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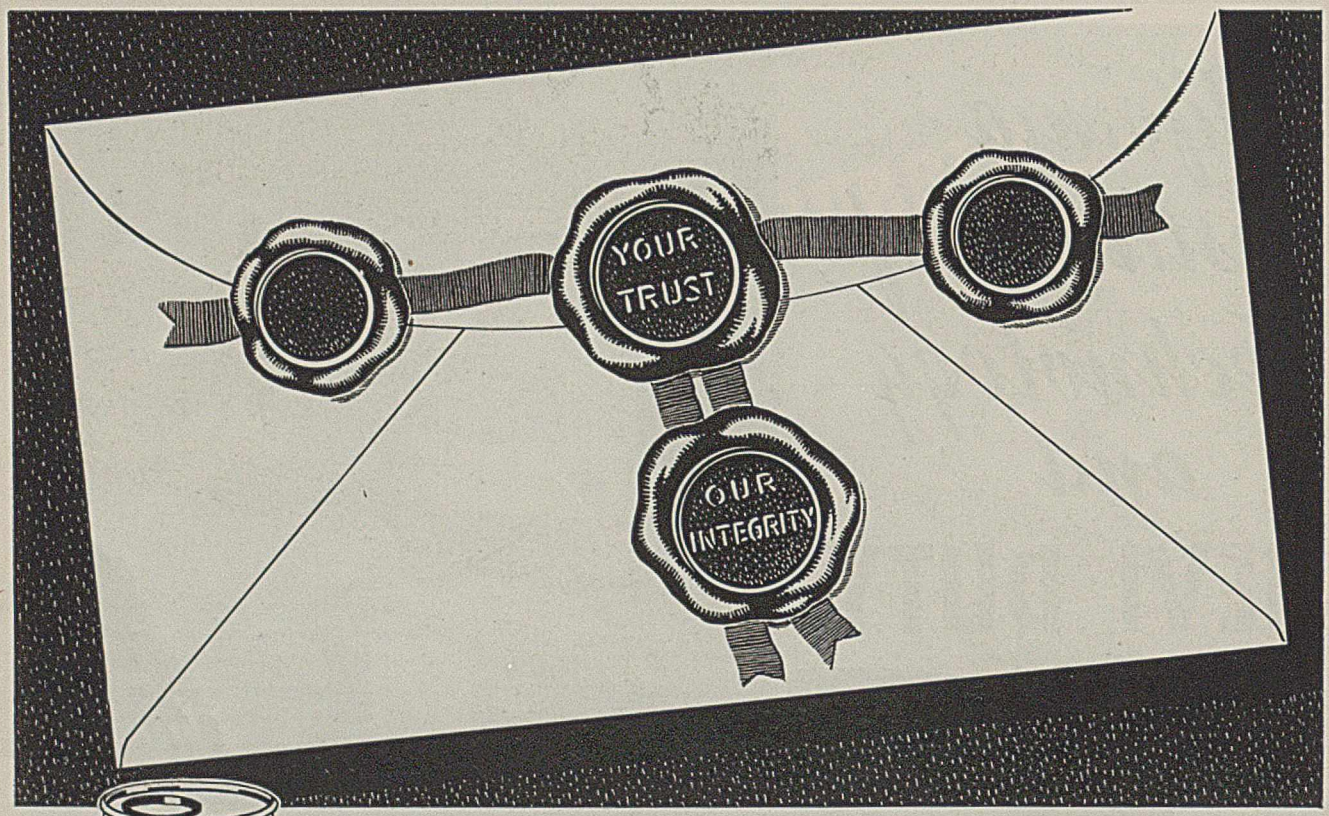

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Organic Halogen Compounds in Mineral Oils

Detection, Determination, and Identification

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The Pure Oil Company, Chicago, Ill.

DURING the past fifteen years, and particularly during the past five years, addition of organic halogen compounds to various types of mineral oils has become a practice in the petroleum industry. These mixtures include a wide range of products, such as fireproof cleaning solvents, cutting oils, break-in oils, and special lubricants. In almost every case the halogen compound used in the mixture is a chlorine compound. Methods for the detection, determination, and identification of the organic halogen compound or compounds present in mineral oils are therefore necessary in the examination of such products.

Methods are included in the present paper for (1) the determination of total halogen (such as chlorine) in an oil, (2) the determination of the type of halogen compound present—i. e., whether the chlorine is or is not attached directly to an aromatic ring—and (3) the possible identification of the halogen compound present.

Determination of Total Halogen

A number of well-known methods have been advanced for the determination of halogen in organic compounds or in mineral oils, but these consume too much time to be used in ordinary laboratory operations. The following method, in which the hydrogen chloride formed during combustion of the mineral oil in a Parr oxygen bomb is absorbed by sodium bicarbonate solution (in the bomb), which is in turn titrated with standard silver nitrate solution by either the Mohr or Volhard procedure, is rapid and accurate and has given excellent results over a period of several years.

PROCEDURE. About 20 ml. of distilled water, in which is dissolved 0.500 gram of C. P. sodium bicarbonate, are placed in a Parr oxygen bomb of 400-ml. capacity. From 0.6 to 0.8 gram of the oil to be tested is placed in the weighed oil cup and the weight of the charge is determined to the nearest milligram. The cup is then placed in the bomb, which is previously arranged so that the iron firing wire (10 cm.) touches the oil sample when the cup is placed in position. The bomb is tightly closed and oxygen at 30 atmospheres is admitted, after which the ignition is carried out in the usual manner in a container of cold water. After ignition the bomb is allowed to cool (10 minutes) and the pressure is released at a uniform rate such that the operation requires not less than 1 minute.

The bomb is opened and the inside is examined for traces of unburned oil and sooty deposit. If either is found, the determination is discarded. If complete combustion has taken place, the interior of the bomb, including the oil cup, is rinsed with a fine jet of distilled water into a 400-ml. beaker. The washings usually do not exceed 350 ml. To the beaker is added 1 ml. of 2.5 per cent potassium chromate indicator solution and the halogen content is determined by titration with 0.05 *N* silver nitrate solution. In order to obtain a sharp end point, it is essential that the solution be viewed through bright yellow glass during the titration with silver nitrate solution. A blank determination must be carried out, in which 0.500 gram of the C. P. sodium bicarbonate, dissolved in exactly the same volume of water used in the beaker during the test, is titrated under the same conditions as when the sample was used. The halogen content is calculated in the usual manner after the quantity of silver nitrate solution used in the blank is subtracted from that used for the sample.

If the particular halogen present—bromine, chlorine, or iodine—is unknown, a second sample is burned in the bomb and the contents are examined by the usual qualitative procedure.

Typical results by the above procedure are shown in Table I.

TABLE I. DETERMINATION OF TOTAL HALOGEN IN LUBRICATING OIL

Halogen Compound Added	Halogen Calculated	Halogen Found
	%	%
n-Heptyl bromide	0.045	0.044
	0.450	0.466
n-Amyl chloride	0.333	0.324
Triphenylmethyl chloride	0.014	0.014
	0.142	0.140
Ethylene dichloride	0.069	0.067
	0.690	0 695
Benzyl chloride	0.028	0 029
	0.280	0.294
o-Dichlorobenzene	0 479	0 470
	0.048	0.050

Determination of Type of Halogen Compound Present

Procedures given in the literature and well-known qualitative organic texts are very indefinite with respect to experimental details for the determination of the type of halogen compound present. Potassium and sodium hydroxide solutions, both aqueous and alcoholic, have long been suggested as reagents for this purpose. It has been found that 1 *N* alcoholic potassium hydroxide solution when boiled 5 minutes with the sample does not react with halogen attached to an aromatic ring except in the case of iodine where a positive group such as a carboxyl or aldehyde group is in the ortho position. Practically all other organic halogen compounds are attacked by the alcoholic potassium hydroxide solution.

PROCEDURE. From 0.1 to 0.2 gram of the halogen compound or 8 cc. of mineral oil containing a halogen compound is placed in a 15-cm. (6-inch) test tube with 8 cc. of 1 *N* alcoholic potassium hydroxide solution (prepared from special potassium hydroxide containing not more than 0.002 per cent chlorine and 95

TABLE II. REACTIVITY OF ORGANIC HALOGEN COMPOUNDS

(With 1 *N* alcoholic potassium hydroxide and saturated silver nitrate solutions)

Positive Reaction with 1 *N* Alcoholic KOH		
Positive reaction with alcoholic $AgNO_3$	Negative reaction with alcoholic $AgNO_3$	Negative Reaction with 1 *N* Alcoholic KOH
Isopropyl bromide	Acetylene tetrachloride	Chlorobenzene
n-Butyl chloride	Trichloroethylene	Trichlorobenzene
Isobutyl bromide	Tetrachloroethane	*o*-Dichlorobenzene
n-Butyl bromide	Pentachloroethane	*α*-Chloronaphthalene
n-Amyl chloride	Tetrachloroethylene	*p*-Chlorodiphenyl
n-Amyl bromide		*p*-Bromoaniline
Isoamyl bromide		Chlorohydroquinone
Isoamyl chloride		Bromobenzene
n-Heptyl bromide		*α*-Bromonaphthalene
Triphenyl methyl chloride		Tetrachloronaphthalene
Benzyl chloride		*p*-Dibromobenzene
Benzyl bromide		*p*-Dichlorobenzene
Hexachloroethane		Iodobenzene
p-Phenylphenacyl bromide		
Chloroacetal		
Dichloropentane		
Chlorocyclohexane		
p-Nitrobenzyl bromide		
Butadiene tetrabromide		
Trichloroacetic acid		
Carbon tetrachloride		
Iodoform		
Ethylene dibromide		
Ethylidene bromide		
o-Iodobenzoic acid		
Slightly positive reaction with alcoholic $AgNO_3$		
Propylene dichloride		
Chlorex		
β-Phenoxy-*β'*-chloroethyl ether		
Monochloroacetic acid		
Methylene chloride		
Chloroform		
Ethylene dichloride		
1,1,2-Tribromoethane		

per cent ethyl alcohol). The test tube is placed in a steam bath for exactly 5 minutes; then 5 cc. of distilled water are added and the contents are filtered through Whatman No. 42 filter paper. The filtrate is acidified with dilute nitric acid; if turbid it is filtered through sufficient filter paper to give a clear solution. To this solution is added 0.5 cc. of 0.05 *N* silver nitrate solution and it is examined for turbidity due to silver halide. Blank tests on the reagents are carried out in the same manner. Any excess of turbidity over that given by a blank test on the reagents used indicates the presence of organic halogen not attached to an aromatic ring (except with a few iodine compounds as stated above).

The results obtained with a number of organic halogen compounds are shown in Table II. For purposes of comparison, results obtained by heating the halogen compound with a saturated solution of silver nitrate in absolute alcohol are also included.

The sensitivity of the test for the determination of organic halogen compounds in mineral oil is shown in Table III.

In case the two types of organic halogen compounds are present in the same sample of mineral oil, the halogen not attached to an aromatic ring may usually be removed from the oil by continued refluxing with 1 *N* alcoholic potassium hydroxide until the halogen content of the mineral oil decreases to a constant value, this latter value indicating the amount of halogen attached directly to an aromatic ring.

TABLE III. SENSITIVITY OF TEST FOR HALOGEN IN LUBRICATING OIL

(Where halogen is not attached to an aromatic ring)

Compound Present	Minimum Halogen Detected by Test %	Compound Present	Minimum Halogen Detected by Test %
n-Amyl chloride	0.002	Hexachloroethane	0.005
n-Heptyl bromide	0.002	Trichloroethylene	0.004
Benzyl chloride	0.001	Pentachloroethane	0.004
Chloroform	0.005	Ethylene dichloride	0.004
Carbon tetrachloride	0.005	Monochloroacetic acid	0.002
Triphenylmethyl chloride	0.001	Trichloroacetic acid	0.003
		Chlorex	0.003

Identification of Halogen Compound Present

Mineral oils containing halogen compounds may be distilled *in vacuo*, the halogen compounds usually being concentrated in one or more of the fractions obtained. Upon examining the results obtained by the methods mentioned above and the physical constants of the fractions, the halogen compound present usually is readily identified and a derivative may be prepared if desired. This examination of the fractions is time-consuming and may be replaced in many instances by a simple procedure by which the halogen compound may be extracted from the mineral oil as a thiuronium salt and identified by means of the corresponding thiuronium picrate.

PROCEDURE. One hundred grams of mineral oil and 50 cc. of C. P. benzene are refluxed for 2 hours with 10 cc. of 95 per cent ethyl alcohol containing 1 gram of thiourea. The alcohol layer is separated and the oil extracted with 25 cc. of hot 95 per cent alcohol, after which the two alcohol solutions are combined and evaporated to dryness on a steam bath. The resulting thiuronium salt is washed with cold hexane to remove traces of oil, then dissolved in 10 cc. of 95 per cent alcohol, and 1 gram of picric acid is added. The mixture is heated on the steam bath until complete solution is attained, then allowed to cool slowly. The thiuronium picrate is filtered off, washed with 5 cc. of cold 95 per cent alcohol, then repeatedly recrystallized from 95 per cent alcohol until the melting point becomes constant. Usually two recrystallizations are sufficient.

Table IV shows the melting points of the thiuronium picrates obtained by this procedure from several portions of lubricating oil to which had been added the compounds listed.

TABLE IV. MELTING POINTS OF THIURONIUM PICRATES DERIVED FROM HALOGEN COMPOUNDS IN LUBRICATING OIL

Halogen Compound Added to Lubricating Oil (1% in Each Case)	Melting Point of Thiuronium Picrate Found (picrate derived from halogen compound in oil) ° C.	Literature value (*1*) ° C.
n-Amyl chloride	151	154
n-Heptyl bromide	138	142
Triphenylmethyl chloride	170	...
Ethylene dichloride	259	260
Benzyl chloride	188	188

Since no value for the melting point of *s*-triphenylmethyl thiuronium picrate was available in the literature, this compound was prepared by refluxing triphenylmethylchloride with thiourea in alcoholic solution, followed by addition of picric acid and recrystallization from alcohol. The melting point was 172° C. and analysis of the compound indicated 5.4 per cent of sulfur (theoretical, 5.85 per cent).

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PRESENTED before the Division of Petroleum Chemistry at the 100th Meeting of the American Chemical Society, Detroit, Mich.

Note on Determination of Silica in Calcined Alumina

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THE determination of traces of silica in calcined alumina required for the manufacture of aluminum presents a number of difficulties. The author has found that silica may be determined in calcined alumina colorimetrically as silicomolybdate in acid solution and that this method is quicker and more accurate than the gravimetric method. It may also be adapted to many estimations of small amounts of silica or silicon.

A Rapid Method for Determining Ferric and Ferrous Iron

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Using precipitated copper as a reducing agent, determinations of ferrous and ferric iron are carried out in less than 10 minutes, with a minimum of equipment and skill, with common stable reagents, in the presence of large amounts of cupric, ferrous, manganous, zinc, nickel, and chromic ions, and with a precision and accuracy of around 3 parts per thousand.

WITH many mills turning to the use of ferric sulfate for pickling brass and other copper alloys, the need for a simple rapid method for the determination of ferric ion in the baths has arisen. (Metals are pickled after high-temperature annealing to remove the oxide film and to produce a bright uniform appearance.)

Standard methods are unsatisfactory for this purpose because mills demand a method so simple and rapid that it can be carried out beside the bath by regular workmen without special training and without taking much time from their other duties. Thus, all methods are eliminated which take more than 10 minutes, which require laboratory skill, special apparatus, and unusual or unstable reagents, and which are expensive or inaccurate in the presence of large amounts of cupric ion.

The method developed to meet these requirements comprises reduction by finely divided copper, removal of the excess, and titration with standard permanganate.

Metallic copper has been used before for reducing ferric salts (*1*, *3–5*, *7–9*), but in all cases copper with a relatively small surface area per unit of weight, such as gauze, turnings, or foil, has been used. This makes the procedures several times longer than the present one and necessitates reduction in a boiling solution, which is an undesirable complication in itself. For example, Hendel (*5*), who made a study of the time required to reduce various amounts of iron, found 30 minutes' boiling with 32 sq. cm. of copper gauze was required to reduce 125 mg. of iron. This quantity, and more, is reduced in 2 minutes at room temperature by the method described.

No one has previously pointed out that this method is capable of determining the ferric-ferrous ion ratio in the presence of large amounts of cupric ion.

Materials

A glass-stoppered 250-cc. flask is preferable. Filtration facilities are desirable but not essential.

Potassium permanganate, 0.1 *N*. Commercial anhydrous ferric sulfate (Ferrisul).

Copper metal precipitated powder (a reagent electrolytic product was purchased, 98 per cent of which passed through a 325-mesh certified Tyler sieve and 84 per cent of which is in the range 10 to 35 microns as determined by a Wagner turbidimeter).

Other reagents used in tests were of ordinary laboratory C. P. grade.

Method

Add 50 cc. of water, 15 cc. of 20 per cent sulfuric acid, and a 4-cc. sample of filtered pickle bath to the 250-cc. flask. Titrate with 0.1 *N* potassium permanganate, add a slight excess of copper metal precipitated powder (0.1 to 0.2 gram), shake violently 2 minutes, filter, and titrate again. One half the net titer gives per cent of ferric sulfate present. For convenience in titration the size of sample may be adjusted to give a titer of 15 to 25 cc. There must be an excess of copper present at the end of the shaking, but an extremely large excess is not recommended in accurate work. For cases where an error of 1 per cent is not serious, the filtration may be eliminated by shaking 30 seconds with 2 cc. of mercury and titrating rapidly.

CALCULATIONS.

1 cc. of 0.1 *N* $KMnO_4$ = 0.019993 gram of $Fe_2(SO_4)_3$
= 0.02 gram of $Fe_2(SO_4)_3$ with error of only 0.035%
= 0.01519 gram of $FeSO_4$
= 0.0152 gram of $FeSO_4$ with error of only 0.066%

% salt = grams of salt in 100 cc. of solution

Cc. of $KMnO_4$ for 4 cc. of sample × 25 = cc. of $KMnO_4$ for 100 cc. of solution

Net cc. of 0.1 *N* $KMnO_4$ × 25 × 0.02 = cc. of 0.1 *N* $KMnO_4$ × 0.5 = % of $Fe_2(SO_4)_3$

(Cc. of 0.1 *N* $KMnO_4$, 1st titer, − Blank) × 25 × 0.0152 = cc. of 0.1 *N* $KMnO_4$ × 0.38 = % of $FeSO_4$

The three different Ferrisul solutions used in testing the method are designated as I, II, and III. Elaborate precautions or unusual care are not required and were not taken in this work except where necessary in the two standard procedures used. Duplicate analyses were made in all cases. The greatest deviation of the duplicate analyses from their average was ±0.5 per cent. The average deviation was 0.3 per cent. (These are percentages of actual value found; in terms of per cent ferric sulfate they are, for solutions I and II, 0.03 and 0.04 per cent, respectively.) Since this precision is entirely satisfactory for the purpose at hand, no attempt was made to determine the limits of precision attainable by this method. All per cent concentrations given are equivalent to grams of salt per 100 cc. of solution at room temperature.

TABLE I. COMPARISON WITH STANDARD METHODS

	$Fe_2(SO_4)_3$ Found	
	Solution I	Solution II
	%	%
SO_2 method	8.73	..
Cu method	8.72	12.74
$SnCl_2$ method	..	12.71

COMPARISON WITH STANDARD METHODS (TABLE I). The sulfur dioxide reduction was carried out according to a standardized procedure used here for plant control work.

Add the sample containing about 0.75 gram of ferric sulfate to 200 cc. of distilled water in a 500-cc. flask; raise the pH to point of precipitation and pass in a rapid stream of sulfur dioxide for 15 seconds. Bring to boil over 5-minute period (must be colorless) and add 10 cc. of 1 to 1 sulfuric acid. Boil rapidly until there is no odor of sulfur dioxide, or at least 10 minutes. Cool and titrate with 0.1 *N* potassium permanganate. Make blank correction. About 30 minutes are required.

The stannous chloride reduction and titration using the Zimmerman-Reinhardt preventive solution followed the directions of Swift (*10*). The agreement of the present method within about 2 parts per thousand with these standard methods is thus less than experimental error, and shows that the new method is accurate.

EFFECT OF OTHER IONS. Table II shows that large amounts of ferrous, cupric, manganous, zinc, nickel, and chromic ions

Table II. Effect of Other Ions

(Ferrisul Solution III)

Added Salt	%	$Fe_2(SO_4)_3$ Found %
...	...	8.83
$FeSO_4.7H_2O$	25	8.86
$CuSO_4.5H_2O$	25	8.81
$MnSO_4.2H_2O$	62	8.78
$ZnSO_4.7H_2O$	100	8.87
$NiSO_4.6H_2O$	42.5	8.87
$Cr_2(SO_4)_3$	3	8.81
NaCl	25	13.1

do not affect the method outside of experimental error. Chloride, as would be expected, caused interference.

Discussion

Aside from the simplicity of a copper reduction method, it has the all-important advantage here of being applicable directly to brass pickling baths containing large amounts of cupric ions, which would not be the case with a zinc or stannous chloride reduction. The fact that chloride is not required as in a mercury (*2*, *6*) or silver (*11*) reduction makes it possible to use a simple permanganate titration. The absence of special apparatus, such as a reductor tube or colorimetric standards, makes the method particularly suited to millwork where such equipment could be subjected to careless handling and breakage. The simple equipment used in this method is easily replaced without the attention of a trained chemist.

The method requires no special skill and the accuracy and precision of the results show that interference by cuprous compounds and air oxidation is negligible. The use of precipitated copper powder so accelerates the reduction that the method becomes one of the fastest and simplest of analytical methods, which should recommend its use in other applications. The precision of the results is consistent with the technique used and no results were obtained which would indicate that the method could not be made even more precise than 3 parts per thousand, if desired, by introducing refinements.

Acknowledgment

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Volumetric Determination of Iron and Aluminum in Cement with 8-Hydroxyquinoline

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In the usual procedure (*2–10*, *11*, *14*) for the determination of iron and aluminum with 8-hydroxyquinoline, the acidity is generally set with acetic acid and ammonium acetate at a pH close to 7, and an acetic acid solution of the 8-hydroxyquinoline is added to precipitate the metals.

Results obtained by this procedure are erratic. While the gravimetric results are satisfactory, the volumetric results are consistently low; although the metals are completely precipitated, they do not come down completely as the oxine. This is due to the fact that the pH for precipitation as the oxine is the same as for precipitation of these elements as the basic acetates. Moyer and Remington (*12*) have shown that iron is precipitated completely with oxine at a pH of 3.45 to 4.00 and aluminum at a pH of 5.0. At both these points the basic acetate precipitates. When the metals are ignited to the oxides the results are satisfactory, but when the oxine precipitate is weighed, the results are a little low because the basic acetates are lighter than the oxines. In the volumetric determination the metal which comes down as the basic acetate is not determined. The amount of error which this procedure can cause will depend on the dilution, the temperature, and the time of heating, all of which favor the formation of the basic acetate.

To precipitate both iron and aluminum, the pH must be above 5.0. The addition of tartaric acid prevents the formation of the basic acetate at this pH, and the metals can then be precipitated completely as the oxine.

Since divalent iron reacts with only 2 instead of 3 moles of the oxine, it is necessary to boil the solution with bromine water before adding the oxine.

Two methods for the titration of the oxine precipitate are used. The first—to titrate the oxine directly with a bromate solution before using neocarmine, methyl red, or other dye as the indicator—is not generally used because the color of the dye fades gradually and the end point is not sharp (*7*, *13*). The second method—to add an excess of the bromate and back-titrate with potassium iodide and thiosulfate—cannot be used in the titration of the iron oxine because the potassium iodide also reacts with the ferric ion.

The poor end point with the first method was therefore studied. It was found that the color produced in this titration was not reversible. A very small amount of the bromate solution is able to decolorize a large amount of the indicator. Thus, during the titration, the indicator is being used up by the bromate. To eliminate this it is only necessary to add more indicator when the solution starts to become decolorized. Another reason for the poor end point is that when methyl red, for example, is used in a weak acid solution, the color change is from red to orange to green to yellow. The final transition from green to yellow is very indistinct and it is easy to overrun the end point by as much as 1 cc. If, however, the acid concentration is above 15 per cent, the green color is eliminated and a sharp transition from orange to yellow will be observed, the red changing to orange just before the end point is reached. A satisfactory end point can then be obtained by direct titration with the bromate solution.

Results obtained with these modifications as shown in Table I are typical of many similar determinations.

To determine the iron and aluminum volumetrically the two metals can be precipitated together as the oxine and titrated as indicated above. The iron can then be determined volumetrically on a separate portion in the usual way, or determined on the same portion oxidimetrically.

To determine iron and aluminum in the same solution, both were precipitated with oxine and titrated with the bromate solution; then the iron was titrated oxidimetrically in the same solution. The first titration gave satisfactory reproducible results, but the second did not. When the iron was titrated with potassium permanganate by the Zimmerman-Reinhardt method, the very high results were due, apparently, to an oxidation of the oxine. An oxidizing agent with lower potential (potassium dichromate) gave better results but still high. The iodometric method, which has a much lower potential, was then tried and, after modification, gave satisfactory results as shown in Table I.

TABLE I. DETERMINATION OF IRON AND ALUMINUM

Sample	Metal Present		Bromate Titration	Thiosulfate Titration	Error
		Gram			*Mg.*
1	Al_2O_3	0.0107	0.0108	....	+0.1
2	Al_2O_3	0.0270	0.0267	....	−0.3
3	Al_2O_3	0.0480	0.0480	....	0.0
4	Fe_2O_3	0.0080	0.0081	....	+0.1
5	Fe_2O_3	0.0186	0.0185	....	−0.1
				0.0187	+0.1
6	Fe_2O_3	0.0362	0.0363	....	+0.1
				0.0361	−0.1
7	Al_2O_3	0.0261	0.0267	....	+0.6
	Fe_2O_3	0.0196	....	0.0195	−0.1
8	Al_2O_3	0.0262	0.0259	....	−0.3
	Fe_2O_3	0.0170	....	0.0173	+0.3
9	Al_2O_3	0.0346	0.0341	....	−0.5
	Fe_2O_3	0.0116	....	0.0114	−0.2

Procedure

Dissolve 0.5 gram of cement in perchloric or hydrochloric acid, depending on whether the silica is determined by the perchloric acid or the ammonium chloride method. After removal of the silica, add an excess of 10 ml. of hydrochloric acid, 2 drops of methyl red, and 5 ml. of 20 per cent tartaric acid. Make just alkaline with concentrated ammonium hydroxide and add 1 to 10 hydrochloric acid till a slight red color appears. Add 20 ml. of a 25 per cent solution of ammonium acetate and bring the solution to a boil. This procedure sets the pH at 5.8; however, it drops to 5.0 after precipitation, because of the release of hydrogen ions in the formation of the oxine complex and because the reagent contains acetic acid.

Cool slightly and add an excess of the 8-hydroxyquinoline reagent (shown by the yellow color of the supernatant liquid). Prepare the reagent by dissolving 12.5 grams of oxine in 30 ml. of glacial acetic acid and diluting with water to 1 liter. Let the precipitate stand 15 to 30 minutes, filter through a coarse filter paper, such as Whatman 41H, keeping the precipitate at least 1.25 cm. (0.5 inch) from the top of the paper because the iron oxine creeps excessively. Wash with warm water. Wash the precipitate back into the original beaker with a stream of hot water and pour 50 ml. of hot 1 to 2 hydrochloric acid over the filter paper to dissolve the remaining precipitate into the original beaker. Heat carefully till the precipitates just dissolve. Cool, add 2 drops of methyl red, and titrate with 0.2 *N* bromide-bromate solution. When nearing the apparent end point, add another 2 drops of methyl red, allowing 15 seconds before continuing the titration. Continue adding 2 drops of the indicator and titrating until the color changes to an orange and then a yellow within 15 seconds after adding the indicator. For greater accuracy, correct for the amount of bromate used up by the indicator by counting the number of drops of indicator added and determining on a blank the bromate equivalent. Fifteen drops of 0.5 per cent methyl red are decolorized by 0.10 ml. of bromate solution and 10 to 15 drops of indicator are generally used up in a titration.

Add a few drops of the oxine and cool to 15° to 20° C. Add about 6 grams of sodium carbonate and then 20 ml. of 15 per cent potassium iodide. After 5 minutes add 10 cc. of concentrated hydrochloric acid and titrate with 0.02 *N* thiosulfate, adding starch when the solution becomes a light yellow. Any color that develops after this titration should be ignored (this is due to the decomposition of the potassium iodide which occurs at the high acidity necessary to keep the iron oxine in solution). The iodometric titration must be run immediately after the bromometric titration because the iron is reduced on standing.

TABLE II. INTERFERENCE

Sample	Al_2O_3 Present	P_2O_5 Present	Al_2O_3 Found
	Gram	*Mg.*	*Gram*
10	0.0267	5	0.0264
11	0.0267	10	0.0261
12	0.0267	15	0.0257
13	0.0267	20	0.0255
14	0.0267	25	0.0257

The bromate solution can be standardized with potassium iodide and thiosulfate. The thiosulfate can be standardized against potassium dichromate, or both solutions can be standardized by using the above procedure with Bureau of Standards' Sibley iron ore 27b or standard iron wire.

The interfering elements are large amounts of silica (which causes slightly lower results), copper (*4*), cadmium, cobalt, nickel, zinc, titanium, and zirconium. Less than 5 mg. of phosphate do not interfere. Table II shows the interference when more than 5 mg. are present. Magnesium and calcium do not interfere. The only interfering elements in this list, besides silica, that are likely to occur in cements are titanium and zirconium.

This method has the following advantages for routine laboratory analysis over the ammonium hydroxide method. It is free from most of the errors indicated by Hillebrand and Lundell (*7*): phosphate ion interference, traces of silica in the sample and in the reagents (especially ammonia), improper ignition (aluminum oxide requires 1200° C.), absorption of moisture by the ignited oxides, etc.

After the iron and aluminum are determined as above, the calcium can be precipitated at the same pH by the addition of ammonium oxalate and also determined volumetrically. Magnesium can then be precipitated by heating to 70° C., adding more oxine, then 30 ml. of concentrated ammonium hydroxide, stirring for 15 minutes, and letting settle 10 minutes. It is determined volumetrically in the same manner as the iron and aluminum. Cements contain up to 1 per cent of P_2O_5. This amount does not interfere with the oxine method but is carried down in the ammonium hydroxide method. Consequently, this method, in the presence of this ion, will give lower results than the A. S. T. M. method (*1*). Samples 7, 8, and 9 are cements with only a trace of P_2O_5.

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Spectrophotometric and Biological Assay of Vitamin A in Oils

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THE discovery in 1928 by Morton and Heilbron (*15*) that vitamin A has a characteristic absorption band in the region 320 to 330 $\mu\mu$ has been followed by numerous publications on the use of this physical fact as a means of quantitative measurement of the amount of vitamin A in oils. Such literature covers the improvement in experimental technique, irrelevant absorption, proper solvents, forms of vitamin A, and, dependent on all these, the correlation of results obtained by physical tests with biological assay through the proper conversion factor.

There is now available a wide variety of spectrophotometers and photoelectric photometers used in the physical assay of vitamin A. The Vitamin Assay Committee of the American Drug Manufacturers' Association (*1*) in 1937 and again in 1939 made an attempt to correlate results obtained by such instruments. An examination of these reports reveals a divergence among the values of extinction coefficients obtained by different instruments on the same oils, attributed partially to errors in the instruments and partially to the technique of the operator and experimental conditions.

The question of irrelevant absorption, or the presence in oils of substances which show ultraviolet absorption, not necessarily selective, in the region of 328 $\mu\mu$ is of great importance in the physical assay of vitamin A. Attempts to correct for such absorption have involved the application of correction factors (*3, 12, 16*) or the suggestion of Hume and Chick (*9*), who state that the irrelevant absorption bears no constant relationship to the amount of vitamin A present, that it is less for the unsaponifiable fraction than for the oil itself, but that in oils with large concentrations of vitamin A the error is insignificant. On the other hand, the problem is further complicated by the question as to whether the ultraviolet absorption at 328 $\mu\mu$ is a measurement of all the substances which cause vitamin A activity in rats.

There are two aspects to this problem. In the first place Gillam *et al.* (*5*) and Edisbury *et al.* (*4*) report the presence in oils of a new vitamin, vitamin A_2, which also has biological activity, but with ultraviolet absorption maximum at 345 to 350 $\mu\mu$. One other vitamin in whale liver oil with absorption maximum at 290 $\mu\mu$ has been reported by Willstaedt and Jensen (*17*), and it is possible that other forms of the vitamin exist. In the second place, Gray, Hickman, and Brown (*6*) report that vitamin A in fish liver oils is present mainly in the ester form. It is further implied by Hickman (*7*) that the esters of vitamin A have a greater biological activity than the alcohol. This may be due to the fact that the ester is more stable than the alcohol, as Mead, Underhill, and Coward (*13*) have found.

All these factors influence the value of the conversion factor. The normal procedure of obtaining the conversion factor is by direct computation from the E and biological values of the pure substance. In the case of vitamin A this procedure is not logical because of the instability of the vitamin and because some substances which have biological activity similar to vitamin A do not have absorption maxima at 328 $\mu\mu$. The value of 1600 first reported (*10*) was based not on pure vitamin A but on the richest concentrate known at the time. Since that time many values have been reported.

Hume and Chick (*9*) state that the values vary from 1300 to 2580. Morgan *et al.* (*14*) indicate that the factor may not be the same for cod liver oils as for oils of higher potency. Holmes and Corbett (*8*) report on tests made on crystalline vitamin A values from 1600 to 1800; one test by Darby gave a value of 2100. The 1939 report of the A. D. M. A. committee gave conversion factors ranging from 1920 to 2780. Barthen and Leonard (*2*) on tests on U. S. P. standard oil report a value of 2222; Mead, Underhill, and Coward (*13*) report values of 1920 and 2150 for two esters and, having made the correction factor for the acid fraction of the ester molecules, compute 2000 as the conversion factor for vitamin A. Hickman (*7*), using data obtained mainly from molecular distillation studies, states that the conversion factor for the esters of vitamin A is higher than for the alcohol, that each preparation has a characteristic conversion factor, and that cod liver oils in general have higher factors than most other fish liver oils.

It would thus seem that, until some of these matters are further clarified, each laboratory should obtain its own conversion factors by direct comparison between the E and biological values of a great variety of oils. Such a procedure has been adopted in this laboratory.

Experimental Procedure

PHYSICAL. Two instruments, the Hilger vitameter and the ultraviolet spectrophotometer, were used in the physical assay of the oils. Experience with the vitameter has shown that variations in its performance lead to rather inaccurate results. The vitameter, however, is a simple and speedy instrument to operate and has been found excellent for measurements preliminary to the biological and more accurate physical assays.

The spectrophotometer used was a Judd Lewis ultraviolet photometer with vanes carefully refinished and reset and the various units rigidly mounted to the frame of a 10 × 25 cm. (4 × 10 inch) Bausch and Lomb ultraviolet spectrograph. The original mounting of the Judd Lewis photometer has been re-

TABLE I. U. S. P. REFERENCE COD LIVER OIL

Oil No.	$E^{1\%}_{1\text{ cm.}}$	$\Delta E^{1\%}_{1\text{ cm.}}$ [a]	Conversion Factor [b]
	Full Bottles		
1	1.40 (unsap.)	..	2140
2	1.36 (unsap.) 1.49 (whole)	0.13	2210 2010
3	1.39 (unsap.) 1.49 (whole)	0.10	2160 2010
4	1.36 (unsap.) 1.49 (whole)	0.13	2210 2010
	Partially Filled Bottles [c]		
5	1.24 (unsap.)	..	2420
6	1.12 (unsap.)	..	2680
7	1.15 (unsap.)	..	2610
8	1.17 (unsap.)	..	2560
9	1.17 (unsap.) 1.26 (whole)	0.09	2560 2390
10	1.14 (unsap.) 1.26 (whole)	0.12	2630 2390
11	1.24 (unsap.) 1.32 (whole)	0.08	2420 2270

Average conversion factor for unsaponified fraction
Full bottles 2180 ± 1.4%
Partially filled bottles 2550 ± 3%

Average conversion factor for whole oil
Full bottles 2010 ± 0%
Partially filled bottles 2350 ± 2%

[a] $E^{1\%}_{1\text{ cm.}}$ whole − $E^{1\%}_{1\text{ cm.}}$ unsap.
[b] Biological value taken as 3000 U. S. P.
[c] Flushed with carbon dioxide and allowed to stand in refrigerator for several weeks.

TABLE II. COD LIVER OILS

Oil No.	$E^{1\%}_{1\ cm.}$	$\Delta E^{1\%}_{1\ cm.}$ [a]	U. S. P. XI (Biological)	Conversion Factor
			Units per gram	
1872 (unsap.)	0.75	0.25	2050	2730
(whole)	1.00			2050
1891 (unsap.)	1.10	0.26	3100	2820
(whole)	1.36			2280
1870 (unsap.)	0.76	0.07	2300	3030
(whole)	0.83			2770
1885 (unsap.)	0.87	0.08	2400	2760
(whole)	0.95			2530
1847 (unsap.)	0.75	0.10	2400	3200
(whole)	0.85			2820
1838 (unsap.)	0.63	0.25	1800	2860
(whole)	0.88			2050
1859 (unsap.)	0.84	..	2000	2380
1857 (unsap.)	0.66	0.18	2300	3490
(whole)	0.84			2740
1928 (unsap.)	0.64	0.06	1650	2580
(whole)	0.70			2360
1667 (unsap.)	0.66	0.06	1800	2730
(whole)	0.72			2500
1850 (unsap.)	2.33	0.42	6000	2570
(whole)	2.75			2190
1833 (unsap.)	0.77	0.08	2250	2920
(whole)	0.85			2650
1697 (unsap.)	0.87	0.04	2050	2360
(whole)	0.91			2250
1699 (unsap.)	0.82	0.09	2150	2620
(whole)	0.91			2360
1903 (unsap.)	0.70	0.09	1700	2430
(whole)	0.79			2150
1879 (unsap.)	0.80	..	2150	2690
1978 (unsap.)	0.84	0.10	2150	2560
(whole)	0.94			2290
1987 (unsap.)	0.68	0.18	1600	2350
(whole)	0.86			1860
2013 (unsap.)	0.57	0.09	1550	2720
(whole)	0.66			2350
2004 (unsap.)	0.55	0.03	1400	2550
(whole)	0.58			2410
2021 (unsap.)	0.78	0.02	2100	2700
(whole)	0.80			2620
2022 (unsap.)	0.82	0.11	2000	2440
(whole)	0.93			2150
Average conversion factor for unsaponifiable fraction				2700 ± 8%
Average conversion factor for whole oil				2370 ± 9%

[a] $E^{1\%}_{1\ cm.}$ whole $- E^{1\%}_{1\ cm.}$ unsap. One per cent refers to concentration of whole oil before saponification.

placed by a more rigid and better aligned single unit mounting designed by the Squibb staff. In this mounting all the photometer units, including the source, are attached to the same unit. With such a mounting it has been the experience in this laboratory that in the 8 months of use, once the new photometer setup had been brought into adjustment (using approximately thirty plates), the only subsequent adjustments have been incidental to the removal and sharpening of the electrodes as they have worn away with use. On replacement of the electrodes a single plate taken varying the height of the arc was sufficient to reset the source.

The light source used was a tungsten-steel spark. On each assay plate one exposure with both apertures fully open and no absorbing cells in position was made as an adjustment check on the equality in intensity of the two beams. The density scale of the lower sector of the photometer was calibrated by comparing values found with solutions of potassium chromate and potassium nitrate with the values recorded in literature. Such concentrations were used as would include the entire scale. All absorption maxima of these absorbing salts were used. Such calibration was checked on the average after every ten plates had been exposed.

Eastman spectroscopic plates No. II O were used. Exposures with such plates, using Bausch & Lomb 1-cm. cells and slit width of 0.03 mm., varied from 2 to 10 seconds. Plates were developed for 5 minutes (at 20° C.) in Eastman D19 developer, washed and fixed for 15 minutes, then washed in running water for at least 30 minutes.

The actual procedure in the assay of any oil was to weigh out directly into a 100-ml. flask a quantity of the fresh oil accurate to a 0.1 mg. This was then diluted with isopropanol and a vitameter reading was taken. Dilutions that would give a match at a density reading of between 0.50 and 0.95 were used. A similar concentration was then placed in one of the photometer cells with isopropanol in the compensating cell, and a plate was taken varying the aperture of the density scale from 0.15 to 0.95 by steps of 0.05. On such a plate it was possible to read the match point at 328 $\mu\mu$ to 0.05 density readings and also to plot the absorption curve of the oil. When such a density reading was obtained the value of $E^{1\%}_{1\ cm.}$ was calculated from Beer's law and a second plate was taken. On this second plate two or three concentrations were used, each covering from six to nine exposures. The test solution was thus exposed to the ultraviolet light for not more than 1.5 minutes.

Tests made for deterioration of the oils due to irradiation from the source when the cells were in their normal position with respect to the source and the aperture was fully open indicated that for exposures up to 3 minutes the decrease in the E value was well within the error of the instrument. A second weighing was also made and a third plate taken with two or three concentrations. If the extinction coefficients computed from the plates agreed to within 5 per cent for the different weighings no further data were taken; if not, a third weighing was made and plate exposed.

Plates, when dry, were placed on a well-illuminated viewing stand and examined visually with a jeweler's loop for density match points at 328 $\mu\mu$. If there was doubt about any match point, the plate was projected on a screen using a Bausch & Lomb Balopticon projector and the projected image was examined for the match point.

With the lower potency oils assays were made on both the whole oil and the unsaponifiable fraction. Two methods of saponification were used—one, that suggested by the Vitamin Assay Committee of the A. D. M. A. of 1937 (*1*) with the additional procedure that the ether was evaporated under carbon dioxide, and the other a modified form of this procedure by which in the process of saponification and extraction of 1 gram of oil larger amounts of ether, alcohol, and water were used than in the first procedure.

Following this procedure, an accurate determination on a cod liver oil can be made in about 6 hours and on an oil of higher potency in 4 hours.

BIOLOGICAL. The U. S. P. XI procedure was followed in all cases. A master curve was used as an aid in interpreting the results and to increase the accuracy. Precautions were taken in the biological assay to avoid the use of U. S. P. reference oil which had been exposed to air or had been stored in partially filled bottles any appreciable length of time.

Fresh bottles of the oil, as distributed by the U. S. Pharmacopœia Vitamin Committee, were taken at intervals of 2 to 3 months, and subdivided into three or five small vials which were thoroughly flushed, sealed, and stored in a refrigerator. These small vials were then consumed in periods not exceeding 2 to 3 weeks. These vials and various diluted solutions which were used in the tests were always thoroughly flushed with carbon dioxide after being opened and then returned to the refrigerator. Fresh dilutions were prepared weekly.

Results

In Table I values obtained for the U. S. P. reference cod are tabulated. Each E value recorded represents an average value obtained by following the procedure outlined above for the assay of cod liver oils. In some cases more than two saponifications were made. In Tables II to IV the results for a series of oils are recorded. The conversion factors were obtained by dividing the biological value by the E value. The average conversion factors for the various types of oils and the average per cent errors were calculated.

Many absorption curves of the oils have been plotted. For the oils of higher potency the curves are fairly symmetrical about a pronounced peak around 328 $\mu\mu$. In some of these there is evidence of a "flat" in the curve at 310 and 340 to 350 $\mu\mu$. A comparison of the curves of the whole oils and unsaponifiable fractions of cods is interesting. In both cases there is a pronounced peak at approximately 328 $\mu\mu$, also evidence of a flat at 310 and 340 to 350 $\mu\mu$, but the curves for the whole oils are more irregular, showing evidence of selec-

TABLE III. TUNA AND HALIBUT LIVER OILS

No.	$E^{1\%}_{1\text{ cm.}}$	U. S. P. XI (Biological)	Conversion Factor
		Units per gram	
		Tuna Liver Oil	
1959	58	120,000	2070
1902	12	26,000	2170
1897	34	80,000	2350
1909	49	75,000	1530
1623	56	145,000	2590
1741	12	30,000	2500
1916	23	55,600	2420
1917	26	55,300	2130
2016	55	110,000	2000
2023	44	92,000	2090
Average conversion factor		2180 ± 10% 2260 ± 9% (omitting 1909)	
		Halibut Liver Oil	
1617	67	150,000	2240
1619	33	70,000	2120
1863	56	140,000	2500
1860	41	110,000	2680
1936	46	100,000	2180
1937	50	105,000	2100
1914	26	61,700	2380
1915	18	42,400	2360
1692	31	62,000	2000
1988	26	56,000	2150
1989	37	80,000	2160
1990	93	202,000	2170
Average conversion factor		2250 ± 6%	

tive absorption in the region 260 to 280 $\mu\mu$, and the curves as a whole are higher than those for the unsaponifiable fractions.

Discussion of Results

An examination of Table I reveals the fact that the E values listed for the U. S. P. reference cod liver oil vary over a considerable range from the higher consistent values for the assays on full bottles to the lower varied values on bottles which were only partially filled and were handled as indicated above. A similar falling off of the E values for this reference oil was reported by McFarlan (*11*).

There has been no evidence that any loss of vitamin A has occurred in the original bottles of U. S. P. reference samples as determined by repeated physical tests and biological assays. Whether or not the decrease in the E values which occur in the U. S. P. reference oil after standing for some time in partially filled bottles is also accompanied by a decrease in vitamin A activity has not been determined but is not believed to affect the interpretation of the biological assays, since the use of such oil has been avoided.

In Table II are listed the conversion factors for 22 cod liver oils as obtained by direct computation of the biological and physical values for the individual oils. The average of the unsaponifiable fraction for these oils is 2700 and that for the whole oils 2370. Conversion factors for oils of higher potency—tuna, halibut, and other fish liver oils—are listed in Tables III and IV, giving average values of 2260, 2250, and 2270, respectively.

It would appear that the conversion factors of the cod liver oils or at least a part of them were higher than that of the U. S. P. reference cod liver oil or samples of the more highly active tuna, halibut, and other fish oils which were studied. These differences cannot be explained by variations in the instrument, since the various oils have been run as received in the laboratories. Different oils were run concurrently and results should be comparable. Furthermore, the instrument was calibrated at frequent intervals with standard inorganic solutions. Neither is it considered that these differences are due to the loss of the vitamin in the process of saponification, since two methods of saponification were used and results agree to within 5 per cent. Moreover, the values for the whole oils themselves are greater than those for the other oils.

There is no explanation at the present time for the higher conversion factor, but the inference might be made that there are present in cod liver oils, in proportions greater than in oils of higher potency, substances which have biological activity similar to that of vitamin A but do not have maximum ultraviolet absorption at 328 $\mu\mu$. Further work on this point is in progress.

Summary

The details of a spectrophotometric method of assay of vitamin A in fish liver oils and the results of the biological and physical assay of 53 such oils are recorded. In all cases assays were made on fresh oils and all biological assays were made on the whole oils.

The average conversion factors computed from the measurements on 22 cod liver oils yield values of 2700 and 2370 for the unsaponifiable fractions and whole oils, respectively.

The average conversion factors for oils of higher potency are 2260 for tuna liver oils, 2250 for halibut liver oils, and 2270 for miscellaneous oils as listed, giving an average of 2260 for these oils of higher potency.

Studies on the U. S. P. reference standard have shown that the E value gradually decreases when the oil remains in partially filled bottles, even though they have been flushed with carbon dioxide and stored in a refrigerator.

TABLE IV. MISCELLANEOUS OILS

No.	Type of Oil	$E^{1\%}_{1\text{ cm.}}$	U. S. P. XI (Biological)	Conversion Factor
			Units per gram	
1839	Pollack	3.8	10,500	2760
1878	Pollack	4.5	9,500	2110
1899	Pollack	3.7	8,500	2300
1948	Pollack	3.5	7,800	2230
1977	Pollack	4.2	10,200	2430
1934	Shark	56	135,000	2410
1944	Shark	57	109,000	1910
1979	Sword	28	67,000	2390
2019	Shark	109	206,000	1890
	Average conversion factor		2270 ± 9%	

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Polarographic Determination of Nickel and Cobalt

Simultaneous Determination in Presence of Iron, Copper, Chromium, and Manganese, and Determination of Small Amounts of Nickel in Cobalt Compounds

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THE purpose of this paper is to describe conditions under which nickel and cobalt can be determined simultaneously by the polarographic technique with the dropping mercury electrode (*2*). Heretofore an ammoniacal supporting electrolyte has been recommended for the simultaneus polarographic determination of nickel and cobalt (*5*, *8*, *9*). However, the degree of separation of the two waves in ammoniacal medium is none too good, and accurate determination of the nickel is not possible when a large amount of cobalt is present (compare curve 2, Figure 1). The authors have found that an excellent separation of the nickel and cobalt waves is obtained in supporting electrolytes containing thiocyanate or pyridine. The use of these supporting electrolytes makes possible the rapid determination of small amounts of nickel present as an impurity in cobalt compounds, and greatly improves the simultaneous determination of nickel and cobalt in iron products (steel).

Apparatus and Experimental Technique

The usual polarographic technique was employed, using a Heyrovsky-Shikata type of polarograph for the automatic photographic recording of the polarograms (*1*, *2*). The H-type of cell, with a permanent external calomel electrode as anode, and the arrangement of the dropping electrode described by Lingane and Laitinen (*4*) were used for most of the measurements. Air was displaced from the cell solutions with nitrogen; the commercial tanked gas proved to be sufficiently pure for the present purpose. All measurements were made with the cell in a water thermostat at 25° C.

