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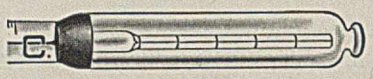

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Patent Applied for

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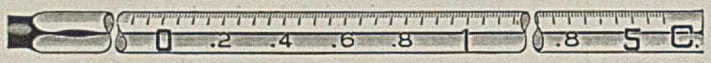

Obviously, the predominant advantage of a differential thermometer lies in the fact that the 5° interval in the region of the anticipated temperature is greatly elongated.

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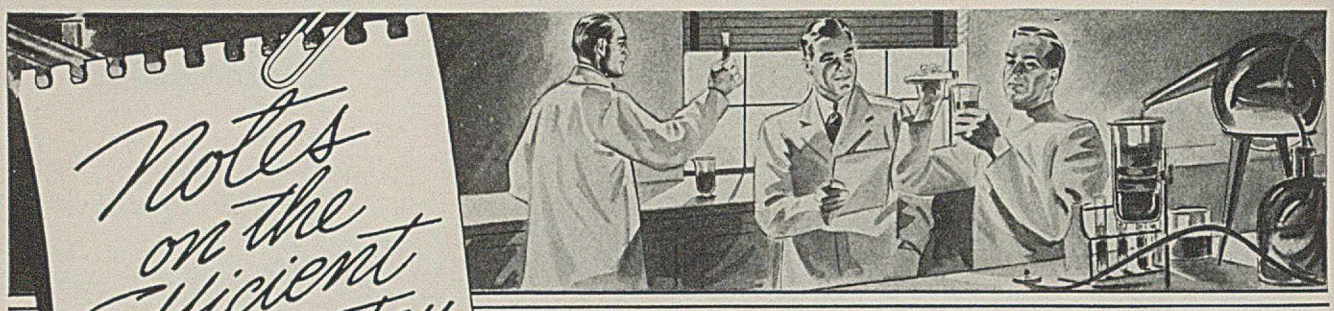

Prepared in cooperation with the technical and research staff of NORTON COMPANY, Worcester, Mass.

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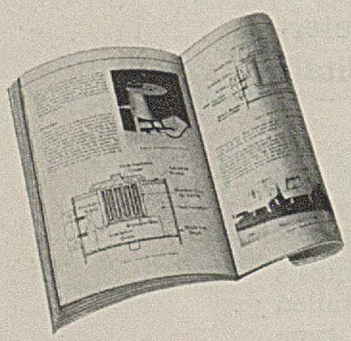

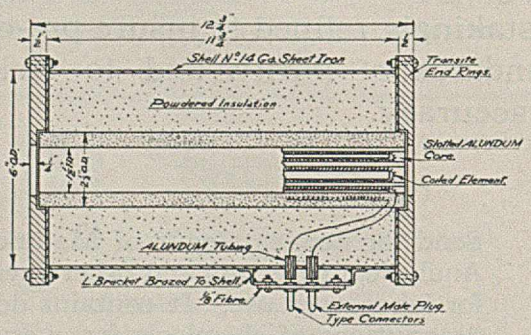

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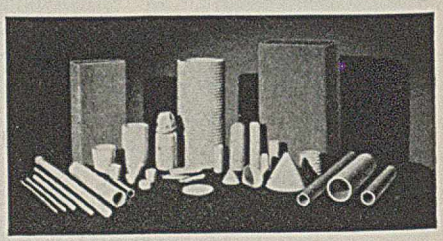

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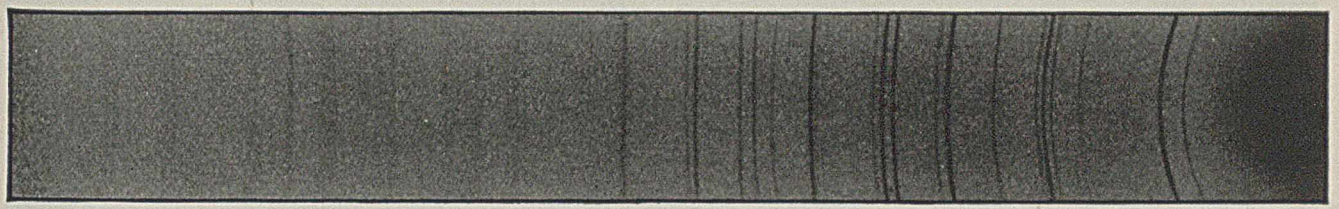

Pattern B

G-E XRD Unit successfully analyzes two different chemical compounds which have identical chemical formulae

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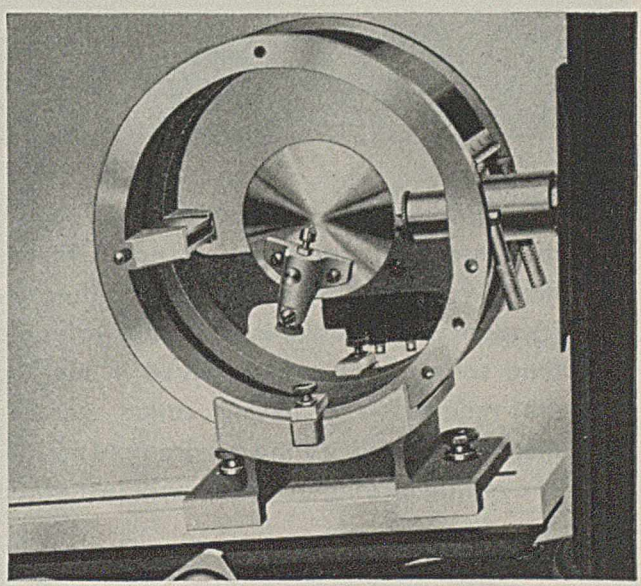

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INDUSTRIAL AND ENGINEERING CHEMISTRY

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Determination of Unsaturation in the Terpene Series

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The usual halogen absorption methods as well as those using standard potassium permanganate or standard perbenzoic acid were found unsatisfactory for the determination of unsaturation in terpenes. Quantitative hydrogenation using either a platinum or a palladium catalyst furnished satisfactory results with a variety of terpenes, but not with the resin acids. High-pressure reduction was also shown to be a suitable quantitative method for terpenes.

THE quantitative determination of unsaturation in the terpene series presents a rather different problem from that met with in determining unsaturation in most other classes of compounds. Because of the ease with which these compounds undergo substitution and isomerization, the usual halogen absorption methods are of very little use. Particularly with the Hanus method (*9*) it was noticed that the value obtained for α-pinene varied regularly with the size of the sample used. When the amount of reagent was kept constant, the smaller samples yielded higher values (Figure 1).

Other halogen absorption methods, some specifically claiming to eliminate substitution, such as a modified Hanus method using mercuric acetate (*8*), the pyridine–bromine–sulfuric acid reagent of Rosenmund and Kuhnhenn (*15*), and the Kaufmann method (*10*), were likewise found to be unsatisfactory. Similar observations have been recorded previously: Kubelka and Zuravlev found the iodine number of pinene and turpentine as determined by the Hanus and two other halogen absorption methods to be dependent upon the weight of sample (*13*) and the time of reaction (*14*); Kranz, Hrach, and Franta (*12*) reported that the iodine number of turpentine increased with increasing concentration of the iodine solution; Gal'pern and Vinogradova (*6*) obtained high values for pinene using the Kaufmann method; and Winkler (*20*) using a bromine–acetic acid solution found almost identical values for α-pinene and *l*-limonene which agreed with the theoretical value of neither.

Two methods not involving halogen absorption—namely, the use of a standard permanganate solution (*11*) and of a standard perbenzoic acid solution—were equally ineffective in solving the problem. The dependence of the double bond value of α- and β-pinene upon the ratio of the amount of reagent to the size of the sample taken, noted with the Hanus method, was again observed when perbenzoic acid was used (Figure 1), suggesting that the abnormal reaction occurred during the back-titration of the iodine liberated by the excess perbenzoic acid rather than during the original reaction of the perbenzoic acid with the terpene.

Quantitative hydrogenation has been used to determine unsaturation in several terpenes by Shaefer (*18*) using a palladium hydroxide catalyst, and a micromethod has been reported by Skarblom and Linder (*19*). The present authors found this general method, modified as described below, to be the most useful one investigated.

Shaefer (*18*) reported difficulty in effecting the addition of more than one mole of hydrogen to dipentene and Conant and Carlson (*4*) found the hydrogenation of this compound to stop after the absorption of about 1.6 moles. We found that dipentene recently fractionated through a good column readily absorbed the calculated amount of hydrogen, whereas low results were obtained with material purified merely by distillation over sodium.

Under the conditions used the method was not satisfactory for the quantitative determination of unsaturation in the

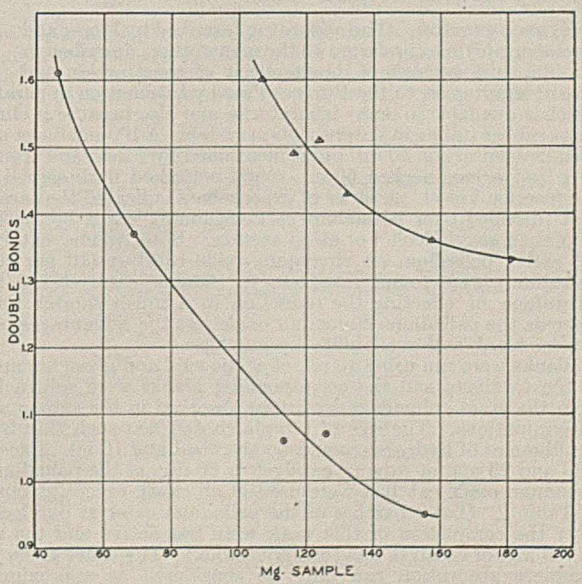

FIGURE 1. DOUBLE BOND VALUES FOR α-PINENE
⊙ Hanus method
△ Perbenzoic acid

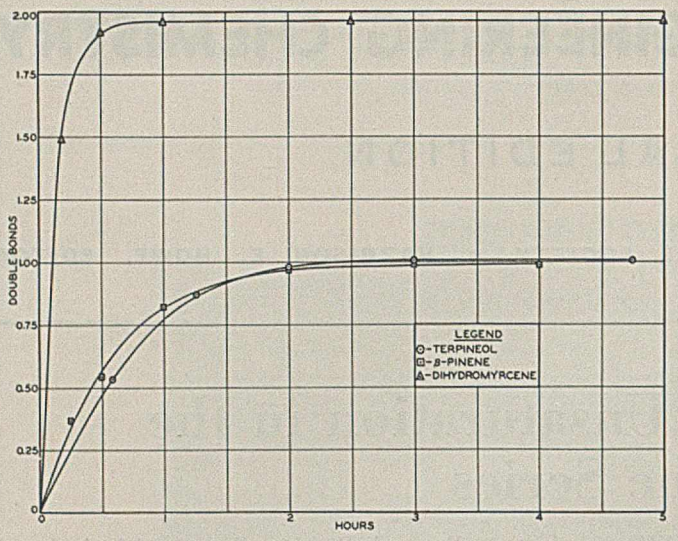

FIGURE 2. HYDROGENATIONS USING PALLADIUM–ZIRCONIUM OXIDE CATALYST

TABLE I. HYDROGENATION RESULTS

Compound[a]	Palladium–Zirconium Oxide: Double bonds	Palladium–Zirconium Oxide: Time, hours	Adams' Catalyst: Double bonds	Adams' Catalyst: Time, hours
α-Pinene (1)	0.99	3	1.03	0.5
β-Pinene (1)	1.00	3	0.99	0.25
Terpineol (1)	1.01	3	1.00	0.5
Pinocarveol (1)	0.94	1	0.99	0.25
Dipentene (2)	1.90	1.5	1.99	0.5
Dihydromyrcene (2)	1.96	1	1.95	0.3
Myrcene (3)	2.98	1	2.93	0.2
Alloöcimene (3)	2.91	1	2.90	0.2
Abietic acid (2)	1.67[b]	23	1.68[c]	23
l-Pimaric acid (2)	..	..	1.84[d]	47
Camphor (0)	0.00	1.5	0.02	3
Fenchone (0)	0.02	2	0.01	2
Borneol (0)	0.02	23	0.00	1
Fenchyl alcohol (0)	0.00	6	0.01	2
Dehydroabietic acid (one aromatic ring)	0.02	21	0.06	1.5

[a] Number in parentheses following name of compound indicates number of double bonds present.
[b] 100 mg. of catalyst used initially and 50 mg. more added after 3 hours. At this time 1.57 moles of hydrogen had already been absorbed.
[c] 50 mg. of catalyst used initially and 25 mg. more added after 2 hours. Hydrogen equivalent to 1.63 moles absorbed during first hour.
[d] Total of 200 mg. of catalyst added in 50-mg. portions used. 1.58 moles of hydrogen absorbed in first hour.

resin acids as exemplified by abietic and *l*-pimaric acids, since in these cases, despite the addition of fresh catalyst, the reduction became extremely slow after the initial rapid absorption of hydrogen corresponding to about 1.6 double bonds. It has been shown that it is possible to introduce two moles of hydrogen into these acids using elevated temperatures and with repeated reactivation of the catalyst (*16*, *17*).

Experiments with terpene alcohols and ketones possessing no double bonds showed that they were not attacked. In a like manner dehydroabietic acid, which contains an aromatic ring, remained unaffected under the conditions used.

Exploratory experiments showed that high-pressure hydrogenation using Raney nickel catalyst was also suitable for the determination of unsaturation in terpenes.

Experimental

All the compounds used were freshly distilled or freshly crystallized and had physical constants closely agreeing with the accepted values.

HYDROGENATION. The apparatus described by Fieser and Hershberg (*5*) was preferred to the many others described in the literature because of its simplicity of construction and ease of adaptation to the Burgess-Parr hydrogenation apparatus which is available in many laboratories and also because it eliminates rubber tubing in the absorption system. A 100-ml. buret was used instead of the 50-ml. buret mentioned by Fieser and Hershberg and a long-necked 50-ml. round-bottomed flask served as the reaction vessel. A series of experiments indicated that acetic acid (distilled over potassium permanganate) was a better solvent than ethyl alcohol or ethyl acetate. Both Adams' catalyst (*1*) and a palladium on zirconium oxide catalyst (10 per cent palladium) were found satisfactory. Adams' catalyst has the advantage of effecting the reduction in a much shorter time, whereas the palladium–zirconium oxide has the advantages of a smaller blank and availability by purchase.

Blanks were run using 10 ml. of acetic acid and given amounts of the catalysts and the corresponding blanks were subtracted from the gross volumes of hydrogen absorbed in the subsequent determinations. The size of sample chosen was such that 3 or 4 millimoles of hydrogen would be absorbed and 10 ml. of acetic acid and 25 mg. of Adams' catalyst or 50 mg. of the palladium–zirconium oxide catalyst were used in all cases, except as noted in Table I. (Later batches of the palladium catalyst purchased after the completion of this work were less active and the use of 100 mg. of catalyst was then advisable.) The times given for complete absorption can only be considered as approximate figures, and the shaking was of course always continued for some time longer to make certain that the absorption of hydrogen had definitely stopped. Some of the values obtained are given in

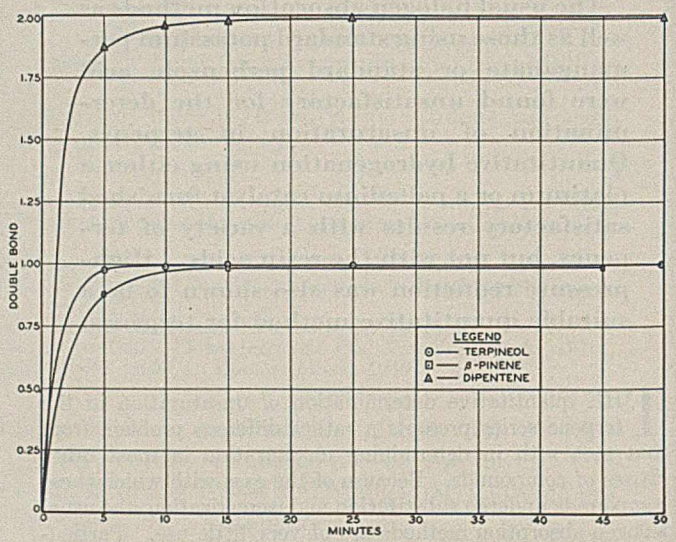

FIGURE 3. HYDROGENATIONS USING ADAMS' CATALYST

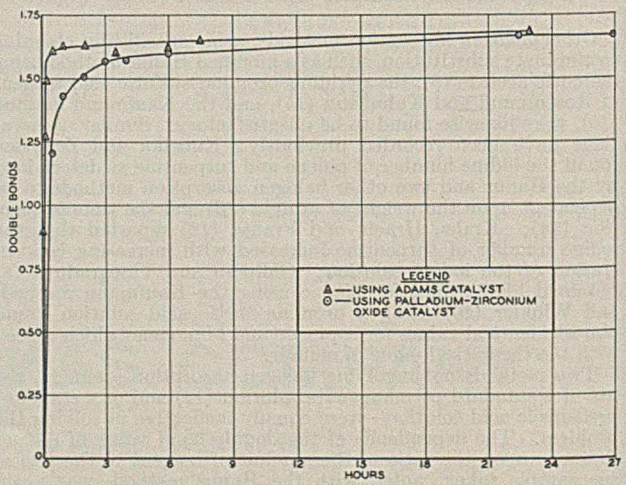

FIGURE 4. HYDROGENATION OF ABIETIC ACID

Table II for comparison with the results obtained by the other methods and a more complete list of results is compiled in Table I. Some typical rates of hydrogenation curves are shown in Figures 2, 3, and 4.

PERBENZOIC ACID TITRATIONS. Perbenzoic acid was readily and reproducibly prepared in good yield by the method of Braun (3) using benzoyl peroxide. One to 1.5 milliequivalents of the terpene were dissolved in 10.00 ml. of a dried, approximately 0.5 *N* solution of perbenzoic acid in chloroform and allowed to stand at 5° for 24 hours and then the excess perbenzoic acid was titrated in the usual manner (3). Blanks were simultaneously run on 10.00-ml. portions of the reagent. Other samples were allowed to stand at 5° from 48 to 96 hours, but the results showed clearly that in all cases the reaction was over at the end of 24 hours.

The other determinations, results of which are indicated in Table II, were carried out as described in the literature.

HIGH-PRESSURE HYDROGENATION. A bomb with a total void of 183 ml. was used, but the use of a glass liner (7) reduced the void to 142 ml. Calibration of this bomb and liner using acetone made up to a volume of 40 ml. with alcohol showed the pressure drop to be 3800 pounds per mole of hydrogen absorbed. Reduction of 17.09 grams of α-pinene made up to 40 ml. with alcohol was complete in about 12 hours at 75° (initial pressure 1670 pounds at room temperature). The pressure-drop was 480 pounds, which corresponds to 1.01 moles of hydrogen per mole of α-pinene. A similar experiment with β-pinene gave a value of 1.06 double bonds. Raney nickel catalyst (2) was used in all these experiments.

TABLE II. COMPARISON OF METHODS INVESTIGATED[a]

Method	α-Pinene (1)	β-Pinene (1)
Hanus	0.95–1.61[b]	1.12
Hanus + mercuric acetate	1.25	1.42
Rosenmund and Kuhnhenn	1.70	1.65
Kaufmann[c]	2.15	2.02
Potassium permanganate	0.90	1.23
Perbenzoic acid[d]	1.33–1.60[b]	1.37–1.63[b]
Hydrogenation (Pd)	0.99	1.00
Hydrogenation (Pt)	1.03	1.05

[a] Results are expressed as number of double bonds found per molecule, and the number in parentheses following name of terpene indicates number of bonds theoretically present.

[b] Depending upon size of sample. Other figures represent averages of check analyses.

[c] Kaufmann method gave a value of 2.15 double bonds for alloöcimene (3) and 1.99 for dipentene (2).

[d] Perbenzoic acid also gave following results: myrtenol (1), 0.89; pinocarveol (1), 1.23; myrcene (3), 2.17; alloöcimene (3), 2.35; dihydromyrcene (2), 2.10; dipentene (2), 2.03; terpineol (1), 0.87.

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Colorimetric Determination of Formaldehyde in the Presence of Other Aldehydes

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THE method of detecting formaldehyde in the presence of higher aldehydes suggested by Denigès (3) can be made semiquantitative in character with the aid of a colorimeter. The errors involved vary from 2 to 10 per cent, becoming greater as the proportions of higher aldehydes are increased. The limit of sensitivity under the experimental conditions described herewith is of the order of 0.02 mg. of formaldehyde in 5 ml. of solution. The test depends upon the fact that the magenta color given by Schiff's reagent with formaldehyde in the presence of sulfuric acid does not fade appreciably during 6 hours, whereas the color given by the higher straight-chain aldehydes, glyoxals, and their polymers fades completely within 2 hours. Trioxymethylene reacts the same as formaldehyde.

The reagent is prepared by first dissolving 0.5 gram of fuchsin in 500 ml. of water, then adding 5.15 grams of sodium bisulfite. Approximately 15 minutes later, 17 ml. of 6 *N* hydrochloric acid are added and the whole solution is allowed to stand for 3 hours. During this time the solution fades to a permanent, pale yellow color.

In a determination, 5 ml. of an aqueous solution of the substance to be analyzed are added to a mixture of 5 ml. of the Schiff's reagent and 1.2 ml. of 75 per cent sulfuric acid. A known comparison solution is made up at the same time using a standard formaldehyde solution and the two solutions are compared after they have stood for 2 hours in stoppered test tubes. Too long a time should not be allowed to elapse before the comparison is made, for even the color due to formaldehyde fades slightly on standing. Before results are considered final, the formaldehyde concentrations of the unknown and standard solutions should be within 5 per cent of each other. Accordingly, an ap-

TABLE I. TYPICAL ANALYTICAL DATA AND RESULTS

Compositions of Standard Comparison Solutions: Per cent H_2CO	Compositions of Standard Comparison Solutions: Ratio of second aldehyde to H_2CO	H_2CO in Unknown: Present	H_2CO in Unknown: Determined	Ratio of Second Aldehyde to H_2CO in Unknown	Error
		%	%		%
Formaldehyde Solutions					
0.0050	..	0.0067	0.0068	..	+1.5
0.0050	..	0.0050	0.0050	..	0.0
0.0040	..	0.0040	0.0039	..	−2.5
0.0015	..	0.0020	0.0019	..	−5.0
Formaldehyde-Acetaldehyde Solutions					
0.0040	50	0.0025	0.0024	40	−4.0
0.0040	50	0.0045	0.0048	20	+6.7
0.0040	5	0.0070	0.0068	3	−2.9
0.0040	10	0.021	0.020	10	−5.0
Formaldehyde-Propionaldehyde Solutions					
0.0040	25	0.0043	0.0040	16	−7.0
0.0040	25	0.0033	0.0031	20	−6.0

proximate calculation of the unknown is made first. If the two solutions differ by more than 5 per cent, one or the other is diluted sufficiently to satisfy this requirement and a new colorimetric comparison is made. Sometimes a third comparison is necessary. The concentration range for the most satisfactory colorimetric comparison is from 0.001 to 0.005 per cent formaldehyde.

Aqueous solutions of pure formaldehyde may be analyzed to a degree of accuracy dependent upon the colorimeter—i. e., within 2 or 3 per cent. However, other aldehydes tend to change the color and solutions containing appreciable quantities of other aldehydes may not be accurately compared with pure formaldehyde standards. In such cases the standard should be made up to contain the higher aldehydes in concentrations approximating those considered to be in the unknown. For example, in solutions containing acetaldehyde and formaldehyde, if the ratio of acetaldehyde to formaldehyde is between 10 and 100 the standard should be made up with a ratio near 50; if between 1 and 10 the standard ratio should be about 5; and if the ratio is less than 1 a pure formaldehyde standard may be used.

The method has been tested on mixtures of formaldehyde with acetaldehyde, propionaldehyde, glyoxal, methylglyoxal, biacetyl, and their polymers. It has been used satisfactorily in photochemical studies of acetaldehyde (*2*) and propionaldehyde. Table I contains a few typical analytical results obtained on unknown solutions.

In addition to the results described above, the Denigès qualitative method works very well in colorimetric capillaries (*1*). In such experiments the limit of sensitivity is about 0.02 microgram in 5 cu. mm. of solution.

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Determination of Sulfur in Organic Compounds

Oxidation of Sulfur of Cystine and Methionine, Combination of Parr Oxygen Bomb and Acidimetric Benzidine Method, and Determination of Small Amounts of Sulfur Compound Present as Contaminant in Organic Material

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IN CONNECTION with investigations concerned with the sulfur-containing amino acids and their derivatives, data pertaining to various analytical problems have accumulated in this laboratory. The material set forth below deals chiefly with the behavior of the sulfur present in the structure of cystine and methionine on oxidation by alkaline permanganate and by other wet-oxidation methods, the development of a practical analytical procedure in which combustion of the organic substance in the Parr oxygen bomb is followed by precipitation of sulfate as the benzidine salt and acidimetric determination of the latter, the effect of certain inorganic substances in this procedure and an investigation of its accuracy when applied to the determination of small amounts of organic sulfur in the presence of large amounts of sulfur-free material.

Alkaline Permanganate Method

The method as used by the authors is based on that reported by Blix (*2*), who showed that the sulfur of cystine was recovered with an accuracy of 98.9 ± 0.6 per cent when the substance was oxidized by an alkaline permanganate solution, the excess permanganate was reduced to manganous ion by hydrochloric acid, and the sulfate was determined as barium sulfate. The authors have compared this procedure with the alternate possibility of reducing the permanganate to manganese dioxide which is filtered off before precipitation of barium sulfate.

Twenty-five cubic centimeter portions of a solution of 1.6132 grams of l-cystine [which optical rotation (*18*) showed to be at least 99.7 per cent pure] and 50 millimoles of hydrochloric acid in a total volume of 250 cc. were analyzed as follows.

a. The sample of solution was heated for 2 hours on the steam bath with 30 cc. of 2 *M* sodium hydroxide and 1.50 grams of potassium permanganate (both of reagent grade) in a total volume of 150 cc. Excess permanganate was then decomposed by addition of 25 cc. of 7 *M* hydrochloric acid and gentle boiling. After partial neutralization (just acid to methyl orange) with sodium hydroxide, sulfate was precipitated by 35 cc. of a 0.05 *M* barium chloride solution and, after leaving on the warm steam bath overnight, filtered, ignited, and weighed.

b. After oxidation as under (*a*) a few cubic centimeters of methanol were carefully added to the hot solution, causing the formation of manganese dioxide and a colorless solution. After addition of 20 cc. of 2 *M* hydrochloric acid, which causes conversion of the manganese dioxide into a more easily filterable condition, the manganese dioxide was at once filtered and washed with hot water (containing about 1 per cent ammonium acetate to prevent colloidal passage of manganese dioxide) until a sample of the filtrate showed absence of chloride ion (about 8 washings). In the combined filtrate and washings sulfate was determined, after adjustment of the acidity, as under (*a*).

c. The determination was carried out as under (*b*) except that 4.5 grams of potassium permanganate were used. In the determination of the sulfate blank attributable to the potassium permanganate the procedure was similar to (*b*), except that cystine was omitted and 10.0 grams of potassium permanganate were used.

The results were as follows: sulfur found in potassium permanganate, 0.0176 and 0.0163 per cent. The sulfur content of the cystine was found, after deduction of the corresponding permanganate blanks, as 27.01 and 26.61 per cent according to procedure (*a*), 26.41 and 26.50 according to (*b*), and 26.51 and 26.71 according to (*c*). The theoretical value is 26.67 per cent. The mean value of procedures (*b*) and (*c*) corresponds to 99.5 ± 0.3 per cent of the theoretical, while procedure (*a*) gave 100.5 ± 0.8 per cent. In further work the procedure involving filtration of manganese dioxide was adopted because of its higher precision and accuracy, and because the color of the barium sulfate obtained in procedure (*a*) indicated the presence of manganese.

d. A semimicromodification (about 50 mg. of substance, 100 mg. of barium sulfate) which the authors have frequently used for the determination of sulfur in certain oxidation products of cystine (*15*) is as follows:

A 50-mg. sample of the substance, weighed into a 125-cc. Erlenmeyer flask to the nearest 0.05 mg., is dissolved in about 50 cc. of a solution containing 10 millimoles of sodium hydroxide and 0.50 gram of potassium permanganate. After heating the solution for 2 hours on the steam bath, 1.5 cc. of methanol are added, followed by 10 cc. of 2 *M* hydrochloric acid when reduction of the permanganate is completed. The precipitate is at once filtered and washed until free of chloride. In this case, where filtration is carried out at an acid reaction, addition of ammonium acetate to the wash water [see (*b*), above] proved to be unneces-

sary. The filtrates amounting to about 200 cc. are evaporated to about 100 cc. and, if the reaction is acid to methyl orange, precipitated with 15 cc. of 0.05 *M* barium chloride. The barium sulfate is isolated as usual and weighed with the same precision as the sample.

The percentage differences found between several duplicate determinations run in this way (*d*) on unknown substances were 0.04, 0.15, 0.59, and 0.08. A pair of determinations on l-cystine (the same material as above) gave 26.32 and 26.26, or a difference of 0.23 per cent and a mean value of 98.6 per cent of the theoretical. The mean precision in this modification is about 0.1 per cent, while the accuracy may be about 99 per cent.

The alkaline permanganate method has further given correct sulfur figures on cystine disulfoxide (*19*) and cysteine sulfinic acid (*6*), and it has been found convenient and productive of concordant results in the determination of the sulfur content of wool and of mixed copper salts obtained from wool hydrolyzates (*16*).

In the case of wool, 1-gram samples, 10 grams of permanganate, and 160 millimoles of sodium hydroxide in 150-cc. total volume were used, and 48 hours of heating on the steam bath were allowed to be certain of complete digestion of the wool. After filtration of the manganese dioxide and acidification some insoluble silica was filtered off before precipitation of barium sulfate. The results were 3.212 and 3.223 per cent of sulfur. In the case of the copper salts a 0.1- to 0.15-gram sample was dissolved in 20 cc. of 0.5 *M* hydrochloric acid and cupric ion was precipitated from the hot solution by hydrogen sulfide. The copper sulfide was washed with 0.005 *M* perchloric acid until free of chloride, the total filtrate (about 80 cc.) freed of hydrogen sulfide by passing a stream of carbon dioxide through it, and it was then subjected to alkaline permanganate oxidation in the manner outlined under (*b*). The differences within five pairs of determinations averaged 1.2 ± 0.7 per cent of the mean value of each pair.

Permanganate Oxidation of Methionine

An obvious advantage of the permanganate method is its direct applicability to dilute aqueous solutions. A sulfur determination method possessing this advantage would be of great value, for instance, in tracing the fate of the sulfur-containing amino acids in the fractionation of protein hydrolyzates by various chemical methods. It was important, therefore, to ascertain the response of methionine to permanganate oxidation, especially since under various other oxidative conditions methionine sulfur has revealed unusual resistance against complete degradation (*14*, p. 343). The results with permanganate were interesting. Two oxidations carried out according to method (*d*), except that all quantities were doubled, produced no measurable amount of barium sulfate. The following experiments make it probable that this result is caused by volatilization of sulfur-containing fragments (methyl sulfide, etc.).

One of the solutions to which barium chloride had been added was evaporated to dryness and redissolved, and a fraction of the solution was treated with aqua regia and magnesium chloride, as described in the next section. No evidence of the formation of sulfate by this oxidative treatment, which as shown below produces 80 to 90 per cent of the theoretical value when applied to methionine directly, could be obtained. The possibility of obtaining a different kind of oxidative cleavage of methionine by permanganate in acid solution was next examined. The procedure was as in the alkaline oxidation, except that 10 millimoles of hydrochloric acid instead of 20 millimoles of sodium hydroxide were used at the start. After heating on the steam bath, one sample for 0.75 hour and another for 1.5 hours, 15 cc. of 2 *M* sodium hydroxide were added and heating was continued for 2 hours, and after adding more permanganate (25 per cent of the original amount, since no unused excess was left) for a further 2 hours. The samples were then worked up as usual; however, no barium sulfate was obtained. Likewise, subsequent oxidation by aqua regia, in the manner applied following the direct alkaline oxidation, produced no sulfate whatever.

The quantitative results obtained by the alkaline permanganate oxidation on cystine and methionine (all of the sulfur and none of it, respectively, oxidized to sulfate) suggest the possibility of utilizing this behavior for the differential determination of methionine and cystine sulfur. If the observed distinction holds true when the compounds are present as parts of a protein structure, the difference between total sulfur and "permanganate sulfur" should correspond to methionine sulfur if no other sulfur is present. Preliminary experiments carried out on egg albumin, in which total sulfur was determined by the bomb method (see below) and the subfractions (inorganic, cystine, and methionine sulfur) according to Kassell and Brand (*5*), while at the same time oxidations with alkaline permanganate were run, showed the expected agreement between the methionine values within reasonable limits. However, no comprehensive comparisons have as yet been run to establish the general validity of the principle or its value compared with the differential oxidation method of Lugg (*8*).

Behavior of Methionine Sulfur in Wet-Oxidation Methods

The practical consequences of the difficulty of completely converting methionine sulfur to sulfate are obvious. Not only would availability of a reliable and simple method for the oxidation of methionine sulfur present in aqueous solution be of definite value in work concerned with the chemical isolation or the metabolism of this amino acid, but doubts about the adequacy of some of the customary methods may reflect more or less seriously, depending on the relative amounts of methionine involved, on the validity of many published analytical figures for the total sulfur content of proteins or of metabolic intake, intermediate, or output materials. The authors have, therefore, studied the response of methionine to some recently published oxidation procedures and some independent modifications.

A wet-oxidation method involving the protracted action, at slowly rising temperatures, of a mixture of nitric and perchloric acid and cupric salt, has been recommended for metabolic products by Pirie (*13*). Except for the statement that "80 to 90 per cent of the sulfur in *S*-benzylcysteine, or *S*-ethylcysteine added to urine, is converted into sulfate by this method", no data on the accuracy of the method are presented. The authors' results, given in Table I, show sulfur recoveries of 70 to 80 per cent in the case of methionine and, in addition, demonstrate that reasonable agreement between duplicates, especially when run simultaneously, cannot be taken as evidence of complete oxidation.

A seemingly simple and promising method is that of Zahnd and Clarke (*20*) in which oxidation is obtained by boiling with nitric acid and potassium chlorate (or nitrate), followed by fusion of the evaporation residue. Applicability is claimed to any type of organic substance except volatile sulfones or compounds yielding them, and good results are reported for various substances, including a close relative of methionine—*S*-ethylcysteine—on which consistent values of 97 per cent of the theoretical were obtained. However, as shown in Table I, application of the method to methionine yielded variable results below 90 per cent of the theoretical. A modified reagent, in which aqua regia is the oxidizing agent, gave likewise low results which regardless of variations of procedure fluctuated between 80 and 90 per cent. No better result was obtained when hydrobromic acid was substituted for hydrochloric acid, and when hydriodic acid was used instead the yield became much lower. The last experiment with hydriodic acid is of special interest because here the first stage of the treatment corresponds closely to the conditions under which, as was shown by Baernstein (*1*), methionine is nearly

Table I. Formation of Sulfate from Methionine by Methods of Wet Oxidation

Oxidation Method	Methionine Sample Substance	Solution	Sulfate Found
	Mg.	*Cc.*	% *methionine sulfur*
HNO_3, $HClO_4$, $Cu(NO_3)_2$; method of Pirie (*13*); initial reagent added: 5 cc. of Pirie's mixture	100.3	Aqueous, 10	80.4[a]
	100.3	10	81.4[a]
	100.3	10	78.7[b]
	100.3	10	77.8[b]
	95.8	0.2 *M* HCl, 10	69.6[b]
	47.4	5	79.1[b]
	9.5	1	78.2
	85.5[c]	None	78.4
HNO_3, $KClO_3$; method of Zahnd and Clarke (*20*); initial reagent added: 2.5 cc. of 10% $KClO_3$ and 1 cc. of concd. HNO_3	10.03	Aqueous, 1	89.8
	10.13	1	89.2
	10.13	1	83.9
Aqua regia, $MgCl_2$[d]	10.03	Aqueous, 1	90.3
	10.03	1	84.9
Aqua regia, $MgCl_2$, $HgCl_2$[e]	10.03	1	85.0
Aqua regia, $MgCl_2$, repeated treatment[f]	10.03	1	84.6
	10.03	1	87.2
Aqua regia, $MgCl_2$, delayed oxidation[g]	9.48	1	82.6
	9.48	1	91.6
HNO_3, HBr, $MgCO_3$[h]	9.48	1	88.7
HNO_3, HI, $MgCO_3$[i]	9.48	1	0.0
HI, followed by aqua regia, $MgCl_2$[j]	9.48	1	15

[a] Determined as $BaSO_4$; all others as benzidine sulfate by titration.
[b] 20-cc. portions of 250 cc. of solution used for titrimetric sulfate determination.
[c] Individually weighed sample; all others are aliquot volumes of solutions.
[d] 0.5 cc. of 0.34 *M* $MgCl_2$ in 4 *M* HCl, 3 cc. of concd. HCl, and 1 cc. of concd. HNO_3 were added to sample in large test tube. Solution, containing freshly heated quartz chip, was briskly boiled to dryness and residue was heated until no more nitric vapors were evolved. Sulfate was determined in aqueous or slightly acid (HCl) solution of residue.
[e] Procedure identical with [d] except that 0.2 cc. of 1 *M* $HgCl_2$, as a possible catalyst, was added to oxidizing mixture.
[f] After evaporation to dryness as under [d], 3 cc. of concd. HCl and 1 cc. of concd. HNO_3 were added, followed by [d].
[g] Treatment as under [d] except that sample with reagent mixture added was left in steam bath at 80° to 90° overnight before proceeding with evaporation.
[h] Sample, with 4.4 cc. of concd. HBr (d 1.38), 1 cc. of concd. HNO_3, and 60 mg. of $MgCO_3$ added, was left in warm steam bath overnight, evaporated to dryness, and after addition of 1 cc. of concd. HNO_3 again evaporated and heated. Residue was dissolved in minimum amount of concd. HCl and after addition of 20 cc. of H_2O benzidine sulfate was determined as usual.
[i] Procedure as under [h] except that instead of HBr 5.8 cc. of HI (d 1.70) were used.
[j] Sample was heated overnight in steam bath with 5.8 cc. of HI (d 1.70); then 3 cc. of 0.7 *M* $MgCl_2$ in 4 *M* HCl, 2 cc. of concd. HCl, and 2 cc. of concd. HNO_3 were carefully added, and evaporation to dryness was obtained by heating step by step (130–140°, 180–190°) in air bath, followed by evaporation, etc., with 3 cc. of concd. HCl and 1 cc. of concd. HNO_3, as under [d].

completely converted into homocysteine thiolactone. Apparently this compound on further degradation tends more toward the formation of volatile oxidation products than the unmodified methionine.

