Determination of Mercury in Organic and Inorganic Compounds

Stannous Chloride Reduction Method

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The stannous chloride reduction method of determining mercury has been successfully applied to the determination of mercury in organic and inorganic compounds. Macro- and semimicromethods are presented for inorganic compounds. For organic compounds a semimicromethod is given in which the mercury is precipitated with stannous chloride from dioxane solutions of the compound. The mercury is collected on a filter, dissolved in nitric acid, and titrated with standard thiocyanate solution. Preliminary oxidation of the organic matter is eliminated. Results compare favorably with umpire methods.

THE determination of inorganic mercury by its reduction to the free state with stannous chloride has not been too successful, whether the mercury was determined gravimetrically or volumetrically. The Krieckhaus method (*4*), modified by Fenimore and Wagner (*3*), proved satisfactory when samples containing 0.2 to 0.3 gram of mercury were analyzed but with samples containing 0.1 gram or less of mercury the results were too low. Anfinsen (*1*) and later Staple (*7*) were unable to improve upon the results of Fenimore and Wagner. The low results obtained by Willard and Boldyreff (*8*), who reduced mercury with stannous chloride and weighed the free metal, were attributed to loss of mercury by volatilization during the drying process

Most of the methods used in determining mercury in organic compounds depend upon preliminary oxidation of the organic matter followed by determination of the resulting inorganic mercury by a suitable method. Using ethanolamine alone or with metallic sodium as reducing agents, Rauscher (*5*) successfully determined mercury in organic compounds by a direct reduction of the mercury to the free state without first destroying the organic matter.

In this investigation methods were successfully developed for the determination of inorganic and organic mercury, using stannous chloride as the reducing agent.

REAGENTS

Standard Mercuric Nitrate Solution. Solutions ranging from 0.05 to 0.025 molar were prepared by dissolving the appropriate amounts of redistilled mercury in 20 ml. of concentrated nitric acid. Saturated potassium permanganate was added to destroy the oxides of nitrogen and to oxidize any mercurous mercury to the mercuric state. The excess permanganate was destroyed by adding a drop or two of ferrous sulfate solution, after which the mercury solution was diluted to volume in a calibrated volumetric flask.

Standard Potassium Thiocyanate Solution. Solutions of the recrystallized salt were standardized by titrating against standard mercuric nitrate solution at 12° to 15° C., using ferric alum as the indicator.

Ferric Alum Indicator Solution. Forty grams of ferric alum were dissolved in 100 ml. of water and nitric acid was added for the usual purpose.

Ferrous Sulfate Solution. Ten grams of ferrous sulfate heptahydrate were dissolved in 100 ml. of 0.3 *N* sulfuric acid.

Stannous Chloride Solution. One hundred twenty-five grams of stannous chloride dihydrate were dissolved in 70 ml. of concentrated hydrochloric acid and diluted to 250 ml. The resulting solution was heated with metallic tin until the precipitate disappeared and was then stored over tin.

Potassium Permanganate Solution. A saturated solution was allowed to stand for several days, after which it was decanted from the manganese dioxide.

Dioxane. The product obtained from the J. T. Baker Chemical Co. was used without further purification.

Organic Mercury Compounds. The percentage of mercury in these compounds was determined by umpire methods.

DETERMINATION OF INORGANIC MERCURY

In the Krieckhaus method (*4*), as modified by Fenimore and Wagner (*3*), the inorganic mercury solution is treated with stannous chloride in an Erlenmeyer flask which is covered with a watch glass and is digested on a steam bath until the mercury is in the form of tiny globules. This digestion requires 1.5 to 2 hours. The mercury is filtered and the filter paper containing the mercury is placed in the Erlenmeyer flask. Concentrated nitric acid is added and the solution is subsequently titrated with standard thiocyanate solution. A study of this method indicated two sources of error—namely, loss of mercury by volatilization during digestion, and incomplete removal of the chloride ions from the filter paper after filtration of the free mercury.

The loss of mercury by volatilization was prevented by inserting a small cold finger into the flask during digestion, as shown in Figure 1, instead of merely covering the flask with a watch glass. The glass flange, *A*, on the cold finger served as an added protection against loss of mercury. Although improved results were obtained with this modification, there was still an apparent loss of mercury. Since mercuric chloride is ionized to a lesser degree than mercuric thiocyanate, the presence of any chloride ions in the mercury solution gives a premature end point when the latter is titrated with potassium thiocyanate solution, thereby yielding low results for mercury. Qualitative tests with silver nitrate showed that under the conditions of this method the complete removal of chloride ions from the filter paper was difficult. Similar tests conducted upon shredded asbestos showed no difficulty in the removal of chloride ions. Therefore a No. 2 Gooch crucible was used instead of filter paper for filtering the mercury and good results were obtained.

