INDUSTRIAL and ENGINEERING CHEMISTRY

ANALYTICAL EDITION

21,000 Copies of This Issue Printed

Vol. 31, Consecutive No. 23

Issued June 15, 1939

Harrison E. Howe, Editor

Vol. 11, No. 6

Publication Office: Easton, Penna.

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

Published by the American Chemical Society, Publication Office, 20th & Northampton Sts., Easton, Penna. Entered as second-class matter at the Post Office at Easton, Penna., 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.

RATES FOR CURRENT NUMBERS: Annual subscription rates: INDUSTRIAL AND ENGINEERING CHEMISTRY complete $6.00; (*a*) Industrial Edition $3.00; (*b*) Analytical Edition $2.50; (*c*) News Edition $1.50; (*a*) and (*b*) together, $5.00. Foreign postage to countries not in the Pan American Union, $2.40; (*a*) $1.20; (*b*) $0.60; (*c*) $0.60. Canadian postage one third these rates. Single copies: (*a*) $0.75; (*b*) $0.50; (*c*) $0.10. Special rates to members.

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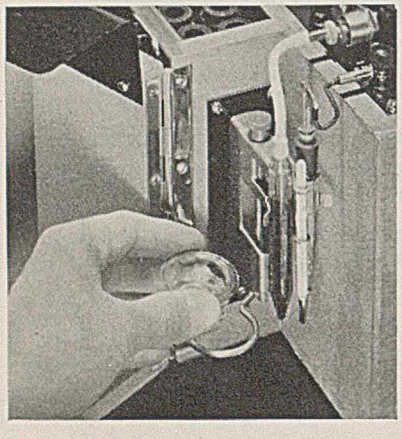

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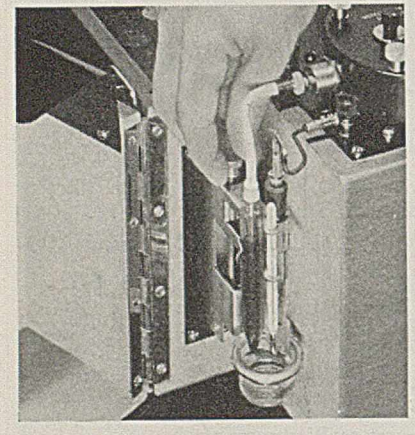

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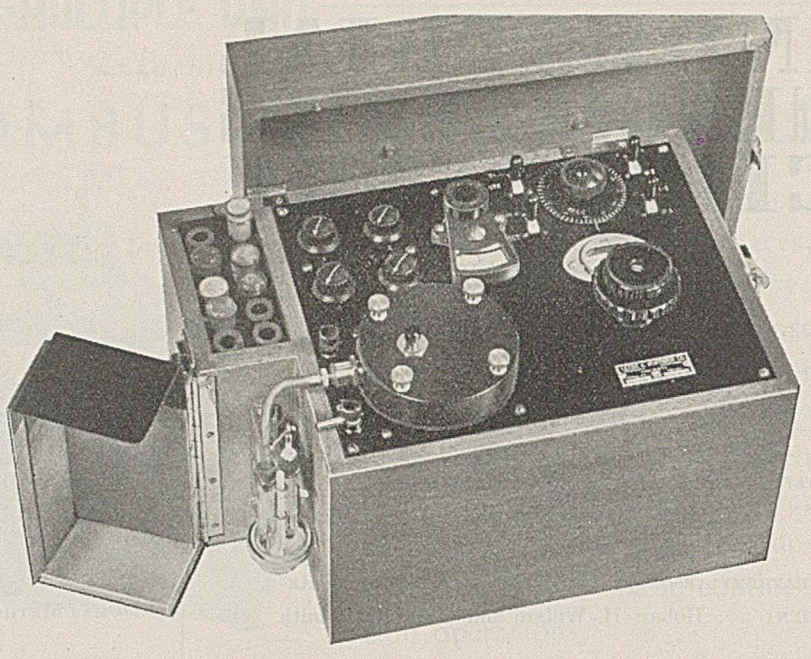

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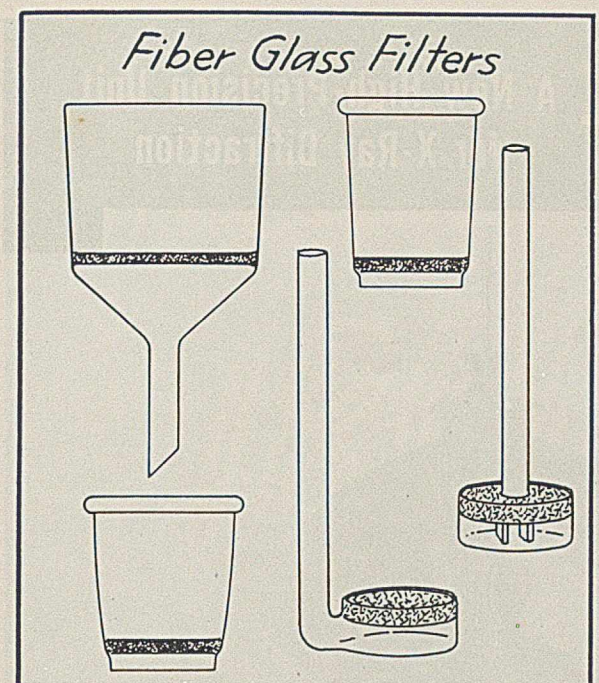

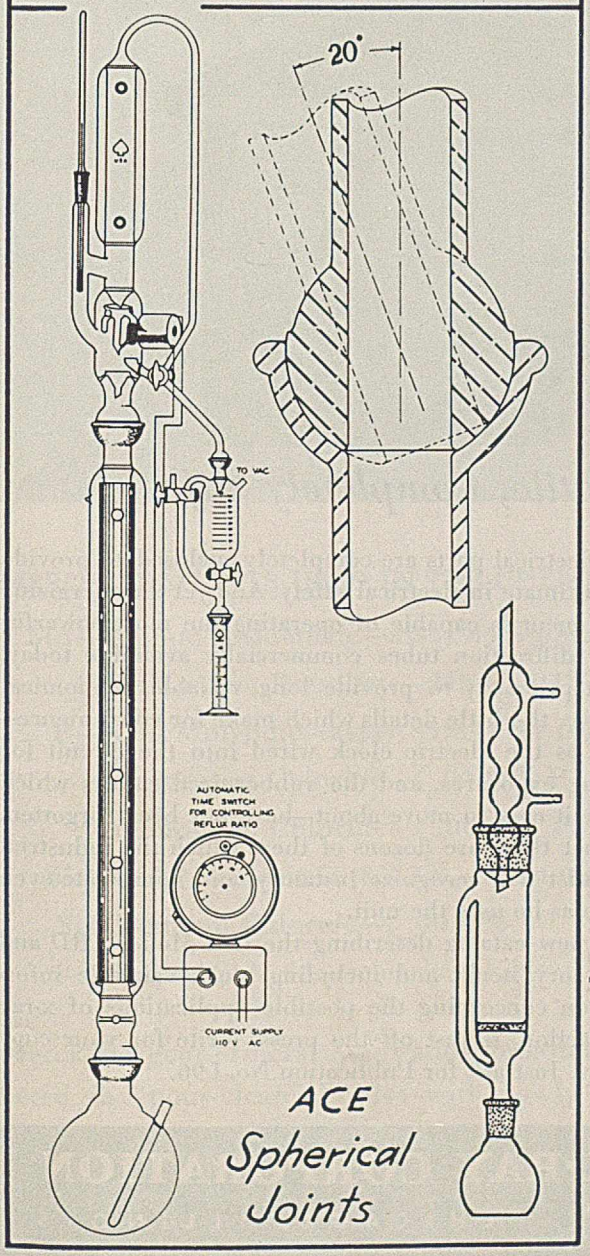

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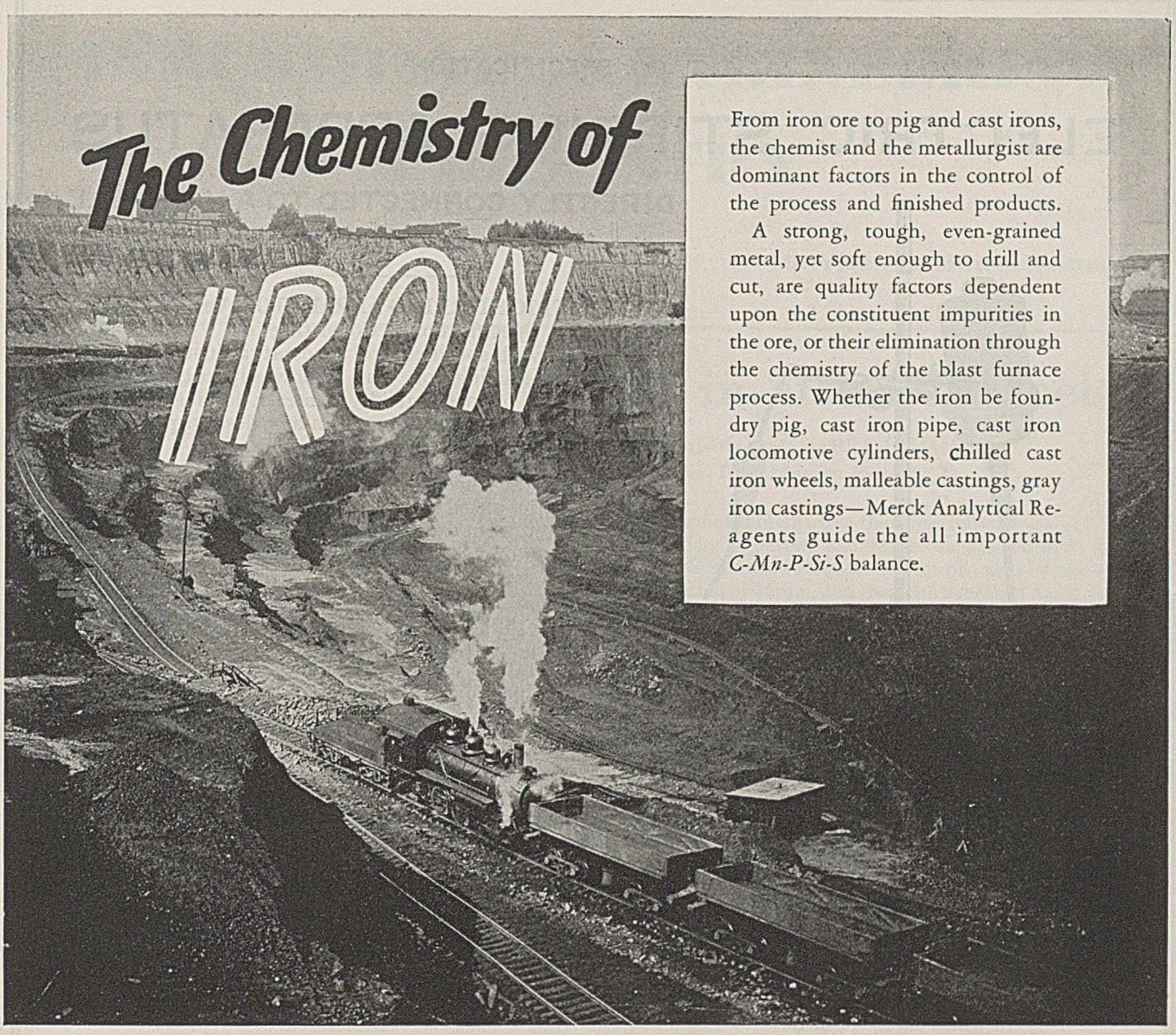

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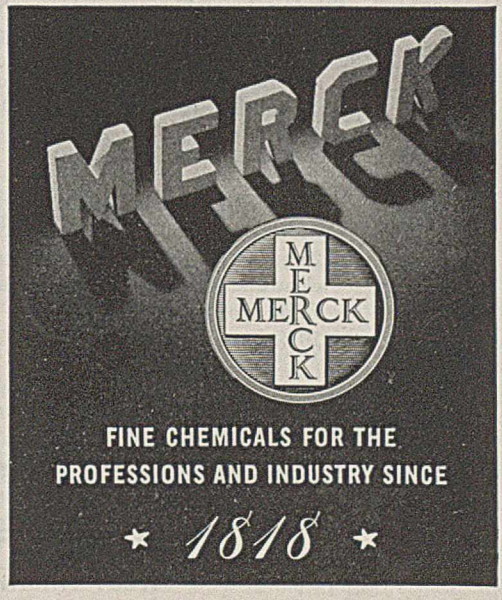

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INDUSTRIAL and ENGINEERING CHEMISTRY

ANALYTICAL EDITION ◆ Harrison E. Howe, Editor

Determination of Nitrogen in Stainless Steels

THOS. R. CUNNINGHAM, Electro Metallurgical Company, AND HARRY L. HAMNER, Union Carbide and Carbon Research Laboratories, Inc., Niagara Falls, N. Y.

THE commercial production of chromium steels containing nitrogen has necessitated the development of an accurate method for determining this element. The Allen method (*1*) for combined nitrogen in plain carbon steels consists in dissolving the sample of metal in the form of drillings or millings in hydrochloric acid (sp. gr. 1.11), making the solution alkaline with calcium oxide, distilling off the ammonia, and completing the determination with Nessler's solution.

Among the authors who have employed modifications of Allen's method are Tschischewski (*5*), Weiss and Englehardt (*6*), Ruff and Eisner (*4*), Johnson (*2*), and Jordan and Swindell (*3*). Jordan and Swindell's modification of Allen's method, which consists in dissolving the sample in hydrochloric acid (sp. gr. 1.11), adding a strong solution of potassium hydroxide, distilling the ammonia over into a measured excess of standard sulfuric acid, and titrating the excess acid with alkali, is applicable to most stainless steels, provided they do not contain any metals such as titanium, columbium, tantalum, tungsten, or vanadium, which form acid-insoluble nitrides. Tschischewski (*5*) stated that in a 0.35 per cent silicon and 0.84 per cent manganese steel he found a residue insoluble in hydrochloric acid that contained 0.000125 per cent of nitrogen. The authors have experienced no interference due to silicon, which undoubtedly is because of the use of hydrofluoric acid in the initial solution of the sample. However, the nitrogen in a titanium-treated steel, provided sufficient titanium is added, will be found almost entirely in the hydrochloric acid–insoluble residue. If the steel is alloyed with columbium, tantalum, or vanadium, only part of the nitrogen will be in the insoluble residue. Since one or more of these elements are frequently present in stainless steels, it is never safe to omit testing any acid-insoluble residue for nitrogen.

The amounts of nitrogen that were found in the acid-insoluble portions of several stainless steels are shown in Table I. This table also shows that results for nitrogen by the authors' modification of Jordan and Swindell's method agree very well with the results obtained by the vacuum fusion method. The method to be described has been used by this and other laboratories since 1932.

Table I. Analysis of Titanium and Columbium (Plus Tantalum) Stainless Steels for Nitrogen

Type of Steel	Nitrogen: Acid soluble	Nitrogen: Acid insoluble	Composition of Steel: Total N	Cr	Ni	C	Cb	Ta	Ti	Mn	Si
	%	%	%	%	%	%	%	%	%	%	%
18-8 + Cb	0.031	0.015	0.046	17.81	9.18	0.07	0.75	0.09	Not over 0.01	0.52	0.36
18-8 + Cb	0.15	0.07	0.21 0.22[a]	18.18	8.80	0.062	1.10	0.04	..	..	..
18-8 + Ti	0.006	0.042	0.048	18.21	9.17	0.158	..	..	1.56	0.31	0.54
24 Cr + Ti	0.009	0.300	0.309 0.309[a]	23.98	..	0.104	..	..	1.36	..	..
24 Cr + Ti	0.011	0.270	0.281	23.67	1.18	0.094	..	..	1.27	..	..

[a] Results obtained by vacuum fusion.

Method

Five grams of the sample are transferred to a large platinum dish (300-ml.) provided with a tight-fitting cover, and treated with 50 to 60 ml. of dilute hydrochloric acid (1 to 1, prepared by mixing ammonia-free water with hydrochloric acid, sp. gr. 1.19, from a fresh bottle that has just been opened), a little at a time, until the violent reaction ceases. Three milliliters of hydrofluoric acid (48 per cent, from a freshly opened bottle) are next added and the dish and its contents are heated on a hot-water bath until the solution of the alloy is practically complete. Should the alloy dissolve completely in the hydrochloric acid, the addition of hydrofluoric acid may be omitted and the solution of the alloy may be effected in a 150-ml. covered beaker.

While the alloy is being dissolved, 100 ml. of sodium hydroxide (500 grams per liter), several small pieces of mossy zinc, about 400 ml. of water, and 20 grams of tartaric acid are transferred to a 500-ml. Kjeldahl flask connected to a spray trap and a block-tin condenser. The apparatus used is shown in Figure 1. Two hundred milliliters of the solution are distilled over and discarded, and the alkaline solution in the Kjeldahl flask is then allowed to cool.

The solution of the alloy is removed from the hot-water bath, allowed to cool, and added to the sodium hydroxide solution in the Kjeldahl flask. The tartaric acid previously added to the alkaline solution serves to hold most of the iron and chromium in solution and thus makes the distillation much easier. The dish is rinsed successively with four 50-ml. portions of ammonia-free water. The contents of the flask are then boiled until 200 ml. of the distillate have passed over. The distillate is collected in 25 ml. or more of standard 0.02 *N* hydrochloric acid, depending upon

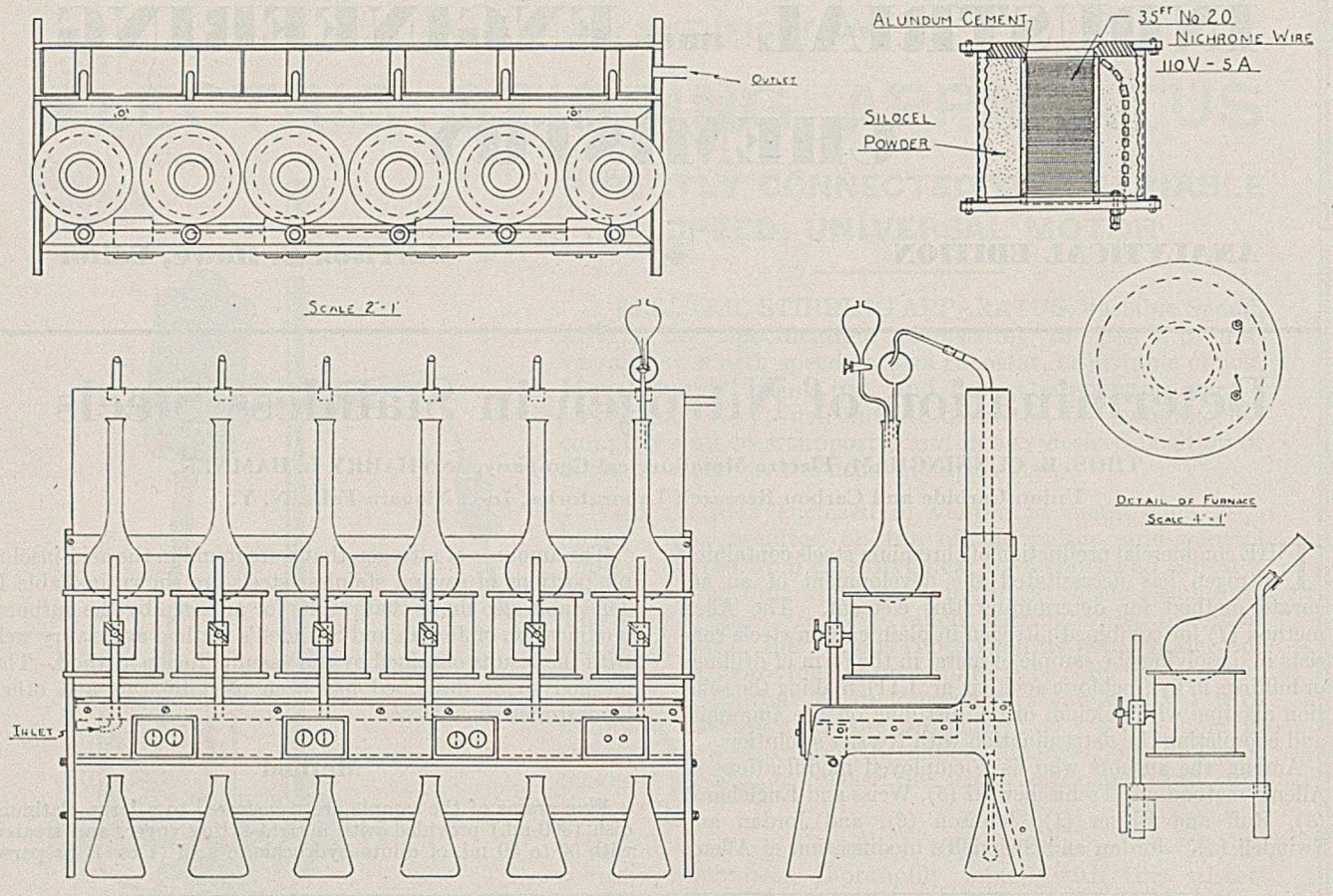

FIGURE 1. DIAGRAM OF APPARATUS

the nitrogen content of the alloy. The excess of acid is titrated with standard 0.02 *N* sodium hydroxide solution. Two drops of a 1 per cent aqueous solution of sodium alizarin sulfonate are used as an indicator. The end point is marked by the complete disappearance of the clear yellowish green color, or the first indication of a brown. Each milliliter of 0.02 *N* hydrochloric solution is equivalent to 0.00028 gram of nitrogen. A blank is run on all the reagents used and any nitrogen found is deducted. For steels containing very low percentages of nitrogen, solutions weaker than 0.02 *N* are used.

Should the steel contain vanadium, titanium, columbium, tantalum, or any other metals known to form a nitride insoluble in hydrochloric acid the solution obtained as described in the first paragraph should be filtered on a 9-cm. filter and the residue washed well with 1 per cent hydrochloric acid. The nitrogen in the filtrate is determined as described in paragraphs 2 and 3. The paper and insoluble residue are transferred to a 500-ml. Kjeldahl flask, 10 grams of potassium sulfate, 1 gram of copper sulfate, and 20 ml. of sulfuric acid (sp. gr. 1.84) are introduced, and the flask and its contents are heated just below the boiling point of the acid until all frothing ceases. At no time during the digestion should the part of the flask above the surface of the liquid be heated. The liquid is next heated to boiling and the boiling continued for from 15 to 30 minutes after the solution has become colorless. The solution is allowed to cool, 200 to 250 ml. of ammonia-free water are added, the flask is connected to the condenser, 150 ml. of 10-per cent sodium hydroxide solution are added, and the nitrogen in this solution is determined as described in paragraph 3. A blank is run on all the reagents used, including the filter paper, and any nitrogen so found is deducted. Any nitrogen found after deducting the "blank" is added to that obtained by acid solution of the sample and distillation, to obtain the total nitrogen.

Ammonia-free water is prepared by dissolving 200 grams of potassium hydroxide and 8 grams of potassium permanganate in 1100 ml. of distilled water and boiling the solution until the volume has been reduced to approximately 1000 ml. This solution is added to the water to be purified in the ratio of 1 to 10. Distillation is then carried on until a test of 100 ml. of the distillate does not require more than 1 or 2 drops of 0.02 *N* hydrochloric acid solution. Two drops of a 1 per cent aqueous solution of sodium alizarin sulfonate are used as the indicator.

Literature Cited

(1) Allen, A. H., *J. Iron Steel Inst.* (London), 8, 181 (1880).
(2) Johnson, C. M., *Iron Age*, 134, (July 26, 1934).
(3) Jordan, L., and Swindell, F. E., Natl. Bur. Standards, *Sci. Paper* 457 (Nov. 8, 1922).
(4) Ruff, O., and Eisner, F., *Ber.*, 41, 2252 (1908).
(5) Tschischewski, N., *J. Iron Steel Inst.* (London), 92, Part II, 47–90 (1915).
(6) Weiss, L., and Englehardt, T., *Z. anorg. Chem.*, 65, 38–104 (1909).

PRESENTED before the Division of Physical and Inorganic Chemistry at the 97th Meeting of the American Chemical Society, Baltimore, Md.

Correction

In an article entitled, "A Modification of the Berl-Kullmann Melting Point Block" [IND. ENG. CHEM., Anal. Ed., 9, 340 (1937)] failure was inadvertently made to mention an article by Matthäus and Sauthoff [*Chem. Fabrik*, 8, 92 (1935)], who have designed a block in which reflections from the surface of the melting point tube are eliminated by illumination from above. Unfortunately, this article was not abstracted by the American or British abstracts, upon which dependence was placed in the literature search (an abstract was later found in *Centralblatt*). The author wishes to acknowledge the priority of Matthäus and Sauthoff in regard to the feature of the block mentioned.

F. W. BERGSTROM

STANFORD UNIVERSITY, CALIF.
April 24, 1939

Tetraphenylarsonium Chloride as an Analytical Reagent

Determination of Rhenium

HOBART H. WILLARD AND GEORGE M. SMITH[1]
University of Michigan, Ann Arbor, Mich.

THE perrhenate ion unites with the tetraphenylarsonium ion, in the reaction

$$(C_6H_5)_4As^+ + ReO_4^- = (C_6H_5)_4AsReO_4$$

to form a white, crystalline precipitate which is insoluble in cold water. This permits the quantitative determination of perrhenate both potentiometrically, by the titration of the excess reagent with iodine (*1*), and gravimetrically. The latter is usually more convenient and is the method described in this paper. The potentiometric titration is, however, equally satisfactory.

The determination is carried out by adding an excess of tetraphenylarsonium chloride to the perrhenate, keeping the volume as small as possible. The precipitate, which is allowed to stand several hours, preferably overnight, is filtered through a Gooch crucible, washed several times with ice water, dried, and weighed as $(C_6H_5)_4AsReO_4$. The precipitation is carried out in a hot solution, in the presence of a neutral salt, such as sodium chloride or sodium sulfate, to make the precipitate more granular and more easily transferred. The most satisfactory precipitation medium is 0.5 molar sodium chloride. Nitrates, except in very low concentration, should be avoided because of the limited solubility of tetraphenylarsonium nitrate.

TABLE I. GRAVIMETRIC DETERMINATION OF PERRHENATE

(Volume, 25 to 60 ml.; NaCl, 0.5 molar)

Perrhenate Present	Perrhenate Found	Error
Mg.	*Mg.*	*Mg.*
0.44	0.40	−0.04
0.89	0.91	+0.02
1.33	1.26	−0.07
1.78	1.82	+0.04
2.22	2.21	−0.01
4.44	4.43	−0.01
13.32	13.33	+0.01
17.76	17.82	+0.06
22.21	22.21	±0.00
22.21	22.17	−0.04
44.41	44.39	−0.02
88.82	89.00	+0.18
133.24	133.18	−0.06

Determinations were attempted in the presence of most of the common anions and cations. Anions, such as permanganate, periodate, perchlorate, thiocyanate, iodide, bromide, and fluoride, which unite directly with the tetraphenylarsonium ion to form insoluble compounds, should be absent. Those cations the halide complexes of which form insoluble tetraphenylarsonium compounds in the presence of 0.5 molar chloride ions interfere except in very low concentration. High concentrations, approaching saturation, of any substance should be avoided to prevent the precipitation of excess reagent. In general, the presence of other substances in solution causes the formation of a precipitate which is more easily transferred.

Procedure

In all determinations a standard solution, made by dissolving pure potassium perrhenate in water containing 8.8824 mg. of perrhenate ion per ml. was used. To a definite volume of the hot solution, containing sufficient sodium chloride to make the final solution about 0.5 molar, a measured excess of tetraphenylarsonium chloride is added. The total volume should be 25 to 60 ml. The mixture is stirred and allowed to stand several hours, preferably overnight.

The precipitate is filtered through a Gooch crucible, washed several times with ice water, and dried at 110° C. It is weighed as tetraphenylarsonium perrhenate, $(C_6H_5)_4AsReO_4$. Multiplying by the factor 0.3952 converts this weight to perrhenate, ReO_4^-.

TABLE II. EFFECT OF ACIDITY AND ANIONS ON PERRHENATE DETERMINATIONS

(ReO_4^-, 22.21 mg.; total volume, 25 to 30 ml.)

Substance Present	Molar Concentration	Perrhenate Error, Mg.
HCl	0.5	+0.04
HCl	4.8	+0.08
HNO_3	0.7	+1.54
H_2SO_4	0.8	+0.09
H_2SO_4	3.6	+1.30
H_3PO_4	0.8	+0.07
$HC_2H_3O_2$	6.9	+0.19
Citric acid	0.2	0.00
Tartaric acid	0.6	+0.08
Oxalic acid	0.3	+0.10
Na_2SO_4	0.5	−0.01
Na_2WO_4	0.1	+0.04
Na_2HPO_4	0.1	−0.11
NH_4OH	6.0	+0.03
NaOH	0.5	−0.26

In this way quantities of perrhenate varying from 0.40 to 133 mg. have been determined with satisfactory accuracy in the presence of various other ions. Typical data are shown in Table I. Many of these gravimetric results were duplicated by the potentiometric titration of the excess of reagent with iodine.

TABLE III. EFFECT OF CATIONS ON DETERMINATION OF PERRHENATE (INCLUDING METAVANADATE, VO_3^-)

(Volume, 25 to 35 ml.; NaCl, about 0.5 molar)

Ion Present	Quantity of Ion	Perrhenate Present	Perrhenate Found	Error
	Mg.	*Mg.*	*Mg.*	*Mg.*
Al^{+++}	112	22.21	22.13	−0.08
Ba^{++}	565	44.41	44.34	−0.07
Ca^{++}	360	44.41	44.34	−0.07
Cd^{++}	440	22.21	22.17	−0.04
Co^{++}	210	1.33	1.26	−0.07
Cr^{+++}	215	44.41	44.50	+0.09
Cu^{++}	255	44.41	44.34	−0.07
Fe^{++}	200	2.22	2.29	+0.07
Fe^{+++}	206	2.22	2.13	−0.09
Fe^{+++}	206	44.41	44.34	−0.07
Mg^{++}	100	22.21	22.21	0.00
Mn^{++}	275	22.21	22.21	0.00
Ni^{++}	250	22.21	22.13	−0.08
Sb^{+++}	366	22.21	22.17	−0.04
UO_2^{++}	635	22.21	22.30	+0.09
VO^{++}	200	44.41	45.01	+0.60
VO^{++}	800	22.21	23.67	+1.46
VO^{++}	1000	4.44	6.97	+2.53
VO_3^-	35	44.41	44.57	+0.16
VO_3^-	650	22.21	22.31	+0.10
VO_3^-	520	4.44	4.55	+0.11
Zn^{++}	260	22.21	22.17	−0.04

Similar determinations were satisfactorily made under conditions of acidity varying from weakly alkaline to fairly strongly acidic. Sodium hydroxide has a solvent action on the precipitate, but a relatively high concentration of ammonium hydroxide is not harmful. Nitric acid or nitrates, except in very low concentration, will cause coprecipitation of tetraphenylarsonium nitrate with the perrhenate. High results are obtained with high concentrations of hydrochloric

[1]Present address, Vanderbilt University, Nashville, Tenn.

acid or other acids, probably because of the decreased solubility of the reagent under such conditions. Bromide, iodide, and fluoride, in more than traces, should be absent. Tungstate does not interfere. Results are shown in Table II.

TABLE IV. EFFECT OF NITRATE AND MOLYBDATE ON PERRHENATE DETERMINATION

(Volume, 20 to 35 ml.; NaCl, about 0.5 molar)

Substance Present	Concentration	Perrhenate Present	Perrhenate Found	Error
		Mg.	*Mg.*	*Mg.*
NH_4NO_3	0.5 *M*	44.41	50.98	+6.57
$NaNO_3$	0.3 *M*	22.21	25.77	+3.56
$NaNO_3$	0.016 *M*	22.21	22.40	+0.19
$NaNO_3$	0.1 *M*	0.89	0.91	+0.02
$NaNO_3$	0.05 *M*	0.44	0.40	−0.04
	Mg. MoO_3			
MoO_3 + 4 ml. NH_4OH	315	22.21	22.13	−0.08
MoO_3 + 3 g. tartaric acid	315	22.21	22.21	0.00
MoO_3 + 4 ml. NH_4OH	210	2.22	2.25	+0.03
MoO_3 + 4 ml. NH_4OH	210	44.41	44.34	−0.07
MoO_3 + 3 g. tartaric acid	210	44.41	44.26	−0.15
MoO_3 + 4 ml. NH_4OH	100	0.44	0.40	−0.04
MoO_3 + 4 ml. NH_4OH	100	2.22	2.21	−0.01
MoO_3 + 2 ml. NH_4OH	100	22.21	22.29	+0.08
MoO_3 + 2 ml. NH_4OH	20	22.21	22.17	−0.04
MoO_3	100	22.21	120.26	+98.05

Interfering Substances

The effect of the presence of various cations is shown in Table III. Only those ions interfere which form insoluble chlorides or whose complex halides form insoluble salts with tetraphenylarsonium ion. These include mercuric, stannic, bismuth, tellurium, lead, vanadyl, and silver. Cadmium and zinc do not interfere if the chloride-ion concentration is low. Metavanadate ion in fairly high concentration does not interfere. All these ions serve to make the precipitate more granular.

The presence of nitrate even in small quantities may cause serious interference if the quantity of perrhenate is fairly large. However, the interference is not so pronounced for very small amounts of perrhenate. It is practically impossible to wash out all traces of tetraphenylarsonium nitrate from the heavier perrhenate precipitate. Typical data are shown in Table IV.

Molybdate ion forms a fairly insoluble precipitate with tetraphenylarsonium ion, but this precipitation is hindered or prevented altogether in the presence of ammonium hydroxide, tartrates, citrates, and their acids. Typical data are shown in Table IV.

Summary

From 0.40 to 133.0 mg. of perrhenate ion can be determined gravimetrically with tetraphenylarsonium ion, in moderate excess, in volumes from 25 to 60 ml.

The presence of a small amount of sodium chloride, about 0.5 molar, or of other salts, and heating before precipitation are very effective in producing a crystalline precipitate which is easily transferred and washed.

Accurate determinations may be made in solutions varying from strongly ammoniacal to fairly strongly acidic.

Permanganate, perchlorate, periodate, iodide, bromide, fluoride, thiocyanate, mercury, tin, vanadyl, and bismuth ions interfere.

Nitrate must be absent in all but very low concentrations. Interference by molybdate may be avoided by the use of ammonium hydroxide or tartaric acid.

Literature Cited

(1) Willard and Smith, IND. ENG. CHEM., Anal. Ed., 11, 186 (1939).

Determination of Riboflavin in Milk

By Photoelectric Fluorescence Measurements

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Riboflavin in milk was determined by adding 50 ml. of acetone to 25 ml. of milk, filtering, and comparing the fluorescence of the filtrate with that of a cube of uranium glass which had previously been calibrated against solutions containing known amounts of riboflavin. The comparison of fluorescence was made with a photoelectric cell and microammeter, using suitable glass filters.

RIBOFLAVIN or lactoflavin, the principal water-soluble pigment of milk and whey, is of interest because of its nutritional value as vitamin G (*8*), its behavior as a co-enzyme (*15*, *17*), as a hydrogen acceptor (*16*), and as a photosensitizer for the oxidation of vitamin C in milk by light (*5*, *6*, *12*). The riboflavin content of milk can be determined rapidly and accurately by using suitable light filters and standards and measuring with a photoelectric cell and microammeter the fluorescence produced in a filtered acetone extract of milk or whey.

Methods for the determination of riboflavin so far described have involved visual estimation of color or fluorescence intensity by comparison with standards, or photoelectric measurement of either light absorption or fluorescence intensity. Charite and Khaustov (*1*) compared riboflavin extracts with a standard solution of potassium chromate in a colorimeter. Kuhn, Wagner-Jauregg, and Kaltschmitt (*11*) determined the chloroform-soluble, photochemical decomposition product with a stage photometer. Koschara (*7*) purified the riboflavin solutions with chromatographic adsorption and oxidation by permanganate, and determined concentrations directly in a stage photometer. Sullivan (*13*) measured the light absorption by the use of filters and a photoelectric cell. Euler and Adler (*3*) and later Supplee, Ansbacher, Flanigan, and Hanford (*14*) and Whitnah, Kunerth, and Kramer (*20*) compared visually the fluorescence of unknown and standard riboflavin solutions. Weisberg and Levin (*19*) described a similar method but used fluorescein solutions as standards. Cohen (*2*) measured the intensity of fluorescence directly with a photoelectric cell and amplifier, using fluorescein as a standard.

The methods based on light absorption have the advantage that the absorption coefficients reported are in absolute units and can be determined very accurately. However, many of the colored materials accompanying riboflavin interfere with the determination. Fluorescence measurements are more

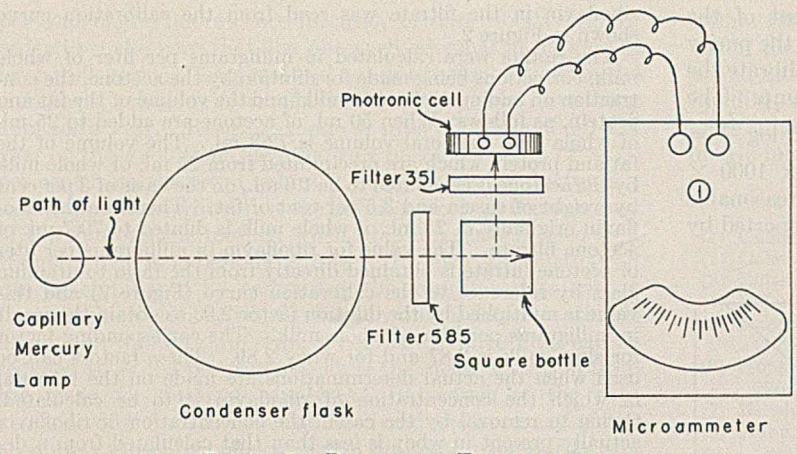

FIGURE 1. DIAGRAM OF FLUOROMETER

specific. Perhaps no other substance present in the acetone extract of the natural products yields a green fluorescence. A blue or violet fluorescence, sometimes observed, can be screened out by means of light filters. More important still, riboflavin occurs in milk in an amount convenient for fluorescence measurement but too small for ordinary direct colorimetric determination. The range for colorimetric determination is 4 to 40 mg. per liter, while the range for the fluorescence method is 0.1 to 4 mg. per liter.

Construction of Apparatus

The author's apparatus consists of a photoelectric cell, a microammeter, a capillary mercury lamp, two glass filters, a block of uranium glass, and a square glass bottle, arranged as shown diagrammatically in Figure 1, and mounted in a light-tight housing which definitely maintains the positions of the various parts. A liter Pyrex flask filled with distilled water serves as a condenser.

[A sufficiently sensitive photronic cell can be obtained from Pfalz and Bauer, Empire State Bldg., New York, N. Y. The microammeter was full-scale 15s, 150 ohms internal resistance. The capillary mercury lamp was G. E. vertical type H-4, 100-watt, nonex envelope. The glass filters were from Corning, N. Y., 5 cm. (2 inches) square and unpolished, Nos. 585 and 351. The 25-mm. uranium glass cube was also from Corning. The square bottle was a 30-ml. dropping bottle, A. H. Thomas No. 2248.]

The filters were selected to give maximum intensity of fluorescence and to absorb all stray light which might strike the photocell. The transmission of the first filter corresponds to the absorption of riboflavin between 3000 and 5000 Å.; the second filter absorbs nearly all the light in the range transmitted by the first filter, cutting the light off sharply below 5000 Å. Thus no blue or violet fluorescence can interfere and the effect of scattered light from slightly turbid solutions is minimized.

The separate units in the instrument were selected so as to provide sufficient fluorescence intensity for direct reading without the use of an amplifier and to minimize the destruction of riboflavin by light during the time of measurement. In the apparatus described here a 10 per cent destruction of riboflavin occurred in one minute, but since only 2 to 3 seconds are required for a reading, destruction of riboflavin during the determination is considered negligible. If the riboflavin is completely reduced or destroyed by light, readings on the instrument will fall to zero.

A slight fluctuation in the intensity of the light source is unavoidable. The lamp is operated by the 110-volt alternating current with the aid of a transformer and gives a remarkably steady light, much steadier than a tungsten lamp connected to the same line. To eliminate the error due to fluctuations in light intensity, readings for the unknown are compared immediately to the readings produced by the fluorescence of a cube of uranium glass used as a working standard. From the ratio of these two readings the riboflavin concentration is calculated. It is impracticable to use riboflavin or fluorescein directly as standard solutions because both are destroyed by light. Riboflavin is used as an ultimate standard for comparison with the uranium glass and the uranium glass is used as a working standard for comparison with the unknown solutions.

The uranium glass can be mounted conveniently in a square bottle identical with that used to hold the solutions to be tested. The bottle is first cut in two just below the shoulder. A piece of black paper containing a centered opening 1 cm. square should be pasted to the surface of the uranium glass facing the photocell. The base of the uranium glass is then imbedded in sealing wax and the bottle is cemented together. (The bottles are very easily cut after scratching all the way around, by touching each corner momentarily with a hot glass rod. The cracks are then led together by means of the hot rod. LePage's waterproof cement has proved useful in cementing the pieces together. The seal is permanent if kept dry.)

Calibration

The fluorescence intensity of pure riboflavin was obtained by comparing the fluorescence of four purified riboflavin preparations with the standard uranium glass. (L. C. Norris kindly gave the author a sample of synthetic riboflavin supplied by R. Kuhn. Three commercial preparations of pure riboflavin were obtained from the Vitamin Products Company, Emoryville, Calif., and Borden Company, Bainbridge, N. Y.) These preparations were weighed in duplicate on a microbalance and stock solutions containing 0.1 mg. per ml. in 20 per cent alcohol were prepared and kept in the dark at 10° C. Table I gives the results. Since any deterioration of riboflavin would weaken its fluorescence, it is likely that the higher results with preparation 2 are more nearly correct. Moreover, preparation 2 was obtained more recently than the other three and was the only one of the four which dissolved without leaving a residue. A sample purchased a year later was found to be identical with the earlier sample. These stock solutions of riboflavin did not deteriorate in a year's time.

The fluorometer was calibrated by determining the variation of fluorescence intensity with concentration of riboflavin in a 66 per cent acetone filtrate from milk. For this calibration curve the stock solution of preparation 2 was added to an acetone filtrate from milk from which the riboflavin had been completely removed by exposure to sunlight. In this way the necessity of correcting for the riboflavin originally in the milk was avoided. Results are shown in Figure 2.