The general conclusion suggested by the results listed in Table I is that the oxidation of methionine in acid media is accompanied by the formation of volatile sulfur compounds. The extent to which sulfate is formed varies in individual experiments without any evidence of simple dependence on controllable working conditions. Occasional identical results in duplicate determinations are no evidence of completeness of oxidation to sulfate. Attention should be called to a recent publication by Masters (*9*) in which a wet-oxidation method is described which, according to the single determination on methionine reported, yields 101.4 per cent of the theoretical amount of sulfate with this compound. The method uses the same reagent as Pirie's (*13*, nitric acid, perchloric acid) except for the omission of copper salt, but the oxidative digestion is carried out much more slowly, being spread over approximately 30 hours with frequent additions of reagent.

Use of the Oxygen Bomb

In employing the oxygen bomb method the authors have based their technique on the procedure of Garelli and Saladini (*4*).

The combustion sample is weighed directly into a small silica crucible (4 cc.). Safe and complete combustion can be expected if the sample does not weigh more than 1 gram nor occupy more than one half of the crucible space. Depending on the sulfur content, the sample size has varied between 0.1 and 1 gram. The sample is thoroughly wetted with Decalin (decahydronaphthalene Eastman, practical, was found free of sulfur) using enough to bring the level of the liquid to the surface of the solid matter, without permitting the total charge of organic matter to exceed 2 grams; for about 0.1 gram of crystalline organic substances 0.5 cc. of Decalin was used as a rule. Finally 25 mg. of ammonium nitrate (reagent grade) are added and mixed in by means of a short piece of fuse wire which is left in the crucible. Ten cubic centimeters of water are put in the bottom of the bomb (Parr oxygen bomb) the crucible is wired (*12*) and placed in the bomb, and the bomb is carefully closed. It is filled with oxygen to a pressure of 30 atmospheres, placed under water in a pail, and if no gas leakage is evident, connected with a 110-volt circuit. The circuit contains, in addition to a knife switch, a resistance board with three lamp sockets, which are connected in parallel. A 25-watt bulb is inserted and if the switch is momentarily closed completeness of the circuit is established by the glow of the lamp filament. The charge is best ignited by placing two 200-watt bulbs in the circuit and closing it for a second—i. e., by using a 4-ampere current. Although under these conditions there is no visible glow of the filament (or cessation to indicate occurrence of the ignition), the use of the invisible but stronger current is preferable to the visible one obtained with bulbs of higher resistance (lower wattage). In certain special cases (substances containing inert inorganic matter or material otherwise difficult to ignite), a low-amperage current will not induce a full explosion in the bomb but merely a slow combustion, indicated by duration of the glow of the lamp filament for as long as one minute, and usually resulting in incomplete oxidation. If ignition has taken place, the bomb is warm to the touch.

Although the authors have used the bomb with complete safety for over 6 years, the electric controls are located at a safe distance, as a matter of precaution. At least one hour is permitted to elapse before the bomb is removed from the pail. It is then rinsed from the outside and the gases are slowly released by means of the valve control. The 1-hour waiting period is essential to permit completion of the slow reaction $SO_3 + H_2O \rightarrow H_2SO_4$, as was demonstrated by Garelli and Saladini (*4*). The authors' evidence confirms this: cystine (sulfur calculated, 26.67 per cent) gave 26.34 and 26.18 per cent of sulfur when the bomb was opened after 10 minutes, while with a 1-hour waiting period 26.57, 26.60, and 26.68 per cent were obtained, or 99.8 ± 0.2 per cent of the theoretical, while optical rotation (*18*) indicated a purity of 99.7 ± 0.2 per cent

After opening the bomb, the valve in the cover and the bomb interior are carefully washed out with water which contains 8 drops of methyl orange (0.1 per cent aqueous solution) per liter, until all traces of acid reaction have been removed. To the combined washings 1 cc. of concentrated ammonia is added and the solution is heated. A small amount of iron hydroxide, together with any insoluble particles, is then removed by filtration, and after proper acidification the solution is ready for the determination of sulfur, either as barium sulfate or volumetrically as benzidine sulfate. If the latter method is to be employed, the bomb washings are ordinarily made to 200 or 250 cc. and determinations, in the manner described in the following section, are run on 20-cc. portions. When the amount of sulfate present is small, 40-cc. portions may be used, or all of the bomb washings may be used after evaporation to about 20 cc.

Acidimetric Benzidine Method

The authors' procedure is based on the method of Fiske (*3*) in which acetone is employed to reduce the solubility of the benzidine sulfate and to facilitate filtration and washing. The acidimetric determination of benzidine sulfate is much better suited for microanalytical quantities than for macro amounts, because of the sluggishness with which benzidine sulfate goes into solution on titration with alkali, even in the heat. The authors have found 20 cc. of an approximately 0.003 *M* sulfate solution, corresponding to about 2 mg. of sulfur, a convenient quantity for an accurate determination, when titrating with 0.05 *N* sodium hydroxide from a 5-cc. microburet graduated to 0.01-cc. (equal to about 1-mm.) intervals. The buret is calibrated and the alkali is standardized against potassium acid phthalate with phenolphthalein as indicator.

For the precipitating reagent purified benzidine hydrochloride is prepared from benzidine (reagent grade) according to

the method of Letonoff and Reinhold (*7*), and a solution of 0.086 *M* benzidine in 0.23 *M* hydrochloric acid is prepared.

To 20 cc. of the sulfate solution in a 100-cc. beaker, 2 drops of bromophenol blue (0.1 per cent) are added and the solution is adjusted to a slight acidity (indicator just yellow, *11*) by approximately *N* hydrochloric acid or ammonia. Then 4 cc. of benzidine reagent and, after 2 minutes, 8 cc. of 95 per cent acetone are added. After 15 minutes the precipitate is filtered on the pad of a Fiske filtration tube (*3*) which is permanently lodged in a clean rubber stopper that fits a suction flask. It is desirable to have several of these units. The hole in the authors' tubes has a diameter of 2 or 3 mm., and it is convenient to form a thin but firm filter pad by using a disk of 10-mm. diameter of fluffy blotterlike filter paper (oil filter, Durieux No. 127) which is sealed at the edges by a small amount of an aqueous paper pulp suspension. The beaker and the interior of the filter tube are carefully washed, successively with three 2-cc. and one 10-cc. portion of 95 per cent acetone.

The filter tube is now placed over a 125-cc. Erlenmeyer flask (the rubber stopper sits on the rim of the flask) and precipitate and pad are poked through the hole into the flask. For this operation a platinum rod 10 cm. long and 2.5 mm. in diameter is used, tapered at one end over a length of 1 cm. to a blunt tip. The use of copper wire instead of platinum led to erroneous results (see below). Rod and tube are rinsed with a little water, the rod is returned to the beaker, and the flask, with the tube remaining on it, is heated on a hot plate until the contents boil. After addition of 2 drops of phenol red (0.1 per cent), approximately one half of the amount of sodium hydroxide which is estimated to be required is run from the buret through the tube into the flask, followed by rinsing with about 5 cc. of water. After about 0.5 minute of gentle boiling the inside of the tube and the outside below the stopper are carefully rinsed with about 10 cc. of water from the wash bottle and the tube is removed and placed in the beaker. The total volume should now be approximately 20 cc. Titration is then continued with intermittent boiling until no visible amounts of undissolved benzidine sulfate are left and the solution retains a pink shade on boiling. At this point the hot solution is carefully poured back into the original beaker, passing through the filter tube, and after thorough swirling and complete dispersion of any remaining paper wad, returned to the flask. The titration is then continued to a definite pink which must persist on continued boiling.

The principles of this procedure were originally proposed by Fiske (*3*) for metabolic sulfur determinations and the limits of precision and accuracy were not examined, except that in the twelve cited comparisons between titrimetric and gravimetric determination the titrimetric values averaged 100.7 ± 0.9 per cent (average error) of the gravimetric ones. In order to obtain information on these points the authors have, in a number of actual analyses in which the organic substance was burned in the oxygen bomb, performed the sulfate determination by both the Fiske procedure and the conventional barium sulfate method, using separate portions of the bomb washings. The results of these determinations are listed in Table II. The mean difference between the results of the two procedures is −0.4 ± 0.7 per cent.

In 82 cases where duplicate determinations by the benzidine method have been carried out on portions of the same solution, the average difference was 0.009 cc. (±0.006, average deviation). The lowest difference was 0.000 (8 times), the most frequent 0.002 (15 times), and the highest 0.026 (2 times); 0.009 cc. of 0.05 *N* sodium hydroxide corresponds to 0.007 mg. of sulfur or 0.05 mg. of barium sulfate. It therefore seems that under favorable circumstances (dependable buret calibration, alkali standardization, practiced operator, etc.) the benzidine results are at least the equals of barium sulfate results in accuracy and precision. The benzidine method is superior in that it is much less time-consuming and requires only a fraction of the material needed for the barium sulfate method.

Effect of Inorganic Substances in Oxygen Bomb Combustion and Benzidine Sulfate Titration

It has long been recognized (*11*) that the presence of excessive amounts of various ions, such as chloride or phosphate, can be the cause of disturbances in the precipitation of benzidine sulfate. Since in the combustion products of ordinary organic substances such salts are not present, the benzidine method seems eminently suited as an adjunct to the oxygen bomb method of combustion. When inorganic matter is present in the substance to be analyzed the applicability of the combination of the two methods may be expected to encounter limits which should depend on the one hand on the effect of the inorganic component on the combustion process in the bomb, and on its interference in the precipitation or titration of benzidine sulfate on the other. Some information on these points has come to light in the course of sulfur determinations which the authors have run on certain crude mixtures of amino acids and sodium chloride and on combinations of mercury salts and amino acids.

Disturbances were encountered when a rod of copper instead of platinum was used in the handling of the benzidine sulfate. In the course of sulfur determinations the authors used a stout copper wire for a considerable time. During this period some entirely satisfactory results were interspersed with a number of cases in which multiple determinations by the benzidine method differed by as much as 11 per cent, and others in which the benzidine result was as much as 14 per cent lower than the barium sulfate result. Since these difficulties ceased entirely as soon as platinum was substituted, some interaction of the acid precipitate with copper must be suspected as their cause.

Turning to the effect of sodium chloride, a 1-gram sample of a crude amino acid precipitate which contained 50 per cent of sodium chloride was successfully ignited in the usual manner, leaving a slightly colored residue in the crucible. Two benzidine sulfate determinations on fractions of the solution gave 1.020 and 1.041 per cent of sulfur. Another ignition, of a 500-mg. sample, gave 1.036 and 1.040 per cent of sulfur. A different product of similar nature, containing 45 per cent of sodium chloride, yielded by the benzidine method 1.28 and 1.23 per cent of sulfur on two separate ignitions of 343 and 251 mg., respectively. Fiske

Table II. Comparisons between Gravimetric and Volumetric Results of Sulfate Determination

Sample Substance		$BaSO_4$ Method $BaSO_4$	Benzidine Method NaOH	Benzidine Method S	Difference between Benzidine and $BaSO_4$ Results
	Mg.	*Mg.*	*Cc.*	%	*% of $BaSO_4$ result*
l-Cystine (S calcd., 26.67%)	201.8[a]	155.6	1.276	26.38	−0.75
		157.1	2.506	26.40	
S-(*p*-tolylmercapto)-cysteine[b]	105.2[c]	163.5	2.585	26.01	−2.50
	101.1[c]	155.3	2.493	26.10	−1.02
Mixture 3B[d]	895.2[c]	109.9	2.108	1.800	2.093	−0.75
Mixture 3A[d]	392.9[e]	56.2	2.456	0.327	2.475	+0.77
Mixture 4[d]	662.8[c]	163.9	4.246	2.682	4.212	−0.80
Impure *l*-methionine 4	97.9[c]	80.4	14.10	1.336	14.20	+0.71
Impure *l*-methionine 2A	101.3[c]	108.9	18.46	1.802	18.51	+0.27
Impure *l*-methionine 2B	99.8[c]	121.1	20.83	...	...	...
	109.6[c]	...	...	1.640	20.74	...
				1.648	20.84	−0.19
l-Methionine (S calcd., 21.49%)	99.8	125.2	21.50	2.060	21.48	−0.09

[a] Combined washings of two bomb combustions of about 100 mg. each made to 500 cc. and weighed. Weighed 200-cc. portions used for $BaSO_4$ method, and weighed 10- and 20-cc. samples for benzidine method.
[b] Prepared by T. F. Lavine (unpublished).
[c] Bomb washings made to 250 cc.; 200 cc. used for $BaSO_4$ precipitation and 20 cc. for benzidine method.
[d] Methionine-containing amino acid mixture from hydrolysis of egg albumin.
[e] Same as [c] except that only 7 cc. used for benzidine procedure.

TABLE III. DETERMINATIONS OF SULFUR IN PRESENCE OF MERCURY

Substance or Mixture	Weight of Sample Ignited	Bomb Washings Treated with Ammonia	Fraction of Bomb Washings Used for Benzidine Sulfate Determination	Sulfur Found	
	Mg.		%	*Wt. %*	*% of S present*
5H 2[a]	262.8	Yes	40	0.264	..
	289.2	Yes	40	0.260	..
	310.0	No	16	0.573	..
5H 5[a]	299.2	Yes	40	1.82	..
	658.3	Yes	40	1.40	..
l-Methionine + $(CH_3COO)_2Hg$	696[b]	Yes	8	14.48[c]	71
l-Methionine + $HgCl_2$ + $(CH_3COO)_2Hg$	582[d]	Yes	8	15.07[e]	74
	698[e]	No	8	21.45[e]	103
Benzoic acid + $HgCl_2$ + $(CH_3COO)_2Hg$	699[f]	No	16	0.00	..
l-Methionine mercury compound[g]	318.3	No	8	4.18	103
	357.7	No	8	4.20	104

[a] Methionine-containing precipitate obtained from protein hydrolyzate by mercuric chloride and mercuric acetate.
[b] Mixture of 115.0 mg. of *l*-methionine and 581 mg. of mercuric acetate.
[c] Calculated for methionine added; latter, isolated from egg albumin, gave on separate determinations 20.74, 20.84% S (Table II), indicating purity of 97 per cent.
[d] Mixture of 108.9 mg. of *l*-methionine, 365 mg. of mercuric chloride, and 108 mg. of mercuric acetate, approximating molar ratio 2:4:1 of methionine mercury compound (*17*).
[e] Mixture of 122.3 mg. of *l*-methionine, 445 mg. of mercuric chloride, and 131 mg. of mercuric acetate, approximating molar ratio 2:4:1 of methionine mercury compound (*17*).
[f] Mixture of 123 mg. of benzoic acid, 445 mg. of mercuric chloride, and 131 mg. of mercuric acetate, run as control for possible effect of mercury compounds.
[g] $(CH_3—S—CH_2—CH_2—CH(NH_2)—COO)_2Hg + 4HgCl_2$ (*17*, p. 368).

obtained good results by his method when the ratio of chloride (as sodium chloride) to sulfur was as high as 60, and the authors' results, in which that ratio is about 30, conform with his. Absence of carbonized residues in the bomb and the agreement between samples of different size further indicate that satisfactory results may be expected from oxygen bomb combustion in the presence of considerable inorganic matter.

A special situation was encountered in the presence of mercury salts. In connection with work on the isolation of methionine from protein hydrolyzates by means of mercury salts (*14*, *17*) determination of the sulfur content of a number of crude precipitates containing 60 to 65 per cent of mercury and about 15 per cent of chlorine became necessary. No difficulty was encountered in obtaining complete combustion in the bomb of samples as large as 650 mg. A considerable amount of ash, consisting largely of mercury and mercurous chloride was, of course, found in the bomb after ignition. Addition of excess ammonia to the bomb washings led to the precipitation of additional black mercury salts. Precipitation of benzidine sulfate in the filtrate and its titration offered nothing unusual. However, while duplicate ignitions resulted in substantially identical values when the size of the sample was similar, a decrease of 23 per cent in the percentage of sulfur found was encountered when the size of the sample was doubled. Investigation showed that in the presence of mercury salts a large fraction of the sulfate escapes determination by being incorporated in the ammonia precipitate, probably in the form of basic salts, thus producing results 25 to 55 per cent too low. However, when the treatment of the bomb washings with ammonia was omitted, and only the insoluble residue was filtered off, consistent results were obtained which exceed the theoretical value by about 3 per cent. The reason for this inaccuracy has not been ascertained. As is indicated in Table III, no evidence was found of a blank contribution of the mercury salts. However, results within 3 per cent of the actual value are satisfactory for many purposes, and the oxygen bomb–benzidine titration method can in such cases be recommended, on account of its simplicity, for the determination of sulfur in the presence of mercury.

Application of Bomb-Benzidine Combination to Determination of Methionine in Leucine

The difficulty of completely removing methionine from leucine specimens of protein origin is well known (*10*). The

TABLE IV. DETERMINATION OF SMALL AMOUNTS OF SULFATE AND SULFUR

Compound	H_2SO_4, 0.000253 *M*	H_2O	Time for Precipitation	0.04544 *N* NaOH Used	0.04544 *N* NaOH Corrected	0.04544 *N* NaOH Calculated
	Cc.	*Cc.*	*Min.*	*Cc.*	*Cc.*	*Cc.*
Sulfate determination only	0.00	80	15	0.004		..
	0.00	80	15	0.000	0.001 ±	..
	0.00	80	15	0.000	0.001	..
			Hours, 0° C.			
	0.00	80	18	0.024		..
	0.00	80	18	0.018	0.021 ±	..
	0.00	80	18	0.022	0.002	..
	0.00	20	18	0.030		..
	0.00	20	18	0.026	0.022 ±	..
	0.00	20	18	0.010	0.008	..
	5.00	15	18	0.083		..
	5.00	15	18	0.097	0.064 ±	0.056
	5.00	15	18	0.079	0.008	..
	10.00	10	18	0.136		..
	10.00	10	18	0.126	0.108 ±	0.111
	10.00	10	18	0.128	0.008	..
Bomb combustion and sulfate determination						
Reagent blank[a]	...	200	18	0.165	...	..
	...	200	18	0.155	...	..
dl-Leucine, 698.8 mg.	...	200	18	0.157	...	..
dl-Leucine, 803.7 mg.	...	200	18	0.165	...	..
dl-Leucine, 701.4 mg. + *dl*-methionine, 0.959 mg.[b]		200	18	0.423	0.431 ±	0.444[c]
dl-Leucine, 702.3 mg. + *dl*-methionine, 0.959 mg.[b]		200	18	0.439	0.008	..
Purified *l*-leucine, 499.0 mg.	...	200	18	0.284	...	..
Purified *l*-leucine, 481.7 mg.	...	200	18	0.270	...	..

[a] In these and following determinations bomb ignition was performed using 1.0 cc. of Decalin and 25.0 ± 0.5 mg. o ammonium nitrate.
[b] Methionine and leucine mixed as described in text.
[c] Average of the two reagent blanks and the two *dl*-leucine runs served as blank value.

authors have applied the combination of oxygen bomb combustion and benzidine sulfate titration to the problem of determining the residual methionine present in a highly purified sample of natural leucine.

The procedure was as described above, with a few modifications. The bomb washings were evaporated to dryness on the steam bath after they had been filtered following the addition of ammonia. The evaporation residue was then redissolved in 20 cc. of water and benzidine sulfate was precipitated after proper acidification. Further, in order to ensure completeness of crystallization of benzidine sulfate even near its solubility borderline the precipitate was filtered only after it had stood overnight at about 0° C. Finally, each determination was accompanied by an exactly corresponding blank determination.

Table IV shows that with these precautions small amounts of sulfate are determined with an accuracy equal to the analytical precision and that amounts of the order of magnitude of the reagent blank may escape determination when insufficient time is permitted for crystallization.

Finally, complete determinations were run on the reagents only, as well as on a sample of synthetic leucine (*dl*-). The identical values of the two pairs of determination show that the synthetic product is, as would be expected, free of sulfur. The magnitude of the blank values is noteworthy. Systematic experiments in which one component was varied at a time showed that the various components are responsible approximately as follows: 1 cc. of Decalin, 0.00 cc. (0.05 *N* sodium hydroxide); 25 mg. of ammonium nitrate, 0.12 cc.; 200 cc. of water, 0.02 cc.; and 4 cc. of benzidine hydrochloride solution, 0.01 cc. Careful control of the amount of ammonium nitrate added as oxidation catalyst and use of the purest grade available are advisable because of its large contribution to the blank value and because erratic values and evidence of incomplete combustion were encountered when excessive amounts (more than 100 mg.) were used. The role of the blank value in the benzidine determinations of the preceding sections is greatly diminished by the fact that not more than 8 per cent of the total bomb washings were employed there.

For the next pair of determinations synthetic mixtures of *dl*-leucine and *dl*-methionine were prepared by adding 0.400-cc. portions of a solution of 120 mg. of *dl*-methionine in a volume of 50 cc. to quartz crucibles containing the 700-mg. samples of *dl*-leucine; after mixing with a piece of fuse wire the crucible and contents were dried overnight at 100°. The results show that the added methionine sulfur, corresponding to a 0.03 per cent sulfur contamination, is recovered with an accuracy of about 96 ± 3 per cent. The final pair of determinations shows the results obtained on the natural leucine. Calculation yields the figures 0.0180 and 0.0166 per cent for the sulfur content. A conservative evaluation of these results and the control and blank determinations seems to justify the statement that the leucine in question contains 0.017 ± 0.002 per cent of sulfur.

Summary

A convenient procedure for the oxidation of organic sulfur compounds, preparatory to sulfur determination, by alkaline permanganate is described. No sulfate is formed from methionine by this procedure. Incomplete and variable oxidation of methionine sulfur is shown to occur in some other wet-oxidation methods. A tested procedure for the determination of sulfur in organic compounds is described in which the substance is burned in a bomb by compressed oxygen and the sulfate formed is determined acidimetrically as benzidine sulfate. The presence of sodium chloride and mercury salts is shown within certain limits not to interfere in this method. Its successful application to the accurate determination of a few hundredths of 1 per cent of sulfur present in an organic substance is described.

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Simple Condenser for Use during Digestion Operations

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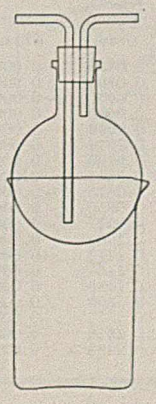
WHEN it is necessary to employ beakers for longtime digestions, a simple condenser of the type described below presents several advantages over the watch glass conventionally used as a cover. In the first place, evaporation is practically eliminated—a rather important factor when expensive reagents, such as 30 per cent hydrogen peroxide, are involved. Furthermore, where a mixture of reagents is used, their initial ratio is well maintained despite any wide difference which may exist between their boiling points. One who has had occasion to digest solids in a mixture of nitric and sulfuric acids for extended periods will appreciate the difficulty in keeping reasonably constant the composition of such a mixture.



The condenser in question is nothing more than a round-bottomed, short-necked, Pyrex flask carrying a two-hole rubber stopper fitted with two pieces of glass tubing bent at right angles. One serves as an intake and the other as an outlet for water. It is desirable that the intake tube be extended to a point close to the bottom of the flask. This improvised condenser may be of any convenient diameter, dependent upon the size of the beaker with which it is to be employed.

The apparatus depicted may, of course, be employed as a single unit or in series.

Determination of Sulfate in the Presence of Chromate

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IN A STUDY of the system sodium sulfate–sodium chromate–water (*1*) it was found necessary to analyze solutions and solid mixtures containing both sodium sulfate and sodium chromate. Accurate analysis of sulfate-chromate mixtures for sulfate is made difficult by contamination of the barium sulfate precipitate with coprecipitated barium chromate, which forms mixed crystals with barium sulfate. To prevent this coprecipitation, the chromate may be reduced to chromic salt, acetic acid added, and the sulfate precipitated in the usual way. Willard and Schneidewind (*3*) describe and discuss this method in some detail.

In this paper, an alternative method for the determination of sulfate in the presence of chromate is described. Sulfate is precipitated from acid solution with barium chloride in the usual manner, and the necessary correction for coprecipitated barium chromate is determined and applied.

Reagents and Solutions

SODIUM SULFATE. Reagent grade sodium sulfate was twice recrystallized as decahydrate, the crystals being separated centrifugally from the mother liquor. They were then placed in a platinum dish and dehydrated by heating, gently at first, then strongly in a muffle furnace. The mass was powdered in a mortar, heated again, and kept in a weighing bottle in a desiccator until used.

SODIUM CHROMATE. Pure sodium chromate was prepared from reagent grade sodium dichromate by the method of Richards and Kelley (*2*), which differs from the more usual methods of purification in that it removes sulfate, the sodium salt of which is isomorphous with sodium chromate in all its hydrated forms (*1*). The salt thus prepared was dehydrated and kept in a weighing bottle in a desiccator.

SODIUM CARBONATE. Reagent grade anhydrous salt was used without further purification.

BARIUM CHLORIDE SOLUTION. Reagent grade salt, without further purification, was dissolved in distilled water.

SODIUM THIOSULFATE SOLUTION, 0.05 *N*. About 32 grams of reagent grade salt were dissolved in 2.5 liters of freshly boiled distilled water in a bottle which had been thoroughly steamed. This solution was standardized against twice recrystallized reagent grade potassium dichromate.

IODINE SOLUTION. About 16 grams of iodine were dissolved in 2.5 liters of potassium iodide solution containing about 20 grams of potassium iodide per liter.

STARCH SOLUTION. This solution was freshly prepared from potato starch each day on which it was needed.

TABLE I. REPRESENTATIVE RESULTS

Na_2SO_4 Taken	Na_2CrO_4 Taken	Wt. of Ppt.	Na_2SO_4 (Uncorrected)	$BaCrO_4$ in Ppt.	Na_2SO_4 Found	Error
Gram	*Grams*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
0.0162	1.93	0.0424	0.0258	0.0162	0.0159	−0.0003
0.0291	2.18	0.0646	0.0393	0.0159	0.0294	0.0003
0.0468	0.345	0.0810	0.0493	0.0045	0.0466	−0.0002
0.0550	0.340	0.0935	0.0569	0.0031	0.0550	0.0000
0.0596	0.155	0.0992	0.0604	0.0012	0.0596	0.0000
0.0608	0.124	0.1023	0.0623	0.0017	0.0612	0.0004
0.0760	0.339	0.1297	0.0789	0.0039	0.0766	0.0006
0.0811	0.11	0.1370	0.0834	0.0032	0.0814	0.0003
0.0827	0.166	0.1373	0.0836	0.0018	0.0825	−0.0002
0.0850	0.328	0.1475	0.0898	0.0078	0.0850	0.0000
0.0935	0.268	0.1581	0.0962	0.0058	0.0927	−0.0008
0.1000	0.102	0.1665	0.1013	0.0009	0.1008	0.0008
0.1127	0.802	0.2017	0.1227	0.0179	0.1119	−0.0008
0.1153	0.758	0.2080	0.1266	0.0178	0.1157	0.0004
0.1490	1.020	0.2581	0.1571	0.0135	0.1489	0.0001
0.1503	0.093	0.2485	0.1512	0.0015	0.1503	0.0000
0.1868	0.398	0.3098	0.1885	0.0020	0.1873	0.0005
0.2213	0.285	0.3770	0.2295	0.0126	0.2218	0.0005
0.2482	0.169	0.4093	0.2491	0.0005	0.2488	0.0006

Procedure

Dissolve the sample in about 75 ml. of water and add 5 ml. of concentrated hydrochloric acid. Heat to boiling, and add very slowly, dropwise, from a separatory funnel, about 75 ml. of barium chloride solution of such concentration as to give a small excess of barium ion over that needed to precipitate all the sulfate. After adding the precipitant, digest the mixture just below the boiling point for at least 3 hours. Cool, filter off the precipitate on a quantitative paper, and wash thoroughly to remove all traces of chromate from the paper. The chromate in the form of mixed crystals with barium sulfate cannot be removed by washing.

Place the moist paper with the precipitate in a weighed platinum crucible and place the crucible in a cold electric muffle furnace. Adjust the current so that the furnace attains a temperature of about 800° C. in an hour. With this rate of heating, the paper burns off evenly and completely, without at any time bursting into flame. After an hour, remove the crucible from the furnace, cool in a desiccator, and weigh again. The difference in weight is the weight of barium sulfate plus the coprecipitated barium chromate.

Add to the crucible about 5 grams of anhydrous sodium carbonate and heat strongly in the oxidizing flame of a blast lamp until the precipitate has dissolved in the melt. Remove the flame, and after the melt has solidified, but while it is still hot, place the crucible in a 400-ml. beaker containing about 150 ml. of gently boiling water. When the melt has disintegrated, which takes about 10 minutes, remove the crucible from the beaker and wash thoroughly, pouring the washings back into the beaker. Filter off, wash, and discard the precipitated barium carbonate, collecting the filtrate and washings in a beaker containing about 20 ml. of concentrated hydrochloric acid. The filtrate now contains sulfate, chromate, dichromate, chloride, hydrogen, and sodium ions.

When the solution has cooled, add freshly prepared potassium iodide solution. Titrate with standard sodium thiosulfate solution the iodine set free by the chromate, adding only a slight excess of thiosulfate. Titrate back with standard iodine solution, using starch solution as indicator. Subtract the weight of barium chromate thus obtained from the weight of the mixed precipitate to obtain the weight of barium sulfate.

Discussion of Procedure

In the procedure described, the concentration of hydrochloric acid in the solution to which barium chloride is added is higher than is usually recommended for sulfate precipitations. This was found to be necessary in order to keep the coprecipitation of barium chromate from assuming too large proportions.

A Gooch crucible with asbestos filter cannot be used for the filtration, since when asbestos has been fused in sodium carbonate and the melt extracted with water, the solution contains substances which liberate iodine from potassium iodide. Consequently filter paper (Whatman No. 40) was used and was found satisfactory. Tests showed that chromate was not reduced when ignited in contact with filter paper in a muffle furnace.

Other methods for determining the correction to be applied, in which the precipitate was dissolved in concentrated sulfuric acid prior to further treatment, were tried and found unsatisfactory.

Results

Samples of known weight prepared from the purified reagents were dissolved in water and analyzed by the above

procedure. The quantity of sodium sulfate used was varied from about 0.05 to 0.25 gram, and the ratio of sodium sulfate to sodium chromate was varied from 2.5:1 to 1:75. Table I gives data selected at random from the results obtained.

Columns 1 and 2 give, respectively, the weights of sodium sulfate and of sodium chromate taken. Column 3 gives the weight of the precipitate of mixed barium sulfate and barium chromate obtained. Column 4 gives the weight of sodium sulfate found, calculated on the assumption that all the precipitate is barium sulfate. This quantity, by comparison with the corresponding quantity in column 1, is considerably in error. Column 5 gives the weight of barium chromate in the precipitate, determined as described above. Column 6 gives the weight of sodium sulfate found after the correction has been applied, and column 7 gives the difference between this quantity and the weight of sodium sulfate taken.

It is evident from these results that this method of determining sulfate adequately meets the ordinary analytical requirements.

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Platinized Silica Gel as a Catalyst in Gas Analysis

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Previous work showed that hydrogen, carbon monoxide, and hydrocarbons can be oxidized quantitatively over a platinized silica gel catalyst containing 0.075 per cent of platinum. The commercial catalyst now available contains 0.125 per cent of platinum and is considerably more reactive. The changes in technique from the use of the copper oxide tube give more rapid results, as the catalyst tube replaces the copper oxide tube and slow combustion pipet. Hydrogen is oxidized at 115° C., methane and other hydrocarbons at 510° C. Oxidation temperatures are determined for carbon monoxide, ethylene, acetylene, ethane, and propane. Ethylene may be hydrogenated over this catalyst at 375° C. Nitrous oxide may be determined by reduction with hydrogen at 515° C.

THE previous papers in this series (*2*, *3*, *4*) have shown that hydrogen, carbon monoxide, and hydrocarbons can be quantitatively oxidized by air or oxygen over a platinized silica gel catalyst and have given the conditions necessary for the oxidation. The catalyst used in the previous work, the commercial platinum catalyst of the Silica Gel Corporation, containing 0.075 per cent of platinum, has now been replaced by a more active catalyst containing 0.125 per cent of platinum. This paper states the conditions necessary for its use to replace the copper oxide tube and combustion pipet in the determination of hydrogen, carbon monoxide, hydrocarbons, and nitrous oxide.

Apparatus

The U. S. Steel Corporation gas analysis apparatus used in the previous work was modified by replacing the usual heater for the copper oxide tube with one made from the heating element of a volatile matter furnace (Figure 1). The heating element, *B*, is surrounded by magnesite insulation, *C*, within a sheet-iron shell. The two ends, *D*, are of 0.94-cm. (0.375-inch) Transite, held in place by the straps, *E*.

The technique of the copper oxide tube (*5*) is to cool the tube to room temperature after each analysis. This cannot be done when a catalyst tube is used, as the platinized silica gel will adsorb an appreciable quantity of gases at room temperature. This error is avoided and the time required for an analysis is shortened by maintaining the tube at the temperature of analysis and adjusting the pressure in the tube to the standard pressure after each analysis. It is advisable to keep the catalyst tube

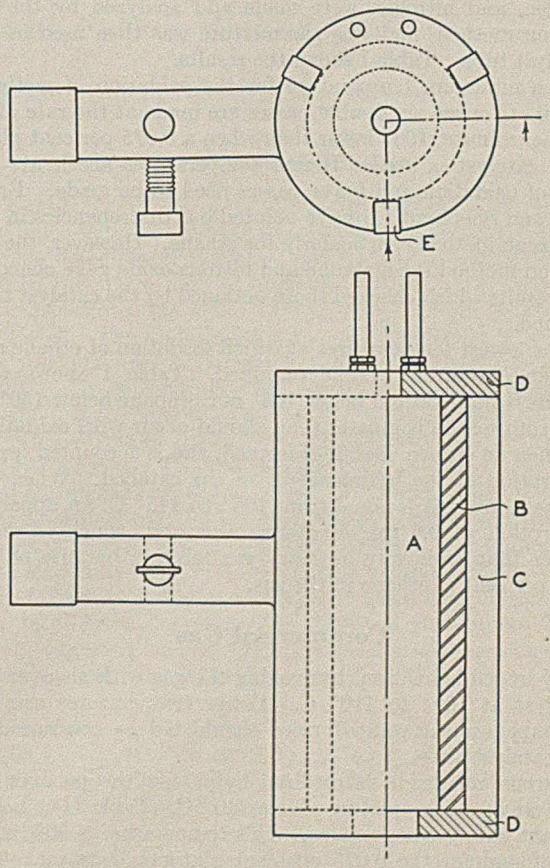

FIGURE 1. DIAGRAM OF APPARATUS

TABLE I. OXIDATION OF METHANE, ETHANE, AND PROPANE

Analysis	1	2	3	4	5	6
Oxidation of Methane						
Temperature, ° C.	497	510	510	510	510	510
No. of passes	9	4	6	9	6	6
Rate, ml. per min.	40	40	40	40	40	40
CH_4 by explosion, %	11.2	7.2	7.2	7.2	7.2	7.2
CH_4 by catalyst, %	10.8	6.7	7.3	7.3	7.2	7.2
Oxidation of Ethane						
C_2H_6, %	12.9	..	..	..	..	..
Temperature, ° C.	224	195	170	166	165	145
No. of passes	4	4	4	4	4	4
Rate, ml. per min.	40	40	40	40	40	40
Sample, ml.	92.8	90.4	89.3	88.4	88.3	88.7
Contraction, ml.	1.4	0.9	0.6	0.1	0.0	0.6
CO_2, ml.	1.0	0.2	0.0	0.0	0.0	0.0
Oxidation of Propane						
C_3H_8, %	5.5	..	..	..	..	..
Temperature, ° C.	235	179	150	135	..	..
No. of passes	8	8	6	8	..	..
Rate, ml. per min.	30	30	30	30	..	..
Sample, ml.	98.4	99.2	99.2	95.6	..	..
Contraction, ml.	1.4	1.0	0.0	0.0	..	..
CO_2, ml.	0.2	0.2	0.0	0.0	..	..

open to the buret during heating and cooling, as a large change in temperature (as from room temperature to 510° C.) greatly changes the pressure in the tube.

