

TECHNICAL RESEARCH PROJECT TERMINATION REPORT

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METHYLATION OF MERCURY BY MICROORGANISMS

Submitted by

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6/72

ABSTRACT

Both organic and inorganic forms of mercury are analyzed by a modification of the classical dithizone method in the range 0-50 μg . The separation of mercury from the bulk of interfering ions such as Cu^{++} , Fe^{+++} , Bi^{+++} , Mg^{++} , Ca^{++} and Na^{+} can be accomplished simply by use of chelating resins. No satisfactory method of separating inorganic mercury compounds from organomercurial compounds by differential elution was found. Mercury is not appreciably volatile in hot oxidizing acidic solution and the elaborate refluxing systems of the standard dithizone method are unnecessary.

Key Words:

chelating resin

dithizone

inorganic mercury

organomercurial

INTRODUCTION

As was stated in our last report, in order to study the pathway of conversion of inorganic mercury to organomercurial compounds a reliable method for determining both total mercury^{1,2,3,4} and the relative amounts of inorganic and organic mercury⁵ is needed. As was explained earlier, lack of specialized equipment such as a flameless atomic adsorption spectrophotometer and a gas chromatograph with electron-capture detection device caused us to elect the classical analytical methods for these determinations. Several colorimetric spectrophotometric methods were investigated and a modification of these was adopted for routine work for determination of the total mercury content of samples.

In order to separate the organic and inorganic forms of the metallic compounds several methods have been proposed. Westoo^{6,7,8} in Sweden and Tatton and Wagstaffe⁹ in England have used gas chromatographic methods, while Gage¹⁰ extracted the sample with benzene and used a titrimetric modification of the dithizone colorimetric procedure for his analyses. Polley and Miller¹¹ used a dithizone spectrophotometric method after chloroform extraction that is specific for methyl mercury compounds, while Clarkson and Greenwood¹² have utilized radioactive mercury which is selectively reduced to differentiate between the two forms of mercury compounds. A paper chromatographic method has been employed by Bartlett and Curtis¹³ for the separation of the various organomercurials and a selective chelating resin for collecting methyl mercury and inorganic mercury has been reported by Law¹⁴. This last paper seemed to merit investigation for application to our problem and it was hoped that a simple

separation of the two forms of mercury could be obtained in this way.

Since it might prove necessary to quickly identify and determine the presence of organomercurials in mixtures, some of the methods using thin layer chromatography (TLC)⁹ and paper chromatography¹³ were tried to evaluate their adaptation to semiquantitative preliminary studies.

The difficult problems of matrix and sampling were considered and test mixtures were made to find the effect of sampling on mixtures spiked with mercury compounds.

EXPERIMENTAL

A. The determination of mercury with crystal violet according to the method of Kothny¹.

This method was tried with a mercury standard in the range of 0-10 μg of mercury. A standard curve was prepared (Fig. I) and samples spiked with mercury were carried through the procedure as outlined (Table I). This method seemed to be satisfactory but it was felt that the standard dithizone method ought to be tried and used as the reference method.

B. The determination of mercury with dithizone according to the Official Methods of Analysis of the Association of Official Agricultural Chemists⁴.

Using the procedure outlined in the reference (4) two standard curves were made covering the range of 0-10 μg and 0-50 μg of mercury (Fig. 2). Spiked samples were then analysed after digestion and extraction (Table II). This proved more difficult as the dithizone reagent is unstable to oxidation and sensitive to light and heat. The acidity of the solution must be closely controlled for proper extraction of the mercury compounds and the cleavage of the mercury thiosulfate complex is carried out only under rigid conditions.

C. The attempted separation of methyl mercury compounds from inorganic mercury compounds using chelation resins.

1. Preparation of methyl mercury chloride.

Methyl mercury chloride was prepared from a commercial preparation of methyl mercury dicyandiamide used as a fungicide in the seed industry. The preparation had 2.2% of the organomercurial along with a brilliant red dye to make detection of the compound simple. To 100 ml of this

preparation in a separatory funnel 10 ml of conc. HCl and 50 ml of reagent grade chloroform were added. After shaking vigorously and allowing the phases to separate, the chloroform layer was removed. Three more extractions with 50 ml portions of chloroform were made and the combined organic layer taken to dryness on a rotary flash evaporator. The residue was recrystallized from hot methanol, and the precipitate filtered and washed with cold methanol until free from the dye additive. This compound was found to sublime at temperatures over 100°C and melt at 171°C . It was insoluble in water but soluble in methanol. When a sample of methyl mercury chloride (m.p. 170°C) and this compound were examined by both paper chromatography and TLC, the two co-chromatographed. This compound was used to make a standard stock solution of methyl mercury chloride as follows: 31.3 mg were dissolved in app 12 ml methanol and diluted to 25 ml with water containing 8 ml conc. HCl/L. (1 mg Hg/ml). The final solutions used for salting experiments were diluted with N/20 HCl and made fresh every few days.