The absorption coefficients of riboflavin with various filter combinations were also measured as a further check on the purity of the preparations. As shown by Table I, the measurements of absorption coefficients agreed with the fluorescence measurements in indicating that the other preparations

TABLE I. COMPARISON OF RIBOFLAVIN PREPARATIONS

Preparation No.	Fluorescence[a] Intensity	Absorption Coefficient[b] 5 mg./liter	10 mg./liter
1	1.26	0.104	0.210
2	1.36	0.124	0.244
3	1.21	0.106	0.215
4	1.30	...	0.223
No. 2 in acetone - milk filtrate	1.20	...	...
Fluorescein	1.62	...	...

[a] Fluorescence intensity equals the ratio of stock solution readings to uranium glass readings with filters 585 and 351. The stock solutions (20 per cent alcohol) diluted to 1 mg. per liter in water. Fluorescein was Kahlbaum's, dissolved in 0.0011 *N* NaOH.

[b] Absorption coefficient equals $\log_{10} \frac{I_0}{I}$, in water solution, tubes 1.0 cm. in internal diameter, 6-volt tungsten lamp, filters 585 + 556, photronic cell, microammeter of 150 ohms.

were less pure than preparation 2. Measurement of the absorption coefficient provides a valuable check on the purity of a riboflavin standard when it is necessary to recalibrate the apparatus—e. g., after replacing the mercury lamp. The molar extinction coefficient can be calculated from $\log \frac{I_0}{I} =$ 0.0244 for 1 mg. per liter by multiplying by $364 \times 1000 \times 2.303$ and is found to be 20.5×10^3. This is in reasonable agreement with the value of 24×10^3 at 445 mμ, reported by

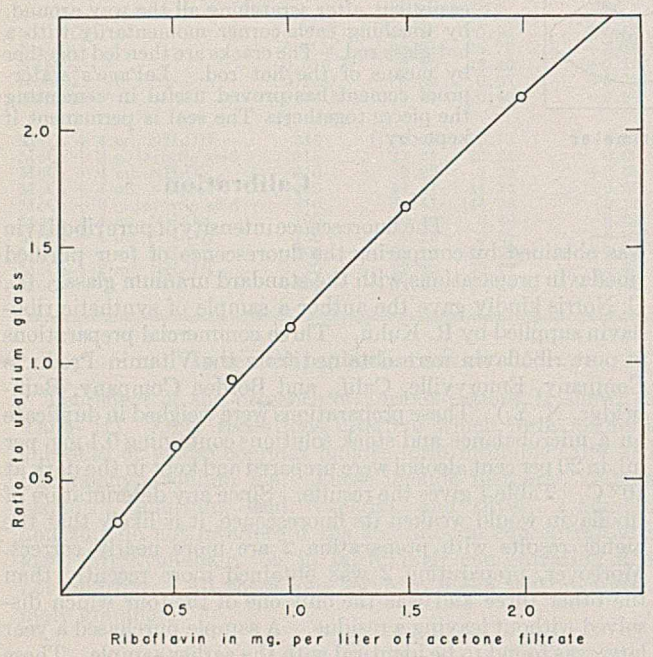

FIGURE 2. CALIBRATION OF FLUOROMETER
For determining riboflavin in 66 per cent acetone filtrate from milk

Kuhn, György, and Wagner-Jauregg (*9*). A value approaching the maximum value for monochromatic light indicates that the riboflavin is pure and that the transmission of the filter and sensitivity of the photocell give a "band" corresponding closely to the absorption band of riboflavin between 400 and 500 mμ.

Since the fluorescence of riboflavin depends on the nature of the solvent, it is necessary to calibrate the apparatus for each particular solvent. For example, the fluorescence of riboflavin is 13 per cent weaker in the 66 per cent acetone-milk filtrate than it is in pure water (Table I). Intensity decreases below pH 3 and above pH 9. The microammeter readings for fluorescence are nearly proportional to riboflavin concentration, as shown in Figure 2, in which the microammeter readings for riboflavin in 66 per cent acetone-milk filtrate divided by the microammeter reading for the uranium glass are plotted against concentration of riboflavin. The color of the fluorescence is almost but not exactly like that of uranium glass, as shown by the fact that the ratios are slightly different with different filters. There is a little more blue in the fluorescence of the uranium glass, but this difference is not visible in a pocket spectroscope.

Procedure

Fifty milliliters of acetone were added to 25 ml. of whole milk and the mixture was filtered through a coarse 15-cm. filter. The first part of the filtrate was poured through the filter a second time. The clear filtrate was placed in the special bottle for measuring fluorescence and the ratio of its fluorescence to that of the standard uranium glass was determined. The concentration of riboflavin in the filtrate was read from the calibration curve shown in Figure 2.

The results were calculated in milligrams per liter of whole milk, corrections being made for dilution by the acetone, the contraction on adding acetone to milk, and the volume of the fat and protein, as follows: When 50 ml. of acetone are added to 25 ml. of whole milk the total volume is 72.3 ml. The volume of the fat and protein which are precipitated from 25 ml. of whole milk by the acetone is calculated to be 1.6 ml., on the basis of 3 per cent by weight of casein and 3.5 per cent of fat. Therefore the riboflavin originally in 25 ml. of whole milk is diluted to 70.7 ml. of acetone filtrate. The value for riboflavin in milligrams per liter of acetone filtrate is obtained directly from the ratio to uranium glass by reference to the calibration curve (Figure 2) and this value is multiplied by the dilution factor 2.83 to obtain the result in milligrams per liter of whole milk. The corresponding factor for skim milk is 2.87 and for whey 2.89. These factors can be used when the actual determinations are made on the material in which the concentration of riboflavin is to be calculated. Owing to removal by the casein the concentration of riboflavin actually present in whey is less than that calculated from a determination of riboflavin in whole or skim milk.

Practically the entire fluorescence of these clear solutions can be destroyed by irradiation or reduction. Less than 10 per cent of the fluorescence (through filter 351) is a bluish (or white) fluorescence or turbidity which is not destroyed by reduction or irradiation. Table II shows that riboflavin added to milk can be recovered quantitatively by this method. The milk in these experiments was first exposed to sunlight to cause the destruction of nearly all of the original riboflavin. Souring of the milk slows filtration, but has no measurable effect on the fluorescence. Pasteurizing, or holding for one week in the dark or for one hour in ordinary diffuse room light, has no effect.

Variation of the normal riboflavin content of milk has been found to be from 1.20 to 3.40 mg. per liter in a series of 400 determinations. In view of the wide variation in individual milk samples, due to feed, breed, and especially to differences among individual cows in the same breed, the assignment of a normal value to the riboflavin content of milk would be misleading. The riboflavin content of milk can be best described in the form of a distribution curve which involves a large number of samples. Therefore to be of use in this connection a method should be designed for large numbers of determinations rather than for extreme accuracy.

The fluorometric method in addition to being extremely rapid is reliable to ±5 per cent. The mean deviation for 30 duplicate analyses on the same milk was found to be ±2.2 per cent. While the accidental error is small, there is a chance of greater systematic error in impurities in the standard riboflavin, and in some change in the optical system of the apparatus such as replacement of the light or alteration in the photocell. It is much simpler to get relative values for the different samples than to determine absolute quantities of riboflavin in milk. In order to get absolute values as accurately as possible, four riboflavin preparations have been examined and found to agree within 10 per cent (Table I). The fluorescence intensity of the best preparation is probably within ±5.0

TABLE II. RECOVERY OF ADDED RIBOFLAVIN
(Showing that all the riboflavin in milk is extracted from the curd by 66 per cent acetone. Riboflavin values in mg. per liter)

Present in Original Milk (Determined)	Amount Added	Present in Milk after Addition (Calcd.)	Found by Analysis	Per Cent Error
	Mg. per liter			
0.55	..	0.55	0.55	...
0.55	0.25	0.80	0.81	+1.3
0.55	0.50	1.05	1.04	−0.9
0.55	0.75	1.30	1.32	+1.5
0.55	1.0	1.55	1.58	+1.9
0.55	1.5	2.05	1.91	−6.8
0.55	2.0	2.55	2.51	−1.6
0.55	3.13	3.68	3.81	+3.4

per cent of the fluorescence intensity of pure riboflavin. In order to make sure that the observed seasonal variations in fresh milk samples were real and not due to fluctuations in the standards, solutions of riboflavin and fluorescein have been kept for a year. Readings made at intervals throughout the year on these solutions have shown a mean deviation of ±5.0 per cent from the mean values. This day-to-day fluctuation in the apparatus probably is the limiting factor in the accuracy of the fluorometric method when uranium glass is used as a standard over long periods of time. However, the fluctuation can be eliminated by comparing the uranium glass with a fresh stock solution of riboflavin each time a determination is made. In this way the error of comparative readings can be reduced to ± 2.2 per cent, which is the mean deviation from the mean for duplicate analyses. But much of the convenience of the method is sacrificed and the accuracy of the absolute values of riboflavin is still limited by the purity and reproducibility of the standard riboflavin preparations.

The method gives the value for the total riboflavin regardless of whether it is free or combined with protein. Preparations of the combined form of riboflavin (yellow enzyme) have been made from yeast according to the directions of Warburg and Christian (*18*). Analyses of this material yielded the same results by precipitation with acetone as by refluxing 10 minutes in 75 per cent methyl alcohol with or without 0.2 per cent glacial acetic acid. Precipitation by acetone yields higher readings and less turbid solutions than precipitation by trichloroacetic acid. Apparently 66 per cent acetone at room temperature is sufficient to separate the riboflavin from the protein, presumably by denaturing and precipitating the protein. Moreover, Kuhn and Kaltschmitt (*10*) state that riboflavin in milk occurs in the free form. Since they succeeded in removing 90 per cent by water dialysis there is no convincing evidence that any bound flavin occurs in milk (*4*, *10*). Because of its accuracy, rapidity, and specificity the fluorometric method is perhaps more suitable than either the biological method or the colorimetric method for determining riboflavin in milk.

Literature Cited

(1) Charite, A. J., and Khaustov, N. W., *Biochem. J.*, 29, 34 (1935).
(2) Cohen, F. H., *Rec. trav. chim.*, 54, 133 (1935).
(3) Euler, H. von, and Adler, E., *Z. physiol. Chem.*, 223, 105 (1934).
(4) Euler, H. von, Adler, E., and Schlötzer, A., *Ibid.*, 226, 88 (1934).
(5) Hand, D. B., Guthrie, E. S., and Sharp, P. F., *Science*, 87, 439 (1938).
(6) Hopkins, F. G., *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, 22, 226 (1938).
(7) Koschara, W., *Z. physiol. Chem.*, 232, 101 (1935).
(8) Kuhn, R., György, P., and Wagner-Jauregg, T., *Ber.*, 66, 317 (1933).
(9) *Ibid.*, 66, 1034 (1933).
(10) Kuhn, R., and Kaltschmitt, H., *Ibid.*, 68, 386 (1935).
(11) Kuhn, R., Wagner-Jauregg, T., and Kaltschmitt, H., *Ibid.*, 67, 1452 (1934).
(12) Martini, E., *Boll. soc. ital. biol. sper.*, 10, 1235 (1934).
(13) Sullivan, R. A., thesis, Cornell University, 1937.
(14) Supplee, G. C., Ansbacher, S., Flanigan, G. E., and Hanford, Z. M., *J. Dairy Sci.*, 19, 215 (1936).
(15) Theorell, H., *Biochem. Z.*, 288, 317 (1936).
(16) Wagner-Jauregg, T., and Ruska, H., *Ber.*, 66, 1298 (1933).
(17) Warburg, O., and Christian, W., *Biochem. Z.*, 254, 438 (1932).
(18) *Ibid.*, 266, 379 (1933).
(19) Weisberg, S. M., and Levin, I., IND. ENG. CHEM., Anal. Ed., 9, 523 (1937).
(20) Whitnah, C. H., Kunerth, B. L., and Kramer, M. M., *J. Am. Chem. Soc.*, 59, 1153 (1937).

PRESENTED before the Division of Biological Chemistry at the 97th Meeting of the American Chemical Society, Baltimore, Md.

Determination of Nickel and Cobalt in Silicate Rocks

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THE method here described for the determination of nickel is based on the extraction of nickel dimethylglyoxime with chloroform from the ammoniacal citrate solution of the rock sample. By shaking the chloroform extract with dilute hydrochloric acid the dimethylglyoxime compound is decomposed and nickel is brought into the aqueous phase, in which it is then determined colorimetrically by Rollet's method (*3*).

This method is particularly designed for rocks of such low nickel content that the nickel cannot well be determined by the gravimetric method of Harwood and Theobald (*2*).

Procedure

Weigh 0.25 gram of finely powdered basic rock (0.01 to 0.05 per cent nickel), or 0.5 gram or more of acidic rock, into a platinum dish, add a few milliliters of water, 0.5 ml. of 70 per cent perchloric acid, and 2.5 ml. of hydrofluoric acid (for a sample greater than 0.25 gram these amounts should be correspondingly increased). Evaporate the mixture to dryness, take up the residue in 0.5 ml. of perchloric acid and 2 or 3 ml. of water, and again evaporate to dryness. To the residue add 0.5 to 1 ml. of concentrated hydrochloric acid and 5 ml. of water. Heat to bring all soluble material into solution, add 5 ml. of 10 per cent sodium citrate solution, neutralize the cold solution with concentrated ammonium hydroxide using litmus paper, and add a few drops in excess. If there is an appreciable amount of precipitate or residue in the solution at this point, filter through a small paper, wash with small portions of water, and ignite the paper and its contents. Fuse the residue with approximately 0.1 gram of sodium carbonate, add an excess of dilute hydrochloric acid to the cooled melt, and heat to effect as complete solution as possible. Add 2 or 3 ml. of 10 per cent sodium citrate solution, make slightly ammoniacal, and reserve the solution.

To the main solution (filtrate from any insoluble material) add 2 ml. of 1 per cent alcoholic dimethylglyoxime solution, and shake vigorously for one-half minute with two or three portions of reagent-quality chloroform, each having a volume of 2 or 3 ml. In a similar manner extract the ammoniacal solution of the sodium carbonate melt. Combine all the chloroform extracts and shake vigorously with 10 ml. of 1 to 50 ammonium hydroxide solution. Draw off the chloroform, taking care that no drops of the aqueous phase accompany it, and shake the water layer with a milliliter or two of chloroform to recover any suspended drops of chloroform solution.

Shake the chloroform solution of nickel dimethylglyoxime vigorously for 1 minute with two portions of 0.5 *N* hydrochloric acid, each having a volume of 5 ml. (or slightly less if the solution is finally to be made up to 10 ml.). Transfer the hydrochloric acid solutions to a volumetric flask of suitable size or a flat-bottomed color comparison tube (1.8 × 15 cm.), taking care that no appreciable amount of chloroform is carried over. For color comparison in a colorimeter the nickel concentration of the final solution should be at least 1 microgram per ml. For most acidic rocks the standard series method of color comparison will

usually have to be applied because of the low nickel content. A suitable series of standards for a silicic rock is 0, 1, 2..... 10 micrograms of nickel for a 0.5-gram sample. Whether a colorimeter or tubes are used, the final nickel concentration should not exceed 5 micrograms per ml. or else a precipitate of nickel dimethylglyoxime may be produced.

The unknown nickel solution and the standard nickel solution diluted to about 10 ml. with 0.5 *N* hydrochloric acid are treated simultaneously as follows: Add 5 drops of freshly prepared saturated bromine water, mix, and then add concentrated ammonium hydroxide dropwise with shaking until the color of bromine disappears; finally add an excess of 3 or 4 drops. Next add 0.5 ml. of 1 per cent alcoholic dimethylglyoxime solution, mix, and dilute to volume with water if a volumetric flask is used. The color comparison may be made immediately. The color intensity of the solutions increases slowly on standing; the unknown and standard solution should therefore be treated with the reagents at the same time.

If necessary apply a correction for nickel in the reagents.

TABLE I. DETERMINATION OF NICKEL

No.	Sample	Addition	Ni Taken[a] %	Ni Found %	Error %
1	Extracted[b] solution of granite	.....	0.0003	0.0003	0.0000
2	Synthetic basic rock	.....	0.0020	0.0018	−0.0002
3	Extracted solution of synthetic basic rock	.....	0.0020	0.0019	−0.0001
4	Synthetic basic rock	.....	0.0060	0.0060	0.0000
5	Synthetic basic rock	.....	0.011	0.011	0.000
6	Synthetic basic rock	.....	0.021	0.020	−0.001
7	Synthetic basic rock	.....	0.042	0.041	−0.001
8	Synthetic basic rock	0.04% Co	0.0030	0.0028	−0.0002
9	Synthetic basic rock	0.03% Cu	0.010	0.010	0.000
10	Synthetic basic rock	0.1% Cu, 0.05% Co	0.009	0.010	+0.001
11	Synthetic basic rock	1.0% Mn	0.011	0.011	0.000
12	Synthetic basic rock	0.2% Cr^{VI}, 0.05% V^{V}	0.020	0.020	0.000

[a] Includes nickel originally present in synthetic basic rock (0.0010%). For composition of synthetic basic rock see (*4*).
[b] Solution of sample extracted with chloroform after addition of dimethylglyoxime to remove nickel originally present, and nickel then added to extracted solution.

Discussion

The results obtained by applying the foregoing directions are given in Table I. One-fourth gram samples were used and the color comparison was made in a Duboscq colorimeter when the nickel content was 0.005 per cent or greater. The sensitivity of the method is great enough to allow the detection of less than 0.0001 per cent of nickel when a 0.5-gram sample is taken.

Copper, cobalt, manganese, chromium, and vanadium in the amounts that are likely to be encountered in most igneous rocks do not interfere. It may be expected that much copper and cobalt will lead to high results. One hundred micrograms of cobalt carried through the procedure gave a color corresponding to about 1.5 micrograms of nickel, and 100 micrograms of copper gave no color. Manganese in large quantities may cause trouble by oxidizing nickel to the nickelic condition in the ammoniacal solution during shaking, and the results for nickel will then be low, because nickelic dimethylglyoxime is not extracted by chloroform.

Under the conditions specified above for the final determination of nickel, Beer's law is closely followed up to a concentration of about 6 micrograms of nickel per milliliter. Above this concentration a precipitate may separate.

The solubility of nickel dimethylglyoxime in chloroform at room temperature corresponds to approximately 50 micrograms of nickel per milliliter.

Cobalt

The following method for the determination of cobalt in silicate rocks is based on the extraction of the element with a carbon tetrachloride solution of dithizone from the ammoniacal citrate solution of the sample. The carbon tetrachloride extract, which also contains the dithizonates of copper and other heavy metals, is evaporated to dryness, the residue is ignited to destroy organic matter, and the metal oxides are dissolved in aqua regia. The solution is treated with stannous chloride to reduce copper to the cuprous condition, and cobalt is then determined colorimetrically by the addition of ammonium thiocyanate and acetone, essentially according to the directions of Tomula (*5*). Nickel accompanies cobalt to a greater or less extent in the dithizone separation, but because of the low concentration it usually does not markedly affect the determination of cobalt even when the ratio of nickel to cobalt in the final solution is 10 to 1. Alternatively, cobalt can be determined by the thiocyanate–amyl alcohol method (*1*) in which large amounts of nickel do not interfere. By using a 1-gram sample, 0.0001 per cent of cobalt can be detected by either method.

Tables II and III contain some of the results obtained in applying the procedure described below.

PROCEDURE. Decompose 0.25 gram of basic rock, or 0.5 to 1 gram of acidic rock, as described above, making a sodium carbonate fusion of any insoluble material.

To the main solution (filtrate from any insoluble material after the hydrofluoric acid decomposition), containing 5 ml. of 10 per cent sodium citrate and at least 0.2 to 0.25 ml. of concentrated ammonium hydroxide in excess (these quantities are for a 0.25-gram sample), add 5 ml. of 0.01 per cent (weight by volume) dithizone in carbon tetrachloride. Shake vigorously for one-half minute and draw off the carbon tetrachloride extract. Add 2 or 3 ml. of dithizone to the solution, shake as before, and continue in this manner until the last portion of dithizone does not become red after shaking for 1 minute. In like manner extract the ammoniacal citrate solution of the sodium carbonate melt with a milliliter or two of dithizone. Wash the combined carbon tetrachloride extracts with 5 ml. of water, and run the carbon tetrachloride layer into a small silica dish, being careful to avoid the transfer of any aqueous phase.

Evaporate the carbon tetrachloride, rinse the upper portion of the dish with a few drops of carbon tetrachloride to wash down any residue, and ignite at redness to destroy organic matter. Care must be taken to burn off all organic material, but too prolonged heating or too high a temperature should be avoided. Add 2 or 3 drops each of hydrochloric and nitric acids, distribute

TABLE II. DETERMINATION OF COBALT BY THIOCYANATE-ACETONE METHOD

No.	Sample[a]	Addition	Co Taken[b] %	Co Found %	Error %
1	Synthetic basic rock	.....	0.0012	0.0010	−0.0002
2	Synthetic basic rock	.....	0.0048	0.0048	0.0000
3	Synthetic basic rock	.....	0.0100	0.0100	0.0000
4	Synthetic basic rock	.....	0.0250	0.0235	−0.0015
5	Synthetic basic rock	0.05% Ni	0.0048	0.0045	−0.0003
6	Extracted solution of synthetic basic rock	0.02% Cu	0.0048	0.0048	0.0000
7	Synthetic acid rock	.....	0.0004	0.0004	0.0000
8	Synthetic acid rock	.....	0.0009	0.0008	−0.0001
9	Extracted solution of synthetic acid rock	0.03% Ni	0.0003	0.0003	0.0000

[a] 0.25 gram of synthetic basic rock, 1.0 gram of synthetic acid rock.
[b] Includes Co present in synthetic rock samples (0.0002% in basic rock, 0.0001% in acid rock).

TABLE III. DETERMINATION OF COBALT BY THIOCYANATE–AMYL ALCOHOL METHOD

No.	Sample[a]	Addition	Co Taken %	Co Found %	Error %
1	Synthetic acid rock	....	0.0003	0.0002	−0.0001
2	Extracted solution of synthetic acid rock	....	0.0005	0.0005	0.0000
3	Synthetic acid rock	0.03% Ni	0.0001	0.0001	0.0000
4	Extracted solution of synthetic acid rock	0.02% Ni	0.0005	0.0005	0.0000
5	Synthetic acid rock	0.03% Cu	0.0004	0.0003	−0.0001
6	Synthetic acid rock	0.05% Ni, 0.01% Cu	0.0006	0.0006	0.0000
7	Granite	....	0.00075[b]	0.0007	−0.00005

[a] 1.0-gram sample.
[b] 0.00025% Co present in sample, 0.0005% Co added.

the liquid over the interior of the dish with the aid of a stirring rod, and evaporate to dryness on the steam bath. Add to the cold dish 0.5 ml. of water and 3 or 4 drops of stannous chloride solution (20 grams of $SnCl_2.2H_2O$ in 100 ml. of 2 *N* hydrochloric acid). Transfer the solution to a small glass-stoppered flat-bottomed tube (1 × 8 cm.) or a small vial. (A colorimeter may be used for the color comparison when the cobalt concentration is greater than 2 micrograms per milliliter of final solution.) Rinse the dish with 0.5 ml. of ammonium thiocyanate (50 grams in 100 ml. of water), then with 2 ml. of reagent-quality acetone, and transfer these washings to the tube. The concentration of acetone in the final solution must be at least 50 per cent by volume. After mixing, make the color comparison against a series of standards containing the same amounts of stannous chloride, ammonium thiocyanate, and acetone as the unknown. The percentage of cobalt in a basic rock such as a gabbro or diabase is likely to be less than 0.01 and the standards can be prepared accordingly. In acidic rocks the percentage is likely to be less than 0.001. The color of the sample solution should be pure blue, differing little if at all in hue from the standards. A greenish hue may be due to incomplete destruction of organic matter, insufficient stannous chloride to reduce copper, or the presence of much nickel.

Alternatively the cobalt determination can be made by the amyl alcohol extraction method. This method should be used when much nickel is present in the sample, and it is also recommended for acidic rocks. Transfer the cobalt solution, treated with stannous chloride as before, to a 1 × 8 cm. glass-stoppered tube, and add 1.5 ml. of ammonium thiocyanate solution (50 grams in 100 ml. of water) and 0.50 ml. of amyl alcohol. Treat the standards similarly. Shake vigorously for 10 to 15 seconds and compare the colors of the amyl alcohol layers by viewing the tubes transversely against a white background.

Run a blank on the reagents and apply a correction if required.

Literature Cited

(1) Hackl, O., *Chem.-Ztg.*, 46, 385 (1922).
(2) Harwood, H. F., and Theobald, L. T., *Analyst*, 58, 673 (1933).
(3) Rollet, A. P., *Compt. rend.*, 183, 212 (1926).
(4) Sandell, E. B., Ind. Eng. Chem., Anal. Ed., 8, 337 (1936).
(5) Tomula, E. S., *Z. anal. Chem.*, 83, 6 (1931).

Control of pH in Peroxide Solutions

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Control of pH is important in processes involving the production and applications of the peroxygen compounds. The applicability of colorimetric and potentiometric methods for measuring the pH of peroxide solutions is discussed and data are presented showing relationships between pH and normality of hydrogen peroxide solutions in concentrations up to 200 volume. Hydrogen peroxide behaves like a weak acid and increases the hydrogen-ion activity of sulfuric acid in proportion to the peroxide concentration.

PEROXYGEN compounds vary in their behavior with the pH of the solutions in which they are employed. The methods available for measuring and controlling the pH of oxidizing solutions are contributing markedly to the development of the chemistry of peroxygen compounds and to the extension of their commercial applications. In processes employing sodium peroxide, hydrogen peroxide, or sodium perborate, pH control has become fully as important as control of temperature, time, and concentration.

The pH of peroxide solutions has an important bearing on their stability. Peroxides are most stable in acid solutions and their rate of decomposition increases with the hydroxyl-ion concentration. Stability is furthermore influenced by inhibitors and catalysts whose activity depends upon the pH of the peroxide solution.

Rate and degree of bleaching with peroxides depend largely on the pH maintained in the particular bleaching processes. The catalytic effect of peroxides on hydrolytic reactions may be inhibited or accelerated by pH control. Rates of corrosion of structural materials are affected by changes in pH. In general, the desired properties of the peroxygen compounds can be promoted and their undesirable reactions can be repressed by proper pH control.

Methods for pH Determination

Some of the colorimetric indicators are affected by oxidizing compounds. However, with a proper choice of indicators, colorimetric methods can be satisfactorily applied in many cases. Among the electrode systems, the hydrogen gas electrode, the quinhydrone electrode, and the metal–metal ion electrode, such as the antimony electrode, are subject to large errors in the presence of oxidizing agents (*6*). The glass electrode, one of the more recent developments in pH measurement, is well adapted to potentiometric pH measurements in oxidizing and reducing systems, and is suitable for determining the pH of peroxide solutions. It has the advantage over colorimetric methods that it can be employed successfully in highly colored or turbid solutions.

The pH values presented in this article have been corrected for sodium-ion concentration in accordance with instructions supplied by the manufacturer of the pH meter. These corrections correspond closely with those given by Dole (*4*). No corrections were made at pH values below 9.3; the maximum correction of 0.4 pH unit was applied at the highest pH value shown—i. e., to the solution containing no hydrogen peroxide at a pH of 12.3.

Comparison of Colorimetric and Potentiometric Methods

Figure 1 shows the agreement between colorimetric and glass electrode pH determinations for clear, colorless hydrogen peroxide solutions varying in concentration from 0 to 200 volumes. [A commonly used term for expressing the active oxygen content of peroxide solutions is "volume concentration", which is defined as the number of cubic centimeters of oxygen gas, measured at 0° C. and 760-mm. pressure, liberated from 1 cc. of the solution (measured at 20° C.) when the peroxide is completely decomposed.] The potentiometric determinations were made with a Beckman pH meter which provides for temperature corrections. The colorimetric determinations were made at a temperature of 20° to 30° C.; in this range variations in pH with changes in temperature

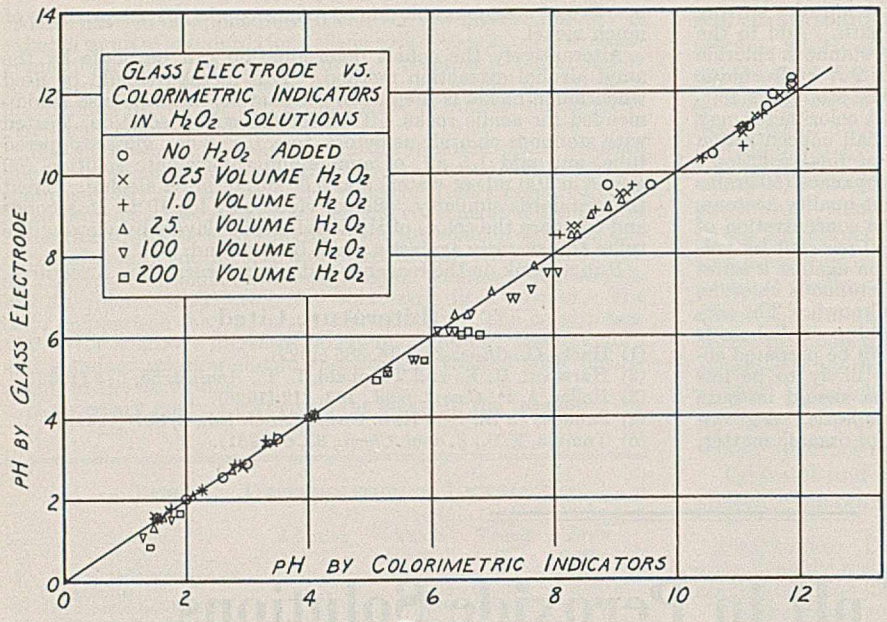

FIGURE 1

are negligible. The following La Motte colorimetric indicators were employed:

Indicator	pH Range
m-Cresol purple	1.2–2.8
La Motte yellow	2.6–4.2
Bromophenol blue	3.0–4.6
Bromocresol green	3.8–5.4
Chlorophenol red	5.2–6.8
Bromocresol purple	5.2–6.8
Bromothymol blue	6.0–7.6
Phenol red	6.8–8.4
Cresol red	7.2–8.8
Thymol blue	8.0–9.6
La Motte oleo red	8.6–10.2
La Motte purple	9.6–11.2
La Motte sulfo orange	11.0–12.6

In general, the values obtained by the two methods are in fair agreement, though in some cases the variation is as much as 0.8 pH unit. The closest agreement was obtained in the acid range with *m*-cresol purple, La Motte yellow, bromophenol blue, and bromocresol green. With these indicators the maximum difference between observed colorimetric and potentiometric pH values was less than 0.2 pH unit at peroxide concentrations up to and including 100 volume. In concentrations up to one volume the pH values obtained with La Motte sulfo orange also coincided within 0.2 pH unit with the potentiometric values. With the remaining indicators, the colorimetric pH values coincided in most cases within 0.4 pH unit with the potentiometric values. With some indicators differences in color tone made color matching between the standards and the unknown difficult.

Relation between pH and Normality

The pH curves shown in Figure 2 are derived from the data presented in Tables I and II. Each value in these tables is based on a separately prepared solution. The requisite amounts of hydrogen peroxide solution and standard sodium hydroxide or sulfuric acid solution were diluted in volumetric flasks, from which the samples were then removed for pH measurement. In this way errors due to dilution of the peroxide and of the acid or alkali were avoided.

A number of pH measurements were made on 100-volume solutions containing the usual small amount of inorganic salts and on 100-volume solutions freed from these salts by redistillation. Comparisons were made at and near the vertical and most sensitive section of the curve. The two sets of values coincided closely, indicating that no appreciable change in the location of the curves resulted from the use of redistilled peroxide.

The curves obtained illustrate the weakly acid property of hydrogen peroxide as reported by Bredig and Calvert (*1*, *2*). In the alkaline range the pH decreases with increase in peroxide concentration at a fixed sodium hydroxide concentration. Assuming that hydrogen peroxide in aqueous solution undergoes primary dissociation according to the equation

$$H_2O_2 \longrightarrow H^+ + OOH^-$$

it should react with caustic soda according to the equation

$$H_2O_2 + NaOH \longrightarrow NaOOH + H_2O$$

In Figure 2 the neutralization of the hydrogen ions resulting from the primary dissociation of hydrogen peroxide is theoretically complete in 0.25-volume hydrogen peroxide with 0.0223 *N* sodium hydroxide. A slight break, similar to the breaks encountered in the titration of a weak acid with a strong base, might be expected at this point in the curve; however, no such break is apparent. With the higher concentrations of hydrogen peroxide (1.0, 25, 100, and 200-volume) the highest concentration of sodium hydroxide used (0.05 *N*) is too low to neutralize completely the first hydrogen ion; consequently, no breaks are to be expected in the curves for these concentrations of hydrogen peroxide within the normalities presented. In an exploratory trial with 25-volume hydrogen peroxide the concentration of sodium hydroxide was increased to extend the curve in Figure 2 beyond

TABLE I. RELATION BETWEEN PH AND NORMALITY

H_2O_2 Volume Concentration	H_2SO_4 Normality	pH Glass Electrode
0.0	0.0001	4.12
0.0	0.0004	3.49
0.0	0.0016	2.90
0.0	0.0064	2.28
0.0	0.0128	2.02
0.0	0.0512	1.55
0.25	0.0001	4.07
0.25	0.0004	3.40
0.25	0.0016	2.82
0.25	0.0064	2.24
0.25	0.0256	1.78
0.25	0.0512	1.55
1.0	0.0001	4.10
1.0	0.0004	3.46
1.0	0.0016	2.88
1.0	0.0064	2.28
1.0	0.0256	1.80
1.0	0.0512	1.55
25	0.0001	4.08
25	0.0004	3.40
25	0.0016	2.75
25	0.0064	2.12
25	0.0256	1.60
25	0.0512	1.35
100	0.0004	3.05
100	0.0064	1.55
100	0.0256	1.00
100	0.0512	0.80
200	0.0004	2.80
200	0.0016	1.63
200	0.0064	0.85
200	0.0256	0.20
200	0.0512	0.00

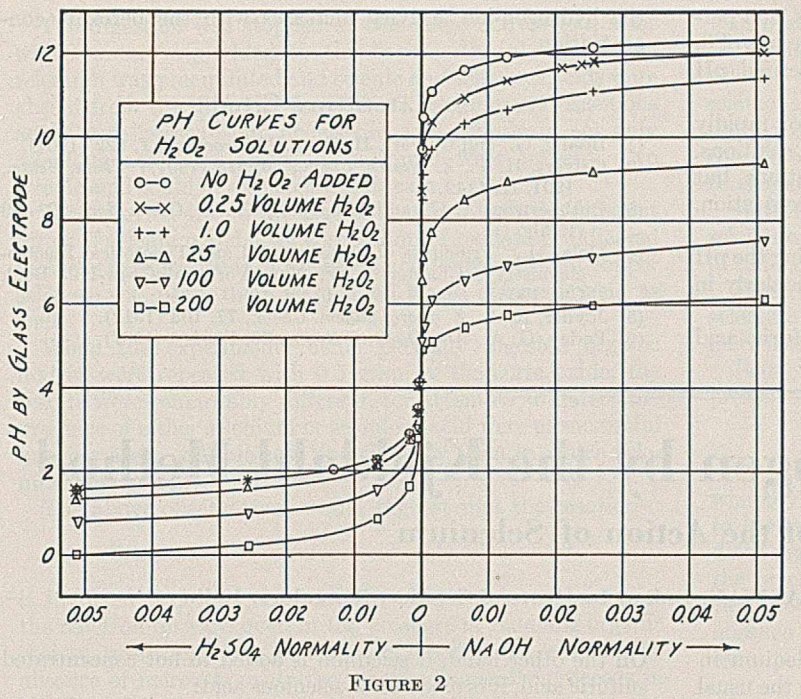

FIGURE 2

the point of neutralization (89 grams of sodium hydroxide per liter). A slight break was apparent in the curve at the point of equivalence. However, potentiometric pH measurements at these high sodium-ion concentrations are subject to considerable error and the slight break observed at this point in the curve may not be significant.

In the acid range of the curves the pH of the solution is abnormally lowered as the peroxide concentration is increased. With complete dissociation and an activity coefficient of 1, the calculated pH is 1.3 at an acid concentration of 0.05 *N*. A 200-volume hydrogen peroxide solution showed a measured pH of 0.0 at a sulfuric acid concentration of 0.05 *N*. A dissociation constant of 2.4×10^{-12} for hydrogen peroxide at 25° C., as determined by Joyner (*5*), is not sufficient to account for this lowering in the observed pH value.

The dielectric constants for hydrogen peroxide solutions are considerably greater than the dielectric constant for water (*3*). Hence, the ionizing power of peroxide solutions should be greater than that of water, and this might be expected to result in an increased hydrogen-ion activity. Cuthbertson and Maass (*3*) did not find this to be the case in their studies of the conductivity of potassium chloride and acetic acid in aqueous hydrogen peroxide solutions. The authors' present data, however, show an increased hydrogen-ion activity for sulfuric acid dissolved in aqueous solutions of hydrogen peroxide.

At a constant concentration of sulfuric acid the observed pH values decrease as the peroxide concentration increases. This direct relationship is illustrated by the straight line shown in Figure 3, which represents the variation of pH with increasing peroxide concentration at a constant sulfuric acid concentration of 0.05 *N*. A similar curve illustrating the conditions at 0.025 *N* sulfuric acid produces a straight line with the same slope as that in Figure 3. This relationship, as derived from Figure 3, is represented by the equation

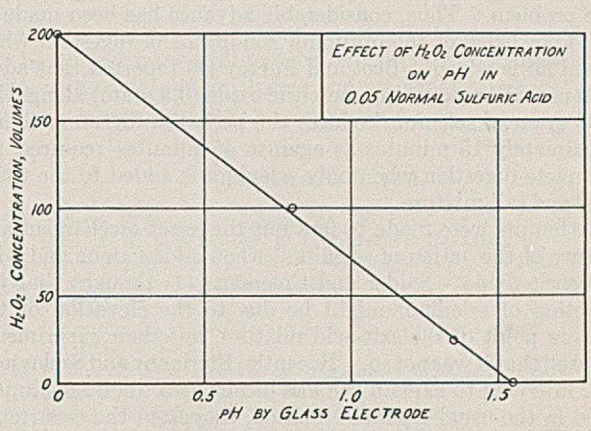

FIGURE 3

$$pH_{H_2O_2} = pH_{H_2O} - K \text{ (concentration of } H_2O_2)$$

where $pH_{H_2O_2}$ is the pH of a sulfuric acid solution in aqueous hydrogen peroxide, pH_{H_2O} is the measured pH of an aqueous solution of the same sulfuric acid concentration, and K is a constant. If the strength of the peroxide solution is expressed as volume concentration, the value of 0.008 is found to apply for K at acid concentrations of 0.015 to 0.05 *N*. The value for K decreases slightly with decreasing acid concentrations; it is 0.0072 at 0.0064 *N* sulfuric acid and 0.0064 at 0.0016 *N* acid.

TABLE II. RELATION BETWEEN PH AND NORMALITY

H_2O_2 Volume Concentration	NaOH Normality	pH Glass Electrode
0.0	0.0001	9.70
0.0	0.0004	10.44
0.0	0.0016	11.07
0.0	0.0064	11.59
0.0	0.0128	11.91
0.0	0.0256	12.16
0.0	0.0512	12.29
0.25	0.0001	8.70
0.25	0.0004	9.50
0.25	0.0016	10.35
0.25	0.0064	10.94
0.25	0.0128	11.35
0.25	0.0210	11.64
0.25	0.0240	11.73
0.25	0.0256	11.82
0.25	0.0512	12.02
1.0	0.0001	8.47
1.0	0.0004	9.08
1.0	0.0016	9.69
1.0	0.0064	10.32
1.0	0.0128	10.62
1.0	0.0256	11.08
1.0	0.0512	11.40
25	0.0001	6.55
25	0.0004	7.08
25	0.0016	7.70
25	0.0064	8.45
25	0.0128	8.93
25	0.0256	9.15
25	0.0512	9.37
100	0.0001	5.13
100	0.0004	5.43
100	0.0016	6.10
100	0.0064	6.55
100	0.0128	6.93
100	0.0256	7.14
100	0.0512	7.52
200	0.0004	4.90
200	0.0016	5.07
200	0.0064	5.40
200	0.0128	5.75
200	0.0256	5.98
200	0.0512	6.10

Summary

Determination and control of pH are important factors in processes employing peroxygen compounds, such as sodium peroxide, hydrogen peroxide, and sodium perborate.

The relationships between pH values, normalities, and peroxide concentrations, as presented in this article, are useful in the preparation of peroxide solutions with the desired pH values.

The glass electrode provides a simple method for rapidly and accurately determining the pH of peroxide solutions. This method is applicable to colored or turbid solutions, but in solutions of high pH and high sodium-ion concentration, corrections are necessary.

Colorimetric methods are applicable for determining the pH of peroxide solutions under many conditions, particularly in clear and colorless solutions.

In hydrogen peroxide solutions containing sulfuric acid the hydrogen-ion activity increases with the peroxide concentration.

Literature Cited

(1) Bredig, G., and Calvert, H. T., *Z. Elektrochem.*, 7, 622 (1901).
(2) Calvert, H. T., *Z. physik. Chem.*, 38, 513 (1901); *Chem. Zentr.*, 1901, II, 1142.
(3) Cuthbertson, A. C., and Maass, O., *J. Am. Chem. Soc.*, 52, 489 (1930).
(4) Dole, M., "Principles of Experimental and Theoretical Electrochemistry", Chapter XXV, New York, McGraw-Hill Book Co., 1935.
(5) Joyner, R. A., *Z. anorg. allgem. Chem.*, 77, 103 (1912).
(6) Perley, G. A., *Am. Dyestuff Reptr.*, 26, P832–6 (1937).

Estimation of Nitrogen by the Kjeldahl Method

Nature of the Action of Selenium

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FOLLOWING Lauro's (*6*) observation that selenium in small quantities reduces the time of digestion in the usual Kjeldahl method, numerous workers (*1, 3, 9, 10*) have studied the problem. Thus, considerable advance has been made in our knowledge of the optimum conditions of digestion when selenium is added. Beet and Furzey (*2*) reported that addition of small amounts of mercuric oxide (0.5 gram) along with 0.05 gram of selenium reduces the period of digestion to approximately 15 minutes as against 45 minutes required for complete digestion when only selenium is added to the usual acid and salt mixture.