Comparison of Nickel-Cobalt Waves in Various Supporting Electrolytes

The polarogram in Figure 1 shows the waves obtained with a mixture of 0.001 *M* nickel chloride and 0.002 *M* cobalt chloride in different supporting electrolytes. Each solution contained 0.01 per cent gelatin as a maximum suppressor (*2*). The half-wave potentials of nickel and cobalt in these media are listed in Table I.

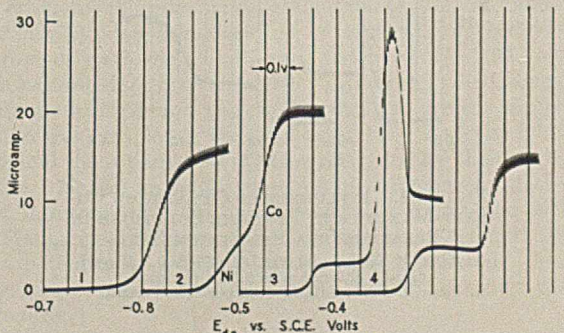

FIGURE 1. WAVES OF A MIXTURE OF NICKEL AND COBALT IN VARIOUS SUPPORTING ELECTROLYTES IN PRESENCE OF 0.01 PER CENT GELATIN

1. 0.001 *M* $NiCl_2$ and 0.002 *M* $CoCl_2$ in 1 *N* KCl
2. 0.001 *M* $NiCl_2$ and 0.002 *M* $CoCl_2$ in 1 *N* NH_4Cl–1 *N* NH_4OH
3. 0.0007 *M* $NiCl_2$ and 0.0013 *M* $CoCl_2$ in 1 *N* KCl containing 0.5 *M* pyridine
4. 0.001 *M* $NiCl_2$ and 0.002 *M* $CoCl_2$ in 1 *N* KCNS

Starting potential of each curve indicated on abscissa, and distance between each vertical line corresponds to 0.1 volt

TABLE I. HALF-WAVE POTENTIALS OF NICKEL AND COBALT IN DIFFERENT SUPPORTING ELECTROLYTES

(Half-wave potentials in volts with respect to the saturated calomel electrode at 25° C.)

Supporting Electrolyte	$E_{1/2}$ Ni	$E_{1/2}$ Co	$E_{1/2}$ Difference
1 *N* KCl	−1.1	−1.2	0.1
1 *N* NH_4Cl–1 *N* NH_4OH	−1.12	−1.30	0.18
1 *N* KCl + 0.5 *M* pyridine	−0.78	−1.07	0.29
1 *N* KCNS	−0.70	−1.03	0.33

In a non-complex-forming supporting electrolyte the half-wave potentials of nickel and cobalt are so close together that a mixture of the two produces only a single wave, as shown by curve 1 in Figure 1. The coalescence of the waves in a non-complex-forming supporting electrolyte is further favored by the fact that the reduction of the aquo nickel and aquo cobaltous ions is irreversible, and the waves have an abnormally small slope.

In ammoniacal medium the difference between the half-wave potentials of the complex nickel and cobalt ammonio ions is large enough so that a double wave is obtained, as shown by curve 2 in Figure 1. However, the waves are still not sufficiently separate to allow an accurate measurement of their individual heights, especially if the concentrations of nickel and cobalt are disproportionate.

In pyridine or thiocyanate supporting electrolytes the half-wave potentials of both the nickel and the cobalt are shifted markedly to a more positive value but the nickel wave is shifted more than that of the cobalt; hence, in such solutions an excellent separation of the two waves is obtained, as shown by curves 3 and 4 in Figure 1. The complex ions formed in pyridine and thiocyanate solutions are reduced reversibly, as is evidenced by the normal slopes of the waves. Furthermore, the half-wave potentials in pyridine or thiocyanate solutions are about what one would expect from the ordinary standard potentials of nickel and cobalt combined with a reasonable estimate of the dissociation constants of the complex ions. This matter will be discussed in a later paper.

It will be noted from Figure 1 that the wave of cobalt in pyridine solution has a very prominent maximum, in spite of the presence of gelatin, but the diffusion current following the maximum is well defined. The authors attempted to suppress the cobalt maximum in 0.5 *M* pyridine by increasing the concentration of gelatin, with the results shown in Figure 2. The small nickel maximum is easily eliminated with only 0.01 per cent gelatin, but the cobalt maximum is not suppressed even with 0.05 per cent gelatin.

The cobalt maximum in pyridine solution in the presence of 0.05 per cent gelatin is not obtained when the concentration of pyridine is smaller than about 0.2 or 0.3 *M* (Figure

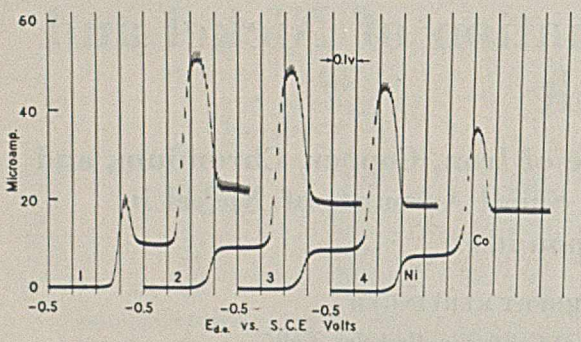

FIGURE 2. INFLUENCE OF GELATIN CONCENTRATION ON NICKEL AND COBALT MAXIMA IN PYRIDINE SOLUTION

0.002 *M* $NiCl_2$ and 0.002 *M* $CoCl_2$ in 1 *N* KCl containing 0.5 *M* pyridine. Concentrations of gelatin: (1) none, (2) 0.01, (3) 0.02, (4) 0.05 per cent

3). With concentrations of pyridine less than about 0.2 or 0.3 *M* the double nickel-cobalt wave is very well defined with no maxima, but with increasing concentration of pyridine the cobalt maximum appears and it increases with increasing pyridine concentration.

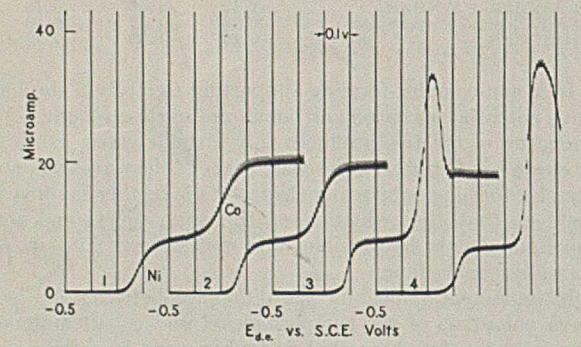

FIGURE 3. INFLUENCE OF PYRIDINE CONCENTRATION ON NICKEL AND COBALT WAVES

0.002 *M* $NiCl_2$ and 0.002 *M* $CoCl_2$ in 1 *N* KCl containing 0.05 per cent gelatin. Concentrations of pyridine: (1) 0.05, (2) 0.1, (3) 0.5, (4) 1 *M*

When a pyridinium salt is also present in the pyridine solution, the cobalt maximum is even larger than it was in the foregoing experiments in the absence of the pyridinium ion. This is shown by curve 1 in Figure 4, obtained with a mixture of nickel and cobalt in 0.5 *M* pyridine containing 0.5 *M* pyridinium chloride as supporting electrolyte. However, in the presence of pyridinium ion the cobalt maximum is easily suppressed by 0.05 per cent gelatin or even less, as shown by curve 2.

It will be noted from Figure 4 that the final current rise occurred at a more positive potential in the presence of pyridinium ion. This final current rise at −1.4 volts is due to the reduction of the pyridine, which has been investigated by Shikata and Tachi (*6*). The reduction potential of the pyridine is shifted to a more positive value in the presence of pyridinium salt, owing to the decrease in pH resulting from the buffering effect of the pyridinium ion. However, even in the presence of an equivalent concentration of pyridinium ion, the reduction potential of the pyridine is more negative than that of the cobalt-pyridine complex and does not interfere with the wave of the latter.

The authors have also made a detailed investigation of the nickel and cobalt waves in supporting electrolytes containing thiocyanate, in which medium the waves are slightly more separated than in pyridine solutions (compare Table I). When only nickel is to be determined in neutral solutions, or for the determination of small amounts of nickel in cobalt salts, thiocyanate serves very well as supporting electrolyte. However, a thiocyanate supporting electrolyte is not very suitable for the simultaneous determination of both nickel and cobalt, because in such solutions the diffusion current of the cobalt is not well defined and shows peculiar irregularities, especially in acid solutions or in the presence of ammonium salts. These are the most likely conditions in a practical analysis. For these reasons the use of thiocyanate supporting electrolytes cannot be recommended for the simultaneous determination of nickel and cobalt.

Simultaneous Determination of Nickel and Cobalt in Presence of Iron, Chromium, Manganese, and Copper

Ferric iron is reduced at a potential considerably more positive than the calomel zero, and hence its diffusion current interferes with the waves of practically all other metal ions when it is present in large excess. The authors have found that precipitation as hydrous ferric oxide in pyridine solution affords an excellent separation of relatively large quantities of iron from nickel, cobalt, and copper. Pyridine is a very weak base ($K_b = 1.4 \times 10^{-9}$) and a solution containing equal concentrations of pyridinium ion and pyridine has a pH of about 5.2. At this pH the precipitation of ferric hydroxide and its separation from nickel and cobalt are quantitatively complete.

The outstanding advantage in the use of pyridine is that the precipitation of the iron can be made at a lower pH than when ammonia is used, and hence the possibility of coprecipitation of nickel, cobalt, and copper is greatly lessened.

The separation of small quantities of nickel and cobalt from a relatively large amount of iron by the pyridine method is demonstrated by the polarogram in Figure 5, which was obtained under conditions that simulate the determination of small amounts of nickel and cobalt in steel.

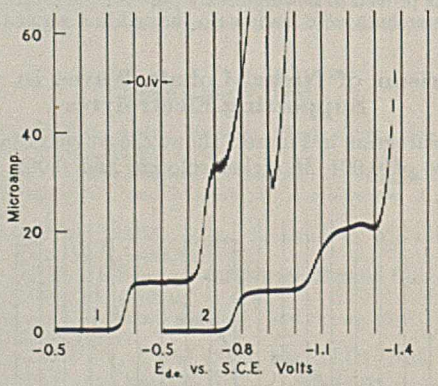

FIGURE 4. INFLUENCE OF GELATIN ON COBALT MAXIMUM IN PYRIDINE SOLUTIONS IN PRESENCE OF A PYRIDINIUM SALT

1. 0.002 *M* $NiCl_2$ and 0.002 *M* $CoCl_2$ in 0.5 *M* pyridinium chloride plus 0.5 *M* pyridine
2. Repeated after adding 0.05 per cent gelatin

Curve 1 was obtained with a solution prepared by diluting 5.00 ml. of 0.01 *M* nickel chloride, 5.00 ml. of 0.01 *M* cobalt chloride, 2.0 ml. of 12 *N* hydrochloric acid, 5.0 ml. of pure pyridine (*ca.* 13 *M*), and 5 ml. of 0.2 per cent gelatin to 100 ml. in a volumetric flask. Hence this solution was 5×10^{-4} *M* in respect to both nickel and cobalt (about 3 mg. of each metal per 100 ml.), 0.24 *N* in pyridinium chloride, 0.4 *M* in pyridine, 0.01 per cent in gelatin, and had a pH of about 5.4. Curve 2 was obtained with a solution prepared in the same way except that 0.3 gram of

iron (as ferric chloride) was added before the addition of the pyridine. The composition of this solution would correspond to about 1 per cent each of nickel and cobalt in a steel. It was not necessary to filter off the precipitated ferric hydroxide; it was simply allowed to settle for a few minutes and a 25-ml. sample of the clear supernatant solution was taken with a pipet and transferred to the polarographic cell for determination of the nickel and cobalt.

The heights of the nickel and cobalt waves were exactly the same after the precipitation of the ferric hydroxide as in the absence of iron, which demonstrates that there was no appreciable coprecipitation of these metals with the iron under these conditions.

For the determination of nickel and/or cobalt in steel a 0.3- to 0.5-gram sample is dissolved in about 5 ml. of concentrated hydrochloric acid in a small beaker, and after addition of a few drops of concentrated nitric acid to oxidize the iron, the solution is evaporated to incipient dryness. The residue is dissolved in 2 ml. of concentrated hydrochloric acid, and transferred to a 100-ml. volumetric flask with 50 to 75 ml. of water. Then 5 ml. of pure pyridine are added, followed by sufficient gelatin solution to give a concentration of 0.01 per cent, and the mixture is made up to the mark and shaken thoroughly. After allowing a few minutes for the ferric hydroxide to settle, a sample of the clear supernatant solution is transferred with a pipet to the polarographic cell and the polarogram is obtained. The dropping electrode is calibrated with known amounts of nickel and cobalt, or with a standard steel sample, under exactly the same conditions.

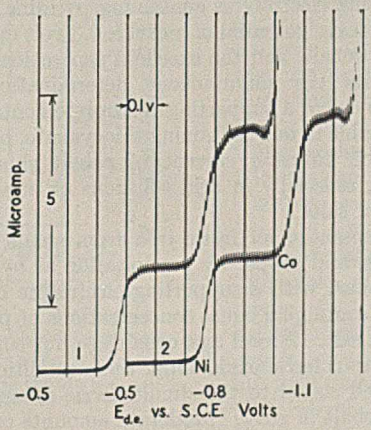

FIGURE 5. SEPARATION OF NICKEL AND COBALT FROM IRON IN PYRIDINE SOLUTION

In the absence of iron, chromic ion is not precipitated in a pyridinium chloride–pyridine solution of pH 5.4, and the chromic-pyridine complex that is formed is reduced to the chromous state with a half-wave potential of −0.95 volt *vs.* the saturated calomel electrode. However, when considerably more ferric iron than chromium was present the chromium was completely coprecipitated with the ferric hydroxide, and the supernatant solution did not show the chromium wave. Hence, moderate amounts of chromium in steel will not interfere with the determination of cobalt and nickel.

Manganous ion in pyridine solution is not reduced below the potential at which pyridine itself is reduced; hence, manganese does not interfere with the nickel-cobalt determination.

Copper in pyridine solution shows two waves, very similar to the double waves obtained in ammoniacal medium (*7*), with half-wave potentials of +0.05 and −0.25 volt *vs.* the saturated calomel electrode (compare Figure 6). The first wave is due to the reduction of the cupric-pyridine complex to the cuprous-pyridine complex, and the second to the reduction of the cuprous complex to the metal (see Figure 6). The copper waves are sufficiently far in advance of the nickel and cobalt waves so that copper does not interfere with the nickel-cobalt determination when its concentration is about the same as (or smaller than) the concentrations of the nickel and cobalt. This is demonstrated by the polarogram in Figure 6 obtained with equal concentrations of copper, nickel, and cobalt in a pyridine solution.

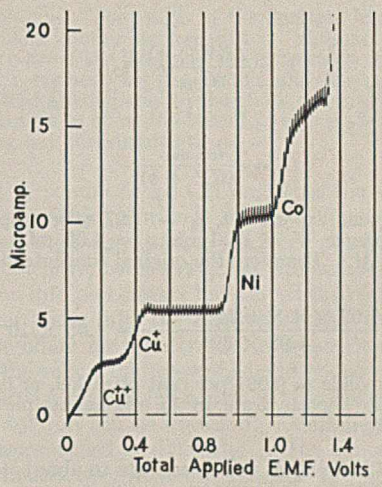

FIGURE 6. SIMULTANEOUS DETERMINATION OF COPPER, NICKEL, AND COBALT IN PYRIDINE SOLUTION

0.001 *M* $CuSO_4$, 0.001 *M* $NiCl_2$, and 0.001 *M* $CoCl_2$ in 0.13 *M* pyridinium perchlorate plus 0.13 *M* pyridine containing 0.02 per cent gelatin

When the concentration of copper is not more than about ten or twenty times that of the nickel and/or cobalt, its diffusion current can be balanced out, and the nickel and cobalt determined, by the "compensation method" (*3*). When a larger excess of copper is present, the bulk of it must be removed before nickel and cobalt can be determined. In such a case it is not necessary to remove the copper completely, but only to reduce its concentration to the same order of magnitude as the concentration of the nickel and cobalt.

Determination of Small Amounts of Nickel in Cobalt Compounds

The use of pyridine or thiocyanate supporting electrolytes makes possible the rapid and accurate determination of small amounts of nickel present as an impurity in cobalt and cobalt compounds. This is illustrated in Figure 7.

Curve 1 in this polarogram was obtained from a solution prepared by dissolving a 3.00-gram sample of reagent quality cobalt sulfate heptahydrate in about 50 ml. of water in a 100-ml. volumetric flask, adding 2 ml. of 12 *N* hydrochloric acid, 5 ml. of pure pyridine, and 5 ml. of 0.2 per cent gelatin, and diluting to 100 ml. A 75.0-ml. portion of this solution was used for obtaining curve 1, which shows a well-defined wave of the nickel impurity. After the first curve was obtained, 4.00 ml. of a 9.24×10^{-3} *M* nickel chloride solution were added to the solution in the cell and curve 2 was recorded. The diffusion current of the nickel is directly proportional to its concentration, and hence from the increase in the height of the nickel wave resulting from the addition of the standard nickel solution the original concentration of nickel can be easily computed.

Let V = original volume of solution in cell
v = volume of standard nickel solution added
C_1 = original molar concentration of nickel
$C_{Std.}$ = molar concentration of standard nickel solution
i_1 = original diffusion current of nickel
Δi = increase in diffusion current resulting from standard addition
ΔC = increase in concentration due to standard addition

We then have the following relations

$$C_1 = \frac{i_1}{k} \quad (1)$$

and

$$\Delta C = \frac{\Delta i}{k} = \frac{v}{V + v} C_{\text{Std.}} \quad (2)$$

or

$$k = \frac{\Delta i(V + v)}{v C_{\text{Std.}}} \quad (3)$$

Therefore,

$$C_1 = \frac{i_1 v C_{\text{Std.}}}{\Delta i(V + v)} \quad (4)$$

In the present experiment, i_1 = 1.97 microamperes, Δi = 1.98 microamperes, V = 75.0 ml., v = 4.00 ml., and $C_{\text{Std.}}$ = 9.24×10^{-3} M. Therefore the original concentration of nickel was

$$C_1 = \frac{1.97 \times 4 \times 9.24 \times 10^{-3}}{1.98 \times 79} = 4.65 \times 10^{-4}\ M$$

This corresponds to 0.091 per cent of nickel, or 0.44 per cent of nickel sulfate heptahydrate, in the original sample of cobaltous sulfate heptahydrate.

This result is believed to be accurate to about ±3 per cent, and is at least as accurate as the determination of this small amount of nickel would be by the classical dimethylglyoxime procedure. Furthermore, the polarographic method is more rapid and less troublesome than the dimethylglyoxime method.

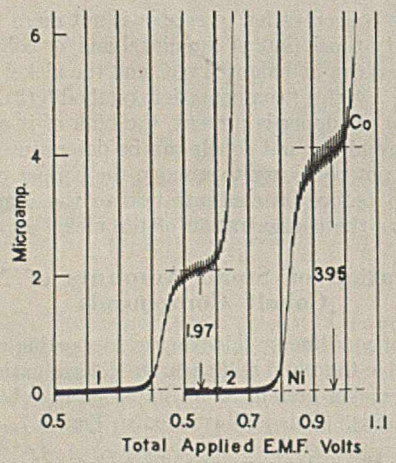

FIGURE 7. DETERMINATION OF NICKEL IMPURITY IN COBALTOUS SULFATE

It will be noted from Figure 7 that in the presence of a large excess of cobalt the reduction of cobalt begins before the diffusion current of the nickel has become entirely constant. For this reason it is necessary to use a standardized technique for measuring the nickel diffusion current. The authors have found that reliable and consistent results are obtained when the wave height of the original solution and the wave height after addition of the standard nickel solution are both measured at the same potential as shown in Figure 7. In the present case the optimum potential for this measurement is 0.10 to 0.12 volt beyond the half-wave potential. Correction for the "residual current" is made by extrapolation as indicated by the dotted lines.

For maximum precision the amount of standard nickel solution added should be sufficient just about to double the height of the original nickel wave. After a little experience with a given capillary, the amount to add can be readily estimated from the height of the original wave. Furthermore, in routine applications of this method, with the same capillary and with all other conditions constant, the calibration by addition of standard nickel solution would have to be made only once, and in subsequent analyses the concentration of nickel could be read directly from the height of the original nickel wave.

The application of this method to other cobalt compounds will be obvious. For determining nickel in metallic cobalt, cobalt oxide, etc., a sample of the material would be dissolved in hydrochloric acid, excess pyridine added, and then the foregoing procedure followed. Incidentally, the removal of nickel from cobaltous chloride by recrystallization from water is very ineffective. Only 35 per cent of the original amount of nickel (0.15 per cent) present in a sample of cobaltous chloride was removed by two recrystallizations.

The foregoing procedure can also be used for the rapid determination of small amounts of copper, either alone or simultaneously with nickel, in cobalt compounds.

Summary

In supporting electrolytes containing pyridine or thiocyanate the half-wave potential of nickel is 0.3 volt more positive than that of cobalt, and the excellent separation of the two waves permits the simultaneous determination of both metals. The use of a supporting electrolyte containing pyridine is preferable to one containing thiocyanate, because with the latter the diffusion current of cobalt shows peculiar irregularities, especially in acid solutions or in the presence of ammonium salts.

A sharp separation of ferric iron from small amounts of nickel, cobalt, and copper by precipitation as hydrous ferric oxide is obtained with a supporting electrolyte of pH equal to about 5.4 containing equal concentrations of pyridine and a pyridinium salt. Nickel and cobalt are not coprecipitated with the hydrous ferric oxide under these conditions, and the method is well suited to the simultaneous determination of nickel and cobalt in steel. Moderate amounts of chromium in steel are completely coprecipitated with the hydrous ferric oxide, and hence do not interfere with the determination of nickel and cobalt. Manganese and small amounts of copper do not interfere with the nickel-cobalt determination. Copper gives a double wave at +0.05 and −0.25 volt *vs.* the saturated calomel electrode in pyridine solutions, and when present in large excess the bulk of it must be removed prior to the nickel-cobalt determination.

Small amounts of nickel impurity in cobalt compounds can be rapidly and accurately determined in a supporting electrolyte containing pyridine or thiocyanate.

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Colorimetric Determination of Phosphorus in Iron Ore

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COLORIMETRIC methods have been described for the determination of phosphorus in iron and steel.

Zinzadze (*5*) described a method for determining phosphorus in the presence of silica, iron, and nitrates, but because of the sensitive nature of the molybdenum blue and the time necessary to develop it, it does not seem applicable to iron ore. Murray and Ashley (*3*) and Bogatzki (*1*) described methods for the determination of phosphorus in iron and steel by converting it into a yellow complex phosphovanadomolybdate, which according to Misson (*2*), who first suggested this method, has the formula $(NH_4)_3PO_4.NH_4VO_3.16MoO_3$. The color is very stable and undergoes no appreciable color change after 14 days. Murray and Ashley (*3*) used the Pulfrich photometer and worked at 430 mμ. They found, however, that high silica interfered with the determination, because of the formation of yellow silicomolybdic acid.

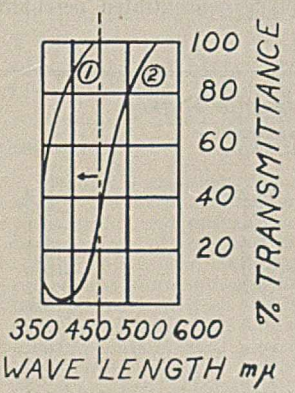

FIGURE 1. TRANSMITTANCE CURVES
1. Ferric perchlorate
2. Ferric chloride

Of all the methods studied this modification of Misson's method seemed the most promising, but it has two serious objections: (1) Iron ores are usually high in silica as compared to irons and steels, and therefore the formation of silicomolybdic acid would interfere seriously; (2) the iron content of ores varies considerably more than that of irons and steels, and since ferric chloride shows a large absorption of light at 430 mμ, the interference of iron would be serious. The method which is described here eliminates both of these difficulties.

Experimental

In attempting to determine phosphorus by measuring the intensity of a reduced solution of molybdenum blue, it was found that the color which had once been developed could not be diluted, since its intensity depended on the pH, and that a variation in the concentration of stannous chloride added as a reducing agent caused a change in the color. Moreover, considerable time was required for the maximum color to develop. For these reasons this method was abandoned.

It was necessary to find a reagent which would completely remove the silica and would also remove the yellow color of ferric iron. The hydrochloric acid solution of the ore was therefore evaporated with perchloric acid, which dehydrated the silica and rendered it easily filtered (*4*). The resulting ferric perchloric solution is practically colorless and at the same time the acidity of the solution is correct for the formation of the yellow complex.

Apparatus

A Coleman Model 10 regional spectrophotometer was used to measure the amount of monochromatic light transmitted through the yellow solution. The width of the spectral band used was 30 mμ. A blank containing iron but no phosphorus was first placed in the path of the light and the instrument was balanced. This was then replaced by the sample and the percentage of light transmitted as compared to the blank was measured.

It was necessary to allow the lamp to warm up for 10 minutes before making any measurements.

The effect of perchloric acid in removing the yellow color characteristic of ferric chloride is shown in Figure 1, in which curve 2 shows the transmittance for ferric chloride solutions and curve 1 for ferric perchlorate. It will be noted that conversion of iron into perchlorate has moved the region of maximum transmittance into the ultraviolet and this colorless solution now offers no interference in measuring the transmittance of the yellow phosphovanadomolybdate complex.

The nearer the ultraviolet region is approached the more interference by iron can be expected. On the other hand, if 550 mμ is approached a difference in the concentration of phosphorus makes very little difference in the percentage of light transmitted, as shown by Figure 2. Therefore, 450 mμ was chosen as the correct wave length because it is as near to the ultraviolet as possible without interference from the iron content.

Effect of Varying the Concentration of Perchloric Acid

Past a certain minimum the maximum color was developed at a low acid concentration. If more than 13 ml. of perchloric acid was used the color did not develop a full maximum. Less than that amount allowed the formation of a precipitate on the addition of ammonium molybdate.

The amounts of ammonium vanadate and ammonium

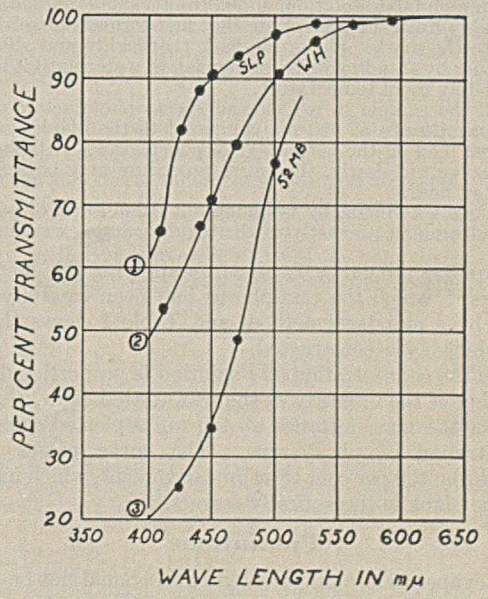

FIGURE 2. TRANSMITTANCE–WAVE LENGTH CURVES FOR THREE IRON ORES
Phosphorus (1) 0.019 per cent, (2) 0.0705 per cent, (3) 0.243 per cent

[1] Present address, Oliver Iron Mining Company, Hibbing, Minn.

molybdate were always in excess of the calculated values for a 0.500-gram sample high in phosphorus.

Reagents

AMMONIUM VANADATE SOLUTION. To 2.350 grams of ammonium metavanadate dissolved in 500 ml. of hot distilled water add 20 ml. of 1 to 1 nitric acid and dilute to 1 liter.

AMMONIUM MOLYBDATE SOLUTION. Dissolve 50 grams of molybdic acid in a mixture of 200 ml. of distilled water and 40 ml. of concentrated ammonium hydroxide. Filter the solution, boil the filtrate 20 minutes, and dilute to 500 ml.

TABLE I. DATA USED IN PLOTTING TRANSMITTANCE-CONCENTRATION CURVE

Sample	Phosphorus, Per Cent	Transmittance at 450 mμ
SLP standard, OIMC[a], National Bureau of Standards	0.019	90.6
11 R, OIMC[b]	0.030	87.0
28 Cargo, OIMC[b]	0.035	84.4
114 S, OIMC[b]	0.049	78.3
WH standard, OIMC, and other laboratories	0.0705	70.5
Soudan standard, OIMC, and other laboratories	0.118	57.1
39 MB, OIMC	0.149	50.8
03 MB, OIMC	0.196	40.2
29 MB, OIMC	0.224	35.9
52 MB, OIMC	0.243	34.4

[a] Oliver Iron Mining Co., Hibbing, Minn.
[b] Samples checked at OIMC for soluble and insoluble phosphorus.

Procedure

Weigh out a 0.5-gram sample of iron ore, place it in a 150-ml. beaker, add 10 ml. of concentrated hydrochloric acid, and heat the covered solution on a hot plate until the ore is dissolved. It may be necessary to add more hydrochloric acid. When the ore is in solution, evaporate the solution almost to dryness (do not bake), and add 13 ml. of 70 to 72 per cent C. P. perchloric acid. Boil the mixture on the hot plate until the dark ferric solution has changed to a straw yellow color. This usually takes 4 or 5 minutes, and at this point the sample should be fuming strongly. (If a Méker burner is used instead of a hot plate, the time required to reach fumes of perchloric acid can be reduced to 2 or 3 minutes.) Cool the solution slightly and add from a pipet 10 ml. of ammonium vanadate solution. Boil for about 30 seconds to remove any chlorine present, remove the beaker from the hot plate, and place it in a pan of cold water until it can be held in the hand. Wash off the cover glass and sides of the beaker with a little distilled water and filter the solution into a 100-ml. volumetric flask. Wash the beaker and paper with 15 ml. of ammonium molybdate solution, added from a small graduate, shake thoroughly until the precipitate that first forms is dissolved, and dilute to the mark. Shake the flask thoroughly and allow it to cool either by standing or by immersion in water until it is at approximately room temperature.

Place the sample in a Coleman spectrophotometer, measure the transmittance at 450 mμ compared to the blank, and from the curve read off the percentage of phosphorus. If the ore does not dissolve too slowly, the entire procedure may be completed in half an hour.

The blank is made by weighing out 0.255 gram of pure iron wire and running it exactly as the regular sample was run.

Figure 3 is the curve obtained by applying this method to ten ores in which the phosphorus had been carefully determined by a standard method, and Table I shows the data from which it was constructed.

Since the concentration of a solution is proportional to the logarithm of the intensity of the transmitted light, the logarithm of the transmittance at 450 mμ is plotted against the concentration of phosphorus. The curve passes almost through the 100 per cent transmittance point, which indicates that the blank is theoretically correct.

Precautions

The evaporation with perchloric acid should not be carried beyond the color change because acid will be lost by evaporation and if the acidity of the solution is too low the precipitate first formed on the addition of ammonium molybdate will not dissolve on shaking.

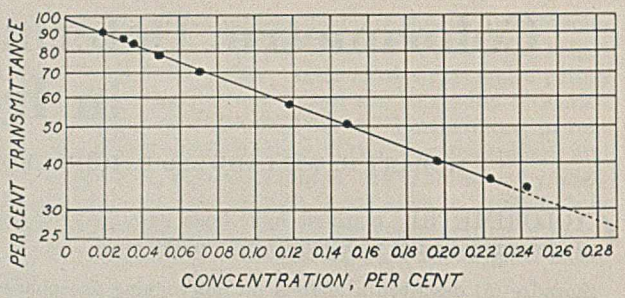

FIGURE 3. CONCENTRATION OF PHOSPHORUS *vs.* TRANSMITTANCE

After the development of the color the solution should be allowed to stand at least 4 minutes before measuring the per cent of transmittance.

If chlorine is not boiled off, it has a tendency to dissolve the filter paper and also causes variations in the intensity of the developed color.

Stannous chloride cannot be used to hasten the solution of the ore in hydrochloric acid because it causes a turbidity.

Reasonable care should be taken that no organic matter comes in contact with the boiling concentrated perchloric acid during the evaporation.

TABLE II. PRECISION OF METHOD

	Transmittance at 450 mμ	Deviation
	%	%
WH standard	70.2	−0.3
	69.1	−1.5
	70.4	−0.1
	71.4	1.1
	70.6	0.1
	70.2	−0.3
	70.7	0.2
	71.3	0.8
	Av. 70.5	
SLP standard	90.6	0
	90.8	0.2
	91.0	0.4
	91.0	0.4
	89.8	−0.8
	90.5	−0.1
	Av. 90.6	

Precision of the Method

Of the ten samples used in constructing the curve, two are selected to show the precision of the method (Table II). By the method of least squares the average deviation for the WH samples is calculated to be approximately 0.6 and that of the SLP samples 0.35. The two deviations are typical of all samples run.

On examining Figure 3 it is seen that for a sample low in phosphorus 1 per cent in transmittance corresponds to 0.002 per cent of phosphorus, and for a sample high in phosphorus 1 per cent transmittance corresponds to approximately 0.006 per cent of phosphorus. Thus for the WH standard the deviation would be, for high phosphorus, $0.006 \times 0.6 = 0.0036$ per cent phosphorus, and for low phosphorus, $0.002 \times 0.6 = 0.0012$ per cent phosphorus. The average is 0.0024. For the SLP standard the corresponding values are 0.0021 and 0.0007 per cent, the average being 0.0014 per cent. The average for both samples is, in round numbers, 0.002 per cent. This precision is about the same as that obtained by usual methods.

Summary

The colorimetric determination of phosphorus in iron ore by the phosphovanadomolybdate method has been greatly improved by converting the iron to colorless ferric perchlorate. This not only removes interfering silica but shortens the time required, and makes possible the use of the more favorable

wave length of 450 mμ. The transmittance at this wave length is determined and from a standard curve the percentage of phosphorus is calculated.

Acknowledgment

The authors are indebted to Eberbach & Son Co., Ann Arbor, Mich., for the use of the spectrophotometer.

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Basic Sulfates of Iron and Aluminum in Analytical Separations

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AS DESCRIBED in a previous paper (*3*), a solution of ferric sulfate was used to form a basic ferric alum with potassium which was thereby separated from cesium. In a similar manner, after the addition of potassium sulfate an excess of iron can be separated from divalent metals, which are then determined by the usual methods. In this procedure a basic sulfate of iron and potassium is formed at a definite pH and at steam bath temperature, 90° C. The precipitation of iron is nearly complete, while divalent metals remain in solution. A small part of any aluminum present is also precipitated.

Mellor (*7*) mentions as products of the hydrolysis of ferric alum a few basic sulfates of iron and potassium which vary in composition and are microcrystalline. Krueger (*5*) obtains a basic sulfate of iron and ammonium in separating iron from cobalt, nickel, zinc, and manganese. He fails to consider the presence of aluminum and completes the separation of iron as a basic acetate. Ardagh and Bongard (*1*) obtain good separations of nickel and zinc from iron and aluminum in a small volume of solution containing a little hydrochloric acid and 5 grams of ammonium chloride, to which strong ammonia is added in excess. Noyes and Bray (*9*) separate 2 to 50 mg. of nickel or zinc from 100 mg. of iron very satisfactorily by the above procedure, but with cobalt 1 mg. in 50 is caught in the precipitate of iron. This separation is troublesome and requires repetition, especially when excessive quantities of iron and aluminum are present. Lundell and Knowles (*6*) find that nickel only may be satisfactorily separated by the Blum method in a single precipitation. The writer has observed that the separation from aluminum is by far the more uncertain. Nickel only can be separated in a faintly acid solution containing ammonium sulfate.

Aluminum, like iron, forms a basic sulfate with potassium. A patent on basic alum has been issued to Fleischer (*4*), who heats a solution of alum above 60° C. in a continuous system. Titanium forms no double sulfate, as tested by experiment. According to Mellor (*8*) magnesium, zinc, or manganese may enter the basic sulfate molecule, which resembles alunite in composition but contains the same proportion of potassium sulfate as ordinary alum. Cobalt also forms a double sulfate with aluminum. Aluminum may be separated from beryllium by this method satisfactorily without the use of ammonium sulfate. Britton (*2*) obtains a 90 per cent separation of aluminum from beryllium as potassium alum.

Procedure

Treat a slightly acid solution of ferric sulfate, free from fluoride, chloride, and nitrate, with potassium acid sulfate and partially neutralize with dilute ammonia until a precipitate begins to persist. Heat this solution for several hours in a flask lowered directly into the steam bath; a dense, microcrystalline, orange-red precipitate is produced. Potassium sulfate yields a less soluble precipitate than the ammonium salt. The presence of titanium or phosphate renders subsequent filtration slow and the filtrate yellow with too much iron. In such a case, further neutralize the filtrate and repeat the hydrolysis. About 5 mg. of iron remain in solution. If appreciable quantities of titanium are present, first hydrolyze at 0.1 *N* acidity. Proceed in like manner for phosphate after adding titanium sulfate.

DETERMINATION OF ZINC AND NICKEL. Place the solution of iron and zinc sulfate in a 500-ml. Kjeldahl flask, add potassium acid sulfate equal in weight to the ferric oxide probably present, dilute to 300 ml., and slowly add dilute ammonia until a slight permanent precipitate remains. Place a small funnel in the neck of the flask and heat to 90° C. in the steam bath for at least 4 hours, or overnight. Hydrolysis with a wired-in stopper yields a more granular basic sulfate. Filter while hot. The filtrate should be colorless and have a pH close to 2.8, just yellow to thymol blue. Evaporate the filtrate and wash water to 150 ml. Filter off any precipitate of iron, add methyl orange, and neutralize until the color just remains red, at a pH of 3.1, which is about right for the precipitation of zinc sulfide. Transfer the solution to an Erlenmeyer flask, add 2 ml. of 5 per cent mercuric chloride solution, and precipitate zinc and mercury with hydrogen sulfide. Ignite the filtered and washed sulfides and weigh as zinc oxide in the usual way. If cobalt or nickel was originally present the precipitation of zinc may require repetition.

After removing the greater part of the iron as above, concentrate the filtrate and wash water to 300 ml., add methyl orange, and neutralize the filtrate until faintly red. Add 5 grams of ammonium sulfate and hydrolyze in steam for at least 4 hours. This second hydrolysis removes more iron, together with half of any aluminum present. Ammonium sulfate keeps nickel in solution. Concentrate the filtrate from aluminum to 150 ml., add 2 grams of tartaric acid, and precipitate nickel with dimethylglyoxime in the usual way.

The hydrolysis of ferric sulfate begins at about pH 1.2 and some iron remains in solution at pH 3.4. This figure was first calculated, then verified after the hydrolysis by direct determination in a pH meter. As the pH is increased to 3.8 the basic sulfates become slimy and filter slowly. At pH 3.8 the solubility for either iron or aluminum is still about 5 mg.

The hydrolysis of aluminum sulfate begins at about pH 2.8 and is one-half complete at pH 3.1. The solubility at 3.4 in terms of aluminum oxide is increased to 35 mg. in the presence of 10 grams of ammonium sulfate.

In the analysis of meteorites, stainless steel, and some minerals, a few per cent of the divalent metals are present with large percentages of iron or both iron and aluminum. Numerous quantitative tests were made of the behavior of such combinations involving cobalt, nickel, zinc, or beryllium.

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Biometry in the Service of Biological Assay

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SOME chemists may wonder how biometry, or statistics applied to biology, is involved in the biological assay of drug potency. The biometrician or statistician on the other hand may be equally puzzled that the pharmacologist and chemist have been willing to work for so long without his help. Sometimes he is asked to evaluate experimental data after the laboratory work has been completed and the possibility of modifying the original technique has passed. This is no more logical than to hold an analytical chemist responsible for determining the original sugar content of a sirup which may have partly fermented before it reaches him, owing to improper preservation. To realize its full possibilities, statistical control must also start from the beginning, with the original experimental design. Then modern statistical methods may be expected to give more precise results with fewer animals and a measure of just how far a given determination should be trusted, whether it occurs in a routine assay or in developing a new medicinal product.

For a quantitative approach to biological assay, one must first adjust himself to an order of variation in the original measurements far greater than that customary in analytical chemistry. Because in most cases this variation agrees closely with the normal curve of error, many advantages are at once available to the biologist. One distinction must be emphasized. Consistency with the normal curve of error does not imply a faulty technique on the part of the experimenter or a mistake in his measurements. This is an inherent characteristic of many animal populations and the more carefully the animals are selected and handled and the more accurately each response is measured, the more nearly they will adhere to the normal curve of error.

All-or-None Effects of Drugs

The normal curve can be demonstrated most readily from measurements of the toxicity of the digitalis glucosides to individual cats by slow intravenous infusion.

In a long series of tests at the Lilly Research Laboratories, a single worker infused the laboratory standard of digitalis or Digiglusin simultaneously into each of 4 cats until its heart stopped. The results on 52 of these groups of 4 have been reported recently by Bliss and Hanson (*3*). In their group No. 2, for example, the 4 replicated analyses gave the following lethal doses: 29.6, 25.8, 29.2, and 19.9 cc. per kg. of cat. An analytical chemist might consider the first and third replicates in satisfactory agreement, the second questionable, and the fourth probably an error in analysis. Let us take another set of 4 from the same series, No. 52, for example, where the 4 replicates gave 28.1, 26.2, 22.5, and 34.4 cc. per kg., respectively. Here it would be more difficult to separate the "good" from the "bad" determinations and it is evident that a rule-of-thumb approach is not of much use. Instead we will assume that mistakes in titrating the individual cats were negligible when compared with the variations in their susceptibility to the drug and center our attention upon the nature of this variation. For such a study selected groups of 4 are clearly less satisfactory than an analysis based upon the entire series without selection.

The first question concerns the unit of computation, for any unit which may be adopted commits us at once to specific biological assumptions as to the nature of digitalis action. The better the basis for these assumptions, the greater is the chance that the computation will lead to a satisfactory conclusion. Digitalis acts directly upon heart tissue and in relating the quantity of drug which poisons the heart of each individual to its gross body weight, it is assumed that the mass of specific heart tissue is directly proportional to that of the rest of the animal. For the present data this has proved to be a valid assumption. However, if the dose in cubic centimeters of extract per kilogram is used directly in arithmetic units, we assume further that the effect of a given increase in dosage is constant, regardless of an individual's inherent susceptibility or of previous dosage. The weight of pharmacological evidence shows that to obtain a uniform increment in effect, the dosage of most drugs must be increased by a constant proportion or percentage. It is better, therefore, to base our analysis upon this more likely assumption by transforming the just-lethal dose for each cat to logarithmic units. If the assumption is ill-founded, statistical analysis will disprove it.

Only 4 cats were tested in parallel, although the 52 assays covered a period of 18 months. More often than not, the susceptibility of animals to the same sample of drug varies from one test day to the next. To control these unpredictable variations pharmacologists have adopted standard preparations for many drugs with which new samples can be compared by running both at the same time. If the reaction of cats to digitalis were to vary similarly, the means of the groups of 4 animals should vary more than would be expected from the variation within groups of 4. This possibility has been tested by one of the most flexible and valuable of all statistical tools, the analysis of variance. The differences between the log-dose per kilogram for each individual cat and the general mean for all 52 × 4 or 208 cats are squared and summed to obtain a total sum of squares which is then separated into two portions, that due to differences between the groups of 4 and that arising from variations in susceptibility within these groups. In terms of squared deviations these sources of variation are additive and we have the separation shown in Table I, where the mean square or variance

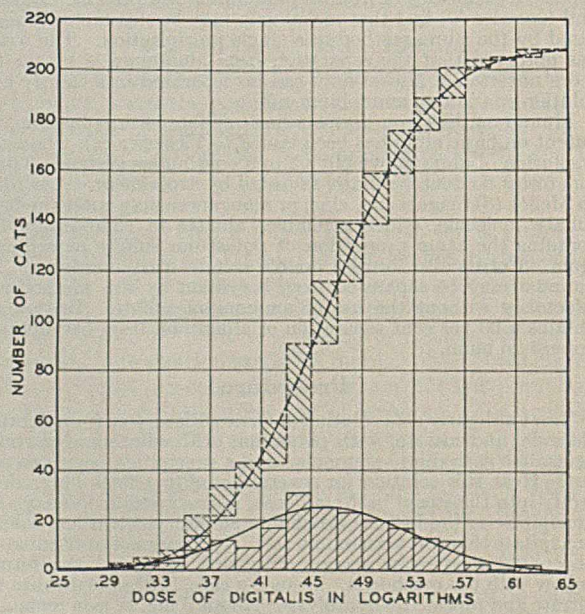

FIGURE 1. VARIATION IN SUSCEPTIBILITY OF INDIVIDUAL CATS TO DIGITALIS AFTER CORRECTION FOR DIFFERENCES BETWEEN DAYS

From Bliss and Hanson (*3*)

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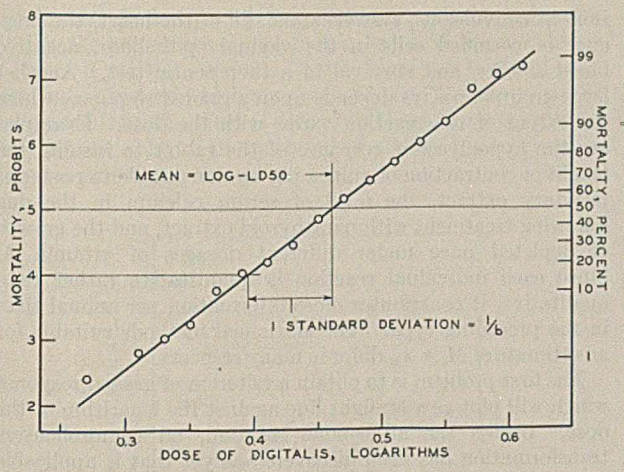

FIGURE 2. CUMULATIVE CURVE OF FIGURE 1 PLOTTED IN LINEAR FORM

between assays is 2.44 times as great as that within assays or groups of 4, a difference which would not be expected by chance alone even once in 1000 similar experiments. By avoiding the larger variation between groups and always comparing an unknown with a standard upon cats tested in parallel, it is apparent that fewer animals will suffice in assaying the potency of an unknown in terms of the laboratory standard of digitalis for any required degree of precision. The same principle holds throughout all biological assay.

TABLE I. PARTITION BY ANALYSIS OF VARIANCE OF VARIATIONS IN TOXICITY OF DIGITALIS TO CATS

[In terms of deviations from the mean log-dose in cc. per kg. of cat; data from Bliss and Hanson (3)]

Variation	Degrees of Freedom	Sum of Squares	Mean Square or Variance	Variance Ratio	Standard Deviation
Between assays	51	0.52992	0.010391	2.44	0.10193
Within assays	156	0.66478	0.004261	1	0.06528
Total	207	1.19470			

We are now ready to examine the nature of the variation in susceptibility between cats. The differences between the group means and the general mean have been multiplied by the ratio of the standard deviation ($= \sqrt{\text{mean square}}$) within assays to that between assays and these corrections added to or subtracted from each individual measurement to bring all of the determinations to a common basis. Then they could be studied just as if all 208 cats had been run simultaneously with the same precision as was obtainable from groups of 4. The number of cats dying at adjusted doses within each of 20 equal log-dose intervals has been plotted along the base of Figure 1 in a block diagram. The smooth curve drawn through the tops of these blocks is the computed normal curve of error. It evidently fits well, as can be shown also by statistical test. The fact that the variation in susceptibility is symmetrical and normal when measured in terms of the logarithm of the just-lethal dose justifies the original assumption underlying the transformation to logarithms.

By a simple transformation the "frequency distribution" along the base of Figure 1 can also be plotted as a straight line and in this form it is comparable to the much larger group of bioassays for which the just-lethal dose cannot be measured individually. The data are converted first to a cumulative form by moving the block for each dosage interval along the base of Figure 1 vertically upward until its lower edge is contiguous with the upper edge of the next preceding block. They then describe a symmetrical sigmoid curve conforming to the cumulative normal curve of error which intersects them. From the intersection of each observed cumulative number of cats with the sigmoid normal curve in Figure 1, one could interpolate in abscissal units a log-dose predicted from the response. Plotting these predicted log-doses against those observed experimentally should define a straight line if the data conform to the normal curve.

The same result can be accomplished more easily. Frequencies are changed to the percentage of individuals reacting at all dosages up to and including each successive log-dose. Then these percentages of effect can be transformed by tables based upon the normal curve to hypothetical "dosages" in units of the standard deviation, such as the "normal equivalent deviations" of Gaddum (7) or the "probits" of Bliss (1). When these are plotted against the corresponding observed log-doses, a straight line should result as in the data replotted in Figure 2 from Figure 1. When the points fall along a straight line, the mean of the observed log-doses is the log-LD50 or the log-dose for 5 probits or 50 per cent kill and the reciprocal of their standard deviation fixes the slope of the line from which the log-dose can be interpolated for any required percentage effect or vice versa.

