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2-(o-Hydroxyphenyl)-benzimidazole as a Reagent for the **Determination of Mercury**

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2-(o-Hydroxyphenyl)-benzimidazole, a compound synthesized in the organic analytical reagents program, was found to be selective for mercury. As few of the organic reagents now in use for mercury are selective, it was decided to develop analytical methods based upon the use of the new reagent. It was found possible to determine, either gravimetrically or volumetrically, from 10 to 85 mg. of mercury with an average accuracy of 0.3 mg. using this reagent. Ferric ion is the main interference. The methods described are simple, rapid, and accurate. The use of 2-(o-hydroxyphenyl)-benzimidazole makes possible the determination of mercury in the presence of many metals which heretofore were serious interferences.

THE reagent, 2-(o-hydroxyphenyl)-benzimidazole, well deserves to take its place among the highly selective reagents for the determination of mercury. The most popular reagents now in use for the determination of macro amounts of mercury are mercaptobenzimidazole, tetraphenylarsonium chloride, the azo derivatives of 8-quinolinol, and thionalide (2, 6). These reagents may be used with success only in the absence of most other heavy metals. Even when thionalide is used, the most selective of those mentioned, no copper or silver may be present, the chloride ion concentration must be exceedingly low, and a threefold excess of reagent must be used. 2-(o-Hydroxyphenyl)benzimidazole may be used for the determination of mercury in the presence of most of the ions commonly interfering with mercury. The only ion that seems to cause interference in the determination, if present in amounts greater than a few milligrams, is iron.

When an alcoholic solution of the reagent is added to a slightly acid solution containing mercuric ions, a precipitate is formed which when dried at 130° C. corresponds to the formula Hg $(C_{13}H_9N_2O)_2$. The following equation, with the mercury compound represented as a possible chelate, best describes the reaction involved.



In the development of a volumetric method for the mercury determination, use was made of the fact that the reagent, a phenol, could be quantitatively brominated.

REAGENTS AND APPARATUS

2-(o-Hydroxyphenyl)-benzimidazole is a new organic compound, first prepared in this laboratory by a procedure analogous to that used for preparation of 2(o-hydroxyphenyl)-benzoxazole (5).

One mole of o-phenylenediamine and 1 mole of salicylamide were mixed in a mortar until finely powdered, then heated in a round-bottomed flask immersed in an oil bath. The mixture melted at about 160° C. and evolved ammonia and water. After the melting was complete, the temperature was raised to 200° C. and maintained there until the ammonia evolution ceased. The total heating period was about 4 hours. The molten mixture was transferred into a distilling flask and distilled under atmospheric pressure. All the material was distilled over to give a yellow colored mass which was recrystallized three times from an ethyl alcohol-water mixture. The pure compound, obtained in 30% yield, melted at 242° C. (corrected). The solid compound fluoresces strongly in the ultraviolet with a blue color. Its alcoholic solution has a strong blue fluorescence. Analysis: Calculated for C₁₃H₁₀N₂O, 13.32% N; found, 13.62% N. **Standard Mercury Solution**. Exactly 3.1990 grams of C.P. mercury metal obtained from Eimer and Amend was dissolved

Standard Mercury Solution. Exactly 3.1990 grams of C.P. mercury metal obtained from Eimer and Amend was dissolved in dilute nitric acid and the volume brought to 1000 ml. with distilled water in a calibrated volumetric flask. Aliquots of this solution were taken as needed.

Reagent Solution. A 1% w./v. solution of the reagent in $95\tilde{c}_c$ ethyl alcohol was used.

Metal Ion Solutions. Reagent grade nitrates of barium, magnesium, zinc, mercury, aluminum, chromic, ferric, manganous, silver, cupric, cobaltous, nickelous, bismuthous, cadmium, lead, arsenic, sodium, potassium, and stannous chloride were used to make solutions which contained approximately 50 mg. of metal per milliliter for use in testing the specificity of the reagent.