2. Preparation of columns.

Since it had been reported that the chelating resin Chelex 100 (Dow Chemical Company) would quantitatively bind inorganic mercury but not organomercurial compounds¹⁴ two columns were prepared with Chelex 100 [H^+ , 40x10, 1 ml/min] and pre-equilibrated to both N/20 HCl and 5% thiourea in N/20 HCl. After washing free of thiourea 20 μg of Hg^{++} were introduced in 5 ml N/20 HCl washed with 10 ml N/20 HCl, eluted with 10 ml of thiourea solution and washed free of the elution compound with 10 ml N/20 HCl. The various fractions were then analysed for mercury according to the method of the Ass. of Official Agr. Chemists

(ref. 4). It was hoped that the digestion step could be eliminated but the mercury thiourea complex is not easily broken.

Thiourea can be oxidized by N-Bromo-succinimide¹⁵, but at the concentration employed in the elution step the oxidation did not proceed to the sulfate form but precipitated free sulfur. Since the amount of mercury to be analysed is of trace quantities, it is not feasible to have large masses of precipitate at any step of the analysis which must be filtered from the solution. Only if the precipitate can be redissolved could this be made practical.

3. Method of Analysis of Eluents.

According to an abstract of a Japanese paper by Naito et al.¹⁶, benzene is found to be superior to either chloroform or carbon tetrachloride as a solvent for dithizone. Modifications of the digestion and analysis are also proposed by Gage¹⁰ and Polley and Miller¹¹. A combination of these methods was adopted and is described below.

Reagents.

(a) Mercury std. soln.-Prep. stock soln. contg. 1 mg Hg/ml from reagent grade HgCl_2 (67.7 mg/50 ml) or CH_3HgCl (62.6 mg/50 ml) in N/20 HCl. Prep. dil. std. soln. as needed in N/20 HCl. The CH_3HgCl stock soln. must also contain CH_3OH in app. 50% v/v.

(b) Dithizone soln.-Prep. stock soln. in benzene (200 mg/L.) using reagent grade chemicals. Store in the dark. Prep. soln. 20 mg/L. benzene as needed.

(c) Potassium permanganate soln. - 5% w/v in H_2O .

(d) Hydroxylamine hydrochloride soln. - 20% w/v in H_2O .

Method.

To the eluent containing 10 ml thiourea carefully add 10 ml conc. HCl and 5 ml 1:1 $\text{HNO}_3:\text{H}_2\text{SO}_4$. Bring to simmering temperature and continue to heat until fumes of H_2SO_4 just appear. To ensure complete removal of HCl and HNO_3 add a few drops of permanganate solution until a permanent pink color persists. The solution should progress from a brown to golden to colorless color before the addition of the permanganate. Cool slightly, wash down with water to ensure complete elimination of all acids except sulfuric. Remove the excess permanganate and any precipitated manganese dioxide with the dropwise addition of hydroxylamine hydrochloride solution. Cool to below room temperature. Neutralize to pH 4 with conc. NH_4OH using bromphenol blue as an indicator and cool. Dilute to 50 ml and add 10 ml 6N H_2SO_4 and 0.5 ml hydroxylamine hydrochloride. If Ag^+ Cu^{++} Bi^{+++} Sn^{++} ions are suspected to be present they can be removed according to the method outlined in ref. 15 or masked with EDTA as in ref. 10. Transfer the solution to a 125 ml separatory funnel. Add 10 ml dithizone reagent (20 mg/L.) in benzene. Shake for 1 minute. Allow phases to separate. Read the absorbance of the organic phase on a Zeiss PMQ II spectrophotometer at 490 nm against a dithizone blank. Compare the absorbance reading with those of a standard curve to obtain the amount of mercury contained in the sample.

Reproducibility of Results of Modified Dithizone Method.