In most assays depending upon an all-or-none reaction, the just-effective dose cannot be determined for each individual and separate lots of animals are treated uniformly with predetermined dosages of drug. The positive reactors in each lot, expressed as a percentage, include of course those that would have reacted at all smaller doses. Hence the initial data are recorded in a cumulative form and the relation between percentage effect and log-dose of drug is typically a symmetrical sigmoid curve. A good example has been reported by Morrell, Chapman, and Allmark (10) on the therapeutic assay of neoarsphenamine from the incidence of negative blood smears in the male rat. Six series have been adjusted for differences in over-all susceptibility, plotted in Figure 3, and fitted by the cumulative normal curve. Statistical test shows that these observations agree with the curve rather better than would be expected by chance. Yet even the present amount of scatter would inject an appreciable subjective error in a sigmoid curve drawn through these points merely by inspection. By transforming percentages to probits the same data can be plotted as shown in Figure 4 and fitted by the simplest curve of all—a straight line. Moreover, the reliability of the curve or of the log-LD50 or

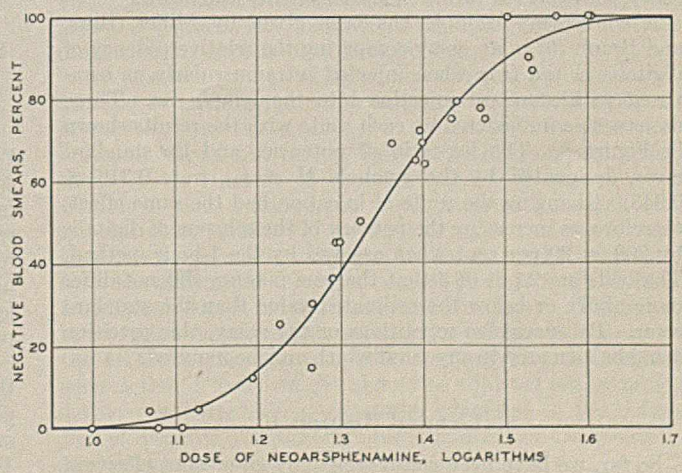

FIGURE 3. SIGMOID DOSAGE-EFFECT CURVE FOR THERAPEUTIC ACTION OF NEOARSPHENAMINE IN MALE RATS

From data of Morrell, Chapman, and Allmark (10). Six independent curves superimposed by adjustment of dose.

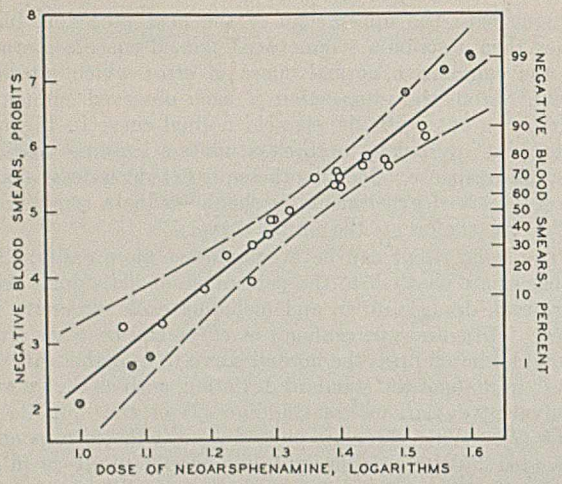

FIGURE 4. DOSAGE-EFFECT CURVE OF FIGURE 3 PLOTTED IN LINEAR FORM

Zone of error, designated by broken lines, shows expected precision for one of six component series.

of any other point upon it can be estimated for any given odds, as shown in the diagram by the broken parabolas. Here they indicate the average limits which would apply to one of the 6 series, involving 5 lots each of 20 to 25 rats, and would enclose the true curve in 19 out of 20 determinations ($P = 0.05$).

Since the biological indicators of drug action vary in an orderly manner, we can use these curves and the techniques for their computation to measure relative potency. Two or more dosages of both standard and unknown are administered and the data plotted separately on linear coordinates. If the unknown produces the same biological effect as the standard, their respective curves should be parallel within the limits of the experimental error; if the unknown has the same potency as the standard, the two parallel lines should coincide, again within the limits of the experimental error. When the curves do not coincide but are parallel, the horizontal distance between them, of course, is the same at all levels of effect and, being a difference on a logarithmic scale of dosage, measures in logarithms the ratio of their potencies. By adjusting the concentration of the unknown, its potency can be made equal to that of the reference standard. Since the error of the ratio is computed as an integral part of the assay, the expected variation in this potency will be known.

A convenient example has been given by Miller, Bliss, and Braun (*9*) in an assay comparing the relative potency of digitalis in the frog when injected intramuscularly as compared with standard injection into the lymph sac. Three doses were administered by each route with the results shown in Figure 5. The log-ratio of potencies and its standard error, designated by the symbols $M \pm s_M$, were 0.319 ± 0.043. Changing the route of injection had the same effect, therefore, as increasing the potency of the solution of digitalis by 209 ± 20 per cent when assayed by the 1-hour method. The odds are 2 out of 3 that the true potency did not differ more above or below the estimated value than the standard error. By successive repetitions of the assay, this precision could be increased to any point which may be required.

Graded Response

So far, we have been considering only all-or-none effects of drugs, where a given reaction is either present or absent. Although perhaps death occurs more frequently than other end points, the method has been applied to many criteria, such as convulsions, systolic standstill of the heart, the presence of cornified cells in the vaginal epithelium, negative blood smears, and survival in a therapeutic test. Another large group of assays depends upon a graded response, where the extent of the reaction varies with the dose. Examples are the hypoglycemic response of the rabbit to insulin, the height of contraction of guinea pig uterine muscle to posterior pituitary extract, the level of serum calcium in the dog following treatment with parathyroid extract, and the growth of depleted mice under different dosages of vitamin A. Since each individual reaction is quantitative rather than qualitative, it contributes more information per animal than in the preceding type. The statistical methods suitable for an estimate of $M \pm s_M$ differ in many respects.

The first problem is to obtain a criterion of graded response which will plot as a straight line against the logarithm of the dose. Unlike the all-or-none reaction, no comprehensive transformation has been discovered as yet that is applicable to a wide variety of responses. Since the effect that it is practicable to measure is usually an empirical composite of several factors, which differ widely from one type of drug to another, a transformation as general as Gaddum's N. E. D. or the probit is relatively improbable. When graded reactions are studied over a wide enough range of dosages, they tend to flatten out toward a lower limit or floor and toward an upper limit or ceiling, leading to a sigmoid form. Frequently, however, an extended central section does not differ appreciably from a straight line or other factors intervene, so that the curved portion approaching a limit is never reached.

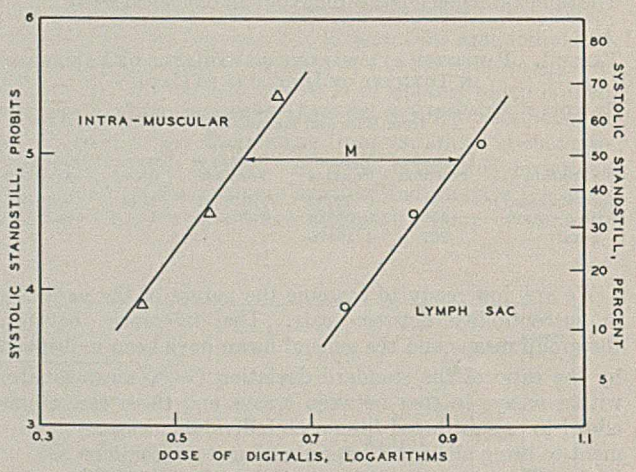

FIGURE 5. ASSAY OF EFFECT OF ROUTE OF INJECTION UPON THE POTENCY OF DIGITALIS

Data of Miller, Bliss, and Braun (*9*)

When dosages are restricted to this central section, the data can be handled by a flexible statistical procedure known as factorial analysis, which has been adapted for biological assay by Bliss and Marks (*4*). Factorial analysis not only tests whether the dosage-response curves for standard and unknown can be considered as parallel straight lines but leads directly and easily to an estimate of the log-ratio of potencies and its error, $M \pm s_M$.

Factorial arrangements have the added advantage that they are usually coupled with designs that screen out important sources of potential error, leading to unbiased estimates of drug action and marked improvements in precision. Two statistical techniques contribute to this end. The first is the balancing of known and suspected variations in susceptibility between individual animals, litters, days of the experiment, and other qualitative sources, so that they occur

equally at all dosages of the standard and the unknown. Within these restrictions the assignment of dosages to living material is strictly random. Then when the test has been completed, factors which are extraneous to the assay can be segregated from the effect of dosage and from the experimental error by the analysis of variance, usually with a marked increase in precision.

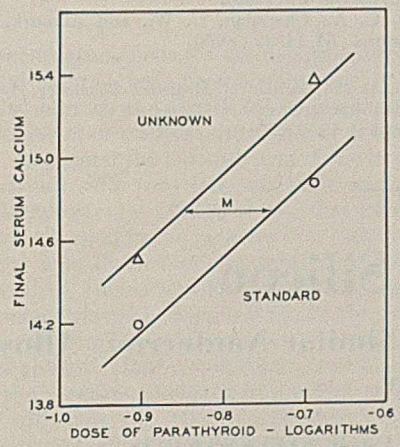

FIGURE 6. ASSAY OF AN UNKNOWN SAMPLE OF PARATHYROID EXTRACT FROM THE SERUM CALCIUM OF THE DOG
Data of Bliss and Rose (*5*)

The second technique is the use of covariance to correct graded variations in initial or concomitant measurements from the internal evidence of each assay. In the past these have been adjusted by arbitrary corrections, often involving unrecognized and doubtful assumptions which sometimes may even increase the error.

TABLE II. ANALYSIS OF VARIANCE OF A PARATHYROID ASSAY

[Arranged in five 4 × 4 Latin squares, in terms of the final calcium level 16 hours after injection; data from Bliss and Rose (*5*)]

Cause of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio
Differences between days	3	0.941	0.314	...
Differences between dogs	19	43.188	2.273	4.49
Unknown *vs.* standard	1	3.280	$3.280 = D^2$	6.49
Slope of dosage-response curve	1	11.705	$11.705 = B^2$	23.14
Departure from parallelism	1	0.162	0.162	...
Experimental error	54	27.312	$0.506 = s^2$	1
Total	79	86.588		

An example of an arbitrary correction is the rise in the serum calcium of the dog as a criterion for the assay of parathyroid extract. First segregating the differences between individual dogs, which can be used repeatedly, Bliss and Rose (*5*) showed by covariance that all the information needed for the assay is contained in the final measurement of serum calcium 16 to 18 hours after injecting the parathyroid extract and nothing is contributed by a knowledge of the initial level prior to treatment. Here statistical analysis has demonstrated that the number of bleedings and of chemical analyses can be cut in half without any loss of precision. Another instance is furnished by the assay of vitamin D from the bone ash of rats, where the use of the percentage ash as the criterion of response requires an unproved assumption as to the relation between the organic matter and the ash in the bone. By shifting from the percentage ash to the log-weight of ash for measuring the activity of vitamin D, Bliss (*2*) showed by covariance that the weight of bone lost in ashing contributed negligibly to the assay and that the estimate of potency was improved to the same extent as if twice as many rats had been used.

The numerical form of some of these procedures may be illustrated by the assay of parathyroid extract from the serum calcium of 20 dogs as recorded 16 hours after injection, each tested with 2 doses of standard and 2 doses of unknown in a Latin square design on 4 different days. The analysis of variance is given in Table II. To judge the relative importance of each factor in the assay, we are especially interested in the ratio of its mean square to that for error. It is evident that the dogs differed but little in their mean serum calcium on the 4 days of this particular test, but that precision was improved substantially by segregating the over-all differences between individual dogs. Since the departure from parallel dosage-response curves for standard and for unknown had a smaller mean square than the error, the assay was clearly a valid one, as shown graphically in Figure 6. The variance ratio shows that the unknown produced a significantly greater effect than the standard. Its estimated potency was computed readily from D, B, and s as $M = 0.1137 \pm 0.0505$ or 130 ± 15 per cent.

Many other applications of statistical methods to biological assay still remain and there are many possibilities yet to be explored. All the above illustrations have been drawn from experiments upon laboratory animals, but in the final analysis, no drug or assay technique can be passed until it has been checked clinically. Clinical experiments offer opportunities for improved statistical design and control fully as great as those conducted in the laboratory. In many cases relatively small changes in procedure would enable the clinician to double the reliability of his evidence or to obtain quantitative conclusions from what would otherwise constitute a roughly qualitative result. Time does not permit a further examination of this topic.

Characteristics of a Valid Biological Assay

1. Different samples of the same drug must show the same relative potencies in biological assay as under clinical test. Since the products assayed biologically are frequently complex or impure mixtures, the patient may not react to the same components as the laboratory test animal. Gold and Kwit (*8*), for example, have shown a threefold difference in the reaction of man to related cardiac glucosides judged as equipotent when tested in cats and frogs.

2. On the coordinates used for biological assay, the curve relating response to log-dose should be a straight line and relatively steep when compared with the variation about the line. Either the curve should have been shown to have a constant, known slope by repeated test over a considerable period of time or the slope should be determined as an integral part of each assay. Assumed relations between dose and effect are to be avoided.

3. The potency of the unknown or sample should be determined by comparative test with a stable reference standard and expressed in units of this standard. In a determination of potency the biological reaction has the same function as a chemical indicator and biological terms, such as the "cat unit" for digitalis, have neither the uniformity nor the correct dimensions for expressing quantity of drug.

4. The living material exposed to different doses of standard and of unknown must be as nearly equivalent as it can be made. Potential sources of variation, such as differences between individuals, litters, dates of treatment, and sexes, should never coincide or be confounded with differences in treatment but within these limitations the dosages and samples must be assigned at random. The analysis of variance or an equivalent technique should be used to segregate from the estimate of error the sources of variation that have been quarantined by the design of the assay. Variations in an initial measurement, such as the initial blood sugar in the rabbit insulin assay, or in a concomitant measurement, such as that of body weight, should be adjusted not by an assumed relation which is sometimes concealed in the definition of response but rather from the internal evidence of self-contained experiments by covariance.

5. A determination of potency should always include an estimate of its error, computed as an integral part of the assay. Not only do the assays of different drugs vary widely

in their average precision—one of those in the British Pharmacopœia (*6*) has limits of 92 to 108 per cent (Staphylococcus antitoxin), another limits of 37 to 272 per cent (vitamin A)—but the precision of the assay of a single drug varies from laboratory to laboratory and from one run to another in the same laboratory. No assay with an indeterminate error can be considered satisfactory.

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Determination of Silicon

In Monel Metal, Copper-Silicon Alloys, and Similar Nonferrous Alloys Containing Silicon

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IN THE determination of silicon in Monel metal, copper-silicon alloys, and similar nonferrous alloys containing silicon, a rapid, accurate method, to replace the longer and more commonly used methods of dehydration by nitric-sulfuric acids or hydrochloric acid and potassium chlorate, is needed for the analysis of a large number of samples. Little attention appears to have been paid to the use of perchloric acid for the dehydration of silica in these materials. Willard and Cake (*2*) proposed the use of perchloric acid as a dehydrating agent in silicon determination. Both they and Fowler (*1*) have pointed out the disadvantages of nitric, sulfuric, and hydrochloric acids in the dehydration of silica in steels, silicates, and some other metals and alloys. These same disadvantages (slow evaporation, slow solution of soluble salts, bumping and spattering of the solution, and the necessity of recovering silica from filtrates) apply to Monel metal and other nonferrous alloys containing silicon.

The following procedure, evolved by this laboratory gives accurate results in from 2 to 3 hours, eliminates the disadvantages of the nitric-sulfuric acid and hydrochloric acid procedures mentioned above, and avoids the bumping that occurs when the metal is first dissolved in nitric acid and then fumed with perchloric acid.

Procedure for Monels

Weigh 2 grams of fine drillings into a 400-ml. Pyrex beaker, add 25 ml. of 70 per cent perchloric acid and 1 ml. of concentrated nitric acid, place the beaker on a hot plate, and heat until solution of metal is complete. Add a few more drops of nitric acid if necessary to ensure complete solution. Place the beaker on an extremely hot electric or gas plate and boil the solution for about 10 minutes to ensure the complete dehydration of silica. The beaker may be heated over an open gas flame towards the end of this period and the contents of the beaker slowly revolved. Cool slightly, add water, and heat until all soluble salts are dissolved, boiling the solution at the end. Filter hot through a Whatman No. 41H or similar filter paper. Wash 5 times alternately with a solution containing a mixture of 1 per cent nitric acid and 1 per cent hydrogen peroxide, and with boiling water.

Remove filtrate for the subsequent determination of other elements and wash the silica residue with hot 10 per cent hydrochloric acid and boiling water alternately 5 times and then with water until free from acid. Place filter paper in a 30-ml. platinum crucible, ignite, cool, and weigh. Volatilize silica with hydrofluoric acid plus 3 drops of sulfuric acid, ignite again, cool, and reweigh. The loss in weight is silica (SiO_2). The residue remaining in the crucible will consist of traces of nickel, iron, and titanium. This can be fused with pyrosulfate, dissolved in water, and added to the filtrate. Some platinum will be introduced during this fusion and will be subsequently deposited with the copper and weighed as such. This has been found to be extremely small and in routine analysis can be disregarded.

The filtrate from the silicon determination can be used for the determination of copper, iron, and titanium by the following procedure:

Boil filtrate strongly to remove free chlorine, and add 1 ml. of concentrated nitric acid and 5 ml. of concentrated sulfuric acid. Keep the volume of solution at approximately 300 ml. Electroplate copper on the platinum cathode at 0.5 ampere and 3 volts. After copper is removed, make the solution weakly ammoniacal and determine iron and titanium in the usual manner. Excess ammonia will form a precipitate with perchloric acid.

Table I gives results by the three methods for determining silicon in samples of Monel metal. In each instance the filtrates were evaporated to dryness twice to recover silica. No silica was found in the filtrates from the perchloric-nitric acid treatment. In the other two cases sufficient silica was found to make these evaporations necessary.

TABLE I. DETERMINATION OF SILICON

	Perchloric-Nitric Acid	Sulfuric-Nitric Acid	Hydrochloric Acid–Potassium Chloride
	%	%	%
Sample 1	3.02–3.03	2.99–3.01	3.07–3.10
Sample 2	1.49–1.49	1.50–1.46	1.48–1.46
Sample 3	3.08–3.09	3.04–3.19	3.08–3.12

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THE views presented in this article are those of the writers and should not be construed as the official views of the Navy Department.

Determination of Ascorbic Acid in Citrus Fruit Juices

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THE chemical methods for the determination of ascorbic acid (vitamin C) have been mainly based on the method of Tillmans, Hirsch, and Hirsch (*5*) employing 2,6-dichlorophenolindophenol or on some modification of the iodometric titration. In general the iodometric methods have been subject to criticism since they lack specificity and a sharp end point, although Stevens (*3*) has shown that by employing a double back-titration in the presence of a high concentration of hydrogen ions the iodine end point may be made very sharp. Further, Tauber and Kleiner (*4*) have demonstrated that the iodometric method is adequate for the determination of ascorbic acid in citrus fruit juices, since interfering substances are absent. However, in common with all iodometric methods, the reagents employed in Stevens' method change their titer with time, requiring frequent standardization, and the double back-titration increases the volumetric error.

In the method presented here, a direct titration with iodate in acid-iodide gives an end point comparable in sharpness with that of the above double back-titration method, and in addition avoids the necessity for frequent standardization. Only one accurately prepared solution is required, and this is stable over long periods of time.

In addition to the iodate titration described below, the ascorbic acid content of the citrus juices was determined by the method of Stevens (*3*). The juices were also titrated by the Mack and Tressler (*1*) modification of the Tillmans method (*5*). The indophenol dye, recrystallized from the Eastman product, was standardized by the method of Menaker and Guerrant (*2*). All the reagents used in the three methods were referred to a single primary standard solution, 0.1 *N* potassium iodate.

REAGENTS. Standard iodate, 0.1 *N* (0.0167 *M*), contains 3.567 grams of potassium iodate per liter. For use this stock solution is diluted to 0.01 *N*.

Potassium iodide, 10 per cent solution fresh daily.

Sulfuric acid, 2 *N*.

Starch indicator is made fresh daily from improved Lintner's soluble starch, using 1 gram per 100 ml. with 2 grams of potassium iodide added.

PROCEDURE. Add to 5 ml. of citrus fruit juice 1 ml. of 10 per cent potassium iodide and 2 ml. of 2 *N* sulfuric acid. Titrate the resulting solution with 0.01 *N* iodate, adding the reagent dropwise near the end point. It is best not to add the starch until very near the end point.

1 ml. of 0.01 *N* iodate = 0.88 mg. of ascorbic acid.

Results and Discussion

A preliminary comparison of the three methods employed for the determination of ascorbic acid was carried out on solutions of synthetic, crystalline ascorbic acid (Merck). The results, presented in Table I, show that the indophenol and the iodate titration are in agreement, although the absolute amounts recovered were slightly high. On the other hand, the double back-titration of Stevens gave slightly low values for the ascorbic acid content.

Analyses on citrus juices are recorded in Table II. The results obtained by the Stevens and iodate methods are compared with the ascorbic acid content as determined by the Mack and Tressler indophenol titration as a standard. Stevens' method gives variable results that differ rather widely from those determined by the indophenol method, being up to 12 per cent too high. On the other hand, the ascorbic acid content as determined by the iodate method agrees with that found by the indophenol titration. On the basis of these results the iodate method can be recommended for routine determinations of ascorbic acid in citrus juices.

The iodate titration has the following advantages over the double back-titration method of Stevens: increased accuracy, stability of reagents, greater simplicity and rapidity, and more reproducible results.

TABLE I. DETERMINATION OF ASCORBIC ACID

Ascorbic Acid Taken	Indophenol Titration		Stevens' Iodine Titration		Iodate Titration	
	Ascorbic acid found	Recovery	Ascorbic acid found	Recovery	Ascorbic acid found	Recovery
Mg.	*Mg.*	%	*Mg.*	%	*Mg.*	%
1.75	1.81	103.4	1.80	102.8	1.77	101.7
2.00	2.09	104.5	1.88	94.0	2.08	103.9
2.00	2.09	104.5	1.90	95.0	2.07	103.6
3.00	3.10	103.3	2.96	98.6	2.97	99.0

TABLE II. ANALYSES OF CITRUS JUICES

Material	Indophenol Titration	Stevens' Iodine Titration		Iodate Titration		
			Difference from indophenol titration		Difference from indophenol titration	Difference from Stevens' iodine titration
	G./100 ml.	*G./100 ml.*	%	*G./100 ml.*	%	%
Lemon juice, 1	0.0460	0.0470 0.0471	2.1	0.0460 0.0461	0.0	−2.1
Lemon juice, 2	0.0427	0.0465 0.0461	8.4	0.0434 0.0438	2.1	−6.2
Lemon juice, 3	0.0462	0.0460	− 0.5	0.0462	0.0	0.5
Lemon juice, 4	0.0396	0.0397	0.2	0.0392	−1.0	−1.2
Lemon juice, 5	0.0416	0.0458 0.0448	8.8	0.0424	1.9	−6.4
Orange juice, 1	0.0416	0.0420 0.0420	0.9	0.0419 0.0417	0.5	−0.4
Orange juice, 2	0.0388	0.0390 0.0388	0.2	0.0387 0.0387	−0.3	−0.5
Orange juice, 3	0.0399	0.0346 0.0344	1.7	0.0342 0.0341	0.6	−1.1
Grapefruit juice, 1	0.0424 0.0430	0.0461 0.0470	9.8	0.0446 0.0442	4.0	−4.5
Grapefruit juice, 2	0.0370 0.0370	0.0412 0.0416	11.8	0.0372 0.0378	1.4	−9.4

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Determination of Bromine Addition Number

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A method is described for indicating degree of unsaturation quickly, by direct titration of sample dissolved in chloroform with a standard solution of bromine in glacial acetic acid, using the color of the bromine itself as indicator. It has given correct results when applied to pure hydrocarbons of known unsaturation, such as cyclohexene, diisobutene, triisobutene, etc., and reproducible results on unknowns. It was found that the solvents used in the analysis are factors affecting the results.

The method may also find utility in fat analysis.

SINCE it is practically impossible to separate the numerous components of complex hydrocarbon mixtures, such as naphtha and gasoline, one must still be content with an analysis that indicates the proportions of classes of hydrocarbons. Methods have been published for analytically resolving the components of gasoline into four major groups—unsaturates, aromatics, naphthenes, and paraffins. A good degree of accuracy is frequently claimed for such methods of analysis when the latter three classes comprise the gasoline; much less is claimed when unsaturated hydrocarbons are also present.

Modern processes for manufacturing gasoline have made unsaturated hydrocarbons more important than ever before, and accurate knowledge of degree of unsaturation an urgent requirement.

The many published methods for determining unsaturation of oils, etc., fall into two major groups:

1. Those in which the unsaturates are dissolved or chemically transformed and separated, such as methods involving the use of mercuric acetate (*15*), sulfur chloride (*2*), and sulfuric acid (*17*).
2. Methods based on reactions with halogen, in which unsaturates are not separated but merely reacted for titrations.

None of the methods in group 1 is entirely satisfactory. Brame and Hunter (*1*) found the mercuric acetate (*15*) method unreliable because different unsaturated hydrocarbons react differently with the reagent. The sulfur chloride method (*2*) is rather involved for routine use, and procedures which employ sulfuric acid (*17*) are particularly unsuited for modern cracked gasoline because of alkylation reactions which sulfuric acid may induce (*7*), causing aromatics and isoparaffins, at least, to appear in the analytical result for olefins.

Most methods of group 2 were developed for the analysis of saponifiable fats and oils and their application to hydrocarbons meets with very limited success, the magnitude of the result being influenced to a large extent by the excess of reagent, as can be seen from Figures 1 and 2. The Hubl, Hanus, and Wijs procedures are well-known examples of such methods. Many attempts have been made to devise more reliable methods for determining unsaturation. Margosches (*12*) allows an ethyl alcoholic iodine solution to react with hydrocarbons in presence of water. However, other investigators (*9*) report that this method gives values that are too low. Grosse-Oetringhaus (*5*) reviewed and investigated numerous methods and concluded that Kaufmann's method (*8*) is reliable. This method was investigated by the present authors and is discussed below. Kaufmann employs an excess of bromine solution in methyl alcohol saturated with sodium bromide and makes no correction for substitution.

The bromine method of McIlhiney (*11*) gained prominence, apparently because it provided means for distinguishing bromine consumed for addition reactions from that for substitution, the substituted bromine being calculated from the resultant hydrobromic acid. Evidence has been accumulating, however, which discredits the general suitability of this procedure. Pure hydrocarbons of known unsaturation have yielded results for bromine addition number by the McIlhiney method which were not only too low but in numerous instances were negative values, indicating that reactions other than addition and substitution occurred during the analysis. Grosse-Oetringhaus (*5*) concludes that the McIlhiney method is of value only in those cases where substitution has not occurred as indicated by the absence of hydrobromic acid in the reaction products. The same limitation appears to apply to the other methods mentioned—a condition probably commonly experienced with saponifiable fats, but rarely with hydrocarbon mixtures.

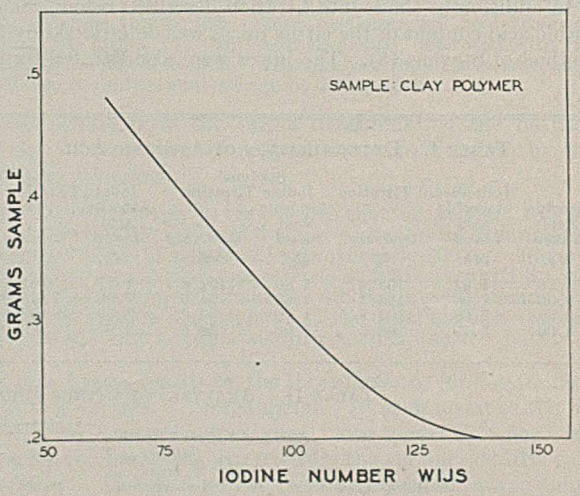

FIGURE 1. EFFECT OF SAMPLE SIZE ON MAGNITUDE OF IODINE NUMBER

Amount of reagent constant and in every case so large that at least half was left unconsumed at end of test.

Most methods for determining unsaturation employ an excess of the halogen, apparently to ensure complete halogenation, the excess being titrated after the reaction period. Francis (*3*) seeks to avoid substitution by keeping the excess of bromine low through controlled addition of sulfuric acid to his potassium bromide-bromate reagent which upon acidification liberates bromine.

To study the effect of excessive bromine on bromine addition number, triisobutene was tested both by Francis' and Kaufmann's methods, using various excesses. The results of these tests are shown in Table I. Triisobutene was chosen because its bromine addition number could not be deter-

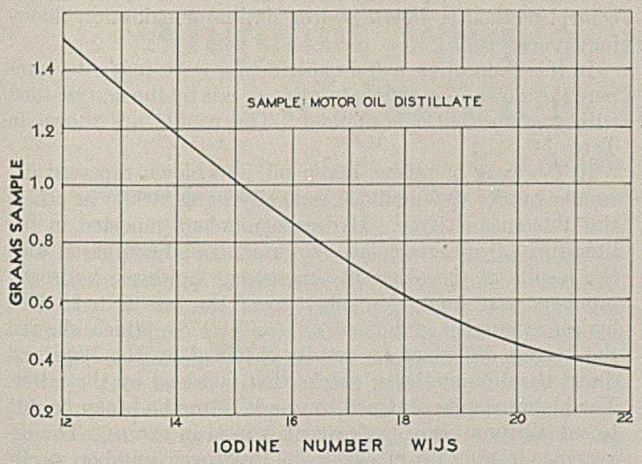

FIGURE 2. EFFECT OF SAMPLE SIZE ON MAGNITUDE OF IODINE NUMBER

Amount of reagent constant and in every case so large that at least half was left unconsumed at end of test.

mined simply and accurately by methods previously employed by the authors.

These results clearly indicate a relationship between excess of bromine and magnitude of bromine number. Though theoretical values are obtainable by such methods, they suffer the disadvantage of requiring a series of trial titrations to determine the optimum excess of bromine.

Thomas, Bloch, and Hoekstra (*16*) have improved the Francis method by cooling in many steps. However, the present authors have found even this improved procedure to yield low results for triisobutene (58 to 62 *vs.* theoretical 95) and tetraisobutene (45 to 50 *vs.* theoretical 71). These low values have been confirmed by Lewis and Bradstreet (*10*) in their further improvement of the Francis method, which was published after the present paper had been submitted for presentation at the September, 1940, meeting of the AMERICAN CHEMICAL SOCIETY.

The data shown in Table I caused the authors to investigate the feasibility of titrating with bromine, since by such means the bromine would never be in excess. Morrell and Levine (*13*) described a method based on this principle, titrating in diffused light with bromine solution (4 per cent by volume in carbon tetrachloride) which has been standardized against a pure olefin, olefin-free naphtha being recommended as solvent for the sample.

Normann (*14*) and Hofmann (*6*) find the nature of the solvent to be an important factor in bromination reactions, and the present authors find that carbon tetrachloride tends to retard the reaction and frequently renders the end point uncertain. Chloroform as solvent for bromine and sample was not consistently satisfactory; nor was olefin-free naphtha (A. S. T. M. precipitation naphtha and Kahlbaum's normal benzine) an improvement when employed as sample solvent. The bromine addition number of triisobutene, for example, could not be obtained with naphtha as sample solvent, though it was easily determined with chloroform as sample solvent, the titration in each case being made with a solution of bromine in glacial acetic acid, using bromine color for end point. The chloroform–glacial acetic acid solvent combination proved very satisfactory, end points being sharp and absorption of bromine rapid.

The question of whether or not substitution occurs in the proposed method was not directly investigated because of the intricacy of the problem. However, since Kaufmann's and Francis' methods give bromine numbers which approach theoretical addition values as the excess of bromine employed becomes smaller, and since the proposed method employs no excess of bromine and yields theoretical values, it seems a justified conclusion that substitution does not occur.

The following are the details of the method adopted by the authors.

Apparatus and Reagents

A 10-ml. buret divided in 0.05 ml., a 50-ml. buret divided in 0.1 ml., 25-ml. Erlenmeyer flasks, a 5-ml. pipet, a 1-ml. pipet divided in 0.1 ml., and 250-ml. glass-stoppered iodometric flasks.

Bromine (2 per cent by volume) in C. P. glacial acetic acid (keep in a dark, glass-stoppered bottle), 0.1 *N* sodium thiosulfate, C. P. chloroform, 10 per cent potassium iodide solution, and starch solution.

TABLE I. INFLUENCE OF EXCESS BROMINE ON APPARENT BROMINE ADDITION NUMBER

(Hydrocarbon, triisobutene)

Size of Sample	Excess of Bromine	Bromine Addition Number Found	Theoretical
Grams	*Mg.*		
	Francis' Method		
0.382	121	130	
0.382	93	127	
0.382	70	119	
0.382	64	113	95
0.382	54	103	
0.382	8	97	
0.382	4	87	
0.382	2	77	
	Kaufmann's Method		
0.151	215	120	
0.151	144	117	
0.151	77	110	95
0.151	10	99	
0.151	0	94	

Procedure

Weigh 0.100 to 1.000 gram of sample, depending on the degree of unsaturation, into a 250-ml. Erlenmeyer flask and to it add 5 ml. of chloroform. Volatile samples are best measured (0.1 to 1.0 ml.) into 5 ml. of chloroform, and the weight of the sample calculated from its gravity. Titrate the sample directly (no indicator) with bromine reagent, adding it until a distinct orange-yellow color is obtained which persists for about 15 seconds. With a little experience it is possible to add the bromine solution rapidly at first and in 0.05-ml. portions toward the end of the titration.

The end point is obscure with some dark samples. In these cases, add the bromine reagent slowly, especially when approaching the end point, and add with a glass rod one drop of the chloroform solution of the sample to about 1 ml. of potassium iodide–starch solution kept in the cavity of a spot plate. The appearance of the blue color marks the end point. This latter procedure is recommended only for dark samples with which the end point cannot otherwise be ascertained.

Determine the titer of the bromine reagent by introducing 5 ml. of the bromine solution into 25 ml. of 10 per cent potassium iodide solution and 5 ml. of C. P. chloroform in a 250-ml. iodometric flask, and titrating promptly with 0.1 *N* thiosulfate in the usual manner, using starch as indicator toward the end of the titration.

CALCULATIONS:

1 ml. of 0.1 *N* thiosulfate = 7.992 mg. of bromine

$$\frac{\text{Mg. of Br per ml. of Br reagent} \times \text{ml. of Br reagent consumed} \times 100}{\text{wt. of sample in mg.}} = \text{bromine addition number}$$

Precision and Accuracy

The precision and accuracy of the proposed method, applied in a routine manner, are evidenced by Table II.

Some samples of diisobutene have been encountered where reproducibility has not been too good—for example, 10 de-

TABLE II. BROMINE ADDITION NUMBERS

Actual Composition of Samples: Kahlbaum normal benzine	Cyclohexene	Diisobutene	Triisobutene	Bromine Found	Addition Number Theoretical
100	...	...	...	0	0
100	...	...	...	0	
...	100	...	...	198	194
...	100	...	...	193	
...	...	100	...	140	143
...	...	100	...	144	
...	...	...	100	93	95
...	...	...	100	93	
50	50	...	...	100	97
50	50	...	...	100	
50	...	50	...	71	72
50	...	50	...	71	
50	...	...	50	43	
50	...	...	50	45	
50	...	...	50	46	47
50	...	...	50	46	
50	...	...	50	46	
50	25	25	...	80	84
50	25	25	...	83	
50	...	25	25	61	59
50	...	25	25	57	
25	25	25	25	104	107
25	25	25	25	106	
Styrene				153	154
Benzene, C. P.				0	0
Toluene, commercial				2–2	0
Xylenes, commercial				3–3	0
Triisopropylbenzene, commercial				1–1	0
Oleic acid, C. P.				58	57
Cyclohexane, C. P.				0–0	0
Isopentane, C. P.				0-0	0
Tetraisobutene				69–72	71

terminations on a sample varied from 131 to 142 *vs.* a theoretical bromine addition number of 143. It was found, however, that if one drop of water was added to the contents of the flask just before commencing the titration, excellent reproducibility and improved accuracy were obtained, the same sample run 10 times yielding results of 139 to 142. The recommendation to add a drop of water is made for this hydrocarbon only, as it is generally unnecessary and may prove detrimental with some.

The results presented in Table II indicate that although the method is primarily intended for hydrocarbons of the gasoline range, it can successfully be applied to other compounds, such as fatty acids and aromatic compounds having an olefin linkage; in the latter the aromatic double bond is not affected.

The possible interference of such substances as sulfur dioxide, hydrogen sulfide, acids, or mercaptans, all of which may conceivably be present in naphthas at some stage of their processing, can be eliminated by treating the naphtha with an alkaline lead solution (doctor solution) and filtering prior to making the bromine number test.

The proposed method was found also to give reproducible and correct values on finished gasolines, neither the dye nor the lead (3 cc. of tetraethyllead per gallon, 3.785 liters) interfering.

TABLE III. COMPARATIVE BROMINE ADDITION NUMBERS BY DIRECT TITRATION AND MCILHINEY METHODS ON UNKNOWNS

Sample No.	Bromine Addition Number: Direct titration	McIlhiney method
1	95	39
2	140	−5
3	151	24
4	0	0
5	16	5

Other Applications

The authors feel that it may be possible to advance the proposed method as a rapid substitute for the usual procedure for iodine value, as it requires only a few minutes, compared with over an hour. It might thus become a useful control method to follow hydrogenation or oxidation (blowing) processes.

In this connection a few experiments were made to compare the indications of unsaturation of fats by the new method with that by the Wijs method. The results are shown in Table IV.

In the cases of tallow, castor oil, and blown rapeseed oil, results by the two methods generally agree, while in others the difference is large. Hydrogen numbers reported in the literature (*4*) were calculated to bromine and compared with the results of the other two methods; however, hydrogen numbers were found for only two of the fats tested. The hydrogen number of linseed oil (≏ Br 78), which showed the biggest difference for results obtained by the Wijs and direct titration methods, checks that obtained by the latter. The higher results obtained by the Wijs method may be due to substitution, since the reagent is used in excess. The divergence in the case of castor oil (hydrogen number ≏ Br 50) is unexplained at this writing. Saturation by bromine of only one double bond of fats containing conjugated double bonds appears a partial explanation, confirmed by the values obtained for tung oil whose acids have three conjugated double bonds with a theoretical bromine addition number of 172.

The experiments on fats were not exhaustive but are believed sufficient to indicate utility for the proposed method in the fatty oil industries.

TABLE IV. COMPARATIVE UNSATURATION OF FATS BY WIJS AND DIRECT TITRATION METHODS

Nature of Fat	Unsaturation by Wijs Method: As % I_2	As % Br_2	Unsaturation by Direct Titration Method: As % I_2	As % Br_2
Tallow	42	26	44	28
Cottonseed oil	112	71	84	53
Castor oil	90	57	88	55
Lard oil	75	47	67	42
Linseed oil	182	115	123	79
Rapeseed oil	105	66	88	55
Blown rapeseed oil 1	63	40	62	39
Blown rapeseed oil 2	59	37	61	38
Blown rapeseed oil 3	62	39	53	33
Tung oil	...	...	89	56

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Aliquant Samples in the Determination of Fluorides in Mixed Foods

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IN A STUDY of the fluoride content of the normal diet it became necessary to ash total 24-hour mixed food samples, including solids, semisolids, and liquids that were not true solutions. (Fluids that were true solutions were collected and analyzed separately.) Ashing the sample in its entirety was difficult, fats in the foods causing particular difficulty, and the time required for drying and ashing the entire sample was found to be prohibitive. Consequently an attempt was made to devise a method of macerating the sample, mixing it to homogeneity, and aliquanting.

Since acid digestion could not be used, mechanical maceration was tried. Grinding and thorough mixing with an electric food mixer produced such apparent homogeneity as to justify the attempt to use aliquants.

A series of 27 samples ranging from 900 to 1500 grams and representing the food intake of an experimental subject on 27 successive days, was ground and mixed, and 300-gram aliquants were removed, both aliquants and remainders being ashed and analyzed. The 300-gram sample was usually completely ashed and ready for analysis after 6 to 12 hours in the muffle furnace. In the case of the large remainder, 4 to 6 times as much time was required for drying and ashing.

In Table I the mean value of the results on the aliquants is compared with that on the remainders. The mean value of the results obtained from the aliquants did not differ significantly from that from the remainders. Especially significant is the greater coefficient of variability for fluoride values obtained from the remainders. As the latter were bulky, the greater variability of these results is attributed to the difficulties inherent in handling and ashing such large samples.

Since the 4.5 to 7.0 grams of ash (and magnesium oxide) from 300 grams of food were greater than the amount necessary for distillation, an attempt was made to reduce the size of the aliquant. Food samples for 25 successive days were used to compare 150-gram aliquants with 300-gram quantities. Comparison of the mean value of results obtained by analysis of the smaller aliquants with that of the large aliquants is given in Table II.

TABLE I. COMPARATIVE RESULTS

	No. of Samples	Mean Fluoride Content	Standard Deviation	Coefficient of Variability
		Micrograms	*Micrograms*	
300-gram aliquants	27	248 ±9.2	±70	28
Remainders after removal of 300-gram aliquants	27	249 ±13.1	±101	40

No significant differences exist between the mean values for the results obtained with the two sizes of aliquants. The means are stable and there is no significant difference between their coefficients of variability. Therefore, the use of aliquants of 150 grams or more will yield satisfactory results.

The second series of samples was obtained some time after the first. Variation in the diet probably explains the lower mean fluoride content of the second series.

TABLE II. COMPARATIVE RESULTS

(Remainders not analyzed)

	No. of Samples	Mean Fluoride Content	Standard Deviation	Coefficient of Variability
		Micrograms	*Micrograms*	
300-gram aliquants	25	158 ±6.2	±46	29
150-gram aliquants	25	161 ±6.8	±50	31

FIGURE 1. FOOD MIXER

Method

The 24-hour food sample after collection in glass jars is ground in a household electric mixer with grinding attachment (Figure 1). The wet weight is determined and magnesium peroxide is added at the rate of 1.0 gram per 100 grams of wet food. (Magnesium peroxide having the lowest fluoride blank obtainable should be procured.) The magnesium peroxide is mixed with the food for from 0.5 to 1.5 hours, depending upon the time required to obtain a homogeneous mixture. Two 150-gram aliquants are weighed into 200-ml. nickel evaporating dishes and dried on an electric hot plate. The fats are driven out by charring on an electric burner and the sample is then placed in the automatically controlled electric muffle furnace, set at 570° C. After complete ashing, the ash is weighed and prepared for distillation.

The fluoride is separated from the ash by distillation with perchloric acid according to MacIntire and Hammond's (*3*) modification of the method of Willard and Winter (*4*). Silver sulfate, as recommended by McClure (*2*), is added to the ash to prevent volatilization of the chlorides.

The back-titration method of Dahle, Bonnar, and Wichman (*1*) is employed from this point on; the unconcentrated distillate is used.

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Determination of Iron by the Zimmerman-Reinhardt Method

Effects of Temperature and Determination of Blank

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IN THE various procedures for determination of iron by the Zimmerman-Reinhardt method as outlined in standard texts on analytical chemistry, there seems to be a fairly general impression that the treatment with mercuric chloride and the titration with permanganate are favored by having the solutions at a low rather than a high temperature, and that the determination of the blank does not require the presence of ferric chloride in the solution, although it is one of the end-point products.

Regarding the mercuric chloride treatment one reads such statements as "cool at least to room temperature", "cool completely", "the temperature should not be over 25° C.", etc. Concerning the permanganate titration the instructions vary from "titrate the cool solution" to "dilute with ice-cold distilled water". In the matter of the blank the following quotation is typical: "A blank should be carried out on the reagents used in the analysis." In the many articles dealing with the Zimmerman-Reinhardt method evidently the temperature effects were not considered worthy of special consideration or comment, and there appears to be no detailed information as to the proper method of obtaining a blank correction (*1–5, 7–10*). It is of significance to note that Meineke has shown that it is possible for the ferric chloride to be reduced by the mercurous chloride and that the extent of the reaction depends upon the amount of mercurous chloride present (*6*).

It has been the experience of the present authors that within the range of 10° to 40° C. the results are little affected by the temperature during the mercuric chloride treatment, but that during the permanganate titration there is a noticeable advantage in having the temperature around 30° rather than 10° C. In work of approximately 0.1 per cent accuracy the ferric chloride has an effect on the value of the blank by no means negligible. Tables I and II are offered in support of these statements.

TABLE I. DEVIATIONS OF ZIMMERMAN-REINHARDT FROM JONES REDUCTOR METHOD

(At different temperatures of addition of mercuric chloride and permanganate)

Temperature of $HgCl_2$ Addition, ° C.	Temperature of Titration				
	10° C.	20° C.	25° C.	30° C.	40° C.
	Ml. of 0.1122 *N* $KMnO_4$				
10	0.05	0.05	0.04	0.04	0.05
20	0.04	0.05	0.05	0.05	0.05
25	0.05	0.04	0.04	0.04	0.05
30	0.04	0.03	0.03	0.03	0.06
40	0.04	0.03	0.03	0.04	0.06

Table I shows the effects of the temperature of the solutions during the mercurous chloride precipitation and the titration with permanganate within the temperature limits of 10° to 40° C. The deviations are the number of milliliters of 0.1122 *N* permanganate by which the Zimmerman-Reinhardt method exceeds the Jones reductor method in the titration of 25.00 ml. of approximately 0.17 *N* ferric chloride solution. Under the conditions of the experiments the results are apparently little affected by the temperature of the mixture during the mercuric chloride treatment, since the deviations at 10° C. are so little different from those at 40° C. If there is any advantage at all it is probably at the higher temperature. As for the titration temperature, the deviations do not indicate that there is any advantage in cooling the solution to 10°, nor does there appear to be any harm in having the temperature as high as 30° C. In the actual titrations, however, the end points at 30° and 40° were somewhat sharper and did not fade so rapidly as at the lower temperatures. It is probably inadvisable to have the titration temperature as high as 40°, as column 6 begins to show a definite trend toward higher results. In Tables I and II all values are the results of 3 to 5 determinations whose average deviations were not greater than 0.01 ml.

TABLE II. DETERMINATION OF BLANK

Experiment	Temp.	Total Volume of Solution	Volume of $SnCl_4$ Solution	Volume of $FeCl_3$ Solution	Volume of Preventive Solution	Volume of 0.1022 *N* $KMnO_4$ Required
	° *C.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*
1	10	500	10	20	25	0.16
2	20	500	10	20	25	0.16
3	40	500	10	20	25	0.16
4	26	500	5	10	25	0.12
5	26	300	5	10	25	0.10
6	26	100	5	10	25	0.09
7	26	500	5	0	25	0.10
8	26	500	5	5	25	0.11
9	26	500	5	20	25	0.16
10	26	500	5	10	10	0.14
11	26	500	5	10	40	0.12

Table II shows the effects of temperature, total volume of solution, and amounts of stannic chloride, preventive solution, and ferric chloride on the size of the blank for the amounts of mercurous chloride, mercuric chloride, and hydrochloric acid that might be present in a typical analysis. The values of the blank are expressed in milliliters of 0.1022 *N* permanganate. The results indicate that under the procedure used the value of the blank is not affected by temperature (experiments 1, 2, and 3) nor by the amount of stannic chloride (experiments 3 and 9), but that it does depend upon the volume of the solution (experiments 4, 5, and 6), the amount of preventive solution until a certain limit is reached (experiments 4, 10, and 11), and the amount of ferric chloride (experiments 4, 7, 8, and 9). The important point to observe is the definite effect of the ferric chloride.

The fact that the Zimmerman-Reinhardt method is slightly higher than the Jones reductor method, even under optimum conditions (0.08 per cent), may be due to an inherent difference between the two methods or to personal factors. Mixer and Dubois have reported results obtained by the two methods in which the Zimmerman-Reinhardt method was slightly lower (0.07 per cent, *6*). Barneby's work indicates that this method shows no appreciable error (*1*), while results of Jones and Jeffrey (*4*), Harrison and Perkin (*2*), and Skrabal (*9*) show trends that are distinctly high.

Reagents

Preventive solution: 67 grams of manganous sulfate ($MnSO_4 \cdot 4H_2O$), 175 ml. of phosphoric acid (sp. gr. 1.7), and 133 ml. of concentrated sulfuric acid in 1 liter of solution. Stannous chloride: 50 grams of the iron-free dihydrate to 1 liter of solution 2 *N* in hydrochloric acid. Mercuric chloride: a saturated solution containing 5 ml. of 6 *N* hydrochloric acid in each liter. Ferric chloride: a 0.1695 *N* solution 3 *N* in hydrochloric acid. Potassium permanganate: 0.1122 *N* and 0.1022 *N* solutions.

Procedure

To standardize the ferric chloride solution by the Jones reductor method, 25.00 ml. of solution were fumed with 10 ml. of concentrated sulfuric acid, 150 ml. of water were added, and the ferric sulfate was dissolved, cooled to room temperature, and rinsed through a Jones reductor until the final volume was about 400 ml. Titration with permanganate followed, the end point taken being the appearance of faint pink which persisted for at least 30 seconds.

The blank was determined as follows: First, the blank of the reductor was determined from the difference between the permanganate titers at room temperature of two 400-ml. solutions containing 10 ml. of concentrated sulfuric acid, one of which had been passed through the reductor and the other had not. The total blank for the process was obtained by adding this blank to that obtained on 400 ml. of solution containing 25 ml. of the iron solution which had been fumed with 10 ml. of concentrated sulfuric acid. Titrations of reduced iron solutions and blank determinations made with the addition of 25 ml. of preventive solution produced the same results but yielded more satisfactory end points.

In the procedure for the Zimmerman-Reinhardt method, which closely conformed to that of Barneby (*1*), 25.00 ml. of the ferric chloride solution were heated to 90° C., and stannous chloride solution was added until the yellow color disappeared, followed by 2 drops in excess. The solution was adjusted to the desired temperature and 10 ml. of mercuric chloride at the same temperature were added with thorough mixing. After about 2 minutes the resulting solution was washed into a beaker containing about 400 ml. of water and 25 ml. of preventive solution. The solution was adjusted to the desired temperature and with thorough stirring titrated with permanganate at an average rate of about 3 seconds per ml. but more slowly toward the end, until a faint pink was obtained which persisted for 15 to 30 seconds.

