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DETERMINATION OF FORMATION CONSTANTS FOR THE BINDING OF METHYLMERCURY BY THE AMINO GROUP OF SELECTED AMINOCARBOXYLIC ACIDS

by

© CHIRAPA SUVANPRAKORN

A THESIS

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FALL, 1973

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled DETERMINATION OF FORMATION CONSTANTS FOR THE BINDING OF METHYLMERCURY BY THE AMINO GROUP OF SELECTED AMINOCARBOXYLIC ACIDS submitted by CHIRAPA SUVANPRAKORN in partial fulfilment of the requirements for the degree of Master of Science.

.....
Supervisor

.....

.....
External Examiner

Date:....., 1973

To my parents.

ABSTRACT

The aqueous solution chemistry of methylmercury and the binding of methylmercury by the amino group of fourteen selected aminocarboxylic acids has been investigated by the pH-titration method. Equilibrium constants for the reaction of CH_3Hg^+ with hydroxide ion to form CH_3HgOH and with CH_3HgOH to form $(CH_3Hg)_2OH^+$ were determined from pH-titration data. Previous studies using nuclear magnetic resonance spectroscopy have shown that methylmercury binds to the carboxylate group of aminocarboxylic acids at pH less than 5, and to the amino group at pH greater than 9. From pH-titration data obtained at pH greater than 9, the formation constants for the binding of CH_3Hg^+ by the amino group of the aminocarboxylic acids were determined. The logarithm of the formation constants is in the range 7.4 to 9.4. The magnitude of the formation constants for coordination of CH_3Hg^+ by the amino dentate of an aminocarboxylic acid is found to be dependent on the number of carboxylate groups in the aminocarboxylic acid, the degree of substitution on the nitrogen atom and the basicity of the nitrogen atom. Use of nitrilotriacetic acid as a therapeutic reagent for treating methylmercury poisoning is discussed.

ACKNOWLEDGEMENT

I would like to express my most sincere appreciation and deep gratitude to Dr. D. L. Rabenstein for his encouragement, supervision, and personal understanding throughout the course of this research.

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CHAPTER I

INTRODUCTION

Mercury Poisoning

It is well known that mercury and its compounds are poisonous to man and other animals. The toxic properties of mercury and its compounds result from their ability to bind to protein. As a result, they can inactivate enzymes and disrupt cell membranes, leading to cellular death and destruction of any tissue with which they come into contact in sufficient concentration. Mercury poisoning can result from the inhalation of mercury vapor, from long term administration of mercury drugs, by continued exposure in industries that utilize mercury or its salts, and from ingestion of mercury contaminated foodstuffs.[1]

Mercury and its compounds are often classified as inorganic mercury and organic mercury.[2] Inorganic mercury includes the elemental form and the salts of mercurous and mercuric ions. Compounds in which mercury is covalently bonded to carbon are organomercurials and mercury in this state of cor nation is described as organic mercury. Table I summarizes some of the different forms of mercury.[3]

TABLE I

Inorganic and Organic Forms of Mercury

INORGANIC MERCURY

Hg^0	Hg_2^{++}	Hg^{++}
ELEMENTAL	MERCUROUS ION	MERCURIC ION

ORGANIC MERCURY

$C_6H_5Hg^+$	$H_3C\text{-}Hg^+$
PHENYLMERCURIC ION	METHYLMERCURIC ION
$H_3CCH_2Hg^+$	CH_3HgCH_3
ETHYLMERCURIC ION	DIMETHYLMERCURY

All of the forms of mercury shown in Table I are toxic. However, both the toxicity and the symptoms of poisoning by the different forms are different. Methylmercury is the most toxic form. Its propensity for the nervous system, its long retention within the body, and its effects on developing tissue pose a particularly serious problem.[1] In two outbreaks of mercury poisoning in Japan, the mercury was identified as methylmercury. The first of these occurred in Minimata where there

were 121 cases with 46 deaths reported as of 1970. The second occurred in the riverside villages of the Agano River in Niigata prefecture where 47 cases with 6 deaths were reported through 1970.[4] Both incidents were traced back to consumption of fish contaminated with methylmercury. Mercury vapor and short chain alkyl mercurials affect the central nervous system but in a completely different way. Of particular significance is the fact that the symptoms of alkylmercurial poisoning are irreversible. Little is known about the chronic toxicity of mercuric salts to humans.[1]

Sources of Methylmercury in the Environment

Most of the mercury discharged into the environment is in the form of inorganic salts or the metal itself. Until several years ago, the major source of alkylmercury compounds in the environment was thought to be seed treatments used to protect against fungus, for example, methylmercury dicyanodiamide (Panogen).[5] Recently, however, it has been discovered that inorganic mercury can be converted directly or indirectly to methylmercury or dimethylmercury by microorganisms in the environment. It has been

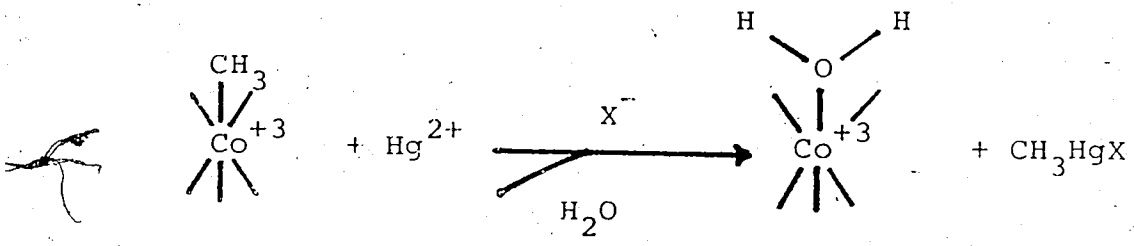
reported by Kurland that inorganic mercury issued with [illegible] fluent into the sea may be alkylated by p[illegible] other marine life.[6] Jensen and Jernlo[illegible]strated that microbial methylation of mercuric chloride to both methyl- and dimethylmercury occurs in cultures from aquaria sediments and from decaying fish.[7] They postulated that these reactions could occur by the following two reaction paths.

a) $Hg^{2+} \xrightarrow{2R\text{-}CH_3} (CH_3)_2Hg \xrightarrow{X} CH_3Hg^+ + XCH_3$ or

b) $Hg^{2+} \xrightarrow{R\text{-}CH_3} CH_3Hg^+ \xrightarrow{R\text{-}CH_3} (CH_3)_2Hg$

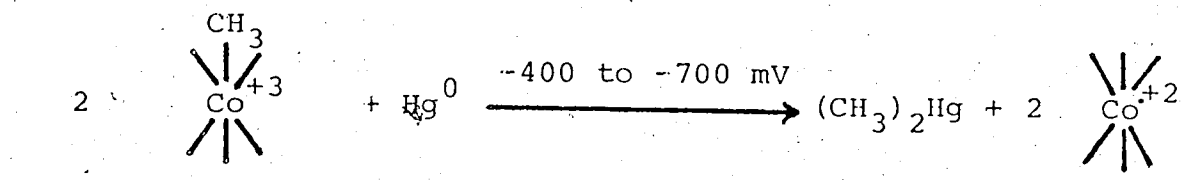
In 1968, using cell extracts of a methanogenic bacteria, Wood et al were able to provide some support for a combination of both a and b.[8] They also reported the formation of methylmercury and dim[illegible]ylmercury from Hg^{2+} by transfer of a methyl group from methylcobalamin, a common coenzyme in both anaerobic and aerobic bacteria.[9] Significant quantities of this coenzyme are present in sediments [10] depending on the microbial population of the sediments. Any microorganism which is capable of synthesizing methylcobalamin will have the potential

to methylate mercury. This group also showed that the reaction mechanism can be both enzymatic and non-enzymatic.[11] In the non-enzymatic reaction, the mechanism proceeds by electrophilic attack[12] (proved by using a spin-label to determine the valency of the cobalt atom during catalysis[13]) by Hg^{2+} on methylcobalamin as follows:



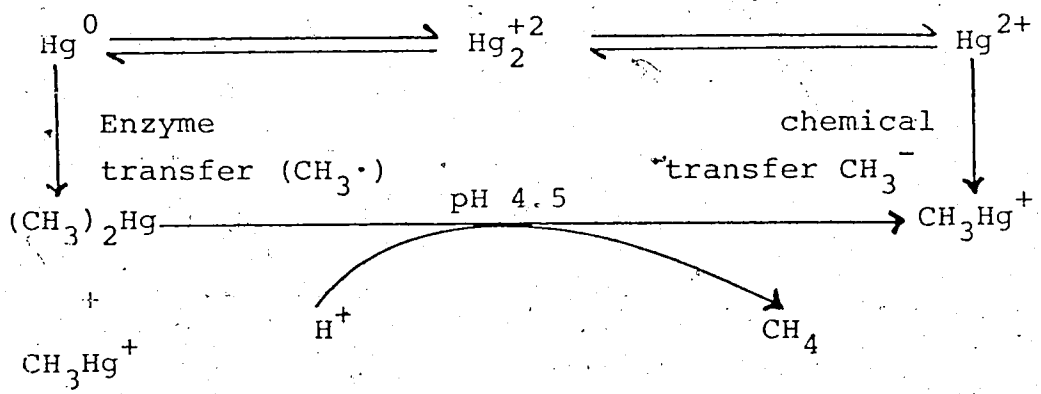
X^- = anion

Under anaerobic conditions Hg^{2+} is reduced to Hg^0 and, depending on the concentration of Hg^0, methylmercury and dimethylmercury are formed as products. Studies of these reactions with radioactive isotopes[14] and with spin-labeled methylcobinamide[13] indicate that methyl groups are transferred as radicals. The following general enzymatic reaction occurs:



From these studies on the methylation of inorganic

mercury, the following scheme for the synthesis of methylmercury in the environment has resulted.



Recently, Desimone has shown that inorganic mercury salts are methylated in aqueous solution by trimethylsilyl salts commonly used as nmr reference compounds.[15] This suggests that an additional pathway for the formation of methylmercury in the environment might be by reaction with naturally occurring methylsilyl compounds.

Methylmercury in Foodstuffs

Fish has been shown to be the most important source of methylmercury in foodstuffs, although it has also been identified in a variety of other foods. Westöö found that 89 ± 6%, 94 ± 6%, 80 ± 4%, and 82 ± 4% of the mercury in samples of the muscle

tissue of perch, pike, haddock and cod respectively were methylmercury. She also found 92%, 73%, 73 ± 5%, 70 ± 5%, and 89 ± 13% of the mercury in samples of ox, hen, pig liver, egg yolk, and egg white respectively to be methylmercury.[16] Bache, Gutanmann, and Lisk found the amount of methylmercury and the relative proportion of methylmercury to total mercury in lake trout of precisely known ages to increase with age. Relative proportions of methylmercury varied between 30 - 80%.[17] Thus, it has been demonstrated that mercury is present in the food chain as methylmercury, presumably entering the food chain after conversion to this form from metallic mercury and inorganic mercury by microorganisms in the environment.

Coordination Chemistry of Methylmercury

In general, the coordination chemistry of methylmercury has not been quantitatively characterized. Such information is potentially useful for an understanding of the behavior of methylmercury in the environment and in biological systems and in the design of therapeutic agents for treating methylmercury poisoning. A variety of therapeutic reagents have

been tested on rats, for example, British Anti Lewisite (BAL), penicillamine, ethylenediamine tetraacetic acid (EDTA), polythiol resins,[1] pyridoxine 5-thiol[18] and nitrilotriacetic acid (NTA),[19-20] with the most successful ones being those that contain at least one thiol group. It is known that methylmercury binds strongly to the sulfhydryl group of amino acids, peptides and proteins[21-23] and this reaction forms the basis for the use of methylmercury as a titrant for free sulfhydryl groups in purified enzyme and as an enzyme inhibitor to establish the involvement of sulfur at the catalytic site of the enzyme. Furthermore, with a detailed knowledge of the coordination chemistry of methylmercury, it may be possible to understand at the molecular level why a diet containing selenium in tuna decreases the toxicity of methylmercury to Japanese quail and rats.[24]

Solubility studies,[25] crystal structure determinations[26] and spectroscopic investigations[27-28] of methylmercury compounds have shown that the principal coordination number of methylmercury is one. Schwarzenbach and Schellenberg determined the formation constants of the methylmercury complexes of several inorganic ligands using the pH-titration technique for measuring formation

constants.[25] Their observation that methylmercury reacts with hydroxide to form CH_3HgOH and $(CH_3Hg)_2OH^+$, as described by equation (1) and (2), is of particular importance to an understanding of the solution chemistry of methylmercury.

$$CH_3Hg^+ + OH^- \rightleftarrows CH_3HgOH$$

$$K_1 = \frac{[CH_3HgOH]}{[CH_3Hg^+][OH^-]} = 2.34 \times 10^9 \qquad (1)$$

$$CH_3Hg^+ + CH_3HgOH \rightleftarrows (CH_3Hg)_2OH^+$$

$$K_2 = \frac{[(CH_3Hg)_2OH^+]}{[CH_3Hg^+][CH_3HgOH]} = 2.34 \times 10^2 \qquad (2)$$

Waters of solvation have been omitted from the reactions for the sake of simplicity. Simpson reported the association constants of methylmercury with compounds containing functional groups common in proteins, including acetate, ammonia, imidazole, the amino group of his idine, the imidazole group of histidine and the sulfhydryl group of cysteine.[23] In evaluating these formation constants, however, he did not take into account the formation of $(CH_3Hg)_2OH^+$ and thus, the validity of his results is questionable.

