Analytical Edition
Vol. 9, No. 10

# INDUSTRIAL and ENGINEERING CHEMISTRY

October 15, 1937

Vol. 29, Consecutive No. 39
Published by the American Chemical Society
Harrison E. Howe, Editor

Publication Office: Easton, Pa. . Editorial Office: Room 706, Mills Building, Washington, D. C. . Telephone: National 0848
Cable: Jiechem (Washington) . Advertising Department: 332 West 42nd Street, New York, N. Y. . Telephone: Bryant 9–4430

## CONTENTS

*19,100 Copies of This Issue Printed*

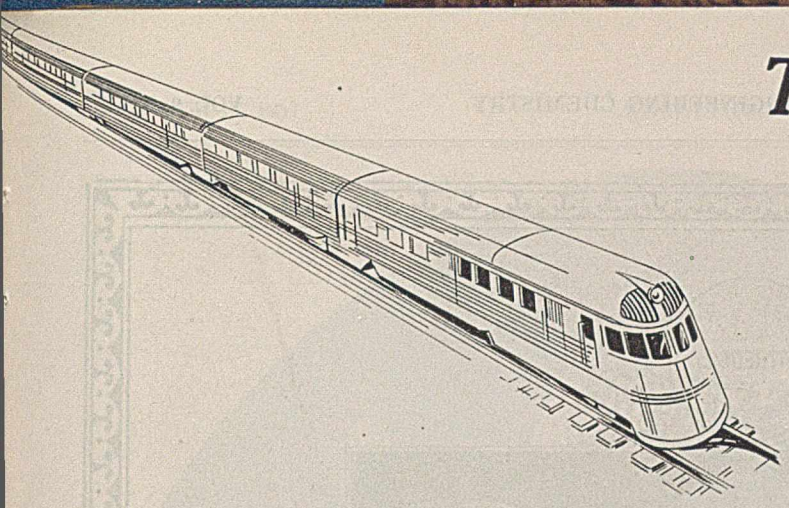




Published by the American Chemical Society, Publication Office, 20th & Northampton Sts., Easton, Pa. Entered as second-class matter at the Post Office at Easton, Pa., under the Act of March 3, 1879, as 48 times a year. Industrial Edition monthly on the 1st; Analytical Edition monthly on the 15th; News Edition on the 10th and 20th. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized July 13, 1918.

Annual subscription rates: (*a*) INDUSTRIAL EDITION $5.00; (*b*) ANALYTICAL EDITION $2.00; (*c*) NEWS EDITION $1.50; (*a*) and (*b*) together, $6.00; (*a*), (*b*), and (*c*) complete, $7.50. Foreign postage to countries not in the Pan American Union, (*a*) $1.20; (*b*) $0.30; (*c*) $0.60; to Canada one-third these rates. Single copies: (*a*) $0.75; (*b*) $0.50; (*c*) $0.10. Special rates to members.

Claims for copies lost in mails to be honored must be received within 60 days of date of issue and based on reasons other than "missing from files." Ten days' advance notice of change of address is required. Address Charles L. Parsons, Business Manager, Mills Building, Washington, D. C., U. S. A.



# *There's a* COLEMAN *in the Cab!*

On speeding streamlined trains . . . in laboratory . . . in industry . . . in a hundred varied fields of pH activity—COLEMAN pH ELECTROMETERS are setting the pace for pH technique.

*Setting the Pace for pH*

**COLEMAN pH Electrometer**

A widely diverging and constantly expanding activity in pH research and control finds, COLEMAN ASSEMBLIES, ready for practical adaptation to nearly every phase of pH work.

With COLEMAN EQUIPMENT, an efficient, reliable pH technique has been established in many industries, assuring the proved profits of pH control.

In accuracy, compactness, rugged reliability, comprehensiveness, and simplicity of operation, COLEMAN EQUIPMENT is setting the pace for pH practice in the hands of both veteran and less experienced operators.

Four battery operated and three AC Models are offered . . . with a complete selection of ELECTRODE ASSEMBLIES to meet all requirements of pH research. Write TODAY for descriptive literature.

*A new catalog showing the complete COLEMAN LINE will be ready for distribution shortly. Write today and Reserve Your Copy.*

**LOOK FOR THE GUARANTEE CARD**
accompanying ALL COLEMAN INSTRUMENTS and ELECTRODES. It is your assurance of our responsibility.

## COLEMAN ELECTRIC CO., Inc.

310 MADISON ST., MAYWOOD, ILLINOIS

**SIMPLICITY . . ACCURACY . . RUGGED PRACTICABILITY**

ANALYTICAL EDITION

INDUSTRIAL and ENGINEERING CHEMISTRY

Harrison E. Howe, Editor

# Determination of Glycol or Glycerol

## In Dilute Solutions Containing Oxidizable Impurities

WILLIAM E. SHAEFER

Hercules Powder Company, Experiment Station, Wilmington, Del.

GLYCEROL and glycol are so similar in their chemical behavior that, generally, a chemical method suitable for the determination of one is also applicable to the determination of the other. Regardless of the type of substance being analyzed for glycerol, the two commonly used methods for its quantitative determination are the dichromate oxidation method and the acetin method, wherein the acetate is formed and saponified. Before the dichromate method can be applied to a sample, all oxidizable impurities and all chlorides must be removed. This is more difficult and time-consuming than is desirable. The acetin method, according to some writers (*3, 5*), cannot be applied to a glycerol solution less concentrated than 40 or 50 per cent.

The problem at hand was to analyze dilute (1 to 5 per cent) aqueous glycol solutions that also contained oxidizable impurities. Since the direct and simple pyridine acetic anhydride method (*1, 2, 4, 6*) possesses manifest advantages, the writer wished to employ it if possible in solving this problem. Evidently such a method cannot be applied directly to the analysis of a 5 per cent glycol solution because the water in a 2-cc. sample, containing 100 mg. of glycol, would require 10.8 grams of acetic anhydride. There seemed to be no hope of removing a sufficiently large proportion of the water by distillation of an aqueous glycol solution through a column, without a serious loss of glycol, so that the pyridine–acetic anhydride method could be applied to the residue. However, it proved possible to remove the water completely by distillation when a suitable liquid was added to form an azeotropic mixture.

### Experimental Work

Several preliminary experiments were made in an effort to remove practically all the water from a dilute glycol solution by distillation after the addition of xylene to form a constant-boiling mixture with water. In further experiments, pyridine was employed to form a one-phase solution for the acetylation. Since results were encouraging, the work was continued and the procedure described below was developed.

Before working out the details, two determinations of glycerol and a number of determinations of glycol were made by the direct pyridine–acetic anhydride acetylation method. The specific gravity of the glycerol used in this work indicated that it was 98.6 per cent pure. In two preliminary experiments, acetylation was found to proceed to the extent of 97.8 per cent in 30 minutes and to the extent of 96.6 per cent in 2 hours. The latter value, although surprising because it was lower than the former, was not confirmed because the author was interested primarily in the determination of glycol. The glycol used in these and subsequent experiments had a specific gravity of 1.1172 at 15.6°/15.6° C., which corresponds to a purity of 99.5 per cent.

The results of acetylation experiments on weighed samples of this glycol are shown in Table I.

On the basis of these results it was concluded that in the acetylation of glycol for 15 minutes under these conditions the reaction proceeds only to the extent of about 97.9 per cent of the stoichiometric value.

Pyridine hydrate, $C_5H_5N \cdot 3H_2O$, has a constant boiling point of 94.4° C. In a number of experiments a 50-cc. sample of dilute glycol solution was distilled slowly through a 3-bulb Snyder column until its volume was reduced to about

TABLE I. EXTENT OF ACETYLATION OF GLYCOL BY PYRIDINE–ACETIC ANHYDRIDE REAGENT

| Refluxing | | | | Standing at Room Temperature for |
|---|---|---|---|---|
| 1 hour | 15 min. | 10 min. | 2 min. | 1 Hour |
| % | % | % | % | % |
| 97.0 | 97.2 | 95.9 | 93.0 | 70.9 |
| 97.7 | 97.7 | 94.9 | 83.9 | .. |
| .. | 98.2 | 96.4 | .. | .. |
| .. | 97.1 | .. | .. | .. |
| .. | 98.7 | .. | .. | .. |
| .. | 98.7 | .. | .. | .. |
| Av. | 97.9 | | | |

TABLE II. PRELIMINARY ANALYSES OF SYNTHETIC GLYCOL SOLUTIONS

| Experiment No. | Glycol Present[a] | Glycol Found |
|---|---|---|
| | *Grams* | *Grams* |
| 1 | 2.46 | 2.42, 2.45 |
| 2 | 2.46 | 2.44, 2.43 |
| 3 | 2.46 | 2.45, 2.47 |
| 4 | 2.46 | 2.44, 2.42 |
| 5 | 0.49 | 0.50 |
| 6 | 0.49 | 0.49 |
| 7 | 0.49 | 0.47 |
| 8 | 0.49 | 0.48 |
| 9 | 0.49 | 0.46 |
| 10 | 2.46 | 2.42, 2.43 |
| 11 | 2.46 | 2.45, 2.45 |
| 12 | 0.49 | 0.49 |
| 13 | 0.49 | 0.49 |
| 14 | 0.10 | 0.10 |
| 15 | 0.10 | 0.09 |

[a] Aliquot portions of a solution containing 5.92 grams of glycol per 100 cc. were measured with pipets.

10 cc. Then it was treated with 50 cc. of pyridine and distillation was continued until the temperature reached 99° C. It was assumed that a negligible amount of water was left in the sample after this distillation. If the residue contained more than 0.5 gram of glycol, it was diluted to 100 cc. with pyridine and an aliquot was analyzed. Some results for glycol obtained by this procedure, calculated on the assumption that the acetylation values were only 97.9 per cent of the theoretical ones, are shown in Table II.

In an effort to obtain results of higher accuracy, the distillation of the pyridine-water solution was continued in some cases until the distillation temperature reached 110°. Better results were obtained, which indicated that the more complete removal of the water was beneficial and did not result in an increased loss of glycol. Results obtained by several different analysts following the improved procedure are given in Table III.

The analyses reported in Table IV were made on portions of an aqueous glycerol solution which contained 4.88 grams of glycerol per 100 cc.

The average accuracy of the results obtained by this method was found to be about 1 per cent when applied to samples containing 0.5 gram or more of glycol. The average precision of the results shown in Table III, where added impurities had to be removed, is about 1.5 per cent; that shown in Table IV is about 1.0 per cent.

## Procedure

Measure out a sample of 50 cc. or less which is thought to contain about 2.5 grams or less of glycol and remove any salt which can form a hydrate or an addition compound with glycol. Neutralize the treated sample with dilute acid or alkali, using phenolphthalein as the indicator. Transfer the sample to a pear-shaped acetylation flask and distill slowly through a 3-bulb Snyder column until the volume of liquid in the flask is reduced to about 10 cc.

Remove the thermometer and stopper at the top of the column and add 50 cc. of pyridine, dried over sodium hydroxide. Continue the distillation slowly until the temperature rises to 110° C. Rinse the Snyder column with approximately 10 cc. of pyridine.

To the acetylation flask containing the residue, or to another acetylation flask containing an aliquot of the residue after dilution to 100 cc. with pyridine, add from a Lowy automatic pipet, which need not be calibrated, 25 cc. of approximately 2.6 *N* pyridine–acetic anhydride reagent, always measured at the same chosen temperature. This reagent is prepared by treating 154 cc. of acetic anhydride with 1 liter of dry pyridine. Acetylate the glycol by boiling the reaction mixture gently for 15 minutes. Heating for 1 hour does no harm. Treat the hot reaction mixture with 20 to 30 cc. water to convert the excess acetic anhydride to acetic acid and to rinse down the condenser, cool the flask somewhat to avoid loss of acetic acid, remove it from the condenser, and further cool it with tap water. Titrate with approximately *N* sodium hydroxide solution, using phenolphthalein as the indicator. Certain precautions should be taken during this titration to avoid the danger of saponification of the glycol acetate or glycerol acetate. The solution should be shaken continuously while being titrated to prevent the accumulation of a local excess of alkali and care should be taken to avoid overtitrating it. Treat 25-cc. portions of acetic anhydride–pyridine reagent by the procedure just described to obtain blank values.

Table III. Analyses of Synthetic Glycol Solutions

| Analyst | Experiment No. | Glycol Present | Glycol Found |
|---|---|---|---|
| | | *G./100 cc.* | *G./100 cc.* |
| 1 | 1 | 2.04 | 2.03, 2.02 |
| 2 | 2 | 2.04[a] | 2.02, 2.04 |
| 2 | 3 | 2.04[a] | 2.05, 2.01 |
| 1 | 4 | 1.74[b] | 1.74, 1.72 |
| 2 | 5 | 1.74[b] | 1.76, 1.72 |
| 2 | 6 | 1.74[b] | 1.76, 1.74 |
| 3 | 7 | 2.04[a] | 2.04, 2.04 |
| 3 | 8 | 2.04[a] | 2.01, 2.06 |
| 2 | 9 | 1.98[c] | 1.94, 2.00 |
| 4 | 10 | 1.54 | 1.52, 1.53 |

[a] Solution also contained 4% of HCl. This was neutralized before distillation was begun.
[b] Solution also contained HCl, ethylene dichloride, and chlorohydrin. The HCl was neutralized before distillation was begun.
[c] Solution also contained 4% of $CaCl_2$ and chlorohydrin. A calculated amount of sodium sulfate solution was added and precipitated calcium sulfate was removed by filtration before distillation was begun.

Table IV. Analyses of Synthetic Glycerol Solution

| Experiment No. | Glycerol Present | Glycerol Found |
|---|---|---|
| | *Grams* | *Grams* |
| 1 | 2.44 | 2.45, 2.42 |
| 2 | 2.44 | 2.44, 2.42 |
| 3 | 2.44 | 2.45, 2.42 |
| 4 | 1.71 | 1.73, 1.73 |
| 5 | 1.71 | 1.71, 1.71 |
| 6 | 1.22 | 1.24, 1.21 |

This method is believed to be applicable to the analysis of dilute solutions of glycol or glycerol in water or any solvent boiling below 110° C., unless the solution contains a higher boiling or nonvolatile impurity that can be acetylated.

## Calculation

$$\frac{(A - B) \times \text{normality of alkali} \times 0.03103 \times 100}{\text{cc. of sample} \times \text{density} \times 0.979} = \% \text{ glycol}$$

where $A$ = cc. of sodium hydroxide required for blank
$B$ = cc. of sodium hydroxide required for sample
0.03103 = gram of glycol equivalent to 1 cc. of *N* alkali
0.979 = factor to represent the extent to which stoichiometric results are approached in the reaction

If the glycol sample contains hydrochloric acid which is neutralized before the distillation is started, and if an aliquot of the residue is analyzed, the volume of the sodium chloride formed on neutralization must be calculated and the result used in calculating the volume of pyridine solution in the volumetric flask. The volume so calculated, divided by the size of the aliquot acetylated, is then applied as a factor to the expression used in calculating per cent of glycol. If a dilute glycerol solution is being analyzed, the factor for the glycerol equivalent to 1 cc. of *N* alkali—viz., 0.03069—should be used in place of 0.03103.

## Summary

A method for the determination of glycol or glycerol in dilute solutions that may contain oxidizable impurities consists essentially in the removal of the water or other low-boiling solvent after the addition of pyridine, and subsequent acetylation of the residue. The method cannot be applied in the presence of high-boiling impurities that can be acetylated. Since the acetylation reaction proceeds only to about 98 per cent of the stoichiometric value, a constant correction must be applied in calculating the results. When applied to synthetic mixtures, the average accuracy and precision of the method are about 1 per cent.

## Acknowledgment

The writer wishes to thank two of his colleagues, V. A. Aluise and W. R. Tomlinson, Jr., for their assistance in some of the analytical work.

## Literature Cited

(1) Freed, M., and Wynne, A. M., Ind. Eng. Chem., Anal. Ed., 8, 278 (1936).
(2) Marks, S., and Morrell, R. S., *Analyst*, 56, 428 (1931).
(3) Meyer, Hans, "Nachweis und Bestimmung organischer Verbindungen," p. 44, Berlin, Julius Springer, 1933.
(4) Peterson, V. L., and West, E. S., *J. Biol. Chem.*, 74, 379 (1927).
(5) Sherman, "Organic Analysis," 2nd ed., p. 281, New York, Macmillan Co., 1917.
(6) Verley, A., and Bölsing, Fr., *Ber.*, 34, 3354 (1901).

Received August 2, 1937.

# Determination of Uncombined Lime in Portland Cement

## The Ethylene Glycol Method

D. R. MacPHERSON AND L. R. FORBRICH
Portland Cement Association, Chicago, Ill.

THE determination of uncombined lime, calcium oxide, in Portland cement has been the subject of much study during the past two decades. At present, the glycerol-alcohol method (*3*) is generally used, but, because of the long time required to conduct a single determination, attention during the last few years has been given to the development of more rapid methods. Among the newer methods is the ethylene glycol method (*2*, *4*), which has been studied in this laboratory in connection with pozzolana investigations. The most important finding of these studies, described below, is that for determining uncombined calcium oxide in Portland cement a modification of the ethylene glycol method of Schläpfer and Bukowski (*4*), requiring about 40 minutes, can be substituted for the glycerol-alcohol method which requires from 1 to 7 hours for completion.

The ethylene glycol method is based on the ability of calcium oxide and calcium hydroxide to react with ethylene glycol to form a soluble calcium glycolate which is titratable with standard hydrochloric acid. The reactions involved may be considered to be as follows:

$$\underset{\text{or } Ca(OH)_2}{CaO} + \underset{\text{Ethylene glycol}}{C_2H_6O_2} \longrightarrow \underset{\text{Calcium glycolate}}{CaC_2H_4O_2} + \underset{\text{or } 2H_2O}{H_2O}$$

$$\underset{\text{Calcium glycolate}}{CaC_2H_4O_2} + 2HCl \longrightarrow \underset{\text{Ethylene glycol}}{C_2H_6O_2} + CaCl_2$$

According to the method described by Schläpfer and Bukowski, a sample of pulverized cement weighing 0.5 gram is placed in a flask with 50 ml. of ethylene glycol and a small amount of quartz sand to prevent the formation of lumps. The flask is closed by means of a rubber stopper and shaken for 30 minutes in a water thermostat at 65° to 70° C. The flask is removed from the thermostat, the solution filtered, preferably by suction, and the residue on the filter washed several times with small amounts of anhydrous denatured alcohol. The filtered solution is then titrated with 0.1 *N* hydrochloric acid, using as indicator a mixture of 0.10 gram of phenolphthalein and 0.15 gram of α-naphtholphthalein dissolved in 100 ml. of anhydrous denatured alcohol.

Schläpfer and Bukowski stated that it is essential that the ethylene glycol be chemically pure and neutral to the above indicators. However, none of the ethylene glycol obtained by the authors was neutral, and when neutralized by addition of calcium oxide it failed to remain so, reversion being slow at room temperature; but, under the conditions of the test procedure, shaking at 65° to 70° C., this reversion was essentially eliminated. That acid impurities were present, or developed during the shaking period, was shown by an experiment in which samples of calcium oxide were treated according to the method described above. The results are shown in Table I.

Since all the calcium oxide was consumed, it was evident that in every case a part of it had been neutralized during the shaking period, presumably by acidic impurities, thus giving low values for titratable calcium oxide.

Manifestly, with the reagent acting in this manner the method could not be used. However, it appeared that if this were merely a matter of neutralizing impurities, a suitable pretreatment of the ethylene glycol should eliminate the trouble.

TABLE I. EFFECT OF ACIDIC IMPURITIES

| CaO Present | CaO Found | | CaO Neutralized by Acidic Impurities in 50 Ml. of Ethylene Glycol |
|---|---|---|---|
| *Mg.* | *Mg.* | % | *Mg.* |
| 67.0 | 56.3 | 84 | 10.7 |
| 54.9 | 45.0 | 82 | 9.9 |
| 30.6 | 23.4 | 77 | 7.2 |
| 19.7 | 13.4 | 68 | 6.3 |
| 6.5 | 3.8 | 58 | 2.7 |

TABLE II. EFFECT OF PRETREATMENT

| CaO Present[a] | CaO Found | |
|---|---|---|
| *Mg.* | *Mg.* | % |
| 19.9 | 19.9 | 100 |
| 24.9 | 24.8 | 100 |
| 29.9 | 29.2 | 98 |
| 37.1 | 36.4 | 98 |
| 41.3 | 40.3 | 98 |
| 41.4 | 41.4 | 100 |
| 50.5 | 49.6 | 98 |
| 51.3 | 50.6 | 99 |
| 53.4 | 52.9 | 99 |
| 55.7 | 54.7 | 98 |
| 63.4 | 61.4 | 97 |

[a] In addition to that added during preneutralization of the ethylene glycol.

Accordingly, a small quantity of calcium oxide, about 0.3 gram per liter, was shaken with the ethylene glycol for 1 hour at 65° to 70° C. This neutralized the reagent effectively without seriously impairing its ability to take up calcium oxide, since it is capable of dissolving a total of about 2.0 grams per liter under the test conditions described.

With the ethylene glycol so treated, the test method became one of measuring the increase in titratable calcium oxide, rather than measuring the total titratable calcium oxide. The results obtained under these conditions are shown in Table II.

### Application to Clinkers and Dry Cements

With the method thus modified, its applicability to Portland cements was studied. In Table III are shown the uncombined calcium oxide contents of a number of cements, clinkers, and the major compounds of Portland cement, as determined by the ethylene glycol and the glycerol-alcohol methods. The average difference between the results of the two methods was less than 0.2 per cent and the maximum difference not greater than 0.4 per cent. Furthermore, the results by the ethylene glycol method were fully as reproducible as those by the glycerol-alcohol method. It appears, therefore, that the modified ethylene glycol method is satisfactory when applied to clinkers and dry cements.

The chemical data for the cements and clinkers are given in Table IV. It is of interest that for clinkers and cements containing less than about 1.5 per cent of uncombined calcium oxide, the ethylene glycol method generally gave higher values than the glycerol-alcohol method; and for samples containing more than 1.5 per cent calcium oxide, the converse was true. The results of studies by Bessey (*1*) show the same tendency. The lack of agreement might be due to faults in either or both methods.

TABLE III. COMPARISON OF THE GLYCEROL-ALCOHOL AND ETHYLENE GLYCOL METHODS

| Material | Reference Number | Uncombined Calcium Oxide[a] Glycerol-alcohol method | Uncombined Calcium Oxide[a] Ethylene glycol method[b] | Deviation from Glycerol-Alcohol Method |
|---|---|---|---|---|
| | | % | % | % |
| Clinker | 1 | 0 | 0 | 0 |
| | 2 | 0 | 0.1 | +0.1 |
| | 3 | 0 | 0.1 | +0.1 |
| Cement | 1 | 0.3 | 0.4 | +0.1 |
| | 2 | 0.4 | 0.4 | 0 |
| | 3 | 0.5 | 0.8 | +0.3 |
| | 4 | 0.5 | 0.9 | +0.4 |
| | 5 | 0.6 | 0.8 | +0.2 |
| | 6 | 0.7 | 0.9 | +0.2 |
| | 7 | 0.7 | 0.7 | 0 |
| | 8 | 0.8 | 1.0 | +0.2 |
| | 9 | 1.0 | 1.0 | 0 |
| | 10 | 1.0 | 1.3 | +0.3 |
| | 11 | 1.1 | 1.0 | −0.1 |
| | 12 | 1.2 | 1.3 | +0.1 |
| | 13 | 1.5 | 1.6 | +0.1 |
| | 14 | 1.8 | 1.6 | −0.2 |
| | 15 | 1.9 | 1.9 | 0 |
| | 16 | 2.0 | 1.6 | −0.4 |
| | 17 | 2.0 | 1.8 | −0.2 |
| | 18 | 2.3 | 1.9 | −0.4 |
| | 19 | 2.4 | 2.3 | −0.1 |
| | 20 | 2.8 | 2.5 | −0.3 |
| | 21 | 3.3 | 3.2 | −0.1 |
| | | | Av. difference | 0.16 |
| Tricalcium silicate | 1 | 0.7 | 0.7 | 0 |
| Dicalcium silicate | 2 | 0 | 0 | 0 |
| Tricalcium aluminate | 3 | 0 | 0.1 | +0.1 |
| Tetracalcium aluminoferrite | 4 | 0 | Trace | 0 |
| $CaSO_4 \cdot 2H_2O$ | 5 | 0 | 0 | 0 |

[a] Each value is the average of two determinations in good agreement.
[b] Modified as described in the text.

## Application to Hydrated Cements

In the study of the ethylene glycol method applied to hydrated Portland cement, the glycerol-alcohol method was again used as the reference method because it was found by Work and Lasseter (*6*) and by the authors to be satisfactory for set cements. The results obtained for several hydrated cements and the pure compounds are shown in Table V.

The ethylene glycol method did not appear to be satisfactory when applied to hydrated cements. It gave low results for cements and tricalcium silicate cured 2.5 to 3.5 years and high results for cements cured only 3 days. Moreover, both tricalcium aluminate and the calcium sulfoaluminate which was formed in the mixture of tricalcium aluminate and gypsum (5th line) appeared to be decomposed, especially the latter. This confirms results obtained by Schläpfer and Esenwein (*5*).

The low results obtained with cements cured 2.5 to 3.5 years are believed to be the result of incomplete solution of the calcium hydroxide which, during the long curing period, formed crystals of such size that they were not readily soluble. This has been partly confirmed by determining the solubility of crystalline calcium hydroxide in glycerol-alcohol following the procedure of the glycerol-alcohol method, and in ethylene glycol. The calcium hydroxide was dissolved almost quantitatively by the glycerol-alcohol, but only 68 per cent was dissolved by the ethylene glycol.

TABLE V. RESULTS WITH HYDRATED CEMENTS

| Hydrated Material[a] | Ref. No. | Uncombined $Ca(OH)_2$[b] Glycerol-alcohol method | Uncombined $Ca(OH)_2$[b] Ethylene glycol method | Deviation from Glycerol-Alcohol Method | Length of Curing Period at 70–75° F. |
|---|---|---|---|---|---|
| | | % | % | % | *Years* |
| $C_3S$ | 1 | 30.1 | 6.3 | −23.8 | 2.5 |
| $C_2S$ | 2 | 0.3 | 0.4 | + 0.1 | 2.5 |
| $C_3A$ | 3 | 0 | 0.7 | + 0.7 | 2.5 |
| $C_4AF$ | 4 | 0 | 0.3 | + 0.3 | 2.5 |
| 81% $C_3A$ + 19% $CaSO_4 \cdot 2H_2O$ | .. | 0 | 5.2 | + 5.2 | 2.5 |
| $Ca(OH)_2$ (crystalline) | 6 | 99.1 | 68.4 | −30.7 | .. |
| Cement | 36 | 23.7 | 12.2 | −11.5 | 3.5 |
| Cement | 37 | 21.0 | 9.1 | −11.9 | 3.5 |
| | | | | | *Days* |
| Cement | 38 | 23.7 | 25.1 | + 1.4 | 3 |
| Cement + 3% $SiO_2$ aq. | 38 | 17.8 | 18.9 | + 1.1 | 3 |
| Cement + 10% $SiO_2$ aq. | 38 | 15.2 | 17.3 | + 2.1 | 3 |

[a] All hydrated materials dried at 150° C. for 2 hours in carbon dioxide–free air except the hydrated mixture of $C_3A$ and $CaSO_4 \cdot 2H_2O$ which was air-dried at room temperature.
[b] Each value is the average of two determinations in good agreement.

The high results for cements cured 3 days may be accounted for by assuming that the calcium hydroxide when first formed is amorphous, or very finely crystalline, and hence readily soluble; and then by assuming further that the additional calcium hydroxide came from the decomposition of the hydrated alumina compounds. This partly confirms the findings of Bessey (*1*).

TABLE IV. CHEMICAL DATA ON CLINKERS AND CEMENTS USED

| Ref. No. | Chemical Analyses $SiO_2$ | $Fe_2O_3$ | $Al_2O_3$ | CaO | $SO_3$ | Loss | Potential Compound Composition[a] $C_3S$ | $C_2S$ | $C_3A$ | $C_4AF$ | MgO | $CaSO_4$ | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | % | % | % | % | % | % | % | % | % | % | % | |
| | | | | | | Clinkers | | | | | | | |
| 1 | 23.76 | 2.07 | 6.08 | 64.28 | 0.07 | 0.17 | 45 | 34 | 12 | 6 | 3.05 | 0 | Lab. prepared[b] |
| 2 | 22.03 | 2.02 | 7.01 | 65.49 | 0.18 | 0.28 | 49 | 26 | 15 | 6 | 2.54 | 0 | Commercial |
| 3 | 23.50 | 3.92 | 5.30 | 63.39 | 0.09 | 0.13 | 45 | 34 | 6 | 12 | 3.10 | 0 | Lab. prepared[b] |
| | | | | | | Cements | | | | | | | |
| 1 | 22.56 | 3.14 | 4.03 | 61.82 | 1.82 | 0.77 | 42 | 33 | 5 | 10 | 5.24 | 3.1 | Commercial |
| 2 | 23.26 | 1.53 | 5.19 | 65.67 | 1.54 | 1.37 | 48 | 31 | 11 | 5 | 1.22 | 2.6 | Commercial |
| 3 | 22.76 | 4.40 | 3.61 | 63.68 | 1.26 | 1.44 | .. | .. | .. | .. | 1.98 | ... | Special cement |
| 4 | 20.71 | 3.59 | 7.00 | 64.95 | 1.87 | 0.71 | 52 | 20 | 13 | 11 | 0.98 | 3.2 | Commercial |
| 5 | 22.72 | 1.70 | 5.36 | 66.52 | 1.51 | 1.13 | 53 | 26 | 11 | 5 | 0.68 | 2.6 | Commercial |
| 6 | 20.36 | 2.48 | 7.44 | 62.71 | 1.84 | 1.04 | 39 | 29 | 16 | 8 | 3.21 | 3.1 | Commercial |
| 7 | 21.48 | 3.20 | 6.61 | 62.85 | 1.87 | 1.05 | 35 | 35 | 12 | 10 | 2.26 | 3.2 | Commercial |
| 8 | 22.92 | 1.85 | 5.05 | 63.64 | 1.72 | 3.00 | 40 | 30 | 10 | 6 | 1.15 | 2.9 | Commercial |
| 9 | 20.50 | 3.35 | 5.07 | 64.49 | 1.84 | 2.13 | 53 | 19 | 10 | 10 | 1.11 | 3.1 | Commercial |
| 10 | 23.72 | 2.78 | 5.35 | 44.43 | 1.66 | 1.95 | .. | .. | .. | .. | 19.03 | ... | Natural cement |
| 11 | 20.84 | 2.13 | 4.75 | 63.06 | 1.67 | 2.25 | 54 | 19 | 9 | 7 | 4.58 | 2.8 | Commercial |
| 12 | 20.10 | 4.46 | 6.48 | 64.55 | 1.60 | 0.81 | 50 | 20 | 10 | 14 | 1.20 | 2.7 | Commercial |
| 13 | 20.46 | 3.30 | 6.68 | 64.37 | 1.80 | 1.69 | 46 | 25 | 12 | 10 | 1.11 | 3.1 | Commercial |
| 14 | 20.17 | 3.10 | 6.66 | 63.90 | 1.61 | 2.35 | 46 | 24 | 12 | 9 | 1.45 | 2.7 | Commercial |
| 15 | 19.94 | 2.90 | 6.06 | 64.09 | 1.89 | 1.70 | 59 | 19 | 11 | 9 | 2.70 | 3.2 | Commercial |
| 16 | 21.34 | 4.13 | 5.51 | 63.27 | 2.26 | 1.47 | 38 | 33 | 8 | 13 | 1.34 | 3.8 | Commercial |
| 17 | 21.24 | 3.28 | 6.18 | 64.27 | 1.92 | 1.27 | 39 | 32 | 11 | 10 | 1.49 | 3.3 | Commercial |
| 18 | 20.30 | 2.10 | 5.72 | 63.47 | 1.85 | 2.44 | 48 | 22 | 12 | 6 | 3.76 | 3.2 | Commercial |
| 19 | 20.22 | 2.58 | 5.24 | 63.84 | 1.72 | 1.15 | 52 | 13 | 10 | 8 | 4.12 | 2.9 | Commercial |
| 20 | 20.00 | 3.73 | 6.05 | 64.84 | 1.57 | 1.92 | 50 | 20 | 10 | 11 | 0.98 | 2.7 | Commercial |
| 21 | 18.58 | 2.93 | 7.07 | 66.23 | 1.67 | 1.68 | 54 | 13 | 14 | 9 | 1.26 | 2.8 | Commercial |

[a] Abbreviations used: tricalcium silicate, $C_3S$; dicalcium silicate, $C_2S$; tricalcium aluminate, $C_3A$; tetracalcium aluminoferrite, $C_4AF$.
[b] The laboratory-prepared clinkers were obtained from the Portland Cement Association Fellowship at the National Bureau of Standards, Washington, D. C.

## General Comments

The use of technical ethylene glycol which contains not more than 0.5 per cent of water was found satisfactory. The cost of the technical grade is approximately one-fifth that of the chemically pure variety.

Since ethylene glycol has a tendency to decompose in sunlight, it must be stored in dark-brown bottles and frequent blank determinations should be made on the pretreated ethylene glycol to determine its alkalinity.

A tendency toward lower results was noticed when the shaking period was extended beyond 30 minutes; however, the results, in most cases, were not greatly affected up to about 1 hour.

Because carbonation during filtration

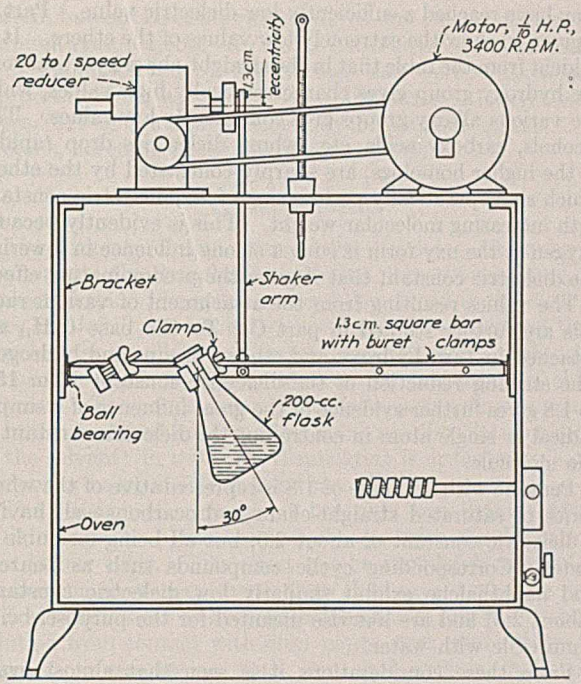



FIGURE 1. AIR THERMOSTAT WITH SHAKER

may cause relatively large errors, it is recommended that the filtering process be carried out as rapidly as possible to minimize this effect. The use of a Gooch crucible with a very thin asbestos mat is satisfactory.

The size of sample of calcium oxide used for standardizing the acid should not greatly exceed 60 mg. when 50 ml. of ethylene glycol containing about 0.3 gram of calcium oxide per liter are used. This limitation is necessary to assure quantitative conversion of the calcium oxide into calcium glycolate during the 30-minute shaking period.

The water thermostat and shaking apparatus proposed by Schläpfer and Bukowski were replaced by the apparatus shown in Figure 1.

## Summary and Conclusions

The applicability of the ethylene glycol method of Schläpfer and Bukowski to the determination of uncombined calcium oxide or hydroxide in Portland cements, clinkers, and hydrated cements was studied.

When modified, the ethylene glycol method, which requires only 40 minutes for a complete determination, is satisfactory for determining uncombined calcium oxide in clinker and Portland cement, and may be substituted for the glycerol-alcohol method which requires from 1 to 7 hours for completion.

The ethylene glycol method cannot be recommended for determining uncombined calcium hydroxide in hydrated Portland cement because, in the older samples, it is not completely dissolved, presumably on account of its coarsely crystalline state, and because hydrates of the alumina compounds decompose in the presence of ethylene glycol.

## Acknowledgment

Acknowledgments are due to C. L. Ford and to the Universal Atlas Cement Co. for chemical analyses of the cements and clinkers used in this investigation.

## Literature Cited

(1) Bessey, G. E., *Cement*, 9, 163–8 (1936).
(2) Bukowski, R., *Tonind.-Ztg.*, 59, 616 (1935); *Rock Products*, 38, 50 (1935).
(3) Lerch, W., and Bogue, R. H., IND. ENG. CHEM., Anal. Ed., 2, 296–8 (1930).
(4) Schläpfer, P., and Bukowskii, R., *Lab. fédéral essais matériaux annexé école polytech. fédérale Zurich*, No. 63 (1933); (*Eidgenöss. materialprüfungsanstalt E. T. H. Zürich*, Rpt. No. 63).
(5) Schläpfer, P., and Esenwein, P., *Zement*, 25, 814–16 (1936).
(6) Work, L. T., and Lasseter, F. P., *Concrete, Cement Mill Ed.*, 38, No. 3, 81–6, No. 4, 89–92, No. 5, 79–84 (1931).

RECEIVED August 6, 1937.

# Intensity and Stability of Ferric Thiocyanate Color

## Developed in 2-Methoxyethanol

H. W. WINSOR, Agricultural Experiment Station, University of Florida, Gainesville, Fla.

OF THE reagents used in colorimetry, thiocyanate for iron is one of the best, but the method has never been completely satisfactory because of the rapid fading of the color when developed in water solution.

Previous to 1900 several workers in Europe found that ether, amyl alcohol, and acetone when used in conjunction with the method, inhibited dissociation of the ferric thiocyanate and helped to preserve the color. The use of acetone for that purpose was introduced in this country by Marriott and Wolf (*4*) in 1906. Miller, Forbes, and Smythe (*5*) reported its successful use in 1929. Fowweather (*2*) employed an acetone-water mixture in which the vapor pressure was lowered by using 50 per cent water in the final volume; but Wong (*10*) avoided the use of acetone because of its very rapid evaporation.

As an alternative, the extraction of ferric thiocyanate by a solvent immiscible with water is, at best, an undesirable technic; and the work of Tarugi (*6*) indicates that such extraction is not strictly quantitative.

Acetone is definitely superior to water as a medium for this determination, yet offers serious disadvantages to practical use. Its rate of evaporation is so great as to be a disturbing factor in precise analysis, and with thiocyanate it slowly forms a flocculent white precipitate which must be filtered out. Moreover, the hypothetical ideal does not obtain, because acetone, a much weaker solvent for inorganic salts, cannot be completely substituted for water. Miller, Forbes, and Smythe used the highest practicable ratio of acetone to water—about 78 per cent of acetone by weight. Yet, because of the high dielectric value of water, the 22 per cent

present in the mixture is enough to lessen in marked degree the effect of acetone in preventing dissociation of the ferric thiocyanate color developed.

Thompson and Nernst, working independently, first suggested (1893) that the ionizing power of solvents is in some way connected with their dielectric nature. Walden began his prolific investigations in this field in 1906, and after years of research confirmed his original findings with the report (*7*) that the greater the dielectric constant of a medium the greater the electrolytic dissociation of dissolved substances. Later research indicates that dissociation in some solvents is influenced by factors other than dielectric constant, but that solvents of the type here considered conform closely to Walden's original premise. Thus the dielectric constant of the solvent has been accepted for this study as a satisfactory criterion for the dissociation of the solute.

TABLE I. DIELECTRIC CONSTANTS

| Material | Formula | Dielectric Constant | Solubility G./100 ml. $H_2O$ |
|---|---|---|---|
| **A. Water and Monohydric Alcohols** | | | |
| Water | HOH | 80.0 | .. |
| Methanol | $CH_3OH$ | 33.7 | ∞ |
| Ethanol | $C_2H_5OH$ | 25.7 | ∞ |
| 1-Propanol | $C_3H_7OH$ | 21.8 | ∞ |
| 1-Butanol | $C_4H_9OH$ | 17.8 | 7.9 |
| 1-Pentanol | $C_5H_{11}OH$ | 15.8 | 2.7 |
| **B. Representative Ethers** | | | |
| Ethoxyethane | $C_2H_5—O—C_2H_5$ | 4.3 | 7.4 |
| 1-Ethoxypentane | $C_5H_{11}—O—C_2H_5$ | 4.0 | Slight |
| 3-Methoxytoluene | $CH_3C_6H_4—O—CH_3$ | 3.6 | Insoluble |
| α-Ethoxytoluene | $C_2H_5—O—CH_2C_6H_5$ | 3.8 | Insoluble |
| Pentyloxypentane | $C_5H_{11}—O—C_5H_{11}$ | 3.1 | Insoluble |
| Ethoxynaphthalene | $C_{10}H_7—O—C_2H_5$ | 3.2 | Insoluble |
| 1,4-Dioxane | $H_2C(—O—CH_2)$ / $H_2C(—O—CH_2)$ | 2.2 | ∞ |
| **C. Substitutions in the Molecule** | | | |
| 1-Pentanol | $C_5H_{11}—OH$ | 15.8 | Slight |
| 1-Ethoxypentane | $C_5H_{11}—O—C_2H_5$ | 4.0 | Slight |
| Pentane | $C_5H_{11}—H$ | 1.8 | Insoluble |
| 1,2-Ethanediol | $CH_2(—OH)$ / $CH_2(—OH)$ | 38.7 | ∞ |
| 2-Methoxyethanol | $CH_2(—OH)$ / $CH_2—O—CH_3$ | 12.0[a] | ∞ |

[a] Preliminary value, courtesy of W. H. Byers, Department of Physics, University of Florida.

Water has the highest dielectric constant of all the common solvents. With the value of 80, it is but natural that it should allow rapid loss of color of ferric thiocyanate through dissociation. Acetone has a much lower dielectric constant than water, but when water is mixed with it, its value of 20 rises rapidly with each addition of the higher dielectric solvent. In a report on the dielectric constants of binary mixtures, Akerlof (*1*) gives 19.56 as the value for pure acetone; but shows that when water is added to the extent of 20 per cent by weight, the dielectric constant is raised to 30.33. Because of its higher amount of water, the 78 to 22 per cent reagent mixture previously discussed would have a still higher dielectric constant. It is obvious that its value of about 31.57 is so high as to permit appreciable dissociation.

It has been the purpose of this study to find, if possible, a solvent having the usual properties requisite for developing and comparing the ferric thiocyanate color, and, in addition, a dielectric constant sufficiently low to prevent loss of that color through dissociation.

Table I gives the dielectric constants (*3*) of a number of solvents, covering the range from 80 for water to 1.8 for pentane. Solubilities have been emphasized throughout, because it is necessary that the solvent be completely miscible with the sample solution and have sufficient power of solubility to prevent precipitation of inorganic salts.

Part A shows that the alcohols are not suitable for the purpose desired, as they become immiscible with water before they have reached a sufficiently low dielectric value. Part B is given to show the extremely low values of the ethers. It is evident from the table that in the straight-chain hydrocarbons the hydroxy group gives characteristically high values, while the various alkoxy groups give consistently low values. The alcohols, carboxy acids, etc., whose dielectrics drop rapidly in the higher homologs, are sharply contrasted by the ethers which show practically no lowering of the dielectric constant with increasing molecular weight. This is evidently because oxygen in the oxy form is such a strong influence in lowering the dielectric constant that it gives the predominating effect.

The values resulting from the attachment of various radicals are further studied in part C. To the base $C_5H_{11}$ are attached in turn hydroxy and ethoxy groups and hydrogen. The striking reduction of the dielectric constant from 15.8 to 1.8 gives further evidence of the great influence of a simple radical or single atom in controlling the dielectric constant of the molecule.

Pentane with its value of 1.8 is representative of the whole series of saturated straight-chain hydrocarbons—all having a dielectric constant of about 2.0, but all being insoluble in water. Corresponding cyclic compounds such as benzene and naphthalene exhibit similarly low dielectric constants (about 2.5) and are likewise unsuited for the purpose, being immiscible with water.

From these considerations it is seen that almost every solvent having a very low dielectric constant is insoluble in water. Dioxane is one of the few exceptions. For a time it was thought this solvent would be ideal, until it was found that it would not dissolve ammonium thiocyanate. Propanoic acid was also deficient in this respect; tertiary butanol dissolved ammonium thiocyanate very slowly, did not give a clear solution, and was not sufficiently miscible with the sample solution. Ethanoic acid also dissolved ammonium thiocyanate very slowly, and with the iron solution gave a color which was purple-red and unstable. The dielectric constants of the above solvents are 2.2, 3.1, 3.8, and 6.4, respectively. Every solvent of extremely low dielectric constant showed some of these faults: (1) failure to dissolve ammonium thiocyanate, (2) formation of a yellow color with ammonium thiocyanate, (3) incomplete miscibility with the sample solution (plant ash in 2.5 *N* hydrochloric acid), (4) precipitation of calcium and similar salts from sample solution, and (5) incomplete development of color.

In respect to (5), the color developed was not the normal deep red of ferric thiocyanate, but was pale and contained more purple than red. It is suggested tentatively that this inhibition may have been because the dielectric constant was too low—i. e., in a medium of this low value, the tendency may be not merely to prevent dissociation but to effect a condition of molecular association (*9*) in which there can be only partial development of color (*8*).

In contrast to the above, 2-methoxyethanol has proved satisfactory in every respect. It has these desirable properties: miscible with water in all proportions, completely colorless, boiling point = 124.3° C., density = specific gravity 0.966, dissolves ammonium thiocyanate readily, easily obtained in pure state, dielectric constant = 12.0, and has no disagreeable odor.

The structural formula is shown at the conclusion of Table I, with that of 1,2-ethanediol. The latter is completely miscible with water, but it has a high dielectric constant. The 2-methoxyethanol, however, has retained with the one hydroxy group an excellent power of solubility; and, with the substitution of a methoxy for the other hydroxy group, has dropped the dielectric constant from 38.7 to 12.0. This lower value has proved very desirable in the colorimetric procedure.

2-Methoxyethanol contains a small amount of iron as re-

ceived, but can be easily rendered iron-free by one distillation through glass. Being very stable it can be stored indefinitely. It is manufactured by the Carbide and Carbon Chemicals Corporation and sold under the trade name "Methyl Cellosolve." The price is considerably below that of the C. P. acetone, heretofore used in routine analysis.

## Experimental

The reagent solution is made by dissolving 10 grams of ammonium thiocyanate in sufficient 2-methoxyethanol to make 250 ml. The solution should be stored in the dark at once and left for 24 hours before using. That length of time is necessary to allow decolorization of the initial pink color formed by the iron present in even the best grade of thiocyanate. The persistence of color from such a small amount of iron is excellent confirmation of the nondissociative nature of the solvent; in water the dissociation is instantaneous, no pink color being visible at any time.

The reagent thus prepared has only one disadvantage: It is subject to photochemical change, with the formation of a yellow color. This effect can be completely prevented by keeping the reagent away from light except when in use. It is also advisable to keep both the solvent and the reagent solution from contact with filter paper, cork, and rubber to prevent contamination with iron and organic materials. If the ammonium thiocyanate is sufficiently clean there will be no need to filter.

In developing the ferric thiocyanate color 4 volumes of reagent are used to 1 of iron solution. For routine analysis the author has followed this plan:

Pipet 2 ml. of unknown solution into a 10-ml. volumetric flask, add 2 drops of 0.1 *N* potassium permanganate, and when decolorized run in the reagent to volume. Develop the standard in the same way and make the color comparison in a Bausch & Lomb biological (semimicro) colorimeter using a 25-watt frosted bulb in a Bausch & Lomb dome-shaped illuminator. Have the mirror at angle of maximum reflection. Standard and unknown iron solutions should contain sufficient hydrochloric acid to give a normality of 2.5. The most suitable concentration for the iron standard is 0.02 mg. of iron per ml.