Methane, Ethane, and Propane

The lowest temperature at which methane could be oxidized completely was first determined. Methane is more resistant to oxidation than the other hydrocarbons, so its oxidation temperature was determined exactly, for here ethane, propane, and higher hydrocarbons will be completely oxidized. The temperature at which oxidation of hydrocarbons begins is of importance, as it shows the temperature below which hydrogen must be oxidized to avoid simultaneous hydrocarbon oxidation. Mixtures of the hydrocarbon, oxygen, and nitrogen were made and analyzed by the explosion method, and this gas mixture was then used in the catalyst tube. Table I shows the results.

The minimum temperature for the oxidation of methane is 510° C. when six double passes are made at the rate of 40 ml. per minute, 100° lower than when a 0.075 per cent platinum catalyst is used. Higher temperatures accelerate the rate of oxidation and fewer passes need to be made. Branham and Shepherd (*1*) have pointed out discrepancies in the explosion method, particularly for ethane. However, the explosion method for methane and nitrous oxide gave concordant results which checked those obtained by the catalyst tube method.

The lowest temperatures at which oxidation of ethane and propane occurred were determined. Table I shows that ethane is not oxidized below 165° nor propane below 150° C. The contraction is greater than should occur with oxidation, or when no carbon dioxide is formed, and is accounted for by adsorption of the hydrocarbon on the catalyst. When the temperature was lowered from 165° to 145° C. an apparent adsorption of 0.6 ml. of ethane occurred, which is much higher than from any ordinary gas mixture because of the 12.9 per cent of ethane in the gas.

Commercial Gas

Hydrogen is oxidized by passing the gas with air over the catalyst at 100° to 110° C. Lower temperatures are not advisable, as the water formed should not be condensed in the catalyst tube.

Carbon monoxide is oxidized by passing the gas over the catalyst at a temperature above 210° C. (Table II); below this the catalyst is poisoned. This temperature is 90° below that found for the 0.075 per cent platinum catalyst. The temperature for the oxidation of carbon monoxide is above the minimum for ethane and propane, so that it cannot be determined by oxidation when these hydrocarbons are present in the gas mixture.

Seattle city gas was analyzed, using three different methods for oxidation. The results (Table III) show that the catalyst tube gives as exact data as the usual combustion methods.

With samples 1 and 2, the hydrogen was removed at 115° C. by five double passes at 40 ml. per minute, and then the hydrocarbons were determined by explosion. With samples 3a and 3b, hydrogen was removed as before, and then the hydrocarbons were determined by six passes through the catalyst tube at 510°. With samples 4a and 4b, hydrogen and hydrocarbons were determined together by six passes at 511° and calculated to hydrogen and methane, which is slightly in error as some ethane is present in Seattle city gas.

TABLE II. OXIDATION OF CARBON MONOXIDE

(Gas mixture 12.7% CO, 18.9% O_2)

Analysis	1	2	3	4	5	6
Temperature, ° C.	320	276	231	210	208	194
No. of passes	6	6	6	6	6	6
Rate, ml. per min.	40	40	40	40	40	40
CO, %	12.6	12.7	12.7	12.6	12.7	7.4

TABLE III. ANALYSIS OF SEATTLE CITY GAS

Sample	1	2	3a	3b	4a	4b
CO_2	5.4	5.3	5.3	5.3	5.5	5.5
Ill.	5.7	5.8	5.8	5.8	5.7	5.7
O_2	1.0	1.1	1.0	1.0	1.1	1.1
CO	14.4	14.2	14.2	14.2	14.1	14.1
H_2	41.7	41.8	41.8	41.9	41.9	41.7
CH_4	15.7	15.8	15.8	16.0	16.8	17.0
C_2H_6	0.7	0.6	0.9	0.7	..	..
N_2	15.4	15.4	15.2	15.1	14.9	14.9
Total	100.0	100.0	100.0	100.0	100.0	100.0

Oxidation of Ethylene and Acetylene

Ethylene and acetylene may be oxidized quantitatively with a limited excess of oxygen. Known mixtures of hydrocarbon, oxygen, and nitrogen were made. Ethylene was determined by absorption, acetylene by explosion. The results with the catalyst tube are shown in Table IV.

Ethylene may be oxidized quantitatively above 310°, and acetylene above 325°. Below these temperatures the catalyst is poisoned by the unsaturated hydrocarbon. Whenever the catalyst is poisoned by ethylene, acetylene, or carbon monoxide, it may be reactivated by heating the tube to about 75° above the minimum oxidation temperature and passing air or oxygen through the tube three or four times.

Hydrogenation of Ethylene

In order to determine the possibility of determining unsaturation by hydrogenation, the activity of the platinized silica gel as a hydrogenation catalyst was studied for ethylene (Table V). In oxidizing ethylene, the catalyst was poisoned

TABLE IV. OXIDATION OF ETHYLENE AND ACETYLENE

Analysis	1	2	3	4	5
	Ethylene				
Temperature, ° C.	450	400	326	310	288
No. of passes	4	4	4	4	4
Rate, ml. per min.	40	40	40	40	40
C_2H_4 by absorption, %	11.0	11.0	11.0	11.0	11.0
C_2H_4 by catalyst, %	11.0	11.1	11.0	11.0	5.7
	Acetylene				
Temperature, ° C.	398	338	325	305	305
No. of passes	4	4	4	4	8
Rate, ml. per min.	40	40	40	40	40
C_2H_2 by absorption, %	7.9	7.9	7.9	7.9	7.9
C_2H_2 by catalyst, %	7.8	8.0	7.9	6.3	7.5

TABLE V. HYDROGENATION OF ETHYLENE

(Gas mixture: C_2H_4 16.5, H_2 83.5 per cent)

Analysis	1	2	3	4	5	6
Temperature, ° C.	375	318	318	286	286	286
No. of passes	4	4	8	4	7	10
Rate, ml. per min.	40	40	40	40	40	40
C_2H_4 by hydrogenation, %	16.5	16.0	16.5	15.8	16.3	16.5

TABLE VI. REDUCTION OF NITROUS OXIDE

(Gas mixture: hydrogen, nitrous oxide by explosion 33.7, 33.8%)

Analysis	1	2	3	4	5	6
Temperature, ° C.	518	518	515	491	491	460
No. of passes	6	12	6	4	8	4
Rate, ml. per min.	40	40	40	40	40	40
N_2O, %	33.7	33.7	33.8	33.2	33.6	30.8

below 310°. In hydrogenating ethylene, the reaction proceeds so rapidly at first that the remaining ethylene does not poison the catalyst, so that hydrogenation can be effected as low as 234°, although the reaction becomes extremely slow.

Nitrous Oxide

The reaction $N_2O + H_2 \rightarrow N_2 + H_2O$ can be carried out by explosion technique. Nitrous oxide was mixed with hydrogen and analyzed by explosion. The gas mixture was passed through the catalyst tube and the oxidation temperature determined (Table VI). Nitrous oxide may be determined by making six double passes at 515° or above. Analysis of a cylinder of nitrous oxide for anesthesia showed 99.7 per cent purity.

Summary

A platinized silica gel catalyst containing 0.125 per cent of platinum lowers the oxidation temperature of methane and carbon monoxide by approximately 100° C. from that obtained with a 0.075 per cent catalyst. Temperatures are given for the complete oxidation of carbon monoxide, methane, ethylene, and acetylene and for the start of oxidation for ethane and propane. Nitrous oxide may be reduced over the catalyst by a limited excess of hydrogen at 515° C.

The catalyst tube can replace the copper oxide tube and explosion (or slow-combustion) pipet in any commercial gas analysis apparatus. Large samples may be used without danger of explosion.

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Detection of Certain Metals in Minerals and Ores

An Ammonium Hypophosphite Fusion Method

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WHEN fused with ammonium hypophosphite many minerals and ores are decomposed, the resulting melt often being highly colored. The color of the melt directly, or after being treated with water or hydrogen peroxide, is the basis of the tests described here for chromium, cobalt, columbium, manganese, molybdenum, tellurium, titanium, uranium, vanadium, and tungsten.

Hypophosphites are powerful reducing agents. They decompose upon being heated, giving off hydrogen and phosphine, which ignite. Ammonium hypophosphite decomposes as follows (*1*):

$$7NH_4H_2PO_2 \longrightarrow 2HPO_3 + H_4P_2O_7 + 7NH_3 + 3PH_3 + 2H_2 + H_2O$$

The clear melt that is obtained serves as an excellent medium for showing any color imparted to it by the reduced mineral. The fused mass has a low melting point, about 60° C., and is readily soluble in water. Sodium and potassium hypophosphites are not suitable for this fusion because upon decomposition they form salts with relatively high melting points, instead of the low melting acids that are obtained from the ammonium salt.

Method

About 0.1 gram of the finely powdered mineral is strongly heated in a small evaporating dish with 2 grams of ammonium hypophosphite. The hypophosphite soon begins to decompose and the gases evolved ignite, forming water and oxides of phosphorus. After 2 minutes a quiet fusion mixture is obtained which is used for the individual tests.

Detection of Metals

COBALT, TITANIUM, AND TUNGSTEN. These three metals give blue melts, but the cobalt melt turns pink upon cooling. For the detection of tungsten, enough water is added dropwise to the warm melt to keep the surface moist. As the water penetrates the melt, the blue color changes to a striking violet. The violet color appears immediately when appreciable amounts of tungsten are present, but 10 to 30 minutes may be required when only very small amounts are present. If the blue color of the melt is due to titanium, the water above the melt becomes a delicate and almost imperceptible rose color. The addition of hydrogen peroxide gives an intense orange-red color. Ammonia causes the rose color to change to blue, but this change is not so sensitive as the color change with hydrogen peroxide. Vanadium also gives a reddish color with hydrogen peroxide, but the vanadium melt is red when hot and green when cool; hence it is easy to distinguish between titanium and vanadium. Since cobalt, titanium, and tungsten are usually not associated together in minerals, these tests are specific for these three metals.

VANADIUM, CHROMIUM, AND URANIUM. These three metals impart a green color to the melt. Vanadium gives a reddish color to the melt when hot, which gradually changes to yellow and finally to green on cooling. The addition of water gives a pale green solution which turns pink when hydrogen peroxide is added. The addition of ammonium carbonate solution to the green melt until the solution is dis-

TABLE I. SENSITIVITY OF TESTS

Material Tested	Weight of Sample	Metal Detected
	Mg.	
Cb_2O_5 + Ta_2O_5 mixture	50	Cb
Columbite (40% Cb_2O_5)	150	Cb
Columbite	100	Cb
WO_3	25	W
Ferberite (6.26% WO_3)	100	W
Ferberite (26.8% WO_3)	30	W
Na_2WO_4	3 (WO_3)	W
Scheelite	120	W
Wolframite	100	W
Hübnerite	100	W
TiO_2	10	Ti
Brookite	100	Ti
Perovskite	120	Ti
Ilmenite	100	Ti
$Co(NO_3)_2$	2	Co
Smaltite	100	Co
V_2O_5	10	V
NH_4VO_3	2	V
Carnotite (1.28% V_2O_5)	200	V
Roscoelite (5.04% V_2O_5)	50	V
Uranyl acetate	4	U
Carnotite (0.28% U_3O_8)	200	U
$Cr(NO_3)_3$	18	Cr
Chromite	100	Cr
MoO_3	4	Mo
Molybdenite (4.07% Mo)	150	Mo
Wulfenite	100	Mo
$MnSO_4$	0.05 (Mn)	Mn
Pyrolusite	50	Mn
TeO_2	10	Te
Sylvanite	100	Te

tinctly basic, followed by the addition of hydrogen peroxide, gives a yellow-orange colored solution if uranium is present. It is better to filter off any solid matter in order to observe the color of the filtrate. Neither vanadium nor chromium gives a yellow-orange color in basic solution. If the green color of the melt is due to chromium, no change in color takes place when hydrogen peroxide is added.

MOLYBDENUM. All molybdenum minerals give a reddish-brown melt, except molybdenite, which is not completely decomposed by the fusion. Therefore, in testing for molybdenum, the melt is treated with concentrated nitric acid, the acid is boiled off, and the mixture is heated to fusion again. The resulting melt is green or blue-green, which changes to yellow upon addition of water.

MANGANESE. Manganese minerals give a clear melt. When concentrated nitric acid is added and the excess acid boiled off, the melt takes on the well-known color of permanganate. Nitric acid does not usually oxidize manganese to permanganate, but in the presence of the melt it does this readily. None of the metals in the usual scheme of analysis interferes with this test for manganese.

TELLURIUM. Tellurium minerals are reduced to metallic tellurium by the fusion and small globules of the metal may be seen floating on the surface of the melt. Strong heating for 2 or 3 minutes causes a deep wine color to appear around each globule of molten tellurium. Addition of water to the warm melt causes the wine color to turn black.

COLUMBIUM. Columbium minerals impart no color to the melt but fine black particles are observed throughout the melt. When concentrated hydrochloric acid is added, the mixture heated to boiling, and a small piece of mossy tin added, an intense blue color develops in a few seconds if columbium is present. Since columbium and tantalum are nearly always associated together in minerals, this test can be used for the detection of both metals.

Sensitivity of Tests

Table I shows that these tests are applicable to different minerals containing the elements for which the tests have been devised, and are sufficiently sensitive to detect appreciable quantities of the metals in question.

It is not claimed that by this method any one of these metals can be detected in the presence of any or all of the others, but it is possible to detect any one metal in any of the naturally occurring minerals thus far tested. Tungsten, for example, can be detected in any one of the tungsten minerals which the authors have been able to obtain. Manganese was detected in a mixture containing 0.5 mg. each of silver, lead, mercury, bismuth, copper, cadmium, arsenic, antimony, tin, cobalt, nickel, chromium, aluminum, zinc, and manganese. During the fusion iron and copper are reduced to colorless compounds which in no way interfere with the tests. If a mineral gives no color, all the metals included in these tests are absent, except columbium and manganese.

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Use of Bromate in Volumetric Analysis

Determination of Arsenic and Antimony Using Internal Indicators at Ordinary Temperatures

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GYÖRY'S method (*1*) for the determination of arsenic and of antimony by titration of strong hydrochloric acid solutions of the trivalent elements, using potassium bromate with methyl orange or indigo sulfonate as internal indicators, requires that the reaction be carried out at 80° to 90° C. The same determination can be carried out at lower hydrochloric acid concentration and at room temperature if the reaction is followed potentiometrically, as shown by Zintl and Wattenberg (*5*). More recently benzopurpurin B was proposed by Raĭkhinshteĭn (*2*) for the determination of antimony using bromate, and Utzel (*4*) suggested a colloidal suspension of alpha-naphtholflavone as a reversible indicator for bromate titrations.

Since the determination of arsenic and antimony using the Györy (*1*) method is still preferred by industrial analytical laboratories and has not been discarded in favor of the Zintl and Wattenberg (*5*) or other procedures, suitable modification of the Györy methods, by means of which the reaction is carried out at ordinary temperatures, has been suggested to the authors. This article describes the use of three internal oxidation indicators of the irreversible type in the Györy procedure, by means of which the oxidation of arsenic and of

Table I. Determination of Bromate Required for Oxidation of 1 Ml. of Internal Indicator in Aqueous Hydrochloric Acid Solution

B. C. I. No.	Indicator Concentration	Indicator	0.1 *N* Bromate Required
	%		*Ml.*
88	0.1	Bordeaux	0.08
185	0.2	Brilliant Ponceaux 5R	0.05
246	0.1	Naphthol blue-black	0.03

Table II. Titration of Trivalent Antimony by Bromate at Various Hydrochloric Acid Concentrations

(Bordeaux used as indicator)

Volume of Concd. HCl	No. of Determinations	Antimony Calcd.	Antimony Found	Error
%		*Gram*	*Gram*	*Mg.*
35	3	0.1523	0.1523	0.0
25	3	0.1523	0.1522	−0.1
20	3	0.1523	0.1523	0.0
15	3	0.1523	0.1522	−0.1
10	3	0.1523	0.1521	−0.2
5	3	0.1523	0.1523	0.0

antimony by bromate can be carried out at ordinary temperatures and over a wide range, from low to high hydrochloric acid concentrations. The previous papers in the series (*3*) should be consulted for further bromate analytical procedures.

Disadvantage of Irreversible Internal Oxidation Indicators

The organic dyes, Brilliant Ponceaux 5R, B. C. I. No. 185 (0.2 per cent aqueous solution), Bordeaux, B. C. I. No. 88, and naphthol blue-black, B. C. I. No. 246 (0.1 per cent aqueous solution), are irreversible oxidation indicators. Their intense color permits the use of 0.1 to 0.2 ml. of a 0.1 per cent solution for titration, and the potentials at which they are oxidized and the color destroyed, using hydrochloric acid solutions with bromate as oxidant, are above the equivalence point potential for the oxidation of trivalent to pentavalent arsenic or antimony. Their destruction results from the liberation of bromine from the action of the first minute excess of bromate in the presence of hydrochloric acid. The mechanism of the reaction for the destruction of methyl orange or indigosulfonate in the Györy method is the same, except that the reaction mixture must be hot. With all these irreversible oxidation indicators the destruction of the indicator is often premature to a slight extent and the color of the solution fades before the equivalence point of the reaction is reached. While this is troublesome, the required use of additional indicator incurs no appreciable indicator blank in the present case, as shown by Table I. The indicator blank is negligible in all cases. Additional indicator to counteract "fading" can be used without appreciable blank determination.

Reagents Employed

Tenth normal solutions of potassium dichromate, potassium bromate, iodine, and potassium antimonyl tartrate as well as 0.2 *N* sodium thiosulfate solution were made up in accordance with accepted procedures.

The iodine solution was standardized using Bureau of Standards arsenic trioxide (standard of reference No. 83), the sodium thiosulfate was then standardized by liberation of iodine from measured portions of the bromate in 1 *N* hydrochloric acid solution by addition of excess potassium iodide, and the liberated iodine was titrated using the standard thiosulfate with starch as indicator. The antimony solution was standardized using the iodine solution in the presence of excess sodium bicarbonate with starch as indicator in the accepted manner. In all cases calibrated pipets, burets, and flasks were employed and the determinations were made with sufficient duplications to ensure an accuracy of one or two parts per thousand.

Effect of Variation in Hydrochloric Acid Concentration at Ordinary Temperatures

In the titration of the antimony solution with the bromate, the acid concentration was varied over a wide range to study the applicability of the indicator. The volume at the beginning of the titration was 100 ml. in all cases. One or two drops of the indicator solution were used, and by the time the end point was reached most of the indicator had been oxidized by the local excesses of the bromate during its addition. As the indicator fades another drop can be added without causing any error. At the end point the indicator is irreversibly destroyed and the solution becomes colorless. If the fading of the indicator is confused with the end point, another drop of the indicator may be added. If the indicator has faded, the additional drop will color the solution. If the end point has been reached, the additional drop of indicator will be destroyed by the excess bromate in the last drop added.

The results of the titrations of the antimony solution using Bordeaux as the indicator are found in Table II.

The results of the titrations of the antimonyl solution using naphthol blue-black and Brilliant Ponceaux 5R are given in Table III, which also includes the titration of samples of Bureau of Standards arsenic trioxide using Bordeaux.

Bordeaux, Brilliant Ponceaux 5R, and naphthol blue-black are satisfactory indicators for the titration of trivalent antimony at acidities varying from 5 to 35 per cent of concentrated hydrochloric acid, using bromate as oxidant and with titration at ordinary temperatures. The same indicators were used in the oxidation of trivalent arsenic, using 20 per cent by volume of concentrated hydrochloric acid, the other conditions being the same. The influence of the fading of these irreversible internal oxidation indicators upon the procedures in question has been discussed and the absence of an appreciable titration error shown. The procedures described are superior to the older method of Györy without requiring titration either in the hot or at higher hydrochloric acid concentrations. At the same time the necessity of a potentiometric titration to gain these advantages as in the Zintl and Wattenberg procedure is avoided.

Table III. Titration of Antimonyl Solution and Arsenic Trioxide

Volume of Concd. HCl	No. of Determinations	Indicator Used	Antimony Taken	Antimony Found	Arsenic Taken	Arsenic Found	Error
%			*Gram*	*Gram*	*Gram*	*Gram*	*Mg.*
10, 15, 20, 25, and 35	5	Naphthol blue-black	0.1523	0.1523	...	...	0.0
10, 20, 25, and 35	4	Brilliant Ponceaux 5R	0.1523	0.15225	...	...	−0.05
20	5	Bordeaux	...	...	0.09361	0.09362	+0.05

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Application of a Photoelectric Colorimeter

Determination of Bismuth in Biological Materials

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THE potassium iodide method for the determination of bismuth in biological materials has been used in many forms since first applied by Autenrieth and Meyer (*1*) and modified by Leonard (*4*). It depends upon the formation of a yellow bismuth iodide complex in acid solution and in the presence of a reducing agent. Scholtz and Chaney (*7*), without evaluation of the effect of the reagents on the optical properties of the solution, applied a photoelectric comparator to this method, using standard bismuth solutions for calibration. Baggesgaard-Rasmussen, Jackerott, and Schou (*2*) showed by means of a Koenig-Martens spectrophotometer that the complex involved absorbs blue light up to a wave length of 4500 Å. They found, furthermore, that variation of the acid and potassium iodide within narrow limits had apparently no effect on the light absorption.

In this investigation an attempt has been made to evaluate objectively, by means of a photoelectric colorimeter, the principal factors which affect the application of the iodide method. Revised procedures are proposed for the determination of bismuth in urine, kidney, liver, and muscle, and test analyses are included to indicate the accuracy.

All determinations were made with a photoelectric photometer employing a single cesium cell and standard substitution technique. Approximately monochromatic light was supplied by means of a 6-volt incandescent bulb operating on a storage battery (Exide, Type LXGH), a blue Pyrex glass filter (Corning No. 554 H. R. Lantern Blue), 6.0 mm. in thickness, and a heat filter (Corning No. 396, Light Shade Aklo). The blue filter was chosen because its maximum transmission (*3*) occurs in the region of the prominent absorption band of the bismuth iodide complex (*2*). Other blue filters gave less satisfactory results. Absorption cells of 1.0-cm. or 2.0-cm. depth with optically flat windows were used to hold the solution and the water, respectively. Measurements of the photocurrent were made with a vacuum tube voltmeter working on a 6C6, R. C. A. tube, with a 6E5 tube as null point indicator. This applies the principle of the 6F5 circuit of Müller (*6*). The instrument used in this work gave a precision of ±0.2 millivolt, equal to ±0.1 per cent of the color measured.

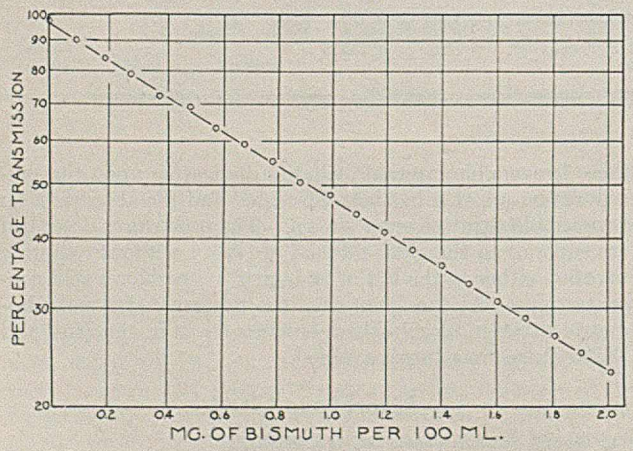

FIGURE 1. RELATIONSHIP BETWEEN LOGARITHM OF PER CENT TRANSMISSION AND BISMUTH CONCENTRATION

For each 100 ml. of solution, 50.0 ml. of 28 per cent (by volume) sulfuric acid, 13.4 ml. of 0.5 per cent Na_2SO_3 solution, and 16.8 ml. of 1.7 per cent KI solution were used with varying amounts of standard bismuth nitrate solution.

Müller (*5*) has shown that the basis for every colorimetric method should be the Lambert-Beer law. In order to establish the justification for the use of this system in a colorimetric procedure the following solutions were prepared, and experiments performed. All reagents were prepared from chemicals of A. C. S. specification.

STANDARD BISMUTH, 0.2321 gram of bismuth nitrate, $Bi(NO_3)_3 \cdot 5H_2O$, dissolved in 50 ml. of 1 to 10 nitric acid and diluted to 1 liter to give a solution containing 10.00 mg. of bismuth per 100 ml.

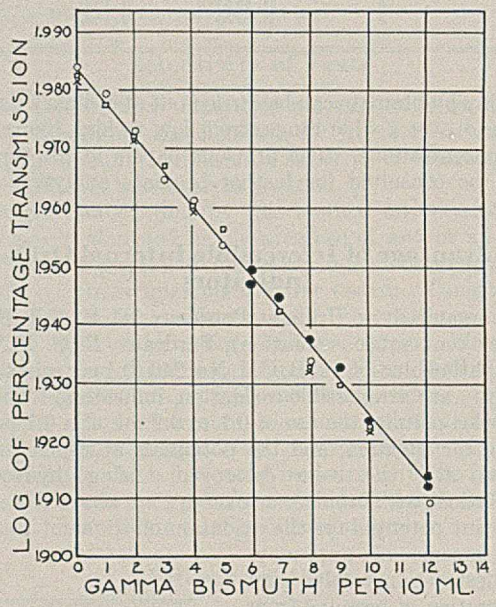

FIGURE 2. RELATIONSHIP BETWEEN LOGARITHM OF PER CENT TRANSMISSION AND 12 MICROGRAMS OR LESS OF BISMUTH

Concentrations of reagents were same as in Figure 1. Cells of 2.0-cm. thickness were substituted for those of 1.0-cm. thickness. Symbols represent determinations made at different times on known concentrations of bismuth. Differences observed at a fixed concentration are a measure of precision of method.

SODIUM SULFITE, 1.00 gram dissolved in water, acidified with 0.8 ml. of concentrated sulfuric acid, and diluted to 200 ml. This solution must be freshly prepared every day.

POTASSIUM IODIDE, 17.0 grams dissolved in 1 liter of water.

SULFURIC ACID, 280 ml. of concentrated sulfuric acid diluted to 1 liter.

Varying amounts of the standard bismuth solution were mixed in each case with 50.0 ml. of sulfuric acid solution, 13.4 ml. of sodium sulfite solution, and 16.8 ml. of potassium iodide solution, and the whole was diluted to 100 ml. in stoppered volumetric flasks. These amounts of reagents were used to get a wide range of bismuth concentration within a measurable transmission. The percentage transmission was then determined for each solution.

Figures 1 and 2 show that the relationship between the logarithm of the percentage transmission and the bismuth concentration is linear; thus, the Lambert-Beer law is obeyed for concentrations up to 2.00 mg. of bismuth per 100 ml. of solution. Figure 2 was prepared as a basis for the determination of from 1 to 10 micrograms of bismuth in 10 ml. of normal blood serum. Absorption cells of 2-cm. thickness and 8-ml.

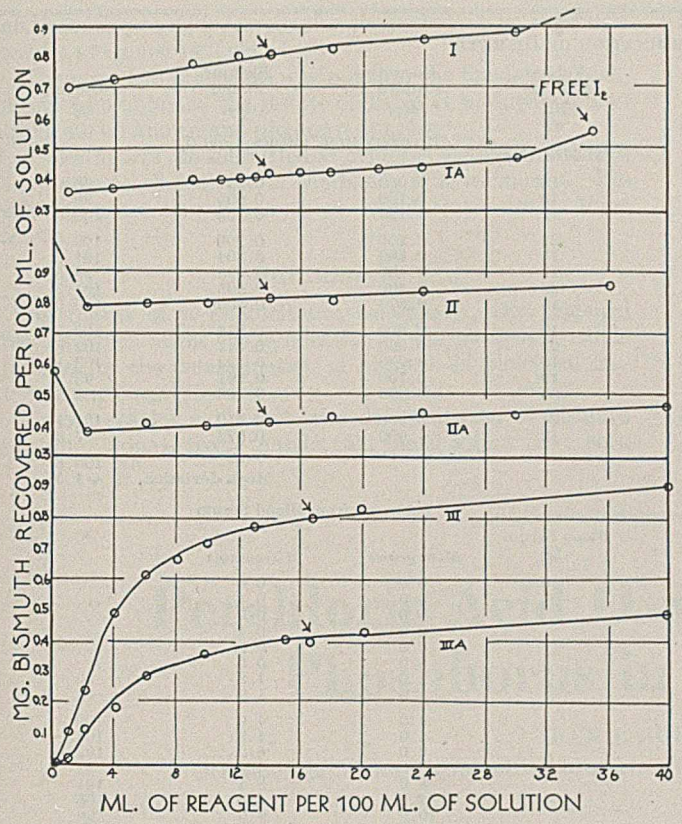

FIGURE 3. INFLUENCE OF VARIATIONS OF REAGENTS UPON RECOVERY OF BISMUTH

One reagent was varied in each curve while others were in same concentrations as in Figure 1.

I, IA. Influence of concentrated sulfuric acid upon recovery of 0.400 and 0.800 mg. of bismuth per 100 ml., respectively

II, IIA. Influence of 0.5 per cent sodium sulfite

III, IIIA. Influence of 1.7 per cent potassium iodide

volume were used. To reduce the accidental errors several measurements were made for each concentration on this curve. The lines do not intercept the axis of ordinates at 100 per cent transmission, because distilled water instead of the reagents (which absorb some light) was used in the reference cell, in order to avoid making up a synthetic mixture of reagents for each determination. Furthermore, if such a mixture were allowed to stand in the open cell it would slowly lose sulfite and free iodine might be liberated. Thus water furnishes a perfectly reproducible reference cell, but by its use no error is introduced into the determination.

To determine the relative effects of the reagents on the color, fixed quantities of standard bismuth solutions were taken, and the concentrations of one of the reagents were varied while all other concentrations were kept the same as in the preparation of the Lambert-Beer's law curve.

Two bismuth concentrations were used for each study—0.400 and 0.800 mg. per 100 ml.—and the results are shown in Figure 3. Curves III and IIIA indicate that the color intensity is a function of the iodide concentration, but as shown by curves I, IA, II, and IIA, it is affected only slightly by the acid and the sulfite. Some sulfite must be present to prevent the precipitation of free iodine, as evidenced by the sudden initial fall (curves II and IIA) in the sulfite curve. If the iodide is in great excess over the sulfite free iodine is liberated, resulting in light absorption. The arrows in Figure 3 indicate the concentrations of the reagents used in obtaining the Lambert-Beer's curve (Figure 1).

To determine the concentration of iron which will interfere, 0.400-mg. and 0.784-mg. samples of bismuth and variable amounts of iron as ferric sulfate were used; the quantities of reagents added were the same as those used in obtaining the transmission curve (Figure 1). Curves IV and IVA, Figure 4, show that the concentration of iron may be as high as 20 mg. without interfering with the determination. Twenty milligrams or less of iron do not cause a change on standing several hours, whereas more than 20 mg. cause the precipitation of iodine.

Separate test analyses with known concentrations of bismuth and various quantities of heavy metals showed that quantitative recovery of bismuth may be obtained in the presence of the following concentration per 100 ml. of heavy metals: 2 mg. of lead, 2 mg. of mercury, 0.5 mg. of copper, and 10 mg. or more of arsenic. As little as 0.5 mg. of silver interferes. Thus it was concluded that the concentration of the above heavy metals normally appearing in tissue will not interfere unless silver is present.

Test analyses showed that, if the solutions are kept securely stoppered after mixing the reagents with the bismuth, the yellow color is stable for as long as 14 days. The same percentage transmission was found at the end of this time as immediately after preparing the color.

Application of the Method to Analyses of Biological Material

URINE. To 100 ml. of urine in a Kjeldahl flask, 10 ml. of concentrated nitric acid and 7.5 ml. of concentrated sulfuric acid are added. This is evaporated to fumes of sulfur trioxide over an open burner. While fuming, a mixture of nitric and perchloric acids (1 to 2) is added dropwise until all color is destroyed. The excess nitric acid is now destroyed by the addition of 1 ml. of 30 per cent hydrogen peroxide, dropwise, to the hot mixture. The peroxide is in turn removed by vigorous boiling. The flask is allowed to cool, about 10 ml. of water are added, and the solution is again evaporated to fumes of sulfur trioxide. The contents of the flask are now rinsed into a 50-ml. volumetric flask, using not more than a total of 25 ml. of water. After cooling, 6.7 ml. of 0.5 per cent sodium sulfite and 8.4 ml. of 1.7 per cent potassium iodide are added and diluted to 50 ml. The solution is centrifuged if not perfectly clear and is then ready for the photometer. The potentiometer is set at zero and the instrument is adjusted to a null point with the phototube dark.

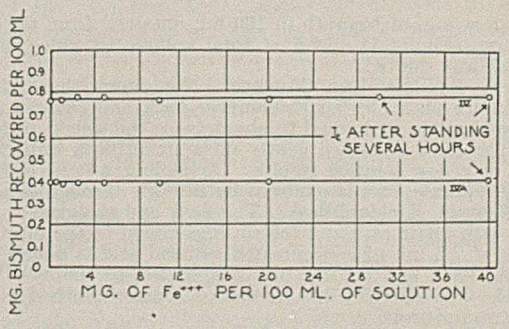

FIGURE 4. INFLUENCE OF IRON, AS FERRIC SULFATE, UPON RECOVERY OF 0.400 AND 0.784 MG. OF BISMUTH PER 100 ML.

The two absorption cells are filled with water, the constant light source is turned on, and the potentiometer is set to a null point. Readings for the right cell, R_0, and the left cell, L_0, are taken alternately by moving them back and forth so as to intercept the beam of light. Three such readings for each cell are, in general, satisfactory. If the readings for the two cells are not the same, a correction is applied in the calculations. The left cell is emptied, rinsed with the solution, and then filled with the solution. Again alternate readings for the unknown, R, and for the water, R_0, are taken.

TABLE I. DETERMINATION OF BISMUTH

Volume of Urine *Ml.*	Bismuth Added *Mg.*	Bismuth Found *Mg.*	Recovery %
	Recovery from Urine		
100	0.000	0.000	...
50	0.000	0.000	...
50	0.000	0.000	...
100	0.000	0.003	...
100	0.050	0.051	102.0
50	0.100	0.098	98.0
100	0.100	0.105	105.0
100	0.150	0.148	98.7
50	0.200	0.202	101.0
100	0.250	0.251	100.4
50	0.300	0.301	100.3
100	0.350	0.348	99.4
50	0.400	0.397	99.3
100	0.450	0.445	98.9
50	0.500	0.493	98.6
50	0.600	0.599	99.8
			Av. 100.1
			Mean deviation, % ±1.3
	Recovery from Tissues		
Kidney *Grams*			
22	0.000	0.000	...
8	0.000	0.000	...
19	0.100	0.102	102.0
10	0.200	0.204	102.0
9	0.200	0.196	98.0
8	0.300	0.315	105.0
9	0.400	0.393	98.3
21	0.600	0.630	105.0
23	0.800	0.806	100.8
Liver			
20	0.000	0.000	...
23	0.000	0.007	...
25	0.000	0.000	...
27	0.200	0.208	104.0
25	0.300	0.306	102.0
Muscle			
13	0.000	0.002	...
17	0.000	0.001	...