Attempts were made to find out the exact mechanism and nature of the action of selenium, when added alone and with mercuric oxide. Snider and Coleman (*11*) thought that the function of selenium might be due to the elevation of the boiling point of the salt-acid mixture, but their experiments showed that it was not so. Recently, Illarionov and Ssolovjeva (*5*), in trying to explain the loss of nitrogen when selenium is used in the usual Kjeldahl method, suggested the occurrence of an oxidation-reduction system of the type

$$\text{Selenium} + \text{oxygen} \rightleftharpoons \text{selenious acid}$$

but they adduced no experimental evidence in support of their view. Therefore, the changes in the added selenium were studied in order to find out the mechanism of the reaction.

Experimental

Selenium was used in the authors' experiments as either elementary selenium or its acids. It was found convenient to use the copper salts, since selenic acid is reduced under ordinary conditions to selenious acid.

The copper salt of selenic acid was prepared as described by Huff and McCrosky (*4*) and recrystallized from 95 per cent alcohol, until free from selenious acid. The presence of selenious and selenic acids was tested by Müller's method (*8*), which was very satisfactory for detecting the presence of either acid in a mixture of the two.

In the following experiments, selenious acid was completely reduced to elementary selenium by adding sulfuric acid and sodium sulfite until the filtrate gave no further red precipitate; the filtrate was then tested for selenic acid.

Magnus (*7*) found that when elementary selenium is added to concentrated sulfuric acid in the cold, it dissolves into a greenish solution from which elementary selenium is precipitated as a red amorphous powder on dilution with water. On the other hand, if selenium is added to hot concentrated sulfuric acid, it is oxidized to selenious acid:

$$Se + 2H_2SO_4 \longrightarrow 2SO_2 + H_2SeO_3 + H_2O$$

With a view to ascertaining the nature of the products formed when selenium is used under the conditions which obtain in the Kjeldahl method, 0.05 gram of selenium was heated in a Kjeldahl flask over a Bunsen burner with 0.2 gram of copper sulfate–potassium sulfate mixture and 20 cc. of concentrated sulfuric acid. At the end of 15 minutes, the reaction mixture was cooled and tested for selenium and selenious and selenic acids. Positive results were obtained for only selenious acid, while both selenium and selenic acid were absent. Similar results were obtained when the copper salt (0.08 gram) of selenious acid was used instead of elementary selenium. When, however, copper selenate (0.09 gram) was used in amount equivalent to the selenium used in the authors' experiments, the reaction mixture contained both selenic and selenious acids at the end of 15 minutes' heating. Obviously, the selenious acid was formed from the decomposition of selenic acid. Thus, under the above conditions, selenious acid is more stable than either selenium or selenic acid.

When the foregoing experiments were repeated with about 0.5 gram of mercuric oxide, a remarkable change was observed. Thus, in all three experiments, tests for selenium and selenious acid were negative and only selenic acid was found to be present, whatever form of selenium was added. These observations show clearly that in the absence of reducing agents, but in the presence of mercuric oxide, hot concentrated sulfuric acid converts selenium into the highest oxide, while without mercuric oxide selenium added in any form is converted into selenious acid.

ORGANIC MATTER. The experiments were repeated with organic matter. In a typical instance, rice flour was employed after it had been partially digested with concentrated sulfuric acid till frothing just ceased. At this stage, portions of the digest were treated with (*a*) selenium, (*b*) copper selenite, and (*c*) copper selenate, respectively, in quantities equivalent to 0.05 gram of selenium, and in each case the digestion was continued for a further period of 10 to 15 minutes. In all the experiments the red amorphous form of selenium was deposited on the cooler sides of the flask, evidently through the reduction of selenious and selenic acids by the organic matter.

The digestion was stopped at this stage and cooled, after which water was added to the flasks. In (*a*) elementary selenium was precipitated and selenic and selenious acids were absent from the filtrate. In (*b*) both selenium and selenious acids were present but no selenic acid; while in (*c*) only selenious acid and traces of selenium were present. No selenic acid could be detected in this case.

Tests carried out on the digests obtained with the different forms of selenium and after completion of oxidation indicated in every case presence of selenious acid only. Where copper selenate was used, there were small traces of selenic acid as well.

When the experiments using partially digested organic matter were repeated with 0.5 gram of mercuric oxide, the results were remarkably different, for attempts to detect the presence of either selenium or selenious acid were unsuccessful in every case. Selenic acid was the only form in which selenium was present.

The above observations would suggest that the reaction

$$\text{Selenium} \longrightarrow \text{selenious acid} \longrightarrow \text{selenic acid}$$

is involved and that the addition of mercuric oxide carries the reaction forward even in the presence of reducing organic matter, resulting in the formation of selenic acid, while in the absence of mercuric oxide the reaction generally proceeds in the direction:

$$\text{Selenium} \longrightarrow \text{selenious acid} \longleftarrow \text{selenic acid}$$

showing that under such conditions selenious acid is the most stable product.

It is therefore clear that in the oxidation of organic matter, the catalytic action of selenium in the presence of mercuric oxide is due to the reaction

$$\text{Selenium} \longrightarrow \text{selenious acid} \rightleftharpoons \text{selenic acid}$$

The velocity of the forward reaction is so great that at no time could selenious acid be successfully detected in the reaction mixture. In the absence of mercuric oxide, another reversible reaction of the type

$$\text{Selenium} \rightleftharpoons \text{selenious acid}$$

is brought about, enabling selenium to act as a carrier of oxygen to the reducing organic matter. But, since both selenium and selenious acid could often be detected in the mixture, this would imply that there is no pronounced difference between the velocities of the forward and reverse reactions; hence the efficiency of oxidation is much less here than when mercuric oxide is also present.

Thus it would appear that selenium acts as a catalyst in the oxidation of organic matter by virtue of setting up a rapid and reversible reaction system in the absence of mercuric oxide of the type $Se \rightleftharpoons SeO_2$ and another of the type $SeO_2 \rightleftharpoons SeO_3$ when mercuric oxide is present, so that oxygen is rendered active for rapid oxidation of the organic matter.

Summary

The reactions between hot concentrated sulfuric acid and different forms of selenium, both with and without reducing organic matter, have been studied as well as the nature of the changes taking place when mercuric oxide is added.

When selenium or selenious acid reacts with hot concentrated sulfuric acid, only selenious acid is present in a stable condition. Selenic acid is slowly decomposed into selenious acid.

When the above reactions are carried out in the presence of mercuric oxide, selenium in any form is converted into selenic acid, and continues to be present as such.

In presence of reducing organic matter, and in the absence of mercuric oxide, addition of selenium to hot concentrated sulfuric acid results in the formation of small quantities of selenious acid. Selenious acid under such conditions is partially reduced to elementary selenium, while selenic acid is completely reduced to selenious acid and, to a small extent, to elementary selenium.

With the addition of mercuric oxide, however, all forms of selenium tend to exist only as selenic acid even in the presence of reducing organic matter.

When the oxidation of organic matter is complete, added selenium is present solely as selenious acid in the absence of mercuric oxide, but as selenic acid in its presence.

It is concluded that the catalytic action of selenium in the presence of mercuric oxide is due to a reversible reaction:

$$\text{Selenium} \longrightarrow \text{selenious acid} \rightleftharpoons \text{selenic acid}$$

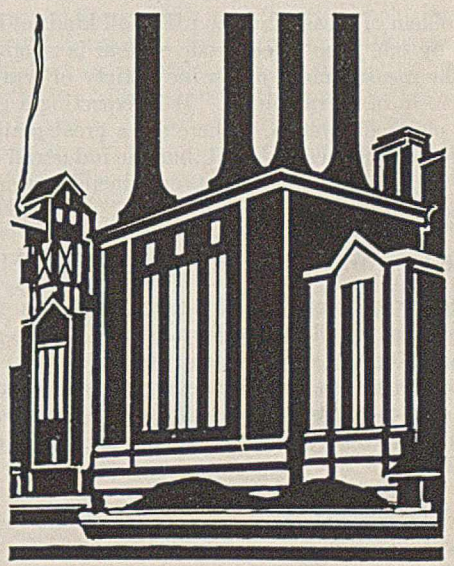
whereby the selenious acid acts as an efficient carrier of oxygen to the reducing organic matter. As long as there is unoxidized organic matter, the forward reaction is more rapid than the reverse reaction. When oxidation is complete, the reaction proceeds to completion in the forward direction. In the absence of mercuric oxide, another oxidation-reduction process

$$\text{Selenium} \rightleftharpoons \text{selenious acid}$$

enables the oxidation of organic matter. There would appear to be no pronounced difference between the velocities of the two reverse reactions; hence the efficiency of oxidation is much less here than with mercuric oxide.

Literature Cited

(1) Ashton, F. L., *J. Soc. Chem. Ind.*, 56, 101T (1937).
(2) Beet, A. E., and Furzey, D. G., *Fuel*, 11, 196 (1932).
(3) Davis, C. F., and Wise, M., *Cereal Chem.*, 10, 488 (1933).
(4) Huff, E. R., and McCrosky, C. R., *J. Am. Chem. Soc.*, 51, 1457 (1929).
(5) Illarionov, V. V., and Ssolovjeva, N. A., *Z. anal. Chem.*, 100, 328 (1935).
(6) Lauro, M. F., IND. ENG. CHEM., Anal. Ed., 3, 401 (1931).
(7) Magnus, G., *Pogg. Ann.*, 14, 328 (1828).
(8) Müller, E., *Z. physik. Chem.*, 100, 346 (1922).
(9) Osborn, R. A., and Krasnitz, *J. Assoc. Official Agr. Chem.*, 16, 110 (1933).
(10) Sandstedt, R. M., *Cereal Chem.*, 9, 156 (1932).
(11) Snider, S. R., and Coleman, D. A., *Ibid.*, 11, 414 (1934).



Antimony Electrode for Industrial Hydrogen-Ion Measurements

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THE continuous determination of the pH of industrial solutions over a limited range has been possible for some time, but no single electrode system has been available over the entire range for all types of solutions. When an automatic record is desired, an electrometric method offers certain advantages. A quinhydrone electrode has been described by Coons (*5*) for use with continuous recording potentiometers; however, this electrode is not applicable to solutions more alkaline than 9 pH. The work in the author's laboratory indicates that the glass electrode can be used to advantage in certain fields of continuous pH measurements, although it has limitations particularly in the presence of sodium, lithium, and potassium salts in the alkaline range and at temperatures in excess of 35° C., and a more reliable electrode is desirable in the alkaline range.

The use of metal–metal oxide electrode systems has appeared inviting to many investigators and many successful applications of this type of electrode are now found in the industries. The work reported in this paper was started over nine years ago.

Requirements of an Industrial pH Electrode

It is desirable to differentiate between the nature of a high-precision pH measurement where a limit of error of ±0.01 pH is desired and the requirements for the average industrial pH control where a limit of error of between 0.1 and 0.2 pH is probably adequate.

The more essential requirements of a desirable pH electrode for continuous industrial measurements are:

1. The electrode system should preferably be applicable over the entire range from 0 to 14 pH, yet a limited range is adequate in the majority of the applications.
2. The potential of the electrode should rapidly attain a reproducible e. m. f. value for a given pH of the solution at the electrode interface.
3. The potential of the electrode should, at the best, be only slightly affected by polarization, salt effects, oxidizing agents, reducing agents, dissolved gases, or movement of the solution at the electrode interface.
4. The electrode system should be of rugged construction.

The problem of measuring the pH of all kinds of industrial solutions by one type of electrode system is roughly analogous to the measurement of a wide variety of temperatures with one form of thermometer. It is expecting a great deal from one electrode system. There are a great many factors entering a true pH measurement, but the industrial control is generally more dependent on a reproducible record of pH than on academic correctness.

Variable Factors of a Metal–Metal Oxide Electrode

The theory of metal–metal oxide electrodes has been briefly discussed by Clark (*4*), Britton (*2*), Getman and Daniels (*6*), and Kolthoff and Furman (*7*).

No extensive theoretical discussion seems necessary; however, the e. m. f.–pH relationships for metal–metal oxide systems have been expressed by Roberts and Fenwick (*9*) as follows:

$$Sb + 3H_2O = Sb(OH)_3 + 3H^+ + 3e^- \qquad (1)$$

for which the e. m. f. expression will be

$$E = E_0 - \frac{RT}{3F} \ln \frac{(Sb\ (OH)_3)(H^+)^3}{(H_2O)^3} \qquad (2)$$

Britton's (*2*, p. 80) viewpoint seems to lead to a better understanding:

$$E = E_0 - \frac{RT}{F} \log K_w + \frac{RT}{nF} \log K_s + \frac{RT}{F} \log (H^+) \qquad (3)$$

where K_s refers to the solubility product of the sparingly soluble metal compound. Hence, the activity of the water must remain constant and the solution must remain saturated with oxide if the following holds true:

$$E = \text{constant} + \frac{RT}{F} \log (H^+) \qquad (4)$$

It is well recognized that the use of a metal–metal oxide electrode depends upon the presence of metal ions in solution from some well-defined source other than the metal itself. A desirable source is a sparingly soluble hydroxide.

The significance of the factors affecting the potential of a metal–metal oxide electrode has not always been fully appreciated. Equation 3 indicates that the potential of a metal–metal oxide electrode is a function of the temperature, the hydrogen-ion concentration, the solubility product of the sparingly soluble hydroxide, the activity of the water, and the electromotive activity of the metal. Some of these factors are influenced by gas and ion adsorption, the type of oxide, foreign oxidation or reduction systems, and the nature or concentration of the salts in solution. Therefore, a considerable number of variables must be carefully controlled before reproducible results can be expected with a metal–metal oxide electrode. The choice of a fundamentally sound metal–metal oxide system does much in the control of these factors.

Choice of the Metal–Metal Oxide

Because of certain conflicting statements in the literature, it seems desirable to review some of the important aspects of this problem. A readily oxidized metal is desired. If the metal is attacked by acid or alkali with the subsequent formation of hydrogen, a combination of a gas electrode and the metal–metal oxide electrode is the resultant. Hence, those metals less electronegative than hydrogen are the most applicable for wide-range operation. However, not all of the metals less electronegative than hydrogen are inviting; it is not the metal which is the fundamental factor, but rather the oxide or a proper sparingly soluble compound of the metal.

If the oxide is to be the source of the metal ion, the fundamental equation assumes that the solubility of the oxide is so small that it does not affect the hydrogen-ion concentrations over the working range of e. m. f. measurements. In other words, the metal oxide must be very sparingly soluble in both acids and alkalies.

The following oxides are those worthy of preliminary consideration: antimony, trioxide, arsenic trioxide, bismuth trioxide, columbium pentoxide, tantalum pentoxide, tungsten trioxide, chromium trioxide, molybdenum trioxide, and tellurium trioxide. Unfortunately, few exact solubility figures for these oxides are available, but by a study of other factors, the metal–metal oxide systems may be narrowed to a consideration of a very small number of elements.

Many metals which have been used for electrode measurements tend to form a surface coating and the nature of many of these coatings has been uncertain. This fact has been ignored in many published accounts. This type of reaction has been erroneously attributed to gas-metal potentials by some investigators. If the action of water upon the oxide produces a hydroxide which is appreciably soluble, the resultant potential at the metal interface will be influenced by the hydroxide.

In order to obtain a qualitative idea of the influence of dissolved oxides upon metal–metal oxide potential measurements, purified oxides were shaken several times with distilled water. The first three lots of water were decanted and the measurements were made upon the fourth extraction. The glass–saturated-calomel electrode system was used to determine the pH of the original water and of the oxide suspensions. The final measurements were made while the oxide was held in suspension by rapid stirring. Table I gives the results of the tests for 0.1 gram of the oxide suspended in 100 ml. of water and in contact with air.

The data of Table I indicate that the use of many oxides as a source of hydroxide in metal–metal-ion electrode measurements will involve serious errors, particularly in unbuffered solutions. This is a limitation to be considered in the use of the metal–metal oxide type of electrode.

TABLE I. CHANGE IN PH OF DISTILLED WATER BY ADDITION OF OXIDES

(Glass-saturated-calomel electrode measurements. Solution in contact with air and stirred. Temperature 23° to 25° C.)

Oxide Added	pH Change	Direction of Change
Sb_2O_3	0.00	Neutral
Sb_2O_5	0.47	Acidic
Bi_2O_3	0.10	Basic
CuO	0.69	Basic
Ag_2O	3.40	Basic
HgO	0.35	Basic
WO_3	1.14	Acidic
Cr_2O_3	0.10	Acidic
Cb_2O_5	0.00	Neutral
Ta_2O_5	0.00	Neutral
TeO_3	1.08	Acidic

Apparently the number of metals and metal hydroxides which are available for the construction of a stable metal–metal oxide electrode adaptable to the range of 0 to 14 pH is limited. Mercury, columbium, and chromium may offer possibilities, if the metal surface can be maintained in equilibrium with the proper insoluble compound. Its moderately low melting point, amphoteric characteristics, and ease of electrolytic purification give antimony an advantage over the other metals.

Although there may be a question as to the existence of the various antimonious acids, there is another chemical equilibrium which may occur in the solution, which results in the formation of a sparingly soluble antimony compound. We may have a reaction of the following general type:

$$4Sb + 3O_2 + x\ H_2O \rightleftharpoons 2Sb_2O_3\ x\ H_2O \qquad (5)$$

Milbauer and Slemr (*8*) found that at room temperature metallic antimony was oxidized when submerged in water, but if tartrates or citrates were present the oxidation was greatly increased. Ruff and Albert (*10*) found that antimony was attacked by solutions of alkalies and their salts. Clark (*3*) showed that neutral hydrogen peroxide was without action on antimony, but in the presence of alkali an antimonate was formed.

Evidently in aqueous solutions in contact with air there may be an ample source of an insoluble antimony compound from the interaction as indicated by Equation 5. This confirms the observations of other investigators that the addition of oxide to aqueous solutions is not required for an industrial electrode of moderate accuracy.

Antimony Metal

It seems desirable to present a brief summary of the development of a practical form of antimony electrode. One important phase of this work involved the purity of the metal. The data of Table II were obtained upon electrodes cast in the form which was finally adopted.

TABLE II. EFFECT OF METAL PURITY UPON ANTIMONY ELECTRODE POTENTIAL

Make of Antimony	Impurities				Antimony–Saturated-Calomel Electrode at 25° C.	
	Fe	As	Sn	Pb	3.8 pH buffer	10.7 pH buffer
	%	%	%	%	*E. m. f.* (*volt*)	
B.	0.015	Trace	...	0.010	0.2096	0.5846
P.	0.010	0.050	0.007	...	0.2158	0.5938
L. & N. electrolytic	000	000	000	000	0.2161	0.5982

Work upon the relative merits of the castings with the B. antimony as compared to the L. & N. electrolytically prepared metal on continuous recording tests showed that in a 2.58 pH buffer, as well as in a 7 pH buffer solution, the metal with small amounts of impurities tended to be less stable in the acid range than the pure electrolytic metal. The presence of small amounts of copper in the metal causes distinct errors.

The possibility of plating antimony upon platinum or gold wires was studied at great length. Some investigators in this field considered that the method of Shukov and Awsejewitch (*11*) gave permanence, reproducibility, and exact linearity over the whole pH range. Their method consisted in electroplating antimony on amalgamated platinum wires from a 25 per cent antimony trichloride solution in acetone.

Anhydrous antimony trichloride was dissolved in a large number of ionizing solvents. The solvents for antimony trichloride which were studied as electrolytes for the metal deposition were water, acetonitrile, nitrobenzene, methyl alcohol, acetone, lactic acid, amyl alcohol, ethyl acetate, and furfural. The best deposits resulted from solutions in acetonitrile, but very good results were obtained in the methyl alcohol and the acetone solutions.

Antimony salts are so easily hydrolyzed in aqueous solutions that care must be exercised to prevent contamination of the deposit with basic salts. All attempts in which aqueous solutions were used to build up a heavy antimony deposit resulted in nonadherent or very black deposits of antimony.

The electrodes of antimony electroplated upon platinum checked very well among themselves, yet their potential was somewhat lower than the ordinary cast-antimony electrode. The electroplated electrode was found to hold its potential for only 5 to 8 hours in a 10.8 pH buffer which was in contact with air and which was in continuous circulation. However, owing to the solubility of the antimony, the life of such an electrode is short. A serious drift begins after about 10 hours of continuous operation when a portion of the base metal becomes exposed sufficiently to set up auxiliary reactions of the air-electrode type.

Because of the comparatively high solubility of antimony in solutions containing dissolved oxygen or air, the electroplated type of electrode is not inviting for continuous measurements, although it may be used for a limited number of indicating measurements.

It was evident that a form of electrode must be employed which had an appreciable thickness of metal to withstand the gradual solvent action caused by continued use. A supply of pure metal was also required. Accordingly, the preparation of electrolytic metal was undertaken.

Antimony trioxide which had been purified by solution in hydrochloric acid and reprecipitation with sodium carbonate was

dissolved in hydrofluoric acid. A 12 per cent solution of antimony fluoride was used. Electrolytic antimony was deposited from this solution at a platinum electrode. A platinum anode of 20-sq. cm. surface was immersed within an extraction thimble compartment containing an excess of antimony oxide. A platinum cathode of 1-sq. cm. surface was used. A current of 5 amperes produced excellent crystals of antimony which settled on the bottom of the container. This metal was washed, dried, and melted and was then ready for final casting and assembly.

Methods of Mounting Antimony

A metal electrode involves not only a metal surface in contact with the solution, but also some means of making electrical contact between the metal surface and the potentiometer.

A great many types of electrodes were made and certain objections were found to most of these. Figure 1 shows a few of the types which were studied.

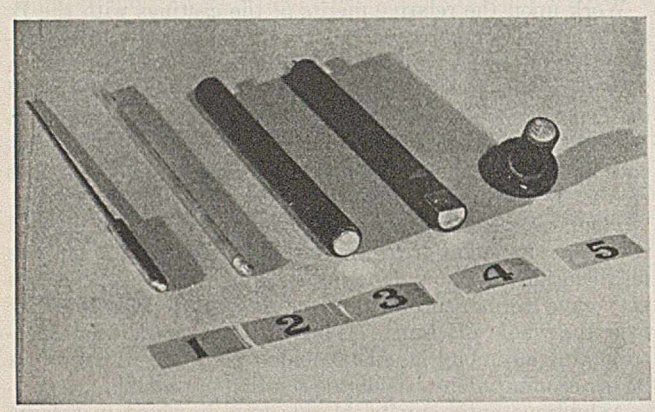

FIGURE 1. TYPES OF ANTIMONY ELECTRODES

No. 1 is a plain antimony metal stick, into which a metal connection had been threaded. This type of electrode behaved erratically on continued use, as a uniform metal surface could not be maintained. No. 2 consists of antimony fused within a glass tube. The active lower end is ground flat and polished. Connection to the external circuit is made through a layer of mercury resting on the top surface of the antimony. This electrode was found to give erroneous results on continuous operation due to occlusion of impurities at the glass–antimony metal seal. Nos. 3, 4, and 5 illustrate the general design of an electrode formed by molding rubber around an antimony casting connected to an external lead wire. The exposed bottom metal surface is ground and polished to a uniform smooth surface. No. 4 shows a section of the mounting, while No. 3 and No. 5 show types of finished commercial antimony electrodes. A rugged type of electrode has been produced.

Basis for Choice of Electrode Form

The molded form of antimony electrode appears to be superior to other types because of three primary considerations.

1. The active electrode surface is completely immersed in the solution. This prevents secondary reactions where the metal enters the solution, as may be the case where a plain stick of antimony is used. The author believes that continuous measurements using an unprotected stick of antimony give erroneous results.

2. The active portion of the metal surface is flat and the exposed metal surface has no rough edges or crevices, which may collect sediment from the liquid. Secondary potential effects are thereby prevented. The antimony metal is slowly etched and from time to time it is necessary to resurface the exposed metal in order to reduce absorption errors. It is essential that the wire lead and the upper portion of the metal be protected from the action of the solution.

3. An electrode assembly is obtained which can withstand rough usage in the industrial field.

Information as to the most desirable size of metal surface, the influence of annealing, and the relative effect of a polished and sandblasted surface was also obtained. The area of the exposed metal surface which is required is governed by the conductance of the solutions in which the measurements are to be made. The larger the exposed surface, the lower is the sensitivity of the galvanometer required in the potential measurement. The author adopted an exposed surface 13 mm. in diameter for average conditions. His tests on the value of annealing at 573° C. indicated that there was little advantage in this treatment for commercial electrodes.

Beilby (*1*) has shown that the flowed layer of a metal on a polished surface is vitreous rather than crystalline. When the solutions are in contact with air the polished antimony surface becomes etched within a short time. Hence, for continuous e. m. f. measurements there is apparently little difference between starting with a polished or rough surface, if an adequate time is provided for the surface equilibrium and oxide formation to be established. However, the scratched sections of an antimony surface are the first to be etched and the deep crevices may hold sparingly soluble salts and introduce false potentials. Accordingly, it is wise to utilize an initially polished surface and thereby provide an even crystal etching. The polished surface must be etched by solution immersion, preferably in a 7 to 9 pH buffer for an hour or two prior to use.

Over five hundred different industrial recording pH installations now utilize the molded antimony electrode.

Literature Cited

(1) Beilby, *Proc. Roy. Soc.* (London), (A) 82, 599 (1909).
(2) Britton, H. T. S., "Hydrogen Ions", 2nd ed., p. 80, Chapman and Hall, Ltd., London, 1932.
(3) Clark, J., *J. Chem. Soc.*, 63, 886 (1893).
(4) Clark, W. M., "The Determination of Hydrogen Ions", 3rd ed., p. 424, Baltimore, Williams and Wilkins, 1928.
(5) Coons, C. C., IND. ENG. CHEM., Anal. Ed., 3, 402 (1931).
(6) Getman and Daniels, "Outlines of Theoretical Chemistry", 5th ed., p. 474, John Wiley & Sons, New York, 1931.
(7) Kolthoff and Furman, "Potentiometric Titrations", 2nd ed., New York, John Wiley & Sons, 1931.
(8) Milbauer and Slemr, *Chem. Listy*, 20, 392 (1926).
(9) Roberts and Fenwick, *J. Am. Chem. Soc.*, 50, 2125 (1928).
(10) Ruff and Albert, *Ber.*, 38, 54 (1905).
(11) Shukov and Awsejewitch, *Z. Elektrochem.*, 35, 349 (1929).

Characteristics of the Antimony Electrode

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A STUDY of the methods of making a desirable form of industrial antimony electrode has been presented in the previous paper (*1*). The work discussed in the present paper was carried out with a molded hard-rubber flat-surface type of antimony electrode, using a saturated-calomel reference electrode in all the tests.

Essential Factors

The e. m. f.–pH relationships of the antimony electrode are influenced by several factors, a knowledge of which makes it possible to employ this electrode to distinct advantage in the continuous recording of the pH of industrial solutions. The stability and the limit of error of the e. m. f. measurement depend upon (1) the nature of the electrode surface, (2) the concentration of dissolved air or oxygen, (3) the agitation prevailing at the electrode surface, (4) the nature and concentration of the dissolved salts, and (5) the temperature of the system.

By proper standardization of these variables, it is possible to obtain continuous pH measurements well within the limit of error demanded by the average industrial application. With proper maintenance a reproducibility of ±0.15 pH may be obtained.

Nature of Electrode Surface

Whenever an antimony electrode is immersed in an aqueous solution that is saturated with air at approximately 25° C., a slow etching action occurs whereby the crystalline nature of the antimony becomes clearly evident after approximately 2 hours of immersion. The e. m. f. of the electrode system gradually drifts until the electrode surface has become thoroughly etched. At least sufficient amounts of a reproducible and sparingly soluble antimony compound must be formed to set up an equilibrium involving the antimony ion.

When an electrode with a polished antimony surface is immersed in an aqueous solution free from dissolved oxygen or air, there is very little change in the nature of the surface. This is in great contrast to the case where dissolved oxygen or air is present. The higher the dissolved oxygen content the more rapid is the etching reaction.

Long-continued use of an antimony electrode in aqueous solutions that are in contact with air results in a surface that is deeply etched. The etching becomes deepest at points of abrasion. Initially smooth surfaces are desirable. A too deeply etched antimony surface tends to occlude impurities and it is then necessary to repolish.

In order to avoid uneven corrosion or excessive secondary reactions, it is desirable to wipe off the antimony electrode surface after 24 hours of continuous service. It is essential to pretreat the polished antimony surface prior to use, in order to secure the proper type of sparingly soluble antimony compound.

An extensive series of automatically cleaned electrode tests has been carried out in order to establish the influence of maintaining a surface which was always free from secondary reaction products. An automatically operated surface cleaner is helpful in certain cases where crystalline salts tend to separate out of the solution or where secondary reactions occur, but is not essential for many commercial applications.

Concentration of Dissolved Air or Oxygen

The e. m. f.–pH relationship of the antimony–saturated-calomel electrode system depends upon the oxygen concentration in the solution. The potential of the pretreated system when buffers were saturated at 25° C. with various gases, and when the solution was constantly stirred, was found to be as follows:

Saturating Gas	3.95 pH *Volt*	6.91 pH *Volt*	11.04 pH *Volt*
Nitrogen	0.261	0.416	0.655
Air	0.224	0.398	0.622
Oxygen	0.201	0.375	0.594

The stability of the electrode in 3.95 pH buffer in the presence of nitrogen gas is not good. There is always a tendency to drift to lower voltage values on continued operation.

The author's studies indicate the following millivolt change per pH change at 25° C. for the antimony–saturated-calomel electrode system:

Saturating Gas	4.0 to 7.0 pH *Volt*	7.0 to 11.0 pH *Volt*
Nitrogen	0.052	0.058
Air	0.059	0.051
Oxygen	0.059	0.050

The Nernst equation at 25° C. indicates that there should be a change of 0.0591 volt per pH change.

The tendency towards instability in the acid range when the dissolved oxygen concentration is low and the failure of the electrode to follow the Nernst equation in the alkaline range when dissolved oxygen is present are characteristics of the antimony electrode. The relative ease of oxidation of the antimony in the alkaline range produces secondary products at the metal interface with the result that the equilibrium conditions are disturbed. In the acid range, in the absence of any dissolved oxygen, the amount of antimony oxide maintained at the metal interface is inadequate to establish equilibrium conditions.

Agitation at Electrode Surface

The e. m. f.–pH relationships of the antimony–saturated-calomel electrode system depend upon the condition of the solution at the metal-solution interface. When the solution is agitated, the e. m. f. of the air-saturated system is different from that when there is no motion at the electrode interface. However, a small agitation at the electrode surface has nearly the same disturbing influence as a vigorous agitation.

The extent of the influence of agitation depends upon the pH, the temperature, and the nature of the solution, and the concentration of dissolved oxygen.

When a buffered solution is saturated with air at temperatures below 15° C., there are only small differences between the readings in agitated and nonagitated solutions over the range of 4 to 11 pH. The influence of agitation is more critical in the case of a solution with a very low salt content. Certain salts, such as citrates or tartrates, favor a greatly increased solubility of antimony metal by oxygen, as well as an increased solubility of the oxide, and these present exceptions to the general case. As the temperature is raised above 15° C., the higher the temperature the greater is the difference between the agitated and nonagitated readings, but with moderately well-buffered solutions below 9.0 pH the error is less than 0.1 pH even at 45° C. In any instance, the nonagitated solution approaches the true equilibrium potential in accordance with the Nernst equation for the particular pH and temperature condition.

Stable e. m. f. readings for the antimony–saturated-calomel electrode system were obtained while the buffer solution was agitated in contact with air with a mechanical stirrer. The stirring was stopped and after 5 minutes another set of readings was taken. The following gives a qualitative idea of the differences noted:

	3.95 pH	6.91 pH	11.04 pH
35° C.	5-mv. rise	5-mv. rise	15-mv. rise
25° C.	2-mv. drop	No change	12-mv. rise
10° C.	3-mv. drop	2-mv. drop	2-mv. drop

However, when buffer solutions are saturated with pure oxygen rather than air, very stable readings are obtained over the temperature ranges of 10° to 35° C. and for ranges of 4 to 11 pH. When using oxygen, the difference between agitated and nonagitated readings is not greater than 0.1 pH over the whole range. This fact is particularly interesting since the slope of the e. m. f.–pH curve with alkaline solutions saturated with oxygen does not follow the Nernst equation. The author's data show that a very reproducible pH electrode results when using a properly designed antimony electrode in solutions saturated with oxygen. This system may be applied to a wide range of pH and the results are not greatly affected by the agitation of the solution, at least over the temperature range of 10° to 40° C.

Considerable differences were noted between the readings for agitated and nonagitated buffer solutions which were saturated with nitrogen in the range of 3 to 7 pH. The differences were smaller in the 7 to 11 pH range. Acid solutions with a deficiency of dissolved oxygen cause an unfavorable operation of an antimony electrode after a few hours of immersion.

The author feels that any condition favoring the reduction of dissolved oxygen in acid solutions, such as temperatures above 40° C., saturation with nitrogen, or the presence of certain salts, tends to give the most pronounced differences between the e. m. f. measurements of the antimony–saturated-calomel electrode system in agitated and nonagitated solutions. Since antimony metal is soluble in aqueous solutions in the presence of dissolved oxygen, there is a tendency to reduce the oxygen concentration of the solution close to the antimony surface. The oxygen depletion is less in acid solutions than in alkaline solutions. Secondary reactions in the alkaline range involving antimonates will change the normal equilibrium conditions. The use of a nitrogen atmosphere in the alkaline range limits this oxidation. When using a nitrogen atmosphere, it is necessary to employ an electrode surface which has been etched by the interaction of dissolved oxygen and thereby has an excess of sparingly soluble antimony compound at the metal-solution interface. An excess of antimony trioxide is desirable in alkaline solutions which are saturated with nitrogen. A continuous movement of solutions past the metal surface produces an unsaturated surface condition unless more oxygen is available to oxidize more of the antimony metal.

When a high concentration of oxygen is available, as at low solution temperatures or in the presence of an oxygen atmosphere, there is sufficient oxidation at the metal surface to ensure saturation, and sufficient excess of dissolved oxygen present at all times to continue the equilibrium conditions even for rapid flows of fresh solution past the electrode surface, as is the case when the solution is agitated. Equilibrium conditions are not obtained when dealing with alkaline solutions saturated with air, since the partial pressure of oxygen is too low to supply all the oxygen required for the various reactions which occur with a continuously changing solution at the metal surface. However, this results only in a change in slope of the e. m. f–pH curve. This also indicates why those solutions which are saturated with oxygen gas show small differences between the agitated and nonagitated conditions, while the alkaline solutions saturated with air are responsive to agitation.

Nature and Concentration of Dissolved Salts

The basic equation for a metal–metal ion electrode involves a consideration of the activity of the water and the solubility of the oxide. As expected, the antimony electrode exhibits definite salt effects.

An e. m. f.–pH relationship may be obtained by the use of certain well-known buffer solutions which will apply to a large number of industrial solutions. Such buffers as potassium hydrogen phthalate, mixtures of this with hydrochloric acid or sodium hydroxide (Clark and Lubs), sodium acetate and hydrochloric acid mixtures (Walpole), primary potassium phosphate and sodium hydroxide mixtures, disodium phosphate and primary potassium phosphate mixtures (Sørensen), boric acid and sodium hydroxide mixtures (Clark and Lubs), and disodium phosphate and sodium hydroxide mixtures (Ringer) have been used to obtain an e. m. f.–pH relationship over the range of 3 to 12 pH and for temperatures from 10° to 70° C., and consistent results have been obtained over the above pH range. These solutions have a specific resistance at 25° C. varying from 100 to 300 ohms. This feature will be discussed in connection with temperature coefficients.

When the calibration curve obtained with the above solutions is used with buffer solutions containing tartrates, citrates, oxalates, or phenyl acetic acid (the Prideaux and Ward buffer), there may be a wide divergence over the 3 to 12 pH range at 25° C. Since this error does not involve the RT/F pH term, or slope portion of the fundamental equation, it is possible to make a constant correction. The influence of temperature upon these solutions is markedly different from that on the previously mentioned buffer solutions. Reproducible results are obtained in solutions containing these disturbing salts, yet a special calibration curve must be used. The use of many so-called universal buffers involves the above error.

When dealing with water solutions in which the salt concentration is low, such as the average city water in the eastern United States, the calibration curve obtained by the first mentioned group of buffers has been found to give erroneous results. It is necessary to use a different type of e. m. f.–pH relationship for solutions of low salt content, yet for these specific conditions reproducible results may be obtained. The specific resistance of this type of solution varies from 3000 to 20,000 ohms at 25° C.

An e. m. f.–pH curve based upon buffers free from citrates, tartrates, etc., has been used in many industrial types of recording pH installations since 1932. As the constituents of a given type of application remain fairly constant, it is possible to make an initial correction for any specific application, by making a supplementary indicating pH measurement with a hydrogen gas electrode or its equivalent. The deviation is thus originally obtained and the recording equipment may be set to correct for the specific salt effect. Reproducible results within ± 0.1 pH over the whole pH range will result just as long as the general nature of the solution does not change.

We should not expect that the antimony electrode would respond only to pH changes in the presence of oxidation-reduction potentials and such is the case. However, fairly reproducible results have been obtained in the presence of mild oxidizing agents as well as mild reducing agents. Dilute solutions of hydrogen peroxide, potassium permanganate, and sodium chromate cause disturbing secondary reactions. In these cases, a low pH value results when using an antimony electrode. However, the electrode behaves in a normal

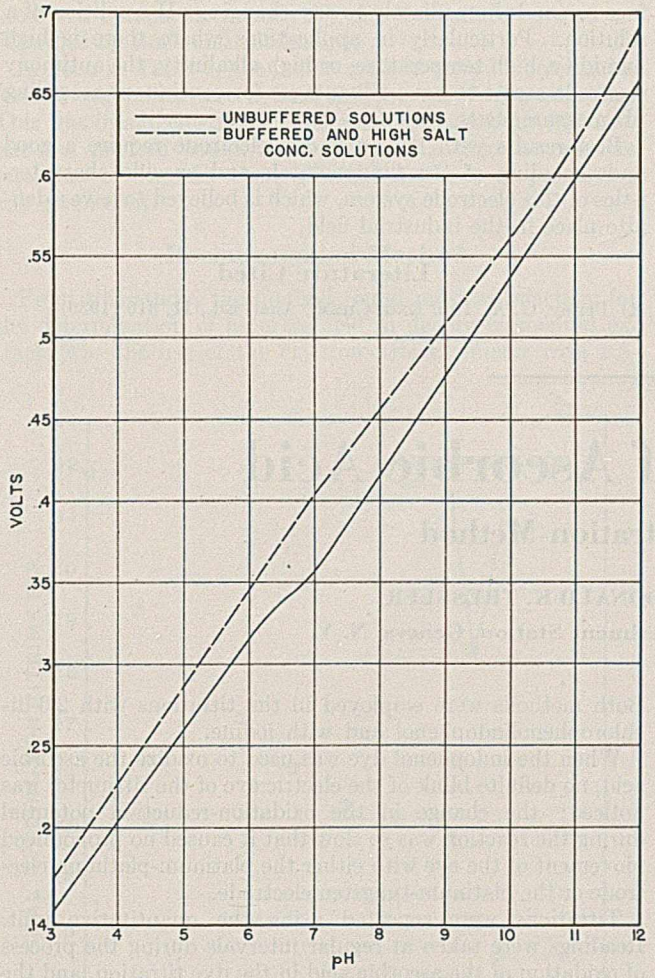

FIGURE 1. E. M. F.–pH FOR ANTIMONY–SATURATED-CALOMEL ELECTRODE SYSTEM

Solutions saturated with air at 25° C. and in motion

manner in solutions with a residual chlorine content of as much as 0.5 part per million. It was found that the antimony electrode recovered from the abnormal effects of oxidizing agents after these had been removed. Good results have been obtained in dilute solutions of sodium sulfite and sodium sulfide.

The continuous use of the antimony electrode in solutions more alkaline than 10 pH with a sodium content in excess of 1 molar results in a slow but gradual deposition of white crystals (probably sparingly soluble sodium antimonates) on the antimony surface. The error is not great, but unless these crystals are occasionally removed, an abnormally high pH reading will result.

Even traces of copper in solution involve a limitation of the antimony electrode. The presence of copper in solutions coming in contact with an antimony electrode establishes secondary reactions which result in abnormal potentials, which would be interpreted as low pH values for a given solution. The presence of 0.5 part of copper per million in a 6.92 pH buffer causes an immediate error of 0.2 pH. When one part of copper per million is present, the coating of copper on the antimony surface may be clearly seen within a half hour after immersion. Apparently there are two types of error: an oxidation-reduction error which occurs immediately, and a secondary copper–copper-ion potential which increases with increased immersion of the electrode. A given concentration of copper produces a greater error the more acid the solution. Accurate results can be obtained on continuous recording installations only when the copper content of the solution is below 0.1 part per million. Fortunately, the solubility of copper in solutions more alkaline than 7 pH is so low that we encounter little trouble in this range of application; however, in the acid range the presence of mere traces of copper may be serious.

Solutions of metals less electropositive than antimony will behave like copper solutions. In other words, when we get immersion deposition of some other metal on the antimony surface, we are no longer involved with an antimony electrode.

A systematic study of the many possible salt errors of the antimony electrode has been undertaken, but this forms material for a separate paper.

Influence of Temperature

The temperature coefficient of the antimony electrode has been determined when the electrode is used in the series of air-saturated buffers mentioned in the preceding section. The coefficient of the antimony–saturated-calomel electrode system varies with the pH value of the buffer and is of the order of 0.00115 volt per 1° C. at 3 pH, 0.00210 volt per 1° C. at 7 pH, and 0.00344 volt per 1° C. at 12 pH. The temperature coefficient for unbuffered solutions is different from that of buffered solutions.

As the problem of temperature coefficients is somewhat involved, yet of great importance to the interpretation of the e. m. f. values of the antimony electrode, this subject will be presented in a separate paper.

Industrial Applications

The use of air-saturated solutions for the continuous recording of pH is more practical than the use of oxygen-saturated solutions. The author's early results indicated that it was possible to diagnose the average industrial conditions and to apply the proper calibration curve.

When dealing with an industrial solution, a calibration curve may be based upon that obtained with the buffers mentioned in the section on the nature and concentration of dissolved salts. For the antimony–saturated-calomel electrode system, used in agitated buffer solutions saturated with air at 25° C., this follows the general relationship:

From 3 to 7 pH	$E = -0.008 + 0.059$ pH
From 7 to 11 pH	$E = +0.050 + 0.051$ pH

When involved with low salt concentrations as in many water-treatment applications at 25° C., a different relationship results:

From 3 to 6 pH	$E = -0.024 + 0.056$ pH
From 6 to 8 pH	A nonlinear relationship
From 8 to 11 pH	$E = -0.071 + 0.060$ pH

Figure 1 shows the e. m. f.–pH relationships at 25° C. for air-saturated solutions.