The blank was determined as follows: The same volume of stannous chloride solution as that used in reducing the iron (approximately 10 ml.) was oxidized with permanganate until the solution was faintly pink, and 2 drops of stannous chloride solution were added, followed by 10 ml. of mercuric chloride solution and, after 2 minutes, 420 ml. of water containing 25 ml. of preventive solution. Finally 25 ml. of the ferric chloride solution were introduced, and the solution was allowed to stand 2 minutes (the approximate duration of the titration) and titrated with permanganate as in the regular determinations. With the exception of the conditions indicated, the blanks in Table II were obtained by essentially the same procedure.

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Distillation of Foaming Solutions under Vacuum

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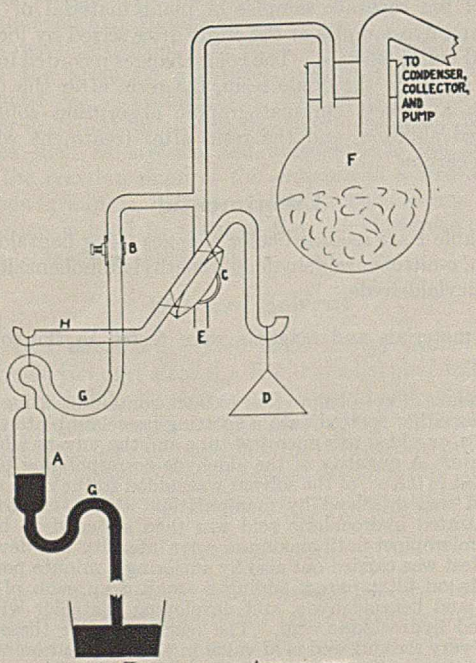

DIAGRAM OF APPARATUS

A. Glass bulb connected with rubber tubes, *G*, and supported by lever arm, *H*, by means of wire connected at gooseneck
B. Rubber connection and pinchcock
C. Glass stopcock with lever arm, *H*, attached by wire binding. Between *H* and the stopcock handle are two small rubber blocks, one at each end of the handle, making the attachment less rigid and less liable to break
D. Counterbalance attached by wire to *H*
E. Air intake
F. Distillation flask
G. Easily flexible rubber tubing (vacuum)
H. Glass lever arm (bent from glass rod)

THIS device for the prevention of excessive foaming operates on the principle that, in the distillation of foaming solutions under vacuum, a slight decrease in vacuum temporarily arrests bubble formation and destroys built-up foam masses.

The height of bulb *A* in respect to the mercury dish is adjusted so that as the vacuum approaches the point of bubble formation, the mercury will have been drawn up into *A*. The weight of the counterbalance, *D*, is such that when *A* is full, or nearly so, the total weight of the mercury, *A*, and attached tubing, *G*, overbalances *D* and pulls the lever arm, *H*, down, opening stopcock *C*, and allowing a small amount of air to enter the system through opening *E*. The vacuum of the system is thereby lowered, allowing the mercury to fall in its column out of *A*. The loss in weight allows *D* to raise the empty bulb, *A*, and reclose *C*. The pump, operating continuously, then raises the vacuum again and the process is repeated.

The pinchcock, *B*, aids in regulating the frequency of the operation by partly closing off the *A* portion of the system from the main vacuum system of which the distillation flask, *F*, is a member. The partial closure of *B* makes a lowering of the vacuum in distillation flask, *F*, system less quickly felt in the *A* system, so that, once down, the bulb tends to remain down longer than if the connection at *B* were left clear. If desired, a similar pinchcock (not shown) may be placed in the system between *C* and *F* above the side connection leading to *A*. In this case, an opening of *C* produces more quickly a drop in vacuum in the *A* system, allowing the mercury to fall faster out of *A*, thus raising the frequency of the operation but allowing less air to enter the system per phase.

The final bend in lever arm, *H*, at the point supporting *A* should not be made until the optimum point for the particular apparatus is found. If the arm from *C* toward *A* is too long, a lowering of that end of the lower arm will produce an undue lowering of the height of the mercury column in respect to the mercury dish and the bulb will remain full until the vacuum of the system, measured in height of mercury, has fallen to a point equal to the new height of the mercury column. On the other hand, if the arm is too short, there will be no appreciable lowering of the mercury column and the bulb will seek a point of equilibrium in which *C* will remain partially open and a constant vacuum will be pro-

duced. The length of *H* from the central axis of *C* to the point of attachment to *A* in the apparatus is about 14 cm.

The degree of vacuum may be regulated by adjusting the height of the mercury column—i. e., raising or lowering the mercury dish and/or decreasing the weight, *D*.

With the apparatus described, evacuated to 12 mm. of mercury with a glass water pump, it was possible to produce a fluctuation of vacuum ranging from 5 mm. to several centimeters. The same fluctuations could be produced as well at lower vacuums. The frequency of the operation could be adjusted from 8 to 20 phases per minute.

Inasmuch as a lowering of vacuum raises the boiling point of a substance, fluctuation of vacuum in this apparatus will produce a corresponding fluctuation in the boiling point of the solvent. The same precaution should be taken with this apparatus as with a manually controlled apparatus—i. e., heat should be applied to the distillation flask in such a way that, with the decreases in vacuum, the temperature does not rise above the decomposition point of the material being treated. With this apparatus a water bath was used, the temperature of which was maintained at a point below the decomposition temperature of the most heat-sensitive substance in the solution being distilled.

If it is desired to carry on the distillation in the presence of an inert atmosphere (carbon dioxide was used in this case), appropriate connections may be made from the gas cylinder or generator to *E*.

If desired, capillary tubes may be used in *F*. With badly foaming solutions, little advantage was realized, the tubes tending to "blow up" bubbles formed. If an inert gas is used, gas connections must also be made to the capillary tubes.

The apparatus has been successfully used in the distillation of water and 50 per cent water–alcohol extracts of animal tissues. The speed of the distillation is approximately the same as for a carefully controlled manual operation.

New Color Reactions for *Cannabis sativa* Resin

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THE recognition of the resinous constituent of *Cannabis sativa* (*Cannabis indica*) has been rendered difficult by the absence of reliable and specific chemical tests.

A clue to the nature of suspected material can often be obtained by microscopic examination; the odor developed by heating the residue of a petroleum ether extract at the temperature of the water bath is also of diagnostic value, provided the residue is free from lipoid and other materials extracted with the resin during toxicological investigations.

Beam (*1*) developed a delicate test, using alcoholic potassium hydroxide, and for many years this test has been of distinct service to the analyst in the East. Doubt has been expressed in regard to its specificity, and moreover it has failed on occasions with genuine plant material and pharmacopoeia preparations.

Recently, several new color reactions have been developed, notably those of Munch (*4*) and Ghamrawy (*3*) using *p*-dimethylaminobenzaldehyde and (*2*) Duquénois and Moustapha using vanillin and acetaldehyde in the presence of ethyl alcohol.

In the Duquénois and Moustapha method the colors are developed in the presence of hydrochloric acid, and in the technique of Ghamrawy and of Munch sulfuric acid is used.

In this laboratory, a considerable number of samples of ganja, charas, and bhang come under observation and it was considered that an examination of these new methods would be of distinct service in connection with forensic chemical work.

The reactions of Ghamrawy, Duquénois and Moustapha, and Munch were given by fifteen samples of *Cannabis sativa* resin examined in this laboratory and appear to be delicate tests for the resin; definite color reactions are also obtained with benzaldehyde, *o*-nitrobenzaldehyde, and salicylaldehyde; the color is best developed with hydrochloric acid; the reaction is not obtained with aliphatic aldehydes and therefore appears to be specific for aromatic aldehydes of the type studied; the reaction can be carried out in the presence of solvents other than ethyl alcohol; the presence of an aromatic aldehyde group is essential for the reaction as described in this paper; and finally of 104 substances tested by the technique, 15 samples of *Cannabis sativa* resin alone gave the reaction.

In this paper detection methods only are considered although it is believed that the test as carried out by the author using vanillin, salicylaldehyde, and *p*-dimethylaminobenzaldehyde could be developed into a quantitative colorimetric estimation.

The samples of *Cannabis sativa* resin examined in this investigation were obtained by the extraction with petroleum ether of ten separate samples of plant material of Indian origin and five samples of the crude drug seized by local custom and police officials. The color changes recorded in Table I were given by the crude resin, the resin after the passage through a Tswett chromatographic absorption column of activated alumina, and the resin after treatment with activated carbon.

Experimental

In Table I are recorded color changes using benzaldehyde, vanillin, *o*-nitrobenzaldehyde, *p*-dimethylaminobenzaldehyde, and salicylaldehyde.

The materials and reagents were tested in the following manner:

A volume of petroleum ether extract containing 2 mg. of the resin was either spotted onto a spotting porcelain plate with depressions, or added to a microtest tube and the solvent allowed to evaporate. A quantity of the aldehyde corresponding to 3 mg. contained in 0.5 cc. of the solvent was added to the resin and the material was dissolved by manipulation with a microspatula. Concentrated hydrochloric acid was then added drop by drop from a micropipet until maximum color intensity was developed.

The test was carried out also by smearing a minute portion of the resin on filter paper, adding a small drop each of benzyl alcohol and benzaldehyde, and developing the color with concentrated hydrochloric acid. The reaction under these conditions is very striking and is of value in the examination of stains and minute quantities of the resin.

Color Changes

In general under the conditions of testing described above benzaldehyde gave an intense violet color which faded rapidly. Vanillin gave a persistent green–blue-green coloration and in some cases violet tinges were noted. The blue-green color faded gradually, but in all cases the color was clearly perceptible after 20 minutes. *o*-Nitrobenzaldehyde,

TABLE I. COLOR CHANGES OF *Cannabis sativa* RESIN

Alcohol Solvent	Reagent	Color	Persistency	Remarks
Methyl alcohol	A	Violet	1 minute	Fades rapidly
	B	Green–blue green	Slow change	Perceptible green after 20 minutes
	C	Little change	.....	Yellow-green precipitate
	D	Violet red	Slow change	Cloudy violet after 20 minutes
	E	Deep green	Slow fade	Yellow green after 20 minutes
Ethyl alcohol	A	Violet	3 minutes	Fades in 5 minutes
	B	Deep blue green	Slow fading	Deep green after 20 minutes
	C	Brown yellow	Slow fading	Pale yellow after 20 minutes
	D	Violet red	Slow fading	Pink after 20 minutes
	E	Deep green	Slow fading	Green after 20 minutes
Isobutyl alcohol	A	Violet	5 minutes	Fades rapidly to slate
	B	Green–blue green	Slow fading	Blue green after 20 minutes
	C	Brown yellow	5 minutes	Slow fading to yellow
	D	Violet red	Slow fading	Pink after 10 minutes
	E	Deep green	Slow fading	Green after 20 minutes
Acetone	A	Cloudy violet	1 minute	Complete fading in 5 minutes
	B	Cloudy green	Slow fading	Light green after 20 minutes
	C	Cloudy brown	5 minutes	Yellow in 15 minutes
	D	Cloudy pink	Slow fading	Light pink after 20 minutes
	E	Cloudy green	Slow fading	Light green after 20 minutes
Benzyl alcohol	A	Violet	3 minutes	Fades to slate in 5 minutes
	B	Green–blue green	Slow fading	Green after 20 minutes
	C	Orange	Slow fading	Yellow after 20 minutes
	D	Violet red	Slow fading	Pink after 20 minutes
	E	Deep green	Slow fading	Pink after 20 minutes

A. Benzaldehyde. B. Vanillin. C. *o*-Nitrobenzaldehyde. D. *p*-Dimethylaminobenzaldehyde. E. Salicylaldehyde.

depending on the solvent used, gave light to brown-yellow colorations which persisted up to 20 minutes. With *p*-dimethylaminobenzaldehyde, beautiful violet-red colorations were developed which faded slowly, the color however being still perceptible after 20 minutes. With salicylaldehyde a beautiful emerald green color was developed immediately and persisted with slow fading for over 20 minutes. The color faded to a yellow brown in 2 hours. The color changes were not affected by the addition of acetaldehyde as recorded by Duquénois and Moustapha.

The reaction did not take place if aliphatic aldehydes replaced the aromatic aldehydes. When experiments were performed using acetaldehyde and formaldehyde in the manner described, in no case was color developed. Similarly ketones such as acetone and acetophenone fail to react. That the reaction requires the presence of a free aromatic aldehyde group is indicated with salicylaldoxime. When this oxime is used in place of the free aldehydes recorded above, no reaction takes place.

Effect of Solvent

The best solvents appeared to be benzyl alcohol and, in most cases, isobutyl alcohol. Ethyl alcohol gave fair results with all the aldehydes, but methyl alcohol and acetone were definitely inferior under the condition of test. This appeared to be due to either the insolubility of the reaction products in the solvents named or the cloudiness developed by the excess acid required to develop the maximum color in these solvents.

Effect of Acid

Neither sulfuric, nitric, nor acetic acid was able to develop color under the conditions of the test. With methyl alcohol and acetone as much as 1 cc. of acid was required to develop the color, whereas with isobutyl and benzyl alcohols 0.3 to 0.4 cc. was sufficient.

Specificity

These reactions, which are similar in type, appear to be specific for *Cannabis sativa* resin. Ghamrawy has tested 60 drugs, Munch 107 drugs, and Duquénois and Moustapha have also recorded the specificity of their technique. In this investigation several substances extractable from plant material with petroleum ether and other plant products which might appear in extracts were tested by the methods described above. The following substances failed to give the reactions recorded in Table I.

ALKALOIDS. Atropine, aconitine, berberine, brucine, caffeine, cinchonine, codeine, colchicum, cocaine, ecgonine, homatropine, hydrastine, morphine, narceine, nicotine, narcotine, papaverine, strychnine, theobromine.

ESSENTIAL OILS. Rosemary, ti-tree, croton, verbena, vetiver, nutmeg, amygdalus, bay, citronella, lemongrass, eucalyptus, cajuput, lavender, clove, chenopodium, sandalwood, turpentine.

FIXED OILS. Candlenut, tung, coconut, linseed, mustard dilo, peanut, cottonseed, castor, sesame, cedarwood, olive, hydnocarpus.

RESINS. Gurjun balsam, colophony, dammar, guiacum, Canada balsam, derris, kauri gum.

TERPENES AND SESQUITERPENES. Pinene, cedrene, carvene, camphene, vetivene, cadinene.

SUGARS. Dextrose, levulose, sucrose, mannose, lactose, galactose.

UNCLASSIFIED. Meconic acid, crude opium, citronellol, citronellal, camphor, ethyl hydnocarpate, salicin, barbitone, nicotinic acid, phytosterol, carotene, ascorbic acid, abietic acid.

It is considered, therefore, that the reactions recorded in Table I take place with a constituent or constituents present in *Cannabis sativa* resin. The reactions have not been carried out as yet with material of known physiological activity, so that it is not possible to attribute the reaction to a physiologically active constituent or constituents.

Sensitivity

Although the color is fleeting, the most sensitive reagent is benzaldehyde in benzyl alcohol as solvent. A deep violet color is obtained immediately with amounts of the order of 0.03 mg. Definite reactions can be obtained with 0.1 mg. using vanillin, *p*-dimethylaminobenzaldehyde, and salicylaldehyde in benzyl alcohol. *o*-Nitrobenzaldehyde does not appear to be as sensitive as the other aldehydes. The slow fading in color of the vanillin, *p*-dimethylaminobenzaldehyde, and salicylaldehyde reaction products suggest the possibility of colorimetric determination, but this point has not as yet been investigated.

Conclusion

A definite chemical reaction takes place between one or more active constituents of Indian hemp and certain aromatic aldehydes dissolved in suitable solvents and in the presence of concentrated hydrochloric acid. The color changes are characteristic of the aldehydes and *Cannabis sativa* resin. Of the materials tested *Cannabis indica* resin alone reacted in the manner described.

Acknowledgment

The author is indebted to the Director of Agriculture for permission to publish these results.

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Determination of the Acetyl Group

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THE determination of acetyl content by means of trans-esterification in ethanol, followed by distillation and determination of the ethyl acetate so formed, was first described by Perkin (*3*), who employed sulfuric acid to catalyze the reaction. The original procedure has since been modified with a view to lessening the decomposition of residues and driving the reaction to completion, as well as assuring quantitative recovery of ethyl acetate.

Sudborough and Thomas (*5*) substituted aromatic sulfonic acids for sulfuric acid as catalyst, eliminating charring of residues together with attendant errors due to formation of sulfur dioxide during distillation. Freudenberg (*2*) employed an intermittent refluxing-distillation technique, adding fresh ethanol to the reaction flask after the first ester had been distilled off and repeating the refluxing-distillation sequence. Phillips (*4*) introduced alcohol vapors into the reaction flask from an external generator during the distillation. These methods require the accumulation of considerable volumes of distillate.

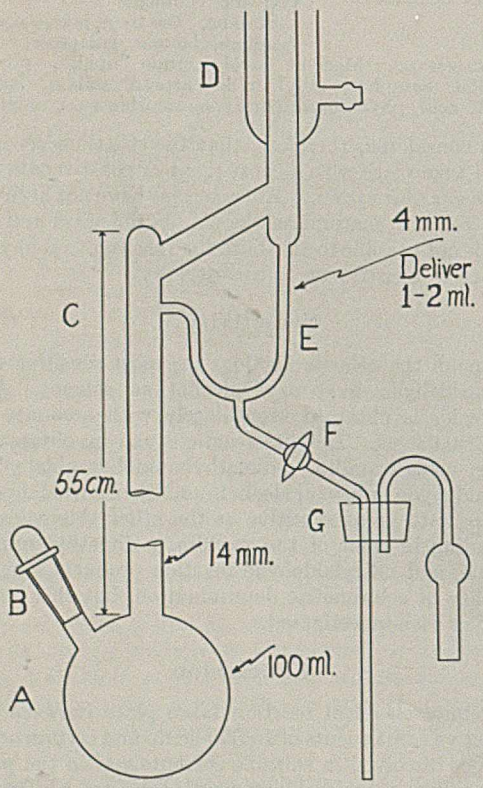

FIGURE 1. DIAGRAM OF APPARATUS

The method herein described accomplishes the removal of ethyl acetate in a minimum volume by distillation through an efficient fractionating column, arranged for operation under total reflux with intermittent small volume take-offs. A nearly constant volume is retained in the reaction chamber, thereby avoiding concentration of the acid in the solution, and enhancing the likelihood of recovery of the deacetylated residue if desired. Advantage is taken of the difference in boiling points of the ternary azeotrope ethyl acetate–ethanol-water (b. p. 70.2°) and alcohol (b. p. 78.3°), which is sufficiently large to permit ready quantitative separation. Concentrated aqueous hydrochloric acid is used as catalyst in place of the less readily available aromatic sulfonic acids or the objectionable sulfuric acid. It is equally effective under the specified conditions and no chloride resulting from the distillation of either hydrochloric acid itself or ethyl chloride may be detected in the hydrolyzed distillate.

TABLE I. RESULTS

Compound	Weight	Vol. of 0.1 *N* NaOH Consumed	Acetyl Found	Acetyl Calcd.
	Gram	*Ml.*	%	%
Diacetyl morphine HCl	0.8064	37.7	20.10	20.30
Calcium acetate.H_2O	0.3250	36.8	48.69	48.84
Aspirin	0.5025	27.8	23.79	23.88
	0.7247	40.2	23.85	
	0.7187	39.8	23.81	
	0.7243	40.3	23.93	
	0.7118	39.65	23.95	
Acetanilide	0.1900	14.0	31.68	31.85
	0.2038	14.95	31.54	
	0.2013	14.95	31.93	
	0.2015	15.0	32.01	
Acetphenetidine	0.2080	11.45	23.67	24.01
	0.1897	10.5	23.80	
	0.2018	11.2	23.87	
	0.2006	11.1	23.79	

The apparatus (Figure 1) used is simple and readily constructed, and the procedure involves a minimum number of operations. By suitable modification, the method can be adapted to semimicrooperation. The small final volume in the receiving flask, in contrast to that obtained by other procedures, permits use of an initially more dilute standard alkali solution for the saponification of the ethyl acetate, while retaining a sufficient final concentration to bring about the saponification. Objections to trans-esterification methods hitherto published, noted by Clark (*1*) on these grounds, are thus overcome.

Apparatus

A 100-ml. round-bottomed flask, *A*, fitted with a ⅋ 12/30 joint, *B*, serving as inlet is attached to a 14-mm. tube, *C*, 55 cm. in length, packed with 4-mm. single-turn glass helices for 50 cm. of its length. The condenser, *D*, is set off the top of this column, causing the condensate to return to the head of the column through the 4-mm. U-tube, *E*, of 1- to 2-ml. capacity. The take-off, *F*, consists of a tube and stopcock sealed into the lowest point of the U-tube and passing through a two-holed stopper, *G*, to which a 125-ml. Erlenmeyer flask is attached. The tube is almost long enough to reach the bottom of the latter flask. Through the second hole of the stopper is a vent protected by a soda-lime tube. The apparatus may be washed by flushing through the condenser, removing the wash-liquid through opening *B* by means of a tube attached to a suction flask.

Procedure

O-ACETYL. Introduce a suitable sample (equivalent to 10 to 30 ml. of 0.1 *N* alkali) into *A* through *B*, washing it in with a total of 50 ml. of pure absolute ethanol, previously freed from ester and aldehyde by refluxing and distillation over potassium hydroxide and aluminum powder. Add 2 ml. of concentrated aqueous hydrochloric acid and several Carborundum chips to serve as boiling points. Attach a 125-ml. flask containing an excess of 0.1 *N* sodium hydroxide solution to *G*, with the end of *F* extending below the surface of the solution. Immerse the receiving flask in an ice bath.

Distill the solution in *A* as rapidly as possible without flooding the column, keeping the stopcock in *F* closed. Drain *E* through the stopcock after each of eight 15-minute intervals. Remove the receiving flask, wash the outside of the tube with 5 ml. of ethanol, and allow 2 to 3 ml. of distillate to wash through the inside. Reflux the solution 0.5 hour. Cool and titrate the excess sodium hydroxide with 0.1 *N* acid, using phenolphthalein as indicator. Each milliliter of 0.1 *N* sodium hydroxide consumed is equivalent to 0.0043 gram of acetyl. A blank run on the alcohol should not consume more than 0.1 ml. of 0.1 *N* sodium hydroxide.

N-ACETYL. Proceed in the manner described above but use 3 ml. of hydrochloric acid as catalyst and space the take-offs at intervals of 30 minutes rather than 15.

The velocity of the trans-esterification reaction is, of course, dependent on the structure of the compound concerned as well as the concentration of acid, and in general is greater for O-acetyl than for N-acetyl compounds. The time of refluxing and amount of catalyst indicated have been found suitable for the substances studied. These include acetphenetidine, which reacts with considerable difficulty; hence, it is presumed that the conditions specified would suffice for most compounds.

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Mineral Analysis of Biological Materials

Use of the Lundegårdh Spectrographic Method

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IN 1929 and 1934 Lundegårdh (*1*, *2*) published the details of an accurate quantitative spectrographic method for the determination of small quantities of metals by using an air-acetylene flame into which was sprayed a solution of the substance under investigation. Since that time, many improvements in the apparatus (*4*, *10*) have simplified the procedure without affecting the accuracy and reproducibility of the results. This method of excitation for the production of emission spectra has been shown to be very dependable, and moreover it "is characterized by a remarkable absence of interactions between the elements in the course of the analysis" (*9*). This is true of both anions and cations. The method has proved to be especially valuable for the analysis of biological substances of all kinds because, in comparison with chemical methods, it requires very small amounts of materials, and the determinations are more rapid and usually of a higher degree of accuracy.

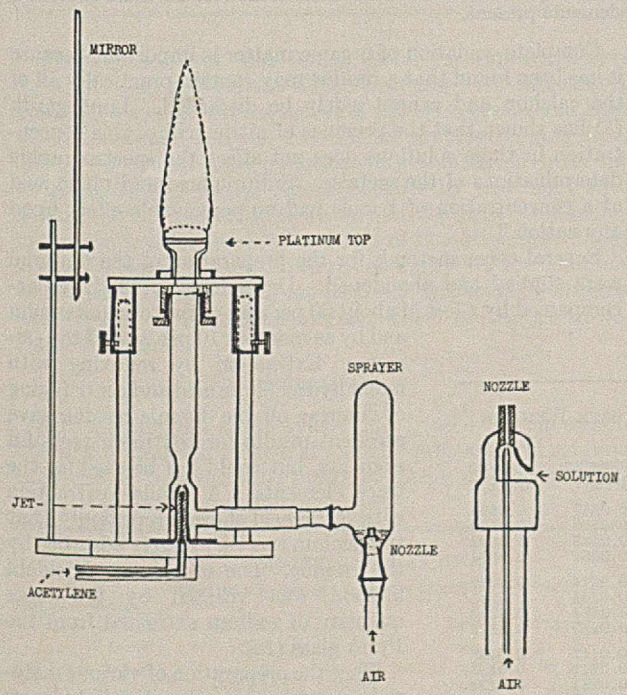

FIGURE 1. DIAGRAM OF BURNER

In order to make a thorough study of the possible uses of the Lundegårdh technique, various types of problems have been investigated. At this time final reports on the special problems are not being made, but such results are included as are necessary to show the accuracy and adaptability of the method.

Apparatus

The apparatus consists of three distinct parts: a Lundegårdh air-acetylene burner and the sprayer through which the solution is introduced into the flame, a Hilger medium quartz spectrograph for photographing the spectra, and a Zeiss spectrum line photometer for measuring the intensity of the spectral lines.

The burner (*11*), sprayer, and nozzle (*6*) are shown diagrammatically in Figure 1. The inverted position of the sprayer permits the use of as little as 2 ml. of the solution.

The arrangement of the burner and the spectrograph is shown in Figure 2. In the background are the two manometers that indicate accurately the air and acetylene pressures, which can be controlled by slight adjustments of the reducing valves on the cylinders. The rack at the left is used for draining the sprayers, and the switch box, shown on the table, controls the solenoid which operates the shutter in front of the slit.

The burner is set up about 45 mm. in front of the slit and exactly in the line of the optical axis of the spectrograph. The intensity of the light entering the slit is greatly increased by the reflection of the flame from a mirror, which is placed so that the reflected image is also in the line of the optical axis of the spectrograph. Lundegårdh found that the most complete emission of most elements takes place in the part of the flame 15 mm. above the top of the inner blue cone (*4*). For this reason the height of the burner is adjusted so that this portion of the flame is directly in front of the slit.

Experimental Procedure

The conditions which produce the maximum intensity of the lines without too much background are obtained by exposures of 2 minutes with an air pressure of 26,366 kg. per square meter (37.5 pounds per square inch) and an acetylene pressure of about 26 cm. of water. Compressed air and acetylene are used from the cylinders as purchased. The slit width is 0.05 mm. and the length of the slit is only 1 mm., so that forty-two exposures can be made on each plate.

Eastman plates No. 33 are used. They are developed for 6 minutes in a tank with Eastman high-contrast developer, formula D-19, and fixed for 12 minutes with Eastman acid fixer, formula F-5.

The intensities of the spectral lines are determined by examination of the plate in the microphotometer. A device for reading "near the line" similar to that used on the Lundegårdh photometer (*7*) has been attached to the Zeiss instrument. The ratio of the reading of the line to the reading of the background is called the intensity ratio (*3*, *5*) and is used for the construction of the intensity ratio–concentration curve. The values for the concentrations are those of four different standard solutions whose

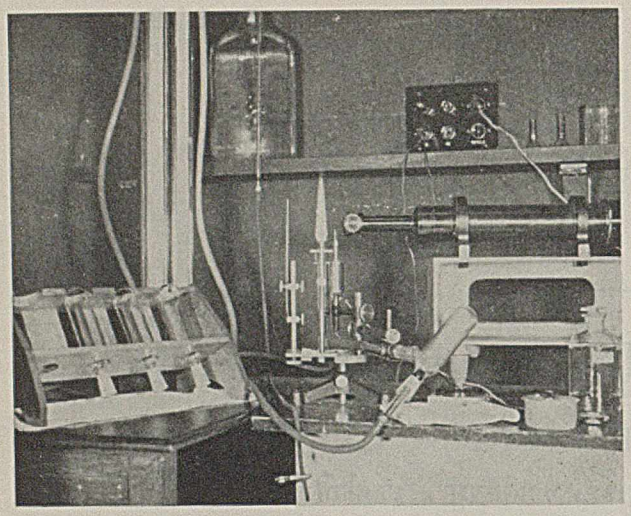

FIGURE 2. SET-UP OF BURNER

spectra are photographed on every plate. Two exposures are taken of every solution and the average of the two intensity ratios is used. At three different intervals on the plate, the spectrum of one of the standards is repeated; this makes it possible to construct a correction curve if the values show any variations that might be due to emission conditions or to the plate emulsion.

In Table I the wave lengths and the maximum and minimum concentrations which are suitable for the construction of the concentration–intensity ratio curves, expressed both in molarity and in grams per liter of solution, are given for each of the metals used.

Some typical intensity ratio–concentration curves are shown in Figure 3.

Accuracy

In order to investigate the accuracy of this method, solutions of known concentrations of a number of the metallic ions were prepared and the ions then determined by the Lundegårdh method, using a single plate, with the results presented in Table II.

Table I. WAVE LENGTHS AND CONCENTRATIONS OF METALS

Metal	Å.	Molarity	Gram/l.
Calcium	4226.7	0.0000125–0.0001	0.00050–0.0040
Cobalt	3526.8	0.00025–0.002	0.0147–0.1179
Copper	3247.5	0.000025–0.0002	0.00159–0.0127
Iron	3720.1	0.00025–0.002	0.01395–0.1116
Magnesium	2852.1	0.00025–0.002	0.0061–0.0486
Manganese	4030.8	0.0000125–0.0001	0.00069–0.0055
Nickel	3524.5	0.00025–0.002	0.0147–0.1174
Potassium	4044.2	0.00025–0.002	0.0098–0.078
Silver	3383.9	0.000025–0.0002	0.0027–0.0216
Sodium	3302.3	0.0005–0.004	0.0115–0.0920

TABLE II. COMPARISON OF WEIGHTS OF ELEMENTS PRESENT WITH WEIGHTS FOUND BY LUNDEGÅRDH METHOD

	Calcium Gram/l.	Copper Gram/l.	Iron Gram/l.	Magnesium Gram/l.	Manganese Gram/l.	Potassium Gram/l.	Sodium Gram/l.
Present	0.00301	0.0095	0.0837	0.0365	0.00412	0.0587	0.0690
Found	0.00299	0.0092	0.0822	0.0356	0.00412	0.0583	0.0690
	0.00302	0.0092	0.0860	0.0352	0.00408	0.0535	0.0682
	0.00296	0.0090	0.0850	0.0341	0.00413	0.0561	0.0674
Present	0.00140	0.0045	0.0391	0.0170	0.00192	0.0274	0.0322
Found	0.00142	0.0042	0.0404	0.0178	0.00192	0.0264	0.0345
	0.00140	0.0047	0.0366	0.0158	0.00192	0.0284	0.0295
	0.00135	0.0044	0.0390	0.0158	0.00199	0.0270	0.0333
Present	0.00060	0.0019	0.0167	0.0073	0.00082	0.0117	0.0138
Found	0.00057	0.0017	0.9166	0.0065	0.00078	0.0111	0.0123
	0.00062	0.0022	0.0181	0.0081	0.00087	0.0111	0.0134
	0.00065	0.0017	0.0166	0.0083	0.00094	0.0091	0.0130

Preparation of Materials

Since this method demands that the metallic elements be in solution, the preparation of material for this analysis is a matter of great importance. In the first place accurately calibrated volumetric apparatus and chemicals of the highest purity are essential and have been used throughout this work. Spectrographic tests were made on concentrated solutions used in the preparation of all standard solutions and reagents to ensure the absence of impurities.

In order to avoid a difficulty frequently encountered in correlating the results of several investigators—namely, the uncertainty of the moisture content of the initial material—a study of possible methods of moisture determination has been made. The following procedures have been adopted:

Solid materials are pulverized, weighed, and dried to constant weight at 70° C. Those substances which become darkened or charred at this temperature are spread in thin layers in large flat weighing bottles and allowed to stand in desiccators over concentrated sulfuric acid until the weight is constant. This usually requires about 72 hours. The samples of liquids such as orange juice or blood are weighed, and also when possible the volumes are measured. Thus the final results can always be definitely expressed in relation to the original material.

Various methods for the actual treatment of the material have been tried and digestion with nitric acid and Perhydrol has been found to be very satisfactory. Complete oxidation of even large quantities of organic material can be accomplished in relatively short periods and the residues are readily soluble in water or in very dilute nitric acid.

The method adopted for all purposes has been to weigh the material directly into 70-ml. Pyrex test tubes calibrated at 20 ml. Nitric acid is added and the tubes are then heated in boiling water until any moisture present, as in orange juice, is nearly evaporated off. The tubes are then heated in a concentrated sulfuric acid bath at a temperature between 120° and 140° C. until the solution is clear. Perhydrol is next added and the tube is carefully heated over a microburner. If necessary, small additional portions of nitric acid and Perhydrol are added to decolorize the solution completely and the solution is finally evaporated nearly to dryness. It can then be diluted to the 20-ml. mark or transferred to any small volumetric flask and diluted to volume. It may be used both for the spectrographic determination of the trace metals and for the various dilutions needed for the more abundant elements present.

Complete oxidation of organic matter is important because it has been found that a residue may contain practically all of the calcium and cannot safely be discarded. Lundegårdh (*8*) has shown that the presence of nitric acid of this concentration in these solutions does not affect the spectrographic determinations of the metals. "Sulfuric acid and nitric acid at a concentration of 1 mole had no perceivable effect upon any cation."

Several other methods for the preparation of the material were studied and abandoned. Dry-ashing at 400° was accompanied by a loss of about 30 per cent of the total potassium and by as much as 10 per cent of the calcium. Extraction by refluxing with molar hydrochloric acid and centrifuging or filtering off the organic residue gave excellent results for the more plentiful elements, but could not be used for the trace elements. A similar extraction with nitric acid showed repeatedly that the calcium was very largely adsorbed by the residue. The use of any Kjeldahl method was vitiated by the large quantity of sodium extracted from the Pyrex glass (*13*).

Since the preparation of various materials for analysis may involve slight losses of some of the metals, the possible re-

covery of such substances, added in known amounts at the beginning of the experiment, was studied. Table III shows such results for some metallic ions added to 50-ml. portions of orange juice.

For a dry material the recovery was tested by adding 0.00023 gram of calcium ion to a 2.4032-gram sample of butter-beans before wet-ashing. The calcium recovered was 0.00020 gram.

TABLE III. RECOVERY OF METALS ADDED TO ORANGE JUICE

	Metal Average	Added Metal	Total Metal Found	Added Metal Recovered
	Gram/l.	*Gram/l.*	*Gram/l.*	*Gram/l.*
Calcium	0.156	0.274	0.4365	0.281
		0.274	0.4320	0.276
		0.274	0.4290	0.273
Potassium	2.61	4.69	7.49	4.88
		4.69	7.23	4.62
		4.69	7.49	4.88
Magnesium	0.110	0.199	0.290	0.180
		0.199	0.290	0.180
		0.199	0.299	0.189
Sodium	0.0152	0.0230	0.0383	0.0231
		0.0230	0.0375	0.0223
		0.0230	0.0356	0.0204

Applications

The plant materials investigated were mainly oranges, butter beans, and pectinates. Much of the preliminary work was done on oranges furnished by the Agricultural Experiment Station at Orlando, Fla. Triplicate analyses of one variety of Temple oranges are given in Table IV. It is of interest to compare these results with those of Roberts and Gaddum (*12*).

TABLE IV. ANALYSIS OF TEMPLE ORANGES

(Grams of metal per liter of juice and pulp)

Calcium	Copper	Iron	Magnesium	Manganese	Potassium	Sodium
Gram/l.	*Gram/l.*	*Gram/l.*	*Gram/l.*	*Gram/l.*	*Gram/l.*	*Gram/l.*
0.158	0.000465	0.00198	0.112	0.000372	2.00	0.0201
0.153	0.000466	0.00187	0.108	0.000380	2.06	0.0194
0.156	0.000451	0.00209	0.104	0.000377	2.25	0.0218

This method was also found useful for the analysis of aquarium and natural sea waters and has been successfully applied to animal tissues, lenses of rat eyes, bones, and both whole blood and serum. Rat blood samples ranging from 0.2 to 1.7 grams were quantitatively transferred from the weighing bottles to the digestion tubes by treatment with hot nitric acid and distilled water and the usual wet-ashing procedure was followed. One gram of blood required about 1 ml. of acid and about 20 drops of Perhydrol for complete oxidation. Table V gives the results of triplicate determinations made on an experimental rat which probably had developed anemia.

Summary

The Lundegårdh air-acetylene burner used for the excitation of the emission spectra of metallic substances in solution is described, together with the Lundegårdh method of calculation of the concentration of these ions in the solutions used. A series of results for calcium, copper, iron, magnesium, manganese, potassium, and sodium shows a very satisfactory recovery of these elements when solutions containing known concentrations are analyzed.

Methods of drying the biological materials are given, followed by a discussion of methods of preparation of these substances for analysis. Complete oxidation with nitric acid and Perhydrol at 120° to 140° C. is recommended. Recovery of metals added to these materials before the above treatment is excellent.

The wide applicability of the method is evident from its successful use in the analysis of such substances as oranges, beans, pectinates, aquarium water, rat lenses, bones, and blood.

TABLE V. ANALYSIS OF RAT BLOOD

(Per cent by weight in whole blood)

Rat No.	Weight of Sample	Calcium	Sodium	Iron	Potassium
	Gram	%	%	%	%
7	0.3444	0.00505	0.200	0.0358	0.2065
	0.3440	0.00515	0.198	0.0359	0.2045
	0.3979	0.00504	0.189	0.0382	0.1855

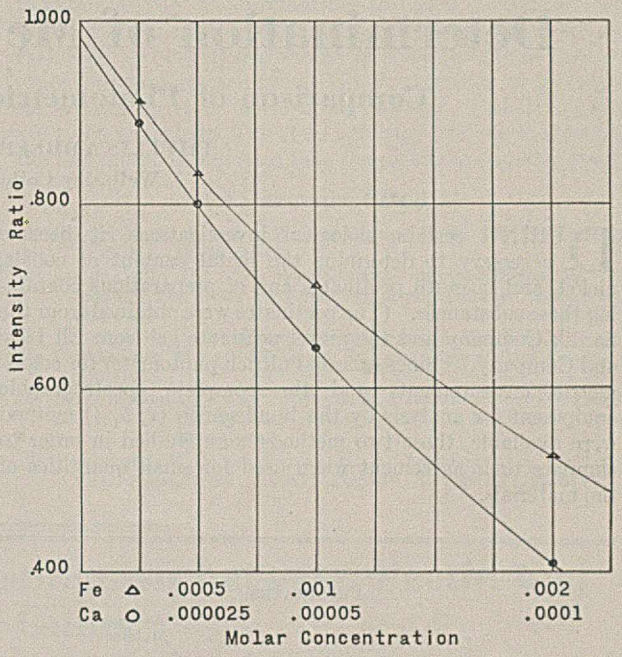

FIGURE 3. CONCENTRATION-INTENSITY RATIO CURVES FOR CALCIUM AND IRON

Acknowledgment

The authors wish to acknowledge their indebtedness to Miss Ruth Abbott for her share in the experimental work.

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Determination of Metals in Some Pectinates

Comparison of Photometric and Spectrographic Methods

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DURING certain biological investigations, it became necessary to determine the metal content of cobalt, nickel, and bismuth pectinates and of preparations containing these substances. (The pectinates were obtained from the Sardik Company and the nickel pectinate gel from Eli Lilly and Company.) Since a Zeiss-Pulfrich photometer for colorimetric measurements and the complete spectrographic equipment for analysis by the Lundegårdh (*2, 3, 4*) method were available, these two methods were studied in order to compare their agreement when used for small quantities of the materials.

TABLE I. AVERAGE PER CENT MOISTURE IN PECTINATES

	Average Moisture, %
Nickel pectinate I	7.64
Nickel pectinate II	7.91
Nickel pectinate III	4.53
Nickel pectinate IV	5.52
Nickel pectinate gel	70.28
Cobalt pectinate	9.43
Bismuth pectinate	7.55

Experimental Methods and Results

As it was found possible to dry the materials to constant weight at 70° C. without any change in appearance, this temperature was used for the determination of moisture.

In general, when the dry material was prepared for analysis by ashing at 500° C. before treatment with nitric acid, there was a slight loss of the metals. For this reason and also because the percentage of total ash was not important, all solutions were prepared by wet-ashing with concentrated nitric acid at 125° to 145° C. until all organic material was destroyed (*1*). Most of the acid was then expelled, and after necessary dilutions were made the solutions were ready for analysis.

The Lundegårdh spectrographic method as used in this study has been described in detail by Griggs, Johnstin, and Elledge (*1*). The spectrum lines used for nickel and cobalt were 3524.5 and 3526.8 Å., respectively. The concentration curves for both the nickel and cobalt were constructed from standard solutions containing from 0.002 to 0.00025 mole per liter. The results of these analyses are given in Tables II, III, and IV. As Lundegårdh found, this method of excitation does not produce spectrum lines suitable for the determination of bismuth, so that no spectrographic results could be obtained.

NICKEL. The photometric method used by Murray and Ashley (*6*) for the determination of nickel in steel was chosen for the analysis of nickel pectinates. The concentration curve was prepared by the use of solutions containing 0.2560 to 0.0512 mg. of nickel. To each solution citric acid, saturated bromine water, ammonia, and dimethyl glyoxime were added before dilution to the final volume of 100 ml. The nickelic complex solution has a pale wine color. It was found necessary to take the photometer readings after 5 minutes had elapsed and to complete them within the next 5 minutes in order to avoid change in transmission.

Nickel pectinates I and II were found to contain some silica, which evidently was precipitated when ammonia was added and adsorbed the colored nickel complex. It was therefore necessary to remove the silica by treatment with hydrofluoric and sulfuric acids before making the photometric determinations.

COBALT. The photometric method developed by MacPherson and Stewart (*5*) for the determination of cobalt was adopted. The concentration curve was prepared by the use of solutions containing 0.02585 to 0.002585 mg. of cobalt. The cobalt was oxidized by nitric and hydrochloric acids and a complex yellow cobaltic compound was formed by the addition of nitroso-R salt and sodium acetate with careful control of temperature and pH of the solution, the final volume of which was exactly 25 ml. Since the color was not permanent, it was necessary to make all photometer readings at a definite interval (such as 1 hour) after the addition of the dye. It was also found that the concentration-transmission curve passed through the origin only when a blank solution containing all reagents was used in the comparison cup instead of water.

BISMUTH. For the analysis of bismuth pectinate the colorimetric method of Rasmussen, Jackerott, and Schou (*7*) was adapted for use with the photometer. It had been found that dilute solutions of bismuth iodide were yellow in color and that their light absorption was proportional to the concentration of the bismuth. Filter S47 and 20-mm. cups were used in the photometer and solutions containing 0.1820 to 0.0455 mg. of bismuth were prepared for the construction of the concentration curve. In the case of these solutions as well as solutions of the pectinate, prepared as described above, it was necessary to remove nitric acid by evaporation in order to prevent oxidation of the iodide. One drop of sulfuric acid and 10 ml. of a 10 per cent

TABLE II. NICKEL IN NICKEL PECTINATES

Nickel Pectinate	Weight of Sample	Nickel Found: Photometric method	Nickel Found: Spectrographic method
	Gram	%	%
I	0.2272	0.22	0.27
	0.1994	0.24	0.26
	0.2161	0.21	0.27
	0.2475	0.19	0.24
		Av. 0.22	0.26
II	0.3554	0.27	0.25
	0.2653	0.26	0.27
	0.1884	0.26	0.25
	0.3093	0.27	0.26
	0.3256	0.28	0.25
		Av. 0.27	0.26
III	0.2299	0.46	0.48
	0.2131	0.43	0.41
	0.2631	0.44	0.41
	0.1693	0.45	0.43
	0.2159	0.45	0.44
		Av. 0.45	0.43
IV	0.2221	0.68	0.68
	0.2840	0.68	0.68
	0.1603	0.67	0.62
	0.1433	0.66	0.65
	0.1822	0.67	0.67
		Av. 0.67	0.66
Gel	20.4860	0.0048	0.0041
	20.0854	0.0046	0.0043
	18.8688	0.0046	0.0044
	19.9490	0.0048	0.0039
	19.4773	0.0047	0.0045
		Av. 0.0047	0.0042

TABLE III. COBALT IN COBALT PECTINATE

Weight of Sample	Cobalt Found: Photometric method	Cobalt Found: Spectrographic method
Gram	%	%
0.2997	0.42	0.36
0.2791	0.41	0.34
0.2111	0.36	Not determined
0.2891	0.37	Not determined
	Av. 0.39	0.35

solution of potassium iodide were then added before diluting to a volume of 25 ml. The concentration was plotted against per cent transmission on a semilogarithmic scale; the graph was a straight line passing through the ordinate at 100 per cent, showing that Beer's law held for these dilute solutions, and that the compound was suitable for use in the photometer.

TABLE IV. BISMUTH IN BISMUTH PECTINATE

Weight of Sample	Bismuth Found by Photometric Method
Gram	%
0.2155	0.57
0.2660	0.53
0.1801	0.53
0.2540	0.55
0.2834	0.55
	Av. 0.55

Conclusions

The photometric methods presented in this paper show excellent agreement and therefore high precision for the determination of very small quantities of nickel, cobalt, and bismuth in organic materials. The spectrographic method used for nickel and cobalt has almost as good agreement and precision but requires somewhat larger quantities of the materials. The latter is more rapid, however, if many samples are to be analyzed. The close agreement of the analyses by the two methods indicates that the results are very accurate.

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Use of Thorium Nitrate to Distinguish between Pectin and Certain Gums

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IDENTIFICATION of the individuals commonly grouped as water-soluble gums is at times difficult, as many of the substances react alike to the same reagent. Pectin is one of the most difficult of this group to identify, because it is usually precipitated by the reagents which also precipitate the other gums. Differentiating between pectin and other gums (*2, 3*) requires more time and labor than a simple precipitation test. In attempting to carry out work on pectin similar to that done by Bonner (*1*) on pectate it was found that precipitation with thorium nitrate exhibits a novel property of pectin not shown by the other gums tested.

If the substance under examination is a powder containing no added materials, such as sugar or acid, it is made up to a 1 per cent aqueous sol. Any admixture should first be extracted with 50 per cent alcohol, or can be eliminated by dissolving the substance and then precipitating with an equal volume of alcohol. If the sample is a liquid preparation, the gum should be precipitated as above, so that a pure sol of known strength can be made.

Irish moss, agar, gum arabic, and karaya gum give no precipitate with 50 per cent alcohol; gum tragacanth gives only a slight precipitate; but pectin, quince seed, and locust-bean gums give good precipitates.

Of the gums listed in Table I, only Irish moss, quince seed, and pectin give precipitates with thorium nitrate. The precipitate from Irish moss is stringy and opaque and easily differentiated, but those from quince seed and pectin are alike, being of a firm, gelatinous nature. The pectin precipitate is easily dispersed or in some cases actually dissolves on adding an excess of the thorium nitrate solution or dilute acetic acid; the quince-seed precipitate with thorium nitrate is unaffected by this treatment. If the dilute acetic acid is first added to the gum sol, the quince seed will yield a firm gel and the pectin shows only a thickening or very slight gel.

A confirmation of the thorium nitrate test for pectin may be made with neutral lead acetate. In unacidified sols pectin and quince seed yield gels, while if acetic acid is added to the sol before the lead acetate, pectin forms a firm, clear, brittle gel but quince-seed gum gives only a very weak gel or a viscous sol. This is just the reverse of the thorium nitrate test.

TABLE I. REACTIONS OF GUMS AND RELATED SUBSTANCES WITH THORIUM NITRATE AND NEUTRAL LEAD ACETATE

	Thorium Nitrate		Neutral Lead Acetate	
Material Used	10% solution	10% solution and 5 *N* acetic acid	10% solution	10% solution and 5 *N* acetic acid
Gum arabic	[a]	[a]	[a]	[a]
Locust-bean gum	[a]	[a]	Slight thickening	[a]
Gum tragacanth	[a]	[a]	[a]	[a]
Irish moss	Stringy, white precipitate	White granular precipitate	Cloudy	Cloudy
Karaya gum	[a]	[a]	[a]	[a]
Quince-seed gum	Firm opaque gel	Firm gel	Fairly firm gel	Very weak gel, a thickening
Pectin	Firm, transparent gel	Very weak gel, a thickening	Firm, transparent gel	Firm, brittle, clear gel
Agar (0.5 instead of 1%)	Slight haze	[a]	[a]	[a]
Methyl cellulose	[a]	[a]	[a]	[a]
Starch	[a]	[a]	[a]	[a]

[a] No apparent reaction.

To 10 ml. of the aqueous solution of the gum (1 in 100) add 1 ml. of 10 per cent thorium nitrate solution, stir, and allow to stand 2 minutes. If a gel results, the gum is either pectin or quince-seed gum. If no gel results it is not pectin.

To differentiate between the two gums: To 10 ml. of the sol add 1 ml. of 5 *N* acetic acid, then 1 ml. of 10 per cent thorium nitrate solution, stir, and allow to stand 2 minutes. If no firm gel results the gum is pectin; if a gel forms it is quince-seed gum.

To check the reaction, a 10 per cent solution of neutral lead acetate is used and the same procedure carried out as for thorium nitrate.

Table I shows the gums tested and the results obtained.

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Determination of Oxygen in Tank Hydrogen

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IN RESEARCH employing tank hydrogen it is often desirable to determine the presence of traces of oxygen. A method is here described for measuring these traces with an error of less than 10 per cent for oxygen concentrations of 0.05 to 0.20 per cent by volume. While the principle involved is far from new (*1*), the apparatus shown in the figure is considerably simpler and more accurate than those previously described. In principle, the traces of oxygen are burned with hydrogen on a glowing platinum wire. The water which forms condenses, thereby effecting a disappearance of three volumes, two of hydrogen and one of oxygen, for each volume of oxygen originally present.