Over a period of three months the method described was used to prepare standard curves. Tables III and IIIa indicate the values for absorbance obtained at various times. These values were analysed for correlation and simple linear regression using the Oregon State University

Control Data Corporation 3300 Computer. The corresponding values for A (intercept of Y axis) and B (slope of line) were then analysed for correlation and the mean values and standard deviations of these values are reported in Table III and IIIa also.

4. Results of Column Separations.

When Hg^{++} is introduced on the Chelex 100 column and washed with N/20 HCl, the effluent was collected. This was designated 'a'. The column was then eluted with thiourea and washed with N/20 HCl. This eluent was collected and designated 'b'. If CH_3Hg^+ was introduced the effluent was designated 'c' and the eluted thiourea eluent 'd'. Similarly a mixture of the two forms when introduced was labelled 'e' and the eluent 'f'. Table IV summarizes the results. The Srafion NMRR (Ayalon Water Conditioning Co., Ltd., Haifa) columns did not stabilize to give consistent results.

5. Difficulties Encountered with Column Separations.

While working with the Chelex 100 columns several difficulties were encountered. The $[\text{H}^+]$ form of Chelex 100 is not stable over long periods of time. However, if the resin is returned to the $[\text{Na}^+]$ form between operations, the volume change is approximately 100% greater in the salt form. This makes reversal to the $[\text{H}^+]$ form impractical due to channeling and packing of the resin.

In order to separate the organic and inorganic forms of mercury from the chelating resin a differential elution method had to be found. Various concentrations of hydrochloric acid and thiourea were tried but were unsuccessful. In order to remove the organic form, 80 mole% methanol was tried as an eluent, but the methanol in the effluent made the dithizone method of analysis inaccurate. An attempt was made to separate the methanol

in the eluent out of the Chelex 100 column from the methyl mercury by passing the eluent through a Dowex-50-W-X-8 column. It was hoped that the mercury would be retained to be eluted later with 4N HCl, while the methanol would pass through in the effluent. Unfortunately, the mercury is not retained by the Dowex 50. This is verified by Samuelson¹⁷. Moreover, after elution with 80 mole % methanol, the Chelex 100 resin does not return easily to the equilibrated state with N/20 HCl. Consequently, the method becomes impractical.

Berg and Truemper¹⁸ have reported the absorption of mercury in the presence of chlorides and 25% v/v methanol on the anion exchange resin Dowex-1. Using a 6 cm column with a flow rate of 1.5 mls/min they have absorbed 100 mg quantities of mercury. It can then be quantitatively eluted with 0.1 M thiourea in 0.01 M HCl. There has been no attempt to utilize this information with respect to the separation of organomercury compounds from their inorganic counterparts.

D. Sampling Problems.

Since the problem of mercury methylation by microorganisms would entail mercury analysis of aquatic sludges, an attempt was made to determine the effect of sampling on the accuracy of the procedure. Garden soil was air dried to constant weight, mixed well and analysed for total mercury content by the dithizone method (ref. 4). On three separate determinations for total mercury using 10 gram samples no mercury was found. To two additional samples 5 μg of Hg^{++} were added and in each case 3.8 μg were recovered. This would be equivalent to 0.5 p.p.m.

In order to determine the variability in sampling of sludge, 250 μg of Hg^{++} were added to 50 grams of soil. This was diluted to 100 ml and

the mercury determined on 10 ml samples taken immediately after mixing using an inverted 10 ml Mohr pipette. The results are compared with samples taken from a similar slurry and then salted with mercury. The results are summarized in Table V.

E. Results of TLC and Paper Chromatography of Mercury Compounds.

Since it would be necessary to recognize quickly the presence of organomercurials in sludges, the work by Bartlett and Curtis¹³ on paper chromatography of mercury compounds and Tatton and Wagstaffe's⁹ work with TLC methods were tried. Both methods have the advantage of overall simplicity and both have the disadvantages of sample size limitation. The R_f value obtained on methyl mercury chloride did not correspond with the value reported in the paper, but a sample of known methyl mercury chloride co-chromatographed with the compound used. It is possible conditions of the experiment were not identical and the problem was not pursued.

SUMMARY

It is the opinion of the writer that a gas chromatograph with the appropriate modifications for the determination of organomercurial compounds is necessary to evaluate simply the concentrations of these substances. Total mercury can be determined relatively easily by the standard spectrophotometric methods or slight modifications of these procedures. The separation of mercury from the bulk of interfering ions such as Fe^{+++} , Cu^{++} , Bi^{+++} , Mg^{++} , Ca^{++} and Na^+ can be accomplished simply by use of chelating resins. Mercury is not appreciably volatile in hot oxidizing acidic solution and the elaborate refluxing systems of the standard dithizone method⁴ are unnecessary.

