
B - Blank

value for the 4M hydrochloric acid experiment corresponds to 29ppb of mercury. This blank value originates as an impurity in the 4M hydrochloric acid solvent and not from the cellulose column. Because of this large blank value, samples of lower than 10ppb will be difficult to distinguish from the blank value.

Tables 13 and 14 indicates that the conditions for quantitative retention on the column are so critically dependent on the chloride concentration at pH 5.8 that this method is not a practical separation method. It should be noted that a change in the chloride ion concentration of 0.001M at 0.01M chloride will cause bleeding of the mercury(II) sample from the column. Experimentally, it would be almost impossible to control the chloride concentration within this narrow limit of $\pm$ 0.001M.

The last set of experiments were performed to determine the effects of the acid wash prior to sample loading and the effect of the sample pH in 0.01M sodium chloride. In these experiments, shown in tables 15 and 16 the pH of the sample and solvent were varied from 1.9 to 7.2. Values of pH greater than 7 were not studied because of the reduction of mercury(II) in basic media. As seen in table 15, mercury(II) was retained on the column at pH 5.8 or 7 when the column was pre-washed with 0.1M hydrochloric acid. When the column was not preconditioned, seventeen percent of the mercury(II) sample bled through

TABLE 15

ELUTION CHARACTERISTICS OF MERCURY(II) IN 0.01M SODIUM CHLORIDE AT VARIOUS HYDROGEN ION CONCENTRATIONS FROM PRECONDITIONED ECTEOLA CELLULOSE.[a]

FRACTION NUMBER	UV RESPONSE, CHART UNITS				
	pH 3[b]	pH 3.5	pH 4.5	pH 5.8	pH 7
1	1	5	2	0.5	0
2	4	3	12	1	5
3	24	6	23	0.5	4
4	22	6	21	1	4
5	12	7	14	1	2
6	8	21	11	1	1
7	6	23	8	1	1
8	5	14	7	1	0.5
9	4	11	6	1	0.5
10	(13%)[c]	8	(18%)	1	0.5
11		7		1	0.5
12		(14%)		1	0.5
13				1	0.5
14				(0%)	0.5
15					(2%)

[a] A 1 ml column was prepared by washing with 20 ml of 0.1M hydrochloric acid followed by 10 ml of the 0.01M sodium chloride at the stated pH. A 1 ml sample of 104ppb mercury(II) in the 0.01M sodium chloride of the stated pH was loaded on the column.

[b] The analyses were performed by the injection of 0.2 ml sample aliquots into the basic reducing aeration cell containing 5 ml of the basic reducing agent.

[c] The percentage in parenthesis is the total mercury species removed from the column.

TABLE 16

ELUTION CHARACTERISTICS OF MERCURY(II) IN 0.01M SODIUM CHLORIDE AT VARIOUS HYDROGEN ION CONCENTRATIONS FROM A NON-PRECONDITIONED ECTEOLA CELLULOSE.[a]

FRACTION NUMBER	UV RESPONSE, CHART UNITS[b]						
	pH 2	pH 2.75	pH 4	pH 5	pH 6	pH 6.5	pH 7
1	4	2	2	9	6	7	2
2	35	4	30	13	6	5	8
3	148	69	43	176	37	5	42
4	45	50	31	90	23	6	14
5	18	22	23	18	9	10	4
6	9	14	16	14	7	6	2
7	7	10	12	11	(17%)	5	2
8	7	10	9	11		(8%)	2
9	6	8	7	(24%)			2
10	(46%)[c]	(34%)	(24%)				(14%)
11							
12							
13							
14							
15							

[a] A 1 ml column was prepared by washing with 10 ml of 0.01M sodium chloride at the stated pH. A 1 ml sample of 104ppb mercury(II) in 0.01M sodium chloride at the stated pH was loaded on the column.

[b] The analyses were performed by the injection of 0.2 ml sample aliquots into the basic reducing aeration cell containing 5 ml of the basic reducing agent.

[c] The percentage in parenthesis is the total mercury species removed from the column.

the column when loaded at pH 6, as seen in table 16. This points out the need for an acid wash to convert the active group to the hydrochloride form. The difference between a preconditioning with sulfuric acid or hydrochloric acid was also studied. After a pre-wash with sulfuric acid or hydrochloric acid, the experiments were performed at pH 7. As seen in table 17, almost one-half of the mercury(II) sample bled through a column in the sulfate form. The amount of bleeding in the hydrochloride form was only two percent compared to a column which had not been preconditioned, which bled about fourteen percent of the mercury(II) sample. This points out the need of the active group to be in the hydrochloride form for the mercury(II) sample to be retained on the column. The last point to note on table 17 was that when the pH was varied below 5.8, the mercury(II) sample bled through the column upon continued washing with the loading solvent.

In summary, 1 ml of 104ppb mercury(II) will be retained on a 1 ml cellulose column in 0.01M sodium chloride at pH 5.8. The column must be preconditioned with a wash of 20 ml of 0.1M hydrochloric acid followed by a wash of 10 ml of 0.01M sodium chloride at pH 5.8. The wash with sodium chloride returns the column to the correct pH before the sample is loaded. The mercury(II) was then quantitatively eluted from the column by washing with 4M hydrochloric acid in about 15 column volumes. One problem with this elution was the large blank value

TABLE 17

ELUTION CHARACTERISTICS OF MERCURY(II) IN 0.01M SODIUM CHLORIDE AT pH 7 FROM VARIOUS TYPES OF PRECONDITIONED ECTEOLA CELLULOSE.[a]

FRACTION NUMBER	UV RESPONSE, CHART UNITS		
	0.01M NaCl wash[b]	0.1M HCl wash	0.1M H_2SO_4 wash
1	2	0	3
2	8	5	117
3	42	4	73
4	14	4	16
5	4	2	9
6	2	1	10
7	2	1	12
8	2	1	11
9	2	1	8
10	(14%)[c]	1	(47%)
11		1	
12		1	
13		1	
14		1	
15		(2%)	

[a] A 1 ml column was prepared by washing with 20 ml of the stated solvent followed by 10 ml of 0.01M sodium chloride at pH 7. A 1 ml sample of 104ppb mercury(II) in 0.01M sodium chloride at pH 7 was loaded on the column.

[b] The analyses were performed by the injection of 0.2 ml sample aliquots into the basic reducing aeration cell containing 5 ml of the basic reducing agent.

[c] The percentage in parenthesis is the total mercury species removed from the column.

of the 4M hydrochloric acid eluting solvent. This limits this method to samples of at least 30ppb mercury(II). Another problem with this separation method is the extremely narrow limits of pH and chloride concentration which have to be controlled to allow the retention of mercury(II). Experimentally these solvent conditions would be difficult, if not impossible, to control. The limits of chloride and pH may be less stringent at more basic pH values, but at this point reduction of mercury(II) prevents the investigation of these variables. The above reasons, along with the fact that mercury(I) is retained under the same solvent conditions, eliminate this exchange polymer as a possible technique for the separation of mercury(0), mercury(I), and mercury(II).

To determine if mercury(0) would be retained on this cellulose polymer, a 1 ml sample of 60ppb mercury(0) in 0.01M sodium chloride was loaded on a 1 ml cellulose column as prepared above. When the column was washed with an additional 10 ml of the sodium chloride solution, the mercury(0) eluted through the column. The sample was recovered in quantitative yield in four column volumes following the dead volume. This demonstrates that mercury(0) was quantitatively recovered from the ECTEOLA cellulose ion exchange material.

For an ion exchange separation method using this material to be successful, a mercury(I) sample must not be retained under the same conditions as those for mercury

(II). To determine if mercury(I) was retained under the set of retention conditions for mercury(II), a 1 ml sample of 170ppb mercury(I)-mercury(0) mixture was loaded on a 1 ml cellulose column. The sample was followed by an additional 30 ml wash of 0.01M sodium chloride at pH 5.8. As shown in figure 18, the mercury(0) in the sample was collected in the first 5 fractions following the dead volume of the column. A second broader peak of mercury(0) was then eluted from the column as seen in figure 20 and confirmed to be mercury(0) in a non-reducing analysis. The quantity of mercury(0) in these fractions corresponded to one-half of the original mercury(I) concentration in the sample that was loaded on the column. This suggests that the mercury(I) disproportionated on the ion exchange column. Later experiments will show that chloride ions prevent the disproportionation of mercury(I) in acid solution (pH 1). The pH in these experiments was 5.8. The fact that the mercury(I) disproportionated on the column eliminated this ion exchange material as a possible separation technique for the inorganic forms of mercury.

The ion exchange properties of organo mercury on ECTEOLA cellulose matrix Methyl- and phenylmercury(II) chloride were also investigated for the cellulose resin at various chloride concentrations. Experiments with these species were performed in a similar manner as those used for mercury(II) with the preconditioned column. The results

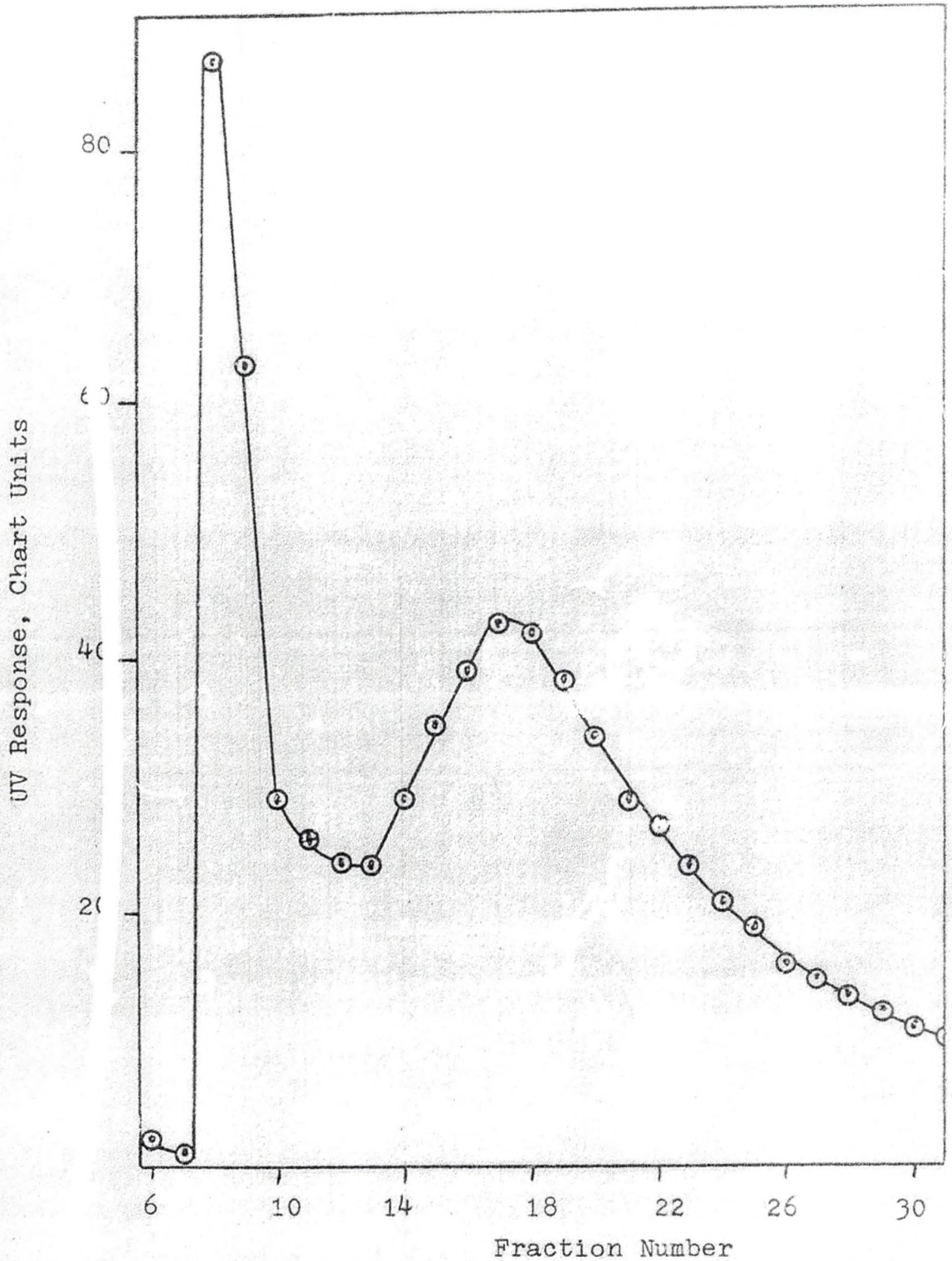


Figure 18 -- Elution of mercury(0)-mercury(I) mixture from ECTEOLA cellulose in 0.01M NaCl pH 5.8.

in tables 18 and 19 show that methyl-and phenylmercury(II) were quantitatively recovered in the fractions following the dead volume in all chloride concentrations studied. These species were not investigated more fully because the resin was of little use for the separation of the inorganic forms of mercury.