Recently, nuclear magnetic resonance spectroscopy has been used to evaluate the formation constants of methylmercury complexes of selected carboxylic acids,[29] amines, and amino acids,[30] and to elucidate the binding of methylmercury by sulfur containing amino acids and by glutathione.[31]

The nuclear magnetic resonance studies with model compounds have shown that the functional group to which methylmercury binds in amino acids containing only amino and carboxylic acid functional groups is highly pH-dependent.[30] To illustrate the fractional concentrations of methylmercury containing species a solution containing 0.200 M methylmercury and 0.200 M acetic acid are shown as a function of pH in the upper half of Figure 1. For comparison, the fractional concentrations of methylmercury containing species in a solution containing 0.200 M methylmercury and 0.200 M methylamine are shown in the bottom half of Figure 1. The formation of the methylmercury complex of acetic acid is described by equation (3),

$$CH_3Hg^+ + CH_3COO^- \rightleftarrows CH_3HgO_2CCH_3$$

$$K_f = \frac{[CH_3HgO_2CCH_3]}{[CH_3Hg^+][CH_3COO^-]} = 3.2 \times 10^3 \qquad (3)$$

and that of the methylmercury amine complex by

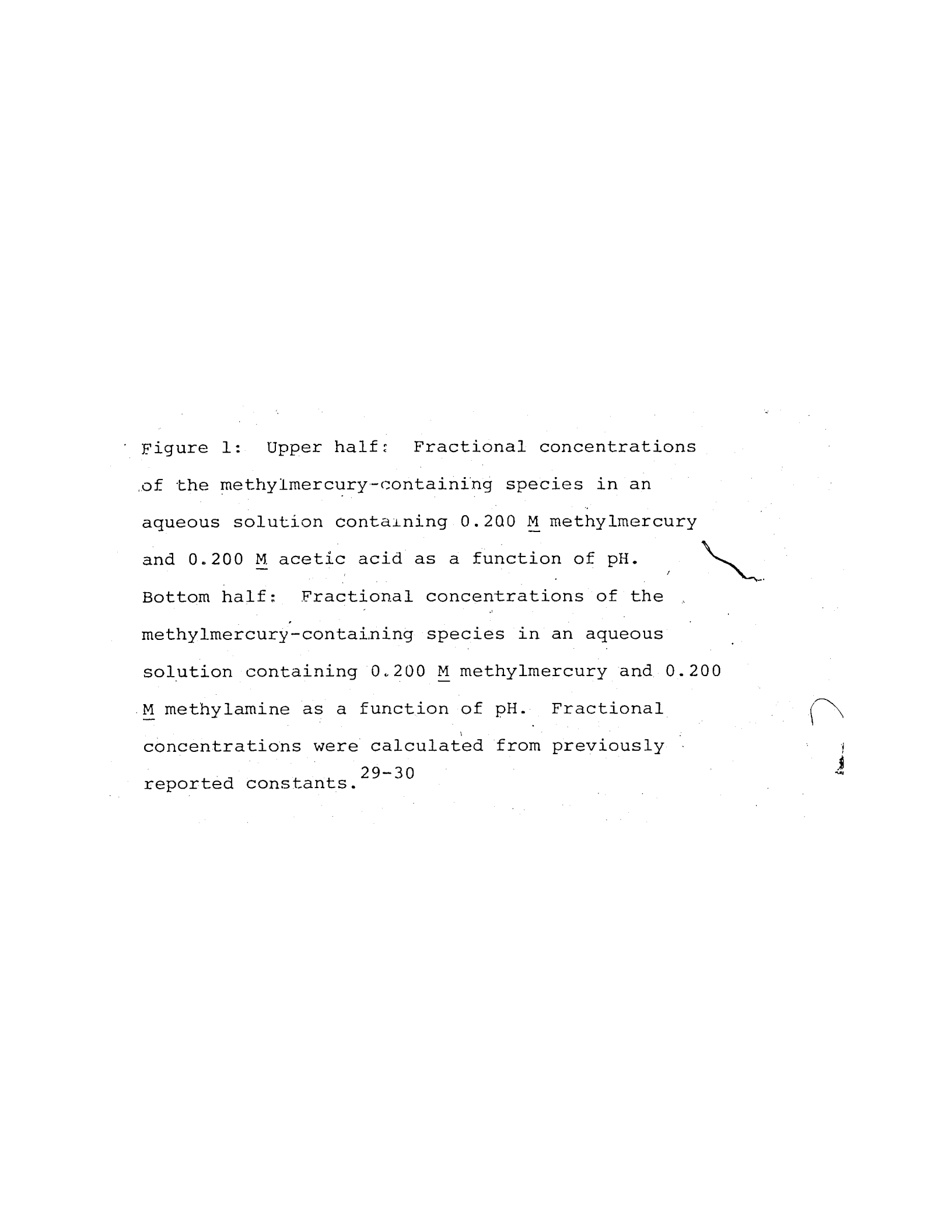
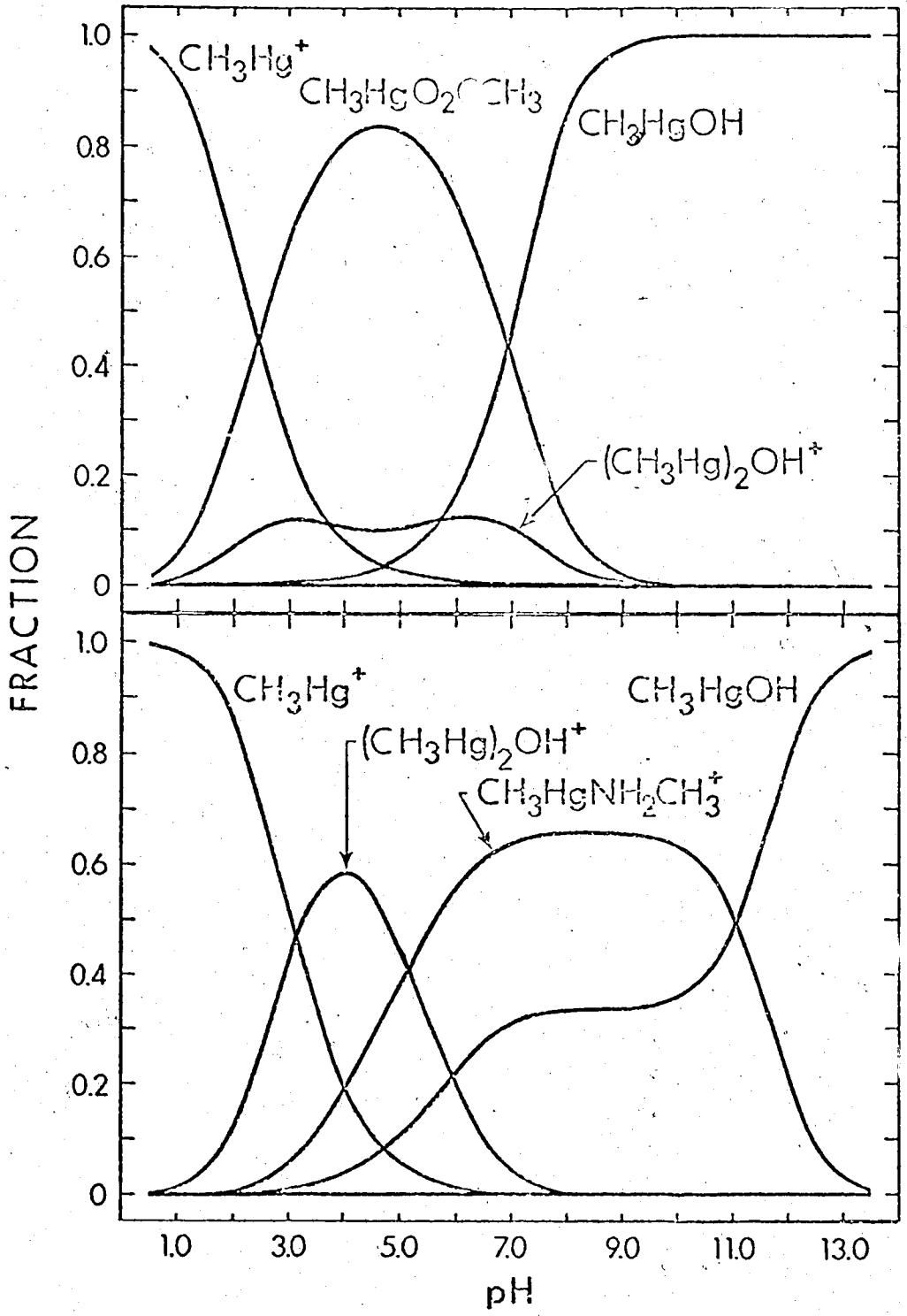


Figure 1: Upper half: Fractional concentrations of the methylmercury-containing species in an aqueous solution containing 0.200 M methylmercury and 0.200 M acetic acid as a function of pH. Bottom half: Fractional concentrations of the methylmercury-containing species in an aqueous solution containing 0.200 M methylmercury and 0.200 M methylamine as a function of pH. Fractional concentrations were calculated from previously reported constants.[29-30]


FRACTION
1.0
0.8
0.6
0.4
0.2
0
CH_3Hg^+
$CH_3HgO_2CCH_3$
CH_3HgOH
$(CH_3Hg)_2OH^+$
CH_3Hg^+
CH_3HgOH
$(CH_3Hg)_2OH^+$
$CH_3HgNH_2CH_3^+$
1.0
3.0
5.0
7.0
9.0
11.0
13.0
pH

equation (4).

$$CH_3Hg^+ + CH_3NH_2 \rightleftarrows CH_3Hg(NH_2CH_3)^+$$

$$K_f = \frac{[CH_3Hg(NH_2CH_3)^+]}{[CH_3Hg^+][CH_3NH_2]} = 3.7 \times 10^7 \qquad (4)$$

Qualitatively, the data in Figure 1 shows that the methylmercury complexes of acetic acid and methylamine form at intermediate pH values. In acidic solution, protons compete with CH_3Hg^+ for the ligand, that is, the complex is dissociated due to protonation of the ligand.

$$CH_3HgX + H^+ \rightleftarrows CH_3Hg^+ + HX$$

In basic solution, hydroxide ion competes with the ligand for CH_3Hg^+

$$CH_3HgX + OH^- \rightleftarrows CH_3HgOH + X^-$$

Acetic acid is approximately six orders of magnitude more acidic than methylamine, however, so that the pH range over which the above reactions take place is different for carboxylic acid complexes and for amine complexes. These results indicate that, in multidentate ligands containing amino and carboxylate donor groups, the site to which coordination occurs

will depend on the solution pH. This has been found to be the case in the nmr studies of binding of CH_3Hg^+ by aminocarboxylic acids.[30] These studies have shown that, in general, the methylmercury-carboxylate complex is the major species at pH's less than 5 whereas at pH's greater than 9 the amino group is the main site of interaction with methylmercury. In the pH region 5 to 9, the methylmercury is shifting from the carboxylate group to the amino group.

The Present Study

The nmr studies have defined the pH region over which the amino group is the predominant binding site in the methylmercury complexes of aminocarboxylic acids.[30] Formation constants for these complexes with several aminocarboxylic acids have also been determined by nmr. Because of the high concentrations necessary in the nmr experiment, however, the ionic strength is high and difficult to control.

In the present study, the formation constants for the binding of methylmercury by the amino group of fourteen different aminocarboxylic acids were determined using the pH-titration technique. The aminocarboxylic acids and their structures are given

in Table II.

The advantage of the pH-titration method is that measurements can be made at lower concentration and at well-defined ionic strengths. The method can be used, however, only when the exact nature of the complexation reaction is known, as it is in this case from the nmr studies, since the measurement is of a macroscopic property which does not provide definitive information at the molecular level.