APPARATUS. The whole apparatus is mounted on a wooden base 37.5 × 25 cm. (15 × 10 inches) with a backboard of the same dimensions. Bulbs *C*, *D*, *F*, and *G* are all of 100-ml. capacity. The slanting manometer, *E*, is of 2-mm. capillary tubing fastened to a 20-cm. length of meter stick, which is inclined to give a drop of 2 cm. over its 20-cm. length. A capillary stopcock may be conveniently inserted at *E*, although this is not essential. The water jacket surrounding *C* is made of 50-mm. tubing and a cork. A rubber stopper, rather than a glass-tungsten seal, is provided for the ignition vessel, *D*, so that the platinum wire can be easily replaced if burned out. Two stout tungsten lead-in wires are insulated from each other by a 5-mm. glass tubing, not shown, which is embedded in the rubber stopper and extends up over one wire to the platinum coil. The platinum spiral is made from No. 28 wire, 20 cm. long.

A scratch is made on *BD* about 3 cm. from *B*. *A* and *B* are small, two-way capillary stopcocks. Section *AI* should be bent out from the board. Leveling bulb *F* is set in a 5 × 5 cm. (2 × 2 inches) wooden block, hollowed out and filled with plaster of Paris, so that *F* may be returned to exactly the same position each time. The block is fastened to a rod which may be clamped into position. Bulb *G* rests in an ordinary ring. *CE* is filled with mercury and *D* with water. Bulb *C* and manometer *E* must be kept clean and dry at all times.

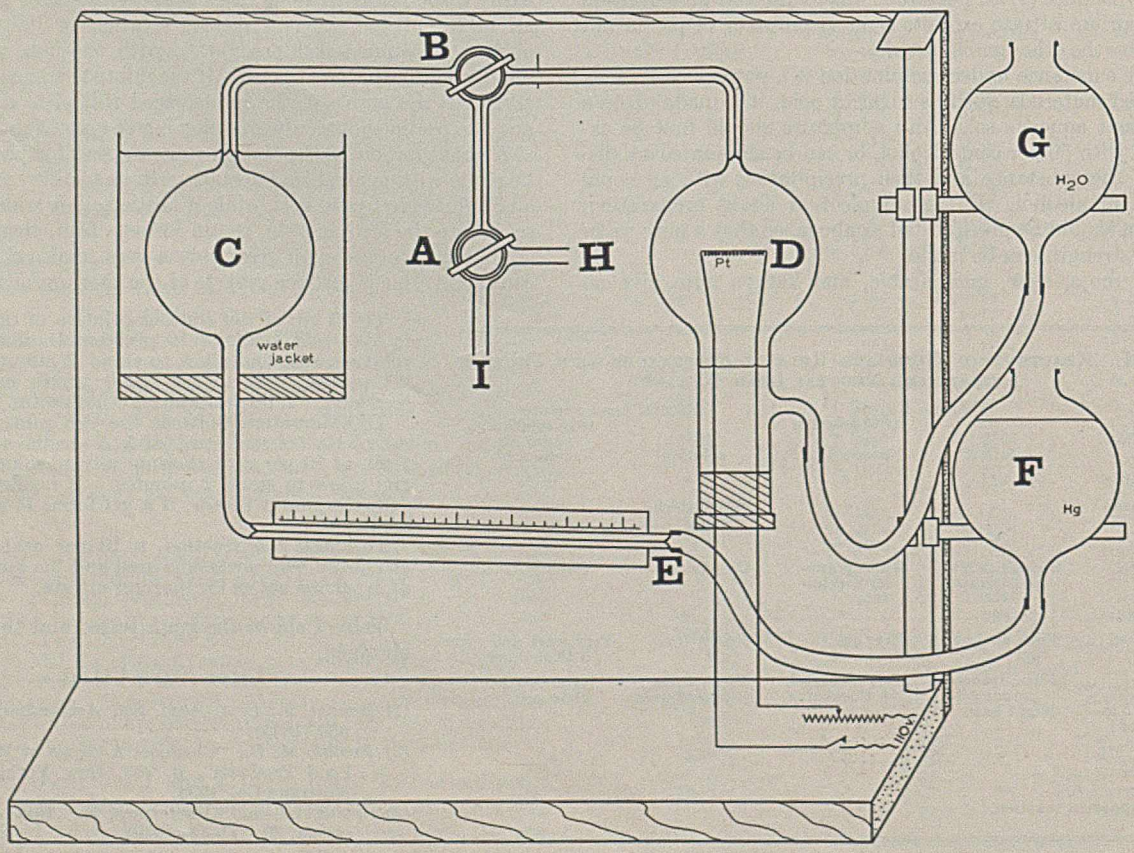
PRELIMINARY OPERATIONS. The residual air in the apparatus must first be replaced by tank hydrogen. Once this has been attained the steps in this section may be omitted.

Open passage *DBI* and raise *G* until the water nearly reaches *B*. Open passage *CBI* and raise *F* until the mercury gushes into *BI*. Excess mercury may be caught in a beaker held at *I*. Attach the hydrogen sample at *H*, open *HAI*, and flush out with the hydrogen. Then turn the cocks to open passages *HABC*, filling *C* with hydrogen. Flush out the residual air in *BD* by opening *CDB*, running in hydrogen by raising *F* or lowering *G*, and finally opening *DBAI* and *CBAI*. Both water and mercury will now be a few centimeters from *B*, and the tiny volume of gas between them will be nearly pure hydrogen. Therefore all subsequent measurements may omit these preliminary operations.

MAKING AN ANALYSIS. Open *CBAI*, and raise *F* to empty the gas out of *CB* until mercury again spills out at *I*. Open *HAI* to flush stopcock; then open *HABC* and run in the sample to be analyzed, stopping when the mercury reaches a point near *E* on the manometer. At this point, switch to *HAI*, and finally close

TABLE I. CONSECUTIVE READINGS ON MIXTURES

Composition of Gas by Volume Hydrogen	Oxygen	Movement in Manometer	Oxygen Found
%	%	*Mm.*	%
99.949	0.051	27	0.054
99.900	0.100	48	0.096
99.864	0.136	70	0.140
99.937	0.063	30	0.060
99.906	0.094	50	0.100



stop cocks *A* and *B*. Record the position of the mercury on the manometer scale. Open passage *CBD*, and run the sample over into *D*, stopping when the mercury is a few centimeters from *B*. The water level will be well below the platinum wire. Close *B* and switch on the electricity, keeping the platinum spiral heated to a dull red, not white, heat. With hydrogen containing about 0.05 per cent oxygen 10 seconds are sufficient; for 0.20 per cent oxygen heat for 1 minute, but never any longer. Allow the gas to cool for 2 minutes, return it to *C*, stopping the water at the scratch 3 cm. from *B*, and record the new manometer reading. The calibration of the manometer can be calculated from its slant, but it is simpler to make a direct measurement with empirical mixtures of oxygen and hydrogen.

Table I shows that a movement of the mercury along 1 mm. of the manometer corresponds to 0.002 per cent of oxygen by volume in the sample.

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Tungsten-Nickel and Tungsten-Silver Electrode Systems in Neutralizations

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THE bimetallic electrode systems, tungsten-nickel and tungsten-silver, were examined to ascertain their value for precise work in neutralizations involving dilute solutions. Kahlenberg and co-workers (*1*, *4*, *5*) have especially recommended tungsten as an indicator electrode for neutralizations, and among the bimetallic electrode systems suggested by these investigators for such reactions are tungsten-nickel and tungsten-silver. After a precise study of the tungsten-nickel system in neutralizations Furman and Low (*3*) reported this system of value in titrations of strong acid with strong base, and *vice versa*, for solutions 0.1 *N* and above. According to their observations nickel undergoes an abrupt change in potential at a pH about that at which the transition of methyl orange occurs, but is relatively insensitive in the region at which phenolphthalein changes.

It is the purpose of the present paper to show that these characteristics of the nickel electrode make the tungsten-nickel pair unsuitable for precise work in the titration of solutions as dilute as 0.01 *N*, and to describe the results of a detailed study of the tungsten-silver system in neutralizations involving solutions the approximate concentrations of which are between *N* and 0.001 *N*.

Materials and Procedure

Solutions of about the normality desired were prepared from reagents of analytical grade, the alkali solutions being nearly free of carbonate and the usual precautions being taken to protect them from atmospheric carbon dioxide.

Electrodes were of the following B. & S. wire gages: tungsten, 19; nickel, 8 and 24; silver, 24. After each titration the electrodes were cleaned with sandpaper, then washed with 6 *N* nitric acid and several changes of distilled water. Occasional omission of the nitric acid washing seemed to have no bearing upon the results.

A definite volume, between 15 and 40 ml., of the solution to be titrated was placed in a closed container and the neutralization was carried out at room temperature in an atmosphere of nitrogen free of carbon dioxide. While the solution was being stirred by a motor-driven stirrer, the course of the titration was followed by means of a Leeds & Northrup students' type potentiometer and accessories. Readings were taken after the e. m. f. appeared virtually constant. In the neighborhood of the end point these were recorded after each drop of the solution was added. The maximum of the Δ e. m. f./Δ ml. was taken as the electrometric end point and compared with end points obtained either simultaneously or independently with phenolphthalein, methyl orange, or methyl red indicators. The color change for methyl red was made more distinctive by the presence of methylene blue. Each of the end-point ratios (ml. of acid per ml. of base) tabulated in the following section represents the mean of values derived by three or more titrations. The average deviation from this mean is also given for the electrometric end-point ratios.

In many of the titrations employing the tungsten-silver system, the potential of each electrode was checked repeatedly against a reference electrode, a 0.1 *N* calomel cell—i. e., after each addition of reagent in the course of a titration, e. m. f. readings were taken for the systems tungsten-silver, tungsten-calomel, and silver-calomel.

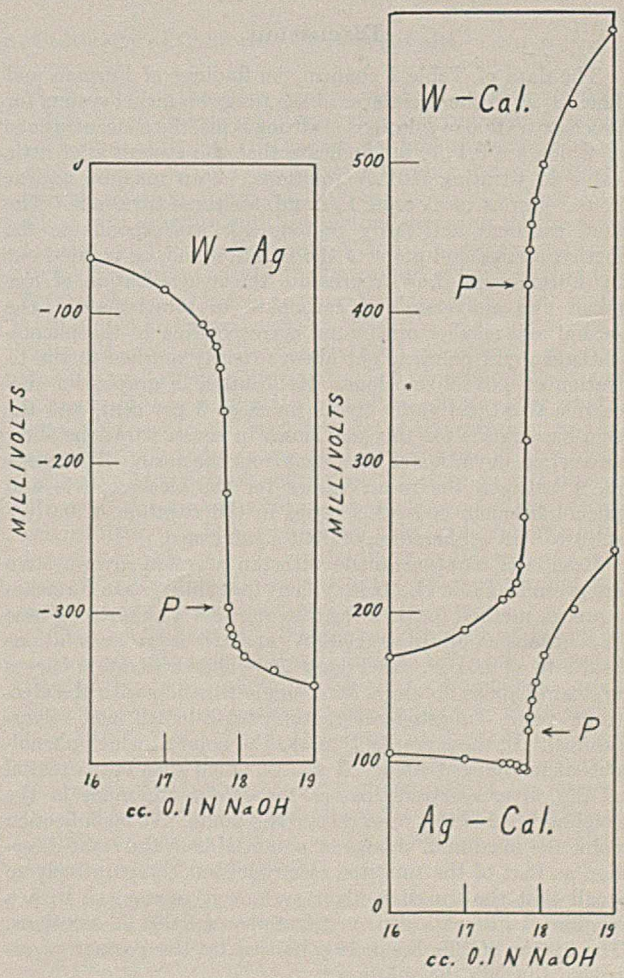

FIGURE 1. TITRATION OF 0.1 *N* HYDROCHLORIC ACID
Using tungsten-silver, tungsten-calomel, and silver-calomel system

TABLE I. TUNGSTEN-NICKEL SYSTEM

(Solution added: NaOH, normality approximately that of solution being titrated)

Solution Titrated (Approximate Normality)	End-Point Ratios: Methyl orange	Electrometric	Phenolphthalein	Electrometric
		Ml. acid per ml. base		
0.1 *N* HCl	1.136	1.138 ± 0.002	1.131	1.130 ± 0.001
0.1 *N* H_2SO_4	1.128	1.129 ± 0.004	1.118	1.120 ± 0.002
0.01 *N* HCl	...	1.062 ± 0.003	1.015	1.037 ± 0.01
0.01 *N* H_2SO_4	...	1.069 ± 0.003	1.043	1.045 ± 0.01

TABLE II. TUNGSTEN-SILVER SYSTEM

Solution Titrated (Approximate Normality)	Solution Added (Approximate Normality)	End-Point Ratios: Methyl red	Phenolphthalein	Electrometric
			Ml. acid per ml. base	
N HCl	*N* NaOH	...	1.162	1.162 ± 0.001
N H_2SO_4	....		0.7745	0.7741 ± 0.0006
0.1 *N* HCl	0.1 *N* NaOH	...	0.8380	0.8388 ± 0.0006
0.1 *N* H_2SO_4	....		0.8294	0.8297 ± 0.0006
0.01 *N* HCl	0.01 *N* NaOH	...	1.037	1.041 ± 0.001
0.01 *N* H_2SO_4	....		1.060	1.064 ± 0.002
0.001 *N* HCl	0.001 *N* NaOH	...	...	1.007 ± 0.002
0.001 *N* H_2SO_4	....	...	...	1.088 ± 0.002
N NaOH	*N* HCl	...	1.156	1.156 ± 0.001
....	*N* H_2SO_4	...	0.7726	0.7726 ± 0.0003
0.01 *N* NaOH	0.01 *N* HCl	...	1.037	1.043 ± 0.001
....	0.01 *N* H_2SO_4	...	1.060	1.063 ± 0.001
0.1 *N* HCl	0.1 *N* NH_4OH	0.9431	....	0.9429 ± 0.0003
0.1 *N* H_2SO_4	....	0.6067	....	0.6070 ± 0.0003
0.01 *N* HCl	0.01 *N* NH_4OH	1.131	....	1.132 ± 0.001
0.01 *N* H_2SO_4	....	1.685	....	1.685 ± 0.004
N $HC_2H_3O_2$	*N* NaOH	...	0.7863	0.7863 ± 0.0006
0.1 *N* $HC_2H_3O_2$	0.1 *N* NaOH	...	0.8922	0.8941 ± 0.0006

Discussion

The data of Table I confirm the findings of Furman and Low (*3*) as to the usefulness of the tungsten-nickel system for precise titration of solutions of strong acids, the concentrations of which are 0.1 *N*, but indicate that the system is of little value in titrating 0.01 *N* solutions. Two maxima appear in the graphs of Δ e. m. f./Δ ml. for these titrations. The first, excellent and fairly reproducible, corresponds to the methyl orange end point of the titration and, as pointed out by Furman and Low, represents the neutralization of free alkali and conversion of carbonate to bicarbonate. The second and smaller maximum, corresponding to the phenolphthalein end point, is not always clearly marked in the titration of 0.01 *N* solutions. Its location in graphs for successive titrations varies by as much as 3 per cent, and the acid-base ratios for this maximum in seven titrations show an average deviation of 1 per cent from the mean. The agreement between the mean values for the electrometric and phenolphthalein end-point ratios in the titration of 0.01 *N* sulfuric acid is, therefore, of little significance.

Results of titrations made with the tungsten-silver system are given in Table II. It is evident that this system furnishes a precise method for titrating a strong acid with a strong base in solutions as dilute as 0.001 *N*, and *vice versa* for solutions as dilute as 0.01 *N*. In Figure 1 are shown titration curves typical of those obtained for a single titration with the electrode pairs, tungsten-silver, tungsten-calomel, and silver-calomel. In these graphs *P* marks the point at which phenolphthalein changed color. It will be noted that the potential of the silver electrode passes through a minimum in the neighborhood of this colorimetric end point. At the inflection point the maximum change in potential is in the same direction as that of the tungsten electrode, but comparatively so small that the tungsten-silver system gives a curve with a significant "break" even in titrations of 0.001 *N* solutions. The extent of this break is enhanced by the passage of nitrogen through the solution.

An attempt was made to see whether the tungsten-silver pair permits the precise titration of solutions of strong acids more dilute than 0.001 *N*. To this end 20-ml. samples of approximately 0.01 *N* sulfuric acid and hydrochloric acid, respectively, were added to about 1700 ml. of water, and the titration was made with 0.01 *N* sodium hydroxide. The following data indicate that the use of the tungsten-silver system leads to precise results under the conditions just described:

	End-Point Ratio: Ml. of 0.01 *N* Acid per Ml. of 0.01 *N* Base: Electrometric	Colorimetric
H_2SO_4	0.9786 ± 0.001	0.9789
HCl	0.9640 ± 0.0002	0.9656

The data of Table II likewise indicate that this system is suitable for the titration of strong acids with the weak base, ammonium hydroxide, and for the titration of the weak acid, acetic acid. Titration curves for the former are similar to those of Figure 1, with a break not quite so great. Figure 2 is a typical e. m. f.–ml. graph for the titration of acetic acid, in which the first of two inflection points coincides with the phenolphthalein end point, *P*. Graphs for the behavior of the

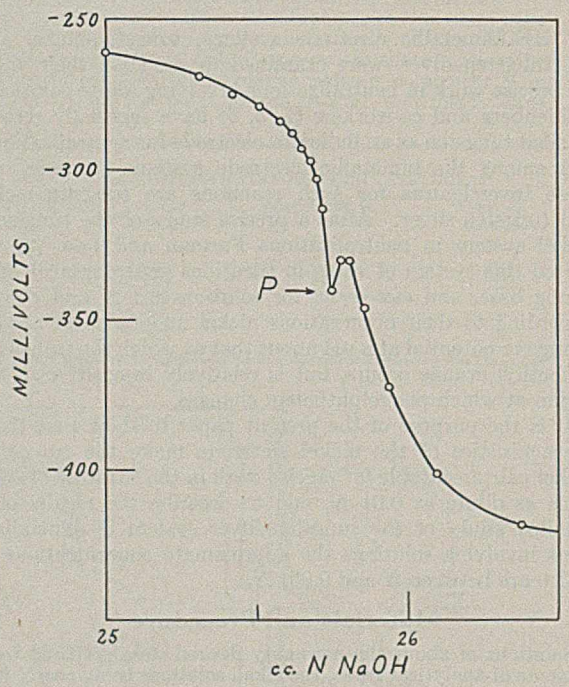

FIGURE 2. TITRATION OF *N* ACETIC ACID
Using tungsten-silver system

silver-calomel and tungsten-calomel pairs during the titration of acetic acid show that to obtain the greatest change in the potential of the silver-calomel system one drop (about 0.03 ml.) more of sodium hydroxide solution is required than for the tungsten-calomel system. Consequently the electrometric end point of the tungsten-silver system arises almost wholly from the behavior of the tungsten electrode; upon addition of base beyond the electrometric end point, changes in the potential of the silver electrode offset those of tungsten to such an extent that two inflection points are observed. Potentiometric readings were not recorded until apparently constant. However, it is possible that in these solutions the electrodes approach equilibrium with extreme slowness and that the behavior observed is due to the system's not having attained equilibrium (*2*). Titration of acetic acid solutions less than 0.1 *N* was not feasible, as the changes in potential were too gradual to permit precise determination of the end point. For the same reason it was not possible in the titra-

TABLE III. COMPARISON OF END POINTS

(Solution added: NaOH, approximately 0.01 *N*)

Solution Titrated (Approximate Normality)	End-Point Ratios: Hydrogen-calomel	Tungsten-silver	Phenolphthalein
	Ml. acid per ml. base		
0.01 *N* H_2SO_4	1.022 ± 0.001	1.021 ± 0.002	1.021
0.01 *N* HCl	0.6918 ± 0.0006	0.6926 ± 0.0006	0.6906

tion of sodium carbonate (0.5 *N*) with hydrochloric acid (*N*) to determine precisely the electrical end point corresponding to the phenolphthalein end point. At the methyl orange end point of the carbonate titration, however, the tungsten-silver system gave a definite, reproducible electrometric end point.

To determine the accuracy, as well as the precision, of the tungsten-silver electrode system in titrations of dilute solutions of strong acids, a comparison was made of end-point ratios obtained by the use of hydrogen-calomel, tungsten-silver, and phenolphthalein, respectively. These data appear in Table III.

Summary

The tungsten-nickel electrode system has not been found satisfactory in neutralizations involving dilute solutions. The tungsten-silver system appears to furnish precise and accurate electrical end points in titrations of strong acids by strong bases as dilute as 0.001 *N*, and *vice versa* for 0.01 *N* solutions. In titrations employing more concentrated solutions the system is of value in the neutralization of strong acids by ammonium hydroxide, of acetic acid by a strong base, and of sodium carbonate.

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Extraction and Determination of Pyrethrin I in Ground Pyrethrum Flowers

An Improved Apparatus

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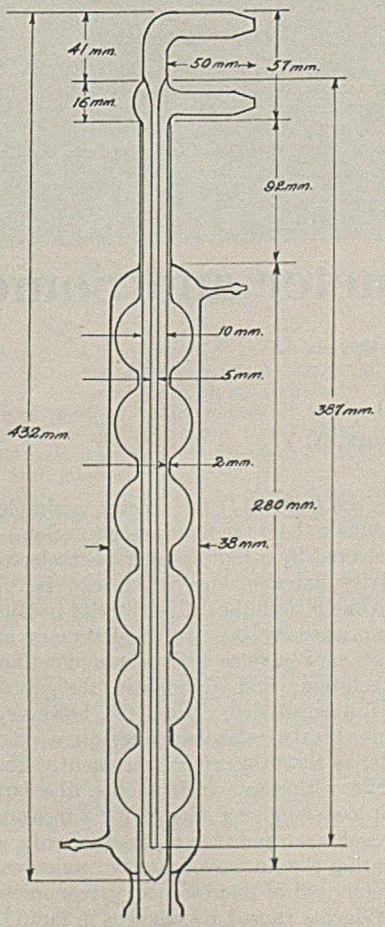

FIGURE 1. AUXILIARY CONDENSER

MANY of the more reliable methods (*1–4*) developed in recent years for the extraction of pyrethrum flowers for analysis recommend the use of low-boiling-point petroleum ether (20° to 40° C.) as a solvent, because it can completely remove the pyrethrins without removing other substances which will interfere with the method subsequently employed for the pyrethrin determination, and this solvent may itself be easily removed with the least amount of heat, so that there is little chance of loss of the pyrethrins.

A simple and inexpensive modification of an apparatus for the extraction and determination of pyrethrin I in ground pyrethrum flowers is proposed. The objects of the changes are primarily to reduce the loss of petroleum ether used for extraction and to eliminate transfer of the extracted and subsequently refluxed solution to a larger container for alcohol removal. Modifications of the condenser amount to insertion of a cold-water column into each condenser and use, as a receiving flask, of a 500-cc. Kjeldahl flask with the same size of interchangeable ground-glass joints as in the rest of the apparatus. Frothing during the evaporation of alcohol is reduced and less attention is required.

The use of a Soxhlet with either an Allihn or a Graham condenser, all with ground-glass joints, in a 7- or 8-hour extraction of ground pyrethrum flowers with low-boiling-point petroleum ether has heretofore resulted in a 60 to 75 per cent loss of a 150-cc. volume of solvent used, especially during warm weather (29.44° C., 85° F., or above) or whenever the temperature of water used for circulating in condensers is much above 25° C. This has been due chiefly to inefficient or insufficient cooling surface in the condenser rather than to leakage in glass connections.

To remedy the difficulty without increasing the length of the condenser so as to make the height of the apparatus too great for convenient handling, a double tube equivalent in length (30 to 42.5 cm., 12 to 17 inches) to the Allihn condenser, and of a diameter to allow a clearance of 2 to 3 mm. at the bulb constrictions, was constructed (Figure 1). It can be inserted into the condenser to the bulblike enlargement that acts as a rest point, and then

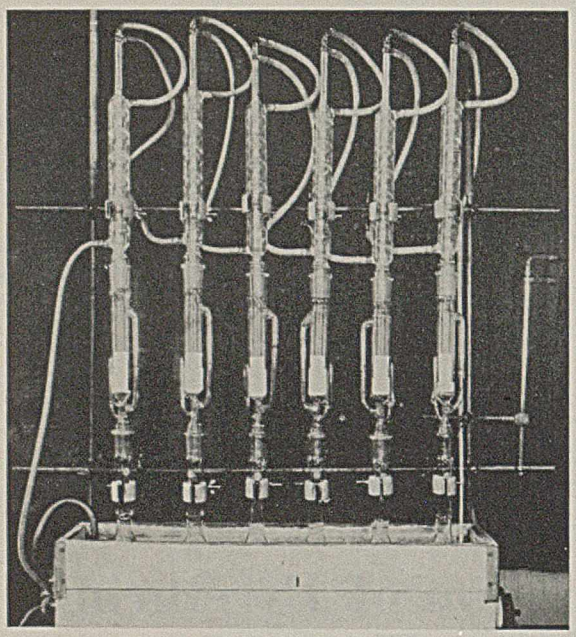

FIGURE 2. SIX EXTRACTORS IN OPERATION WITH CONDENSER MODIFICATIONS
Single unattached condenser modifier at extreme right

connected to receive the outflow of cold circulating water from the Allihn condenser before it goes into the next condenser. This modification has the effect of a double condenser, or a condenser within a condenser.

According to several published analytical methods (*1–4*), the extracted material after complete removal of the solvent must be refluxed with 0.5 *N* alcoholic sodium hydroxide and then, with about 100 cc. of water, transferred to a container (500- to 800-cc. beaker) large enough to accommodate frothing, which always takes place during the removal of alcohol with heat and requires much attention to prevent loss of sample.

If a 500-cc. Kjeldahl flask, specially made with the same size of ground-glass joint as that of the Soxhlet and Allihn condenser, is substituted for a receiving flask in the initial extraction, refluxing and subsequent removal of the alcohol after the addition of water may be accomplished without any transfer, by simply heating the flask with a very low flame in a Kjeldahl digestion rack. Frothing will still exist in the first minutes of boiling and requires some attention, but this soon diminishes as the removal continues, accompanied by a decrease in volume.

Figure 2 shows six complete extractors with the above-mentioned modification. Each Kjeldahl flask used as a receiver is immersed in a water bath (45° to 50° C.) resting on an electric hot plate, with a slow continuous stream of cold water flowing into the bath to replenish the loss of water due to evaporation and at the same time prevent the temperature of the bath from rising very much above the desired range.

Using the modified apparatus, the loss of ether and volume required for extraction have been reduced approximately 50 and 33 per cent, respectively. In addition, little attention is required during the removal of alcohol by heat. These modifications have resulted in greater economy, considerable saving of time, and elimination of a health hazard in the excessive escape and possible accumulation of fumes of a highly volatile solvent.

Acknowledgment

The author is indebted to R. McCready for the suggestions and construction of the type of condenser modifier used.

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New Photoelectric Fluorimeter and Some Applications

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FLUORIMETRIC methods of quantitative analysis have increased in popularity in recent years. These methods must be used when the substances to be determined are present in such small amounts that colorimetric methods cannot be used, but are limited to substances which fluoresce when irradiated by light of the proper wave lengths, usually between 3000 and 4500 Å.

Fluorimeters of two general types are in use. One type of instrument depends on a visual comparison of an unknown with a standard of fluorescence. When the unaided eye is used to determine the match of unknown and standard, the sensitivity of the method is low and decreases with increasing concentration. The use of an instrument such as the modified colorimeter of Josephy (*7*) or a Pulfrich photometer (*12*) increases sensitivity and accuracy of match over those possible by the unaided eye.

The other type of instrument employs a photocell and galvanometer to measure the fluorescence. Most European workers use the Cohen (*1*) fluorimeter, a simple instrument with a test tube to hold the fluorescing solution. The fluorescence is measured by a barrier-layer photoelectric cell and a high-sensitivity galvanometer which can be used only at one tenth to one fiftieth the full sensitivity because of fluctuations of lamp intensity (*4*). In the Hennessy and Cerecedo (*3*) modification, a cuvette with rectangular faces holds the fluorescing solution; and, apparently, the galvanometer can be used at full sensitivity. Hand (*2*), however, uses an instrument in which a relatively insensitive pointer type of galvanometer is the measuring instrument. To obtain consistent results with these instruments, the lamp intensity must remain constant long enough for a measurement to be made of the unknown and then of the standard.

By measuring the fluorescence of an unknown solution in terms of a standard of fluorescence, galvanometer drifts and sudden movements caused by changes in lamp intensity can be eliminated. If the ratio of the fluorescence of an unknown

to that of a standard be measured by a two-photocell balanced-circuit, a high-sensitivity galvanometer can be used at full sensitivity as a null-point indicator; and the range of concentrations measurable can be changed by changing the concentration of the standard. An instrument (made by the Klett Mfg. Co., New York, N. Y.) which incorporated these features was designed and has been used for a variety of measurements for 2 years in this laboratory. Since no other fluorimeter, as yet, employs these principles of design, it seems desirable to describe this one and to indicate how it has performed in some of the quantitative procedures in which it has been tested.

The Fluorimeter

The design of the instrument is such that the potentiometer reading is proportional to the intensity of the fluorescent light emitted by the unknown solution.

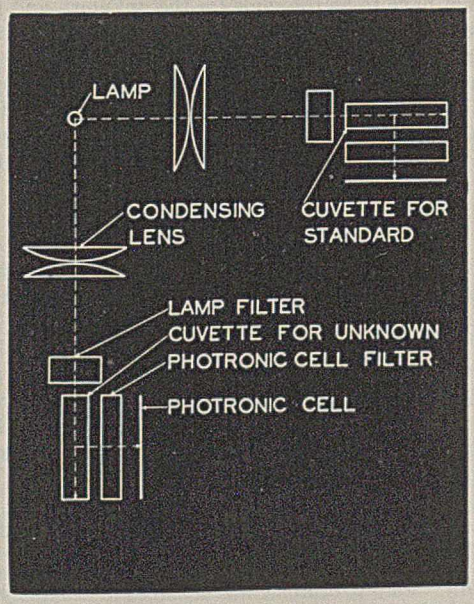

FIGURE 1. ARRANGEMENT OF OPTICAL PARTS OF FLUORIMETER

Ultraviolet or other lines from a type H-4 mercury lamp excite the fluorescence of the solutions in the cuvettes (Figure 1). The lines are isolated by glass filters (the lamp filters). The fluorescent light passes through a face of the cuvette that is parallel to the ultraviolet light beam, then through another light filter (the photocell filter) to a barrier-layer photocell. The two cuvettes are irradiated by the same lamp at the same time; one holds the unknown solution; the other, a standard solution, usually quinine sulfate. The photocells are arranged in a compensated circuit, so that fluctuations in lamp intensity do not affect the operation of the instrument. The ratio of the fluorescence of the unknown to that of the standard is indicated by a built-in potentiometer with a 300-division linear scale.

A portable mirror-type galvanometer with a sensitivity of 0.004 microampere per division is sufficiently sensitive as a null-point indicator for most measurements. All the work reported here was done with a Leeds & Northrup Type R galvanometer used at a sensitivity of 0.001 microampere per millimeter. By making the measurements in terms of a standard, all uncertainties caused by fluctuations and drifts of lamp intensity, nonlinear galvanometer scale, and inconstancy of galvanometer response are eliminated. (Summerson, *9*, gives details concerning the advantages of the two-cell balanced circuit.)

The standard must fluoresce more than the unknown. Because doubling the concentration of the standard halves the potentiometer reading for a particular unknown solution, the range and sensitivity of the instrument can be changed by changing the concentration of the standard. Quinine sulfate U. S. P., 1 or 2.5 mg. per liter in 0.1 *N* sulfuric acid, most often served as a standard solution, although glass standards could have been used.

Filters for a particular determination must be selected with reference to the absorption bands of the fluorescent substance, the concentration range to be measured, and the wave-length limits of the light emitted by the fluorescing solution. The lamp filter must pass lines that are absorbed by the substance which is to fluoresce. For the determination of small amounts of material, the lines that excite the fluorescence should be as near as possible to absorption peaks of the absorption spectrum of the fluorescing substance. To obtain a low and reproducible blank, the photocell filter must absorb those lines of the mercury arc which are passed by the lamp-filter. When this condition is satisfied, a small amount of suspended material in the solution in the cuvette will not affect the response of the instrument.

The determination of some substances can be made more specific by selecting a photocell filter that transmits a band of light not much wider than the band of fluorescent light. For example, the Corning 306 filter can be used in determining thiochrome; but a 306 plus a 430 filter would give a more specific response to thiochrome if other substances which gave fluorescent light of wave lengths longer than about 5000 Å. were present. The filters are all Corning Glass Works polished glass filters and are designated by their Corning numbers. The filters are not necessarily of standard thickness, but the thickness is given for each filter.

By assuming that the intensity of the fluorescent light is proportional to the amount of the exciting light absorbed by the unknown solution and that Beer's law holds for the absorption, the relation between the potentiometer reading, P, and the characteristics of the fluorescing substance is given by

$$P = KI(1 - 10^{-\alpha c b}) = KIf \qquad (1)$$

in which I is the intensity of the exciting line at the lamp end of the cuvette, α is the specific absorption coefficient of the substance for the exciting line, c is the concentration of the fluorescing substance, and b is the length (36 mm.) of the opening in the mask over the photocell. The dimensions of the mask determine the volume of the solution from which the fluorescent light comes and remove the necessity for using accurately measured volumes of solution in the cuvettes. Constant K includes, among other factors, the dimensions of the light beam, the area of the mask opening, the transmission band of the photocell filter, and the spectral sensitivity of the photocell. For a particular instrument, and

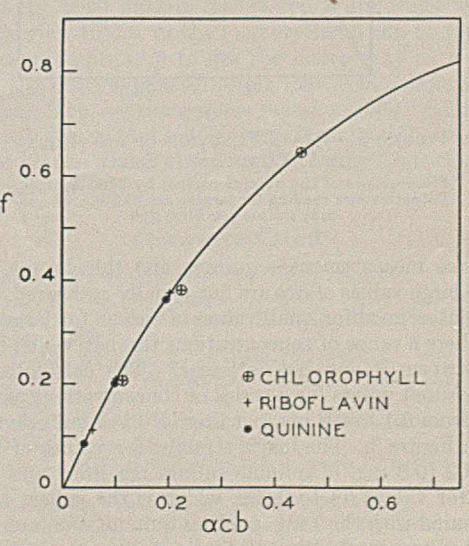

FIGURE 2. f AS A FUNCTION OF αcb (EQUATION 1)
Fluorescence of chlorophyll excited by 4358 Å. line, of quinine by the 3660 Å. lines, and of riboflavin by 4358 Å. line

a given combination of lamp and photocell filters, standard, and substance to be determined, the variables are P and c.

The relation between P and αcb was tested by measuring solutions of quinine, riboflavin, and ethyl chlorophyllide for small and large values of αcb by varying both c and the value of α (by changing the wave length of the exciting line). The results are given in Figure 2, in which the measurements are indicated by the points and the curves are the graph of f against αcb. The points are located in the following manner: The αcb is calculated for each concentration of a series of measurements and a value of f for each concentration is calculated from

$$f = \frac{P}{P_m} f_m \qquad (2)$$

where P_m is the potentiometer reading and f_m is the value of f for the highest concentration. The agreement between the calculated and measured values of f is good for a wide range of concentrations. The concentration, c, is expressed as grams per liter and $\alpha = \frac{\epsilon}{\text{mol. wt.}}$ = specific absorption coefficient.

$\alpha cb = \epsilon Cb$, where ϵ = molecular absorption coefficient and C = moles per liter; b is expressed as cm.

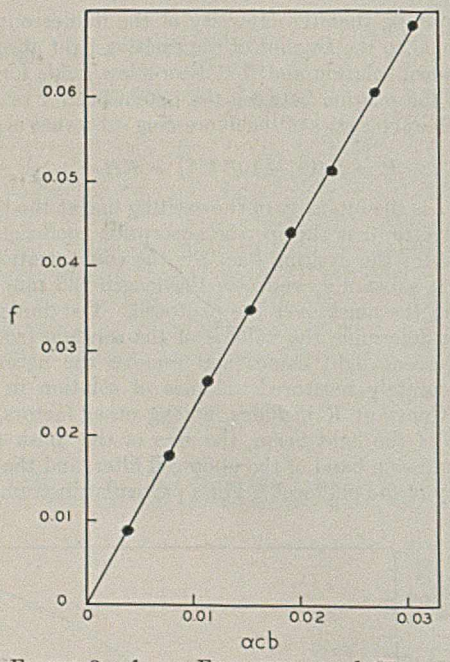

FIGURE 3. f AS A FUNCTION OF αcb (EQUATION 1) WHEN αcb IS SMALL

Fluorescence of the quinine excited by 3660 Å. lines. Potentiometer reading for largest αcb (0.8 mg. of quinine sulfate per liter) 266

In some measurements—quinine and thiochrome, for example—large values of αcb are not usually measured. Is the agreement as good for small values of αcb as for large values, and is there a range of concentrations through which the relation between P and αcb is linear? The calculated curve and measured points for a series of concentrations of quinine sulfate from 0.1 to 0.8 mg. per liter in 0.1 N sulfuric acid are given in Figure 3. The line is straight for values of αcb less than 0.02 (0.5 mg. of quinine sulfate per liter) and slightly curved for values up to 0.032, which is the largest that can be measured with the 1 mg. per liter quinine standard and the particular lamp and photocell filters used.

Whether the potentiometer-concentration curve will be nearly straight or strongly curved depends upon the value of α, the intensity of the exciting lines, the fraction of the absorbed light emitted as fluorescent light, and the spectral sensitivity of the photocell. (The intensity, the wave length of the exciting line, and the spectral sensitivity of the photocell do not have to be the same for the unknown and for the standard.) The concentration range that can be measured for a particular set of conditions always lies between zero and the concentration that gives a potentiometer reading of 300. If the measurable concentration range is large, the potentiometer scale reading–concentration curve will be strongly curved; if the range is short, the potentiometer–concentration curve will approach a straight line.

TABLE I. RANGE OF CONCENTRATIONS OF SUBSTANCES DETERMINED WITH FLUORIMETER

("Lower" concentration gives potentiometer reading of approximately 10 with filters and standard indicated. Filters for quinine standard were lamp, 597; photocell, 306.)

Substance Determined	Concentration		Filters		Quinine Standard
	Lower	Highest	Lamp	Photocell	
	Mg. per liter				*Mg. per liter*
Aluminum	0.04	0.9	554	338	1
	0.1	6	597	306	1
Chlorophyll	0.03	0.6	585	243	1
	0.1	5	597	243	1
Quinine	0.03	0.9	597	306	1
Riboflavin	0.005	0.15	554	351	0.25
	0.02	0.6	554	351	1
	0.05	2	597	351	2.5
Thiochrome	0.005	0.1	597	306	1

Analyses with the Fluorimeter

Methods for determining thiamin, riboflavin, chlorophyll, and aluminum have been tested with this fluorimeter to determine the possibilities of the instrument and the limitations of the methods. The solutions did not necessarily contain accurately measured quantities of the substances and no attempt was made to establish standard curves. The concentration of the standards, the filters, the upper concentration of the unknown, and the concentration for which there is a potentiometer reading of 10 are shown in Table I.

In all the work reported here, the lamp filter for the standard solutions was a 597 (5.25-mm.) and the photocell filter was a 306 (3.2-mm.).

THIAMIN. Pure thiochrome was dissolved in n-butanol. The first test with this compensated circuit fluorimeter showed that thiochrome decomposed rapidly when irradiated by 3660 Å. lines and was about half destroyed in 10 minutes. By using the following procedure accurate measurements were possible:

With the quinine standard in place, put the cuvette containing the thiochrome into the instrument and start a stop watch the moment the cuvette enters the beam of ultraviolet light; at definite intervals after placing the cuvette in the light beam, measure the fluorescence, at least four times in the first 2 minutes; correct the measurements for the fluorescence of the blank; plot the logarithm of the corrected potentiometer readings against time on semilog paper; and extrapolate to zero time to obtain the potentiometer reading of the undecomposed thiochrome. These potentiometer readings at zero time for solutions of different concentrations plotted against the concentration gave a straight line. If an error of 1 or 2 per cent is of no importance, read the potentiometer as quickly as possible and assume that no decomposition has occurred. The upper limit of measurement for the thiochrome method for thiamin with this fluorimeter is about 150 micrograms of thiamin per liter; the lower limit, about 1 microgram per liter.

Caution. Butanol can remove enough grease from an apparently clean stopcock of a separatory funnel to give an appreciable fluorescence, and more fluorescing substance from a Whatman No. 42 filter paper used to remove suspended material than is contained in some solutions of thiochrome.

RIBOFLAVIN. Riboflavin can be determined either fluorimetrically or colorimetrically. Supplee, Bender, and Jensen (*10*) compared visually the fluorescence of an unknown with a series of standards containing known amounts of riboflavin. Josephy (*7*), using a modified Duboscq-type colorimeter with sodium fluorescein as a standard, could determine amounts as small as 0.3 microgram in a 2-ml. sample. The methods using photoelectric fluorimeters for determining the fluorescence of

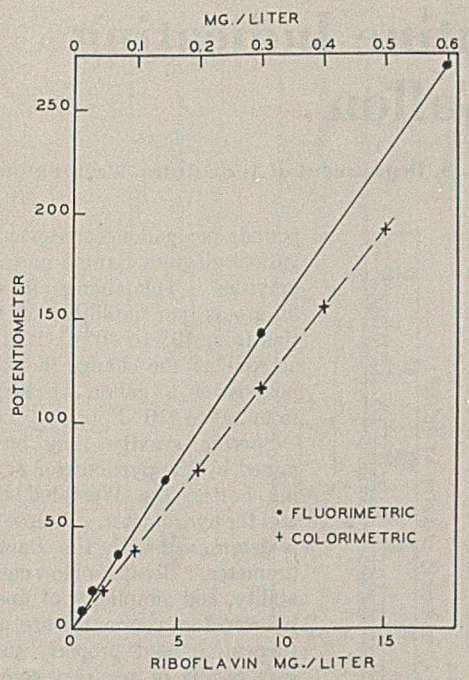

FIGURE 4. COMPARISON OF CALIBRATION CURVES FOR FLUORIMETER AND COLORIMETER IN DETERMINATION OF RIBOFLAVIN

Lower curve and scale of concentrations are for colorimeter (42 filter). Upper curve and scale at top are for fluorimeter using a 554 lamp filter and a 351 photocell filter

riboflavin are more accurate and sensitive than the visual methods and the results can be corrected for the presence of nonriboflavin fluorescing and light-absorbing substances as Hodson and Norris (*5*) have done.

This fluorimeter and a Klett-Summerson colorimeter (*9*) equipped with 42 filter have been compared over a wide range of concentrations of riboflavin dissolved in 66 per cent acetone. The colorimeter was satisfactory for concentrations between 1 and 20 mg. of riboflavin per liter (Figure 4). The fluorimeter could be used for concentrations between 0.005 and 2 mg. per liter (Table I). By the selection of various filters and quinine standards, several ranges of concentrations could be determined with the fluorimeter. Measurements could be made by the fluorimetric method on solutions too dilute to give any response with the colorimeter. Not only did the fluorimeter determine smaller amounts of riboflavin than the colorimeter, but small amounts of suspended material did not interfere with the determinations as they did with the colorimetric determinations.

The riboflavin, or similar substance, produced by several fungi in solutions in which they had grown, was measured by the method of Hodson and Norris (*5*). The results will be published elsewhere.

The corrections of Hodson and Norris for light-absorbing and nonriboflavin fluorescing substances are most easily applied when the potentiometer readings are proportional to the concentration of riboflavin. This condition was satisfied when a 554 (5.2-mm.) lamp filter was used at concentrations below 0.3 mg. of riboflavin per liter (Figure 4).

CHLOROPHYLL. The fluorescence of chlorophyll has been used in characterizing it for many years. Zscheile (*13*) found most of the fluorescence of an ether solution of chlorophyll *a* and of chlorophyll *b* to be between 6200 and 7600 Å. This fluorescence can be used to determine very small quantities of chlorophyll.

A series of dilutions of an unknown mixture of crystalline ethyl chlorophyllide *a* and *b* prepared by F. M. Schertz was made in *n*-butanol and used to determine a calibration curve for the fluorimeter. A 243 (3.3-mm.) filter, which cuts off wave lengths less than 6100 Å. and transmits freely above 6300 Å., was placed over the photocell that measured the fluorescence of the chlorophyll. Results for two lamp filters are given (Table I). The 585 (1.5-mm.) filter transmits the violet and ultraviolet lines of the mercury arc with little absorption. The lines passed by this filter excite the fluorescence of chlorophyll to such an extent that the highest concentration that can be determined with the 1 mg. per liter standard is low (0.6 mg. per liter, Table I); and, consequently, the calibration curve is straight. The 597 (5.25-mm.) lamp filter which passes only the ultraviolet lines excites much less fluorescence than the 585 filter, with the result that the highest concentration that can be determined is 5 mg. per liter; and the calibration curve is strongly curved. The thermocouple photometer method of Johnston and Weintraub (*6*) has a sensitivity almost as high as the fluorimetric method but is more difficult to use and requires more time for its operation. Both the colorimeteric and fluorimetric methods for total chlorophyll probably give different results for different ratios of chlorophyll *a* and *b* for the same total concentration.

If the chlorophyll solution is treated with dilute sulfuric acid, there is a rapid decrease in fluorescence to about 70 per cent of the original value as the result of conversion of chlorophyll into pheophytins. In quantitative work, the precautions recommended by Mackinney (*8*) must be observed.

To demonstrate the value of the fluorimetric method, the chlorophyll content of one *Lemna minor* was determined. The plant (fresh weight 2 mg.) was ground with a few milliliters of *n*-butanol, diluted to 20 ml. with *n*-butanol, and the fluorescence determined. The potentiometer reading was 39. Reference to the appropriate calibration curve indicated that the plant contained 6 micrograms of chlorophyll.

ALUMINUM. White (*11*) has given a review of recent work on fluorimetric methods applied to inorganic analysis. As an example of the application of the instrument to inorganic analysis, a calibration curve was made for the morin method of determining aluminum. The method was slightly different from the one used by White and Lowe (*12*).

To the aluminum in an acetate buffer at pH 4, 1 ml. of aqueous morin solution (0.75 gram per liter of morin, Eastman) was added, and the sample was diluted to 25 ml. with the acetate buffer solution. The results are given in Table I. The calibration curve for the filter combination 597-306 was straight up to 0.6 mg. per liter and then curved toward the concentration axis for higher concentrations. The filter combination 554-338 gives a linear calibration curve to 0.3 mg. per liter and a slightly curved one for higher concentrations. The former filter combination gives about five times the range and the latter filter combination gives at least three times the sensitivity of the instrument of White and Lowe. No effort was made to obtain conditions of maximum sensitivity.

Other Uses for Fluorimeter

The fluorimeter is provided with two photocells, one in each light beam, which can be connected to the potentiometer to form a Klett-Summerson type of colorimeter. For this reason, the potentiometer has the logarithmic scale of that colorimeter in addition to the linear scale of the fluorimeter. The colorimeter can be used with the lines in the visible spectrum and the 3660 lines of the mercury arc which can be isolated by glass filters, or the mercury lamp can be replaced by an incandescent lamp to use the instrument as a colorimeter of the usual type with filters that isolate narrow spectral regions. The colorimeter has the same linear calibration curve for substances that follow Beer's law and the ease of correction for blanks that is characteristic of the Klett-Summerson colorimeter.

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Hydrometer for Turpentine Indicating Pounds per Gallon

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IN COMMERCE turpentine is measured by both volume and weight. Domestic transactions are based on units of the standard gallon and the avoirdupois pound; and transportation charges are computed from weight. A lot of turpentine may be subjected to a considerable variation in temperature. While its weight will be affected but slightly by such changes, its volume will vary with the temperature in accordance with its coefficient of expansion.

The weight of a known volume of water and other liquids of unvarying composition at a definite temperature, or the volume of a known weight when facilities for actual determinations are not available, can be calculated from standard data. For turpentine and other liquids whose composition and properties vary it is necessary to determine the weight of a unit volume before such calculations can be made.

Federal specification LLL-T-791a requires that turpentine for use by the Government be purchased by volume, the unit being a gallon of 231 cubic inches at 60° F., or by weight in units of pounds or hundred pounds, and gives directions for correcting gallonage to the standard temperature of 60° F. and for determining the weight of a gallon in pounds. A gallon of acceptable turpentine must weigh from 7.16 to 7.29 pounds at 60° F. In making volume corrections a coefficient of 0.000945 per degree Centigrade, or 0.000525 per degree Fahrenheit, is used. The specific gravity of a sample is determined at 15.5° C./15.5° C. by any method that is accurate within two points in the fourth decimal place, and the weight of a gallon in pounds is determined by multiplying the figure thus obtained by 8.33.

Tables are available (*1*, *3*, *4*) for converting observed specific gravity and Baumé readings taken at other temperatures to true values at 60° F. and also tables showing the pounds per gallon corresponding to various degrees Baumé and specific gravities. The change in density in grams per milliliter of turpentine from 10° to 40° C. is given (*1*) as well as the change in weight in pounds of a gallon of turpentine from 20° to 110° F. (*4*).