Summary of the ion exchange experiments One disadvantage of using an ion exchange process to separate the mercury species was the time involved in performing the column separation as compared to the short analysis time needed for a direct injection method using selective reducing conditions. Another disadvantage was that neither of the ion exchange materials could be used as a separation technique for the inorganic mercury species. A third disadvantage in using the Bio Rad resin, was the irreversible retention of the inorganic species. The problem with using the cellulose polymer was the retention of mercury(I) and mercury(II) under the same conditions with mercury(I) disproportionating on the column. Another disadvantage of the cellulose polymer was the extremely narrow limits of pH and chloride ion concentration for the retention of mercury(II). Experimentally these limits make the procedure very impractical. The one advantage gained by using ion exchange was the separation of the organic mercury species from each other and inorganic forms of mercury on the Bio Rad AG2X8 resin. This procedure

TABLE 18

ELUTION CHARACTERISTICS OF METHYLMERCURY(II) CHLORIDE IN VARIOUS SODIUM CHLORIDE CONCENTRATION AT pH 5.8 FROM ECTEOLA CELLULOSE.[a]

FRACTION NUMBER	UV RESPONSE, CHART UNITS		
	0.01M NaCl[b]	0.001M NaCl	0.0001M NaCl
1	2.5	3	3
2	36	36	28
3	78	52	45
4	17	11	11
5	4	3 (93%)	6 (105%)
6	6		
7	7 (113%)[c]		

[a] A 1 ml sample of 94ppb methylmercury(II) in the stated sodium chloride concentration at pH 5.8 was loaded on a 1 ml acid washed column with 1 ml fractions being collected.

[b] The analyses were performed by injection of 0.2 ml of each sample into the aeration cell containing 5 ml of the basic reducing solution.

[c] The percentage in parenthesis indicates the total mercury species removed from the column.

TABLE 19

ELUTION CHARACTERISTICS OF PHENYLMERCURY(II) IN VARIOUS CHLORIDE MEDIA FROM ECTEOLA CELLULOSE.[a]

FRACTION NUMBER	UV RESPONSE, CHART UNITS				
	1M HCl[b]	.1M HCl	0.01M HCl	0.001M HCl	0.01M NaCl[c]
1	1.5	0.3	1	7	1
2	10	53	6	6	31
3	79	190	116	94	98
4	143	234	164	158	27
5	180	234	170	180	19
6	186	228	172	174	5
7	186	220	176	176	3
8	158	98	112	126	3
9	86	26	36	28	2
10	39	13	13	22	2
11	22	9	7	16	1.5
12	19	9	6	11	(95%)
13	12	8	5	11(108%)	
14	10	8	4		
15	9	4	4		
16	9	2.5	4		
17	8(10%)[d]	2(10%)	(92%)		

[a] A 5 ml sample for the HCl media or a 1 ml sample for the NaCl media of 88ppb phenylmercury(II) in the stated media was added to a 1 ml column with 1 ml fractions being collected.

[b] The analysis of each fraction was performed by injection of 0.2 ml of each sample into the aeration cell containing 5 ml of the basic reducing solution.

[c] This experiment was performed at pH 5.8.

[d] The percentage in parenthesis indicates the total mercury species removed from the column.

worked well except for the extremely large elution volume and should provide a valuable analytical tool for the separation of methyl-and phenylmercury(II) chloride at trace levels.

Stability of Mercury(II) in Aqueous Solution

Mercury(II) solutions analyzed in acid non-reducing media

Mercury(II) solutions, when analyzed in a non-reducing aeration cell containing dilute acid, should not give an analysis value because no mercury(0) would be present nor can it be produced. This was found to be true when a freshly prepared mercury(II) solution in 0.1M acid was injected into the aeration cell containing 0.1M acid. When a mercury(II) solution several days old was analyzed, a small quantity of mercury(0) was measured in the acid aeration cell. An example of this problem was shown by the injection of 1 ml of 416ppb mercury(II) solution in 0.1M perchloric acid into an aeration cell containing 1 ml of distilled water. A peak of 4 chart units was obtained in the analysis compared to a peak of 226 chart units for a reducing analysis of the same solution. A water blank gave no detectable response.

This problem was studied by Grieble.[90] In his work the production of mercury(I) in mercury(II) solutions in 0.1M perchloric acid was followed as a function of time. When these solutions were analyzed by a non-reducing aeration cell, the mercury(I) disproportionated forming

mercury(0) which was observed in the UV detection cell. The rate of production of mercury(I) is shown in figure 19. As seen in the figure, the reaction came to equilibrium in about 5000 minutes.

When an old mercury(II) solution was exhaustively oxidized, by constant-current electrolysis, the small concentration of mercury(I) would disappear. In the electrolysis experiments, mercury species were oxidized at an anode at a controlled potential of +1.65 volts vs SCE. The cathode was isolated from the anode compartment by use of a cracked test tube filled with the same acid media as the cathode compartment. The electrolysis time was usually 2-3 hours or overnight.

An indication of the extent of the production of mercury(0) was shown in experiments in which mercury(II) solutions were purged with nitrogen at pH 5.5 in the absence of chloride. When this experiment was performed the total concentration of mercury remaining in the solution decreased. This data is shown in figure 20 along with solutions purged in the same manner containing chloride at pH 5.5 or 0.1M sulfuric acid. As seen in figure 20, chloride ion or sulfuric acid prevented the loss of mercury during the purging process. The above experiments are added evidence of the reduction of mercury(II) in near neutral solution.

Mercury(II) solutions analyzed in basic non-reducing media

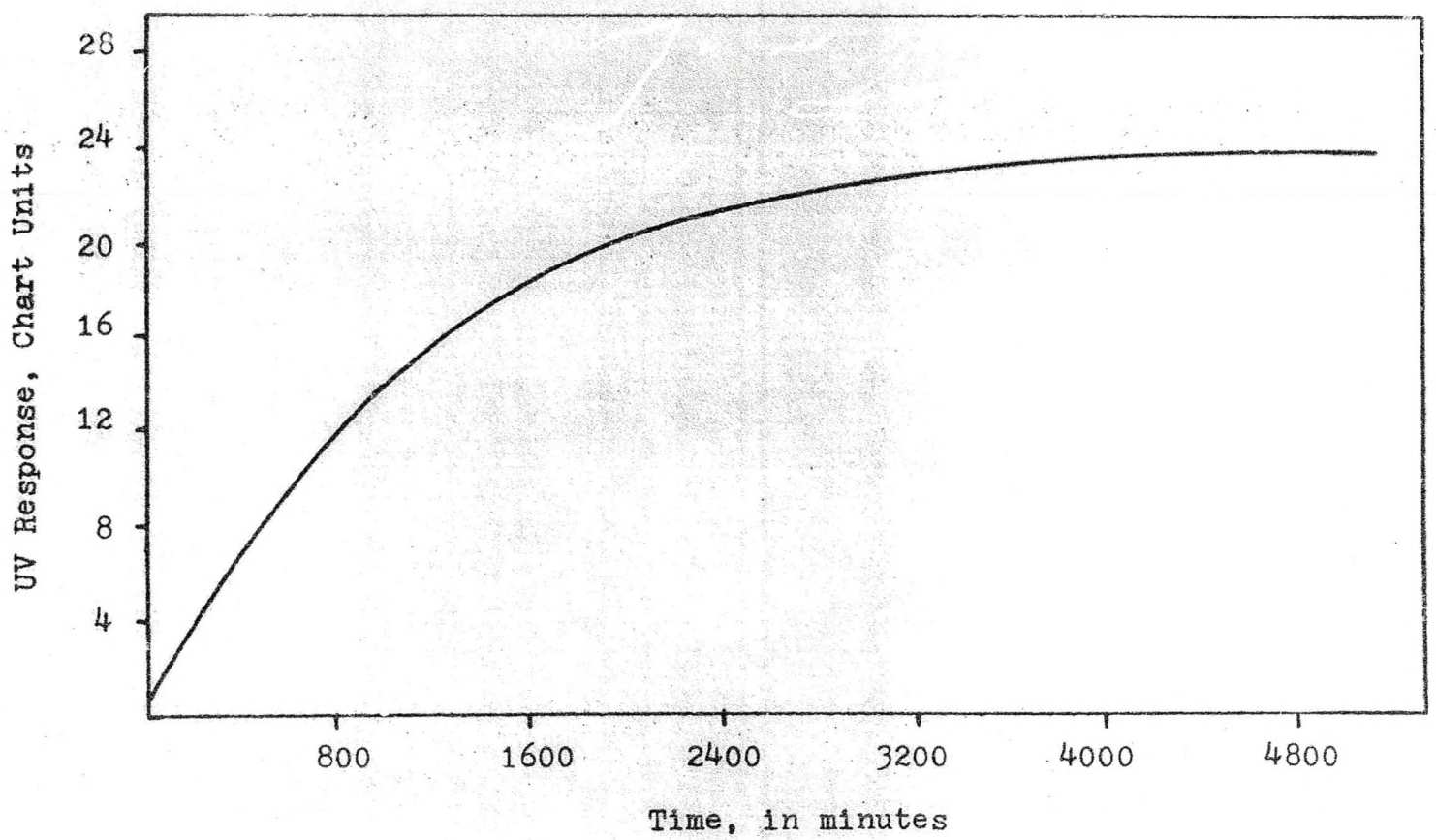


Figure 19 -- The approach to equilibrium of the oxidation of water by mercuric ion.