TABLE II

Names and Structures of the Aminocarboxylic Acids Studied

Ligand	Structure
Glycine	$^{+}NH_3CH_2\ CO_2^-$
α-Alanine	$^{+}NH_3\ CH(CH_3)CO_2^-$
DL-Valine	$^{+}NH_3CH[CH(CH_3)_2]CO_2^-$
L-Leucine	$^{+}NH_3CH[CH_2\ CH(CH_3)_2]CO_2^-$
L-Isoleucine	$^{+}NH_3\ CH[CH(CH_3)C_2H_5]CO_2^-$
L-Phenylalanine	$^{+}NH_3\ CH(CH_2\ C_6H_5)CO_2^-$
Aspartic acid	$^{+}NH_3\ CH(CH_2\ CO_2^-)CO_2^-$
Glutamic acid	$^{+}NH_3CH[(CH_2)_2\ CO_2^-]CO_2^-$
S-Methylmercury cysteine	$^{+}NH_3\ CH(CH_2\text{-}S\text{-}HgCH_3)CO_2^-$
N-Methyl glycine	$^{+}NH_2(CH_3)\ CH_2\ CO_2^-$
Iminodiacetic acid	$^{+}NH_2(CH_2\ CO_2^-)_2$
Methylimidodiacetic acid	$^{+}NH(CH_3)(CH_2\ CO_2^-)_2$
Nitrilotriacetic acid	$^{+}NH(CH_2\ CO_2^-)_3$
Ethylenediaminetetra-acetic acid	$(CH_2CO_2^-)_2NH^+\ (CH_2)_2NH^+\ (CH_2CO_2^-)_2$

CHAPTER II

EXPERIMENTAL

Chemicals

Glycine, α-alanine, L-leucine, L-glutamic acid, disodium ethylenediamine tetraacetic acid (Fischer Scientific Company), DL-valine, aspartic acid (Eastman Organic Chemicals), L-isoleucine (Aldrich Chemical Company), L-phenylalanine (Nutritional Biochemicals Corp.), ethylenediaminetetraacetic acid (J. T. Baker Chemical Co.), N-methyl glycine, iminodiacetic acid, methyliminodiacetic acid, and nitrilotriacetic acid (K & K Laboratories Inc.) were used without further purification. Cysteine (Nutritional Biochemicals Corp.) was recrystallized from water as the free base. Methylmercuric hydroxide (Alfa Inorganics) was purified as described below.

All other chemicals were reagent grade and were used without further purification.

Purification of Methylmercuric Hydroxide

The methylmercuric hydroxide contained an acetate impurity,[29] as indicated by a singlet in

the proton magnetic resonance spectrum at 1.26 ppm upfield from the central resonance of the triplet for the tetramethylammonium ion, and an insoluble material. The methylmercuric hydroxide was purified by first passing an approximately 0.4 M solution in triply distilled water through a 0.2 micron membrane filter two or three times to remove the insoluble fraction. The filtrate was then passed through an anion exchange column (Dowex 1-X8) in the hydroxide form to remove the acetate ions. The acetate impurity in the methylmercuric hydroxide presumably was sodium acetate.

Standardization of the Methylmercuric Hydroxide Solution

The methylmercuric hydroxide solution prepared above was standardized by several methods.

The first method was a titration with sodium thiosulfate in which the end point was located by nuclear magnetic resonance spectroscopy.[29] A value of 0.432 ± 0.012 M was obtained for the concentration by this method.

Because the nmr titration procedure is time consuming, two potentiometric methods for standardizing the solution were investigated. The first of

these involved adding a small excess of sodium thiosulfate to displace the hydroxide from the methylmercuric hydroxide.

$$CH_3HgOH + S_2O_3^{2-} \rightleftharpoons CH_3HgS_2O_3^- + OH^-$$

The hydroxide was then titrated with standard nitric acid; the end point was located by measuring the pH throughout the titration. This method yields accurate results only if the solution contains no cation other than CH_3Hg^+. By flame photometry, it was found that a stock solution containing 0.342 M CH_3HgOH (as determined by the nmr titration) also contained 0.030 Na^+. Since the methylmercuric hydroxide used in the present work was from the same source, the stock solution prepared for use in this work presumably also was a mixture of methylmercuric hydroxide and sodium hydroxide after being passed through the ion exchange column. The pH-titration method yielded a concentration of 0.366 M for the above stock solution, confirming by comparison with the nmr results that the presence of sodium causes high results. Consequently, this method was not investigated further.

The second potentiometric method investigated involved titration with standard sodium chloride

and is based on the reaction of chloride with methylmercuric ion to form methylmercuric chloride.

$$CH_3Hg^+ + Cl^- \rightleftarrows CH_3HgCl$$

$$K_f = \frac{[CH_3HgCl]}{[CH_3Hg^+][Cl^-]} = 1.78 \times 10^5 \quad ^{25}$$

The end point was determined potentiometrically using an Ag/AgCl indicating electrode and a saturated calomel reference electrode. To minimize the competition of hydroxide with titrant for CH_3Hg^+, the titration was performed in acidic solution. Under these conditions the major fraction of methylmercury exists as the hydrated cation, the actual distribution between species depending on the total methylmercury concentration and the pH.[29] Initially, the titration was attempted at pH 2 in aqueous solution, yielding the titration curve shown in Figure 2. The titration curve for these conditions is drawn out because of the small formation constant for the CH_3HgCl complex, making it difficult to obtain precise end points. To increase the formation constant, and thus the sharpness of the break at the end point, the titration was performed in 80% ethanol by volume. A typical titration curve for

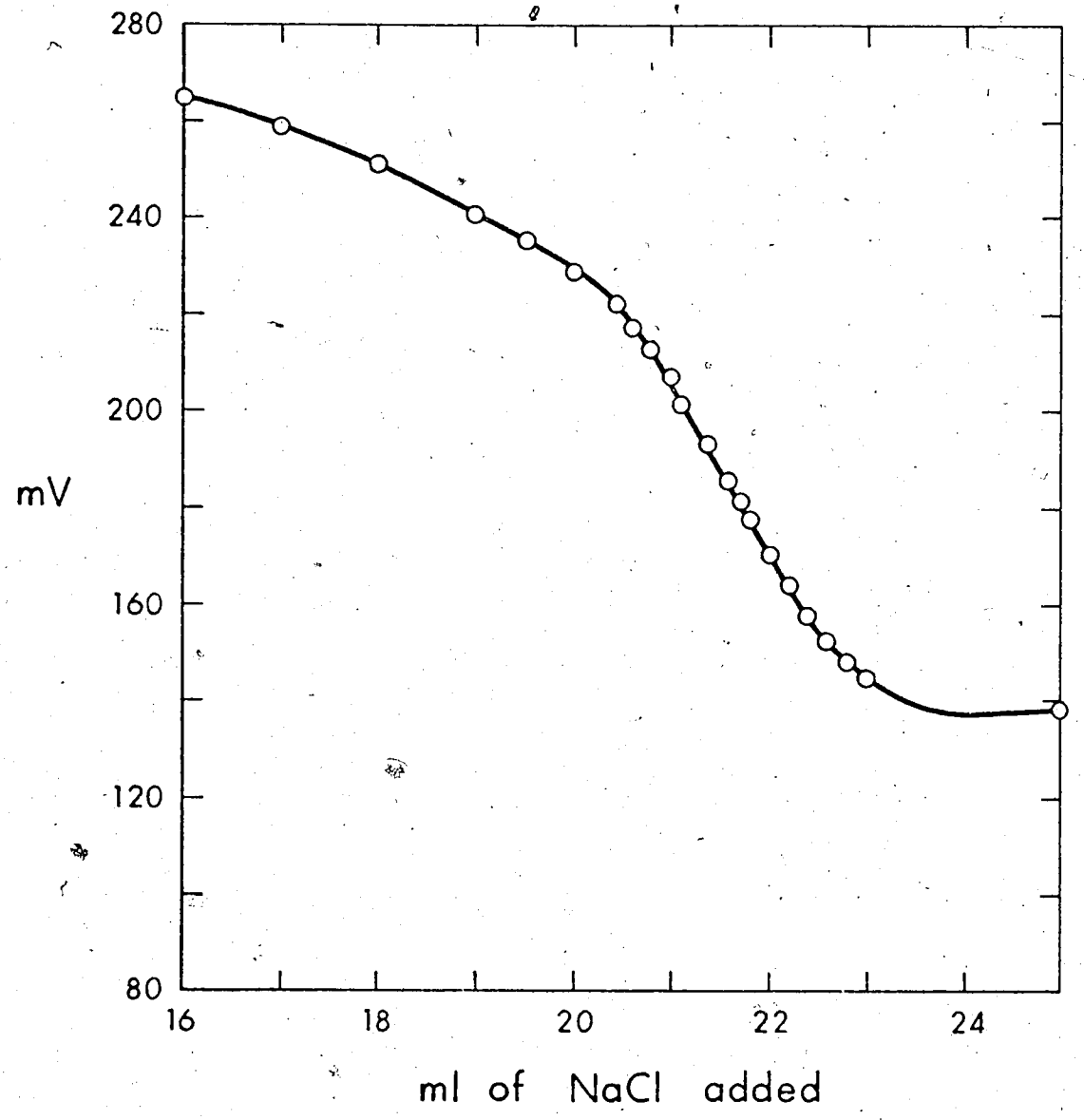


Figure 2: Potentiometric titration curve for 5.00 ml of methylmercury stock solution diluted to 100 ml with distilled water. pH was adjusted to 2 with concentrated nitric acid. The titrant was 0.1024 M sodium chloride.

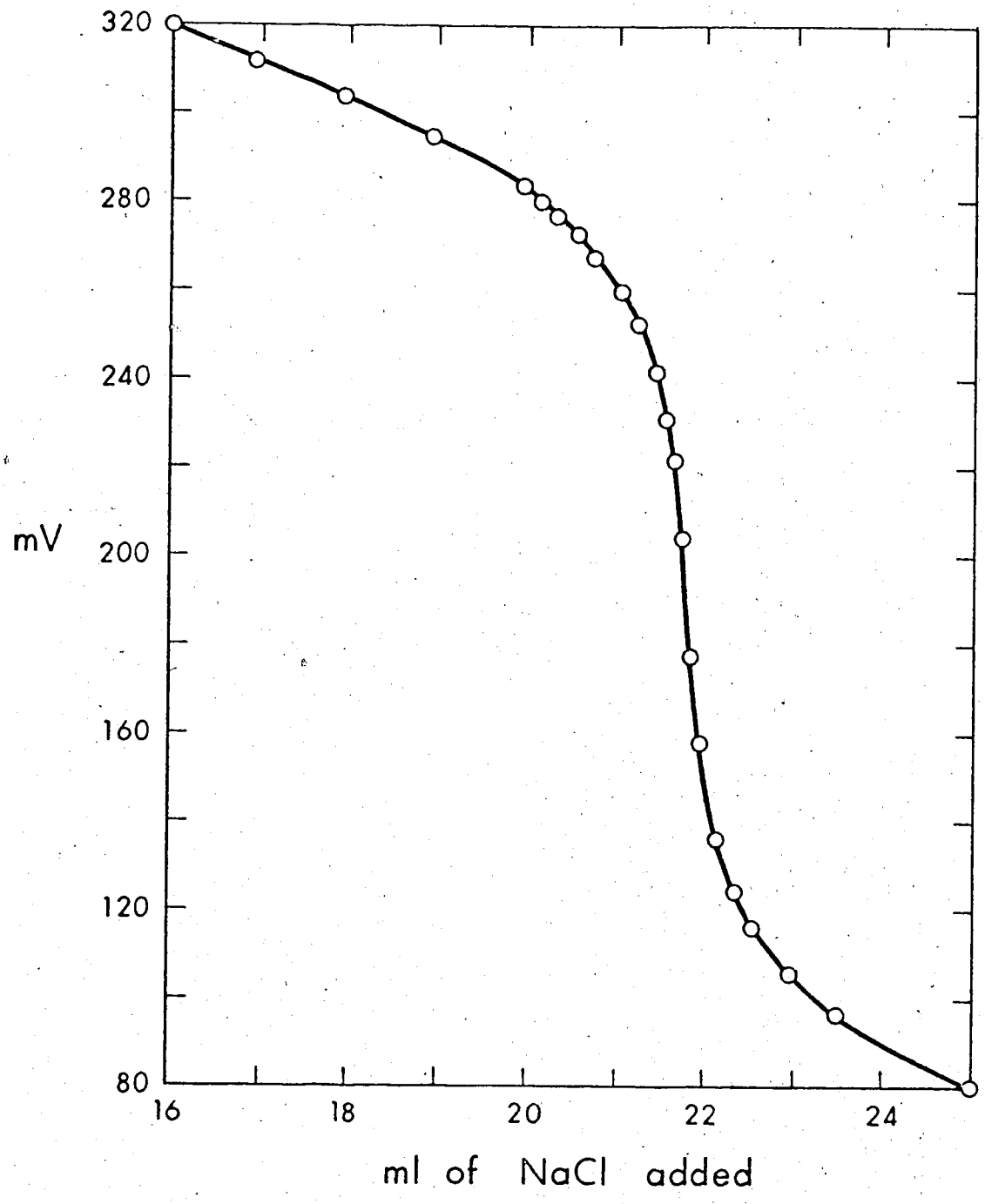


Figure 3: Potentiometric titration curve for 5.00 ml of methylmercury stock solution diluted to 100 ml by the addition of 80 ml of 95% ethanol and 15 ml of 0.3 M nitric acid. The titrant was 0.1024 M sodium chloride.

these conditions is shown in Figure 3. In this titration, the potential changed by 0.194 V on going from 1% before to 1% after the end point. The procedure involved pipetting 5 ml of methylmercuric hydroxide stock solution into a 150 ml beaker and adding to it 15 ml of 0.3 M nitric acid to reduce the pH to approximately 2. 80 ml of 95% ethanol were then added and the solution titrated with 0.1024 M sodium chloride. The concentration of the stock solution was found to be 0.4463 $\pm$ 0.0005 M.