Under the above conditions, several tests were made to determine the relative intensities of color developed with ammonium thiocyanate in the three media under discussion: water, water–acetone mixture, and 2-methoxyethanol. Using the water solution as the standard, and reading immediately, the 2-methoxyethanol gave a comparative intensity of $\frac{20.0}{10.8}$ mm. With the acetone–water mixture as the standard, the average reading was $\frac{20.0}{15.7}$ mm. Thus the intensity of color developed in 2-methoxyethanol is 85.2 per cent deeper than in water and 27.4 per cent deeper than in the acetone–water mixture.

In order to test the optical clarity of the 2-methoxyethanol–ammonium thiocyanate solution, the following comparison was made: In one flask a standard solution was used which contained 0.02 mg. of iron, and in the other flask an equal volume of standard containing 0.04 mg. of iron. Both flasks were developed with the 2-methoxyethanol reagent under identical conditions. At different depths the readings were $\frac{10.0}{5.2}$, $\frac{15.0}{7.7}$, $\frac{20.0}{10.2}$, $\frac{25.0}{12.6}$, and $\frac{30.0}{15.3}$, thus indicating excellent conformity to Beer's law.

The boiling point of 2-methoxyethanol is so high that there is no loss in volume during the process of analysis. The developed solution will stand in an open test tube for weeks without pronounced loss of color or of volume. The evaporation loss from solutions developed by the organic solvent reagents was calculated from decrease in depth of column in open test tubes at average room temperature of 27° C. The values at the end of 24 and 47 hours both showed the evaporation in 2-methoxyethanol to be 96 per cent less than in acetone. The acetone reagent (*5*) used in all the above tests was made by dissolving 10 grams of ammonium thiocyanate in 45 ml. of water and 200 ml. of acetone.

## Summary

2-Methoxyethanol is a superior medium in which to develop and compare the ferric thiocyanate color. It gives a color 85 per cent more intense than that developed in water. Compared with the most effective acetone–water mixture, it shows 27 per cent greater intensity of color and 96 per cent less evaporation.

It is colorless, almost odorless, and can be rendered iron-free by one distillation through glass. Because of its low price and slight evaporation it is very economical. With ammonium thiocyanate it makes a clear colorless reagent which conforms to Beer's law, and, having a low dielectric constant, it effectively inhibits loss of color through dissociation.

Since this new medium makes possible the development of a ferric thiocyanate color of unusual intensity and stability, it should prove especially effective for determination of the minute amounts of iron in biological materials.

## Acknowledgment

The author wishes to express his appreciation to Joseph P. Bain, Department of Chemistry, University of Florida, for valuable advice concerning organic solvents, and to thank W. H. Byers of the Department of Physics for his determination of the dielectric constant of 2-methoxyethanol.

## Literature Cited

(1) Akerlof, G., *J. Am. Chem. Soc.*, 54, 4125 (1932).
(2) Fowweather, F. S., *Biochem. J.*, 20, 93 (1926).
(3) International Critical Tables, Vol. 6, pp. 78–96 (1929).
(4) Marriott, W. McK., and Wolf, C. G. L., *J. Biol. Chem.*, 1, 451 (1906).
(5) Miller, R. C., Forbes, E. B., and Smythe, C. V., *J. Nutrition*, 1, 217 (1929).
(6) Tarugi, N., *Ann. chim. applicata*, 16, 281 (1926).
(7) Walden, P., *Z. physik. Chem.*, 147, A, 1 (1930).
(8) Weigert, F., *Ibid.*, 10, B, 241 (1930).
(9) Williams, J. W., *Chem. Rev.*, 6, 589 (1929).
(10) Wong, S. Y., *J. Biol. Chem.*, 55, 421 (1923).

RECEIVED August 16, 1937.

# The Absolute Turbidity of Raw Sugars

F. W. ZERBAN AND LOUIS SATTLER
New York Sugar Trade Laboratory, New York, N. Y.

IT HAS previously been stated by the writers [IND. ENG. CHEM., Anal Ed., 6, 178 (1934); 7, 157 (1935); 9, 229 (1937)] that the correction factor $f_{(k)}$ of Sauer can be used to calculate the absolute turbidity of white sugars, but that it is not applicable to raw sugars high in turbidity. A reëxamination of the writers' data on raw sugars has shown that the correction factor must be based, not on the extinction coefficient ($-\log T$ for 1 cm. thickness) of the turbid solution as has been done by Landt and Witte, but on that corresponding to the coloring matter alone. If this be done, the absolute turbidity of raw sugars is found to be directly proportional to the turbidity expressed in the system used by the writers. Details will be published later.

RECEIVED September 16, 1937.

# Vitamin A Content of Cod Liver Oil

## A Comparison of Spectrophotometric and Chemical Methods

ARTHUR D. HOLMES, The E. L. Patch Company, Boston, Mass., FRANCIS TRIPP AND G. HOWARD SATTERFIELD, University of North Carolina, Raleigh, N. C.

SOON after the discovery of vitamin A by McCollum and Davis (*5*) and by Osborne and Mendel (*6*) in 1913, investigators reported color tests for measuring the vitamin A potency of liver oils. In 1920 Rosenheim and Drummond (*8*) found that a substance which gave reactions similar to those produced by the lipochromes, but not identical with any of the lipochromes, seemed to be responsible for the well-known color test with sulfuric acid that was extensively employed by analysts for identifying cod liver oil. This substance was reported to be a normal constituent not only of fresh cod liver oil, but of all liver fats. In 1922 Drummond and Watson (*3*) found that the substance present in liver oils which gave a purple coloration with sulfuric acid was found in the following species: man, horse, ox, pig, cat, monkey, rabbit, guinea pig, chicken, duck, pigeon, rat, mouse, frog, shark, cod, haddock, ling, coalfish, dogfish, sprat, and skate.

Three years later Rosenheim and Drummond (*7*) reported that arsenic trichloride gives a brilliant ultramarine blue color reaction with cod liver oil, which like the sulfuric acid test is characteristic for vitamin A. Furthermore this color persists for a sufficient length of time to allow a colorimetric comparison with a suitable standard.

About the same time Carr and Price (*2*) developed an antimony trichloride color test which is used in many laboratories for determining the vitamin A potency of liver fats and oils. Since the biological vitamin A assay method was adopted as the official method for determining vitamin A potency, the antimony trichloride color test has been subjected to severe criticism. Recently the spectrophotometer and the Hilger vitameter have been introduced, with a view to determining the vitamin A value of fish liver oils with a minimum of time and expense. Nevertheless many English concerns still express the vitamin A potency of their cod liver oil in terms of Blue value.

Accordingly it seemed desirable to compare the Blue value as obtained by the British Pharmacopoeia (*1*) method with the E value as determined with the Hilger vitameter. For this study a series of thirty-two medicinal cod liver oils was assembled. Since nearly one-half of these were purchased on the open market, it has been assumed that the oils are typical of commercial medicinal cod liver oil.

### Methods

The method for preparing reagents and determining the Blue value as reported in the British Pharmacopoeia has seldom appeared in the American literature. Accordingly it is reproduced here.

ANTIMONY TRICHLORIDE REAGENT. A solution of antimony trichloride in pure dry chloroform saturated at 20° C.

Wash chloroform two or three times with its own volume of water, and dry it over anhydrous potassium carbonate. Pour off and distill, rejecting the first 10 cc. of distillate. Protect chloroform from light at all times. Wash antimony trichloride with pure dry chloroform until washings are clear. Prepare a saturated solution at 20° C. of the above reagents. The solution contains not less than 21 grams and not more than 23 grams of antimony trichloride per 100 cc. Keep in a well-stoppered amber bottle.

ASSAY. Mix 1 cc. of antimony trichloride solution with a solution of sodium potassium tartrate in 20 cc. of water, rotate, add 2 grams of sodium bicarbonate, and titrate with 0.1 *N* iodine. Each cubic centimeter of 0.1 *N* iodine equals 0.01141 gram of antimony trichloride.

TEST. Weigh 2 grams of oil into a 10-cc. volumetric flask, fill to mark with chloroform at 20° C., and mix. Take 0.2 cc. of this solution at 20° C. in a 1-cc. pipet (graduated part must be 15 cm. long). Put it into a colorless glass cell of 10-mm. internal measurement. Place cell in colorimeter, add rapidly 2 cc. of antimony trichloride, and mix. Match color at the point of maximum intensity (using neutral glasses if necessary and disregard their value). The maximum color may develop in 10 seconds. Keep antimony trichloride and oil out of contact with rubber.

The extinction coefficient (E value) of the oils under consideration was determined by the method employed by the American Drug Manufacturers' Association Vitamin Committee (*4*) in its extensive study of the reliability of the vitameter for assaying the vitamin A potency of fish liver oils and fish liver oil concentrates.

In this study cyclohexane was used as a solvent for the cod liver oils in determining the E values. The oil dilutions were adjusted to such a strength that the vitameter readings were between 0.6 and 0.8 on the vitameter logarithmic scale.

### Comparison of Results

The oils are arranged (Table I) according to the descending E values obtained for them. In general, the E value and Blue value follow the same trend. Oils 1 and 2 which gave the highest E values, 1.69 and 1.65, also gave the highest Blue values, 19.1 and 18.3, respectively. The E values and the Blue values for oils 29, 30, 31, and 32 were of the same order, and were the lowest values of all the samples under

TABLE I. COMPARISON OF E VALUES AND BLUE VALUES OF COD LIVER OILS

| Oil No. | E Value | Blue Value | | | Ratio, Blue Value | Free Fatty Acids | Unsaponifiable |
|---|---|---|---|---|---|---|---|
| | | Blue | Red | Yellow | E Value | | |
| | $1\frac{\%}{cm.}$ | | | | | % | % |
| 1 | 1.69 | 19.1 | 1.0 | 2.0 | 11.30 | 0.97 | 1.00 |
| 2 | 1.65 | 18.3 | 0.0 | 1.5 | 11.10 | 0.73 | 1.02 |
| 3 | 1.62 | 15.5 | 4.0 | 0.0 | 9.57 | 0.38 | 1.16 |
| 4 | 1.56 | 16.0 | 0.5 | 1.0 | 10.25 | 0.70 | 1.10 |
| 5 | 1.54 | 16.5 | 2.5 | 2.0 | 10.71 | 0.80 | 1.12 |
| 6 | 1.54 | 14.5 | 4.5 | 0.0 | 9.41 | 0.95 | 1.24 |
| 7 | 1.52 | 16.5 | 2.0 | 1.0 | 10.85 | 0.87 | 1.07 |
| 8 | 1.46 | 14.5 | 2.0 | 1.5 | 9.94 | 0.86 | 1.23 |
| 9 | 1.46 | 13.0 | 4.0 | 0.0 | 8.90 | 0.17 | 1.22 |
| 10 | 1.45 | 13.0 | 2.5 | 1.0 | 8.96 | 0.10 | 1.14 |
| 11 | 1.43 | 15.0 | 1.5 | 0.0 | 10.49 | 0.34 | 1.12 |
| 12 | 1.43 | 11.6 | 9.0 | 0.0 | 8.11 | 0.67 | 1.32 |
| 13 | 1.38 | 12.7 | 3.0 | 0.0 | 9.20 | 0.13 | 1.20 |
| 14 | 1.36 | 12.0 | 2.5 | 0.0 | 8.82 | 0.14 | 1.13 |
| 15 | 1.34 | 14.0 | 4.5 | 0.0 | 10.45 | 0.86 | 1.24 |
| 16 | 1.33 | 15.0 | 0.5 | 0.0 | 11.27 | 0.23 | 1.04 |
| 17 | 1.29 | 12.1 | 4.0 | 0.0 | 9.38 | 0.11 | 0.90 |
| 18 | 1.19 | 11.6 | 3.5 | 0.0 | 9.75 | 0.54 | 1.28 |
| 19 | 1.18 | 12.1 | 3.0 | 0.0 | 10.25 | 0.56 | 1.24 |
| 20 | 1.15 | 11.1 | 4.0 | 0.0 | 9.65 | 0.11 | 1.06 |
| 21 | 1.14 | 10.6 | 3.0 | 0.5 | 9.30 | 0.21 | 1.17 |
| 22 | 1.12 | 12.5 | 2.0 | 1.0 | 11.16 | 0.32 | 0.99 |
| 23 | 1.07 | 11.3 | 3.0 | 1.0 | 10.56 | 0.18 | 1.17 |
| 24 | 0.99 | 10.6 | 1.5 | 0.0 | 10.70 | 0.32 | 1.32 |
| 25 | 0.99 | 11.1 | 3.0 | 1.0 | 11.21 | 0.19 | 1.15 |
| 26 | 0.97 | 9.1 | 2.5 | 0.0 | 9.38 | 0.09 | 0.92 |
| 27 | 0.96 | 9.1 | 2.0 | 0.0 | 9.48 | 0.73 | 1.11 |
| 28 | 0.83 | 9.1 | 2.5 | 0.5 | 10.96 | 0.40 | 0.80 |
| 29 | 0.79 | 9.1 | 2.0 | 1.0 | 11.52 | 0.41 | 1.15 |
| 30 | 0.78 | 8.1 | 0.5 | 0.0 | 10.38 | 0.45 | 1.04 |
| 31 | 0.58 | 7.6 | 1.0 | 0.0 | 13.10 | 0.24 | 1.11 |
| 32 | 0.50 | 3.3 | 1.0 | 0.5 | 6.60 | 1.20 | 0.98 |
| Av. | 1.23 | 12.4 | 2.7 | 1.1 | 10.08 | 0.47 | 1.12 |

consideration. The E values for the thirty-two samples ranged from 0.50 to 1.69, while the Blue value range was from 3.3 to 19.1.

There was no consistent relationship between the E value and the Blue value for the remaining twenty-six samples. Oil 16, with an E value of 1.33, gave a Blue value of 15.0, while oils 6, 8, 9, 10, and 12 gave Blue values under 15.0 although their E values were much higher than that for oil 16. Oil 12, with an E value of 1.43, had a Blue value of 11.6, while oil 22, whose E value was only 1.12, gave a Blue value of 12.5.

A thorough examination of Table I will also show similar discrepancies for several other samples. The Blue values for the last half of the group of oils under consideration—namely, oils 17 to 32, inclusive—did not exceed 12.5 and the E value did not exceed 1.29; hence there was a tendency for oils with a low E value to give low Blue values also.

The majority of the oils gave a Blue value:E value ratio between 9.0:1 and 11.0:1, although the ratio varied from 6.60:1 for oil 32 to 13.10:1 for oil 31. The average for the ten samples whose Blue value: E value ratio was between 10.25:1 and 10.96:1 was 10.56:1, while ten other samples whose ratio was between 9.20:1 and 9.94:1, gave an average Blue value:E value ratio of 9.51:1. The average ratio for the thirty-two samples was 10.08:1.

It is difficult to explain the inconsistent trend of E values and Blue values for the first sixteen samples. In order to accumulate data which might explain the discrepancies, the free fatty acid and the unsaponifiable content were determined by the official United States Pharmacopoeia methods (*9*), and these results are also reported in Table I.

In general the oils with the highest E values also had the highest free fatty acid values. With the exception of oil 3, the fatty acid content for oils 1 to 8, inclusive, exceeded 0.7 per cent. These seven samples also gave the highest E values. However, oil 3, with a fatty acid content of only 0.38 per cent, gave a high E value of 1.62. Oil 32, whose fatty acid content, 1.20 per cent, was higher than that for any other oil in the series, gave the lowest E value, 0.50, of all the samples under consideration. Twelve of the cod liver oils had a fatty acid content of less than 0.25 per cent and their E values varied from 0.58 to 1.46.

Observations were made to determine whether or not the fatty acid content of the cod liver oils had any tendency to cause a red tinge to the characteristic blue color resulting from the antimony trichloride reaction. As will be noted in Table I, it was necessary to place in the tintometer along with the blue glasses a red Lovibond glass having 4.0 Lovibond red units in order to match the color produced by oil 3 and the antimony trichloride solution. The fatty acid content of this oil was but 0.38 per cent. Oil 1, whose acid content, 0.97 per cent, was approximately three times as large, required only 1.0 Lovibond red unit in the color match. On the other hand, oil 12, whose acid content, 0.67 per cent, was midway between that of oils 1 and 12, required 9.0 Lovibond red units to obtain a color match. Cod liver oils 17 and 20, whose fatty acid content, 0.11 per cent, was extremely low, each required 4.0 Lovibond red units in the Blue value color match. Hence a further survey of the results reported in Table I shows that there is no relationship between the amount of free fatty acid in the oil and the amount of red color produced by the reaction of the oil and the antimony trichloride solution in the Blue value determination.

Attention was also given to the amount of yellow color which was produced during the antimony trichloride test of the oils under consideration. Cod liver oils 1, 2, and 5, whose free fatty acid content was 0.97, 0.73, and 0.80 per cent, necessitated the use of yellow glasses having 2.0, 1.5, and 2.0 Lovibond yellow units, respectively, in order to obtain a satisfactory Blue value determination. On the other hand, cod liver oils 6, 27, and 12 had practically the same fatty acid content as cod liver oils 1, 2, and 5, yet no Lovibond yellow glasses were required to obtain a satisfactory Blue value color match. It appears that there is no correlation between free fatty acid content and the amount of yellow color produced by the action of the antimony trichloride solution on the oil in the process of the Blue value determination.

The amount of unsaponifiable material present in the thirty-two samples of cod liver oils varied from 0.80 per cent for oil 28 to 1.32 per cent for oils 12 and 24. There is obviously no relationship between the amount of unsaponifiable material contained in the oils and their vitamin A potency as determined by the antimony trichloride or vitameter methods. Cod liver oils 1 and 2, which gave the highest E values of the entire group, contained only 1.00 and 1.02 per cent of unsaponifiable material, respectively, while oils 29, 30, 31, whose E values were among the lowest of the entire group, contained more unsaponifiable material than oils 1 and 2. Cod liver oils 12 and 24, whose E values were 1.43 and 0.99, respectively, contained 1.32 per cent of unsaponifiable material. Hence it is evident that there is no consistent relationship between the amount of unsaponifiable material present in the oils and their vitamin A potency.

## Summary

The vitamin A potency of thirty-two samples of cod liver oils was determined by the Hilger vitameter E value and the antimony trichloride Blue value methods.

A comparison of the results obtained by the two methods indicated that in general they were of the same order. The oils that gave high E values also gave high Blue values, while those with the lowest E values also gave the lowest Blue values.

The Blue value: E value ratio was between 9.0:1 and 11.0:1 for the majority of the samples under consideration.

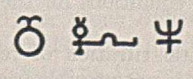
The fatty acid content of an oil has no direct relationship to the amount of red color or yellow color produced by the action of the antimony trichloride solution on the oil.

The results obtained for free fatty acid and unsaponifiable material showed that the amount of free fatty acid and unsaponifiable material present in an oil is not correlated with its E value or Blue value; there is no consistent relationship between the vitamin A potency and the free fatty acid or unsaponifiable material present in cod liver oils.

## Literature Cited

(1) British Pharmacopoeia, London, England, Constable and Co., 1932.
(2) Carr, F. H., and Price, E. A., *Biochem. J.*, 20, 497 (1926).
(3) Drummond, J. C., and Watson, A. F., *Analyst*, 47, 341 (1922).
(4) Holmes, A. D., Black, A., Eckler, C. R., Emmett, A. D., Heyl, F. W., Neilsen, C., and Quinn, E. J., *J. Am. Pharm. Assoc.*, 26, 525 (1937).
(5) McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 15, 167 (1913).
(6) Osborne, T. B., and Mendel, L. B., *Ibid.*, 16, 423 (1913); 17, 401 (1914); 20, 379 (1915).
(7) Rosenheim, O., and Drummond, J. C., *Biochem. J.*, 19, 753 (1925).
(8) Rosenheim, O., and Drummond, J. C., *Lancet*, 1, 862 (1920).
(9) U. S. Pharmacopoeia, XI ed., Easton, Pa., Mack Printing Co., 1936.

Received August 11, 1937. Submitted by Francis Tripp in partial fulfillment of the requirements for the degree of master of science, University of North Carolina.



# Estimation of Total Reducible Sulfur in Caustic Soda

GUSTAVE HEINEMANN AND HENRY W. RAHN, Southern Alkali Corporation, Corpus Christi, Texas

**A method has been developed for the estimation of small quantities of reduced and reducible sulfur compounds (other than sulfate) found in caustic soda. The method involves an alkaline and an acid reduction of the sulfur compounds to the sulfide form with subsequent evolution of hydrogen sulfide and its titration iodometrically.**

**The procedure is applicable to both lime-soda and electrolytic caustic. An accuracy of ±3 per cent is indicated in the range 0.0001 to 0.01 per cent sulfur.**

COMMERCIAL caustic soda ordinarily contains small quantities of reduced and reducible sulfur compounds—namely, sodium sulfide and sodium thiosulfate—in addition to a relatively high concentration of sodium sulfate, which is usually classed as a reducible compound. By the method outlined in this report, however, the sulfate is irreducible, and being so does not enter into the discussion. On the other hand, the polythionates which are normally reducible by the method do not exist in concentrated caustic soda. This is illustrated by the following reaction:

$$2Na_2S_6O_6 + 6NaOH \longrightarrow 5Na_2S_2O_3 + 3H_2O$$

which occurs during the process of evaporation, causing the gradual conversion of the polythionates to the thiosulfate (*1*).

The determination of the total quantity of sulfur (total reducible sulfur) contained in these reduced and reducible sulfur compounds in concentrated sodium hydroxide is not readily accomplished by any one of the usual analytical methods for small quantities of reducible sulfur. The colorimetric method of Yoe (*8*) involving the formation of methylene blue by hydrogen sulfide serves to determine only the sulfide sulfur, and unless the thiosulfate sulfur is reduced to the sulfide, it becomes indeterminable by the method. An added objection to this method, in connection with industrial applications, is the time interval of several days required for the development of maximum color. Similarly, the stain methods of Snell (*6*) determine only the sulfide sulfur unless the thiosulfate form is reduced by some auxiliary treatment. Like most stain methods, they lose accuracy as the sulfur content increases and, in addition, considerable time is involved in the preparation of a fresh set of standards daily. Johnson's (*3*) method for the determination of sulfur in iron and steel has the advantage of being applicable to higher sulfur concentrations than can conveniently be handled by the stain or colorimetric methods. This is true because of the fact that the sulfur is titrated iodometrically. However, in common with the previously mentioned methods, this method does not offer conditions for the complete reduction of the thiosulfate.

The direct iodometric titration of reducible sulfur compounds in caustic soda is not possible because hypoiodite and iodide are formed by the reaction between the caustic soda and the iodine. This reaction would lead to an erroneously high result.

$$2NaOH + I_2 = NaOI + NaI + H_2O$$

In the colorimetric stain method of Scherer and Sweet (*5*) for the determination of reducible sulfur compounds in paper, specific provision is made for the reduction of these compounds with nascent hydrogen. However, this method of reduction proved inadequate when applied to acidified caustic soda solutions containing known quantities of sodium thiosulfate. To reduce the thiosulfate, completely, it was necessary to develop the more vigorous reduction procedure herein described.

If, then, the thiosulfate sulfur is reduced to sulfide and determined as such in an acid solution together with the sulfur originally present as sulfide, the iodine equivalent obtained by titration will be a true measure of the total reducible sulfur in the sample. As yet no method has been developed for the determination of the relative quantities of sulfide and thiosulfate in the low concentrations normally found in caustic soda.

## Principles of Modified Method

The method of the writers is dependent upon the complete reduction of thiosulfate sulfur to sulfide sulfur, which is accomplished in two stages: (1) the partial reduction of the thiosulfate in an alkaline medium with stannous chloride, and (2) completion of the reduction with aluminum metal in an acid solution. The reactions occurring in each stage can be represented by equations of the general type:

*Reduction in the Alkaline Medium:*

$$Na_2SnO_2 + Na_2S_2O_3 + 2NaOH \longrightarrow Na_2SO_3 + Na_2S + Na_2SnO_3 + H_2O$$

*Reduction in the Acid Medium:*

$$Na_2SO_3 + 2HCl \longrightarrow 2NaCl + SO_2 + H_2O$$
$$SO_2 + 3H_2 \text{ (nascent)} \longrightarrow H_2S + 2H_2O$$

*Additional Reactions in the Acid Medium:*

$$2Al + 6HCl \longrightarrow 2AlCl_3 + 3H_2$$
$$Na_2S + 2HCl \longrightarrow H_2S + 2NaCl$$

By this procedure, any sulfide sulfur originally present in the sample becomes included with the reduced thiosulfate sulfur, and as such is indistinguishable from the latter.

In developing the reduction procedure, use was made of the fact that a difference exists between the stoichiometric iodine demands of thiosulfate and sulfide sulfur. These relations are as follows:

$$Na_2S + I_2 \longrightarrow 2NaI + S \qquad (1)$$
$$1S \equiv 2I$$

$$2Na_2S_2O_3 + I_2 \longrightarrow Na_2S_4O_6 + 2NaI \qquad (2)$$
$$1S \equiv \frac{I}{2}$$

The preceding equations indicate that if $X$ grams of thiosulfate sulfur are equivalent to $a$ ml. of a standard iodine solution, then $X$ grams of thiosulfate sulfur completely reduced to sulfide sulfur are equivalent to $4a$ ml. of the same iodine solution. This relation was utilized to determine the completeness of reduction by various methods.

## Outline of the Modified Method

Reduction of the thiosulfate is carried out in two stages: (1) stannous chloride is added to the diluted solution of the sample and boiled for 1 minute; (2) aluminum metal is placed in the cooled solution, which is then acidified with hydrochloric acid to yield nascent hydrogen. The evolved gases—mainly hydrogen sulfide, hydrogen, hydrochloric acid, and carbon dioxide—are passed into an ammoniacal cadmium chloride solution wherein the sulfur is precipitated as cadmium sulfide. When the reaction has gone to completion the cadmium sulfide is titrated with a potassium iodate–iodide solution in an acid medium.

## Apparatus

The reaction vessel consists of a 1000-ml. Erlenmeyer flask fitted with a two-hole sulfur-free rubber stopper into which have been inserted a 150-ml. dropping funnel (with turned-up outlet) extending to the bottom of the flask, and a Pyrex distilling head such as is used for Kjeldahl distillations (Figure 1).

The distilling head outlet is connected to a sintered-glass bubbling unit by means of a short piece of rubber tubing, which has been previously boiled in a strong sodium hydroxide solution (30 per cent) to remove surface sulfur.

## Reagents

Ordinary distilled water is usually sufficiently free of reducible sulfur compounds not materially to affect the accuracy of this determination. A convenient source of distilled water at 15° C. is indispensable for carrying out titrations.

C. P. Analyzed Concentrated Hydrochloric Acid.

Aluminum Reduction Strips: 1.6-mm. (0.062-inch) pure sheet aluminum cut into 1.25 × 10 cm. (0.5 × 4 inch) strips. Just prior to use, the strips are boiled in a dilute hydrochloric acid solution (1 to 5) for 30 seconds and then rinsed with distilled water.

Ammoniacal Cadmium Chloride Solution: A 2 per cent solution of C. P. cadmium chloride to which sufficient ammonium hydroxide is added to dissolve the first precipitate of cadmium hydroxide.

Sodium Hydroxide: 50 per cent sodium hydroxide as made from C. P. sodium hydroxide and which, by a blank determination on all the reagents, has also been shown to be substantially sulfur-free.

Standard Thiosulfate Solution, 0.00624 *N*: Made by dissolving C. P. $Na_2S_2O_3 \cdot 5H_2O$ in distilled water and standardizing against a standard iodine solution. The thiosulfate solution is stabilized by adding 5 grams of sodium hydroxide and 0.1 gram of hydroquinone per liter, and is then protected from the sunlight by covering the bottle with black paper or paint. One milliliter contains 0.0004 gram of reducible sulfur.

Standard Potassium Iodate–Potassium Iodide Solution, 0.00624 *N* with respect to the liberated iodine on acidification: 1 ml. $KIO_3 - KI = 0.0001$ gram of sulfide S ≡ 0.0004 gram of thiosulfate S.

Starch Solution: 5 grams of soluble starch and 50 ml. of concentrated acetic acid per liter.

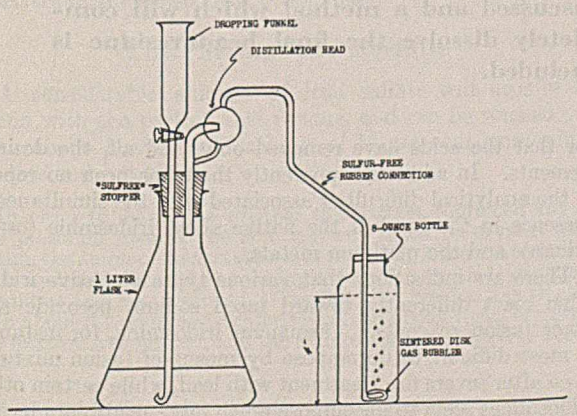



FIGURE 1. ASSEMBLY OF APPARATUS

TABLE I. REDUCTION WITH ALUMINUM (ACID SOLUTION)

| 0.00624 *N* Thiosulfate | Theoretical Iodine Titer Following Complete Reduction to Sulfide (0.00624 *N*) | Actual Iodine Titer |
|---|---|---|
| *Ml.* | *Ml.* | *Ml.* |
| 4.0 | 16.0 | 10.1 |
| 4.0 | 16.0 | 12.8 |
| 4.0 | 16.0 | 11.4 |
| 3.0 | 12.0 | 6.0 |

Stannous Chloride: 10 per cent C. P. stannous chloride solution containing a stick of C. P. tin.

## Operation

A sample containing approximately 50 grams of caustic soda is weighed into a 1000-ml. Erlenmeyer flask, and dissolved in 200 ml. of distilled water. Twenty milliliters of 10 per cent stannous chloride solution are added and the solution is brought to a boil. An absorption unit containing 200 ml. of 2 per cent ammoniacal cadmium chloride solution is prepared; to the cooled caustic stannous chloride solution two aluminum reductor strips are added, the apparatus is immediately assembled as shown in Figure 1, and the caustic is acidified with 200 ml. of concentrated hydrochloric acid.

The cadmium chloride solution, together with any cadmium sulfide adhering to the receiver, is transferred to an 800-ml. beaker. The sintered-glass disk is also placed in the beaker because a considerable quantity of cadmium sulfide usually adheres to the outlet surface. Sufficient cold distilled water (15° to 20° C.) is then added to make the total volume in the beaker about 650 ml.

The titration is carried out by adding an excess of potassium iodate–iodide solution, stirring thoroughly and then acidifying with concentrated hydrochloric acid (5 cc. excess per 100 ml.). A few milliliters of 6 *N* hydrochloric acid are also forced through the bubbling unit to dissolve the adhering cadmium sulfide. Care should be exercised in keeping the outlet well below the surface until this cadmium sulfide has all dissolved. The temperature at this stage should not be more than 20° to 25° C. because of the possible volatilization of the iodine.

Three to five minutes are allowed for the completion of the reaction and then the excess iodine is back-titrated with standard sodium thiosulfate.

The potassium iodate–iodide consumption is a measure of the cadmium sulfide present. The entire reaction and the subsequent titration should be carried out in the absence of direct sunlight (*2*).

For the titration of samples containing from 0.001 to 0.005 gram of total reducible sulfur, it was convenient to use 0.03 *N* potassium iodate–iodide and sodium thiosulfate solutions. The titration of more than 0.005 gram of total reducible sulfur is not accurate by this method, because of the absorption of iodine on the free sulfur liberated during the reaction (*7*).

## Experimental Notes

When appreciable amounts of chlorates are present in the sample (as in electrolytic caustic), they can be eliminated by using larger quantities of stannous chloride for the preliminary reduction and allowing the treated solution to stand at room temperature for 5 minutes prior to boiling. Five grams of stannous chloride are normally sufficient for this purpose.

The reduction of sodium thiosulfate by aluminum in caustic soda solution does not, like the stannous chloride reduction, yield all the sulfur as the sulfide. According to Mellor (*4*), the reaction appears to be as follows:

$$Na_2S_2O_3 + 4NaOH + Al \longrightarrow Na_2SO_3 + Na_2S + Na_2AlO_2 + 2H_2O$$

On acidification the sulfite sulfur (sulfur dioxide) can be reduced to hydrogen sulfide with nascent hydrogen and all the sulfur obtained as the sulfide. However, this procedure is not recommended, for much difficulty is invariably encountered in dissolving the large quantity of aluminum hydroxide which precipitates as the solution approaches neutrality.

In Table I are listed typical results obtained on reducing known quantities of sodium thiosulfate in 20 per cent sodium hydroxide solutions by the method of nascent hydrogen reduction (Scherer and Sweet, *5*). In these experiments the alkaline solutions were immediately acidified following the introduction of two aluminum reductor strips. The remainder of the procedure was as recommended. The results indicate incomplete reduction of sulfur to the sulfide. A probable explanation lies in the reaction between sodium thiosulfate and hydrochloric acid.

$$Na_2S_2O_3 + 2HCl \longrightarrow S + H_2SO_3 + 2NaCl$$

The elevated temperature at which the neutralization is completed no doubt accelerates the above reaction, involving a loss of sulfur.

TABLE II. REDUCTION WITH $SnCl_2$ ONLY (ALKALINE SOLUTION)

| 0.00624 *N* Thiosulfate | Theoretical Iodine Titer Following Complete Reduction to Sulfide (0.00624 *N*) | Actual Iodine Titer |
|---|---|---|
| *Ml.* | *Ml.* | *Ml.* |
| 4.0 | 16.0 | 11.6 |
| 3.0 | 12.0 | 8.3 |
| 4.0 | 16.0 | 13.8 |
| 3.0 | 12.0 | 6.7 |

In Table II are listed typical results obtained from determinations in which the reduction procedure consisted only of adding 20 ml. of a 10 per cent stannous chloride solution to the diluted caustic sample, which was then boiled and cooled previous to acidification with hydrochloric acid. From these results, it is evident that stannous chloride alone does not give the desired results.

Table III presents normal results obtained when the reduction procedure consisted of pretreating the caustic sample with stannous chloride, followed by acidification in the presence of aluminum reductor strips. These results definitely indicate that the combined use of stannous chloride and nascent hydrogen completely reduces sodium thiosulfate. The hydrogen evolved from the aluminum has a triple function: (1) in maintaining a reducing atmosphere, (2) in reducing sulfur dioxide to hydrogen sulfide, and (3) in transferring the liberated hydrogen sulfide from the reaction flask to the absorption liquid.

TABLE III. REDUCTION WITH STANNOUS CHLORIDE AND ACIDIFICATION

(20 ml. of 10 per cent $SnCl_2$, boiled 1 minute; 2 aluminum reductor strips)

| 0.00624 *N* Thiosulfate | Theoretical Iodine Titer Following Complete Reduction to Sulfide (0.00624 *N*) | Actual Iodine Titer |
|---|---|---|
| *Ml.* | *Ml.* | *Ml.* |
| 4.0 | 16.0 | 16.1 |
| 4.0 | 16.0 | 15.9 |
| 4.0 | 16.0 | 15.8 |
| 3.0 | 12.0 | 11.8 |

## Range of Application and Accuracy

A total reducible sulfur content of 0.0001 and 0.01 per cent in caustic soda can be estimated by following the outlined procedure. The accuracy is about ±3 per cent as indicated from results obtained by the reduction of known quantities of sodium thiosulfate in C. P. caustic soda.

## Literature Cited

(1) Ephraim, F., "Inorganic Chemistry," p. 497, New York, D. Van Nostrand Co., 1934.
(2) Hillebrand and Lundell, "Applied Inorganic Analysis," p. 582, New York, John Wiley & Sons, 1929.
(3) Johnson, C. M., "Chemical Analysis of Special Steels," pp. 330–5, New York, John Wiley & Sons, 1930.
(4) Mellor, J. W., "A Comprehensive Treatise on Inorganic and Theoretical Chemistry," Vol. 10, p. 503, New York, Longmans, Green & Co., 1930.
(5) Scherer and Sweet, IND. ENG. CHEM., Anal. Ed., 4, 103–4 (1932).
(6) Snell, F. D., and Snell, C. T., "Colorimetric Methods of Analysis," Vol. I, pp. 593–8, New York, D. Van Nostrand Co., 1936.
(7) Treadwell, F. P., and Hall, W. T., "Analytical Chemistry," Vol. II, p. 583, New York, John Wiley & Sons, 1930.
(8) Yoe, J. H., "Photometric Chemical Analysis," Vol. I, Colorimetry, pp. 375–6, New York, John Wiley & Sons, 1928.

RECEIVED July 14, 1937.

# Analysis of Platinum Metals-Silver Assay Bead

F. E. BEAMISH AND M. SCOTT, University of Toronto, Toronto, Ontario, Canada

**A procedure has been developed for the analysis of the platinum metals–silver-gold assay bead, and the losses of these precious metals at each stage of the analysis are reported.**

**The analytical difficulties associated with the presence of iridosmine in the bead are discussed and a method which will completely dissolve the final bead residue is included.**

THIS report deals with a procedure for the separation and determination of the platinum group of metals and gold after collection from the ore by pot assay and subsequent cupellation of the lead regulus to a silver–platinum metals bead. The analyst has been reminded from time to time that for various reasons the analysis of an assay bead is difficult. The authors contend that few procedures have been published for the analysis of the assay bead which contain sufficient information to permit the inexperienced assayer to complete the analysis in an intelligent manner. The bead and residues are treated with acids, etc., and generally there are very few, if any, data recorded which will assure the operator that the acids have removed only, and all, the desired elements. In addition, apparently there has been no report of the analytical difficulties associated with the simultaneous presence in the bead of the native alloy, iridosmine (osmiridium), and the platinum metals.

There are indications that various types of native iridosmine react differently toward fused sodium peroxide and other fusion reagents. Tasmanian iridosmine, for instance, is most difficult to decompose by means of fusion mixtures even after severe fire treatment with lead, while certain other iridosmines seem to decompose much more readily under the same conditions. In view of these facts and because severe

treatment is necessary to dissolve all the iridium in the final residue, it seems reasonable to assume that any fusion mixture which dissolves all this metal may also attack the iridosmine which is very often present in platinum-bearing material. Also, unless the operator succeeds in dissolving all the cupeled iridium, the undissolved metal will be included in the so-called "insoluble residue" generally reported as "osmiridium." The authors feel that this insoluble residue ought to be so called and the term "osmiridium" eliminated from published reports on the composition of platinum metals–silver beads.

### Preliminary Treatment of the Bead

It has been customary to flatten the bead into a cornet before parting, apparently to ensure thorough contact with the parting acid. Unless great care is taken, this procedure must produce considerable losses—for instance, in the case of beads containing a high proportion of iridium or rhodium, where the metal often appears on the surface of the bead in clumps of dark material which is easily rubbed off during the flattening process. It has been the authors' experience that beads part sufficiently rapidly and clean enough for wet analysis without flattening. Various methods may be used to clean the beads, which are sometimes dipped in caustic solutions or in acetic acid. The authors do not clean the beads before wet analysis.

### The Parting Acid

Beamish and Russell (*1*) have pointed out that boiling sulfuric acid will dissolve appreciable proportions of platinum and produce serious losses of rhodium. The authors find that iridium may very often be found in the sulfuric acid parting solution. It is possible that this metal passes into the filtrate as very finely divided particles. The experiments made for this report indicate that iridium is more consistently found in the parting solution if filtration of the insoluble residue is made with Whatman's No. 40 instead of No. 42 filter paper, although on occasion as much as 0.1 mg. of iridium has passed through the finer No. 42 grade. With the "modified parting method" the dissolving of platinum and rhodium is reduced to inappreciable proportions and the iridium is also more generally absent from the parting acid, probably because the bead residue is more coherent.

Boiling sulfuric acid, of course, dissolves most of the palladium if sufficient silver is present. By the "modified parting method" appreciable proportions of palladium are dissolved and the fact that the parting acid is water-white is no evidence that this metal is absent. A definite precipitate with dimethylglyoxime can often be obtained in such cases. With the "modified parting method" considerable amounts of silver remain with the residue, but this does not seriously interfere in the subsequent procedures.

### The Parting Acid Residue

A considerable amount of lead sulfate will usually be found with the parting acid residue, and can be washed out with about 3 alternate washings of 50 per cent ammonium acetate and hot water.

In order to determine whether this extract contained any of the platinum metals, the solution was taken to dryness over steam, the ammonium acetate was removed by 5 or 6 evaporations with hydrochloric acid, 1 to 3 aqua regia was added, and then the nitric acid was removed. The final dry residue was treated with about 0.1 cc. of hydrochloric acid and about 10 cc. of water, the mixture was filtered, and the filtrate was evaporated to about 2 cc.

A negative test with dimethylaminobenzilidine rhodanine showed the absence of palladium. Stannous chloride reagent confirmed this and proved the absence of platinum. Benzilidine showed the absence of gold. Addition of stannous chloride to about 0.2 cc. of the test solution and boiling proved the absence of rhodium. About 0.5 cc. was treated with sulfuric acid and fumed to about 0.4 cc., then fuming nitric acid was added, and the solution was boiled to about 0.2 cc. The absence of a blue or green color indicated that iridium was not present.

The authors have found no combination of platinum metals in which cupeled rhodium and iridium were appreciably attacked by strong aqua regia (3 to 1). Parting acid residues containing about 8 mg. of iridium and 8 mg. of rhodium have been kept in contact with 3 to 1 aqua regia for 3 to 10 hours, and in every case these metals were found to be absent from the acid solutions. This is in striking contrast to directions included in certain published procedures stating that the cupeled iridium can be dissolved quantitatively by means of strong aqua regia. It was necessary to determine whether the strong aqua regia dissolved all the platinum, palladium, and gold out of the final residue. Many of these final residues were examined and it was found that, even after a single 3-hour treatment with aqua regia, traces of palladium and particularly of platinum remained with the rhodium and iridium. In no case was gold found to be present.

### Determination of Gold

A considerable number of analyses on native platinums derived from Dunite sources were made. A characteristic of this type of material is the low palladium content, which is very often less than 0.5 per cent. When much gold is present the reaction product of hydroquinone interferes with the subsequent precipitation of traces of palladium.

Hydrochloric acid solutions were prepared containing varying relative proportions of gold and palladium. The gold was precipitated with hydroquinone and dimethylglyoxime added directly to the filtrate. This liquid was filtered and the filtrate evaporated to dryness, then a few cubic centimeters of sulfuric acid were added and the organic matter was destroyed in the usual manner. The sulfuric acid was fumed off until the residue was just moist, then 20 to 30 cc. of water were added to dissolve the residue. This solution was filtered, and the palladium was precipitated with dimethylglyoxime. The results obtained are given in Table I.

TABLE I. DETERMINATION OF PALLADIUM IN THE HYDROQUINONE SOLUTION FROM THE GOLD PRECIPITATION

| No. | Palladium Added | Gold Added | Palladium Recovered from Second Precipitation |
|---|---|---|---|
| | *Mg.* | *Mg.* | *Mg.* |
| 1 | 0.2 | 25 | 0.2 |
| 2 | 0.4 | 25 | 0.3 |
| 3 | 0.6 | 25 | 0.6 |
| 4 | 0.8 | 25 | 0.6 |
| 5 | 1.0 | 25 | 0.3 |
| 6 | 1.5 | 25 | 0.1 |
| 7 | 3.0 | 25 | 0.05 |
| 8 | 10.0 | 25 | 0 |
| 9 | 35.0 | 25 | 0 |
| 10 | 75.0 | 25 | 0 |
| 11 | 150.0 | 25 | 0 |

In the case of Nos. 8, 9, 10, and 11 the addition of dimethylglyoxime to the first gold filtrate produced an immediate precipitate. With Nos. 5 and 6, precipitation was induced after considerable stirring, and with Nos. 1, 2, 3, and 4 the addition of dimethylglyoxime to the first gold filtrate produced no precipitate.

When solutions containing 0.2 mg. of palladium and about 15 mg. of gold were treated with sulfur dioxide water to remove the gold it was found that, even after vigorous boiling of the filtrate, the palladium could not be completely removed with dimethylglyoxime. It is common practice to treat

the filtrate from gold–sulfur dioxide precipitations with a few drops of nitric acid after having boiled the solution vigorously, to aid in the removal of sulfites. The authors' results indicate that the nitric acid does not make the precipitation of palladium dimethylglyoxime more complete.

When traces of palladium are present in solution with considerable amounts of gold, there is less interference with palladium dimethylglyoxime precipitation from sulfur dioxide than from hydroquinone treatments. However, the authors recommend that for accurate analysis the gold filtrate be evaporated to remove sulfites, or in the case of hydroquinone that the organic matter be removed, before precipitating palladium.

With the "modified parting method" appreciable amounts of silver remain with the parting acid residue. This is usually dissolved in the strong aqua regia. The final hydrochloric acid solution of gold, palladium, and platinum will sometimes retain traces of this silver.

To determine whether these small amounts of silver would interfere with the gold precipitation by means of hydroquinone, two solutions were prepared, each containing 25 mg. of silver and 25.01 mg. of gold. Each solution was evaporated 3 times in the presence of about 25 mg. of sodium chloride and a few cubic centimeters of hydrochloric acid. The residue was dissolved in about 20 cc. of slightly acid solution and the cold liquid filtered with Whatman's No. 42 filter paper. The residue was washed to a volume of 50 cc., 5 cc. of concentrated hydrochloric acid were added, and the precipitation was made with hydroquinone. The gold recovered weighed 25.01 and 24.99 mg.

## Determination of Palladium

Very little information has been directly recorded in the literature with respect to the danger of loss of metal by burning palladium dimethylglyoxime. Tacit recognition of this difficulty has been made by various suggestions, such as wrapping the precipitate and filter paper in a second paper before ignition. The authors recommend two procedures which will avoid loss of palladium on ignition. The precipitate and paper may be burned very slowly, preferably by first charring in an oven with suitable temperature control. The method generally adopted by the authors is not an orthodox one but is justified, in that there is no appreciable loss of palladium and results are obtained in a much shorter time. The wet paper and the contents of the crucible are subjected to the full flame from a 17.5-cm. (7-inch) Meker burner. Then the paper and the contents are charred and ignited, after which the flame is again applied until the residue is converted to ash. If this manipulation is properly made, there is no visible evidence of the emission of carbon.

It is usually necessary to make two palladium precipitations in order to remove the platinum contamination. The palladium dimethylglyoxime and paper are treated with sulfuric acid and nitric acid to remove the organic matter. If too much sulfuric acid is fumed off, the palladium will bake out as a brown residue, but if there is not too much of this formed it will dissolve when water is added and the solution is boiled.

## Determination of Platinum

Gilchrist (*4*) recommends the separation of palladium from platinum by oxidation in acid solution with sodium bromate, then adjusting the solution to pH 6 and boiling to coagulate the palladium dioxide. The authors attempted to adapt this method to the separation of these elements when they were present together in solutions which had been fumed with sulfuric and nitric acids and the nitric acid had been removed. Solutions such as these have been heated with bromate for more than 10 hours and invariably a heavy brown platinum oxide settled out when the solution was neutralized to pH 6 and boiled. Even after this brown precipitate was dissolved in hydrochloric acid and the bromate added again, some of the brown material again appeared on neutralizing and boiling. Repeated treatments such as this will eventually remove the platinum, but under the above stated conditions the method is not a practical one.

## Residue from Aqua Regia Treatment

This final residue should contain all the rhodium, iridium, and iridosmine. Small traces of platinum and palladium are sometimes retained, but these may generally be neglected. The authors have found that a 10-minute fusion will usually suffice to dissolve the final residue completely; in only one case did any unfused substance remain. A sample of native platinum weighing 110 mg. was assayed and the residue remaining after fusion examined under a microscope. It was found to consist of three very small well-rounded grains of metal resembling iridosmine which together weighed 0.1 mg.