Apparatus. All pH measurements were carried out using a Model G Beckman pH meter. A Beckman Model DU quartz spectrophotometer was used to obtain the absorption spectra of the reagent and the mercury chelate.

In the iodometric titration of the dissociated chelate, the deadstop indicator apparatus was utilized. This apparatus was first introduced by Foulk and Bawden in 1926 (3) and is best described by Willard, Merritt, and Dean (7). Figure 1 shows the modification of the Foulk and Bawden apparatus.

QUALITATIVE REACTIONS

Qualitative tests were performed upon several common ions. In acetic acid-sodium acetate buffered solution, 2-(o-hydroxyphenyl)-benzimidazole precipitates only mercury. It does not form a precipitate with aluminum, bismuth, ferric, ferrous, zinc, cupric, cadmium, manganous, nickelous, barium, lead, magnesium, cobaltous, chromic, silver, stannous, arsenic, sodium, and potassium. Reaction probably occurred with a number of these metals, inasmuch as color changes were observed upon the addition of the reagent to certain of the metal solutions. The analytical significance of these color changes is under study. No discernible precipitate formation was observed with any of the above metals in either ammoniacal or alkaline tartrate solution.

PROPERTIES OF MERCURY PRECIPITATE OF 2-(o-HYDROXYPHENYL)-BENZIMIDAZOLE

Thermal Stability. A quantity of the dry yellow mercury precipitate was introduced into a capillary melting point tube and heated slowly. At 250° C. a darkening of the precipitate was noted. No sign of melting was noted even at 320° C. It was concluded that a temperature between 130° and 140° was a safe drying temperature for the precipitates. It was found that the precipitate could be heated to constant weight in 2 hours at 130° to 140° C.

Composition. A microanalysis was performed at the university organic microanalytical laboratory to determine the amount of nitrogen present in the precipitate. Calculated for $Hg(C_{13}H_{s}-N_{2}O)_{2}$, 9.10% N; found, 9.30% N. Further proof of the validity of this structural formula is found in the fact that 0.3230, the theoretical value of the gravimetric factor for mercury in $Hg(C_{12}-H_{s}N_{2}O)_{2}$, was used in all calculations with good results.

ANALYTICAL CHEMISTRY

Solubility. The solubility of the mercury chelates in various organic solvents was determined in a qualitative manner. The precipitate was found to be insoluble or only very slightly soluble in ethyl alcohol, methanol, hexane, acetone, chloroform, carbon tetrachloride, and benzene. It was very soluble in glacial acetic acid, giving a solution which had a bluish tinge and which fluoresced with a bright blue color under ultraviolet light. As the ultraviolet absorption spectra of both the reagent and the mercury chelate solutions were identical, it was concluded that acetic acid dissociated the chelate.

EFFECT OF pH UPON PRECIPITATION OF MERCURY

In order to investigate further the potentialities of this compound as an organic reagent, the optimum pH range for complete mercury precipitation was studied. The following series of experiments was performed to accomplish this.

A definite amount, 25.00 ml., of the standard mercury solution (containing 0.0800 gram of mercury) was diluted to 100 ml. with distilled water and heated to 60° C., and 25 ml. of the alcoholic reagent solution (containing 0.180 gram of reagent) representing about 5 to 10% excess was added. The pH was then adjusted to the desired value by adding dilute sodium hydroxide. The precipitate was digested at 60° C. for 15 minutes, allowed to cool to room temperature, filtered through a medium porosity sintered-glass crucible, and dried at 130° to 140° to constant weight (at least 2 hours). The per cent precipitate was plotted against pH as shown in Figure 2, A.