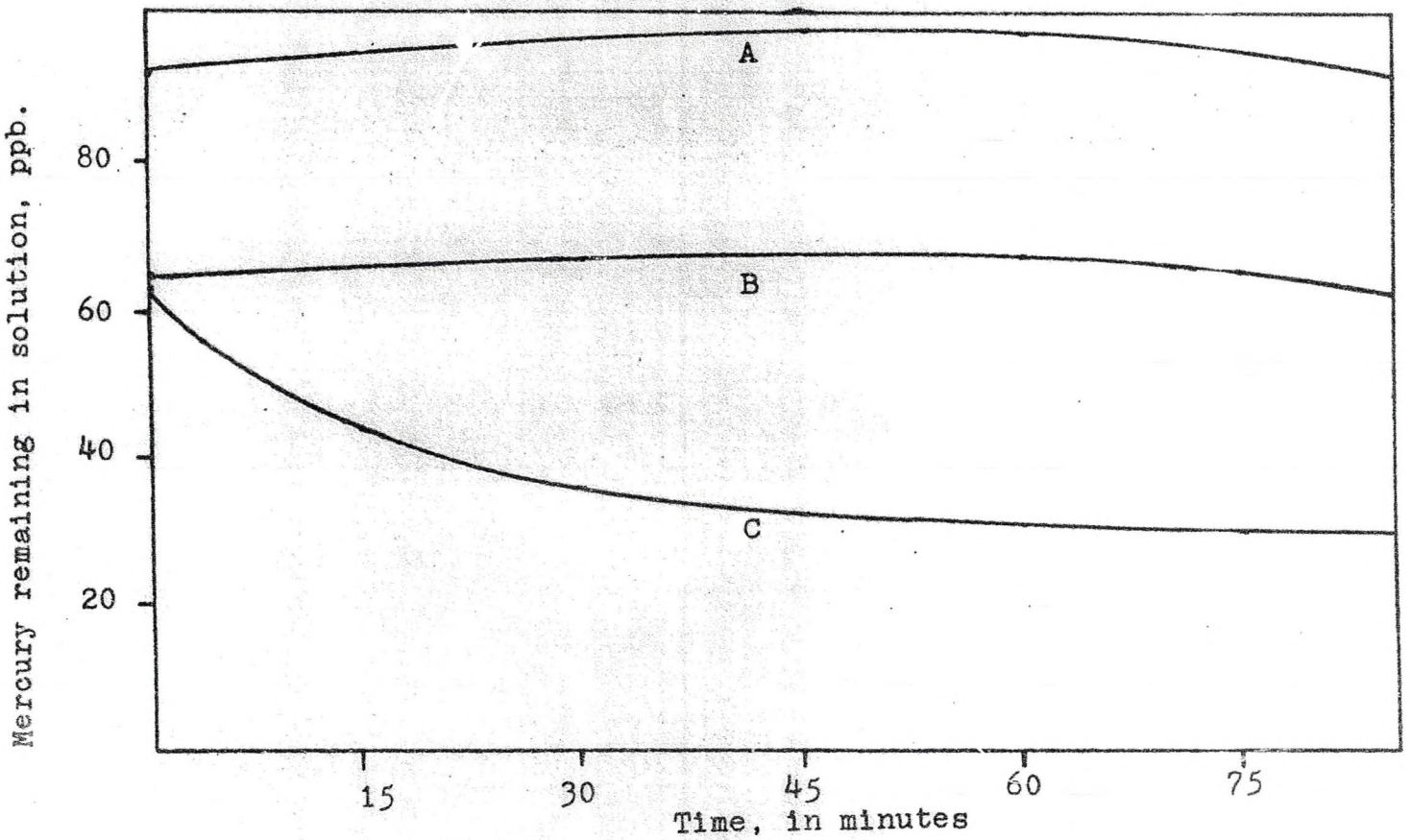


Figure 20 -- Purging of mercury(II) solutions in various media
(A) 0.01M NaCl at pH 5.5, (B) 0.1M H_2SO_4, (C) pH 5.5 with $HClO_4$

When a mercury(II) solution, regardless of its age, was injected into a basic solution in the aeration cell, a large quantity of elemental mercury was released. A typical analysis was performed by injection of a 1 ml sample aliquot of 416ppb mercury(II) in 0.1M perchloric acid into an aeration cell containing 2 ml of 4M sodium hydroxide. A response of 45 chart units was obtained with a sizable tail. When the mercury(II) solution was electrolyzed prior to the analysis to remove any mercury(0) in the sample, the same response of 45 chart units was obtained. The response for an analysis in basic solution was over a factor of ten larger than the response obtained in the acidic analysis. To confirm that these analysis peaks were mercury(0) and not a volatile mercury(II) complex, a special reaction vessel was built.

The vapor from a water aeration cell was passed through a second aeration cell prior to passage into the UV detection cell. The purpose of the second aeration cell was to have available in the system a means of converting any volatile HgX_2 compounds to elemental mercury. The response of the system to a particular mercury(0) quantity was determined in the following manner. Both aeration cells were filled with distilled water with no reducing agent in either cell. A 1ml sample of 416ppb mercury(II) solution in 0.1M perchloric acid was injected in this first aeration cell. The final concentration in the first cell was 0.05M perchloric acid. The response of the

detector was 7 chart units. Immediately after the elemental mercury was through the gas train, a 2 ml aliquot of 8M sodium hydroxide was injected into the cell containing the 416ppb mercury(II) sample. The response was 67 chart units. At this point the two aeration cells were emptied, cleaned, and refilled. However, the second cell was filled with 2 ml of basic reducing mixture. The above analysis sequence was repeated. When no base was added to the first aeration cell, 6 chart units were observed on the recorder. When the basic solution was added to the 416ppb mercury(II) solution in the first cell, 62 chart units were observed. A comparison of the results in chart units with and without a reducing solution in the second aeration cell indicates that only elemental mercury vapor was passing through the system from the first cell. When a 416ppb mercury(II) solution was added to the first cell under acid conditions 6-7 chart units were observed regardless of the solution in the second cell. This indicates that none of this vapor could have been HgX_2. Similarly when the 416ppb mercury(II) was injected into basic media in the first cell, 62-67 chart units were recorded regardless of the nature of the second cell. Again no HgX_2 was possible since no increase was observed in the UV response. Sodium hydroxide appears to retard slightly and broaden the elemental mercury peak since in both experiments the observed peak height was slightly lower, 67:62 and 7:6. This could be due to the high viscosity of the basic

reducing solution relative to water. The experiment confirmed that elemental mercury is present in acidic mercury solution when stored for more than several hours time and that basic solution partially reduces mercury (II) to mercury(0).

Cause of mercury(II) instability in aqueous media It was first suspected both the reduction in water or base occured because of some contamination in one of the reagents. Several sources of base, mercury(II), water, and glassware were investigated and it was found that the source of the reagents or glassware had little effect on the reduction process. Reagents were also electrolyzed for 24 hours by constant-current electrolysis which would decompose any reducing agent in all solutions used. The reduction of mercury(II) still occured in basic solution. It is possible that water or hydroxide ion are responsible for the reduction of mercury(II). Some possible reactions are

$$4Hg^{+2} + 4OH^{-} = 2O_2 + 2Hg_2^{+2} + H_2O \text{ or} \qquad (30)$$

$$4Hg^{+2} + 2H_2O = O_2 + 2Hg_2^{+2} + 4H^{+} . \qquad (31)$$

Further evidence that one of the above reactions was responsible for the reduction was obtained by Grieble.[90] In his work, the production of mercury(I) in mercury(II) solutions was followed as a function of time. He observed that when mercury(II) solutions were saturated with oxygen,

TABLE 20

FORMATION OF MERCURY(I) IN MERCURY(II) SOLUTIONS AS A FUNCTION OF THE AERATION GAS AT 30°C.[a]

AERATION GAS	CONCENTRATION OF MERCURY(I) IN M	
	INITIAL[b]	AT EQUILIBRIUM[c]
Nitrogen	5×10^{-9}	1.35×10^{-7}
Argon	5×10^{-9}	1.3×10^{-7}
Oxygen	2.5×10^{-9}	6×10^{-8}

[a] 5×10^{-6}M mercury(II) solutions in 0.1M perchloric acid were purged with the stated gas at a flow of 300 ml/min.

[b] The analyses were performed by injection of 0.2 ml of the mercury(II) solution into the non-reducing aeration cell containing 5 ml of 0.1M perchloric acid.

[c] The solutions reached equilibrium in approximately 5000 minutes.

argon, and nitrogen, by purging, the oxygen saturated solution contained less mercury(I) by about a factor of 2 compared to the other solutions at equilibrium. This data is shown in table 20. This would be expected if the solution was saturated with one of the products of the reaction.

Storage Problems of Mercury(II)

Surface Adsorption A second problem associated with the properties of mercury(II) solutions is their loss of concentration during storage. A great deal of work has been done in this area.[71,72,74,92,93] Previous investigators have suggested that the loss of mercury(II) from solution occurs by two processes. The first is adsorption onto the walls of the container. Examples of this problem are seen in figure 12, where wax and teflon containers absorbed large quantities of mercury(II) from 0.1M perchloric acid media while solutions were stored in Pyrex containers were relatively stable for the time period studied. Several chloride and hydrogen ion concentrations were also studied for their effectiveness as preservatives for mercury II) solutions. The results shown in figures 9 and 10 were quite erratic. These solutions increased and decreased in concentration. The glass surface was the most probable source or sink for this mercury. For example, the leaching of mercury(II) off of the surface of a mercury (II) contaminated flask, by chloride ions, is shown in

figure 11. To avoid this type of problem, flasks that were used for one concentration level were not used for solutions in which the concentration differed by more than a factor of 10. Due to the efficiency of chloride in leaching mercury(II) from contaminated flasks, a cleaning procedure using 0.01M sodium chloride was adopted. Contaminated flasks were repeatedly leached with freshly prepared 0.01M sodium chloride until the chloride solution no longer developed a mercury concentration.

Reduction and volatility Another process by which mercury(II) is lost from solution was by reduction to mercury(I). Mercury(0) formed by the disproportionation of the mercury(I) is then lost be volatilization. One source of this reduction problem could be the reaction of mercury(II) with water as suggested in this and Grieble's work.[90] The loss of elemental mercury from solution has been well documented in the literature by several authors working with radioisotopic labeled mercury(II).[72,93] These isotopic tracer studies were performed by both trapping the labeled mercury(0) lost from the solution and by counting the remaining activity of the solution and container to accurately determine the fate of the mercury (II). In these studies it was found that 18% of the inital concentration was lost by volatilization from distilled water in 21 days. Seventy-seven percent of the initial concentration was found on the glass surface. These

investigators have demonstrated that the volatilization of mercury(0) was one of the mechanisms of loss of concentration from solution. In another study the high mobility of mercury(0) through polyethylene containers was demonstrated in experiments in which the total concentration of mercury in sea water samples was measured as a function of time.[74] It was shown in this work that mercury(0) from the room air diffused through the walls of polyethylene bottles into the sea water samples. When the samples were isolated from the source of mercury(0), the concentration of the sea water samples remained constant.

Less specific experiments as to the mechanisms of loss have been carried out by measuring total mercury concentration at various times.[71,74,92] These investigators agree that the mercury loss was caused by volatilization of mercury(0) but could only speculate that reducing agents as impurities could have caused the loss. This possibility has never really been investigated because the addition of strong oxidizing agents was used by these authors to store the samples.[71,72,74,92] Another cause of the reduction which has been investigated was bacteria.[94-98] It has been found that mercury(II) salts were converted to elemental mercury during the incubation of pure cultures of certain specific bacteria.[97] This interconversion of the mercury species by bacteria has been of great interest because of mercury transportation in nature. The conversion of mercury(II) to the organo

forms was was already discussed in the introduction.[7,8] It was also found that a different strain bacteria grown in the presence of mercury(II) developed the ability to convert mercury(II) to elemental mercury(0).[94] It was found in all of the studies with bacteria, that a great deal of concentration of mercury in all forms occured in the bacteria cells. Certain forms of bacteria have also been isolated which will convert the organo mercury forms to elemental mercury completing the circle of interconversions.[98] The possible bacterial conversions are mercury(II) to Mercury(0);[94-96] mercury(0) to mercury(II);[97] mercury(II) to organo mercury(II);[7,8] and organo mercury(II) to elemental mercury;[98] Depending on the particular strain of bacteria present in an environmental water sample, any of the mercury species can be interconverted to other forms. Controls were performed in these experiments in which only certain strains of bacteria were found to be active in the conversions of mercury species.[94,95] When the particular strains of bacteria were absent from the culture media the mercury species were not affected.[94,95]

However, it should be remembered that the above experiments were performed in incubated bacteria cultures with large concentrations of nutrients and bacteria present with optimum conditions for bacteria growth. The extent of these reactions occuring in environmental samples would be less than in the cultures because of the lower concentrations of bacteria and nutrients. The problem of reduction in

in laboratory prepared standard solutions can not be explained by a bacterial pathway because solutions would not contain any measureable concentrations of nutrients or bacteria.

Summary The method of storage using strong oxidizing agents would not be usable in a study for the differentiation of mercury species because all of the different mercury species would be oxidized to mercury(II). Considering the many problems experienced in the storage of mercury(II) samples, it is not recommended to store mercury(II) samples in the ppb range for more than one working day. Solutions to be used as a source of only mercury(II) ion must be prepared from the salt on the same working day.