If much iridosmine is present, the solution of the final residue may contain appreciable quantities of platinum derived from the native alloy, and, of course, this metal must be isolated from the rhodium and iridium.

The separation of rhodium adopted by the authors is essentially that described by Gilchrist (*3*). In order to determine whether this method would separate and determine as little as a few tenths of a milligram, a sample of platinum metals–bearing material containing 0.02 mg. of rhodium was salted with 0.50 mg. of rhodium in solution form. The ore was assayed and the cupeled bead was treated as described in the procedure outlined below. The rhodium recovered weighed 0.50 mg. When the acid solution is treated with hydrogen sulfide to precipitate the rhodium, the liquid should be filtered even though it appears clear, because the light-brown precipitate may be finely dispersed.

The presence of osmium and ruthenium introduces no difficulties, as these metals are converted to volatile oxides by the reagents used in the procedure. The authors recommend that the determination of these metals be made on a separate sample, and procedures for their analysis are being prepared.

The following recipe has been used by the authors for a period of a year, and determinations have been made on platinum-bearing materials from most of the important world sources. The quantitative aspect of each stage of the procedure has been reported by Beamish and Russell (*1*) and Beamish, Russell, and Seath (*2*). Various additions have been made with a view to decreasing the time necessary for complete analysis. In many platinum-bearing materials certain of the precious metals are present in very small proportions, sometimes as low as a few hundredths of a per cent—for example, native platinum very often contains only a few hundredths of a per cent of palladium and rhodium. Consequently, the total weight of platinum metals to be used for analysis should not be much less than 100 mg. The proportion of silver should be about 15 to 1 of total platinum metals.

## Procedure

The bead is parted with 30 to 40 cc. of 95 per cent sulfuric acid, keeping the temperature below boiling but sufficiently high to ensure a uniform and rapid rate of parting. About 4 to 7 minutes should suffice for the operation, and complete removal of the silver is not necessary. The acid is cooled and diluted to about 175 cc. with hot water and the residue is filtered and thoroughly washed with hot water. Whatman's No. 42 is a suitable filter paper. The filtrate is evaporated and fumed to a volume of 4 to 5 cc. and hot water added to a volume of 200 cc. About 3 cc. of 10 per cent sodium bromate solution are added and the liquid is then boiled for about 25 minutes. The acidity of the solution is reduced considerably by means of solid sodium bicarbonate

and then 5 cc. more of sodium bromate are added. The liquid is again boiled for about 10 minutes and the neutralization carried to pH 6. The details of this manipulation are given by Beamish, Russell, and Seath (*2*).

The liquid is boiled to precipitate and coagulate the palladium dioxide, which is filtered and washed with freshly boiled distilled water, and the residue is added to a 125-cc. Pyrex beaker with a cover glass. The paper and contents are treated with 5 cc. of sulfuric acid and sufficient fuming nitric acid, and the organic matter is destroyed as usual. About 5 minutes should suffice for this operation. The solution is now diluted to about 40 cc. with water and a few drops of hydrochloric acid are added to precipitate the silver chloride, which is filtered off. If this precipitate is stained brown because of the presence of much palladium in the sample, the operation is repeated. The filtrate, which should now contain not more than 4 cc. of sulfuric acid per 100 cc. of solution, is treated with dimethylglyoxime to remove the palladium. This precipitate can be filtered through the same paper used for the palladium obtained from the aqua regia extract.

The residue obtained from the parting acid is washed several times with 50 per cent by weight ammonium acetate with intermittent hot-water washings. The ammonium acetate filtrate and washings are discarded. The residue and paper are added to a 125-cc. beaker with cover glass, treated with 30 cc. of aqua regia (3 to 1) and placed on a steam bath for about 2 hours. The solution is diluted and filtered and the paper and contents are washed thoroughly with hot water, after which the residue and paper are set aside for the fusion treatment. The aqua regia extract is taken to near dryness in the presence of 100 mg. of sodium chloride. The residue is treated with 3 small additions of hydrochloric acid to remove the nitric acid, then 25 cc. of hot water are added, and the mixture is allowed to stand with occasional stirring. The silver chloride is then filtered off.

If gold seems to have baked out, the paper and contents are burned, the gold is dissolved in aqua regia, the nitric acid is removed, water is added, and the gold solution is filtered into the original filtrate. This solution of 50 to 60 cc. is acidified with about 5 cc. of concentrated hydrochloric acid, and about 1 mg. of hydroquinone in aqueous solution is added for each milligram of gold present. The mixture is boiled gently for 5 minutes and the gold is filtered, washed, and heated. The filtrate and wash water, which should not exceed 175 cc., are treated directly with dimethylglyoxime. The yellow precipitate should appear in a minute or two if much palladium is present. The liquid is allowed to stand for about 30 minutes and then filtered.

Because platinum is very often carried in this precipitate, the paper and contents are dissolved by treatment with sulfuric acid and fuming nitric acid. If much platinum has been carried with the precipitate, it sometimes appears as metal after the treatment to destroy organic matter. In this case the residue is filtered off, burned, and treated with aqua regia, the nitric acid is removed with one or two additions of hydrochloric acid, and water is added. This is filtered into the palladium solution and the filtrate diluted to about 175 cc. Dimethylglyoxime is added to precipitate palladium. This precipitate and that from the parting acid are combined and the palladium is determined as metal.

The combined filtrates and wash water are evaporated and the sulfuric acid is fumed off. The beaker is held over the flame from a Meker burner for about 1 minute. The platinum is dissolved in aqua regia, the nitric acid is removed, water is added, and the solution is then filtered and washed to a volume of about 100 cc. If there is any evidence of undissolved platinum the paper and contents are burned, the residue is dissolved, the nitric acid is removed, and the aqueous solution is filtered into the original platinum solution. If the platinum is to be precipitated as the sulfide, the solution is acidified with 5 cc. of concentrated hydrochloric acid and hydrogen sulfide is passed into the gently boiling liquid for about 40 minutes. The platinum sulfide and paper are first dried, then ignited very slowly to avoid as much as possible the occlusion of sulfur. If the platinum is to be precipitated as metal, sodium formate is used in a solution of pH 6.

Where the gold filtrate shows no evidence of palladium dimethylglyoxime, a capillary drop of the platinum solution from which organic matter and nitric acid have been removed is treated with dimethylaminobenzilidine rhodanine. If a definite purple color is produced the platinum solution is treated with dimethylglyoxime to remove this small amount of palladium.

The final residue remaining after aqua regia treatment is placed in a silver crucible and carefully burned to an ash. The black residue is covered with about 3 grams of sodium peroxide and the mixture is melted and then maintained at a dull red heat for 10 minutes. If thin-walled crucibles are used, less time is required to dissolve the residue. The fused mass is allowed to cool, the crucible is placed in a 250-cc. Pyrex beaker, and water is added to dissolve the solid. The crucible is washed with water and carefully transferred to a small casserole, then cleaned with dilute nitric acid and with water. The liquid in the casserole is washed back into the original solution and heated, and sufficient nitric acid is added to dissolve the silver oxide.

If a heavy brown residue persists, indicating the presence of much rhodium, it is filtered off, the paper and residue are dissolved with about 6 to 7 cc. of sulfuric acid and fuming nitric acid, water is added, and the solution is transferred to the original beaker. If only a little rhodium is present, 6 to 7 cc. of concentrated sulfuric acid are added directly to the original solution, which is then evaporated and fumed to a volume of about 4 cc. About 175 cc. of hot water are added and the dioxides are precipitated and purified in the same manner as with the palladium dioxide from the parting acid. It is important to avoid the introduction of the sulfuric acid solution to the indicator bottle by means of the capillary tube. If this happens the neutralizing of the silver sulfate solution may inadvertently proceed to pH 7, and silver salts will separate.

The final solution is made up to exactly 100 cc., 50 cc. of which are treated with titanous chloride, as described by Gilchrist (*3*). It is best to make two precipitations of rhodium with titanous chloride, in each case treating the rhodium and paper in a 50-cc. beaker with 2.5 cc. of concentrated sulfuric acid. Care must be taken to wash down and fume off any nitric acid on the walls of the beaker before precipitation. The final titanous-rhodium precipitate and paper are dissolved and 20 cc. of water are added, followed by 2 cc. of concentrated hydrochloric acid. The solution is boiled very gently for 15 minutes, then diluted, and filtered. It is important to avoid loss of much water, otherwise the hydrochloric acid will be removed by the sulfuric acid. When very small amounts of rhodium are present, as very often occurs with native platinums, the pink color does not appear. The acid solution is finally diluted to about 75 cc. and a rapid stream of hydrogen sulfide is passed into the boiling liquid for about 40 minutes.

The second 50 cc. are treated with about 5 cc. of filtered 10 per cent sodium bromate solution and boiled for about 20 minutes. If the original liquid was a blue color it will turn to an amber. The acidity of the liquid is reduced somewhat with a filtered sodium bicarbonate solution, a few cubic centimeters of bromate are again added, and the liquid is boiled. Sodium bicarbonate solution is now added to reach pH 6. The dioxides of rhodium and iridium are precipitated and coagulated by boiling for 20 to 25 minutes, filtered, and washed with freshly boiled distilled water. The details of the determination are described by Gilchrist (*3*).

By this fusion with sodium peroxide the final residue from the aqua regia treatment is almost always completely dissolved and the residue obtained on final reduction with hydrogen will be the total rhodium and iridium. Any osmium present will pass off during the process.

## Summary

If the assay bead is parted by the "modified parting method" the amount of platinum, rhodium, and iridium which is dissolved by the sulfuric acid can be neglected.

Strong aqua regia (3 to 1) will not attack cupeled rhodium or iridium, but will dissolve all but traces of platinum and palladium out of the final residue. When traces of palladium are associated with considerable proportions of gold, it is best to destroy the organic matter in the filtrate from gold precipitation before adding dimethylglyoxime. Solutions of platinum which have been fumed with sulfuric and nitric acids will yield a brown precipitate, very similar in appearance to hydrated palladium oxide, when the boiling solution is treated with sodium bromate and subsequently neutralized to pH 6.

The final bead residue of rhodium, iridium, and iridosmine can be completely dissolved by fused sodium peroxide.

## Literature Cited

(1) Beamish, F. E., and Russell, J. J., Ind. Eng. Chem., Anal. Ed., 8, 141 (1936).
(2) Beamish, F. E., Russell, J. J., and Seath, J., *Ibid.*, 9, 174 (1937).
(3) Gilchrist, R., *Bur. Standards J. Research*, 9, 547 (1932).
(4) *Ibid.*, 12, 291 (1934).

Received July 8, 1937.

# Determination of Copper, Zinc, and Lead in Silicate Rocks

E. B. SANDELL, School of Chemistry, Institute of Technology, University of Minnesota, Minneapolis, Minn.

THE colorimetric method here described for the determination of the small amounts of copper, zinc, and lead occurring in rocks is based on the use of dithizone (diphenylthiocarbazone, phenylazothionoformic acid phenylhydrazide), a reagent which is well suited for the purpose because of its great sensitivity. The procedure developed is based on the investigations of Hellmut Fischer and others on the use of dithizone for determining traces of heavy metals.

The sample is decomposed with hydrofluoric-perchloric acid and sodium carbonate, and the resulting solutions are extracted with a carbon tetrachloride solution of dithizone in a slightly basic medium in the presence of citrate. Copper, zinc, and lead form dithizonates which dissolve in the carbon tetrachloride, whereas the major constituents of the rock remain in the aqueous phase. The carbon tetrachloride layer is separated and shaken with 0.01 *N* hydrochloric acid, whereby the dithizonates of zinc and lead are decomposed, giving the chlorides of these metals in the aqueous phase; the copper complex is not decomposed by the dilute acid and remains in the carbon tetrachloride. The latter is evaporated to dryness, and the residue is ignited to cupric oxide, which is dissolved in hydrochloric acid; the copper in this solution can be determined by extractive titration with dithizone–carbon tetrachloride at a pH of approximately 3.5, or by mixed-color colorimetry with the same reagent in acid solution. The 0.01 *N* hydrochloric acid solution containing the zinc and lead is made up to volume and the two metals are determined colorimetrically in separate aliquots with dithizone: zinc by the mixed color technic at a pH of about 4.1 in the presence of thiosulfate to prevent the interference of lead, and lead in an ammoniacal solution containing potassium cyanide to prevent the reaction of zinc.

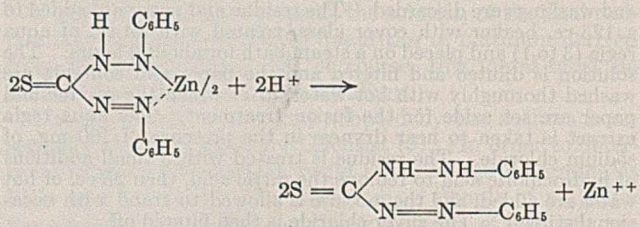
## Discussion of the Procedure

THE DECOMPOSITION. After the hydrofluoric-perchloric acid decomposition of the sample there may remain undecomposed silicates, quartz, iron ores, and barium sulfate. If the latter substance is present it will contain most of the lead in the sample. The undecomposed material is filtered off, fused with sodium carbonate, and the melt is leached with water and filtered; the washed residue is treated with hydrofluoric and perchloric acids to remove any silica that may be present, and is finally dissolved in hydrochloric acid. There are thus obtained three solutions which are extracted separately with dithizone after adjustment of the pH as described in the procedure.

THE EXTRACTION. The separation of copper, zinc, and lead from most of the other constituents of the sample in the main solution is effected by making the dithizone–carbon tetrachloride extraction in a citrate solution containing a slight excess of ammonia (pH approximately 8.5). The extraction of zinc and lead from such a solution is virtually complete when an excess of the reagent is used as described in the procedure. The pH of the solution should not be unnecessarily high, because then copper may be extracted in the enol form of the dithizone complex instead of the keto form, which is undesirable.

The alkalies, alkaline earths, magnesium, ferric iron, aluminum, beryllium, titanium, zirconium, chromium (III and VI), tin (IV), antimony (III and V), arsenic (III and V), thallium (III), cerium, thorium, and platinum (IV) do not react with dithizone and are not extracted. Nickel, cobalt, cadmium, mercury, bismuth, tin (II), thallium (I), and palladium react under the conditions described. Manganese is extracted at least in part and it seems that ferrous iron behaves similarly; silver is extracted partially. Ferric iron oxidizes the reagent to some extent, especially in basic solution, imparting a brownish color to the carbon tetrachloride layer.

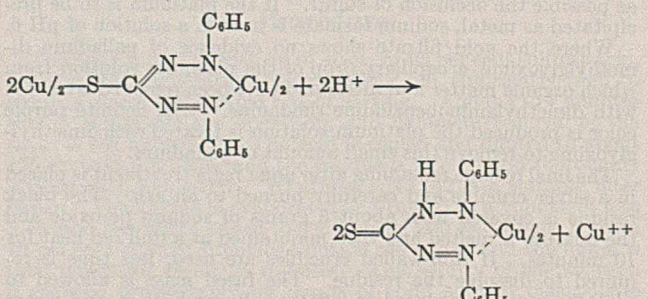
SEPARATION OF COPPER FROM LEAD AND ZINC. When a carbon tetrachloride solution of the keto forms of cupric copper, zinc, and lead dithizonate is shaken with a 1 to 1000 (approximately 0.01 *N*) solution of hydrochloric acid, the copper complex remains virtually unchanged in the carbon tetrachloride, but the zinc and lead complexes are decomposed:



Zinc and lead can thus be recovered quantitatively from the carbon tetrachloride solution even when an excess of dithizone is present. The lead complex is more easily decomposed than the corresponding zinc compound. The acid concentration should not be less than 0.01 *N*, because then the extraction of zinc becomes difficult. The keto form of copper dithizonate is practically not affected when the carbon tetrachloride solution is shaken with the dilute hydrochloric acid, as the following experiment shows:

Ten milliliters of carbon tetrachloride containing copper ketodithizonate equivalent to 100 $\gamma$ of copper were shaken with two successive 10-ml. portions of 1 to 1000 hydrochloric acid (1 minute each time). The total amount of copper in the hydrochloric acid after this treatment was found to be 1.5 $\gamma$. When the experiment was repeated with the addition of 0.5 ml. of 0.01 per cent dithizone to the 10 ml. of the carbon tetrachloride solution of the copper complex, the amount of copper in the 20 ml. of 0.01 *N* hydrochloric acid was found to be 0.5 $\gamma$ or less. The last experiment corresponds to the conditions of an actual determination, because more or less of the excess reagent will be present in the carbon tetrachloride. Accordingly, the amount of copper lost in the separation will be small enough to neglect in the majority of cases.

It is important that the copper be present in the form of the keto and not the enol complex. As shown by Fischer (*1*), copper can form both keto and enol dithizone complexes, the enol tautomer (yellow-brown) being stable in basic solutions and the keto (red-violet) in acid. When a carbon tetrachloride solution of the enol form is shaken with a dilute acid, transformation into the keto form occurs:



The separation of copper from zinc and lead will then fail, unless an excess of dithizone is present to combine with the liberated cupric ions. Although not essential, it is advantageous to prevent the formation of the copper enol complex in the extraction of the sample. This can usually be accomplished by avoiding the addition of too much ammonia, and too long shaking with the first portions of the reagent. The higher the pH of the aqueous phase the greater will be the velocity of migration of the dithizone itself from the carbon tetrachloride into the aqueous phase, and the resulting deficiency of the reagent in the carbon tetrachloride favors the formation of the enol tautomer. However, formation of the enol complex does no harm if sufficient reagent is added before shaking with 0.01 *N* hydrochloric acid to combine with the cupric ions liberated.

Nickel and cobalt remain in the carbon tetrachloride solution on treatment with 0.01 *N* hydrochloric acid. As shown by Willoughby, Wilkins, and Kraemer (*5*), bismuth dithizonate is stable in dilute acid solution (pH = 2) and these authors make use of this fact in separating lead from bismuth. Cadmium accompanies zinc and lead in the acid separation.

DETERMINATION OF COPPER. The first step in the determination of copper is the recovery of the element from the carbon tetrachloride solution which has been shaken with the dilute hydrochloric acid to remove zinc and lead. This can be done by evaporating off the organic solvent, and igniting the residue to destroy organic matter. The cupric oxide so obtained is dissolved in hydrochloric acid. The solution will also contain nickel, cobalt, and more or less silver, if this element is present in the sample. Any reagent which gives a sensitive color reaction with copper, and which does not react with nickel, cobalt, and silver can be used to determine the element. It is convenient to use dithizone itself for the purpose. The determination can be made in a variety of ways. The most accurate method (except for very small amounts) is that of extractive titration (*2*), which is carried out in the following manner:

An aliquot part of the hydrochloric acid solution is adjusted to a pH of 3.5 and shaken with successive portions of 0.001 per cent reagent in carbon tetrachloride, each portion being drawn off before the next is added, until the change in color of the carbon tetrachloride layer shows that all the element has reacted. At this pH the reaction between the reagent and the copper is virtually complete within 30 to 45 seconds when the mixture is vigorously shaken. As long as an excess of copper remains in the aqueous phase the final color of the carbon tetrachloride layer is red-violet (the color of the keto form of the cupric complex). When all the copper has reacted, the color of the carbon tetrachloride remains green after prolonged shaking. The color transition in successive portions of carbon tetrachloride from red-violet, through various shades of purple and blue, to blue-green or green takes place over an interval of about 1.5 ml. of 0.001 per cent reagent, corresponding to approximately 1.5 $\gamma$ of copper, when the mixture is well shaken for 30 to 45 seconds after each 0.5-ml. addition.

The results obtained in applying the extractive titration method to the determination of amounts of copper ranging from 3 to 20 $\gamma$ are indicated in Figure 1. In this graph the points represent the volume of dithizone required for titration to the intermediate blue color representing roughly equal amounts of copper dithizonate and reagent. The straight line showing the relation between the amount of copper and the volume of reagent intersects the Y-axis at approximately 0.5 ml. This then represents the "indicator" blank which must be subtracted from the volume of reagent used to obtain the volume actually reacting with the copper. The correction is empirical, since a small amount of copper remains in solution after the end point represented by the intermediate blue color has been reached, and on the other hand, a certain amount of unreacted reagent is drawn off with the carbon tetrachloride before the end point is attained; these

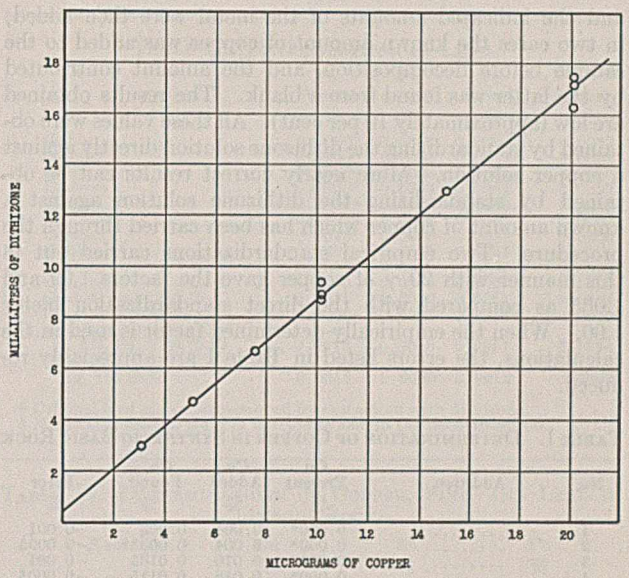



FIGURE 1. DETERMINATION OF COPPER BY EXTRACTIVE TITRATION

amounts approximately cancel each other. The uncertainty in the location of the end point need not exceed 0.5 $\gamma$ of copper, corresponding to $\pm 0.0005$ per cent when an aliquot representing 0.1 gram of sample is used. The largest error in the results given in Figure 1, some of which were obtained by adding nearly all the reagent required in one portion, is about 0.75 $\gamma$. Doubtless the accuracy can be improved by shaking the mixture a little longer with a moderate excess of reagent at the end point, and then determining more exactly the excess of reagent present by adding dithizone solution to a measured volume of copper dithizonate of known concentration (such as the first portions of carbon tetrachloride extract obtained in the titration) until the hues of the two solutions are identical.

Nickel and cobalt do not react at the pH at which the copper titration is made. Bismuth does react, but not until the copper has reacted, so that it does not interfere. In the presence of bismuth the color change in the titration is from red-violet to brownish instead of green; the end point can still be determined with fair precision. Silver does not react if iodide or thiocyanate is added. Zinc reacts slowly at pH 3.5, giving rise to a vague color change in the titration of copper. In the procedure given zinc is removed before the titration is made.

Copper can also be determined by the mixed color method in which the solution of adjusted acidity (pH approximately 3) is shaken with an excess of a 0.001 per cent carbon tetrachloride solution of dithizone, and the hue of the carbon tetrachloride solution, varying from greenish blue to violet-red, is compared with standards similarly prepared. This method is more suitable than the titration method for the determination of a few thousandths of a per cent of copper, and can be used for larger amounts. As little as 0.0002 per cent of copper can be determined in a 10-mg. sample when a suitably small volume of the reagent is used. The mixed-color technic is not applicable when bismuth is present, but this element is not likely to be encountered in silicate rocks in interfering amounts.

The results obtained in the determination of added amounts of copper in a synthetic basic rock by the extractive titration method are given in Table I. In most cases the small amount of copper originally present in the synthetic sample and introduced by the reagents was removed from the solution resulting from the decomposition by extraction with dithizone,

and the indicated amounts of the metal were then added; in two cases the known amount of copper was added to the sample before decomposition, and the amount contributed by the latter was found from a blank. The results obtained are low (approximately 10 per cent). All these values were obtained by standardizing the dithizone solution directly against a copper solution. More nearly correct results can be obtained by standardizing the dithizone solution against a known amount of copper which has been carried through the procedure. Two empirical standardizations carried out in this manner with 20 $\gamma$ of copper gave the factors 1.09 and 1.065 as compared with the direct standardization factor 1.00. When the empirically determined factor is used in the calculations, the errors listed in Table I are appreciably reduced.

TABLE I. DETERMINATION OF COPPER IN SYNTHETIC BASIC ROCK

| No. | Addition | Cu Present % | Cu Added % | Cu Found % | Error % |
|---|---|---|---|---|---|
| 1 | ..... | 0.000[a] | 0.004 | 0.003 | −0.001 |
| 2 | ..... | 0.000[a] | 0.004 | 0.0035[b] | −0.0005 |
| 3 | ..... | 0.0015 | 0.010 | 0.0105 | −0.001 |
| 4 | ..... | 0.000[a] | 0.012 | 0.0115 | −0.0005 |
| 5 | ..... | 0.000[a] | 0.024 | 0.0215 | −0.0025 |
| 6 | ..... | 0.0015 | 0.024 | 0.0235 | −0.002 |
| 7 | ..... | 0.000[a] | 0.050 | 0.046 | −0.004 |
| 8 | ..... | 0.000[a] | 0.050 | 0.0455 | −0.0045 |
| 9 | 0.2% Ni, 0.1% Co | 0.000[a] | 0.010 | 0.009 | −0.001 |
| 10 | 0.1% Zn, 0.05% Pb | 0.000[a] | 0.010 | 0.0095 | −0.0005 |
| 11 | 0.5% $Cr^{VI}$ | 0.000[a] | 0.010 | 0.010 | 0.000 |
| 12 | 0.4% VV | 0.000[a] | 0.010 | 0.0095 | −0.0005 |
| 13 | 0.1% $Sn^{II}$, 0.01% Ag | 0.012[c] | 0.000 | 0.012[d] | 0.000 |

[a] Original copper removed by extraction with dithizone.
[b] All of solution titrated; 2/3 aliquot in others.
[c] Sample of gabbro.
[d] Determination by mixed-color technic in the presence of iodide.

DETERMINATION OF ZINC. The determination of zinc in the 0.01 *N* hydrochloric acid extract of the carbon tetrachloride solution of the heavy metal dithizonates is carried out by adjusting the pH of an aliquot portion to 4 to 4.3, shaking with an excess of 0.001 per cent dithizone in carbon tetrachloride, and comparing the hue of the latter solution with a series of standards prepared in the same way. The interference of lead is prevented by adding a complex-forming substance such as an alkali iodide (together with a little sulfite to prevent the liberation of iodine which would react with the reagent) or thiosulfate. The results in Table II were obtained with the use of potassium iodide to prevent the reaction of lead. After the experimental work on zinc had been completed, there appeared a paper by Fischer and Leopoldi (*3*) in which it was shown that sodium thiosulfate was an excellent substance for preventing the interference of lead, and most other metals as well, under proper conditions. Comparison of the use of thiosulfate and iodide in the determination of zinc in ten igneous rocks showed that either can be used with equally good results. The small amounts of lead—of the order of a few thousandths of a per cent—usually encountered in silicate rocks hardly lead to a perceptible coloration of the reagent in the weakly acid solution in which the zinc determination is made, but it is not safe to depend on the adjustment of the pH as the sole means of preventing interference by lead. If the pH of the solution is too high neither thiosulfate nor iodide prevents the reaction of lead with the reagent.

The reaction between zinc ions and dithizone at pH 4 to 4.5 is slow and is moreover incomplete even after long shaking, so that it is not practicable to apply the extractive titration method. However, it is possible to determine zinc by a colorimetric titration in which a standard solution of the metal is added to a comparison solution containing the same amount of a carbon tetrachloride solution of the reagent as the unknown until, after prolonged shaking, the hue of known and unknown match. This method demands care in its application because of the slowness with which zinc reacts, and it is perhaps safer and as rapid to use the standard series method in which the period of shaking is the same for both the unknown and the known.

The results in Table II (obtained by the standard series method) show that zinc can be determined with satisfactory accuracy in the presence of the major and minor rock constituents.

DETERMINATION OF LEAD. Lead is determined in a simple manner in the 0.01 *N* acid extract by adding potassium cyanide and ammonia to an aliquot portion and shaking with a small volume of a 0.001 per cent solution of dithizone. The cyanide prevents the reaction of zinc (and any small amounts of other metals that may be present). The only other elements reacting in alkaline cyanide medium as shown by Fischer are tin(II), bismuth, and thallium(I). Divalent tin or univalent thallium cannot be present at this stage nor can bismuth, which is separated from lead and zinc by treatment of the original carbon tetrachloride extract with 0.01 *N* hydrochloric acid. Copper, except in very small amounts, is an undesirable element in a solution in which lead is to be determined, because it tends to give a yellow-brown oxidation product with dithizone. In the procedure given, copper is not present in the solution used for the lead determination. Ferric iron must not be present because it oxidizes the reagent, giving a yellowish brown carbon tetrachloride layer; the lead determination then becomes difficult or impossible because of the difference in hue between the unknown and the standards.

By adding a little ammonia to the cyanide medium the complete extraction of the excess dithizone from the carbon tetrachloride layer is assured, and the amount of lead is indicated by the intensity of the color imparted by the lead dithizonate which remains dissolved in the carbon tetrachloride. The color comparison is most conveniently made by using a series of standards contained in flat-bottomed glass-

TABLE II. DETERMINATION OF ZINC IN SYNTHETIC ACID AND BASIC ROCK

| No. | Addition | Zn Present % | Zn Added % | Zn Found % | Error % |
|---|---|---|---|---|---|
| | | Acid Rock | | | |
| 1 | | 0.000[a] | 0.0024 | 0.0026 | +0.0002 |
| 2 | | 0.000[a] | 0.005 | 0.005 | 0.000 |
| 3 | | 0.002[b] | 0.002 | 0.0045 | +0.0005 |
| 4 | | 0.002[b] | 0.005 | 0.007 | 0.000 |
| 5 | | 0.002[b] | 0.006 | 0.009 | +0.001 |
| 6 | | 0.000[a] | 0.0105 | 0.0105 | 0.000 |
| 7 | | 0.000[a] | 0.0145 | 0.016 | +0.0015 |
| 8 | 1% Mn | 0.002[b] | 0.002 | 0.005 | +0.001 |
| 9 | 0.05% Cu | 0.002[b] | 0.004 | 0.0065 | +0.0005 |
| 10 | | 0.0045 | 0.001 | 0.0050 | −0.0005 |
| 11 | | 0.0045 | 0.0035 | 0.009 | +0.001 |
| 12 | | 0.0045 | 0.0045 | 0.0105 | +0.0015 |
| 13 | | 0.0045 | 0.007 | 0.012 | +0.0005 |
| 14 | 1% Mn | 0.0045 | 0.0035 | 0.009 | +0.001 |
| 15 | 0.1% Pb | 0.0045 | 0.0035 | 0.0095 | +0.0015 |
| 16 | 0.05% Cu | 0.0045 | 0.0035 | 0.007 | −0.001 |
| 17 | 0.05% Cu, 0.05% Pb | 0.0045 | 0.0035 | 0.007 | −0.001 |
| 18 | 0.05% Pb | 0.000[a] | 0.0024 | 0.0026 | +0.0002 |
| 19 | 0.08% Cu, 0.05% Pb, 0.3% Ni, 0.1% Co, 1% Mn | 0.000[a] | 0.0055 | 0.0065 | +0.001 |
| 20 | 0.05% Cu, 0.05% Pb, 0.25% Ni, 0.1% Co, 0.5% Mn | 0.000[a] | 0.010 | 0.011 | +0.001 |
| | | Basic Rock | | | |
| 21 | | 0.000[a] | 0.005 | 0.005 | 0.000 |
| 22 | | 0.005 | 0.0055 | 0.010 | −0.0005 |
| 23 | | 0.000[a] | 0.010 | 0.010 | 0.000 |
| 24 | | 0.005 | 0.010 | 0.015 | 0.000 |
| 25 | 0.2% Pb | 0.005 | 0.000 | 0.004 | −0.001 |
| 26 | 0.1% Cd | 0.000[a] | 0.005 | 0.006 | +0.001 |
| 27 | 0.05% Cu, 0.05% Pb | 0.000[a] | 0.005 | 0.006 | +0.001 |
| 28 | 0.05% Cu, 0.1% Pb, 0.2% Ni, 0.05% Co | 0.000[a] | 0.005 | 0.006 | +0.001 |
| 29 | 0.03% $Cr^{VI}$, 0.05% VV, 0.02% $Mo^{VI}$ | 0.000[a] | 0.0055 | 0.0055 | 0.000 |

[a] Original zinc removed by extraction with dithizone.
[b] Sodium carbonate fusion not made.

stoppered tubes of 25- to 30-ml. capacity. In using an aliquot corresponding to 0.05 gram of sample as little as 0.0002 per cent of lead can be detected. The main hindrance to the accurate determination of very small amounts of lead is the existence of an appreciable blank.

In testing the method for lead, natural rock samples were used because the synthetic samples showed much higher amounts of the metal. The results are summarized in Table III. The blank for lead in the reagents amounted to 0.0010 ± 0.0001 per cent.

The reproducibility of the method proposed can be judged from the data in Table IV, which are a part of those obtained in determining copper, zinc, and lead in a number of Minnesota rocks. It is more difficult than might be expected to obtain good agreement in duplicates. Differences of 20 per cent in the determination of copper and zinc can occur.

Table III. Determination of Lead

| No. | Sample | Pb Present % | Pb Added % | Pb Found % | Error % |
|---|---|---|---|---|---|
| 1 | Gabbro | 0.0000[a] | 0.0000 | 0.0000 | 0.0000 |
| 2 | Granite | 0.0000[a] | 0.0004 | 0.00025 | −0.00015 |
| 3 | Gabbro | 0.0000[a] | 0.0004 | 0.0004 | 0.0000 |
| 4 | Diabase | 0.0000[a] | 0.0004 | 0.0002 | −0.0002 |
| 5 | Granite | 0.0000[a] | 0.0008 | 0.0008 | 0.0000 |
| 6 | Diabase | 0.0000[a] | 0.0008 | 0.0007 | −0.0001 |
| 7 | Granite | 0.0015 | 0.0020 | 0.0035 | 0.0000 |
| 8 | Gabbro | 0.0007 | 0.0040 | 0.0041 | −0.0006 |
| 9 | Granite | 0.0015 | 0.0040 | 0.0057 | +0.0002 |
| 10 | Granite | 0.0015 | 0.0080 | 0.009 | −0.0005 |
| 11 | Granite + 0.2% Zn | 0.0015 | 0.0040 | 0.0057 | +0.0002 |
| 12 | Granite + 0.05% Cu | 0.0000[a] | 0.0040 | 0.0037 | −0.0003 |
| 13 | Granite + 0.4 % $Cr^{III}$, 0.6% Ba, 0.25% Sb | 0.0015 | 0.0040 | 0.0051 | −0.0004 |
| 14 | Granite + 0.01% $Tl^{II}$, 0.02% Ag, 1% $Sn^{II}$ | 0.0015 | 0.0000 | 0.0012 | −0.0003 |

[a] Original lead of sample removed by extraction with dithizone.
[b] Nitric acid used in place of perchloric acid in the decomposition.

Table IV. Determinations of Copper, Zinc, and Lead in Igneous Rocks

| Rock | Cu % | Zn % | Pb % |
|---|---|---|---|
| Granite | 0.002[a] | 0.003[a] | 0.0015 |
| | 0.0015[b] | 0.006 | 0.0016 |
| Granite | 0.013[b] | 0.007[a] | 0.0027 |
| | 0.0105[b] | 0.007 | 0.0026 |
| | | 0.007 | |
| Granodiorite | 0.0045[a] | 0.0055[a] | 0.002 |
| | 0.004[b] | 0.007 | 0.0022 |
| Gabbro | 0.012[b] | 0.0095 | 0.0008 |
| | 0.0135[b] | 0.0105 | 0.0006 |
| | | 0.0105 | |
| Diabase | 0.0255[a] | 0.0175 | 0.0025 |
| | 0.023[b] | 0.015 | 0.0021 |
| | | 0.016 | |
| Diabase | 0.022[b] | 0.016 | 0.0005 |
| | 0.021[b] | 0.0135 | 0.0004 |
| | | 0.015 | |

[a] Sodium carbonate fusion not made.
[b] Copper determined by mixed-color colorimetry, others by colorimetric titration.

## Apparatus

The only special apparatus needed is a set of flat-bottomed glass-stoppered color-comparison tubes of approximately 30-ml. capacity (diameter 1.8 cm., height 15 cm.).

The glassware used should be zinc- and lead-free.

## Reagents and Standard Solutions

Dithizone, 0.01 per cent solution. Dissolve 10 mg. of the pure product in 100 ml. of reagent quality carbon tetrachloride. Some products of dithizone may be used as purchased without purification. A product which meets the following test is satisfactory for use in the method: To 2 or 3 ml. of an approximately 0.001 per cent solution in carbon tetrachloride in a glass-stoppered tube add 5 ml. of water, a few drops of 5 per cent potassium cyanide solution, and 2 drops of concentrated ammonium hydroxide. Shake vigorously for 10 to 15 seconds and allow the carbon tetrachloride to settle. If the lower layer is colorless or shows only an extremely faint yellowish tinge when viewed against a white background, the reagent is sufficiently pure for the purpose. Impure dithizone can be purified as described by Fischer and Leopoldi (*4*).

Dithizone, 0.001 per cent solution. Dilute the 0.01 per cent solution with nine times its volume of reagent quality carbon tetrachloride. The dilute solution decomposes slowly, especially if exposed to light, and it is best to prepare the solution the same day it is to be used.

Water. Redistill in glass (preferably Pyrex) apparatus and preserve in a Pyrex flask.

Perchloric Acid, 70 per cent. If lead is to be determined it is preferable to vacuum-redistill the analytical grade acid.

Hydrofluoric Acid. The analytical reagent is likely to contain lead and is best purified by distillation from platinum apparatus.

Hydrochloric Acid, 1 to 1. It is preferable to use freshly distilled constant-boiling acid, stored in Pyrex.

Hydrochloric Acid, 1 to 1000. Dilute the constant-boiling acid with 500 times its volume of redistilled water.

Ammonia, specific gravity 0.9. Saturate redistilled water with ammonia gas and keep the solution in a ceresin-coated bottle.

Sodium Citrate, 10 per cent. Dissolve 10 grams of trisodium citrate in 100 ml. of water and add 1 ml. of concentrated ammonium hydroxide. Shake the solution with small portions of 0.01 per cent dithizone in carbon tetrachloride, drawing off each portion before the next is added, until the carbon tetrachloride is colored only faintly pink and the aqueous layer is a strong yellow-brown. Then continue the extraction with carbon tetrachloride alone until the latter shows only a pinkish tinge on prolonged shaking. Preserve the solution in a wax-lined bottle. The excess dithizone in the solution need not be removed; it soon decomposes, giving a nearly colorless solution.

Sodium Acetate, 2 *N*. Prepare from a good grade of salt, containing minimal amounts of copper and zinc. The solution can be purified if desired by shaking with dithizone in carbon tetrachloride, but generally this is unnecessary.

Sodium Acetate–Acetic Acid Buffer, pH ca. 4.1. Mix equal volumes of 1 *N* sodium acetate and 4 *N* acetic acid.

Sodium Thiosulfate, 50 grams $Na_2S_2O_3 \cdot 5H_2O$ in 100 ml. of water.

Potassium Cyanide, 5 per cent. Dissolve 5 grams of lead-free salt in 100 ml. of water. The solution should be tested for lead as follows: Dilute 1 ml. with 2 to 3 ml. of water, add 2 drops of pure ammonia water, and shake with 1 or 2 ml. of 0.001 per cent dithizone solution. The carbon tetrachloride layer should show no trace of pink color.

Sodium Carbonate, anhydrous. If necessary, the reagent quality salt can be freed from lead by shaking a 5 to 10 per cent solution with precipitated calcium carbonate (0.5 gram per 100 ml.) for 5 or 10 minutes, filtering, evaporating the solution in platinum, and weakly igniting the residue.

Standard Copper Solution, 0.001 per cent. Prepare by diluting a stronger copper solution which has been obtained by weighing out unefflouresced copper sulfate pentahydrate crystals; add sufficient hydrochloric acid to make the acidity approximately 0.01 *N*.

Standard Zinc Solution, 0.001 per cent. Prepare by diluting a stronger zinc solution which has been obtained by dissolving pure zinc metal in excess hydrochloric acid; add sufficient hydrochloric acid to make the acidity 0.01 *N*.

Standard Lead Solution, 0.001 per cent. Prepare from a stronger lead solution obtained by weighing out dry lead nitrate. Add enough hydrochloric acid to make the final acid concentration 0.01 *N*. Preserve the solution in a Pyrex bottle. It is important that the final solution be slightly acid, because otherwise lead will be adsorbed by the glass.

## Procedure

Weigh 0.25 gram of 100-mesh rock powder into a small platinum dish, and add 0.5 ml. of 70 per cent perchloric acid and 5 ml. of 1 to 1 hydrofluoric acid. Heat on a low-temperature hot plate, stirring occasionally with a platinum wire if the powder cakes, until the solution has evaporated to dryness and the excess of perchloric acid has been expelled. Then add 0.5 ml. of perchloric acid together with 0.5 to 1 ml. of water, again evaporate to dryness and expel the excess of acid. To the residue add 2 ml. of 1 to 1 hydrochloric acid and 5 ml. of water, and warm gently, if necessary, to bring all soluble material into solution. Next add 5 ml. of 10 per cent sodium citrate solution, cool to room temperature, and add dropwise concentrated ammonium hydroxide until the solution becomes basic to litmus paper, and finally an excess of 0.25 to 0.3 ml. If the solution became appreciably turbid on the addition of ammonia it is well to let it stand for 10 or 15 minutes before beginning the filtration.

Filter the solution through a small (4 or 5 cm. in diameter) filter paper of medium texture, transfer any residue in the dish to the paper, and wash with three or four 1-ml. portions of cold water containing a drop of ammonia and sodium citrate solution. If the filtrate is turbid or becomes so on standing, refilter it. Reserve the filtered solution (solution 1). Ignite the paper and its contents in a platinum crucible, avoiding an unnecessarily high temperature. Cool, mix the residue with 0.15 gram of sodium carbonate, fuse, and add 2 or 3 ml. of water to the cooled melt. Warm to hasten disintegration of the melt, and filter through a 4-cm. paper, keeping most of the insoluble material in the crucible. Rinse the crucible and wash the filter well with small portions of cold water totaling 8 to 10 ml. Reserve the filtrate (solution 2) and transfer the filter paper and its contents to the platinum crucible previously used. Char the paper, burn off the carbon at a low temperature, add 1 to 1.5 ml. of 1 to 1 hydrofluoric acid and 2 drops of 70 per cent perchloric acid, evaporate to dryness, and expel the excess perchloric acid. Add a few drops of water and 1 or 2 of perchloric acid, again evaporate to dryness, and expel the excess acid. To the residue add 0.5 to 1 ml. of 1 to 1 hydrochloric acid and 2 or 3 ml. of water. Warm to bring all the residue into solution. (If any insoluble material remains, filter it off and fuse again with sodium carbonate.) Add 0.5 to 1 ml. of 10 per cent sodium citrate to the solution and then ammonia in a 3- to 4-drop excess over that required to give a basic reaction with litmus (solution 3).

Transfer the main solution (solution 1) to a separatory funnel of suitable size, add 5 ml. of 0.01 per cent dithizone solution, and shake for 0.5 minute; if the carbon tetrachloride is green at the end of this time shake for an additional half minute. Allow the carbon tetrachloride to settle and draw it off into another separatory funnel, leaving a few drops in the first funnel to prevent loss of aqueous phase. Unless the separated carbon tetrachloride is distinctly green, showing that an excess of reagent is present, add another 5-ml. portion of 0.01 per cent reagent to the aqueous solution, and shake vigorously for 30 to 45 seconds, draw off the carbon tetrachloride, and continue in this manner until the last 5-ml. portion of dithizone remains distinctly green after being shaken vigorously for 45 to 60 seconds. Next extract the solution of the leached sodium carbonate melt (solution 3) in the same manner, using 2-ml. portions of reagent, and combine these extracts with those from the main portion. There now remains the filtrate from the sodium carbonate leach (solution 2) which had best be extracted as a precautionary measure. Add to it 0.5 ml. of sodium citrate solution and a drop of thymol blue, and then dilute hydrochloric acid until the indicator shows the greenish intermediate color. Extract with 2 ml. of reagent, shaking for 1 minute, and add this extract to the others.

If the combined extracts from solutions 1, 2, and 3 contain any drops of aqueous phase, draw off the carbon tetrachloride layer into a clean separatory funnel and wash any small volume of aqueous solution with a few drops of carbon tetrachloride. It is important that the separated carbon tetrachloride be free from droplets of aqueous solution, because iron so introduced will oxidize the reagent and lead to difficulties in the determination of lead. To separate zinc and lead from copper, add 10 ml. of 1 to 1000 hydrochloric acid to the combined carbon tetrachloride extracts and shake vigorously for 1 minute. If the carbon tetrachloride remains red on shaking and does not become greenish or purple, add sufficient dithizone solution to change the red to a bluish or purplish color.

Draw off the carbon tetrachloride layer into another separatory funnel and again shake vigorously for a full minute with 10 to 12 ml. of 1 to 1000 hydrochloric acid. If the volume of the carbon tetrachloride phase is greater than 15 or 20 ml. it is advisable to shake for 2 minutes the last time to ensure the complete extraction of zinc. Combine the second portion of acid extract with the first, add a few drops of carbon tetrachloride to remove any droplets of colored carbon tetrachloride, transfer to a 25-ml. volumetric flask, and make up to the mark with 1 to 1000 hydrochloric acid. This solution contains all the zinc and lead of the sample. Run the carbon tetrachloride solution which has been washed with the dilute hydrochloric acid into a silica dish. This solution contains the copper of the sample.

Determination of Copper. Evaporate to dryness the carbon tetrachloride solution containing the copper dithizonate (which has been extracted with 0.01 *N* hydrochloric acid to remove zinc and lead), add 3 or 4 drops of 1 to 1 nitric acid to moisten the residue, evaporate, and ignite gently to destroy all organic material. To the cold dish add 0.5 to 0.75 ml. of concentrated hydrochloric acid, and by means of a stirring rod rub the acid over the interior of the dish so that all the cupric oxide will be dissolved. The intensity of the yellow color of the solution gives an indication of the amount of copper, provided that much nickel is not present; with less than about 0.01 mg. of copper (corresponding to 0.004 per cent in the sample) hardly any color is apparent. Add water, transfer the solution to a 25-ml. volumetric flask, and make up to the mark.

Copper may be determined in this solution by either procedure (*a*) or (*b*). The first procedure is better suited for the determination of very small amounts of the metal, and the second for larger amounts which are to be determined as accurately as possible. However, procedure (*a*) will generally be found satisfactory; it is not applicable when bismuth is present.

(*a*) Mixed Color Method. Unless the color of the concentrated hydrochloric acid solution indicated that only a very small quantity of copper is present, it is advisable to determine approximately in a preliminary test the amount of the metal in the solution in order to avoid the necessity for preparing a large number of standards. To do this, measure 1 ml. of the mixed solution into a color-comparison tube, dilute with 3 or 4 ml. of water, add a drop of methyl orange indicator solution, and then sodium acetate (conveniently a 2 *N* solution is first used and then one which is 0.1 or 0.2 *N*) until the color of the indicator just begins to deviate from the full red of the acid form (pH approximately 3). Run in 5 ml. of 0.001 per cent dithizone solution and shake vigorously for 1 minute. The quantity of copper can be estimated from the hue of the carbon tetrachloride layer: 1 $\gamma$, green with bluish tinge; 2 $\gamma$, blue-green; 3 $\gamma$, purplish gray; 4 $\gamma$, violet-purple; 5 $\gamma$, red-violet or violet.