Preparation of Other Solutions

An approximately 0.3 M solution of nitric acid was prepared by diluting 19.5 ml of concentrated nitric acid to 1 liter. This solution was standardized with primary standard mercuric oxide. The procedure involved weighing accurately about 0.2 gm of mercuric oxide into an erlenmeyer flask and then adding about 1.5 gm of potassium iodide which had been dissolved in a small volume of water. The iodide reacts with the mercuric oxide to displace an equivalent amount of hydroxide according to the following reaction.

$$HgO + 4I^- + H_2O \rightleftarrows HgI_4^= + 2OH^-$$

The nitric acid solution was found to have a concentration of 0.2937 ± 0.0004 M by titration of hydroxide to a phenolphthalein end point.

A stock solution of sodium hydroxide prepared from a saturated carbonate free sodium hydroxide solution was standardized by titration against the standard nitric acid. The concentration was found to be 0.1017 ± 0.0001 M.

A stock solution of ammonia was prepared from concentrated ammonium hydroxide. The solution was standardized by titration with standard nitric acid; the end point in the titration was located from the pH titration curve. The concentration was found to be 0.0840 ± 0.0001 M. This solution was used immediately after standardization to avoid any change in concentration due to evaporation.

Equipment

All pH measurements were made at 25°C with an Orion Model 801 pH-meter equipped with a standard glass electrode and a fiber-junction saturated calomel reference electrode. Saturated potassium acid tartrate, 0.050 M phosphate, and 0.01 M sodium tetraborate solutions, pH values 3.56, 7.00,

and 9.18 at 25°C were used to standardize the pH-meter.

Procedure

The acid ionization constants of the amino-carboxylic acids were determined at an ionic strength of 0.15 M by titration of a 0.0100 M solution of the ligand with the standardized sodium hydroxide solution. The ionic strength was adjusted to 0.15 M with KNO_3. Stirring of the solutions was accomplished using a magnetic stirrer.

The formation constants were determined by titration of a solution containing 0.0100 M methyl-mercuric hydroxide and 0.0100 M aminocarboxylic acid in the deprotonated form with standardized nitric acid. Standard sodium hydroxide was added to convert the aminocarboxylic acid to the fully ionized form. For example, in the determination of the formation constants of the methylmercury complex of an aminocarboxylic acid containing one amino group and one carboxylic acid group, 10.00 ml of 0.1017 M NaOH was added to titrate the acidic proton of the carboxylic acid group. In the determination of the formation constants of the

complex of an aminocarboxylic acid containing two carboxylic acid groups, 20.00 ml of 0.1017 M sodium hydroxide was added. In the calculation of the formation constants, the excess amount of sodium hydroxide added to the solution and sodium hydroxide formed from passing the methylmercury solution through the anion exchange column were accounted for. The concentration of sodium hydroxide in a 0.0100 M methylmercuric hydroxide solution prepared from the 0.4463 M stock solution was found to be 0.0006 M by pH-titration with standardized nitric acid. In the formation constant titrations, the initial volume of solution was 100 ml. Although chemical equilibrium is reached very rapidly, at least two minutes were allowed between addition of the acid and measurement of the pH in order to permit thorough stirring and temperature equilibrium.

CHAPTER III

RESULTS AND DISCUSSION

Determination of the Equilibrium Constants of CH_3HgOH and $(CH_3)_2OH^+$

The equilibrium constants for the formation of CH_3HgOH and $(CH_3Hg)_2OH^+$ from CH_3Hg^+ and OH^-, defined by equation (7) and (8), were determined from pH-titration data for the experimental conditions used in this work.

$$CH_3Hg^+ + OH^- \rightleftarrows CH_3HgOH$$

$$K_1 = \frac{[CH_3HgOH]}{[CH_3Hg^+][OH^-]} \qquad (7)$$

$$CH_3Hg^+ + CH_3HgOH \rightleftarrows (CH_3Hg)_2OH^+$$

$$K_2 = \frac{[(CH_3Hg)_2OH^+]}{[CH_3Hg^+][CH_3HgOH]} \qquad (8)$$

The titration curve obtained for the titration of 0.0100 M methyl mercuric hydroxide with nitric acid is shown in Figure 4. The initial pH is high due to the NaOH formed upon passing the CH_3HgOH-NaOAc solution through the ion exchange column. When nitric acid is added, the sodium hydroxide is titrated first, and then the pH decreases rapidly to that of 0.0100 M methyl-

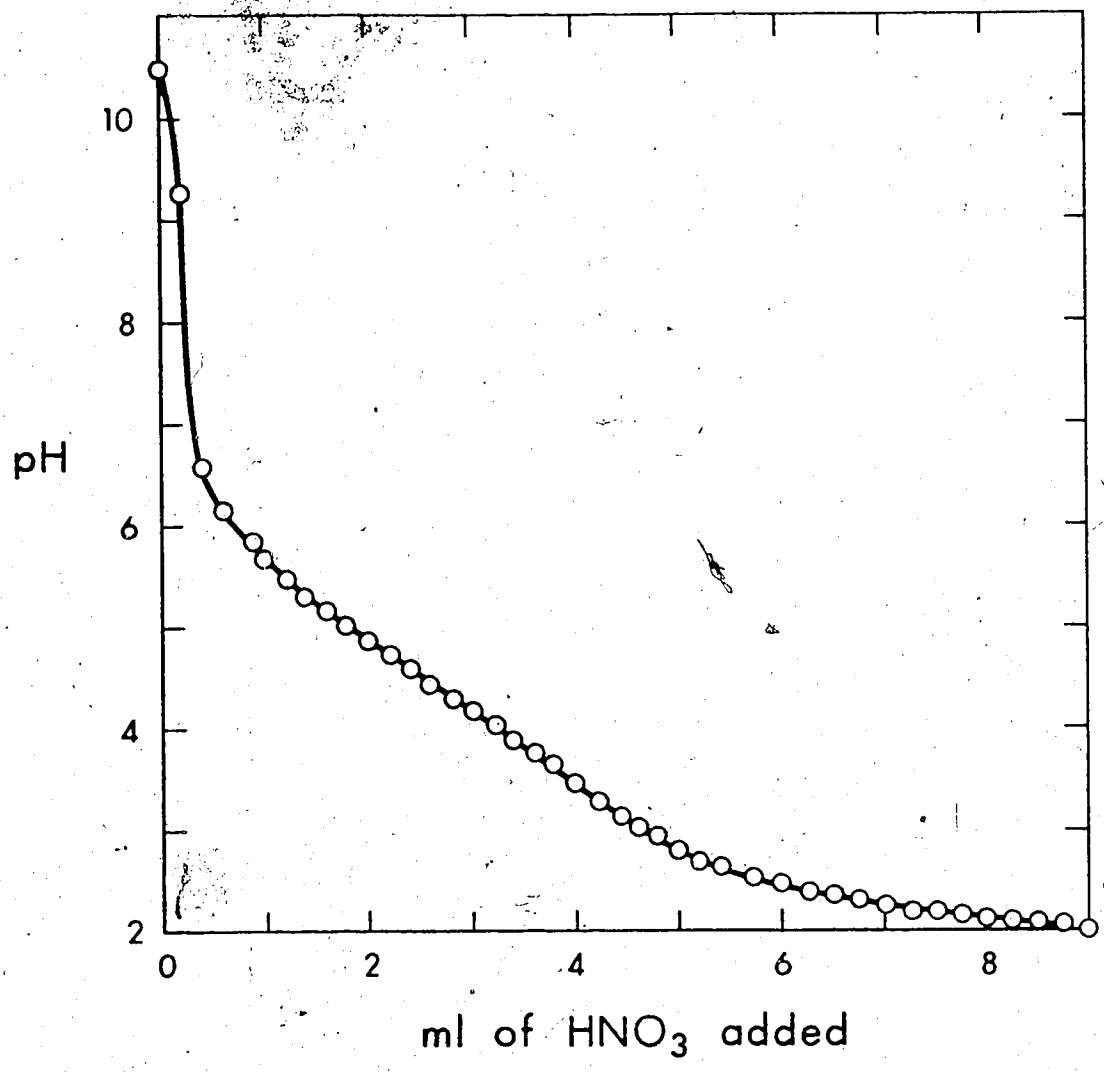


Figure 4: pH-titration curve for the titration of 100 ml of 0.0100 M methylmercuric hydroxide with 0.2937 M nitric acid. Ionic strength adjusted to 0.15 M with potassium nitrate.

mercuric hydroxide solution. Using a value 2.34×10^9 for K_1^{25} the pH of a 0.0100 M CH_3HgOH solution is predicted to be 8.35. After titration of the sodium hydroxide, the shape of the remainder of the titration curve is governed by the equilibria represented by equation (7) and (8).

K_1 and K_2 were evaluated from the pH-titration data by relating the pH at each point on the titration curve to K_1 and K_2. The mass balance equation for the methylmercury species is

$$[CH_3Hg]_t = [CH_3Hg^+] + [CH_3HgOH] + 2[(CH_3Hg)_2OH^+] \quad (9)$$

and the charge balance expression is

$$[CH_3Hg^+] + [H^+] + [(CH_3Hg)_2OH^+] = [OH^-] + [NO_3^-] \quad (10)$$

Substituting equation (7) and (8) into equation (9), equation (11) is obtained.

$$[CH_3Hg]_t = [CH_3Hg^+] + K_1[CH_3Hg^+][OH^-] + 2K_1K_2[CH_3Hg^+]^2[OH^-] \quad (11)$$

Substituting equation (7) and (8) into equation (10), equation (12) is obtained.

$$[CH_3Hg^+] + [H^+] + K_1K_2[CH_3Hg^+]^2[OH^-] = [OH^-] + [NO_3^-] \quad (12)$$

Equation 12 can be solved for $[CH_3Hg^+]^2$

$$[CH_3Hg^+]^2 = \frac{[OH^-] + [NO_3^-] - [H^+] - [CH_3Hg^+]}{K_1K_2[OH^-]} \quad (13)$$

Similarly, equation (11) can be rearranged to give

$$[CH_3Hg^+]^2 = \frac{[CH_3Hg]_t - [CH_3Hg^+] - K_1[CH_3Hg^+][OH^-]}{2K_1K_2[OH^-]} \quad (14)$$

By equating equation(13) and equation (14), collecting terms, and solving for $[CH_3Hg^+]$, equation (15) is obtained.

$$[CH_3Hg^+] = \frac{[CH_3Hg]_t - 2\{[OH^-] - [NO_3^-] - [H^+]\}}{K_1[OH^-] - 1} \quad (15)$$

In the region where the equilibria represented by equation(7) and (8) govern the pH, the pH is 7 or less so that $[OH^-] << [NO_3^-]$.

$$[CH_3Hg^+] = \frac{[CH_3Hg]_t - 2[NO_3^-] + 2[H^+]}{K_1[OH^-] - 1} \quad (16)$$

The equilibrium constants were evaluated by choosing a value for K_1 close to the literature value

and then calculating the concentration of CH_3Hg^+ at each point on the titration curve using equation (16). The concentration of other methylmercury species at each experimental point were then calculated using equation (7) and (9), from which a value for K_2 was calculated at each point. K_1 was then varied slightly and the entire calculation was repeated. By this method, best values were obtained for K_1 and K_2, as judged by the smallest standard deviation in K_2. This method yielded $K_1 = 2.00 \times 10^9$ and $K_2 = (2.11 \pm 0.45) \times 10^2$. For comparison, Schwarzenbach and Schellenberg reported $K_1 = 2.34 \times 10^9$ and $K_2 = 2.34 \times 10^2$ at 20°C and an ionic strength of 0.1 M KNO_3.[25] Rabenstein and Libich reported $K_1 = 2.00 \times 10^9$ and $K_2 = 2.34 \times 10^2$ at 25°C and an ionic strength of 0.2 M.[29]

Determination of the Formation Constant of $CH_3HgNH_3^+$

The methylmercury-ammonia complex was considered to be the simplest model system with which to develop the pH titration method for evaluating the formation constants for the binding of CH_3Hg^+ by the amino group of aminocarboxylic acids. In this section, the determination of the formation constant for the

$CH_3HgNH_3^+$ complex is described.