Volume of Urine *Ml.*	Bismuth Added *Mg.*	Bismuth Found *Mg.*	Recovery %
	Recovery from Tissues, Cont'd		
Muscle, Cont'd			
12	0.050	0.048	96.0
13	0.100	0.099	99.0
13	0.150	0.153	102.0
22	0.200	0.200	100.0
13	0.300	0.304	101.3
19	0.400	0.398	99.5
20	0.400	0.405	101.3
15	0.500	0.500	100.0
19	0.600	0.616	102.7
22	0.600	0.617	102.8
17	0.700	0.722	103.1
19	0.700	0.691	98.7
20	0.800	0.788	98.5
24	0.900	0.910	101.1
12	1.000	0.973	97.3
			Av. 100.8
			Mean deviation, % ±1.5
	Recovery from Blood Serum		
Blood Serum *Ml.*	*Micrograms*	*Micrograms*	
10	0.0	0.0	...
	0.0	0.3	...
	0.0	0.0	...
	0.0	0.4	...
	0.0	0.0	...
	0.0	0.0	...
	2.0	1.8	90
	2.0	1.9	95
	4.0	4.0	100
	4.0	4.3	108
	6.0	6.2	103
	6.0	5.0	83
	8.0	8.1	101
	8.0	8.6	108
	10.0	9.6	96
	10.0	10.0	100
			Av. 98
			Mean deviation, % ±6

The per cent transmission of the sample is calculated as follows: Cell correction = R_0/L_c, per cent transmission = $R_0/L_0 \times R/R_0 \times 100$, where this R_0/L_0 is usually equal to 1.

The bismuth concentration in mg. per 100 ml. is found by interpolation of the Beer's law curve (Figure 1). The total bismuth content of the sample analyzed is given by:

$$\text{Mg. of Bi} = a \times \frac{b}{100}$$

where a = mg. of bismuth in 100 ml. obtained from the Beer's law curve (Figure 1) and b = total volume to which digested material was diluted.

LIVER, KIDNEY, AND MUSCLE. Not more than 25-gram samples are placed in an Erlenmeyer flask and covered with a volume of nitric acid equal to about twice the weight of the tissue. The flask is heated gently on a steam bath until a clear brown or yellow solution results. On cooling, a layer of fat will usually separate, and is removed by filtering through glass wool into a 500-ml. Kjeldahl flask. The flask and the glass wool are rinsed with nitric acid and the rinsings added to the filtrate. A volume of 7.5 ml. of concentrated sulfuric acid is added to the Kjeldahl flask and the mixture evaporated to fumes of sulfur trioxide. The digestion and estimation are now finished as in the procedure for urine.

BLOOD SERUM. To 10 ml. of normal serum in a 100-ml. Kjeldahl flask, 1.5 ml. of concentrated sulfuric acid and 5 ml. of concentrated nitric acid are added. This is digested as described for urine. By means of 5 ml. of water the residue is rinsed into a 10-ml. volumetric flask and cooled, 1.4 ml. of 0.5 per cent sulfite and 1.7 ml. of 1.7 per cent potassium iodide are added, and the solution is diluted to 10 ml. This is centrifuged and the percentage transmission determined as for urine, substituting cells of 2.0-cm. depth for those of 1.0-cm. depth. The bismuth concentration in micrograms per 10 ml. is found by interpolation of the Beer's law curve (Figure 2).

For the determination of such small quantities of bismuth all glassware used should be first washed with hot nitric acid, all flasks should be kept covered to protect against dust particles, and the rigorous precautions for exact photometry must be followed. Less than 1 microgram of bismuth should not be estimated by the method.

Known amounts of bismuth were added to a series of tissues (cat and rabbit), urine (human), and blood serum (dog) samples. Control experiments using normal tissue, to which

TABLE II. DETERMINATION OF BISMUTH

Total Volume *Ml.*	Bismuth Added *Mg.*	Aliquot Recovery from Urine	Total Bismuth Found *Mg.*	Recovery %
1513	2.50	50	2.54	101.6
		50	2.48	99.2
		50	2.57	102.8
		100	2.51	100.4
		100	2.51	100.4
		100	2.50	100.0
908	2.50	50	2.38	95.2
		50	2.37	94.8
				Av. 99.3
			Mean deviation, %	±2
100	Unknown	10	3.10	...
		10	3.08	...
200	Unknown	10	5.08	...
		10	5.20	...

Total Weight *Grams*	Recovery from Tissue (Liver) *Grams*		
242	25	2.57	...
		2.59	...
262		1.98	...
		1.98	...
291		2.58	...
		2.51	...

no bismuth was added, were also conducted. The analytical results are given in Table I.

The complete procedure was put into the hands of a technician who obtained the results of Table II in duplicate and triplicate on known and unknown samples.

These figures are submitted as evidence that the method is adaptable to clinical use in the hands of a technician. Results on other tissues have been found as precise as these shown.

Summary

By means of a photoelectric colorimeter, the principal factors which affect the application of the potassium iodide method for the determination of bismuth in biological materials were evaluated.

Procedures for a photoelectric determination of bismuth in urine, kidney, liver, muscle, and blood serum have been devised with test analyses included to indicate the accuracy of such procedures.

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Perchloric Acid Oxidation of Organic Phosphorus in Lake Waters

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THE utilization of phosphate, dissolved in lake water, by growing phytoplankton attaches considerable importance to this element and to the phosphorus in the lake. Various studies have shown that only a small portion of the total phosphorus content is present as the readily available phosphate but a large potential supply exists organically bound.

The Denigès (*1*) colorimetric method estimates only the phosphate phosphorus. The amount of organic phosphorus is derived by deducting the phosphate phosphorus from the total phosphorus content. The method as described by Robinson and Kemmerer (*2*) has served very well for the determination of total phosphorus. Briefly, the organic material in a 100-ml. water sample is digested with 0.2 ml. of concentrated sulfuric acid and 0.5 ml. of concentrated nitric acid to convert the organic phosphorus to phosphate. Shortly before sulfuric acid fumes appear 3 ml. of concentrated hydrochloric acid are added to destroy the excess nitric acid.

Although the above method has proved its value, the following alternate procedure is proposed because of its greater simplicity.

To the 100-ml. water sample contained in a 125-ml. Erlenmeyer flask, 0.2 ml. of 72 per cent perchloric acid is added and the sample is evaporated to fumes of perchloric acid. (More acid must be added to hard waters to gain an excess of the acid.) If oxidation of the organic material is slow, as is sometimes the case with water from extractive lakes, the flask may be covered with a watch glass to prevent loss of perchloric acid and the heating continued until the coloration disappears.

The use of perchloric acid as the oxidizing agent has a number of advantages. Only one acid, rather than three, is measured. Undesirable fumes are not inhaled during pipetting or discharged during digesting. The excess nitric acid must be completely removed, as it causes fading of the blue phosphomolybdate complex; the excess perchloric acid does not cause fading. The flask may be covered with a watch glass during digestion to prevent loss of perchloric and phosphoric acids; this cannot be done with the nitric acid because of the retention of the nitric acid. Consequently, with a covered flask there is less need for careful temperature regulation in the digestion to prevent loss of phosphoric acid. The nitric acid is destroyed and expelled at a low temperature, the perchloric acid is present throughout; this means that difficultly oxidizable substances may be subjected to a higher oxidation temperature. Lastly, there is less phosphate impurity in perchloric acid than in the sulfuric-nitric-hydrochloric acid mixture.

The perchloric acid oxidation has proved an efficient method of oxidizing organic phosphorus compounds. First it has been checked by the analysis of Eastman's C. P. glycerophosphate, the phosphorus content of which had been verified by oxidation and gravimetric analysis of macrosamples. Amounts of glycerophosphate approximating the phosphorus content normally found in 100 ml. of lake water were used to simulate lake water conditions. Complete recovery was obtained, as indicated in Table I.

Secondly, the efficiency of the method has been checked by analysis of lake waters which were also analyzed by the sulfuric-nitric-hydrochloric acid method. A comparison of the two methods is included in Table II. Trout Lake water was particularly suited to a critical comparison of the two methods, because in it there was enough extractive organic material to make it more difficult to oxidize than the average lake water. The organic material was as quickly and efficiently oxidized by the perchloric acid as by the nitric-sulfuric acid mixture. During the progress of this

TABLE I. ANALYSIS OF GLYCEROPHOSPHATE

Phosphorus Taken	Phosphorus Found
Mg./l.	*Mg./l.*
0.028	0.029
0.028	0.027
0.028	0.027
0.028	0.027

Table II. Total Phosphorus in Lake Water

	Phosphorus Found	
	$HClO_4$	H_2SO_4-HNO_3-HCl
	Mg./l.	*Mg./l.*
Trout Lake	0.028	0.026
	0.027	0.027
Lake Washington	0.018	0.017
	0.017	0.017
Green Lake	0.024	0.026
	0.026	0.025

Table III. Total Phosphorus in Trout Lake, San Juan Islands, Wash.

	Phosphorus Found	
	Unneutralized	Neutralized
	Mg./l.	*Mg./l.*
H_2SO_4 + HNO_3 + HCl	0.024	0.026
	0.024	0.027
	0.024	
0.2 ml. $HClO_4$	0.023	0.028
	0.022	0.027
0.5 ml. $HClO_4$	0.019	0.026
	0.018	

work the oxidation always proceeded quietly and never with violence, probably because of the quantity and nature of the organic compounds present in lake water, together with the gradual oxidation during evaporation and digestion.

It is known that the residual acid from the digestion has a small but definite inhibiting effect upon the formation of the blue reduction complex. This effect may be prevented by neutralization of the excess acid before development of the blue reduction color. Analyses of Trout Lake are given in Table III to show the effect of neutralization. Using either methyl red or phenolphthalein as the indicator, the acid was carefully titrated with *N* ammonium hydroxide, followed by *N* hydrochloric acid until the acid form of the indicator was just prevalent. The colorimetric comparisons were made with the Zeiss-Pulfrich photometer as described by Wirth and Robinson (*3*). Interference by the methyl red was entirely eliminated by the S-72 color filter (average wave length 7200 Å.). Estimations were also made in Nessler tubes when phenolphthalein had been used as the indicator in the neutralization. Although the inhibiting effect of the excess acid is not large, greater accuracy is undoubtedly obtained when the acid is previously neutralized.

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The Mixed Indicator Bromocresol Green–Methyl Red for Carbonates in Water

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The mixed indicator bromocresol green–methyl red has been applied to the detection of the end point of bicarbonate titrations in concentrations which are found normally in natural or commercial waters. Electrometric titration curves for waters of various compositions show that the final equivalence point pH is definitely dependent on the carbon dioxide content there existing. A method is presented whereby the pH value to be obtained at the final end point can be ascertained before that point is reached. This allows the selection of the proper indicator color at the end point. The glass electrode, bromocresol green–methyl red, methyl orange, and methyl orange–xylene cyanole are compared in titrations of carbonates of various concentrations.

THE estimation of carbonates in extremely dilute solution with methyl orange as indicator is unsatisfactory for two reasons: The color change is difficult to observe in solutions whose pH is changing slowly, and the equivalence point in the majority of titrations of this type comes at pH values too high to permit this indicator to respond correctly. The pH at the final equivalence point is a function of the carbonic acid concentration existing there and this in turn is dependent on the neutralized bicarbonate as well as on the initial carbon dioxide content. In the titration of such solutions methyl orange leads to high results if the carbonic acid content at the final equivalence point is lower than about 1.2 millimoles per liter (73 p. p. m. of carbonic acid). This limit is realized if the end point color is read when the indicator shows its first definite cast of orange (pH = about 4.6). For example, if 0.0200 *N* acid is used this indicator shows a minimum positive error of 4.6 per cent for total carbonate and of 7.2 per cent for bicarbonate when used in a solution initially containing 21.6 p. p. m. of carbonate and 17.5 p. p. m. of bicarbonate.

In the official method the carbonic acid end point is determined by the use of methyl orange (*1*, *3*, *4*), methyl orange–xylene cyanole (*3*), or erythrosin (*1*, *5*). Methyl orange is subject to the errors pointed out above and erythrosin requires special technique in its use. For low carbonates, methyl orange–xylene cyanole leads to higher results than does methyl orange alone. Furthermore, the official method using methyl orange has been shown to be unreliable when carbonates are low (*12*). Therefore, an indicator which would respond more nearly at the carbonic acid equivalence point, when solutions of carbonates or bicarbonates are titrated yielding less than 1.2 millimoles of carbonic acid per liter, would prove useful. In such titrations the solutions are fairly well buffered as the equivalence point is crossed. Any indicator which is to reveal this point correctly must respond over a very narrow pH range. A distinct characteristic of mixed indicators is that they tend to show sharp color changes within narrow pH limits.

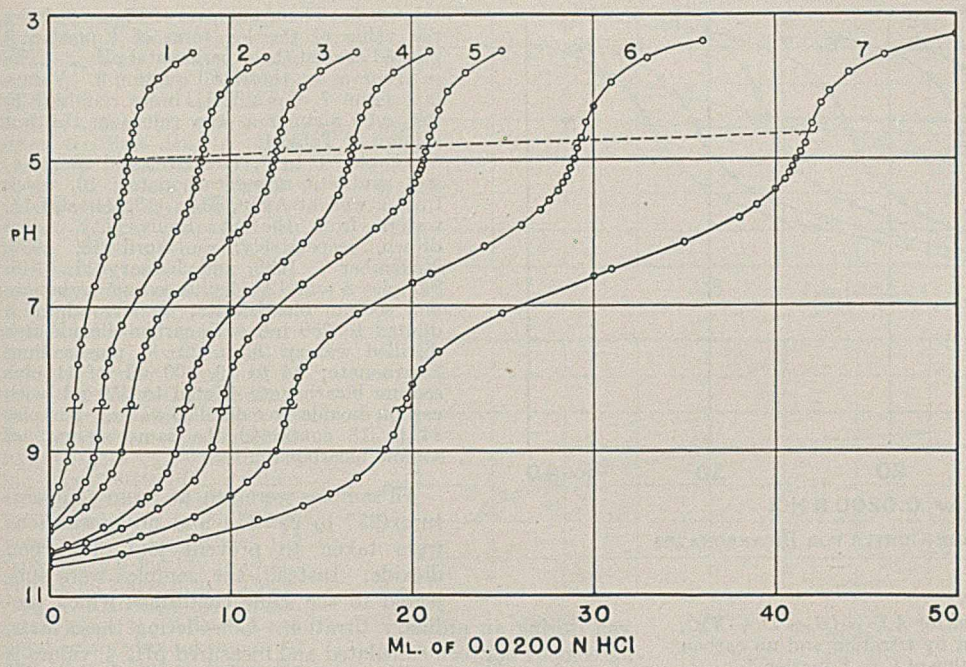

FIGURE 1. TITRATION CURVES OF MIXED CARBONATES

The mixed indicator bromocresol green–dimethyl yellow (*7*) has been recommended for end points in the titration of mixed carbonates. Methyl red and sodium alizarin sulfonate containing certain color screens (*8*) are good indicators for ammonia titrations, showing their maximum response between pH 5.0 and 5.4. Methyl red–bromocresol green (1 to 1) has been recommended as an excellent mixed indicator for titrations in the presence of ammonium ion (*6, 11*). Methyl red (0.05 per cent)–bromocresol green (0.075 per cent) in alcohol (*9, 10*) shows a marked color change near pH 5.1. By lowering the ratio of methyl red to bromocresol green, the maximum response can be made to occur at lower pH values.

Establishment of Equivalence Point pH

A number of solutions containing both sodium carbonate and sodium bicarbonate were prepared and 250 ml. of each were titrated electrometrically with 0.0200 *N* hydrochloric acid (standardized electrometrically against sodium carbonate) using the glass electrode as pH indicator.

The composition of solutions titrated and the equivalence point pH ($pH_{e.p.}$) values are recorded in Table I. The entire titration curves are shown in Figure 1.

These data indicate that, whereas the pH at the first equivalence point (bicarbonate) is constant (8.35) over a final range (after titration with 0.020 *N* hydrochloric acid) of bicarbonate from 0.199 to 1.650 millimoles per liter, the pH of the second equivalence point (carbonic acid) changes from 4.98 to 4.63 for final carbonic acid concentrations of 0.197 to 1.525 millimoles per liter, respectively.

The pH values listed in Table I were obtained by plotting $\Delta pH/\Delta ml.$ as a function of ml. of hydrochloric acid, whereby the volume of hydrochloric acid used to the equivalence point was determined, and the pH equivalence point was read from the curves in Figure 1.

TABLE I. ELECTROMETRIC TITRATION OF SODIUM CARBONATE-BICARBONATE SOLUTIONS WITH HYDROCHLORIC ACID

Solution No.	Na_2CO_3	$NaHCO_3$	0.0200 *N* HCl to Equivalence Point HCO_3^-	0.0200 *N* HCl to Equivalence Point H_2CO_3	pH at Equivalence Point HCO_3^-	pH at Equivalence Point H_2CO_3
	Millimoles per liter		*Ml.*	*Ml.*		
1	0.151	0.049	1.89	4.39	8.35	4.98
2	0.231	0.215	2.88	8.45	8.35	4.93
3	0.360	0.286	4.50	12.58	8.34	4.87
4	0.601	0.140	7.51	16.77	8.35	4.83
5	0.765	0.141	9.57	20.90	8.35	4.79
6	1.065	0.212	13.31	29.27	8.35	4.72
7	1.567	0.210	19.59	41.81	8.35	4.63

Selection of Indicator

Since methyl orange or methyl orange–xylene cyanole responds visually at pH values lower than approximately 4.6, the indicator needed must show its response in the range of pH between 4.60 and 5.00. Three drops of a mixed indicator, made by dissolving 0.02 gram of methyl red and 0.10 gram of bromocresol green in 100 ml. of 95 per cent alcohol, when used in either 100 or 250 ml. of solution, show a maximum color change over this pH range. Table II gives the colors which this mixed indicator shows in terms of the pH of the solution; as the pH changes from 5.0 to 4.6 the indicator color changes from light blue or lavender gray to a light pink. With practice, pH values may be determined without comparison to 0.1 pH unit within this range by the use of this indicator.

TABLE II. COLOR OF THREE DROPS OF MIXED INDICATOR IN 100 OR 250 ML. OF SOLUTION

pH of Solution	Color
5.2 and above	Blue with trace of green
5.0	Light blue with lavender gray
4.8	Light pink gray with cast of blue
4.6	Light pink
Below 4.6	Pink or rose

Estimation of pH at Carbonic Acid Equivalence Point

It is possible to estimate the pH at the carbonic acid equivalence point before that point is reached, provided the original solution contains no excess carbon dioxide.

Let the final concentration of carbonic acid equal *C;* then from the dissociation: $H_2CO_3 \rightleftarrows H^+ + HCO_3^-$, it follows that

$$[H^+]_{e.p.} = [K_1(C - [H^+])]^{1/2}$$

or

$$pH_{e.p.} = {}^1/_2\, pK_1 - {}^1/_2 \log (C - [H^+])$$

If it is assumed $C - [H^+] = C$, then

$$pH_{e.p.} = {}^1/_2\, pK_1 - {}^1/_2 \log C \qquad (1)$$

If the solution at the equivalence point contains 0.196 millimole of carbonic acid per liter (sample 1, Table I) an error of 0.04 pH unit is introduced and if the carbonic acid content rises to 0.431 millimole per liter (sample 2, Table I) the error is only 0.01 pH unit by this assumption. If *B* ml. of mixed carbonate require *D* ml. of *A* normal acid to reach the bicarbonate equivalence point and *X* additional ml. of the same acid to reach the carbonic

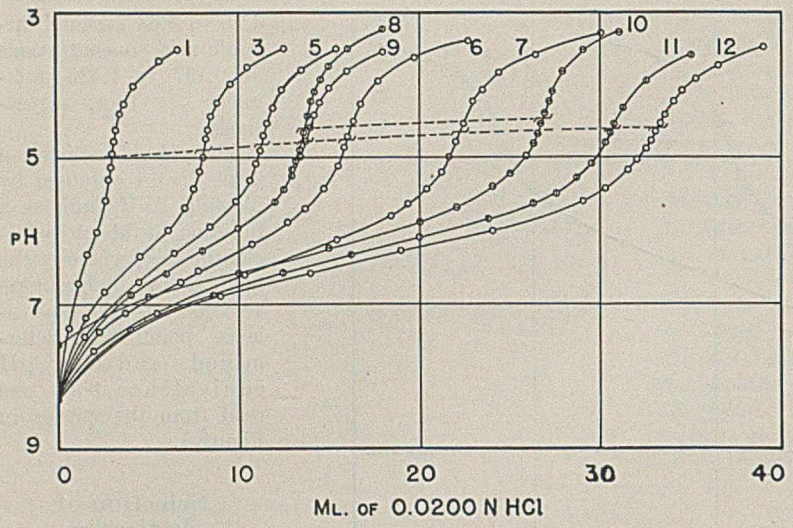

FIGURE 2. TITRATION CURVES FOR BICARBONATES

acid equivalence point, then at the latter $AX = (B + D + X)C$, if all the carbonic acid is developed by titration and no carbon dioxide is lost. Substituting this value of C in Equation 1,

$$pH_{e.\,p.} = {}^1/_2\,pK_1 - {}^1/_2 \log A + {}^1/_2 \log\left(\frac{B+D}{X} + 1\right) \quad (2)$$

If $A = 0.0200\ N$ acid and $pK_1 = 6.50$, Equation 2 becomes

$$pH_{e.\,p.} = 4.10 + {}^1/_2 \log\left(\frac{B+D}{X} + 1\right) \quad (3)$$

Table III shows the results of 24 separate electrometric titrations.

Headings for columns 2, 3, and 4 have the same significance as in Equation 3. The values in column 5 are those in column 4 adjusted to a common basis of 250 ml. of bicarbonate titrated. Columns 6 and 7 show, respectively, the pH at the carbonic acid equivalence point as calculated by Equation 3 and as determined from the titration curves. In Figure 2 titration curves of bicarbonate to carbonic acid for the first twelve solutions of Table III are plotted as milliliters of 0.0200 N hydrochloric acid against pH. In the lower half of Figure 3 the values of experimental $pH_{e.\,p.}$ (column 7 of Table III) are plotted against the values in column 5. The upper part of Figure 3 shows the value of the log term of Equation 3 plotted against the experimental $pH_{e.\,p.}$. The solid curves are those of Equation 3. Values in column 7 of Table III were obtained in the same manner as the values in the last column of Table I.

Samples 1 to 7, inclusive, and 23 represent synthetic mixed carbonates; 10, water from a well at Avon, Mo.; 13, 24, and 11, waters from the St. Louis City mains drawn, respectively, on April 18, 1939, November 7, 1939, and January 11, 1940. Samples 8 and 12, 11 plus sodium carbonate and sodium bicarbonate; 9, 100 ml. of 8 diluted to 250 ml. with carbon dioxide–free distilled water; 20 to 22, 11 plus sodium bicarbonate; 14 to 19, 100 ml. of 11 plus sodium bicarbonate diluted to 250 ml. with carbon dioxide–free distilled water. Samples 14 to 18 contained the same amount of sodium bicarbonate.

All samples were run at room temperature (23° to 25° C.) and no precautions were taken to prevent loss of carbon dioxide. Instead, the samples were subjected to the same conditions which prevail during an ordinary titration. Considering these facts, agreement between calculated and measured $pH_{e.\,p.}$ values is very close. This agreement allows Equation 3 to be used to predict the final $pH_{e.\,p.}$ and permits the selection of the proper indicator color for the end point.

TABLE III. COMPARISON OF pH AT CARBONIC ACID EQUIVALENCE POINT

(Calculated with experimental values)

Sample No.	B	0.0200 N HCl D	0.0200 N HCl X	$\frac{250}{B+D}X$	Equivalence Point pH — Equation 3	Equivalence Point pH — Experimental ±0.03
	Ml.	*Ml.*	*Ml.*			
1	250	1.39	2.99	2.98	5.07[a]	4.98
2	250	2.88	5.55	5.49	4.93	4.93
3	250	4.50	8.08	7.94	4.86	4.87
4	250	7.51	9.27	9.01	4.83	4.83
5	250	9.57	11.30	10.90	4.79	4.79
6	250	13.31	15.98	15.20	4.72	4.72
7	250	19.59	22.21	20.65	4.66	4.63
12	250	1.00	33.12	32.99	4.57	4.58
8	100	0.40	13.25	32.99	4.57	4.58
9	250	0.30	13.40	13.39	4.75	4.76
10	100	0.00	26.80	67.00	4.44	4.45
11	250	0.00	30.60	30.60	4.58	4.60
13	250	0.00	6.95	6.95	4.88	4.85
14	250	0.00	13.40	13.40	4.75	4.83
15 to 18[b]	250	0.00	13.50	13.50	4.75	4.74
19	250	0.00	14.45	14.45	4.73	4.74
20	100	0.00	12.75	31.97	4.56	4.58
21	100	0.00	13.20	33.00	4.57	4.61
22	100	0.00	13.64	34.10	4.56	4.59
23	100	0.00	21.55	53.87	4.48	4.44
24[c]	250	2.10	10.20	10.12	4.81	4.83

[a] This value should be 5.11, since $C - [H^+] \neq C$ for this solution.
[b] Four separate samples of same composition gave identical results.
[c] Value not included in Figure 3.

TABLE IV. COMPARATIVE TITRATIONS

(Ml. of 0.0200 N hydrochloric acid completely to titrate different waters as determined electrometrically and with mixed indicator, 0.02 gram of methyl red plus 0.10 gram of bromocresol green per 100 ml. of 95 per cent alcohol)

Sample No.		0.0200 N HCl Electrometric	0.0200 N HCl Indicator (arithmetic mean)	Number of Titrations	Deviation of Arithmetic Mean from Electrometric	Maximum Difference from Electrometric	Average Deviation in Single Titration
	Ml.	*Ml.*	*Ml.*		%	%	± *Ml.*
1	250	4.38	4.33	15	1.1	2.3	0.03
2	250	8.43	8.39	12	0.5	1.4	0.03
3	250	12.58	12.53	12	0.4	1.0	0.03
3	100	5.03	5.04	10	0.2	0.4	0.01
4	250	16.78	16.69	11[a]	0.5	0.9	0.02
5	250	20.87	20.85	13	0.1	0.6	0.04
5	100	8.35	8.34	10	0.1	0.5	0.02
7	100	16.72	16.68	5[b]	0.2	0.4	0.01
13	250	6.95	6.85	7	1.4	2.8	0.05
24	250	12.30	12.25	11	0.4	0.7	0.02
25[c]	100	12.52	12.53	7[d]	0.1	0.4	0.03

[a], [b], [d] One value eliminated in each case on the basis that deviation from mean was greater than 4 × average deviation of a single titration. Values eliminated: [a], 16.86; [b], 16.58; [d], 12.42.
[c] Sample not included in Table III. Synthetic carbonate-bicarbonate.

Titrations with Mixed Indicator

Samples of bicarbonates and of mixed carbonates as well as waters from the St. Louis City mains were titrated using 2 drops of 1 per cent phenolphthalein followed by 3 drops of mixed indicator. If the solution assumed a pink color with phenolphthalein, titration was carried on until the last trace of pink was discharged. Three drops of mixed indicator were added to the solution reacting neutral to phenolphthalein and the titration was continued to the end point. The color of the mixed indicator at the end point was taken as that consistent with the $pH_{e.\,p.}$ read from the curve in the lower part of Figure 3. All titrations were made with 0.0200 N hydrochloric acid (standardized electrometrically against sodium carbonate) with the solutions in beakers resting on a white background. The indicator color change is best observed by looking downward through the solution. If the solution is placed in a white porcelain casserole or evaporating dish the end point is more distinct. Results of these titrations as compared to the electrometric values are shown in Table IV.

In order to compare the results obtained by use of the mixed indicator with those given with methyl orange and methyl

orange–xylene cyanole, six synthetic sodium carbonate solutions were titrated using each of the three indicators (Table V). End points for methyl orange (*3*) were taken when the first definite shade of orange was observed and those for methyl orange–xylene cyanole (*3*) when the first trace of gray appeared in green. When phenolphthalein was used in each sample it became colorless at a volume of acid approximately one half of that used when methyl red–bromocresol green began to change in color. One half of the total methyl red–bromocresol green titer, when referred to the curve in the lower half of Figure 3, gave the following approximate $pH_{e.\,p.}$ values for the separate solutions: 4.85 for 30 and 50 p. p. m. of carbonate; 4.75 for 70 and 100 p. p. m.; 4.6 for 150 p. p. m.; and 4.5 for 250 p. p. m. Therefore with methyl red–bromocresol green the following end point colors were taken: in 30 and 50 p. p. m. of carbonate the lavender cast was discharged and a pink developed on a background of light blue; in 70 and 100 p. p. m. the bluish cast was discharged leaving a pinkish gray; in 150 p. p. m. the pinkish gray was converted to a pink; and in 250 p. p. m. the pinkish gray was converted to pink and the solution vigorously stirred for 30 seconds, during which time a bluish cast appeared. The removal of carbon dioxide during stirring causes the pH to rise to about 4.8, which is within the range of the indicator. This color was then converted to pink. (If this solution was again stirred for 30 seconds a grayish pink developed which could be converted to pink with 1 or 2 drops of acid. Therefore, one vigorous stirring of 30 seconds removed sufficient carbon dioxide to allow the indicator, methyl red–bromocresol green, to give the correct end point in the titration of the 250 p. p. m. of carbonate.)

The hydrochloric acid used in these titrations was standardized by diluting 0.8835 gram of pure sodium carbonate to 1 liter with carbon dioxide–free distilled water, diluting 10.00 and 25.00-ml. portions of this solution to 100 and 250 ml., respectively, with carbon dioxide–free distilled water, and titrating both electrometrically and in the presence of the mixed indicator. Results: electrometric, 8.35 and 20.87 ml.; using mixed indicator, 8.34 and 20.85 ml. of hydrochloric acid. Normality of hydrochloric acid; electrometric, 0.01996; using mixed indicator, 0.0200.

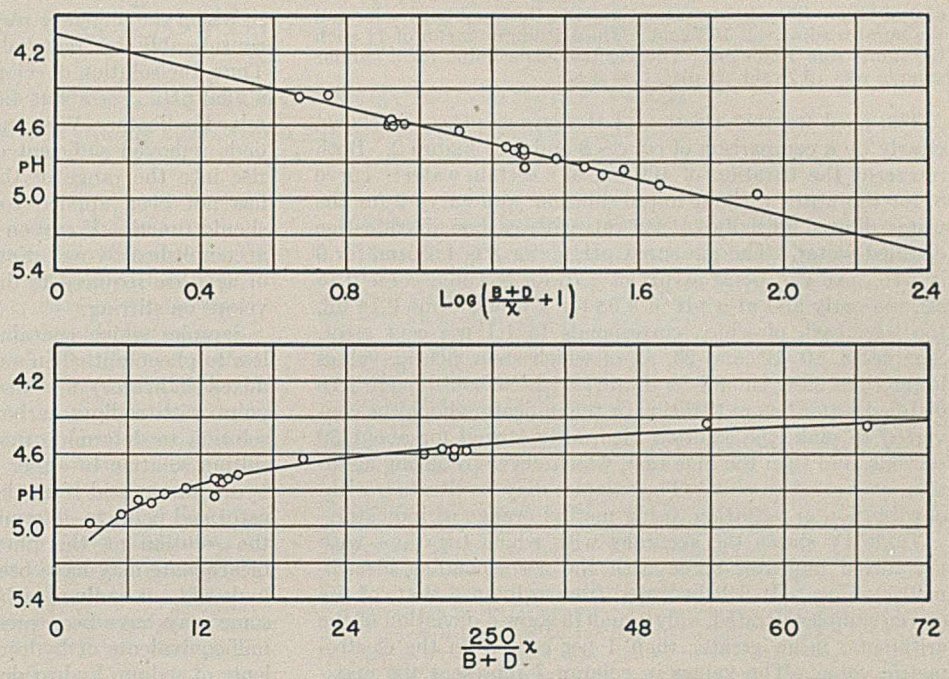

FIGURE 3. EQUIVALENCE POINT pH IN MIXED CARBONATE TITRATIONS

Discussion of Results

Figures 1 and 2 and especially Figure 3 show that, if the carbonic acid equivalence point is to be determined with reasonable accuracy in very dilute solutions, titrations must be carried to pH values consistent with the carbonic acid concentration.

Each electrometric titration was carried out with 3 drops of mixed indicator in the solution and for each sample whose $pH_{e.\,p.}$ was higher than approximately 4.60 the indicator showed a color change as the equivalence point was crossed. In samples 10 and 23 ($pH_{e.\,p.}$ = 4.45 and 4.44, respectively) the indicator developed a pink before the equivalence point was reached. Sample 10, which contained excess carbon dioxide initially, gave a $pH_{e.\,p.}$ value higher than would have been expected if all carbon dioxide had been retained by the solution. In samples 11, 20, 21, and 22 the indicator passed from light pink gray to light pink as the equivalence point was approached.

The differential method applied to the titration curves gave $pH_{e.\,p.}$ values which could be estimated to 0.03 pH unit. Variations in these values from those calculated by Equation 3 are caused by at least two factors: loss of carbon dioxide during titration, and error in estimation of the value.

The method used for estimating the $pH_{e.\,p.}$ before it was reached may be best shown by quoting a specific example.

During one of the titrations of 250 ml. of sample 4 of Table IV, the color of 2 drops of 1 per cent phenolphthalein was discharged with 7.50 ml. of 0.0200 *N* acid and 3 drops of the mixed indicator had lost its green and developed a cast of lavender (pH = 5.0 from Table II) with 16.30 ml. of acid. From Figures 1 and 2 it can be seen that at this pH the volume of acid added is within 0.5 ml. of the equivalence point. The values B = 250, D = 7.50, and X = 8.8 (approximately), when substituted in Equation 3, give $pH_{e.\,p.}$ = 4.84. From Table II it is seen that the color to be expected at a pH of 4.8 is a light pink gray with a

TABLE V. TITRATION WITH DIFFERENT INDICATORS

(Comparison of methyl red–bromocresol green, methyl orange, and methyl orange–xylene cyanole indicators in titrations of carbonates with 0.0200 *N* HCl)

CO_3^{--} in water, p. p. m.	30	50	70	100	150	250
Water titrated, ml.	250	100	100	100	50	50
HCO_3^- developed, p. p. m.	29.8	48.6	67.2	93.9	136	210
0.0200 *N* HCl required, ml.	12.50	8.33	11.67	16.67	12.50	20.83
Methyl red–bromocresol green, ml. of HCl, mean[a]	12.55(19)	8.33(15)	11.67	16.70(10)	12.52	20.86
Error, %	+0.4	0	0	+0.2	+0.2	+0.2
Maximum deviation from mean, ml.	0.08	0.03	0.02	0.08	0.04	0.04
Methyl orange[b], ml. of HCl, mean[a]	13.06	8.48	11.73	16.74(6)	12.51	20.85
Error, %	+4.5	+1.8	+0.5	+0.4	+0.1	+0.1
Maximum deviation from mean, ml.	0.18	0.07	0.09	0.06	0.05	0.03
Methyl orange–xylene cyanole[c], ml. of HCl, mean[a]	13.17	8.60	11.92	16.92	12.68	20.96
Error, %	+5.3	+3.2	+2.1	+1.5	+1.4	+0.6
Maximum deviation from mean, ml.	0.17	0.03	0.08	0.11	0.03	0.01

[a] Arithmetic mean of five titrations unless indicated by number of titrations in parentheses.
[b] End point taken when first definite shade of orange appeared.
[c] End point taken when first trace of gray appeared in green.

cast of blue. Titration was continued to this quality of color and the buret reading was 16.72 ml. The arithmetic mean of 11 such titrations was 16.69 ml. The electrometric value for a similar sample was 16.78 ml. at $pH_{e.\ p.} = 4.83$.

The need for the selection of the proper $pH_{e.\ p.}$ is shown clearly by a comparison of curves 8 and 9 of Figure 2. Both represent the titration of 100 ml. of a certain water: curve 8, for the water without initial dilution, and curve 9 for the water diluted initially to 250 ml. with carbon dioxide–free distilled water. The measured $pH_{e.\ p.}$ for 8 is 4.58 and for 9 is 4.76. An end point at pH of 4.76 for 8 would come 0.15 ml. too early and at a pH of 4.58 for 9 would come 0.15 ml. too late, each of which corresponds to 1.1 per cent error. Samples 8, 10, 12, and 23, all of which show $pH_{e.\ p.}$ values lower than 4.60, should be diluted with carbon dioxide–free distilled water before titration or the indicator should be converted to pink, the solution vigorously stirred for about 30 seconds, and then the blue cast, which develops during agitation, converted to pink. The samples may be titrated, without dilution or agitation, using methyl orange as indicator.

Table IV shows the accuracy with which titrations with the mixed indicator agree with the corresponding electrometric values. It will be noted from column 6 that, of the eleven samples titrated, only 1 and 13 show a deviation of the arithmetic mean greater than 1 per cent from the electrometric value. The values in column 7 represent the maximum difference (a single determination) shown by any titration from the respective electrometric value. Of the 113 titrations represented in Table IV, no value obtained differed from the electrometric value by more than 2.8 per cent.

The titration data recorded in Table V show that the mixed indicator is more reliable than either methyl orange or methyl orange–xylene cyanole except in solutions in which the bicarbonate developed is high. The bicarbonate is the component which is titrated to the final end point. It is apparent from Table V that the error of the mean for titrations in the presence of mixed indicator is less than 0.5 per cent for all concentrations of bicarbonate titrated. The corresponding errors for methyl orange as indicator are greater than 0.5 per cent for solutions developing bicarbonate less than about 67 p. p. m. The errors are greater for all solutions in which methyl orange–xylene cyanole was used.