Having determined the approximate percentage of copper, transfer 5 ml. (for less than 0.01 per cent) or 2 ml. (for 0.01 to 0.02 per cent or slightly more) of the mixed solution to a color-comparison tube, and in similar tubes prepare a series of standards of which adjacent numbers differ by 0.002 per cent of copper. Add sufficient hydrochloric acid to the standards to make their acidity roughly the same as that of the unknown, and adjust the volumes so that all are approximately equal. Add sodium acetate solution until methyl orange shows a slight deviation from the full acid color. Then add 5.0 ml. of 0.001 per cent dithizone solution to standards and unknown, shake all the tubes simultaneously for 1 to 1.5 minutes, and compare transversely against a white background. If silver or bismuth is present the hue of the unknown cannot be matched with the standards. The interference of silver can be prevented by adding 2 or 3 drops of 1 per cent sodium bisulfite solution and 25 mg. of potassium iodide to each of the tubes, again shaking for 0.5 minute and comparing.

It appears that the results obtained by following the above procedure are slightly low (see Table I). Possibly the values can be corrected by increasing them by 10 per cent, but it is better to obtain the correction factor by carrying 50 $\gamma$ of copper through the procedure. The final percentage is to be corrected for the copper in the reagents as determined in a blank.

(*b*) Extractive Titration. To save time it is advisable to make a rough preliminary titration or to proceed as in (*a*) to find the approximate amount of copper present.

Pipet 10 ml. (for less than 0.03 per cent of copper) of the hydrochloric acid solution which has been diluted to 25 ml. into a separatory funnel of suitable size, add a drop of methyl orange, and adjust the pH to approximately 3.5 by adding sodium acetate solution; it is preferable to use a buffer solution of pH 3.5 containing methyl orange for comparison in adjusting the acidity of the solution to be titrated. Now run in nearly the required volume of 0.001 per cent dithizone solution from a buret, shake vigorously for 1 minute, allow the carbon tetrachloride to settle, and draw it off as completely as possible. Finish the titration by adding the reagent in 0.5-ml. portions, shaking vigorously for 45 seconds each time. Near the end point it is convenient to draw off each successive small portion of carbon tetrachloride into a small vial so that the change in color can be noted better.

Take as marking the end point the 0.5-ml. portion of reagent which becomes blue after shaking the specified time. The next 0.5-ml. portion should show a distinct green tinge after thorough shaking. Deduct 0.5 ml. from the volume of standard solution required as the "indicator" correction. Standardize the dithizone solution in the same way against a known amount of copper which has been carried through the procedure. This may be done conveniently by adding 50 $\gamma$ of copper to the extracted solution of the sample, and using a 10/25 aliquot of the final hydrochloric acid solution for the titration. Apply the correction for the copper in the reagents as determined in a blank.

Determination of Zinc. It is advantageous to determine first the approximate percentage of zinc present. Transfer 1 ml. of the 0.01 *N* hydrochloric acid extract, which has been diluted to 25 ml., to a color-comparison tube, add 1 ml. of acetic acid–acetate buffer of pH approximately 4.1, 3 ml. of water, and 0.10 ml. of sodium thiosulfate solution. Shake vigorously with 5 ml. of 0.001 per cent dithizone for 1 minute. The quantity of zinc present can be roughly estimated from the color of the carbon tetrachloride: 1 $\gamma$, blue; 2 $\gamma$, purplish violet; 3 $\gamma$, violet; 4 and 5 $\gamma$, red-violet.

Having determined the range of the zinc percentage, measure 5 ml. (for less than 0.01 per cent of zinc) or 2 ml. (for 0.01 to 0.025 per cent) of the 0.01 *N* hydrochloric acid solution into a tube, and prepare a series of standards differing, for example, by 0.002 per cent of zinc. Dilute the standards with 0.01 *N* hydrochloric acid to the same volume as the unknown. Then to each of the tubes add 1 ml. of buffer solution of pH approximately 4.1 and 0.10 ml. of sodium thiosulfate solution. Run in 5.0 ml. of 0.001 per cent dithizone solution and shake vigorously for 1 to 1.5 minutes. Compare the tubes transversely against a white background. Apply the correction for zinc in the reagents as determined in a blank.

DETERMINATION OF LEAD. Transfer 5 ml. of the 0.01 *N* hydrochloric acid extract to a color-comparison tube, add 1 drop of 10 per cent sodium citrate solution, 2 drops of concentrated ammonium hydroxide, and 1 ml. of 5 per cent potassium cyanide. Prepare a series of standards (containing for example 0.5, 0.75, 1 . . . 2 $\gamma$ of lead) in 5 ml. of 0.01 *N* hydrochloric acid, and treat with citrate, ammonia, and cyanide. From a buret add 2.0 ml. of 0.001 per cent dithizone in carbon tetrachloride to the unknown and the standards. Shake the tubes vigorously for 10 to 15 seconds and compare the colors by viewing the carbon tetrachloride layers transversely against a white background. If desired, adjacent members of the standards may be mixed for a more precise comparison after the first comparison has been made. Correct the lead value obtained for the amount in the reagents.

## Literature Cited

(1) Fischer, H., *Z. angew. Chem.*, 47, 685 (1934).
(2) *Ibid.*, 47, 691 (1934).
(3) Fischer, H., and Leopoldi, G., *Z. anal. Chem.*, 107, 241 (1937).
(4) Fischer, H., and Leopoldi, G., *Z. angew. Chem.*, 47, 90 (1934).
(5) Willoughby, C. E., Wilkins, E. S., Jr., and Kraemer, E. O., IND. ENG. CHEM., Anal. Ed., 7, 285 (1935).

RECEIVED July 16, 1937.

# Influence of Solvent on the Saponification Number of Rosin

W. C. SMITH, Naval Stores Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.

SINCE about two-thirds of the rosin consumed industrially goes into the production of paper size and soap, these users are especially interested in the determination of the so-called saponification number of rosin as the means by which they decide what quantity of alkali is needed to combine with rosin to convert it into a soap or a size. Because of the different results obtained on saponifying oils, fats, and waxes with different solvents, as reported in the literature, this investigation was undertaken to ascertain what solvent or solvents should be used in determining the saponification number of rosin.

Rosin is usually described as consisting of abietic acid, natural esters of abietic acid, and unsaponifiable materials. It is described in more detail (*8*) as consisting of about nine parts of isomeric resin acids having the empirical formula $C_{20}H_{30}O_2$ and one part of neutral bodies consisting of esters, sesquiterpenes, oxidation products, and unidentified components. Earlier investigators (*5*) held conflicting opinions as to the presence of lactones and anhydrides.

Abietic and isomeric resin acids are monobasic. One gram of these acids will therefore require for neutralization alkali equivalent to 185.6 mg. of potassium hydroxide. Esters of these acids will require for saponification smaller quantities of alkali, dependent on the molecular weight of the alcohol combined with the acid.

It would appear that the sum of the acids in rosin, calculated as abietic from the saponification number, and the unsaponifiable matter should not exceed 100 per cent, and if water-soluble alcohols or light volatile matter were present such sum would be less. The results obtained on a large number of samples of gum rosin by the proposed American Society for Testing Materials methods (*1*) show that on the average, the calculated abietic acid plus the unsaponifiable matter totals a little over 102 per cent and, in some instances, is as much as 105 per cent. FF wood rosin (*13*) is reported to contain 89 per cent acid as abietic, and 13 per cent unsaponifiable matter. Matlack and Palkin (*7*) observed this apparent anomaly and suggested the presence of acids of lower molecular weight than abietic as an explanation. The presence of volatile water-soluble acids in rosin has been reported previously. Tomeo and Garcia-Viana (*18*) found Spanish rosin to have a Reichert-Meissl number of about 1.5. Terpougoff (*17*) saponified 14.4 grams of WW rosin, and after 24 hours decomposed the soap with excess sulfuric acid. In the aqueous layer were found 0.228 gram of acetic acid and possibly traces of formic and butyric acids. This percentage of acetic acid in rosin would increase its saponification number by 11.9 and its apparent resin acid content by 6.4 per cent.

It does not necessarily follow from the high free-acid content of rosin and its ready solubility in aqueous and alcoholic alkalies that complete saponification of the esters present is easily effected. In fact, certain esters of abietic acid are very difficult to saponify. The manufacturers of methyl abietate state that ordinary saponification methods have little effect on this product. The standard method yields a saponification number of 11 on neutral methyl abietate.

Standard methods for determining the saponification numbers of oils, fats, and waxes, such as have been adopted by the Association of Official Agricultural Chemists and the American Society for Testing Materials, specify that 95 per cent ethyl alcohol be used for dissolving the sample and also for preparing the saponification solution. In order to conform as closely as possible to standard methods, 95 per cent ethyl alcohol was specified for use in determining the saponification number of rosin (*3*). Some industrial laboratories substitute for ethyl alcohol a tax-free denatured alcohol, such as specially denatured No. 30. Gardner (*4*) recommends the use of absolute methyl alcohol for preparing a saponifying solution for the resins on account of its better keeping qualities. Schamelhaut (*14*), without presenting any data, states that isopropyl alcohol can satisfactorily replace ethyl in determining the acid and saponification numbers of fats, essential oils, and balsams. The comparison of other solvents with ethyl alcohol in the saponification of oils, fats, waxes, and esters has been the subject of many investigations.

Pollmann (*12*) concluded that methyl alcohol cannot replace ethyl for saponification of esters because the reaction is incomplete after 0.5 hour's boiling. Leaper (*6*) found that a Carbitol or diethylene glycol monoethyl ether solution of potassium hydroxide would completely saponify oils, fats, and waxes in 1 hour, whereas

3 hours often were required when ethyl alcohol was used. Steet (*16*), using a Cellosolve or ethylene glycol monoethyl ether solution of potassium hydroxide, obtained the same results on saponifying pure fatty oils as were obtained when an ethyl alcohol solution was used. The time required for saponification was much shortened by using Cellosolve. Anderson and Brown (*2*) reported that castor oil is saponified more rapidly in amyl than in ethyl alcohol and more rapidly in ethyl than in methyl. Schuette and Davis (*15*) obtained slightly higher results with isopropyl than with ethyl alcohol in saponifying vegetable oils. Pardee and Reid (*11*) showed that, because of alcoholysis, in the saponification of esters of volatile acids, especially formic, there is a loss of ethyl formate when ethyl alcohol is used, but that when normal butyl alcohol is used theoretical results are obtained. Pardee, Hasche, and Reid (*10*) obtained higher saponification numbers on several oils, fats, and waxes in *n*-butyl alcohol than in ethyl. The greatest differences were found in linseed oil, tallow, and neat's-foot oil. The results reported for these in ethyl and *n*-butyl alcohol, respectively, are: linseed oil, 185.6 and 198.5; tallow, 183.7 and 198.3; neat's-foot oil, 111.5 and 131.7.

For the present investigation methyl, isopropyl, and *n*-butyl alcohols were selected for comparison with ethyl alcohol in the saponification of rosin because methyl alcohol has been recommended for use with resins and isopropyl for use with balsams and because isopropyl and *n*-butyl alcohols gave higher results than ethyl in saponifying oils, fats, and waxes. Specially denatured alcohol No. 30 was also used for comparison with ethyl because it is now being used by some industrial laboratories.

All samples used in the test were gum rosin varying in grade from X to D. With the exception of samples 18 and 19, which were prepared in the laboratory by the method of Palkin and Clark (*9*), all were considered to be normal rosin. No. 18 was unusually high in resene or unsaponifiable matter, and No. 19 unusually low in resene. The precautions recommended by Veitch and Sterling (*19*) were taken to prevent changes due to oxidation.

The alcohols used as solvents and for preparation of the 0.5 *N* saponification solutions were as follows:

Methyl, C. P. grade, almost absolute, used without any treatment.

Ethyl, 95.2 per cent after distillation from potassium hydroxide.

S. D. No. 30, prepared by adding one volume of methyl to 10 volumes of ethyl.

Isopropyl, commercial "Petrohol" refined as described by Schuette and Davis (*15*).

*n*-Butyl, technical grade refined as described by Pardee and Reid (*11*).

Two-gram portions of the sample were placed in 300-cc. Erlenmeyer flasks and 50 cc. of the selected solvent and 25 cc. of a corresponding saponification solution added. The flasks were then placed on a steam bath for 1 hour under air reflux. After cooling, the excess alkali was titrated with 0.5 *N* hydrochloric acid. Great care was taken in determining the end point. It was found necessary in some cases to reheat and cool and again neutralize the solution after the first end point had been reached, in order to dissolve the alkali which apparently had combined with the glass. The greatest difference between the first and final end points corresponded to 0.3 cc. of 0.5 *N* hydrochloric acid. The maximum difference in duplicate determinations was 1.4 units in saponification number. The average results on the individual samples are listed in Table I.

TABLE I. SAPONIFICATION NUMBERS OF ROSINS IN VARIOUS ALCOHOLS

| Sample No. | Methyl | Ethyl | S. D. No. 30 | Isopropyl | *n*-Butyl |
|---|---|---|---|---|---|
| 1 | 167.7 | 174.3 | 174.6 | ... | ... |
| 2 | 167.7 | 174.8 | 173.9 | ... | ... |
| | 171.3[a] | | | | |
| 3 | 165.0 | 170.7 | 170.9 | ... | ... |
| | 167.3[a] | | | | |
| 4 | 167.5 | 174.3 | 173.8 | ... | ... |
| 5 | 168.8 | 173.7 | 173.9 | ... | ... |
| | 173.0[a] | | | | |
| 6 | .. | 172.3 | 173.0 | ... | ... |
| 7 | .. | 175.1 | 175.4 | ... | ... |
| 8 | .. | 170.9 | 170.9 | ... | ... |
| 9 | .. | 168.7 | 169.5 | ... | ... |
| 10 | .. | 173.7 | 172.6 | ... | ... |
| 11 | .. | 175.4 | 175.4 | ... | ... |
| 12 | 170.9 | 176.0 | 175.4 | 179.0 | 194.0 |
| | 173.2[a] | | | | |
| 13 | 168.8 | 174.0 | 173.9 | 176.0 | 189.4 |
| 14 | .. | 176.5 | 176.0 | 176.0 | 176.9 |
| 15 | .. | 172.8 | ... | 175.3 | 191.9 |
| 16 | .. | 176.4 | ... | 181.0 | 196.1 |
| 17 | .. | 173.8 | ... | 175.8 | 190.3 |
| 18 | .. | 166.3 | ... | ... | 184.2 |
| 19 | .. | 179.9 | ... | ... | 182.6 |

[a] 4 cc. of water added along with saponification solution.

## Discussion of Results

The higher results obtained with *n*-butyl alcohol indicate that it in some way promotes the saponification of rosin components not saponified in the lower alcohols.

The average unsaponifiable matter of a large number of samples of commercial gum rosin was found to be about 8 per cent, with very few samples having less than 6 per cent. A saponification number of 175 would account for a total abietic or isomeric resin acid content of slightly more than 94 per cent. The average saponification number in butyl alcohol for the normal gum rosins was 189.8. This suggests the presence in the rosins of low molecular weight acids, esters of such acids, polybasic acids, or their esters.

In order to determine whether there was any loss of light volatile esters when ethyl alcohol was used, several samples were saponified in pressure flasks immersed in a steam bath. On all samples except No. 18 the saponification numbers obtained when the determination was carried out under pressure were essentially the same as obtained under air reflux. On this sample the pressure determination gave a value only 2.4 units higher than did the air reflux determination.

An attempt was made to recover from some of the samples having high saponification numbers in *n*-butyl alcohol sufficient volatile acids to account for the high values. A prolonged steam distillation of the rosin yielded but negligible quantities of volatile acids. The addition of a small quantity of sulfuric acid did not increase the yield of volatile acids. A 50-gram portion of the sample was then saponified in *n*-butyl alcohol solution with an excess of alkali, and after saponification as much as possible of the solvent was removed by distillation. A calculated small excess of sulfuric acid was then added and the mass was subjected to prolonged steam distillation. The greatest quantity of acid recovered by this means would represent about 7 units in the saponification number of the sample taken. When the saponification was made in aqueous alkali, similar results were obtained. Slightly smaller quantities of acid were recovered when ethyl alcohol was used. Acids of the abietic type, prepared from rosin by recrystallization, treated in this manner yielded only a trace of volatile acid.

Commercial methyl and ethyl abietates were used to test the relative effectiveness of ethyl and *n*-butyl alcohols in saponifying esters of abietic acid. The saponification numbers in ethyl and *n*-butyl alcohol, respectively, were for methyl abietate 18.2 and 37.8, and for ethyl abietate 11.2 and 19.6. All these values are far short of the theoretical.

Finally three samples of abietic-type acids, prepared as described above, were saponified in ethyl and *n*-butyl alcohol solutions. The average saponification number in each solvent was approximately 185. The acid number of the acids determined in ethyl alcohol was 184.

## Summary and Conclusions

The saponification number of rosin is influenced by the solvent used in the determination, alcohols of the higher

molecular weights giving the higher values. This is in harmony with the findings of earlier investigators on the saponification of oils, fats, and waxes.

Since known esters of abietic acid are not completely saponified in *n*-butyl alcohol and it is generally assumed that rosin contains esters of the resin acids, it is probable that rosin is not completely saponified even in this medium, although the results obtained are higher than with the other solvents tested.

Since the results obtained using specially denatured alcohol No. 30 are essentially the same as those obtained with 95 per cent ethyl, this denatured alcohol may be used in the place of the 95 per cent ethyl in the saponification of rosin as customarily carried on.

A method for the complete saponification of rosin will be of scientific value in determining its composition and nature. However, from a commercial standpoint it is not essential, since industrial saponification of rosin is carried out in aqueous solutions of alkali. A method to be of maximum commercial value should simulate the procedure used industrially.

Attempts by the writer to determine the saponification number of rosin by saponifying in aqueous alkali have not met with complete success. However, results obtained by this method also indicate that saponification is not complete.

The presence in rosin of volatile water-soluble acids, reported by others, has been confirmed. The quantities found, however, are not sufficient to account for the high saponification numbers obtained on certain samples.

The presence in rosin of nonvolatile polybasic acids, or their esters, such as are found in copals, would increase its saponification number.

Rosins having very low resene content appear to contain only isomeric resin acids of the abietic type. A study of the resene or neutral portion of rosin may reveal the cause of the high saponification numbers obtained with certain solvents.

### Literature Cited

(1) Am. Soc. Testing Materials, *Proc.*, 33, 438 (1933).
(2) Anderson, A., and Brown, H. L., *J. Phys. Chem.*, 20, 195 (1916).
(3) Assoc. Official Agr. Chem., Official and Tentative Methods of Analysis, 4th ed., 75 (1935).
(4) Gardner, H. A., "Physical and Chemical Examination of Paints, Varnishes, Lacquers and Colors," p. 846, Washington, Institute of Paint and Varnish Research, 1935.
(5) Haller, Hans, doctorate dissertation, University of Bern, 1907.
(6) Leaper, J. M. F., *Textile Colorist*, 55, 601, 636 (1933).
(7) Matlack, M. B., and Palkin, S., *J. Assoc. Official Agr. Chem.*, 18, 466–71 (1935).
(8) Palkin, S., *J. Chem. Education*, 12, 35 (1935).
(9) Palkin, S., and Clark, C. K., Ind. Eng. Chem., 26, 720 (1934).
(10) Pardee, A. M., Hasche, R. L., and Reid, E. E., *Ibid.*, 12, 481 (1920).
(11) Pardee, A. M., and Reid, E. E., *Ibid.*, 12, 129 (1920).
(12) Pollmann, F., *Seifensieder-Ztg.*, 55, 375 (1928).
(13) Romaine, E. V., *Ptg. Ink Maker*, June, 1931.
(14) Schamelhaut, A., *J. pharm. Belg.*, 9, 401 (1927).
(15) Schuette, H. A., and Davis, L. E., *J. Am. Pharm. Assoc.*, 15, 166 (1926).
(16) Steet, W. R., *Analyst*, 61, 687 (1936).
(17) Terpougoff, J., *Bull. inst. pin*, No. 17, 103 (1931).
(18) Tomeo, M., and Garcia-Viana, J., *Inst. forestal investigaciones experiencias (Madrid) Bol.*, 4, 88 (1931).
(19) Veitch, F. P., and Sterling, W. F., Ind. Eng. Chem., 15, 576 (1923).

Received July 31, 1937.

# Determination of Coumarin in Sweet Clover

## A Comparison of the Steam-Distillation and Alcoholic-Extraction Methods

IRA J. DUNCAN AND R. B. DUSTMAN
West Virginia Agricultural Experiment Station, Morgantown, W. Va.

IN 1934 the authors (*3*) proposed a method for the quantitative determination of coumarin in plant material, based upon the removal of the coumarin by steam distillation and subsequent titration with potassium permanganate according to the method of Obermayer (*6*). In 1935 Clayton and Larmour (*1*) published a tentative colorimetric method for the determination of coumarin and melilotic acid in Melilotus species, that consists in extracting the sweet clover material with 95 per cent alcohol and determining the coumarin and melilotic acid colorimetrically in aliquot portions of the extract. The color is produced when the extract is treated with sodium carbonate and heated, and a solution of diazotized *p*-nitroaniline is added. Color comparisons are made with standards prepared from pure coumarin in alcoholic extracts of some noncoumarin-bearing plant, such as western rye grass or alfalfa. With colorless solutions the method was regarded as quantitative, but only comparative results were claimed when it was applied to alcoholic extracts of sweet clover. In 1936 Stevenson and Clayton (*8*) reported results obtained with sweet clover, using certain modifications of the Clayton-Larmour method, and extracting with 50 per cent methyl alcohol instead of 95 per cent ethyl alcohol.

Recently the steam-distillation method was applied to the removal of coumarin from vanilla extracts (*4*), and it was found that the colorimetric procedure of Clayton and Larmour could be used for determining the coumarin in the distillates with excellent results.

Because some of the results reported by Stevenson and Clayton were at variance with results obtained in this laboratory, it seemed desirable to make a comparison of the analytical procedures involved. While this work was in progress the authors' attention was drawn to a still more recent modification of the alcoholic-extraction method by Roberts (*7*), and this modification has been included in the comparison trials reported here.

### Comparison of Colorimetric and Titration Methods

The colorimetric method of Clayton and Larmour, with slight modifications (*4*), gave quantitative results when applied to the clear sweet clover distillates obtained by the steam-distillation method. Table I shows that approximately identical results are obtained when coumarin is determined in aliquot portions of the same distillates by the colorimetric and permanganate titration methods.

The colorimetric method is superior to the titration method because less time is required and because small amounts of noncoumarin reducing substances present in the distillates do not interfere with the color produced. Treatment of the distillates with lead acetate can, therefore, be omitted. For

TABLE I. COMPARISON OF COLORIMETRIC AND TITRATION METHODS FOR SWEET CLOVER DISTILLATES

| Sample No. | Coumarin Content: Colorimetric method | Coumarin Content: Titration method |
|---|---|---|
| | % | % |
| 1 | 1.19 | 1.17 |
| 2 | 1.28 | 1.29 |
| 3 | 1.07 | 1.10 |
| 4 | 1.52 | 1.59 |
| 5 | 1.33 | 1.34 |
| 6 | 1.72 | 1.73 |
| 7 | 0.31 | 0.32 |
| 8 | 0.22 | 0.24 |
| 9 | 0.35 | 0.35 |
| 10 | 0.36 | 0.36 |
| Av. | 0.94 | 0.95 |

TABLE II. DETERMINATION OF COUMARIN

(In green, in room temperature–dried, and in oven-dried sweet clover by extraction and by distillation with and without added pure coumarin)

| | Extraction | Distillation |
|---|---|---|
| | % | % |
| Dried at room temperature | | |
| Sweet clover alone | 0.54 | 1.11 |
| Dried at 66° | | |
| Sweet clover alone | 0.53 | 1.06 |
| Sweet clover plus 0.4% of added coumarin | 0.94 | .... |
| Recovery of added coumarin | 103.0 | .... |
| Green | | |
| Sweet clover alone | 0.46 | 0.42 |
| Sweet clover plus 0.28% of added coumarin | 0.47 | 0.71 |
| Recovery of added coumarin | None | 103.1 |

these reasons the colorimetric method was used throughout the work reported in this paper.

## Extraction and Steam-Distillation Methods

Preliminary trials, which were made to compare the extraction method of Stevenson and Clayton with the steam-distillation method, showed wide differences in the coumarin content of samples taken from the same sweet clover material. Additional trials were therefore made on carefully sampled green, room temperature–dried, and oven-dried sweet clover in an attempt to find an explanation for the differences. The samples were prepared as quickly as possible in the following manner:

A large quantity of second-year-growth white sweet clover was cut at the early bloom stage, the cut ends were placed in a vessel of water to prevent wilting, and the whole was carried to the laboratory. Samples of green material were then prepared in duplicate by cutting the stems into sections just below each leaf axis and collecting alternate sections for samples. Three such pairs of alternate-section samples were prepared, and after weighing, each sample was cut into thin sections with scissors. One sample from each pair was extracted and the remaining three were distilled.

At the same time a set of three additional pairs was prepared in a similar manner. To three samples 0.005 gram of pure coumarin was added, and to the other three 0.01 gram was added. The first three were extracted and the last three were distilled. Samples for moisture determination were prepared at the same time. The sweet clover plants remaining were divided into two large samples. One sample was placed before an electric fan and dried for 5 days at 27° to 31° C. (room temperature). The other sample was placed in a forced draft oven and dried for 8 hours at 66° C. Coumarin was then determined in the dried material by extraction and by distillation. Samples of the oven-dried material to which 0.004 gram of pure coumarin was added were also extracted.

The average values for these coumarin determinations, calculated to a dry-weight basis, are given in Table II. The most noticeable fact is the failure to recover added pure coumarin from green sweet clover samples by extraction. By distillation the added coumarin was recovered fairly well, but, because the samples had not been ground, the recovery was somewhat variable. The values for dry sweet clover show wide differences, the extraction method giving values approximately one-half those obtained by the distillation method. Since the coumarin in extracts and distillates was determined by the same colorimetric procedure, it appears that the extraction was incomplete or that some of the coumarin was present in a form not soluble in alcohol or was destroyed in the process of extraction. That the former was not the case was shown by failure to recover an appreciable amount of coumarin from the residues by further extraction and by steam distillation. In other trials extraction gave values which were considerably lower than those obtained by steam distillation. The added pure coumarin was completely removed from the dry sweet clover samples by extraction.

TABLE III. EXTRACTION OF COUMARIN FROM GREEN SWEET CLOVER WITH AND WITHOUT ADDED PURE COUMARIN

(Second trial)

| Sample No. | Sweet Clover Alone | Sweet Clover + Added Coumarin | Recovery of Added Coumarin |
|---|---|---|---|
| | % | % | % |
| 1 | 0.64 | 0.59 | .. |
| 2 | 0.54 | 0.57 | .. |
| 3 | 0.19 | 0.18 | .. |
| Av. | 0.46 | 0.45 | None |

Recovery of added coumarin from green sweet clover by extraction was repeated on samples prepared as before. The added coumarin amounted to 0.0125 gram in each sample, which was approximately equal to that originally present in the samples as shown by the extraction procedure.

The results shown in Table III agree with those in Table II, in that the coumarin added to green samples is not accounted for when subjected to alcoholic extraction and subsequent colorimetric determination of coumarin in the extract. Just what happened to the added coumarin is not apparent. It did not remain in the samples following extraction, since further extraction of the residues gave only a very slight trace of color-producing substance when the extracts were treated with the diazotizing reagent. Likewise, steam distillation of some of the residues failed to recover any appreciable amount of coumarin. Other similar residues were washed with a little cold water to remove the adhering substances which had been extracted by the alcohol, 0.01 gram of pure coumarin was added to each residue, and the extraction procedure was repeated. The added coumarin was recovered quantitatively in each trial.

These results indicate that in the alcoholic extraction of green material something is removed which destroys, renders nonreactive, or interferes with the final color determination of the added coumarin.

In addition to errors resulting from extraction, considerable difficulty arose when the actual color comparisons were made. The sweet clover extract itself was somewhat colored, and the extracts of alfalfa and of other noncoumarin-bearing plants developed a decided reddish brown color when carried through the colorimetric procedure. When sodium carbonate was added to the sweet clover extract a pronounced yellow color was produced, and on heating, a cloudiness due to the precipitation of impurities resulted. Finally, because of off colors produced by impurities in both the standards and the unknowns, accurate color comparisons in a colorimeter were difficult if not impossible. For these reasons it appears that only approximate values may be obtained by the extraction method.

Repeated trials have shown that coumarin is removed quantitatively from sweet clover by the steam-distillation method. The distillates are clear and apparently contain no substances which interfere with the colorimetric estimation of the coumarin present.

## Behavior of Melilotic and *o*-Coumaric Acids in the Distillation Process

Since coumarin and melilotic acid are thought to occur together in sweet clover (*2*), and since *o*-coumaric acid also may be closely associated with coumarin in the plant, it seemed desirable to investigate the behavior of these substances when subjected to the steam-distillation process. Either substance would interfere with the colorimetric determination of coumarin in the sweet clover distillates, since both react with the diazonium reagent and produce a red color similar to that given by coumarin. *o*-Coumaric acid was prepared by the method of Fittig and Ebert (*5*), and melilotic acid was prepared according to the method of Tieman and Herzfeld (*9*). After several recrystallizations each was obtained in pure form. When a given weight of melilotic or *o*-coumaric acid was treated with the diazonium solution, a color was produced similar to that given by coumarin and found to be 92.8 and 79.4 per cent as intense, respectively, as that produced by an equal concentration of pure coumarin.

In order to determine the extent to which melilotic acid and *o*-coumaric acid are carried over into the distillates, 0.025-gram samples of each in 100 ml. of distilled water were subjected to four distillations by the steam-distillation procedure. The distillates from the *o*-coumaric acid gave no color test, showing that it is not volatile with steam under the conditions of the method. The melilotic acid distillates gave an average percentage recovery of 22.9, 17.7, 13.2, and 8.5 for the first, second, third, and fourth distillations, respectively. Pure coumarin in solution was recovered quantitatively by one distillation. Even though melilotic acid is distilled over appreciably from pure aqueous solution, when added to 5-gram samples of alfalfa, only a very small amount (2 to 3 per cent) was distilled over in four distillations. Distillates from the alfalfa samples alone gave negative color tests.

TABLE IV. DETERMINATION OF COUMARIN

(In dry samples of sweet clover in the presence of sodium acetate with and without added melilotic acid and *o*-coumaric acid)

| Sample No. | Substance Added | Coumarin |
|---|---|---|
| | | % |
| 1 | None | 0.72 |
| 2 | None | 0.72 |
| 3 | None | 0.68 |
| 4 | None | 0.70 |
| 5 | None | 0.70 |
| | Av. | 0.70 |
| 6 | Melilotic acid, 0.025 gram | 0.70 |
| 7 | Melilotic acid, 0.025 gram | 0.69 |
| 8 | Melilotic acid, 0.025 gram | 0.69 |
| | Av. | 0.69 |
| 9 | Melilotic and *o*-coumaric acids, 0.025 gram of each | 0.69 |
| 10 | Melilotic and *o*-coumaric acids, 0.025 gram of each | 0.68 |
| | Av. | 0.69 |

TABLE V. COMPARISON OF ROBERTS' MODIFICATION AND STEAM-DISTILLATION METHODS

| Sample No. | Coumarin by Roberts' Modification | Coumarin by Steam Distillation[a] |
|---|---|---|
| | % | % |
| 1 | 1.07 | 1.10 |
| 2 | 1.13 | 1.04 |
| 3 | 0.97 | 1.10 |
| 4 | 1.14 | 1.01 |
| 5 | 1.68 | 2.59 |
| 6 | 0.99 | 0.90 |
| 7 | 0.98 | 0.88 |
| 8 | 1.35 | 1.90 |
| 9 | 1.34 | 1.99 |
| 10 | 1.41 | 2.05 |
| 11 | 1.61 | 2.24 |
| 12 | 2.36 | 2.27 |
| 13 | 1.76 | 2.25 |
| Av. | 1.37 | 1.64 |

[a] Distillation samples also ground in mortar with sand.

In an effort to prevent entirely the transfer of melilotic acid without interference with the removal of coumarin, a series of trials was made in which 0.025-gram quantities of melilotic acid, or 0.025 gram each of melilotic acid and coumarin, were placed in the distillation flasks, 0.5 to 1 gram of a suitable salt and 100 ml. of distilled water were added, and the contents were distilled to dryness. The distillates were diluted to 250-ml. volumes and colorimetric determinations were made on 25-ml. aliquots. One-half gram of sodium acetate gave the desired result: The distillates from melilotic acid showed only a very slight trace of that substance present, while the distillates from the melilotic acid–coumarin mixture gave complete recovery of coumarin.

Similar trials on the removal of melilotic acid and coumarin from 5-gram samples of alfalfa by four distillations gave analogous results. The sodium acetate prevented the transfer of melilotic acid but did not hinder the passage of coumarin. Finally, trials were made on the distillation of sweet clover samples, with and without addition of melilotic and *o*-coumaric acids, to which 0.5 gram of sodium acetate was added. Five-gram samples were used and coumarin was determined in 20-ml. aliquots from 1000-ml. volumes after four distillations. The results of these trials are given in Table IV. Samples 1 and 2 were each subjected to a fifth and sixth distillation without increasing the quantity of coumarin found.

It was concluded that the addition of 0.5 gram of sodium acetate to the sweet clover samples in the distillation flasks would prevent melilotic acid or *o*-coumaric acid from interfering with the coumarin determination. Four distillations are required to remove the coumarin completely.

## Comparison of Roberts' Modified Extraction Method with Steam Distillation

Recently Roberts modified the extraction procedure of Stevenson and Clayton by grinding the green leaves in a mortar with sand and extracting the coumarin with 50 per cent methyl alcohol, at room temperature, while shaking for 30 minutes. Coumarin was determined in aliquot portions by comparing with standards containing pure coumarin to which an extract of alfalfa leaves was added. Comparison of this method with the steam-distillation method was made on samples of sweet clover leaves.

Table V shows considerable differences in the coumarin content as determined by the two methods. Why these differences were obtained is not apparent. Roberts' modification is a decided improvement over the original extraction method—for example, pure coumarin added to green sweet clover samples was recovered quantitatively in numerous trials. Determinations of coumarin on green material without previously grinding with sand were both low and variable. Determinations made on samples similar to Nos. 1, 2, and 3 by the distillation method without grinding gave an average value of less than half (0.38 per cent) of that reported in Table V for these samples, showing that with green material grinding is essential for accurate quantitative determinations.

After the coumarin is separated by extraction from the sweet clover samples, the same difficulty prevails with color comparisons as for the original method. Moreover, the extraction of any melilotic acid, *o*-coumaric acid, or other substance which would react with the color reagent interferes with the coumarin determination. A large number of sweet clover samples, from which the coumarin was removed completely by distillation, were extracted by Roberts' modification and the amount of color-producing substance present in the extracts was determined. These values ranged from a very slight trace to 0.32 per cent calculated as coumarin.

TABLE VI. DETERMINATION OF COUMARIN

(Percentage of total coumarin removed from dry sweet clover material by one distillation, estimated total coumarin content on the basis of one distillation, and deviation of the estimated value from the true value for 5- and 2-gram samples)

| | Five-Gram Samples | | | Two-Gram Samples | | |
|---|---|---|---|---|---|---|
| | Removed | Estimated total | Deviation | Removed | Estimated total | Deviation |
| | % | % | % | % | % | % |
| | 57.3 | 102.0 | 2.0 | 77.2 | 103.0 | 3.0 |
| | 56.4 | 100.3 | 0.3 | 74.9 | 99.8 | 0.2 |
| | 57.5 | 102.3 | 2.3 | 75.8 | 101.2 | 1.2 |
| | 54.8 | 97.5 | 2.5 | 72.3 | 96.5 | 3.5 |
| | 59.0 | 105.0 | 5.0 | 76.5 | 102.1 | 2.1 |
| | 54.0 | 96.1 | 3.9 | 77.3 | 103.2 | 3.2 |
| | 55.0 | 97.9 | 2.1 | 77.9 | 104.0 | 4.0 |
| | 57.7 | 102.7 | 2.7 | 72.7 | 97.1 | 2.9 |
| | 57.1 | 101.6 | 1.6 | 72.1 | 96.3 | 3.7 |
| | 53.9 | 95.9 | 4.1 | 74.7 | 99.7 | 0.3 |
| | 57.1 | 101.6 | 1.6 | 71.9 | 96.0 | 4.0 |
| | 57.1 | 101.6 | 1.6 | 76.0 | 101.5 | 1.5 |
| | 54.1 | 96.3 | 3.7 | 74.6 | 99.6 | 0.4 |
| | 55.0 | 97.9 | 2.1 | .. | ... | ... |
| | 56.5 | 100.6 | 0.6 | .. | ... | ... |
| | 54.6 | 97.2 | 2.2 | .. | ... | ... |
| | 53.8 | 95.7 | 4.3 | .. | ... | ... |
| | 57.6 | 102.5 | 2.5 | .. | ... | ... |
| | 59.0 | 105.0 | 5.0 | .. | ... | ... |
| | 56.8 | 101.0 | 1.0 | .. | ... | ... |
| Av. | 56.2 | | 2.6 | 74.9 | | 2.3 |

This would lead one to expect slightly higher values, on the average, by Roberts' method than by steam distillation, whereas Table V shows the opposite result.

To obtain more information as to the specific nature of the color produced by pure coumarin, sweet clover distillates, melilotic acid, *o*-coumaric acid, sweet clover extracts, and alfalfa extracts plus added coumarin when each is treated with the diazonium solution, the percentage of transmittance of light of various wave lengths by these solutions over the range at which light is transmitted (560 to 700 millimicrons) was measured with a Keuffel and Esser spectrophotometer. These measurements showed that solutions containing pure coumarin, melilotic acid, *o*-coumaric acid, and sweet clover distillates gave nearly identical transmittance curves for solutions of equal color intensity. However, when the spectrophotometric curves produced by alcoholic extracts of sweet clover were compared with those produced by alcoholic extracts of alfalfa plus pure coumarin, considerable differences in transmittance were noted in the orange and red parts of the spectrum. The transmittance values for alfalfa extract plus pure coumarin fell below those for sweet clover extract in this region. This may account for the difficulty in matching the two solutions in a colorimeter and may cause some error in the estimation of the coumarin content. Sweet clover distillates are not subject to this difficulty when compared with standards of pure coumarin.

## Short Method for Comparative Purposes

Numerous trials have shown that under the conditions described four distillations will remove all the coumarin from 5-gram samples of dry sweet clover material. If the sample is reduced, the number of distillations necessary to remove the coumarin completely is also reduced. Trials were made to determine how constant the percentage removal is by one distillation from 5-gram and from 2-gram samples. Total coumarin was determined in samples chosen from six different stock materials, one of which was a sample of leaves, ranging from 0.43 to 1.31 per cent. Two to six samples from each stock material were distilled once and the coumarin present in the distillates was determined. The average percentage removal of coumarin from the 5-gram and 2-gram samples was found to be 56.2 and 74.9, respectively.

The factors for converting these average values to 100 per cent are 1.779 and 1.336, respectively. Applying the appropriate factor to the percentage of total coumarin removed from each sample by one distillation gives as a product the estimated total coumarin content. Comparison of the values thus found with 100 per cent gives the deviation from the true value as determined by four distillations. The results of these determinations and calculations are summarized in Table VI, and show that the approximate coumarin content of sweet clover samples may be found by one distillation. If other sample weights are employed, a suitable factor for each must be determined. When permissible, this shortening of the distillation procedure speeds up the method and is sufficiently accurate for sorting sweet clover strains.

## Summary

A comparison of the alcoholic-extraction and steam-distillation methods for removing coumarin from sweet clover samples showed considerable differences in the coumarin content as determined by the two methods. The Stevenson and Clayton method of extraction failed to recover pure coumarin added to green sweet clover, and when dry samples were extracted values of less than half those given by the distillation method were obtained. Roberts' modification of the extraction method showed a decided improvement, but likewise gave lower values in most cases than did the distillation method.

Spectrophotometric curves obtained from sweet clover extract did not agree closely with those obtained from alfalfa extract plus pure coumarin in the orange and red region. This probably accounts for the difficulty encountered when these solutions were compared in a colorimeter, but this difficulty was not experienced when the sweet clover distillates were compared with pure coumarin standards.

Pure melilotic acid added to alfalfa samples and distilled gave 2 to 3 per cent recovery in four distillations. The addition of sodium acetate to the sample in the distillation flask prevented melilotic acid from distilling over, but did not reduce the rate of coumarin distillation. *o*-Coumaric acid was not volatile under the conditions of distillation.

Green samples should be ground with sand prior to distillation.

For approximation purposes, where a high degree of accuracy is not necessary, the steam-distillation method may be shortened considerably. Whereas four distillations are required to remove the coumarin completely from the sweet clover samples, one distillation removed, on the average, 56.2 per cent of the coumarin from 5-gram samples and 74.9 per cent from 2-gram samples. Multiplied by suitable factors, these values can be converted to fairly satisfactory estimates of the total coumarin content. In a number of trials this method showed an average deviation of 2.6 per cent for 5-gram samples and 2.3 per cent for 2-gram samples. Where more accurate results are desired the longer procedure should be used.

## Literature Cited

(1) Clayton, J. S., and Larmour, R. K., *Can. J. Research*, **13**, C, 89–100 (1935).
(2) Czapek, Friedrich, "Biochemie der Pflanzen," Vol. 3, p. 471, Jena, Gustav Fischer, 1922.
(3) Duncan, I. J., and Dustman, R. B., IND. ENG. CHEM., Anal. Ed., 6, 210–13 (1934).
(4) *Ibid.*, 9, 416 (1937).
(5) Fittig, R., and Ebert, G., *Ann.*, **226**, 351–4 (1884).
(6) Obermayer, E., *Z. anal. Chem.*, **52**, 172–91 (1913).
(7) Roberts, W. L., unpublished method, Dept. Agr. Chem., University of Wisconsin.
(8) Stevenson, T. M., and Clayton, J. S., *Can. J. Research*, **14**, C, 153–65 (1936).
(9) Tieman, Ferd., and Herzfeld, H., *Ber.*, **10**, 286–8 (1877).

RECEIVED July 6, 1937. Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper 190.

# Determination of Osmium in a Lead Assay Button

J. J. RUSSELL, F. E. BEAMISH, AND J. SEATH, University of Toronto, Toronto, Ontario, Canada

**A method has been devised for the determination of osmium in a lead button. The influence of each of the platinum metals, silver, and gold on the collection of osmium by fire assay is described. Evidence is submitted to show that certain native platinums, after fire-assaying and subsequent cupellation to a small lead button, will yield osmium directly from the nitric acid parting solution.**

IN SO FAR as the authors are aware, no procedure for the quantitative determination of osmium in a lead regulus has ever been recorded in the scientific literature. This lack of a recipe may result from two conceptions: (1) that osmium occurs only as the mineral iridosmine (osmiridium), which is generally given the somewhat vague definition of a solid solution of the platinum metals with predominant proportions of osmium and iridium; (2) that, during the process of fire assay with lead as collector, the original osmium alloy passes into the lead button as a mechanically mixed constituent.

In connection with statement 1, the authors have found that samples of an Abyssinian native platinum would give off traces of osmium by direct ignition in air, but on fire assay with lead and silver and subsequent cupellation no osmium whatever could be distilled from the nitric acid parting solution. Beamish and Russell (*1*) have shown that if a platinum metals–lead button has been salted with osmium, all this metal is not given off when the resulting bead is parted, but that an appreciable proportion of it will appear after the final residue is fused with sodium peroxide and the fused mass treated with nitric acid. And so it may be true that even more osmium might be evolved from the parting acid in the cases stated in this report, were it not for the restraining effect of certain other platinum metals brought on by the fire treatment with lead.

Concerning statement 2, apparently no suggestion has been recorded of the possibility that some of the natural alloys of platinum, in which osmium is present, may give off this metal as a result of fire treatment with lead and subsequent parting with nitric acid.

The following is a report of a successful attempt to devise a method for the determination of total osmium in a lead button. In all cases of fire assay the temperature of the furnace pot was 1200° ± 50° C. The cupellation temperature in all cases was 950° ± 25° C.

## Determination in Nitric Acid Solutions

Ammonium bromosmate was prepared and analyzed by burning under hydrogen. Samples of 33.89 and 28.31 mg. were found to contain 27.00 and 27.01 per cent of osmium, the calculated percentage being 27.01.

The first method employed for osmium determination was nitric acid distillation into sulfur dioxide–hydrochloric acid solution.

Because small amounts of osmium were to be determined, it was decided to reduce the distilling apparatus to about one-tenth the size of that described by Gilchrist (*2*). The first connecting tube was lengthened, however, to avoid collection of too much nitric acid in the distillate.

A sample weighing 22.30 mg. of the pure ammonium bromosmate (6.024 mg. of osmium) was dissolved in 15 cc. of water and 7 cc. of 6 *N* nitric acid were added. The first collection flask contained 15 cc., the second and third 10 cc., of hydrochloric acid freshly saturated with sulfur dioxide. Air was passed through the solution at a rate just sufficient to prevent bumping. The osmium solution was boiled for 40 minutes, after which time no osmium remained in the distilling liquid. The total volume of 35 cc. of sulfur dioxide absorbing solution was evaporated over steam. With the addition of hydrochloric acid to decompose sulfites, the thick dark-brown sirup became yellow and yielded 3.454 mg. of osmium. These facts are significant because Gilchrist (*2*) was successful in collecting weights of osmium twenty times as large, in volumes of sulfur dioxide–acid solution ten times as great as those used by the authors.

An unsuccessful attempt was made to determine osmium which had been distilled into 10 per cent sodium hydroxide solution.

The authors then decided to distill the milligram samples of osmium into large volumes of absorbing solution. The first receiving flask contained 150 cc. of this solution, the second and third 50 cc. each. The apparatus used was a standard distilling equipment sold by the Scientific Glass Apparatus Co., Bloomfield, N. J.

Samples of 0.513 and 23.38 mg. of ammonium bromosmate were treated with nitric acid and distilled. The osmium was determined according to the procedure described below, which is identical with that used for all osmium determinations recorded in this report.

The 250 cc. of sulfur dioxide–acid solutions were evaporated over steam to a sirup. The sulfites were removed by three additions of 12 *N* hydrochloric acid solution. The liquid was then diluted to 75 cc. with water and filtered by means of a filtering crucible. The 125 cc. of liquid were brought to pH 4 by means of a filtered 10 per cent sodium bicarbonate solution with bromophenol as indicator and boiled to precipitate and coagulate the black osmium dioxide. The precipitate was filtered through an A2 grade filtering crucible and washed with 100 cc. of a 1 per cent aqueous solution of ammonium chloride. Then about 50 mg. of wet solid ammonium chloride were added to prevent decrepitation. The ammonium chloride was slowly removed in an atmosphere of hydrogen and heating was continued in this atmosphere for 20 minutes. The crucible was cooled for 20 minutes in carbon dioxide and the residue weighed as metal. The results obtained were 0.14 and 6.37 mg. of osmium, respectively. By calculation the osmium content is 0.14 and 6.32 mg.

## Determination in Partly Cupeled Lead Buttons

As mentioned above, there was a loss of osmium when Abyssinian native platinum was fire-assayed and subsequently cupeled to the silver bead. Samples of this material were assayed in the regular manner. Cupellation was stopped when the lead buttons weighed between 1 and 2 grams. The lead regulus was placed in the distilling flask, treated with 6 *N* nitric acid solution, and the distillate was collected in 250 cc. of sulfur dioxide–acid solution. This solution yielded a very considerable quantity of osmium.

It was then decided to determine the efficiency of lead as a collector in the fire assay for osmium. Blank ores were salted with spectroscopically pure osmium and ammonium bromosmate, then fluxed and cupeled to smaller buttons. The osmium content was determined as above described and the results obtained are given in Table I.