The formation of $CH_3HgNH_3^+$ is represented by equation (17)

$$CH_3Hg^+ + NH_3 \rightleftarrows CH_3HgNH_3^+$$

$$K_f = \frac{[CH_3HgNH_3^+]}{[CH_3Hg^+][NH_3]} \qquad (17)$$

The dissociation of the ammonium ion is given by equation (18)

$$NH_4^+ \rightleftarrows H^+ + NH_3$$

$$K_A = \frac{[H^+][NH_3]}{[NH_4^+]} \qquad (18)$$

The mass balance expression for methylmercury species is

$$[CH_3Hg]_t = [CH_3Hg^+] + [CH_3HgOH] + 2[(CH_3Hg)_2OH^+] + [CH_3HgNH_3^+] \qquad (19)$$

and for the ligand species is

$$[NH_3]_t = [NH_4^+] + [NH_3] + [CH_3HgNH_3^+] \qquad (20)$$

The charge balance expression is

$$[H^+] + [CH_3Hg^+] + [(CH_3Hg)_2OH^+] + [NH_4^+] + [CH_3HgNH_3^+] =$$

$$[OH^-] + [NO_3^-] \qquad (21)$$

By substituting equation (7), (8), and (20) into equation (19), equation (22) is obtained

$$[CH_3Hg]_t = [CH_3Hg^+] + K_1[CH_3Hg^+][OH^-] + 2K_1K_2[CH_3Hg^+]^2[OH^-]$$

$$[NH_3]_t - [NH_4^+] \quad [NH_3] \qquad (22)$$

Solving equation (18) for $[NH_4^+]$, followed by substitution of the resulting equation into equation (22) and then rearranging, equation (23) is obtained.

$$[NH_3] = \left\{\frac{K_A}{K_A + H}\right\}\{[CH_3Hg^+] - K_1[CH_3Hg^+][OH^-]$$

$$+ 2K_1K_2[OH^-][CH_3Hg^+]^2 + [NH_3]_t - [CH_3Hg]_t\} \qquad (23)$$

By substituting equation (7), (8), and (20) into equation (21), equation (24) is obtained

$$[NH_3] = [H^+] + [CH_3Hg^+] + K_1K_2[CH_3Hg^+]^2[OH^-] + [NH_3]_t$$

$$- [OH^-] - [NO_3^-] \qquad (24)$$

By equating equation (23) and (24) and rearranging all the terms according to the power of CH_3Hg^+,

equation (25) is obtained.

$$\{K_1K_2K_A[OH^-] - K_1K_2K_w\}[CH_3Hg^+]^2 + \{K_1K_A[OH^-] - [H^+]\}$$
$$[CH_3Hg^+] - K_A\{[CH_3Hg]_t + [H^+] - [OH^-] - [NO_3^-]\}$$
$$- [H^+]\{[H^+] + [NH_3]_t - [NO_3^-]\} + K_w \qquad (25)$$

where $K_w = [H^+][OH^-]$.

The only unknown in equation (25) is the concentration of CH_3Hg^+. Using equation (25), the concentration of CH_3Hg^+ can be calculated at each point on the titration curve for a solution containing CH_3HgOH and NH_3. From the concentration of CH_3Hg^+ at a particular point, the concentration of CH_3HgOH can then be calculated from the pH at that point using equation (7). The concentration of $(CH_3Hg)_2OH^+$ can then be calculated from the concentration of CH_3HgOH using equation (8), and finally the concentration of the complex $CH_3HgNH_3^+$ can be calculated by difference from the mass balance expression [equation (19)]. The sum of the concentrations of NH_4^+ and NH_3 are then obtained by difference using equation (20), from which the concentration of NH_3 and of NH_4^+ can be obtained using equation (18). When the concentrations so obtained for CH_3Hg^+,

$CH_3HgNH_3^+$ and NH_3 are substituted into equation (17), they yield a value for the formation constant. In this way a value for K_f can be obtained from each point on the titration curve.

The titration curve for a solution containing equimolar concentration of NH_3 and CH_3HgOH is shown in Figure 5. The usable portion of this curve is from 0.5 ml of titrant to 6.75 ml of titrant; at less than 0.5 ml of titrant the NaOH in the CH_3HgOH solution is being titrated while at titrant volume greater than 6.75 ml, both the ammonia and the CH_3HgOH have been titrated. This corresponds to the pH range from about 10.7 to 2.8. The logarithms of formation constants calculated from the titration curve shown in Figure 5 are listed in Table III. In the region of the titration curve where the pH changes rapidly with the addition of nitric acid, the formation constants show large random variations and are not listed in Table III. The average value for the logarithm of the formation constant calculated from the results listed in Table II is 7.33 with a standard deviation of 0.10. For comparison, Simpson reported the log K_f value to be 8.4 at 25°C.[23] The ionic strength at which his measurements were made was not given, and, as mentioned in the introduction, he did not consider the formation of the $(CH_3Hg)_2OH^+$ species.

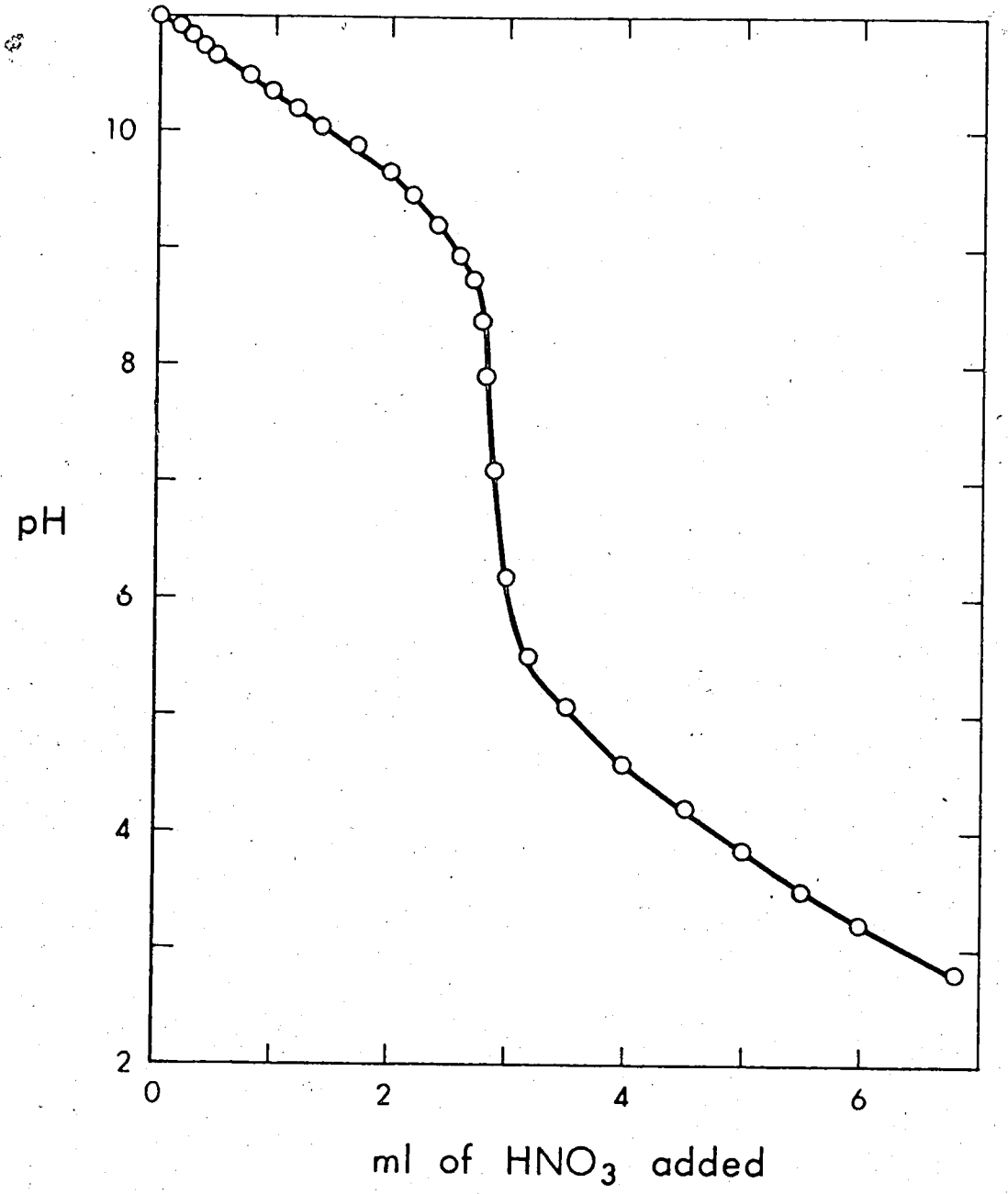


Figure 5: pH-titration curve for the titration of 100 ml of a solution containing 0.010 M methylmercury and 0.010 M ammonia with 0.2937 nitric acid. Ionic strength adjusted to 0.15 M with potassium nitrate.

TABLE III

pH Titration Data for Determination of the Formation Constant of the Methylmercury-Ammonia Complex

pH	ml of HNO_3	log K_f
10.685	0.500	7.24
10.611	0.610	7.24
10.540	0.700	7.27
10.474	0.805	7.29
10.404	0.900	7.29
10.349	1.00	7.32
10.276	1.100	7.32
10.195	1.205	7.31
10.138	1.300	7.32
10.061	1.400	7.31
9.853	1.700	7.31
9.631	2.010	7.31
9.461	2.200	7.28
9.213	2.420	7.20
8.967	2.600	7.11
5.107	3.500	7.55
4.840	3.750	7.48
4.601	4.000	7.46
4.402	4.250	7.44
4.236	4.500	7.40
4.029	4.750	7.45
3.860	5.000	7.44
3.709	5.250	7.42
3.527	5.505	7.44
3.364	5.750	7.44
3.209	6.000	7.42
2.912	6.500	7.35
3.798	6.750	7.19

Thus the accuracy of his value is questionable. Schwarzenbach and Schellenberg reported the value to be 7.60 at 20°C and an ionic strength of 0.1 M.[25] Rabenstein and coworkers reported the value to be 7.25 ± 0.05 at 25°C and an ionic strength of approximately 0.2 M.[30]

The results in Table III demonstrate that the pH-titration method is applicable to the study of the binding of methylmercury by the amino group of aminocarboxylic acid ligands.

Determination of the Acid Ionization Constants for the Amino Groups of the Aminocarboxylic Acids

In order to determine the formation constants for the binding of methylmercury by the amino group of aminocarboxylic acids, the acid ionization constants for the amino groups must be known at the appropriate ionic strengths. The acid ionization constants for the carboxylic acid groups are not needed in the present work; the formation constants are determined in basic solutions where the carboxylic acid groups are completely ionized and binding of CH_3Hg^+ by the carboxylic acid group is not a competing reaction. The acid ionization constants for each

of the aminocarboxylic acids listed in Table IV were determined by the pH-titration technique. The derivations of the equations used in the evaluation of the acid ionization constants from the pH-titration data follow. These derivations are generalized according to the number of amino and carboxylic acid groups in the ligand.

The dissociation of the ammonium proton of an aminocarboxylic acid containing one amino group and one carboxylic acid group can be represented by equation (26)

$$H_3\overset{+}{N}HRCO_2^- \rightleftarrows H^+ + H_2NCHRCO_2^-;$$

$$K_A = \frac{[H^+][H_2NCHRCO_2^-]}{[H_3\overset{+}{N}CHRCO_2^-]} \qquad (26)$$

The mass balance expression for the ligand species is

$$L_t = HL + L^- \qquad (27)$$

and the charge balance expression is

$$Na^+ + H^+ = OH^- + L^- \qquad (28)$$

Combining equation (26), (27), and (28) leads to

$$K_A = \frac{[H^+][Na^+ + H^+ - OH^-]}{[L_t - Na^+ + H^+ - OH^-]} \qquad (29)$$

TABLE IV

Acid Ionization Constants for the Amino Groups of the Aminocarboxylic Acids

Ligand	pK_A this work (a)	pK_A literature (b)
Glycine	9.68	9.70
α-Alanine	9.79	9.86
DL-Valine	9.62	9.65
L-Leucine	9.69	9.69
L-Isoleucine	9.70	9.69
L-Phenylalanine	9.16	9.15
Aspartic acid	9.75	9.63
Glutamic acid	9.61	9.64
S-Methylmercury-cysteine	9.07	9.05[c]
N-Methylglycine	10.13	10.20
Iminodiacetic acid	9.42	9.33
Methyliminodiacetic acid	9.68	9.65
Nitrilotriacetic acid	9.73	9.73
Disodiumethylenediamine-tetraacetate	pK_3 = 6.15	6.16
	pK_4 = 10.19[d]	10.23

(a) Potentiometrically determined constants at ionic strength = 0.15 M, T = 25°C, unless otherwise indicated.