Analysis of Mercury(I) and Mercury(0)

The analysis of a solution containing only mercury(0) was performed by injection of a sample aliquot into a non-reducing aeration cell containing 0.1M perchloric acid. The mercury(0) was aerated into the gas stream and measured in the UV detection cell. The analysis becomes considerably more complicated when mercury(I) is present in the sample. Under the highly ideal conditions of a sample containing mercury(0) and mercury(I) in the absence of any complexing agents, the quantitiy of mercury (0) observed was equal to the sum of the mercury(0) originally dissolved in the sample and that portion of the mercury(I)

that will disproportionate during the analysis sequence. In the analysis of real mixtures of mercury(0) and mercury (I), the amount of disproportionation varied with the sample composition. When chloride was present in the sample there was retarded disproportionation. Ideally the mercury(I) should either not disproportionate at all or completely in all media; but this is not the case. In the following experiments various ligands will be investigated for their ability to either stabilize mercury(I) from disproportionation or cause it to disproportionate. The fact that strong base, ammonia, is used in the quantitative analysis sequence to disproportionate mercury (I) led to the investigation of the analysis of mercury(I) in basic media

Mercury(I) disproportionation in basic media The first study of disproportionation was carried out in basic media; mercury(I) was found to disproportionate rapidly to mercury (II) and mercury(0). A typical UV response for the analysis of a mercury(I) solution is shown in figure 21. An initial rapid release of mercury(0) resulted which was nearly equal to one-half the mercury(I) concentration, but a continual production of mercury(0) occured resulting in a broad peak with considerable tailing. The area under the curve contained fifty percent more mercury than could have resulted from disproportionation. The analysis response of a mercury(II) solution shown in figure 22

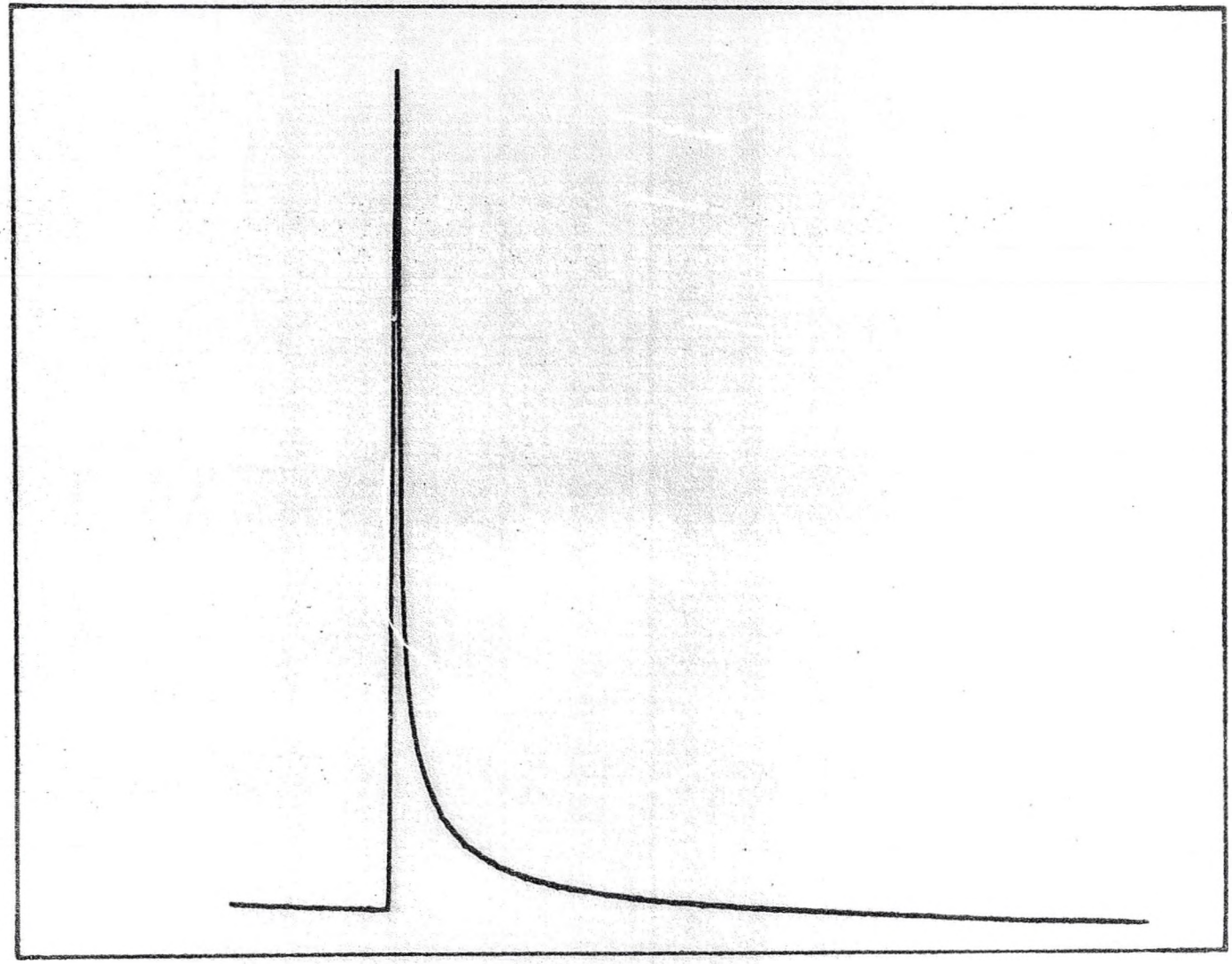


Figure 21 -- A typical UV response of the analysis of mercury(I) in 4M NaOH

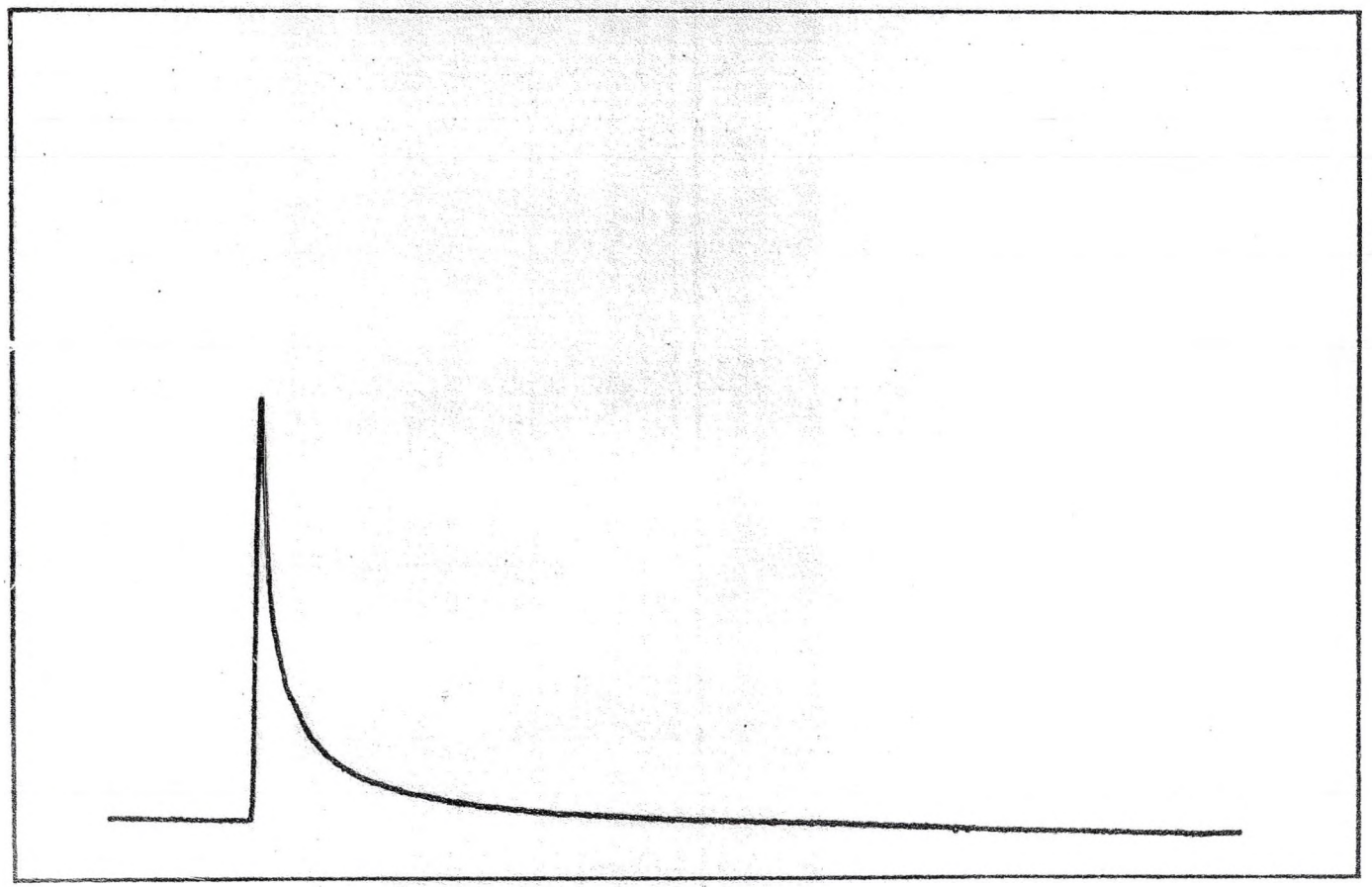


Figure 22 -- A typical UV response of the analysis of mercury(II) in 4M NaOH

gave a similar type of response without as large an initial peak. Due to the broadness of the response for the analysis of mercury(I) and mercury(II) in basic media, it was difficult to obtain an accurate measure of the mercury(0) produced. The extent of this reduction reaction was determined in an experiment in which the total mercury remaining in the aerated sample was measured in a separate analysis. This experiment was performed by aeration of a 10 ml sample of the mercury(I) solution in which the total mercury concentration remaining in the solution was followed as a function of time. Initially, the sample was aerated in 0.01M sodium chloride at pH 5.5 with negligible loss of mercury because the chloride prevents the disproportionation of mercury(I) as seen in tables 21 and 22. At a chosen time (10 or 30 min) a volume of sodium hydroxide solution was added to the mercury(I) solution. The amount of reduction or disproportionation was then detected by the continued sampling and analysis of the sample as a function of time. If disproportionation occured upon addition of the base, the remaining mercury(II) concentration of the solution would have been equal to one-half of the original mercury(I) concentration. This would be true only if the mercury(II) remaining in the solution would be stable in basic media. As shown in tables 21 and 22, the loss of concentration was more than half of the original concentration and seemed to increase with the amount of base added. This indicates that a sizable quantitiy of the mercury(II) formed by a rapid

TABLE 21

AERATION OF MERCURY(I) SOLUTION IN 0.01M SODIUM CHLORIDE AT pH 5.5

MINUTES	Total mercury remaining,[a] CHART UNITS
0	65.3
2.6	65.3
8.6	70.6
12[b]	NaOH added
12.5	21
15.8	23
21.9	18
29.1	13.2
78.7	8.7

[a] The analyses were performed by injection of 0.2 ml sample aliquots into the basic aeration cell containing 5 ml of the basic reducing mixture.

[b] Time when a 1 ml sample aliquot of 14M NaOH was added for a final solution concentration of 1.3M. Aeration and analysis of the sample was continued.

TABLE 22

AERATION OF 10 ml OF MERCURY(I) SOLUTION IN 0.01M SODIUM CHLORIDE AT pH 5.5.

MINUTES	Mercury remaining,[a] CHART UNITS
0	70
3	69.3
6	70.5
10	68.4
15	67
20	72.8
25	74
26.7[b]	NaOH added
30	46
35	45
40	42.9
45	43
50	39.5
56.6	40.2
60	39.5
70	35.5
80	34.9
82	34.2
90	32.3
100	31.7
110	32
120	27.9

[a] The analyses were performed by the injection of 0.2 ml sample aliquots into the basic reducing aeration cell containing 5 ml of the basic reducing mixture.

[b] Time when 0.5 ml of 6M NaOH was added for a final solution concentration of 0.28M.

disproportionation of mercury(I) was reduced by the base added to the solution. The reduction of mercury(II) in base has been clearly demonstrated in previous sections.