In each case the residue, if any, remaining in the distilling flask was filtered out by means of a porous-bottomed filtering crucible. The crucible was then placed in a silica tube 2.5 cm. (1 inch) in diameter, the end of which was drawn down to a narrow outlet immersed in 3 to 4 cc. of a 10 per cent sodium hydroxide solution. Oxygen was passed through the heated tube and any

TABLE I. DETERMINATION OF OSMIUM

| Ammonium Bromosmate Added | Osmium in Bromosmate | Osmium Metal Added | Initial Weight of Button | Final Weight of Button | Osmium Recovered | Error |
|---|---|---|---|---|---|---|
| *Mg.* | *Mg.* | *Mg.* | *Grams* | *Grams* | *Mg.* | *Mg.* |
| 1.394 | 0.377 | | 24.6 | 2.5 | 0.379 | + 0.002 |
| 2.348 | 0.634 | | 25.1 | 0.8 | 0.648 | + 0.014 |
| 10.22 | 2.760 | | 24.5 | 0.6 | 2.683 | − 0.077 |
| 98.12 | 26.50 | | 23.9 | 2.6 | 24.08 | − 2.42 |
| 146.6 | 39.59 | | 24.6 | 2.6 | 29.17 | −10.42 |
| 225.0 | 60.77 | | 23.8 | 2.5 | 53.94 | − 6.83 |
| | | 0.397 | 23.5 | 2.4 | 0.399 | + 0.002 |
| | | 5.534 | 24.0 | 2.6 | 5.559 | + 0.025 |
| | | 29.75 | 24.0 | 3.4 | 20.83 | − 8.92 |

osmium tetroxide formed was collected in the caustic solution which was then transferred to the distilling flask. The residue remaining after the oxidation was scraped out into a silver crucible, 3 grams of sodium peroxide were added, the mixture was melted, and the flux was maintained at a dull red heat for 10 minutes. The fused mass was then washed into the distilling flask along with the above caustic solution, treated with nitric acid, and any osmium tetroxide present was distilled into the original absorbing solution. The results in Table I indicate considerable loss of osmium when 25 mg. of metal were added to the flux.

## Determination in Large Buttons

A 28.5-gram button was obtained from flux salted with 101.2 mg. of ammonium bromosmate (27.33 mg. of osmium). This button was treated directly as above described, except that instead of the usual 20 cc. of 6 *N* nitric acid solution, 70 cc. were required to dissolve the lead. When the evaporated sirup was treated with 12 *N* hydrochloric acid the deep brown color became a light yellow, indicating possible loss of osmium. The osmium recovered weighed 18.96 mg.

A second sample was salted with 198.2 mg. of ammonium bromosmate containing 53.53 mg. of osmium. The lead in this case was allowed to cool in contact with the slag and the button weighing 28.5 grams was removed by breaking the pot. The absorbing solution was treated as above described and the osmium recovered weighed 32.46 mg.

A 30-gram button salted with 55.14 mg. of osmium was placed in the distilling flask and treated directly with 70 cc. of nitric acid and the evaporated absorbing solution was returned to the distilling flask. When 10 cc. of 12 *N* hydrochloric acid were added and the solution was warmed, osmium tetroxide was distilled into the absorbing solution. The osmium recovered weighed 20.99 mg. These results prove, that under the conditions described above, osmium is lost during the evaporation.

A third sample was salted with 4.917 mg. of osmium. The lead button weighing 25 grams was treated directly with nitric acid and the total osmium recovered weighed 2.424 mg. Therefore, a large button must be reduced in size, as indicated in Table I, before treatment to recover the osmium.

## Fusion of Osmium with Sodium Peroxide

In order to determine whether any osmium was lost during the process of sodium peroxide fusion, 4.926 mg. of osmium were placed on the bottom of the silver crucible and covered with 3 grams of sodium peroxide. The peroxide was slowly melted and then maintained at a dull red heat for 10 minutes. The cooled melt was washed into the distilling flask and the tetroxide distilled into 6 *N* hydrochloric acid saturated with sulfur dioxide. The osmium recovered weighed 4.734 mg. A second sample of 0.321 mg. similarly treated yielded 0.325 mg. of osmium.

## Determination of Total Osmium in Beads Containing Platinum Metals

A sample of blank ore and a suitable flux were salted with 16.77 mg. of ammonium bromosmate (4.531 mg. of osmium), 83 mg. of platinum, 0.8 mg. of iridium, 0.8 mg. of rhodium, 0.5 mg. of palladium, and 0.5 mg. of gold. The lead button of 25 grams was cupeled to about 3 grams. On distillation the lead nitrate solution yielded 1.402 mg. of osmium. The residue was fused with sodium peroxide as described above and yielded 3.061 mg. of osmium—a total osmium recovery of 4.463 mg. and a loss of 0.068 mg.

A second sample, prepared as described above, contained 17.69 mg. of ammonium bromosmate (4.781 mg. of osmium), 57.5 mg. of palladium, 17.5 mg. of gold, 4.3 mg. of iridium, 4.5 mg. of rhodium, and 4.2 mg. of ruthenium. The button was cupeled to about 3 grams. The osmium recovered from the parting acid weighed 0.230 mg., and the peroxide fusion yielded 4.464 mg.—a total recovery of 4.694 mg. and a loss of 0.087 mg.

## Retention of Osmium by Silver, Gold, and the Remaining Platinum Metals

It has been shown by Beamish and Russell (*1*) that lead buttons containing platinum metals and silver, salted with osmium, produce silver beads which on parting do not yield all of their osmium content, and that much of this metal is retained until treatment of the final fusion.

The experiments described above also indicate that certain, if not all of the platinum metals and perhaps silver and gold, somehow protect the osmium from attack by nitric acid. It was decided to determine which of the metals exerted this pronounced effect.

SILVER-OSMIUM. The button seemed to cupel in a normal manner until practically all the lead was removed. At the finishing temperature the silver bead broke into a fine spray of metallic globules and rapidly decreased in size. A lead button containing 500 mg. of silver and 25 mg. of osmium was cupeled and the cupel removed 30 seconds after the blick. The bead then weighed 175 mg. and the cupel, examined under the microscope, was found to be covered with innumerable silvery globules. The bead was placed in the distilling flask and dissolved in nitric acid. The solution was boiled and the distillate collected in 3 cc. of 10 per cent sodium hydroxide solution. There was no residue in the flask and no osmium could be found in the caustic solution by treatment with thiourea, which is sensitive to 0.01 mg. of osmium per cc. A number of buttons prepared in this manner produced identical results.

SILVER-GOLD-OSMIUM. A 30-gram lead button was made up to contain 360 mg. of silver, 10 mg. of osmium, and 20 mg. of gold. The button was cupeled and the bead removed 30 seconds after the blick and weighed 181 mg. At the finishing temperature the bead gave off a spray of metal, but this effect was not so pronounced as with the silver-osmium beads.

SILVER-PLATINUM-OSMIUM. A 30-gram lead button containing 360 mg. of silver, 10 mg. of osmium, and 20 mg. of platinum when treated as described above yielded a bead weighing 9 mg. The cupel depression was completely covered with minute globules of metal.

SILVER-PALLADIUM-OSMIUM. A 30-gram lead button containing 360 mg. of silver, 10 mg. of osmium, and 20 mg. of palladium, treated exactly as described for silver-gold-osmium, yielded a bead weighing 183 mg. The cupel was thickly covered with minute beads.

SILVER-RUTHENIUM-OSMIUM. A 30-gram lead button containing 360 mg. of silver, 10 mg. of osmium, and 20 mg. of ruthenium produced a bead which was coherent, black in color, and did not blick. The bead was somewhat flattened.

SILVER-IRIDIUM-OSMIUM. A 30-gram lead button containing 360 mg. of silver, 10 mg. of osmium, and 20 mg. of iridium cupeled to a residue which was spread out in a flat irregular black mass.

SILVER-RHODIUM-OSMIUM. A 30-gram lead button containing 360 mg. of silver, 10 mg. of osmium, and 20 mg. of rhodium produced a bead which was coherent, somewhat round, with a rough black surface. It was characterized by a deep indentation and there was a rather indefinite blick.

The quantitative results obtained from the above experiments are given in Table II.

In the cases of 8, 9, 10, 11, and 12 the distillate was collected in 3 cc. of a 10 per cent sodium hydroxide solution. A drop of this solution was put on a spot plate and a drop of hydrochloric acid saturated with sulfur dioxide added. This gave no pink color on standing when a drop of 9 per cent aqueous solution of thiourea was added, indicating absence of osmium.

## Assay of Native Platinum

A lead button obtained from a blank ore and flux, salted with 263.5 mg. of Abyssinian native platinum and 3 grams of silver, cupeled to the silver bead, yielded no osmium whatever in the distillate from the nitric acid parting solution.

A second sample, salted with 256.6 mg. of this native platinum and containing no silver, was reduced in weight by

TABLE II. RETENTION OF OSMIUM

| No. | Osmium | Ruthenium | Rhodium | Iridium | Silver | Initial Weight of Button | Osmium Recovered | Loss of Osmium |
|---|---|---|---|---|---|---|---|---|
| | *Mg.* | *Mg.* | *Mg.* | *Mg.* | *Mg.* | *Grams* | *Mg.* | *Mg.* |
| 1 | 6.178 | .. | 5.445 | .. | 150 | 24 | 1.310 | 4.868 |
| 2 | 6.155 | .. | 26.53 | .. | 450 | 24 | 0.666 | 5.489 |
| 3 | 27.56 | .. | 5.569 | .. | 450 | 24 | 0.300 | 27.26 |
| 4 | 5.513 | 6.113 | .. | .. | 150 | 25 | 0.950 | 4.563 |
| 5 | 5.311 | 26.13 | .. | .. | 450 | 25 | 0.246 | 5.065 |
| 6 | 6.318 | .. | .. | 26.27 | 450 | 24 | 1.293 | 5.025 |
| 7 | 26.33 | .. | .. | 5.146 | 450 | 25 | 2.487 | 23.84 |
| | | Platinum | Palladium | Gold | | | | |
| 8 | 25.0 | .. | .. | .. | 500 | 25 | None | |
| 9 | 10.0 | .. | .. | .. | 20 | 25 | None | |
| 10 | 10.0 | 20.0 | .. | .. | 360 | 25 | None | |
| 11 | 10.0 | .. | 20.0 | .. | 360 | 25 | None | |
| 12 | 10.0 | .. | .. | 20.0 | 360 | 25 | None | |

cupellation to a 3-gram lead button, and 0.629 mg. of osmium was distilled from the parting acid.

A 150.0-mg. sample of British Columbian native platinum, after fire assay and subsequent reduction of the lead button to about 3 grams, produced 0.325 mg. of osmium by distillation from the nitric acid parting solution.

## Summary

Osmium is not collected in a fire-assay bead by silver, silver-platinum, silver-palladium, or silver-gold.

The presence of ruthenium, iridium, or rhodium in a silver bead causes the retention of small amounts of osmium.

Collection by lead may be used for the quantitative determination of osmium, provided the weight of sample to be analyzed is adjusted to contain about 5 mg. or less of osmium.

Sulfur dioxide–hydrochloric acid solution is not a good reagent for the collection of osmium for large lead buttons.

Small amounts of osmium can be fused in sodium peroxide and the fused mass heated with nitric acid and distilled without loss of the metal.

Osmium can be distilled from the nitric acid solution which was used to part lead buttons obtained by fire assay of certain native platinums.

## Literature Cited

(1) Beamish, F. E., and Russell, J. J., IND. ENG. CHEM., Anal. Ed., 8, 141 (1936).
(2) Gilchrist, R., *Bur. Standards J. Research*, 6, 421 (1931).

RECEIVED June 29, 1937.

# Determination of Mercurous Chloride and Total Mercury in Mercury Ores

JOSEPH J. FAHEY, Geological Survey, Washington, D. C.

IN THE analysis of mercury-bearing rocks and mercury ores, the determination of total mercury is usually all that is required. This can be done by any of several well-known methods, in one of which (*2*) the mercury is vaporized by heating the fluxed sample and amalgamated with a previously weighed gold plate or foil.

In a few places, especially in Texas, calomel and oxychlorides of mercury are found associated with mercury ore. No method for the quantitative determination of calomel in rocks was found in the literature. The procedure described here combines a modified form of the Eschka method (*2*) for total mercury with one that permits a quantitative measurement of calomel associated with the mercury ore. This is done by fixing the chlorine of the sublimed mercurous chloride as sodium chloride, determining it with silver nitrate, and computing the percentage of the calomel. The reaction may be represented by the following composite equation:

$$4HgCl + 2Na_2CO_3 \xrightarrow{650^\circ\ C.} 4NaCl + 4Hg + 2CO_2 + O_2$$

The mercurous chloride is sublimed in a current of air and decomposed by a layer of hot sodium carbonate. The mercury passes over it and is collected as amalgam on a gold coil; the chlorine is retained by the sodium carbonate. Any sulfur present as cinnabar is oxidized and does not interfere with the other determinations.

## Procedure

The material to be analyzed is ground to pass a 60-mesh sieve and air-dried, and a sample is weighed, the quantity depending on the apparent richness of the ore. A Pyrex tube open at both ends and about 50 cm. long, having an inside diameter of approximately 0.8 cm., is charged first with the sample and then with dry granulated sodium carbonate sufficient to fill about 2.5 cm. of the tube, the two being separated by a wad of asbestos and each terminated by a similar asbestos plug. The sample and the sodium carbonate do not touch each other.

A tightly rolled gold sheet 6 or 7 cm. long is inserted into the end of the tube next to the sodium carbonate. The diameter of this roll is such that it will just go in without binding. The other end of the tube is connected to an air line, and air, dried by sulfuric acid, is passed first through the sample, then through the sodium carbonate, and finally over the gold coil.

The tube is placed in a horizontal electric furnace 15 to 25 cm. long in such a way that the end containing the gold protrudes about 15 cm. The temperature at the position of the sample in the furnace is determined by a thermocouple placed at that point against the outside of the tube.

Air is passed through the tube at the rate of 4 to 6 bubbles per second, noted in the sulfuric acid drying flask, and the temperature in the furnace is raised to 650° C. and held constant for 5 minutes. The total time required for heating is from 30 to 45 minutes.

The mercury minerals volatilize and passing into the sodium carbonate are broken down and the mercury condenses on the cool wall of the tube outside the furnace. By means of a gently applied Bunsen flame the mercury is forced along the tube and amalgamates with the gold coil. An intermediate product, mercuric oxide (*1*), which forms as a red coating on the grains of sodium carbonate and decomposes to mercury and oxygen at 630° C., makes it necessary to raise the temperature to a point above this figure. Dry air is allowed to continue passing through the tube until any moisture that has condensed on the gold coil, resulting from the heating of the ore, is swept out of the system.

After cooling, the gold coil containing the mercury is removed from the tube and weighed. It is then heated under a hood, at first gently and finally by the full heat of the Bunsen flame, to drive off the mercury, allowed to cool, and weighed again. The difference between these two weights represents the total mercury content of the sample. The weight of the gold coil before

TABLE I. DETERMINATION OF MERCUROUS CHLORIDE AND MERCURY

| HgCl Taken | HgCl Found | Error | Hg Equivalent of HgCl Taken | Hg Found | Error |
|---|---|---|---|---|---|
| *Mg.* | *Mg.* | *Mg.* | *Mg.* | *Mg.* | *Mg.* |
| 50.0 | 49.6 | −0.4 | 42.5 | 42.3 | −0.2 |
| 10.0 | 9.7 | −0.3 | 8.5 | 8.3 | −0.2 |
| 1.0 | 1.2 | +0.2 | 0.8 | 0.8 | 0.0 |

TABLE II. ANALYSES OF MERCURY ORES

| Location | Total Hg | HgCl |
|---|---|---|
| | % | % |
| Terlingua, Tex. | 60.28 | 0.21 |
| | 0.20 | 0.02 |
| Pike County, Ark. | 11.13 | 0.13 |
| | 0.90 | None |
| | 1.83 | None |
| | 0.14 | 0.03 |

it was inserted into the tube serves as a check on the final figure obtained after expulsion of the mercury.

The tube when cool is broken at the asbestos wad between the sample and the sodium carbonate. The latter is transferred to a 250-ml. beaker, put in solution in about 50 ml. of water, and made acid to litmus with nitric acid. After filtering to remove any asbestos, the chlorine is precipitated with silver nitrate and the resulting silver chloride is filtered and weighed. The quantity of mercurous chloride in the sample is computed from this figure. If the silver chloride precipitate is very small it may be necessary to arrive at its estimation by the nephelometer. The need of a blank correction for chlorine in the sodium carbonate depends on the purity of the reagent used.

The method gives excellent results on samples containing up to 50 mg. of mercurous chloride. None of the minerals found in mercury ores, including halite and sylvite, interferes with the determination. The rare mercury minerals, terlinguaite and eglestonite, if present, would yield chlorine and be reported along with calomel as mercurous chloride.

Table I contains results of analyses of samples of pure mercurous chloride.

Table II is made up of analytical data on mercury-bearing rocks from Terlingua, Tex., and Pike County, Ark. The samples are not representative of ore bodies at these localities. The quantity of calomel found in these particular samples was small and was not visible under a hand lens. Calomel has been reported from Terlingua, however, in crystals up to 1 cm. in length, and is generally present in the earthy matrix of veins carrying the rarer mercury minerals (*3*).

## Summary

A method for the determination of mercurous chloride and total mercury on the same sample is described.

The mercury minerals are volatilized in a glass tube and brought into intimate contact with granulated sodium carbonate. The chlorine is fixed as sodium chloride, determined with silver nitrate, and computed to mercurous chloride. The mercury is collected on a previously weighed gold coil and weighed.

## Literature Cited

(1) Echols, W. H., *Chem. News*, 44, 189 (1881).
(2) Eschka, *Z. anal. Chem.*, 11, 344 (1872).
(3) Hillebrand, W. F., and Schaller, W. T., U. S. Geol. Survey, *Bull.* 405, 9, 159 (1909).

RECEIVED June 8, 1937. Published by permission of the Director, U. S. Geological Survey.

# A Simple Fume Absorber for Kjeldahl Digestions

HAROLD G. CASSIDY, Oberlin College, Oberlin, Ohio

IN THE Kjeldahl digestion the unpleasant and corrosive fumes which are evolved, sometimes in copious amounts, are a source of inconvenience. Many devices for disposing of them have been developed. A common practice is to carry out the digestion in a hood, or to insert the neck of the digestion flask in a pipe leading either to an exhaust fan or to a water pump. Henwood and Garey (*2*) have described a flanged, porous alundum tube, closed at the bottom, which prevents the escape of the sulfuric acid mist yet allows the free exit of steam, sulfur dioxide, and carbon dioxide formed in the process of digestion. This tube is fastened in the open end of the digestion flask (*1*). Under these conditions the digestion may be performed on the laboratory table.

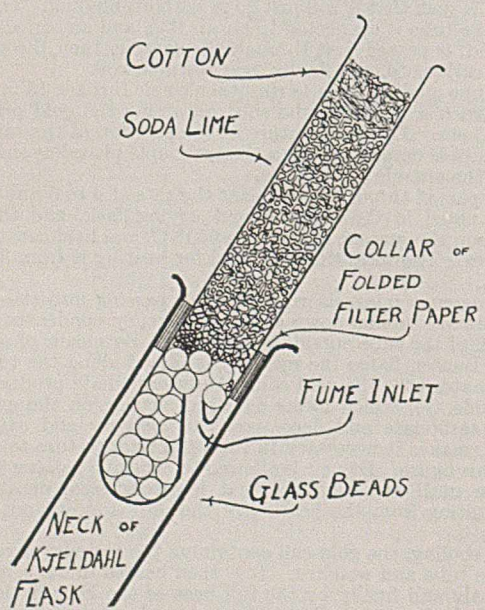



FIGURE 1. FUME ABSORBER IN POSITION IN NECK OF KJELDAHL FLASK

The device described below is simple and inexpensive, can be made from materials found in the laboratory, and is useful where ordinary types of fume-disposal equipment are not available. It absorbs, completely in most cases, both sulfur dioxide and the sulfuric acid mist. The absorption tube is the most satisfactory of many which have been designed, made, and tested. It may be modified for use in other reactions which give off noxious gases—for example, organic reactions in which hydrogen chloride is evolved.

## The Absorption Tube

A test tube (preferably, though not necessarily, of Pyrex) is chosen of the largest size which easily slips into the neck of the Kjeldahl flask to be used. The tube is heated at a point somewhat above the rounded end and the wall is pushed inwards and upwards to form an invagination, or deep depression. This is most easily done with a stout wire flattened a little at the end. The wire is held in the depression until the glass sets, then it is given a sharp twist: The flattened portion acts as a lever and shatters the glass at the upper point of the invagination, leaving a hole

in the position shown (fume inlet). The tube is ready for use as soon as it has cooled.

The tube is first filled with glass beads or glass wool to a point somewhat above the fume inlet. The glass beads or wool support a column of loosely packed soda lime or other suitable absorbent. A wad of cotton may be placed above this.

The filled tube is fitted tightly into the neck of the Kjeldahl flask by means of a collar of folded filter paper, as suggested by Henwood and Garey (*1*). The tube must not project down too far into the neck of the flask; otherwise spray may collect on it. Before beginning the digestion, the cotton wad is removed and the soda lime moistened with a little distilled water. The wad may then be replaced.

The charge in one tube may last for several digestions. The filling and fitting of the tube require only a few minutes.

This type of absorber has been used with Kjeldahl flasks of 100- to 500-cc. capacity and with a variety of digestions, including feeds, sugars, and pure organic compounds. It has shown itself satisfactory, enabling the digestions to be carried out in the open room without inconvenience. Occasionally a small amount of sulfuric acid mist has escaped from the tube at the beginning of a digestion, but this has always ceased after a few minutes.

Because of the shape of the opening, any spray which spatters up onto the bottom of the tube may readily be washed off the rounded end with the stream from a wash bottle, and any small condensate which forms inside the tube will collect safely inside the rounded end.


### Summary

A test tube, perforated near the bottom, is filled with moist soda lime or other suitable absorbent, the perforation being first shielded with glass beads or glass wool, and is then tightly fastened into the neck of the Kjeldahl flask by means of a collar of folded filter paper. This device absorbs and prevents the escape of practically all irritating fumes during the digestion, and thus permits it to be carried out in the open room without inconvenience. It may be modified for use in other reactions which evolve unpleasant fumes.


### Literature Cited


(1) Henwood, A., and Garey, R. M., *J. Franklin Inst.*, **221**, 531–8 (1936).

(2) Henwood, A., and Garey, R. M., *Science*, 76, 524 (1932).


RECEIVED July 9, 1937.

# A Self-Filling Pycnometer


G. F. HENNION

University of Notre Dame, Notre Dame, Ind.


THE pipet type of pycnometer is widely used for the determination of densities, particularly of organic liquids, but has the serious inconvenience that filling requires suction. As this is frequently accomplished orally, obnoxious vapors may be drawn into the mouth. Precise adjustment of the liquid volume is also frequently troublesome.

By making a few minor changes in construction, pycnometers have been made in this laboratory which are entirely self-filling and extremely easy to adjust. Figure 1 illustrates one of these. The dimensions are somewhat variable except for the capillary tip, *A*, and tube *D*. The pycnometer is used as follows:

Hold with thumb and second finger at point *F*, place index finger over opening *G*, and dip capillary tip *A* beneath the surface of the liquid whose density is to be determined, contained in a small crucible or beaker. Remove pressure at *G*. The pycnometer fills automatically, owing to capillary rise of liquid at *A* and overflow at *B*. When the liquid reaches the calibration mark, apply pressure at *G* and withdraw *A* from the liquid. Wipe the capillary tip, rock the pycnometer gently to withdraw the liquid from *AB*, suspend in a balance, and weigh.

If desired, the pycnometer may be filled beyond the calibration mark and suspended in a constant-temperature bath maintained at the desired temperature. Excess liquid may be subsequently withdrawn at *A* in the usual manner.

The capillary section at *AB* must extend beyond both sides of bend *B*. Too fine a capillary causes very slow filling. A 2-ml. pycnometer used by the author fills with water in 18 seconds at 25° C. If tube *D* has an internal diameter greater than 2 mm. the descending column of liquid tends to break, causing air bubbles. Naturally the pycnometer must be cleaned and dried before use. The presence of a small slug of liquid at *E* prevents liquid rise at *A*.

A number of these pycnometers have been constructed by the author, with volumes varying from 0.75 to 10 ml., and have been used with organic liquids of various densities and surface tensions. The pycnometers are readily constructed from selected pipets and are calibrated without difficulty in the usual manner. A fine wire, connected at *C* and *F*, serves for suspending the pycnometer in the balance.

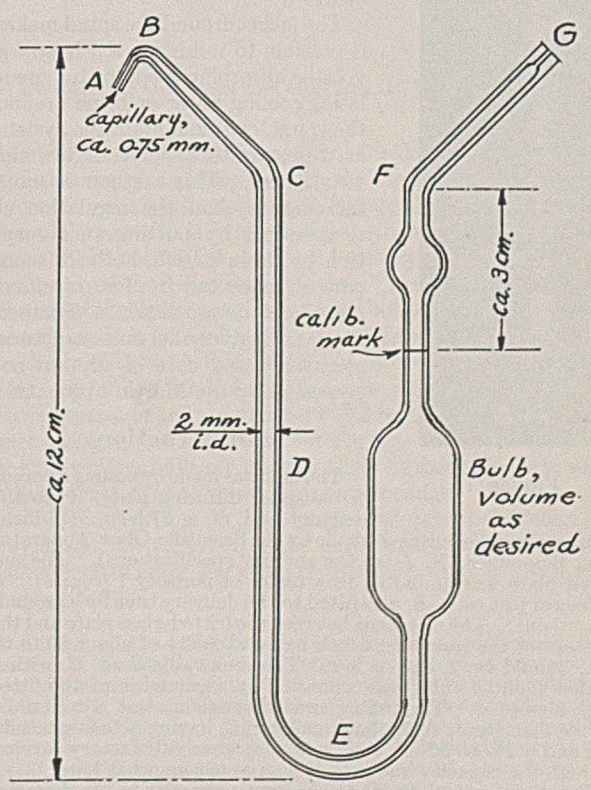



FIGURE 1. SELF-FILLING PYCNOMETER

RECEIVED August 13, 1937.

# A Laboratory Gas-Washing or Absorption Unit

M. W. BRENNER AND G. L. POLAND
Research Laboratories, United Fruit Company, New York, N. Y.

THE absorbing unit described herein was developed in this laboratory for complete absorption of various gases or vapors by volatile solvents. The literature contains many references to gas-absorption vessels, such as those described by Milligan (*4*), Martin (*2*), Shaw (*5*), and Martin and Green (*3*), which seem to be satisfactory for general laboratory use. However, it is believed that the apparatus illustrated in this article has certain advantageous features not found in the units mentioned above.

This apparatus, Figures 1 and 2, about 45 cm. (18 inches) high from the fritted-glass diffusing plate to the top of the spiral, gives a path of travel of about 225 to 250 cm. (90 to 100 inches), compared with approximately 175 cm. (70 inches) in the Martin unit, 100 to 112 cm. (40 to 45 inches) in Milligan gas-washing bottles, and considerably less in other types. The side leakage which is found in Milligan bottles, particularly when the spiral and sleeve do not fit perfectly, and the channeling characteristic of packed bead columns, especially at low rates of gas flow, are avoided in this equipment.

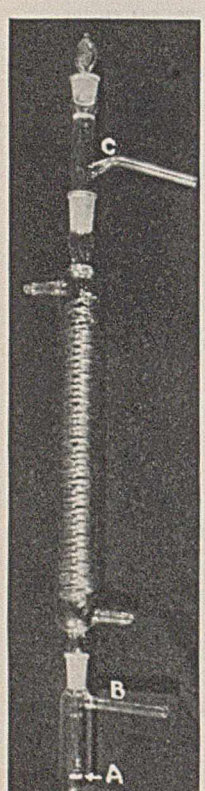



FIGURE 1

The jacket around the spiral makes it possible to minimize the loss of a volatile absorbing medium by circulating cooling water or brine around the spiral, or to maintain the system at a temperature above that of the atmosphere. This method is more satisfactory than the regulation of temperature by putting an absorption bottle in a bath, both for economy of space and freedom of movement and manipulation. Further, the jacket gives the unit structural stability and ease of support not possessed by the Martin apparatus.

## Construction

The unit was made by sealing a porous fritted-glass diffusing plate, *A*, to the delivery end of a 37.5-cm. (15-inch) Graham spiral condenser (made by the Scientific Glass Apparatus Co., Bloomfield, N. J., to the authors' specifications). The sintered plate was similar to Jena plates of porosity 1 (coarse). A side-arm test tube, *B*, was fitted to the delivery tube by a ground-glass joint. The clearance between the fritted-glass plate and the bottom of the reservoir, which had a capacity of about 50 to 60 ml., should be 1 cm. or less. The removable head, *C*, with a sealed-in outlet tube, was connected by a ground joint and fitted with a stopper. The tubing used throughout was 8 to 9 mm., inside diameter. A similar, smaller unit, having 5- to 6-mm. tubing and a 25- to 30-ml. reservoir, also gave satisfactory service, though the capacity and rate of flow were somewhat lower.

It is necessary to match the reservoir capacity to the diameter of tubing used. The volume of liquid between points *A* and *B* should be almost sufficient to fill the internal spiral. In this way, by varying the height to which the reservoir is filled, before the passage of the gas to be washed, it is possible to regulate the height to which the liquid will be forced. Thus the effective path of the gas through the absorbing medium may be set anywhere between 12.5 and 250 cm. (5 and 100 inches).

## Operation

Sufficient solvent is added to fill the reservoir at the bottom to just below the side-arm inlet tube, *B*. Suction is applied to the outlet tube, *C*, in the head (or positive pressure on the inlet side). The liquid is forced up into the spiral, and the gas, broken into small bubbles by the fritted-glass plate, *A*, travels up through the solvent. The liquid is carried up to some extent but descends by gravity, resulting in constant mixing of the absorbing medium and washing of the walls of the spiral. If the solvent is definitely volatile, loss may be largely prevented by passing cooling water or brine through the jacket, the level of liquid being adjusted to give sufficient condensing surface on the upper portion of the spiral.

The efficiency and operating characteristics were determined in a series of simple tests, similar to those employed by Shaw (*5*). The rate at which a gas may be passed is dependent upon the character of the absorbing medium, being influenced by the gravity, viscosity, and surface tension. A rate of approximately 80 liters per hour has been found to be the maximum possible. With water as the liquid absorbent a rate greater than 60 liters per hour had a tendency to carry the liquid up out of the spiral into the head. The head is of sufficient volume so that no liquid is carried over, but absorption at these extreme rates becomes inefficient.

This rate (maximum 60 to 80 liters per hour) is not as high as the maximum possible with a Milligan bottle. However, two Milligan bottles were tested and it was found that one showed considerable side leakage. This first became appreciable at a rate of 10 liters per hour and it was estimated that at 20 liters per hour more than 50 per cent of the gas was not following the spiral, but was passing vertically through the liquid between the spiral and the sleeve. The other Milligan

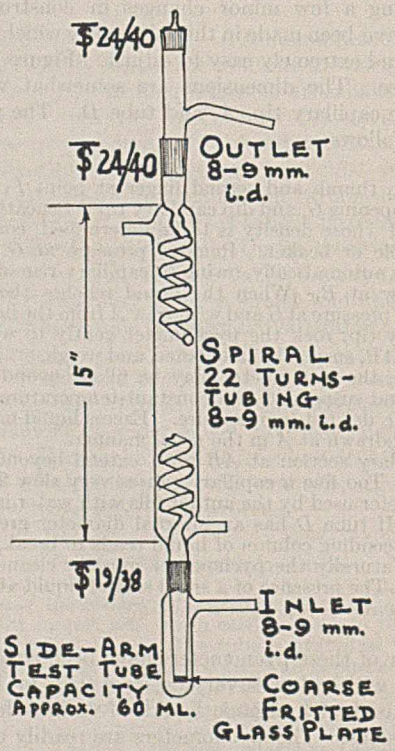



FIGURE 2

bottle performed well up to 60 liters per hour, and by-passing first became significant at this rate.

The time of contact in a well-matched Milligan bottle at a rate of 60 liters per hour is roughly 0.7 second, whereas the time of passage through the absorber described herein is about 5 to 6 seconds at that rate.

Carbon dioxide solutions in air were passed through potassium hydroxide solutions (approximately 0.5 *N*) at varying rates and the gas stream was then checked by means of the method of Higgins and Marriott (*1*) and found free of carbon dioxide.

Air containing 8 to 10 per cent of ammonia was passed through the unit which had been charged with 50 ml. of 0.1 *N* sulfuric acid to which a few drops of methyl orange had been added. A trap containing 2 drops of 0.1 *N* sulfuric acid in water colored with methyl orange was connected to the outlet. The rate of flow was 60 liters per hour. Flow was maintained for 60 seconds and the absorbing liquid was washed out of the unit by adding several small portions of water through the opening in the head. The solution was then titrated to the neutral point with 0.35 ml. of 0.1 *N* sodium hydroxide. The color of the solution in the trap had not changed and 2 drops of 0.1 *N* sodium hydroxide were required for neutralization.

In a second similar test, with the same setup except that the trap contained 5.00 ml. of 0.1 *N* sulfuric acid, the ammonia-air solution was passed through until the absorbing solution had just passed the neutral point. Titration of the acid in the trap showed that only 0.12 ml. of the acid in the trap had been consumed.

The apparatus has also been found to give satisfactory results in the absorption of unsaturated hydrocarbons in liquid bromine, as well as in bromine solutions in petroleum ether, carbon tetrachloride, and potassium bromide–water solution.

The volume of absorbing liquid is small, only 20 to 50 ml. being required, and the absorbing medium or any soluble or liquid products of absorption may be quantitatively recovered by rinsing the spiral from the top with a small amount of wash liquid.

### Literature Cited

(1) Higgins, H. L., and Marriott, W. McK., *J. Am. Chem. Soc.*, 39, 68–71 (1917).
(2) Martin, W. McK., IND. ENG. CHEM., Anal. Ed., 8, 396 (1936).
(3) Martin, W. McK., and Green, J. R., *Ibid.*, 5, 114–18 (1924).
(4) Milligan, L. H., IND. ENG. CHEM., 16, 889 (1924).
(5) Shaw, J. A., *Ibid.*, Anal. Ed., 6, 479–80 (1934).

RECEIVED May 29, 1937.

# A Thermoregulator

## For Direct Control of the Electric Circuit in a Heating Bath

FREDERIC E. HOLMES

**Children's Hospital Research Foundation and Department of Pediatrics, University of Cincinnati, Cincinnati, Ohio**

IT IS OFTEN satisfactory to control the temperature of a water bath or other heating bath to within 1° or 2°. Such operations as digestions, saponifications, and evaporations may not require closer control. The thermoregulator described here fulfills such requirements, and in addition eliminates the use of the relay usually needed in a highly sensitive temperature-control system.

The regulator employs the mercoid principle, the current being broken between mercury and mercury in an atmosphere of hydrogen. The spark occurring at the break is isolated within the apparatus, affording protection against the danger of igniting volatile inflammable liquids. There are no outside contacts. Further protection is given in case of breakage by the location of the switch below the surface of the liquid of the bath.

Two of these regulators have been in constant operation, 24 hours a day, for more than a year, one maintaining an average temperature of 85° (for the saponification of esters) and the other a maximum temperature of 60° (for evaporation of petroleum ether) in the cholesterol method of Kirk, Page, and Van Slyke (*1*). The one which maintains a temperature of 85° makes and breaks the current supplying a 600-watt immersion heater.

Breaking the mercury circuit in a nearly horizontal capillary tube (Figure 1) minimizes the change of pressure due to change in length of the mercury column, thus in turn reducing the change in temperature required to produce a wide break in the mercury.

The details of construction may be obtained from Figure 1, which is made to scale. Considerable latitude is permissible in most of the dimensions. The most critical part of the glass blowing is the making of the circuit-breaker tube, particularly the shaping of its junction with the vertical tube. Without increasing the width of the opening into the vertical tube, the top of the junction is rounded slightly to avoid trapping hydrogen and the bottom is kept sharp to ensure breaking the circuit uniformly at the desired temperature. The tube is slanted from the horizontal only enough to prevent bubbles of hydrogen from remaining in it (slope 2 to 13, angle 8°, approximately). The best size of bore for this tube is 1.5 mm. The most satisfactory size for the vertical tube is 5-mm. inside diameter. The large expansion and manometer bulbs reduce undesirable pressure changes, and help to reduce the lag. For some purposes the manometer bulb and plunger might be made longer to allow adjustments over a wider temperature range.

The construction of the plunger and terminal blocks can obviously be varied to suit individual preference. The method of making the plungers actually used is given as a suggestion.

A 3-mm. (0.125-inch) threaded brass rod, or a rod threaded over enough of its length to allow vertical movement of the plunger through the entire length of the manometer bulb, is cut off at about 5 mm. less than the length required to extend to the bottom of the glass plunger. A platinum electrode long enough to dip into the mercury when the bath is cold is fused directly to the brass rod. A glass tube having about one-third the cross-section area and about half the length of the manometer bulb is slipped over the electrode and lower end of the threaded rod and then fused to the platinum wire about 5 mm. from its junction with the brass rod. The space between the open top of the glass tube and the rod is sealed with wax.

When a threaded iron rod is used as an alternative, a bead of copper is fused to the lower end of the rod, using borax as a flux, and the platinum electrode is fused into this bead. The threaded

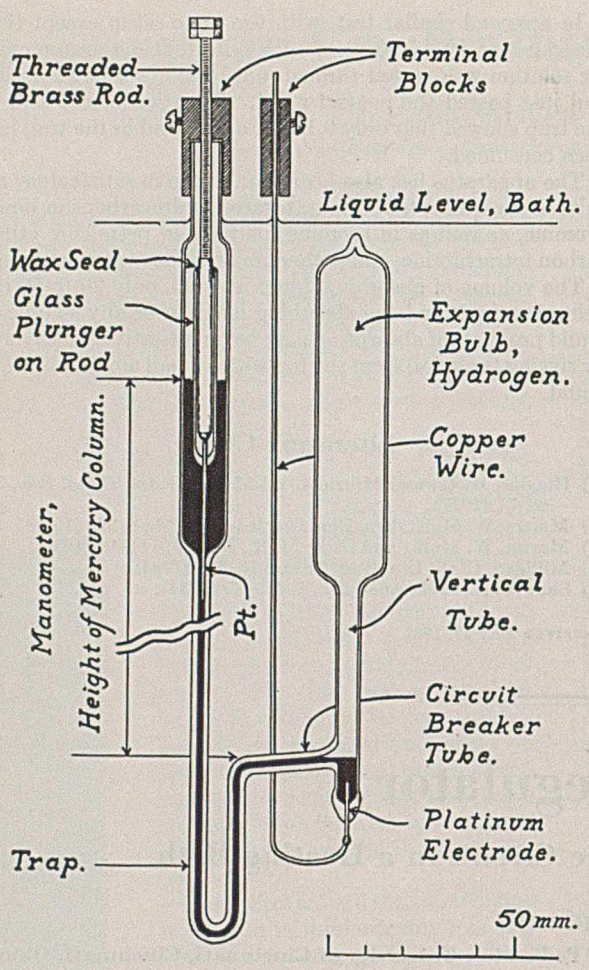



FIGURE 1. DIAGRAM OF APPARATUS

rod, plunger, and electrode may be made entirely of a metal which does not amalgamate with mercury.

The seal-off tube at the top of the hydrogen bulb is constricted to a hairlike capillary with very thick walls, because it is to be sealed off with positive pressure in the apparatus. Mercury is dropped into the manometer until the trap, the circuit-breaker tube, and the electrode well at the bottom of the vertical tube are filled, and the mercury is at the same level in both sides of the U, with the mercury just broken in the circuit-breaker tube. With a cotton plug in the top of the manometer to prevent loss of mercury, the expansion bulb is flushed with hydrogen until all air has been displaced. The rubber tube from the hydrogen supply to the seal-off tube is closed by a clamp and disconnected from the supply. The clamp is opened cautiously, allowing hydrogen to escape slowly until the mercury shows that it is at atmospheric pressure, and is then clamped tight again. More mercury is added through the manometer, any bubbles of air being teased out with a wire. The seal-off tube is then heated just to the softening point and its bore is closed by pulling and twisting. The heat is increased and the seal completed.

The regulator is connected in series with the immersion heater. Mercury is added until the desired temperature is obtained.

This regulator has a slight disadvantage common to the gas-filled type, in that adjustment must be made to compensate for changes in barometric pressure, except when a deviation of 5° or 6° is permissible. If closer regulation is required, some device, such as the plunger shown, may be used. The effects of pressure changes are so constant that adjustment can be made to known barometric pressure against a scale held behind the nut on the threaded rod and resting on the top of the terminal block. With a little experience adjustment can be easily and quickly made, without the scale, from the observed deviation of the thermometer from the desired temperature.

In order that the regulator may operate at a given temperature and within the range of adjustment for barometric changes, the length of the manometer should be such that, at maximum local barometric pressure, the top of the mercury column will reach the middle of the bulb when the plunger is raised above the mercury. The height of mercury column required for the desired temperature, at this pressure, is calculated by means of the following equation:

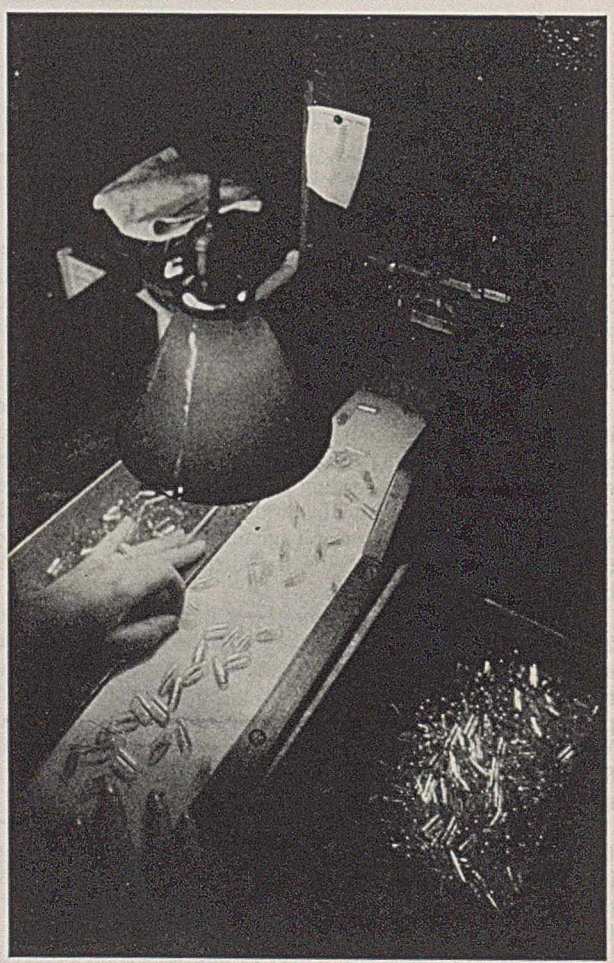
$$\frac{273 + \text{desired temperature}}{273 + \text{room temperature}} \times \text{maximum local barometric pressure} = \text{height of Hg column}$$

A plunger 45 mm. long and having an outside diameter of 7 mm., operating in a bulb of 11-mm. inside diameter, will compensate for a drop in barometric pressure of approximately 28 mm. below the maximum.

## Literature Cited

(1) Kirk, E., Page, I. H., and Van Slyke, D. D., *J. Biol. Chem.*, **106**, 203 (1934).

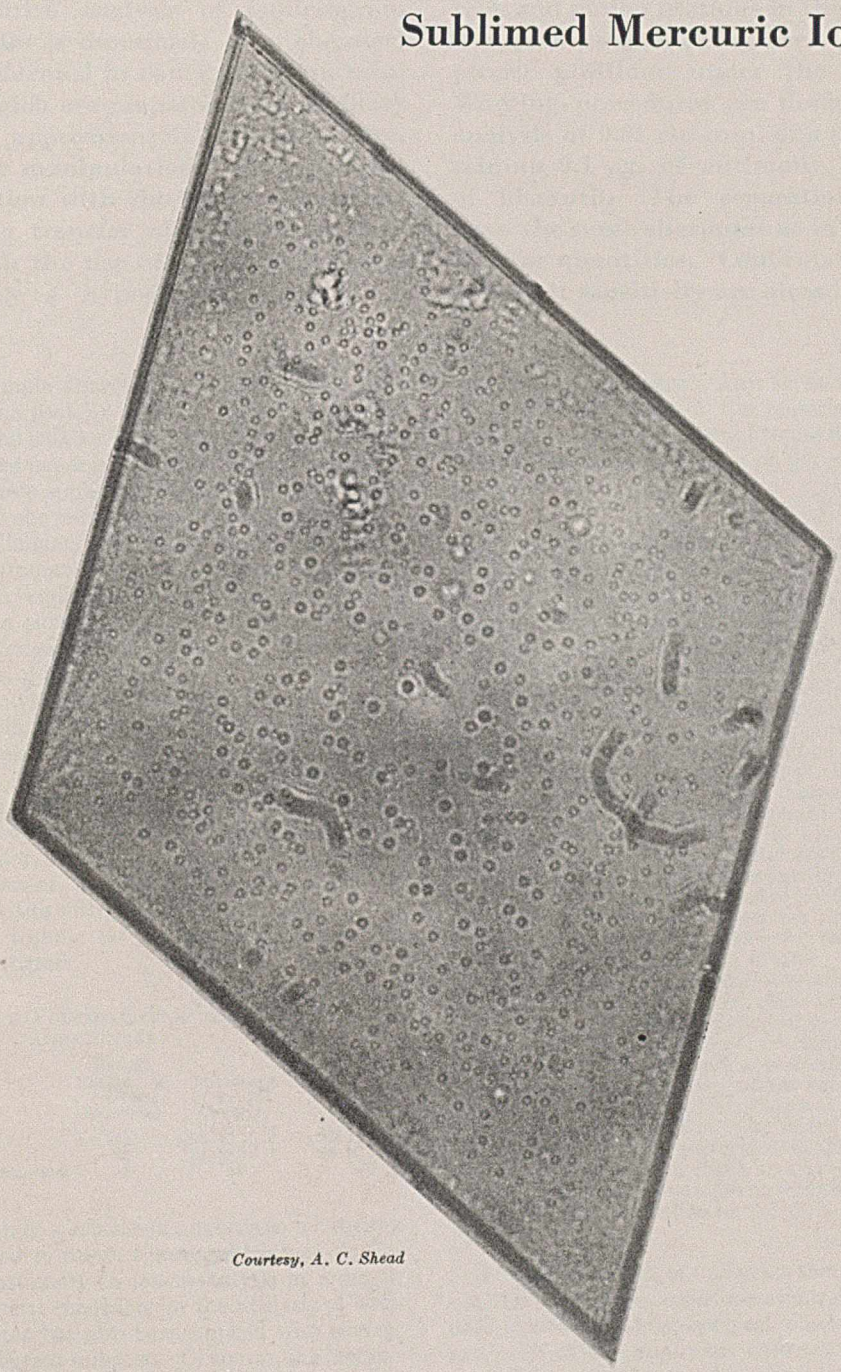
RECEIVED July 30, 1937.



INSPECTION OF FINISHED EMPTY GELATIN CAPSULES UNDER A STRONG LIGHT, ELI LILLY AND CO., INDIANAPOLIS, IND.