Sampling problems are a possibility and care must be taken to obtain not only a large enough sample but also a representative sample.

It is possible that some practical applications of the binding of mercury to the chelating resins could be developed for the elimination of mercury in waste waters.

Table I

<u>$\mu\text{g Hg}^{++}$ salted</u>	<u>$\mu\text{g H}^{++}$ recovered</u>	<u>% recovery</u>
2.0	1.6	90
4.0	3.8	95
6.0	6.0	100
8.0	8.5	106
10.0	10.8	108

Analyses according to the method of E. L. Kothny (ref. 1)

Table II

<u>µg Hg⁺⁺ added</u>	<u>µg Hg⁺⁺ recovered</u>		<u>% recovery</u>	
	Series A	Series B	Series A	Series B
2.0	0	1.0	0	50
4.0	4.0	3.9	100	98
6.0	6.0	3.5	100	59
8.0	6.8	7.0	85	89
10.0	8.5	8.3	85	83

Analyses according to the Ass. of Off. Agr. Chemists, (ref. 4)

Table III

Absorbance at 490 nm
Hg⁺⁺

	I	II	III	IV	V	VI	VII
µg Hg ⁺⁺ 10		.237	.136	.195	.166	.254	.235
20	.413	.440	.356	.388	.392	.386	.394
30		.632	.418	.552		.590	.651
40	.788	.842	.682	.750		.770	.817
50		1.04	1.02	.840		.980	.948

Using the equation $Y = A + B * X$ where X represents the amount of mercury and Y the absorbance

A = Y intercept B = slope of line

A	.006333	.017048	-.050381	.023524	-.010000	.021524	.025857
B	.019700	.020591	.019429	.017226	.019600	.019006	.019266
Standard Error A	.014162	.009330	.060356	.024274	.022361	.018857	.027017
Standard Error B	.000548	.000308	.001993	.000802	.001732	.000623	.000892

Mean of A = .00484

Std. Dev. of A = .02736

Std. Error of Mean = .01034

Mean of B = .01926

Std. Dev. of B = .00103

Std. Error of Mean = .00039

Table IIIa

Absorbance at 490 nm
 CH_3Hg^+

	Not Oxidized		Oxidized	
	I	II	III	IV
$\mu\text{g Hg}^{++}$ 10		.074	.164	.150
20	.147	.118	.347	.368
30		.196		.460
40	.262	.267	.682	.654
50		.338	.900	.780
	Using the equation $Y = A + B * X$ where X represents the amount of mercury and Y the absorbance A = Y intercept B = slope of line			
A			-.008767	.008857
B			.017807	.015726
Standard Error A			.012717	.020612
Standard Error B			.000419	.000681

Note the stoichiometric relationship when the CH_3Hg^+ ion is not oxidized to Hg^{++} . The dithizonate with Hg^{++} gives approximately twice the absorbance that the CH_3Hg^+ dithizonate does.

Table IV

Recovery of Mercury from Chelating Columns

Column Number	1	2	3	4
Type	Chelex 100	Chelex 100	Srafion NMRR	Srafion NMRR
Form	H ⁺	H ⁺	H ⁺	H ⁺
Size	40 x 10	40 x 10	40 x 10	40 x 10
Rate of Flow	1 ml/min	1 ml/min	1 ml/min	1 ml/min

Pre-equilibrated all columns to 5% thiourea and N/20 HCl

Spiked samples 20 µg in each case. Recoveries in parentheses.

'a'	< 1	< 1		
Hg ⁺⁺ 'b'	16 (80%)	16 (80%)		
'a'	0	0	10	3
Hg ⁺⁺ 'b'	20 (100%)	17.5 (88%)	15	20
'c'	0	< 1	10½	11½
CH ₃ Hg ⁺ 'd'	18.5 (92%)	20.5 (102%)	7½	8½
'c'	< 1	< 1		
CH ₃ Hg ⁺ 'c'	14 (70%)	19.5 (98%)		
'a'	< 1	< 1		
Hg ⁺⁺ 'b'	19 (95%)	13 (75%)		
'c'	< 1	< 1		
CH ₃ Hg ⁺ 'd'	22 (110%)	21 (105%)		
'e'	< 1	< 1		
Hg ⁺⁺ +CH ₃ Hg ⁺ 'f'	34 (85%)	34 (92%)		

Pre-equilibrated to N/20 HNO₃. All N/20 HCl substituted with N/20 HNO₃.