(b) From L. G. Sillen and A. E. Martell, "Stability Constants of Metal-Ion Complexes," The Chemical Society, London, 1964, unless otherwise indicated.

(c) D. L. Rabenstein and M. T. Fairhurst, submitted for publication.

(d) Determined by nmr.

Using equation (29), K_A can be calculated from each point on the titration curve where the amino proton is being titrated. For example, the titration curve for glycine is presented in Figure 6. The amino proton is being titrated in the region from 0.0 ml to 6.75 ml of the titrant. In the calculation, the concentration of H^+ is obtained from the pH at that point and the concentration of Na^+ from the volume of NaOH added.

The acid ionization constants for glycine, α-alanine, DL-valine, L-leucine, L-isoleucine, L-phenylalanine, N-methyl glycine, and S-methylmercury cysteine were calculated from pH-titration data using equation (29). The results are summarized in Table IV. To illustrate the precision of the K_A values so obtained, the K_A values calculated at each point of the titration curve for glycine are listed in Table V.

The dissociation of the ammonium proton of an aminocarboxylic acid containing one amino group and two carboxylic acid groups can be represented by

$$HL^- \rightleftarrows L^= + H^+ ;$$

$$K_A = \frac{[L^=][H^+]}{[HL]} \qquad (30)$$

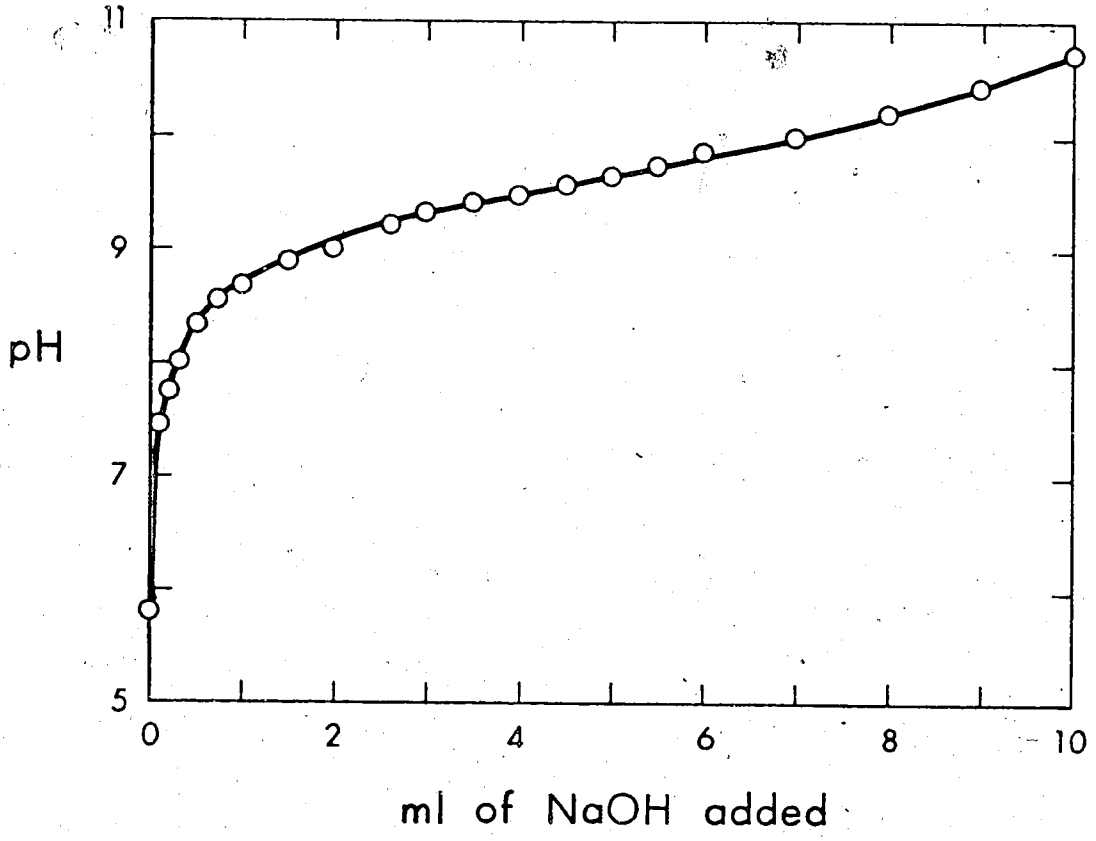


Figure 6: pH-titration curve for the titration of 100 ml of 0.0100 M glycine with 0.1017 M sodium hydroxide. Ionic strength adjusted to 0.15 M with potassium nitrate.

TABLE V

pH Titration Data for Determination of the Acid Ionization Constant for the Amino Group of Glycine

pH	ml of NaOH	K_A
9.017	1.800	2.11×10^{-10}
9.075	2.000	2.11×10^{-10}
9.129	2.200	2.10×10^{-10}
9.181	2.400	2.09×10^{-10}
9.273	2.800	2.08×10^{-10}
9.317	3.000	2.07×10^{-10}
9.370	3.250	2.06×10^{-10}
9.442	3.500	2.05×10^{-10}
9.467	3.750	2.06×10^{-10}
9.514	4.000	2.05×10^{-10}
9.556	4.250	2.07×10^{-10}
9.596	4.500	2.09×10^{-10}
9.635	4.750	2.11×10^{-10}
9.671	5.000	2.15×10^{-10}
9.716	5.250	2.14×10^{-10}
9.758	5.550	2.14×10^{-10}
9.800	5.750	2.15×10^{-10}
9.840	6.000	2.18×10^{-10}
9.883	6.250	2.19×10^{-10}
9.924	6.500	2.21×10^{-10}
9.970	6.750	2.20×10^{-10}

From equations similar to those outlined above,

$$K_A = \frac{[H^+][Na^+ + H^+ + L_t - OH^-]}{[2L_t - Na^+ - H^+ - OH^-]} \qquad (31)$$

Using equation (31), K_A can be calculated at each point on the titration curve where the ammonium proton is being titrated. For example, K_A can be calculated at each point in the region from 11.5 ml to 15 ml of titrant in the titration of aspartic acid shown in Figure 7.

The acid ionization constants for aspartic acid, glutamic acid, iminodiacetic acid, and methyliminodiacetic acid were calculated from pH-titration data using equation (31). The results are summarized in Table IV.

The dissociation of the ammonium proton of NTA can be represented by equation (32).

$$HL^{2-} \rightleftarrows H^+ + L^{3-}$$

$$K_A = \frac{[H^+][L^{3-}]}{[HL^{2-}]} \qquad (32)$$

From equations analogous to those presented above, equation (33) was derived for the evaluation of K_A for NTA.

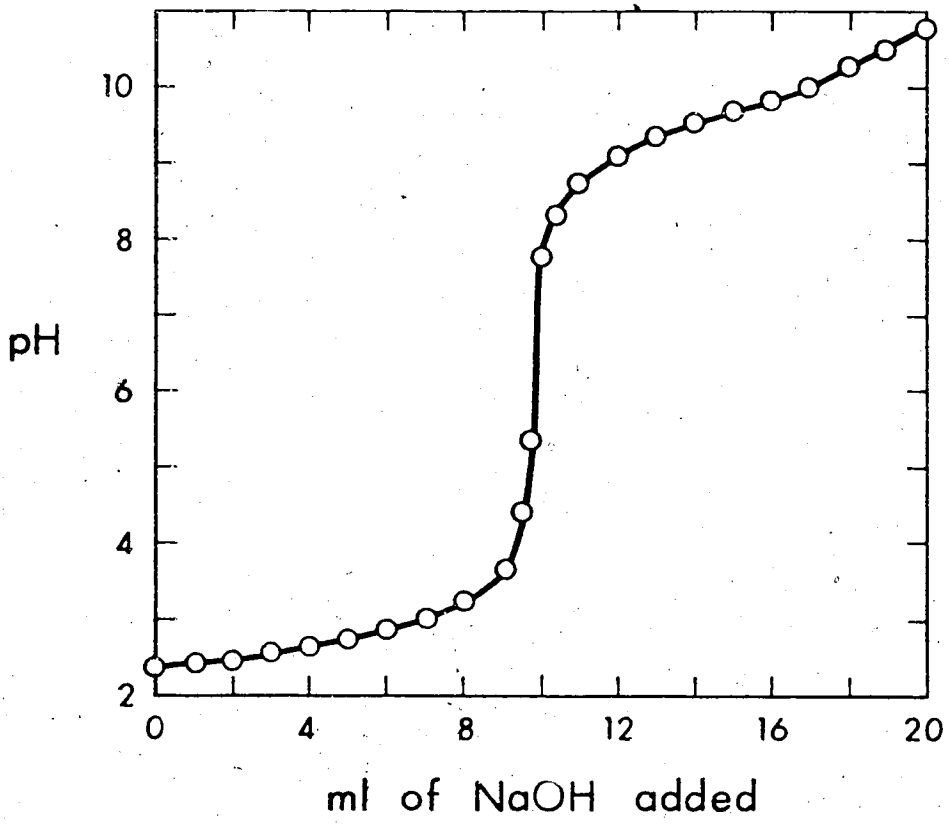


Figure 7: pH-titration curve for the titration of 100 ml of 0.0100 M aspartic acid with 0.1017 M sodium hydroxide. Ionic strength adjusted to 0.15 M with potassium nitrate.

$$K_A = \frac{[H^+][Na^+ + H^+ - OH^- + 2L_t]}{[3L_t - Na^+ - H^+ + OH^-]} \qquad (33)$$

The acid ionization constant of NTA was calculated from each point on the titration curve where the ammonium proton is being titrated. The result is listed in Table IV.

The acid dissociations represented by equations (34) and (35) are important in the EDTA ligand system in the region of interest.

$$H_2L^= \rightleftarrows H^+ + HL^{3-};$$

$$K_3 = \frac{[H^+][HL^{3-}]}{[H_2L^=]} \qquad (34)$$

$$HL^{3-} \rightleftarrows H^+ + L^{4-};$$

$$K_4 = \frac{[H^+][L^{4-}]}{[HL^{3-}]} \qquad (35)$$

K_3 was measured directly from the titration curve for EDTA at the point where $[HL^{3-}] = [H_2L^{2-}]$. The acid ionization constant K_4 can be obtained from pH-titration data in the region where HL^{3-} and L^{4-} are present using equation (36)

$$K_4 = \frac{[H^+][Na^+ + H^+ - 3L_t - OH^-]}{[4L_t - Na^+ - H^+ + OH^-]} \qquad (36)$$

The value so obtained from the titration of 0.0100 M Na_2H_2EDTA with standard NaOH was 9.90, somewhat less than the value of 10.23 reported for similar conditions.[33] This was found to be due to the reaction of sodium ions with HL^{3-} as described by equation (37), causing the pH to be displaced to lower values.