# MICROCHEMISTRY

## Sublimed Mercuric Iodide



*Courtesy, A. C. Shead*

# Qualitative Analysis of Microgram Samples

## General Technic

A. A. BENEDETTI-PICHLER, Washington Square College, New York University, New York, N. Y.

**A general working technic applicable to the qualitative analysis of 1-microgram solid samples is described. The chemical work is performed in cones of 0.5-cu. mm. capacity which are prepared from capillary tubing of approximately 0.5-mm. bore. Most of the manipulations are carried out by observation with the low-power microscope. The transfer of solutions is performed with the use of micrurgical pipets operated by a hypodermic syringe. A mechanical stage and a manipulator with rack and pinion motions in the three directions are used to bring the tools into the proper positions under the microscope. Working procedures are described in the analysis of 0.01 cu. mm. of a solution containing 0.1 μg. of antimony and 0.01 μg. of bismuth. The separations obtained have the same sharpness as in the analysis of large quantities. Confirmatory tests of sufficient sensitivity are already available.**

RELATIVELY simple analytical procedures have been carried out in the past by different workers with solid samples as small as and smaller than 1 microgram (*1*, *4*, *6*, *7*, *8*, *10–13*). The present paper is concerned with the development of a general technic applicable to the qualitative analysis of quantities of the above-mentioned order, where "analysis" refers to the preliminary separation of the constituents rather than to the performance of the final confirmatory tests. It seems reasonable to require that a qualitative analysis for the identification of a substance should permit the detection and estimation of constituents representing 1 per cent or more of the total mass of the sample. Accordingly, a microgram procedure of analysis should be sensitive to 0.01 microgram. A very large number of the confirmatory tests used at present in microanalysis possess limits of identification equal to and less than this quantity, a fact which eliminates the necessity of providing suitable confirmatory tests. The performance of chemical tests in the hanging drop with the use of the micromanipulator, as recommended by Chambers (*4*) as a students' experiment, should allow the attainment of extremely high sensitivities with most reactions. A systematic investigation of this technic for the performance of confirmatory tests is in progress.

TABLE I. SURFACE/VOLUME RATIOS OF SOLUTIONS IN CONTAINERS

| | 100-Ml. Beaker of 4.4-Cm. Diameter | Microcone of 0.7-Ml. Capacity | Capillary Cone |
|---|---|---|---|
| Volume of solution, ml. | 25 | $25 \times 10^{-3}$ | $25 \times 10^{-6}$ |
| Surface of solution, sq. cm. | 26 | $24 \times 10^{-3}$ | $58 \times 10^{-4}$ |
| Surface, sq. cm. per ml. of solution | 1 | 10 | 230 |

As to the application of a scheme of analysis to microgram samples, it was decided to retain the concentrations of solutions and reagents customary in macroanalysis in order to approximate the chemical conditions in the analysis of large samples. This principle has also been applied with success to the analysis of milligram samples. Of course, the maintenance of the customary concentrations implies a reduction of the volumes of solutions and reagents proportional to the decrease of the mass of the solid sample. A simple calculation shows that on a microgram scale the volumes of solutions and reagents will range between 0.001 and 0.2 cu. mm. It becomes imperative that the design of any apparatus used on such a scale permit the confinement of the solutions and precipitates in a small space. Any spreading out would lead to the irretrievable loss of the material under investigation. After an unsuccessful attempt to use fine capillaries (*3*), these considerations led to the use of the capillary cone described below.

In spite of the maintenance of the customary concentrations, there remain, however, two factors of possibly decisive influence on the outcome of chemical reactions as seen by the observer, which must vary when the volume of the reacting solution is decreased. One of these factors is the surface area of the solution; the other is the time required for the reaction to start.

The relative surface of a spherical droplet varies inversely with the cube root of its volume: $s/s_0 = 4.83\, v^{-1/3}$. Keeping the concentrations identical, the relative surface areas of the solutions of the microgram procedures will be approximately 10 times larger than those of the solutions of milligram procedures and approximately 100 times larger than those of the solutions of gram procedures (macroanalysis). These figures are only approximate, but they are in satisfactory agreement with the results of more accurate calculations (Table I), in which the actual forms and dimensions of the respective containers are considered.

The time lapsing, on the average, until a reaction starts will depend on the number of effective collisions between the molecules of the reacting substances per unit of time, or, in other words, on the probability of the occurrence of a sufficient number of effective collisions per unit of time. There can be no doubt that this probability must decrease when the absolute mass of reacting substances decreases, other conditions remaining constant. A delay in the formation of crystal nuclei and a consequent delay in the formation of precipitates might reasonably be expected when chemical work is performed on a very small scale. However, no such phenomenon has been observed in the capillary cone up to the present; neither was there any evidence of an influence exerted by the increase in the ratio of surface area to volume. Experiments performed in Chambers' laboratory (*4*) indicate that with even much smaller quantities than are met with in a microgram procedure no serious disturbances are caused by the two factors considered.

While the analysis of milligram samples hardly requires the use of a microscope, most operations on the microgram scale need continuous microscopical observation. The reagents and most of the apparatus used are assembled on a small glass slide, which is placed in a moist chamber to prevent the otherwise rapid evaporation of the small volumes of solutions. The solution under investigation remains most of the time in this moist chamber on the stage of the microscope. For centrifuging, heating, or treatment with gases, the capillary cone containing the solution is transferred into capillaries which are also designed to retard evaporation.

## Apparatus

CAPILLARY CONE. The chemical reactions are carried out in a small centrifuge cone which is prepared from a short piece of capillary, and thus may reasonably be called a "capillary cone" (Figure 1). The cone has a capacity of approximately 0.5 cu. mm., and is made from a thin-walled capillary of about 0.5-mm. bore. The center portion of a 6- to 10-cm. piece of the capillary is heated in the edge of a nonluminous Bunsen flame until the glass fuses together, forming an elongated bead. Outside the flame, the bead is drawn out to a rod 2 to 5 cm. long and approximately 0.2 mm. in diameter. Cutting in the proper places gives two capillary cones with handles. The length of the cone proper should be 2 mm., and one-half of this length should be formed by the taper of the capillary cone. The handle may be from 0.5 to 2 cm. long.

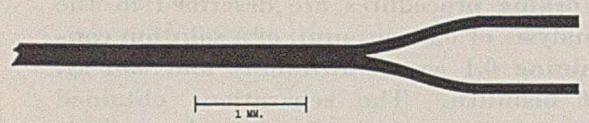



FIGURE 1. CAPILLARY CONE

The capillaries used for the preparation of the cones may be of any kind of glass, but they must be perfectly clean inside and outside. The best policy is to use capillaries freshly drawn out from clean glass tubing. These capillaries and the apparatus made of them should never be touched with the fingers, since on using the apparatus in the moist chamber fingerprints develop into a pattern of small droplets which greatly interfere with the microscopic observation of the contents of capillary cones, reagent containers, or measuring capillaries. Gloves or finger cots may be worn when preparing or handling any device to be used in the moist chamber. The finished capillary cones are kept in a screw-cap vial.

REAGENT CONTAINERS. Containers for reagents and wash liquids are given the same general form as the capillary cones. They may possess far greater capacities than the capillary cones, and 5- to 8-mm. lengths of the original capillary are retained with the handles (Figure 2, *A*, *B*, *C*). The reagent containers are prepared from a long piece of capillary of a uniform bore of 0.5 to 1 mm., which bore is measured under the microscope (*2*) and recorded on the vial in which the reagent containers are kept until used.

MEASURING CAPILLARIES. Very small volumes (0.001 to 0.01 cu. mm.) of solutions are measured in capillaries of 0.05- to 0.2-mm. uniform bore. Such fine capillaries are drawn out from thin-walled capillaries of 0.8- to 1-mm. bore (*2*) and cut into lengths of 2 to 3 cm. each. To control the uniformity, the diameter of the bore is measured at both ends of the capillary pieces and recorded. Finally one end of each short piece of fine capillary is fused shut. The measuring capillaries (Figure 2, *E*) thus obtained may be kept in pieces of wide capillary, sealed at both ends.

CAPILLARY CARRIER. A strip of glass, 10 × 30 mm., cut from a microscope slide, is used for the assembly of the apparatus and reagents used in the analytical procedure. One-half of the top surface of the carrier is covered with a layer of vaseline approximately 1 mm. thick, by placing small portions of vaseline on the slightly heated carrier slide. The capillary cones, reagent containers, and measuring capillaries needed are then placed side by side on the carrier, so that the handles are embedded in the vaseline. Those parts of the capillaries, cones, and containers in which the actual work is performed project beyond the edge of the carrier (Figure 2).

MOIST CHAMBER. All the work requiring microscopical control is carried out within a moist chamber. The form used with the micromanipulator of Chambers (*4*, *9*) was found satisfactory.

MICROSCOPE. The microscope must be fitted with a revolving mechanical stage which can be moved so far back that the coverslip roofing of the moist chamber completely clears the opening of the stage. A revolving nosepiece, permitting the rapid interchange of 5 ×, 10 ×, and stronger objectives, is recommended. The illumination should allow the change without much delay from observation with transmitted light to observation with reflected light. A small microscope lamp held by an adjustable stand may serve for this purpose.

MECHANICAL MANIPULATOR. A Leitz manipulator, permitting motions in the three directions by means of rack and pinion, is used for the operation of the micropipets.

MICROPIPETS AND INJECTION APPARATUS. The micropipets used for the transfer of liquids are prepared according to the directions of Chambers (*4*, *9*) and operated following his instructions with the use of a hypodermic syringe (*5*). Pipets with a very gradual, fine, straight taper are best suited for this type of work. The micropipets must be straight in order to be easily introduced into the horizontally lying capillary cones and reagent containers. The diameter of the bore should be approximately 1 $\mu$ at the tip to restrict the flow of liquid sufficiently to permit dispensing definite, small volumes of solutions. The syringe is filled with water as in micrurgical work. The point of the micropipet is rinsed after use by repeatedly taking in a suitable cleaning fluid and expelling it. When working with pipets having a relatively wide opening, the rinsing can often be performed with the water contained in the injection apparatus (syringe, connecting copper tube, and adapter for the micropipet). It is usually sufficient to press out a small amount of this water through the point of the pipet. After rinsing in this way, the whole pipet is filled with water, which must be sucked back before resuming work so that the meniscus is located in the part of the pipet close to the adapter. This operation sometimes causes trouble because of the surface tension force holding the liquid in the point of the pipet. Approaching the point of the pipet with a hot glass rod while applying suction with the plunger of the syringe will easily overcome this difficulty.

## Experimental Procedure

The analysis of 0.01 cu. mm. of a solution containing 0.1 $\mu$g. of antimony and 0.01 $\mu$g. of bismuth, corresponding to 10 and 1 per cent of the mass of a 1-$\mu$g. solid sample, provides a suitable example for the description of the working technic. According to the customary procedure, the solution of antimony and bismuth is to be diluted with 0.1 cu. mm. of water and then saturated with hydrogen sulfide. The precipitate of the sulfides is to be separated from the solution and washed with 0.02-cu. mm. portions of 0.3 *M* hydrochloric acid containing ammonium chloride. The washed sulfides are to be treated with 0.01 cu. mm. of 48 per cent $Na_2S \cdot 9H_2O$ solution. The solution of the antimony sulfide is finally to be separated from the residue of bismuth sulfide, and the antimony sulfide precipitated by the addition of 0.1 cu. mm. of 1 *M* sulfuric acid.

PRELIMINARY PREPARATIONS. The performance of the experiment is begun by selecting a suitable micropipet, fastening it in the adapter held by the manipulator, and testing the proper operation of micropipet and syringe.

As the volumes of all the solutions are to be measured with the use of the eyepiece micrometer and capillaries of known

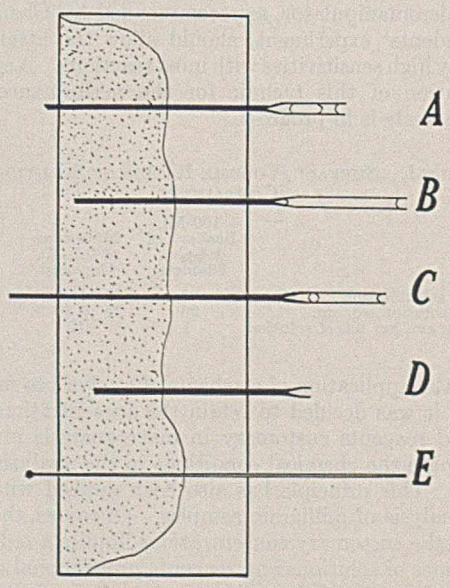



FIGURE 2. CAPILLARY CARRIER WITH ASSEMBLY OF APPARATUS

*A*, *B*, *C*. Reagent containers
*D*. Capillary cone
*E*. Measuring capillary

bore, preparation of a table of the required micrometer readings is recommended. If the capillary to be used for measuring the 0.01 cu. mm. antimony-bismuth solution has a bore of 0.132 mm., calculation shows that a 0.73-mm. length will hold 0.01 cu. mm. If one division of the eyepiece micrometer corresponds to 33$\mu$, 0.73 mm. is equal to 22 divisions. The capillary, from which the reagent containers have been made, may have possessed a bore of 0.417 mm. The lengths of reagent containers holding 0.1, 0.02, and 0.01 cu. mm. are then equal to 0.736, 0.147, and 0.074 mm., or 22, 4.5, and 2 divisions of the micrometer scale, always assuming that 1 division equals 33$\mu$.

The required apparatus is assembled on a capillary carrier as shown in Figure 2: three reagent containers, *A*, *B*, and *C*, one empty capillary cone, *D*, and a measuring capillary *E*. The reagent containers are filled by capillary pipets operated with the mouth previous to placing them on the carrier. Container *A* holds the acid antimony-bismuth solution (10 mg. per ml. of antimony and 1 mg. per ml. of bismuth). *B* is filled with water, and a 1 per cent solution of ammonium chloride in 0.3 *M* hydrochloric acid is introduced into *C*.

The filter-paper lining of the moist chamber is soaked with distilled water, and the carrier with the apparatus assembly is placed in the rear of the cell which is immediately roofed with the cover slip. The chamber is transferred to the microscope and clamped into the mechanical stage.

Measuring the Sample. Using transmitted light and a total magnification of the microscope of 40 to 60 ×, the opening of reagent capillary *A*, containing the antimony-bismuth solution, is focused so that the plane containing the axis of the capillary is in sharp focus. The reagent container is oriented parallel to the axis of the micropipet; it should lie in a diameter of the field of vision and extend into only one-third of the field.

Using the unaided eye, the point of the micropipet is brought close to the opening of the reagent container. Looking into the microscope, one should see a blurred image of the point of the pipet. The point is now brought in focus by raising or lowering it with the manipulator, which is also used for bringing it finally close to the opening of the reagent container (Figure 3). The tip of the pipet and the axis of the container

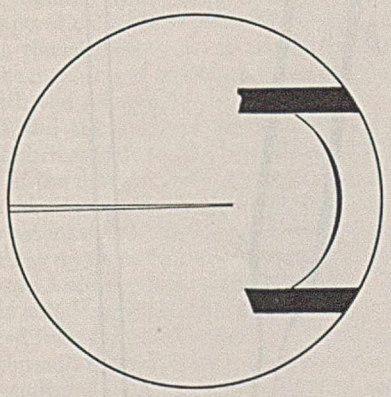


Figure 3. Micropipet Ready for Insertion in Reagent Container

are made parallel by rotation of the stage. The point of the pipet will now be located in the axis of the capillary, if the adjustment is always performed in such a way that the microscope is focused on the axial plane of the container and the pipet is brought in focus by the use of the manipulator only.

The following procedure, which is used here only for the preliminary measurement of the sample, is of sufficient accuracy to be used for the measurement of reagent solutions.

The micrometer eyepiece is inserted and rotated to make the scale appear parallel to and in the image of the container. The container is moved forward with the use of the mechanical stage until the opening of the pipet is immersed in the solution beyond that length which is to be taken up by the pipet and until the front meniscus of the solution coincides with a convenient division of the micrometer scale. A length of 3 divisions is to be taken up this time to have approximately 50 per cent more than the required volume (2 divisions = 0.01 cu. mm.). Accordingly, the point of the pipet is immersed beyond division 13 of Figure 4, and suction is applied carefully with the plunger of the syringe.

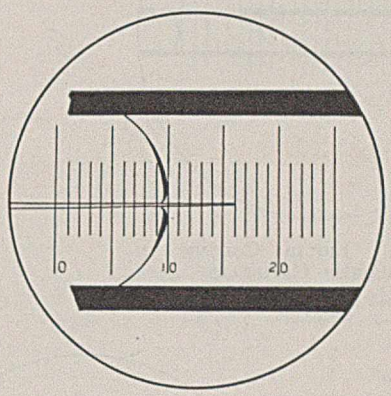



Figure 4. Micropipet in Position for Taking Up Definite Volume of Reagent

The meniscus of the solution slowly recedes as the liquid enters the micropipet. When the meniscus has moved 3 divisions of the micrometer scale (coincides with division 13 of Figure 4), the reagent container is rapidly withdrawn into its original position (Figure 3) with the use of the mechanical stage. The micropipet contains now approximately 0.015 cu. mm. of the solution.

Use of the Measuring Capillary. The micropipet is backed up horizontally for approximately 1 cm., then, with the use of the mechanical stage, the measuring capillary is brought into the diameter of the field of vision so that it extends to the center of the field. Rotation of the stage makes capillary and micrometer scale coincide. The microscope is focused on the axial plane of the measuring capillary and then the opening of the capillary is approached with the point of the micropipet, observing with the unaided eye. The position of the micropipet is corrected until the blurred image of the tip appears in the microscopic field. The point of the pipet is moved into focus, using the vertical adjustment of the manipulator. The tip of the micropipet is introduced into the bore of the measuring capillary with the use of the mechanical stage; the opening of the pipet should enter the measuring capillary to a length of at least 75 per cent of that of the liquid column to be measured off. In general, it does not matter whether or not the point of the pipet touches the wall of the measuring capillary. When pressure is supplied to the plunger of the syringe, the solution first forms a droplet at the point of the pipet which finally fills the bore of the capillary. As the measuring capillary fills, the micropipet is gradually withdrawn. Using pressure and suction supplied by the syringe, the volume of the solution is changed until the two menisci are the proper distance (22 divisions of the micrometer scale, Figure 5) apart, after which the micropipet is completely withdrawn.

Cleaning the Micropipet. The micropipet may be cleaned outside the moist chamber by blowing its contents into a narrow strip of thin filter paper, and repeatedly taking in small volumes of dilute hydrochloric acid from a drop hanging from the end of a glass rod and discharging upon filter paper. The strip of paper is best attached to the same glass rod which carries the drop of acid; the rod should be held by a stand.

TRANSFER OF SOLUTIONS TO THE CAPILLARY CONE. The cleaned micropipet is inserted into the measuring capillary as described above, and all the solution is taken up by suction

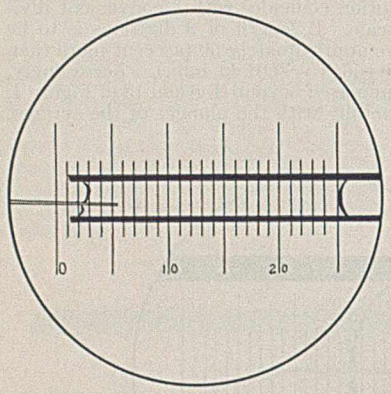



FIGURE 5. LIQUID COLUMN IN MEASURING CAPILLARY
Length adjusted to 22 divisions of micrometer scale

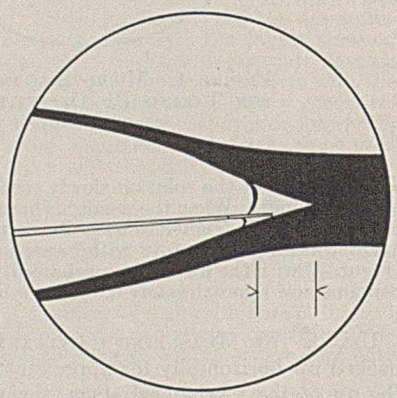


FIGURE 6. TRANSFER OF SOLUTION TO CAPILLARY CONE

supplied by the syringe. Maintaining a slight suction all the time, the micropipet is slowly withdrawn from the measuring capillary. Now the capillary cone is brought in position with its opening close to the center of the field. The microscope is focused on the point of the taper, which lies in the axis of the cone. The micropipet is inserted in such a way that its opening touches the inside wall of the capillary cone close to the point of the taper of the cone. When pressure is supplied with the syringe, the point of the cone fills with the solution (Figure 6). When all the solution is discharged, as indicated by the meniscus in the point of the pipet or by the appearance of air bubbles in the liquid, the capillary cone is immediately withdrawn with the use of the mechanical stage without releasing the pressure on the plunger of the syringe. The micropipet is cleaned as before.

MEASURING REAGENT SOLUTIONS. Reagent container *B* (Figure 2) is brought into the field of the microscope, the micropipet is inserted, and a column of water corresponding to a volume of 0.1 cu. mm. (22 divisions of the micrometer scale under the conditions stated in the beginning) is taken up with the pipet, using the same procedure as in the preliminary measurement of the sample solution. The water is immediately transferred to the solution in the capillary cone. Use of a black background and observation with reflected light permit the observation of the white precipitate of basic chlorides.

TREATMENT WITH HYDROGEN SULFIDE. A simple calculation shows that only 0.275 cu. mm. of hydrogen sulfide measured under normal conditions, is required for the precipitation of 1 $\mu$g. of antimony as antimony sulfide. The following procedure obviously ensures the presence of a sufficient excess of hydrogen sulfide to accomplish complete precipitation:

The capillary cone is removed from the moist chamber with the use of forceps, the handle is wiped free of vaseline, and then the cone is placed in the capillary of the device shown in Figure 7. The opening of the capillary is drawn out to a fine tip, *A*, and the wide tube, *B*, which contains a plug of cotton, is connected to the hydrogen sulfide generator. The air in the capillary is displaced by hydrogen sulfide, and when the latter begins to escape from tip *A*, the point of the tip is sealed. Finally the capillary is fused shut at the constriction, *C*, drawn off from the wide part of the tube, and placed for 3 minutes in water at 70° C. The formation of an orange precipitate in the capillary cone can be observed with the unaided eye. The precipitation is allowed to stand for 5 to 30 minutes at room temperature; then the capillary is cautiously cut open at *D*, placed in a microcone, as shown in Figure 8, and centrifuged with the use of an ordinary hand centrifuge.

ESTIMATION OF QUANTITIES. The capillary is taken out of the microcone, and the capillary cone is pulled out of the capillary and transferred back onto the carrier in the moist chamber. Microscopic inspection with reflected light will show a clump of orange precipitate in the point of the capillary cone. The volume may be roughly estimated with the use of the micrometer scale.

SEPARATION OF SOLUTION AND PRECIPITATE. The point of the micropipet is inserted into the clear liquid with the opening of the pipet close to the precipitate, and then the solution is taken up into the pipet by the slow application of suction. This operation may be observed with reflected light, using a white background. A nearly quantitative removal of the solution can be accomplished without difficulty and without disturbing the precipitate. The filtrate is rejected and the pipet is cleaned.

WASHING THE PRECIPITATE, MEASURING THE WASH LIQUID. A volume of 0.02 cu. mm. of the ammonium chloride–hydro-

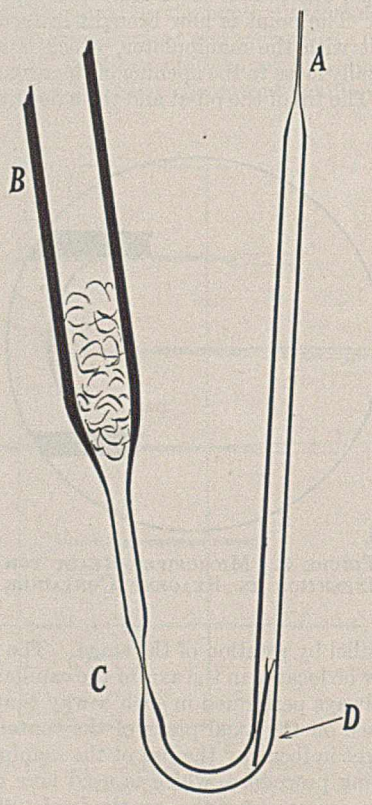



FIGURE 7. TREATMENT WITH HYDROGEN SULFIDE

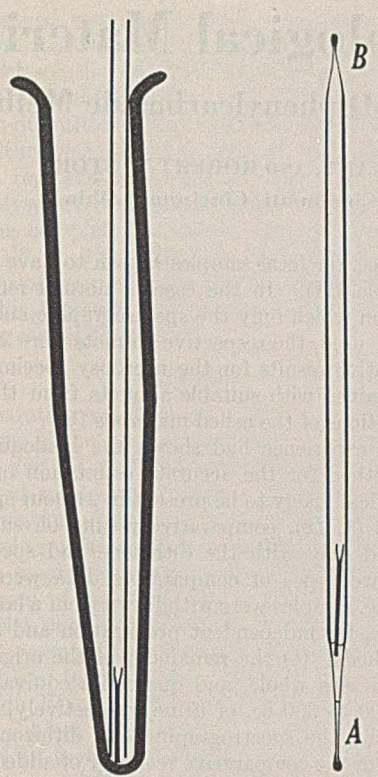



FIGURE 8 (*left*). CAPILLARY CONE IN MICROCONE FOR CENTRIFUGING
FIGURE 9 (*right*). HEATING OR PRESERVING SOLUTIONS IN CAPILLARY CONE

chloric acid solution is taken up from reagent container *C* (Figure 2) and transferred to the precipitate in the capillary cone. The precipitate may be stirred up with the use of a fine glass thread operated with the manipulator. Then the capillary cone is again removed from the moist chamber, inserted into a wide capillary open at both ends, placed in a microcone (Figure 8), and centrifuged. Inserting the cone into the capillary not only facilitates handling, but also retards evaporation of the solution in the capillary cone. After returning the capillary cone to the moist chamber, the position of the meniscus of the wash liquid is determined by measuring with the micrometer scale the distance between the meniscus and the point of the cone. This position of the meniscus corresponds to 0.02 cu. mm. of wash liquid. After removal of the first portion of the ammonium chloride–hydrochloric acid solution, the second portion is added without preliminary estimation of its volume in the reagent container by simply filling up to the 0.02-cu. mm. mark of the capillary cone.

STARTING ANOTHER SET OF PROCEDURES. When the washing of the sulfide precipitate is complete, the capillary carrier is removed from the moist chamber. The capillary cone containing the sulfide precipitate is transferred to another carrier already equipped with an empty capillary cone and two reagent containers, one holding sodium sulfide solution and the other containing dilute sulfuric acid. These reagents are to be used for the separation of the copper and arsenic groups. After placing the new capillary carrier in the moist chamber, the sulfide precipitate is treated with 0.01 cu. mm. of the sodium sulfide solution.

HEATING AND PRESERVING SOLUTIONS IN CAPILLARY CONES. The capillary cone is placed in a capillary containing a small volume of water in the tip of its sealed end, *A* (Figure 9). Then the open end of the capillary is fused shut, and the capillary is placed in water at 70° C. After 5 minutes it is taken out of the water bath, transferred to a microcone, and centrifuged with end *A* down. A tiny speck of black precipitate collects in the point of the capillary cone and can be seen with the unaided eye. Solutions may be kept in capillary cones for days without evaporation taking place, if the cone is sealed in a capillary as shown in Figure 9.

The separation of the sodium sulfide extract from the bismuth sulfide and the precipitation of the antimony sulfide from the extract with dilute sulfuric acid are performed with the use of procedures described above.

In an actual experiment with 0.08 μg. of antimony and 0.008 μg. of bismuth, the volume of the bismuth sulfide residue was estimated under the microscope to approximate that of a sphere of a diameter of 2 divisions of the micrometer scale. The volume of the mixed antimony and bismuth sulfides had been estimated as corresponding to a sphere of a diameter of 4 divisions of the same scale. Considering the observation that equal quantities of different metals give sulfide precipitates of approximately identical volumes (*2*), it was derived that the concentrations of antimony and bismuth in the original solution stood in the ratio of 1 to 7, which is in satisfactory agreement with the ratio in which they were given.

In another experiment using the proposed technic 0.01 μg. of silver was precipitated as chloride; the precipitate was washed repeatedly and could finally be recrystallized from ammonia furnishing crystals, the form of which was recognizable under the microscope with the use of a magnification of 1000 ×.

The working technic described permits performance of analytical operations, in which concentrations and volumes of the various solutions are specified in a precise and controlled manner on a microgram scale as in macroanalysis. The operations are rather time-consuming at the present stage of development, but the technic has the merit of giving convincing results with the use of chemical procedures familiar to every analyst. Finally, it seems valuable to know that with such small quantities of material the reactions of analytical chemistry take place in the usual way. This knowledge should inspire increased confidence in the results of milligram procedures.

## Acknowledgment

The author is greatly indebted to Robert Chambers and his co-workers, C. G. Grand and M. J. Kopac, for advice and for instruction in the use of the micromanipulator and the injection apparatus.

## Literature Cited

(1) Benedetti-Pichler, A. A., IND. ENG. CHEM., Anal. Ed., 9, 149 (1937).
(2) Benedetti-Pichler, A. A., and Spikes, W. F., "Introduction to the Microtechnique of Inorganic Qualitative Analysis," pp. 26, 36, and 139, Douglaston, N. Y., Microchemical Service, 1935.
(3) *Ibid.*, p. 1, footnote 1.
(4) Chambers, R., in McKlung's "Handbook of Microscopical Technique," New York, P. B. Hoeber, 1929.
(5) Chambers, R., Kopac, M. J., and Grand, C. G., IND. ENG. CHEM., Anal. Ed., 9, 143 (1937).
(6) Emich, F., in "Abderhaldens Handbuch der biologischen Arbeitsmethoden," Abt. I, Teil 3, p. 278, Vienna and Berlin, Urban and Schwarzenberg, 1921.
(7) Emich, F., *Monatsh. Chem.*, 22, 670 (1901); 23, 76 (1902).
(8) Emich, F., *Z. anal. Chem.*, 54, 493 (1915); 56, 10 (1917).
(9) Howland, R. B., and Belkin, M., "Manual of Micrurgy," New York, New York University Press Book Store, 1931.
(10) Hudson, C. L., and Mudd, S., *J. Immunology*, 28, 311 (1935).
(11) Richards, A. N., Bordley, J., III, and Walker, A. M., *J. Biol. Chem.*, 101, 179 (1933).
(12) Richards, A. N., and Walker, A. M., *Ibid.*, 87, 479 (1930).
(13) Titus, R. N., and Gray, H. Le B., IND. ENG. CHEM., Anal. Ed., 2, 368 (1930).

RECEIVED June 5, 1937. Presented before the Microchemical Section at the 93rd Meeting of the American Chemical Society, Chapel Hill, N. C., April 12 to 15, 1937.

# Determination of Lead in Biological Materials

## Comparison of Spectrographic, Dithizone, and *s*-Diphenylcarbazide Methods

JACOB CHOLAK, DONALD M. HUBBARD, ROBERT R. MCNARY, AND ROBERT V. STORY
Kettering Laboratory of Applied Physiology, University of Cincinnati, Cincinnati, Ohio

IN THE COURSE of investigations of the extent and significance of human lead absorption and excretion (*7–12*) three distinct analytical methods have been employed in this laboratory. The earliest (referred to here as the carbazide method) consisting of numerous time-consuming chemical separations and a final estimation of lead colorimetrically by means of *s*-diphenylcarbazide (*10*), was known to yield low results (*9*, *12*). A spectrographic method requiring few manipulations (*2*, *3*) provided a more satisfactory means for dealing with minute quantities of lead and has been used extensively in the past few years. The desirability of supplementing the latter method with a chemical one of corresponding sensitivity and accuracy led to an intensive study of current methods depending on the estimation of lead by means of dithizone, diphenylthiocarbazone (*1*, *4*, *5*, *14–17*). The method finally adopted (*6*), a modification of the "mixed color" method of Clifford and Wichmann (*4*), has proved very satisfactory in the authors' hands.

Since the facilities of the laboratory permitted the simultaneous use of all three methods, data have accumulated which demonstrate the relative accuracies of the methods and indicate their individual field of usefulness in the determination of the lead content of various types of biological material.

It is the purpose of this paper to record illustrative data and to discuss their significance.

Table I. Results on Samples with Added Known Amounts of Lead

| Lead Added | Lead Recovered | | | | | |
|---|---|---|---|---|---|---|
| | Spectrographic[a] | | Dithizone[a] | | Carbazide | |
| *Mg.* | *Mg.* | *Mg.* | *Mg.* | *Mg.* | *Mg.* | *Mg.* |
| Nil | Trace | Trace | Nil | Nil | Nil | Nil |
| 0.030 | 0.022 | 0.024 | 0.035 | 0.026 | Nil | Nil |
| 0.060 | 0.054 | 0.050 | 0.070 | 0.055 | Nil | Nil |
| 0.090 | 0.083 | 0.095 | 0.095 | 0.095 | 0.03 | 0.02 |
| 0.130 | 0.124 | 0.130 | 0.125 | 0.135 | 0.07 | 0.06 |
| 0.170 | 0.164 | 0.160 | 0.160 | 0.165 | 0.11 | 0.09 |
| 0.210 | 0.184 | 0.200 | 0.210 | 0.195 | 0.13 | 0.13 |
| 0.310 | 0.294 | 0.320 | 0.300 | 0.305 | 0.24 | 0.24 |
| 0.440 | 0.470 | 0.460 | 0.410 | 0.425 | 0.37 | 0.35 |
| 0.620 | 0.620 | 0.600 | 0.620 | 0.610 | 0.48 | 0.50 |

[a] In practice the results would be expressed to the second place, since the quantities of lead usually encountered are such that the third figure becomes insignificant. These results were calculated from amounts found in 1/10 aliquots.

### Procedure

In comparative studies of the three methods, materials commonly handled in the laboratory, such as urine, feces, mixed food samples, and tissues obtained from human necropsies, were used. The methods of preparing samples for analysis were as previously described (*2*, *10*), except that some (especially feces and mixed food) were found to contain such large quantities of tin that metastannic acid was formed on treatment of the ash with nitric acid. To prevent this, the ashed materials were dissolved in hydrochloric acid and distilled water and the volumes of the clear solutions were adjusted to 250 cc. for mixed food samples and 100 cc. for fecal samples. When all three methods were employed in analyzing the same solution, the aliquots chosen for the spectrographic, dithizone, and carbazide method, respectively, were 2, 10, and 238 cc. for the mixed food samples (Table II), and 1, 5, and 94 cc. for fecal samples known to have a high lead content (Table III). In the case of normal fecal samples (Table IV), on which only the spectrographic and dithizone methods were used, the respective aliquots were 2 and 10 cc. The comparative results for the necropsy specimens (Table VI) were obtained with suitable aliquots from the properly adjusted solutions of the ashed materials (*2*).

Since past experience had shown the inadequacy of the carbazide method for the accurate estimation of the small quantities of lead likely to be present in 24-hour specimens of normal urine (*9*, *12*), comparative results on such samples were obtained only with the dithizone and spectrographic methods. Two types of comparative data were obtained; (*a*) two 100-cc. samples were withdrawn from a larger volume of clear urine, for independent preparation and analysis by the two methods; (*b*) the remainder of the original sample was prepared as a whole, and quantities equivalent to 100 cc. and to 200 to 300 cc. of urine, respectively, were taken for analysis by the spectrographic and dithizone methods.

The study of the comparative recovery of added quantities of lead (Table I) was carried out with synthetic samples

Table II. Frequency Distribution for Mixed Food Samples

(Fifty samples obtained by normal subject)

| Lead *Mg.* | Spectrographic | Dithizone | Carbazide |
|---|---|---|---|
| 0.00–0.049 | .. | .. | 2 |
| 0.05–0.099 | .. | .. | 2 |
| 0.10–0.149 | 4 | 4 | 8 |
| 0.15–0.199 | 4 | .. | 5 |
| 0.20–0.249 | 7 | 8 | 8 |
| 0.25–0.299 | 10 | 8 | 9 |
| 0.30–0.349 | 5 | 10 | 7 |
| 0.35–0.399 | 8 | 7 | 3 |
| 0.40–0.449 | 6 | 6 | 5 |
| 0.45–0.499 | 3 | 3 | .. |
| 0.50–0.549 | 2 | .. | 1 |
| 0.55–0.599 | .. | 3 | .. |
| 0.60–0.649 | 1 | 1 | .. |
| 0.65–0.699 | .. | .. | .. |
| Number of samples | 50 | 50 | 50 |
| Total lead found, mg. | 15.42 | 16.41 | 12.30 |
| Calculated mean | 0.316 ± 0.011 | 0.334 ± 0.011 | 0.248 ± 0.011 |
| Standard deviation | ± 0.115 | ± 0.116 | ± 0.113 |
| Differences between means: | Carbazide and spectrographic | | 0.068 ± 0.015 |
| | Carbazide and dithizone | | 0.086 ± 0.015 |
| | Spectrographic and dithizone | | 0.018 ± 0.015 |

Table III. Frequency Distribution for Fecal Samples

(Fifty samples from a subject exposed to lead)

| Lead (Daily) *Mg.* | Spectrographic | Dithizone | Carbazide |
|---|---|---|---|
| 0.20–0.399 | 2 | 3 | 6 |
| 0.40–0.599 | 5 | 7 | 6 |
| 0.60–0.799 | 9 | 7 | 13 |
| 0.80–0.999 | 10 | 9 | 5 |
| 1.00–1.199 | 5 | 3 | 5 |
| 1.20–1.399 | 5 | 6 | 3 |
| 1.40–1.599 | 1 | 2 | 4 |
| 1.60–1.799 | 4 | 2 | 3 |
| 1.80–1.999 | 2 | 3 | 4 |
| 2.00–2.199 | 2 | 3 | .. |
| 2.20–2.399 | 1 | 1 | 1 |
| 2.40–2.599 | 3 | 3 | .. |
| 2.60–2.799 | 1 | 1 | .. |
| Number of samples | 50 | 50 | 50 |
| Total lead found, mg. | 58.48 | 59.80 | 48.25 |
| Calculated mean | 1.184 ± 0.060 | 1.188 ± 0.062 | 0.976 ± 0.049 |
| Standard deviation | ± 0.625 | ± 0.646 | ± 0.517 |
| Differences between means: | Carbazide and spectrographic | | 0.208 ± 0.077 |
| | Carbazide and dithizone | | 0.212 ± 0.079 |
| | Spectrographic and dithizone | | 0.004 ± 0.086 |

simulating ashed samples of urine. The use of synthetic samples permitted an accurate control of the lead content since the stock solution employed could be prepared free from lead (*2*). The strength of the stock solution was such that when known quantities of lead were added to 500-cc. portions and the mixture was diluted to 1000 cc. with distilled water, each 100 cc. represented the ash of 1 liter of average normal urine. Analyses were carried out in duplicate by each method following the complete procedure employed for samples of fresh urine (*3*, *6*, *10*). Aliquots corresponding to 100-cc. samples of urine were used for analysis by the dithizone and spectrographic methods and the equivalent of 1000 cc. of urine for the carbazide method.

## Results

The comparative results obtained by the different methods in the analysis of the synthetic samples are listed in Table I. In Tables II and III are listed the frequencies of distribution, the calculated means with their probable errors, and the standard deviations from the means for the results on mixed food and fecal samples by all three methods. The differences between the means obtained by the different methods, the probable errors of these differences, and the total lead recovered in each case have also been recorded. The material employed represented fifty 24-hour fecal samples and a like number of mixed food samples. Each of the latter samples consisted of duplicates of all meals eaten during each 24 hours by a human subject under study.

The high lead findings in the fecal series in Table III are due to known conditions of lead exposure on the part of a human subject under observation. In Table IV are listed similar data for a series of normal fecal samples on which only the dithizone and spectrographic methods were employed. In Table V are recorded the results obtained by the dithizone and spectrographic methods on independent 100-cc. samples of the same urine and the results obtained by each method with aliquots from the prepared remaining sample, as well as the total volume of urine originally available. Table VI lists the comparative results for necropsy specimens from a suspected case of lead poisoning. The data are recorded not only to give the actual comparative results for each organ, but also to show the types and quantities of material and the lead ranges which can be conveniently handled by either method.

## Discussion

The comparative findings in the analysis of synthetic samples containing known quantities of lead (Table I) show agreement for the dithizone and spectrographic results and substantiate the magnitude of the average loss occurring with the carbazide method (*9*). That this loss of approximately 0.07 mg. per sample also occurs in dealing with the usual run of samples is evident from inspection of the differences between the respective means recorded in Table II. The application of the carbazide method to the determination of larger quantities of lead (Table III) is likely to result in an increased loss as a consequence of the simultaneous carrying of a large number of samples through the numerous steps of the method. The maintenance of the average loss at a uniform low level requires careful attention to detail which is possible only when a few samples are analyzed at one time. Nevertheless the carbazide method is satisfactory in those cases in which the loss inherent in the method becomes negligible either as a consequence of the use of large samples or because of the high lead content of the material (*9*, *12*).

The close agreement between the findings for the dithizone and spectrographic methods is evident not only from the results listed in Tables I and VI but, most strikingly, from the comparative recoveries and from the differences between the means of the two sets of results recorded in Tables II, III, and IV. A statistical evaluation of the ratios of these differences to their probable errors indicates values of 1 or less, and offers a further proof of the similarity of the findings by the two methods (*13*).

The apparent discrepancies which may be observed in the results on samples of urine in which the lead levels lie between 0.01 and 0.02 mg. per 1000 cc. become understandable with a recognition of the possible errors of analysis by each method. The error in estimating concentrations of lead from 0.01 to 0.10 mg. per 1000 cc. of urine is ±0.01 mg. for the spectrographic method (*3*). Since all samples are adjusted so that each cubic centimeter of the final solution is equivalent to 10 cc. of the original urine, the relative error remains constant regardless of the size of sample employed (*3*). On the other hand, the relative error of analysis by the dithizone method varies with the size of the aliquot and equals that of the spectrographic method, for aliquots corresponding to 100 cc. of urine, but decreases as the aliquot is increased. The determination of the total quantity of lead from the analysis of an aliquot can therefore be attended by a relatively large error in both methods, especially in the extremely low lead ranges. While the use of larger aliquots in working with the dithizone method results in an increased analytical accuracy, the results, as is evident from Table V, are not significantly different from

TABLE IV. FREQUENCY DISTRIBUTION FOR NORMAL FECES

(Fifty samples from a normal subject)

| Lead | Spectrographic | Dithizone |
|---|---|---|
| *Mg.* | | |
| 0.00–0.049 | 1 | 1 |
| 0.05–0.099 | 1 | 1 |
| 0.10–0.149 | 8 | 8 |
| 0.15–0.199 | 9 | 7 |
| 0.20–0.249 | 3 | 9 |
| 0.25–0.299 | 12 | 12 |
| 0.30–0.349 | 10 | 4 |
| 0.35–0.399 | 2 | 3 |
| 0.40–0.449 | .. | 2 |
| 0.45–0.499 | 3 | 2 |
| 0.50–0.549 | 1 | 1 |
| Number of samples | 50 | 50 |
| Total lead found, mg. | 12.10 | 12.12 |
| Calculated mean | 0.252 ± 0.010 | 0.248 ± 0.010 |
| Standard deviation | ± 0.108 | ± 0.106 |
| Difference between means: | 0.004 ± 0.014 | |

TABLE V. ANALYSIS OF URINE

(Showing the reproducibility of results by each method)

| Sample | Independent 100-Cc. Samples | | Aliquots from Prepared Remainder | |
|---|---|---|---|---|
| | Spectrographic | Dithizone | Spectrographic | Dithizone |
| *Ml.* | *Mg.* | *Mg.* | *Mg.* | *Mg.* |
| 1800 | 0.03 | 0.05 | 0.04 | 0.04+ |
| 1900 | 0.02 | 0.05 | 0.02 | 0.05 |
| 1650 | 0.08 | 0.08 | 0.08 | 0.07 |
| 930 | 0.04 | 0.04 | 0.04 | 0.04 |
| 1500 | 0.02 | 0.04 | 0.03 | 0.04+ |
| 2200 | 0.02 | 0.04 | 0.03 | 0.04 |
| 1040 | 0.07 | 0.08 | 0.06 | 0.07 |
| 1020 | 0.06 | 0.07 | 0.05 | 0.05 |
| 1300 | 0.01 | 0.04 | 0.01 | 0.03 |
| 930 | 0.05 | 0.05+ | 0.05 | 0.04 |
| 1200 | 0.05 | 0.06 | 0.06 | 0.06 |

TABLE VI. ANALYSIS OF NECROPSY SPECIMENS (CASE R)

| Tissue | Sample | Lead Found By spectrograph | Lead Found By dithizone |
|---|---|---|---|
| | *Grams* | *Mg.* | *Mg.* |
| Kidney | 30.5 | 0.29 | 0.31 |
| Liver (portion of left lobe) | 38.9 | 1.17 | 1.20 |
| Liver (portion under gall bladder) | 38.9 | 1.15 | 1.20 |
| Brain (fiber tracts—midbrain) | 21.3 | 0.10 | 0.11 |
| Brain (cellular—midbrain) | 19.8 | 0.08 | 0.09 |
| Spleen | 37.1 | 0.20 | 0.18 |
| Bone (femur) | 105.6 | 3.10 | 3.10 |
| Spicules (from medulla of rib) | 2.2 | 0.015 | 0.025 |
| Spicules (from medulla of femur) | 1.7 | 0.010 | 0.010 |
| Total lead | | 6.115 | 6.225 |

the results obtained with smaller aliquots. Moreover, since these variations in the analysis of low urinary lead concentrations (0.01 to 0.02 mg. per 1000 cc.) can hardly be of practical importance, either method can be considered satisfactory. The comparative data, therefore, indicate that the dithizone and spectrographic methods may be used with equal assurance as to the reliability of the results in the estimation of amounts of lead in excess of 1 gamma.

While the two methods are equally satisfactory for the analysis of the general run of samples, the spectrographic method must be considered superior in certain cases in which the quantity of lead is less than 1 gamma, as in the analysis of spinal fluid (where the available samples vary from 3 to 10 cc.), and other materials available only in small quantities. The superiority of the spectrographic method here is due to the following factors: the constancy of the relative error of analysis—0.02 gamma on the arc (*3*); the lack of need for blanks because of the use of specially purified reagents (*2*, *3*); the minimal number of manipulations; and the remarkable sensitivity of the spectrographic detection of lead (*3*). Conversely, the estimation of these minute quantities by the dithizone method is rendered uncertain partly because of the opportunities for contamination occasioned by the manipulative procedures, but principally because of the necessity for correcting the result by a "rack blank" which may be greater than the lead content of the material under analysis.

In dealing with such sensitive methods as the dithizone and spectrographic, it is necessary to take every possible precaution against lead losses and contaminations, especially when the total quantity of lead in the aliquot is of the order of 1 to 10 gamma. In general, losses are negligible and the most serious factor is contamination. This may occur from the use of improperly cleaned apparatus and from lead in dust-laden air. The lead contamination from the latter source may be reduced appreciably by the removal of dust from the air of the laboratory, and chance contamination in general may be ruled out for practical purposes by the analysis of duplicate samples when sufficient material is available.

### Summary

Comparative data for determinations of lead in biological material by means of a spectrographic, a dithizone, and a chemical separatory (carbazide) method have been given. The specific field of usefulness of each method has been defined as follows:

In dealing with the general run of biological samples in which the quantity of lead present exceeds 1 gamma, the spectrographic or dithizone method may be used with equal assurance of the reliability of the results.

The spectrographic method is superior in dealing with quantities of lead less than 1 gamma.

The chemical separatory (carbazide) method is chiefly useful in the case of large samples, and is satisfactory when the lead content of the samples is such that the loss inherent in the method (approximately 0.07 mg. per sample) is insignificant in either a chemical or a physiological sense.