Modified Procedure of Krieckhaus Method. Measured volumes of standard mercuric nitrate solution containing about 100 mg. of mercury, diluted to 50 ml., were treated with 10 ml. of concentrated hydrochloric acid and 10 ml. of stannous chloride solution in a 125-ml. Erlenmeyer flask. A small cold finger (Figure 1) was inserted into the flask, so that the bottom of the finger was about 2 cm. from the surface of the liquid, and the flask was heated on a steam bath until the mercury formed into globules. The mercury was filtered through a No. 2 Gooch crucible and washed by decantation with 2 *N* sulfuric acid followed by water until no chloride was detected in the wash water. The crucible was removed from its holder and, using a small stirring rod, the asbestos mat containing the mercury was transferred to a 125-ml. Erlenmeyer flask. The crucible was placed over the opening of the flask and 5 ml. of hot concentrated nitric acid were poured around the inside of the crucible in such a way as

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to dissolve any finely divided mercury clinging to the wall. The acid, draining through the bottom of the crucible, then dissolved the bulk of the mercury already in the flask. The solution was diluted to about 35 ml. and potassium permanganate solution was added drop by drop in slight excess, destroying the excess with a drop of ferrous sulfate solution. After the solution was cooled to about 15° C., 0.4 ml. of ferric alum indicator was added and the solution was titrated with standard potassium thiocyanate solution.

The results given in Table I indicate that the method is satisfactory for samples containing 100 mg. of mercury. Larger samples were analyzed with equal success.

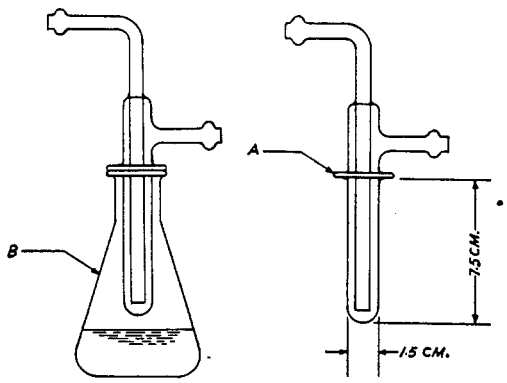

Figure 1. Details of Digestion Apparatus

A. **Glass flange**
B. **Completed assembly**

The reason for digesting the mercury on the steam bath before filtration was to increase the particle size of the mercury thus making it more filterable. Various attempts were made to accomplish the same end by shaking the freshly reduced mercury to eliminate the lengthy digestion. This met with little success until macerated filter paper was added to the flask before the stannous chloride was added. Finely shredded asbestos could not be used instead of the macerated paper. This procedure was substantially the same as when digestion was used, except that the reduction was made at room temperature and the flask containing the mercury and macerated filter paper was shaken for 5 to 8 minutes in a mechanical shaker before filtration. The average error in seven analyses of samples of mercuric nitrate containing 250 mg. or more of mercury was ±0.18%. For some unknown reason the errors in the analysis of samples containing 100 mg. of mercury were proportionately greater than would have been expected. It was suspected that the difficulty was due to incomplete removal of chloride from the macerated filter paper during the washing process, or to a loss by volatilization during filtration because the mercury was in such a finely divided state.

Even though the macerated filter paper method gave results which were satisfactory for fairly large amounts of mercury, the problem of lengthy digestion on a steam bath was not solved for samples containing small amounts of mercury—i.e., 100 mg. or less. Therefore a semimicromethod was developed in which the reduced mercury was centrifuged and filtered with an immersion filter.