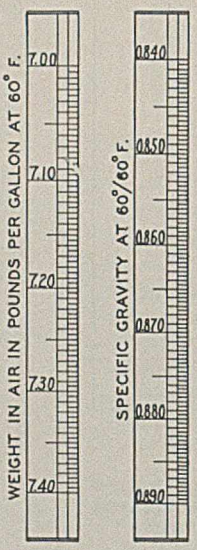


FIGURE 1

Specific gravity may be determined by the pycnometer or weighing bottle, the Westphal balance, and the hydrometer. Baumé gravity is determined with the Baumé hydrometer. Because of low cost, portability, and simplicity of operation, hydrometers commonly are used for turpentine, and properly used will give results of accuracy commensurate with that obtained in commercial weighing and gaging of liquids.

A method for weight-volume conversion of turpentine which will eliminate or reduce the use of tables is obviously desirable. A hydrometer indicating weight in air in pounds per gallon at 60° F. was not available, although hydrometers for reading per cent of alcohol and sugar and other special scales are in common use. An instrument manufacturer constructed four experimental hydrometers having 5-inch scales graduated as shown in Figure 1; over-all length, 12 inches; diameter of float chamber, 1 inch; and length of stem, 7 inches. These instruments, on standardization by the

TABLE I. WEIGHT IN AIR IN POUNDS PER GALLON OF TURPENTINE FROM 32° TO 95° F.

Temperature of Reading, ° F.														
32	7.261	7.271	7.281	7.290	7.300	7.310	7.320	7.330	7.340	7.350	7.360	7.369	7.379	7.389
34	7.254	7.264	7.274	7.283	7.293	7.303	7.313	7.323	7.333	7.343	7.353	7.562	7.372	7.382
36	7.247	7.257	7.267	7.276	7.286	7.296	7.306	7.316	7.325	7.335	7.345	7.365	7.375	7.385
38	7.249	7.239	7.249	7.259	7.269	7.279	7.289	7.299	7.308	7.318	7.328	7.338	7.348	7.358
40	7.232	7.242	7.252	7.262	7.272	7.282	7.292	7.302	7.311	7.321	7.331	7.341	7.351	7.361
42	7.225	7.235	7.245	7.255	7.265	7.274	7.284	7.294	7.304	7.314	7.324	7.334	7.344	7.354
44	7.218	7.228	7.238	7.247	7.257	7.267	7.277	7.287	7.297	7.307	7.317	7.327	7.337	7.347
46	7.210	7.220	7.230	7.240	7.250	7.260	7.270	7.280	7.290	7.300	7.310	7.319	7.329	7.339
48	7.203	7.213	7.223	7.233	7.243	7.253	7.263	7.273	7.283	7.293	7.303	7.302	7.322	7.332
50	7.196	7.206	7.216	7.226	7.236	7.246	7.256	7.266	7.276	7.286	7.296	7.305	7.315	7.325
52	7.189	7.199	7.209	7.219	7.229	7.239	7.249	7.259	7.269	7.278	7.288	7.298	7.308	7.318
54	7.182	7.192	7.202	7.211	7.221	7.231	7.241	7.251	7.261	7.271	7.281	7.291	7.301	7.311
56	7.174	7.184	7.194	7.204	7.214	7.224	7.234	7.244	7.254	7.264	7.274	7.284	7.294	7.304
58	7.167	7.177	7.187	7.197	7.207	7.217	7.227	7.237	7.247	7.257	7.267	7.277	7.287	7.297
60	7.160	7.170	7.180	7.190	7.200	7.210	7.220	7.230	7.240	7.250	7.260	7.270	7.280	7.290
62	7.152	7.162	7.172	7.182	7.192	7.202	7.212	7.222	7.232	7.242	7.252	7.26	7.272	7.282
64	7.145	7.155	7.165	7.175	7.185	7.195	7.205	7.215	7.225	7.235	7.245	7.25	7.265	7.275
66	7.137	7.147	7.157	7.167	7.177	7.187	7.197	7.207	7.217	7.227	7.237	7.247	7.257	7.267
68	7.130	7.140	7.150	7.160	7.170	7.180	7.190	7.200	7.210	7.220	7.230	7.240	7.250	7.260
70	7.122	7.132	7.142	7.152	7.162	7.172	7.182	7.192	7.202	7.212	7.223	7.233	7.243	7.253
72	7.114	7.124	7.134	7.144	7.154	7.164	7.174	7.185	7.195	7.205	7.215	7.225	7.235	7.245
74	7.107	7.117	7.127	7.137	7.147	7.157	7.167	7.178	7.188	7.198	7.208	7.218	7.228	7.238
76	7.099	7.109	7.119	7.129	7.139	7.149	7.159	7.170	7.180	7.190	7.200	7.210	7.220	7.230
78	7.092	7.102	7.112	7.122	7.132	7.142	7.152	7.162	7.172	7.183	7.193	7.203	7.213	7.223
80	7.084	7.094	7.104	7.114	7.124	7.134	7.144	7.155	7.165	7.175	7.185	7.195	7.205	7.215
82	7.076	7.086	7.096	7.106	7.116	7.127	7.137	7.147	7.157	7.167	7.177	7.188	7.198	7.208
84	7.069	7.079	7.089	7.099	7.109	7.119	7.129	7.140	7.150	7.160	7.170	7.180	7.191	7.201
86	7.061	7.071	7.081	7.092	7.102	7.112	7.122	7.132	7.142	7.152	7.162	7.173	7.183	7.193
88	7.054	7.064	7.074	7.084	7.094	7.104	7.114	7.125	7.135	7.145	7.155	7.165	7.175	7.186
90	7.046	7.056	7.066	7.076	7.087	7.097	7.107	7.117	7.127	7.137	7.148	7.158	7.168	7.178
92	7.039	7.049	7.059	7.069	7.079	7.089	7.099	7.109	7.120	7.130	7.140	7.150	7.160	7.171
94	7.031	7.041	7.051	7.061	7.071	7.082	7.092	7.102	7.112	7.122	7.132	7.143	7.153	7.163
95	7.027	7.037	7.047	7.058	7.068	7.078	7.088	7.098	7.108	7.119	7.129	7.139	7.149	7.159

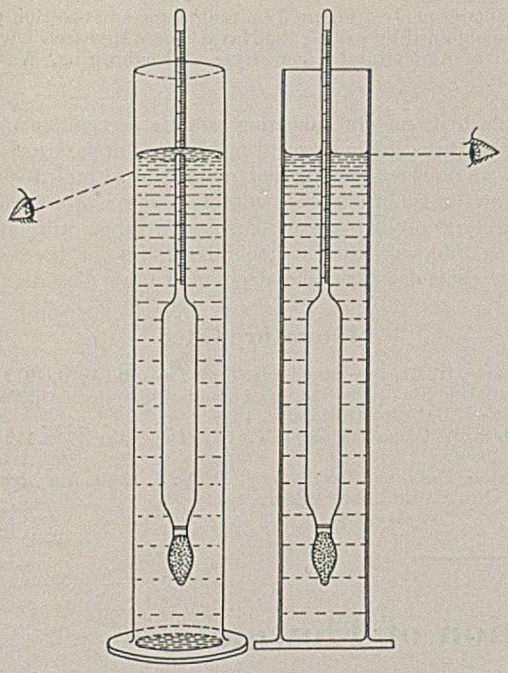


FIGURE 2. READING HYDROMETERS

National Bureau of Standards, were found to require maximum scale corrections of 0.002. The comparison liquid used in the standardization was mineral oil, which has a surface tension very close to that of turpentine. The coefficient of cubical expansion of the glass used in the instruments was stated by the manufacturer to be 0.000027 per degree Centigrade, or 0.000015 per degree Fahrenheit.

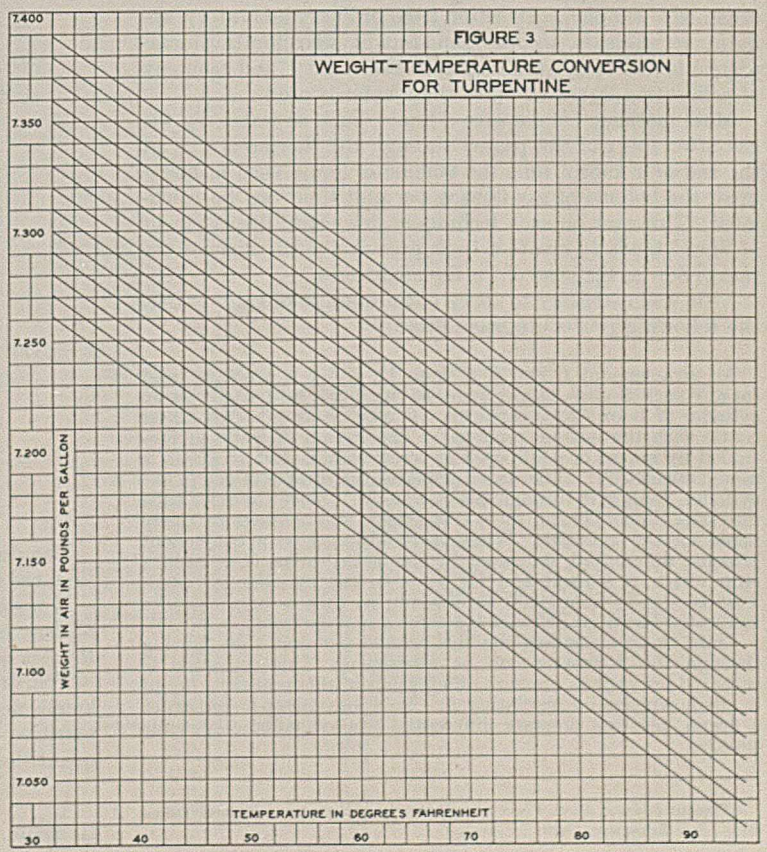
The difference between the observed reading and correct value is approximately 0.001 pound per gallon for each 10° F. above or below 60° F., subtracted from observed readings made above 60° F. and added to those made below this temperature. A sample on which a hydrometer reading at 80° F. showed 7.150 pounds per gallon has a true weight in air at 80° F. of 7.148 pounds per gallon. Such small corrections generally need not be applied, since the limit of accuracy in reading the hydrometer is ±0.001, and a change of only 0.3° F. in the sample will result in a change of 0.001 in hydrometer reading.

Table I and Figure 3 are designed primarily to convert observed hydrometer readings at other temperatures to pounds per gallon at 60° F. They may be used, with corrections above noted, for determining change in weight of a gallon of turpentine with temperature. The data used as a guide in preparing Table I (*3*) were obtained experimentally from 14 samples of gum spirits of turpentine and two samples of steam-distilled wood turpentine using a specific gravity hydrometer made of the same type of glass used in the pounds per gallon hydrometer and constructed by the same manufacturer.

To obtain the true weight in air of turpentine at the standard temperature of 60° F. from observed readings taken at other temperatures on a pounds per gallon hydrometer standardized for 60° F., locate observed reading, by interpolation if necessary, in horizontal line opposite temperature at which reading was made, and follow in vertical column to corresponding reading at 60°, which is the true weight in air at 60°.

The thermal expansion of destructively distilled or wood spirits of turpentine has been indicated (*1*) as about 10 per cent greater than that of either gum spirits of turpentine or steam-distilled wood turpentine. It is probable that the material produced at that date (1911) differed markedly from present-day destructively distilled wood turpentine. The writer has found aromatic hydrocarbons in very old destructively distilled wood turpentine, the presence of which would increase the thermal expansion of the turpentine. No data have been found on the thermal expansion of sulfate wood turpentine. In order to determine the relative thermal expansions of the four kinds of turpentine and to test the new hydrometer, three samples of sulfate wood, three of destructively distilled wood, two of steam-distilled wood, and two of gum spirits representing current production were obtained. Readings on both the specific gravity and the pounds per gallon hydrometer were taken on each sample at 34°, 68°, and 95° F. These tests showed that thermal expansion of turpentine is independent of kind. Therefore, the tables for specific and Baumé gravity hydrometers (*3*), and Table I and Figure 3 may be used for any of the four kinds of turpentine.

The accuracy of a determination will depend on the accuracy of the hydrometer and thermometer and the care with which they are used. To obtain the highest degree of accuracy, both the hydrometer and the thermometer should be standardized by the National Bureau of Standards.



Method of Reading Hydrometers

Fill a clear glass jar or cylinder having a height equal to the length of the hydrometer and an inside diameter at least 1 inch greater than the diameter of the hydrometer bulb to within about 2 inches of the top with the turpentine to be tested; place a thermometer in the jar, and set it on a table in a sheltered place. Carefully immerse the hydrometer in the turpentine to a point slightly below that to which it naturally sinks and then allow it to float freely. Be sure the hydrometer is not in contact with the jar or the thermometer. When the temperature as registered by the thermometer has become stationary and the turpentine and the hydrometer are free from air bubbles and are at rest, place the eye slightly below the plane of the surface of the turpentine (Figure 2, left) and raise the eye slowly until this surface seen as an ellipse appears to be a straight line (Figure 2, right). Take the reading of the instrument at the point at which this line cuts the hydrometer scale (*2*). The third decimal on the scale must be determined by interpolating (estimating) from the smallest division on the scale. Record the reading of the hydrometer and the thermometer (first making corrections if instruments have been standardized). If the temperature of the turpentine is not 60°, the weight per gallon at 60° and other temperatures may be obtained from Table I or Figure 3.

An enlargement of Figure 3 on millimeter cross-section paper may be obtained by writing the Naval Stores Research Division, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

While hydrometers indicating pounds per gallon are not at present listed by instrument makers and apparatus supply firms, a number of instrument makers have indicated that they can furnish instruments suitable for use with turpentine.

This type of hydrometer of suitable range with accompanying tables or graphs should be useful for the weight-volume conversion of mineral oils and other liquids.

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Rapid Method for Calibration of Flowmeters

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IT OFTEN becomes necessary to construct and calibrate a flowmeter or to calibrate a capillary tube for a flowmeter with interchangeable capillaries in a laboratory where no calibration apparatus is immediately available.

Under such circumstances the most useful method of calibration for laboratory meters using relatively low pressures consists of displacement of the gas by a liquid, thus forcing the gas through the flowmeter. The following advantages are obtained: simple construction from ordinary laboratory apparatus; precision, accuracy, and rapidity of calibration measurements due to upward displacement of gas; and economy of calibrating liquid (if other than water is used).

Most methods (*1*) displace a liquid, such as water, by the gas after the gas has passed through the flowmeter. If at the end of a known time the volume of liquid used is measured, the volume of gas flowing per unit time can be calculated. However, in such techniques downward displacement of liquid tends to create a back pressure which changes the rate of flow as the head of the liquid changes.

As it was necessary to carry out a number of calibrations, the following procedure was devised:

An arrangement *B* and *C* (Figure 1), giving a constant head (or any such device), allows water to run into a calibrated mixing cylinder of 1- or 2-liter capacity. *B* may be an inverted 2-liter bottle with the bottom removed. The stream of water is controlled by means of a stopcock or screw clamp, *A;* a stopcock is more satisfactory. The water displaces the sample of gas in the cylinder, forcing it through the flowmeter. Since the inlet tube into the cylinder is bent, there is no splash, and readings are easily made on the cylinder. When *A* is adjusted to a convenient pressure differential, indicated by the liquid in the flowmeter, a stop watch is started and the level in the cylinder is noted. This is usually about 200 to 300 ml., so that a large cylinder calibrated like a graduate of the same size is desirable.

From these measurements the volume per unit time flowing through the flowmeter can be calculated. This operation is repeated for various pressure differentials of the indicating liquid in the flowmeter by regulating *A*. It is convenient to construct a graph, plotting pressure differential against volume per unit time.

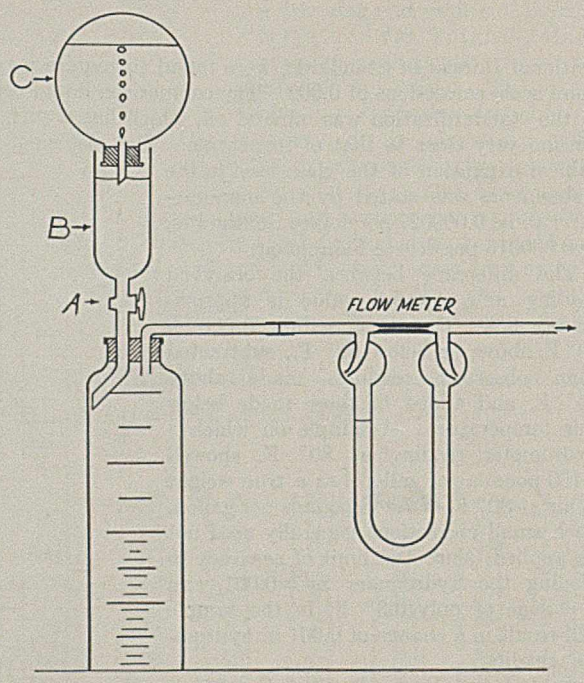

FIGURE 1

The design of the apparatus permits, with slight modification, use of any gas desired.

If water is undesirable as a calibration liquid, mercury or oil may be substituted, the apparatus being designed so that there is no loss of liquid.

The dropping mercury method, on a smaller scale than herein described, is especially adapted to the calibration of sensitive meters, measuring the continuous flow of small quantities.

Literature Cited

(1) Smith, G. W., IND. ENG. CHEM., Anal. Ed., 4, 244 (1932).

[1] Present address, U. S. Food and Drug Administration, Federal Office Bldg., San Francisco, Calif.

Carbon Train for Control Analysis

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OF THE methods now being used for the determination of carbon in steel, the combustion method has proved most satisfactory from the point of view of accuracy and general applicability. On occasion, however, as for some purposes of control work, greater speed is demanded than that afforded by the ordinary combustion method. It is the purpose of this paper to show how, by an adaptation of the ordinary combustion train, more rapid analyses may be obtained.

Practically all texts of quantitative analysis include discussion of the ordinary combustion method for carbon in steel and a description of a carbon train. This being the case, only those modifications of the train will be discussed which serve to speed up the analysis.

Modifications of Train

HASTENING THE SWEEPING PROCESS. A large part of the time taken for an analysis is used in the sweeping process, in which the gaseous products formed in the combustion of the sample must be swept out of the combustion tube and absorbed. This sweeping period may be considerably shortened by reducing the volume through which the gas must be passed. Such volume reduction is effected by inserting in the exit end of the tube a quartz plug (*A*, Figure 1), made by sealing off both ends of a piece of quartz tubing. The outside diameter of the plug should be only slightly less than the inside diameter of the combustion tube. In the train described, the quartz plug was 2.34 cm. (0.906 inch) in outside diameter and the inside of the combustion tube was 2.5 cm. (1 inch).

REMOVAL OF SULFUR GASES AND WATER VAPOR. Following the combustion tube is an absorption tube, *B*, of about 50- to 60-cc. capacity which is attached to the combustion tube by means of a short piece of heavy-walled rubber tubing. It is half filled with 20-mesh zinc to remove sulfur gases (*1*) and the balance is packed with Anhydrone.

ARRANGEMENT OF TRAIN. Considerable time may be saved by setting up the carbon train for easy manipulation. Following the three-way stopcock (Figure 2) is a 0.47-cm. (0.188-inch) copper tube, *E*, rigidly secured by means of a table clamp, *F*, rod, *G*, and clamp holder, *H*, and extended into and close to the base of the balance. With this arrangement it is necessary to hold the balance door only slightly open during weighing. This narrow opening does not sensibly affect the weighing.

The copper tube in the balance terminates in a one-hole rubber stopper, *C*. The tube extends half way into the stopper, leaving the other half to accommodate the weighed absorber, *B*. The combination of copper tube and stopper should be rigid enough to act as the only support of the weighed absorber. Using this support, the absorber can be handled with one hand.

A sketch of the weighed absorber, including dimensions, is seen in Figure 3. Another absorber of the same dimensions, also filled with Ascarite and Anhydrone, is used as a counterpoise (*A*, Figure 2). This counterpoise is necessary, as the effluent gas, oxygen, exerts an increased buoyant effect on the weighed absorber (*B*, Figure 2) over that of air. The absorbers may be interchanged when the weighed absorber is exhausted. Both are left unstoppered during the entire time of an analysis. Weighing with the absorber open introduces no error.

METHOD OF WEIGHING. Since stress is placed upon speed, it

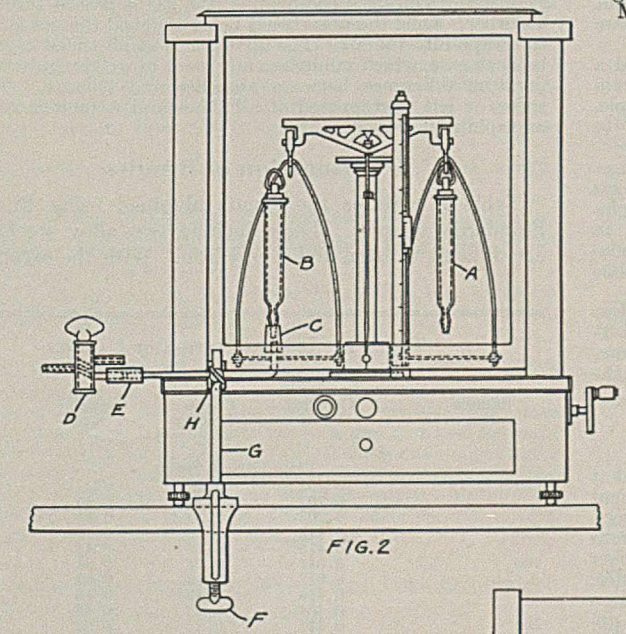


FIG. 2

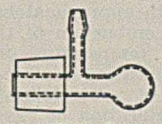


FIG. 4

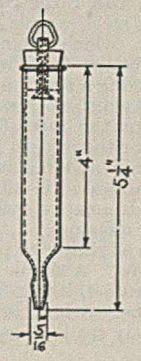


FIG. 3

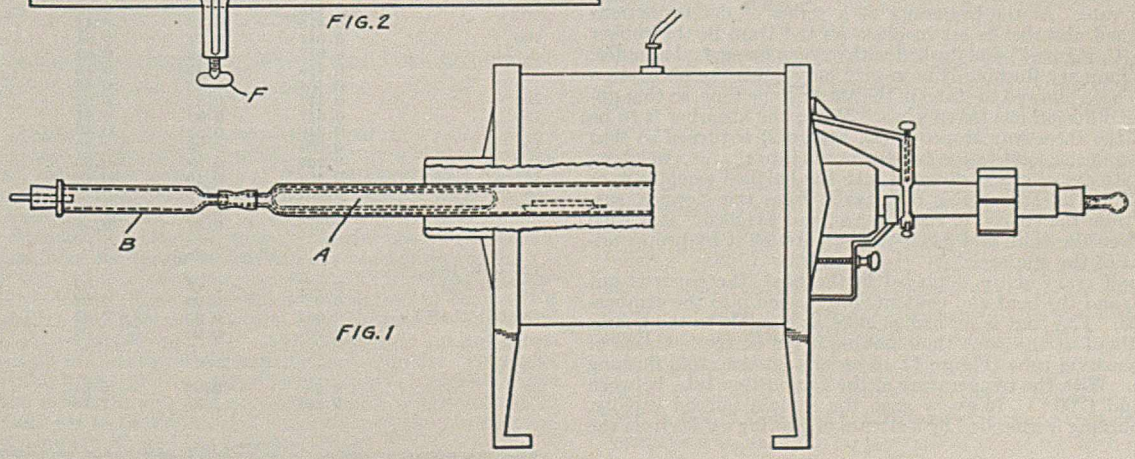
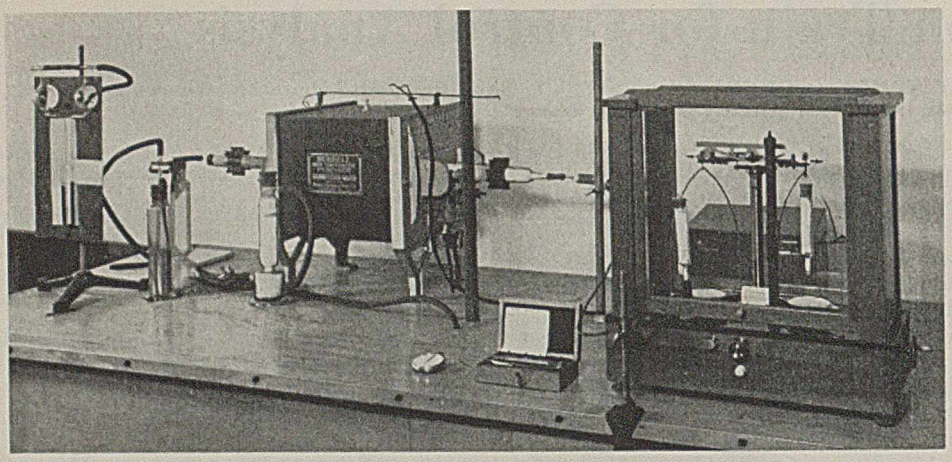


FIG. 1



SET-UP OF CARBON TRAIN

is necessary to use a fast method of weighing; accordingly, the single deflection method (*2*) was employed. This method is rapid and does not involve a loss in sensitivity as when using a magnetic damper. The authors used a chainomatic balance of ordinary sensitivity (0.25 division per 0.1 mg).

BOATS AND COVERS. For combustion of the sample an Alundum boat 8.75 cm. (3.5 inches) long, 1.25 cm. (0.5 inch) wide, and 0.78 cm. (0.31 inch) deep is used. The boat is provided with an Alundum cover to protect the combustion tube from flying particles of ignited oxide. By eliminating the bedding material, a more rapid ignition of the sample is obtained, but it is necessary to use a new boat every third or fourth analysis. Prior to use, a large number of boats and covers are burned out at 900° to 1000° C. for about 0.5 hour in a muffle furnace, and are then stored in a large desiccator until ready for use.

LOADING THE BOAT FOR ANALYSIS. In the boat is placed a 0.500-gram steel sample together with approximately 0.5 gram of tin shot, which promotes rapid combustion of the sample. The combustion tube is kept at a temperature of 1150° to 1200° C., using a furnace with globar heating elements.

PURIFICATION AND METERING OF GAS. The following devices are used for the regulation and purification of oxygen: a gas regulator for the oxygen tank (an Air Reduction Company combination gage and regulator was found very satisfactory); an ordinary calibrated U-tube gage to measure the gas flow; a soda-lime tower to remove carbon dioxide; and a tower of Anhydrone to remove moisture.

It is also advisable to provide the combustion tube with a clear glass window. A simple and inexpensive device is the glass T-tube of Figure 4, which fits into the rubber stopper of the combustion tube. A bulb is blown on the glass T-tube, so that the operator may see the boat in the combustion tube.

Procedure for Analysis

The gas flow from the oxygen cylinder is adjusted so that it is between 250 and 300 cc. per minute. No further adjustment of gas flow is made, except that necessary to maintain the specified flow rate. At the beginning of a series of determinations the weighed absorber is set in place on the train in the rubber stopper (*C*, Figure 2) and flushed with oxygen for several minutes. This preliminary flushing is necessary only when the absorption tube has been allowed to stand in the air for some time, so that appreciable diffusion has taken place. When the absorber is to be weighed the three-way stopcock (*D*, Figure 2) is turned so that oxygen from the combustion tube exhausts into the air; weighing while a stream of gas is directed into the balance would not be advisable. The absorber is now taken from the stopper, suspended from the stirrup of the balance, and weighed. It is then replaced on the train and gas is passed through it by proper adjustment of the stopcock.

One-half gram of tin is placed in the boat, the cover is put in place, and the boat and contents are pushed into the combustion tube. The boat is pushed as close as possible to the quartz plug without at the same time moving it out of the hot zone of the combustion tube (Figure 1), in order to make rapid flushing possible. With the temperature of the combustion tube between 1150° and 1200° C. in every case the sample burned rapidly. Rapid burning is attended by a stream of glowing oxides from the sample.

The time that elapses after introduction of the boat into the combustion tube, before rapid ignition sets in, varies somewhat with the composition of the steel, the size of the sample, and its form (shot, drillings, etc.). In the case of drillings, for plain carbon steels, rapid ignition sets in within 30 seconds after introduction of the sample. Using the Bureau of Standards samples, which are millings, between 30 and 60 seconds elapse before rapid combustion sets in. In the case of shot, which is the form in which the control chemist usually receives the sample for analysis, a sample of 12- to 14-mesh is suitable. Usually one minute is necessary to burn the sample completely after ignition once starts. At the end of this time, the tube must be flushed for 30 seconds. After flushing, the oxygen is exhausted into the air and the absorber is immediately weighed.

Thus the absorber is ready for weighing 2.5 minutes after introduction of the sample into the combustion tube. The total time of an analysis will depend on the skill of the operator. However, using the carbon train described and weighing by single deflection, an analysis can be made in 4 minutes.

In the manipulation of the train, several features represent a departure from conventional practice. No attempt is made to regulate the gas flow during the analysis. The flow rate is set at the start of a series of analyses and the only change is the path the gas takes, as controlled by the three-way stopcock (*D*, Figure 2). During a combustion, the gas is passed through the absorber; while the absorber is being weighed the gas is allowed to escape into the air. The absorber is at all times kept in the balance case, which minimizes any error in weighing due to temperature difference between absorber and balance. The absorber is left unstoppered at all times and weighings are made as rapidly as possible.

Discussion of Results

Table I contains the results obtained using Bureau of Standards samples. These include two alloy steels which are difficult to burn and a cast iron. With the exception of

TABLE I. DETERMINATION OF CARBON

Sample No.	Bureau of Standards Certified Value	Carbon Present	Carbon Found	Error
		%	%	%
		Plain Carbon Steels		
11d	0.202	0.20	0.21	+0.01
	0.202	0.20	0.20	None
	0.202	0.20	0.21	+0.01
12d	0.418	0.42	0.40	−0.02
	0.418	0.42	0.41	−0.01
100	0.617	0.62	0.63	+0.01
	0.617	0.62	0.62	None
130	0.454	0.45	0.45	None
	0.454	0.45	0.46	+0.01
72a	0.317	0.32	0.32	None
14b	0.817	0.82	0.81	−0.01
	0.817	0.82	0.80	−0.02
72	0.294	0.29	0.30	+0.01
	0.294	0.29	0.30	+0.01
15b	0.101	0.10	0.10	None
	0.101	0.10	0.10	None
13c	0.573	0.57	0.55	−0.02
	0.573	0.57	0.57	None
		Alloy Steels		
121 (CR 18%, Ni 8%)	0.057	0.06	0.07	+0.01
	0.057	0.06	0.07	+0.01
126 (Ni 36.4%)	0.034	0.03	0.03	None
	0.034	0.03	0.04	+0.01
		Cast Iron		
5g	2.86	2.86	2.91	+0.05
	2.86	2.86	2.88	+0.02
				Av. ±0.01

the cast iron, which showed in one determination an error of +0.05 per cent, the greatest error for any determination was 0.02 per cent. Most of the analyses show either an error of 0.01 per cent or no error at all.

The certificate values of the second column are rounded and listed as percentage of carbon present in column 3. It is these latter values that are compared with the results obtained, because the magnitude of the weight change in the absorber restricts expression of results to two significant figures.

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(2) Brinton, P. H. M. P., *J. Am. Chem. Soc.*, 41, 1151 (1919).

OPINIONS expressed in this article are those of the writers and do not necessarily reflect the ideas of the Naval Service.

Simple Tests to Indicate the Condition of an Analytical Balance

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IT OFTEN falls to the lot of a teacher of quantitative analysis or an industrial chemist to determine whether a balance is in satisfactory weighing condition. This paper presents a simple and rapid method of balance testing that is sufficiently exacting for most purposes. The fundamental principles upon which this brief series of tests is based are well recognized, but the method of application is believed to be new. A recent paper (*1*) gives valuable additional information from the instrument repairman's point of view.

The following points should be considered:

1. The general condition of the balance must be acceptable.
2. The rest point should be constant for any particular load when the mass on each of the pans is equal.
3. The balance must be of the proper degree of sensitivity. (Sensitivity is understood in this paper to be numerically equal to the deflection on the pointer scale caused by the addition of a 1-mg. load to a single pan of the balance.)
4. The balance must give weighings that are closely reproducible.
5. The balance must have lever arms of nearly equal length.

These points are intimately interrelated—for example, an imperfect knife-edge may cause the balance to perform poorly in respect to points 2, 3, and 4.

Point 1 is not readily tested quantitatively, but a careful inspection will usually suffice.

Make sure that the beam releases, pan rests, rider carrier, chain weight devices, and other moving parts are in good mechanical condition. See that the knife-edges are separated from their bearing plates by the beam lift to a gap of about 0.1 mm. and that when the beam lift is released all three knife-edges make contact with their plates over the whole edge gently and simultaneously. This is essential to the life of the edges. Metal parts well finished and free from corrosion are desirable, but do not necessarily indicate an accurate balance.

The information sought in points 2 and 3 can be obtained quantitatively by the following method:

Place the balance on a firm support in a part of a room where a fairly constant temperature prevails (away from radiators, open windows, and other drafts, out of direct sunlight, and removed from other hot light sources). Level the balance with the setscrews provided in the base. If the balance has recently been moved from another location, open the door of the case and allow at least an hour for the balance to attain room temperature.

After the balance has been brought to the same temperature as its environment, determine the data required to construct a table similar to Table I. For this purpose select two sets of analytical weights, W_1 and W_2. It is convenient, but not necessary, to have the sets agree within fairly narrow limits. They need not be calibrated. If two sets of weights are not available, one can make shift with only one set—for example, if the data at 20 grams' load are to be determined, one could call the two 10-gram weights together W_1 and the 20-gram weight W_2.

Table I records data obtained in applying this method to a typical student balance. The principle involved is that of double weighings first devised by Gauss.

TABLE I. DATA FOR STUDENT BALANCE

Weight of W_1 and W_2 (Each) *Grams*	Rest Point[a], *A*	Rest Point[b], *B*	Average Rest Point, *C* $(A + B)/2$	Rest Point[c], *D*	Sensitivity, *E* $(B - D)$
0	9.0	9.0	9.0	6.9	2.1
10	9.5	9.1	9.3	7.3	1.8
20	10.2	8.6	9.4	7.2	1.4
50	9.7	9.5	9.6	8.5	1.0
100	9.3	11.1	10.2	10.3	0.8

[a] W_1 on left pan, W_2 on right pan.
[b] W_2 on left pan, W_1 on right pan.
[c] W_2 on left pan, W_1 + 1 mg. on right pan.

Constancy of the values in column *C* would satisfy point 2. If the rest point in *C* should shift by as much as two or three pointer scale divisions between loads of zero weight and 100 grams' weight on each pan, the balance would not be acceptable for determining absolute mass values, but might prove acceptable for certain types of gravimetric analysis where the determination of small differences in mass only is required. The balance tested (Table I), where the rest point shifts 1.2 scale divisions between zero weight and 100 grams' weight load on each pan on the basis of a sensitivity of 0.8 at 100 grams' load, would cause an error of 1.5 mg. in determining a 100-gram load. This amounts to a deviation of only 0.0015 per cent, which would be negligible for most work.

It is worth while to test the effect of changing the position of the masses from the centers of the pans to the edges and see if the value of the rest point is thereby changed. Defects of the end knife-edges may sometimes be detected by this method, whereas they may remain unnoticed when the masses on the pans are perfectly centered. The point of rest should also be checked by using swings of small amplitude and then swings of considerably greater amplitude. Difference between the two values indicates worn, nonparallel, or otherwise faulty knife-edges.

If the balance is to be used where the requirements are only moderately exacting (point 3), the sensitivity (column *E*) should have a numerical value of at least 2 and preferably 3 or 4 at zero load on the balance pans. The sensitivity of a balance should remain nearly constant or should decrease slowly and regularly with increasing load on the balance pans. The fall in sensitivity is usually due to a difference in level between the middle and the end knife-edges, and may be caused by bending of the beam under the load, by wear of the knife-edges, or by not sharpening them uniformly. In general, it is not safe to use a balance for loads that reduce the sensitivity to less than 40 per cent of the value with zero load.

Weights of 90 to 100 grams should be the maximum allowed on each pan of the balance that is serving as our example.

Point 4 could be determined by again getting the rest point of the balance with pans empty after securing the data of Table I. If this checks with the corresponding value in the table within 0.2 pointer scale division, the balance is satisfactory. If further checks on this point are desired, one can repeat the weighings at any pan load.

Point 5 is easily tested as follows:

An object of mass M (a 20-gram weight is convenient) is placed on the left-hand balance pan and counterbalanced with other weights from the set and their sum, S, is recorded. M is then transferred to the right-hand balance pan and again counterbalanced with weights from the set and their sum, S', is recorded. If L equals the length of the left lever arm and R equals the length of the right lever arm of the balance, from the principle of the lever

$$ML = RS \quad (1)$$

and

$$MR = LS' \quad (2)$$

If we divide Equation 2 by Equation 1 we get

$$R/L = \sqrt{S'/S} \quad (3)$$

On developing the quantity under the radical sign in terms of a series of powers of x and S, where x represents the difference between S' and S ($S' = S \pm x$), we get the series

$$\frac{R}{L} = 1 \pm \frac{x}{2S} \mp \frac{x^2}{8S^2} \pm \frac{x^3}{16S^3} \cdots\cdots \quad (4)$$

In applying this to the balance, x is always very small as compared to S; so the equation reduces essentially to

$$\frac{R}{L} = 1 \pm \frac{x}{2S} \quad (5)$$

The upper sign is used where S' is greater than S and the lower sign is used when S' is less than S. In a good balance the R/L value should be 1.0 ± 0.00002. In dealing with comparative values, as in gravimetric analysis, an R/L value of 1.0 ± 0.0002 can be tolerated without appreciable error in the final result.

In deciding whether a balance is suitable for the work at hand, one must also know the probable limits of error introduced by factors other than the balance. The balance may be used without hesitation if it is twice as accurate as the least accurate of any of the other measurements involved. It is very probable that more errors in student and commercial work are due to uncalibrated or poorly calibrated weights than to inaccurate balances. Moreover, manipulative techniques, aside from weighing, usually introduce far larger errors than can be accounted for by the inaccuracy of weighing; and the percentage of error of many analytical methods, due to such things as end-point errors, solubility of precipitates, adsorption, deliquescence, inability to measure volumes accurately, etc., is far greater than most of us would tolerate in an analytical balance.

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Useful Centrifuge Accessories

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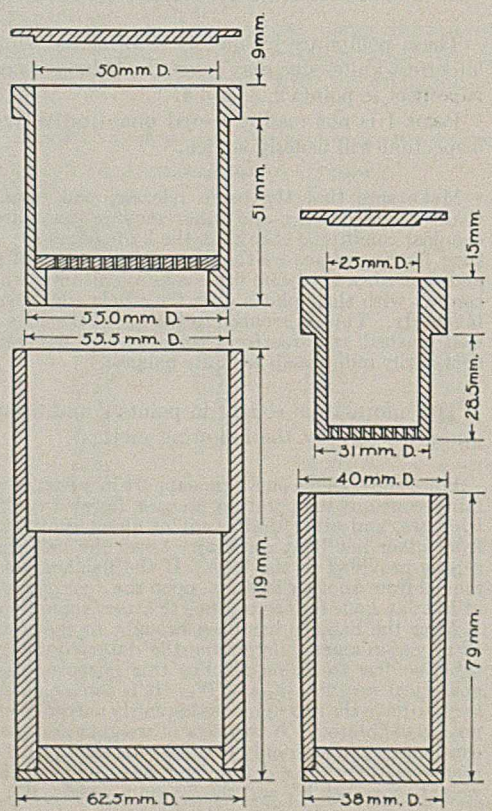
WHEN reagents are to be purified rapidly and efficiently by crystallization, centrifugal draining of the crystals is essential. Unfortunately, convenient and inexpensive centrifuge accessories for this purpose are scarce, particularly for crystallizations which must be carried out on a small laboratory scale. The basket-head type of apparatus is expensive, cannot easily be made without special equipment, and is not very convenient for use with small quantities of material, or in any case where very high purity is required. Accessories which permit the adaptation of various sizes of Büchner funnels or Gooch crucibles as baskets are not quite so expensive, but are unsatisfactory in other respects.

The perforated cup type of accessory, in which both crystals and liquid are completely enclosed during centrifuging, is especially suitable for small quantities of material. Made from platinum or gold, such an accessory is universally useful, but the cost is prohibitive for most laboratories. A plated apparatus might seem to offer a suitable substitute, but inquiries indicate that the cost of any practical design is rather high. Accessories of this type made from the methyl methacrylate resin Lucite are inexpensive and for many purposes fully as useful as if they were made from one of the noble metals. Lucite is almost completely insoluble in water solutions of salts, acids, and dilute alkalies, and in straight-chain hydrocarbons. However, it cannot be heated much above 70° C. and is soluble in many organic solvents.

The authors have designed two accessories which may be machined from stock sizes of Lucite sheet, rod, and tubing to fit standard centrifuge cups. The inner cup of the small model has 61 holes drilled in a hexagonal pattern with a No. 70 B. & S. gage drill. The inner cup of the larger model has interchangeable bottom plates, each with 169 No. 60 or No. 70 holes.

Eight complete accessories were made for $3.50 each, including the cost of labor and material. This is one eighth the lowest quotation obtained for a single set made from any suitable combination of base metals, and one fifteenth the cost of a small basket-head accessory made from manganese bronze. The economy is even greater, since the Lucite accessories are more generally useful with water solutions than those made from base metal alloys.



Ultraviolet Photometer

Quantitative Measurement of Small Traces of Solvent Vapors in Air

V. F. HANSON, E. I. du Pont de Nemours & Co., Inc., Niagara Falls, N. Y.

An ultraviolet photometer has been developed for rapidly measuring concentrations of trichloroethylene as low as 10 p. p. m. and perchloroethylene as low as 0.3 p. p. m. in air. It is applicable to many toxic organic vapors in concentrations as low as 0.13 p. p. m. Its operation depends on the partial opacity of these vapors to ultraviolet light. It is specific for vapors having a strong absorption in the 2537 Å. region of the spectrum (Table I). Special measures must be taken when mixtures of such substances are present.

The instrument is portable, direct-reading, and capable of making at least two determinations per minute on substances for which it has been previously standardized.

Details of principles of operation, construction, and application are given.

THE increasing use of chlorinated solvents, particularly trichloroethylene and perchloroethylene, in degreasing, dry cleaning, and extraction has led to an urgent need for a rapid and reliable analytical method for determining solvent concentrations in the air in the working areas.

Such an analytical method is needed in studying the causes of solvent losses for both economic and health hazard reasons. Results of analyses should be instantly available to check changes in concentrations during the various operations. The method should be simple, so that special technicians are not required, and the equipment should be portable and sensitive to concentrations as low as 20 parts per million. Health, insurance, and labor agencies of several states have requested some of the operators using these solvents to check their equipment to preclude the possibility of health hazards arising as a result of excessive solvent losses, which are generally due to faulty operation or defective equipment.

Methods of Analyses Extant

The following analytical methods have been used with varying degrees of success:

1. Thermal decomposition of the solvent followed by absorption and measurement of the products of decomposition (*4, 8, 9, 12*)
2. Alcoholic absorption-colorimetric method (*1*)
3. Specific gravity measurements (*5*)
4. Refractive index measurement (*6, 10*)
5. Weight gained by charcoal absorption (*2*)
6. Thermal conductivity (*14*)
7. Vapor pressure of condensed solvent (*3, 7, 11*)
8. Flame tests (*13*)

A critical inspection of these methods indicated that none fully met the requirements set forth above. Therefore, the R. & H. Tri-Per-Analyzer was developed to determine microquantities of trichloroethylene and perchloroethylene (known as "Tri" and "Per" to the trade) in air.

It consists of an extremely sensitive and stable ultraviolet photometer with a built-in sampling and standardizing system. Its operation depends on the partial opacity of solvent vapors to certain bands of light in the ultraviolet region of the spectrum. Ease of operation makes it possible for a layman to make a complete survey of solvent concentrations in an area of a plant in a half hour's time. Concentrations from 10 to 2000 p. p. m. of trichloroethylene and 0.3 to 500 p. p. m. of perchloroethylene can be measured, and higher concentrations can be determined by making slight modifications in the instrument. It is common practice to make two determinations per minute of trichloroethylene vapor in concentration as low as 10 p. p. m. (by volume) or as low as 0.3 p. p. m. of perchloroethylene vapor in air. The analyses are made as rapidly as samples can be pumped through the instrument, the results of the analyses being instantly available.

TABLE I. SENSITIVITY OF PHOTOMETER

	Sensitivity, per Scale Division *P. p. m.*
Mercury	0.0001 (approx.)
Tetraethyllead	0.13
Xylene	0.2
Monochlorobenzene	0.3
Aniline	0.3
Perchloroethylene	0.5
Chloroprene	0.5
Toluene	1.0
Benzene	1.2
Vinylacetylene	1.6
Phosgene	5
Acetone	5
Ethylbenzene	5
Pentachloroethane	7
Hydrogen sulfide	8
Trichloroethylene	10
Carbon disulfide	12
Gasoline (Blue Sunoco)	50

Photometer Insensitive to:

Methylene chloride	Ethyl alcohol
Carbon tetrachloride	Amyl alcohol
Ethylene dichloride	Ethyl acetate
Tetrachloroethane	Ethyl Cellosolve
Chloroform	Methyl Cellosolve
Methyl chloride	Dowtherm A
Vinyl chloride	Water vapor
Methyl alcohol	

While the instrument was developed primarily for trichloroethylene and perchloroethylene, it may be used equally well on many toxic vapors, but is insensitive to others (Table I).

An instrument operating on similar principles has been used for measuring mercury concentrations in air (*15*).

Theory of Operation

All substances absorb light at some region of the spectrum. If a spectral region exists where a vapor has a high absorption and the diluent a high transmission, the concentration of the vapor can be measured in terms of light absorbed. The ideal instrument would be one using a monochromatic source of light of wave length corresponding to the maximum absorption of the vapor and a minimum absorption of the diluent and a highly sensitive and stable light-measuring device sensitive only to this wave length.

The operation of this analyzer is based on the Beer-Lambert law which states that the light absorption depends on the distance traversed and the molar concentration of the light absorbent. Thus, a unit layer of unit concentration absorbs as much light as a layer of twice the thickness and half the concentration. The Beer-Lambert law is then

$$I = I_0 10^{-dca}$$

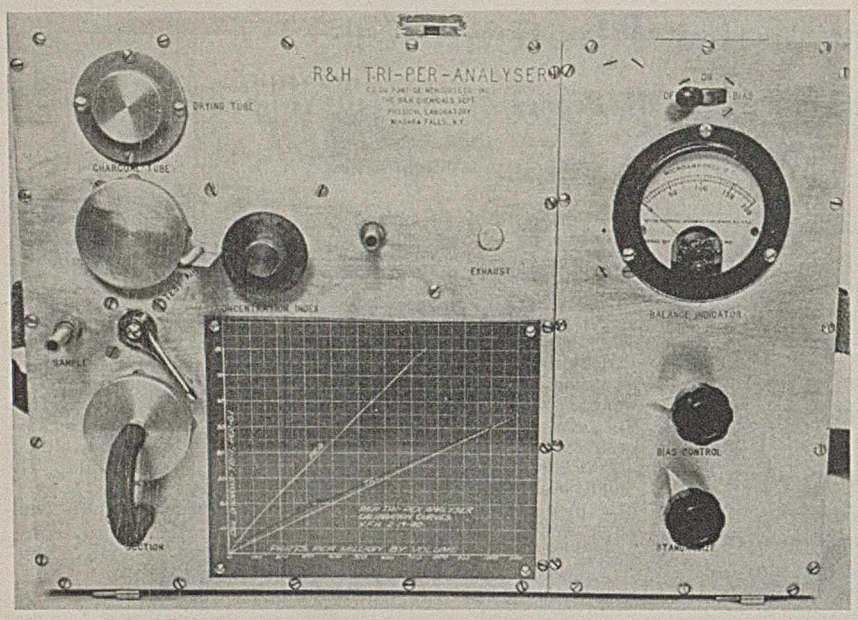


FIGURE 1. PHOTOMETER

where I is the intensity of the transmitted light, I_0 is the intensity of the incident light, a is the extinction coefficient for common logarithms, c is the molar concentration of the light absorbent, and d is the thickness of the layer measured in centimeters.

It is very important to note that these relations favor measurements of low concentrations of the light absorbent—i. e., there is a greater percentage difference in light absorbed by small quantities than by larger quantities of absorbent.

The other analytical methods used for measuring small traces of vapors operate on a linear relationship, and have the obvious disadvantage for low concentrations of giving the same incremental differences regardless of concentration. Thus, while a unit absolute error in a reading at high concentrations might result in a low relative error, the same absolute error at low concentrations would result in a high relative error.

The following average extinction coefficients were found to hold for a light consisting of 90 per cent 2537 Å. radiation (extinction coefficients, a, are calculated for log base 10): trichloroethylene, 1.9; perchloroethylene, 45.

Selecting the following requirements:
Light cell length, d, 15 cm.
Minimum concentration of trichloroethylene to be detected, 10 p. p. m.
Minimum molar concentration

$$\frac{10^{-5}}{22.4} = 4.45 \times 10^{-7} \text{ mole per liter}$$

then $$I = I_0 10^{-acd} \quad (3)$$

$$\text{Log } 10 \frac{I_0}{I} = acd$$

so that

$$\text{Log } 10 \frac{I_0}{I} = 1.9 \times 4.45 \times 10^{-7} \times 15 = 1.27 \times 10^{-5}$$

the ratio of light transmitted by solvent-free air to air containing 10 p. p. m. of trichloroethylene, is approximately $\frac{I_0}{I} = 1.00003$.

It is thus evident that the photometer must be capable of detecting changes in light intensity of 0.003 per cent in order to detect 10 p. p. m. of trichloroethylene.