The hydrochloric acid used was standardized electrometrically and with mixed indicator. The indicator method gave a normality which was within 0.2 per cent of the electrometric value. The concentration of sodium carbonate used for standardization corresponded to 50 p. p. m. of initial carbonate. If the acid had been standardized with methyl orange its normality would have been 0.01966 (from values in Table V), which would have given an error in the mean of +2.7 per cent for 29.8 p. p. m. of bicarbonate and of −1.6 per cent for 210 p. p. m. of bicarbonate. If the acid is standardized against sodium carbonate, with methyl orange, where the carbonate is 70 p. p. m. or greater it may be used for solutions in which the bicarbonate at the phenolphthalein end point is 67 p. p. m. or greater, but not for more dilute solutions without the introduction of a positive error. Therefore one should standardize the acid against a solution containing approximately the same concentration of bicarbonate at the phenolphthalein end point as the solution which is to be titrated when methyl orange is used. Methyl orange–xylene cyanole is less desirable from this standpoint than methyl orange alone.

The curve in the lower half of Figure 3 explains the reason this type of variation is to be expected. The final $pH_{e.\ p.}$ is a function of the bicarbonate titrated and no indicator can show the correct end point unless the indicator color is selected to correspond to the correct $pH_{e.\ p.}$ for the solution in question, or unless the final pH value is adjusted to that to which the indicator responds. The final pH may be raised considerably by removal of carbon dioxide from solution. Thus, the solution developing bicarbonate of 210 p. p. m. has a final $pH_{e.\ p.}$ of about 4.5, which is outside the range of the mixed indicator. Vigorous agitation of the solution for 30 seconds removed sufficient carbon dioxide to allow the pH to rise into the range of the indicator. The mixed indicator has not been applied to more concentrated solutions but should function if carbon dioxide is removed. This could be accomplished by agitation of the solution until 1 or 2 drops of acid are required to discharge the cast of blue which develops on stirring.

Samples which contain free carbon dioxide (react colorless to phenolphthalein and alkaline to methyl orange, or the mixed indicator) are titrated to the phenolphthalein end point with sodium carbonate (*4*) or sodium hydroxide (*2*) solution to determine the carbon dioxide content. The resulting solution in either case can be titrated with 0.020 *N* hydrochloric acid from the phenolphthalein to a mixed indicator end point to determine the total bicarbonate present in the solution at the phenolphthalein end point. All this bicarbonate may have been developed by reaction of sodium hydroxide or sodium carbonate on carbon dioxide, although some may have been present in the original solution. The milliequivalents of hydrochloric acid minus the milliequivalents of sodium hydroxide or sodium carbonate will give the milliequivalents of bicarbonate in the original solution.

The mixed indicator can be used to detect the end point in all titrations of bicarbonate with acid provided the $pH_{e.\ p.}$ is not lower than 4.60. If materials are present which show buffer action other than that of bicarbonate with carbonic acid the above principles will not apply. The indicator, however, does show the correct end point for phosphates (pH = 4.6) and may be used in their presence.

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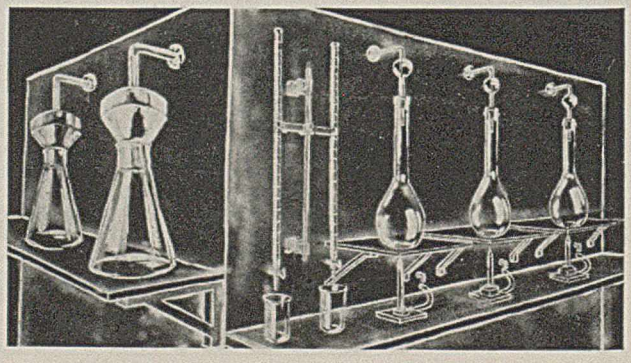

Studies on Soybean Carbohydrates

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Neither the identity of all carbohydrates occurring in soybeans nor their percentage distribution has yet been well established. Evidence that this is so is to be found in the scanty data in the literature, in the diversity in manner of reporting carbohydrate forms, and in the lack of agreement of figures given. This paper presents analytical data on amounts of sugars, galactans, and pentosans found in several edible varieties of soybeans at different times during their maturation from a very immature green vegetable stage to the mature seed. The three carbohydrates mentioned were determined because preliminary data indicated that they might be interrelated during growth. Total carbohydrates and total and reducing sugars tended to decrease as the beans became more mature, while galactans and pentosans tended to increase.

Qualitative separation of hemicellulose fractions was attempted by methods which have heretofore been employed with woody and herbaceous materials. The relatively large amount of protein present in soybeans interfered greatly in this procedure, but evidence was obtained that the hemicelluloses of soybeans are a mixture of galactoarabans containing galacturonic acid.

A SUMMARY is given in Table I of such data on the percentages of different carbohydrates in soybeans as could be found in the literature. Since each investigator has specified certain carbohydrate forms, some of the data are scarcely comparable. In addition, other workers have identified certain of the carbohydrates—for example, Kraybill, Smith, and Walter (*12*) isolated crystalline sucrose from soybeans. Tanret (*21*, *22*) claimed to have separated and identified stachyose. Herissy and Sibassié (*11*) indicated the presence of stachyose and raffinose. Although the authors have never identified starch in soybeans by microscopical methods, it has been reported by others to be present in amounts varying from none to 5 per cent or more, as determined by chemical analysis. Certainly the percentage of starch is highly insignificant in comparison with the quantities present in Lima, navy, and other kinds of beans. In the present investigation, sugars, galactans, and pentosans were determined, since preliminary data (*26*) indicated that these carbohydrates might be interrelated during growth.

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Analyses for Sugars, Pentosans, and Galactans

MATERIAL. The soybeans used were grown by the Agronomy Department of the University of Illinois, at Urbana. Sugar analyses were run on beans of the 1936 and 1937 crops; analyses for pentosans and galactans on those of the 1938 crop. The seven varieties for which sugars, pentosans, and galactans are being reported included Emperor, Funk Delicious, Hokkaido, Imperial, Jogun, and Willomi, six varieties rated "very good" for edible purposes by Woodruff and Klaas (*25*), and also Illini, a field variety which received a rating of "good" from the same investigators. Sugars only are being reported for Fuji and Higan, which also received a rating of "good".

Samples were analyzed at four stages of maturity. The very immature stage was designated as "precooking". The "cooking" stage refers to beans which could be shelled out and used as green vegetables, similar to peas. The "postcooking" stage is that in which the beans were too old to be suitable as green vegetables, but were not yet mature. The fourth was the mature stage.

PREPARATION OF SAMPLES. The green samples were blanched in the pods 2 minutes to facilitate shelling, as well as to arrest enzyme activity. They were cooled immediately under running water, shelled out at once, and weighed. Two 100-gram samples of shelled beans of each variety were preserved for sugar analyses by boiling for 10 minutes in 500 ml. of 75 per cent alcohol, to which 3 grams of calcium carbonate had been added to keep the pH sufficiently high to prevent hydrolysis of the complex sugars. The samples were cooled, stoppered, and stored in the dark until analyses were made.

The green shelled soybeans to be used for pentosan and galactan analyses were immediately ground through a Russwin No. 1 handmill, and dried at once for 48 hours at 80° C. They were then reground to pass 32-mesh, weighed, extracted for 24 hours by refluxing with petroleum ether (b. p. 30–60° C.) to remove oil, dried, weighed, and reground to pass 100-mesh.

The mature beans were prepared for sugar analysis by merely grinding to pass a No. 32 grit gauze. Otherwise the mature beans were treated like the green beans—i. e., ground to pass 32-mesh, oil-extracted, and reground to pass 100-mesh.

All samples used for pentosan and galactan determinations were treated as follows: Portions of approximately 5 grams each were weighed accurately into 250-ml. centrifuge bottles. Each was extracted four times with distilled water, to remove proteins; four times, 1 hour each, with boiling 80 per cent alcohol, to remove sugars; four times, 0.5 hour each, with cold 10 per cent alcohol to remove dextrins; and four times, 0.5 hour each, with boiling water, to remove any residual dextrins, etc. The wash liquor was centrifuged off after each extraction. The last three sets of extractions were those recommended by the Com-

TABLE I. PERCENTAGES OF CARBOHYDRATE CONSTITUENTS OF SOYBEANS

Form of Carbohydrate Reported	Summary of Analytical Data Given						
	Street and Bailey (*20*)[a]	Burrell and Wolfe (*5*)[b]	Bailey, Capen, and LeClerc (*4*)[c]	Woodruff and Klaas (*25*)[d]	Satow (*18*)[e]	Chiu (*6*)[f]	Gerhardt (*10*)[g]
Sugars	..	...	7.0	7.18–9.62	..	..	11.12
Sucrose	3.31	4.0–6.39	..	..	5.90	..	..
Invert sugar	0.07	...	..	..	..	..	..
Reducing sugars	..	0.053–0.229	..	Trace	..	..	..
Dextrose after hydrolysis	..	3.27 –4.45	..	..	..	..	..
Stachyose	..	...	..	..	3.52	..	..
Raffinose	1.13	0.86–1.12	..	..	..	..	..
Starch	0.50	0–0.16	5.6	0.0	..	..	
Dextrins	3.14	2.39–5.53	..	..	..	..	2.84
Pentosans	4.94	2.51–3.24	4.4	..	3.80	4.41–5.24	..
Galactans	4.86	1.15–2.08	..	..	4.62	..	..
Total hemicellulose	..	...	..	..	..	..	5.95
Undetermined hemicellulose	0.04	...	..	..	..	..	..

[a] Hollybrook variety, 12.6 per cent moisture.
[b] Five edible varieties.
[c] Average of many varieties, 8 per cent moisture.
[d] Fourteen edible varieties, 7 per cent moisture.
[e] Analyses by Yakawa.
[f] Four varieties.
[g] Varieties not stated, 5.36 per cent moisture.

TABLE II. PERCENTAGES OF SUGARS, PENTOSANS, AND GALACTANS IN SOYBEANS DURING MATURING

(Vacuum-dried basis)

Variety of Soybeans	Stage of Maturity	Total Carbohydrates (by Diff.) 1936	Total Carbohydrates (by Diff.) 1937	Total Sugars 1936	Total Sugars 1937	Reducing Sugars, 1937	Pentosans, 1938	Galactans, 1938
Emperor	Precooking	...	...	...	...	..	3.05	1.21
	Cooking	...	41.67	...	18.27	5.66	2.39	2.02
	Postcooking	...	...	...	...	..	3.65	2.13
	Mature	...	43.91	...	9.11	..	3.53	2.03
Funk Delicious	Precooking	45.63	45.88	25.45	26.54	8.11	2.71	1.55
			44.25		20.30	6.84		
	Cooking	42.89	41.93	21.34	16.04	5.22	2.43	2.68
		38.87	39.66	17.93	16.80	5.13		
		38.01		14.83				
	Postcooking	35.17	39.17	13.90	16.24	5.03	2.91	2.35
			38.72		13.28	3.98		
	Mature	32.41	33.35	10.35	10.28	..	3.66	2.56
Hokkaido	Precooking	42.94	46.37	26.90	19.44	7.42	2.38	1.38
	Cooking	42.75	44.31	20.67	19.44	6.97	3.09	1.84
		39.07	44.49	16.44	18.29	6.39		
	Postcooking	35.71	41.69	9.02	14.19	5.63	3.78	3.14
			36.36		14.55	5.34		
			34.77		14.07	4.89		
	Mature	33.31	37.53	9.40	7.56	..	3.79	2.60
Imperial	Precooking	...	...	...	...	..	2.36	1.30
	Cooking	40.48	38.98	16.28	17.19	5.28	2.98	1.71
	Postcooking	...	...	...	...	..	2.81	2.55
	Mature	32.23	35.94	10.32	9.46	..	3.65	2.26
Jogun	Precooking	...	...	...	...	..	2.46	1.37
	Cooking	31.68	49.70	12.38	16.94	5.98	2.64	1.79
	Postcooking	...	...	...	...	..	3.39	2.24
	Mature	33.86	37.26	9.96	9.02	..	3.73	1.99
Willomi	Precooking	44.87	43.84	31.66	27.26	9.70	2.50	1.20
	Cooking	42.91	42.25	22.01	22.83	7.40	2.98	2.24
		42.23	37.24	20.02	20.21	6.31		
		35.56	33.00	14.76	15.38	4.96		
	Postcooking	33.28	32.19	10.24	14.76	4.45	3.58	2.33
		34.02	31.94		14.27	4.23		
	Mature	31.70	35.69	9.27	9.79	..	3.40	2.72
Fuji	Precooking	38.44	51.27	23.34	20.70	6.60	..	..
		36.58		21.19				
	Cooking	37.27	49.64	17.40	18.88	6.05	..	..
		34.75	46.54	16.42	16.68	5.15		
	Postcooking	31.61	35.76	15.29	14.39	4.52	..	..
			36.50		5.94	1.77		
	Mature	35.47	36.82	8.78	8.01	..	..	..
Higan	Cooking	34.33	43.92	11.66	17.85	5.66	..	..
	Mature	34.94	35.18	10.29	8.49	..	..	..
Illini	Precooking	47.01	47.72	21.22	16.46	5.78	2.58	1.34
	Cooking	39.41	46.61	19.20	16.37	5.39	2.43	1.81
		39.20	38.60	15.25	14.64	4.79		
	Postcooking	35.22	42.18	12.30	13.08	3.98	3.76	1.51
			39.55		9.23	1.80		
	Mature	31.08	40.50	10.34	9.11	..	3.58	2.21

mittee on Methods of Chemical Analysis of the American Society of Plant Physiologists (*23, 24*). These exhaustive extractions were sufficient to have removed all sugars. From the nature of the extraction liquors used, it is felt that the probability of the loss of the two polysaccharides at this stage was slight. Loss would have caused low final values, furthermore, and the actual values obtained are well within the range of those reported by other investigators.

DETERMINATION OF SUGARS. The alcohol was drained from the green soybeans, as the first extract. The beans were ground in a mortar and extracted with boiling 70 per cent alcohol for 1 hour. The suspension was cooled and filtered. This was repeated three times. The five extracts were combined, and evaporated on a water bath to remove the alcohol. The alcohol-free extract was clarified with neutral lead acetate and diluted to volume by the official method (*3*, pp. 134–5). The mature beans were extracted four times and the combined extracts treated as above. Sugars were determined according to the Lane and Eynon titration method (*3*, p. 477). Reducing sugars were determined on the clarified solutions. Aliquots of the solutions were inverted with hydrochloric acid (*3*, p. 341) before determination of total sugars. All sugar determinations were run in duplicate. The amount of reducing sugars present in mature soybeans was so small that no results could be obtained with this method.

DETERMINATION OF PENTOSANS AND GALACTANS. Methods of determination were those chosen by the Committee on Methods of Chemical Analysis of the American Society of Plant Physiologists (*23, 24*). Galactans were determined by Dore's method (*8*), which differs from the official method in omitting solution and reprecipitation of the mucic acid. The precipitate obtained was found to be pure mucic acid, as indicated by analysis and by melting point. Pentosans were determined by the official method (*3*, pp. 344–5), by which they are calculated from the weight of furfural phloroglucide obtained after distillation of the furfural. It was necessary, however, because of excessive foaming, especially of immature samples, to substitute a 500-ml. distilling flask for the 300-ml. flask designated by the official method. All analyses were run in quadruplicate.

DETERMINATION OF TOTAL CARBOHYDRATES. Total carbohydrates were calculated by difference after moisture, protein, fat, and total ash of soybeans had been determined.

DISCUSSION OF ANALYTICAL DATA. Analytical data are shown in Table II. The cooking stage is that at which the soybeans are suitable for use as green vegetables, similar to peas. Where two or more values are reported for one type of determination at a given stage, the data are in chronological order for samples of increasing maturity.

While there was some variation in constituents of soybeans grown in different years, the fluctuations were no greater than would be expected with samples not grown under identical conditions. Comparison of the 1936 and 1937 values for total sugars and for total carbohydrates shows that the fluctuations, in general, were not greater from year to year than from variety to variety. Unpublished preliminary data show this to be true, also, for pentosans and galactans.

Both reducing and total sugars decreased with increasing maturity. The changes were often marked even during the comparatively brief period during which the beans remained in the cooking stage. The total sugar content of the soybeans when in the green vegetable stage was but little less than that of shelled green peas, and was nearly twice that of the mature soybeans.

Attention is called to the average percentage of total sugars found in soybeans at different stages of maturity. Even on a moist, fresh basis the amount of sugar in green soybeans is about 4.2 per cent of the total weight. There is reason to believe that most if not all of these sugars can be assimilated by the body. In fact, Adolph and Kao (*1*) reported that about 40 per cent of the total carbohydrates of soybeans could be utilized by the animal body. According to this statement, 14 per cent by weight of mature dry soybeans (40 per cent of the 35 per cent of total carbohydrates) represents assimilable carbohydrate. This fact is significant in view of a popular tendency to speak of soybeans as a "diabetic food". The term implies that soybeans are essentially carbohydrate-free, and has been used especially to designate the low starch content of soybeans. The term "diabetic food" has itself lost its earlier significance since the advent of insulin therapy.

In general, both pentosan and galactan content increased with increasing maturity of the beans. It seems probable that the decrease in sugars with increasing maturity is directly related to the simultaneous increase in pentosan and galactan content. The high values for pentosans obtained at the precooking stage of Emperor, Funk Delicious, and Illini varieties are as yet unexplained. The fact that other varieties studied (unpublished data) showed this peculiarity makes it improbable that the analytical data are affected by some undetermined experimental factor. Pringsheim (*17*) states that the pentosan content of seeds decreases with age. It is possible, therefore, that a portion of the pentosans and galactans may be metabolized during the maturing processes. Several varieties showed a decrease in one or both of these constituents from the postcooking to the mature stage. The general increase in pentosans and galactans with increasing maturity of the soybeans would, however, be expected if these compounds were functioning as reserve carbohydrates.

Plants of the same variety grown under different conditions often show distinct variations in chemical constituents. Plants of the same species, but of different varieties, exhibit even greater variations. Both types of variations are illustrated by the data obtained in this investigation. The sea-

Table III. Summarized Data on Percentages of Soybean Carbohydrates Found

(Vacuum-dried basis)

Stage	Total Carbohydrates (by Diff.) Range	Av.	Total Sugars Range	Av.	Reducing Sugars Range	Av.	Pentosans Range	Av.	Galactans Range	Av.
Precooking	38.44–51.27	44.57	16.46–31.66	23.37	5.78–9.70	7.41	2.36–3.05	2.58	1.20–1.55	1.34
Cooking	31.68–49.70	40.60	11.66–22.83	17.41	4.79–7.40	5.76	2.39–3.09	2.71	1.71–2.68	2.01
Postcooking	31.61–42.18	36.11	5.94–16.24	12.80	1.77–5.63	4.15	2.81–3.78	3.41	1.51–3.14	2.32
Mature	31.08–43.91	35.36	7.56–10.35	9.38	..[a]	..	3.40–3.79	3.62	1.99–2.72	2.34

[a] Too low for analysis by method used.

sonal differences were as great as the varietal differences. At maturity, the one stage which could be determined without subjective observations, and, therefore, the one at which the beans were most strictly comparable, there was a 24 per cent variation from the average for total carbohydrates, 10 per cent for total sugars, 5 per cent for pentosans, and 16 per cent for galactans, for the samples studied.

The general trends are strikingly shown by the ranges and averages in Table III. Although in every case there is an overlapping of the limits from stage to stage for any given determination, when the averages are compared there is a regular decrease for total carbohydrates, total sugars, and reducing sugars, and a similar, though less marked, increase for pentosans and galactans through the successive stages from precooking to mature.

Comparison of Tables I and III will show that the average value found for pentosans in mature soybeans is lower than values reported by Street and Bailey (*20*), by Bailey, Capen, and LeClerc (*4*), and by Chiu (*6*). The value is only slightly lower than that given by Satow (*18*), and appreciably higher than those found by Burrell and Wolfe (*5*). Both Street and Bailey (*20*) and Satow (*18*) reported galactans in nearly twice as great amount as was found by the present writers, whose results, however, are considerably higher than the values reported by Burrell and Wolfe (*5*).

Qualitative Separation of Hemicellulose Fractions of Soybeans

Chemical analyses for pentosans and galactans give only partial information about the hemicellulose complex of soybeans, since many hemicelluloses are known to be polyuronides, similar to pectin. Pectic substances may themselves occur in as important amount as hemicelluloses. It was hoped that a qualitative separation of the hemicellulose fractions might throw further light on the nature of that portion of soybeans to which is assigned the name "carbohydrates".

Other workers have shown that pectic compounds are related to softening changes which vegetable tissues undergo as they are cooked. Personius and Sharp (*16*) state "that the intercellular material of potato tissue is pectic in nature and that the softening of tissue characteristically obtained during cooking is the result of an extensive decrease in cell adhesion which accompanies the weakening of the intercellular cementing substance". Simpson (*19*) found that the tissues of carrots and parsnips tend to disintegrate during cooking, and that this disintegration follows the change of protopectin to pectin. The relation of hemicelluloses to such a disintegration seems possible in view of the fact that hemicelluloses often have structural as well as reserve functions in the plant (*13*).

Soybeans soften noticeably less than many kinds of plant tissue when they are cooked to a degree that makes them acceptable for eating. This point was thought to lend added interest to a study of their hemicellulose fractions.

Extraction. Mature Willomi soybeans of the 1939 crop were used. The beans were ground through a Hobart mill to pass 32 mesh, and were then fat-extracted by refluxing with petroleum ether, boiling point 30° to 60° C., for 24 hours. After evaporation of the ether, the beans were reground through the Hobart mill to pass 100 mesh.

The pectin and hemicellulose extractions were based upon the methods of Norris and Preece (*15*), with certain modifications suggested by Norman (*14*). The method as finally adopted for this work was as follows:

The 100-mesh sample was extracted twice, for 2-hour periods, on the steam bath with 0.5 per cent ammonium oxalate. The extraction liquors were centrifuged off and combined, the calculated amount of acetic acid was added to precipitate protein, which was centrifuged off, and the pectin was then precipitated by the addition of alcohol, washed, and dried in the usual manner. The residue was suspended in 2 per cent sodium carbonate solution and extracted on the steam bath for 4 hours. The extraction liquor, containing a portion of the hemicelluloses, was centrifuged off and reserved. The residue was suspended for 15 minutes in 1500 ml. of distilled water with 75 ml. of "Purex" (5.25 per cent sodium hypochlorite) and 30 ml. of 20 per cent sulfuric acid added, then centrifuged. The residue was extracted on the steam bath for 35 minutes with 0.5 per cent sodium hydroxide, filtered hot through glass wool, and the residue was extracted at room temperature for 18 hours with 0.5 per cent sodium hydroxide. After filtration through glass wool, the 0.5 per cent sodium hydroxide extracts were combined with the 2 per cent sodium carbonate extract.

A series of tubes was set up with varying ratios of acetic acid to combined hemicellulose extracts, to determine the optimum amount of acid to be used for precipitation of hemicellulose A, a procedure recommended by Angell and Norris (*2*). Hemicellulose A was precipitated by addition of the calculated amount of glacial acetic acid. The crude hemicellulose A was washed four times with distilled water, and dried in the usual way with alcohol and ether. It was worked out of ether on a porous plate and dried over phosphorus pentoxide in a vacuum desiccator.

After the A fraction had been removed from the combined hemicellulose extracts, a half volume of acetone was added to the solution to precipitate hemicellulose B. There was practically no precipitation at this point. After clarification with the aid of Filter Cel, another volume of acetone was added, equal to that used to precipitate hemicellulose B. This precipitated hemicellulose C, which was washed and dried like the A fraction.

The solution from which hemicellulose fractions A, B, and C had been removed was treated with a large excess of acetone. A precipitate was obtained which was designated "fraction D".

From 500 grams of soybeans (7.82 per cent moisture), 14.38 grams of crude hemicellulose A were obtained, 0.16 gram of crude hemicellulose C, and 1.24 grams of the D fraction. The amount of pectin, precipitated from the 0.5 per cent ammonium oxalate extracts, was so slight as to be practically negligible, but it is possible that pectin was lost during removal of the protein. Other series of extractions gave similar results.

The fractions accounted for only about one half of the hemicelluloses present, according to the analytical data previously given. The methods of extraction, precipitation, and fractionation, however, have never been considered to be strictly quantitative. For material with as many interfering components as are encountered in soybeans, further refinement of the methods will be necessary before more nearly quantitative recovery of hemicelluloses can be anticipated. No hemicelluloses, as these are defined by Norman (*13*, p. 37), would have been removed prior to the alkaline extractions. The losses were doubtless due to incomplete extraction.

In spite of the preliminary extractions, considerable protein was carried over into the hemicellulose extracts, as much as 30 per cent in some cases, and was precipitated with the various fractions.

Purification. The crude hemicellulose A was dissolved (5 grams to 500 ml.) in 4 per cent sodium hydroxide. Glycerol and cupric sulfate were added to precipitate the A_1 fraction, according to the method of Angell and Norris (*2*). No precipitate of

A_1 was obtained. Upon neutralization with acetic acid, a voluminous precipitate of A_2 separated. Repeated washings with acetone failed to break down the copper complex. Alcohol washings proved equally unsuccessful. After solution in 4 per cent sodium hydroxide and reprecipitation, repeated several times, the copper-hemicellulose complex was still persistent. It could be decomposed with acetic acid, but on reprecipitation the copper was still in combination with the hemicellulose. No method has yet been found which will completely free the hemicellulose from copper. This is probably due to the protein content of the hemicellulose fraction. A portion of the crude hemicellulose A, therefore, was treated with pepsin. After the treatment and subsequent washing and drying, 4.38 grams of crude hemicellulose A yielded 3.19 grams of "purified" hemicellulose A (72.83 per cent), which was still highly impure, containing 13.5 per cent of nonprotein nitrogen.

Three grams of crude hemicellulose C, similarly treated with pepsin, yielded 2.56 grams of the "purified" product (85.33 per cent). The sample was not large enough to furnish material for nitrogen determination, but undoubtedly considerable quantities of protein degradation products were present in it also. Preliminary experiments, using glycerol and cupric sulfate to effect precipitation, showed no C_1 fraction present. The precipitate of hemicellulose C_2 was a copper-hemicellulose complex as difficult to break up as the A_2 complex described above. The C_2 fraction was, therefore, treated with pepsin, washed, and dried, as the A_2 fraction had been.

IDENTIFICATION. Each hemicellulose fraction was hydrolyzed by heating with hydrochloric acid. The solutions were then tested by heating with concentrated sulfuric acid, by heating with phloroglucinol, by treatment with potassium permanganate, and by heating with Disch's reagent, to determine which sugars had been produced by the hydrolysis of each fraction (*7*). Osazones were also prepared.

A portion of each solution was heated with basic lead acetate to test for the presence of galacturonic acid (*9*). This test was not satisfactory; it was very difficult to check results upon the samples. Portions of the solution were heated with 12 per cent hydrochloric acid and the evolved gases tested for presence of carbon dioxide, to determine the presence of uronic acids (*13*, p. 64).

RESULTS AND CONCLUSIONS. Hemicelluloses A_2, C_2, and the D fraction each contained galactose, arabinose, and uronic acid. The uronic acid was undoubtedly galacturonic acid, as this is the acid usually occurring in the presence of galactose. Both galactose and uronic acid contents progressively increased in proportion to arabinose from A_2 to C_2 to D fraction, as indicated by the speed of reaction, etc., of the qualitative tests used. Although the test used for galacturonic acid was not satisfactory, it gave positive results with the A_2 fraction.

It seems probable that the soybean hemicelluloses comprise a mixture of galacto-arabans containing galacturonic acid. This is a combination frequently encountered (*13*, p. 37). The three fractions obtained are considered to be portions of this mixture which have been separated arbitrarily on the basis of their physical properties.

Only a very small amount of pectin was obtained by precipitation from the ammonium oxalate extract, but it is possible that pectin was lost in the precipitation of the protein. A low content of pectic compounds, which other workers (*16*, *19*) have shown to be concerned in the softening of vegetable tissues during cooking, may explain the firmness which persists in soybeans even after they are cooked.

Summary

The sugar, pentosan, and galactan contents of edible varieties of soybeans were determined at different stages of maturity.

Both reducing and total sugars decreased with increasing maturity. Pentosan and galactan contents tended to increase as the soybeans matured. It is suggested that the decrease in sugars may be related to the increase in pentosans and galactans, and that the latter function as reserve carbohydrates in the seeds.

In the soybean varieties studied, both seasonal and varietal differences in carbohydrate constituents were marked.

The hemicelluloses of Willomi variety soybeans were extracted and fractionated. The large percentage of protein present in soybeans rendered purification exceedingly difficult. Fractions A_2 and C_2 were obtained, as well as a D fraction precipitated by excess acetone from the liquor from which A_2 and C_2 fractions had been removed. Qualitative tests indicated that each of the three fractions obtained was composed of arabinose, galactose, and a uronic acid, undoubtedly galacturonic acid. The ratio of galactose and uronic acid to arabinose appeared to increase from A_2 to C_2 to D fraction.

The hemicelluloses of the soybean are considered to be a mixture of galacto-arabans containing galacturonic acid. The fractions obtained differed in physical properties and in relative proportions of the constituents.

A low pectin content of soybeans may explain their failure to soften greatly during cooking.

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A Pendulum Method for Measuring Settling Velocities

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IN THE preparation and study of liquids containing finely divided particles it is frequently desirable to know how fast the particles are settling out of the suspension. This is especially true when a stable mixture or a mixture which is expected to endure for a long time is being investigated. It is a relatively simple matter to measure the settling rate when the liquid involved is transparent, for then one needs only to observe the rate at which the surface between the clear liquid and the suspension is moving. If, however, the liquid is opaque, the task of measuring the settling rate is a difficult one and a new method of measurement needs to be devised.

To meet this problem of opaque suspensions, a method was developed in this laboratory in which the stability of a suspension and the rate of particle fall could be obtained from a few relatively simple measurements. The method utilized the principles of a compound pendulum and was an extension of a method first introduced by Manning and Taylor (*1*), which was somewhat limited in its scope, since it was employed only to detect settling and did not measure the rate. In the present method it was possible to measure not only the rate at which the center of gravity of the mixture moved, but also the speed with which the individual particles fell. The liquid with its suspended particles was placed in a long tube and mounted so that the tube swung like a pendulum from a support just above the center of gravity of the suspension. When the particles settled, the center of gravity of the system was lowered, causing the period of oscillation to shorten. The tube was also equipped with a second support just above the first one and the periods of oscillation were measured for both supports. The data on these periods for various times of settling were then used to calculate the rate at which the center of gravity of the entire mixture was lowered, which gave a measure of the suspension's stability.

It was also possible by making certain simplifying assumptions regarding the nature of the settling process to calculate the rate at which the particles themselves were settling. In developing the theory for this calculation, it was assumed that all the particles in the suspension fell with the same constant velocity, that there was no interference, and that the particles did not agglomerate. While it is generally known that these effects occur to some degree in most cases of settling, they were ignored in this instance to keep the mathematical theory as simple as possible. Since the experimental results for the samples investigated agree reasonably well with the derived equations, there is some justification for the simple picture of the settling process as adopted in this analysis.

The double support pendulum method as described here was developed primarily to study suspensions of finely divided coal particles in fuel oil, but has also been used to measure the settling of milk in cream and the settling of paint pigments.

To facilitate the calculations of settling rates when a great number of samples are being studied, certain approximate expressions for these velocities have also been developed.

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Experimental Procedure

The essential parts of the apparatus consisted of a 100-ml. Nessler tube fastened in a holder which in turn was suspended from one of its supports by means of two sharp points resting on a flat plate (Figure 1). The holder, which was 32 cm. long, was made of brass and either one of two sets of points could be used as the support. The pendulum unit was enclosed in a constant-temperature box equipped with an observation window. This box also served to eliminate errors due to air currents. The period of oscillation was measured with a stop watch.

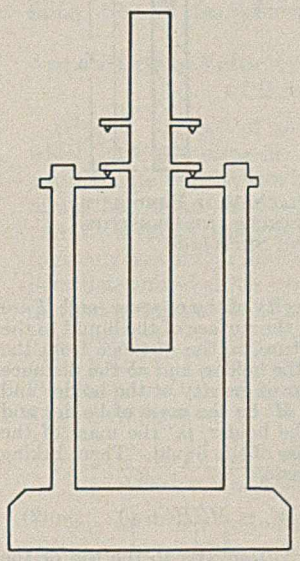

FIGURE 1. DIAGRAM OF PENDULUM SHOWING BASE AND TWO SETS OF SUPPORT POINTS

The oils used in this investigation were virgin gas oil, cracked gas oil, and several cracked residue fuel oils. The coal was washed Kansas bituminous, ground so that all passed a 200-mesh screen. Mixtures of 10 to 50 per cent coal by weight were investigated.

The oil-coal mixtures were poured into the Nessler tube until the surface level was about the same distance above the lower axis of suspension as the axis was above the bottom of the tube. As the coal particles settled, the periods of vibration were measured for both support axes. These measurements were made at 0.5-hour intervals, which were later lengthened to 1-hour and 2-hour intervals. Readings were stopped when the measured periods showed no further change, since it was apparent the particles had completely settled.

Theory of Stability Determinations

The period of vibration, T, for a compound pendulum is given by

$$T^2 = \frac{4\pi^2 I}{MgH}$$

where I is the moment of inertia of the pendulum about its support axis, M its mass, g the acceleration due to gravity, and H the distance from the axis of suspension to the center of gravity. Since a well-known theorem of mechanics states that the moment of inertia about any axis, I, is related to the moment of inertia about the center of gravity, I_0, by the expression

$$I = I_0 + MH^2$$

where H is the distance between the gravity and support axes, the period about the lower axis T_2 may be expressed as

$$T_2^2 = \frac{4\pi^2 (I_0 + MH^2)}{MgH}$$

and about the upper axis, T_1, as

$$T_1^2 = \frac{4\pi^2 I_0 + M(H + h)^2}{Mg(H + h)}$$

where h is the distance between the two support axes. Subtracting these two expressions and solving for H gives

$$H = \frac{4\pi^2h^2 - ghT_1^2}{-8\pi^2h - g(T_2^2 - T_1^2)} \quad (1)$$

The period for the lower axis, T_2, is greater than that for T_1 and, as the settling proceeds, decreases much more rapidly than T_1. H increases with settling and at a rate which is proportional to the lowering of the center of gravity of the suspension.

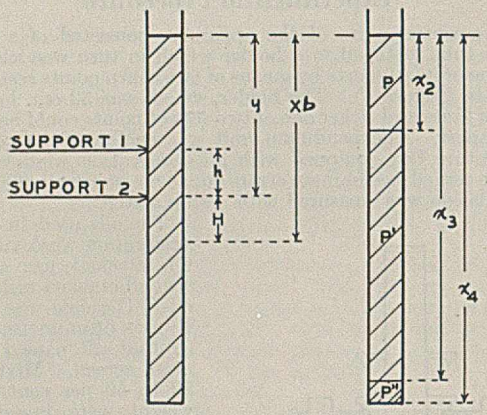

FIGURE 2. SCHEMATIC DIAGRAM OF PENDULUM SHOWING IMPORTANT DISTANCES AND DENSITIES OF LIQUID DURING SETTLING

To find how x'', the center of gravity of the mixture, and H are related, let x'' be the distance from the surface of the liquid to the center of gravity of the liquid mixture, xa the distance from the surface to the center of gravity of the holder, and xb the distance between the surface and the center of gravity of the holder and liquid mixture together. Also let M' be the mass of holder and liquid mixture, M'' the mass of the holder, m' the mass of the suspended particles, and m the mass of the liquid. Then, taking moments about the surface as an axis,

$$M'xb = M''xa + (m' + m)x'' = M'(H + y) \quad (2)$$

where y is the distance from the bottom axis to the top of the liquids (Figure 2). Combining Equations 1 and 2 yields

$$(m' + m)x'' = \frac{(4\pi^2h^2 - ghT_1^2)M'}{-8\pi^2h - g(T_2^2 - T_1^2)} + M'y - M''xa \quad (3)$$

This gives at once the center of gravity of the mixture in terms of the measured periods of vibration. The center of gravity of the holder may be determined in a separate experiment with an empty holder in the same manner that x'' is determined above for the holder filled with the liquid mixture.

Sample Data on Stability

An example of the results obtained is shown in the reproduction of the data for a 10 per cent mixture of coal and virgin gas oil.

The measured period, T_2, is plotted in Figure 3 and a smooth curve drawn through the experimental points. At 8.5 hours T_2 reached a constant value and remained so for readings taken up to a total elapsed time of 57.5 hours. T_1 happened to be constant for this sample and had the value of 1.40 seconds.