Ethylenediamine was also investigated for its ability to cause disproportionation of mercury(I). A fifty percent by volume aqueous solution of ethylenediamine was used as a reaction solution in the non-reducing aeration cell. The mercury(I) solution was injected into the amine solution with an observed analysis of 137ppb. The total analysis of the mercury(I) sample by injection into the basic reducing aeration cell gave a value of 135ppb. It is obvious from the above analysis that the amine solution caused a total reduction of the mercury(I) instead of a disproportionation. In an attempt to find out if the reduction reaction was occuring with mercury(I) or mercury (II), a mercury(II) sample was analyzed in a similar amine solution. An analysis of 123ppb was obtained for a 208ppb mercury(II) solution. This was a sixty percent reduction of the mercury(II) sample compared to a complete reduction of the mercury(I) sample. Due to the difference in the quantity of reduction, the reaction with the mercury(I) sample was probably a direct reduction of mercury(I). The use of other amines gave varing amounts of reduction. The use of amines and other bases were discontinued as reagents to force disproportionation for the analysis of any mercury mixture under non-reducing conditions.

Mercury(I) disproportionation in acidic media The analysis of mercury(I) in acidic media depends if complexing ligands are present. In perchloric or sulfuric acid media, in the absence of complexing agents, it was possible to disproportionate mercury(I) quantitatively to mercury (0). In this analysis a sample containing mercury(I) was injected into a non-reducing aeration solution of 0.1M perchloric or sulfuric acid and the resulting mercury(0) analyzed by UV detector. The quantity of mercury(0) found corresponded to the sum of the mercury(0) solubility and the amount that would have been formed by a complete disproportionation of the mercury(I). The determination of a 288ppb mercury(I) solution in 0.18M sulfuric acid was performed by dividing the solution into three portions. (A) The first portion was analyzed for the total mercury concentration by injection of a 0.2 ml sample aliquot into a basic reducing aeration cell run under standard conditions. This analysis gave a response of 264 chart units. (B) A second portion was analyzed by injection of a 0.2 ml sample aliquot into a non-reducing aeration cell containing 5 ml of 0.18M sulfuric acid. A response of 143 chart units obtained in the analysis was equal to the soluble mercury(0) concentration and one-half of the mercury(I) concentration in the sample. (C)The third portion was adjusted to be 0.01M in chloride ion and allowed to react for one hour to stabilize the mercury(I)

from disprportionation. The stabilizing effect of chloride on mercury(I) solutions will be demonstrated in detail later. The third portion was then analyzed by injection into a non-reducing aeration cell which gave a response of 33 chart units equal to the solubility of mercury(0) in the sample. The quantities measured in this experiment were,

$$\left[Hg^{+2}\right] + \left[Hg^{o}\right] + \left[Hg_2^{+2}\right] = \left[Hg\right]_A , \quad (32)$$

$$\left[Hg^{o}\right] + \tfrac{1}{2}\left[Hg_2^{+2}\right] = \left[Hg\right]_B , \quad (33)$$

and

$$\left[Hg^{o}\right] = \left[Hg\right]_C , \quad (34)$$

where the subscripts denote the type of analysis. All concentrations are in ppb. The mercury(I) concentration in the sample can be calculated by two different methods;

$$\left(\left[Hg\right]_A - \left[Hg\right]_B\right) \times 2 = \left[Hg_2^{+2}\right] = 220 , \quad (35)$$

and

$$\left(\left[Hg\right]_A - \left[Hg\right]_C\right) = \left[Hg_2^{+2}\right] = 231 . \quad (36)$$

The difference in the two methods of calculation is related to the amount of the mercury(II) in the sample. This difference was equal to the experimental error of the method of analysis and so no significance can be attached to the number. The mercury(II) concentration in the sample is less than five percent when equilibrated over a drop of elemental mercury.

Mercury(I) disproportionation in acidic chloride media

The difficulty with the above approach for the analysis of a mercury(0)-mercury(I) mixture was that a complexing ligand such as chloride in the original sample prevents the quantitative disproportionation of mercury(I). Table 23 shows the analysis of a mixture of mercury(I) and mercury (0) in various chloride and perchloric acid concentrations. The analyses were performed in a non-reducing aeration cell. Table 23 demonstrates that 0.01M chloride prevented the disproportionation of the mercury(I) from a mercury(0)-mercury(I) mixture equilibrated over a drop of mercury(0). The mercury(0) vaporized from chloride media was equal to the solubility of mercury(0) at the experimental temperature demonstrating that no disproportionation occured. When no chloride was added to the perchloric acid media, a partial disproportionation occured. If the disproportionation was quantitative, the total mercury(0) analyzed would be equal to

$$\frac{[Hg]_{total} - [Hg^{o}]_{solubility}}{2} + [Hg^{o}]_{solubility} = [Hg^{o}]_{observed} \quad . \qquad (37)$$

Experimentally the total mercury(0) vaporized was measured to be 97 and 268ppb; for quantitative disproportionation the value should have been 327ppb for the solution. Reagent grade perchloric acid contains trace quantities of chloride which interfered with the disproportionation

TABLE 23

ANALYSIS OF MERCURY(0) IN MERCURY(I) SOLUTIONS[a]

SOLUTION	UV RESPONSE CHART UNITS[b]	UV RESPONSE ppb	MERCURY SOLUBILITY LITERATURE VALUE[c]	TEMP.
0.001M NaCl	48	43	43.2	20.6°C
0.1M HCl	65.4	58.5	55.3	23.3°C
0.01M NaCl	63.5	56.8	55.3	23.3°C
0.1M NaCl	64.2	57.4	55.3	23.3°C
0.1M $HClO_4$	108	96.9	55.3	23.3°C
0.01M $HClO_4$	293	268	55.3	23.3°C

[a] The total mercury concentration of these mercury(I) solutions stored over mercury(0) was 600ppb. These solutions were prepared in the presence of air.

[b] The analysis was performed by injection of 0.2 ml sample aliquots into the non-reducing aeration cell.

[c] Reference 37.

reaction. Because chloride would be expected to be present in most samples, the above approach for the analysis of mercury(I) was not possible. However, the mercury(0) content of a solution containing mercury(I) and mercury(0) was measureable if chloride was added to complex the mercury(I) present in the solution.

Tha analysis was performed by adjusting a 104ppb mercury(I) sample stored over mercury(0) at 25°C to be 0.01M in sodium chloride. After a one hour reaction time the sample was divided into two parts. The first part of the sample was analyzed for the total mercury concentration by injection of a 0.2 ml sample aliquot into a basic aeration cell. The second portion of the sample was analyzed for the mercury(0) concentration by injection of a 0.2 ml sample into a non-reducing aeration cell containing 0.01M perchloric acid. The analysis in the non-reducing aeration cell gave a value of 62ppb which agrees with the literature value for the mercury(0) solubility at 25°C.[37] The analysis in the basic reducing cell gave a value of 172ppb for the total mercury content of the solution. The quantitiy of mercury(I) was determined by subtraction of the two analysis values to be 110ppb. This value is within the experimental error of the quantity of the mercury(I) originally added to the solution under the airless conditions.

In summation, it was possible to analyze a mixture of mercury(0) and mercury(I). The chloride concentration

was adjusted to 0.01M which allowed the detection of only the quantity of soluble elemental mercury. After performing a total mercury analysis the quantity of mercury(I) was determined by a subtraction of the two analysis values. This method of analysis is based on the assumption that as long as there is soluble mercury(0) in the sample, the mercury(II) concentration is less than five percent of the total as required by the disproportionation equilibrium constant. This type of analysis will not be usable on a mixture of mercury(II) and mercury(I) because the sample will give only a single analysis, the mercury total. This is because mercury(I) will not disproportionate in the presence of chloride, preventing any mercury(0) from being formed in the non-reducing analysis.

Analysis of Mercury(I) in the Presence of Mercury(II)

The analysis of mercury(I) in the presence of mercury (II) was performed by Grieble in the absence of complexing agents.[90] In his work various ratios of mercury(II) to mercury(I) were prepared from 0 up to 50 in 0.1M perchloric acid. These solutions were analyzed in the non-reducing aeration cell for the quantity of mercury(0) formed by disproportionation. These solutions were prepared in the absence of any chloride to avoid the retarding effect on the disproportionation reaction. As seen in table 24, when the ratio became as large as 20 the observed mercury(0) became substanially reduced and continued to decrease as

TABLE 24[a]

ANALYSIS OF MERCURY(I) IN THE PRESENCE OF MERCURY(II)[b]

Ratio, $[Hg^{+2}] / [Hg_2^{+2}]$ [c]	$[Hg^{+2}]$, M/1	UV RESPONSE, CHART UNITS[d]
0	0	95
0.2:1	1.0×10^{-7}	95
1:1	5.0×10^{-7}	95
10:1	5.0×10^{-6}	95
15:1	7.5×10^{-6}	95
20:1	1.0×10^{-5}	77.8
30:1	1.5×10^{-5}	55.1
50:1	2.5×10^{-5}	34

[a] This work taken from MS thesis, D. Grieble, University of North Dakota, December 1976.

[b] The analysis of mercury(I) was done by FAA.

[c] The concentration of mercury(I) was kept constant at 5×10^{-7} M.

[d] These values are the instrument response for the analysis of mercury(I).

the ratio increased. An explanation of this decrease in the observed mercury(0) via mercury(I) disproportionation was that a large excess concentration of mercury(II) in a solution limits the mercury(0) to a very small concentration. This concentration is the limit below which mercury(0) can not be purged efficiently from solution in the time span of an aeration-cell analysis. Some indication of this limiting concentration was determined to be about 0.01ppb in tin(II) reducing media by Howley and Ingle.[99] This analysis was not a study of a disproportionation reaction but the analysis of a total mercury content by a reducing solution. The value of 0.01ppb was their limit of detectability at which point further evaluation of mercury could not be detected.

At a ratio of 20 the observed mercury(0) was only thirty six percent of the total quantity that could have formed. When thirty six percent of mercury(0) was removed from solution, the ratio of mercury(II) to mercury(I) became 79 and prevented the detection of the remainder of the mercury(0). At the ratio of 79 there is a mercury(0) concentration of 0.014ppb remaining in the solution calculated from

$$K_d = 5.5 \times 10^{-9} M = \frac{[Hg^{+2}]\,[Hg^{o}]}{[Hg_2^{+2}]} \qquad (38)$$

which when rearranged is equal to

$$\left[Hg^{o}\right] = (5.5 \times 10^{-9}M)\frac{\left[Hg_2^{+2}\right]}{\left[Hg^{+2}\right]} = \frac{5.5 \times 10^{-9}}{79} =$$

$$7 \times 10^{-11}M = 0.014ppb \quad . \quad (39)$$

This is approximately the limit below which mercury(0) can not be efficiently aerated from solution. Two very important points should be noted in this experiment. The first point is that a ratio of 20 to 1 for a mercury (II)-mercury(I) mixture is the point at which the observed mercury(0) is greater than the expected experimental error of $\pm 3\%$. The second point is that these are ideal solutions in which a great deal of care was taken to eliminate the presence of chloride. The presence of chloride would prevent the use of this method for the analysis of a mercury(II)-mercury(I) mixture.

Study of the chloride stabilization of mercury(I) Because of the stabilizing effect of chloride in the analysis of a mercury(I)-mercury(II) mixture, the quantity of mercury (I) can not be quantitated by measurement of the mercury(0) formed by disproportionation. To develop an analytical technique for the analysis of such a mixture, it is necessary to understand exactly what is causing the stabilization and to devise a method of overcoming and circumventing the stabilization. The effects of other ligands that could also be present in the sample were

investigated to learn how to analyze real environmental water samples. The first part of this problem to be investigated was the stabilization of chloride on mercury (I) toward disproportionation.