### Literature Cited

(1) Assoc. Official Agr. Chem., Official and Tentative Methods, 4th ed., 1935.
(2) Cholak, J., Ind. Eng. Chem., Anal. Ed., 7, 287 (1935).
(3) Cholak, J., *J. Am. Chem. Soc.*, 57, 104 (1935).
(4) Clifford, P. A., and Wichmann, H. J., *J. Assoc. Official Agr. Chem.*, 19, 130 (1936).
(5) Fischer, H., and Leopoldi, G., *Wiss. Veröffent Siemens-Konzern*, 12, 44 (1933).
(6) Hubbard, D. M., Ind. Eng. Chem., Anal. Ed., 9, 493 (1937).
(7) Kehoe, R. A., Edgar, G., Thamann, F., and Sanders, L. W., *J. Am. Med. Assoc.*, 87, 2081 (1926).
(8) Kehoe, R. A., and Thamann, F., *Ibid.*, 92, 1418 (1929).
(9) Kehoe, R. A., Thamann, F., and Cholak, J., *Ibid.*, 104, 90 (1935).
(10) Kehoe, R. A., Thamann, F., and Cholak, J., *J. Ind. Hyg.*, 15, 257–340 (1933).
(11) *Ibid.*, 16, 100 (1934).
(12) *Ibid.*, 18, 42 (1936).
(13) Pearl, R., "Introduction to Medical Biometry and Statistics," 2nd ed., p. 287, Philadelphia, W. B. Saunders and Co., 1930.
(14) Tompsett, S. L., and Anderson, A. B., *Biochem. J.*, 29, 1851 (1935).
(15) Wilkins, E. S., Jr., Willoughby, C. E., Kraemer, E. O., and Smith, F. L., 2nd, Ind. Eng. Chem., Anal. Ed., 7, 33 (1935).
(16) Willoughby, C. E., Wilkins, E. S., Jr., and Kraemer, E. O., *Ibid.*, 7, 285 (1935).
(17) Winter, O. B., Robinson, H. M., Lamb, F. W., and Miller, E. J., *Ibid.*, 7, 265 (1935).

Received July 16, 1937. Presented before the Microchemical Section at the 94th Meeting of the American Chemical Society, Rochester, N. Y., September 6 to 10, 1937.

# An Inexpensive Microchemical Filter Crucible

A. J. BAILEY
Division of Forestry, University of Minnesota, St. Paul, Minn.

FILTRATION micromethods are numerous but often handicapped by special considerations in quantitative work. Hemmes' filtration procedure (*1*), drawing off liquid by filter paper, is only of qualitative value. Strzyzowski's glass funnel (*2*), Pregl's microfunnel (*3*), Pregl's filter stick (*4*), and Schwinger's suction filter (*3*) are instruments of distinct quantitative utility but in certain cases may be handicapped by the impossibility of wiping clean the interior of the funnel tube. Further, igniting may be impossible or difficult. Donau dishes (*5*) and similar commercial crucibles with a platinum-iridium sponge are excellent but expensive.

A simple, inexpensive, and easily made crucible that has given excellent results was made by heating the end of a Pyrex glass tube 10 mm. in outside diameter until the glass, through surface tension, formed a smooth orifice of approximately the shape shown in Figure 1. While still plastic, the end of the tube was flattened by pressing on an asbestos mat.

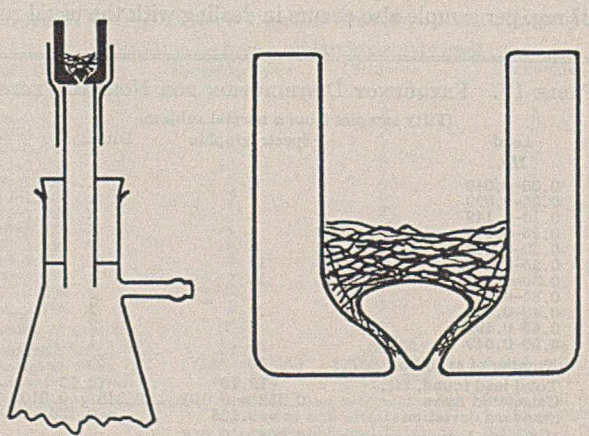


Figure 1

After cooling, the lower centimeter of the tube was cut off and the edges were fire-polished. Small "tear drops" of Pyrex glass were made approximately to fit the interior of the orifice. The final preparation of the crucible consisted in connecting it to a suction tube with a short section of heavy rubber tubing, and forming an asbestos mat above the tear drop. The tip of the tear drop should not extend below the bottom of the crucible; otherwise the filter mat would be disturbed when the crucible was set down.

Checked in analytical work by comparison with commercial platinum crucibles with a platinum-iridium sponge filter plate, it showed little difference in performance. It successfully withstood ignition, if heated gradually at first. Loss in weight was practically nil. The exterior could be easily cleaned by wiping, preventing possible retention of solid material apt to "creep" into the tube of a tube-type filter. Hygroscopic materials were easily transferred from the desiccator to a small weighing bottle in equilibrium with the atmosphere of the balance case. The total load was still less than half the capacity of a 20-gram microbalance. The weighing bottle had the further advantage of obviating the difficulty of obtaining constant weight (anhydrous surface, electrical charges?). Its general performance attested its value for analyses requiring a number of crucibles at one time or for use in class instruction.

### Literature Cited

(1) Emich, F., "Microchemical Laboratory Manual," p. 25, New York, John Wiley & Sons, 1932.
(2) *Ibid.*, p. 28.
(3) *Ibid.*, p. 30.
(4) *Ibid.*, p. 68.
(5) *Ibid.*, p. 75.

RECEIVED June 9, 1937. Released for publication by courtesy of the Director, Agricultural Experiment Station, University of Minnesota.

# Microdetermination of Organic Sulfur

WILLIAM SASCHEK, College of Physicians and Surgeons, Columbia University, New York, N. Y.

THE Pregl gravimetric method (*8*) of determining sulfur in organic compounds by catalytic oxidation in the spiral combustion tube occurs in two main operations: combustion of the substance and determination of the sulfuric acid formed.

The general advantage of this method is somewhat impaired by experimental difficulties encountered in the second part of the original procedure, which involves washing out the combustion tube, evaporating the collected wash liquid to a very small volume or to dryness before precipitation, and transferring the precipitate to a Neubauer filter crucible. If precipitation is done before evaporation, an evaporation ring of precipitated barium sulfate tends to form and adhere tenaciously to the wall of the vessel, causing difficulty in quantitative transfer. These difficulties have led to the application of various devices such as alternate washing with water and alcohol, use of a "feather," use of automatic transfer devices (*7*, *10*, *11*), or precipitation with a "celluloid sol" (*2*). All these devices for facilitating quantitative transfer of barium sulfate precipitate, which is inherently difficult, are inconvenient and include possible sources of error. The various open fusion and bomb fusion methods (*3*, *12*) tend to increase the possibilities of error, as they require precipitation of the small amounts of barium sulfate in the presence of proportionately large amounts of other salts. These conditions of high nitrate-ion concentration (anion most highly coprecipitated with barium sulfate), insufficient digestion, and the impossibility of reprecipitation are most favorable for extensive co-precipitation.

To circumvent difficulties connected with the quantitative transfer of barium sulfate, titrimetric methods of determining the sulfuric acid after the combustion have been recommended (*4*, *5*, *8*). These have only limited applicability to halogen-free and nitrogen-free substances, or are cumbersome and time-consuming.

Heller and Meyer (*6*) suggest transferring the barium sulfate after evaporation into a large crucible of 25-cc. capacity and drawing off the wash liquid by means of a porcelain filter stick (*9*), but fail to give sufficient detail.

During the past 7 years the following technic, which avoids many of the above difficulties, has been practiced and taught to students in this laboratory, and has, therefore, already found its way into many other laboratories. As the necessity of transferring the barium sulfate precipitate from one vessel to another is eliminated, it is generally preferred to the Neubauer crucible technic.

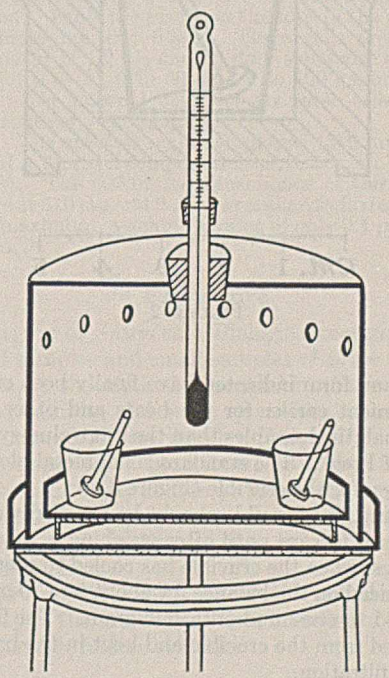


FIGURE 1

### Procedure

For precipitation a thin-walled porcelain crucible (preferably with black interior glazing) of about 15-cc. capacity (*1*) and a small porcelain filter stick (*9*) are used. The crucible should be cleaned of precipitate from previous analyses and rinsed with distilled water, the exterior wiped with a clean cloth. The filter stick may be cleaned by brushing off any adhering precipitate and then washed by sucking water

through it in both directions to rid the porous filter surface of precipitate as much as possible. Reverse washing is facilitated by the use of a suitable "adapter."

The crucible, containing the filter stick, should be dried at 150° to 200° C. for about 10 minutes. For this purpose an oven, which can be made easily and practically without expense from a tin can, is convenient and adequate; Figure 1 explains some of its details. An electric hot plate will also serve nicely as a heating base for the oven. The crucible is then placed on a piece of clean metal, covered, and allowed to cool. A metal cooling block (Figure 2), with glass cover for protection against dust, allows more secure handling of the crucible.

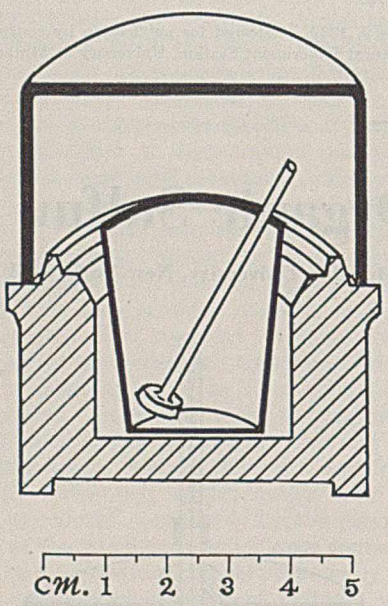



FIGURE 2

The size and form indicated have finally been evolved as a more convenient carrier for the boats and other small containers of analytical samples than the more clumsy glass hand desiccator of Pregl. The standard small metal block fits over the depression for the crucible (Figure 3).

After drying, the crucible should be handled with suitable (curved) metal forceps until after weighing. After about 15 to 20 minutes, when the crucible has cooled to room temperature, it is placed on the balance for about 5 to 10 minutes and then weighed to one-hundredth milligram. The filter stick is then removed from the crucible and kept in the balance until needed for filtration.

Combustion is carried out according to Pregl. The absorption liquid of the combustion tube is transferred quantitatively to the weighed crucible in the following manner:

The combustion tube is either clamped in a vertical position over the crucible, or held horizontally, and 1 to 2 cc. of water acidulated with hydrochloric acid (1 to 300) are blown into the wide end from a wash bottle fitted with a fine nozzle. For convenience a length of thin rubber tubing may be attached to the mouthpiece of the wash bottle. The combustion tube is rotated in such a manner as to wash the entire inside surface, and the wash liquid is then transferred to the crucible by holding the tube vertical with the capillary end in the crucible. Care must be taken not to lose wash liquid outside the crucible. Since the liquid flows out through the capillary rather slowly, expulsion may be hastened by blowing into the combustion tube through an air filter to which a length of flexible rubber tubing is attached for more convenient operation. This process is repeated four to five times to ensure complete rinsing. The amount of wash liquid employed should be so adjusted that the crucible will not become much more than about two-thirds filled.

For checking the completeness of the rinsing, another larger washing of the combustion tube may be collected in the protecting test tube and tested for sulfuric acid. If a precipitate of barium sulfate forms, it may be added to the crucible during subsequent filtration. It has been found, however, in several hundred sulfur determinations, that complete removal of the sulfuric acid produced in the combustion can easily be effected with three to four washings of 1 to 2 cc. as described. For precipitation of the barium sulfate the crucible is placed on a suitable steam bath, and about 0.5 cc. of 10 per cent barium chloride solution is added dropwise.

Any combustion residue in the boat is extracted with small portions of wash liquid and filtered into (the reduced contents of) the crucible. After about 15 minutes the solution is allowed to cool and filtration is carried out as described below. In case of very low percentages of sulfur, the volume should be reduced before filtration.

For filtration the filter stick is attached to a suitable suction device, and sufficient suction is applied so that the liquid filters at a rate of 1 to 2 drops per second. The precipitate is then washed in such a manner that the whole inside surface of the crucible is thoroughly moistened and rinsed three to four times with small portions of the acidulated water, making a total wash volume of about 3 cc. The filtrate should be collected and examined in a separate vessel of the suction device, and must be perfectly clear. Traces of barium sulfate may pass through a filter used for the first time; in this case the filtrate is poured back into the crucible and refiltered before washing. The filter is then detached and left in the crucible, which is wiped outside with a clean moist cloth and placed in the oven. With moist precipitate in the crucible, drying should be started below 100° C. to allow the moisture to be driven off slowly without spattering.

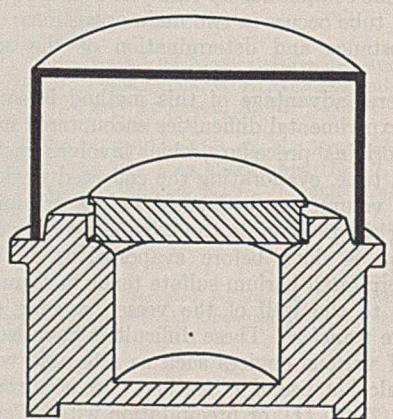


FIGURE 3

When thoroughly dried, the crucible is allowed to cool and is weighed as before. Many trials have shown that another washing with 2 to 3 cc. as before should not cause a decrease of weight of more than 0.01 to 0.02 mg.

## Literature Cited

(1) Benedetti-Pichler, A., *Z. anal. Chem.*, 64, 412 (1924).
(2) Eigenberger, E., *Z. angew. Chem.*, 68, 22 (1926).
(3) Elek, A., and Hill, D. W., *J. Am. Chem. Soc.*, 55, 3479 (1933).
(4) Friedrich, A., "Die Praxis der quantitativen organischen Mikroanalyse," 1st ed., Leipzig, F. Deuticke, 1933.
(5) Friedrich, A., and Watzlaweck, O., *Z. anal. Chem.*, 89, 401 (1932).
(6) Heller, K., and Meyer, K., *Ibid.*, 71, 117 (1927).
(7) Meixner, A., and Krocker, F., *Mikrochemie*, 5, 128 (1927).
(8) Pregl-Roth, "Die quantitative organische Mikroanalyse," 4th ed., Berlin, Julius Springer, 1935.
(9) Schwarz-Bergkampf, E., *Z. anal. Chem.*, 69, 327 (1926).
(10) Wagner, O., *Z. angew. Chem.*, 36, 494 (1923).
(11) Wintersteiner, O., *Mikrochemie*, 2, 14 (1924).
(12) Zahnd, H., and Clarke, H. T., *J. Am. Chem. Soc.*, 52, 3275 (1930).

RECEIVED August 10, 1937.

# Determination of Lead

## A Photometric Dithizone Method as Applied to Certain Biological Material

DONALD M. HUBBARD, Kettering Laboratory of Applied Physiology, University of Cincinnati, Cincinnati, Ohio

THE introduction of dithizone by Fischer (*9*) and its later adaptation as a special reagent for the determination of minute quantities of lead by Fischer and Leopoldi (*10*), have led to its use in the development of numerous methods applicable to the determination of lead in biological materials (*1, 2, 8, 12, 13, 14, 15*). A study of these methods has resulted in the development of a photometric dithizone method, based essentially on the so-called "mixed color" method of Clifford and Wichmann (*8*) but incorporating ideas from Wilkins, Willoughby, Winter, and their co-workers (*13, 14, 15*). This method has proved reliable for the accurate evaluation of lead in almost every range of concentration and has been tested extensively by comparison with the spectrographic method (*4, 5*) used in this laboratory in recent years.

It is the purpose of this paper to present the details of this method and to discuss its application to the analysis of certain biological material.

### Reagents

High-grade chemicals are used throughout the analysis to ensure a low initial lead content.

Whenever possible and feasible, reagents are further purified to reduce the lead content to a minimum, either by the aid of hydrogen sulfide or by distillation. These methods of purification as previously described (*4, 5*) are used for acids, distilled water, and ammonium citrate solution. Ammonia and chloroform are freshly distilled according to the technic of Clifford and Wichmann (*8*), and used chloroform containing dithizone and lead dithizonate in solution is reclaimed by the method of Biddle (*3*). Dithizone used for the final extraction is obtained in a state of exceptionally high purity (*2*).

The analysis of reagents commonly used is shown in Table I. No attempt is made to purify potassium cyanide. [According to Clifford (*7*), the presence of phosphates in sodium and potassium cyanides is harmful and purification of these salts by recrystallization is advisable. In the case of potassium cyanide the effect is negligible, in his experience, if the color is developed immediately after the addition of the ammonium cyanide reagent to the 1 per cent acid (*7*), as done in this laboratory.]

### Glassware

All glassware (Pyrex) before use is washed thoroughly with hot 50 per cent nitric acid, followed by rinsing with distilled water to ensure the removal of lead which may be present as surface contamination.

The same procedure is used for silica or porcelain ware.

### Apparatus

The neutral wedge photometer as described by Clifford and Wichmann (*8*) is used, with slight modifications.

LIGHT BOX. The light source consists of nine 32-candlepower, 12- to 16-volt, automobile headlight bulbs (Mazda "Tung Sol" T-1144) wired in series from a 122-volt alternating current circuit and arranged in a circle around two 2.5-cm. (1-inch) circular apertures in the interior of the box. The light is reflected from a piece of solid opal glass 15 × 15 cm. (6 × 6 inches) mounted about 2.5 cm. (1 inch) from the light source, so as to give diffuse reflected light.

EYEPIECE. Prism system and housing complete, from the Bausch & Lomb No. 3600 hemoglobinometer.

WEDGE. A neutral density wedge constructed of Bausch & Lomb smoked "C" neutral glass, length 150 mm., height 20 mm., thickness at thick end 8.0 mm. and at thin end not more than 0.5 mm. The wedge is compensated and reinforced by a wedge of clear glass of the same dimensions cemented to the wedge proper.

TABLE I. ANALYSIS OF REAGENTS COMMONLY USED IN DITHIZONE METHOD

| Reagent | Lead Content *Gamma/liter* |
|---|---|
| Water (regular double-distilled, Barnstead still) | 2 |
| Water (triple-distilled, third distillation in Pyrex) | Trace[a] |
| $HNO_3$ concentrated | 10–30 |
| $HNO_3$ concentrated (triple-distilled, in quartz) | Trace[a] |
| HCl concentrated | 10–30 |
| HCl constant-boiling (triple-distilled, in quartz) | Trace[a] |
| $H_2SO_4$ concentrated | Ca. 800 |
| KCN solution, 10 per cent W/V | 80 |
| Ammonium citrate solution, 40 per cent W/V | Trace[a] |

[a] Less than 1 gamma.

CELLS. All cells are constructed of Pyrex glass with ground and polished fused-on windows of Corex glass. The windows are parallel and optically plane. No flux or cement is used in the construction. Vents are placed in the tops of the cells to permit of ready emptying and filling. Three cells are used having internal lengths of 12, 25, and 50 mm. (tolerance of ±0.1 mm.) for ranges of 0 to 10, 0 to 50, and 0 to 100 gamma of lead, respectively. The internal diameter for these cells is 14.5 mm. with a tolerance of ±0.5 mm.

FILTER. The spectral region employed (510 mu) is obtained by means of a Wratten compound filter (Eastman Kodak No. 45 and No. 58). The maximum transmission of this filter (10 per cent) occurs at 510 mu and it decreases sharply for regions on either side of the maximum, reaching a value of only 0.1 per cent at 478 and 550 mu.

### Procedure

PREPARATION OF SAMPLES. Biological material other than mixed food samples and small samples of urine are prepared for analysis by the method described by Kehoe et al. (*11*). Mixed food samples and small quantities of urine are prepared by the method described by Cholak (*5*). In preparing samples of bone it has been found advisable to isolate the lead in the form of its sulfide as the first step, in order to eliminate the use of large amounts of ammonium citrate, otherwise required to keep the calcium in solution during the dithizone extraction.

The clear prepared solutions of the ashed materials or the solutions of the sulfides are then transferred to graduated glass-stoppered cylinders or flasks.

EXTRACTION 1. A suitable aliquot of the prepared sample is delivered by pipet into a properly graduated Squibb separatory funnel equipped with glass stopper held on by a rubber tie. (The aliquot chosen depends upon the extraneous salt and lead content of the sample, one-tenth of the prepared sample generally being satisfactory. Whenever possible the lead range selected should be 0 to 100 gamma in order that the greatest accuracy may be obtained in the determination of large amounts of lead.) Fifteen milliliters of purified ammonium citrate solution, equivalent to 6 grams of citric acid (monohydrate), and 2 drops of phenol red (Clark and Lubs) are next added to the solution, which is then partially neutralized to a distinct yellow color by the addition, dropwise, of concentrated redistilled ammonia, the funnel being swirled meanwhile for mixing. At this point 5 ml. of potassium cyanide solution (10 per cent W/V) are added and the pH of the mixture is adjusted to 7.5 by further addition of ammonia.

The solution is now ready for the initial extraction of lead with dithizone. The dithizone solution for this purpose is prepared by dissolving 30 mg. of the regular grade of dithizone (approximately 86 per cent pure) in 1 liter of freshly distilled or

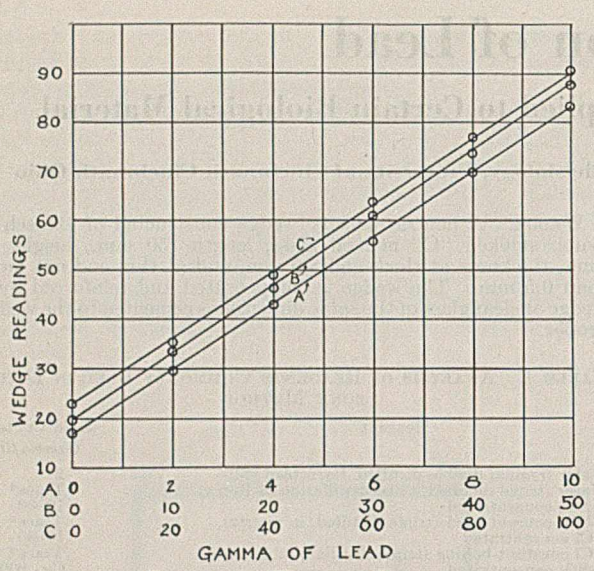



FIGURE 1. WORKING CURVES

reclaimed chloroform. The strength is such that 1.0 ml. of solution is equivalent approximately to 10 gamma of lead. (In order to remove traces of lead, the dithizone solution to be used is shaken with 1.0 per cent nitric acid immediately prior to use.) The extraction is made by adding 5-ml. portions of the dithizone solution until the color of added dithizone remains unchanged, the color ranging from a bright red for large amounts of lead, through purple, to the bluish green of the dithizone solution itself. The separatory funnel is shaken well after each 5-ml. addition, the chloroform layer being drawn off and saved in a second separatory funnel. To the collected lead-dithizone complex in the second separatory funnel 20 ml. of 1.0 per cent nitric acid are now added, the funnel is shaken well, and the chloroform lead-free phase is discarded, except for 1 ml.

EXTRACTION 2. To the contents remaining in the funnel, 2 drops of *m*-cresol purple (Clark and Lubs) are added and the pH of the aqueous phase is adjusted to 2 by the addition of weak ammonia (10 per cent). The funnel is shaken well and the phases are allowed to separate. If the chloroform, dithizone-containing phase shows no change of color, bismuth is absent (*14*) and the aqueous phase is readjusted to pH 7.5 by further addition of weak ammonia, one drop of phenol red being used as indicator. Again the funnel and contents are shaken and the phases permitted to separate. Inspection of the chloroform phase at this point will indicate whether the amount of lead present is more or less than 10 gamma.

The lead extraction is carried to completion by the repeated addition of 5-ml. portions of dithizone solution as in the first extraction, the number of portions used definitely fixing the lead range. The separated and collected chloroform fraction, containing all the lead as chloroform-soluble lead dithizonate plus uncombined dithizone, is then shaken with 40 ml. of 1.0 per cent nitric acid (nitric acid adjusted to specific gravity 1.40 should be used for dilution). The chloroform-dithizone phase is discarded and the last traces are driven out with pure chloroform. The nitric acid solution is now filtered into another clean funnel through a carefully cleaned pledget of cotton placed in the stem of a small funnel. (The pledget is washed with hot 50 per cent nitric acid followed by successive washing with hot redistilled water and with chloroform-saturated, 1.0 per cent, redistilled nitric acid.) The original separatory funnel is rinsed twice with 5-ml. portions of 1.0 per cent nitric acid, as above, and the rinsings are filtered. All the lead is now present in 50 ml. of 1.0 per cent chloroform-saturated nitric acid, ready for the final estimation.

If bismuth is present it seriously interferes and must be removed. If the amount is less than 0.5 mg. this is easily accomplished, according to Willoughby et al. (*14*), by extracting with dithizone at the point of extraction, when pH 2 is obtained (see first paragraph under Extraction 2). When large amounts of bismuth (greater than 0.5 mg.) are present a fresh aliquot must be taken from the remainder of the prepared sample, which has been freed from bismuth by a method described earlier (*11*), which step is necessary for occasional samples of feces.

EXTRACTION 3 (FINAL ESTIMATION OF LEAD). For the final estimation lead is again extracted by means of chloroform dithizone-containing solutions made up by using purified dithizone (*2*). To the 50 ml. of 1.0 per cent chloroform-saturated nitric acid containing the lead, 10 ml. of ammonia cyanide mixture are added (20 grams of potassium cyanide plus 150 ml. of redistilled concentrated ammonia, specific gravity 0.9, or equivalent, diluted to 1 liter with triple-distilled water). Upon shaking, a pH of approximately 9.5 is obtained. Next, 10 or 25 ml. of the proper dithizone solution are added and the mixture is shaken vigorously for 1 minute. Enough of the chloroform phase to fill the proper-sized cell is filtered through a Whatman No. 42 filter paper and the density of the water-free phase is determined by means of the wedge photometer. The linear displacement of the wedge, as read from a millimeter scale with vernier attachment, can be correlated to the lead content by comparison with the working curves shown in Figure 1.

The lead content of the sample is then determined by subtracting a blank designated as the "rack blank" run with each series of samples and by multiplying this result by the aliquot factor. In the case of samples requiring large amounts of acids for their preparation, it is also necessary to make a further correction by subtracting an additional blank representing the lead content of the acids used. This blank rarely exceeds 20 gamma.

The working curves are obtained by using 50-ml. portions of pure lead nitrate solutions in 1.0 per cent chloroform-saturated nitric acid, the procedure being essentially that given by Clifford and Wichmann (*8*). Concentrations of the various dithizone solutions and pertinent data regarding the quantity to be used as well as the size of cell employed are listed below.

| Range *Gamma* | Dithizone Concn. *Mg./l.* | Volume Used *Ml.* | Cell Length *Mm.* |
|---|---|---|---|
| 0–10 | 4 | 10 | 50 |
| 0–50 | 8 | 25 | 25 |
| 0–100 | 16 | 25 | 12 |

RECLAMATION OF CHLOROFORM. A modification of importance is the use for the preparation of the above dithizone solutions, of chloroform reclaimed by the method of Biddle (*3*) and given a special treatment in order to ensure the stability of the solutions. One liter is placed in a large separatory funnel and is shaken with 100 ml. of 0.5 per cent aqueous hydroxylamine hydrochloride solution neutralized with 1.0 per cent ammonia to pH 7.5, phenol red being used as indicator. Traces of water are removed by filtering through a dry fluted filter paper. Dithizone solutions prepared with chloroform so treated have been found to maintain their strength for at least 2 months. It has been found good practice to test the particular dithizone solution for stability each time that it is used in a series of analyses, by checking the wedge reading for zero lead concentration.

## Analytical Results

In Table II are listed results obtained for known amounts of lead prepared from pure lead nitrate solutions and analyzed directly by the step of final estimation as outlined above.

TABLE II. DIRECT ANALYSIS OF PURE LEAD NITRATE SOLUTIONS (FINAL STEP ONLY)

| Range Used *Gamma* | Lead Added *Gamma* | Lead Found *Gamma* |
|---|---|---|
| 0–10 | 0.5 | 0.7 |
| 0–10 | 1.3 | 1.3 |
| 0–10 | 3.4 | 3.4 |
| 0–10 | 5.5 | 5.6 |
| 0–10 | 7.2 | 7.2 |
| 0–10 | 8.9 | 9.1 |
| 0–100 | 12.0 | 11.0 |
| 0–100 | 57.0 | 57.0 |
| 0–100 | 62.0 | 63.0 |
| 0–100 | 75.0 | 75.0 |
| 0–100 | 86.0 | 87.0 |
| 0–100 | 96.0 | 96.0 |

In Table III are listed results obtained by adding known amounts of lead to a suitable quantity of salt stock solution such as that described by Cholak (*5*). Ten samples in duplicate were made up as unknowns, each having a volume of 100 ml. and containing urine salts equivalent to 1 liter of normal urine (*5*). The solutions were analyzed with the same technic as that outlined for regular urine samples, an aliquot being taken equivalent to 100 ml. of urine.

A study of the reproducibility of results gave the findings

presented in Table IV. The data show results for like aliquots of the same prepared sample run at weekly intervals.

A large number of analytical results obtained from the analysis of various kinds of biological material has been collected during the past year. In many cases the samples could be analyzed simultaneously by the *s*-diphenylcarbazide (*11*) and spectrographic (*4, 5*) methods, and these comparative results are reported elsewhere (*6*).

## Discussion

The reliability of the method reported is clearly demonstrated by the results listed in Tables II, III, and IV. From Table II it is apparent that pure lead nitrate solutions of lead content 1 to 100 gamma can be analyzed with an error of 1 to 2 per cent except when the amount is less than 1 gamma or within the lower part of the range selected, where the percentage of error becomes somewhat greater. To obtain these values only the last step of the method has been used. Such accuracy, however, is not attained when samples are carried through the complete analytical procedure, as the results listed in Table III clearly show. This is due principally to the fact that the greater number of manipulations offers opportunities for losses and contamination.

TABLE III. ANALYSIS OF SYNTHETIC URINE SAMPLES (BY COMPLETE PROCEDURE)

| Range Used | Lead Added | Lead Found[a] |
|---|---|---|
| *Gamma* | *Mg.* | *Mg.* |
| 0–10 | Nil | Nil |
| 0–10 | Nil | Nil |
| 0–10 | 0.030 | 0.035 |
| 0–10 | 0.030 | 0.026 |
| 0–50 | 0.060 | 0.055 |
| 0–10 | 0.060 | 0.070 |
| 0–50 | 0.090 | 0.095 |
| 0–50 | 0.090 | 0.095 |
| 0–50 | 0.130 | 0.125 |
| 0–50 | 0.130 | 0.135 |
| 0–50 | 0.170 | 0.160 |
| 0–50 | 0.170 | 0.165 |
| 0–50 | 0.210 | 0.210 |
| 0–50 | 0.210 | 0.195 |
| 0–50 | 0.310 | 0.305 |
| 0–50 | 0.310 | 0.300 |
| 0–50 | 0.440 | 0.410 |
| 0–50 | 0.440 | 0.425 |
| 0–100 | 0.620 | 0.620 |
| 0–100 | 0.620 | 0.610 |

[a] Calculated from result obtained with 1/10 aliquots.

Losses may occur when surface lead absorbed from ashed material is incompletely removed from the container used in ashing. Surface lead in glassware and the lead from dust-laden air which inevitably get into the sample are sources of contamination. The effect of the latter can be reduced considerably in the case of small samples by carrying out the complete procedure in a laboratory equipped with a dust-removal system.

For the estimation of amounts of lead below 10 gamma—amounts such as in practice may be present in 100 ml. of urine—the final accuracy depends upon the magnitude of the "rack blank" which is a variable, for reasons stated above, and must be determined in conjunction with each series of analyses. An average value of 0.8 gamma has been obtained, with deviations between 0.5 and 1.5 gamma.

TABLE IV. REPRODUCIBILITY OF ANALYTICAL RESULTS

| Time | Lead Found in Mixed Food | Lead Found in Feces |
|---|---|---|
| | *Mg.* | *Mg.* |
| Initial analysis | 0.26 | 1.18 |
| 1 week later | 0.28 | 1.24 |
| 2 weeks later | 0.25 | 1.13 |
| 3 weeks later | 0.25 | 1.18 |
| 4 weeks later | 0.25 | 1.18 |

The method described above should prove highly satisfactory for the analysis of practically all types of material likely to be encountered. In the analysis of material such as the ash of mixed foods and feces containing considerable quantities of extraneous salts in addition to large amounts of tin, copper, aluminum, and manganese, the introduction of two extraction steps before the final estimation permits the isolation of lead as pure lead dithizonate in the final step. The role of the first extraction step is to remove complexes of such metals as tin, copper, and zinc and to separate the lead from extraneous salts. Because of the presence of these salts, the lead extraction at this step proceeds gradually and not quantitatively, making an accurate fixing of the range difficult. The second extraction is therefore useful not only for fixing the range definitely after the extraneous salts have been completely removed, but also for detecting the presence of bismuth and removing this element totally if it is present. Of the known interfering elements, thallium has thus far not been encountered, while bismuth can be readily identified and removed, as just stated. Stannous tin if present as such is oxidized and removed during the course of analysis. Although samples frequently contain large quantities of tin, numerous spectrographic tests of the final dithizone extract have shown it to be entirely free from this element.

The wedge photometer as described has given satisfaction. Wedge readings as obtained by the type of wedge used have been found to be a linear function of corresponding density measurements.

It is planned to continue experimental work, particularly in the lower range of lead concentration (0 to 10 gamma), the spectrophotometer being used to attain greater accuracy in density measurements. Of decided advantage will be the elimination of light filters, since this will simplify the application of the method to the estimation of other metallic dithizone complexes.

## Summary

A photometric "mixed color" dithizone method has been applied to the analysis of certain biological materials. The introduction of three extraction steps permits the ready isolation of lead in a state of high purity. The first extraction step removes extraneous salts, the second eliminates bismuth, if present, and the third extraction step is used for the final estimation. Other metals usually found in biological material do not affect the results.

Reclaimed chloroform, as such, and the "specially treated" product have given complete satisfaction in the method described, thus eliminating waste.

The method is rapid. Prepared samples can be analyzed completely on the same day that they are received.

## Literature Cited

(1) Allport, N. L., and Skrimshire, G. H., *Analyst*, 57, 440 (1932).
(2) Assoc. Official Agr. Chem., Official and Tentative Methods, 4th ed., p. 378 (1935).
(3) Biddle, D. A., IND. ENG. CHEM., Anal. Ed., 8, 99 (1936).
(4) Cholak, J., *J. Am. Chem. Soc.*, 57, 104 (1935).
(5) Cholak, J., IND. ENG. CHEM., Anal. Ed., 7, 287 (1935).
(6) Cholak, J., Hubbard, D. M., McNary, R. R., Story, R. V., *Ibid.*, 9, 488 (1937).
(7) Clifford P. A., *J. Assoc. Official Agr. Chem.*, 20, 191 (1937).
(8) Clifford, P. A., and Wichmann, H. J., *Ibid.*, 19, 130–56 (1936).
(9) Fischer, H., *Wiss. Veröffent. Siemens-Konzern*, 4, Heft 2, 158 (1925); *Z. angew. Chem.*, 42, 1025 (1929).
(10) Fischer, H., and Leopoldi, G., *Wiss. Veröffent. Siemens-Konzern*, 12, Heft 1, 44 (1933).
(11) Kehoe, R. A., Thamann, F., Cholak, J., *J. Ind. Hyg.*, 15, 257 (1933).
(12) Tompsett, S. L., and Anderson, A. B., *Biochem. J.*, 29, 1851 (1935).
(13) Wilkins, E. S., Jr., Willoughby, C. E., Kraemer, E. O., and Smith, F. L., 2nd, IND. ENG. CHEM., Anal. Ed., 7, 33 (1935).
(14) Willoughby, C. E., Wilkins, E. S., Jr., and Kraemer, E. O., *Ibid.*, 7, 285 (1935).
(15) Winter, O. B., Robinson, H. M., Lamb, F. W., and Miller, E. J., *Ibid.*, 7, 265 (1935).

RECEIVED July 16, 1937. Presented before the Microchemical Section at the 94th Meeting of the American Chemical Society, Rochester, N. Y., September 6 to 10, 1937.

# Angular Constants of Microcrystalline Profiles and Silhouettes

## In the Conclusive Identification of Substances

ARTHUR CURTIS SHEAD, University of Oklahoma, Norman, Okla.

ANGULAR measurements on such facies of crystals as fortuitously presented themselves have been made sporadically for the last three-quarters of a century by mineralogists, botanists, and a few chemists. Drawings and photomicrographs illustrative of tests and purported tests have also circulated in the literature since the time of Wormley (*18*). The value of these, for comparative purposes, has been unquestioned but so preponderant were the nondiagnostic glasses, isotropic substances, rectangular prism or pinacoidal facies, regular hexagonal aspects, crystallites (*15*), and the like, that the existence of diagnostic crystals with characteristic, mathematically expressible angular constants was almost overlooked. Such meager quantitative data of this nature as were submitted were discredited by petrographic crystallographers, familiar with the outlines of random rock cross sections, and by other mineralogists, who while recognizing the law of constancy of interfacial angles on crystals, failed to comprehend fully that any observed angle on a crystal must necessarily be some function of a corresponding interfacial angle, and as such can itself serve directly as a constant under proper conditions. The dearth of this sort of data is proof of the oversight.

This discrepancy was noted by Shead (*12*) who proposed a corollary to the law mentioned with the purpose of extending its usefulness to practical analytical chemistry. This modification may be thus stated: Any functional angle of an interfacial angle on a crystal must be also a constant. This functional angle is called a "profile" or "silhouette" angle to emphasize its relationship with the actual interfacial angle as being the same as that between the "shadow and substance" of, for example, the human face or form. As distinguished from its profile or silhouette angle the profile or silhouette itself is a flat crystal of such a tubular habit as to exhibit sensible dimensions in two directions with subordinate extension in the third. Such a thin crystal, settling through a solution, will generally assume a constant position upon a microscopic slide or other flat surface, under the influence of gravity. Here any characteristic angles it may have can be measured in profile by recognized methods. Microscopic inspection will usually disclose individual crystals in orientation suitable for accurate measurement even in cases where dry (sublimation) preparational methods have been employed.

The profile or silhouette angle as a constant is invaluable to the microchemist because frequently the actual interfacial angle cannot be readily determined on microcrystals because of unfavorable orientation and the difficulty of manipulation to a more suitable position on account of minute size, whereas its function, the profile angle, can be directly measured on crystals of the habit above considered. In such cases, the functional angle may be directly employed as data, whereas the corresponding interfacial angle would have to be laboriously calculated before it could serve that purpose. Such constants are indispensable in characterizing substances that decompose or sublime before the more familiar melting or boiling points are attained. Furthermore, the silhouette or profile angle is a unique constant in that it can be photographed, preserved, and exhibited, especially in important forensic cases, to a jury that can usually appreciate simple geometrical differences and varying angular magnitudes.

Illustrations in Figure 1 depict the value of photomicrographs of single crystals, when of adequate size and perfection, as an ocular demonstration of visible quantitative data. A closely allied application is the detection of local variation in a lot of crystals that show chemical purity on the average. In such cases the condition is revealed by inconsistency of angular measurements on individual crystals taken from different parts of the lot. This lack of uniformity has been noted especially in C. P. acid potassium tartrate and is explained as localizations of normal potassium tartrate or tartaric acid in individual cream of tartar crystals. Such circumstances may explain in part some reported difficulties in accurately duplicating angular measurements.

Crystals suitable for profile angular constant measurement are obtained by a combination of proper preparational methods and discriminating selection of material that is adequate in perfection, simplicity, and orientation. The individual crystal should be isolated from all other specimens so as to lie flat, from its tabular habit, on the supporting microscope slide, in order that its whole periphery may be simultaneously brought to focus at moderate magnifications; it should approach the perfection and simplicity of a line drawing, and it should preferably exhibit the statistically common geometrical form of a parallelogram or hexagon. A casual survey of illustrations to be found in the literature will disclose that these specifications are not unduly rigid.

### Preparational Methods

Preparational methods for the production of crystals suitable for profile angular measurements necessarily vary with the physical and chemical properties of the different substances concerned, and consequently must be classified accordingly. Owing to the profound effect of speed of formation upon the character of the resulting crystals, methods may be roughly classified as rapid or slow according to whether production requires but a few seconds or takes place over an extended period of time. As Walcott (*17*) concludes, crystals growing at a rapid rate have a tendency to develop as simple forms while complex forms result as a rule from long-continued operations. Sometimes a rapid method produces nondiagnostic crystallites where a slower one provides optimum results. With other substances a rapid process produces the desired simplicity, where a slower one furnishes diagnostic but complex forms. In still other cases method seems to have little influence on results. In all cases frequent inspection of work in progress and the judgment of the operator are invaluable in determining the proper course to pursue.

Crystallization of substances, 0.5 to 5.0 per cent soluble in water, a typical rapid procedure, is ordinarily effected by a combination of sudden cooling and violent agitation of a hot solution at such concentration as will be definitely supersaturated at room temperature. The cooling is usually produced by simply holding the container under the tap while the violent agitation is effected by striking it forcefully against the hand or some wooden object. If nondiagnostic crystallites

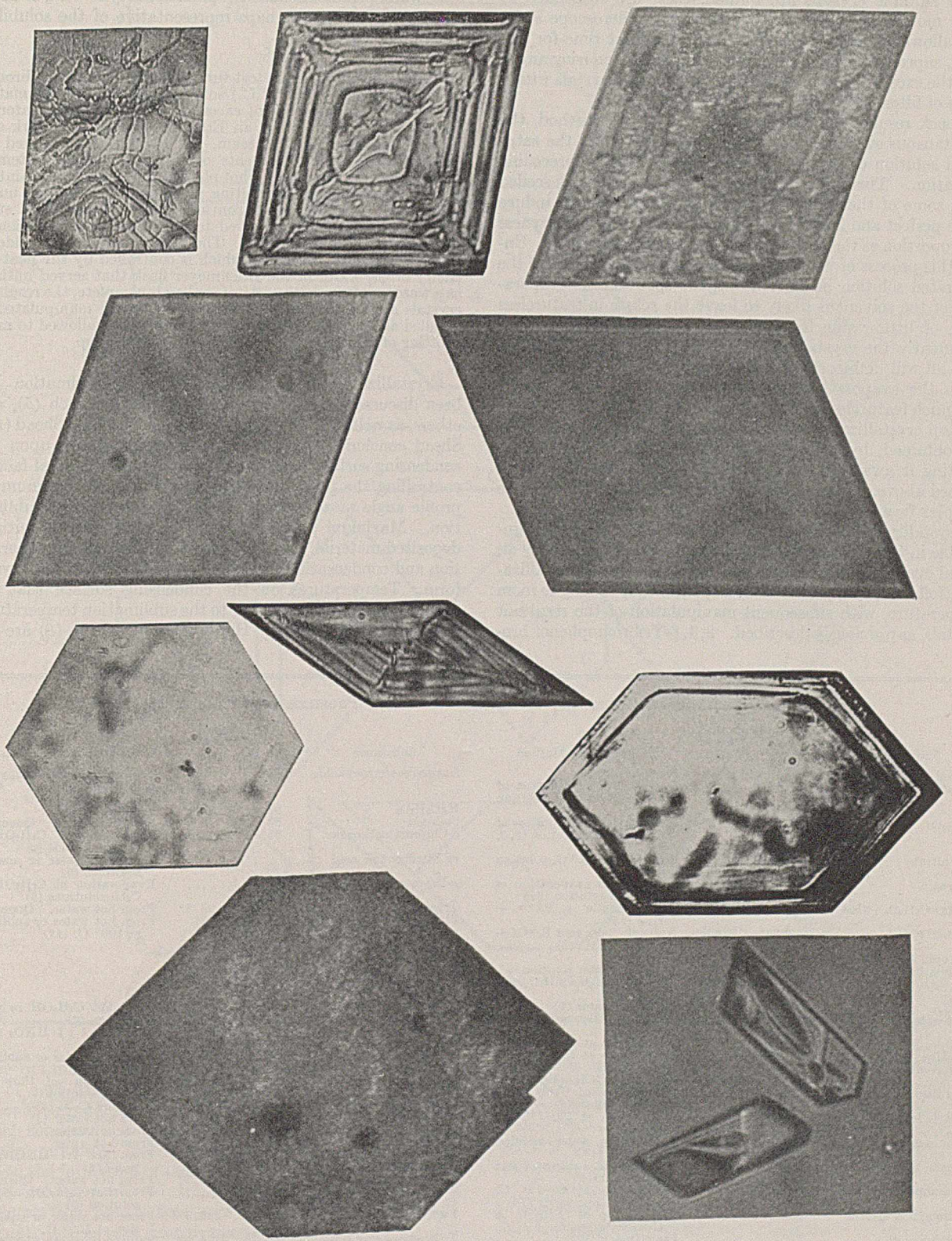


FIGURE 1. PHOTOMICROGRAPHS

Upper row: trional, potassium chlorate, 2,4,6-tribromophenol bromide
Second row: mercuric bromide, mercuric iodide
Third row: veronal, aspirin, acetanilide
Lower row: picric acid, morphine

form under this treatment, milder measures may be adopted. After suitable crystals are formed, these are suspended in their mother liquor, poured upon a glass microscope slide, and allowed to subside thereon. After a short time for settling, supernatant liquid is drained off at a low pouring angle, and the excess is gently blotted away from the crystals with a piece of filter paper held taut between the fingers.

Check results may be obtained by a slower method, the spontaneous evaporation of the solvent water from the saturated solution or mother liquor resulting from the preceding operation. This solution, placed upon a glass slide, is seeded with some of the separated solute, which appears to induce more perfect and larger crystals upon evaporation to dryness in proportion as the seed particles are fewer in number. Unless this process of what may be termed "autoseeding" of the saturated solution be carried out, the spontaneous evaporation of the solvent is likely to leave the solute in featureless films of little value for quantitative angular measurement. Frequently the crystallizing power of the solute is so great that all will be incorporated in crystals, leaving a clear field when the evaporation of the solvent is complete. But if too much featureless debris tends to form, it may be advisable to stop crystallization, when it is seen that optimum results are obtained, by pouring off the excess mother liquor and blotting it away from the crystals with filter paper, as described above. This slower method is likely to produce more complex forms than the preceding faster one.

Crystallization of substances, soluble in hot water but insoluble in the cold, may perhaps best be accomplished by an initial evaporation of the hot solution to incipient crystallization, followed by a period of slow spontaneous cooling to room temperature, with subsequent manipulation of the resultant crystals, as previously described. 2,4,6-Tribromophenol bromide (Figure 1) prepared by the action of excess warm bromine water on a hot solution of phenol, salicylic acid, or other allied compound, is perhaps representative of the solubility group under discussion.