Most industrial solutions are in contact with air, and generally information is available as to their nature and salt content; hence, it is relatively easy to apply the correct scale law for any given application. It is possible to supply a recording potentiometer with a manually adjustable rheostat in order to fix the scale to an exact value at the most important point on the pH range. In the absence of the chief disturbing features, such as oxidation-reduction potentials or copper, a pH measurement reproducible to ±0.15 pH may be obtained, provided reasonable consideration is exercised in the choice, installation, and care of the electrode system. Oxidation-reduction potentials or copper poisoning are present in a distinct minority of industrial applications.

The antimony electrode has given satisfactory industrial performance in widely different types of commercial applications, such as in sugar-mill liquors, paper-mill solutions, water-treatment systems, aqueous starch suspensions, various silicate solutions, clay suspensions, sulfite solutions, phosphate solutions, ammonium hydroxide solutions, lime treatment, soda ash neutralization, alum solutions, beer, etc.

The antimony electrode is rugged. Since the antimony–saturated-calomel electrode system represents a relatively low electrical resistance type of pH measuring equipment, the problems of electrical pickup and leakage are practically absent. An antimony electrode with a properly prepared surface responds immediately to any changing pH condition of a solution. Particularly in applications where there is high humidity, high temperature, or high alkalinity, the antimony electrode seems to have advantages for continuous recording pH measurements.

Best results with the antimony electrode require a good understanding of the fundamentals and specific characteristics of this electrode system, which is believed to have a definite place in the industrial field.

Literature Cited

(1) Perley, G. A., Ind. Eng. Chem., Anal. Ed., 11, 316 (1939).

Determination of Ascorbic Acid

Electrometric Titration Method

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UNTIL recently no chemical method for the determination of vitamin C has proved adequate in the presence of colored pigments. A new method using 2,6-dichlorophenolindophenol in electrometric titrations is presented herewith.

In making vitamin C determinations on fruits and vegetables the titration method of Tillmans (*10*), as modified by Bessey and King (*2*) and Mack and Tressler (*7*), has been used extensively. These authors titrate the ascorbic acid with 2,6-dichlorophenolindophenol. The complete oxidation of the ascorbic acid is indicated by a change in the color of the solution titrated from colorless to a faint pink in the acid solution. This color change is obscured in juices with a natural red or pink pigment. The animal assay method can be employed in such cases, but it is expensive, must be continued through a long time interval, is not sensitive to small differences in concentration, and is not applicable to many problems where titration methods can be used satisfactorily.

Since the present investigation was completed, two publications (*1*, *4*) have appeared describing the use of the photoelectric colorimeter which also can be used in determinations on colored extracts. The chief difficulty in most of the methods of determination lies in the fact that the oxidizing agents are nonselective and will oxidize compounds other than ascorbic acid. The 2,6-dichlorophenolindophenol dye seems to have a high specificity for reduction by vitamin C. Very few other compounds react rapidly with the dye in acid solution. Since this dye is considered a satisfactory indicator for the estimation of vitamin C in colorless extracts, a method employing it in pigmented solutions where the end point is masked was considered advantageous. The color change at the end point in the titration with the dye is accompanied by a decrease in oxidation-reduction potential; hence a variation in e. m. f. should be easily measured by means of suitable equipment. This is possible with an electrometric titrimeter such as the one used in the present investigation (*5*).

Methods Used

The electrometric titrimeter can be used in two ways. If the qualitative unit is used alone, the end point of the reaction is indicated by a distinct blink of the electric eye. A titration curve can be plotted using the quantitative unit. Both methods were employed in the titrations with 2,6-dichlorophenolindophenol and with iodine.

When the indophenol dye was used to oxidize the ascorbic acid, no definite blink of the electric eye of the titrimeter was noticed; the change in the oxidation-reduction potential during the reaction was so slow that it caused no pronounced movement of the eye with either the platinum-platinum electrode or the platinum-tungsten electrode.

Titrations were repeated using the quantitative unit. Readings were taken at regular intervals during the process of oxidation of the ascorbic acid in the dye titration, and the curve was plotted. The addition of dye causing the first large change of potential was taken as the end point of the reaction. This point was easily found by plotting the differences between consecutive readings.

The Stevens iodine method (*9*) was also tried on the titrimeter. The visual color change coincided exactly with a distinct closure of the eye, giving a definite end point. No titration curve could be obtained, since a back-titration was used. However, the curve was unnecessary, since a distinct end point could be found more quickly with the blink of the eye using only the qualitative unit. Frequent standardization of the iodine and thiosulfate was unnecessary, but the dye

Table I. Ascorbic Acid Content of Vegetables

Vegetable	Visual Titration	Electrometric Titration		Iodine Titration	
			Difference from visual titration		Difference from visual titration
	Mg./g.	*Mg./g.*	%	*Mg./g.*	%
Yellow Tomato					
Yellow Plum	0.229	0.245	+7.0	0.288	+25.8
Golden Queen	0.274	0.269	−1.8	0.320	+16.8
Ripley	0.181	0.185	+2.2	0.263	+45.3
Yellow Marigold	0.287	0.285	−0.7	0.353	+23.0
Golden Ball	0.213	0.213	0.0	0.203	−4.7
Golden Dwarf Champion	0.206	0.194	−5.8	0.253	+22.8
Peas (frozen)	0.130	0.130	0.0	...	...
Kale, German Dwarf (leaf)	1.170	1.160	−0.9	...	...
Spinach, New Zealand	0.192	0.199	+3.6	...	...
Lettuce					
Iceberg	0.102	0.096	−5.9	...	...
Mignonette	0.109	0.105	−3.7	...	...
Lemon juice	0.352	0.349	−0.9	...	...
Orange juice	0.348	0.365	+4.9	...	...

used was standardized daily by the thiosulfate method as described by Buck and Ritchie (*3*).

As seen in Table I, the iodine method gave higher results for the vegetables tested than did the visual dye titration. This was in agreement with the results obtained by Stevens, but the method could be used for following the relative retention of vitamin C in different methods of processing any given fruit.

Recommended Method

From this study, the method found most satisfactory for the determination of ascorbic acid in deeply pigmented extracts was the use of the electrometric titrimeter with 2,6-

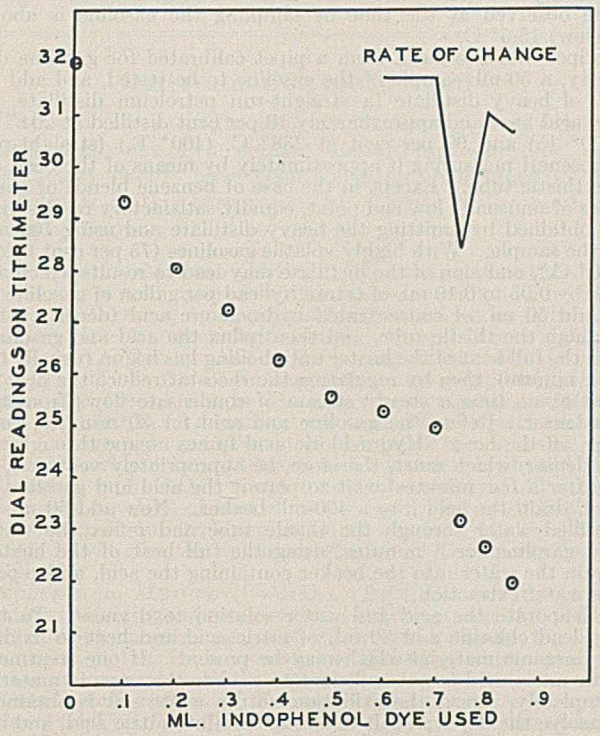

FIGURE 1. DETERMINATION OF ASCORBIC ACID

dichlorophenolindophenol to determine points on a titration curve. A platinum-tungsten electrode stirrer was found to be more sensitive than a platinum-platinum electrode in the determination of the potential.

Extracts were made of peas, kale, lettuce, and spinach by placing weighed samples in mortars containing 30 cc. of the extracting solutions, and grinding with acid-washed sand. They were centrifuged for at least 5 minutes and the supernatant liquid was poured off into 100-cc. volumetric flasks. Washing of the material was repeated with 30-, 20-, and 20-cc. portions of the fresh extracting solution. The extract was made up to volume with distilled water. Tomatoes were run through a Sep-ro-siv equipped with a 20-mesh screen instead of grinding with sand. This removed skin and seeds. Although only 75 cc. of the extractant were used with 25 cc. of the pulpy tomato juice, four extractions were made. Sulfuric acid (*N*, 5 per cent) was used in the case of tomatoes, lettuce, and spinach, while 8 per cent trichloroacetic acid was used for peas and kale. As suggested by several workers (*6*, *7*, *8*), 2 per cent metaphosphoric acid was used in all acid extractants to prevent catalytic oxidation of ascorbic acid. Varying concentrations of ascorbic acid dissolved in 8 per cent acetic acid were tested as checks, using both the visual titration and electrometric titration methods.

For titration with the titrimeter, a 10- or 20-cc. aliquot of the extract was transferred into a 50-cc. beaker and about 25 cc. of 8 per cent acetic acid were added to increase the volume. Dye was added in 0.1-cc. aliquots until the end point was approached, after which 0.05-cc. portions were added. Readings were taken

TABLE II. DETERMINATION OF PURE ASCORBIC ACID

Visual titration, mg./100 cc.	1.54	2.38	3.14	4.19
Electrometric titration, mg./100 cc.	1.57	2.52	3.32	4.29
Difference, %	+1.95	+5.88	+2.55	+2.39

on the titrimeter about 15 seconds after each addition; if taken immediately or much after 15 seconds, reliable results were not obtained. The titration curve was plotted and the first large change of potential found. Figure 1 is a typical curve. The rate of change of readings on the titrimeter dial appears at the top of the graph. The results of the visual dye titration and electrometric titration methods agree to within experimental error for both vegetables (Table I) and pure ascorbic acid (Table II). Dilution of the dye results in more accurate determinations.

Since the method was proposed for colored extracts, some work was also carried out with strawberry juice. The color made visual titration difficult, but results obtained with very dilute solutions agreed with titrimeter results. The titrimeter gave 0.489 mg. of ascorbic acid per cc. of juice, while visual titration gave 0.495 mg. of ascorbic acid per cc.

In order to test the specificity of the titrimeter method for vitamin C, some of the strawberry juice was filtered through Norite. This oxidized the ascorbic acid to dehydroascorbic acid which does not reduce the dye. Known quantities of ascorbic acid were added to 10 cc. of this oxidized juice plus 10 cc. of 10 per cent metaphosphoric acid. Titration showed that the presence of oxidizing or reducing agents found in the juice did not interfere with the end points. Results are given in Table III.

TABLE III. DETERMINATION OF ASCORBIC ACID ADDED TO OXIDIZED STRAWBERRY JUICE

Ascorbic Acid		
Added	Found by titrimeter	Difference
Mg.	*Mg.*	%
0.125	0.121	−3.2
0.250	0.252	+0.8
0.500	0.494	−1.2

Summary and Conclusions

Because of the obvious difficulty in obtaining a rapid check on the use of the electrometric titrimeter to determine ascorbic acid in colored extracts, substances were studied with which it was possible to use the usual indophenol method. If a titration curve is drawn using the titrimeter, the values derived therefrom agree consistently with those of the ordinary visual titration. The indicating eye alone cannot be used, since the change in e. m. f. in the reaction is not rapid enough to cause a noticeable blink. Reducing substances, other than ascorbic acid, which are usually found in fruit juices, did not interfere with the end point.

Literature Cited

(1) Bessey, O. A., *J. Biol. Chem.*, 126, 771 (1938).
(2) Bessey, O. A., and King, C. G., *Ibid.*, 103, 687 (1933).
(3) Buck, R. E., and Ritchie, W. S., IND. ENG. CHEM., Anal. Ed., 10, 26 (1938).
(4) Evelyn, K. A., Malloy, H. T., and Rosen, C., *J. Biol. Chem.*, 126, 645 (1938).
(5) Fischer Scientific Co. *Laboratory*, 9, 46 (1938).
(6) Fujita, A., and Iwatake, D., *Biochem. Z.*, 277, 293 (1935).
(7) Mack, G. L., and Tressler, D. K., *J. Biol. Chem.*, 118, 735 (1937).
(8) Musulin, R. R., and King, C. G., *Ibid.*, 116, 409 (1936).
(9) Stevens, J. W., IND. ENG. CHEM., Anal Ed., 10, 269 (1938).
(10) Tillmans, J., Hirsch, P., and Jackisch, J., *Z. Untersuch. Lebensm.*, 64, 276 (1932).

APPROVED by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 303. Taken from the report to be submitted by Mary Mann Kirk to the Graduate School of Cornell University in partial fulfillment of requirements for the degree of doctor of philosophy.

Determination of Tetraethyllead in Gasoline

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Tetraethyllead in gasoline is determined quantitatively by refluxing the gasoline with concentrated hydrochloric acid, extracting the lead chloride with water, and determining the lead found by any of the standard methods.

AN EARLIER publication from this laboratory (*1*) described a method for the determination of tetraethyllead in gasoline, based on bromination of the gasoline and separation of the lead as bromide. This method has been in wide use since its publication, and appears to give satisfactory results in a majority of cases. It presents, however, certain difficulties when applied to highly cracked gasolines, on which results are apt to be low even if the operator is particularly skilled and careful, and it cannot be applied at all to such fuels as alcohol blends. These drawbacks, coupled with the recent demand for greater accuracy, have led to the development of an improved method which is described below. The principle of this method, treatment of the gasoline with concentrated hydrochloric acid, was first suggested by Ferreri (*2*) and has recently been proposed again by the Imperial Oil Company of Canada, in Sarnia, Ontario (*3*). The method described here is a modification of these earlier suggestions, aimed at an increase in rapidity and ease of handling without sacrifice of accuracy.

Apparatus

The apparatus (Figure 1) is made of heat-resistant glass and consists of a 500-ml. boiling flask; a Hopkins reflux condenser, the vapor outlet of which is vented by a rubber tube to an outside vent or to a hood; a thistle tube of approximately 70-ml. volume with bead to indicate approximately 50 ml. of volume; a heating tube with a chimney for increasing convection in the liquid; a heating coil, 250 watts, made of 2.7 meters (9 feet) of No. 30 B. and S. Nichrome wire; and a rheostat of 25 ohms' resistance with a current carrying capacity of at least 2.0 amperes, for regulating the heater. (This apparatus is available through several makers of glassware and laboratory supply houses.)

Method

The reaction consists in converting the tetraethyllead to lead chloride by refluxing the sample of gasoline with concentrated hydrochloric acid, and extracting the lead chloride with water, for determination by any standard method of analysis for lead.

Procedure

Obtain the temperature of the sample of gasoline to be tested. If it is desired to follow the practice of the oil industry to refer measurements to the standard temperature of 60° F. (15.5° C.), the true tetraethyllead content of the gasoline at 15.5° C. is obtained by adding (subtracting) 0.1 per cent of the milliliters of tetraethyllead present for each degree Centigrade the temperature observed at the time of sampling the gasoline is above (below) 15.5° C.

Pipet into the flask, from a pipet calibrated for gasoline delivery, a 50-ml. sample of the gasoline to be tested, and add 50 ml. of heavy distillate [a straight-run petroleum distillate, of low acid heat and approximately 10 per cent distilled at 204° C. (400° F.) and 90 per cent at 238° C. (460° F.) (straight-run kerosene)] measuring it approximately by means of the bead on the thistle tube. Except in the case of benzene blends or gasolines of unusually low end point, equally satisfactory results may be obtained by omitting the heavy distillate and using 100 ml. of the sample. With highly volatile gasolines (75 per cent below 100° C.), omission of the distillate may lead to results which are low by 0.05 to 0.10 ml. of tetraethyllead per gallon of gasoline.

Add 50 ml. of concentrated hydrochloric acid (density 1.19) through the thistle tube, and then reflux the acid and gasoline. Use the full heat of the heater until boiling has begun (usually 0.5 to 1 minute), then by regulating the rheostat reduce the heat so that at no time a steady stream of condensate flows from the condenser. Reflux the gasoline and acid for 30 minutes, then turn off the heat. Hydrochloric acid fumes escape through the condenser, which must, therefore, be appropriately vented.

After a few minutes' wait to permit the acid and gasoline to cool, drain the acid into a 400-ml. beaker. Now add 50 ml. of distilled water through the thistle tube and reflux the water and gasoline for 5 minutes, using the full heat of the heater. Drain the water into the beaker containing the acid, and repeat the water extraction.

Evaporate the acid and water solution to dryness. To the dry lead chloride add 30 ml. of nitric acid and heat to oxidize any organic material which may be present. If one treatment with nitric acid is not sufficient to oxidize the organic material completely, repeat the oxidation until a white salt is obtained. Dissolve the dry lead salt in 10 ml. of dilute nitric acid, and determine the lead present in the solution by any standard procedure for lead.

(When the concentration of tetraethyllead is expressed in volume, the density of the pure material is taken as 1.65, which is equivalent to: 1 ml. of tetraethyllead = 1.0570 grams of lead.)

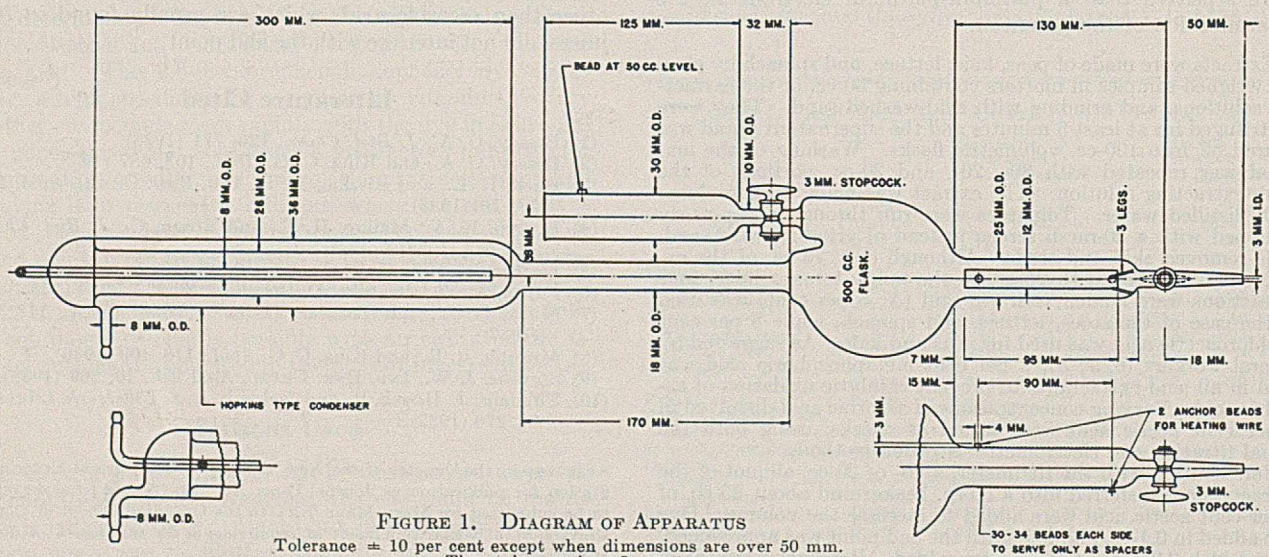

FIGURE 1. DIAGRAM OF APPARATUS

Tolerance ± 10 per cent except when dimensions are over 50 mm. Then tolerance is ±5 mm.

TABLE I. DETERMINATION OF TETRAETHYLLEAD IN GASOLINE

(Results in ml. of $Pb(C_2H_5)_4$ per gallon at 15.5° C.)

	Mean Error (Observed − Calculated)								Precision[d]	
Type of Gasoline:	Very volatile		Partly cracked			Highly cracked	Mean	Reliable Limits[c]	Same laboratory	Different laboratories
Calculated Composition:	2.820	0.978	2.989	1.430	0.286	2.293	1.80	....	...	...
Regular[a] HCl reflux	−0.003[b]	−0.010	−0.003[b]	−0.011[b]	−0.006[b]	−0.039	−0.012	±0.006[c]	0.026	0.050
No. of determinations	24	20	18	15	13	13				
No. of laboratories	9	8	7	7	6	7				
Optional[a] HCl reflux	−0.028	−0.014	−0.019	−0.008[b]	−0.005[b]	−0.039	−0.019	±0.007[c]	0.023	0.032
No. of determinations	28	32	42	33	31	34				
No. of laboratories	12	13	14	14	14	13				
Bromine	−0.043	−0.013[b]	−0.062	−0.041	−0.041	−0.124	−0.054	±0.011[c]	[e]	0.075
No. of determinations	10	10	10	10	10	8				
No. of laboratories	6	6	6	6	6	5				

[a] Regular method, 50 cc. of sample + 50 cc. of heavy distillate. Optional method, 100 cc. of sample, no heavy distillate.
[b] Error is not significant—i. e., does not differ reliably from zero.
[c] Twice standard deviation of mean.
[d] Twice standard deviation of individual results.
[e] Depends upon care taken in bromination; one laboratory with extreme care obtained a precision of 0.012, three other laboratories with ordinary care obtained a precision of 0.062.

Determination of the Lead

The lead present in the lead nitrate solution can be determined by any one of the regular standard procedures for lead. Satisfactory results have been consistently obtained in the laboratories of this corporation by the use of the methods described below.

GRAVIMETRIC METHOD ($PbCrO_4$). Neutralize the nitric acid solution of the lead nitrate first with dilute ammonium hydroxide, adding 5-ml. excess, using *p*-nitrophenol or litmus as indicator, and then with dilute acetic acid, adding 1- to 2-ml. excess. Dilute the solution to 200 ml., and to the boiling solution add drop by drop 10 to 15 ml. of a 10 per cent solution of potassium dichromate. Boil the solution until a deep orange colored precipitate is obtained (10 to 15 minutes), cool, and allow to settle several hours or overnight. Collect the precipitate on a dried and weighed Gooch crucible, wash well with hot water, dry at 110° C., and weigh as $PbCrO_4$. (On an original sample of 50 ml. of gasoline, the weight of lead chromate multiplied by 48.533 will give the grams of lead per gallon of gasoline; multiplied by 45.915, it will give the milliliters of tetraethyllead per gallon of gasoline.)

VOLUMETRIC METHOD ($PbMoO_4$). *Lead Nitrate.* Prepare a solution containing approximately 4.8 grams per liter of C. P. lead nitrate. Standardize the solution by precipitating the lead from an aliquot, as lead chromate, using the procedure described for the gravimetric method.

Tannic Acid Indicator. Prepare a 0.5 per cent solution in water of the U. S. P. fluffy tannic acid. This solution deteriorates on prolonged standing, and should be prepared at frequent intervals.

Ammonium Molybdate. Prepare a solution containing 2.38 grams per liter C. P. ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$. For standardization, take an aliquot of the standard lead nitrate solution, preferably 25 ml., make ammoniacal with dilute ammonium hydroxide to slight excess, and then make acid with dilute acetic acid, using 1- to 2-ml. excess. Dilute to 150 ml., and titrate hot with the ammonium molybdate solution, using tannic acid as an external indicator. (A small electric hot plate may conveniently be used, as the sample must be kept above 90° C. during the titration.) For the end-point tests care must be taken always to use the same amount of solution (4 drops) to add to the indicator solution (2 drops) contained in the depression of a spot plate. Determine a blank on the same amount of water and ammonium acetate, and subtract the amount of molybdate solution used (about 0.3 ml.) from the result of the titration.

The molybdate solution may be adjusted to be equivalent to 2.7924 mg. of lead per milliliter, in which case 1 ml. of this solution used on an original 50-ml. sample of gasoline corresponds to 0.2 ml. of tetraethyllead per gallon of gasoline.

The concentration of lead nitrate solution may be adjusted to be equivalent to the molybdate solution, so that it may be used for adding known quantities of lead to the sample to complete the titration in case the sample is overtitrated.

Procedure. Determine the lead present in the nitric acid solution by the method described for the standardization of the ammonium molybdate solution.

For samples titrating, initially, less than 5 ml. of the molybdate solution, 10 ml. of the lead nitrate solution should be added before the titration is completed. The volume of the ammonium molybdate solution equivalent to the lead acetate solution is subtracted from the titration volume before calculating the results.

Discussion

The equipment and procedure as described above are the result of a fairly exhaustive investigation, and no obvious simplification of this method was found which would not sacrifice convenience, or accuracy, or both.

In addition to exhaustive tests in this laboratory on widely different types of gasolines, the method was tested by fourteen laboratories on 6 samples of gasoline made from 3 different base stocks. The results obtained are compared in Table I with the results obtained simultaneously by the bromination method (*1*).

Comparing the three methods, it will be observed that the mean errors for the hydrochloric acid reflux methods are considerably less than for the bromine method. This corresponds to a more complete lead recovery by the hydrochloric acid reflux methods. The regular hydrochloric acid reflux method has less mean error than the optional method.

The precision, which is a measure of how closely results check each other, is approximately equal for results obtained in the same laboratory by either the regular or optional hydrochloric acid reflux methods. When comparing results obtained in different laboratories, the precision is somewhat better for the optional method than for the regular method and both are considerably better than the bromine method. The superiority of the optional method in this respect, which is under consideration only when comparing a result obtained in one laboratory with a result obtained in another laboratory, is ascribed to the variations in technique and burets in different laboratories. The titration error introduced by such variations has a higher relative effect in the regular method because the sample is only half as large as in the optional method.

Conclusions

Table I indicates that the new method yields results with a mean error of only −0.012 ml. of tetraethyllead per gallon of gasoline, as against −0.054 for the bromination method, the improvement being particularly noticeable in the case of cracked gasolines. Furthermore, the precision of the new method is greater than for the bromine method, the upper limit for the variation between laboratories being 0.050 ml. of tetraethyllead per gallon of gasoline by the new method, as compared with 0.075 by the bromine method.

Literature Cited

(1) Edgar, Graham, and Calingaert, George, IND. ENG. CHEM., Anal. Ed., 1, 221 (1929).
(2) Ferreri, *Giorn. chim. ind. applicata*, 1, 625 (1925).
(3) Private communication.

Separation of Cobalt from Manganese

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THE quantitative separation of cobalt from manganese by any known method is difficult. Manganese dioxide precipitated by potassium chlorate and nitric acid tends to carry down some cobalt with it. Precipitation of cobalt sulfide in acetic acid solution does not always give a sharp separation; either some manganese remains with the cobalt or some cobalt with the manganese, or both. The separation here proposed has been suggested by Prescott and Johnson (*5*) and by Lundell and Hoffman (*3*), but no figures to show its accuracy have been published. The usual procedure of separating cobalt from manganese as potassium cobaltinitrite appears somewhat longer than the proposed method, as it requires the removal of ammonium salts and is intended for a few milligrams of cobalt only.

Although cobalt phosphate by itself is soluble in ammonia in the presence of manganese both metals are quantitatively precipitated by ammonium phosphate unless citrate ions are present. As little as 2 mg. of cobalt can be quantitatively separated from 0.1 gram of manganese after the addition of 2 grams of citric acid, when the phosphate precipitation is made by the method of W. Gibbs (*1*, *2*), in which manganese phosphate is slowly precipitated at boiling temperature by the addition of dilute ammonia. The concentration of manganese should not exceed 0.1 gram of manganese in 100 ml. containing 1 to 2 grams of ammonium phosphate and 20 grams of ammonium chloride.

Ammonium citrate appears to act as a differential retarding agent by permitting only a partial retention of cobalt, while only slightly retarding the precipitation of manganese. This permits a fairly clean-cut separation of the two metals in from one to three precipitations of manganese phosphate. The color of the precipitate is helpful in judging the extent of separation. Pure manganese phosphate, which is flesh-colored, becomes lilac in the presence of 1 mg. or more of cobalt.

It is permissible to determine cobalt quantitatively as the sulfate, which may be dehydrated at 600° C. without dissociation (*4*). Consistently accurate results were obtained with quantities up to 0.4440 gram of cobalt sulfate. It is advisable to re-ignite the sulfate as a check for constant weight without an intervening evaporation with water and to stir it with a platinum wire before ignition. This avoids decrepitation and gives the same final weight of cobalt sulfate as that obtained by evaporation with water. No manganese was found in the cobalt sulfate by the colorimetric test.

Any cobalt retained by the manganese phosphate is conveniently determined colorimetrically as the blue cobalt chloride (*6*). The slight green color of cobalt blue, which is perhaps due to iron or some other slight impurity, may be partly overcome by reduction with a little sodium sulfite.

Reagents and Solutions

COBALT NITRATE SOLUTION. Filter a concentrated solution of the C. P. crystals in hot water, add an equal volume of concentrated nitric acid, and allow the cobalt nitrate to crystallize out overnight. Use the wet crystals to prepare a stock solution containing 1 mg. of cobalt per milliliter. The actual strength was established by evaporating 30 ml. with 2 drops of 1 to 1 sulfuric acid and igniting the residue at 600° C.

CITRIC ACID SOLUTION. Dissolve 20 grams of the crystals in 100 ml. of water.

AMMONIUM PHOSPHATE. Use the solid diammonium reagent.

AMMONIUM CHLORIDE. Use the solid reagent.

MANGANESE SULFATE SOLUTION. Use C. P. manganese dioxide to prepare a solution containing 10 mg. of manganese per milliliter.

Procedure

The solution of cobalt and manganese, which has been freed from other members of the ammonium sulfide group as well as the alkaline earths, may be the filtrate from the basic acetate precipitate if only minor quantities of calcium and magnesium are present. More than a few milligrams of the alkaline earths are undesirable because of their pronounced retentive action on cobalt when a group precipitation of phosphates is made. Magnesium phosphate has a remarkably high adsorptive power and is particularly objectionable.

The solution is treated by the Gibbs method for the precipitation of manganese phosphate, except that it also contains 2 grams of citric acid in each 125 ml. The precipitation should proceed slowly. The final precipitate of manganese phosphate is filtered off and washed twice with very dilute ammonia and dissolved in just enough 1 to 1 hydrochloric acid. This solution is caught in the precipitation beaker and evaporated to dryness on the steam bath. For the colorimetric determination of cobalt, this residue is dissolved in 20 ml. of 1 to 1 hydrochloric acid and transferred to a porcelain crucible of 30-ml. capacity, reduced with a few crystals of sodium sulfite, and heated on the steam bath till its volume is about 10 ml. A series of standards from 0.3 to 1.2 mg. of cobalt at 0.3 mg. intervals is similarly treated on the steam bath. From it the quantity of cobalt in the manganese phosphate may be closely determined.

The combined filtrates from the manganese phosphate are concentrated to a volume of 200 to 300 ml. Bromocresol purple is added until the cobalt solution is decidedly colored, and it is then made slightly acid with 50 per cent acetic acid, or to the complete disappearance of the purple color at pH 5.2. Precipitation of cobalt sulfide will be complete at this acidity. It is saturated cold with hydrogen sulfide in an Erlenmeyer flask of suitable size, provided with the usual inlet and outlet tubes; then the tubes are closed and the flask is heated to about 70° C. on the steam bath until the cobalt sulfide coagulates, about one hour.

The filtered and washed cobalt sulfide is dried and ignited slowly in a 30-ml. porcelain crucible. After all carbon is gone, the cobalt oxide is dissolved in just enough 1 to 1 hydrochloric acid and evaporated to dryness. The precipitation flask is cleaned with a little hot 1 to 1 nitric acid which is added to the cobalt chloride in the crucible, together with 2 to 8 drops of 1 to 1 sulfuric acid—the latter quantity for 0.2 gram of cobalt. When all liquid is evaporated, the crucible is slowly heated in a radiator until the excess of sulfuric acid is apparently gone. The bottom of the crucible is then heated directly in the Bunsen flame to a dull red for about one minute. The ignition at 550° to 600° C. should be repeated until the cobalt sulfate reaches constant weight.

The experimental results appear in Table I.

Experiments 1 to 7, which cover a range from 2 to 169 mg. of cobalt, show that the quantitative separation of cobalt and manganese phosphates in the presence of ammonium cit-

TABLE I. DETERMINATION OF COBALT IN THE PRESENCE OF MANGANESE

Expt.	Taken				Cobalt Found in Phosphate Precipitate	Cobalt as $CoSO_4$	Total Cobalt Found	Error
	Co	Mn	Ca	Mg				
	Gram	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
1	0.0021	0.1000	....	....	0.0000[a]	0.0021	0.0021	0.0000
2	0.0060	0.1000	....	....	0.0005	0.0058	0.0063	+0.0003
3	0.0123	0.1000	....	....	0.0008	0.0114	0.0122	−0.0001
4	0.0249	0.1000	....	....	0.0003	0.0254	0.0257	+0.0008
5	0.0562	0.1000	....	....	0.0001	0.0560	0.0561	−0.0001
6	0.1045	0.1000	....	....	0.0006	0.1043	0.1049	+0.0004
7	0.1687	0.2500	....	....	0.0006[b]	0.1676	0.1682	−0.0005
8	0.0509	0.2500	0.1000	....	0.0003	0.0518	0.0521	+0.0012
9	0.0509	0.2000	0.0400	0.0400	0.0024	0.0497	0.0521	+0.0012
10	0.0509	0.1000	....	0.0400	0.0008[b]	0.0511	0.0519	+0.0010

[a] One precipitation.
[b] Three precipitations.

rate is fairly satisfactory. Experiments 8 to 10 show the adverse effect of the alkaline earths, which are not precipitated as completely as manganese phosphate and tend to accompany cobalt sulfide. An electrolytic determination of cobalt would be better in such cases.

If the alkaline earths are likely to be present in only very minor quantities, they may be precipitated jointly with manganese phosphate. This step shortens the procedure by avoiding the previous separation of cobalt and manganese with ammonium sulfide. The presence of an appreciable quantity of magnesium is strongly suggested by the deepened lilac color of the first phosphate precipitate. In such case, the precipitate should be dissolved in just enough hydrochloric acid, the quantity of citric acid doubled to 4 grams in 125 ml., and the precipitation repeated. Three precipitations should be sufficient in any case.

This procedure, which is reasonably rapid, is especially adaptable to the analysis of cobalt-bearing psilomelane, which is very high in manganese and low in alkaline earths. The manganese in the phosphate precipitate may be readily determined by the bismuthate method, if it contains only a negligible quantity of cobalt.

By igniting cobalt sulfate at 550° to 600° C. all free acid may be safely expelled, thus permitting a rapid determination and the handling of larger quantities than have been thought advisable heretofore. The ignited sulfate is completely soluble in cold water.

Literature Cited

(1) Blair, "Chemical Analysis of Iron", 8th ed., p. 106, Philadelphia, J. P. Lippincott Co., 1918.
(2) Hillebrand and Lundell, "Applied Inorganic Analysis", p. 349, New York, John Wiley & Sons, 1929.
(3) Lundell and Hoffman, "Outlines of Methods of Chemical Analysis", p. 70, New York, John Wiley & Sons, 1938.
(4) Mellor, "Comprehensive Treatise on Inorganic and Theoretical Chemistry", Vol. XIV, Pt. 3, p. 765, New York, Longmans, Green & Co., 1935.
(5) Prescott and Johnson, "Qualitative Chemical Analysis", p. 334, New York, D. Van Nostrand Co., 1933.
(6) Snell, "Colorimetric Methods of Analysis", Vol. I, p. 321, New York, D. Van Nostrand Co., 1936.

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Warder's Method for the Titration of Carbonates

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A critical survey of Warder's method and of the literature pertaining to it shows that titration of carbonate to bicarbonate must be performed in a closed system to avoid loss or gain of carbon dioxide. A titration procedure which is essentially identical with that of Tillmans and Heublin is described in detail so as to assure acceptable results. Its accuracy and precision have been tested on a carbonate-bicarbonate solution having a carbon dioxide tension of approximately 0.0003 atmosphere so as to render the solution reasonably stable in contact with air.

The relative average deviation of the titration of carbonate to bicarbonate has been found approximately equal to 1.5 parts per thousand. The determination of the titratable base can be performed with a precision of 0.5 part per thousand. Calculations based on these figures show that in the application of Warder's method to the determination of hydroxide, carbonate, and bicarbonate in the presence of one another the precision becomes poor whenever the mass of the constituent determined is less than one tenth of the mass of the major component. Traces of carbonate in hydroxide may be determined by the use of a refined titration technique, but it would be hopeless to attempt with Warder's method the determination of traces of hydroxide in carbonate, traces of carbonate in bicarbonate, or traces of bicarbonate in carbonate.

A SURVEY of the literature on Warder's method (*42*) reveals great differences in the performance of the titration (*6, 12, 17, 22, 27, 30, 31, 36, 37, 39*), and the reliability of the method appears a matter of controversy. Theoretical analysis of the problem permitted an adequate evaluation of the individual publications, but an experimental investigation seemed necessary for the confirmation of the conclusions drawn. A review of the literature at the close of the investigation finally showed that, while all the required precautions were known, not one of the authors customarily consulted had succeeded in describing an entirely satisfactory procedure.

The principal aspects of the titration of carbonates may be derived from Figure 1, in which it is assumed that 10-ml. portions of sodium carbonate solutions are titrated with standard acids of the same molar concentrations as the carbonate solutions. pH curves I, II, and III have been calculated for 1 molar, 0.1 molar, and 0.01 molar solutions, respectively. The pH at the bicarbonate equivalence point is the same for all concentrations (*28*), while the pH at the carbon dioxide equivalence point varies considerably with changes of the concentration. The curves through E_1 and E_3 show the continuous change of the carbon dioxide tension of the titrated solutions. The carbon dioxide tension has been calculated as a function of the hydrogen-ion concentration (*11*) and the sodium-ion concentration by means of the equation

$$P_{CO_2} = 8.2 \times 10^7 [H^+] \frac{[H^+]^2 + [H^+][Na^+] - 10^{-14}}{7.8 \times 10^{-11} + [H^+]} \text{ atmosphere}$$

The common logarithms of the carbon dioxide tensions have been plotted against the volumes of standard acid added. The horizontal line E_1E_3 indicates the tension 0.0003 atmosphere, which corresponds to the partial pressure of carbon dioxide in air containing 0.03 per cent by volume of this gas. The curves, through E_1 for molar solutions and through E_3 for 0.01 molar solutions, show clearly that the titrated solu-

tions are in general not in equilibrium with the atmosphere (*7, 11, 24, 39*).

Carbon Dioxide Tension and Performance of Titration

It is obvious that production of correct results with Warder's method requires that the carbon dioxide content of the titrated material be kept constant until the bicarbonate end point is adjusted (*24*); losses or gains of significant quantities of carbon dioxide must be prevented.

The slow rate of hydration in the interval from pH 11 to 8 determines the conditions which actually prevail during a titration to the bicarbonate end point. The addition of every portion of standard acid creates in the titrated solution locally, close to the surface, a strongly acid region containing a high concentration of carbonic acid which immediately dehydrates to carbon dioxide. Considerable losses of carbon dioxide are imminent at this stage, and quick dissipation of the acid region by mixing gives no radical improvement, since considerable time is required until the carbon dioxide is

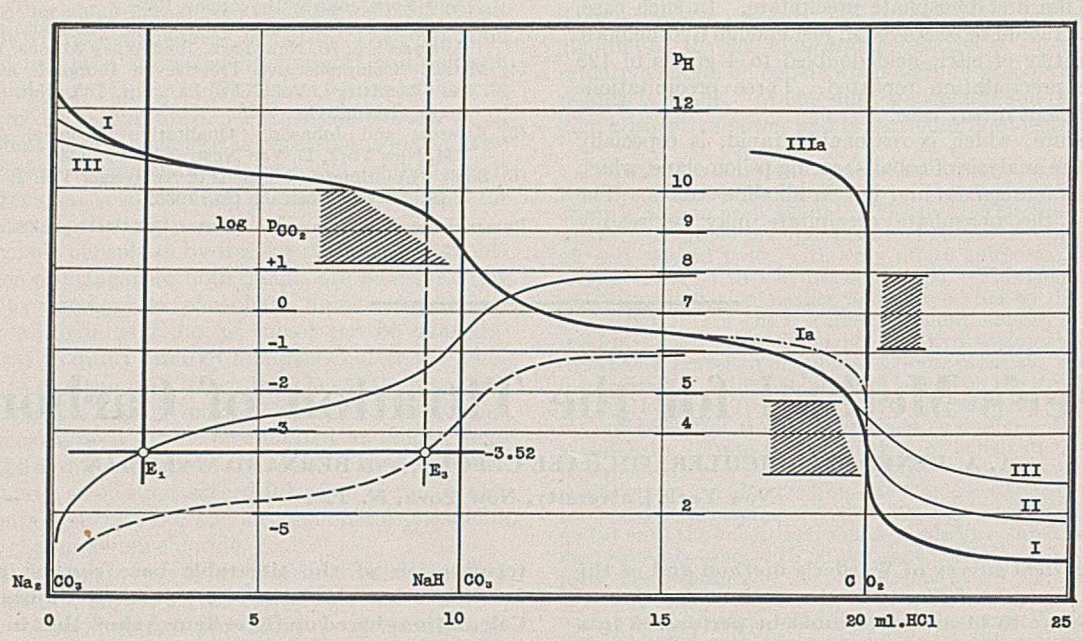

FIGURE 1. TITRATION OF CARBONATES

The instability of solutions of carbonate and bicarbonate in contact with air is shown by Figure 1. At the bicarbonate equivalence point the carbon dioxide tension exceeds 0.1 atmosphere with molar solutions and 0.001 atmosphere with 0.01 molar solutions (*11, 21, 39*). It is true that bicarbonate solutions lose carbon dioxide very slowly when standing in contact with air, but the rate of loss is greatly increased by the mixing operations which are necessary during titrations. Losses averaging 9 per cent of the carbon dioxide present were observed with 0.1 molar and 0.01 molar solutions of pure sodium carbonate decahydrate when, in the authors' experiments, titrations were carried out at freezing temperature in open 250-ml. Erlenmeyer flasks. The occurrence of losses of such magnitude is explained by the fact that, during titration, the carbon dioxide tensions for most of the time exceed those shown in Figure 1, which have been calculated for conditions of equilibrium.