In this experiment the total mass of holder and mixture M' was 181.33 grams, of the holder M'' 114.52 grams, and of the mixture $(m' + m)$ 66.81 grams. Period measurements with the empty holder gave a value of 12.96 cm. for xa. The distance between supports h was 2.06 cm., and the distance between the surface of the liquid mixture and the lower support, y, was 12.90 cm.

From the smooth curve of Figure 3, new values of T_2 were taken, and by substitutions in Equation 2, x'' was calculated. The calculated x'' values, representing the distances from the liquid surface to the center of gravity of the mixture, are graphically shown in Figure 4.

In this sample with its low concentration of coal, the center of gravity of the system was not altered much, even when settling had been completed. This sample was obviously not stable, having completely settled out in 8.5 hours.

Theory of Velocity Determination

In setting up the expression for the velocity of settling, it was assumed that the suspended particles fell with a uniform velocity. At some time, t hours after settling begins, the particle-free liquid extends to x_2 and the layer of settled matter has risen to x_3, both distances being measured from the surface of the liquid (Figure 2). If p'' stands for the linear density of the settled layer, p' the linear density of the mixture, and p the linear density of the particle-free oil, moments about the liquid surface may be taken, giving

$$(m' + m)x'' = \tfrac{1}{2}px_2^2 + \tfrac{1}{2}p'(x_3 - x_2)(x_3 + x_2) + \tfrac{1}{2}p''(x_4 - x_3)(x_4 + x_3)$$

If v stands for the velocity of fall, then $x_2 = vt$ and $x_3 = x_4 - kvt$. Here k is the ratio between the rates at which the two surfaces, x_2 and x_3, move and it can be shown that $k = p'c/p''$, where c is the concentration of coal in the mixture.

If x_2 and x_3 are replaced in the above equation by these expressions involving the velocity and time, the equation becomes

$$(m' + m)x'' = e + fvt - qv^2t^2 \quad (4)$$

where $e = \tfrac{1}{2}p'x_4^2$
$f = (p'' - p')kx_4$
$q = \tfrac{1}{2}(p' - p) + \tfrac{1}{2}(p'' - p')k^2$

Equation 4, when used with Equation 3, permits the evaluation of k and v for each measured T_1 and T_2. In measurements on 10 per cent virgin oil, $p'' = 4.4$ grams per cm., $p' = 2.63$ grams per cm., $p = 2.54$ grams per cm., and $x_4 = 25.4$ cm. The values of f and q which best fit Equations 3 and 4 gave for k in this investigation a value of 0.0442. The calculated data on the velocity of settling for various time intervals are shown in Figure 5. The velocities are constant and give no evidence of a trend with time, showing that the assumption regarding uniform velocity of fall which was employed in the derivation was justified. Two points on the curve fall outside the rest and were discarded in determining the average velocity, which was 2.36 cm. per hour.

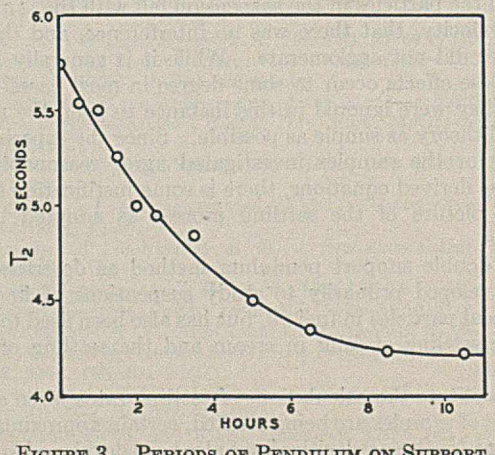

FIGURE 3. PERIODS OF PENDULUM ON SUPPORT 2 FOR INTERVALS OF TIME t AFTER SETTLING BEGINS

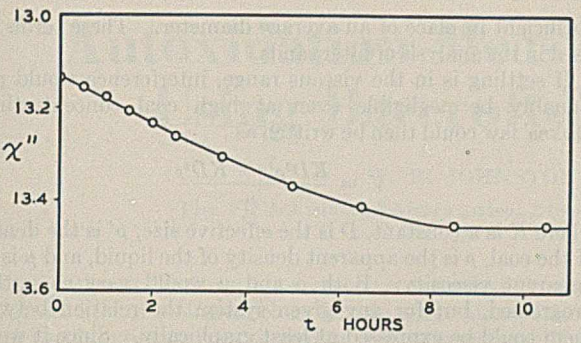

FIGURE 4. LOWERING OF CENTER OF GRAVITY OF LIQUID WITH TIME OF SETTLING

Sensitivity of Pendulum Method

An examination of the authors' data on the coal-in-oil suspension shows that the calculated velocity is sensitive to small changes in the measured periods. This is a disadvantage, since it means that small errors in measurement introduce large errors in the calculated velocity. Obviously the measurement of settling by direct observation in transparent liquids is more accurate than those made with the pendulum. With a few simplifying assumptions one can show that an error in the period ΔT introduces an error in the calculated velocity ΔV given by

$$\Delta V = \frac{2vM'HT_2}{(2qt^2 - ft)\,(T_2^2 - T_1^2)}\Delta T \qquad (5)$$

Here ΔV is measured in centimeters per hour and ΔT in seconds. The term in Equation 5 before ΔT is greater than 1 and plays the role of a magnifying factor. Taking the data on virgin gas oil with 10 per cent coal at $t = 2$ hours and inserting it in Equation 5 gives $\Delta V = 3.97\ \Delta T$. Part of this unfavorable ratio between ΔV and ΔT is due to the large mass, M'. The experiment could have been improved by a reduction in M' through a reduction in the mass of the holder. An aluminum holder would have had a decided advantage.

Expression for Maximum Velocity from Duration of a Constant Period

If the suspension is quite stable, so that there is no change in the period of vibration over a rather long time interval, it is possible to determine the maximum velocity of settling with very little mathematical calculation. In testing samples this offers a rapid method of finding the sample's upper limit velocity. Let V_m be this maximum velocity and t_0 the time interval during which no change in period T_2 is detected. At $t = 0$, Equation 4 becomes

$$(m' + m)x'' = e$$

and at $t = t_0$, it becomes

$$(m' + m)x'' = e + fvt_0 - qv^2t_0^2$$

Since the periods are constants, both left-hand sides of these equations are the same and we have for the maximum velocity

$$V_m = \frac{f}{q} \times \frac{1}{t_0} = \frac{2x_4p'c(p'' - p')}{p''(p' - p) + c^2(p')^2\,(p'' - p')} \times \frac{1}{t_0} \qquad (6)$$

All the quantities in this expression are easily determined and the maximum, V_m, is readily calculated.

Expression for Approximate Velocity of Settling

Often it is desirable to sacrifice accuracy for speed in calculating the settling velocity. The equations for the approximate velocity, V_a, follow from Equations 3 and 4. If h is small, $4\pi^2h^2$ and $8\pi^2h$ may be neglected in Equation 3, and if t is small, qv^2t^2 may be neglected in Equation 4. By combining Equations 3 and 4, we have

$$(m' + m)x'' = \frac{hT_1^2}{T_2^2 - T_1^2}M' + M'y - M''xa = e + fvt$$

Solving for v gives

$$V_a = \frac{M'y - M''xa - e}{ft} + \frac{hT_1^2}{T_2^2 - T_1^2} \times \frac{M'}{ft} \qquad (7)$$

Now let T_{21} be the period (at lower axis) at time t_1, and T_{22} that at time t_2. Inserting these values in Equation 7 and subtracting, we have

$$V_a = \frac{(T_{21}^2 - T_{22}^2)T_1^2}{(T_{22}^2 - T_1^2)\,(T_{21}^2 - T_1^2)} \times \frac{2p''M'h}{(p'' - p')\,p'cx_4^2\,(T_2 - t_1)} \qquad (8)$$

where f has been evaluated and where it has been further assumed that T_1 changes very little during the settling process.

Approximate Velocity Using a Standard Specimen of Known Velocity

When a number of samples are being examined, all of which have the same density and concentration, the above approximate velocity can be expressed in terms of the velocity of any one of the samples, such as a standard sample. This permits a rapid examination and calculation of the velocity for many samples in terms of one standard whose velocity has been determined either by direct observation or by the longer method given above. Let V_s be the velocity of settling for the standard, T_{21s} and T_{22s} the periods of this standard at times t_1 and t_2, V_u the velocity of the unknown, and T_{21u} and T_{22u} its periods. Substituting these quantities in Equation 8 and dividing gives

$$V_u = \frac{(T_{21u}^2 - T_{22u}^2)\,(T_{22s}^2 - T_1^2)\,(T_{21s}^2 - T_1^2)}{(T_{21s}^2 - T_{22s}^2)\,(T_{22u}^2 - T_1^2)\,(T_{21u}^2 - T_1^2)}V_s \qquad (9)$$

Thus with four period readings on the standard and four on the unknown, the velocity of the unknown, V_u, may be readily calculated in terms of the standard, V_s.

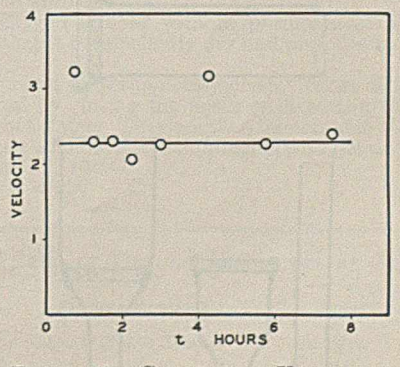

FIGURE 5. CALCULATED VELOCITIES OF PARTICLE FALL AT INTERVALS OF TIME t AFTER SETTLING BEGINS

Conclusion

In the above analysis, the expression for the center of gravity of a settling mixture, which is contained in Equation 3, was an exact one free from assumption as to the mechanism of the settling process. From it the rate at which the center of gravity changes may be calculated. When this expression

was extended to determine the velocity of particle fall, by means of Equation 4, it was necessary to assume that all the particles fell with the same velocity and that this velocity was constant with time. In other words, Stokes' law was assumed to hold and the particles were assumed to have the same diameter.

For those cases where the particles do not fall with uniform velocity, the theory given above must be modified. However, since the method gives an almost instantaneous value for velocity, it can be used to determine just when the mixture failed to settle according to Stokes' law. This suggests that the method could be applied successfully to a study of the interval between the time that settling begins and the time at which "conglomeration" or interference occurs.

In this experiment no special precautions were taken to ensure uniformity of particle size, and consequently there seemed to be no justification for using Stokes' law to calculate particle diameter. It was known that the sizes varied from 1 to 74 microns, but only the 325-mesh screen was available between these sizes. If size distribution could have been determined, it would have been possible to relate this method to other methods of sedimentation. Probably this could best be done by the use of an effective size or uniformity coefficient in place of an average diameter. These terms are used in the analysis of filter sands.

If settling is in the viscous range, interference would presumably be negligible even at high coal concentrations. Stokes' law could then be written as

$$V = \frac{KD^2\rho' - KD^2\rho}{\mu}$$

where K is a constant, D is the effective size, ρ' is the density of the coal, ρ is the apparent density of the liquid, and μ is the apparent viscosity. Both ρ and μ would vary as settling progressed, but for any given system the relation between them could be expressed at least graphically. Since it would be extremely difficult to determine the rate of settling of coal particles in oil by any other method, the samples could not be checked. However, the pendulum method as a method could be tested, using lead shot falling through a transparent liquid and observing the rate of fall visually. A study of settling theory by means of the pendulum is contemplated using these materials.

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Preparing Gas Distributors Using Alundum Disks

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A NUMBER of methods have been described in the literature for the preparation of sintered- or fritted-glass filters from Pyrex glass (*1–8*). These filters are very satisfactory and are especially valuable for use as gas distributors in systems where intimate contact between a gas and a liquid is desired. These methods require considerable time to prepare the glass, and the equipment needed is not always available in chemical laboratories.

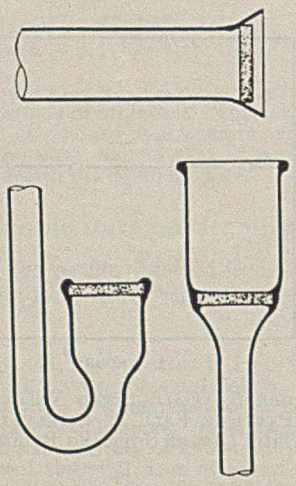

FIGURE 1. SEALING ALUNDUM DISKS TO PYREX GLASS

Attempts have been described to seal Alundum disks directly to Pyrex glass (*2*). While no details have been given regarding the method of preparation of these seals, the results are stated to have been unsatisfactory.

During the last several years, the author has used gas distributors prepared by sealing Alundum disks to Pyrex. These distributors have been very satisfactoy.

A Pyrex tube of slightly smaller diameter than the disk to be used is flared until the disk will fit inside the flared portion as shown in Figure 1 (top). The glass is then heated in the flame of a blast lamp and, as the glass is softened, it is worked against the disk and slightly rolled over the outside of the disk. For use as an aerator, a small connecting tube is sealed on and bent so that the gas is delivered upwards (Figure 1, left).

Alundum thimbles may be used in place of disks.

Filters may be prepared by constricting a Pyrex tube of suitable diameter so that the disk will be held at right angles to the axis of the tube (Figure 1, right). By heating and pulling the tube, a seal is obtained. Filters of this type are convenient for sealing into all-glass systems.

This method requires no special equipment and may be carried out by anyone with an elementary knowledge of glass blowing. Careful annealing of the Alundum-to-glass seal is not required.

Alundum disks are made by the Norton Company, Worcester, Mass., and may be obtained in at least two degrees of porosity. For ordinary laboratory usage, disks 0.75 and 1 inch in diameter are most suitable.

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Autoxidation Measurements on Fatty Oils Using Barcroft-Warburg Apparatus

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THE Barcroft-Warburg apparatus has been widely used in the field of biological chemistry to study autoxidation reactions of various types, including a few cases involving fatty oils, but so far as the authors are aware all measurements have been made at temperatures below 40° to 50° C.

A projected study of antioxidants required a relatively precise and convenient method of measuring autoxidation induction periods by direct oxygen absorption. Because of complexity and lack of flexibility, none of the numerous assemblies previously used seemed satisfactory for the purpose. With the Barcroft-Warburg equipment it was possible to measure induction periods at any temperature from 50° to 110° C., and probably at higher temperatures, with considerable precision. In order to effect oxidation in a satisfactory time interval, most of the determinations have been made at 100° C. in an atmosphere of oxygen.

The equipment permits exact control of experimental conditions, which must be rigidly reproduced if the greatest precision is desired.

Apparatus and Procedure

The Barcroft-Warburg equipment and technique are described by Dixon (*1*). The apparatus, except the flasks, was constructed by the American Instrument Co., Silver Spring, Md., and is shown in Figure 1. The flasks, of special design (Eck & Krebs, New York, N. Y., Catalog No. 210), have volumes of approximately 70 cc. and are fitted with capillary exit valves for gas flushing. Each flask has a central cup about 24 mm. in diameter in which the oil under test is placed. As pointed out by Stephens (*2*), the extent of surface has a pronounced effect on the oxidation rate, and for greatest precision each central cup should have the same dimensions. This is readily achieved by using glass tubing of the same diameter for all reaction flasks. A flask-manometer assembly is shown in Figure 2.

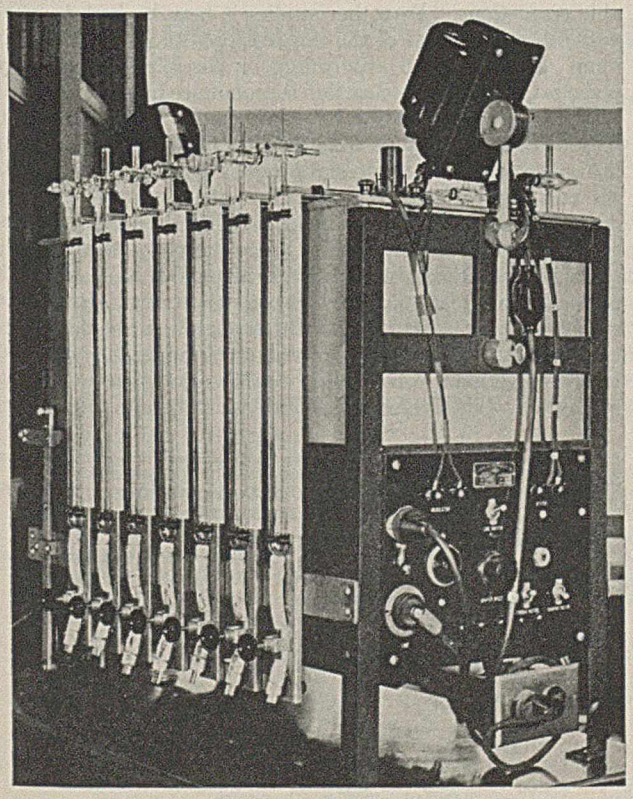

FIGURE 1. BARCROFT-WARBURG APPARATUS (14-MANOMETER MODEL)

Preliminary work indicated that a 0.50-cc. sample of oil is just sufficient to permit completion of the autoxidation induction period when the full scale (30.0 cm. of Brodie solution) is utilized for pressure measurements. The rate of oxygen absorption by 0.50 cc. of oil at 100° C. usually becomes markedly accelerated when the pressure drop amounts to 17 to 21 cm. of Brodie solution, thereby enabling an induction period measurement to be obtained without resetting the manometer. Evidently larger oil samples can be used if manometric resettings are made or denser manometric fluids are employed. A 0.50-cc. oil sample is ideal from the standpoint of gas equilibration, since it forms a thin film about 1 mm. thick which is suitably agitated by the usual shaking conditions of 110 to 120 oscillations per minute at an amplitude of 3 cm.

Temperatures from 50° to 100° C. have been employed, water being used in the bath up to 80° C., and glycerol above 80° C. The temperature control must be precise (at least 0.05° C.). The following procedure has been devised for comparative measurements:

The temperature is set at 100° C., the shaking rate at 110 oscillations per minute, and the amplitude at 3 cm. The shaking rate and the amplitude are sufficient to agitate the oil-gas interface without causing splashing, which would extend the glass-oil interface and prevent the maintenance of reproducible conditions. The determination is made by pipetting or weighing 0.50 cc. of oil into the central cup of a flask, flushing the flask with a rapid stream of cylinder oxygen at a pressure of 10 to 15 cm. of Brodie solution for 15 minutes at room temperature, and then equilibrating at 100° C. for 10 minutes before sealing the system and beginning pressure measurements. The manometers are set at 29 cm. during equilibration. Depending on the rate of absorption, pressure readings are made at various intervals, usually 5 to 30 minutes apart, until about 1500 cu. mm. of oxygen have been absorbed.

Cylinder oxygen is used without purification. Experiments have shown that it is sufficiently dry and provides a reproducible atmosphere.

The flasks must be scrupulously clean. Most of the oxidized fat is removed by rinsing the flasks with carbon tetrachloride, alcohol, and hot water. They are then immersed in cleaning solution for at least 5 hours, rinsed thoroughly with hot water, steamed out, and dried at 110° C.

TABLE I. RATE OF OXYGEN ABSORPTION BY SESAME OIL

Time	Pressure Readings		Volume of Oxygen Absorbed	
	A	*B*	*A*	*B*
Min.	*Mm. of Brodie Solution*		*Cu. Mm.*	*Cu. Mm.*
30	− 3.1	− 3.3	15	17
60	− 5.9	− 7.0	28	36
90	− 14.9	− 15.4	72	78
120	− 20.1	− 21.3	96	108
150	− 29.5	− 30.2	142	154
180	− 35.9	− 38.2	173	194
210	− 46.1	− 46.6	222	237
245	− 56.9	− 58.4	274	297
270	− 68.1	− 68.4	328	348
300	− 80.7	− 83.0	389	423
330	− 97.1	−102.0	468	519
360	−126.9	−143.9	611	733
375	−161.6	−207.7	779	1058
385	−202.9	−272.4	978	1387
390	−232.4	−323.8	1120	1649
400	−326.7	...	1574	....

Induction period, minutes: *A*, 391; *B*, 378

TABLE II. REPRODUCIBILITY OF OXIDATION MEASUREMENTS ON SESAME OIL

Determination Number	Induction Period *Min.*	Deviation from Mean *Min.*
1	420	0
2	422	+ 2
3	426	+ 6
4	427	+ 7
5	433	+13
6	427	+ 7
7	416	− 4
8	414	− 6
9	413	− 7
10	417	− 3
11	417	− 3
12	420	− 0
13	418	− 2
14	416	− 4
15	419	− 1

Arithmetic mean, 420 minutes
Average deviation from mean, 4.3 minutes
Standard deviation, 5.5 minutes
Standard error of mean, 1.5 minutes
Probable error of mean, 1.0 minute

The apparatus includes fourteen manometers and flasks, two sets being used as thermobarometers. Thus a maximum of twelve oil samples can be measured at one time, though at 100° C. it is often possible to measure 24 samples per day.

At 100° C. the choice of a lubricant for the ground joints is very important. Celvacene (heavy), a vacuum grease manufactured by the Distillation Products Company, seems to be excellent for the purpose, since suitable controls have shown no appreciable oxygen absorption by the grease as normally exposed at the edges of the ground joints. Its high melting point is also an important property, since the grease does not tend to leak out of the joints.

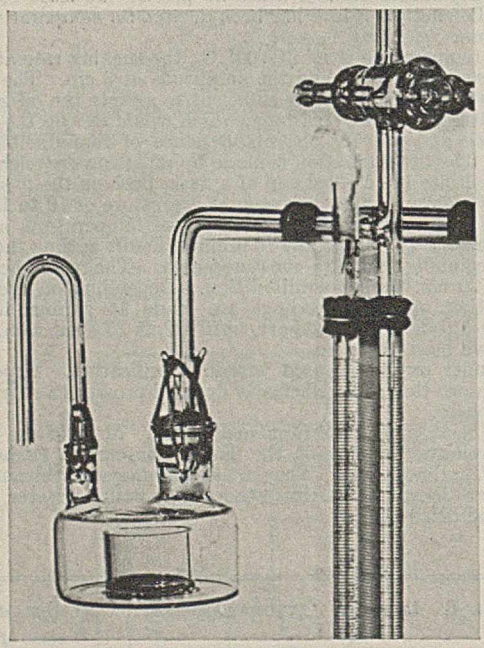

FIGURE 2. FLASK-MANOMETER ASSEMBLY

Discussion of Results

The measurements may be evaluated in the customary manner by converting the pressure readings to cubic millimeters of oxygen absorbed, utilizing calibration data, or the results may be interpreted directly from the pressure measurements. Table I presents the results of duplicate measurements on a refined sesame oil at 100° C. The divergence in volume of oxygen absorbed after the rate becomes accelerated is typical, but this effect is greatly minimized when referred to the time scale.

In Figure 3, results *A* are plotted, showing the rapid acceleration at the end of the induction period. For purposes of comparison the induction period has been taken as the time required for 0.50 cc. of oil to absorb 1000 cu. mm. of oxygen at 100° C. under the experimental conditions previously defined. This choice satisfies, in essentially all cases, the customary definition of the induction period as the time required for the absorption rate to become rapidly accelerated. This procedure also permits one to complete an induction period measurement without resetting the manometers. The time required to reach a maximum absorption rate can be measured by resetting the manometers and making suitable corrections. For other studies it may be best to compare oils on the basis of the time required to reach the maximum rate of oxygen absorption, or possibly to reach any given rate after the acceleration is pronounced.

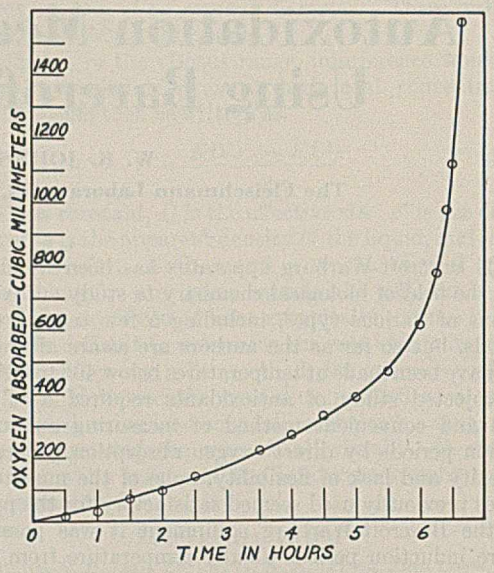

FIGURE 3. AUTOXIDATION OF 0.50 CC. OF SESAME OIL IN OXYGEN AT 100° C.

The time corresponding to an absorption of 1000 cu. mm. may be evaluated by plotting a curve, or it may be obtained by interpolation with adequate precision for general analytical purposes. From a given flask constant the pressure drop corresponding to an absorption of 1000 cu. mm. of oxygen is calculated and then by interpolation of the pressure measurements the induction period is found. Since the flask constants approach 5.0, several pressure readings in the neighborhood of 200 mm. of Brodie solution should be recorded in order to attain the best precision. The measured induction period is corrected for equilibration time by adding 5 minutes. The systems approach temperature equilibrium

TABLE III. REPRODUCIBILITY OF OXIDATION MEASUREMENTS ON COTTONSEED OIL

Determination Number	Induction Period *Min.*	Deviation from Mean *Min.*
1	139	+2
2	135	−2
3	142	+5
4	140	+3
5	138	+1
6	145	+8
7	133	−4
8	133	−4
9	135	−2
10	132	−5

Arithmetic mean, 137 minutes
Average deviation from mean, 3.6 minutes
Standard deviation, 4.1 minutes
Standard error of mean, 1.3 minutes
Probable error of mean, 0.9 minute

after 5 minutes but to ensure complete equilibration a 10-minute period is used.

An estimate of the precision of the measurements may be obtained from Table II wherein a series of induction periods measured on the same sesame oil is listed. The determinations were made over a 3-day period using 10 different flasks. It is evident that the precision of a single determination will probably be between ±1 and ±3 per cent. By using the same flask for each measurement and carefully controlling conditions, a precision of 1 per cent is easily realized in measuring sesame oil.

Determinations on a commercial sample of refined corn oil were similar to those with sesame oil with respect to precision and induction period, except that the rate after absorption of 1000 cu. mm. of oxygen was about 50 per cent less. Comparisons might better be made at corresponding rates, but the calculations required are probably not worth while for routine analysis.

Refined cottonseed oil is much less resistant to oxidation than sesame or corn oil as shown by measurements on a typical commercial sample (Table III). Because of the higher oxidation rate, the induction period measurements are not so precise as on the more stable oils.

However, cottonseed oil and other unsaturated oils can be measured with high precision if care is taken to select matched flasks of nearly the same dimensions. The measurements listed in Table IV were made at the same time on six aliquots of a sample of cottonseed oil, using six different flasks and manometers. When the surface factor is thus controlled, the divergence in absorption values noted in Table I is largely eliminated and the induction period is measured with an error of less than one per cent.

Table IV. Oxidation of Cottonseed Oil

Time	Oxygen Absorbed					
	A	*B*	*C*	*D*	*E*	*F*
Min.	*Cu. mm.*	*Cu. mm.*	*Cu. mm.*	*Cu. mm.*	*Cu. mm.*	*Cu. mm.*
5	0.0	0.0	0.0	0.0	0.0	0.0
10	3.6	1.0	1.0	1.0	3.5	2.7
30	30	23	27	24	23	16
60	107	95	84	101	93	79
90	369	360	332	356	353	351
100	739	744	691	716	725	738
105	999	1015	895	974	987	1011
108	1152	1168	1091	1120	1124	1165
100	1280	1292	1212	1243	1268	1278
112	1390	1401	1315	1347	1370	1388
115	1576	....	1486	1526	1560	1599

The tabulated data presented show that the Barcroft-Warburg technique can be extended to elevated temperatures with convenience and relatively good precision.

The accelerated autoxidation of fats and oils and many other substances may, therefore, be studied under reproducible conditions with standard and readily available equipment.

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

An Improved Automatic Continuous Percolator

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Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.

THE investigation of plant materials for possible insecticidal properties has necessitated the construction of apparatus suitable for the percolation of the ground plants in quantities ranging from several hundred grams to several kilograms. A simple apparatus (*1*) meeting these requirements has been described and used successfully for some time in this laboratory. A number of improvements have now been made, and the redesigned extractor is shown in Figure 1, diagram I.

Like the previously described extractor, this apparatus is comparatively simple and inexpensive, the condensers and three-necked flasks being standard laboratory equipment. In the old extractor the bottom of a bottle was cracked off to make the percolator, while in the new one a hole was drilled through the bottom of a bottle. It is thus possible to reduce the loss of solvent to a minimum, since the new apparatus is open to the atmosphere only through condenser *I*.

Another improvement has been made in the arrangement of the overflow tube, *B*, which is now slipped over the outlet tube and allowed to rest on stopper *P*, so that excess solvent is returned to the flask through tube *O*. In the old apparatus the rates of distillation and percolation had to be so regulated that the solvent distilled into the percolator faster than the percolate ran into the flask; consequently there always was a return of some solvent to the flask through the overflow tube. If the source of heat happened to be discontinued for some time, the entire percolate would drain down into the flask. The adjustment of the new apparatus is not so critical, particularly if the material to be extracted has not been ground too fine, in which case the only adjustment necessary is to leave stopcock *D* closed.

The percolator, *A*, was made by drilling a hole through the bottom of a bottle. Bottles varying from the common 2.5-liter acid bottles to 5-gallon (18.9-liter) water bottles have been used. In the smaller bottles the hole was placed in the center of the bottom, while in the larger bottles it was near the side. The three-necked flask, *F*, ranged in capacity from 1 to 5 liters; for a 5-gallon bottle it should be 5 liters or larger. The smaller percolator bottles, up to about 5 or 6 liters capacity, may be supported by a sturdy clamp at the neck, but larger bottles, such as the 5-gallon size, should be supported more firmly. An excellent support for the larger bottles is a small table with a hole cut through the top, through which the neck of the bottle is inserted, diagram IV. The table should be of the same diameter as the bottle or slightly smaller, so that tubes *O* and *G* can be brought close to the sides of the bottles for the sake of compactness.

The hole was drilled through the glass bottle with the simple tool shown in diagram III. It was made by wrapping a sheet of galvanized iron 0.8 mm. ($^1/_{32}$ inch) thick and 3.8 cm. (1.5 inches) wide around a cylindrical wooden block about 2.5 cm. (1 inch) thick, through the center of which a metal rod was bolted, the iron sheet being fastened to the block with screws. The tool should be about 0.64 cm. (0.25 inch) smaller in diameter than the hole desired. The hole was cut through the bottom of the securely held bottle by alternately applying and releasing pressure on the tool, which was held in a drill press, with care that not too much heat was produced during the drilling. The cutting edge of the tool was kept well lubricated with a slurry of No. 320 Carborundum powder and water. Owing to the unevenness of most bottles the drill will come through at one place

first. The pressure applied from then on should not be too great. A hole to accommodate a No. 10 stopper was found most convenient for the smaller bottles, and a No. 14 stopper for the 5-gallon bottle.

The glass blowing can be done by anyone having a moderate degree of skill. Instead of the No. $^{12}/_{5}$ ball joint at *C*, a rubber connection may be used, as shown in diagram II, with the rubber tubing wired in place. Stopper *R* should be placed on the tube before bends *S* and *T* are made. Rubber or neoprene stoppers which fit well into the neck of the flask and the other openings have been used satisfactorily, in spite of considerable swelling with such solvents as petroleum ether. As a precaution stopper *P* should be wired in place. Stopper *K* should not be inserted too far into the bottle; if the bottom of the stopper extends more than about 0.3 cm. (0.125 inch), there may be difficulty in removing it at the end of the extraction. The bottom of the stopper should be sliced off as much as necessary. After exposure to the air for a day the stoppers return to their normal size. The steam coil, *E*, was made of glass, but metal pipe may be used where the extract is not of interest or is not harmed by contact with hot metal.

The percolator is filled and operated as follows:

A is supported by a sturdy clamp at the neck. Tube *O*, flask *F*, and steam coil *E* are then put in place. For compactness *O* should be placed against the side of *A*, like the siphon tube of a Soxhlet extractor. [For the sake of clarity in the diagram, it has not been illustrated this way.] *F* can be conveniently supported on a cork ring on the table top. The overflow tube, *B*, is slipped over the outlet tube and allowed to rest on stopper *P*. The top of *B* should be 1.88 to 2.5 cm. (0.75 to 1 inch) higher than the level of bend *N*. Cotton is packed loosely around the overflow tube in the neck of the bottle. A small, very loose plug of cotton may be placed at the top of tube *B* to keep floating particles from flowing into it, but care should be taken not to obstruct the overflow of solvent into this tube.

Stopcock *D* is closed, the percolator bottle is filled about half full with solvent, and the material to be extracted is put into the bottle and allowed to settle evenly by falling through the solvent. The percolator bottle should not be filled above the level of *N* with the material to be extracted. Usually the percolate will be clear, unless the material to be extracted has been ground very fine. In this case the stopcock may be turned to allow the percolate to run out through side arm *Q* into a beaker until it is clear. It is then shut off and the turbid liquid is poured back into the top of the bottle. Enough solvent is poured into the three-necked flask to keep it from half to three-quarters full during operation. The rest of the apparatus, tubes *G* and *J* and condensers *H* and *I*, is then put in place. The opening of *H* was reamed to receive the stopper on *G*. *J* has a small hole at *L* to keep the pressure at the top of the percolator bottle equal to that at the condenser outlets. The side arm of *J* is attached to *M* by a piece of rubber tubing, to prevent siphoning around *O*. The diameter of the glass tubing used will vary with the size of the apparatus. The dimensions shown in diagram I are for a 1-gallon percolator bottle and a 2-liter three-necked flask. Tube *G* should be as wide as will fit into the neck of the flask and should be beveled sharply at the lower end.

A very slow stream of steam is sufficient to boil ether or petroleum ether vigorously. The vapors ascend *G* and are condensed in *H* and *I*, from which the fresh solvent flows into the top of the percolator bottle. *F* may be heated externally by a water, steam, or oil bath if desired, in which case the steam coil, *E*, may be omitted. However, internal heating with a steam coil has proved to be far more convenient than any form of external heating. The arrangement of condensers as shown is extremely efficient, and *H* will condense practically all the vapors. Care should be taken not to boil the solvent so vigorously that all the vapors cannot be condensed in the two condensers. In the summer ice water may be circulated through the condensers by means of a circulating pump, in which case *I* should be protected by a calcium chloride tube.

The adjustment of stopcock *D* depends on how fine the material to be extracted has been ground. With coarsely ground materials *D* may be left shut completely, but with finely ground materials it may be left open. In any case the stopcock should not be left open so much that the solvent level in the percolator bottle drops below the level of *N*. If the source of steam, or other heat, is likely to be interrupted during the night when no one can take care of the apparatus, *D* should be left closed. In normal operation the solvent level in the percolator bottle will always be kept between the level of *N* and the top of *B*. When the material to be extracted has been ground very fine, the supernatant solvent may at first be slightly murky from suspended

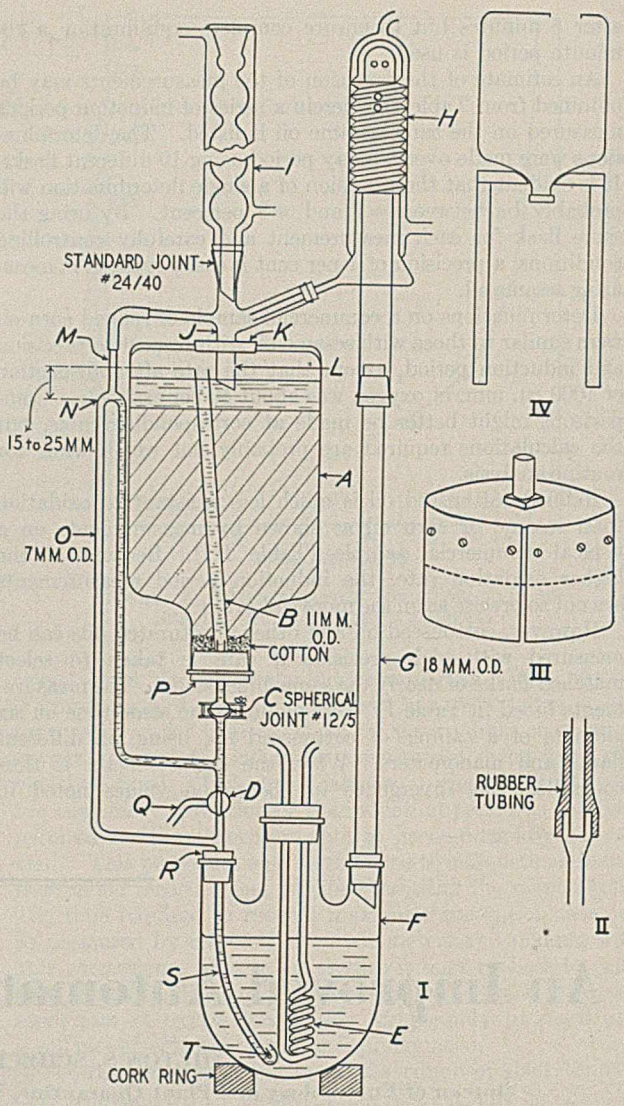

FIGURE 1. AUTOMATIC CONTINUOUS PERCOLATOR

particles. The settling of these particles may be hastened by opening stopcock *D* temporarily to allow the level of the solvent in the percolator to drop a little below the surface of the material to be extracted and shutting off the steam temporarily, if necessary.