Table I. Determination of Inorganic Mercury by Modified Krieckhaus Method

[Mercury taken as $Hg(NO_3)_2$ = 0.10088 gram]

Mercury Found	Difference	Error
Gram	*Gram*	%
0.10074	−0.00014	−0.14
0.10063	−0.00025	−0.25
0.10066	−0.00022	−0.22
0.10075	−0.00013	−0.13
0.10059	−0.00028	−0.28

Table II. Semimicrodetermination of Inorganic Mercury

Compound[a]	Mercury Taken	Mercury Found	Error
	Gram	*Gram*	%
$Hg(NO_3)_2$	0.05014	0.05003	−0.22
	0.05014	0.05009	−0.10
	0.05014	0.05004	−0.20
	0.05014	0.05007	−0.14
$HgCl_2$	0.06881	0.06884	+0.04
	0.07201	0.07196	−0.07
	0.07754	0.07749	−0.07
$HgBr_2$	0.04400	0.04413	+0.29
	0.02979	0.02990	+0.37
$HgSO_4$	0.04052	0.04047	−0.12
	0.04036	0.04031	−0.12
	0.04008	0.04010	+0.05
$Hg(C_2H_3O_2)_2$	0.03895	0.03888	−0.18
	0.03750	0.03746	−0.10

[a] Purity of compounds determined by method of Sloviter, McNabb, and Wagner (*6*).

Procedure for Determining Semimicro Amounts of Mercury. Samples containing 30 to 100 mg. of mercury were poured into a 15-ml. centrifuge tube and 5 ml. of water containing 2 to 3 drops of hydrochloric acid were added. The solution was heated to 50° C. and a mixture of 3 ml. of concentrated hydrochloric acid and 3 ml. of stannous chloride was added at the same temperature. The mercury precipitated rapidly and completely. The precipitated mercury was allowed to stand for 5 minutes, reheated to 50° C., and centrifuged for 5 minutes at 1500 r.p.m. At this point all the mercury was generally in globular form, but if not it was reheated in a water bath, allowed to stand for a few minutes at 50° C., and then centrifuged for 5 to 8 minutes more.

The supernatant liquid was removed by means of an immersion filter, 10 mm. in diameter and of "fine" porosity. When the level of the liquid in the apex of the centrifuge tube fell below the filter, the tube was raised to a horizontal position, so that all the liquid was removed. The mercury was washed 3 to 4 times with 2-ml. portions of 2 *N* sulfuric acid and then with water until no chloride was detected in the wash water. The wash water was removed with the immersion filter each time.

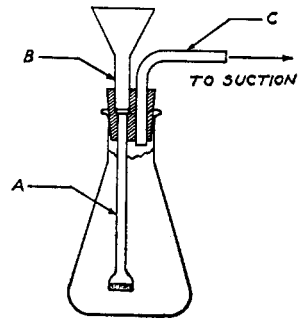

Figure 2. Assembly for Washing Immersion Filter

The immersion filter was disconnected and placed in the centrifuge tube with 2 ml. of concentrated nitric acid, in which it was allowed to stand for 3 to 4 minutes until all the mercury had been dissolved from the pores of the filter. The contents of the centrifuge tube were transferred to a 125-ml. Erlenmeyer flask and the filter was washed as shown in Figure 2. Water was added to the buret funnel, *B*, and drawn through the immersion filter, *A*, into the flask by applying suction at *C*. The filter was removed and potassium permanganate was added as in the previous procedures, and the solution was diluted to 30 ml. and titrated with standard potassium thiocyanate solution at 12° to 15° C., using 0.4 ml. of ferric alum indicator.

The results of the analysis of several inorganic mercury compounds are to be found in Table II.

Table III. Determination of Mercury in Organic Compounds

Compound	Sample	Mercury Found	Mercury Found, Umpire Method[a]
	Gram	%	%
Phenyl mercuric acetate	0.18924	59.73	59.71
	0.09028	59.79	59.54
		Av. 59.76	59.63
Phenyl mercuric bromide	0.09720	55.76	55.69
	0.08287	55.81	55.64
	0.12172	55.80	...
	0.07034	55.71	...
	0.10180	55.89	...
		Av. 55.79	55.67
Pheny mercuric chloride	0.12075	64.01	63.79
	0.08410	64.00	...
	0.08452	63.96	...
		Av. 63.99	63.79
Pheny mercuric nitrate	0.08807	63.24	63.09
	0.05219	62.96	62.96
		Av. 63.10	63.03
Di-*p*-tolyl mercury	0.10770	52.01	52.16
	0.08881	51.88	52.01
	0.09333	52.01	51.92
		Av. 51.97	52.03
p-Dimethylamino phenyl mercuric acetate	0.09523	53.16	53.24
	0.10494	53.19	53.24
	0.11674	53.26	...
	0.10679	53.35	...
		Av. 53.24	53.24
Pyridine mercuric chloride	0.10777	60.83	60.82
	0.10265	60.77	60.97
		Av. 60.80	60.89
Mercurochrome[b]	0.27441	22.81	22.87
	0.28178	22.74	22.70
	0.35346	22.82	22.95
		Av. 22.79	22.84

[a] Umpire method used for first five compounds was method of Sloviter, McNabb, and Wagner (*6*). Remaining three compounds analyzed by Rauscher's method (*5*).