The rate of hydration of carbon dioxide is far lower than the rate of dehydration of the carbonic acid, and even dilute solutions of carbon dioxide contain approximately 99 per cent anhydride (CO_2), 0.9 per cent bicarbonate ion, and only 0.1 per cent carbonic acid (H_2CO_3) (*5, 35*). Thiel (*34*), Faurholt (*5*), and Brinkman, Margaria, and Roughton (*4*) came to the conclusion that in alkaline solutions the reaction $CO_2 + OH^- \rightarrow HCO_3^-$ prevails, the rate obviously depending upon the hydroxyl-ion concentration. The times required for approach within 10 per cent of equilibrium are given as follows (*5*):

At pH 14 to 12, a small fraction of a second
At pH 12 to 11 and 4 to 0, a few tenths of a second
At pH 11 to 10 and 6 to 4, 1 to 10 seconds
At pH 8, 80 seconds

bound by reaction with the hydroxyl ion of the weakly alkaline solution. Efficient stirring, which would prevent the temporary formation of strongly acid regions, is not admissible, for it would greatly facilitate the exchange of carbon dioxide between solution and atmosphere.

The necessity of avoiding loss of carbon dioxide was recognized from the very beginning. Warder himself (*42*) recommended titration of dilute solutions, a provision which is not sufficient, however, for the titration of mixtures of carbonate and bicarbonate. Thomson (*36*) recommended in 1883 "keeping the point of the buret in the liquid, so that no carbonic acid escapes." A buret was used "with a long capillary spit reaching nearly to the bottom of a tall narrow cylinder in which the liquid was titrated with continual stirring by a circular glass rod, which was never lifted above the surface of the liquid" (*29, 30*). Kippenberger (*12*) seems to have been the first to recommend performance of the titration in a stoppered flask (*7, 11, 37, 39*).

Adjustment of Bicarbonate End Point

The adjustment of the bicarbonate end point requires (1) the use of a color standard for the adjustment of the end point (*3, 23*), and (2) allowance of sufficient time after each addition of standard solution for the establishment of equilibrium. The slow hydration of carbon dioxide, which is responsible for the "fading of the phenolphthalein color" (*20, 40*), has been discussed.

The color standard for the adjustment of the end point, which is easily prepared with the use of pure bicarbonate, must be protected against loss of carbon dioxide and, for obvious reasons, is kept in a stoppered flask of the type used in the titrations. The color standard must contain the same quantity of indicator as the titrated solution, and it must

approximate the solution which has been titrated to the bicarbonate end point with respect to volume, bicarbonate concentration, total ion concentration, and temperature (*10, 14*).

Phenolphthalein is recommended for the indicator; thymol blue (*27*) appeared definitely less satisfactory. Excellent results were obtained with Simpson's indicator (*31*), but its usefulness may be expected to show great variations, depending upon the ability of the experimenters to match shades of orange. The use of a one-color indicator, such as phenolphthalein, requires special attention to the selection of the proper indicator concentration. The intensity of coloration produced by such indicators in a solution of a definite pH is directly proportional to the stoichiometric concentration of the indicators (*1*). Thus, it is absolutely necessary to add equal masses of indicator to the titrated solution and color standard. As a matter of common sense, the quantity of indicator is chosen so as to produce a tint permitting precise colorimetric matching (*22*).

The coloration produced by phenolphthalein in a bicarbonate solution depends on the total ion concentration and the temperature of the solution (*10, 14*). Cooling and addition of neutral salts were used (*17*) in order to obtain a "colorless" solution at the phenolphthalein end point. The shape of the pH curve makes it impossible, however, to obtain an abrupt change of color, and the adjustment to a definite shade of "colorless" is, of course, far more difficult than the reproduction of a pink which has been intentionally chosen because of its suitability for colorimetric matching. Comparison of the "colorless" solution with a sample of water reveals the definitely pink hue of the former.

Adjustment of Carbon Dioxide End Point

The procedure for the adjustment of the carbon dioxide end point must depend essentially upon the concentration of the titrated solution (Figure 1). Tenth molar and stronger carbonate solutions are best titrated with the use of bromophenol blue to a greenish gray coloration, pH 4, taking care to remove from the titrated solution by agitation as much carbon dioxide as possible. More dilute carbonate solutions are best treated with a slight excess of standard acid, boiled for the removal of the liberated carbon dioxide, cooled to room temperature, and then titrated back with standard alkali, using an indicator acting at approximately pH 7. The use of an end point close to pH 7 is essential for precise determinations of the titratable base of very dilute solutions. In the back-titration the pH of the solution follows the steep curve, IIIa, of Figure 1 and, around pH 7, a small volume of standard solution is able to change the color of the indicator. Curve III for the titration in the presence of carbon dioxide indicates low precision of the adjustment of the end point and the necessity of using a color standard.

The suitability of the proposed procedures may be derived from the results of the following experiments. Eleven 50-ml. portions of an approximately 0.1 molar solution of pure sodium carbonate decahydrate were titrated with 0.5 molar standard acid, using bromophenol blue as indicator. As the arithmetical mean of 11 determinations, 21.53 ± 0.01 ml. of acid were required for the neutralization of the titratable base. Then the 0.5 molar standard solutions and the 0.1 molar carbonate solution were diluted ten times, using the same volumetric apparatus in the dilution of all the solutions. Fifty-milliliter portions of the 0.01 molar sodium carbonate solution obtained were now titrated with the use of 0.05 molar standard acid and standard alkali. The carbon dioxide was eliminated by boiling and bromothymol blue was used as indicator for the back-titration to pH 7. As the mean of 7 titrations 21.53 ± 0.02 ml. of the 0.05 molar acid were required, which is in satisfactory agreement with the result of the former series of determinations.

The traditional use of methyl orange (*17, 42*) offers no decided disadvantages in the titration of strong carbonate solutions. The change of color occurs in the pH range between 4.8 (yellow) and 3.0 (red). Curve I of Figure 1 indicates that with 1 molar solutions the color will gradually change before the equivalence point is reached. Beyond that point, one drop of standard acid will suffice to change the color from orange to red, and the appearance of red should, therefore, be chosen as end point. In the calculation of curves I, II, and III it was assumed that no carbon dioxide is given off during the titration. If care is taken, however, to eliminate by agitation most of the carbon dioxide formed, the pH of the solution will approximately follow curve Ia, and a far more sharply defined change of the color of methyl orange will be obtained at the end point.

Reinitzer (*26*) was probably the first to recognize the influence of carbon dioxide on the color of methyl orange, but even today there seem to be differences of opinion concerning the suitability of this indicator. From Figure 1 it is obvious that methyl orange can be employed for the titration of approximately 0.1 molar and stronger carbonate solutions, especially if a color standard is used (*3, 15, 17, 23*). The titration of 0.01 molar or even 0.001 molar carbonate solutions with the use of methyl orange should not be attempted; it must be kept in mind that 100 ml. of distilled water need 1 ml. of 0.01 molar acid and more than 10 ml. of 0.001 molar acid to acquire a pH of 4 (*3*).

Procedure

The titration is carried out in a 250-ml. volumetric flask with long narrow neck and glass or cork stopper (*37*). Two more flasks of the same size, shape, and make are needed; one holds the color standard, the other is filled with plain water.

Of solid materials and strongly alkaline solutions, an amount that will require approximately 40 ml. of 0.5 *N* acid to neutralize all the titratable base is transferred into the volumetric flask and treated with 50 ml. of distilled water which has been freed from carbon dioxide. If the sample contains large or moderately large quantities of carbonate, the distilled water may be "freed" from carbon dioxide by shaking it in a large flask while suction is applied. Special precautions (*9*) are required for the determination of traces of carbonate. For the titration of dilute alkaline solutions it is advisable to use standard acid of such normality that approximately 10 ml. are required for neutralization of the titratable base of 80 ml. of sample.

The color standard for the bicarbonate equivalence point must resemble the titrated solution at this point in volume, bicarbonate concentration, total ion concentration, and temperature; furthermore, it must contain the same mass of indicator as the titrated solution. Obviously, an approximate knowledge of the composition of the sample is required.

Some U. S. P. sodium bicarbonate is stirred for about 3 minutes with distilled water. The mixture is transferred to a Büchner funnel, strong suction is applied, and the cake of salt is washed once with cold distilled water. When the bicarbonate begins to dry, the required quantity of the still slightly moist salt is weighed out on a horn-pan balance and transferred to the volumetric flask. The calculated quantity of neutral salt and distilled water, previously freed from carbon dioxide, are added. The flask is immediately stoppered and then shaken until all solids have dissolved. Finally, a 1 per cent solution of phenolphthalein in alcohol is added from a medicine dropper or graduated pipet until the solution assumes a pink coloration. Approximately 0.1 ml. of the indicator solution will produce a tint satisfactory for colorimetric matching.

Exactly the same amount of phenolphthalein, as used in the preparation of the color standard, is added to the solution to be titrated. The titrated solution and the color standard are always kept stoppered. The flask containing the titrated solution is opened only for the addition of standard solution and then im-

mediately closed again. At the start of the titration, the standard acid is added in relatively large portions and, after every addition, the contents of the flask are vigorously shaken for about 20 seconds so as to establish equilibrium between the gaseous and the liquid phases. When the color of the titrated solution has brightened to pink, the standard acid is added in portions of 0.2 ml., single drops, and eventually fractions of drops. Simultaneously the time of shaking is the more prolonged the nearer the titration approaches the end point. When this point seems to have been reached, it becomes necessary to shake one full minute and to wait another minute before comparing with the color standard. It is advisable to shake the color standard just as often and as long as the titrated solution, to make certain that the equilibrium with the gaseous phase is established in the standard also. For the close matching of colors it is convenient to place the flasks on a large sheet of white paper. The flask with plain water will be found helpful when comparing the pink solutions. Strongly colored solutions cause fatigue of the eyes and must be kept away from the titration table.

For the titration to the carbon dioxide end point the stopper is removed and rinsed off into the titrated solution with 10 or 15 drops of distilled water. After addition of the proper indicator, the titration is continued, shaking the solution from time to time to accelerate the escape of carbon dioxide. The choice of indicator and procedure for the adjustment of the end point have been discussed above. The use of standard alkali is permissible in the adjustment of both end points.

TABLE I. TITRATION OF TEST SOLUTIONS

		Na_2CO_3 G./l.	$NaHCO_3$ G./l.
Solution I	Calculation	12.715 ± 0.0032	13.446 ± 0.0027
	Warder's method	12.78 ± 0.013	13.38 ± 0.019
	Winkler's method	.....	12.97 ± 0.017
Solution II	Calculation	12.708 ± 0.0051	13.445 ± 0.0027
	Warder's method	12.60 ± 0.023	13.63 ± 0.034
	Winkler's method	.....	13.38 ± 0.0083

Accuracy of the Suggested Procedure

The test material was a solution that contained known quantities of carbonate and bicarbonate in such proportion that the carbon dioxide tension of the solution approximated the partial pressure of carbon dioxide in the atmosphere. Thus it was hoped to overcome the difficulties arising from the variation of composition due to the exchange of carbon dioxide between liquid and air. Since the reliability of simple procedures for the preparation of pure sodium bicarbonate and pure sodium carbonate could be subject to controversy (*18*, *19*, *32*, *41*), it was decided to base all calculations on the analyses of these substances.

U. S. P. sodium bicarbonate was repeatedly washed with water and quickly dried. The salt thus purified was free from ammonium, chloride, and sulfate ions, as could be demonstrated by qualitative tests. Part of this bicarbonate was used for the preparation of anhydrous carbonate. Use of a platinum crucible and heating in a Stähler block eliminated the possibility of contamination by combustion products of the Bunsen flame.

STANDARD ACID AND ALKALI. The 0.5 *N* standard solutions were prepared and kept in 7-liter stock bottles which were permanently connected with the calibrated burets. An arrangement of washers and soda-lime tubes, similar to that employed by Lindner (*18*), prevented changes of the standard solutions by either evaporation of water or absorption of carbon dioxide. The stock bottles were filled with distilled water and then, using a gas diffuser stone of cylindrical form, air free from carbon dioxide was bubbled through the water for 7 hours. After this treatment for the removal of carbon dioxide the calculated amounts of hydrochloric acid and oily lye, respectively, were added, the bottles were closed, and the contents were mixed by shaking. Finally, the stock bottles were permanently connected to the burets.

For the determination of the titer, 25 ml. of the acid were measured with the buret and precipitated with silver nitrate. The weight of the silver chloride was corrected for the buoyant effect. As the arithmetical mean of three determinations, 0.48695 ± 0.00007 (±0.14 ‰) gram-equivalent weight per liter was found for the normality of the acid. The titer of the standard alkali was determined at short intervals by titration with the standard acid. All titrations were carried out at a temperature of approximately 25° C.; on very few occasions did it deviate from this norm as much as ±4° C. With the temperature nearly constant and the solutions always dispensed by the same burets, any errors caused by the use of the 0.5 *N* standard solutions are automatically eliminated.

TITRATION OF PURIFIED SODIUM BICARBONATE. The determination of the bicarbonate content was tried by weighing the carbon dioxide obtained on decomposition. In one series of experiments carbon dioxide and water were liberated by heating the bicarbonate at 290° C. in a combustion tube; in the other series, the bicarbonate was decomposed with dilute acid. The precisions of both methods proved inadequate for the establishment of the degree of purity of sodium bicarbonate, and it was finally decided to measure the total alkalinity, which can be determined with high precision.

Five 1.4- to 1.8-gram portions of bicarbonate were titrated using methyl red as indicator. An excess of acid was added first and the carbon dioxide was removed by boiling for 2 minutes. After cooling to room temperature the end point was adjusted by titrating with standard alkali to yellow. The weight of the sodium bicarbonate was corrected for the buoyant effect. In 5 determinations 24.443 ± 0.003 (±0.12 ‰) ml. of standard acid were required for the titration of 1.00000 gram of bicarbonate. From this figure the bicarbonate content was calculated (*2*) as 100.00 ± 0.02% $NaHCO_3$. The presence of 0.1 per cent of sodium carbonate in the bicarbonate would increase the above value by 0.06 per cent.

TITRATION OF SODIUM CARBONATE. Three 1-gram portions of the sodium carbonate were titrated with standard acid and alkali, using the same procedure as in the determination of the titratable base in sodium bicarbonate. The weight of the sodium carbonate was corrected for the buoyant effect. In 3 titrations 38.718 ± 0.008 (±0.2 ‰) ml. of standard acid were required per 1.00000 gram of carbonate. Calculation gives a content of 99.93 ± 0.024% Na_2CO_3.

PREPARATION OF TEST SOLUTIONS. Approximately 160 millimoles of sodium bicarbonate and 120 millimoles of sodium carbonate were dissolved to 1 liter of solution. The distilled water was boiled beforehand to expel the carbon dioxide dissolved; for cooling the water to room temperature the flask was closed with a stopper carrying a soda-lime tube. The following quantities of salts were taken for the preparation of 1.00000 liter of test solution:

Test solution I	13.4461 grams of $NaHCO_3$
	12.7236 grams of Na_2CO_3
Test solution II	13.4450 grams of $NaHCO_3$
	12.7236 grams of Na_2CO_3 (99.88%)

All weights were corrected for the buoyant effect, and the solutions were kept in stoppered flasks.

TITRATION OF TEST SOLUTIONS. All these titrations were carried out in 250-ml. volumetric flasks with long narrow necks, and for each experiment a 49.967 ± 0.0015-ml. portion of test solution was taken. During the titrations the scale of the acid buret was kept covered so as to eliminate bias in the adjustment of the end points.

In 10 titrations of test solution I, using the suggested procedure for the performance of Warder's method the following volumes of standard acid were required:

12.38 ± 0.011 (±0.9 ‰) ml., bicarbonate end point
41.100 ± 0.0057 (±0.14 ‰) ml., carbon dioxide end point

These 10 titrations were carried out within 7 days from the preparation of the test solution. Twelve days later this solution was titrated, using Winkler's method (*43*) as described by Kolthoff and Sandell (*16*), but employing strontium chloride in the place of barium chloride for the precipitation of carbonate. In 4 titrations the volumes of sodium hydroxide standard solution required for the conversion of the bicarbonate to carbonate corresponded to 15.84 ±0.02 (±1.3 ‰) ml. of the standard acid.

Since the time interval between the two series of titrations was obviously too long, another series of experiments was started and all the titrations were carried out within 3 days from the preparation of test solution II. In 5 determinations using Warder's method the following volumes of standard acid were required:

12.20 ± 0.02 (±1.75 ‰) ml., bicarbonate end point
41.056 ± 0.006 (±0.14 ‰) ml., carbon dioxide end point

In 4 titrations with the use of Winkler's method, a volume of hydroxide solution was required which was equivalent to 16.345 ± 0.01 (±0.62‰) ml. of the standard acid.

The results of the titrations are compiled in Table I. As indicated by the average deviations of the means, the significance of the deviations between experiment and calculation is, in general, doubtful. The deviations of the values for carbonate and bicarbonate obtained with Warder's method are probably of an accidental nature, and it appears that the method is able to give correct results (*33*). The limitations spring from its lack of precision as outlined below.

The results obtained with Winkler's method deviate significantly from the calculated values. As already mentioned, the figure 12.97 is explained by loss of carbon dioxide while test solution I was standing for 12 days. It is obvious that carbonate-bicarbonate solutions can be stable only at a definite temperature in contact with air of a definite carbon dioxide pressure. Actually neither of these factors was under control.

Precision of Warder's Method

The following calculations are based upon the assumption that sodium salts are titrated, but substitution of the proper equivalent weights in the final equations permits their application to other carbonates and hydroxides. Titration to the phenolphthalein end point and the determination of the titratable base allow calculation of the hydroxide, carbonate, bicarbonate, and total carbonic acid. Bicarbonate–carbonic acid mixtures are not considered here, and it is understood that hydroxide and bicarbonate cannot occur simultaneously.

The Titratable Base, P_{Na}, is calculated as a function of the volume, M, of standard acid required to reach the carbon dioxide end point. If S represents the amount of sample, N the normality of the standard acid, and 2.3 one tenth of the equivalent weight of the constituent determined, the exact relation between P_{Na} and M is given by

$$P_{Na} = \frac{2.3\,NM}{S}$$

The precision of the determination of titratable base depends mainly upon the precision, μ, of M—i. e., on the precision of the adjustment of the carbon dioxide end point. The relative average deviation, μ', of a single observation will not exceed 0.5 ‰, if proper care is exercised and proper judgment is used in the selection of buret and concentration of the standard solution. In the two series of titrations listed in the preceding section, μ' was found equal to ±0.45 ‰ (10 determinations) and ±0.32 ‰ (5 determinations), respectively. With the use of special precautions (*8, 9*) the precision of M may be considerably improved, but $\mu' = \pm 0.5$ ‰ must be considered a fair figure, if a standard procedure of titration is used. It follows (*2*) that the relative average deviation, π'_{Na}, of a single determination of the content P_{Na} on titratable base is

$$\pi'_{Na} = \mu' = 1000\,\frac{\mu}{M} = \pm 5\ ‰ \qquad (1)$$

From the above two equations are derived the following relations which are needed later:

$$\mu = \pm\frac{M}{2000} \qquad (2)$$

$$M = \frac{SP_{Na}}{2.3\,N} \qquad (3)$$

The Total Carbonic Acid is always a direct function of $M - M_1 = M_2$, the volume of standard acid required for the titration from the bicarbonate end point to the carbon dioxide end point:

$$P_{CO_2} = \frac{4.4\,NM_2}{S} \qquad (4)$$

This relation holds for mixtures of hydroxide and carbonate and for mixtures of carbonate and bicarbonate. Thus, the equation

$$M_2 = \frac{SP_{CO_2}}{4.4\,N} \qquad (5)$$

is valid for the whole of the following discussion.

The precision of the result for total carbon dioxide obviously must depend upon precision μ_1 of M_1 as well as on precision μ of M. In other words, the precision of the determined content of carbon dioxide will depend upon the precisions of the adjustments of the bicarbonate end point and the carbon dioxide end point. The latter precision is a function of the amount of titratable base present, as shown in Equation 2. The absolute precision of the adjustment of the phenolphthalein end point depends altogether on the amount of bicarbonate present at this stage of the titration and is the same as obtained in the titration of an equivalent quantity of carbon dioxide with the use of standard alkali (*13, 38*). It has been demonstrated (*13*) that the relative precision, μ_1/M_2, does not depend on the absolute amount of carbon dioxide present, if proper judgment is used in the selection of buret and concentration of the standard solution; the relative average deviation of a single adjustment of the phenolphthalein end point has been calculated from the titrations discussed above, and the values ±1.2 ‰ (10 titrations) and ±1.6 ‰ (5 titrations) were found. A value of ±1.5 ‰ appears a fair assumption, if the standard procedure outlined in this paper is carefully followed:

$$1000\,\frac{\mu_1}{M_2} = \pm 1.5\ ‰$$

or

$$\mu_1 = 1.5\,\frac{M_2}{1000} \qquad (6)$$

The precision of the determination of the total carbon dioxide follows from Equation 4:

$$\pi'_{CO_2} = \mu_2' = 1000\,\frac{\sqrt{\mu_1^2 + \mu^2}}{M_2}$$

Substitution of the values obtained for μ, μ_1, and M_2 in Equations 2, 5, and 6 leads to

$$\pi'_{CO_2} = \pm\sqrt{1.5^2 + \left(0.5\,\frac{44}{23}\,\frac{P_{Na}}{P_{CO_2}}\right)^2}\ ‰ \qquad (7)$$

The first item under the root represents the average deviation, ±1.5 ‰, introduced by the adjustment of the phenolphthalein end point, while the second item takes care of the average deviation, ±0.5 ‰, of the determination of the titratable base.

Determination of Sodium Carbonate in Presence of Hydroxide. The carbonate content is calculated as a direct function of M_2:

$$P_{Na_2CO_3} = \frac{10.6\,NM_2}{S}$$

The relative average deviation, $\pi'_{Na_2CO_3}$, which is equal to π'_{CO_2}, is given by Equation 7.

DETERMINATION OF SODIUM HYDROXIDE IN PRESENCE OF CARBONATE. The hydroxide content is calculated as a function of the difference, $M_1 - M_2 = 2M_1 - M$. The error, μ_1, of the adjustment of the phenolphthalein end point will be doubled in the calculation of the determination. The average deviation is given by

$$\pi'_{NaOH} = 1000 \frac{\sqrt{(2\mu_1)^2 + \mu^2}}{M_1 - M_2}$$

$$\pi'_{NaOH} = \pm \sqrt{\left(2 \times 1.5 \frac{40 P_{CO_2}}{44 P_{NaOH}}\right)^2 + \left(0.5 \frac{40 P_{Na}}{23 P_{NaOH}}\right)^2} \text{ ‰} \quad (8)$$

DETERMINATION OF SODIUM CARBONATE IN PRESENCE OF BICARBONATE. The carbonate content is a direct function of M_1, and the relative precision, $\pi'_{Na_2CO_3}$, is determined by

TABLE II. RELATIVE PRECISIONS OF DETERMINATIONS OF SODIUM HYDROXIDE, SODIUM CARBONATE, AND SODIUM BICARBONATE

(In samples containing sodium carbonate and sodium hydroxide or sodium carbonate and sodium bicarbonate)

NaOH-Na_2CO_3 Ratio	Na_2CO_3-$NaHCO_3$ Ratio	±π' ‰ of the Determination of: NaOH	Na_2CO_3	$NaHCO_3$
Grams	*Grams*			
10,000:1	.....	0.50	13,800	.....
1000:1	.....	0.50	1300	.....
100:1	.....	0.50	130	.....
10:1	.....	0.56	13	.....
1:1	.....	1.4	2.8	.....
1:10	.....	11	1.8	.....
1:100	.....	120	1.8	.....
1:1000	.....	1200	1.8	.....
1:10,000	.....	12,000	1.8	.....
.....	10,000:1	.....	1.5	25,000
.....	1000:1	.....	1.5	2500
.....	100:1	.....	1.5	250
.....	10:1	.....	1.7	25
.....	1:1	.....	3.4[a]	5.6[b]
.....	1:10	.....	19	3.3
.....	1:100	.....	190	3.0
.....	1:1000	.....	1900	3.0
.....	1:10,000	.....	19,000	3.0

[a] Observed, 2.8 and 4.0 parts per thousand.
[b] Observed, 4.3 and 5.5 parts per thousand.

the precision of the adjustment of the phenolphthalein end point only. The average deviation of a single determination is expressed by

$$\pi'_{Na_2CO_3} = \mu_1' = 1000 \frac{\mu_1}{M_1} = \pm 1.5 \frac{106 P_{CO_2}}{44 P_{Na_2CO_3}} \text{ ‰} \quad (9)$$

DETERMINATION OF SODIUM BICARBONATE IN PRESENCE OF CARBONATE. The bicarbonate content is a direct function of the difference, $M_2 - M_1 = M - 2M_1$. The uncertainty, μ_1, of the adjustment of the phenolphthalein end point is doubled in the calculation of the result of the determination. The average deviation of a single determination is given by

$$\pi'_{NaHCO_3} = 1000 \frac{\sqrt{(2\mu_1)^2 + \mu^2}}{M_2 - M_1}$$

and

$$\pi'_{NaHCO_3} = \pm \sqrt{\left(2 \times 1.5 \frac{84 P_{CO_2}}{44 P_{NaHCO_3}}\right)^2 + \left(0.5 \frac{84 P_{Na}}{23 P_{NaHCO_3}}\right)^2} \text{ ‰} \quad (10)$$

The relative precisions of Table II have been calculated with the use of the above equations and Equations 11 and 12, which require no comment:

$$P_{CO_2} = \frac{44}{106} P_{Na_2CO_3} + \frac{44}{84} P_{NaHCO_3} \quad (11)$$

$$P_{Na} = \frac{23}{40} P_{NaOH} + \frac{46}{106} P_{Na_2CO_3} + \frac{23}{84} P_{NaHCO_3} \quad (12)$$

The precisions of Table II represent average deviations for single observations, and there is a chance of approximately 3 in 1000 that deviations occur which are four times as large as those listed. The table shows that Warder's method becomes unreliable whenever the mass of the determined component is a small fraction of the mass of the second component. An improvement of the precision by refinement of the working technique is possible only for that type of mixtures of little carbonate with much hydroxide, which is indicated in the table by framing with a broken line. The required changes of procedure have been described by Rather (*25*) and by Han and Chao (*8*, *9*).

Literature Cited

(1) Auerbach, Fr., *Z. angew. Chem.*, 25, 1722 (1912).
(2) Benedetti-Pichler, A. A., IND. ENG. CHEM., Anal. Ed., 8, 373 (1936).
(3) Bjerrum, N., *Z. anal. Chem.*, 56, 81 (1917).
(4) Brinkman, R., Margaria, R., and Roughton, F. J. W., *Trans. Roy. Soc.* (London), A232, 65 (1933).
(5) Faurholt, C., *J. chim. phys.*, 21, 400 (1924).
(6) Greenberg, D. M., Moberg, E. G., and Allen, E. C., IND. ENG. CHEM., Anal. Ed., 4, 309 (1932).
(7) Greenfield, R. E., and Buswell, A. M., *J. Am. Chem. Soc.*, 44, 1435 (1922).
(8) Han, J. E. S., IND. ENG. CHEM., Anal. Ed., 6, 208 (1934).
(9) Han, J. E. S., and Chao, T. Y., *Ibid.*, 4, 229 (1932).
(10) Hastings, A. B., and Sendroy, J., Jr., *J. Biol. Chem.*, 65, 445 (1925).
(11) Johnston, J., *J. Am. Chem. Soc.*, 38, 947 (1916).
(12) Kippenberger, C., *Z. angew. Chem.*, 7, 495 (1894).
(13) Kolthoff, I. M., "Die Massanalyse"; Vol. I, p. 120, and Vol II, p. 141, Berlin, Julius Springer, 1931.
(14) Kolthoff, I. M., *J. Phys. Chem.*, 32, 1820 (1928).
(15) Kolthoff, I. M., *Z. anorg. Chem.*, 100, 143 (1917).
(16) Kolthoff, I. M., and Sandell, E. B., "Quantitative Inorganic Analysis", p. 528, New York, Macmillan Co., 1936.
(17) Küster, F. W., *Z. anorg. Chem.*, 13, 127 (1897).
(18) Lindner, J., *Mikrochemie*, Molisch-Festchrift, p. 301 (1936).
(19) Lindner, J., and Figala, N., *Z. anal. Chem.*, 91, 105 (1932).
(20) McBain, J. W., *J. Chem. Soc.*, 101, 814 (1912).
(21) MacCoy, H. N., *J. Am. Chem. Soc.*, 29, 437 (1903).
(22) Noll, H., *Z. angew. Chem.*, 25, 998 (1912).
(23) Noyes, A. A., *J. Am. Chem. Soc.*, 32, 815 (1910).
(24) Partridge, E. P., and Schroeder, W. C., IND. ENG. CHEM., Anal. Ed., 4, 271 (1932).
(25) Rather, J. B., *J. Assoc. Official Agr. Chem.*, 1, 317 (1915).
(26) Reinitzer, B., *Z. angew. Chem.*, 7, 547 (1894).
(27) Scales, F. M., *Science*, 51, 214 (1920).
(28) Schroeder, W. C., IND. ENG. CHEM., Anal. Ed., 5, 389 (1933).
(29) Seyler, C. A., *Analyst*, 22, 312 (1897).
(30) Seyler, C. A., *Chem. News*, 70, 187 (1894).
(31) Simpson, St. G., IND. ENG. CHEM., 16, 709 (1924).
(32) Smith, G. F., and Croad, G. F., *Ibid.*, Anal. Ed., 9, 141 (1937).
(33) Straub, F. G., *Ibid.*, 4, 290 (1932).
(34) Thiel, A., *Ber.*, 46, 241, 867 (1913).
(35) Thiel, A., and Strohecker, R., *Ibid.*, 47, 945, 1061 (1914).
(36) Thomson, R. T., *Chem. News*, 47, 123 (1883).
(37) Tillmans, J., and Heublin, O., *Z. angew. Chem.*, 24, 874 (1911).
(38) Tizard, H. T., and Boeree, A. R., *J. Chem. Soc.*, 119, 132 (1921).
(39) Truog, E., J. IND. ENG. CHEM., 7, 1045 (1915).
(40) Vorlaender, D., and Straube, W., *Ber.*, 46, 172 (1913).
(41) Waldbauer, L., McCann, D. C., and Tuleen, L. F., IND. ENG. CHEM., Anal. Ed., 6, 336 (1934).
(42) Warder, R. B., *Chem. News*, 43, 228 (1881).
(43) Winkler, L. W., *Z. anal. Chem.*, 40, 523 (1901); 42, 735 (1903); 52, 421 (1913).

ABSTRACTED in part from a thesis to be submitted by Michael Cefola to the faculty of the Graduate School of New York University in partial fulfillment of the requirements for the degree of doctor of philosophy.

Dumas Method for Organic Nitrogen

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DIFFICULTY is frequently experienced in obtaining accurate results in the determination of nitrogen by the Dumas method, errors sometimes being introduced by too rapid combustion of the sample and by impurities in the carbon dioxide. The error due to too rapid combustion is especially encountered in the analysis of liquid compounds of nitrogen of a semi-explosive nature which tend to dissociate suddenly on heating, and which cannot be analyzed accurately by the Kjeldahl method because of losses during digestion.

After considerable experimenting, several modifications of the standard Dumas assembly have been made, with the result that the apparatus described below can be depended upon to give consistently good results even with relatively unstable liquid compounds. Compounds giving "nitrogenous charcoal" and requiring additional oxygen as described by Spies and Harris (*1*) are not included in the scope of the present apparatus, since these compounds are at the opposite end of the scale so far as ease of combustion is concerned. The present method is concerned with the analysis of compounds which burn too readily rather than too slowly. The modified apparatus makes use of both gas and electric heating, the former to secure the very low, easily controlled heat essential for the proper burning off of the sample. Near the exit end of the combustion tube, the electric furnace is preferable for heating the copper reduction section of the assembly. The complete setup is shown in Figure 1.

Recommended Modifications

The combustion boat is filled with a mixture of 50 per cent by weight of powdered copper oxide and 50 per cent by weight of calcium carbonate instead of with pure copper oxide. The use of this mixture allows the sample to burn off more slowly, because of the inhibiting effect of the calcium carbonate, and prevents a sudden spurt of gas from forcing its way through the tube immediately after ignition of the sample.

Volatile liquids should be weighed in glass ampoules. The ampoule is then laid in the combustion boat and covered with the calcium carbonate–copper oxide mixture, taking care to have the open end of the stem beneath the surface. High-boiling liquids may be weighed directly onto a layer of the mixture in the boat; then, after re-weighing, the boat should be filled to cover the sample.

Pure copper in wire form is used instead of rolled copper gauze in the end of the combustion tube nearest the azotometer, to ensure tight packing of the tube and to obtain close contact of the gases with the surface of the hot copper. Copper in the form of short lengths of wire is more convenient to use than the spiral form and can be more effectively packed in.

The copper is easily made by reducing the regular 0.94-cm. (0.375-inch) length of copper oxide wire with hydrogen gas in a combustion tube.

An electric furnace is used to heat the copper oxide and the pure copper wire in the azotometer end of the tube to a temperature of 650° C., a dull red heat. Gas burners are used to ignite and burn off the sample, starting cold and gradually heating to a maximum of 550° C. The burner under the copper oxide spiral is lighted first. This prevents the gas from receding towards the rear end of the tube. After the spiral is red hot, the second burner is lighted with a low flame to allow the sample to burn off slowly. In this way the rate of gas flow from the burning sample can be regulated very satisfactorily. Towards the end the third burner is lighted to ensure carrying the last traces of gases from the sample into the furnace section.

Temperatures may be determined in advance of actual introduction of the sample by placing a thermocouple in the combustion tube and noting approximate setting of the rheostats and gas flames. A little experience will quickly familiarize the operator with the control of temperature in different sections of the tube.

A convenient method of checking the complete removal of nitrogen from the combustion tube is to take readings at 3- or 4-minute intervals after the combustion is apparently complete, recording the time and volume on a scratch pad. In this way a waste of time is avoided, as the increase in the volume of gas in the azotometer rapidly ceases and a constant final volume varying within only 0.1 ml. is obtained. Determinations may be made in from 35 to 50 minutes, using samples with a total nitrogen content of 0.03 to 0.05 gram of nitrogen.

A two-way stopcock is attached at each end of the train. These are very helpful for the following reasons:

The combustion tube may be swept out with carbon dioxide while the whole apparatus is completely assembled without having to pass the carbon dioxide through the azotometer and reduce the absorptive power of the alkaline solution. Also the carbon dioxide itself may easily be tested for impurities when desired, simply by diverting the flow through the two-way stopcock into the azotometer.

The carbon dioxide supply can be connected to the opposite end of the train during the insertion of the sample. This allows one to reverse the flow of carbon dioxide and prevents air from entering the apparatus while the sample is being pushed into place.

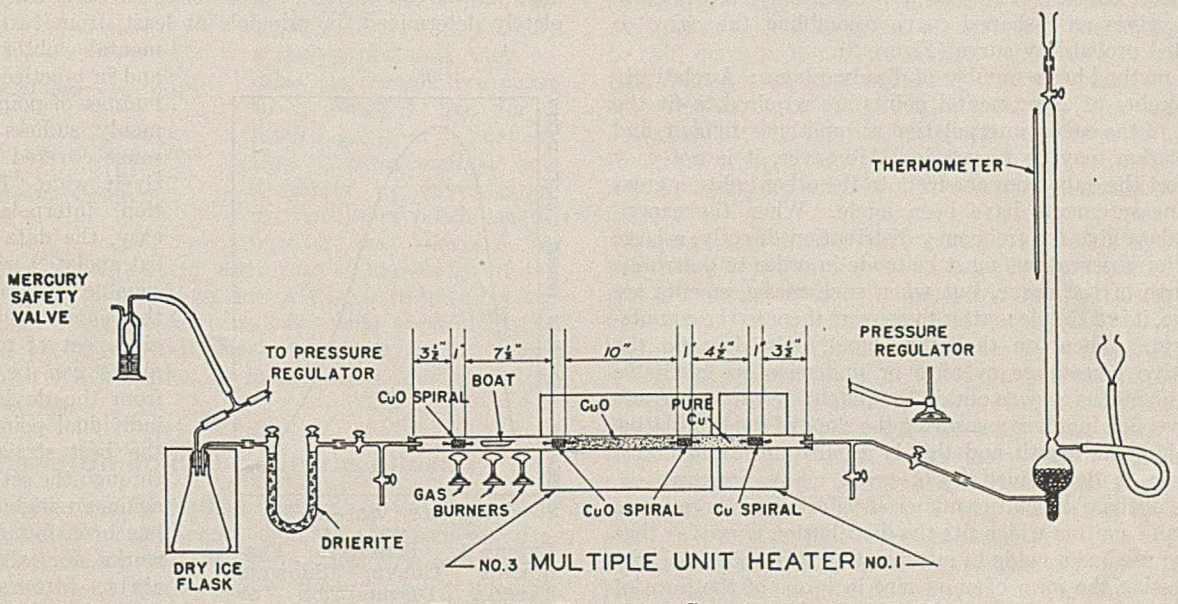

FIGURE 1. DIAGRAM OF SETUP

By careful use of both two-ways, at least 25 determinations may be made without repacking the train, although it may be necessary to burn off the copper spiral plug directly above the first gas burner every 4 or 5 determinations.

The stock azotometer tube has been cut off below the graduations and a bulb sealed on between the graduations and the mercury trap. This bulb is approximately 5 cm. (2 inches) in diameter and is filled with small glass beads. The extra space in the bulb allows the use of an excess of caustic solution and the beads serve to break up the gas bubbles and thus facilitate complete absorption of the carbon dioxide by the alkaline reagent. With this modified azotometer there is no danger of exhausting all the alkali during one determination; in fact, the same solution has been used for two or more determinations.

A satisfactory source of carbon dioxide is dry ice, finely broken up and packed tightly in a 1-liter Pyrex Erlenmeyer flask. Care should be taken in filling the flask to avoid visible air spaces. The mouth of the flask is connected to the rear end of the combustion tube by a piece of bent glass tubing. The flow of carbon dioxide is regulated through a Tirrill burner connected by another piece of glass tubing as shown in Figure 1. A mercury safety valve obviates the risk of breaking the dry ice flask by inadvertent closing of the screw valve of the burner. The rubber stopper through which the outlet tubing passes is wired into the flask to withstand moderate pressure. For safety against possible breakage and to prevent too rapid evolution of carbon dioxide, the flask is wrapped in a towel or placed in a container or shield of some sort.

This carbon dioxide generator, when set up and adjusted properly, will furnish a dependable supply of pure carbon dioxide for a period of about 10 hours.

The following typical results have been obtained using this assembly:

	Nitrogen Found %	Nitrogen Theoretical %
2-Nitro-3-hexanol	9.42 9.45	9.52
2-Methyl-2-nitro-1-butanol	10.51 10.53	10.53
3-Nitro-4-heptanol	8.65 8.72	8.70
3-Methyl-3-nitro-2-pentanol	9.39 9.42	9.52

Literature Cited

(1) Spies and Harris, IND. ENG. CHEM., Anal. Ed., 9, 304 (1937).

PRESENTED before the Division of Physical and Inorganic Chemistry at the 96th Meeting of the American Chemical Society, Milwaukee, Wis.

Methods of Representing Distribution of Particle Size

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DISTRIBUTION of size in a particulate material is commonly represented by plotting either a frequency distribution curve showing the relative number of particles within each range of diameter, or a cumulative curve giving the fraction of the total number of particles which have a diameter greater, or less, than that indicated. The curve from the first method, which is essentially a differential method, resembles a probability curve (see Figure 1), but is usually skewed rather than symmetrical as is the normal probability curve. The second, essentially an integral method, gives an S-shaped curve resembling the ogive or integrated probability curve (Figure 2).

Each method has a number of disadvantages: A relatively large number of experimental points are required to fix the position of the curve, interpolation is sometimes difficult, and extrapolation may be uncertain. Moreover, it is not easy to convert the data from one form to the other unless a great many measurements have been made. When the experimental data give the frequency distribution directly, a large number of observations must be made in order to determine the course of the curve, but when such measurements are available, it is a simple matter to convert them to the cumulative form. When, on the other hand, only data on the cumulative percentage oversize or undersize are available it is by no means easy to obtain the frequency curve, because the conversion involves measuring the slope of the cumulative curve along its length and this is usually uncertain unless the curve is determined by a great many observations. Because of these difficulties numerous efforts have been made to find an equation which fits the distribution curves so that it can be used as a guide in interpolation, extrapolation, and in expressing the form of one curve in terms of the form of the other. The results of these efforts which have been reviewed by Work (*14*), leave much to be desired. The simpler relations are not satisfactory, whereas the more successful ones, such as that derived by Rosin and Rammler (*12*) for broken coal, are somewhat cumbersome to use. It is also clear that no single expression will fit the distribution in size for all types of material.

An alternative approach to the problem is to use a graphical rather than an analytical method and to devise a means of plotting which reduces the distribution curves to a straight line. If this can be done, the course of the curve can be completely determined, in principle at least, from two experimental observations, and in practice a small number of points commonly suffices if the range covered is relatively wide. In addition, interpolation is easy, the data can be extrapolated with reasonable certainty, and the consistency of a given set of measurements can be judged from the deviation of individual points from the best straight line through the set. Here again, no single method has been found which works for all materials, but several methods of plotting,

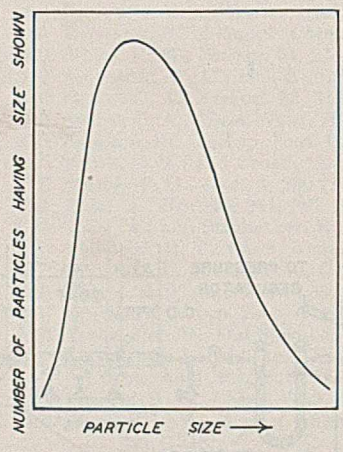

FIGURE 1. SCHEMATIC FREQUENCY CURVE, SHOWING TYPICAL DISTRIBUTION OF PARTICLE SIZE

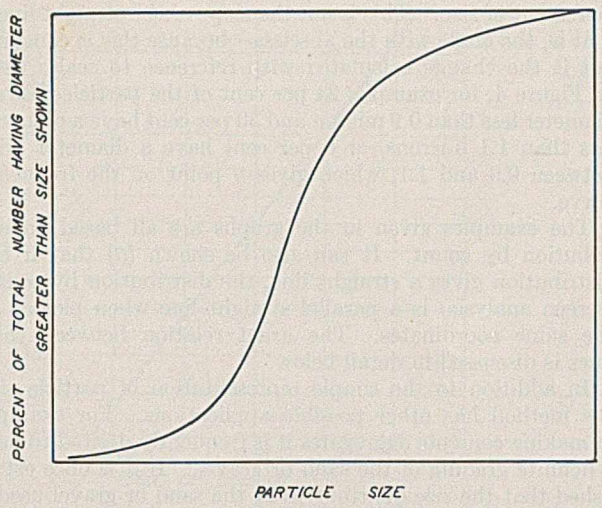

FIGURE 2. SCHEMATIC CUMULATIVE CURVE, SHOWING TYPICAL DISTRIBUTION OF PARTICLE SIZE

method is very convenient. It was first used by Drinker (*3*) for the size distribution of dusts, was discussed further by Loveland and Trivelli (*9*), and has been studied in some detail by Hatch and Choate (*7*) and by Hatch (*6*). Careful tests by Hatch and Choate show that it holds with satisfactory accuracy for pulverized silica, granite, calcite, and limestone. The author has also used it successfully for a number of other materials, as is illustrated by the lines in Figures 3, 4, 5, and 6, which show typical cumulative curves plotted on log-probability coordinates.