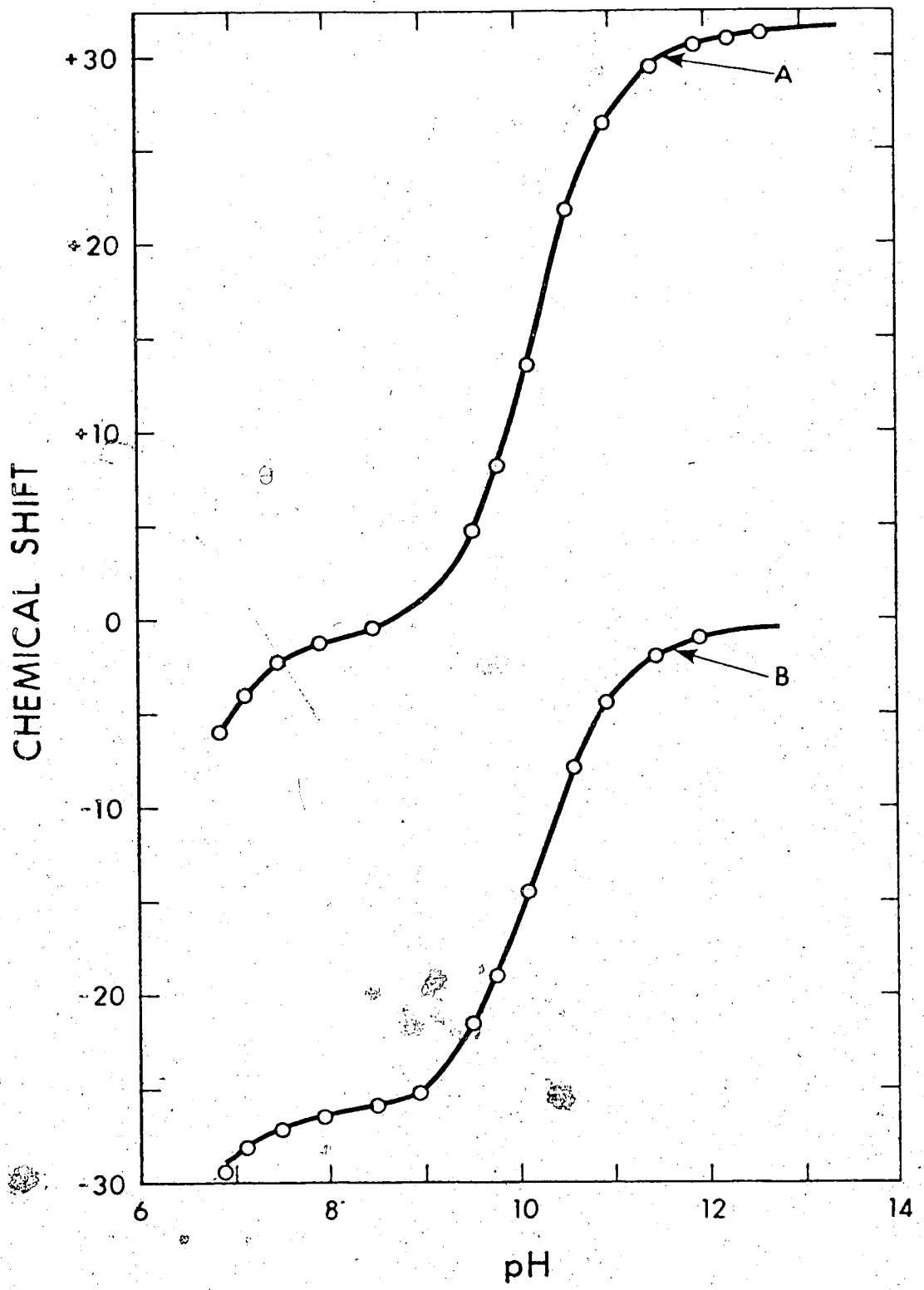
$$Na^+ + HL^{3-} \rightleftharpoons NaL^{3-} + H^+ \qquad (37)$$

Thus, the value obtained for K_4 from the titration of Na_2H_2EDTA is in error due to the formation of the Na-EDTA complex.

To avoid this interference, K_4 was determined by nmr by a procedure described previously.[34] A solution containing 0.15 M KNO_3 and 0.0100 M H_4EDTA was titrated with KOH. The chemical shifts of the carbon bonded protons of EDTA were measured at several pH's in the pH region where HL^{3-} is being titrated to L^{4-}. The nmr titration curves are given in Figure 8. The value obtained for K_4 from the data in Figure 8 is 10.19 ± 0.01.

Literature values for the acid ionization constants[33] are also listed in Table IV for comparison with the results of the pH titration studies.

Figure 8: pH-dependence of the chemical shift of the methylene protons of 0.010 M EDTA in an aqueous solution. pH adjusted by the addition of potassium hydroxide. Curve A represents the protons of the methylene groups between two nitrogen atoms of EDTA. Curve B represents the protons of the methylene groups bonded to the carboxylate dentate of EDTA.


+30
+20
+10
0
-10
-20
-30
CHEMICAL SHIFT
6
8
10
12
14
pH
A
B

Determination of the Formation Constants for the Binding of Methylmercury by the Amino Group of Aminocarboxylic Acids

The formation constants for the binding of CH_3Hg^+ by the amino group of aminocarboxylic acids were determined from pH-titration curves for solutions containing methylmercury and aminocarboxylic acid. The titration curves, in general, are similar to that shown in Figure 5 for the $CH_3HgNH_3^+$ system. The formation constants were evaluated from data at pH values greater than pH 9.5 to eliminate errors due to the binding of some methylmercury by the carboxylic acid group. Figure 1 indicates that binding to the carboxylic acid group would occur at pH less than 9 and this has been confirmed in nmr studies on the binding of methylmercury by several aminocarboxylic acids.[30] The method used in the evaluation of the formation constants will be described according to the number of amino groups and carboxylic acid groups in the ligand.

The reaction describing the formation of the methylmercury complex of an aminocarboxylic acid containing one amino group and one carboxylic acid group is represented by equation (38).

$$CH_3Hg^+ + L^- \rightleftarrows CH_3HgL \ ;$$

$$K_f = \frac{[CH_3HgL]}{[CH_3Hg^+][L^-]} \qquad (38)$$

The mass balance expression for methylmercury species is:

$$[CH_3Hg]_t = [CH_3Hg^+] + 2[(CH_3Hg)_2OH^+] + [CH_3HgOH] + [CH_3HgL] \qquad (39)$$

and for the ligand species is:

$$[L]_t = [HL] + [L^-] + [CH_3HgL] \qquad (40)$$

The charge balance expression is:

$$[Na^+] + [H^+] + [CH_3Hg^+] + [(CH_3Hg)_2OH^+] = [OH^-] + [L^-] + [NO_3^-] \qquad (41)$$

By making substitutions similar to those made in the derivation of equation (25) for the ammonia system, equation (7), (8), (26), (38)-(41), were combined to yield equation (42).

$$\{K_1K_2K_AOH^- - K_1K_2K_w\}\{CH_3Hg^+\}^2 + \{K_1K_AOH^- - H^+\}\{CH_3Hg^+\} - K_A\{CH_3Hg_t + H^+ - OH^- - NO_3^-\} - H^+\{L_t + H^+ - NO_3^-\} + K_w = 0 \qquad (42)$$

At each point on the titration curve, the concentration of CH_3Hg^+ can be calculated using equation (42) from which the concentrations of other species in solution can be calculated as described in the determination of K_f for $CH_3HgNH_3^+$ complex. From these concentrations K_f can then be calculated at each point on the titration curve. The formation constants obtained in this way for the binding of CH_3Hg^+ by the amino group of glycine, α-alanine, DL-valine, L-leucine, L-isoleucine, L-phenylalanine and N-methyl glycine and the structures of the complexes are given in Table VI. To illustrate the precision of the formation constants obtained in this way, the formation constants calculated from each point on the titration curve at pH greater than 9.5 in the glycine system are listed in Table VII. For comparison, Rabenstein and coworkers reported the value of 7.88 at 25°C and an ionic strength of 0.2 M for the logarithm of the formation constant of the glycine complex and the value of 7.44 for the same conditions for the DL-valine complex. These are the only literature values available for comparison with the formation constants determined in this work for the binding of methylmercury by the amino group of aminocarboxylic acids.

TABLE VI

Structures and Formation Constants of the Methylmercury Aminocarboxylic acid Complexes

Ligand	Structure of Complex	log K_f
Glycine	$CH_3Hg\overset{+}{N}H_2CH_2CO_2^-$	7.59 ± 0.03
α-Alanine	$CH_3Hg\overset{+}{N}H_2CH(CH_3)CO_2^-$	7.61 ± 0.02
DL-Valine	$CH_3Hg\overset{+}{N}H_2\ CH[CH(CH_3)_2]CO_2^-$	7.37 ± 0.02
L-Leucine	$CH_3Hg^+\ NH_2CH[CH_2\ CH(CH_3)_2]CO_2^-$	7.63 ± 0.02
L-Isoleucine	$CH_3Hg\overset{+}{N}H_2\ CH[CH(CH_3)C_2H_5]CO_2^-$	7.55 ± 0.02
L-Phenylalanine	$CH_3Hg\overset{+}{N}H_2\ CH(CH_2\ C_6H_5)CO_2^-$	8.00 ± 0.03
Aspartic acid	$CH_3Hg\overset{+}{N}H_2\ CH\cdot(CH_2\ CO_2^-)CO_2^-$	7.82 ± 0.07
Glutamic acid	$CH_3Hg\overset{+}{N}H_2\ CH[(CH_2)_2\ CO_2^-]CO_2^-$	8.40 ± 0.06
S-Methylmercury-cysteine	$CH_3Hg\overset{+}{N}H_2CH(CH_2\text{-}S\text{-}HgCH_3)CO_2^-$	8.56 ± 0.03
N-Methyl glycine	$CH_3Hg\overset{+}{N}H(CH_3)\ CH_2\ CO_2^-$	7.16 ± 0.07
Iminodiacetic acid	$CH_3Hg\overset{+}{N}H(CH_2CO_2^-)_2$	7.81 ± 0.03
Methyl iminodiacetic acid	$CH_3Hg\overset{+}{N}(CH_3)(CH_2\ CO_2^-)_2$	7.81 ± 0.03
Nitrilotriacetic acid	$CH_3Hg\overset{+}{N}(CH_2CO_2^-)_3$	8.33 ± 0.11
Disodiumethylenediamine-	$CH_3Hg\overset{+}{N}(CH_2CO_2^-)_2\ (CH_2)_2\ N\ (CH_2CO_2^-)_2$	
		log K_{f_1} = 4.42 ± 0.03
	$CH_3Hg\overset{+}{N}(CH_2\ CO_2^-)_2\ (CH_2)_2\ \overset{+}{N}H(CH_2CO_2^-)_2$	
		log K_{f_2} = 9.36 ± 0.04

TABLE VII

pH Titration Data for Determination of the Formation Constant of the Methylmercury-Glycine Complex

pH	ml of HNO_3	log K_f
10.798	0.900	7.56
10.750	1.000	7.55
10.703	1.100	7.56
10.651	1.200	7.58
10.614	1.300	7.59
10.545	1.400	7.62
10.496	1.500	7.60
10.442	1.600	7.61
10.386	1.700	7.61
10.334	1.800	7.53
10.278	1.900	7.62
10.215	2.005	7.62
10.161	2.100	7.61
10.110	2.200	7.61
10.048	2.310	7.62
9.979	2.400	7.63
9.920	2.500	7.60
9.858	2.600	7.61
9.774	2.700	7.[illegible]
9.685	2.815	7.58
9.613	2.900	7.57
9.521	3.015	7.55

The reaction describing the formation of the methylmercury complex of an aminocarboxylic acid containing one amino group and two carboxylic acid groups is represented by equation (43).

$$CH_3Hg^+ + L^= \rightleftarrows CH_3HgL^-$$

$$K_f = \frac{[CH_3HgL^-]}{[CH_3Hg^+][L^=]} \qquad (43)$$

The mass balance expression for methylmercury species is:

$$[CH_3Hg]_t = [CH_3Hg^+] + 2[(CH_3Hg)_2OH^+] + [CH_3HgOH] + [CH_3HgL^-] \qquad (44)$$

and for the ligand species is:

$$[L]_t = [L^=] + [CH_3HgL^-] + [HL^-] \qquad (45)$$

and the charge balance expression is:

$$[Na^+] + [H^+] + [CH_3Hg^+] + [(CH_3Hg)_2OH^+] = [OH^-] + 2[L^=] + [NO_3^-] + [CH_3HgL^=] + [HL^-] \qquad (46)$$

ing substitutions similar to those made in the derivation of equation (25) for the ammonia system, equations (7), (8), (30), (43)-(46) when combined also yield equation (42).

Using equation (42), the formation constants for the binding of CH_3Hg^+ by the amino group of aspartic acid, glutamic acid, iminodiacetic acid and methyl iminodiacetic acid were calculated by a procedure similar to that described for the determination of K_f for $CH_3HgNH_3^+$. The results are listed in Table VI. There are no literature values available for comparison with these formation constants.

The reaction describing the formation of the methylmercury complex of an aminocarboxylic acid containing one amino group and three carboxylic groups is represented by equation (47).

$$CH_3Hg^+ + L^{\equiv} \rightleftarrows CH_3HgL^{=}$$

$$K_f = \frac{[CH_3HgL^{=}]}{[CH_3Hg^+][L^{\equiv}]} \qquad (47)$$

The mass balance expression for methylmercury species is:

$$[CH_3Hg]_t = [CH_3Hg^+] + [CH_3HgOH] + 2[(CH_3Hg)_2OH^+] + [CH_3HgL^{=}] \qquad (48)$$

and for the ligand species is:

$$[L]_t = [L^{\equiv}] + [CH_3HgL^{=}] + [HL^{=}] \qquad (49)$$

The charge balance expression is:

$$[Na^+] + [H^+] + [CH_3Hg^+] + [(CH_3Hg)_2OH^+] =$$

$$[OH^-] + 3[L^{\equiv}] + 2[CH_3HgL^{=}] + [NO_3^-] + 2[H \qquad (50)$$

By making substitutions similar to those made in the derivation of equation (25) for the ammonia system, equation (7), (8), (32), (47)-(50) were combined to yield once again equation (42). The formation constants for the binding of CH_3Hg^+ by the amino group of NTA was calculated using equation (42). The result is listed in Table VI.