In order not to subject the major portion of the extractive to prolonged heating, it is advisable after several hours to withdraw the percolate from flask *E* and replace it by fresh solvent.

When the extraction is completed, most of the solvent may be recovered directly from the apparatus by removing the outlet of *H* from *J* and rotating the condenser so that the solvent can be received in a suitable container. If the condenser water is warm, the distillate from *H* may have to be passed through an auxiliary condenser to make sure that all the vapors have been condensed.

This type of apparatus has been used to extract large amounts of plant material and has worked satisfactorily in every case with very little loss of solvent and very little attention after the apparatus has been adjusted.

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A Powder Compactor for Air-Permeation Experiments

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IN EXPERIMENTS involving the permeability of granular beds, a prime requisite is uniform compaction of the sample. In cases where the permeating fluid is air or some other gas, the compaction methods that have been used are of two main types, vibration and compression.

TABLE I. AIR-PERMEATION RESULTS IN DUPLICATE

(Surface mean diameters in microns taken at random from routine determinations, to indicate degree of reproducibility)

First Run	Second Run	First Run	Second Run
<1	<1	6.9	6.9
1.1	1.1	16	14
1.8	1.8	16	16
2.1	1.9	18	17
3.2	3.2	31	31

The vibration type of method is the more common. The procedure is to place the sample in the cylinder in which it is to remain during the permeation experiment, and either shake or tap the cylinder, laterally or vertically, by hand or by machine, until any channels or pockets in the sample have disappeared and the bulk volume has reached equilibrium. Vibrational compaction was used by Dalla Valle (*1*) for coarse sand; by Traxler and Baum (*4*) for slate, silica, and other materials in pulverized form; and by Gooden and Smith (*2*) for silica powder and other similarly fine materials. This general method is satisfactory for free-flowing samples.

Compression methods are sometimes used alone and sometimes supplemented by vibration. The compacted mass is either formed in the dry state (as is preferable) or formed wet and then dried before use. Traxler and Baum (*4*) experimented with wet-formed briquets for studies of average pore diameter, but found them unsatisfactory because of the tendency to cracking in the process of desiccation. Lea and Nurse (*3*), in determining specific surface of portland cement, consolidated the powder by tapping and then compressed it to a predetermined volume.

In the use of the self-calculating air-permeation apparatus for determination of average particle diameter, described by Gooden and Smith (*2*), it was found that the vibrator originally constituting an integral part of the apparatus was ineffective for certain low-density materials of extreme fineness—that is, around 2 or 3 microns in surface mean diameter. The compression method of Lea and Nurse was also inapplicable here. For reasons involving the wide variety of samples and the automatic calculating feature of the instrument, there is required a column of sample considerably taller than wide, as used, for instance, by Traxler and Baum, rather than the broad, low bed described by Lea and Nurse. The result is that pressure applied to the top of the sample is not transmitted freely all the way to the bottom.

The necessity for a new method of compaction gave rise to the designing of the simple device here described, which works well for all the many types of powders for which it has been tried.

Construction and Use

The compactor, as shown in Figure 1, *a*, is a plunger consisting of two parts, a rod and a foot. The rod is a piece of stiff wire between 1 and 2 mm. in diameter, with kinks distributed throughout its length except for a space of several centimeters near the top reserved as a handle. To the bottom end of the rod is attached the foot, which is shown in detail in Figure 1, *b*, *c*. The foot is of rubber, in the general shape of an inverted stopper with a portion (less than half) cut away on one side. The bottom of the foot (before removal of the segment) must fit smoothly inside the sample tube. If regular rubber stoppers are available in an appropriate size, the required cone frustum may be formed from a stopper by cutting off the large end at the level having the required diameter. Otherwise a piece may be cut out with a crank-type cork borer; a little practice may be required to obtain a suitable taper. The method of joining the two parts of the plunger is to pierce the foot about three fourths through without removing any of the rubber, and force the rod into the pore thus formed.

In use, the compactor is inserted in the empty sample tube, to the top of which a funnel is connected as closely as possible by a short piece of rubber tubing. The funnel stem and the sample tube should have practically the same inside diameter. The sample is poured in on top of the compactor foot. Then the plunger is alternately raised and lowered in short strokes and meanwhile twirled slowly between thumb and forefinger, so that the powder gradually works down past the foot, the latter packing the bed as fast as it is formed. Firm but gentle pressure is applied on each downward stroke. The kinks in the rod assist in feeding the powder and guard against clogging. The twirling motion serves both to increase the effectiveness of the kinks and to prevent compactional inequality due to the failure of the foot to cover the whole cross section. When the bulk of the sample has been packed in place, the plunger is used as a policeman to scrape down any powder left clinging to the wall of the tube.

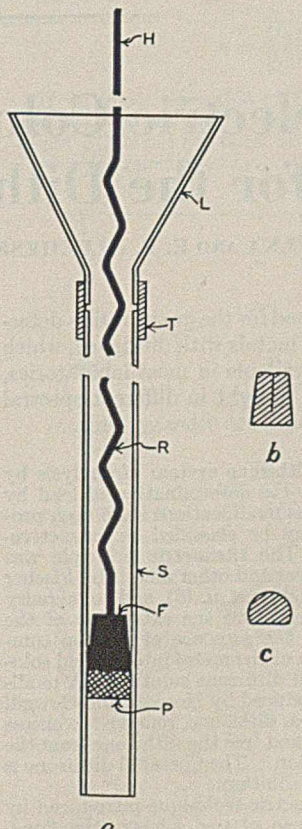

FIGURE 1. COMPACTOR

a. Compactor in sample tube, in position for loading
P. Porous support for sample
F. Foot of compactor
S. Sample tube
R. Rod of compactor
T. Rubber tubing
L. Loading funnel
H. Handle
b. Foot in longitudinal section
c. Foot in cross section

Tests

Only moderate care is required to produce practical uniformity of porosity, as judged by reproducibility of permeation results (Table I).

Comparison of results by this compaction method with those by vibration, as expressed in the respective values obtained for surface mean diameter by air permeation, is afforded by Figure 2. The six samples represent four materials—talc, diatomaceous earth, size fractions of commercial white arsenic, and a coarse fraction of synthetic cryolite. These samples were not in general subjected to size measurements by other methods than air permeation, and in some cases the existence of extraordinary shape characteristics would raise complications

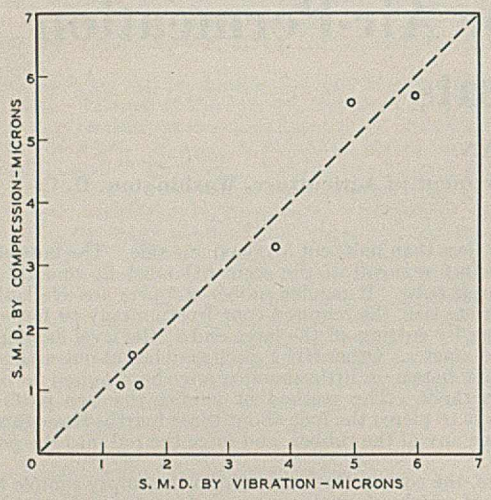

FIGURE 2. GRAPH FOR COMPARING RESULTS OF VIBRATIONAL COMPACTION AND COMPACTION BY COMPRESSION DEVICE

as to the definition of surface mean diameter. The purpose of presenting the comparison is to show that where both methods are usable, substantially the same results are obtained by the new and more convenient method as by the old. Materials coarser than those in the range of the graph usually offer little difficulty of compaction by any method, and many samples of the order of the finest samples here indicated cannot be compacted satisfactorily by any ordinary means of vibration. The system here described not only fills a special need but is suitable for very general use.

Summary

A new device for compacting powders builds up within the sample tube a column of any desired height, the compacting process proceeding from bottom to top concurrently with the deposition of the material. The combined process of loading and packing involves little more work than the simple task of loading alone. Designed particularly for use with the self-calculating air-permeation apparatus for measuring surface mean diameter of powders, it gives promise of equal usefulness in other fields involving permeability of powder beds to gases.

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Photoelectric Colorimetric Technique for the Dithizone System

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A SIMPLE and accurate method for the quantitative determination of lead and other metals with dithizone, which may be used with equipment available in most laboratories, involves the relative absorption of light in different spectral regions and can be readily generalized to other systems.

Since the introduction of the dithizone system of analysis by Fischer (*2*) and its application to the determination of lead by Fischer and Leopoldi (*3*), numerous modifications have been proposed. The current methods may be classified as extractive-titrimetric and as colorimetric. The titrimetric principle was applied to the determination of silver and other metals by Fischer (*4*), to lead by Wilkins and Willoughby *et al.* (*8*), and to mercury by Winkler (*9*). These methods involve the extraction of the metal from the original digest in the presence of fixation complexes when necessary, reliberation of the metal into an acid solution, and titration with a standard dithizone solution. A modification of this technique was introduced by Horwitt and Cowgill (*5*), who extract the lead with excess dithizone, remove the excess dithizone with a cyanide solution, and free the dithizone from the lead dithizonate with an acid solution. The liberated dithizone is then titrated with a standard lead solution.

The modifications of the colorimetric technique introduced by Fischer and Leopoldi (*3*) have been of two types: the "one-color" methods in which the excess dithizone must be removed; and the "mixed-color" method, proposed by Clifford and Wichmann (*1*), in which it is not necessary to remove the excess dithizone. The dithizone is allowed to partition between the aqueous and solvent phases and so modifies the color of the extracted dithizonate according to the relative amounts of dithizone and metal dithizonate. This method has the advantage of not requiring the removal of the excess reagent but has the disadvantage of requiring a series of standard dithizone solutions for the various lead ranges and careful control of the volume relationships between the solvent and aqueous fractions. This procedure is time-consuming if lead determinations are made at irregular intervals, since dithizone is relatively unstable.

The adaptation of the two-color methods to photometric measurements by the construction of standard curves avoids the repeated preparation of standards but has the disadvantage of requiring standard dithizone solutions for the extraction of the metal.

Spectrophotometric methods are selective and permit the determination of each of a series of colored constituents of a mixture. Weigert (*7*), who originated this technique, has determined as many as four constituents in a solution. This method, however, requires an instrument with a monochromatic light which is not generally available. A method adapted to photoelectric colorimeters and which does not require the removal of the excess dithizone or a standard solution of the reagent with which to extract the metal should have a distinct advantage for routine work. With this proposed technique, dithizone of any concentration (approximately 10 mg. per 100 ml. of carbon tetrachloride), but sufficient to ensure complete extraction of the metal from the solution, can be employed. The extract can then be diluted to a definite volume and the necessary photoelectric colorimeter readings taken.

The amount of lead present in the specimen can be determined from a nomograph adapted to the particular photoelectric colorimeter available.

For this work the Evelyn photoelectric colorimeter was employed. The spectrophotometer used for the absorption spectrum studies was described by James and Birge (*6*). A ribbon filament lamp served as the source of light. The slit was kept at a

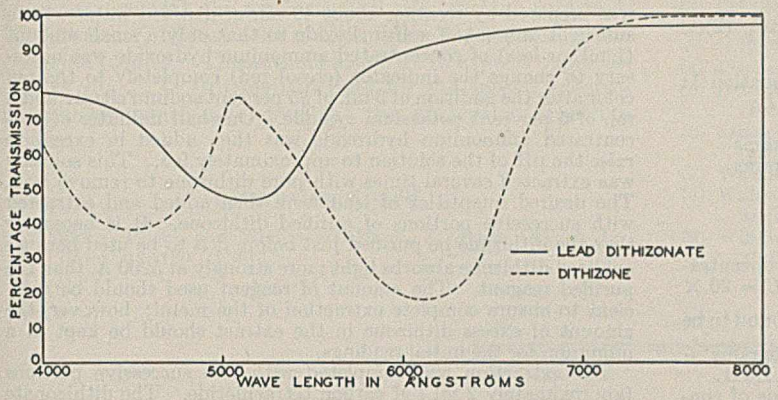

FIGURE 1. TRANSMISSION OF LIGHT

minimum and varied from almost completely closed to approximately 1 mm., depending upon the amount of light energy. The width of the light band was approximately 30 Å. The thickness of the absorption cell was 1 cm.

Principle of Technique

The percentage transmission of light of different wave lengths through solutions of pure lead dithizonate and of pure dithizone is plotted in Figure 1. It will be noted that lead dithizonate (1 microgram of lead per milliliter) in carbon tetrachloride has a maximum absorption in the region of 5200 Å. (45 per cent transmission) and that the absorption of dithizone in carbon tetrachloride is at a minimum in this region. Consequently, a maximum sensitivity to slight changes in the concentration of lead is obtained. The region centered at 6100 Å. is the most sensitive to slight changes in the concentration of dithizone. However, a filter centered at 6600 Å. was selected for this work because the readings in this region are not significantly affected by changes in the concentration of lead dithizonate. The transmission of light through lead dithizonate in this region is approximately 95 per cent.

Monochromatic light of a given wave length passing through a solution containing a mixture of dithizone and lead dithizonate should obey Beer's law, which is expressed by the relationship

$$\log_{10}\frac{\text{intensity of transmitted light}}{\text{intensity of incident light}} = k_d C_d + k_1 C_1$$

where C_d and C_1 are the concentrations of dithizone and of lead dithizonate, respectively, in any convenient unit. The k_d and k_1 are constants for any particular wave length which could be determined from the transmission coefficients of Figure 1. From this equation it follows that when the $\log_{10}$ of the 5200 Å. transmitted light is plotted against the $\log_{10}$ of the 6600 Å. transmitted light for a solution containing a particular value of C_1 and different amounts of excess dithizone, as in Figure 2, the curve should be a straight line. Furthermore, the straight lines for equal increments of C_1 should be equally spaced. However, slight deviations from this rule are observed, since Beer's law is not strictly applicable to conditions of a white light source passing through filters such as are used in photoelectric colorimeters. Other deviations from Beer's law are to be expected at high concentrations because of the effect of solvation. These deviations necessitate the calibration of the apparatus but do not decrease the accuracy of the determination.

The instrument can be calibrated by the preparation of a series of 3 to 5 standard lead dithizonate solutions for each increment of lead. Each successive member of this series must contain an increased amount of excess dithizone. The calibration can also be made with a single standard of lead dithizonate for each increment of lead and the excess dithizone added by 0.1-ml. quantities. The colorimeter readings are taken through the 5200 Å. and 6600 Å. filters on the initial solution and after each addition of the dithizone. The corrections of the 5200 Å. readings for the dilution of the lead dithizonate by the additions of the dithizone would be made as follows:

Owing to the additions of excess dithizone, the volume of the solution is no longer 10 ml. and the concentration of lead is changed correspondingly. L_2, the logarithm of the 5200 Å. reading, must be corrected to be on the basis of an even number (original concentration) of micrograms of lead per 25 ml. of the original solution. To do this we recognize that to a first approximation (corresponding to the assumption of Beer's law), the change in L_2 with the concentration of lead is a constant, k.

$$\frac{\partial L_2}{\partial C_1} = k$$

This derivative can be taken either at constant concentration of excess dithizone or at constant L_1 (logarithm of the 6600 Å. reading), since the lead dithizonate does not absorb the light significantly at this wave length. To evaluate k, it is only necessary to take two concentrations of lead, $(C_1)_a$ and $(C_1)_b$, which have essentially the same values for L_1.

Then

$$k = \frac{(L_2)_a - (L_2)_b}{(C_1)_a - (C_1)_b} \qquad (1)$$

If we know the value of L_2 for a particular concentration of lead, C_1, and we wish to know it for a concentration $C_1 + \Delta C_1$, where ΔC_1 is not too large, it suffices to use the first two terms in Taylor's expansion of L_2.

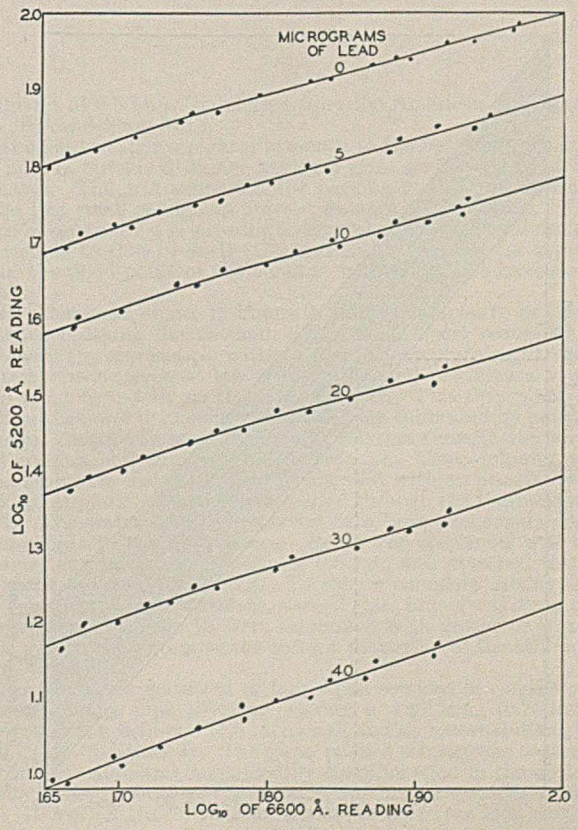

FIGURE 2

$$L_2(C_1+\Delta C_1) = L_2(C_1) + \frac{\partial L_2}{\partial C_1}\Delta C_1 + \ldots = L_2(C_1) + k\Delta C_1 \quad (2)$$

The value of k is determined by substitution in Equation 1: Taking two solutions, a and b, with essentially the same L_1,

a	$L_1 = 910$	$L_2 = 1.735$	$C_a = 10$ micrograms
b	$L_1 = 910$	$L_2 = 1.825$	$C_b = 5$ micrograms

Then $$k = \frac{1.735 - 1.825}{10 - 5} = -0.018$$

L_2 is corrected by substitution in Equation 2: The concentration, C, after the addition of 1 ml. of excess dithizone is $C = 20 \times \frac{10}{11} = 18.2$ micrograms, and L_2 for this concentration is found to be 1.473. Then, L_2 for 20 micrograms = L_2 for 18.2 micrograms + $k(20 - 18.2) = 1.473 - 0.018 \times 1.8 = 1.473 - 0.032 = 1.441$.

The nomograph, Figure 3, was constructed on a basis of concentration of lead per 25 ml. Final dilutions of lead dithizonate of different volumes must be multiplied by the appropriate factor.

Since dilutions of the lead dithizonate have an insignificant effect on the 6600 Å. readings, corrections of L_1, the logarithm of the 6600 Å. readings, are unnecessary.

Construction of Graphs

The standard lead solution containing 10 micrograms of lead per milliliter was prepared from metallic lead 99.98 per cent pure. The extraction of the standard lead solutions was done under conditions similar to those employed for biological specimens. Sulfur dioxide is used on digests of biological material to reduce the iron and oxidize the tin. Other interfering metals are removed chemically because this technique is applicable only to a two-component system. The details of the method for the determination of lead will be described in a subsequent publication.

The desired quantities of lead were extracted from approximately 50 ml. of an aqueous solution of pH 9.5. The solution was prepared by treating about 40 ml. of distilled water with a sufficient amount of sulfur dioxide so that only a small amount (1 ml. or less) of concentrated ammonium hydroxide was necessary to change the indicator (cresol red) completely to the red color after the addition of 5 ml. of 35 per cent sodium citrate and 5 ml. of 5 per cent potassium cyanide. One-half milliliter of concentrated ammonium hydroxide was then added in excess to raise the pH of the solution to approximately 9.5. This solution was extracted several times with pure dithizone to remove lead. The desired quantities of lead were then added and extracted with successive portions of purified dithizone. It is necessary that the dithizone be purified just before it is to be used because oxidized dithizone absorbs light more strongly at 5200 Å. than the purified reagent. The amount of reagent used should be sufficient to ensure complete extraction of the metal; however, the amount of excess dithizone in the extract should be kept at a minimum for the initial readings.

The extraction was completed with two successive portions (approximately 2 ml.) of carbon tetrachloride. The dithizonate was diluted to 25 ml. and filtered through a dry metal-free filter paper, and a 10-ml. aliquot was taken for the readings. Readings were taken through the 5200 Å. and 6600 Å. filters on the initial solution and after each addition of the dithizone. The excess dithizone was added in 0.1-ml. increments. It is essential that the dithizone solution for the additions be washed free of acid to prevent dissociation of the lead dithizonate.

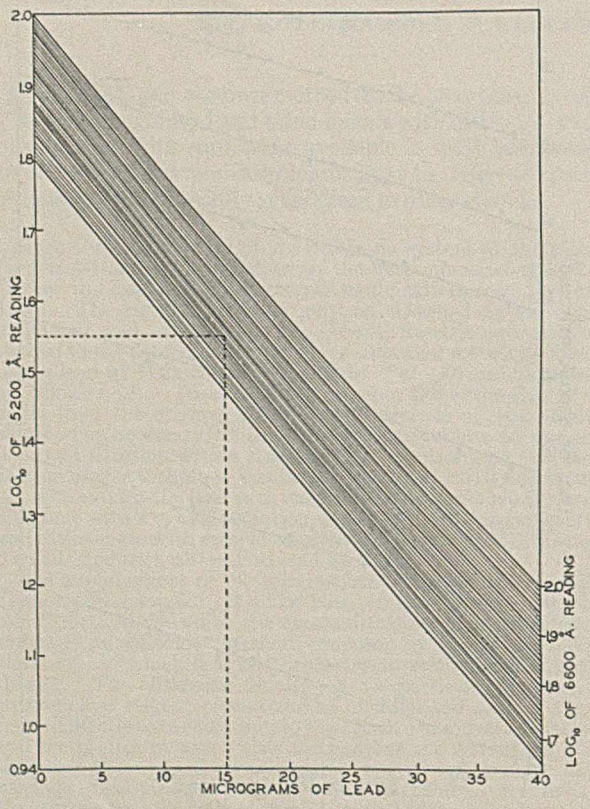

FIGURE 3. NOMOGRAPH

After the logarithms of the 5200 Å. readings had been corrected, the graph (Figure 2) was constructed by plotting these corrected values on the ordinate axis and the logarithms of the 6600 Å. readings on the abscissa axis. This graph should be of sufficient size to permit obtaining the logarithms of the 5200 Å. readings to the third decimal place.

The nomograph (Figure 3) was constructed from data obtained from Figure 2. The logarithms of the 5200 Å. filter readings were plotted as ordinates and micrograms of lead as abscissas. Millimeter paper was found to be the most convenient for this purpose. The most convenient scale was to have 1 cm. on the abscissa axis represent 1 microgram of lead and 1 cm. on the ordinate axis represent a 0.02-variation in the logarithm value. For each quantity of lead indicated in Figure 2 a series of 35 ordinate values (logarithms of the 5200 Å. readings) was obtained. Each reading corresponded to 1 cm. on the abscissa axis where 1 cm. represented a 0.01-variation in the logarithm values of the 6600 Å. readings. The graph was completed by drawing curves through the points which had the same logarithm values for the 6600 Å. readings. From this graph the micrograms of the metal can be read directly after readings have been taken at the two appropriate wave lengths.

Example (Figure 3): If the log of the 5200 Å. reading is 1.550 and the log of the 6600 Å. reading is 1.780, there are 15 micrograms of lead contained in a 25-ml. volume. If the volume of the final extract is 50 ml., the amount of lead in the sample would be twice 15 micrograms, or 30 micrograms.

Summary

A technique adapted to photoelectric colorimeters for the determination of one component in a two-component system is described. The technique is especially adaptable to the dithizone system because it eliminates the necessity of removing the excess dithizone and the use of standard dithizone solutions. It simplifies considerably the procedure for routine analysis. Recoveries of ±1 microgram of lead are obtainable.

Acknowledgments

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The authors are indebted to E. E. Miller, Department of Physics, and J. O. Hirschfelder, Department of Chemistry,

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A 60-Plate Low-Holdup Laboratory Fractionating Column

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The column described has a packed section of 10.5 feet of tubing 8 mm. in inside diameter, filled with glass helices. An efficiency of about 60 theoretical plates, H. E. T. P. of 2.0 inches, and holdup of less than 0.1 ml. per plate make it useful in the laboratory separation of complex mixtures. The simplicity of the all-glass construction, insulation, and heating elements makes it valuable to the laboratory which must consider ease and economy of construction.

A flexible and effective still head permits operation under total reflux, or variable or total take-off, and at atmospheric or reduced pressure.

IN ATTEMPTING to separate the components of a complex mixture resulting from the hydrogenation of lignin, it became essential to employ a column of high efficiency, low holdup, and moderate cost. Because of the large holdup of columns of the bubble-cap type (*3*), the difficulty of construction and high cost of some types such as the Stedman (*2*), and failure to duplicate the high efficiencies of several columns of the wet-wall type, an experimental study of packings, insulations, operating variables, and over-all efficiencies resulted in the design and construction of a column packed with glass helices, having a flexible head, and a holdup of less than 0.1 ml. per plate. This column has proved itself versatile and successful in the precise analytical fractionation of small quantities of material, and should be of value to any organic laboratory. For a comparison of this column with those previously reported, reference is made to a discussion of laboratory columns (*8*) and a recent review of the literature on laboratory fractionating columns (*14*).

Construction of Column

The principal features of the still are shown in Figure 1.

The glass column was constructed of Pyrex tubing 8 mm. in inside diameter. The packed section was 315 cm. (126 inches, 10.5 feet) long and was attached to a liquid-vapor separator at the bottom, and a still head of the total condensation, variable take-off type. The packing consisted of 210 ml. of single-turn glass helices having an internal diameter of 3 mm. and a fiber diameter of 0.5 mm. (purchased from the American Instrument Co., Silver Spring, Md.).

The liquid-vapor separator attached to the bottom was a bulb 20 mm. in outside diameter having a glass grid sealed in at the equator. The grid supported the packing in the tube and had holes just small enough to prevent passage of the helices. This type of separator was superior to several of conventional design, as well as to that of Snell (*12*), and had a capacity far greater than the flood point of the column, with but slight increase in holdup.

The still head, shown in Figure 2, allowed samples to be taken without breaking the vacuum, except for a short reduction to evacuate the new sample bottle, permitted accurate registration of temperature even at low reflux rates, and provided a visual drip point to determine the rate of reflux. This drip point, as well as the lower drip point (Figure 1), was calibrated by pouring a large, known volume of warm test mixture through the warm wet column and counting the drops. The head, column, and separator were sealed together as one unit with no possibility of leakage at joints. Packing was loaded through the thermometer well. The water condenser served as a heat exchanger, dissipating heat to the atmosphere. Below the condenser was the receiver cup having a capacity of 0.5 ml., and attached to the stopcock by capillary tubing. To ensure complete mixing and sweeping out of early distillate, a small glass funnel was set in the receiver cup. Stopcocks were lubricated with grease or water-base jelly in opposition to the solvent properties of the still mixture.

The insulation consisted of four equal sections of commercial asbestos steam pipe covering, having a 1.87 cm. (0.75-inch) central hole, a 3.75-cm. (1.5 inch) wall and an external diameter of 8.75 cm. (3.5 inches). The glass, packed column was centered in the insulation and supported by corks inserted in the central cavity of the steam pipe insulation. The heater consisted of 300 cm. (10 feet) of No. 26 Nichrome wire in each of the four heating units. It was inserted longitudinally in the steam pipe covering,

2.5 cm. (1 inch) from the outer skin, running parallel to and about 1.25 cm. (0.5 inch) from the surface of the packed tube. The one continuous heater wire was carried through the insulation, brought back in the next quadrant, carried down in the third and back in the fourth quadrant, with the ends brought out through the sides about 2.5 cm. (1 inch) from the end for connection to the line.

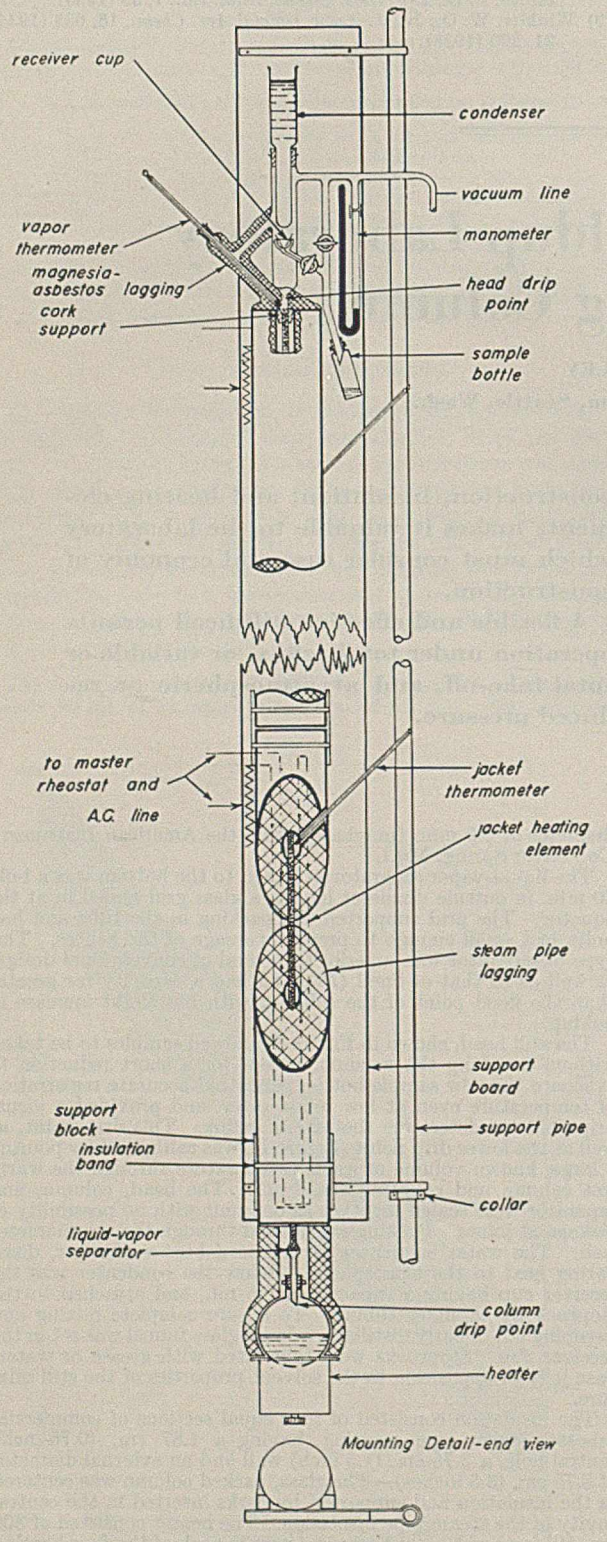

FIGURE 1. ASSEMBLY DRAWING OF COLUMN

A short piece of No. 30 Nichrome wire was attached to one end of each heating element to serve as an individual rheostat, providing an independent heat adjustment for each section of the heater-insulation to maintain a temperature gradient from top to bottom of the column. The four sections of the heater-insulation, each with its individual rheostat, were connected in parallel through a master rheostat to the 110-volt alternating current line to control the temperature of the entire jacket. Preliminary experiments with helically wrapped heating elements showed no advantage over the longitudinal type. Isotherms were determined in an empty section of insulation, with the longitudinal heater installed and ends sealed to eliminate end effect. Temperature variation in any part of the central hole of a section of insulation with the heating element 1.87 cm. (0.75 inch) from the axis and parallel to it in the 3, 6, 9, and 12 o'clock positions (on a cross section) was not more than 0.5° C. In the final, assembled column, thermometers were inserted in each of the four sections to measure jacket temperature, and each section of insulation was protected from heat loss by sealing both ends with asbestos-magnesia mixture. Similar lagging was applied to the glass column projecting below the insulation and to the vapor conduits in the still head.

The heater jacket with the glass column mounted in the center by cork spacer-supports was assembled and attached to a board (2.5 × 15 × 360 cm., 1 × 6 inches × 12 feet, long) by means of standard insulation bands held to the board under wooden cleats which also kept the insulation units aligned with each other. As a final support, a cradle was placed between the board and the still head and the latter fastened to it by a metal strap. The entire board was then mounted on a vertical pipe in the laboratory by two metal U-shaped stirrups at top and bottom, with adjustment for height provided by a metal collar clamped on the pipe. This arrangement permitted lifting and swinging the entire column, replacing the flask with another, and swinging and lowering into its former position over the heater. The cost of all the material used in this column, including lamp-bank rheostats, was less than fifteen dollars.

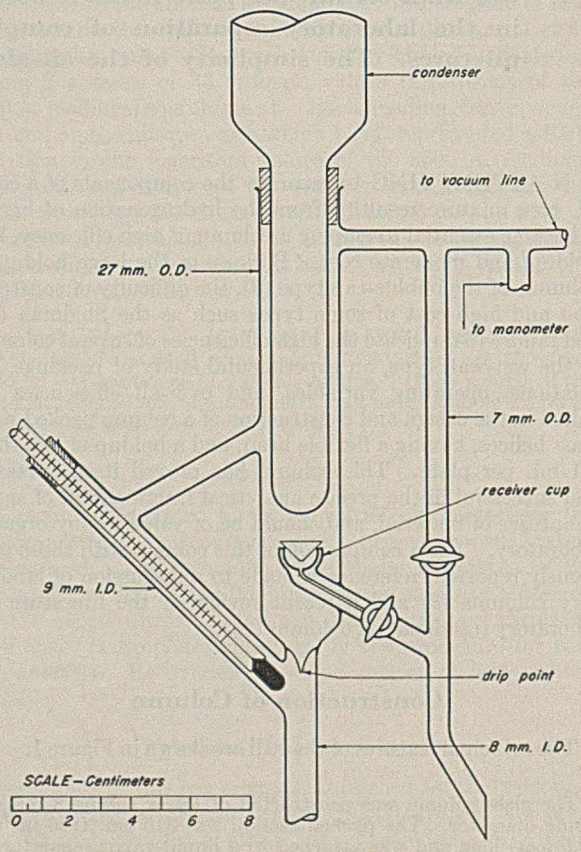

FIGURE 2. TOTAL CONDENSATION, VARIABLE TAKE-OFF STILL HEAD

Operation of Still

The liquid to be distilled was placed in a flask, attached to the column, and protected by an insulating hood, and boiling commenced at a moderate rate. The jacket required several hours to come to temperature and could be adjusted before or after boiling was begun. After reflux from the top of the column was established, the proper temperature of the jacket was attained by the master rheostat and the top-to-bottom gradient adjusted by means of the individual rheostats.

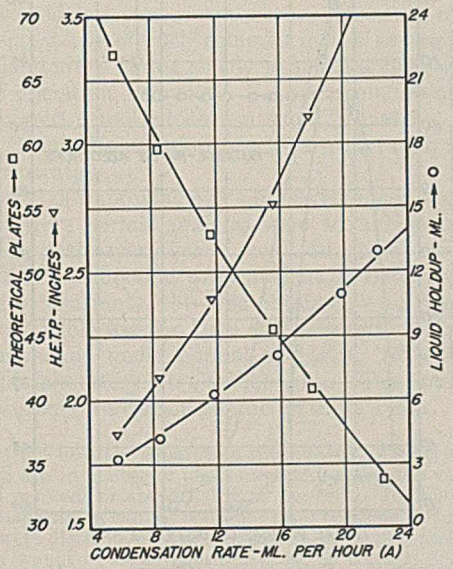

FIGURE 3. OPERATING CHARACTERISTICS OF COLUMN

(A) measured at upper drip point; lower condensation rate approximately constant at 60 ml. per hour. Moderate variations in lower condensation rate from this value had slight effect; variations in the upper condensation rate controlled efficiency.

Data were obtained chiefly on benzene–ethylene chloride mixtures but some values from carbon tetrachloride–benzene. Differences in two mixtures were very small. All tests were made at atmospheric pressure and total reflux. Jacket temperature was adjusted by comparison of upper and lower condensation rates. Take-off was about 1.0 ml. per hour except at 67-plate efficiency when it was 0.5 ml. per hour.