Perhaps the most severe test is that made in Figure 3 with data on ground quartz reported by Martin, Bowes, Coleman, and Littlewood (*10*). These measurements include observations at relatively short intervals over the whole range of sizes and the final values are the average of nine gradings of a single powder.

Another test on clay, using data reported by Norton and Speil (*11*), is shown in Figure 4. Again the points fall on a straight line, except for sizes below 0.5 micron. This deviation, which is systematic, may represent a slight systematic error of measurement which is magnified by the extension

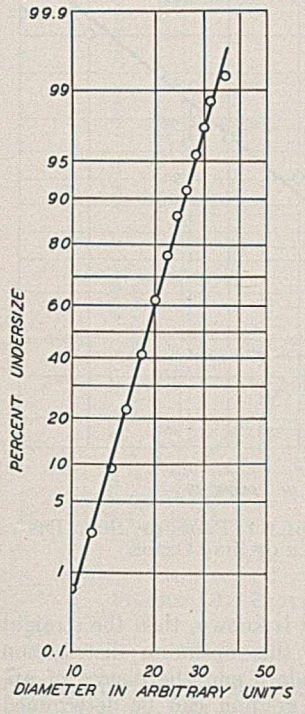

FIGURE 3. LOG-PROBABILITY PLOT OF SIZE DISTRIBUTION IN A SAMPLE OF CRUSHED QUARTZ

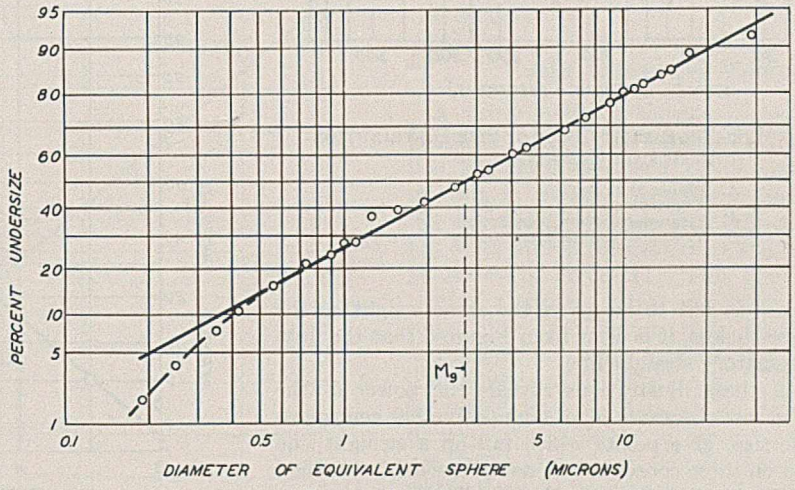

FIGURE 4. LOG-PROBABILITY PLOT OF SIZE DISTRIBUTION IN A SAMPLE OF CLAY

based on the use of special coordinates, have been devised and have been partially successful in that they reduce the cumulative curve to a straight line for a limited group of substances. One of these methods was developed by investigators in the field of public health, another was devised by coal technologists, but in each case a knowledge of the method and of its usefulness does not seem to have become widespread. As these methods have wide applicability in industrial chemistry and chemical engineering, it seems desirable to call attention to them, and to compare their usefulness.

Logarithmic-Probability Coordinates

The most successful of these methods is to plot particle size on a logarithmic scale and cumulative per cent oversize, or undersize, on a probability scale—that is, a scale whose intervals are based upon values of the probability integral. As graph paper with these coordinates is available, this

of the scale, or it may represent a true departure from the straight-line relationship. Even if it is a real departure, no serious error is introduced by assuming the straight line to hold down to the smallest sizes. For example, at a particle diameter of 0.2 micron the observed value is 2.5 per cent, whereas linear extrapolation from the larger sizes gives 5 per cent. Although this difference appears to be large on the graph because of the extension of the probability scale, it is in fact quite small and in many applications would be negligible.

Figure 5 gives data for soda ash and for sodium bicarbonate reported by Weber and Moran (*13*). The scatter of the points is greater than in the preceding tests, but there can be little doubt that the data are best represented by a straight line. Curve D in Figure 5 illustrates one advantage of this method of interpretation of the data. Measurements were not made over the whole range of sizes but were confined to particles of diameter greater than 40 microns. When these data are plotted in the ordinary way it is difficult to extend them very far beyond the limit of actual observations, but when plotted as in Figure 5, they can be extrapolated to smaller sizes with some degree of certainty.

Data for powdered alumina reported by Jones (*8*) are shown in Figure 6. In this case the observations were not tabulated, so that it was necessary to read values from curves;

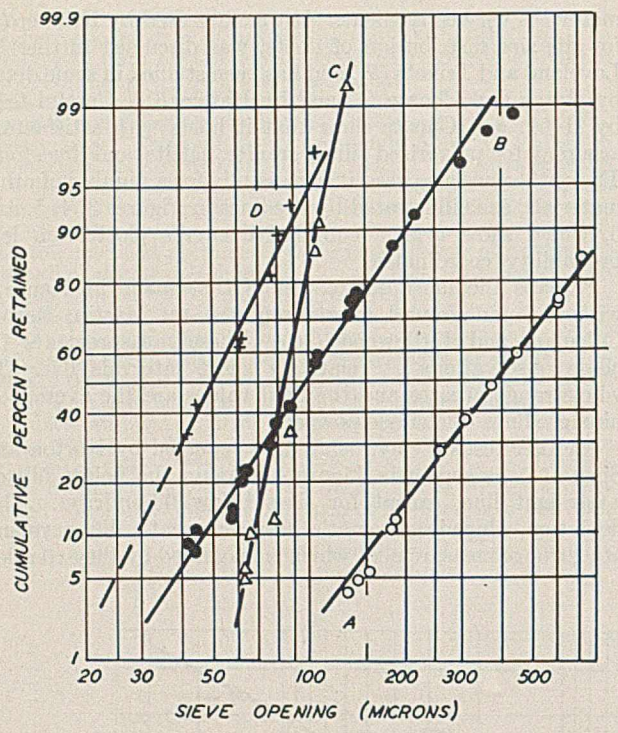

FIGURE 5. LOG-PROBABILITY PLOT OF SIZE DISTRIBUTION IN ALKALI CARBONATES

A. Dense soda ash
B. Light soda ash
C. Granular sodium bicarbonate
D. Powdered sodium bicarbonate

hence, this test is not to be given the same weight as the others. Nevertheless, it is clear from Figure 6 that the data fall on a satisfactory straight line.

A study of these illustrations reveals the power of the method. If a few observations of cumulative per cent oversize, or undersize, give points which fall on a straight line when plotted on these coordinates, one is reasonably justified in taking this line for the cumulative distribution curve. The frequency distribution curve can then be constructed by taking the change in cumulative percentage for each small increment of size. This is not the slope of the straight line—that is, the angle with the abscissa—because this is constant, but is the change calculated with reference to scales used. In Figure 4, for example, 24 per cent of the particles have a diameter less than 0.9 micron and 30 per cent have a diameter less than 1.1 microns, or 6 per cent have a diameter lying between 0.9 and 1.1, which gives a point on the frequency curve.

The examples given in the graphs are all based on distribution by count. It can also be shown (*6*) that if this distribution gives a straight line, the distribution by weight (screen analysis) is a parallel straight line when plotted on the same coordinates. The exact relation between these lines is discussed in detail below.

In addition to the simple representation of particle size, the method has other possible applications. For example, in making concrete aggregates it is frequently desired to have a definite grading of the sand or gravel. If it is once established that the size distribution in the sand or gravel used is represented by a straight line on log-probability paper, and

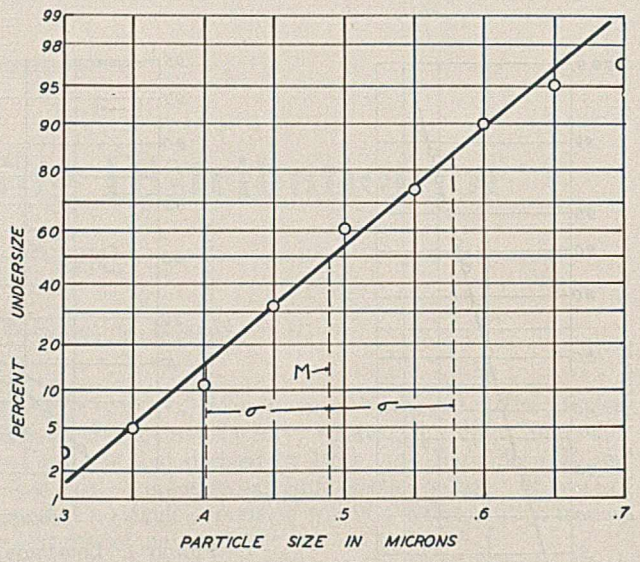

FIGURE 8. ARITHMETIC-PROBABILITY PLOT OF SIZE DISTRIBUTION IN A SAMPLE OF ZINC OXIDE

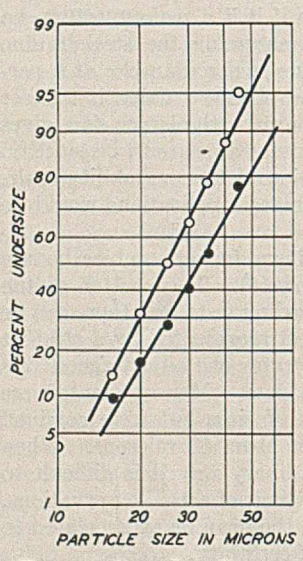

FIGURE 6. LOG-PROBABILITY PLOT OF SIZE DISTRIBUTION IN TWO SAMPLES OF POWDERED ALUMINA

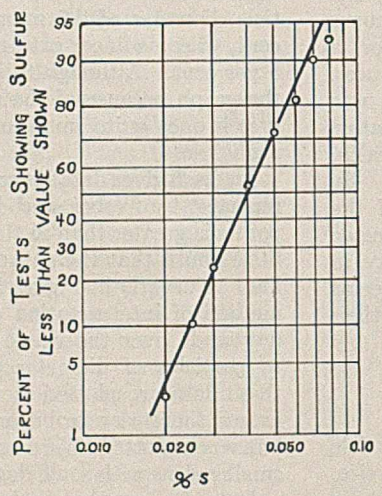

FIGURE 7. LOG-PROBABILITY PLOT OF VARIATION OF SULFUR IN IRON FROM AN "ACTIVE MIXER"

if the optimum grading is known, then the straight line corresponding to this optimum distribution can be used as a standard and the degree of approach to the desired grading can be determined from a small number of sieve measurements. Again, the straight-line plot may be useful in determining whether or not one has a representative sample of a material for chemical analysis. In some materials, such as certain iron ores, the composition is not uniform but varies with the size of the lumps, so that if a sample contains a larger or smaller proportion of fines than the ore as a whole, an erroneous analysis is obtained. If the grading of the ore as a whole is once established and if this grading plots on a straight line, then this line can be used as a standard for other samples. Thus, if two or three screening measurements on a particular sample give points which do not lie upon this straight line, the sample is not representative and should be discarded. It is not impossible that a proper selection of fine or coarse particles will give a correct analysis even though the size distribution of the sample does not

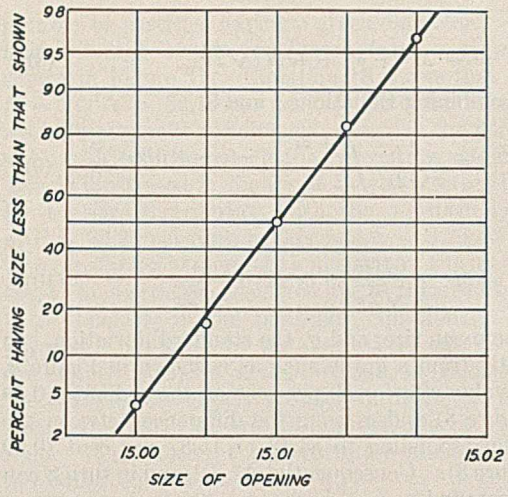

FIGURE 9. ARITHMETIC-PROBABILITY PLOT OF DISTRIBUTION OF DIAMETER AMONG DRILLED HOLES

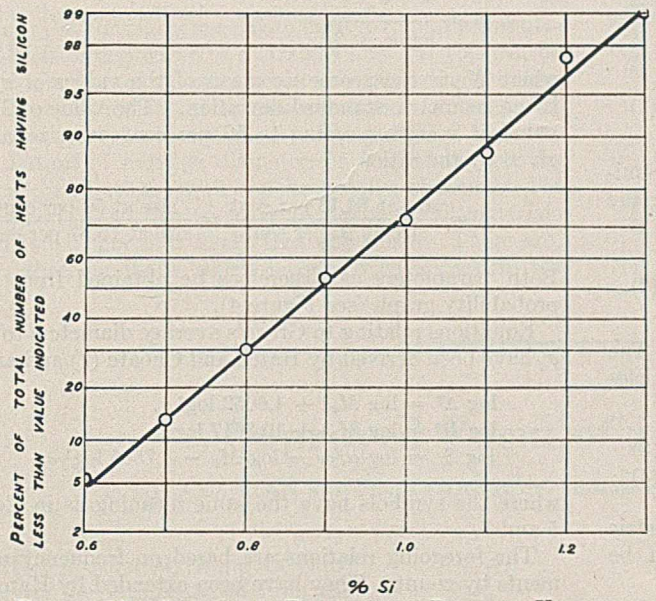

FIGURE 10. ARITHMETIC-PROBABILITY PLOT OF VARIATION OF SILICON IN PIG IRON

conform to the standard, but the chance of obtaining just this selection is very small and it is much safer to use only samples which give a fairly close approach to the grading of the material as a whole.

Logarithmic-probability paper can be applied to distributions other than size. For example, Figure 7 shows the distribution of sulfur in iron taken at different times from an "active mixer." The curve was obtained with far less labor than that necessary in plotting a frequency or cumulative curve in the ordinary manner.

Arithmetic-Probability Coordinates

For a few substances, distribution of size approaches the normal probability distribution fairly closely. When this happens the cumulative curve plots as a straight line when particle size is plotted on a linear scale and cumulative per cent oversize, or undersize, is represented on the probability scale. This method differs from the preceding one in the substitution of a linear scale of particle size for the logarithmic scale. Graph paper having arithmetic-probability coordinates is easily obtainable and is very convenient for this purpose. Materials which show an approximately normal distribution are relatively rare and are found chiefly among substances produced by a chemical process in which the particles tend to a uniform size rather than among those produced by crushing or grinding. One example is shown in Figure 8 in which data on zinc oxide reported by Green (*4*) are plotted on arithmetic-probability coordinates.

This method can, of course, be applied to any measurement of size, or indeed, to any variation which follows the normal probability law. This is illustrated by two examples given in Figures 9 and 10. Figure 9, which is based upon data given by Daeves (*2*), shows the observed variation in size of a number of drilled holes whose nominal diameter was 15.00 mm. In this case, a relatively few observations on the number of holes with diameter above a certain size, which can readily be made by means of several rods of known diameter, enable one to draw the complete distribution curve. Figure 10 shows the variation in silicon content in pig iron produced by a blast furnace over a period of 6 months, and illustrates a close approach to a normal distribution.

Rosin-Rammler Relation

A third method, which reduces the Rosin-Rammler relation for broken coal to a straight line has been described by Bennett (*1*). It consists in plotting cumulative per cent oversize on a log-log scale and size of lump on a log scale and is illustrated by the typical example shown in Figure 11. This distribution represents a greater departure from the normal probability distribution than one which gives a straight line on log-probability coordinates. This method of plotting has had notable success for broken coal and Bennett has suggested that it may be applicable to other materials which are brittle and which contain minute cracks at which cleavage may start, but it almost certainly does not have the wide applicability of the logarithmic-probability graph.

In principle, all three of these methods cover the entire range of sizes from zero to infinite diameter—that is, the cumulative per-

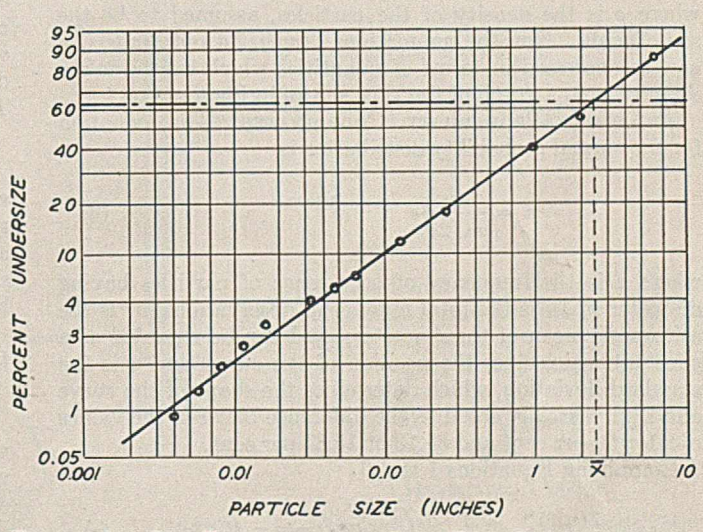

FIGURE 11. SIZE DISTRIBUTION IN RAW COAL PLOTTED ACCORDING TO ROSIN-RAMMLER RELATION (AFTER BENNETT)

centage reaches 100 only when the particle diameter becomes zero or infinite—whereas in all measurements the observations cover but a finite range of size, since the cumulative percentage oversize becomes 100 for the smallest particle in the sample, whose diameter is greater than zero, and becomes zero for the largest particle, whose diameter is some finite size. This means that the methods cannot hold rigorously for the extremes in particle size. Experience shows, however, that in practically every case measurable departure occurs only above 99 per cent or below 1 per cent, where observations are least reliable and where the extension of the probability scale becomes enormous. This limitation is, therefore, of little practical significance. It is possible that the deviation at small sizes which appears in Figure 4 may arise from this cause.

Calculation of Average Diameters

It has been shown by Green (*5*) that a number of properties of a powder—as, for example, the specific surface (surface area per unit weight)—can be calculated in terms of certain average diameters. The calculation of these diameters is greatly facilitated by the use of these methods of plotting.

The diameter, Δ, of a hypothetical particle having average surface area is

$$\Delta = \sqrt{\frac{\Sigma(nx^2)}{\Sigma n}} \qquad (1)$$

where n is the number of particles having diameter x. Similarly, the diameter, D, of a hypothetical particle having the average volume is

$$D = \sqrt[3]{\frac{\Sigma(nd^3)}{\Sigma n}} \qquad (2)$$

where n is the number of particles having volume d. The average surface, $\bar{s}$, and the average volume, $\bar{v}$, of the particles are then given by

$$\bar{s} = \alpha\Delta^2 \qquad (3)$$
$$\bar{v} = \nu D^3 \qquad (4)$$

where α and ν are shape factors depending upon the geometric shape of the particles. The specific surface, S, can be written

$$S = \alpha\Delta^2/\nu\rho D^3 \qquad (5)$$

where ρ is the density of the particles, assumed to be the same for all. Now the summations $\Sigma(nd^2)/\Sigma n$ and $\Sigma(nd^3)/\Sigma n$ can be expressed in terms of quantities which can be taken directly from a graph of the type shown in Figures 8 or 4.

Arithmetic-Probability Coordinates. The equation for the normal probability distribution is

$$n = \frac{\Sigma n}{\sigma\sqrt{2\pi}} e^{-(x - M)^2/2\sigma^2} \qquad (6)$$

where n is the frequency of occurrence of particles having diameter x, Σn is the total number of observations, M is the arithmetic mean of the diameters, and can be regarded as a constant which fixes the position of the curve, and σ is the standard deviation, which determines the shape of the curve and is numerically equal to the difference between the values of x for 50 per cent and 84.13 or 15.87 per cent.

Combining Equations 1 and 6

$$\Delta^2 = \frac{\Sigma(nx^2)}{\Sigma n} = \frac{1}{\sigma\sqrt{2\pi}} \int_{-\infty}^{+\infty} x^2 e^{-(x - M)^2/2\sigma^2} dx \qquad (7)$$

whence

$$\Delta^2 = M^2 + \sigma^2 + 4\sigma M/\sqrt{2\pi} \qquad (8)$$

Similarly, combining Equations 4 and 8

$$D^3 = \frac{\Sigma(nx^3)}{\Sigma n} = \frac{1}{\sigma\sqrt{2\pi}} \int_{-\infty}^{+\infty} x^3 e^{-(x - M)^2/2\sigma^2} dx \qquad (9)$$

whence

$$D^3 = M^3 + \frac{6M^2\sigma}{\sqrt{2\pi}} + 3\sigma^2 M + \frac{8\sigma^3}{\sqrt{2\pi}} \qquad (10)$$

Now M, the mean size, and σ, the standard deviation, can be read directly from a graph such as is shown in Figure 8, M being the value of x for 50 per cent on the ordinate (0.49 micron in Figure 8) and σ being the difference between the values of x corresponding to 84.13 or 15.87 per cent (0.09 micron in Figure 8). Consequently, Δ^2, D^3, and in turn S can be quickly computed.

Logarithmic-Probability Coordinates. The equation for the frequency curve using a logarithmic scale for size becomes

$$n = \frac{\Sigma n}{\log \sigma_g \sqrt{2\pi}} e^{-(\log x - \log M_g)^2/2 \log^2 \sigma_g} \qquad (11)$$

where M_g is the geometric mean of the values of x, and σ_g is the geometric standard deviation. The value of M_g is the value of x corresponding to 50 per cent oversize and σ_g is given by the ratios

$$\sigma_g = \frac{\text{size at 84.13 per cent}}{\text{size at 50 per cent}} = \frac{\text{size at 50 per cent}}{\text{size at 15.87 per cent}}$$

Both parameters can therefore be obtained from the log-probability graph (see Figure 4).

Equations relating to Green's average diameters to M_g and σ_g have been derived by Hatch and Choate (*7*) and are

$$\log \Delta^2 = \log M_g^2 + 4.6052 \log^2 \sigma_g \qquad (12)$$
$$\log D^3 = \log M_g^3 + 10.3617 \log^2 \sigma_g \qquad (13)$$
$$\log S = \log \alpha/\rho\nu - \log M_g - 5.7565 \log^2 \sigma_g \qquad (14)$$

where the symbols have the same meaning as in Equations 5 and 7.

The foregoing relations are based on frequency measurements by count. They have been extended by Hatch (*6*) to cover size-frequency distributions based on screen analysis and measurement by weight. He has shown that if a given distribution follows the logarithmic-probability relation by count it must also follow it when measurements are made by weight (screen analysis); moreover, the curves for the two distributions plot as parallel lines on log-probability paper. The conversion can be made on the basis that

$$\log M_g = \log M_g' - 6.9078 \log^2 \sigma_g' \qquad (15)$$

where M_g' and σ_g' are, respectively, the geometric mean and geometric standard deviation derived from the weight (screen-analysis) curve. Screen measurements for this purpose should be carried out with sieves which have been calibrated by methods which are described in detail by Hatch.

Rosin-Rammler Equation. The Rosin-Rammler equation as given by Bennett (*1*) is

$$R = 100\, e^{-(x/\bar{x})^b} \qquad (16)$$

where R is the percentage of the total number of particles which have a diameter greater than x, and $\bar{x}$ and b are constants which characterize the distribution for each sample. (The symbol b has been substituted for the n used by Bennett in order to avoid confusion with the symbol used for the

number of particles having a given diameter.) The parameters $\bar{x}$ and b can be evaluated from a graph such as is shown in Figure 11. Equation 18 shows that when $x = \bar{x}$, $R = 100/e = 36.79$ per cent, so that the value of x can be read directly as that value of x at which the percentage oversize is 36.79, or at which the percentage undersize is 63.21. This is illustrated in Figure 11 by the intersection of the line for the size distribution with the dot-dash horizontal line at $R = 63.21$ per cent, in this case, $\bar{x} = 6.25$ cm. (2.5 inches). The constant $\bar{x}$ is therefore a measure of the magnitude of the size of the particles considered and in this respect is analogous to M and M_g in the preceding equations. The value of parameter b is given by the slope of the curve. It is a measure of the dispersion of the distribution, that is, if b is large, the particles are closely grouped in diameter, whereas if b is small, the particles are distributed over a relatively wide range. The constant b is therefore analogous to σ in the preceding equations.

The form of Equation 17 makes it difficult to express Green's average diameters, or the specific surface, in terms of $\bar{x}$ and b.

Summary

It is desirable for many reasons to be able to plot a cumulative size-distribution curve as a straight line. If this can be done, the number of measurements can be reduced, interpolation becomes easier, extrapolation becomes more reliable, the consistency of the observations can be judged at a glance, the calculation of average diameters is facilitated, and the frequency distribution curve can be readily obtained. No single method of plotting which is applicable to all materials has been found, nor is it likely that one exists. There are, however, three methods which have proved successful for certain classes of substances. The most widely applicable one is to plot particle size on a logarithmic scale and cumulative per cent on an integrated probability scale. It gives satisfactory results for crushed or ground materials such as silica, granite, limestone, clay, sodium carbonate, and alumina. The second is the Rosin-Rammler method which plots size on a log scale and cumulative per cent on a log-log scale; it has been notably successful with broken coal. The third method, which appears to have only a very limited applicability, is to plot size on a linear scale and cumulative per cent on the integrated probability scale.

These methods have a possible application to the determination of whether or not one has a representative sample on a material whose composition varies with size of lump, and can even be extended to distributions other than size, such as the variation with time of the composition of pig iron from a given furnace.

Literature Cited

(1) Bennett, *J. Inst. Fuel*, preprint, 1936.
(2) Daeves, "Praktische Grosszahl-Forschung", VDI Verlag, Berlin, 1933.
(3) Drinker, *J. Ind. Hyg.*, **7**, 305 (1925).
(4) Green, *J. Franklin Inst.*, **192**, 637 (1921).
(5) *Ibid.*, **204**, 713 (1927).
(6) Hatch, *Ibid.*, **215**, 27 (1933).
(7) Hatch and Choate, *Ibid.*, **207**, 369 (1929).
(8) Jones, IND. ENG. CHEM., Anal. Ed., **10**, 45 (1938).
(9) Loveland and Trivelli, *J. Franklin Inst.*, **204**, 193, 377 (1927).
(10) Martin, Bowes, Coleman, and Littlewood, *Trans. Ceram. Soc.*, **25**, 240 (1926).
(11) Norton and Speil, *J. Am. Ceram. Soc.*, **21**, 89 (1938).
(12) Rosin and Rammler, *J. Inst. Fuel*, **7**, 29 (1933).
(13) Weber and Moran, IND. ENG. CHEM., Anal. Ed., **10**, 180 (1938).
(14) Work, *Bull. Am. Ceram. Soc.*, **17**, 1 (1938).

Improved Form of Jones Reductor

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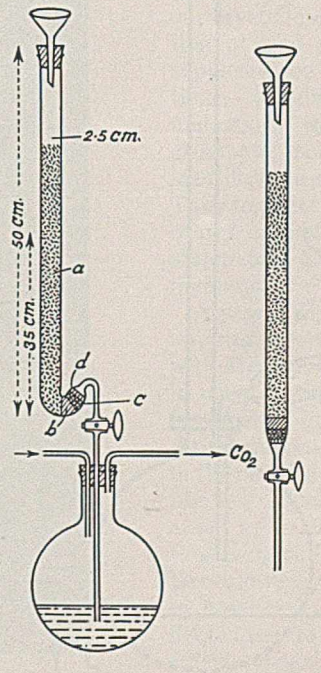
IN THE course of analytical work which involved the estimation of iron, the authors have found a modified form of Jones reductor very convenient.

In the usual form (*1*), shown at right of Figure 1, hydrogen collects just below the zinc column and cannot escape freely. This gives rise to two disadvantages in manipulation: (1) The free and steady flow of the acid solution is impeded. (2) Since in resetting the reductor for a fresh experiment the zinc has to be washed thoroughly and all the accumulated gas displaced by distilled water, and since this cannot be accomplished easily either by passing a swift stream of water through the reductor or even by applying suction, it often becomes necessary to remove the reductor from its support to displace the gas. The improved form obviates both these difficulties. The wide tube is bent round at the bottom, as shown, and the gas which collects below the zinc rises in the narrow tube and is automatically pushed out by the solution. Hence, it cannot accumulate to any undesirable extent and thereby impede the flow. For washing the apparatus finally and for displacing any remaining gas bubble before commencing a fresh experiment, all that is necessary is to run down water in a brisk stream through the tube with the stopcock fully open.



a. Amalgamated zinc granules
b. Glass wool or asbestos pulp
c. Glass beads
d. Perforated porcelain disk

FIGURE 1. JONES REDUCTOR

Literature Cited

(1) Treadwell and Hall, "Analytical Chemistry", Vol. II, p. 554, New York, John Wiley & Sons, 1935.

Large-Size Extractor for Liquids

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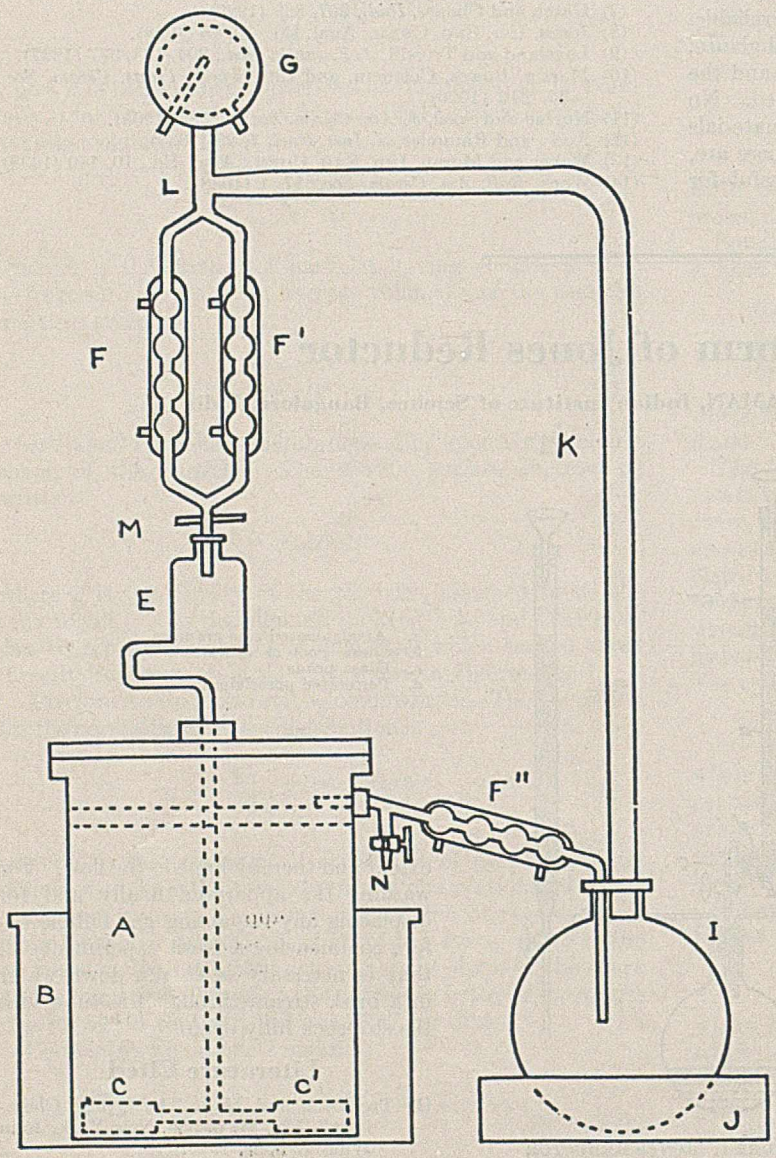
DURING the isolation of certain alkaloids from aqueous concentrates, it became necessary to employ some method whereby sizable quantities of solution could be extracted continuously with ether until alkaloid-free. Although many authors (*1*, *2*, *4*, *5*, *7*) have described large-scale apparatus for the extraction of solid material, the writer has been unable to find in the literature any description of a plant-size extractor for liquids. Employing the principles embodied in the liquid extractor described by Palkin (*6*), an apparatus has been built for the extraction of 40-liter batches of liquid by lighter-than-water solvents.

Description of Extractor

As shown in Figure 1, *A* is a glazed earthenware 60-liter crock equipped with lid. A hole 25 mm. in diameter has been drilled in the center of the lid, and a similar hole drilled in the side of the crock about 15 mm. above the 40-liter level. *B* is an ice bath used when the material being extracted is unstable at room temperature. *C* and *C'* represent six hollow cylinders sealed at one end and constructed of Aloxite brand porous corundum (medium grade). These cylinders are attached by corks to the six legs of a radial glass manifold, which, in turn, is connected by means of 15-mm. glass tubing to the 500-cc. aspirator bottle, *E*. The remaining parts of the extractor consist of three Allihn condensers, *F*, *F'*, *F''*, a spherical condenser, *G*, a 12-liter Pyrex balloon flask, *I*, and miscellaneous glass fittings such as are found in every laboratory. *K* is made of 40-mm. glass tubing, the short leg of which has been tapered to meet the T-tube at *L*. *M* is a cork "umbrella" to prevent condensed moisture from running into *E*.



Operation of Extractor

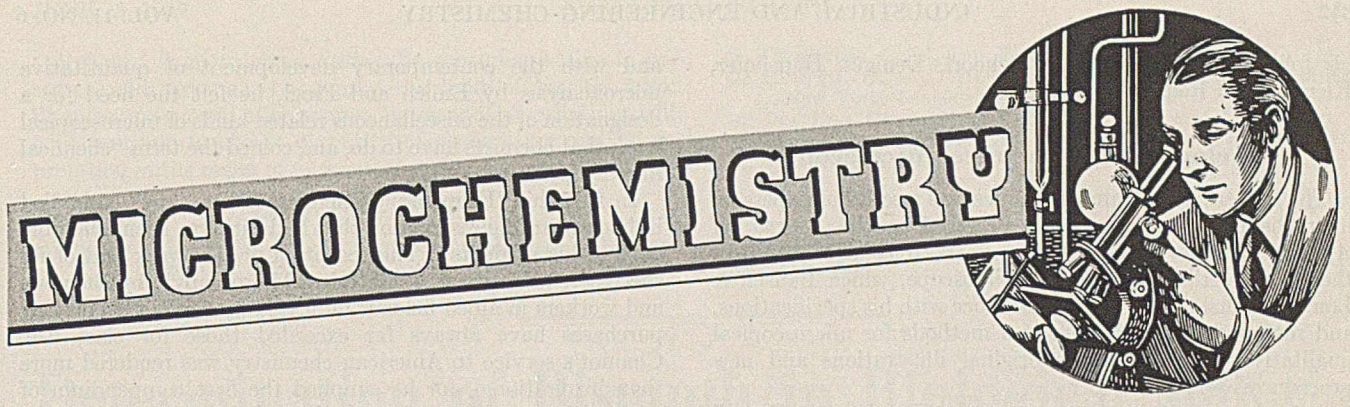
The aqueous concentrate to be extracted is placed in *A* and, if necessary, is diluted to 40 liters. The solvent (ether in this case) is placed in *I* along with the necessary acid solution, if the alkaloid is to be converted immediately into a salt. The solvent is brought to a boil by means of water bath *J*, the vapor passes up through *K*, is condensed by *F*, *F'*, and *G*, and drops into *E*. The purpose of *E* is to enable the operator to follow the rate of flow. The solvent passes from *E* down into the porous cylinders, from which it emerges as a fine spray. As this spray rises through the aqueous liquid, it extracts the alkaloid and spills through the side outlet back into *I*. *F''* prevents hot vapors from *I* from passing into *A* and heating the liquid. Portions of solvent are drawn off at *N* from time to time and tested chemically to determine completion of extraction.

This extractor has been in use in the writer's laboratory for over a year for the extraction of the recently discovered ergot alkaloid, ergonovine. At an approximate concentration of 0.025 per cent this particular alkaloid is completely extracted by ether in 10 to 12 hours' operation of the extractor, as evidenced by a negative test with Glycart's modification of Smith's reagent (*3*). Less than 500 cc. of ether is lost during an 8-hour extraction. The size of the setup can be varied to accommodate the volume of liquid to be extracted.

Literature Cited

(1) Cameron, A. E., Ind. Eng. Chem., Anal. Ed., 4, 394 (1932).
(2) Drake, N. L., and Spies, J. R., *Ibid.*, 5, 284 (1933).
(3) Glycart, C. K., *J. Assoc. Official Agr. Chem.*, 20, 566 (1937).
(4) Grant, L. S, and Pilling, W. M., Ind. Eng. Chem., Anal. Ed., 6, 285 (1934).
(5) McCay, C. M., *Ibid.*, 5, 213 (1933).
(6) Palkin, S., Murray, A. G., and Watkins, H. R., Ind. Eng. Chem., 17, 612 (1925).
(7) Quick, A. J., *Ibid.*, Anal. Ed., 5, 76 (1933).

Figure 1. Diagram of Extractor

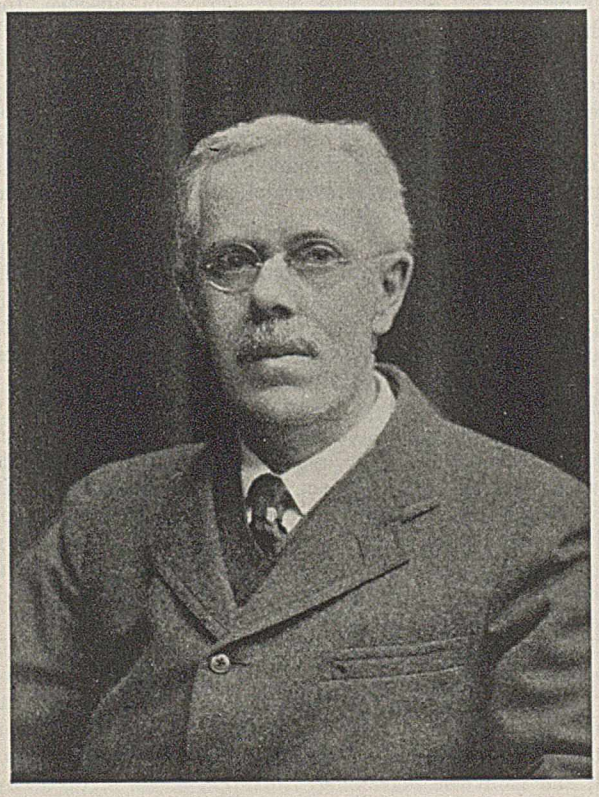


The Services of Émile M. Chamot to Chemical Microscopy

THE origin and advancement of a field of science are rarely to be ascribed to one man; Émile M. Chamot's modesty would prevent him from accepting credit for creating chemical microscopy, but his pioneer work in developing it and in encouraging its application certainly entitles him to be called its foremost exponent if not its father.

It was not merely a case of the time's being ripe; although valuable methods and facts were already available in chemistry and in allied sciences, and microscopes were beginning to be used in chemistry and technology, the work was scattering and unrelated, its value was not generally recognized, and chemists showed little interest in the microscopical approach to their problems.

Chamot's work involved the organization of existing information and its presentation in a form usable by chemists, together with the development of methods and instruments suitable for the variety of work encountered in technical laboratories. Besides this, he had to do a great deal of what he calls "preaching", by precept and example, to show chemists how illuminating microscopical studies can be, and how economical of time and labor. All this was inspired, not by any desire to glorify micromethods for their own sake or to monopolize a specialty, but simply because of his tireless personal enthusiasm for the work and because he was convinced of its general value.



ÉMILE M. CHAMOT

Chamot's services as a chemist, a teacher, and a citizen have previously received tribute (*2*), but his unique role in the development of chemical microscopy can best be appreciated in terms of his career.

Education

As a boy in Buffalo, his birthplace, he enjoyed the formative hobby of nature study with the Naturalists' Field Club, and was encouraged in biological and mineralogical pursuits that proved invaluable adjuncts to chemistry and microscopy.

In his student days at Cornell, his life interest was probably conditioned by his senior thesis under G. C. Caldwell in 1891, for it involved a great deal of microscopical work on the various crystalline phases obtained in the hydrolysis and reduction of lead nitrate and nitrite. During the summer he supplemented this study by photomicrography of his preparations. [Caldwell's appreciation of the value of chemical microscopy was evidenced by more than one instance—a classmate, Lomax, had as a thesis topic the microscopical identification of alkaloids based on Wormley's methods (*5*).] This, together with emphasis on plant constituents and toxicology in analytical chemistry under Caldwell, undoubtedly stimulated Chamot's interest in the organic aspects of microscopy.

A year in Europe, after receiving his doctorate in 1897, was important, not primarily because of the training in toxicology with Macé, its primary objective, but because it brought him in contact with Behrens at Delft. Chamot had previously familiarized himself with Behrens' system of inorganic qualitative microscopical analysis (*1*) but was fortunate in sharing in the detailed instructions which Behrens was giving to Kley, his new assistant, in preparation for the course that was about to be offered formally for the first time. Chamot was thus perhaps Behrens' first pupil. On leaving, he asked how he could repay his master; the reply was "Start some courses in America." Chamot has repeatedly acknowledged his indebtedness to Behrens, whose novel methods and reagents still stand as the greatest contribution to chemical microscopy and have been improved but not supplanted by the work of

his followers, Emich, Donau, Schoorl, Denigès, Haushofer, Klement and Renard, and others.

Development of Chemical Microscopy

The first fruit of Chamot's studies was a series of papers on microchemical analysis which appeared in the *Journal of Applied Microscopy* between the years 1899 and 1902. In them he described a chemical microscope, which Bausch & Lomb had just produced in accordance with his specifications, and reviewed the equipment and methods for microscopical qualitative analysis; many original illustrations and new procedures were included.