G. Galvanometer, sensitivity 0.1 µa. per mm.

From curve A, Figure 2, it can be seen that the pH range of complete precipitation is between 6 and 7. A pH of 7.3 was not exceeded, as above this pH mercuric oxide would precipitate and seriously interfere in the determination. Because in this pH range other metals might interfere by the precipitation of some of the hydrous oxides, the precipitation must be carried out in the presence of some complexing material. Cyanide was immediately ruled out as the mercury cyanide complex is very stable. Tartrate was tried, but too many metals gave interference in the determination of mercury. Citrate was found very effective and therefore the effect of its presence upon the precipitation characteristics of the mercury salt was also investigated.

The procedure described above was followed, except that 2 grams of sodium citrate was added before the solution was heated to 60°. The per cent precipitated is plotted against pH in Figure 2, B.



Figure 2. Effect of pH on Precipitation of 2-(o-Hydroxyphenyl)-benzimidazole Complex of Mercury A. No citrate present B. Citrate present

Introduction of citrate did not seem to affect the pH range of complete precipitation. However, at higher pH's the mercury complex is broken up to form either a soluble complex or mercuric oxide.

As the mercury precipitate was insoluble in alcohol, it was found convenient to wash the precipitate free of excess reagent with 50% alcohol.

RECOMMENDED PROCEDURE

A number of mercury determinations in which varying amounts of mercury were present were run by the procedure used in the previous experiments, with the pH adjusted after precipitation to 6.5 using the pH meter. The data, represented in Table I, show the determination to be accurate to an average of about 0.2 mg. in samples containing from 10 to 85 mg. The weight of mercury was determined in each case by multiplying the weight of the precipitate by the gravimetric factor 0.3230.

Procedure for Mercury. To a solution containing from 10 to 100 mg. of mercury, which may contain large amounts of most

Mercury Taken,	Weight of Frecipitate,	Mercury Found,	Error,
Grani	Gram	Gram	Gram
0.0800	0.2470	0.0798	-0.000
0.0800	0.2506	0.0807	+0.000
0.0800	0.2500	0.0806	+0.000
0.0800	0.2482	0.0799	-0.000
0.0800	0.2470	0.0798	-0.000
0.0800	0.2482	0.0801	+0.000
0.0800	0.2499	0.0805	+0.000
0.0800	0.2498	0.0805	+0.000
0.0782	0.2423	0.0783	+0.000
0.0782	0.2426	0.0783	+0.000
0.0153	0.0470	0.0150	-0.000

metals but no appreciable amount of iron, is added about 2 grams of sodium citrate. The solution is heated to 60° C. and the pH is brought to 5 with dilute sodium hydroxide or dilute acetic acid, depending upon which is required. Sufficient reagent solution is added, containing a slight excess of the amount necessary to precipitate completely the mercury present in solution. In the procedure used, only a small (6 to 10%) excess of reagent was used; however, up to 90% excess may be washed completely free of the precipitate with a 50% alcohol solution. The pH is then raised to 6.5 with 0.3 N sodium hydroxide solution and the resulting precipitate is digested at 60° for about 15 minutes, and allowed to cool to room temperature. The precipitate is then filtered through a weighed medium porosity sintered-glass crucible, washed three or four times with 15 ml. of 50% alcohol, and dried at 130° to 140° to constant weight. As before, the weight of mercury present in the sample was calculated by multiplying the weight of precipitate by the gravimetric factor 0.3230.

Some of the results of the precipitation of mercury with this reagent in the presence of interfering ions can be found in Table II. As this table shows, the reagent is highly selective for mercury, giving results which are accurate to somewhat better than 0.3 mg. in samples containing from 10 to 85 mg. of mercury and in the presence of most metals. Iron in either the ferrous or ferric state is a notable exception. Attempts made to "mask" iron completely by complex tartrate or citrate formation were of no avail.

Volumetric Procedure. Alternatively, the mercury may be determined volumetrically.