[b] Samples weighed without drying. Samples used for umpire method weighed at same time and under same conditions as for method described.

An immersion filter was necessary for the removal of the supernatant liquid from the mercury after centrifuging, even though the bulk of the mercury was in globular form in the bottom of the centrifuge tube. A scarcely perceptible film of mercury remained floating on the surface and was lost when the liquid was removed with a capillary tube instead of an immersion filter. The successful use of sintered-glass immersion filters depended upon the globular form of the mercury. If much of the mercury was finely divided, filtration of the wash water became slow with increased washing because the filter became partially clogged with mercury.

Since the amount of ferric alum indicator used in titrating mercury with thiocyanate solution was found to be critical by Danford, McNabb, and Wagner (*2*), the conditions present in the standardization of the thiocyanate solution were carefully duplicated in all titrations of mercury.

In testing for the absence of chloride while washing the mercury, sufficient time must be allowed for the supersaturated solution of silver chloride to break down. The absence of any opalescence immediately following the addition of silver nitrate to the wash water did not necessarily mean a complete absence of chloride ion. Sometimes as long as 3 to 4 minutes elapsed before a faint opalescence of silver chloride appeared.

This method is not recommended for the determination of mercury in mercuric iodide. Willard and Boldyreff (*8*) showed that the presence of iron, lead, cadmium, copper, antimony, or bismuth did not interfere with the reduction of mercury to the free state with stannous chloride.

DETERMINATION OF ORGANIC MERCURY

The success attained by Rauscher (*5*) in precipitating free mercury from organic compounds without previous destruction of the organic matter suggested the possibility of attaining the same end by the stannous chloride method. The relative insolubility in water of most organic compounds was a factor which, though nonexistent in the Rauscher method, had to be considered in this case. It became necessary to find a water-soluble solvent in which the organic compounds were sufficiently soluble, so that the diluting effect of the stannous chloride–hydrochloric acid mixture would not throw them out of solution, either before or after precipitation of the mercury. Acetone, ethyl alcohol, glacial acetic acid, dioxane, and hydrochloric acid were tried with varying degrees of success.

Seven different aromatic compounds, all belonging to the type in which the mercury was directly attached to the benzene ring, were tested qualitatively to determine whether or not stannous chloride would reduce the mercury to the free state. Each compound was dissolved in one of the above-mentioned solvents and a stannous chloride–hydrochloric acid mixture added. In all cases, mercury was precipitated regardless of the solvent used. However, quantitative tests later indicated that reduction of the mercury was very incomplete in some instances. After the samples were dissolved in a suitable solvent, attempts were made to apply the centrifuge tube–immersion filter method, which had been used for inorganic mercury, but this proved unsuccessful except in the case of *o*-chloromercuriphenol and pyridine mercuric chloride. There were two outstanding difficulties—namely, the slow and incomplete reduction of the mercury and the finely divided state of the metal after reduction. When water was added to the centrifuge tube after the precipitated mercury had been centrifuged, a further reduction of the mercury resulted. If the reduction was carried out between 80° and 100° C., the rate of the reaction increased and the mercury was much more globular. Since the amount of water that could be added to the centrifuge tube was limited by its size and there was danger of losing mercury by volatilization if the reduction was carried out at a high temperature, the centrifuge tube–immersion filter method was discarded. Instead, the reduction was carried out in a large test tube containing a cold finger and the mercury was filtered through a Gooch crucible.

Procedure for Determining Mercury in Organic Compounds. The sample containing 50 to 70 mg. of mercury was placed in a Pyrex test tube, 200 by 25 mm., and 3 ml. of dioxane containing 2 drops of hydrochloric acid were added in such a way as to wash down any of the sample clinging to the wall. (In the case of mercurochrome the sample was dissolved in 2 ml. of water before the acidified dioxane was added.) If the sample did not readily dissolve, heat was gently applied until solution was complete. A mixture of 2 ml. of stannous chloride and 2 ml. of hydrochloric acid was added and a 180 by 15 mm. cold finger was inserted to a point directly above the liquid. The test tube was lowered into an oil bath and, after the mixture had digested for 3 minutes at 115° C., the tube was removed from the bath and 7 ml. of water were added and mixed thoroughly with the dioxane solution. The digestion was resumed at 115° C. until the supernatant liquid became clear and the mercury was in globular form; this required 25 to 30 minutes, depending upon the compound being analyzed. Two milliliters of water were added and if the supernatant liquid remained clear after 3 minutes, reduction of the

mercury was considered complete. If a cloudiness resulted digestion was continued until the supernatant liquid was clear.