Mercury(I) stabilization in various chloride concentrations

To investigate the effect of the chloride concentration on the amount of stabilization, a set of mercury(I) solutions in 0.18M sulfuric acid were prepared containing various amounts of chloride. These solutions were analyzed by injection into the non-reducing aeration cell containing 2 ml of 0.18M sulfuric acid. The response obtained in this analysis was equal to the sum of the elemental mercury dissolved in the solution and the mercury(0) produced by the disproportionation of the mercury(I). In this study, as seen in figure 23, mercury(I) disproportionated quantitatively below a chloride concentration of 10^{-10}M. Above concentrations of 10^{-7}M, chloride seriously retarded the disproportionation. The chloride concentration in most environmental samples of 10^{-3} and 10^{-4}M would prevent the analysis of environmental mercury(I) samples.

Mercury(I) chloride stability during aeration The stability of a mercury(I) solution in 0.01M sodium chloride at pH 5.5 toward disproportionation was also investigated as a function of time. In this experiment a 10 ml sample of 20ppb mercury(I) solution in 0.01M sodium chloride

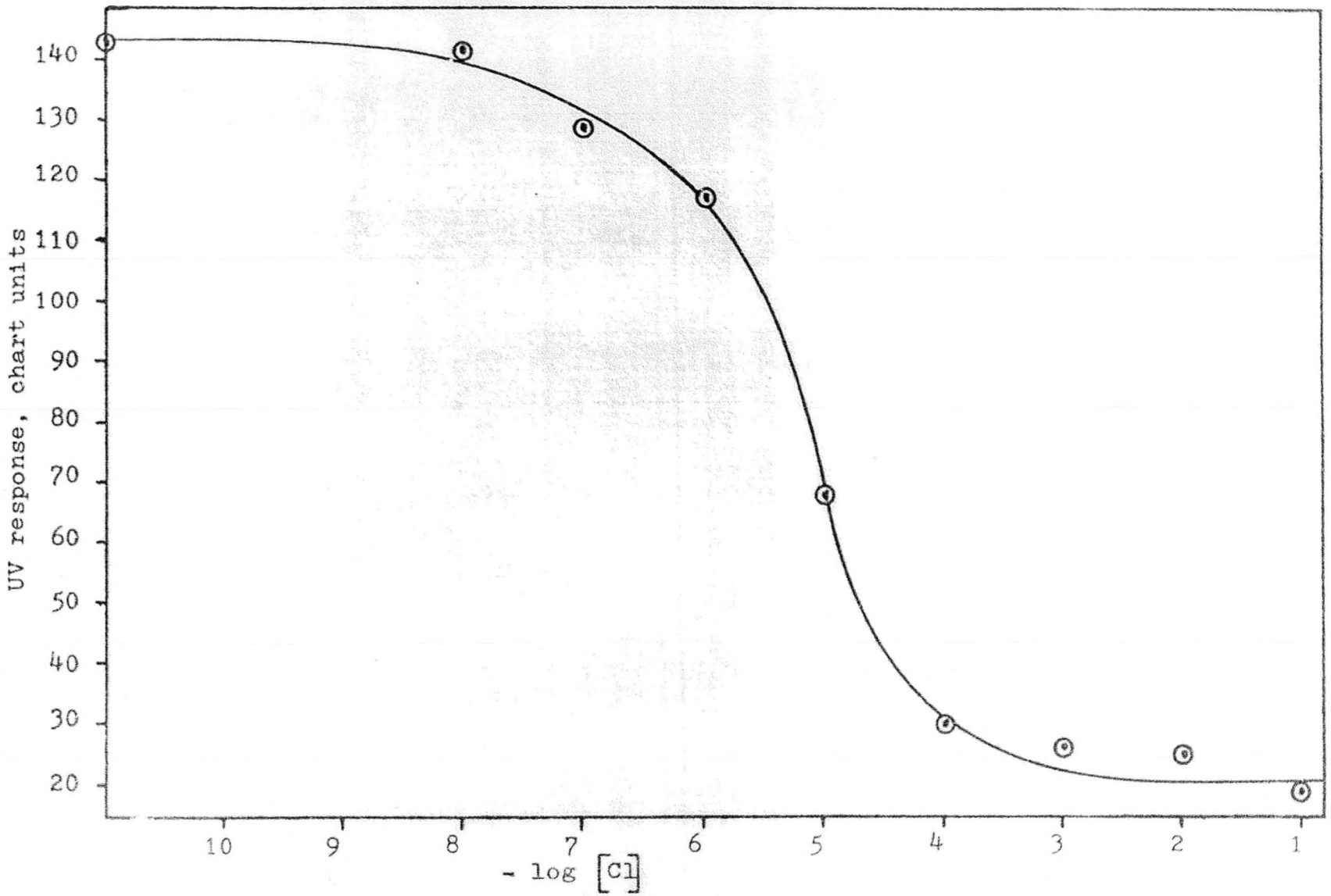


Figure 23 -- Effect of chloride concentration on the amount of disproportionation.

was purged with nitrogen for 325 minutes. The total concentration of the solution was followed as a function of time by sampling the solution and analyzing for total mercury content in the basic aeration cell. As seen in table 25, the concentration of the solution decreased less than ten percent during 325 minutes of purging indicating that mercury(I) disproportionated rather slowly compared to aeration analysis time. This data shows that during the normal analysis time of two minutes in a non-reducing aeration cell essentially no mercury(0) will be observed from the disproportionation of mercury(I) in 0.01M sodium chloride.

Chloride removal by precipitation If the chloride present in samples could be removed by some method, the analysis of a mixture of mercury(II) and mercury(I) would become possible. The quantity of mercury(I) in a mixture could then be determined by the amount of mercury(0) formed by disproportionation. The possibility of removing the chloride from the samples by the addition of silver nitrate was investigated. Silver nitrate was added to a mercury(I) solution to remove any chloride by precipitation,

$$2Ag^{+} + Hg_2Cl_2 = Hg_2^{+2} + 2AgCl \qquad (40)$$

Equation 40 has an equilibrium constant of

$$K = \left[Hg_2^{+2}\right] / \left[Ag^{+}\right]^2 = 10 \qquad .941)$$

TABLE 25

AERATION OF A MERCURY(I) SOLUTION IN 0.01M SODIUM CHLORIDE AT pH 5.5.

MINUTES	UV RESPONSE, CHART UNITS[a]
0	92
10.3	92
16.7	92
26.8	90
40.2	89.5
142	86
206	90.8
222	86
279	80
325	85

[a] The analyses were performed by injection of 0.2 ml sample aliquots into the basic aeration cell containing 5 ml of the basic reducing mixture.

The value for this constant was calculated from the solubility constants of 1.3×10^{-18} for mercury(I) chloride and 1.8×10^{-16} for silver chloride.[45, 78] When the silver ion concentration is a factor of 10 higher than the mercury(I) concentration, the formation of silver chloride is favored by a factor of 10^3 over that of mercury(I) chloride. The addition of silver nitrate should remove the chloride from the solution and the mercury(I). This study was performed by first analyzing a mercury(I) solution containing no added chloride in 0.18M sulfuric acid in the non-reducing aeration cell. The value measured was 430ppb for quantitative disproportionation of the sample. The value is the sum of the mercury(0) dissolved in the sample (70ppb) and the quantity of mercury(0) (330ppb) formed by the disproportionation reaction. The chloride and silver ion concentration were then adjusted to 10^{-4} and 2×10^{-4}M, respectively, and the solutions were allowed to react in the dark for two hours. This mixture contained only a light haze of silver chloride with no attempt being made to separate it from the sample prior to analysis. The solution was reanalyzed in the non-reducing aeration cell with a response of 722ppb. The analysis of a silver nitrate blank solution gave a response of less than one chart unit. The total mercury concentration was determined in the basic reducing aeration cell to be 763ppb. These analyses indicate almost a complete reduction of the mercury(I) to mercury(0) had occured in the solution. The mercury(I) solution initially contained 790ppb total

mercury. The decrease in the total concentration of the sample to 763ppb after a two hour reaction time was not unexpected considering that most of the sample was in the very volatile mercury(0) form at the end of the reaction. The previous study that indicates a chloride concentration above 10^{-9}M can not be tolerated, the silver ion concentration would have to be almost 1M. The addition of silver nitrate to the solution also reduced the mercury (I) to mercury(0). Because of these problems, the use of silver ions as a reagent was not possible and no further studies were carried out.

Measurement of the thermodynamic stability constant for chloro mercury(I) complex ions To determine the exact nature of the chloride stabilization of the disproportionation, a comparison of the disproportionation constants was made in the presence and absence of chloride. If the constant in the presence of chloride is smaller than the constant for the absence of chloride, the stabilization is caused by thermodynamic factors. The magnitude of the decrease in the stability constant required to prevent the disproportionation reaction can be calculated. As shown earlier a ratio of mercury(II) to mercury(I) of 79 prevented further disproportionation because the mercury(0) concentration was below a value of 0.014ppb (7×10^{-11}M) and could not be removed from the solution by aeration. For chloride to prevent the disproportionation of mercury(I),

the mercury(0) concentration in the sample must be below 0.014ppb. Based on the experimental error of ± 3 percent the limit of detectable change of mercury(II) concentration would occur at a ratio of 97 to 3 as shown in equation 44. The disproportionation in the absence of chloride is,

$$Hg_2^{+2} = Hg^{+2} + Hg^{o}(aq) \qquad (42)$$

and the disproportionation constant at 25°C is,

$$\frac{[Hg^{+2}]\,[Hg^{o}(aq)]}{[Hg_2^{+2}]} = K_d = 5.5 \times 10^{-9} M \quad . \qquad (43)$$

Therefore,

$$\frac{[Hg_2^{+2}]}{[Hg^{+2}]} = \frac{97}{3} = 32 \quad . \qquad (44)$$

The conditional disproportionation constant containing all of the terms for the chloride complexes of the two mercury species is,

$$K_{d,Cl} = \frac{C_{Hg^{+2}}\,[Hg^{o}(aq)]}{C_{Hg_2^{+2}}} \qquad (45)$$

where $C_{Hg^{+2}} = [Hg^{+2}] + [HgCl^{+}] + [HgCl_2] + [HgCl_3^{-}] + [HgCl_4^{=}]$, (46)

and $C_{Hg_2^{+2}} = [Hg_2^{+2}] + [Hg_2Cl^{+}] + [Hg_2Cl_2] + [HgCl_3^{-}] + [Hg_2Cl_4^{=}]$. (47)

Using the ratio of mercury(I) to mercury(II) of 32 for a $C_{Hg_2^{+2}}/\ C_{Hg^{+2}}$ and a mercury(0) concentration of 7×10^{-11}M, the conditional stability value must be 2×10^{-12} in order that no measurable mercury(0) be formed by vaporization during aeration. The fraction of the uncomplexed mercury(II) is given by,

$$B_{Hg^{+2}} = \frac{\left[Hg^{+2}\right]}{C_{Hg^{+2}}} \qquad (48)$$

where $$1/\ B_{Hg^{+2}} = 1 + K_{21}\left[Cl^-\right] + K_{21}K_{22}\left[Cl^-\right]^2 + K_{21}K_{22}K_{23}\left[Cl^-\right]^3 + K_{21}K_{22}K_{23}K_{24}\left[Cl^-\right]^4 \qquad (49)$$

The values of K_{21} to K_{24} are the step wise formation constants for the mercury(II) chloro complexes as defined in table 26. A similar set of equations can be obtained for mercury(I) chloro complexes for K_{11} to K_{14} as given in table 27. The conditional stability constants is equal to

$$K_{dCl} = \frac{\left[Hg^{+2}\right]\left[Hg^{o}\ (aq)\right]}{\left[Hg_2^{+2}\right]} \frac{B_{Hg_2^{+2}}}{B_{Hg^{+2}}} = K_d \frac{B_{Hg_2^{+2}}}{B_{Hg^{+2}}} \qquad (50)$$

This derivation is based on the assumption that there was no mercury(I) chloride precipitate formed. The solubility of mercury(I) chloride in water is 5.9×10^{-6}M as shown in table 27.[79] All experiments performed in this study were carried out at mercury(I) concentrations below this value. Therefore no solid mercury(I) chloride was formed

in this study.