The change in transmitted light due to the presence of solvent vapors is measured by the change in resistance of a sodium photocell whose peak sensitivity is at about 3000 Å. Instead of measuring the voltage drop across a fixed resistor in series with this photocell, a second photocell is used as both a series resistor and as a means for partially compensating for changes in the light output from the low-pressure mercury vapor lamp. The photocell voltage is impressed on the grid of a battery-operated pentode which amplifies the changes in photocell voltage. These changes are indicated on a microammeter in the plate circuit of the tube. All measurements are made when the light to the two photocells is equal, to eliminate errors due to changes in battery voltage and tube characteristics.

R. & H. Tri-Per-Analyzer

The instrument and accessories are self-contained in a carrying case weighing 16 kg. (35 pounds). Figure 1 shows the instrument set up for use. Figure 2 shows the panels removed from the case.

ANALYZER UNIT. The analyzer unit is composed of the following elements:

Optical and Light-Measuring Systems. Low-pressure 5-watt mercury vapor lamp emitting 2537 Å. ultraviolet light, lamp stabilizer networks, 15-cm. sample cell provided with quartz windows, two General Electric FJ76 sodium phototubes operating in a bridge network, vacuum tube electrometer with a microammeter balance indicator, and micrometer "light valve".

Sampling System. Charcoal absorption tube, porous filter, sampling pump, and control valve.

The schematic drawing (Figure 3) shows the arrangement of the equipment.

ELECTROMETER BALANCE INDICATOR. An RCA32 tube operating at reduced heater and plate voltage to reduce grid current and increase stability is mounted near the phototubes. The tube control leads pass through the partition into the battery compartment. Grid bias control and phototube balancing potentiom-

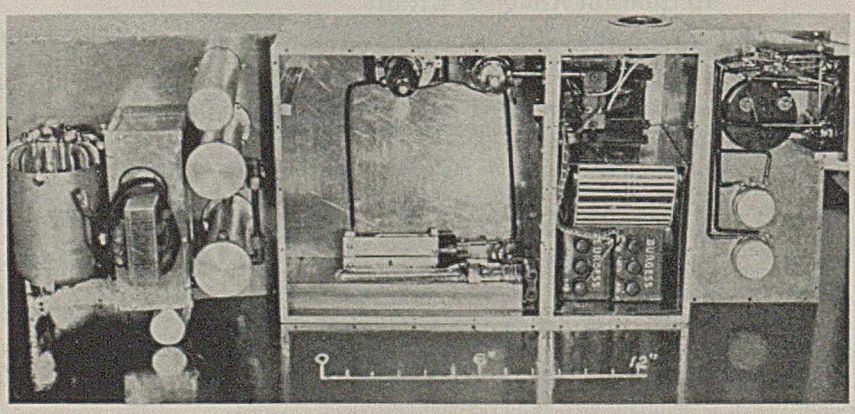


FIGURE 2. INSTRUMENT WITH PANELS REMOVED FOR INSPECTION

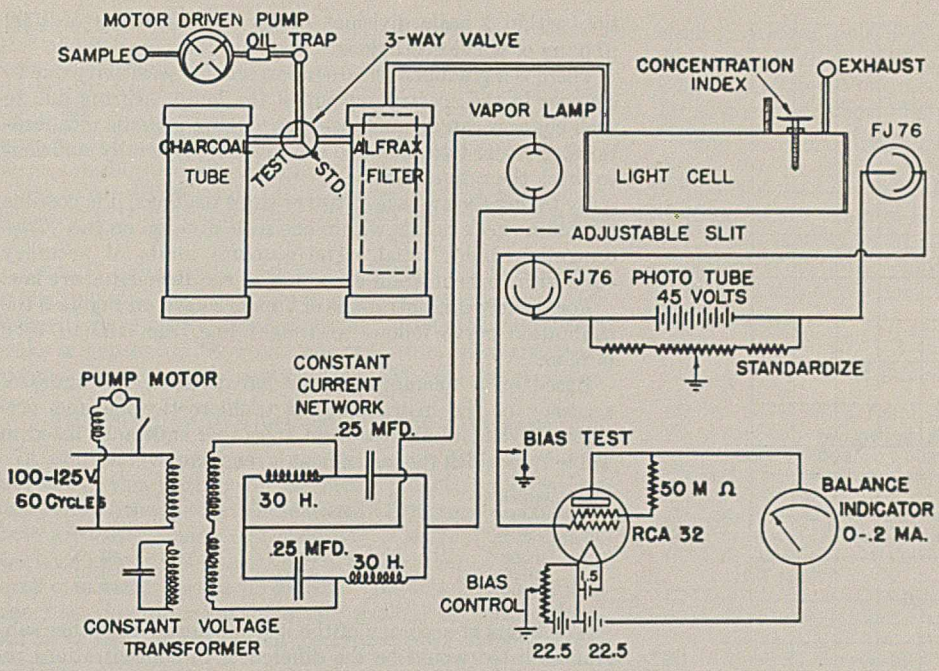


FIGURE 3. SCHEMATIC DIAGRAM OF PHOTOMETER

eters with the plate microammeter are mounted in the battery compartment. A switch is provided to ground the grid to standardize the electrometer.

The combination of voltage and current control with the compensating reference photocell, integral sample cell, and light valve and rapid sampling and standardizing systems were all found necessary to provide the sensitivity and stability to measure changes in light intensity of less than 0.003 per cent.

OPTICAL AND LIGHT-MEASURING SYSTEMS. After attempting to use light-splitting, collimating, and optical wedges for increasing and distributing the light to the two photocells, the simple optical system shown in Figures 2, 3, and 4 was adopted. While greater sensitivity could be obtained with a longer light cell than the 15 cm. used, the decrease in light intensity on the photocells reduced the instrument stability, more than offsetting the advantage of increased sensitivity. Space was also considered an important factor in designing a portable rugged field instrument.

The light from the 5-watt General Electric sterilization lamp passes through the light cell (Figure 4) which is mounted on the instrument panel. Light is also transmitted to the reference phototube through an adjustable slit below the lamp (Figure 2). The light to the two phototubes is approximately equalized by adjusting the light slit on the reference photocell. The fine balance is attained by the voltage divider ("Standardize", Figure 3) across the phototube battery. This fine balance consists of a radio potentiometer in series with two fixed resistors to limit the range of the electrical adjustment. The light is thus balanced when the voltage drop from the common wire of the phototubes to the ground is zero.

The simple micrometer screw proved to be a very satisfactory method for measuring the light that was absorbed.

In operating the instrument, the two phototubes are first balanced as described, with solvent-free air in the test chamber and the "Concentration Index" set at zero. Then the instrument three-way valve is switched to "Test Air", and the "Concentration Index" micrometer is raised until the original balance is restored. Thus, by decreasing the area of the shadow cast by the "Concentration Index" screw on the phototube, the amount of light that is absorbed by the test air is compensated for in a manner that permits very simple measurement.

One complete turn of the "Concentration Index" dial elevates the screw 1 mm. The dial, being divided in 100 parts, makes a change of 0.0031 per cent per dial division on the photocell based on the ratio of the area of the shadow cast by the screw on the phototube to the area of the phototube element.

Concentrations of various vapors in terms of "Concentration Index" dial divisions are plotted on Figure 5. For convenience, calibration curves for the trichloroethylene and perchloroethylene are mounted on the instrument panel.

Since it is impossible to secure equally balanced phototubes, special care was required to stabilize the ultraviolet lamp, which was found to be extremely sensitive to voltage and temperature changes. The lamp has the negative volt-ampere characteristic of an electric arc, making it necessary to provide a constant current network to maintain a steady current regardless of changes in lamp voltage and impedance arising from changes in lamp pressure and temperature. A resonant circuit consisting of equal inductive and capacitive reactances connected in the form of a square was found to give excellent current control as long as the line voltage was constant. This network was made up from radio chokes and bypass condensers, the impedance of each component being equal to the lamp impedance. These elements were selected to operate the lamp at about half the normal current to minimize changes in lamp characteristics with time.

A Sola constant-voltage transformer proved satisfactory for controlling the lamp voltage in spite of normal variations in line voltage.

SAMPLING SYSTEM. The successful use of the analyzer depended as much on the sampling system adopted as on the optical system.

The sample is pumped first through a charcoal tube which removes all traces of solvent vapors. A tube 3.12 cm. (1.25 inches) in inside diameter and 15 cm. (6 inches) long was found effective in reducing solvent concentrations from 600 to less than 1 p. p. m. After the instrument is balanced at the zero point, the three-way valve is turned so that the sample by-passes the charcoal tube, passing through a porous Alfrax filter before it enters the sample cell. This filter is mounted on the screw top of the filter tube, from which it is readily removable for cleaning and inspection. The frequency of cleaning depends on the type of atmosphere to which the instrument is subjected. Unless paint spray, oil, or similar contaminant is encountered, it has been necessary to clean the cell about three times per year.

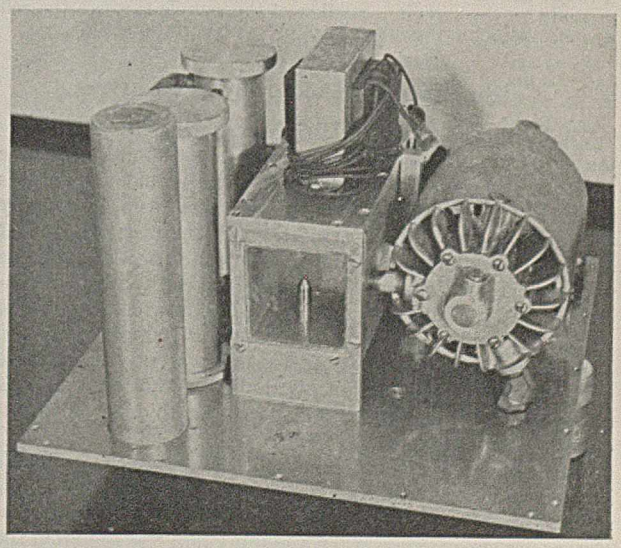


FIGURE 4. DETAILS OF SAMPLING SYSTEM

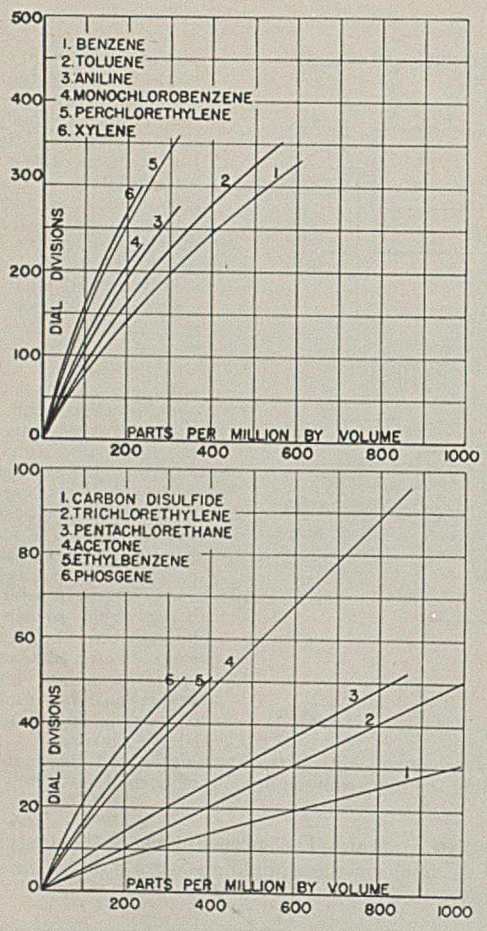


FIGURE 5. CALIBRATION CURVES

Accurate results depend on rapid circulation of the sample through the instrument. Changing the sample about 20 times per minute permits frequent checks of the zero point, which is important for attaining high accuracy at low concentrations giving up to 10 scale division deflection. Motor-driven pumps were troublesome because of lubrication; however, a small cotton filter was effective in keeping the oil from being blown by the pump into the instrument. It was found necessary to force the sample through the cell in order to maintain substantially atmospheric pressure on the sample. Suction of the sample through the cell was unsatisfactory because of cell pressure variations.

Calibration and Accuracy of Analyzer

The analyzer is periodically checked by adding carefully measured quantities of liquid solvent to a 850-liter (30-cubic foot) fumatorium in which an electric circulating fan is mounted. After the solvent sample has been thoroughly mixed with the air, it is passed through the instrument. At least three readings are made at each concentration, and the average dial readings are then plotted against the known concentrations. Figure 5 shows the calibration curves for the materials listed in Table I, at 22° C. and 760-mm. pressure. Unless pressure and temperatures differ greatly from these values, corrections need not be applied. The instrument is about 25 times as sensitive to perchloroethylene as to trichloroethylene.

The instrument has been checked six times in the year that it has been in service, during which it has traveled over 50,000 miles without serious accidents that affected its accuracy. Except for one occasion when a gummy film was found on the quartz windows, the recalibration checked the initial calibration within 2 scale divisions up to concentrations of 1000 p. p. m. of trichloroethylene.

There is a gradual decrease in instrument sensitivity due to changes in the spectral output of the lamp. Errors due to such changes can be minimized by making periodic recalibrations. One or two points on the curve are generally sufficient to check the calibration.

By taking the average of two or three readings, it is possible to obtain check results within one scale division on the "Concentration Index" dial. The absolute limits of accuracy vary with concentration according to the Beer-Lambert law.

For the low concentrations of vapors shown on Figure 5 the calibration curve follows a straight line from 100 to 1000 p. p. m.

Based on a tolerance of one dial division, the limits of accuracy of the instrument for trichloroethylene and perchloroethylene in air free from any other substance listed in Table I, to which the instrument is sensitive, will be:

Concentration of Vapor	Limit of Error	
	Trichloroethylene	Perchloroethylene
P. p. m.	*P. p. m.*	*P. p. m.*
0–50	±15	±0.65
50–200	±20	±0.91
200–600	±24	±0.97

The limits of accuracy of the instrument for the other substances listed would be the differences in concentrations required to produce one scale division change on the "Concentration Index".

TABLE II. FIELD TEST REPORT ON VAPOR CONCENTRATIONS NEAR DEGREASER

(Solvent used, trichloroethylene. Degreaser type, vapor-slush. Use, cleaning metals, steel. Capacity operation, 75%. Vapor concentrations, p. p. m. by volume)

Location	Machine		Exhaust System On	Exhaust System Off
			P. p. m.	*P. p. m.*
45 cm. (18 inches) above left front end	Idling		125	200
			300	170
			250	160
			220	125
		Av.	224	164
45 cm. (18 inches) above center front	Idling		190	170
			110	150
			125	150
			125	150
		Av.	128	155
45 cm. (18 inches) above right front end	Idling		170	150
			125	200
			125	220
			100	240
		Av.	128	202
Operator's nose level	Slushing		150	125
			150	300
				250
				220
		Av.	150	249
45 cm. (18 inches) above left front end	Working		360	
			200	
			380	
			310	
			340	
			2000	
			1200	
			400	
			875	
		Av.	674	

The charcoal should be changed when the zero cannot be checked when comparing clean and contaminated air. This is ordinarily about once in 20 operating hours.

Operation of Analyzer

In making a survey of concentrations in a working area near equipment using solvents to which the instrument is sensitive, as listed in Table I, the following procedure is recommended:

Allow instrument to warm up for 10 to 15 minutes. Turn on amplifier lever switch. Press lever switch to right, and set meter pointer to mid-scale (100 on a 200-microampere meter) by ad-

justing "Bias Control". Start pump, set valve on "Standard Air" and "Concentration Index" on zero. Bring meter to mid-scale by adjusting light slit for coarse adjustment and "Standardize" for fine adjustment. After meter reaches equilibrium, shift valve to "Test Air". Turn "Concentration Index" dial until meter returns to mid-scale. Return valve to "Standard Air" to check zero. Determine concentration by applying average readings to calibration curves.

The motor-driven pump should not be operated in explosive atmospheres because of sparking brushes. A hand pump is provided for such conditions.

Table II indicates the detailed data that can be obtained in less than one hour in a survey of the concentrations of trichloroethylene near a degreasing unit. The abnormally high peaks of concentration would not appear in other analytical methods listed above, except possibly with the interferometer.

Miscellaneous Applications for Analyzer

In addition to the routine analyses of air for solvent concentration, this instrument has many potential applications because of its sensitivity and speed of response. Some of these are determination of efficiency of solvent absorbents, checking efficacy of gas masks, determination of concentrations of certain vapors in gaseous products from chemical reactions, determination of concentrations of certain flammable vapors, and checking performance of fume-disposal systems.

The instrument may be modified to operate continually and may be adapted for automatic control of chemical processes or for use with other vapors.

By using selective absorbents, it is possible to determine the concentration of various components of mixtures of vapors.

Acknowledgment

The writer wishes to acknowledge the technical assistance of P. M. Hackett of this company and F. H. Shepard, Jr., electronics consultant, in developing the instrument. He also wishes to thank F. A. Patty of the Fidelity and Casualty Company of New York, Leonard Greenburg of the New York Department of Labor, Philip Drinker of Harvard University, William P. Yant of the Mine Safety Appliances Company, A. D. Brandt of the Willson Products Company, C. R. Williams of the Liberty Mutual Insurance Company, Allan L. Coleman of the State of Connecticut Department of Health, and H. D. McKinley, M. Marean, A. A. Levine, and B. H. Vollertsen of E. I. du Pont de Nemours & Company, Inc., for sponsoring this development and for conducting field tests on the instrument.

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Presented before the Division of Physical and Inorganic Chemistry at the 100th Meeting of the American Chemical Society, Detroit, Mich.

Simply Constructed Color Comparator

RICHARD H. WILHELM, Princeton University, Princeton, N. J.

THE object of a color comparator is to bring color images from reflecting or luminous surfaces into closely adjacent fields, thereby enabling the eye to compare the colors with sensitivity. These instruments are generally constructed with optical prisms. During the course of a rate study in which the progress of the process was followed by color changes, the inexpensive color comparator herein described was constructed. Its operation depends upon the fact that curved bars of certain resins will transmit light without appreciable loss through the curved surfaces. Although Plexiglas was used, any acrylate or methacrylate resin with similar optical properties would be equally satisfactory.

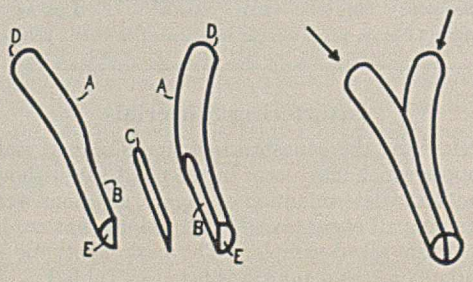


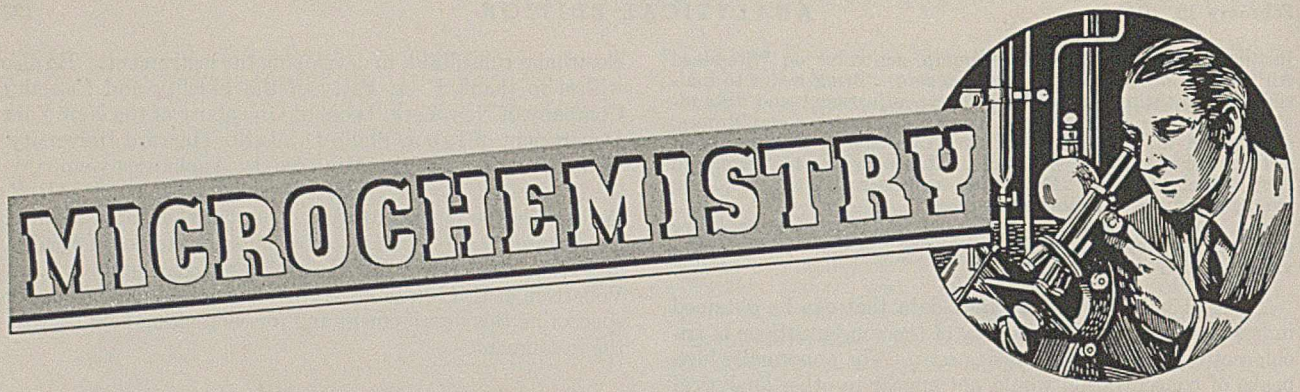
Figure 1. Parts and Assembly of Comparator

The component parts and the assembly of the comparator are shown in Figure 1. Two straight cylindrical rods of resin, *A*, 6 inches long and 0.75 inch in diameter, were softened by heating to about 110° C. and one end of each was bent through an angle of about 30°. One half of the remaining straight portion of each rod was removed by milling, leaving flat surfaces, *B*, as shown. With a strip of reflecting aluminum foil, *C*, between them the two surfaces were cemented together by an adhesive made from some of the resin dissolved in equal parts of carbon tetrachloride and chloroform. A strong bond and excellent internal reflection at the surface of the bond were thus obtained.

To prevent stray light from entering the lateral surfaces of the instrument, it became necessary to coat these with an opaque lacquer which still allowed internal reflection to take place. A coating made from aluminum powder dispersed in the above-mentioned resin solution was satisfactory. Flat surfaces, perpendicular to the resin rod at each point, were ground with fine emery at *D*, where the light enters the comparator, and at *E*, the eyepiece, where it emerges. A final polish on these surfaces was obtained with a finely divided abrasive metal polish.

A small convex lens in a brass tube (not shown) was fitted to the eyepiece of the comparator. By focusing this lens upon the resin surface, *E*, the two half-circle fields appeared to be uniformly illuminated with diffused light. In using the comparator the usual care was taken to provide illumination of equal intensity upon both colored surfaces.

An angle of 60° between incident light sources was the only one tried and is not necessarily the maximum practical angle for a comparator of this type.



Determination of Copper in Plant Materials

Using the Dropping Mercury Electrode

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THE use of the dropping mercury electrode in analysis for copper has received some attention and should be adaptable to the determination of copper in plant material and in soils. The earlier investigations of Shikata (*8*), Mandi (*3*), and Roncato and Bassani (*7*) were of a preliminary nature, did not include limits of copper or of interfering materials, and were not conclusive. Thanheiser and Maassen (*11*) reported the polarograph to be very useful in analysis of steel for copper in the presence of other metals and Hohn (*2*) in his monograph included an outline for copper determination in brass and metal alloys. Suchy (*10*) used the dropping mercury electrode for simultaneous estimation of copper, bismuth, lead, and cadmium, and Stout (*9*) suggested a procedure for polarographic estimation of copper.

In this investigation the regulating solutions suggested by the above authors were tried in the presence of any interfering materials that might be present in plant materials and a method was worked out for the polarographic determination of copper in plant materials.

Tests of Regulating Solutions

Since ferric iron interferes with the determination of copper, it must be eliminated quantitatively from solution. This may be done with inappreciable loss of copper by adding an excess of ammonium hydroxide to the boiling solution and filtering. In testing the suitability of the various regulating solutions for copper, direct polarographic determinations were made of copper (as copper sulfate pentahydrate) added to these solutions. Then, in addition, copper standards were acidified with sulfuric acid and carried through the ammonium hydroxide treatment, the filtrates were evaporated to dryness, and the residues were taken up in the regulating solutions and polarographed.

Stout's solution of ammonium acetate–tartaric acid and Hohn's Grundlösung A were eliminated as giving unsatisfactory curves. In neither case could the curves be repeated with any quantitative precision. Hohn's Grundlösung C is a strongly ammoniacal solution of ammonium chloride containing methyl cellulose as a stabilizing colloid. In order to pass nitrogen through this solution to free it of oxygen it was necessary to add caprylic alcohol to prevent foaming over (*5*). Although there is some irregularity in the curves produced in this solution, it is possible to use it for higher concentrations of copper. At concentrations below 4 micrograms of copper per ml. of solution, the curves were unsatisfactory because the linear spread of the copper "break" (along the voltage axis) was so great as to make measurement difficult where quantities of copper are small and the wave height is correspondingly small.

Suchy (*10*) recommends as a regulating solution a 10 per cent solution of sodium potassium tartrate. This solution is alkaline owing to hydrolysis. Other solutions used by Suchy were acid and alkaline solutions of sodium citrate and sodium tartrate. All these solutions of Suchy's were tried with various copper concentrations. Where the copper solution was acidified with sulfuric acid, neutralized with an excess of ammonium hydroxide, filtered, washed, evaporated to dryness, and taken up in the various solutions of Suchy, free ammonia was liberated on addition of any of the alkaline solutions and unsatisfactory curves resulted.

The most satisfactory curves were obtained with acid sodium citrate (pH 3.8 to 4.2), made by mixing equal quantities of 0.5 *M* sodium hydroxide and 0.5 *M* citric acid. It was comparatively easy to free this solution of dissolved oxygen, requiring 10 to 20 minutes of bubbling in pure nitrogen, whereas the alkaline solutions required 60 to 80 minutes. Where copper alone was added to the regulating solution and polarographed (without the acidification, neutralization, filtration, etc.) there was a tendency toward a slight maximum. This was overcome by the addition of 1.0 ml. of 0.05 per cent acid fuchsin, $C_{20}H_{17}N_3(SO_2ONa)_2$, to every 10 ml. of regulating solution.

TABLE I. BASE MIXTURE

Material	Element	% in Plant[a]	Material	Element	% in Plant[a]
	Mg.			*Mg.*	
KCl	10.0 K	1.0	$MnSO_4$	0.2 Mn	0.02
Na_2CO_3	1.0 Na	0.1	$NiSO_4.7H_2O$	0.05 Ni	0.005
$CaCl_2$	10.0 Ca	1.0	$CoCl_2.6H_2O$	0.05 Co	0.005
$MgSO_4.7H_2O$	5.0 Mg	0.5	$PbCl_2$	0.05 Pb	0.005
H_3PO_4	2.0 P	0.2	$CdCl_2.2H_2O$	0.05 Cd	0.005
$FeCl_3.6H_2O$	1.0 Fe	0.1	$ZnSO_4.7H_2O$	0.05 Zn	0.005
$Al_2Cl_6.12H_2O$	0.2 Al	0.02	$CuSO_4.5H_2O$	0.05 Cu	0.005
SiO_2 (silicic acid)	20.0 Si	2.0	As_2O_5	0.05 As	0.005

[a] Assuming 1.0-gram plant sample represented in 10-ml. solution.

Interfering Materials

To determine the concentration limits of materials other than copper which may occur in the plant ash without interfering with the determination of copper, solutions were made up containing all of those cations or anions that are likely to appear in an ashed sample. The basic synthetic solution which approximates plant ash is listed in Table I.

Now a series of solutions was prepared in which each con-

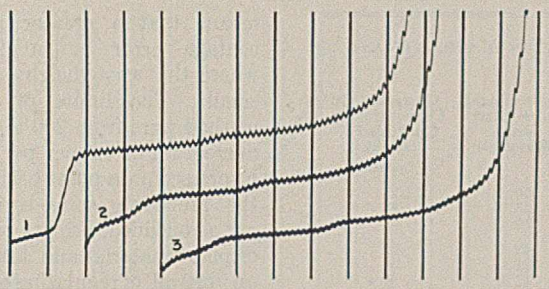

FIGURE 1. RECOVERY OF COPPER ADDED TO PLANT MATERIAL
1, 2, 3. Ashing methods referred to in text. Sensitivity 1/30, drop rate 2.5 seconds, distance between abscissas 0.15 volt

stituent was varied in concentration, holding the other constituents constant. The solutions were then treated with ammonium hydroxide, filtered, washed, and evaporated to dryness, the residue was taken up in the acid sodium citrate, fuchsin was added, and copper was determined polarographically.

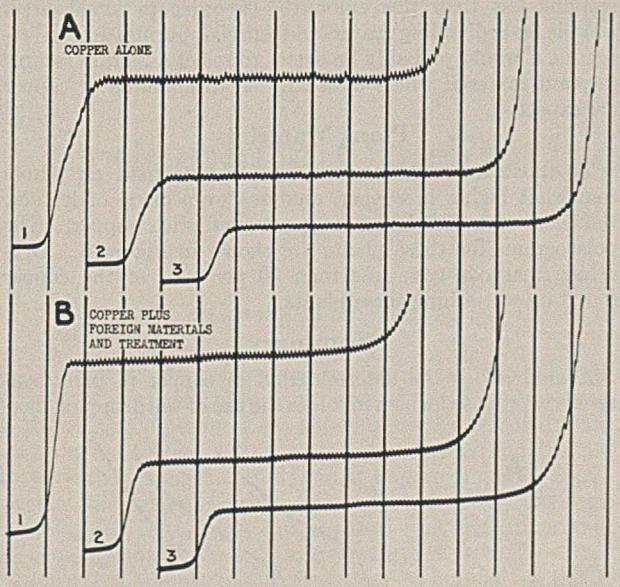

FIGURE 2. COMPARISON OF COPPER ALONE AND COPPER PLUS FOREIGN MATERIALS AND TREATMENT
Sensitivity 1/150, drop rate 2.5 seconds, distance between abscissas 0.15 volt

When the copper concentration was maintained at 5 micrograms per ml. (assuming 1.0 gram of plant material ashed and taken up in 10 ml. of regulating solution, this is equivalent to 0.005 per cent copper), there was no interference from any of the materials considered even when present at concentrations ten times as great as listed in Table I. From this it was evident that there would be no interference from any of these elements at concentrations that greatly exceed their normal concentration in plant material.

The anode potential was measured against the saturated calomel half-cell so as to correct the half-wave potential for it. There was little variation in anode potential and this operation might well be omitted in routine determinations. In the regulating solution used, the corrected half-wave potential for copper was −0.15 volt.

Ashing Procedure

After the method proved satisfactory for copper determinations in synthetic plant ash, a number of different plant materials were ashed in the following ways and copper was determined polarographically by the method used with the synthetic ash:

1. A wet-ashing procedure involving nitric, sulfuric, and perchloric acids.
2. Ignition in a muffle furnace at 450° C. for 12 to 16 hours.
3. Addition of nitric acid followed by ignition at 450° C. for 12 to 16 hours, addition of nitric acid again followed by reignition in the muffle.
4. Ignition in a muffle furnace at 650° C. for 12 to 16 hours.

To determine the effectiveness of the ashing procedures for recovery of added copper, 160 micrograms of copper as $CuSO_4 \cdot 5H_2O$ were added to the plant materials and the above ashing procedures were repeated. In all the dry-ashing trials, separate samples after ignition were taken up independently in hydrochloric and in sulfuric acid. In only the wet-ashing procedure was added copper recovered. This supports the conclusions presented by Mitchell (*4*) with regard to the determination of traces of copper. In Figure 1 are given curves illustrating the ineffectiveness of two dry-ashing procedures commonly used. A blank was run on the reagents used in the wet-ashing procedure to see what part of the "recovered" copper was in the reagents themselves. The acids carried through the procedure and taken up in regulating solution gave 4 micrograms of copper, and the plant material with unadded copper gave 10 micrograms of copper. These are small in comparison with the amount added for recovery, 160 micrograms of copper.

Proposed Method

Place 0.5 to 2.0 grams of plant material in a 30-ml. Kjeldahl flask. Add 5 ml. of concentrated nitric acid, heat until brown fumes are evolved, add 1 ml. of concentrated sulfuric acid, and heat until charring begins, all the nitric acid being driven off. Add 1 to 2 ml. of 60 per cent perchloric acid and continue heating until the solution is colorless or a pale yellow and the excess perchloric acid is driven off. Dilute to 15 to 20 ml., heat to boiling, add a slight excess of concentrated ammonium hydroxide, boil for a minute, filter, and wash with slightly ammoniacal water. Evaporate the filtrate to dryness, take up the residue in 9 ml. of acid sodium citrate (made by mixing equal quantities of 0.5 *M* sodium hydroxide and 0.5 *M* citric acid), add 1 ml. of 0.05 per cent acid fuchsin, and determine the copper polaro-

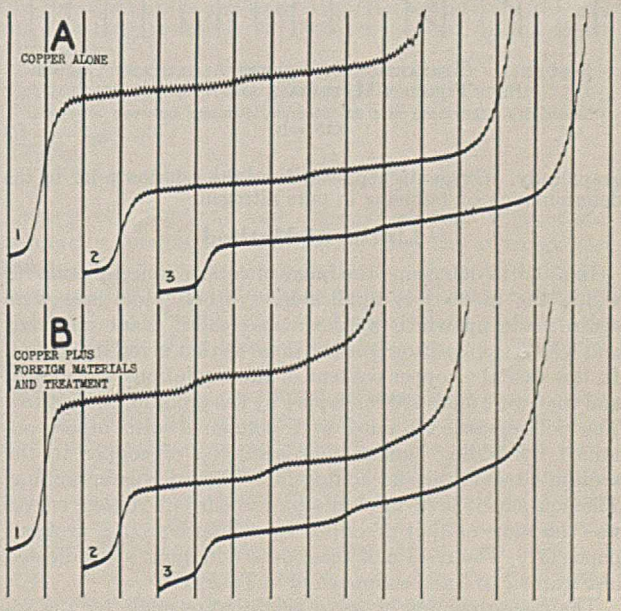

FIGURE 3. COMPARISON OF COPPER ALONE AND COPPER PLUS FOREIGN MATERIALS AND TREATMENT
Sensitivity 1/30, drop rate 2.5 seconds, distance between abscissas 0.15 volt

TABLE II. COMPARISON OF COPPER ALONE AND COPPER PLUS FOREIGN MATERIALS AND TREATMENT

	Copper Added γ/ml.	Galvanometer Sensitivity	Wave Height, Cu Alone Mm.	Corrected Cu Concentration, Cu Added + Blank γ/ml.	Wave Height, Cu + Interfering Materials	Corrected Cu Concentration, Cu Added + Blank γ/ml.
Figure 2	200	1/150	50.0	200	50.0	200
	100	1/150	26.0	100	25.0	100
	64	1/150	15.5	64	16.0	64
Figure 3	32	1/30	43.5	32	42.0	32
	16	1/30	22.0	16	21.0	16
	8	1/30	12.0	8.3	11.0	8.4
Figure 4	4	1/10	21.0	4.3	20.0	4.4
	1	1/10	9.5	1.3	10.5	1.4
	0.5	1/10	5.5	0.84	5.5	0.87
Figure 5	0.5	1/5	12.0	0.84	11.0	0.87
	0.2	1/5	9.5	0.54	8.5	0.57
	Blank	1/5	6.0	0.34	5.5	0.37

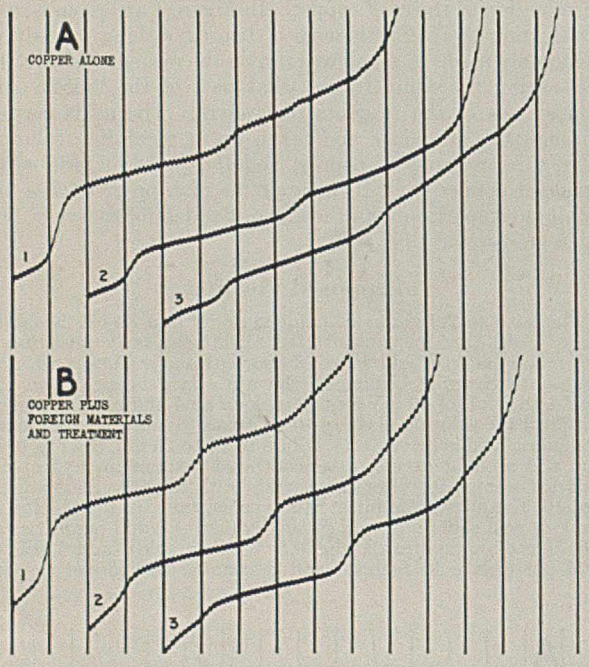

FIGURE 4. COMPARISON OF COPPER ALONE AND COPPER PLUS FOREIGN MATERIALS AND TREATMENT
Sensitivity 1/10, drop rate 2.5 seconds, distance between abscissas 0.15 volt

graphically. Oxygen is removed from the solution prior to the determination by bubbling in pure nitrogen.

Limits of Method

In order to determine the range of copper concentration for which the method is applicable, synthetic solutions were again made up which approximated ashed plant materials and whose composition was the same as that given in Table I. In this case the copper content of these solutions was varied, and they were analyzed for copper by the procedure suggested. The polarograms obtained were compared with others obtained by adding similar concentrations of copper to the sodium citrate solution, adding fuchsin, and polarographing. The equipment used in obtaining the current-voltage curves was the same as that described by the authors in a previous paper (*6*). The results of these determinations are indicated in Figures 2 to 5 and summarized in Table II.

The measurements of wave height were made by the so-called "intersection point" method denoted as method C by Borcherdt *et al.* (*1*). The accuracy, if several measurements are made and averaged, is about 0.5 mm. This obviously means that a greater percentage error is involved where the wave heights are small. The limits of the method were from 200 to 0.2 microgram of copper per ml. Expressed on a plant basis, if the procedure were carried out as outlined, using 1 gram of plant material and taking up in 10 ml. of regulating solution, this would amount to a range of from 0.2 to 0.0002 per cent of copper in the plant. The upper limit could be extended by using a lower galvanometer sensitivity or by diluting the solution.

Even in the case of the copper alone the "blank" was as high as 0.37 microgram per ml. It is evident that a blank is necessary for correction purposes and that with a blank of this magnitude it would not be possible to determine concentrations less than this amount. In order to avoid extra trouble in redistilling water and special purification of reagents, it would probably be better to select a 2-gram sample of plant material where unusually small amounts of copper are expected.

Plant Materials

To illustrate the effectiveness of the method on plants somewhat higher in copper, analyses were made of lettuce, the seeds of which had been pretreated with copper. The polarograms for these plants are shown in Figure 6. Dry-ashing methods gave less than 50 per cent of the copper found by wet-ashing these plants.

Summary

A method for the determination of copper in plant materials is proposed which involves the use of the dropping mer-

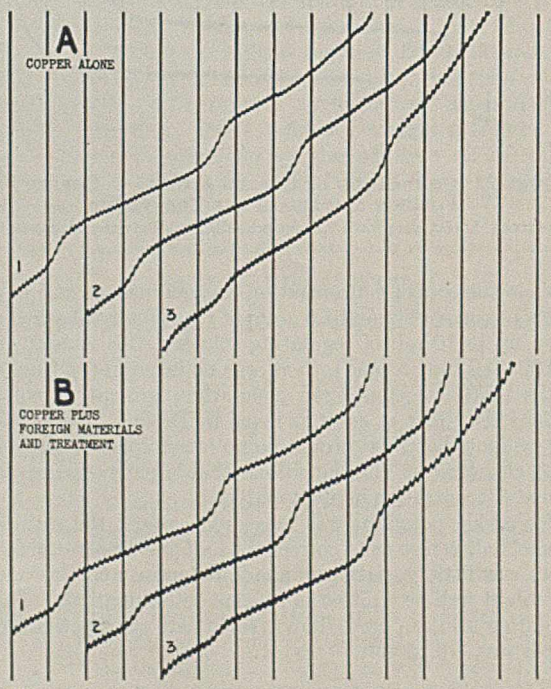

FIGURE 5. COMPARISON OF COPPER ALONE AND COPPER PLUS FOREIGN MATERIALS AND TREATMENT
Sensitivity 1/5, drop rate 2.5 seconds, distance between abscissas 0.15 volt

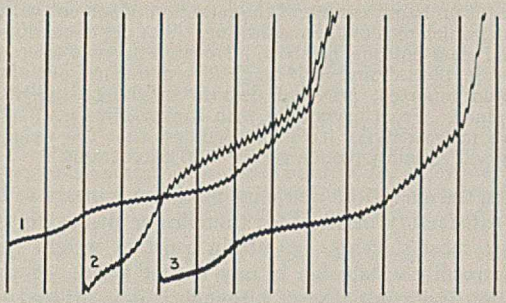

FIGURE 6. COPPER IN LETTUCE
(1) Sensitivity 1/30, 0.004% Cu; (2) sensitivity 1/10, 0.003% Cu; (3) sensitivity 1/30, 0.006% Cu. Drop rate 2.5 seconds, distance between abscissas 0.15 volt

cury electrode. Copper is determined in the presence of all the constituents ordinarily present in plant ash except those that are removed by addition of a slight excess of ammonium hydroxide. No interference is offered by any of the cations or anions likely to be found in plant ash even when present in comparatively large quantities.

Copper added to plant materials as copper sulfate could not be recovered by any of the dry-ashing methods used. A wet-ashing procedure was adopted involving final solution in sulfuric acid.

Limits of the method using a 1-gram sample of plant material are from 0.2 per cent or greater to 0.0002 per cent of copper in the plant.

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Systematic Qualitative Organic Microanalysis

Comparative Study of Procedures of Microextraction

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EXTRACTION procedures constitute an important step in the scheme of organic analysis on a micro scale (*1*, *2*). These procedures are of two types: the removal of an active ingredient from a heterogeneous mixture and the removal of undesirable material from an impure product. Separation of the active principle from a crude drug mixture is an example of the first type; the preparation of derivatives and their consequent purification, an example of the second.

In control laboratories, where time is limited, methods which require less sample and consequently less extraction time are of distinct advantage, provided that the results are comparable to those obtained when using conventional apparatus. This investigation includes only methods for extraction of solid substances by heated solvents and excludes liquid-liquid extraction processes. The merits of a standard macro-Soxhlet, a semimicro-Soxhlet extractor and representative microextractors are compared.

Several microextractors, developed in recent years, were constructed for special investigations. The authors are not cognizant of any study dealing with the use of an extractor for general purposes involving the quantitative recovery of both extractive and residue.

The two types of extraction apparatus are siphoning and percolating; these may be of simple or complex design.

The macro-Soxhlet, its semimicro counterpart (commercially listed as "micro" Soxhlet extractor), the Colegrave (*7*), and the Wasitzky (*21*) extractors belong to the siphoning group. Extractors using the principle of percolation have been developed by Titus and Meloche (*20*), Gorbach (*13*), Hetterich (*14*), and Slotta.

Slotta's extractor (*18*), which is not described in the literature, is shown in Figure 4. A glass crucible with sintered-glass bottom holds the material to be extracted. Crucibles of different porosities may be substituted to correspond with the particle size of the substance and surface tension of the solvent. A Dimroth condenser reduces the over-all height of the apparatus. The receiving flask is connected to the extraction chamber by a ground-glass joint; an apparent disadvantage is the weight of this flask, approximately 15 grams, which reduces the accuracy when very small amounts of extracted material are weighed. When solution is transferred there is always the added risk of accidental loss. Two glass inserts of 5- and 10-ml. capacity, respectively, together with a distribution tube, serve for the extraction of small amounts of liquid.

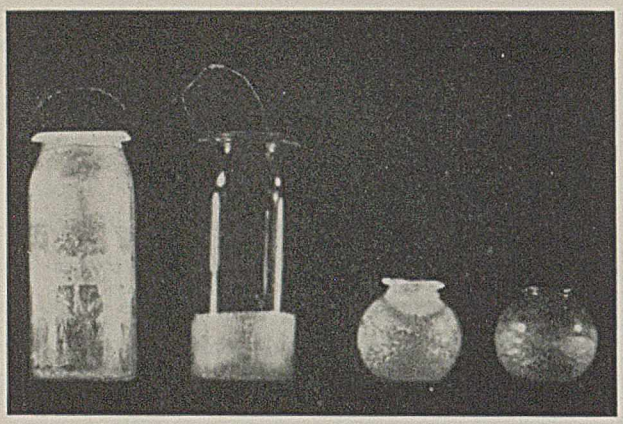
Because of similarity of design to one or another of the microextractors mentioned, the apparatus of Blount (*5*), Browning (*6*), Fulton (*10*), Garner (*11*), and Gettens (*12*) were not included in this investigation.

Factors Influencing Extraction

SOLVENTS. Solvents were selected so that volatile and moderately volatile, low and high boiling, low and high surface tension menstrua were included. A solvent having special affinity for an extractable material likely to be present is preferable. The influence of atmospheric moisture on the solvent is reduced by using drying tubes on top of the condensers.

The range of boiling points of solvents tried was between 35° and 100° C. at normal pressure. The boiling point in a completely enclosed extracting system, or one with a comparatively small condenser, may be slightly higher, especially in the case of a very volatile liquid. Decomposition of natural products when extracted with common solvents may occur if the boiling points, at atmospheric conditions, are high. These two dangers may be avoided by the application of reduced pressure in the extraction apparatus (*13*, *20*, *21*).

The surface tension is another influence in obtaining a smooth-running extraction. The range of surface tensions encountered in common solvents for liquid-air interface, ex-



A B C D

FIGURE 1. COMPARISON OF METHODS OF EVAPORATION

A. Heating from underneath
B. Heating from above, with Chromolox ring
C. Spontaneous evaporation
D. Heating from underneath but removing vapor phase by a jet of air

pressed in dynes per cm., includes: ethyl ether 20° C. = 17, 35° C. = 15; ethyl alcohol 20° C. = 22, 70° C. = 18; and water 20° C. = 73, 100° C. = 59. In the lower range of surface tensions the effect is mainly that of penetrability of the solid; Lehrecke (*16*) states that the penetrability of an organic solvent into a solid is inversely proportional to the surface tension. In the higher range, difficulties with the refluxing liquid are encountered.

SYSTEMS STUDIED. Salicylic acid and kieselguhr extracted with ether, anthracene and starch extracted with ether, and sodium chloride and aluminum oxide extracted with water, were the mixtures and solvents tested in comparing the macro- and semimicroextractors. Because of mechanical difficulties, salicylic acid mixed with sand and extracted with ether, caffeine and barium sulfate extracted with alcohol, and sodium chloride and sand extracted with water, were used for comparing the microextractors. The salicylic acid–sand mixture was not used in the macroapparatus because of the great variation in bulk, which was appreciable but less noticeable when used in the smaller extractors.

All the mixtures were tested in various ratios, so as to have the solid components approximately equal in weight, the soluble part in excess, and the insoluble portion in excess. The last mentioned would correspond to the extraction of material in which the percentage of soluble matter is small (towards the lower limits of the weighing range of a microchemical balance).

Container for Sample

To compare the merits of the materials from which containers are commonly constructed, filter paper in the form of dishes, disks, and thimbles; Alundum thimbles; vessels with sintered glass bottoms; and platinum as well as Monel metal containers were studied.

It is very difficult to obtain constant weight on large masses of filter paper owing to its hygroscopicity. By using standardized procedures (*15*) or certain precautions to prevent access of moisture, the weights of paper thimbles can be reproduced within the limits of accuracy of either method, but these procedures are very cumbersome. In the majority of cases, this difficulty in obtaining constant weights of the filter paper used in some extractors prevented accurate determination of the residue. Even though the filter paper thimbles were subjected to a preliminary extraction, fibers were carried over during the extraction proper and had to be removed by centrifuging—a step that involves two unnecessary transfers.

Although it was possible to obtain constant weight with filter thimbles of Alundum, they were unsatisfactory for use with the apparatus on hand because of the retention of solvent within the thimble, which resulted in overflowing. The use of crucibles with sintered glass bottoms has been advocated in some microextractors—e. g., Slotta's and Browning's (*6*)—and constancy of weight has been reported, provided definite weighing conditions are maintained. From experience with similar apparatus in quantitative microanalysis, it can be deduced that the weight constancy will certainly remain within ±20 micrograms.

From the analytical viewpoint platinum appears to be the most satisfactory material for thimbles or dishes, since it is inert to most solvents, can attain constant weight quickly, and is readily obtainable in any desired form. A possible objection is its initial cost, although if it is utilized over a long period of time the cost is well distributed, for it seldom needs replacing. Preliminary experiments indicate that other metals may be substituted, but they lack the general applicability of platinum.

POROSITY. Conflicting statements have been made regarding the suitable porosity of sintered glass plates when the solvent is removed by gravity. Blount (*5*) recommends G_2, while Browning (*6*) suggests G_3.

Tests conducted in the present investigation, using Jena products, showed that sintered glass plates of porosity G_3 were satisfactory for use with alcohol, while G_4 filters were not sufficiently fast if accumulation of solvent was to be avoided (for porosity gradings see Prausnitz, *17*). The porosities as listed by Ace Glass, Incorporated, would correspond as follows: Jena O to Ace A; 1 to B; 2 to C; 3 to D; and 4 to —.

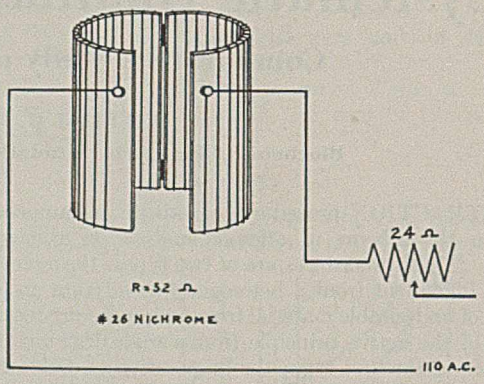


FIGURE 2. HINGED CIRCULAR HEATER

The platinum filter dishes of Donau (*8*), or the Neubauer filter crucibles, both having a mat between two perforated platinum surfaces, may be made up by adjusting the thickness of the mat to suit the conditions of the experiment.

TIME REQUIRED FOR COMPLETE EXTRACTION. The time required for the exhaustion of the extractable material from the sample is best determined by successive extractions until the weight of the last flask substituted shows no further increase.

Another method, frequently used in alkaloidal assaying, consists of removing a little of the freshly siphoned or percolated solution and testing it with the proper reagent until negative results are obtained.

In order to distribute the solvent evenly upon the surface of the sample and prevent channeling, Prausnitz (*17*) constructed the "icicle crown" condenser tip, which was later incorporated in Gorbach's extractor (*13*). The more effective distribution of the condensate results in a shorter extraction time.

Special Procedures and Apparatus Developed

EVAPORATION OF SOLVENT AFTER EXTRACTION. To distill off the solvent and at the same time prevent creeping of the solid

material, the vapors above the surface of the liquid are removed by blowing air onto it or by applying heat from the top (*1*, *4*). In the latter method, a Chromolox resistance ring of 150 watts (outer diameter 65 mm., inner diameter 25 mm., and 5 mm. thick) is placed around the upper part of the vessel and gradually lowered as evaporation proceeds.