The chelate, containing about 80 mg. of mercury, after being precipitated as previously described and washed thoroughly with 50% alcohol solution, is dissolved in about 50 ml. of hot glacial acetic acid, transferred to an iodine flask, and diluted with a little distilled water. Sixty milliliters of a 0.1 N potassium bromate-potassium bromide solution is pipetted into the flask. The flask is immediately stoppered and a few milliliters of potassium iodide solution is added to the reservoir of the iodine flask to prevent any loss of bromine (4).

The reaction is allowed to proceed from 1 to 1.25 hours while the flask is immersed in a beaker of water kept at about 35° . Then a solution containing about 1.5 grams of potassium iodide is added. The liberated iodine is titrated with standard 0.1 N sodium thiosulfate using the dead-stop indicator apparatus to detect the end point.

This apparatus is used in the procedure because of the difficulty encountered in detecting the end point visually, as the precipitate of the bromo compound gives a color to the solution identical with that of an iodine solution.

From the experimental results it was found that three bromines were consumed per molecule of benzimidazole; however, a microanalysis of the bromo compound showed that it contained two bromines. Hence, the third molecule of bromine served to oxidize the compound. Furthermore, this compound had lost the alkali solubility of the parent imidazole, indicating loss of the phenolic function. It is believed that this oxidation was accomplished by loss of the phenolic hydrogen and the imino hydrogen with accompanying ring closure to give the compound illustrated below.



A compound having this particular ring system was not to be found in the Ring Index; however, it can be termed a benzimidazo-benzoisoxazole. There is precedence for this type of oxidative ring closure in the literature (1).

The following equations describe the steps involved in the volumetric procedure.

The mercury is precipitated as the chelate,

Metal Ion Present	Gram	Mercury Taken, Gram	Mercury Found, Gram	Error, Gram
$\begin{array}{c} Co(II)\\ Pb\\ Zn\\ Ca\\ Mg\\ Ni\\ Al\\ Cu(II)\\ Cl\\ Sn(II)\\ Fe(III)\\ Fe(III)\\ Fe(III) \end{array}$	$\begin{array}{c} 0.100\\ 0.100\\ 0.100\\ 0.100\\ 0.100\\ 0.100\\ 0.100\\ 0.100\\ 0.100\\ 0.070\\ 0.100\\ 0.005\\ 0.100\\ 0.005\\ 0.100 \end{array}$	$\begin{array}{c} 0.0800\\ 0.0800\\ 0.0810\\ 0.0810\\ 0.0810\\ 0.0810\\ 0.0800\\ 0.0782\\ 0.0782\\ 0.0782\\ 0.0782\\ 0.0782\\ 0.0782\\ 0.0782\\ 0.0782\\ 0.0810 \end{array}$	$\begin{array}{c} 0.0805\\ 0.0806\\ 0.0813\\ 0.0820\\ 0.0816\\ 0.0809\\ 0.0793\\ 0.0783\\ 0.0783\\ 0.0783\\ 0.0783\\ 0.0783\\ 0.0783\\ 0.0783\\ 0.0783\\ 0.0856\\ \end{array}$	$\begin{array}{c} +0.0005\\ +0.0006\\ +0.0003\\ +0.0010\\ -0.0001\\ -0.0001\\ +0.0001\\ +0.0001\\ +0.0001\\ +0.0001\\ +0.0001\\ +0.0001\end{array}$
		Average	(Run 12 omitte	ed) ± 0.0004
Table III.	Volume	tric Detern	nination of	Mercury
Mercury	Standar	d Mei	cury	-