The cold finger was washed down with water and removed. The mercury was filtered through a No. 2 Gooch crucible and washed by decantation 2 or 3 times with 10-ml. portions of water and then with one 5-ml. portion of 2 *N* sulfuric acid. The washing was continued with water until the filtrate gave no indication of chloride ion with silver nitrate solution. Two milliliters of concentrated nitric acid were added to the test tube and the resulting mercury solution was transferred to a 125-ml. Erlenmeyer flask. Using a small stirring rod the asbestos was transferred from the crucible to the Erlenmeyer flask. The crucible was placed over the opening of the flask, and 2 ml. of hot concentrated nitric acid were poured around the inside of the crucible in such a way as to dissolve any finely divided mercury which might be clinging to the wall and permit the acid to drain directly into the flask. The solution was diluted to about 30 ml., potassium permanganate solution added drop by drop in slight excess and the excess destroyed with a drop of ferrous sulfate solution. After the solution had been cooled to about 15° C., 0.4 ml. of ferric alum indicator was added and the solution was titrated with standard potassium thiocyanate solution.

The results obtained in the analysis of eight organic compounds are shown in Table III.

An oil bath was necessary for the digestion because severe bumping resulted when the test tube containing the mercury was heated directly with a microburner. Boiling stones were ineffective. Paraffin oil contained in a 150-ml. beaker served as a convenient bath for the digestion. The temperature of the bath was safely maintained between 115° and 120° C. without danger of bumping in the test tube. When water was substituted for the oil low results were obtained in the analysis.

Samples larger than those designated are not recommended because too much water must be added to the reduction mixture to ensure complete precipitation of the mercury. This would decrease the effectiveness of the cold finger and would also necessitate the use of a rather cumbersome oil bath.

In some instances when water was added to the clear supernatant liquid during digestion to determine whether or not reduction was complete, a two-phase liquid system resulted on cooling and was at first mistaken for finely divided mercury. However, upon reheating, the phases merged into one, indicating that the cloudiness was due to the organic reduction product which came out of solution at the lower temperature.

Since good results were obtained for the organic compounds analyzed, and since the mercury was attached directly to the benzene ring in most cases, it is believed that this procedure is general in application.

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Colorimetric Microdetermination of Antimony with Rhodamine B

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Modifications of a method for the colorimetric determination of antimony in biological material are reported, a second technique is presented which has certain advantages over the original, and the reactions involved are discussed.

IN A preliminary report from this laboratory (*13*), a method for the colorimetric determination of antimony in biological material was described. The purposes of the present paper are to present and modify that procedure in the light of more extensive experience, to report a second technique which has certain advantages over the original, and to discuss the reactions involved in these methods.

The impetus for the research described in these publications came from the use of antimony compounds in the treatment of several tropical diseases, which were of particular importance to this country during the war. Antimony therapy has been fairly widespread in the tropics since about 1910, but the pharmacological data to govern its clinical use have been inadequate, largely because of the lack of a suitable analytical tool. The colorimetric methods presented here have proved useful in laboratory and clinical work in this direction. It has been possible to study the distribution of antimony in animal organs (*18*) and to follow blood levels and excretion rates in experimental animals and in patients undergoing therapy (*19*).

Up to the present decade, chemical methods of determining the distribution of antimony in biological material were carried out chiefly by estimation of antimony sulfide or by adaptations of the Gutzeit procedure. Goodwin and Page (*8*) have summarized this early work, and have developed a polarographic procedure which was suitable for fairly small (10 micrograms) amounts of antimony in plasma and urine. Since the completion of the colorimetric methods reported here, McChesney (*12*) has described the application of the iodoantimonite reaction to samples of biological material containing a minimum of 10 micrograms of antimony.

From known blood levels of other heavy metals it seemed reasonable to assume that antimony blood levels during the course of therapy might well fall below 1 microgram per gram. The data obtained with the method described here and the studies by Brady *et al.* (*1*) and Cowie *et al.* (*3*) using radioactive antimony have amply confirmed this assumption.

The best opportunity to develop a simple method effective at

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