The value of K_{dCl} can be estimated from the literature value for the formation constants of the chloro complexes for a given chloride concentration. These reactions and their formation constants are shown in table 26 and 27. At a chloride concentration of 0.01M and using the formation constants in table 27 the term, $1/ B_{Hg}+2$, is equal to 1.8×10^{9}. Before this fraction, $1/ B_{Hg_2}+2$, can be calculated, the formation constants K_{11} to K_{14} have to be determined or estimated. The term $K_{11}K_{12}$ for mercury(I) is equal to

$$Hg_2Cl_{2(s)} = Hg_2Cl_{2(aq)} \quad , (51)$$

and $$Hg_2Cl_{2(s)} = Hg_2^{+2} + 2Cl^- \quad , (52)$$

to give $$Hg_2^{+2} + 2Cl^- = Hg_2Cl_{2(aq)} \quad , (53)$$

$$K_{11}K_{12} = 4.5 \times 10^{12} \quad , (54)$$

was calculated from the literature values given in table 27. Due to the similarities in the K_{21} and K_{22} for mercury (II), the assumption will be made in the calculation of $1/ B_{Hg_2}+2$ that K_{11} and K_{12} are also equal. The constants were calculated to be 2.1×10^{-6}. The value for K_{13} was calculated from the reactions,

$$Hg_2Cl_{2(s)} + Cl^- = HgCl_3^- \quad , (55)$$

and $$Hg_2Cl_{2(s)} = Hg_2Cl_{2(aq)} \quad , (56)$$

TABLE 26

REACTIONS OF MERCURY(II) WITH CHLORIDE AT 25°C

REACTION	CONSTANT	REFERENCE
$Hg^{+2} + Cl^- = HgCl^+$	$K_{21} = 5.5 \times 10^6$	75 and 78
$HgCl^+ + Cl^- = HgCl_2$	$K_{22} = 3 \times 10^6$ 2.8×10^6	75 78
$HgCl_2 + Cl^- = HgCl_3^-$	$K_{23} = 7$ 7.9	75 78
$HgCl_3^- + Cl^- = HgCl_4^=$	$K_{24} = 10$	75

TABLE 27

REACTIONS OF MERCURY(I) AT 25°C

REACTION	CONSTANT		REFERENCE
$Hg_2^{+2} + Cl^- = Hg_2Cl^+$	K_{11}	not reported	
$Hg_2Cl^+ + Cl^- = Hg_2Cl_{2(aq)}$	K_{12}	not reported	
$Hg_2Cl_{2(s)} + Cl^- = Hg_2Cl_3^-$	$K_{13(s)}$ =	7.9×10^{-5}	79
		1.2×10^{-5}	75
$Hg_2Cl_3^- + Cl^- = Hg_2Cl_4^=$	K_{14} =	11	75
$Hg_2Cl_{2(s)} = Hg_2^{+2} + 2Cl^-$	K_{sp} =	1.3×10^{-17}	75
		1.1×10^{-18}	45
		1.3×10^{-18}	78
$Hg_2Cl_{2(s)} = Hg_2Cl_{2(aq)}$	S^o =	5.9×10^{-6}	79
$Hg_2^{+2}Cl_2 = HgCl_2 + Hg^o_{(aq)}$	K_{dCl} =	1.7×10^{-6}	75
$Hg_2^{+2} = Hg^{+2} + Hg^o(liq)$	K_r =	0.0077	75

to give $Hg_2Cl_{2(aq)} + Cl^- = HgCl_3^-$. (57)

$K_{13} = 13.4$. (58)

Again due to the similarities between K_{23} and K_{24}, it was assumed that K_{13} equals K_{14} which equals 13.4. The value for $1/ B_{Hg_2^{+2}}$ was calculated for a chloride concentration of 0.01M to be 5.1×10^8. The value of K_{dCl} calculated at 10^{-2}M chloride was 1.9×10^{-8}. The estimated value for K_{dCl} was slightly larger than the value for K_d in the absence of chloride. This does not explain why mercury(I) did not disproportionate in the presence of chloride. As previously estimated the K_{dCl} value would have to be at least 10^{-12} to prevent the disproportionation from occuring.

Due to some of the methods used for the determination of S^o and $K_{13(s)}$ there is some doubt as to the accuracy of the values obtained by the investigators.[79] An experiment was performed in this study to redetermine the values of K_{11}, K_{12}, and K_{13}.

Determination of mercury(I) chloride formation constants

To determine K_{11}, K_{12} and K_{13}, a series of saturated mercury(I) chloride solutions were prepared by equilibration over mercury(I) chloride solid and mercury(0) liquid in 0.1M perchloric acid. The chloride concentration was varied from 10^{-1} to 10^{-3}M and the solutions were prepared under nitrogen. The solutions were stored over mercury(0) to prevent the formation of more than the equilibrium

quantity of mercury(II) and to fix the mercury(0) concentration at the solubility value. After an equilibration time of 5 days, the solutions were analyzed in a basic aeration cell for the total mercury concentration. These analyses are plotted in figure 24. The intercept of the graph is 1.5×10^{-6} and the slope of the line is 5.8×10^{-5}. The mercury(0) concentration in these samples was determined to be 2.33×10^{-7}M at 21°C by analysis in a non-reducing aeration cell. The total concentration of mercury in these solutions, S, is equal to the solubility concentration of mercury(0) and the sum of the concentrations in all of the forms of mercury(I) and mercury(II).

$$S = \left[Hg^{o}\right] + \left[Hg_2^{+2}\right]_T + \left[Hg^{+2}\right]_T \quad . \quad (59)$$

In terms of the stepwise formation constants,

$$S = \left[Hg^{o}\right] + \frac{K_{sp}}{\left[Cl^-\right]^2}(1 + K_r) + \frac{K_{sp}}{\left[Cl^-\right]}(K_{11} + K_{21}K_r)$$

$$+ K_{sp}(K_{11}K_{12} + K_{21}K_{22}K_r) + K_{sp}(K_{11}K_{12}K_{13} + K_{21}K_{22}K_{23}K_r)\left[Cl^-\right] + K_{sp}(K_{11}K_{12}K_{13}K_{14} + K_{21}K_{22}K_{23}K_{24}K_r)\left[Cl^-\right]^2 \quad . \quad (60)$$

Figure 24 indicates that only those terms in S with zero order and first order dependence on the chloride are important concentrations to the total. Therefore, the intercept, 1.1×10^{-6}, is equal to $Hg^{o} + K_{sp}(K_{11}K_{12} +$

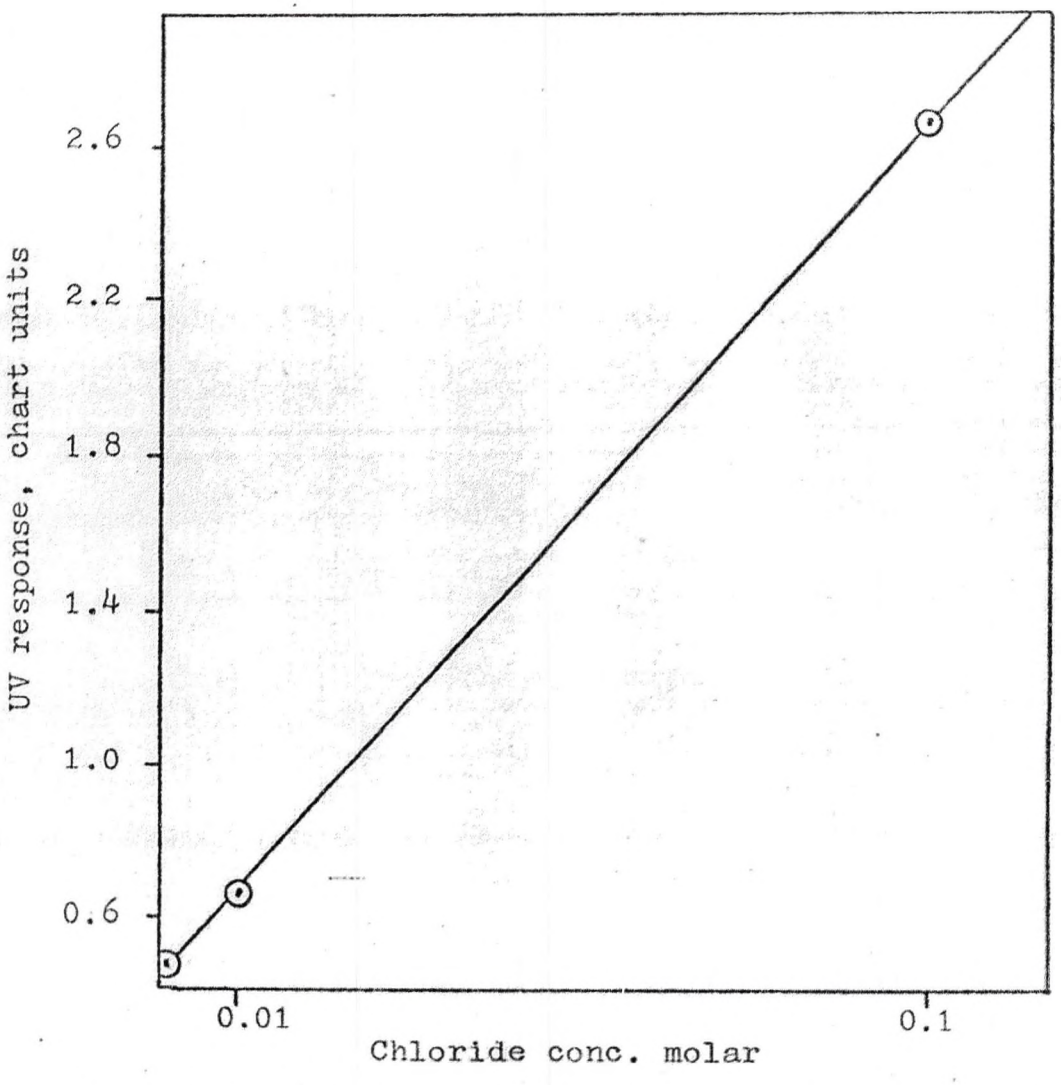


Figure 24-- Total reduction analysis of a series of saturated mercury(I) chloride solutions at varicus chloride concentrations.

$K_{21}K_{22}K_r$) and the slope of $5,8 \times 10^{-5}$ is equal to $K_{sp}(K_{11}K_{12}K_{13} + K_{21}K_{22}K_{23}K_r)$. Since the solubility of mercury(0) was found to be 2.3×10^{-7}M, the value of $K_{sp}(K_{11}K_{12} + K_{21}K_{22}K_r)$ is 5.7×10^{-5}M. Each of the terms is composed of a portion due to the mercury(I) complex and to the mercury(II) complex. If one assumes that the mercury(II) complex concentration is negligible compared to the concentration of the mercury(I), the intercept and slope can be used to calculate a maximum value of K_{11}, K_{12}, and K_{13} using the known value of K_{sp}.

$$K_{11}K_{12} = 7 \times 10^{11} \quad , \qquad (61)$$

$$K_{11}K_{12}K_{13} = 4.4 \times 10^{13} \quad . \qquad (62)$$

Assuming K_{11} is equal to K_{12} the value of these two constants were 8.4×10^5 and K_{13} is equal to K_{14}, then K_{14} is equal to 64. The value of the conditional stability constant in the presence of chloride calculated from the above data is 6.6×10^{-8}. This number is nearly the same as that calculated from the literature values on tables 26 and 27. The increased stability towards disproportionation of mercury(I) in the presence of chloride is not due to the increased stability of the mercury(I) chloro complex relative to mercury(II) ions. In fact, the stability constants values are nearly the same for mercury(I) and mercury(II).

Rate of the induced disproportionation in the presence of chloride The results of chloride stability study indicates

hat, thermodynamically, the addition of chloride will not s ift the position of equilibrium in favor of mercury(I) or mercury(II). A second explanation of the chloride st bilization of mercury(I) is that the rate of rec ystallization of an aged mercury(I) chloride prec pitate is slow, which then prevents the induced disp oportionation of mercury(I) upon aeration.