A small 7.5-cm. (3-inch) test tube full (4 ml.) of this tribromophenol bromide (m. p. 96° C.) solution, hot and approximately saturated in the presence of excess (colored) bromine water, is supported in the mouth of an Erlenmeyer flask by a cork segmented for the escape of steam. The test tube is bathed exteriorly by the water contents of the flask. This system is placed on a wire gauze over a hot plate (105° C.) so as to maintain a temperature below the melting point of the compound under treatment. Evaporation is continued (overnight) until sufficient solvent has been removed to permit initial separation of solute from the hot solution. The system is then set aside to cool spontaneously at a rate which is controlled by the heat capacity of the water in the Erlenmeyer flask that served initially as a water or steam bath. After cooling is complete, the resulting crystals are removed to a microscope slide and manipulated as detailed above. Trichites result if the solute be allowed to melt. Blotting of the crystals is not required in this group.

Crystallization of substances by microsublimation has been discussed by Chamot and Mason (*4*), Emich (*5*), and others, as well as by Hoffman and Johnson (*8*) and Shead (*13*). Shead concluded that conditions of temperature upon the condensing surface and time appear to be the critical factors controlling the production of crystals that are optimum for profile angle measurement, by the process of microsublimation. Maximum periods of time, during which the initially deposited material reworks by repeated cycles of vaporization and condensation, are conducive of perfection of crystal form. Temperatures on the condensing surface that approach as closely as possible to the sublimation temperatures defined and tabulated by Hoffman and Johnson (*8*) are apt

TABLE I. ANGULAR CONSTANTS OF MICROCRYSTALLINE PROFILES AND SILHOUETTES

| Substance | Parallelogram Acute angle | Parallelogram Obtuse angle | Preparational Method |
|---|---|---|---|
| Veronal | 36.4° | 144.4° | From hot water |
| *o*-Nitrobenzoic acid | 39.5° | ... | Spontaneous evaporation of $C_2H_5OH$ or $C_6H_6$ solution (*6*) |
| $Ag_2Cr_2O_7$ (43°) | 44.5° | 136.3° | Ag + $CrO_4$ + evaporation of dilute $HNO_3$ solution (*2, 3, 12*) |
| Urea nitrate | 49.8° | 130.2° | From hot 1:1$HNO_3$ solution (*12*) |
| Asparagin | 50°42′ | 129°18′ | Spontaneous evaporation of $C_2H_5OH$ solution (*19*) |
| $Ba(SbO\cdot C_4H_4O_6)_2\cdot 2H_2O$ | 52° | 128° | Tartar emetic + $Ba^{++}$ + $H_2O$ (*14*) |
| $CaSO_4\cdot 2HOH$ | 52°29′ | 127°31′ | Natural: 2 per cent $H_2SO_4$ + $Ca^{++}$ in $H_2O$ (*7*) |
| Urotropin + $HgCl_2$ | 54° | 125° | Special (*1*) |
| Phenobarbital | 56.7° | 123.3° | From hot water solution |
| $CaC_4H_4O_6\cdot 4H_2O$ | 57°30′ | 122°30′ | $Ca^{++}$ + $H_2C_4H_4O_6\cdot H_2O$ solution (*11*) |
| $Ag_2C_2O_4$ | 58° | 122° | From hot water (*3*) |
| *m*-Nitrophenol | 58° | ... | From hot water or hot $C_6H_6$ (*6*) |
| *p*-Dichlorobenzene | 59° | 121° | Sublimation at 50° C. (*12*) |
| Morphine | 59.1° | 120.9° | Sublimation at 190° C. (*13*) |
| *p*-Chloronitrobenzene | 60° | ... | Evaporation of $C_2H_5OH$, $C_6H_6$, or $CCl_4$ solution (*6*) |
| *o*-Nitrobenzyl alcohol | 62° | ... | Evaporation of $C_2H_5OH$ or $C_6H_6$ solution (*6*) |
| Mercuric iodide | 64.6° | 115.4° | Sublimation at 100–110° C. (*12*) |
| Antipyrene | 66°? | ... | Salted from water solution by NaCl (*16*) |
| Mercuric bromide | 69° | 111° | Spontaneous cooling of hot water solution |
| Tribromophenol bromide | 69.7° | 110.3° | Sublimation at 85–90° C. and from hot water (*13*) |
| *p*-Nitrobenzyl alcohol | 75° | ... | Evaporation of $C_2H_5OH$ or $C_6H_6$ solution (*6*) |
| *p*-Nitrophenol | 77° | ... | From fusion (*6*) |
| $KClO_3$ | 79.8° | 100.3° | From hot water. Quenched |
| $KNO_3$ | 79.8° | 99.4° | Spontaneous evaporation of water solution (*12, 19*) |
| *o*-Nitrophenol | 80° | ... | From hot $C_2H_5OH$ or $C_6H_6$ solution (*6*) |
| *p*-Nitrotoluene | 80° | ... | From hot $C_2H_5OH$ or $C_6H_6$ solution (*6*) |
| Urea nitrate (82°) | 81.8° | 98.2° | From hot 1:1 $HNO_3$ solution (*5, 12*) |
| Antipyreneferrocyanide | 82° | ... | Acid solutions of antipyrene + ferrocyanide. Cold (*16*) |
| $HgC_2H_3O_2$ | 83.5° | 97° | From hot water |
| Sulfonal | 85° | 95° | From hot water. Quenched |
| *o*-Chloronitrobenzene | 85° | ... | Evaporation of $C_2H_5OH$ or $C_6H_6$ solutions (*6*) |
| *m*-Nitrobenzoic acid | ... | ... | From hot water or acetone solutions (*6*) |
| *p*-Bromonitrobenzene | 86° | ... | Evaporation of $C_2H_5OH$ or $C_6H_6$ solutions (*6*) |
| Trional | 86.5° | 93.5° | From hot water. Quenched |
| Picric acid | 87° | 93° | From hot water or sublimed at 100° C. (*13*) |
| | Hexagon: Two apex angles | Hexagon: Four nonapex angles | |
| *m*-Nitroaniline | 80° | ... | From hot $C_2H_5OH$ or $CCl_4$ solution (*6*) |
| Urea nitrate | 81.8° | 139.4° | From hot 1 : 1 $HNO_3$ solution |
| Picric acid | 87° | 136.7° | From hot water or sublimed at 100° C. |
| $Ag_2Cr_2O_7$ | 87.4° | 136.3° | Evaporation of hot 1:1 $HNO_3$ solution |
| *p*-Nitrobenzoic acid | ... | 135° | From hot $C_6H_6$ (*6*) |
| $AgC_2H_3O_2$ | 90° | ... | From hot water (*4*) |
| Sulfonal | 95° | 132.5° | From hot water |
| $HgC_2H_3O_2$ (100°) | 97° | 131.5° | From hot water |
| Urea nitrate | 99.5° | 130.2° | From hot 1:1 $HNO_3$ solution (*12*) |
| Acetanilide | 99.5° | 130.2° | From hot water. Quenched |
| *m*-Nitroaniline | 100° | ... | From hot $C_2H_5OH$ or $CCl_4$ solution (*6*) |
| Picric acid | 108.5°? | 126.3°? | From hot water or sublimation at 100° C. (*13*) |
| Tribromophenol bromide | 110.3° | 124.5° | Sublimation at 85–90° C. (*13*) |
| Phenobarbital | 113.4° | 123.3° | From hot water |
| Morphine | 118.2° | 120.9° | Sublimation at 190° C. (*12*) |
| Aspirin | 119.7° | 120.3° | From hot water. Quenched |
| Phenobarbital | 122.1° | 119.5° | From hot water. Quenched |
| Antipyrene | 128° | 116° | Salted from water solution by NaCl (*16*) |
| *p*-Nitroaniline | 125° | ... | From hot $C_2H_5OH$, $C_6H_6$, or $CCl_4$ solution (*6*) |

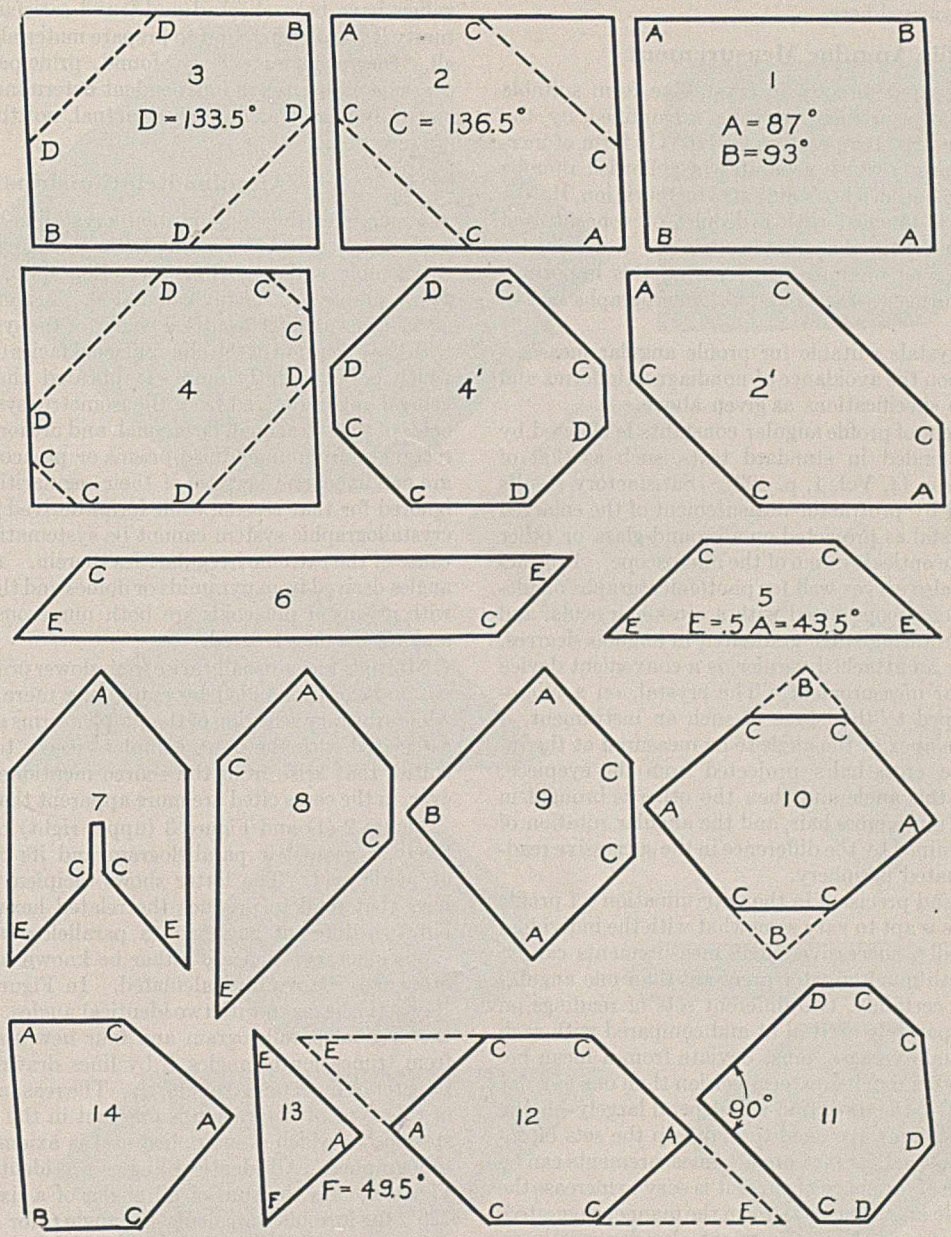



FIGURE 2

to be optimum. Regulation of these temperatures by control of heat, rather than by application of cooling water to the condensing surfaces, is recommended, especially should initial condensation take place at optimum temperature. Finally, minimum amounts of material sublimed are conducive to the formation of individual crystals suitable for profile angle measurement. Fortunately, direct inspection of the sublimate from time to time permits suitable modifications of the procedure as such become necessary.

### Apparatus for Microsublimation

Apparatus for microsublimation at atmospheric pressure has also been described by Shead (*13*) who uses a sublimation cell consisting of a metallic ring for the walls, closed below by metallic foil for the floor and above by a glass microscope slide or cover slip as a condensing surface, to receive the deposit. The metal parts are preferably of platinum or gold for most purposes. The metallic ring is about 1 mm. high and approximately 1 cm. in diameter. One advantage of the metal parts is better heat conduction from the supporting hot plate, which is bored diametrically to furnish a thermometer well, containing preferably a short Anschütz thermometer to avoid emergent stem corrections. Besides the avoidance of temperature lag, another advantage of the metal cell is the prevention of condensation of sublimate on the cell wall, which is a difficulty often encountered when the wall is of glass. A third desirable feature is the lenslike action of the metallic cell in converging a very small deposit at a focus on the condensing surface with the attendant gain in sensitivity for critical cases.

Miscellaneous methods of crystallization including crystallization from nonaqueous solvents and from fusion, have

been reported by others, notable by Hartshorne (*6*) but will not be further discussed here.

### Profile Angular Measurement

Substances prepared directly in crystalline form suitable for profile angle measurement may be exemplified by the production of the high temperature (+126° C.) form of mercuric iodine (Figure 1) which separates in yellowish rhombs (obtuse angle, 115.4 from a hot solution of mercuric ion, $HgCl_2$) when the latter is touched with a droplet of concentrated hydriodic acid on the tip of a splinter of glass. Kohlschutter (*9*) gives several good methods of preparing this important compound and furnishes excellent photomicrographs of the product.

Selection of crystals suitable for profile angular measurements is based upon the avoidance of nondiagnostic forms and the adherence to specifications as given above.

The measurement of profile angular constants is effected by methods recommended in standard texts, such as that of Chamot and Mason (*4*, Vol. 1, p. 423). Satisfactory results may be obtained by protractor measurement of the enlarged image of the crystal as projected on a ground-glass or other screen through the optical system of the microscope. Protractor mensuration also serves well for photomicrographs of adequate size. A microscope fitted with a cross-hair ocular and provided with a rotating stage graduated in angular degrees, read to tenths by an attached vernier, is a convenient device for profile angular measurement. The crystal, on a microscope slide clamped to the stage of such an instrument, is centered with the apex of the angle to be measured at the intersection of the cross hairs projected from the eyepiece. First one side of this angle and then the other is brought in alignment with a given cross hair, and the angular rotation of the stage is determined by the difference in the successive readings on its graduated periphery.

The accuracy and precision in the determination of profile angular constants is apt to vary somewhat with the individual case. Undoubtedly, successive single measurements cannot be duplicated with much greater precision than one angular degree. Just as certainly, two different sets of readings on a given angle, separately arrived at and compared with each other as individual averages, must deviate from a mean between the two with a much greater precision than one angular degree. Evidently, the deviation will depend largely on the number of observations averaged to make up the sets of determinations compared. Since angular measurements can be quickly made in a short space of time, it is easy to increase the precision of profile angular constants in the manner suggested.

The accuracy of a single measurement also depends upon the size of the angle measured, as the percentage error involved decreases as the angle concerned increases in magnitude. For this reason the largest angle available should be most suitable for the determination of the constant. Thus, if but a single value is to be tabulated, the obtuse angle of a parallelogram should be selected rather than its smaller supplement.

An accuracy and precision of 0.2° is tentatively suggested as a reasonable tolerance for permissible deviations pending further developments. This proposal is based as an example upon a representative set of observations on the acute angle of the tribromophenol bromide (Figure 1) parallelogram. An average mean of 69.9° was found for sixteen individual determinations: 70.4°, 70.2°, 70.5°, 69.3°, 70.2°, 69.6°, 69.9°, 69.7°, 70.5°, 69.5°, 69.7°, 70.2°, 69.6°, 70.0°, 68.7°, and 70.1°. These were taken from different specimens and each represents, approximately, an average of four readings, one in each quadrant defined by the cross hairs in the ocular of the microscope.

The proposed accuracy and precision of 0.2° cannot be claimed for Table I, as it has been compiled in part from various sources, no doubt of varying reliability. Some of the values have been checked and found satisfactory, but opportunity has not been found to prepare material and verify them all. Discrepancies will be found, principally from slight experimental errors in independent determinations on mathematically related angles, where actual, not theoretical, values are recorded.

### Angular Relationships

Angular relationships on microcrystalline profiles and silhouettes of crystals belonging to the tetragonal, hexagonal, orthorhombic, and monoclinic crystallographic systems are relatively simple and readily calculated. A method for arriving at definite values is discussed below for the systems mentioned with the exception of the clinopinacoid facies of the monoclinic, which is but slightly more complicated than the rest. In general angles derived from the isometric system, the *c* pinacoids of the tetragonal, hexagonal, and orthorhombic systems, rectangles from unmodified prisms or pinacoids, and the like are nondiagnostic because of their regularities, and are to be ignored for that reason, while forms derived from the triclinic crystallographic system cannot be systematically treated because of the extreme irregularities therein. As a general rule, angles derived from pyramids or domes and their combinations with prisms or pinacoids are both numerous and diagnostic and are the ones treated.

Multiple forms usually arise from slower preparational methods and can be avoided by employing more rapid processes, while arbitrary selection of the simpler forms almost invariably associated with the more complex, serves to avert the difficulties that arise from the source mentioned. These, however, in the cases cited are more apparent than real.

Figure 2 (1) and Figure 3 (upper right) represent, respectively, a geometric parallelogram and its crystalline analog of picric acid. The latter shows incipient development of lines that tend to produce the related hexagon. There are but two different angles on a parallelogram and these are supplementary; hence if either be known and recorded, the other may be readily calculated. In Figure 2 (2 and 2′) is shown the hexagon with two identical angles, $A$, retained from the original parallelogram and four new angles, $C$, resulting from truncation of angles $B$ by lines drawn parallel to the bisector of the retained angle, $A$. The reason for the necessity of this type of truncation is inherent in the symmetry of the systems to which it is applied and is axiomatic to the crystallographer. All identical angles are identically lettered in Figure 2. As the sum of all angles of a six-sided polygon is 720°, the formula for calculating angle $C$ for a hexagon of this general type is $\frac{720° - 2A}{4} = C$. Figure 3 (upper left) is such an actual hexagon of picric acid. If angle $A$ were truncated and $B$ retained, an analogous hexagon would result by the same process. It is evident that either angle $A$ or angle $B$ must be common to the parallelogram and one or the other of its derived hexagons. The octagon (Figure 2, 4 and 4′) results when both angles $A$ and $B$ of the original parallelogram or of either derived hexagon are truncated in the same manner. It is noteworthy that there are only two different angles in the octagon and that these are identical with one or the other of the larger angles found on a hexagon. The trapezoid (Figure 2, 5, and Figure 3, left center) results when the two like angles of a hexagon are bisected, leaving two of the original four identical angles of the last-named figure intact. Close inspection of the rest of Figure 2 shows some of the infinite number and variety of forms that might result by continuing indefinitely the processes indicated.

It is there also indicated how few and simply related are the angles encountered. Figure 3 is to be consulted for crystalline forms analogous to the theoretical figures of Figure 2.

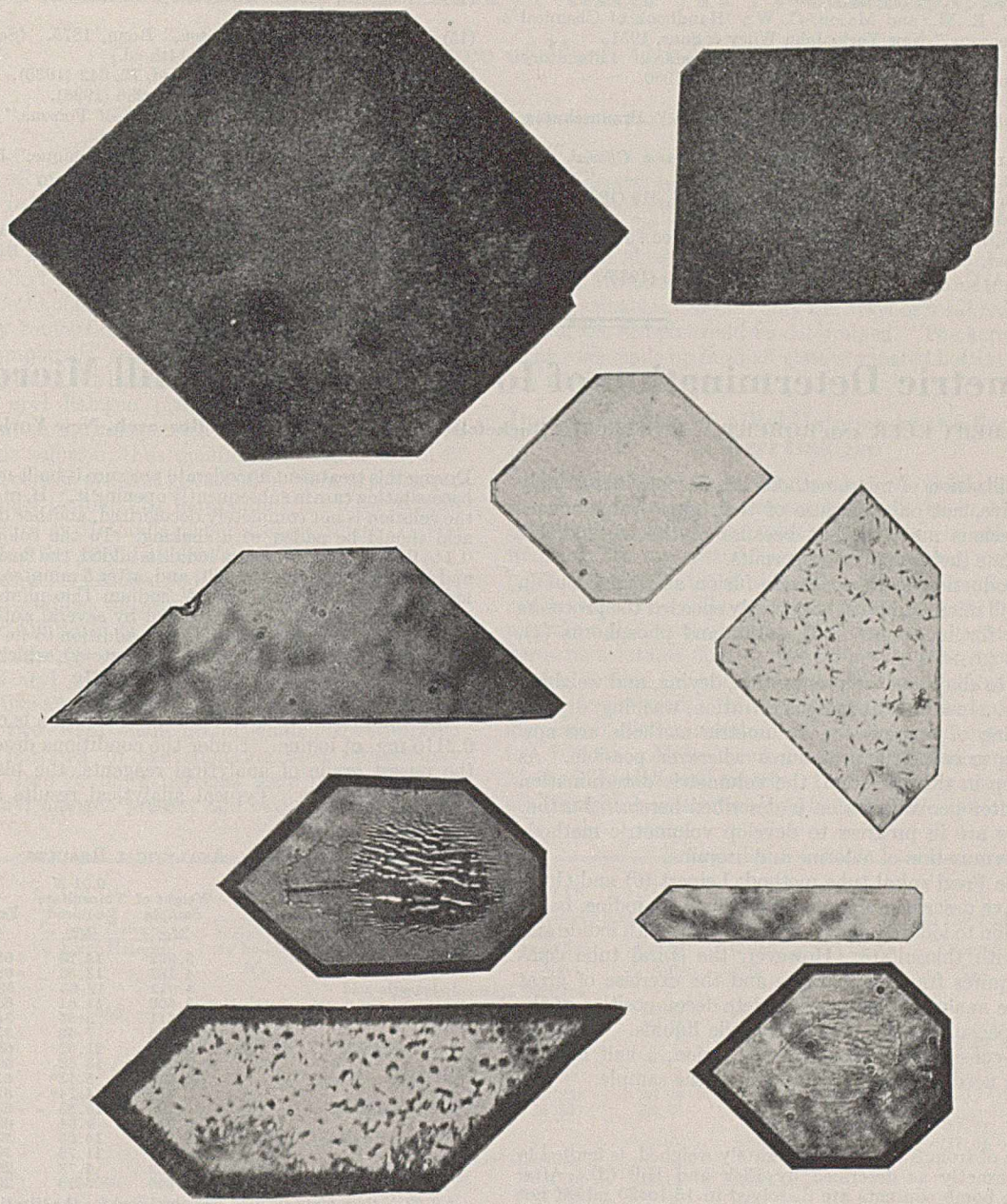


FIGURE 3. PICRIC ACID CRYSTALS

Figure 1 illustrates the usual simplicity of profiles and silhouettes apt to result from the more rapid analytical procedures.

## Summary and Conclusions

The foregoing seems to indicate the validity of a corollary law of the constancy of profile or silhouette angles, corresponding to that of interfacial angles and applicable to chemically precipitated or sublimed crystals with appreciable extensions in two dimensions and restricted extension in the third; the facility with which microcrystals suitable for profile angular measurements may be prepared; the simplicity of profile angle mensuration; the value of profile angular data as constants in cases where the ordinary constants do not exist because of decomposition or sublimation of the compound under examination; the simplicity of profiles and silhouettes produced by the ordinary rapid analytical methods; the comparatively simple numerical and geometrical relationships existing even on complex crystals formed by slower preparational methods; the possibility of individual differentiation by profile angular studies on lots of chemical having a nominal average chemical purity; and the value of photomicrographs of single crystals having adequate size, in the presentation of quantitative ocular proof.

## Literature Cited

(1) Amelink, P., "Schema zur microchemischen Identifikation von Alkaloiden," Amsterdam, N. V. D. D. Centen's Uitgevers Maatschappij, 1934.

(2) Behrens, H., "Manual of Microchemical Analysis," London, Macmillan & Co., 1894.

(3) Behrens, H., "Microchemische Analyse organischen Verbindungen," Voss, Leipzig, 1895–7.
(4) Chamot, E. M., and Mason, C. W., "Handbook of Chemical Microscopy," New York, John Wiley & Sons, 1931.
(5) Emich, F., and Schneider, "Microchemical Laboratory Manual," New York, John Wiley & Sons, 1930.
(6) Hartshorne, N. H., *J. Chem. Soc.*, 1934, 1830.
(7) Haushofer, H., "Microchemische Reaktionen," Braunschweig, 1885.
(8) Hoffman, H., Jr., and Johnson, W. C., *J. Assoc. Official. Agr. Chem.*, 13, 367 (1930).
(9) Kohlschutter, H. W., *Kolloidchem. Beihefte*, 24, 319 (1927).
(10) Labanukrom, Toa, *Ibid.*, 29, 80 (1929).
(11) Molisch, H., "Mikrochemie der Pflanze," 3rd ed., Jena, Gustav Fischer, 1923.
(12) Shead, A. C., *Proc. Oklahoma Acad. Sci.*, 15, 86 (1935).
(13) *Ibid.*, 16, 87 (1936).
(14) Streng, A., "Neues Jahrbuch Mineralogie," 2nd ed., p. 429, 1888.
(15) Vogelsang, "Die Crystalliten," Bonn, 1875. (See crystallites. Encyclopedia Britannica, 14th ed.)
(16) Wagenaar, M., *Pharm. Weekblad*, 72, 642 (1935).
(17) Walcott, A. I., *Am. Mineral.*, 11, 236 (1926).
(18) Wormley, T. G., "Microchemistry of Poisons," Philadelphia, J. B. Lippincott, 1885.
(19) Zimmermann, A., "Botanical Microtechnique," Henry Holt & Co., 1901.

RECEIVED June 7, 1937. Presented before the Microchemical Section at the 93rd Meeting of the American Chemical Society, Chapel Hill, N. C., April 12 to 15, 1937.

# Volumetric Determination of Iodine Using Elek-Hill Microbomb

ADALBERT ELEK AND ROBERT A. HARTE, The Rockefeller Institute for Medical Research, New York, N. Y.

THE utilization of micromethods has become increasingly extensive, not only because of their great value in conserving precious material, but because of the considerable saving in time their application permits.

The introduction of the peroxide fusion as carried out in the Elek-Hill microbomb (*3*) has greatly speeded the processes of analysis for halogens (*1*, *3*), sulfur and phosphorus (*4*), and arsenic (*2*).

In order to eliminate the preparation, drying, and weighing of the filter, as well as the tedious filtration, washing, drying, and weighing of precipitates, volumetric methods are now supplanting gravimetric procedures wherever possible. As another step in this direction, the volumetric determination of iodine after peroxide fusion is described herein. Further experiments are in progress to develop volumetric methods for the determination of chlorine and bromine.

Using the Pregl spiral tube method, Leipert (*6*) and Goldberg (*5*) have described volumetric methods for iodine, based on conversion to iodate followed by treatment with iodide and titration with thiosulfate. However, the spiral tube combustion requires fragile apparatus and the exercise of great care by the analyst to ensure complete decomposition of the sample, especially in the case of volatile liquids. It is also more time-consuming than the bomb fusion, a half hour or more being needed for the combustion of the sample.

## Procedure

A sample of from 3 to 6 mg., accurately weighed, is ignited in the bomb exactly as described by Elek and Hill (*3*). After cooling, the bomb contents are dissolved in 15 to 20 ml. of hot water in a 125-ml. Erlenmeyer flask. When solution is complete, the bomb is removed from the flask and carefully rinsed. To the fluid sufficient boiled-out Alundum, grain size 16, is added to cover about two-thirds of the bottom of the flask, and the liquid is kept in moderate ebullition for 30 minutes, the whole of the free surface being covered with breaking bubbles. (In a preliminary experiment it was found that no peroxide was left in the solution at the end of this time. With shorter boiling, complete destruction of the peroxide could not be assured.) The use of Alundum instead of glass beads completely prevents bumping, even when this alkaline solution becomes very concentrated. After cooling under the tap, the solution is made just acid to methyl orange with 3 *N* sulfuric acid, run in from a 50-ml. buret. About 10 ml. are required. The acid solution is filtered, with suction, into a ground-glass-stoppered 125-ml. Erlenmeyer flask where it is treated with 1 ml. of saturated bromine water to ensure complete oxidation of the iodide to iodate. Iodine is almost quantitatively converted to iodate in the bomb (*3*), but the oxidation with bromine is necessary to make certain the completeness of the conversion.

After the solution has stood, stoppered, for 5 minutes the excess of bromine is destroyed by the addition of 2 to 3 drops of formic acid (*7*) with thorough shaking to remove the last traces of bromine from the vapor phase as well as from the solution. During this treatment a moderate pressure is built up in the flask, necessitating care in subsequently opening it. If, after 2 minutes, the solution is not completely decolorized, another drop of formic acid should be added with shaking. To the colorless solution 0.2 to 0.3 gram of potassium iodide is added, the flask is stoppered and shaken to dissolve the salt, and, after 5 minutes, the liberated iodine is titrated with 0.01 *N* sodium thiosulfate (prescribed instead of 0.02 *N*, as recommended by several authors, because of the greater accuracy it permits, in addition to its more general usefulness in the microanalytical laboratory), which is standardized daily against 0.01 *N* potassium biiodate.

One milliliter of 0.01 *N* thiosulfate solution is equivalent to 0.2115 mg. of iodine. Under the conditions described, using the purest grade of analytical reagents, the blank value is entirely negligible. Typical analytical results are given in Table I.

TABLE I. ANALYTICAL RESULTS

| Substance | Weight of Sample | 0.01 *N* Thiosulfate Required | Iodine Found | Iodine Theory |
|---|---|---|---|---|
| | *Mg.* | *Ml.* | % | % |
| Iodoacetamide | 3.922 | 12.70 | 68.51 | 68.64 |
| | 4.182 | 13.53 | 68.48 | |
| Iodoacetic acid | 3.912 | 12.63 | 68.33 | 68.27 |
| | 3.600 | 11.61 | 68.26 | |
| Iodoacetylsulfanilic acid Na | 5.311 | 8.85 | 35.27 | 35.26 |
| | 4.503 | 7.50 | 35.25 | |
| 2-Iodobutane | 6.633 | 21.62 | 68.99 | 69.02 |
| | 4.183 | 13.68 | 69.18 | |
| *p*-Iodobenzoic acid | 4.220 | 5.13[a] | 51.40 | 51.18 |
| | 3.919 | 4.74[a] | 51.20 | |
| Iodopropionic acid | 5.295 | 15.88 | 63.46 | 63.46 |
| | 3.218 | 9.64 | 63.38 | |
| Iodobutyric acid | 5.220 | 14.63 | 59.33 | 59.30 |
| | 4.202 | 11.76 | 59.23 | |
| 6-Iodo-4-tosyl-2,3-diacetylbenzylglucoside | 4.892 | 4.73 | 20.45 | 20.54 |
| | 5.396 | 5.23 | 20.49 | |

[a] 0.02 *N* thiosulfate solution was employed for the titration.

As seen from the duplicate analyses presented, the precision of the method is high. The results show a very satisfactory accuracy, the mean deviation of the sixteen analyses given from the theoretical values being less than ±2 parts per thousand. The time and labor involved are reduced materially when compared with existing methods, and as the number of analyses to be done is increased the economy of time becomes much more than simply proportionally greater.

## Literature Cited

(1) Beamish, F. E., IND. ENG. CHEM., Anal. Ed., 5, 348 (1933).
(2) Beamish, F. E., and Collins, H. L., *Ibid.*, 6, 379 (1934).
(3) Elek, A., and Hill, D. W., *J. Am. Chem. Soc.*, 55, 2550 (1933).
(4) *Ibid.*, 55, 3479 (1933).
(5) Goldberg, J. L., *Mikrochemie*, 14, 161 (1933).
(6) Leipert, T., *Mikrochem. Pregl Festschr.*, 266 (1929).
(7) Vieböck, F., and Brecher, C., *Ber. deut. chem. Ges.*, 63, 3207 (1930).

RECEIVED July 14, 1937.

# Determination of Alkyl and Aryl Halogen

## In the Presence of Each Other

WILLIAM H. RAUSCHER, Rensselaer Polytechnic Institute, Troy, N. Y.

THE use of monoethanolamine in the determination of halogens in organic combination has been previously reported (*1*). Experience gained in the use of this reagent suggested that it might be used to advantage in the determination of alkyl and aryl halogen in the presence of each other. Monoethanolamine has proved to be capable of quantitatively converting the alkyl or reactive halogen in a number of compounds, solutions, and mixtures, which contain both alkyl and aryl halogen, to the ionic form without affecting the aryl halogen present. The reaction is also quantitative when the halogenated compounds are present in dilute kerosene solution, thus making the reagent useful in the analysis of certain types of insecticides. The aryl halogen in such cases may be found by determining the total halogen by the procedure of Method I as outlined elsewhere (*1*), and then subtracting the alkyl halogen found.

The compounds, solutions, and mixtures listed in Table I were chosen to establish the range of usefulness of the method. The kerosene solutions of carbon tetrachloride and *p*-dichlorobenzene were chosen to simulate an insecticide. Where the alkyl and aryl substances used as pairs had sufficient mutual solubility and fairly close boiling points, known solutions of the two were made up in small glass-stoppered bottles and portions of the solutions used immediately for analysis. Two or more portions were used for determining the alkyl halogen present and two or more portions for the determination of the total halogen present. In those cases where mutual solubility was low, or boiling points widely different, small amounts of the two substances were weighed out in the glass-stoppered vials of Method I or the bomb tubes of Method II (*1*). The entire weighed mixture or solution was analyzed so that only the alkyl or total halogen content of the sample could be determined. The kerosene solutions were made up in small glass-stoppered bottles by weighing all the ingredients.

### Determination of Alkyl Halogen in the Presence of Aryl Halogen

The apparatus, materials, method of weighing samples, and the general procedure are the same as in Method II. Micro- and semimicrosamples of the kerosene solutions are weighed, but macrosamples may be measured with a Mohr pipet. One milliliter of the amine is used for microsamples contained in the small bomb tubes, and 3 to 4 ml. are used for macrosamples contained in the large bomb tubes. The kerosene solutions float on the amine, so that care must be used in sealing the bomb in order to avoid loss of the more volatile constituent. Microsamples are heated at the boiling point of diethanolamine for 0.5 hour and the macrosamples for 1 hour. After opening the bombs the procedure of Method II is followed exactly. Insoluble aromatic substances are brought into solution by the use of alcohol or dioxane.

TABLE I. RESULTS

| Weight of Sample | % Aryl Halogen / % Alkyl Halogen | Total Halogen: Weight of Ag halide | Total Halogen: Found | Total Halogen: Calcd. | Alkyl Halogen: Weight of Ag halide | Alkyl Halogen: Found | Alkyl Halogen: Calcd. | Aryl Halogen (by Difference): Weight of Ag halide | Aryl Halogen (by Difference): Found | Aryl Halogen (by Difference): Calcd. |
|---|---|---|---|---|---|---|---|---|---|---|
| *Mg.* | | *Mg.* | % | % | *Mg.* | % | % | *Mg.* | % | % |
| | | | | | *p*-Bromophenacyl Bromide | | | | | |
| 7.250 | 1.000 | ..... | ... | 57.52 | 4.912 | 28.83 | 28.76 | .... | ... | 28.76 |
| 7.578 | | ..... | ... | | 5.138 | 28.85 | | .... | ... | |
| 6.415 | | 8.669 | 57.51 | | ..... | ... | | .... | 28.67 | |
| 4.509 | | 6.102 | 57.59 | | ..... | ... | | .... | 28.75 | |
| | | | | | *p*-Chlorobenzyl Bromide | | | | | |
| 13.343 | 0.444 | ..... | ... | ... | 12.225 | 38.99 | 38.90 | .... | ... | 17.26 |
| 16.359 | | ..... | ... | | 14.937 | 38.86 | | .... | ... | |
| 7.584 | | 12.215 | ... | | ..... | ... | | 5.276 | 17.21 | |
| 11.164 | | 18.026 | ... | | ..... | ... | | 7.812 | 17.31 | |
| | | | | | *o*-Chlorobenzyl Chloride | | | | | |
| 21.146 | 1.000 | ..... | ... | 44.06 | 18.883 | 22.09 | 22.03 | .... | ... | 22.03 |
| 15.215 | | ..... | ... | | 13.590 | 22.10 | | .... | ... | |
| 12.371 | | 22.070 | 44.13 | | ..... | ... | | .... | 22.04 | |
| 10.031 | | 17.870 | 44.07 | | ..... | ... | | .... | 21.97 | |
| | | | | | *o*-Chlorobenzyl Bromide | | | | | |
| 36.34 | 0.440 | ..... | ... | ... | 33.32 | 39.02 | 38.90 | .... | ... | 17.26 |
| 25.52 | | ..... | ... | | 23.36 | 38.95 | | .... | ... | |
| 57.50 | | 92.70 | ... | | ..... | ... | | 40.03 | 17.22 | |
| 31.15 | | 50.32 | ... | | ..... | ... | | 21.79 | 17.30 | |
| | | | | | *p*-Bromobenzenesulfonyl Chloride | | | | | |
| 15.345 | 2.253 | ..... | ... | ... | 8.848 | 13.67 | 13.88 | .... | ... | 31.28 |
| 19.896 | | ..... | ... | | 11.098 | 13.80 | | .... | ... | |
| 13.868 | | 17.877 | ... | | ..... | ... | | 10.176 | 31.23 | |
| 8.677 | | 11.199 | ... | | ..... | ... | | 6.381 | 31.29 | |
| | | | | | β,β′-Dichlorodiethyl Ether and Chlorobenzene[a] | | | | | |
| 50.25 | 0.641 | ..... | ... | 40.52 | 50.27 | 24.75 | 24.69 | .... | ... | 15.83 |
| 34.95 | | ..... | ... | | 34.95 | 24.74 | | .... | ... | |
| 42.35 | | 69.29 | 40.47 | | ..... | ... | | .... | 15.73 | |
| 38.40 | | 62.90 | 40.52 | | ..... | ... | | .... | 15.78 | |
| 38.98 | 0.0160 | ..... | ... | 49.16 | 76.23 | 48.38 | 48.39 | .... | ... | 0.77 |
| 21.68 | | ..... | ... | | 42.35 | 48.32 | | .... | ... | |
| 46.02 | | 91.40 | 49.13 | | ..... | ... | | .... | 0.78 | |
| 20.60 | | 40.86 | 49.07 | | ..... | ... | | .... | 0.72 | |
| 83.68 | 15.87 | ..... | ... | 32.22 | 6.450 | 1.91 | 1.91 | .... | ... | 30.31 |
| 64.27 | | ..... | ... | | 4.970 | 1.91 | | .... | ... | |
| 15.42 | | 20.002 | 32.10 | | ..... | ... | | .... | 30.19 | |
| 30.40 | | 39.50 | 32.14 | | ..... | ... | | .... | 30.23 | |

[a] Solution.

(*Continued on next page*)

TABLE I. RESULTS (*Continued*)

| Weight of Sample | % Aryl Halogen / % Alkyl Halogen | Total Halogen: Weight of Ag halide | Total Halogen: Found | Total Halogen: Calcd. | Alkyl Halogen: Weight of Ag halide | Alkyl Halogen: Found | Alkyl Halogen: Calcd. | Aryl Halogen (by Difference): Weight of Ag halide | Aryl Halogen (by Difference): Found | Aryl Halogen (by Difference): Calcd. |
|---|---|---|---|---|---|---|---|---|---|---|
| *Mg.* | | *Mg.* | % | % | *Mg.* | % | % | *Mg.* | % | % |
| | | | | Acetylene Tetrachloride and Bromobenzene[b] | | | | | | |
| 7.121 | 0.0114 | ..... | ... | ... | 23.853 | 82.86 | 82.94 | .... | ... | 0.94 |
| 396.45 | 51.25 | ..... | ... | | 15.738 | 0.98 | 0.98 | .... | ... | 50.33 |
| 21.635 | 0.0498 | 70.240 | ... | | | ... | 78.05 | 1.978 | 3.89 | 3.89 |
| 61.754 | 41.68 | 75.860 | ... | | | ... | 1.20 | 72.854 | 50.20 | 50.20 |
| | | | | Hexachloroethane and Iodobenzene[b] | | | | | | |
| 53.28 | 0.122 | ..... | ... | ... | 164.55 | 76.40 | 76.37 | .... | ... | 9.34 |
| 174.70 | 1.880 | ..... | ... | ... | 170.40 | 24.13 | 24.13 | .... | ... | 45.48 |
| 39.361 | 34.73 | 47.17 | ... | ... | ..... | ... | 1.76 | 44.37 | 60.94 | 61.00 |
| 36.746 | 0.164 | 116.05 | ... | ... | ..... | ... | 72.63 | 8.17 | 12.02 | 11.93 |
| | | | | Carbon Tetrachloride and *p*-Dichlorobenzene[b] | | | | | | |
| 73.24 | 11.75 | ..... | ... | 50.13 | 11.820 | 3.99 | 3.93 | .... | ... | 46.20 |
| 64.95 | 5.918 | ..... | ... | 51.83 | 19.644 | 7.48 | 7.49 | .... | ... | 44.34 |
| 16.246 | 0.0275 | ..... | ... | 90.00 | 57.584 | 87.62 | 87.58 | .... | ... | 2.42 |
| 17.959 | 3.035 | 39.675 | 54.64 | 54.72 | ..... | ... | 13.56 | .... | 41.08 | 41.16 |
| 16.162 | 0.0228 | 59.020 | 90.50 | 90.53 | ..... | ... | 88.51 | .... | 1.99 | 2.02 |
| 15.620 | 0.0067 | 57.873 | 91.66 | 91.65 | ..... | ... | 91.03 | .... | 0.63 | 0.62 |
| 33.126 | 0.0074 | 122.65 | 91.59 | 91.59 | ..... | ... | 90.92 | .... | 0.67 | 0.67 |
| 18.192 | 0.0300 | 66.080 | 89.85 | 89.82 | ..... | ... | 87.20 | .... | 2.65 | 2.62 |
| | | | | Carbon Tetrachloride and *p*-Dichlorobenzene (in Kerosene) | | | | | | |
| 7.848 | 1.769 | ..... | ... | 8.03 | 0.924 | 2.91 | 2.90 | .... | ... | 5.13 |
| 8.515 | | ..... | ... | | 0.970 | 2.82 | | .... | ... | |
| 13.405 | | ..... | ... | | 1.460 | 2.69 | | .... | ... | |
| 107.10 | | ..... | ... | | 12.497 | 2.88 | | .... | ... | |
| 141.55 | | ..... | ... | | 16.786 | 2.93 | | .... | ... | |
| 19.94 | | 6.491 | 8.10 | | ..... | ... | | .... | 5.20 | |
| 51.52 | | 16.840 | 8.08 | | ..... | ... | | .... | 5.18 | |
| 851.4 (1 ml.) | | ..... | ... | | 98.55 | 2.86 | | .... | ... | |
| 851.4 | | ..... | ... | | 98.96 | 2.88 | | .... | ... | |
| 425.7 | | 137.30 | 8.01 | | ..... | ... | | .... | 5.11 | |
| 425.7 | | 137.65 | 8.04 | | ..... | ... | | .... | 5.14 | |

[b] Mixture.

No attempt is made to bring the kerosene into solution. Spinning coagulation of the silver halide is the more convenient and rapid method of coagulation.

For compounds of the type *p*-chlorobenzyl bromide and combinations of the type $\beta,\beta'$-dichlorodiethyl ether–chlorobenzene, it suffices to reflux the sample with monoethanolamine alone in the open test tube–cold finger procedure of Method I (*1*). For the first type of compound 10 minutes' refluxing is sufficient to convert the alkyl halogen to the ionic form. In other cases the time allowed should be extended.

## Determination of Aryl Halogen in the Presence of Alkyl Halogen

To determine the percentage of aryl halogen the percentage of total halogen present is found and from this is subtracted the percentage of alkyl halogen found, provided that both halogens present are the same. When the two halogens present are not the same the calculation of aryl halogen must be handled differently. In this case the weight of mixed silver halides from the total halogen present in the sample is first determined. From this weight is subtracted the weight of the silver halide from the alkyl halogen present to get the weight of silver halide from the aryl halogen. The weight of "alkyl" silver halide is obtained by multiplying the sample weight by the percentage of alkyl halogen and dividing by the proper gravimetric factor.

In either case the total halogen is converted to the ionic form by the use of sodium, monoethanolamine, and dioxane exactly as in Method I.

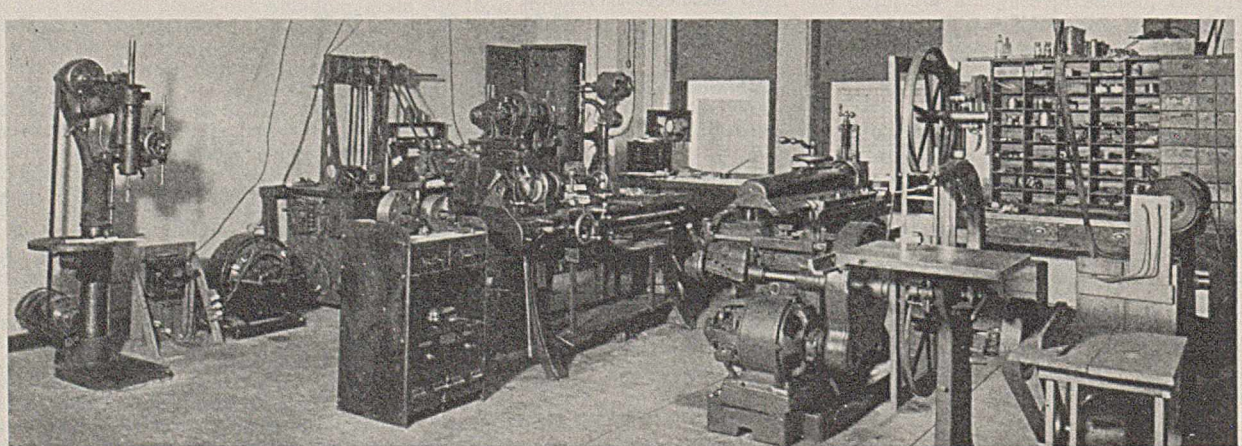
## Summary

A simple and rapid method for the determination of alkyl and aryl halogen in the presence of each other has been developed. It appears to be applicable to a variety of problems and may be used on a micro, semimicro, or macro scale. The procedure may, of course, be used qualitatively.

## Literature Cited

(1) Rauscher, IND. ENG. CHEM., Anal. Ed., 9, 296 (1937).

RECEIVED July 27, 1937. Abstract of part of the Ph.D. dissertation of William H. Rauscher in the Graduate School of Rensselaer Polytechnic Institute.



# *The Book*
# YOU HAVE WAITED FOR


***The first complete and practical book on pH technique and control, written in simple, understandable language.***

## WRITE FOR YOUR COPY *now!*

A de luxe edition showing the full line of Coleman pH electrometers, electrode assemblies and accessories, together with informative discussions of pH theory and practice. An interesting and valuable book for every pH operator or those interested in pH technique.

**56 pages—fully illustrated; from cover to cover—a valuable source book of pH material that will be welcomed by every one who engages in or contemplates pH research and practice.**

**As this de luxe edition is strictly limited, we advise an early reservation. Write today to any of the reliable dealers listed below in order to be sure to get your copy.**

BURRELL TECHNICAL SUPPLY CO.
1936 Fifth Avenue
Pittsburgh, Pa.

CINCINNATI SCIENTIFIC COMPANY
224 Main Street
Cincinnati, Ohio

EBERBACH & SON COMPANY
200 East Liberty Street
Ann Arbor, Michigan

GREENE BROTHERS, INC.
1812 Griffin Street
Dallas, Texas

PHIPPS & BIRD, INC.
915 E. Cary Street
Richmond, Virginia

GEO. T. WALKER & COMPANY
324 Fifth Avenue, South
Minneapolis, Minn.

**TO CONTROL—IS FIRST OF ALL TO KNOW**