'e'	< 1	< 1		
Hg ⁺⁺ +CH ₃ Hg ⁺ 'f'	35 (88%)	35 (88%)		

Table V

Samples taken from slurry salted before sampling	Samples of slurry salted after sampling
Salting 25 $\mu\text{g Hg}^{++}$ for each sample	
1. 14	1. 16
2. 20	2. 16
3. 21	3. 20
4. 11	4. 15
5. 25	5. <u>22</u>
6. 13	Average 18 \pm 3
7. 15	
8. 19	
9. 16	
10. <u>25</u>	
Average 18 \pm 7	

Figure I

Standard Curve for the Determination of Mercury Using Crystal Violet

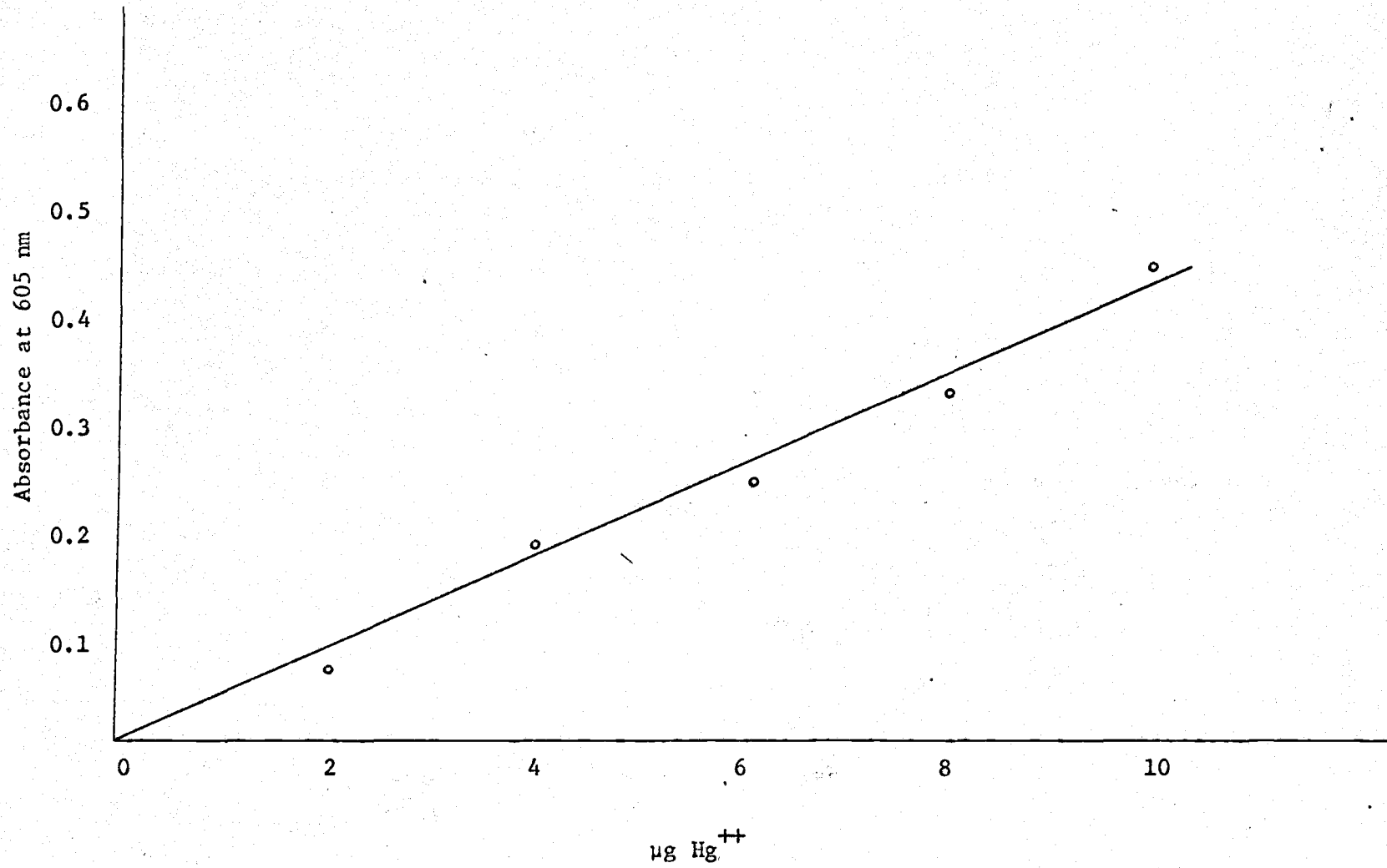
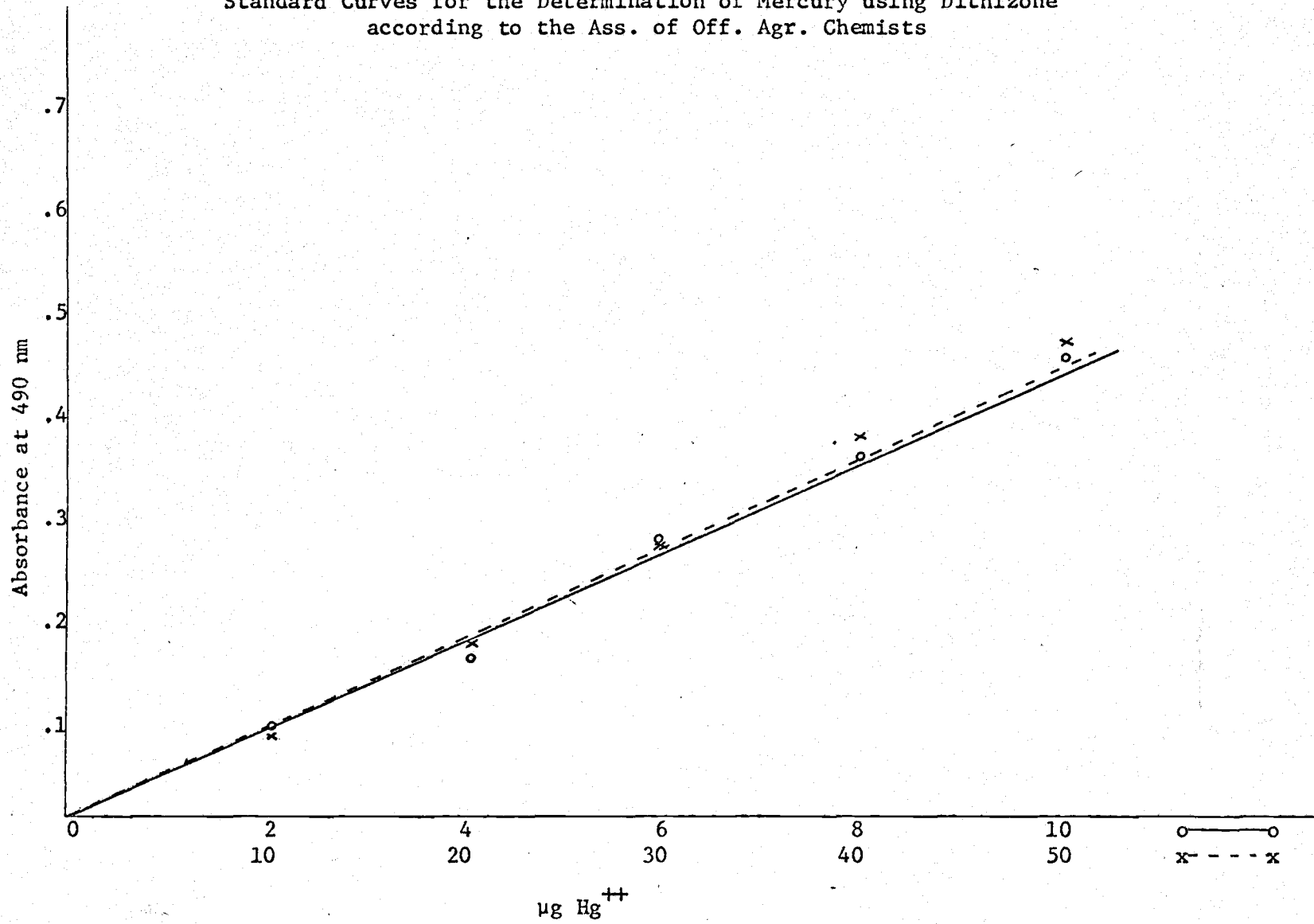


Figure II

Standard Curves for the Determination of Mercury using Dithizone
according to the Ass. of Off. Agr. Chemists



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Reasons for Termination of Project

The project was discontinued because of lack of funds.