Table II. Determination of Mercury in Presence of Other Ions

Volumetric Determination of Mercury				
Standard BrO3-Br Used, Ml.	Mercury Found, Gram	Error, Gram		
46.8 48.8 48.3 48.3 48.3 48.8 46.8 47.0 9.3	$\begin{array}{c} 0.0781 \\ 0.0809 \\ 0.0802 \\ 0.0809 \\ 0.0809 \\ 0.0802 \\ 0.0781 \\ 0.0782 \\ 0.0153 \end{array}$	$\begin{array}{c} -0.0001 \\ +0.0001 \\ -0.0006 \\ +0.0001 \\ -0.0006 \\ -0.0001 \\ 0.0000 \\ +0.0003 \end{array}$		
	Volumetric L Standard BrOp-Br Used, Ml. 46.8 48.8 48.3 48.3 48.3 48.8 46.8 47.0 9.3	Volumetric Determination Standard BrOs-Br Used, Ml. Mercury Found, Gram 46.8 0.0781 48.8 0.0809 48.3 0.0802 48.8 0.0802 46.8 0.0781 9.3 0.0153		

$$Hg^{++} + 2C_{13}H_{10}ON_2 \longrightarrow Hg(C_{13}H_9ON_2)_2 + 2H^+$$
(1)

The mercury chelate is dissolved in acetic acid,

$$Hg(C_{13}H_{9}ON_{2})_{2} + 2HOAc \longrightarrow Hg^{++} + 2OAc^{-} + 2C_{13}H_{10}ON_{2}$$
 (2)

The dissociated chelate is then brominated,

$$2C_{13}H_{10}ON_2 + 6Br_2 \longrightarrow 2C_{13}H_6ON_2Br_2 + 8HBr \qquad (3)$$

$$\therefore 1 \text{ atom of } Hg \equiv 6Br_2$$

From this, one can easily calculate the percentage of mercury present in the sample.

As for each mercury atom is solution 6 molecules of bromine are consumed, a fairly low factor is obtained, giving a high sensitivity. It was found that an accuracy of an average of about 0.2 mg. could be obtained by this procedure. Some of the results can be seen in Table III. In the third column, the number of grams of mercury contained in the samples was calculated by multiplying a quantity, A, which is the volume of thiosulfate used for a blank minus the volume used for the sample, by the normality of the thiosulfate, and finally by the volumetric factor 0.0167.

CONCLUSIONS

The reagent is very easily prepared in good yields. The methods described here are fairly sensitive, and the procedures are simple. The precipitate formed has a comparatively small gravimetric factor, 0.3230. The precipitates are thermally stable and very insoluble in water and most organic solvents. The volumetric factor is low, 1 ml. of 0.1 N sodium thiosulfate corresponding to 1.67 mg. of mercury. The reagent is highly selective for mercury and gives results which are accurate to somewhat better than 0.3 mg. in samples containing from 10 to 100 mg. of mercury, in the presence of most metals. Iron can be tolerated only in very small quantities.

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Measurement of Deuterium Oxide Concentration in Water Samples by the Mass Spectrometer

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THE determination of the deuterium concentration in water samples is becoming an increasingly important measurement in biological tracer studies. In the past the mass spectrometer has been used extensively for making this determination, but in the majority of cases it has been necessary to convert the water sample to gaseous hydrogen and deuterium or to some other compounds, such as a mixture of methane and methyl deuteride (δ) , which in turn are analyzed on the mass spectrometer. It is the purpose of this paper to show how accurate results can be obtained in the determination of small concentrations of deuterium by running water samples directly on a 158° or 180° mass spectrometer. The direct analysis has the advantages of simplicity and small sample requirements.

Some results obtained by analyzing water samples on a 90° sector-type mass spectrometer have been reported previously (7).

These results, however, were of low accuracy, a discrepancy of ± 0.2 atom % concentration of deuterium being indicated for low concentrations of deuterium. In the present experiments it was found that by using a mass spectrometer with a high magnetic field at the ion source and by making proper adjustments of the repeller voltage, electron current, and sample pressure, differences of deuterium concentrations as low as 0.0006 atom % could be determined. These results show, therefore, accuracies comparable with those obtained when the water is first converted to gaseous hydrogen and deuterium.

STATEMENT OF PROBLEM

The problem of determining the deuterium concentration in a water sample containing unknown amounts of deuterium and of