As early as 1900, the Department of Chemistry at Cornell was offering two formal courses in microchemical analysis, a term each of inorganic and organic; in 1902 microscopy of foods was also given. (A photograph of the main chemistry lecture room in 1889 shows large charts of interference figures, and a 1-hour course in microscopy appeared in the announcement for 1890; evidently Caldwell's interest considerably antedated Chamot.) These are probably the earliest courses in America in this field, though Hinrichs (*3*) at St. Louis was active at about this time. Instruction in chemical microscopy has continued at Cornell since the beginning of the present century, and Chamot continually developed and extended its scope; because he held at different times instructorships in almost all the courses of the department he was peculiarly fitted to recognize the value and the relationships of microscopical methods to the various divisions of chemistry in academic, forensic, and technical work.

Fortunately, Caldwell's successor as head of the department, L. M. Dennis, believed strongly in the value of optical methods (courses in polarimetry and refractometry and in spectroscopy were offered as early as 1892), and Chamot was able to build up the equipment of the laboratory as need arose; a photograph shows at least nine microscopes in 1905, and twenty or more in 1915.

At first the courses offered were primarily in microscopical analysis; soon it became evident, from Chamot's own experience and from that of his students, that many other aspects of microscopy were of potential value to chemists in industry and research, and a course in general microscopical methods covering micrometry, quantitative estimations, and crystal studies was offered. At an alumni conference it was recommended that training in microscopical methods be required of all students specializing in chemistry at Cornell and when the degree of bachelor of chemistry was established in 1911, such a course was made part of its curriculum and has continued, although the degree has been superseded by that of chemical engineer. At a rough estimate, between two and three thousand American chemists have had at least an introductory training directly under Chamot, and although only a fraction of these may have done much subsequent microscopical work, they have all had the benefit of his critical thinking and emphasis on careful observation and interpretation, as well as a vivid recollection of the small-scale aspects of chemical phenomena so unforgettably demonstrated in the laboratory experiments which he devised.

Chamot's influence outside his classes was ever widening, by lectures and consultation, continually stressing the importance of direct and rapid microscopical approach to new problems of industry and research. His stories of actual cases from his own vast experience, where a close-up showed holes in what appeared to be a blank wall, are still remembered by those who had the pleasure of hearing him at meetings of local sections and other societies.

At first he had, like Behrens, used the term "microchemistry", but with his growing realization of the importance of physical and physicochemical factors in chemical behavior, and with the contemporary development of quantitative microanalysis by Emich and Pregl, he felt the need for a designation of the miscellaneous related kinds of microscopical work that chemists have to do, and coined the term "chemical microscopy" about 1914.

In 1915, his "Elementary Chemical Microscopy" embodied in book form the instruction that had hitherto been available only to his students. The reception of this work and of its successor, "Handbook of Chemical Microscopy", by chemists and workers in allied fields is indicated by the fact that their purchases have always far exceeded those for class use. Chamot's service to American chemistry was rendered more than inspirational, for he supplied the first compendium of selected information and methods of real utility to the independent investigator, not only for the analysis of minute samples but for technical studies where preparation methods and observations of physical conditions or properties were of primary importance. Chamot's book greatly aided in the spread of instruction in chemical microscopy, by his students or by faculty members in other institutions. By 1920, one or more courses closely patterned after those at Cornell were being offered in at least three other universities, and the number has increased several times since then.

As his students have gone out into industry they have carried his inspiration, and have been willing to "try the microscope first instead of last." In many of the larger research and control laboratories extensive applications of chemical microscopy have been the direct result of his teachings and of his forceful presentation of its potentialities in new fields.

His influence has not been confined to America; students from abroad, correspondence with investigators in other countries, and references to his work in foreign journals attest this. In 1924–25 he was an exchange professor in France, representing seven eastern universities. His lectures and conferences at a score or more of educational institutions aroused much interest in chemical microscopy, and were an important contribution to science in the country of his forebears.

Unfortunately, during this busy period of absence, the Technical Photographic and Microscopical Society, of which he was a founder and the first president, declined and has since been disbanded, but its existence was indicative of the dissemination of microscopical methods in various fields of technology at that time. Their importance received official emphasis in the following statement, from the report of a committee of the National Research Council, on "The Education of the Research Chemist": "The microscope has come to be so valuable a part of research laboratory equipment that every research chemist should be well trained in its use" (*4*). Chamot's "preaching" was the direct cause of this attitude and of the leadership which American chemists have maintained in the broader applications of chemical microscopy.

Modest and shunning publicity, Chamot has given freely of advice and experimental assistance in innumerable investigations, without thought of personal recognition. His knowledge of biology has been invaluable, not only in his early work on toxicology and in his later development of sanitary chemistry and food analysis at Cornell, but also because it enabled him to cooperate with botanists, animal histologists, and bacteriologists, with intelligent insight and novel and convincing microscopical methods of attack.

The services of Professor Chamot received conspicuous recognition by the award in 1937 of the Longstreth Medal of the Franklin Institute "for meritorious work in chemical microscopy", but he derives far greater satisfaction from the contributions his students have made to the extension of this field, and from the increasing utilization of its methods by chemists, geologists, biologists, and metallurgists.

Relieved from teaching duties, he continues active in research and writing. Long may the light of his life work illuminate chemical microscopy; its secondary radiations will ever continue to clarify obscurity of observation and interpretation in the realm of microns.

Literature Cited

(1) Behrens, H., "Manual of Microchemical Analysis", London, Macmillan Co., 1894.
(2) Cavanaugh, G. W., IND. ENG. CHEM., 25, 826 (1933).
(3) Hinrichs, C. G., "First Course in Microchemical Analysis", New York, Lemcke and Buechner, 1904.
(4) IND. ENG. CHEM., News Ed., 7, No. 13, p. 1 (July 10, 1929).
(5) Wormley, T. G., "Microchemistry of Poisons", New York, William Wood, 1869.

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Determination of Bismuth in Biological Material

A Photometric Dithizone Method

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RECENT experimental studies on the physiological behavior of bismuth required an adequate analytical method for dealing with less than 5 micrograms in biological material.

A spectrographic method has been developed by Cholak (*3*) by means of which quantities of 1 microgram or more can be determined, but the literature failed to disclose the existence of a chemical method with the requisite sensitivity. Tompsett (*8*) has described a method for the determination of bismuth in biological material using thiourea following the isolation of bismuth with diethyldithiocarbamate, but he gives no analytical data and does not discuss the application of the method to amounts of bismuth below 5 micrograms. Haddock (*6*) determines bismuth colorimetrically as the iodobismuthite ion following its isolation with dithizone, but his method applies only to values between 5 and 100 micrograms. Fischer (*5*) pointed out the high sensitivity of dithizone in the detection of bismuth, and suggested the possibility of using a "mixed color" technique in the determination of bismuth with dithizone (*4*).

A method based on these suggestions is reported herein. Unlike lead (*7*), however, bismuth is not extracted quantitatively from mixtures of extraneous salts and must therefore be isolated as the sulfide before proceeding with the dithizone extractions. The method has the necessary sensitivity and has proved reliable for all ranges of concentrations likely to be encountered.

Reagents

Ordinary high-grade chemicals may be used for all steps up to the point of separation of bismuth from lead (extraction 2). Since any lead present in the reagents used beyond this point is estimated as bismuth, reagents for the final step must be purified. The precautions to be taken are as follows:

Ammonia used to prepare the ammonia-cyanide mixture is freshly distilled in glass. Potassium cyanide as a 50 per cent (weight by volume) aqueous solution is treated with dithizone in chloroform (20 mg. per liter), the dithizone entering the aqueous phase being reduced to a minimum by repeated treatment with clear chloroform (*2*). The purified solution is then diluted with distilled water to the proper strength as required for the ammonia-cyanide mixture (*7*). The dithizone used to prepare the extraction solutions for extractions 2 and 3 is purified and the chloroform used for solution is redistilled and specially treated to prevent oxidation, by methods previously described (*1, 7*). Nitric acid (1 + 99) used in extraction 2 is prepared from nitric acid once distilled in quartz (specific gravity 1.40) and the distilled water is further redistilled from a Pyrex glass still.

Glassware

The graduated Squibb (Pyrex) separatory funnels used (*7*) must be cleaned meticulously with hot nitric acid (1 + 1) and distilled water to ensure removal of any bismuth present as surface contamination from previous use. This cleansing must be repeated for funnels used in extraction 2 to prevent contamination by surface lead.

Apparatus

A Bausch & Lomb spectrophotometer is employed for density measurements. Readings are taken with the monochromator set of 505 mu. The instrument is equipped with matched pairs of cells, which have been described previously (*7*).

Procedure

PREPARATION OF SAMPLES. Samples of biological material are prepared for analysis by dry or wet ashing methods as described by Cholak (*3*) except that 1 mg. of copper is added to serve as an entraining agent during the isolation of bismuth as the sulfide. The filter paper containing the sulfides is treated with 25 ml. of nitric acid (1 + 9), in the original beaker used for gassing, and the mixture is gently heated to effect solution of the sulfides. The filter paper and free sulfur are removed by filtration through a type 3G4 filter of fritted glass (Jena). After alternate washing of the filter with hot nitric acid (1 + 1) and hot distilled water, the filtrate and washings are returned to the original beaker and evaporated to low volume.

EXTRACTION 1 (REMOVAL OF COPPER). The sample thus prepared is transferred to a 125-ml. graduated Squibb-type separatory funnel by alternately washing the beaker with hot nitric acid (1 + 1) and with hot distilled water. After dilution to 50 ml., 3 drops of thymol blue (Clark and Lubs) and 5 ml. of potassium cyanide solution (10 per cent weight by volume) are added and the pH of the solution is adjusted to 9.5 with concentrated ammonia. Bismuth and lead are extracted as the dithizonates by successive additions of 5-ml. portions of dithizone in chloroform (20 mg. per liter). The number of 5-ml. portions used serves as a preliminary guide to the amount of bismuth present, since each 5-ml. portion extracts approximately 25 micrograms of bismuth. Bismuth dithizonate imparts a bright orange color to the chloroform phase. The last 5-ml. portion, which should show no change in color, is withdrawn as completely as possible. The aqueous layer is shaken twice with 2-ml. portions of clear chloroform and the chloroform phases are added to the previous extracts. The aqueous phase is then treated with 2 ml. of concentrated nitric acid and the solution is readjusted to pH 9.5 with dilute ammonia (1 + 9). Ten milliliters of dithizone solution are then added as above and withdrawn, followed by one 5-ml. portion. The combined extracts containing bismuth and lead dithizonates plus uncombined dithizone in chloroform are next washed with 25 ml. of distilled water. After separation of the two phases the aqueous phase is shaken twice with 2-ml. portions of clear chloroform; the latter are added to the chloroform phase, but the wash water is discarded.

Depending upon the amount of bismuth present, either the whole or an aliquot of the total extract containing not more than 50 micrograms of bismuth is taken for extraction 2. This is shaken twice with 25-ml. portions of nitric acid (1 + 99). The chloroform-dithizone phase is discarded and the collected acid phase is treated with clear chloroform to expell the last traces of the dithizone-chloroform phase.

EXTRACTION 2 (REMOVAL OF LEAD). To the acid aqueous phase obtained above, 3 drops of *m*-cresol purple (Clark and Lubs) are added and the pH of the solution is adjusted to 2 by the addition of dilute ammonium hydroxide (1 + 9). Bismuth is now extracted by successive additions of 5-ml. portions of dithizone in chloroform (20 mg. per liter) in the same manner as in extraction 1, except that the dithizone solution used is treated with nitric acid (1 + 99) to ensure removal of lead. The range is now definitely placed and the chloroform fractions are collected in a clean separatory funnel. (Separatory funnels used for this step as well as the step to follow must be scrupulously clean.) Bismuth free from lead (*9*) is now brought into solution as the pure nitrate in 50 ml. of nitric acid (1 + 99) by the use of two 25-ml. portions of acid as in extraction 1.

EXTRACTION 3 (ESTIMATION OF BISMUTH). For the final estimation, bismuth is extracted a third time by means of chloroform-dithizone solutions of various strengths, depending upon the range. The technique of estimation is identical with that described previously for lead (*7*) except for the following modifications:

1. The 50 ml. of nitric acid (1 + 99) containing pure bismuth nitrate are freed from entrained chloroform either by applying air suction over the surface to remove droplets of chloroform or by normal evaporation. Excess chloroform at the base of the liquid is drawn off as completely as possible (*2*).
2. Potassium cyanide, purified as described under "reagents", is used to make the ammonia-cyanide mixture.
3. To guard against lead contamination the chloroform phase containing bismuth dithizonate plus excess dithizone is removed directly through the funnel stem into the proper-sized cell (*2*), traces of water in the stem having first been removed by swabbing with an ordinary pipe cleaner folded at one end to produce a snug fit.
4. A reagent blank need not be considered since bismuth is not a common contaminant of reagents used (*3*).
5. The ammonia-cyanide mixture and all chloroform solutions of dithizone are stored in a refrigerator to increase their stability (*2*). As a further safeguard the dithizone solutions are stored in lightproof containers.
6. The concentrations of the various dithizone solutions have been increased, as shown in Table I.

TABLE I. DITHIZONE CONCENTRATION

Range	Dithizone Concentration	Volume Used	Cell
Micrograms	*Mg./liter*	*Ml.*	*Mm.*
0– 5	6	10	50
0–25	12	25	25
0–50	24	25	12

Working curves obtained for the various ranges by the use of pure bismuth nitrate solution in chloroform-saturated nitric acid (1 + 99) are obtained in a manner similar to that used for lead (*7*). The slopes of the curves for corresponding ranges are somewhat steeper, however, in the case of bismuth. Pure metallic bismuth is used to prepare the nitrate solutions.

Analytical Results

In Table II are listed results obtained by the analysis of 100-ml. urine samples (in triplicate) containing known added quantities of bismuth. In Table III are listed results obtained by the analysis of 10-gram samples of rabbit blood (in triplicate) containing known added quantities of bismuth.

Discussion

Interfering elements are stannous tin, monovalent thallium, and lead. Stannous tin is oxidized and removed during the course of analysis (*7*). Monovalent thallium and lead are removed during extraction 2; the former, however, has not been encountered in biological material.

In order to prevent the precipitation of Ca^{++}, Fe^{+++}, and PO_4^{---} ions when the solutions are adjusted to pH 9.5, excessive amounts of ammonium citrate must be added. When this has been done the writer has been unable to extract bismuth quantitatively from prepared solutions of biological material such as blood and urine, even though he followed the procedure, outlined by Haddock (*6*), of using a relatively strong chloroform-dithizone solution (1 gram per liter) for extraction. However, no difficulty was encountered when the bismuth was first isolated as the sulfide, copper being used as the entraining agent to effect the removal of minute quantities. The excess copper added does not complicate the method, since it is "complexed out" by means of potassium cyanide in the first extraction.

TABLE II. ANALYSIS OF URINE

Range Used	Bismuth Added	Bismuth Found
Micrograms	*Micrograms*	*Micrograms*
0– 5	Nil	0.1
0– 5	Nil	Nil
0– 5	Nil	0.1
0– 5	1.0	1.1
0– 5	1.0	1.0
0– 5	1.0	1.2
0– 5	5	4.8
0– 5	5	4.9
0– 5	5	5.0
0–25	15	14.5
0–25	15	15.0
0–25	15	15.0
0–50	50	49
0–50	50	50
0–50	50	50
0–50	500	470
0–50	500	470
0–50	500	480

However, excessive amounts of potassium cyanide entrained in the combined extract obtained during extraction 1 subsequently prevent the complete extraction of bismuth as the dithizonate from the acid phase adjusted to pH 2. For this reason the combined dithizone extracts are washed with distilled water before proceeding to the conversion of the bismuth dithizonate to bismuth nitrate with nitric acid (1 + 99). All the potassium cyanide is not removed, but the portion remaining is not harmful.

TABLE III. ANALYSIS OF BLOOD

Range Used	Bismuth Added	Bismuth Found
Micrograms	*Micrograms*	*Micrograms*
0– 5	Nil	Nil
0– 5	Nil	0.1
0– 5	Nil	0.2
0– 5	1.0	1.2
0– 5	1.0	1.0
0– 5	1.0	1.0
0– 5	2.5	2.6
0– 5	2.5	2.4
0– 5	2.5	2.5
0– 5	5.0	5.0
0– 5	5.0	4.9
0– 5	5.0	5.0
0–25	15	15
0–25	15	15
0–25	15	14.5
0–50	50	47
0–50	50	48
0–50	50	48

Particularly important was the discovery that when quantities of bismuth in excess of 50 micrograms are extracted during extraction 1, some bismuth dithizonate as well as some pure dithizone remains in the aqueous phase. This is due, of course, to the partition coefficient of the bismuth dithizonate between the two phases. The greater solubility of the bismuth dithizonate in chloroform suggested a means for partially overcoming this difficulty: The aqueous phase following its initial apparent complete extraction is reacidified with nitric acid, and extracted again with dithizone after again adjusting the pH to 9.5. Preliminary washing of the extracted aqueous phase with the 2-ml. portions of clear chloroform is also beneficial, since an appreciable amount of bismuth dithizonate is removed from the aqueous phase—frequently it is enough to cause a decided color change in the chloroform used. The following experiment indicates the magnitude of the loss likely to occur. When 50-microgram portions of bismuth as the pure nitrate were taken through the extraction procedure without regard to re-extracting the aqueous phase in extraction 1, only 86 per cent of the added bismuth was recovered. On the other hand, when the aqueous phase

from extraction 1 was treated as described above, recoveries of 99 per cent were obtained.

An efficient method was adopted for preparing and keeping dithizone solutions. A chloroform-dithizone solution (6 mg. per liter) prepared from purified dithizone and chloroform redistilled and treated with hydroxylamine (*1*, *7*) has been in use intermittently for a period of 6 months without showing signs of deterioration.

Positive findings for the blank determinations shown in Tables II and III are not due to bismuth, but are due partly to errors in density readings and partly to the fact that minute quantities of lead introduced through contamination after extraction 2 have been estimated as bismuth.

Although the analytical results shown in Tables II and III have been obtained with 10-gram samples of blood and 100-ml. samples of urine, the method can be applied to larger or smaller samples, depending upon the concentration of bismuth present and the amount of material available.

Summary

A photometric "mixed color" dithizone method for the determination of bismuth, applicable to biological material, has been devised. Quantitative extractions are made possible by isolating the bismuth as the sulfide, interference by other metal sulfides being prevented by complex salt formation with potassium cyanide and specific separation of the bismuth at pH 2.

Although used specifically for the analysis of blood and urine samples, the method is applicable to other materials. It is very sensitive; amounts of bismuth below 5 micrograms can be determined with a high degree of accuracy and 95 per cent recoveries have been obtained for quantities above 50 micrograms.

Literature Cited

(1) Assoc. Official Agr. Chem., "Official and Tentative Methods", 4th ed., p. 378, 1935.
(2) Bambach, K., personal communication (data to be published later).
(3) Cholak, J., IND. ENG. CHEM., Anal. Ed., 9, 26 (1937).
(4) Fischer, H., *Z. angew. Chem.*, 50, 919–38 (1937).
(5) Fischer, H., and Leopoldi, G., *Ibid.*, 47, 685 (1934).
(6) Haddock, L. A., *Analyst*, 59, 163–8 (1934).
(7) Hubbard, D. M., IND. ENG. CHEM., Anal. Ed., 9, 493 (1937).
(8) Tompsett, S. L., *Analyst*, 63, 250–2 (1938).
(9) Willoughby, C. E., Wilkins, E. S., Jr., and Kraemer, E. O., IND. ENG. CHEM., Anal. Ed., 7, 285 (1935).

PRESENTED before the Division of Microchemistry at the 97th Meeting of the American Chemical Society, Baltimore, Md.

Microidentification of Metrazole in Mixed Aqueous Solutions

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METRAZOLE (cardiazole) has become recognized as a stimulant, and its identification must be of increasing interest to the toxicologist. The literature contains but little information of this nature.

The method of Wollner and Matchett (*1*, *2*) is well adapted for the extraction and separation of alkaloids and other drugs which the toxicologist may encounter in body fluids. Because of its pronounced solubility in water, metrazole is not so readily extracted by this method as the alkaloids, unless appreciable amounts are present. The faintly ammoniacal, aqueous solution is extracted with ethyl acetate in a special extraction device (*1*) and the solvent is evaporated. The residue is taken up with a few milliliters of chloroform in a special microseparatory tube (*2*). This solution is extracted first with a small portion of 5 per cent potassium hydroxide and then with 0.5 *N* hydrochloric acid.

Amphoteric alkaloids such as morphine are contained in the alkali extract, strongly basic alkaloids such as strychnine appear in the acid extract, and weakly basic alkaloids such as caffeine remain dissolved in the chloroform. When a small amount of metrazole is present it is found entirely in the chloroform fraction. Traces of metrazole occur in the alkali extract when a considerable amount is present in the original solution. Metrazole is recovered by evaporation of the chloroform, and the residue is taken up in a drop of 0.1 *N* hydrochloric acid on a microscope slide for the microcrystalline test.

Metrazole in very dilute solution fails to form microcrystals with the usual alkaloidal reagents. In more concentrated solutions microcrystals and amorphous precipitates are formed with some reagents. Zwikker (*3*) has suggested a hydrochloric acid solution of cuprous chloride as a reagent for metrazole, claiming a sensitivity of 1 in 40,000. He prepares this reagent from cupric chloride and sodium sulfite immediately before use, stating that the reagent cannot be preserved.

The author prepares a satisfactory reagent by dissolving cuprous chloride (Baker's) in dilute hydrochloric acid. This operation requires considerably less time than the preparation of Zwikker's reagent. However, this reagent also must be prepared daily, since it is converted rapidly to cupric chloride.

For routine use a 5 per cent solution of cupric chloride in 0.5 *N* hydrochloric acid seems to be much more satisfactory. This reagent is stable and does not have to be prepared daily; furthermore, its sensitivity is comparable to that of cuprous chloride reagent. One drop of a metrazole solution 1 to 1000 (about 50 micrograms) with a drop of the reagent, upon

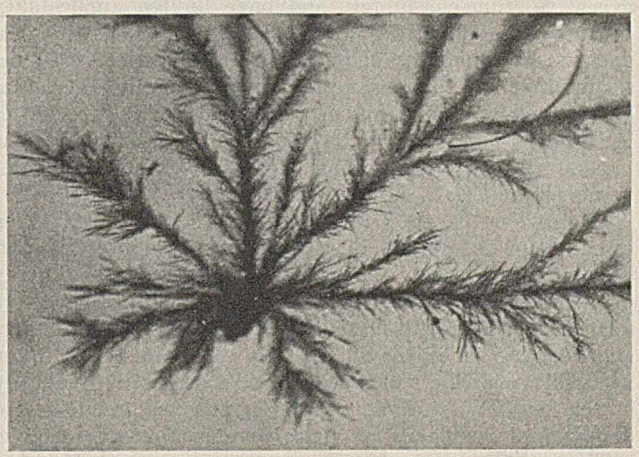

FIGURE 1. METRAZOLE WITH CUPRIC CHLORIDE REAGENT, DILUTION 1 TO 1000 (× 100)

partial spontaneous evaporation, gives an abundance of crystals which are visible even to the naked eye. Even with a metrazole solution so dilute as 1 to 10,000 (about 5 micrograms), a few microcrystals are formed. In these more dilute solutions it is advisable to allow the drop to evaporate completely before examining it under the microscope. Then the addition of a drop of water will dissolve the readily soluble cupric chloride crystals, leaving the well-formed metrazole crystals plainly visible.

The microcrystals are characteristically clusters and rosettes of fine needles, mostly exhibiting arborescent forms resembling a fir tree, and polarizing poorly. Cupric chloride crystals polarize brilliantly and are considerably different in form, so that they scarcely could be confused with metrazole crystals. None of the principal alkaloids or other common drugs which the author has tested with cupric chloride reagent gave microcrystals which might be confused with metrazole. These alkaloids in mixed aqueous solution with metrazole did not appreciably inhibit its separation and identification.

For obvious reasons the results of animal experimentation and data on the mode of elimination of metrazole cannot be made public at the present time.

Modified Method of Wollner and Matchett

Separation and Identification of Metrazole in Body Fluids. Small amounts (1.0, 0.5, 0.1, and 0.05 mg.) of metrazole were added to 100-ml. samples of body fluids (and washings). Twenty-five grams of ammonium sulfate, C. P., were dissolved in each sample (*2*), and the solution was made faintly ammoniacal to litmus paper. The solution was then extracted with ethyl acetate in the special extraction device (*1*), and the solvent was filtered and evaporated to dryness on the steam bath.

The residue was taken up by washing repeatedly with small portions of chloroform (total volume about 3 ml.) and transferred to a microseparatory tube (*2*) by means of a medicine dropper. A 0.25-ml. portion of 5 per cent potassium hydroxide was added to the tube, which then was shaken about 6 minutes in a blood pipet shaker, and centrifuged, and the alkali layer was removed to a second tube by means of a Wright pipet. This alkali extraction was repeated once. Then the chloroform solution was extracted with 0.5 ml. of 0.5 *N* hydrochloric acid by shaking 2 minutes, centrifuging, and transferring the acid layer to a third tube. The acid extraction was repeated once with 0.25 ml. of 0.5 *N* hydrochloric acid, and the chloroform solution was filtered and evaporated to dryness on the steam bath. The residue was taken up in one drop of 0.1 *N* hydrochloric acid and transferred to a microscope slide and one drop of the cupric chloride reagent was added to it.

Microcrystals were formed in the three samples which contained 1.0, 0.5, and 0.1 mg. of metrazole, but not in the sample which contained 0.05 mg. Therefore, the smallest amount of unchanged metrazole which can be extracted and identified by this method is about 0.1 mg. (100 micrograms) per 100 ml.

Summary

A toxicological method for the extraction and separation of metrazole from mixed aqueous solutions with other drugs is described, which is capable of extracting and identifying 0.1 mg. of metrazole in 100 ml. of aqueous solution. Cupric chloride is suggested as a reagent for the detection and identification of metrazole. The sensitivity is 1 part in 10,000.

Literature Cited

(1) Wollner and Matchett, Ind. Eng. Chem., Anal. Ed., **10**, 31 (1938).
(2) Wollner and Matchett, unpublished work.
(3) Zwikker, J. J. L., *Pharm. Weekblad*, **71**, 1170–82 (1934).

A Microbiological Assay for Riboflavin

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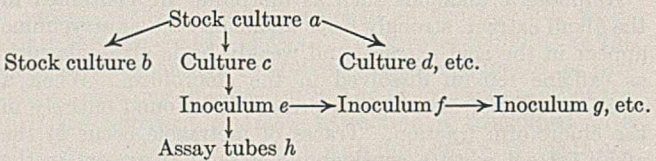
IN A recent critical article, Ellinger (*5*) discussed the errors involved in the chemical and physicochemical methods for the quantitative estimation of riboflavin in biological products, concluding that at present biological assay provides the most reliable method for the estimation of this vitamin. The disadvantages of current biological methods for riboflavin are common knowledge. They are expensive, time-consuming, and have limited applicability because of the quantities of material required for assay. In addition, the reliability and accuracy of such assays are open to question (*3*). For example, the value of the Bourquin-Sherman unit (*3*) in terms of pure riboflavin as reported in the literature (or as it may be estimated from data in various papers) varies from less than 2 to approximately 5 micrograms per unit (*1*, *2*, *9*, *14*).

In previous papers (*16*, *17*) riboflavin was shown to be essential for the growth of certain lactic acid bacteria, and the amount of growth was found in some cases to be directly proportional to the concentration of riboflavin in the culture medium. The growth-promoting activity of various isomers and homologs of riboflavin was also determined (*16*). The naturally occurring substance proved more active than any of the synthetic variants, while degradation products produced from riboflavin by light were completely inactive.

The present paper describes an assay method for riboflavin in natural materials, which has been developed on the basis of the above information.

Stock Culture and Inoculum

The organism used for assay purposes is carried in this laboratory as *Lactobacillus casei;* it is probably identical with the *Bacillus casei* ϵ of Freudenreich (*8*). The requirement of this organism for riboflavin and other growth factors has been previously described (*17*, *18*). Stab cultures of the organism are carried in yeast-water agar containing 1 per cent of glucose. The method of preparing and carrying cultures is indicated in the following diagram:



From the original culture, *a*, a series of stab transfers (*b*, *c*, *d*, etc.) are made into yeast water–glucose agar. After 24 hours' incubation at 37° C. these are stored in the refrigerator. At least one tube, *b*, is reserved as the stock culture. The other tubes (*c*, *d*, etc.) may be used to prepare inoculum as described below. If assays are to be made on each of several successive days, one need not grow inoculum from stock cultures (*c*, *d*) each day, but can transfer a drop of inoculum *e* to a similar tube, *f*, which is incubated for use the next day. Inoculum cultures should not be used after they are more than 36 hours old. One should return to a stock culture about every 5 days to minimize chances of contamination and of bacterial variation. New stock cultures corresponding to *b*, *c*, and *d* are prepared at monthly intervals from a tube such as *b* of the preceding month.

Basal Medium

The riboflavin-free basal medium used is a modification of that developed in previous studies (*16*, *17*). It contains photolyzed, sodium hydroxide-treated peptone, 0.5 per cent; glucose, 1 per cent; sodium acetate, 0.6 per cent; cystine,

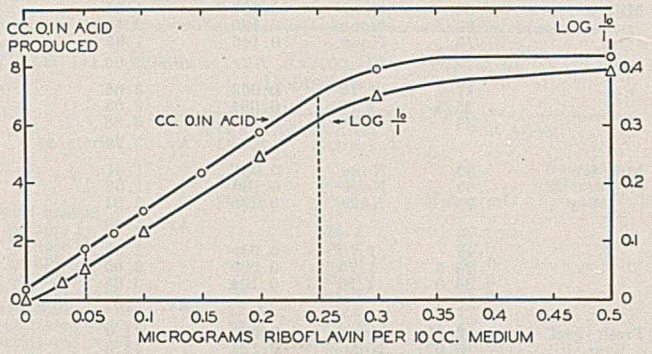


FIGURE 1. RESPONSE OF *L. casei* TO PURE RIBOFLAVIN

0.01 per cent; inorganic salts; and riboflavin-free yeast supplement equivalent to 0.1 per cent yeast extract. The constituents are prepared and kept as follows:

PHOTOLYZED, SODIUM HYDROXIDE-TREATED PEPTONE. A mixture of 40 grams of peptone (Difco Bacto) in 250 cc. of water and 20 grams of sodium hydroxide in 250 cc. of water is exposed in a 25-cm. crystallizing dish to light from a 100-watt bulb with reflector at a distance of approximately 30 cm. for 6 to 10 hours, and is then allowed to stand at room temperature for an additional 18 to 14 hours (24 hours in all). The sodium hydroxide is neutralized with glacial acetic acid (27.9 cc.), 7 grams of anhydrous sodium acetate are added, and the mixture is diluted to 800 cc. This solution contains the equivalent of 5 per cent of peptone and 6 per cent of sodium acetate, which is ten times the concentration of these materials in the final medium. It is preserved under toluene. The above treatment destroys other substances (*18*) besides riboflavin which are essential for growth of the assay organism. These are supplied in the yeast supplement.

YEAST SUPPLEMENT. To a solution of 100 grams of Bacto-yeast extract in 500 cc. of water 150 grams of basic lead acetate (Horne's sugar reagent) dissolved in 500 cc. of water are added, and the precipitate is filtered off. (This yeast extract consists of dry matter from a clarified water extract of autolyzed yeast and is prepared for use in bacteriological culture media. It may be obtained from the Difco Laboratories, Inc., Detroit, Mich.) Ammonium hydroxide is added to a pH of about 10.0, and the precipitate formed is again filtered off. The filtrate is just acidified with glacial acetic acid, the excess lead precipitated with hydrogen sulfide, and the lead sulfide filtered off and discarded. All the riboflavin is removed by this treatment. The filtrate is made to a volume of 1000 cc., and stored under toluene in the refrigerator. One cubic centimeter of this preparation is equivalent to 100 mg. of the original yeast extract.

INORGANIC SALTS. Solution A consists of 25 grams of potassium hydrogen phosphate and 25 grams potassium dihydrogen phosphate dissolved in 250 cc. of water. Solution B consists of 10 grams of magnesium sulfate heptahydrate, 0.5 gram of sodium chloride, 0.5 gram of ferrous sulfate heptahydrate, and 0.5 gram of manganese sulfate tetrahydrate dissolved in 250 cc. of water. Five cubic centimeters of solution A plus 5 cc. of solution B contain inorganic salts for 1000 cc. of basal medium.

CYSTINE. A solution of cystine hydrochloride containing 1 mg. of cystine per cc. is prepared, and kept under toluene.

Standard Riboflavin Solutions

For a stock solution, pure crystalline riboflavin is accurately weighed and dissolved in warm 0.02 *N* acetic acid. A convenient concentration is 100 micrograms per cc. This solution is kept in the refrigerator under toluene. For use from day to day, a more dilute solution (10 micrograms per cc.) is prepared from the stock solution by dilution with 0.02 *N* acetic acid. [Synthetic *d*-riboflavin of at least 99 per cent purity (Merck and Company, Inc.) was used as the primary standard throughout this investigation.]

Procedure

The fermentations are carried out in ordinary chemical or bacteriological test tubes (16 × 150 mm. to 20 × 150 mm.). A metal or wire rack which holds each tube separately and upright and which can be autoclaved without damage is very convenient, but not necessary if the tubes are properly marked. If 50 assay tubes are to be set up from stock solutions of the above concentrations, 50 cc. of photolyzed, sodium hydroxide-treated peptone solution, 50 cc. of cystine hydrochloride solution, 5.0 cc. of yeast supplement, 5.0 grams of glucose, 2.5 cc. of solution A, and 2.5 cc. of solution B are mixed, the pH is adjusted to 6.6 to 6.8 with sodium hydroxide, and the mixture is diluted to 250 cc. Five cubic centimeters are pipetted into each of the 50 tubes, and a suitable aliquot of the riboflavin-containing extract is added. The contents of each tube are then diluted, where necessary, to give a total volume of 10 cc. Thus as much as 5 cc. of liquid may be added with the sample. The volumes indicated should be measured with an accuracy of ±0.1 cc. The tubes are plugged with cotton, sterilized in the autoclave at 1 kg. per sq. cm. (15 pounds per sq. inch) pressure for 15 minutes, and allowed to cool. The tubes are then ready for inoculation.

Inoculation and Incubation

For inoculum, a stab from a stock culture is made into a sterile tube of basal medium to which has been added 0.5 to 1.0 microgram of riboflavin per 10 cc. The culture is incubated 24 hours at 37°, and the cells are centrifuged out aseptically and resuspended in an equal volume of sterile 0.9 per cent sodium chloride solution. One drop (*ca.* 0.05 cc.) of this suspension is used to inoculate each assay tube. The inoculated assay tubes are incubated at 37° to 40° C. for 1 to 3 days.

Standard Curve

With each set of assays there are set up duplicate tubes containing, per 10 cc. of medium, 0.0, 0.05, 0.075, 0.1, 0.15, 0.2, and single tubes containing 0.3 and 0.5 microgram of riboflavin. Titrations or growth data secured from these tubes allow the construction of a standard curve (Figure 1).

Determination of Bacterial Response

Two methods have been used to determine quantitatively the response to added riboflavin. The first involves measurement of the turbidity produced by the growth of the organism.

The Evelyn photoelectric colorimeter (*7*) has been employed for this purpose with excellent results, using the 540 mμ filter. The galvanometer is adjusted to read 100 with the uninoculated basal medium in the colorimeter tube. The assay tubes are well shaken to suspend all bacterial cells uniformly; medium and cells are then transferred to the colorimeter tube and the percentage of incident light transmitted is read directly from the galvanometer. Overhead lights must be off while such readings are being made to prevent stray light from being reflected into the photoelectric cell by the turbid suspension.

This procedure cannot be used when turbid or highly colored extracts are being assayed for their riboflavin content. Corrections for the turbidity of extracts added are not generally valid, since the turbidity is frequently altered by the acid produced during the growth of the organism.

In the second method, which is of general applicability, the acid produced during growth is measured. Contents of the assay tubes are transferred to a 125-cc. Erlenmeyer flask with 10 to 20 cc. of distilled water and titrated to pH 6.8 to 7.0 with 0.1 *N* sodium hydroxide. Bromothymol blue is a satisfactory indicator. A color-comparison flask aids in recognition of the end point, which is reproducible to ± 0.1 cc.

Period of Incubation

Turbidity of cultures grown in the basal medium here described reaches a maximum in approximately 24 hours, remains constant for about 48 hours, and then slowly de-

creases. Turbidity measurements are therefore best made at the end of 24 hours. Acid production, however, increases steadily for 3 to 4 days, so that the method is more sensitive if the cultures are titrated after the longer interval. However, practically the same results are secured when shorter incubation times are used. In the work described below acid production has been measured after 72 hours' incubation except where otherwise stated.

Evaluation of Bacterial Response in Terms of Riboflavin

The response of the organism to pure riboflavin in a typical fermentation is shown in Figure 1. The turbidity measurements were made after 24 hours' incubation. Since within a limited range turbidity and acid production are directly proportional to the concentration of riboflavin, the response elicited by an unknown sample can be evaluated in terms of riboflavin by interpolation on the standard curve. To yield valid results the riboflavin content of the assay cultures must fall on the straight-line portion of the curve above 0.05 microgram per 10 cc.—i. e., between 0.05 and 0.25 microgram per 10 cc. in Figure 1. Preferably each sample should be assayed at three levels which fall within this range with duplicate tubes at each level. The values obtained at the three levels are averaged for the final result.

The results of assays of "unknown" solutions of pure riboflavin by this method are shown in Table I. Similar results have been obtained by several independent observers in this laboratory. No difficulty is experienced in determining to ±5 per cent even a few hundredths of a microgram of riboflavin per cubic centimeter when pure solutions are being analyzed.

TABLE I. RECOVERY EXPERIMENTS WITH PURE RIBOFLAVIN

Acidimetric Assay			Turbidimetric Assay		
Riboflavin present	Riboflavin found	Recovery	Riboflavin present	Riboflavin found	Recovery
Micrograms/cc.	*Micrograms/cc.*	%	*Micrograms/cc.*	*Micrograms/cc.*	%
0.0198	0.0201	101.5	0.013	0.013	100
0.071	0.074	104.2	0.064	0.063	98.4
0.252	0.251	99.6	0.236	0.225	95.5
0.672	0.685	101.9	0.55	0.59	107.0
2.20	2.10	95.5	1.97	2.00	101.5

Applications to Natural Products

For extraction of riboflavin from natural products autoclaving the finely divided material at 1 kg. per sq. cm. (15 lb. per sq. in.) pressure for 15 minutes with a large volume of water is recommended. Extraction with boiling 0.1 *N* hydrochloric acid and subsequent neutralization are also effective. In the latter case not more than 50 mg. of sodium chloride should be added to the assay tubes with the aliquot to be analyzed. Amounts greater than this first increase, then decrease the response of the test organism to a given quantity of riboflavin. It was demonstrated in separate experiments that the biological potency of a dilute solution of riboflavin in 0.1 *N* hydrochloric acid was not detectably affected by autoclaving at 1 kg. per sq. cm. (15 lb. per sq. in.) pressure for 30 minutes.

Several lines of evidence have been used to guard against possible interfering substances:

Assays are made at different levels, and only those assays which give concordant results at the different levels are regarded as reliable.

Recoveries of known quantities of pure riboflavin in the presence of the natural material have been made.

TABLE II. RIBOFLAVIN ASSAYS ON MILK AND LIVER

Sample	Amount Assayed[a]	Riboflavin Added per Gram Sample	Riboflavin Found Per tube	Riboflavin Found Per gram sample	Recovery of Added Riboflavin
	Mg.	*Micrograms*	*Microgram*	*Micrograms*	%
Milk A	50	None	0.097	1.94	
	60	None	0.126	2.10	
	75	None	0.146	1.95	
				Av. 2.00	
	17	1.76	0.062	3.64	
	25.5	1.76	0.094	3.68	
	34	1.76	0.132	3.88	
				Av. 3.70	97
Milk B	50	None	0.081	1.61	
	60	None	0.100	1.68	
	75	None	0.136	1.81	
				Av. 1.69	
	17	1.76	0.061	3.58	
	25.5	1.76	0.092	3.60	
	34.0	1.76	0.124	3.65	
				Av. 3.61	109
Fresh beef liver	2.01	None	0.082	41.0	
	3.02	None	0.134	44.6	
	4.02	None	0.172	42.8	
	6.03	None	0.241	40.2	
				Av. 42.2	
	1.02	24.4	0.070	68.6	
	1.54	24.4	0.100	65.0	
	2.04	24.4	0.146	71.5	
	3.08	24.4	0.214	69.5	
				Av. 68.6	108
Photolyzed milk serum	100	None	0.00	0.00	
	200	None	0.00	0.00	
	200	0.50	0.10	0.50	100
	200	0.75	0.15	0.75	100
Photolyzed liver extract	2.4	None	0.00	0.00	
	2.4	41.6	0.095	39.6	95
	2.4	62.5	0.148	61.6	98.7
	3.2	None	0.00	0.00	
	3.2	31.3	0.100	31.3	100
	3.2	46.9	0.155	48.5	103

[a] Expressed in terms of weight of the original sample.

The riboflavin in various extracts has been destroyed by the least drastic and most selective method available—namely, by the action of visible light on the extract at pH approximately 7.0—and recoveries of pure riboflavin in the presence of the photolyzed material have been made.

Assays have been made on samples which have also been assayed for riboflavin by other biological methods, and the results compared.

The sensitivity of the organism to inhibition by various reagents has been investigated.

Most of this evidence was collected in connection with assays on samples of milk, liver, and yeast.

The data presented in Table II indicate that the apparent riboflavin contents of milk and liver samples as determined at different levels were satisfactorily constant, and that added riboflavin was recovered with an error of 10 per cent or less. The milk was diluted with distilled water and aliquots equivalent to the amounts indicated were added directly to the assay tubes. The milk serum was prepared by making the milks lightly acid with acetic acid, warming briefly to 80° to 90° C. and filtering. The clear serum was photolyzed by readjusting to pH 7.0, adding a few drops of chloroform as a preservative, and exposing a 0.5-cm. layer of the solution for 24 hours at room temperature (25° or less) to the light from a 100-watt bulb with reflector at a distance of 25 cm. Sufficient water approximately to balance evaporation was added during the photolysis, and the final solution was diluted accurately to the original volume.