It has been known for some time that the rate of recry tallization of many freshly prepared precipitates is la ge, but diminishes with time as the particles become perfec ted. Laitimer points out that a three hour old precip tate recrystallizes relatively slowly.[100] The rate o aging is influenced by the presence of common ions in the solvent in a way not always paralleling the solubility produc effect. For example, silver bromide aging is impede by silver ions while bromide ions speedily age the pr cipitate.[101] Kolthoff and von Fischer have shown that r dioactive labeled lead is exchanged rapidly between fresh Lead chromate (15 seconds old) and solution, but a twenty minute old precipitate recrystallizes very slowly.[102] The t enty minute old precipitate required one hour of shaki g to exchange the labeled lead after the addition of the abeled lead.

To investigate this possible explanation of the phe omenon, the rate of release of mercury(0) from a mer ury(I) solution in 0.01M sodium chloride in 0.05M ni ric acid was followed as a function of time. In the

experiment a 300ppb mercury(I) solution was analyzed at various times in a non-reducing cell. The results, shown in table 28, indicate that very rapidly (within 15 minutes) the production of elemental mercury through induced disproportionation was retarded. After 45 minutes the disproportionation was completely prevented. Additional experiments were carried out where the total concentration of mercury was measured in a mercury(I) solution in 0.01M chloride over a period of four days. Initially the concentration of mercury(I) was found to be 300ppb. This analysis value did not change over four days. The results indicate then that a precipitate of mercury(I) chloride, if formed, is of colloidal size so that no loss of mercury(I) chloride from solution was observed. Therefore, the stability of the mercury(I) in chloride media appears to be due to; (1) formation of a colloidal sized particle of mercury(I) chloride, (2) a slow rate of recrystallization of mercury(I) chloride and, (3) the rate of recrystallization of the mercury(I) chloride is so slow compared to the volatilization of elemental mercury that no induced disproportionation occurs during the aeration analysis.

Additional evidence of the slow aging of mercury(I) chloride was found by King.[47] A slow rate of recrystallization of mercury(I) chloride was noted in mercury(II)-mercury(I) exchange studies in chloride media. King found that the labeled mercury was rapidly exchanged between mercury(II) and mercury(I) if mixed immediately

TABLE 28

NON-REDUCING ANALYSIS OF A 300ppb MERCURY(I) SOLUTION IN 0.01M SODIUM CHLORIDE AT 26.7°C AS A FUNCTION OF TIME.[a]

MINUTES[b]	UV RESPONSE, CHART UNITS
0	67.3
5	45.9
15	8.2
25	0.3
45	0.3[c]

[a] The analyses were performed by injection of 0.2 ml samples into the non-reducing aeration cell containing 2 ml of 0.18M sulfuric acid.

[b] Zero time was the analysis prior to addition of sodium chloride.

[c] Total concentration of the solution determined at one hour after addition of chloride was 300ppb.

with hydrochloric acid. However, if labeled mercury(II) was added to a mercury(I) chloride precipitate which was more than a few minutes old, a slow rate of exchange was observed.

Effects of other anions on disproportionation The analysis of mercury(I) in the presence of other ligands was also investigated. This study was performed with and without 10^{-4}M chloride ion present to see which ligands aid or retard the disproportionation reaction. The first part of this study was performed in the absence of chloride by preparing a 300 ppb mercury(I) solution in 0.005M nitric acid. This solution was analyzed in the non-reducing aeration cell to define the response for the quantity of mercury(0) obtained for complete disproportionation. One ml of a 1M stock solution of the anion to be tested was added to 100 ml of the mercury(I) solution and reanalyzed after a 15 minute reaction time. The results of these analyses are shown in table 29. Only EDTA, Na_2S, NaBr, and NaCl had a stabilizing effect on mercury(I) as shown by a lowering of the amount of mercury(0) formed during the analysis in the presence of the complexing ligand.

In the second part of this study, a series of anions were tested in the same manner as above except that the mercury(I) solution also contained 10^{-4}M sodium chloride. In this part of the study, the interference tested was to determine if the added anion caused an increase or decrease

TABLE 29

THE AMOUNT OF DISPROPORTIONATION OF MERCURY(I) IN THE PRESENCE OF VARIOUS LIGANDS.[a]

ANALYSIS WITHOUT INTERFERING ION, CHART UNITS	0.01M INTERFERENCE ADDED	ANALYSIS WITH INTERFERING ION, CHART UNITS
42	H_3PO_4	44
62	$Na_4P_2O_7.10H_2O$	60
45	Na_2SO_3	43
66	NaSCN	63
66	EDTA	47
68	triethanolamine	66
58	cysteinehydrochloride	57
64	NaBr	0.25
67	NaCl	8.2[b]
71	Na_2S	14.5

[a] These analyses were performed in the non-reducing aeration cell containing 5 ml of 0.1M nitric acid.

[b] At 30 minutes, the value for this solution had decreased to 2 units.

in the amount of disproportionation in the presence of 10^{-4}M chloride. As seen in table 30, the ions which seemed to increase the amount of disproportionation were phosphate and pyrophosphate. The effect of the phosphorous containing compounds was easily overcome by increasing the chloride ion concentration to 10^{-2}M eliminating them as a possible source of interference. At the present time no other ions have been found which will increase the amount of disproportionation of a mercury (I) sample in the presence of chloride, except when the solution in made basic. However, at that point total reduction also occurs.

Analysis of mercury(I) chloride in the presence of mercury (II) chloride The analysis of mercury(I)-mercury(II) mixtures in the absence of chloride was based on the disproportionation reaction of the mercury(I) in the sample. The quantity of mercury(0) obtained by the disproportionation in a non-reducing analysis was equal to one-half of the mercury(I) concentration. The mercury (II) concentration was obtained by the difference in the non-reducing analysis and an analysis in reducing media. The presence of chloride makes this approach to the analysis impossible because it prevents the disproportionation from occuring. Attempts at adding basic reagents to increase the rate of disproportionation in the presence of chloride resulted in partial or total reduction of the

TABLE 30

THE AMOUNT OF DISPROPORTIONATION OF MERCURY(I) IN THE PRESENCE OF VARIOUS LIGANDS AND 10^{-4}M SODIUM CHLORIDE.[a]

ANALYSIS WITHOUT INTERFERING ION, CHART UNITS	0.01M INTERFERENCE ADDED	ANALYSIS WITH INTERFERING ION, CHART UNITS
12.8	H_3PO_4	15.2
16.8	$Na_4P_2O_7.10H_2O$	29.7
13.9	NaSCN	10.9
12.3	Na_2S	10.1
11.2	Na_2SO_3	14
12	cysteinehydrochloride	24

[a] These analyses were made in the non-reducing aeration cell containing 5 ml of 0.1M nitric acid.

sample making this method unusable. Attempts at adding ligands to force the disproportionation had little effect in overcoming the stabilization by chloride. The approach to this type of mixture (mercury(I)-mercury(II)) was to add a reagent that would react with mercury(II) only and not the mercury(I) in the sample. The amount of reaction of this reagent must be directly proportional to the mercury (II) concentration and be detectable at the ppb level. The logical reagent that fulfills these requirements is elemental mercury. This is because mercury(0) can only reduce mercury(II) to form mercury(I) and can not reduce mercury(I).

This analysis was performed by dividing the sample into two parts and adjusting the sample to 0.01M in hydrochloric acid and allowing them to react for one hour with the chloride ions. The first portion of the sample was analyzed in the basic reducing aeration cell for total mercury. A specially prepared drop of elemental mercury was added to the second sample aliquot and the solution equilibrated for three hours. The preparation of this mercury(0) was described in the preparation of the oxygen free mercury(I) solutions. The addition of mercury(0) to this mixture reduces the mercury(II) present in the sample to mercury(I).

At equilibrium in this type of solution, the ratio of mercury(I) to mercury(II) will be fifteen in 0.01M chloride solution as calculated from the constants given in tables

26 and 27 and the assumption concerning the values of stepwise constants for mercury(I). This would correspond to a ninety-four percent conversion of all mercury(II) to mercury(I).

The excess mercury(0) was then removed by a four minute aeration with nitrogen prior to the analyis of the second portion. The purpose of removing the mercury(0) was to increase the accuracy of the analysis of low concentration by removing the large solubility quantity of mercury(0). The increase in the total concentration of the second sample was equal to the mercury (II) in the original mixture. In order to obtain the mercury(I) concentration, the quantity of mercury(II) was subtracted from the first total analysis.

$$\left[Hg\right]_1 \text{ (First analysis)} = \left[Hg_2^{+2}\right] + \left[Hg^{+2}\right] \qquad (63)$$

$$\left[Hg\right]_2 \text{ (Second analysis)} = \left[Hg_2^{+2}\right] + 2\left[Hg^{+2}\right] \qquad (64)$$

$$\text{therefore} \quad \left[Hg^{+2}\right] = \left[Hg\right]_2 - \left[Hg\right]_1 \qquad (65)$$

$$\text{and} \quad \left[Hg_2^{+2}\right] = 2\left[Hg\right]_1 - \left[Hg\right]_2 \quad . \qquad (66)$$

To determine the accuracy of this method, a mer y (II) sample prepared in 0.01M hydrochloric acid by the oxygen free method and analyzed in the basic reducing aeration cell giving an average analysis of 76 $\pm$4 chart units for five determinations. Mercury(0) was then added to the

stirred solution under oxygen free conditions. After a three hour reaction time the excess mercury(0) was purged from the solution by a four minute aeration and the solution was reanalyzed for an average value of 150 ±2 chart units for five injections. Within the experimental error of this analysis a quantitative reaction was achieved. To determire the applicability of this method on solutions of lower concentration, this analysis was repeated on a 5ppb mercury (II) solution. The first total analysis gave an average value of 21 ±3 chart units and a second average total analysis for the newly formed mercury(I) of 44 ±2 chart units for five sample injections. The greatest problem for the use of this method was the possible contamination of the sample with ionic mercury dissolved in the added mercury (0) or by air oxidation of the mercury(0). By using the oxygen free preparation and the special method of preparation of mercury(0) these problems can be minimized during the three hour reaction time. This source of ionic mercury will be one of the factors preventing the use of this method on dilute samples of less than 5ppb.

A second problem in the analysis of natural water samples would be the presence of an oxidizing agent with a higher oxidation potential than mercury(I). Presence of an oxidizing agent would mean that all of the mercury would be present as mercury(II), mercury(I) would not be present in the sample. During the analysis of such a sample by this method the oxidizing agent would react with the mercury(0) that was

added to the solution. This would cause an analysis value larger than could be obtained by the reaction of mercury(II) with mercury(0) to form mercury(I). In the absence of an oxidizing agent, the maximum increase in concentration would be double the initial analysis. Such a sample would contain only mercury(II). If the second analysis was less than the initial analysis, the sample would contain a mercury(II)-mercury(I) mixture. If the second analysis were more than double the initial analysis, the sample would contain mercury(II) and an oxidizing agent.

SUMMARY

1. Mercury(0) can be vaporized from an acidic media and measured quantitatively by FAA means.
2. The disproportionation reaction of mercury(I) can not be induced quantitatively by aeration in basic media because of the total reduction of the mercury(I).
3. The disproportionation reaction of mercury(I) in perchloric acid can be analyzed quantitatively in a mercury(0)-mercury(I) mixture or in a mercury(I)-mercury(II) mixture up to a ratio of 20:1 mercury(II) : mercury(I). Complexing agents such as halides or sulfur containing ligands must be absent from the solution.
4. If chloride is present in a mixture of mercury(I)-mercury(0), the mercury(0) was quantitated by a non-reducing analysis. The mercury(I) was quantitated by subtraction of the non-reducing analysis from a reducing analysis.
5. Mercury(I) chloride at the ppb level does not precipitate from solution, but appears to form a colloid.
6. The rate of recrystallization of mercury(I) chloride is so slow after three hours that no disproportionation can be induced by aeration of a solution to remove the mercury(0).
7. A mixture of mercury(I)-mercury(II) can be analyzed for each compound by the addition of chloride and mercury(0) (specially prepared) to convert the mercury(II) quantitatively to mercury(I). The increase in mercury(I) concentration then measured was equal to the mercury(II) concentration in the original sample.

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