A comparison of these methods, with the usual one of heating from underneath, is presented in Figure 1, corresponding receiving flasks containing equal amounts of ether and of salicylic acid were used. The picture, taken with a polaroid screen to remove reflected highlights, illustrates clearly the advantages and disadvantages of the methods applied.

HINGED CIRCULAR HEATER. The condensation of solvent in the ground-glass joints of most of the extractors examined not only resulted in a direct loss, but was also a source of annoyance because of the suction formed when some of the solvent in the joints evaporated. To overcome this, Titus and Meloche (*20*) warmed the joint of their apparatus by winding resistance wire around it and heating by passage of an electric current. To avoid winding all the glass joints, a generally applicable device was constructed (Figure 2). The outside, circular, hinged electric heater consists of a hollow cylinder of hardened asbestos cement, cut in halves in the axial plane and with halves hinged together. Each half of the cylinder is wound vertically with 300 cm. (10 feet) of No. 26 B. and S. Nichrome wire having a resistance of 2.61 ohms per 30 cm. (1 foot), with one of the hinges serving as a contact. A slide-wire resistance of 24 ohms and 3.3 amperes is placed in series with the heater, so that the amount of heat radiated may be controlled. The hinge allows observation of the rate of boiling and permits the heater to be removed from the extractor simply by disconnecting one of the contacts leading to the external wiring.

In experiments using water as a solvent it was extremely difficult to prevent excessive condensation on the walls of the outer shell (as in Titus and Meloche's and Gorbach's apparatus) and on the inner walls of the reservoir in Colegrave's and Wasitzky's extractors. In one experiment some of the condensed solvent dripped onto the hot shell of the Gorbach apparatus and cracked it. The application of the external heater prevented recurrence of this trouble and since its introduction boiling and vaporization have appeared to run smoothly and condensation has been regular.

Modifications of Original Design

Slight modifications were introduced into some of the original designs for the purpose of facilitating certain manipulations.

The ground-glass joint of the thimble in Titus and Meloche's (*20*) extractor was reversed—i. e., the lower portion was made the outside member, so that the filter paper disk would form an upright dish and avoid loss of inert material when removed (Figure 5).

Loss of volatile solvent from the original extractor of Colegrave (*7*) suggested the introduction of a ground-glass joint at the junction of the condenser and the top of the chamber. This modified apparatus (Figure 4) was found more satisfactory in preventing solvent losses. A small glass receiver, placed in the bottom of the chamber, made unnecessary the transfer of solution for evaporation and weighing. A similar device was used for Wasitzky's extractor (*21*).

Experimental

The salicylic acid, sodium chloride, and sand used were Merck's Blue Label reagents which required no purification, but the anthracene and caffeine had to be recrystallized. For precautionary measures the solid insoluble components, kieselguhr, barium sulfate, sand, and starch, were previously boiled in some of the solvent selected for the extraction, so that any solvent-soluble material would be removed.

The organic solvents, ethyl ether and ethyl alcohol, were distilled until the boiling point of each was sufficiently constant. Only those tests for purity (*22*) were performed which would yield information about the solvent properties. The water used was distilled by means of a laboratory still and protected from atmospheric carbon dioxide.

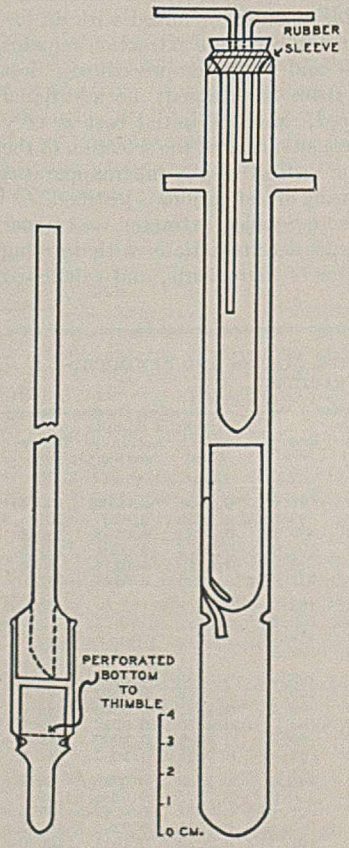


FIGURE 3. MICROEXTRACTORS
Left. Hetterich type
Right. Wasitzky type

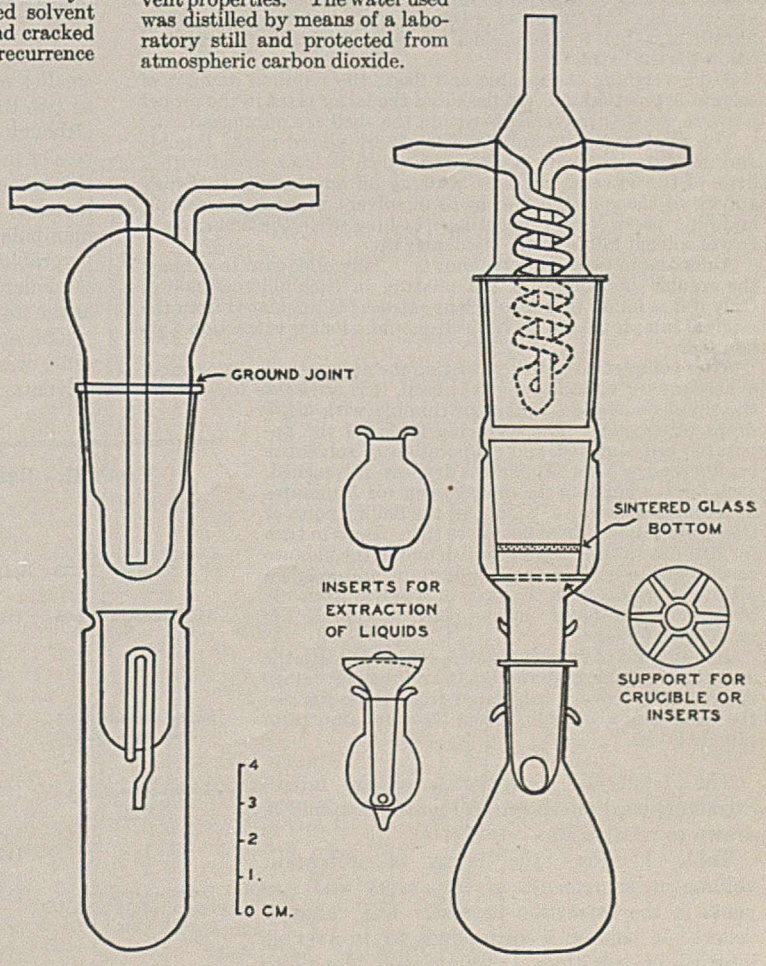


FIGURE 4. MICROEXTRACTORS
Left. Modified Colegrave type
Right. Slotta type

TABLE I. COMPARISON OF VOLUME MEASUREMENTS IN SEMIMICRO- AND MICROEXTRACTORS

(Surface tensions of solvents used, dynes per cm.: ethyl ether: 20° C. = 17, 35° C. = 15; ethyl alcohol: 20° C. = 22, 70° C. = 18; water: 20° C. = 73, 100° C. = 59)

Apparatus	Ratio of Volumes: Volume of whole chamber to initial volume of liquid, ml.	Volume retained in extraction thimble before removal to initial volume of liquid, ml.	Volume of whole chamber to volume retained in extraction thimble before removal, ml.	Contact
Semimicro-Soxhlet	25:20 = 1.2	8:20 = 0.4	3	Long
Titus and Meloche	125:5 = 25	0.2:5 = 0.04	625	Variable
Gorbach	55:2 = 28	0.2:2 = 0.1	280	Short
Colegrave (improved)	60:10 = 6	4:10 = 0.4	15	Long
Wasitzky	60:10 = 6	2.5:10 = 0.25	24	Long
Hetterich	9:1 = 9	0.2:1 = 0.2	45	Medium
Slotta	50:20 = 2.5	0.5:20 = 0.03	100	Variable

PROCEDURE FOR MICROEXTRACTIONS. Each flask, extraction or weighing, is tared with an object of similar material—i. e., a Pyrex flask with a Pyrex beaker and objects of soft glass with soft glass flasks of approximately the same size and shape. The Pyrex flasks for the macro- and semimicro-Soxhlet extractors are carefully tested for electrostatic charges (*19*); otherwise constancy cannot be obtained for the initial weighing. The receiving vessels for the microapparatus are made of either soft or "Jena Geräte" glass. The ingredients for the centigram and milligram extraction processes are weighed separately to obtain the exact weight of each component present. The charging tubes with ground caps (*3*) are employed for the direct introduction of the solid constituents and all weighings are made on a microchemical balance. The weighing flasks, after thorough cleaning and drying, are placed in the balance case to come to temperature equilibrium and then weighed under the same conditions as described below for the extract.

After weighing the sample and flask, the required amount of solvent is pipetted into the flask and the latter is set in the proper position, so that movements within the shell are minimized.

The soluble and insoluble materials are placed in the thimble and gently mixed with a glass rod which is later rinsed with a little of the solvent. Besides washing off any sample that may adhere to the rod, the few drops of solvent moisten the sample and filter paper, thus preventing splashing when the first drop or two of solvent fall from the condenser tip.

A maximum period of one hour is usually adequate to exhaust the soluble portion from the mixture in each microextractor. Only if it is found that insufficient extract (as calculated from the original sample taken) is obtained should a longer extraction time be used.

After completion of the extraction the apparatus is dismantled. Where a very volatile solvent is used, it is advisable to wash off the tip of the siphon, funnel, or thimble with a few drops of solvent. The receiving flasks of the apparatus are removed and evaporation of solvent is commenced. After apparent dryness is reached, each flask is put into the drying oven for 5 minutes at a temperature of 5° C. above the boiling point of the solvent used. The flasks are then placed in turn for 10 minutes each on a Petri dish and in the balance case to attain temperature equilibrium, and weighed after 25 minutes from the time of removal from the oven. All transfers from one place to another are made with forceps.

The amount of extract found is compared with the amount of soluble material taken and the percentage of error of recovery calculated from these figures; the error is not calculated from the total amount of sample taken.

The details of construction of the microextractors used are shown in Figures 3, 4, and 5, drawn to relative size.

Table I shows the ratios of important volume measurements of apparatus and solvents in the extraction process. The value of "short" in column 5 corresponds to an average interval of less than 1 minute and "long" to about 4 or more minutes. From Table I, and from experience in many extraction procedures, the following generalized conclusions may be drawn.

The greater the ratio of the volume of the whole chamber to the initial volume of liquid, the less satisfactory is the extractor for use with high-boiling solvents with high surface tensions.

The smaller (within practical limits) the ratio of volume retained in the extraction thimble before removal to initial volume of liquid, the more satisfactory the extraction process becomes, with regard to the quantitative recovery, especially in the case of the extraction of slightly soluble substances.

The value of the ratio of the volume of the whole chamber to the volume retained in the extraction thimble before removal is dependent upon the method of removal of solution; for percolation the differences are not very significant, whereas for the siphoning method the different values assume importance.

As observed in the extractors of Hetterich and Gorbach, the volume of the reservoir is so small that when evaporation takes place and condensate of liquid of higher surface tension (as water) forms on the inside of the extraction chamber, very little solvent remains in the reservoir.

Comparison of Macro- and Semimicro-Soxhlet Apparatus

Table II shows the results obtained when using a semimicro-Soxhlet extractor; they are at least equivalent to those of the conventional macro-Soxhlet extractor. In addition, however, important advantages are offered by the use of the smaller apparatus: The period of extraction is shorter, up to two thirds of the time for moderately soluble substances, although with a very soluble constituent mixed with an excess of inert material the time of complete extraction remains the same for both macro- and semimicroprocedures; less material is required; less space is occupied; no additional manipulations are introduced; and the initial cost is very favorable. Based upon these advantages, the selection of the semimicro-Soxhlet extractor instead of the macroapparatus as the standard for the following investigations is justified.

The accuracy of the macro-Soxhlet extractor, ±1.9 per cent, was computed from 25 determinations with varying solvents, mixtures, and ratios of ingredients, and calculated

TABLE II. RESULTS OBTAINED WITH MACRO- AND SEMIMICRO-SOXHLET EXTRACTORS

Components and Solvents	Ratio[a]	Macroextractor: Weight of Sample: Total	Macroextractor: Weight of Sample: Active ingredient	Macroextractor: Recovery	Semimicroextractor: Weight of Sample: Total	Semimicroextractor: Weight of Sample: Active ingredient	Semimicroextractor: Recovery
		Grams	*Gram*	%	*Gram*	*Gram*	%
Salicylic acid–kieselguhr with ether	1:9	1.0000	0.1000	97.0	0.2004	0.0200	100.5
		1.8020	0.1802	99.5	0.2036	0.0204	101.5
		1.5002	0.1500	99.5	0.1982	0.0198	100.5
		1.6290	0.1629	100.0	0.2012	0.0201	101.5
		2.0000	0.2000	96.5	0.2112	0.0211	99.0
		1.0054	0.1005	101.0	0.1980	0.0198	101.5
Salicylic acid–kieselguhr with alcohol	1:9	1.1207	0.1121	102.0	....	....	...
		1.0462	0.1046	100.0	....	....	...
		1.1315	0.1132	98.5	....	....	...
		1.0184	0.1018	102.5	....	....	...
Anthracene-starch with ether	1:9	1.0785	0.1079	103.0	0.2232	0.0223	99.5
		1.0519	0.1052	100.0	0.2072	0.0207	99.0
		1.0297	0.1030	98.5	0.2381	0.0238	99.0
		1.0686	0.1069	98.0	0.2570	0.0257	99.0
	1:1	1.0000	0.5000	99.5	0.2000	0.1000	99.5
		1.0004	0.5000	98.5	0.2004	0.1002	99.0
Sodium chloride–aluminum oxide with water	1:9	1.0005	0.1000	103.0	0.2183	0.0218	99.0
		1.0048	0.1005	100.0	0.2075	0.0208	99.5
		....	....	...	0.2068	0.0207	97.5
		....	....	...	0.2051	0.0205	102.5
	1:1	1.0000	0.5000	101.5	0.2022	0.1011	100.0
		1.0109	0.5050	95.0	0.2025	0.1013	97.5

[a] Parts of active ingredient to parts of inert ingredient.

TABLE III. COMPARISON OF MICROEXTRACTORS

(Experimental conditions: ratio of active to inert ingredient, 1:1; extraction time, 1 hour)

Components and Solvent	Semimicro-Soxhlet		Titus and Meloche		Gorbach		Colegrave (Modified)		Wasitzky		Hetterich	
	Extractable substance	Recovery	Extractable substance	Recovery	Extractable substance	Recovery	Extractable substance	Recovery	Extractable substance	Recovery	Extractable substance	Recovery
	Mg.	%	*Mg.*	%	*Mg.*	%	*Mg.*	%	*Mg.*	%	*Mg.*	%
Salicylic acid–sand with ether	15.93	102	16.78	94.0	25.26	94.5	12.66	103	9.70	106	13.17	99.0
	20.96	100	33.33	98.5	28.97	97.0	19.42	101	11.42	108	9.10	114
	24.58	98.5	0.42[a]	64.5	2.14[a]	94.0	0.71[b]	65	...	...	9.76	98.0
	...	...	11.94[c]	99.0	88.25[c]	100	61.31[d]	99.5	...	...	0.91[b]	98.0
Salicylic acid–sand with alcohol	...	...	11.33	100.5	2.97	101	...	...	...	...	...	...
	...	...	25.03	101	4.43	93.0	...	...	...	...	...	...
Caffeine–barium sulfate with alcohol	26.87	100.5	9.66	102	4.58	104	6.38	99.5	26.53	105	2.64	106
	15.24	99.0	9.06	102.5	7.42	100	8.99	99.0	21.23[e]	101	2.84	96.0
	18.54	101	1.06[a]	108	0.61[a]	127[d]	1.42[b]	105[e]	1.88[b]	102.5	1.18[b]	102.5
	...	...	40.94[c]	101	24.37[f]	99.5	43.76[f]	99.5	51.60[f]	101	10.78[f]	99.5
Sodium chloride–sand with water	23.83	100	14.02	100	11.63	99.0	9.97	99.5	...	...	4.37[g]	92.5
	21.46	102	10.57	100	9.81	101.5	11.48	103	...	...	5.74	74.0
	20.16	99.0	1.37[b]	99.0	2.08[b]	104[e]	1.53[b]	101	...	...	...	...
	...	...	24.78[f]	101	8.15[f]	102	96.51[f]	100	...	...	...	...

[a] Ratio 1:100. [b] Ratio 1:50. [c] Ratio 100:1. [d] High results probably due to mechanical carrying-over of inert material. [e] Solution centrifuged and transferred to fresh receiver. [f] Ratio 50:1. [g] Frequent bumping, probably due to shape of receiver.

for the amount extracted. Maximum single deviations from the theoretical values were +3.3 and −9.0 per cent (one case each). Average deviations from the mean value were +1.8 and −3.3 per cent. The accuracy of the semimicro-Soxhlet extractor, ±1.85 per cent, was calculated in the same way from 28 determinations. Maximum single deviations from the theoretical values were +5.9 and −6.7 per cent (one case each). Average deviations from the mean value were +2.1 and −1.7 per cent. Not all the values taken for this evaluation appear in Table II.

Microextractors

A further comparison is made between the semimicroextractor and the various microextractors in Table III, which is self-explanatory.

Similar experiments have been carried out for the ratios of active to inert ingredient: 100 to 1, 50 to 1, 10 to 1, 1 to 10, 1 to 50, and 1 to 100. On the basis of these observations, the following generalized conclusions can be drawn for each individual microextractor:

TITUS AND MELOCHE APPARATUS. Satisfactory for use when the solid components are equally proportioned, when the soluble material is in excess, and with high-boiling solvents.

GORBACH APPARATUS. Satisfactory for use when the solid components are equally proportioned, when the soluble material is in excess, and when the inert material is not too finely divided. This extractor is equipped with an "icicle" distribution crown which permits faster extraction. For liquids having a surface tension near that of water, this apparatus is not recommended.

COLEGRAVE APPARATUS (ORIGINAL AND IMPROVED FORM). Satisfactory for use when the solid components are equally proportioned, when the soluble portion is in excess, and when water is the solvent. This extractor is one of the least expensive to purchase.

WASITZKY APPARATUS. Satisfactory when the soluble material is in excess of the inert. In general, this extractor is similar to the improved Colegrave apparatus with ground-joint condenser and is superior to the simple one.

HETTERICH APPARATUS. Satisfactory for use when the soluble material is in excess and when the solvent is low boiling, but unsatisfactory in the present form for use with water as solvent.

From the values in Table III, one can deduce that the accuracy and precision of the determinations of the extract in the microextractors fall within the range calculated for the macro- and semimicro-Soxhlet extractors—i. e., ±2.0 per cent. Greater errors will occur as one approaches the unfavorable extremes for the microextractors studied; this investigation has suggested the limits of practicability and greater variations should be avoided. An accuracy of 0.75 per cent has been claimed for some microextractors (*9*), but will be obtained only in very special cases. The present investigation has clearly shown that, in general, ±2.0 per cent is a reasonable accuracy to expect.

The advantages of the microextractors, even over the semimicroapparatus, may be summarized as: a shorter total extraction time, the possibility of using smaller amounts of

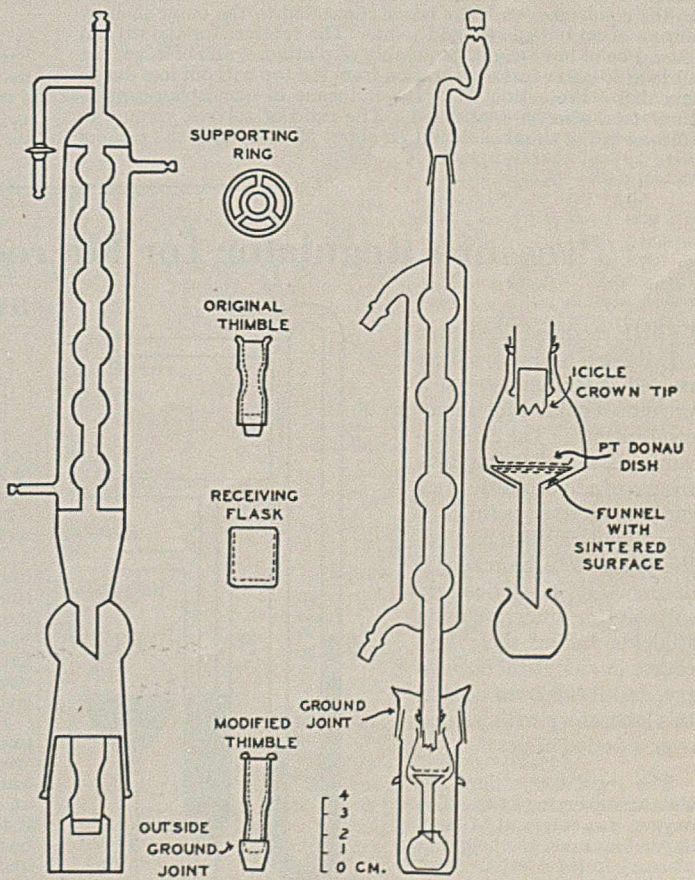


FIGURE 5. MICROEXTRACTORS
Left. Titus and Meloche type
Right. Gorbach type

material (limited only by the weighing accuracy of the available microchemical balance), and the comparative sturdiness of the apparatus. The less commonly used principle of percolation gives better service with respect to general applicability than siphoning (Soxhlet). The apparatus developed by Titus and Meloche is easier to manipulate than the one introduced by Gorbach. One feature, however, which is seldom applied in any of the known macro- or microextractors is incorporated in the Gorbach apparatus—namely, the use of a platinum filter dish as a container for the material to be extracted. Filter devices of metal permit the highest precision in weighing and maximum percentage of recovery of the residual matter without any contamination from the filtering material.

The weighing of milligram amounts of residue on comparatively large filter paper surfaces (extraction thimbles) will not give reliable results, and the microanalyst has to face the fact that all extractors of the siphoning type using paper thimbles are limited to the determination of the extractable matter.

The difficulties in weighing the residual material in the apparatus examined prevented the development of a general formula for the efficiency of an extraction process and of numerical terms for the evaluation of the various factors influencing the extraction.

To accomplish this, a satisfactory microextractor should use the principle of percolation of the solvent through the sample which is held in a platinum container provided with a filter layer of platinum-iridium sponge. The sample should be agitated by means of hot solvent vapors in order to increase the temperature, and condensation of the solvent effected by means of a short-stem water condenser having a device for distributing the refluxed solvent. For convenience the sample container should be attached to the condenser, with the latter connected to the outer shell by means of an outside ground joint. The receiver for the extract should be of low weight, preferably of platinum, and of larger top surface to allow fast evaporation from the top without loss due to creeping. Protection from the influence of atmospheric moisture must also be considered. The ratio of solvent volume to solvent in the thimble should be about 50 to 1, with the ratio of chamber volume to initial volume of solvent as small as practical. A circular hinged heater supplying uniform heat to all sides is recommended for the proposed extractor.

Acknowledgment

The authors express their thanks to Arthur Osol of the Philadelphia College of Pharmacy and Science for his constructive criticism and to Ellice McDonald, director of the Biochemical Research Foundation, for his interest and support throughout the work.

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Pressure Regulator for Microdetermination of Carbon and Hydrogen

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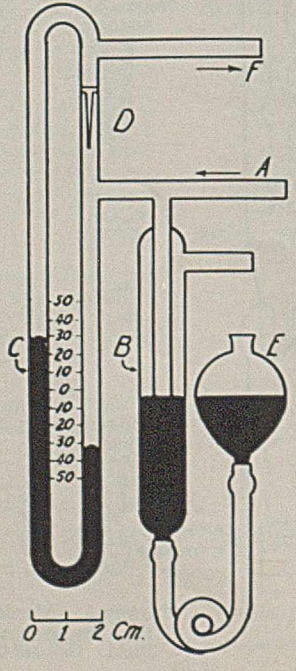
THE modified flowmeter described here has proved satisfactory as a pressure regulator for microcombustions. The chief advantages are that it avoids the use of a hydrostatic pressure head and permits a permanent adjustment of the pressure to be made, if desired. It is particularly suitable for undergraduate students taking their first course in analytical chemistry, and it has been used in this laboratory with success over a period of years.



The regulator is shown in the accompanying figure. The oxygen gas enters at *A* and, depending upon the height of mercury in the overflow tube, *B*, will exert a certain pressure on the capillary, *D*. This pressure is registered on the mercury manometer, *C*, which has a paper millimeter scale behind it. The rate of flow through *F* to the furnace depends upon the size of the capillary as well as upon the head of mercury. A convenient size for the capillary is 0.2 to 0.3 mm.; this will give a flow of 4 to 6 cc. per minute with an appropriate pressure.

The capillary may be drawn out, and the piece of glass tubing into which it is sealed held in the manometer by means of rubber tubing. The rate of flow is then measured; the capillary is removed for alteration and the flow remeasured until the proper size has been found, after which the glass is sealed in place. Once fixed, there should be no need for change, and since the gas flows upward through the capillary, it cannot become clogged by foreign matter.

If desired, the device may be calibrated by measuring the flow under a few different pressure heads. The pressure may be varied by use of the bulb, *E*. The results of such a calibration are reproducible and, when plotted, may be interpolated to give an exact gage of the flow for any pressure. This is a convenience if the sweeping out process at the end of a combustion is to be hastened. If a single flow is sufficient, there is no need for *E*, and the overflow tube may be sealed off at the bottom and filled with the proper amount of mercury.

In operation, the oxygen is permitted to overflow in *B* at a rate of about one bubble a second. At this rate of overflow there is no appreciable variation in the pressure.

Semimicro- and Micro-Kjeldahl Steam-Distillation Unit

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THE steam-distillation of liberated ammonia in the Kjeldahl nitrogen determination has been the subject of considerable study.

The well-known apparatus of Parnas and Wagner, utilized by Pregl (*6*), is familiar to those concerned with microanalyses. Its chief difficulties lie in its bulkiness and the necessity for an elaborately constructed vacuum-insulated distilling chamber.

This latter fault has been eliminated in various ways. Kemmerer and Hallett (*4*) used an improved steam-generating unit, which was only a slight improvement over the original apparatus. Fife (*2*) placed a steam chamber between the steam generator and the distilling chamber. Allen (*1*) clamped his digestion flask to a steam generator. Redemann (*7*) modified Allen's design and adapted it to the semimicrodetermination. The one-piece, all-glass apparatus of Kirk (*5*) was a vast improvement in design. In place of a vacuum-insulated distilling chamber, he used a steam-jacketed chamber, the steam from which entered the distilling chamber and effected the distillation.

The apparatus described here is a modified form of the Kirk apparatus, simpler in construction and made more flexible by the use of standard taper joints.

The construction of the apparatus is apparent from Figure 1. The volume of the distilling chamber is large enough to permit vigorous distillation without frothing and mechanical movement of solution into bulb *A*.

Operation

The procedure follows the method given by Harte (*3*). A sample, sufficient to yield 2 to 5 mg. of nitrogen, is digested in a 100-cc. Kjeldahl flask with sulfuric acid and any of the well-known catalysts. When digestion is complete, the sample is cooled, diluted with 20 cc. of ammonia-free distilled water, and transferred to the distilling chamber, *A*, by means of the funnel, *B*. Both the Kjeldahl flask and the funnel are rinsed successively with three 5-cc. portions of water.

Stopcock *C* is closed and the condenser tube, *D*, is immersed in a known quantity of standard acid in a flask, *E* (not shown). Sodium hydroxide (50 per cent) is added to the sample in *A* through *B* and the funnel is again rinsed down with three 5-cc. portions of water. This is adequate to ensure complete removal of sample and caustic from the funnel and bulb, *A*, to the distilling chamber, *A*.

Although the apparatus has been found entirely satisfactory as described, a tip attached to funnel *B*, extending into bulb *A*, as discussed by Redemann (*7*), represents a possible modification.

The distilling tube, *A*, is now about one-third full. A 200-cc. round-bottomed flask, *F* (not shown), containing 100 cc. of ammonia-free distilled water, is connected to *G*. A flame is applied to *F* and the steam generated heats the jacket, *H*, preventing condensation in *A*, and enters the distilling chamber through tube *I*. This requires about 4 minutes' heating time. After allowing the distillation to proceed for 10 minutes, *E* is lowered and the distillation is continued for another 2 minutes to ensure complete rinsing of the condenser tube. The end of the condenser tube is then rinsed off with two 3-cc. portions of water and the flask is removed. The excess of acid is determined by titrating with standard alkali, using methyl red as the indicator. The flame is removed before the titration is made. As the generating flask, *F*, cools, the residue in *A* is withdrawn through *I*. Successive rinsings with distilled water, introduced at *B*, will be removed through *I* as *F* continues to cool.

Before using, the apparatus is steamed out thoroughly by distilling 100 cc. of water through it. As long as the flame is kept at *F* no sucking back is caused by contact of steam on tube *A* and walls of *G*.

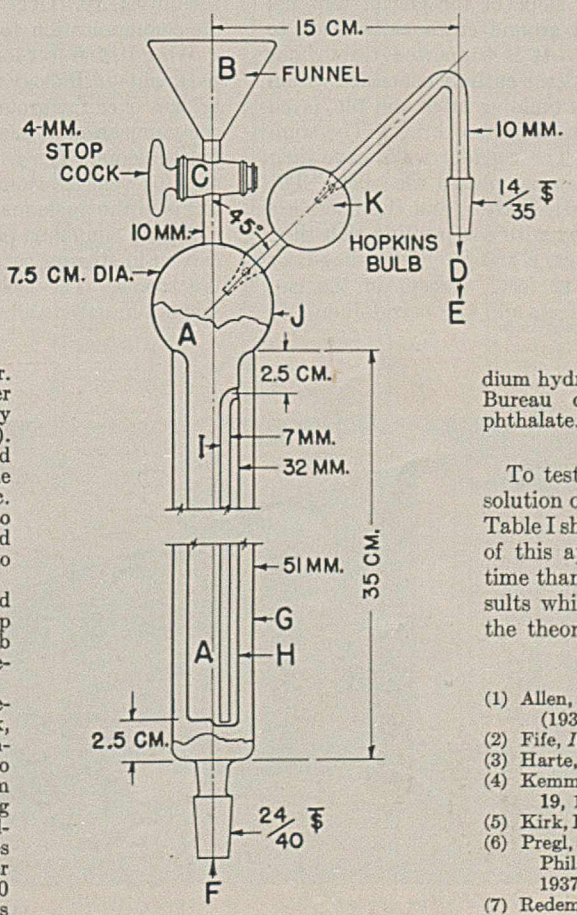

FIGURE 1. APPARATUS

TABLE I. DETERMINATION OF NITROGEN
(Nitrogen present, 4.087 mg.)

Time of Distillation	Nitrogen Found	Error		Deviation from Mean	
Min.	*Mg.*	*Mg.*	*%*	*Mg.*	*%*
10	4.085	−0.002	−0.05	±0.000	±0.00
	4.082	−0.005	−0.12	−0.003	−0.07
	4.088	+0.001	+0.02	+0.003	+0.07
	4.082	−0.005	−0.12	−0.003	−0.07
	4.090	+0.003	+0.07	+0.005	+0.12
	4.080	−0.007	−0.17	−0.005	−0.12
4	4.080	−0.007	−0.17	−0.005	−0.12
	4.082	−0.005	−0.12	−0.003	−0.07
	4.088	+0.001	+0.02	+0.003	+0.07
	4.090	+0.003	+0.07	+0.005	+0.12
Av.	4.085				

The complete outfit may be mounted on one ring stand and thus is completely portable. The total height of 95 cm. (38 inches), including the burner, is no great inconvenience and is compensated for by the simplicity of design and stability.

The water used was distilled off alkaline permanganate until it showed no ammonia by Nessler's reagent. The standard acid was prepared from constant-boiling hydrochloric acid. The standard alkali was prepared from 50 per cent sodium hydroxide and standardized against Bureau of Standards acid potassium phthalate.

Performance

To test the performance, a standard solution of ammonium sulfate was used. Table I shows the results obtained by use of this apparatus. Shorter periods of time than those recommended give results which are in good agreement with the theoretical figures.

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(4) Kemmerer and Hallett, IND. ENG. CHEM., 19, 1925 (1927).
(5) Kirk, P. L., *Ibid.*, Anal. Ed., 8, 223 (1936).
(6) Pregl, "Quantitative Micro Methods", Philadelphia, P. Blakiston's Son & Co., 1937.
(7) Redemann, IND. ENG. CHEM., Anal. Ed., 11, 635 (1939).

MODERN LABORATORIES

McGilvrey Hall at Kent State University

C. F. RUMOLD
Kent State University, Kent, Ohio

OF THE five state universities in Ohio, Kent State University is the unit for the northeastern section of the state. McGilvrey Hall is its new science building, constructed as a joint state and federal project begun late in 1939 and completed by November, 1940, at a cost of $1,153,386.

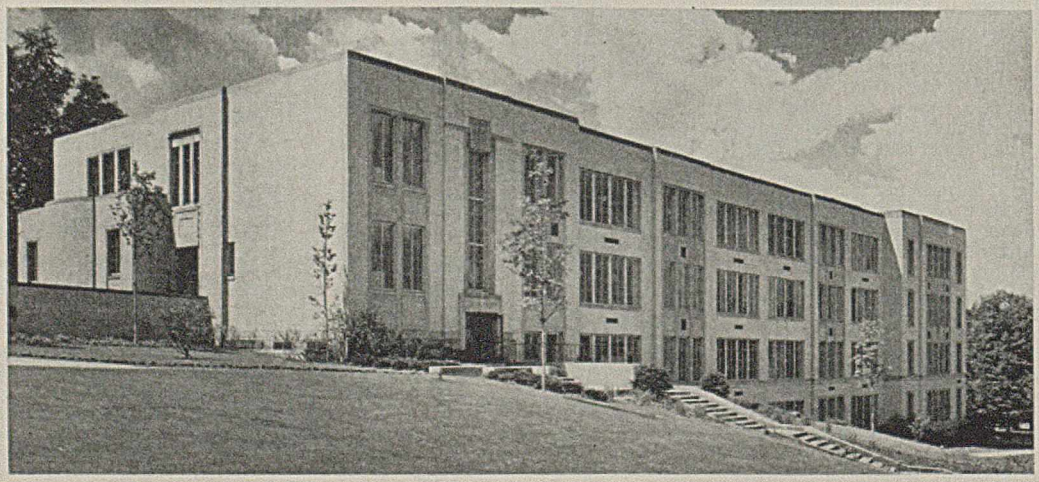
The building has four floors and is in the shape of an L. It is placed on the northerly slope of the glacial terminal moraine in such position that ground entrance is made to each of the first three floors. It is supported throughout by a heavy continuous steel skeleton entirely concealed within the masonry. The walls are of building brick and tile, faced on the outside with brownish-white glazed brick. The trimming is of native sandstone. The corridor walls have their lower two fifths faced with brownish glazed tile brick. The walls and ceilings are of acoustic plaster, and the floors are of heavy concrete base surfaced with composition slab tiles.

McGilvrey Hall has two wings, A and B. The ceiling-floor distance throughout each is 12 feet. Except in the end rooms, the room depth is 24 feet, and the corridors are 10 feet wide. The room partitions are of removable tile or light wood and plaster to admit of ready changes.

Wing A is 236 feet long and has end parts 74 feet wide and mid-bays 65 feet wide. It is used by the Chemistry and Physics Department. The easterly end is connected by a tunnel to Kent Hall to give access to all the main buildings of the university. Over the tunnel is a promenade giving open-air communication to the same buildings.

Wing B, 180 feet long and 65 feet wide, is used by the Departments of Biology and Geography. A connecting passage bridges over the ground floor at the north end and provides communication at each level between the second, third, and fourth floors. The ground space under the bridge affords access for vehicles from Lincoln Avenue to the loading platforms in the angle made by the back walls of the two wings.

All the plumbing, piping, and conduits are exposed and are carried in stirrups suspended 2 feet under the ceilings within the rooms. The heating and ventilating system is of the univent-in-outer-wall type, operated in conjunction with a



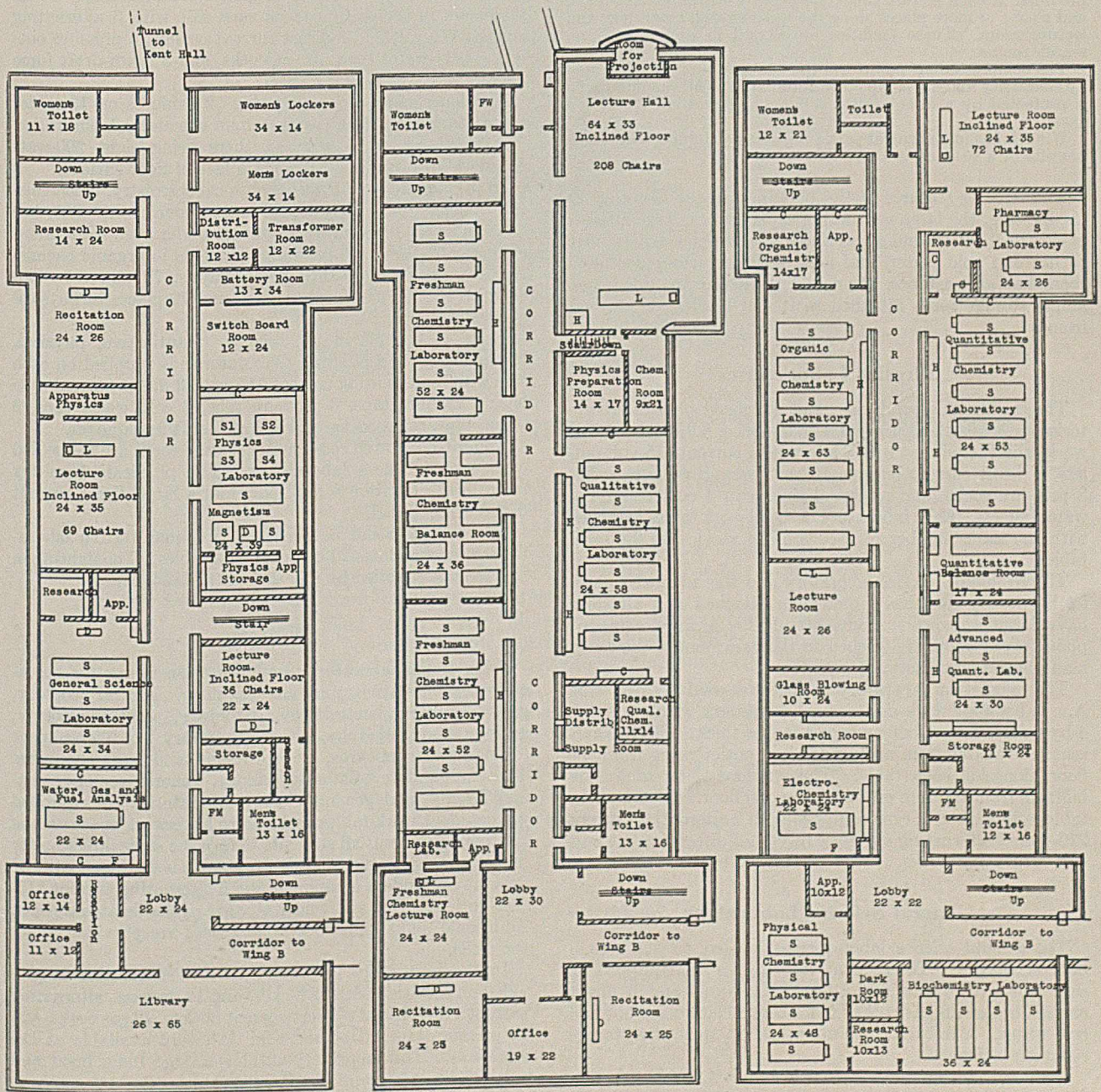
NORTH SIDE VIEW OF WING A, USED BY DEPARTMENTS OF CHEMISTRY AND PHYSICS

system of exhaust and blower fans located in a penthouse on the roof. Heat is supplied by steam from the university's central power plant. The lighting is on a liberal scale and is provided by an indirect system of lamps in white globes, using alternating current at 115 volts.

ELECTRIC POWER. Both alternating and direct current is distributed to all parts of the building. The alternating current is provided by a set of transformers taking energy from the outside commercial high-tension line and delivering it at 220 and 115 volts to a main panel which distributes current to unit distributing panels. The main distribution panel is of the enclosed safety type which permits access to any separate circuit only after it has been disconnected from its current source. Each circuit in the system is protected by an automatic cutout.

High-tension alternating current may be drawn at the main distributing panel and is led to the electrochemistry laboratory and to the advanced physics laboratory.

The direct current is furnished by two storage batteries of the lead accumulator type charged by a motor generator set. One battery delivers a maximum voltage of 220 volts and the other a maximum voltage of 110 volts. Each battery delivers current to the same common distribution panel, from which any desired voltages can be delivered to unit panels in the different



PLANS OF SECOND, THIRD, AND FOURTH FLOORS OF MCGILVREY HALL, WING A

C. Storage cupboard
D. Dumb-waiter
F. Focault pendulum shaft
FM. Faculty men's toilet
FW. Faculty women's toilet
H. Fume hood
L. Lecture table
S. Student work table

rooms, permitting wide ranges of voltages and currents to the terminals on desks and work tables.

DISTILLED WATER. Distilled water is furnished by two completely automatic Barnstead stills, operated with either steam or gas, which have capacities of 5 gallons per hour for wing A and 2 gallons per hour for wing B. These stills are located in penthouses. The distilled water is distributed by gravity through aluminum pipes to one point in each laboratory.

HYDROGEN SULFIDE. Hydrogen sulfide is delivered from a gasometer in the penthouse on the roof of wing A to each hood compartment in the two freshman chemistry laboratories and in the qualitative chemistry laboratory through rubber-fabric pipes. The gasometer is charged from commercial cylinders.

ELEVATOR. The elevator, located in wing A, is an Otis automatic electric type. Access to the carriage is had from the corridor and from the storage room on each floor.

GENERAL FACILITIES. Alternating and direct current, gas, air pressure at 15 pounds, vacuum, and hot and cold water are provided at each lecture table, student's work table, and hood, and at one or more places along the walls of each laboratory and lecture room. These facilities are liberal in each of the research rooms.

The compressed air pump and the vacuum pump are operated by electricity and are completely automatic. The vacuum pump is protected by a filter against solids and corrosive liquids and gases.

Wing A has in its central part a Focault pendulum shaft which permits of a suspension of 80 feet in the clear.

The striking features of the building are the liberality of the lighting, the large ventilating capacity, the large number of electrical outlets and of gas cocks, and the easy accessibility to hot and cold water, and each laboratory and recitation room has at least one commodious research room and an ample storage room attached to it and leading directly off from it.

Physics Laboratories

The freshman, second year, and advanced physics laboratories are located on the ground floor of wing A. Each student work table is equipped with 8 alternating current 115-volt outlets, 8 direct current outlets, 16 gas cocks, 8 vacuum cocks, 8 pressure cocks, and a central sink with 4 cold and 2 hot water faucets. Each laboratory has wall work tables equipped with the same number of each kind of service as the main tables.

The freshman physics laboratory and the advanced physics laboratory each has a darkroom attached and all three have large apparatus rooms attached. A fully equipped photography room is attached to the darkroom of the freshman physics laboratory.

The magnetism laboratory is on the second floor of wing A. It has four work desks of solid masonry without metal, 4 by 6 feet, with Alberene tops 4 inches thick. Electric current is available from an independent outlet rising from the floor alongside each table. These nonmagnetic tables are built up from the firm ground under the floor. Each student table in this laboratory is equipped with 2 alternating current 220-volt, 2 alternating current 115-volt, 2 direct current outlets, and 2 gas cocks.

General Science Laboratory

The general science laboratory is located on the second floor of wing A. The student work tables are equipped on each side with 6 alternating current 115-volt and 6 direct current outlets, 6 gas cocks, and a central sink with hot and cold water. A research laboratory and an apparatus storage room are attached.

Chemistry Laboratories

Each laboratory has liberal hood facilities along the inner walls and on the work tables. All hoods are provided with liberal connections for alternating and direct current, gas, hydrogen sulfide, distilled water, and hot and cold water, and with sinks between the hood compartments. One or two showers in each laboratory are conveniently located for use in case of accidental catching fire of clothing. The lighting in the laboratories is on an unusually liberal scale and is brilliant, one 200-watt 115-volt Mazda lamp per 83 square feet of floor surface, approximately throughout. All drawers and lockers have a master-keyed system of locks for use by the department when the places are not assigned to students; also hasps for student use with locks of their own purchase.

Each student table in the two freshman laboratories and in the qualitative chemistry laboratory has on each side 40 drawers for equipment, giving 960 work places for students in freshman chemistry and 480 in qualitative chemistry. These tables are further equipped on each side with 5 alternating current 115-volt and 5 direct current outlets, 5 pressure outlets, 5 cold water taps, 10 gas cocks, and 3 down-draft fume hoods.

A balance room accommodating 52 balances on 13 tables is located between the two freshman chemistry laboratories. The lighting here is brilliant, there being eight 200-watt Mazda lamps over the 840 square feet of floor surface.

In the organic, quantitative, physical chemistry, biochemistry, and pharmacy laboratories each student work table has on each side 9 drawers with a locker under each for student equipment, giving 126 places for students in organic chemistry, 162 places in quantitative analysis, 72 places in biochemistry, 36 places in pharmacy, and 72 places in physical chemistry.

Each work table in the organic, quantitative, physical, biochemistry, and pharmacy laboratories is supplied on each side with 3 alternating current 115-volt, 3 direct current outlets, 3 vacuum taps, 3 pressure taps, 3 cold water taps, 3 steam taps, 6 gas cocks, and 3 down-draft fume hoods.

An ample research room and a storage room are connected with each of these laboratories. The physical chemistry room has in addition a darkroom and a large direct current distributing panel.

The balance room between the two quantitative laboratories accommodates 24 balances on 6 tables. The lighting in this room is supplied by four 200-watt Mazda lamps.

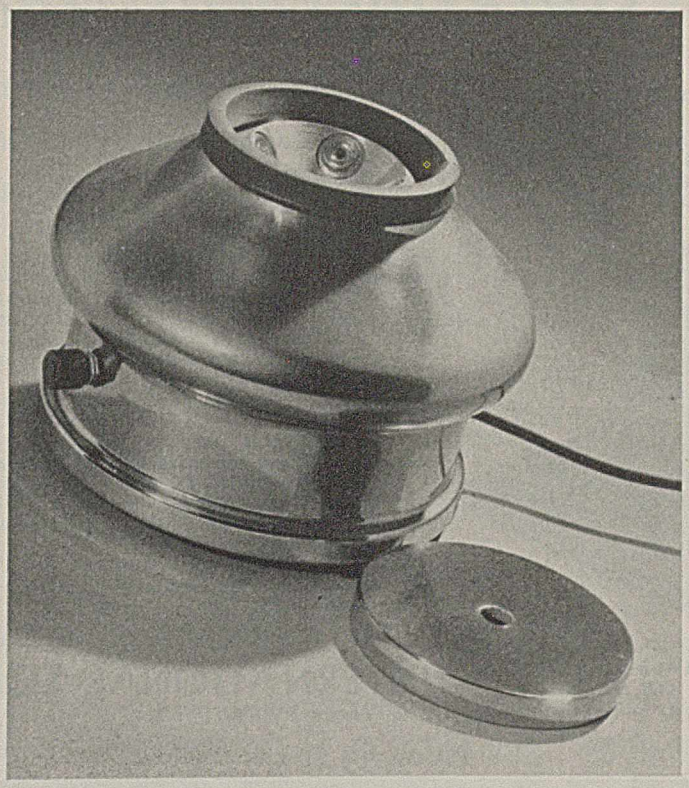
Wing B

Wing B has laboratories and recitation rooms for physiology, comparative anatomy, ecology, general zoology, general botany, bacteriology, pathology, plant physiology, geology, and geography. Each laboratory has liberal space for side-wall cupboards, storage rooms, and research rooms. The comparative anatomy, ecology, general zoology, general botany, and general biology laboratories are equipped with student work tables designed to have work space on one side only, to permit all students to face the same direction for instructional purposes. Each work table is equipped with 24 drawers for student equipment and 6 alternating current 115-volt outlets. Hot and cold water are available at end sinks. Additional sinks and work-table spaces are provided along the walls.

In the physiology laboratory each student table is provided on each side with 12 lockers and 12 drawers, alternating current 115-volt and 3 direct current outlets, 12 gas cocks, and 12 pressure taps. Hot and cold water are available at the end sinks. This laboratory also has a large fume hood and a large direct current distribution panel.

Each student work table in the bacteriology laboratory has 24 drawers on each side, 8 gas cocks, 4 cold water taps, and 4 electrical outlets. Hot and cold water are available at the end sinks.

A NEW SEMI-MICRO CENTRIFUGE



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Amperometric Determination of Cobalt

REAGENT—α-Nitroso-β-naphthol

METHOD—Amperometric titration

REFERENCE—Kolthoff and Langer, *J. Amer. Chem. Soc.*, *62*, 3172 (1940)

COBALT may be determined rapidly and accurately by amperometric titration with α-nitroso-β-naphthol, using the dropping mercury electrode as indicator electrode. An accuracy of 0.5% or better is possible when the quantity of cobalt is of the order of 1 to 20 mg. The titration is best carried out in an acetate buffer. *Eastman P 428 α-Nitroso-β-naphthol* is of a purity that is suitable for this procedure.

Write for an abstract of the article in which the amperometric determination of cobalt is described. . . . Eastman Kodak Company, Chemical Sales Division, Rochester, N. Y.

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