The liver was ground and divided into two parts and the riboflavin to be recovered was mixed into one portion. The samples were then extracted by boiling for 5 minutes with 3 volumes of 0.1 *N* hydrochloric acid, and the residues were centrifuged off and re-extracted twice by the same procedure.

The turbid extracts were neutralized and suitable aliquots taken for assay. The photolysis was carried out as above described on a portion of the neutralized extract from which insoluble matter had been removed by centrifuging.

The results of microbiological and rat assays on the same samples of various materials are summarized in Table III. Extracts for the bacterial assay were prepared by autoclaving

TABLE III. COMPARATIVE RIBOFLAVIN ASSAYS OF NATURAL PRODUCTS

Material	Riboflavin Content[a] Microbiological Assay Direct	Microbiological Assay Extract	Rat Assay	
	Micrograms	*Micrograms*	*Micrograms*	*Bourquin-Sherman units*
Dried grasses				
Sample I	...	24.4	20.0	..
Sample II	...	24.1	25.0	..
Sample III	...	22.6	22.0	..
Sample IV	...	23.9	20.0	..
Dried oat plant	30.3	31.2	35.0	..
Skim-milk powder	17.1	..	17.0	..
Debittered yeast *a*	39.2	38.5	37	..
Debittered yeast *b*	...	36.5	(38.8)	17.7
Debittered yeast *c*	34.7	33.6	(34.4)	15.7
Yeast extract	149.6	..	(145.6)	66.5
Nondebittered yeast	31.8	32.8	(32.0)	14.6

[a] Per gram dry weight.

the weighed sample with 100 times its weight of water at 1 kg. per sq. cm. (15 lb. per sq. in.) pressure for 15 minutes. The extract and residue were then diluted to a volume of 100 cc. for each gram of sample taken, solid material was removed by centrifugation, and an aliquot was assayed for the riboflavin content. In some cases, as indicated in Table III, the suspension was assayed directly. The average value for the Bourquin-Sherman unit calculated from these data is 2.19 micrograms per unit. The values for the riboflavin content of yeast samples given in parentheses in column four were calculated from the Bourquin-Sherman units with this factor.

The data presented in Table IV indicate roughly the sensitivity of *L. casei* to a number of miscellaneous materials. These results indicate that the organism is resistant to a variety of toxic materials in concentrations higher than those ordinarily encountered in biological extracts.

TABLE IV. SENSITIVITY OF *Lactobacillus casei* TO INHIBITION BY VARIOUS SUBSTANCES

Substance Tested	Concentration	Extent of Inhibition
	Micrograms/cc.	
Cu^{++}[a]	40	Complete
Cu^{++}	20	Just noticeable
Mn^{++}	40	None
Fe^{+++}	40	None
Zn^{++}	40	None
Ba^{++}	50	Just noticeable
Pyridine	500	None
Ethanol	2,000	None
Methanol	2,000	None
KCl	1,000	None
NaCl	20,000	None

[a] Cu, Mn, Fe, and Zn were tested in the form of the sulfates; Ba as the chloride.

Riboflavin Content of Various Biological Materials

The riboflavin content of several natural products as determined by the present method is compared in Table V with values given by other investigators for similar products. Where several samples have been analyzed, the range obtained is given. Assays on milk, milk serum, skim-milk powder, dried whey, egg white, and egg yolk were made directly. A hydrochloric acid extract of the beef liver and soybean meal was assayed, while the extract for assay of the dried pork liver was obtained by autoclaving with water and centrifuging off the insoluble residue.

TABLE V. RIBOFLAVIN CONTENT OF BIOLOGICAL MATERIALS

Material	Microbiological Assay	Literature Values	Method of Assay	Reference
	Micrograms per gram			
Beef liver, fresh	44.0	35.6	Colorimetric	*4*
		15–17	Photometric	*13*
Rat liver, fresh	34.0	15–17	Photometric	*13*
Pork liver, vacuum-dried	92–104	100	Chick	*15*
Liver meal, commercial	50.0	...	.....	..
Whole milk	1.5–2.5	1.7–2.4	Rat	*12*
		1.0–1.5	Fluorimetric	*10*
Milk serum	1.3–1.9	...	.....	..
Dried whey	23	30	Chick	*15*
Skim-milk powder	17	20	Chick	*15*
Soybean meal	3.0	3.0	Chick	*15*
Egg white, fresh	3.1	4–5	Colorimetric	*6*
Egg yolk, fresh	7.6	5–6	Colorimetric	*6*

Constancy of Assay Results

The degree of constancy which may be expected when assays are run on the same product at different times is indicated in Table VI. The second assay was made 2 weeks after the first. The maximum difference is about 8 per cent.

TABLE VI. CONSTANCY OF ASSAY RESULTS

Sample	Riboflavin Content Assay 1	Assay 2
	Micrograms per gram	
Beef liver, fresh	42.2	45.7
Rat liver *a*, fresh	33.0	31.2
Rat liver *b*, fresh	36.9	34.3
Skim-milk powder	17.0	17.2

Discussion

RELIABILITY OF THE METHOD. From the results reported in this and previous papers it is evident that riboflavin is indispensable for growth of *L. casei*. A growth response caused by the addition of any material to the riboflavin-free basal medium may therefore be regarded as a reliable qualitative indication of the presence of riboflavin in that material. The high degree of structural specificity required for activity (*16*), the identity of all flavins so far isolated from natural sources (*11*), and the complete inactivity of photolyzed extracts of biological materials (cf. Table II) strongly support this view.

While it has unfortunately not been possible as yet to test such substances as riboflavin phosphate and Warburg's flavin-adenine dinucleotide (*19*), it is very probable that the bacteria respond not only to free but also to the various combined forms of riboflavin. Thus several materials, such as yeast and liver, which are known to contain most of their riboflavin in combined forms, give values by the bacterial assay which are as high or higher than those found by several other methods.

The reliability of the response as a quantitative measure of riboflavin and riboflavin only is more difficult to establish, since inhibition or stimulation of the organism by substances introduced with the sample to be assayed is possible. The question of inhibition seems to be satisfactorily settled by the data in Table IV, by the successful recovery of pure riboflavin added to the sample, and particularly by the recovery of riboflavin in the presence of photolyzed extracts, which would presumably still contain any inhibitory substances originally present.

Stimulation of the organism, which would produce falsely high values for riboflavin, might conceivably result from the presence of food materials or of unknown growth factors in the sample. The former possibility seems remote in view of analyses of known amounts of riboflavin which were carried out with a medium containing twice the usual amount of

glucose and peptone. The apparent riboflavin content was increased less than 10 per cent by the extra food materials available to the organism.

Since the addition of a single pure substance, riboflavin, to the basal medium permits growth through repeated subculture (*16*), it follows that the medium so supplemented contains all the accessory growth factors essential for *L. casei*. The possible existence of other nonessential but stimulatory substances which are not supplied with the yeast supplement cannot be overlooked. The assumption has been made in the above work that the effect on growth of such stimulatory factors will be negligible in a medium which contains suboptimal quantities of an essential growth factor (riboflavin). The validity of this assumption is supported by the quantitative recovery of pure riboflavin in the photolyzed and unphotolyzed extracts as described above. In each case the amount of photolyzed material used was comparable to the amount of the original sample required for the assay. The destruction of stimulatory substances other than riboflavin during photolysis, although possible, appears unlikely in view of the mild conditions employed.

The comparative assays summarized in Table III offer additional support for the reliability of the bacterial method, although the questionable accuracy of the rat assay makes it difficult to evaluate such evidence. Few critical tests of the specificity of the rat or chick assay method for riboflavin have been made.

Sources of Error. The presence in the assay tube of large amounts of solid material from the substance being assayed affects the assay adversely. Thus when recoveries of pure riboflavin were attempted in the presence of the residue from the hydrochloric acid extraction of liver described above the results were high and variable, although the residue alone showed a riboflavin content of zero. Similarly, if substances naturally low in riboflavin, such as the cereal grains, are added directly (after grinding) to the medium for assay, the riboflavin contents calculated from the different levels do not agree. Thus in these cases one must resort to extraction. Obviously, too, the smaller the riboflavin content of a substance, the more extract must be added in order to obtain sufficient riboflavin to fall within the specified range, with the consequent addition of larger amounts of extraneous substances which may affect the assay result. In cases, therefore, where assays at different levels do not check closely enough, or where recoveries of added riboflavin are seriously in error, further purification of the extract assayed is indicated.

On the other hand, incorporation of inert solid material such as Filtercel in the medium did not affect the recovery of added riboflavin. Similarly, the small amount of solid material added in the assay of milk and milk products apparently did not interfere, since accurate recoveries of added riboflavin were obtained in the presence of such material.

The method requires the maintenance of a pure culture of *L. casei*. Contamination of the assay tubes with other microorganisms produces false results through riboflavin synthesis and associated growth phenomena.

Usefulness of the Method. The assay is applicable to crude extracts or in some cases to the whole ground sample, so that extensive preliminary purification with attendant losses of riboflavin is unnecessary. It requires very small amounts of sample, no elaborate or unusual equipment, and is relatively rapid. Routine analyses now under way in this laboratory have shown that one operator can conveniently carry out 15 to 20 assays per working day. The accuracy of the method is of the order of ±10 per cent.

Summary

A biological assay for riboflavin, which is based on the essential nature of this substance for the growth of *Lactobacillus casei*, is described. The reliability of the method is supported by agreement of the assay results at different levels, recovery of added riboflavin, successful determination of riboflavin in the presence of photolyzed extracts, and specificity of structure required for activity.

The results compare well with other bioassays for riboflavin on the same products. The method is rapid, and requires only very small amounts of sample.

Acknowledgment

The authors wish to thank Dr. Levene of the Premier-Pabst Corporation, Milwaukee, for supplying the yeast samples with the rat assay results given in Table III. The rat assays on the other samples were carried out by Professor Elvehjem and Mr. Wagner, to whom the authors also wish to express their thanks.

Literature Cited

(1) Bender, R. C., and Supplee, G. C., *J. Am. Chem. Soc.*, 59, 1178 (1937).
(2) Bessey, O. A., *J. Nutrition*, 15, 11 (1938).
(3) Bourquin, A., and Sherman, H. C., *J. Am. Chem. Soc.*, 53, 3501 (1931).
(4) Charite, A. J., and Khaustov, N., *Biochem. J.*, 29, 34 (1935).
(5) Ellinger, P., *Ibid.*, 32, 376 (1938).
(6) Euler, H. v., Adler, E., and Schlötzer, A., *Z. physiol. Chem.*, 226, 87 (1934).
(7) Evelyn, K. A., *J. Biol. Chem.*, 115, 63 (1936).
(8) Freudenreich, E., and Thöni, T., *Landw. Jahrb. Schweiz.*, 18, 526 (1904).
(9) Hawk, P. B., Bergeim, O., Oser, B. L., and Cole, A. G., "Practical Physiological Chemistry", 11th ed., p. 902, Philadelphia, P. Blakiston's Son & Co., 1937.
(10) Houston, J., Kon, S. K., and Henry, K. M., *Chemistry & Industry*, 57, 276 (1938).
(11) Karrer, P., *Helv. chim. Acta*, 19, E41 (1936).
(12) Kramer, M. M., Gardner, I., Kunerth, B. L., and Riddell, W. H., *J. Agr. Research*, 56, 233 (1938).
(13) Kuhn, R., Kaltschmitt, H., and Wagner-Jauregg, T., *Z. physiol. Chem.*, 232, 36 (1935).
(14) Lepkovsky, S., Taylor, L. W., and Almquist, H. J., *Hilgardia*, 11, 563 (1938).
(15) Norris, L. C., Wilgus, H. S., Ringrose, A. T., Heiman, V., and Heusser, G. F., Cornell Univ., *Agr. Expt. Sta. Bull.* 660 (1936).
(16) Snell, E. E., and Strong, F. M., *Enzymologia* (in press).
(17) Snell, E. E., Strong, F. M., and Peterson, W. H., *Biochem. J.*, 31, 1789 (1937).
(18) Snell, E. E., Strong, F. M., and Peterson, W. H., *J. Am. Chem. Soc.*, 60, 2825 (1938).
(19) Warburg, O., and Christian, W., *Biochem. Z.*, 298, 150 (1938).

Presented before the Division of Biological Chemistry at the 96th Meeting of the American Chemical Society, Milwaukee, Wis. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the Research Fund of the University.

Correction. In the article on "Analytical Balances in Quantitative Microanalysis" [Ind. Eng. Chem., Anal. Ed., 11, 226 (1939)] the following corrections should be made:

In Table I $\frac{\text{"100"}}{P}$ should read $\frac{\text{"1000 } f\text{"}}{P}$.

In Equation 9, substitute "P" for "M".

The last two items in Table II should read as follows:

	G./l.	*f*	*Cu. mm.*	*Cu. mm.*
Cl as AgCl in sea water	20	0.247	75	190
P as phosphomolybdate in serum	0.2	0.016	480	1200

A. A. Benedetti-Pichler

A Modified Buret for Microanalysis of Gases

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IN USING the method for the microanalysis of gases described by Blacet and Leighton (*1*), the author experienced considerable difficulty because of the tendency of the mercury levels to drift up or down the capillary buret during the time required to take the readings. A method of avoiding this difficulty has been described by Blacet, MacDonald, and Leighton (*2*), but an alternative method, used in this laboratory, has proved to have valuable additional advantages.

The drift in the mercury levels is evidently due to the fact that a small displacement of the upper level downward, for example, causes a marked increase in the pressure of the gas and of the pressure of the mercury in the rubber pouch at the base of the buret. This increased pressure makes the gas contract and the rubber pouch distend, both of which cause the upper mercury level to move downward again. If the rubber is not sufficiently stiff, the sample of gas may even be lost into the base of the buret in this manner.

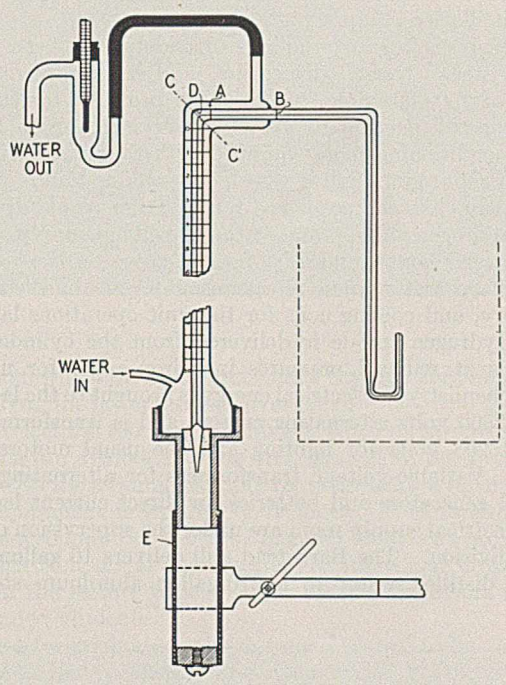


FIGURE 1. DIAGRAM OF BURET

This difficulty may be readily overcome by maintaining the upper mercury level in a horizontal rather than a vertical capillary tube. The same principle has been employed in a different manner in an apparatus described by Swearingen, Gerbes, and Ellis (*3*).

A simple method which does not occupy an undue amount of space is shown in Figure 1, which is similar to the buret described by Blacet and Leighton except that the water jacket now follows the capillary tube up and over the bend at the top. The water jacket is made from a Pyrex buret sealed at the two ends with picene wax (not with de Khotinsky cement, since this slowly disintegrates under water). The capillary tube which runs through the buret is placed near the front surface of the buret, rather than centered, so that the lower mercury level can be read from the buret graduations without danger of serious parallax errors. Two lines, *A* and *B* in Figure 1, are marked on the horizontal portion of the capillary, one close to the bend, the other close to the end of the water jacket. It is convenient to choose positions for these marks such that the volume of the capillary between the marks and the zero of the buret is an integral number of the units of volume employed. This may be readily accomplished if the apparatus is calibrated after assembly but before the horizontal arm of the water jacket is sealed on at *C-C'*.

Readings are taken by drawing the gas into the buret in the manner described by Blacet and Leighton and adjusting the upper level to coincide with *A* or *B*. The lower mercury level is read from the graduations on the buret. Water from a large vessel is circulated through the water jacket. Since its temperature never deviates largely from room temperature, no appreciable error arises from the fact that a small fraction of the gas extends beyond the end of the water jacket when *B* is used. This arrangement has been adopted to relieve the operator from eyestrain during the adjustments. For measuring very small volumes of gas (less than about 20 cu. mm.) *A* must, of course, be used. The temperature of the water in the water jacket is conveniently read from the thermometer arranged as in Figure 1.

In a buret of this type the mercury shows no tendency whatsoever to drift in the manner described. If the mercury in the movable cup (shown by dotted lines) is always brought to a fixed level when a reading is to be made, the pressure on the sample of gas will be the same in consecutive readings unless large barometric changes have occurred. The time required for most analyses is short enough to permit one to ignore entirely all pressure corrections, which is a considerable simplification over the procedure ordinarily followed.

A marked advantage of this type of buret is that it can be cleaned without being dismantled. The "scum" carried into the buret is carried by the upper mercury thread, and since this is not ordinarily drawn into the vertical portion of the capillary tube, only the horizontal portion requires cleaning. To clean the buret the mercury cup is removed and a beaker containing a cleaning solution of potassium dichromate and concentrated sulfuric acid is put in its place. Before the tip of the buret is submerged in the solution, however, the mercury in the buret is drawn back until the level of the thread is about 10 cm. below the bend, *D*. The cleaning solution is then drawn into the buret up to *D* but no farther. After several hours the acid is expelled and distilled water is drawn up in its place. The buret is dried after several such rinsings by evacuating the capillary, an operation which must be done with some care to avoid drawing mercury into the horizontal tube.

Minor advantages of the buret here described are that only one reading is taken from the calibration curve for each measurement and that the volume may be recorded directly without taking differences.

Summary

A modification of the Blacet-Leighton gas microburet is described. The mercury threads are easily adjusted and do not drift from their set positions. Corrections for pressure are not ordinarily required. The buret may be cleaned without dismantling.

Literature Cited

(1) Blacet and Leighton, IND. ENG. CHEM., Anal Ed., 3, 266 (1931).
(2) Blacet, MacDonald, and Leighton, *Ibid.*, 5, 272 (1933).
(3) Swearingen, Gerbes, and Ellis, *Ibid.*, 5, 369 (1933).

MODERN LABORATORIES

BAGLEY HALL, UNIVERSITY OF WASHINGTON

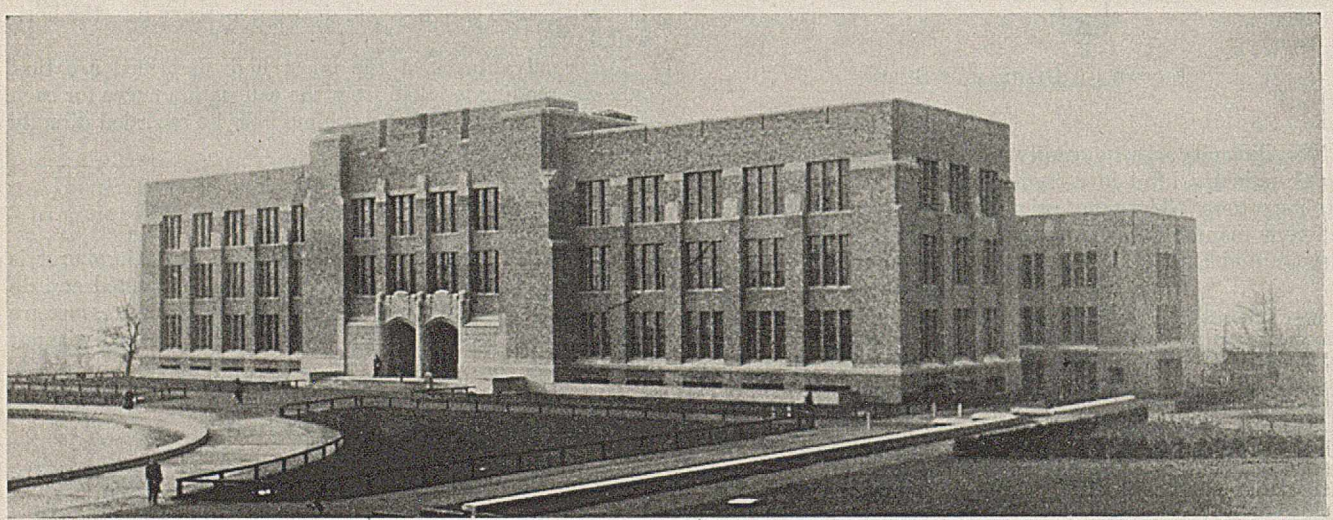
W. L. BEUSCHLEIN, University of Washington, Seattle, Wash.

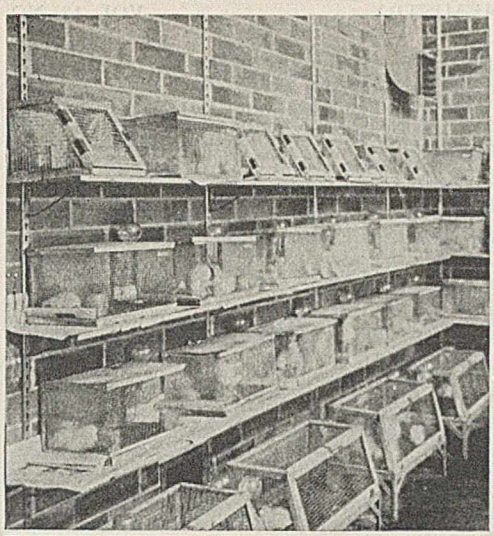
DANIEL Bagley Hall at the University of Washington houses the Department of Chemistry and Chemical Engineering and the College of Pharmacy. Funds for the construction, amounting to $1,250,000, were supplied by state and federal grants. The structure has three and one-half floors with four wings, the over-all dimensions being 260 × 380 feet. Approximately one acre of ground area is covered, the total volume being 2,400,000 cubic feet. The cost has been estimated at 52 cents per cubic foot.

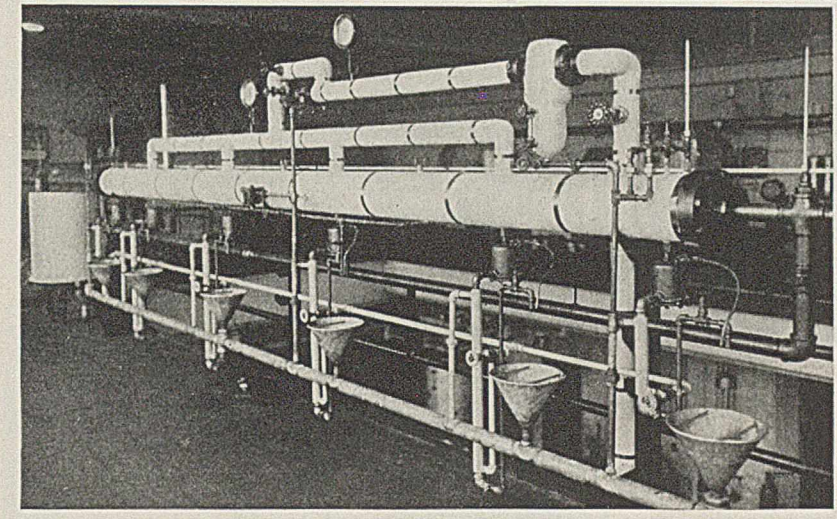
A study of the requirements of a new building under the supervision of S. G. Powell and the author had provided fundamental designs of general laboratory space when funds were allocated for construction. All designs, plans, and acceptances of bids were completed within the ninety days specified under terms of the federal grant. The information presented in the publication of the National Research Council, "Construction and Equipment of Chemical Laboratories", was of great value, as was also a joint report by Carl F. Gould and H. K. Benson on the inspection of eleven recently constructed laboratories in the United States. Special assistance was rendered by Professor Hoover of Wesleyan University and Dr. Coleman of Mellon Institute. The plans and specifications were prepared by F. A. Naramore and Granger and Thomas, associate architects.

THE building is of reinforced concrete construction and surfaced outside with brick. Partitions are of hollow tile, and those forming laboratory walls are to remain unpainted. All laboratories have access to natural lighting, the inner volume of the structure being utilized for corridors, lecture rooms, and storage. A subbasement contains the mechanical equipment, such as air washers and conditioners, ventilation fans, heaters, steam distributors, and reducing valves. The space above the third floor is used for fume ducts, fans, and water-distillation equipment.

The areas enclosed by the inner corridors have been given over to stock storage, dispensing, and lecture rooms. A volume of 150,000 cubic feet has been provided for general storage and dispensing rooms. The service division has a small reconditioning room equipped with the usual facilities, including paint gun, buffing wheel, and soaking tanks. That division also has charge of the refrigeration plant, distilled water, hydrogen sulfide, and variable voltage supply. The refrigeration plant supplies ice for the boxes on the various floors, sharp and normal refrigeration to the biochemistry laboratory, and cooling coils for the unit operations laboratory. Hydrogen sulfide is delivered from the cylinders to manifolds at reduced pressures into iron pipes for use in general chemistry. Electrical energy is brought to the laboratory at 2300 volts alternating current and is transformed to 120 and 208 volts for lighting and the usual motors and heaters. Variable-voltage transformers for alternating current and generators and batteries for direct current located in the electrical supply room are under the supervision of the service division. The Barnstead still delivers 10 gallons per hour of distilled water to a 270-gallon aluminum storage







Upper left. One of Four Animal Rooms. Keyhole Standards Used for Shelving

Upper right. Typical Research Laboratory

Left. Unit Operations Laboratory. Steam to Liquid Heat Exchange

tank from which distribution is made through aluminum pipe and valves, the latter having silver seats.

Eight lecture rooms containing a total of approximately 840 seats are available. The arrangement of the two larger rooms with the adjoining supply room for lecture table experiment equipment is working very nicely. The large lecture hall has a soundproof booth with sound and silent projectors, multiplex controls at the lecturer's station, and down-draft hood on the lecture bench; 3 per cent of the chairs are for left-handed students.

Rooms for graduate research were designed to be occupied by two to four persons. The distribution of these rooms about the building has permitted an economical use of floor space. Facilities available in such rooms include compressed air, oxygen, 15 kw. of 120- to 208-volt 60-cycle alternating current, variable-voltage (0 to 400) alternating and (0 to 150) direct currents, in addition to hot and cold water, gas, steam, fume hood, and fire protection. Vibrationless piers have been installed in some rooms.

Some interesting features were developed in the arrangement of the analytical laboratories. A centrally located room equipped with ball mills, power-driven sieves, and dividers furnishes space for the preparation, storage, and indexing of the 63,000 unknowns carried by this division. The wide corridor connecting the two main analytical laboratories is used for the work in electrolysis. Each laboratory is provided with a Kjeldahl room of 144 digestion capacity.

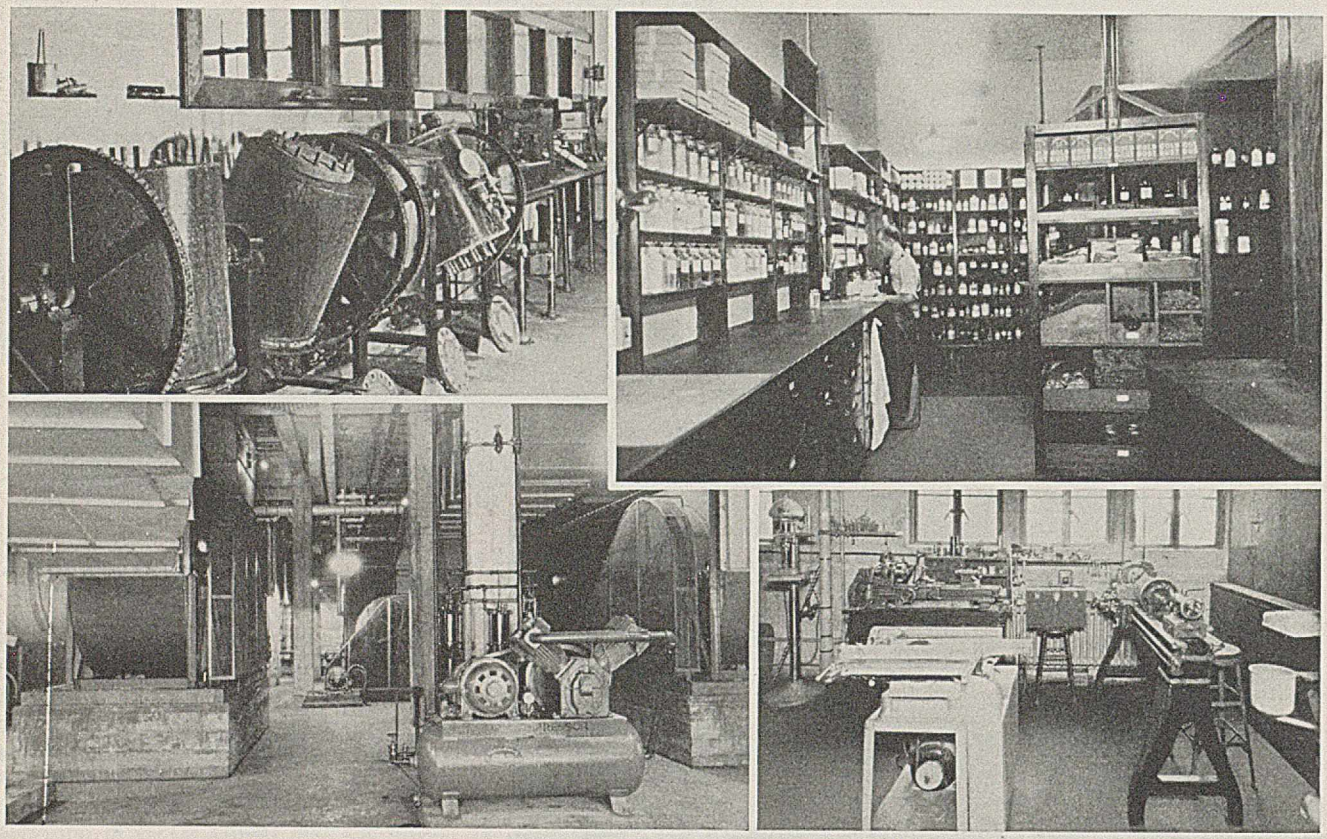
The chemical engineering laboratories contain small- and large-scale equipment for the study of unit operations, unit processes, and technical analysis of industrial materials. The primary services in these rooms furnish steam up to pressures of 175 pounds per square inch, 3-phase 60-cycle alternating current up to 400 amperes at 208 volts, variable-voltage direct current up to 150 amperes, gas, water, compressed air, and fume ducts, so that semiplant equipment can be operated. A machine shop is operated in conjunction with this division.

The laboratory for unit operations has space for such tall equipment as absorption towers, fractionating columns, and

Laboratory Space Distribution

	Maximum Student Capacity	Total Floor Area *Sq. Ft.*
Ground or First Floor		
Physical chemistry	144	2050
Advanced physical chemistry	12	700
Electrochemistry	45	770
Industrial chemistry	84	1650
Unit processes	72[a]	1800
Unit operations	80[a]	5400
Chemical engineering undergraduate research	63	880
Graduate research	56	4655
Total	556	
Main or Second Floor		
Biochemistry	108	1030
Advanced biochemistry	10	450
Microchemistry	30	590
Quantitative chemistry	240	3600
Advanced quantitative chemistry	36	1000
Library	48	1800
Graduate research	24	3000
Total	496	
Third Floor		
Inorganic and general chemistry	2563	8000
Advanced inorganic chemistry	10	520
Organic chemistry	429	4450
Advanced organic chemistry	10	750
Graduate research	25	2500
Total	3037	

[a] Based on six hours per week per student.



Upper left. Battery of Digesters in Wood Pulp Laboratory. *Upper right.* One of Four Rooms in Dispensing Service. *Lower left.* Ventilation and Mechanical Room. *Lower right.* Corner of Machine Shop

evaporators. Equipment for the study of crushing, grinding, and separation of solids is located in a small adjacent room for the localization of any dust problems. Other noteworthy features are positive floor drainage, equipment anchors along the walls, chimney flue through the roof, an ice machine for supplying refrigeration to equipment, and a 5-ton overhead traveling crane. The floors or ceilings over this laboratory have been fitted with cored holes each of approximately 1 square foot area, so that apparatus requiring 60 feet of elevation may be installed.

The importance of the pulp and paper industry in the Pacific Northwest and the need for adequate training of graduates entering that field led to the inclusion of a series of laboratories specially equipped for class work and research in this field. A small digester room contains a waste gutter, a special chimney flue with individual fan to remove relief gases, four rotating digesters for sulfite and alkaline cooking, a semiplant stainless-steel digester with external heating and circulation; also necessary washers, screens, beater, bleacher, thickener, and test equipment for the pulp produced.

Situated between the pulp laboratory and the unit process laboratory is the control laboratory where chemical control tests are performed in the semiplant processes in chemical industry; the student may operate these on a small scale.

A number of special laboratories are provided. A large laboratory for research in unit operations and high-pressure reactions adjoins the unit operations laboratory and has large service lines, floor gutter, keyhole equipment, anchor standards, and other features. A high-pressure compressor furnishes compressed gases for hydrogenation and studies in high-pressure operations. A large-capacity blower furnishes air for submerged combustion investigations. Another laboratory contains space for sixty undergraduate students for senior thesis work. The control laboratory is designed for use by students doing research on pulp and paper, or unit process problems. Many small laboratories for individual students are available for carrying on advanced problems.

THE mechanical equipment of the building is necessarily extensive and intricate. Dictates of economy and maintenance resulted in exposed piping on ceilings with most vertical work concealed in shafts. Ventilation of the entire building is by means of warmed washed air from basement fans and foul air is removed by exhaust fans in roof level rooms. Although no cooling coils have been installed, the temperature of the water used in washing the air and the general climate of the region assure a definite lower differential within the laboratories on the hottest summer days.

The following are required for the operation of the building:

Plenum fresh air, cubic feet per minute	18,500
Total air supply, cubic feet per hour	9,000,000
Hood exhaust fans	46
Transformer room, cubic feet	10,000

Lighting intensity: 15 foot-candles over all laboratory tables and 20 foot-candles in lecture rooms.

Fume exhaust system: vitrified tile pipe.

Waste lines: extra heavy cast iron with ceramic tile for vertical risers in concealed work.

Steam is obtained at boiler pressure from the central plant of the university at 185 pounds and distributed in the building at 125, 15, and 2 pounds.

Black enamel cast-iron sinks are used throughout. These are fitted with Corrosiron plugs and tailpieces to the waste lines.

Laboratory benches were designed by the separate divisions and constructed locally. Birch tops of 2-inch stock and fir frames were selected. Gutters were entirely eliminated, each student having access to a sink. The student capacity of the General Chemistry Laboratories was increased 300 per cent by replacing the drawer and locker type of desk by that of the single drawer.

New

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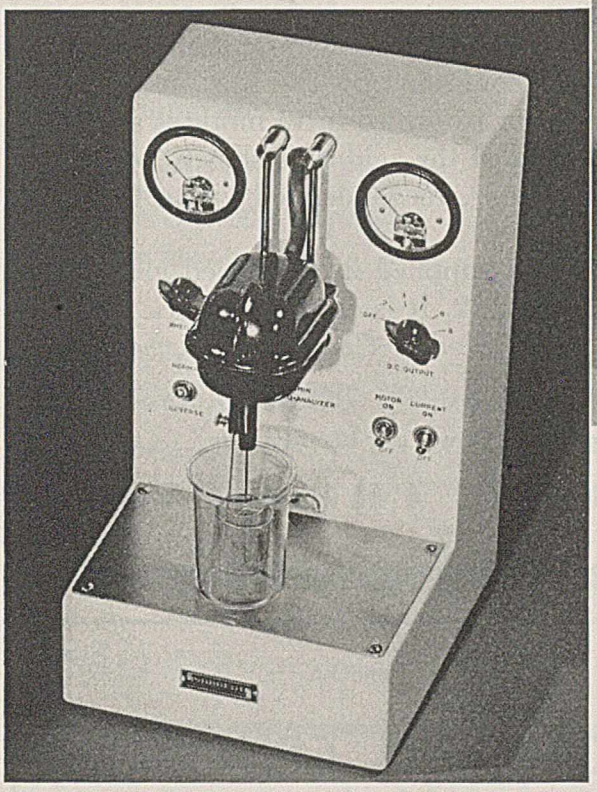

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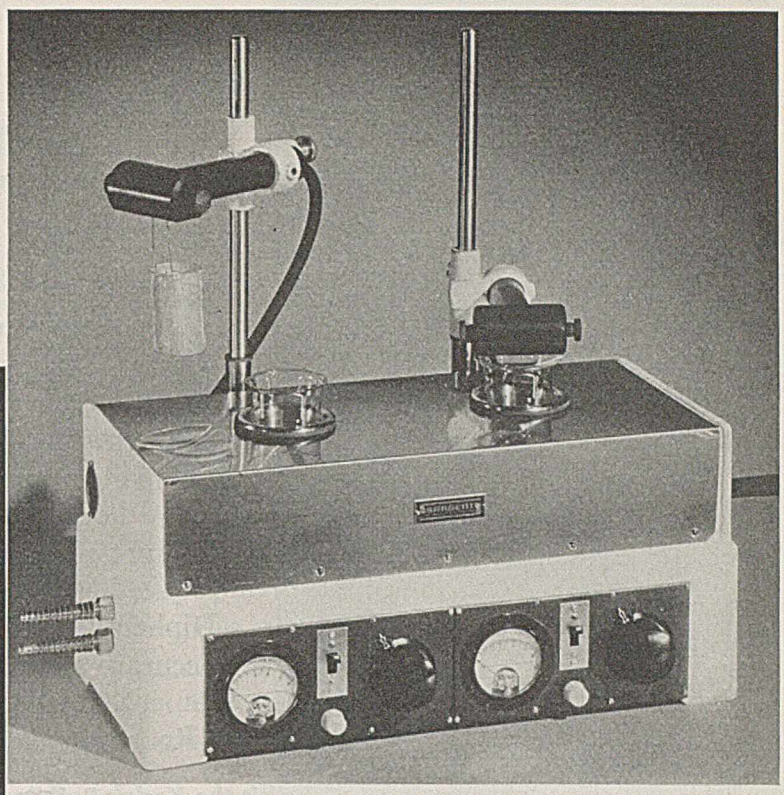

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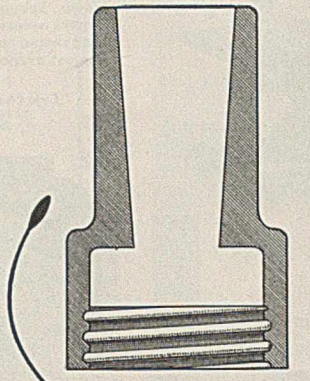

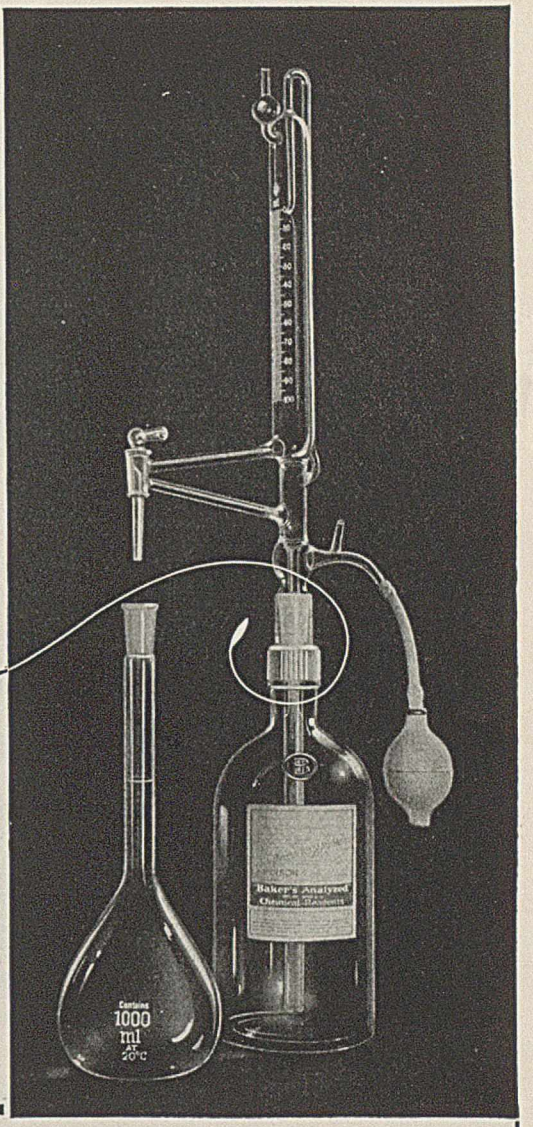

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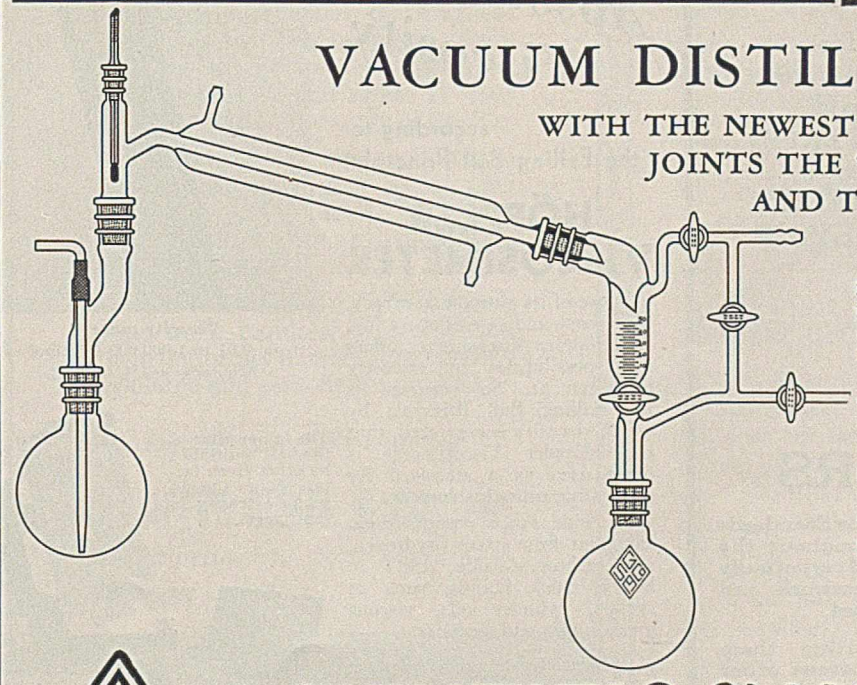

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