The reaction describing the formation of the methylmercury complex of S-methylmercur -cysteine is represented by equation(51).

$$CH_3Hg^+ + CH_3Hg\text{-}S\text{-}CH_2\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}COO^- \rightleftarrows CH_3Hg\text{-}S\text{-}CH_2\text{-}\underset{\displaystyle \overset{+}{N}H_2\text{-}Hg\text{-}CH_3}{\underset{|}{CH}}\text{-}COO^-$$

$$K_f = \frac{[(CH_3Hg)_2L]}{[CH_3Hg^+][CH_3HgL^-]} \qquad (51)$$

The acid dissociation reaction for the ammonium group is:

$$CH_3Hg\text{-}S\text{-}CH_2\text{-}\underset{\displaystyle NH_3^+}{\underset{|}{CH}}\text{-}COO^- \rightleftarrows H^+ + CH_3Hg\text{-}S\text{-}CH_2\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}COO^-$$

$$K_A = \frac{[CH_3HgL^-][H^+]}{[CH_3HgHL]} \qquad (52)$$

The mass balance expression for methylmercury species is:

$$[CH_3Hg]_t = [CH_3Hg^+] + 2[(CH_3Hg)_2OH^+] + [CH_3HgOH] + [CH_3HgHL] + [CH_3HgL^-] + 2[(CH_3Hg)_2L] \qquad (53)$$

and for the ligand species is:

$$[L]_t = [CH_3HgHL] + [CH_3HgL^-] + [(CH_3Hg)_2L] \qquad (54)$$

The charge balance expression is:

$$[Na^+] + [H^+] + [CH_3Hg^+] + [(CH_3Hg)_2OH^+] = [OH^-] + [NO_3^-] + [CH_3HgL^-] \qquad (55)$$

By making substitutions similar to those described earlier, equations (7), (8), (51)-(55) were combined to yield equation (56).

$$\{K_1K_2K_AOH^- - K_1K_2K_w\}\{CH_3Hg^+\}^2 + \{K_1K_AOH^- - H^+\}\{CH_3Hg^+\} + K_A\{L_t - CH_3Hg_t + OH^- + NO_3^- + H^+\} + K_w + H^+\{NO_3^- - H^+ - L_t\} = 0 \qquad (56)$$

At each point on the titration curve, the

concentration of CH_3Hg^+ was calculated using equation (56) from which the concentrations of other species in solution were calculated as described earlier. From these concentrations the formation constant for the binding of CH_3Hg^+ by the amino group of S-methylmercury cysteine was calculated at each point on the titration curve. The value so obtained is listed in Table VI.

EDTA is the most complicated ligand studied; it has two nitrogen atoms which makes possible the following complexation reactions:

$$CH_3Hg^+ + L^{4-} \rightleftarrows CH_3HgL^{3-}$$

$$K_{f_1} = \frac{[CH_3HgL^{3-}]}{[CH_3Hg^+][L^{4-}]} \qquad (57)$$

$$CH_3Hg^+ + HL^{3-} \rightleftarrows CH_3HgHL^{2-}$$

$$K_{f_2} = \frac{[CH_3HgHL^{2-}]}{[CH_3Hg^+][HL^{3-}]} \qquad (58)$$

$$CH_3Hg^+ + CH_3HgL^{3-} \rightleftarrows (CH_3Hg)_2L^{2-}$$

$$K_{f_3} = \frac{[(CH_3Hg)_2L^{2-}]}{[CH_3Hg^+][CH_3HgL^{3-}]} \qquad (59)$$

In the present work, conditions were maintained

so that the complex $(CH_3Hg)_2L^{2-}$ could be neglected.

Considering the equilibria represented by equation(7), (8), (34), (35), (57), and (58), the mass balance expression for methylmercury species is:

$$[CH_3Hg]_t = [CH_3Hg^+] + [CH_3HgOH] + 2[CH_3Hg)_2OH^+] + [CH_3HgL^{3-}] + [CH_3HgHL^{2-}] \quad (60)$$

and for ligand species is:

$$[L]_t = [L^{4-}] + [HL^{3-}] + [CH_3HgHL^{3-}] + [CH_3HgHL^{2-}] \quad (61)$$

The charge balance expression is:

$$[H^+] + [CH_3Hg^+] + [(CH_3Hg)_2OH^+] + [Na^+] = [OH^-] + [NO_3^-] + 3[HL^{3-}] + 4(L^{4-}] + 3[CH_3HgL^{3-}] + 2[CH_3HgHL^{2-}] \quad (62)$$

By the procedure described previously, equation (63) and equation (64) were derived from these equations. The values listed in Table VI for K_{f_1} and K_{f_2} were evaluated from the pH-titration data using these equations.

$$2K_1K_2K_{f_2}K_w[CH_3Hg^+]^3 + \{K_1K_{f_2}K_w - K_1K_2K_4[OH^-] + K_{f_2}[H^+] + K_1K_2K_w\}[CH_3Hg^+]^2 + \{[H^+] - K_1K_A[OH^-] + K_{f_2}[H^+][L]_t - K_{f_2}[H^+][CH_3Hg]_t\}[CH_3Hg^+] + K_4\{[H^+] - [OH^-] - [NO_3^-] + [CH_3Hg]_t\} + [H^+]\{[H^+] + [L]_t - [OH^-] - [NO_3^-]\} = 0 \quad (63)$$

$$2K_1K_2K_{f_1}K_3K_4[OH^-][CH_3Hg^+]^3 + \{K_1K_2K_3K_w + 3K_1K_2K_3K_4[OH^-] + K_{f_1}K_3K_4 + K_{f_1}K_3K_4K_1[OH^-] - K_1K_2K_wH^+\}[CH_3Hg^+]^2 + \{K_1K_3K_w + K_3K_4 + 2K_3K_4K_1[OH^-] + K_{f_1}K_3K_4[L]_t - K_{f_1}K_3K_4[CH_3Hg]_t - [H^+]^2\}[CH_3Hg^+] + K_3K_4\{[OH^-] + [NO_3^-] - [H^+] - 2[CH_3Hg]_t\} + K_3\{K_w - H^+[L]_t - [H^+]^2 + [H^+][NO_3^-] - [H^+][CH_3Hg_t] + [H^+]^2\{[NO_3^-] + [OH^-] - 2[L]_t - [H^+]\} = 0 \quad (64)$$

One purpose of the present study was to determine if any relationship exists between the magnitudes of the formation constants of methylmercury amino-carboxylic acid complexes and the basicity of the nitrogen, as reflected by the magnitude of the

acid ionization constant of the amino group. Rabenstein and Libich have reported a correlation between the formation constant of the methylmercury complexes of selected carboxylic acids and the acid ionization constants of the acids. The formation constant of the methylmercury complexes of simple carboxylic acids increase linearly as the acid ionization constants decrease according to the relationship $pK_A = 1.73 \log K_f - 1.05$.[29] The pK_A's for the amino groups of aminocarboxylic acids containing one amino group and one carboxylic acid group listed in Table III are of similar pK_A ranging from 9.6 to 9.8 except for phenylalanine which is 9.16. The logarithm of the formation constants for the coordination to the amino group of these ligands is in the range 7.4 to 7.6 except for phenylalanine which is 8.00. pK_A values increase in the order pK_A, phenylalanine < pK_A, valine < pK_A, leucine < pK_A, isoleucine < pK_A, glycine < pK_A alanine, whereas log K_f values increase in the order log K_f, valine < log K_f, isoleucine < log K_f, glycine < log K_f, alanine < log K_f, leucine < log K_f phenylalanine. These results suggest that the magnitude of the formation constants for coordination of methylmercury cation by amino dentates

of aminocarboxylic acid complexes containing one amino group and one carboxylic acid group is governed not only by the basicity of the nitrogen atom but also by the nature of the substituent on the alpha carbon. This is particularly true in the case of the L-phenylalanine complex.

The pK_A values for the ammonium group of IDA, MIDA and NTA determined in this work are 9.42, 9.67, and 9.73. The nitrogen of MIDA is more basic than that of IDA, presumably because of the inductive effects of the methyl group in MIDA. The increase in the basicity of NTA over the basicity of MIDA and IDA may result from the higher charge on the NTA. This may also account for the NTA complex with methylmercury being more stable than corresponding MIDA and IDA complexes. Interestingly, the increased basicity of MIDA over IDA does not result in a larger formation constant for the CH_3Hg-MIDA complex. This is likely due to the increased substitution at the nitrogen atom in MIDA. Similar results have been observed for the formation constants of methylamine, dimethylamine and trimethylamine.[30]

The effects of NTA on the toxicity of methylmercury to rats has been investigated.[19-20] From

studies in which rats were given doses of trisodium nitrilotriacetate (Na_3NTA) and methylmercuric chloride in their drinking water, it was concluded that NTA has no effect on the toxicity of methylmercury. The results obtained in the present work would predict this to be the case, since the NTA complex of methylmercury is relatively weak compared to the sulfhydryl complex. The logarithm of the formation constant for binding of methylmercury by the sulfhydryl group of cysteine has been reported to be 15.7,[23] as compared to the value of 8.33 for the logarithm of the formation constant of the NTA complex. Thus, the binding of methylmercury by NTA and the other ligands studied in this work is too weak for these ligands to be effective as therapeutic reagents against methylmercury poisoning.

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APPENDIX

Computer program used for calculation of formation constant of methylmercury-glycine complex.

```
FORTRAN IV G COMPILER(21)      MAIN            03-18-73    14:44.25     PAGE 0001

0001            IMPLICIT REAL*8(A-H,O-Z)
0002            DIMENSION PH(50),V(50)
0003            READ(5,99)NPTS
0004            WRITE(6,102)
0005            DO 1 I=1,NPTS
0006            READ(5,100)PH(I),V(I)
0007            H=1.0D0/10.**PH(I)
0008            OH=1.0D-14/H
0009            GKW=1.0D-14
0010            GLT=0.2000*100.0D0/(100.0D0+V(I))
0011            GKA=.110-10
0012            GK1=2.0000
0013            GK2=2.1102
0014            GMEHGT=0.0100*100.0D0/(100.0D0+V(I))
0015            GNO3=(0.2937)*(V(I)-0.24-0.C001)/(100.0D0+V(I))
0016            A=(GK1*GK2*GKA*OH)-(H*GK1*GK2*OH)
0017            X=GKA*GK1*OH+H
0018            C=-GKA*(GMEHGT+H-OH-GNO3)-H*(H*GLT-GNO3)+GKW
0019            X2=X**2
0020            AC4=4.0D0*A*C
0021            XAC=X2-AC4
0022            IF(XAC)5,6,6
0023          5 WRITE(6,103)PH(I),V(I)
0024            GO TO 1
0025          6 SXAC=DSQRT(XAC)
0026            IF(X)4,4,3
0027          3 GMEHGP=(-X+SXAC)/(2.000*A)
0028            GO TO 2
0029          4 GMEHGP=(-X-SXAC)/(2.000*A)
0030            GO TO 2
0031          2 CHHGOH=GK1*GMEHGP*OH
0032            CHGOH2=GK2*GMEHGP*CHHGOH
0033            GMEHGL=GMEHGT-GMEHGP-CHHGOH-(2.000*CHGOH2)
0034            GRNH2N=GLT+H+GMEHGP+CHGOH2-OH-GNO3
0035            GKF=GMEHGL/(GMEHGP*GRNH2N)
0036            WRITE(6,101)A,X,C,XAC,GMEHGP,CHHGOH,CHGOH2,GMEHGL,GRNH2N,GKF
0037          1 CONTINUE
0038           99 FORMAT(I3)
0039          100 FORMAT(2F6.3)
0040          101 FORMAT(11D12.3)
0041          102 FORMAT(7X,1HA,11X,1HX,11X,1HC,9X,3HXAC,9X,5HMEHGP,7X,6HMEHGOH,5X,7
             *HALEHGOH2,6X,6HMEHGL,6X,5HRNH2N,9X,2HKF,9X,5HLOGKF)
0042          103 FORMAT(3X,10HXAC NEGATIVE FOR PH,2X,D10.3,2X,1HV,2X,D10.3)
0043            STOP
0044            END
```