Most efficient fractionation was obtained at condensation rates of 60 ml. or less per hour at the bottom of the column, 10 ml. or less per hour at the top of the column, and with the jacket temperature within 1° to 2° C. of the vapor temperature. Such adiabatic operation could be established in 3 to 4 hours, starting with a cold boiler and column. Attainment of the initial liquid-vapor equilibrium required about 2 to 3 hours on total reflux for a binary mixture with a 3.5° C. separation of boiling points and about 0.5 to 1 hour to re-establish equilibrium conditions after removing a 0.5-ml. sample. The rate of take-off at 60-plate efficiency was about 1 ml. per hour. Better fractionation was obtained at lower condensation rates with the jacket temperature within a degree or so of the vapor temperature. Even at a condensation rate as low as one drop per minute from the still-head drip point, the thermometer recorded the correct temperature with no fluctuation of the mercury column. Correct temperatures were registered at all points on the boiling curve, "breaks" as well as "plateaus". The initial temperature in the still head often dropped 2°, with a binary mixture whose components boiled 3.5° apart, by the time equilibrium was established; on some experimental mixtures, a drop of 20° from the initial temperature was observed. Removing a small sample after equilibrium was attained caused a temperature rise which decreased to a constant value as equilibrium was re-established.

Efficiency Tests

The column was tested, with equipment and operation as described, at atmospheric pressure and with total reflux.

Binary mixtures of carbon tetrachloride–benzene and benzene–ethylene dichloride were first distilled through a 25-plate column, taking only a heart cut which had the correct and constant boiling point and specific gravity. All measurements of composition of the mixtures were by specific gravity. Efficiencies were calculated by the Fenske equation (*6*), the Dodge and Huffman equation (*5*), and the graphic method of McCabe and Thiele (*7*). Better agreement in results was obtained by the McCabe and Thiele method, probably because neither of the test mixtures is ideal. The liquid-vapor equilibria data were taken from the values of Rosanoff and Easley (*9*). All efficiencies reported here were determined by the McCabe and Thiele method and have one plate subtracted for the still pot.

Graphic summaries of operating variables are shown in Figures 3 and 4.

The flood point was approximately 400 ml. per hour. The pressure drop of the column was not determined.

Holdup

The amount of holdup is of great importance in laboratory fractionation. The bubble cap column has a holdup of about 1 ml. per theoretical plate (*3*), the Stedman 0.17 ml. per plate (*2*), the spinning band 0.12 ml. per plate (*1*), the column reported in this paper 0.06 ml. per plate, and the Selker-Burke-Lankelma column 0.04 ml. per plate (*11*).

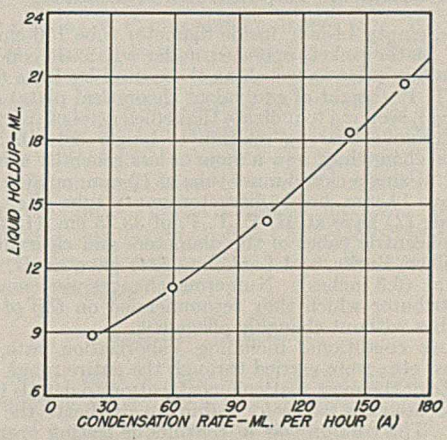

FIGURE 4. LIQUID HOLDUP AS A FUNCTION OF THE RATE OF TOTAL CONDENSATION

(A) measured at lower drip point. Upper condensation rate not accurately controlled but jacket temperature kept within 3° C. of vapor temperature. Data obtained under same conditions as Figure 3.

Holdup was determined with stearic acid by the method of Tongberg, Quiggle, and Fenske (*13*), by the drainage method, and by pouring a known quantity into the top of the warm dry column and measuring the drainable and nondrainable quantities. That reported in Figure 3 is the actual operating holdup (*13*) of the two binary test mixtures. Three factors had an important influence on the quantity of holdup: the surface tension of the mixture, the rate of vaporization (with total reflux), and the temperature of the jacket. Aqueous solutions usually had from two to four times the holdup of organic mixtures. Fortunately, holdup decreased with decreasing rate of vaporization and increasing efficiency.

Furthermore, maintaining the jacket temperature within 1° of the vapor temperature greatly reduced holdup; Selker, Burke, and Lankelma (*11*) also noted a perceptible decrease.

Elimination Curves of Known Systems

In order to show exactly what recovery could be obtained from a known mixture, distillations were carried out when only a small quantity of the more volatile component was present. The final, complete elimination curve gives the shape and magnitude of the boiling curve between plateaus and is a check on the quantity of holdup and a test of the minimum quantity of volatile component which is necessary to yield a pure distillate. The elimination curve is the basis for estimates of quantitative separation of unknown mixtures and a guide to the control of operating variables in such a separation.

Various elimination curves are shown in Figure 5. These data were obtained with the jacket temperature within 1° to 2° C. of the vapor temperature, at about 60-plate efficiency, at total reflux, take-off of 1 ml. per hour, and other conditions substantially as indicated by Figure 3. Figure 4 shows the rate and volume in which the more volatile component was eliminated from a charge of given volume and concentration. All the curves in Figure 4 are really enlargements of the break or boiling curve between two plateaus (representing a pure compound or constant-boiling mixture).

Discussion

A number of interesting efficiencies were experimentally determined during the preliminary fundamental study of analytical columns. The following were determined by benzene–carbon tetrachloride unless otherwise stated.

A single, empty 14-mm. inside diameter tube 150 cm. (5 feet) long with heater jacket, operated under adiabatic conditions at very low reflux rates approaching those used by Rose (*10*), gave an H. E. T. P. (height of equivalent theoretical plate) of 25 cm. (10.0 inches), seeming to indicate that efficiencies of short (30-cm., 12-inch) columns bear no relation to longer columns, presumably due to channeling, now a more or less generally accepted explanation. Using a closed inner tube of 12-mm. outside diameter in the above 14-mm. inside diameter empty tube after the manner of Craig (*4*) gave an H. E. T. P. of 33.75 cm. (13.5 inches). Adding concentric tubes of the diameters and clearances specified by Selker, Burke, and Lankelma (*11*) gave an H. E. T. P. of 15.75 cm. (6.3 inches). Numerous changes were made in the reflux distributor which they recommended on top of the concentric tubes without changing efficiency.

Operating conditions, including vaporization rate, offtake, total reflux, etc., were carried through the entire range specified, but in spite of the most meticulous attention to details it was not possible to assign a cause for failure to duplicate the reported efficiency. The column was in continuous testing operation for about 6 weeks and lack of time made further testing impossible. It may be that differences in wall thickness of the glass caused the failure, since this is reported to have an effect, or that the deviation from ideality of the benzene–carbontetrachloride test mixture did not permit duplication of the ideal mixture used by Selker, Burke, and Lankelma.

Removal of the concentric packing and adding glass helices gave an H. E. T. P. of 5 cm. (2.0 inches). Another column 12.5 cm. (5 feet) long having an inside diameter of 8 mm. instead of 14 mm. and similarly packed with helices also gave an H. E. T. P. of 5 cm. (2.0 inches). This made possible a considerable decrease in the volume of packing, and hence of holdup, and seemed to indicate that irregular packing next to the wall was unimportant or similar in both 14- and 8-mm. tubes and suggests that further reduction in the size of the tube, and hence of holdup, might be achieved. The final 315-cm. (10.5-foot) column was then built as the maximum convenient size and had an H. E. T. P. of 5.25 cm. (2.1 inches). It thus appeared that little difference in channeling existed in the 150-cm. (5-foot) and 315-cm. (10.5-foot) columns. Both had the operating characteristics of the typical wet-wall column described by Rose (*10*) and Craig (*4*)—i. e., increasing efficiency approached zero vaporization asymptotically. In other words, the lower the reflux rate the higher the efficiency, efficiency being limited by ability to maintain a low reflux rate.

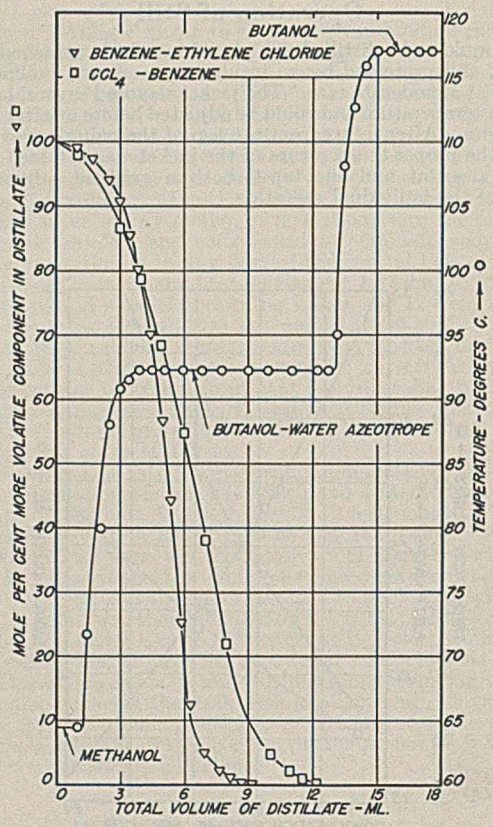

FIGURE 5. ELIMINATION CURVES OF KNOWN MIXTURES IN 60-PLATE COLUMN

Charge for benzene–ethylene chloride and carbon tetrachloride–benzene mixtures was 4.6 grams of more volatile plus 134.0 grams of less volatile component; recovery of volatile component was 99.7 to 99.8 per cent complete. Volume of methanol-water-butanol charge was 1000 ml. Steeper elimination curves were obtained by increasing flask concentration and reducing flask volume.

Voltage variation in the alternating current line used in these tests necessitated laborious manual control and limited the efficiency obtainable.

Other types of packing were also studied in the preliminary tests and although not very efficient are included here for comparison. These efficiencies were determined with benzene–carbon tetrachloride with reflux ratios between 5 to 1 and 10 to 1 unless otherwise noted.

1. Vigreux column with 30-cm. (12-inch) "packed" section and having a double vapor jacket of the Widmer type in addition to external insulation. H. E. T. P. = 11.75 cm. (4.7 inches).
2. Same but with a 107.5-cm. (43-inch) "packed" section. H. E. T. P. = 16.25 cm. (6.5 inches).
3. Raschig rings 7 mm. in diameter and 7 mm. long, packed section 50 cm. (20 inches) long. H. E. T. P. = 10.25 cm. (4.1 inches).
4. Brass jack chain, 100-cm. (40-inch) packed section. H. E. T. P. = 10 cm. (4.0 inches).
5. Glass helices, packed section 150 cm. (60 inches), total reflux, H. E. T. P. = 5 cm. (2.0 inches).

Most of the work on extremely efficient fractionating columns has been done by petroleum chemists who were not handicapped by limited quantities of material. Still-pot charges of at least several liters are commonly reported, and quantities of material between plateaus on the boiling curve of several hundred milliliters are not uncommon. This freedom is reflected in the design of many efficient columns and has prohibited the application of this effective tool to small quantities of material.

Recognition of the great need of efficient columns for the

separation of small quantities of material is shown by the development of 70- to 100-plate columns of extremely small holdup, such as those of Stedman (*2*), Selker, Burke, and Lankelma (*11*), and Baker, Barkenbus, and Roswell (*1*). Since columns of this latter type are still new and not yet widely used, there yet remains to be built up a literature of the specialized and difficult technique of successful operation and the later ingenious stratagems invariably proposed in all types of research to solve specific problems, arriving ultimately at a high level of efficiency, ease of operation, and simplicity, and low cost of apparatus. It would seem that the infrequent use of such columns in the average organic research laboratory is due chiefly to unsuitable design, complexity of operation, and cost. Yet such columns are an extremely powerful tool which can simplify many a formerly involved chemical separation to a clear-cut, rapid, and easy physical separation.

To the end of improving and simplifying operation, several deviations from normal practice were advantageously used in the analytical separation of small quantities. Since less than 1 ml. of a compound was sufficient for determination of the physical constants, this was obtained, even though the quantity available was less than that necessary for obtaining a pure compound under normal operation. Reaction mixtures in this laboratory often amounted to 1 liter or more with only a few grams of a certain product in the mixture.

To illustrate how the separation was effected, suppose 4 grams of benzene are in 1 liter of ethylene dichloride; the concentration is 0.4 per cent or about 0.5 mole per cent. In this dilution, 60 theoretical plates cannot separate pure benzene. However, if 20 grams were distilled, all the benzene would be in it and the concentration would be 24.2 mole per cent; a column of 60 theoretical plates with a sufficiently low holdup can easily separate pure ethylene dichloride from 20 grams of such a 24.2 mole per cent solution.

The column described actually separated pure ethylene dichloride from a solution having a concentration of 5.4 mole per cent.

To avoid the preliminary distillation to concentrate the compound sought, a slightly higher rate of vaporization was established, causing a greater amount of holdup, say 15 ml. The large flask was then removed and a short test tube quickly attached. On slowing down the rate of vaporization, the holdup also dropped to 4 to 5 ml., causing about 10 ml. to drain into the test tube flask.

One particular distillation may be cited as an example of this method. Pure benzene was removed from the top of the column until the distillate showed a trace of ethylene dichloride. The flask composition was a little less than 6 mole per cent benzene. The vaporization rate was increased, with total reflux; a test tube was substituted as a flask; and 8 ml. of holdup were drained back into the flask when reduction in the rate of vaporization permitted the holdup to decrease to 3.5 to 4 ml. This 8 ml. of holdup which returned to the flask had a composition of 78 mole per cent of benzene as compared to the earlier concentration of 6 mole per cent. After obtaining a small sample of the pure compound for determination of the physical constants, the total quantity was determined by the elimination curve.

A variation in the normal elimination curve was obtained by using a nonlinear thermal gradient from top to bottom. For example, in separating small quantities of methanol from a methanol-butanol-water mixture the methanol boiled at 65°, and the water-butanol azeotrope at 92°. Since only about 12 plates were necessary to effect the separation, the jacket temperature in the lower three sections was adjusted to 91°, and in the upper section to 63°. Accordingly, the holdup of methanol was reduced to one fourth of the normal column holdup and not only was more pure methanol obtained but the quantity of the mixture between plateaus correspondingly reduced.

An extremely close approach to adiabatic operation could be made by observation of the condensation rates at top and bottom of the column. The head was designed especially to provide a visual drip point and reflux distributor [although the 5-foot (150-cm.), 8-mm. inside diameter column had the same H. E. T. P. with the reflux running down the walls]. Best efficiencies were obtained when the condensation rates were very low—i. e., 60 ml. per hour at the bottom of the column, and 5 to 10 ml. per hour at the top. They were established by adjusting the heat in the boiler and then the heat in the jacket, and were vastly better guides than the thermometer readings of the jacket temperatures.

As a measure of the efficiency of this type of jacket, the data of Selker, Burke, and Lankelma are of interest. Their 5-foot, silvered vacuum jacket alone, without heater, condensed about 195 ml. per hour at 97-plate efficiency at a reflux rate of 195 ml. per hour plus the take-off rate, 0.16 gram per hour; in other words, the jacket alone condensed 99.9 per cent of the reflux. The jacket alone, described in this paper, operating at condensation rates of 30 to 45 ml. per hour at the bottom and 6 to 12 ml. per hour at the top, condensed only 24 to 33 ml. per hour or 73 to 80 per cent of the reflux and could be adjusted to condense from 0 to 100 per cent of the reflux.

This column is now effectively separating complex hydrogenation reaction mixtures. It is hoped that its utility will not be limited to this laboratory alone. It has given sharp separation of mixtures on which less efficient columns (20 to 25 plates) failed even to show any plateaus in the boiling curve, and hence the location of the boiling points of any compounds. With this degree of mixture, identification of individual compounds is practically hopeless. In common with others who have used efficient columns, the author believes their use is an absolute necessity for precise work and shares the opinion of Bruun and Faulconer (*3*), "It should be particularly noted that with a column containing only 26 plates, the separations to be expected are such that even compounds boiling 12° apart cannot be completely separated. Consequently, the need of columns containing a larger number of plates cannot be emphasized too strongly. Generally speaking, efficient separation of complex mixtures in stills containing less than 30 plates is almost hopeless. Similarly, packed columns with heights below 1.5 meters (5 feet) should be used only for separation of a few of the very simplest known binary mixtures."

Acknowledgment

To the expert skill of Ray G. Newbury, glass blower in the University of Washington, in constructing the more difficult parts of a number of fractionating columns, the author is greatly indebted.

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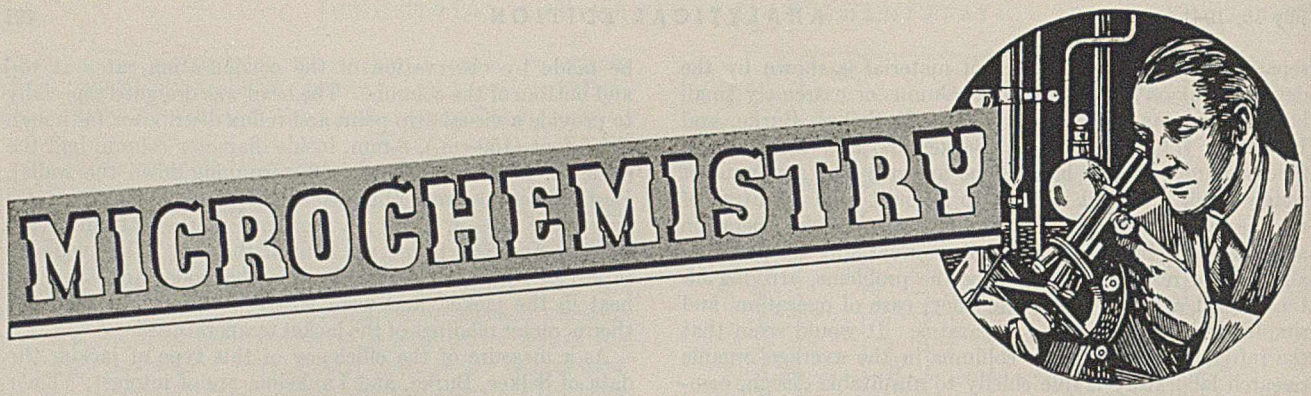

Determination of Lead in Biological Material

A Mixed Color Dithizone Method

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THE principal interfering elements in the determination of lead in biological material with dithizone are ferric iron, stannous tin, thallous thallium, and bismuth.

Ferric iron has an oxidative effect on the dithizone causing considerable decomposition of the reagent. Stannous tin and thallous thallium combine with dithizone under essentially the same conditions as lead.

To overcome the effect of the ferric iron, Behrens and Taeger (*1*) employed hydroxylamine hydrochloride. This reagent, however, is inadequate for specimens containing appreciable quantities of iron and a preliminary extraction of the lead with excess dithizone is necessary. The lead is then stripped from the lead dithizonate solution with a dilute nitric acid solution and re-extracted with purified dithizone.

Since tin can be expected to be present in varying amounts in all biological material upon which lead determinations are made, it is necessary to remove or inactivate it.

Laug (*8*) suggested dry-ashing with nitric acid to change the tin to metastannic acid. Fischer and Leopoldi (*2*) suggested volatilization of the tin as stannic bromide. Wichmann and Clifford (*10*) dissolved the ash with perchloric acid before treating it with hydrobromic acid and bromine in order to facilitate volatilization of the tin as stannic bromide.

While sulfur dioxide (sulfurous acid) was suggested originally by Fischer and Leopoldi (*3*) as a preservative for dithizone, it has not been employed as a reagent for inactivating the ferric iron and stannous tin. Sulfur dioxide will reduce the iron and oxidize the tin according to the following reactions:

$$2Fe^{+++} + SO_3^{--} + H_2O \longrightarrow 2Fe^{++} + SO_4^{--} + 2H^+$$

$$3Sn^{++} + SO_2 + 6H^+ \longrightarrow 3Sn^{++++} + H_2S + 2H_2O$$

The introduction of this reagent has a distinct advantage because it prevents the decomposition of dithizone by the ferric iron regardless of the amount present and at the same time oxidizes any stannous tin which may be present. This reagent eliminates the necessity of a preliminary extraction. In the absence of bismuth the lead can be quantitatively determined with one extraction.

To remove bismuth, Willoughby, Wilkins, and Kraemer (*12*) and Hubbard (*6*) suggested adjusting the solutions containing the lead and the bismuth to pH 2.0 and extracting the bismuth with a large excess of dithizone. Horwitt and Cowgill (*5*) suggested extracting the bismuth at a pH of 3.0 to 3.5. Gant (*4*) eliminated the bismuth by adjusting the stripping acid solution to pH 2.0. The bismuth which partitions into the aqueous phase with the lead is then extracted with excess dithizone.

This separation of the lead from the bismuth was essentially confirmed in this laboratory. It was observed that bismuth is extracted with difficulty with dithizone at pH 2.0; however, very little partitions into the aqueous phase at this pH when present as the dithizonate. A quantitative separation of the lead from the bismuth can be effected by stripping the lead from the dithizonate with a nitric acid solution of pH 2.3 to 2.5. At this pH satisfactory recoveries of both metals can be obtained.

Thallium did not interfere with the determination of lead under the conditions of this procedure. The mechanism by which the thallium is inactivated has not been determined. Apparently the metal is oxidized in the digestion process and is not reduced by the sulfur dioxide.

In the presence of antimony, lead recoveries are low because the two metals form a stable complex during digestion and prevent the lead from combining with dithizone. The character of the complex has not as yet been determined. However, preliminary studies indicate that the lead is combined in the proportion of three atoms of antimony to one of lead when digested in the presence of sulfuric acid. Digestion of the specimen with nitric and perchloric acids alone did not inactivate the lead. In the absence of antimony, a small amount of sulfuric acid is preferred to minimize the hazards of a perchloric acid digestion. Dry-ashing and subsequent solution of the ash with perchloric acid and hydrochloric acid did not inactivate the lead.

Method

REAGENTS AND MATERIALS. Potassium cyanide, 5 per cent solution, C. P. quality. Sodium citrate, 35 per cent solution, C. P. quality. Ammonium hydroxide, concentrated, redistilled. Hydrochloric acid, concentrated, redistilled if necessary.

Dithizone solution, 0.1 mg. per milliliter of redistilled carbon tetrachloride, purified before using. Cresol red indicator, 0.04 per cent solution. Calcium chloride, 10 per cent solution, purified by extraction with dithizone.

Ammonium oxalate, saturated solution. Oxalic acid recrystallized from a 1 to 1 hydrochloric acid solution several times, dissolved in metal-free water, and neutralized with redistilled ammonium hydroxide.

Nitric acid, concentrated, C. P. quality. Sulfuric acid, concentrated, C. P. quality. Sulfur dioxide, obtained in cylinders or generated by treating sodium hydrogen sulfite with concentrated sulfuric acid. Perchloric acid, 60 per cent. Hydrogen peroxide superoxol.

Filter paper, metal-free, Whatman No. 40 extracted with dithizone in carbon tetrachloride and finally with pure carbon tetrachloride to remove the dithizone.

Glassware, cleaned with a nitric acid–hydrochloric mixture

Table I. Logarithms of Readings with 5200 Å. and 6600 Å. Filters for Various Quantities of Lead

(With increasing amounts of excess dithizone)

	Micrograms of Lead					Log10 of 6600 Å. Readings[a]
	0	10	20	30	40	
Log10 of 5200 Å. readings with increasing amounts of excess dithizone	2.000	1.782	1.574	1.376	1.195	2.000
	1.974	1.754	1.542	1.342	1.159	1.950
	1.946	1.724	1.510	1.309	1.124	1.900
	1.920	1.698	1.484	1.282	1.092	1.850
	1.892	1.670	1.458	1.251	1.058	1.800
	1.866	1.644	1.429	1.221	1.022	1.750
	1.832	1.614	1.397	1.187	0.984	1.700
	1.797	1.578	1.360	1.149	0.950	1.650

[a] Each logarithm of 6600 Å. reading corresponds to logarithms of 5200 Å. readings for various quantities of lead.

and rinsed with metal-free water. Previous to using, it is rinsed with dithizone and finally with carbon tetrachloride.

Procedure

Urine. The technique for the separation of lead from urine is essentially the same as that suggested by Ross and Lucas (*9*).

Two hundred milliliters of urine are measured into a 225-ml. Pyrex centrifuge cup and made faintly acid to litmus. Three milliliters of 10 per cent calcium chloride solution are added and the lead and calcium are precipitated by adding 20 ml. of the saturated ammonium oxalate solution. After approximately one hour the specimen is centrifuged and the supernatant fluid carefully decanted. The precipitate is digested with 2 ml. of perchloric acid over a low flame. The digestion is completed by adding hydrogen peroxide dropwise until the solution remains colorless. The digest is then transferred to a 125-ml. separatory funnel. For convenience, the volume of the solution should not exceed 50 ml. Sulfur dioxide is slowly passed through the solution for about 5 minutes, and the solution is permitted to stand for at least one hour.

Twenty milliliters of the 35 per cent sodium citrate, 10 ml. of the 5 per cent potassium cyanide, and 4 to 6 drops of the cresol red indicator are added in the order mentioned. The solution is adjusted to approximately pH 8.4 (indicator completely changed to red color) and extracted with 5-ml. portions of the purified dithizone (shake for about 2 minutes for each extraction) until the carbon tetrachloride phase is no longer discolored by the presence of lead dithizonate. The extraction is completed by washing twice with a few milliliters of pure carbon tetrachloride. The extracts are pooled by draining them into a second separatory funnel. After each extraction it is important to allow a sufficient time for the complete separation of the two phases in order to prevent a transfer of any of the alkaline aqueous solution to the second separatory funnel. Should this occur, it may result in an appreciable loss of lead due to a significant change in the pH of the acid stripping solution. The lead is stripped from the dithizone by shaking for one minute with about 40 ml. of a nitric acid solution, pH 2.3 to 2.5. The dithizone solution is drained off after the two layers have completely separated and the aqueous phase is extracted several times with pure dithizone to remove any bismuth which may be present. A final extraction is made with pure carbon tetrachloride.

The aqueous phase containing the lead is treated with a sufficient amount of sulfur dioxide so that only a small amount of concentrated ammonium hydroxide (1 ml. or less) is necessary to change the indicator (cresol red) completely to the red color after the addition of 5 ml. of sodium citrate and 5 ml. of potassium cyanide. One-half milliliter of concentrated ammonium hydroxide is added in excess to raise the pH of the solution to 9.5. The lead is then extracted with 5-ml. portions of pure dithizone until the carbon tetrachloride layer is no longer discolored by the presence of lead dithizonate. The extraction is completed by washing twice with pure carbon tetrachloride. The extracts are drained into a 25-ml. volumetric flask, diluted to volume, and filtered through a dry metal-free filter paper into a clean colorimeter tube. The readings with the 5200 Å. and 6600 Å. filters are taken and the quantity of lead is determined according to the method described by Kozelka and Kluchesky (*7*).

The data necessary for the construction of the nomogram for an Evelyn colorimeter are given in Table I.

The values for eight points on the ordinate axis with the corresponding 6600 Å. axis values are given for the quantities of lead indicated in the table. After the curves through the points with the same 6600 Å. values have been drawn, the spaces between these curves are subdivided into five equal parts. Each subdivision represents a 0.01 variation in the 6600 Å. value. Satisfactory recoveries for various quantities of lead have been obtained with a similar graph with three different Evelyn colorimeters.

Blood and Other Soft Tissues. The wet-digestion method suggested by Wilkins *et al.* (*11*) with slight modifications was preferred to the dry-ashing method because it is less time-consuming and a complete solution of the inorganic material is more easily effected.

Ten- to 20-gram samples are placed in 300-ml. Kjeldahl flasks and digested with 2 ml. of sulfuric acid with repeated additions of nitric acid until the major portion of the organic material is destroyed. The digestion is continued with 2 or 3 ml. of perchloric acid and additions of small quantities of nitric acid until the solution remains colorless. It was found that addition of the sodium chloride–hydrochloric acid mixture was not necessary. Boiling with about 50 ml. of metal-free water effects a complete solution of the digest. The specimen is transferred to a 125-ml. separatory funnel and the lead is extracted according to the procedure described for urine, except that the solution must stand for at least 2 hours with the sulfur dioxide, and preferably overnight, in order to insure the complete reduction of the ferric iron.

Bone. Specimens (2 to 3 grams) are digested with approximately 25 ml. of nitric acid and repeated additions of small quantities of hydrogen peroxide.

The digestion is completed by the addition of 5 ml. of perchloric acid and repeated additions of small quantities of hydrogen peroxide until the solution remains colorless. The lead is extracted from the bone digests in the same manner as from the

Table II. Recoveries of Known Amounts of Lead

(From 200 ml. of urine and 10 grams of blood with 50 micrograms of stannous tin and thallous thallium added to each specimen)

Lead Present[a]	Lead Added	Lead Recovered	Error
Micrograms	*Micrograms*	*Micrograms*	*Micrograms*
	Urine		
10	10	20.2	+0.2
10	10	20.8	+0.8
10	5	16.2	+1.2
10	5	15.7	+0.7
10	15	24.8	−0.2
10	15	25.9	+0.9
10	20	30.0	0.0
10	20	29.3	−0.7
10	25	35.6	+0.6
10	25	36.0	+1.0
10	17	27.5	+0.5
10	17	26.1	−0.9
	Blood		
2.3	2.5	5.1	+0.3
2.3	7.5	9.2	−0.6
2.3	12.5	14.5	−0.3
2.3	20.0	22.0	−0.3
2.3	25.0	26.8	−0.5
2.3	15.0	17.9	+0.6
2.3	5.0	8.2	+0.9

[a] Reagents contained 1.5 micrograms of lead. Lead present includes reagent blank and lead present in normal specimen.

Table III. Recoveries of Lead from 20 Grams of Blood with Varying Quantities of Added Bismuth

Lead Present[a]	Lead Added	Bismuth Added	Lead Recovered	Error
Micrograms	*Micrograms*	*Micrograms*	*Micrograms*	*Micrograms*
3.0	12.5	12.5	15.8	+0.3
3.0	10.0	10.0	13.6	+0.6
3.0	5.0	10.0	8.6	+0.6
3.0	2.5	15.0	7.0	+1.5
3.0	30.0	20.0	33.8	+0.8
3.0	12.5	15.0	15.2	−0.3
3.0	7.5	12.5	10.0	−0.5
3.0	5.0	25.0	8.8	+0.8

[a] Reagents contained 1.5 micrograms of lead. Lead present includes reagent blank and lead present in normal specimen.

digests of other tissues, except that it is necessary to add 20 ml. more of the sodium citrate to keep the large quantity of calcium in solution and to prevent precipitation of lead as lead phosphate.

Results

The recoveries of lead from urine and blood specimens in the presence of thallium and tin are given in Table II. The recoveries of lead in the presence of bismuth are given in Table III. It will be noted that lead can be separated quantitatively from bismuth with the above technique.

Summary

A simplified mixed-color dithizone method for the determination of lead in biological material is described. The treatment of the digest with sulfur dioxide serves a twofold purpose: It reduces the iron and hence prevents the decomposition of the dithizone, and it oxidizes the tin and hence inactivates one of the metals which combines with the dithizone under essentially the same conditions as lead. The method eliminates the necessity of removing the excess dithizone or preparing standard dithizone solutions. Consistent recoveries of ±1 microgram of lead are obtainable.

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Analysis of a Single Drop of Liquid by Microfractionation

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IT IS frequently desirable and sometimes imperative to determine the composition of a drop of liquid. Fractionation is obviously desirable, but the method (*1*) now in use permits collection of but six to twelve fractions per drop, depending on the skill of the operator. Such limited information is of little value. This paper describes a procedure for collecting thirty to seventy fractions from a single drop. A graph of the boiling point of each fraction against the fraction number shows clearly the existence of one or more components and, with reasonable care, the percentage composition.

The utility of this method has been confirmed by using it as an ordinary experiment in an advanced course in laboratory technique. All graphs reproduced in this article are from the work of students who have first acquired experience on a benzene-xylene mixture of known composition and next shown their proficiency on a mixture whose components and proportions were unknown to them. Over half of the class exhibit a skill comparable to that illustrated by these graphs. An operator, whose performance with the mixture of known composition shows less proficiency, can still make creditable use of the method with unknown mixtures whose boiling points are further apart than those represented here, or with a drop in which one component is present in relatively large proportions. Save for constant-boiling mixtures the method will always reveal whether a drop consists of a single component or a number of compounds.

The basis for the development of this method is the procedure of Emich (*2*, *4*), whose technique has been accepted as standard, but numerous changes have been introduced. The fractionating capillary is tubular in order to provide a high ratio of surface to free space. It is supported in a vertical instead of an inclined position, and is packed with glass wool instead of being left empty. A copper block is substituted for a free flame as a source of heat. The column of the capillary is jacketed by a section of the block instead of being exposed to air.

Apparatus

FRACTIONATING CAPILLARIES. Two types of fractionating capillaries are illustrated in Figure 1. The first is a straight tube whose inside diameter is 1.5 to 2 mm. and over-all length is 13 cm. Soft-glass tubing (14 mm.) can be conveniently drawn to this size. The packing is glass wool, ground in a mortar until small enough to pass easily into the capillary tube, and inserted before the constriction is made. This constriction should be as short as possible, so that no volume of condensate larger than necessary will be trapped. A wet filter paper condenser is illustrated at the top.

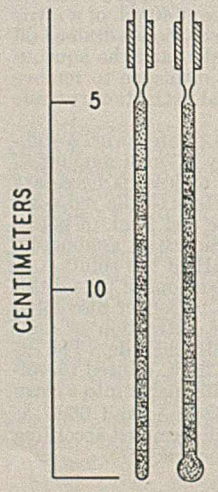


FIGURE 1. FRACTIONATING CAPILLARIES

The second type of fractionating capillary follows closely the conventional pattern of flask, column, and condenser used in macrowork. Construction is more difficult. Advantages are a lower liquid level in the capillary, a resulting increase in effective length of the column, and a larger distilling surface. Moreover, the bulb at the bottom facilitates removal of the insulating jacket.

COPPER HEATING BLOCK. The copper block, pictured in Figure 2, *a*, is 3.8 cm. square and 15 cm. high. Two holes, 6 and 8 mm., respectively, are bored to a depth of 9.5 cm. The thermometer in the first hole is to facilitate control of operations, not to measure the boiling point. In reality, it will record a temperature higher than the boiling point of the lower boiling component. The fractionating capillary shown in the second hole is in practice surrounded by glass or asbestos tubing open at both

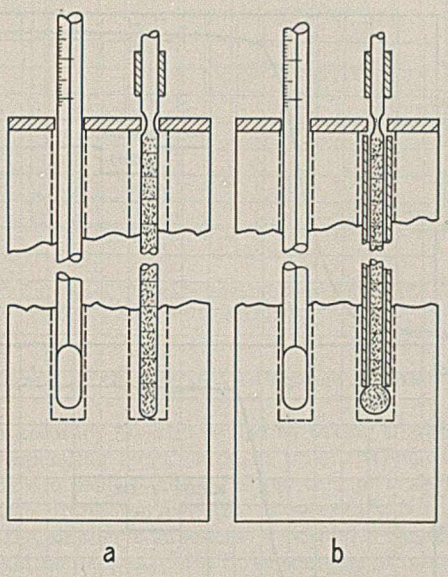


FIGURE 2. COPPER BLOCK USED FOR HEATING FRACTIONATING CAPILLARIES
Insulating jacket around capillary has been omitted from *a* in order to show better the various parts.

ends, which serves to insulate the capillary from the heated copper surface. With fractionating capillaries of the second type, the insulating jacket rests on top of the flask (Figure 2, *b*). A thin (approximately 1-mm.) sheet of asbestos covering the block reduces radiant heat which might otherwise prevent efficient cooling in the condenser. The asbestos fits snugly about the fractionating capillary and is cut from one edge to the center in order to facilitate its removal. A small gas flame directed against the bottom provides satisfactory heating. Electrical heat, produced from 12.2 meters (40 feet) of No. 30 oxidized resistance wire wrapped around the lower 4 cm. of the block and regulated by a variable transformer, is better.

BOILING-POINT CAPILLARIES. The form described by Emich (*2*) has in general been used. He prescribed a bore of 0.5 mm. or more. The authors experience no difficulty with tubes whose diameter is as small as 0.2 mm.; indeed, the smaller the bore the greater the number of fractions which can be collected from a single drop. The smallest permissible bore will vary with the patience and skill of each operator. A fragile boiling point capillary, with the even more fragile tip, which one man can handle safely is broken by another. The corresponding tiny droplet, seen readily by one, cannot be observed so easily by another. Much trouble will be avoided if each experimenter will first construct tubes of varying size and determine with a sample of pure benzene or other liquid the smallest size with which he can obtain consistent results.

The tip should be not less than 1 cm. long. The 2-cm. length prescribed by Emich is satisfactory but harder to make with so small a tube. The longer the fine tip, the less the liquid lost by evaporation during sealing. Soft-glass tubing, 10 mm. in diameter, is suitable for drawing the capillaries. The fine tip at the end is made with the aid of a microflame from a thin-walled capillary tubing whose diameter is about 0.8 mm.

Forty or more of these boiling point capillaries, having as nearly uniform size as possible, are constructed. They can be kept in order by inserting them in small numbered holes bored in a large cork or punched through the two halves of a sheet of notebook paper folded at the middle and standing on the two long edges.

Determination of Boiling Points

The boiling points for Figures 3, 4, and 5 were determined in a liquid bath similar to that described by Emich (*2*) and in greater detail by Gettler (*3*) and co-workers. All other determinations were made in a copper-block apparatus (*5*).

Fractionation

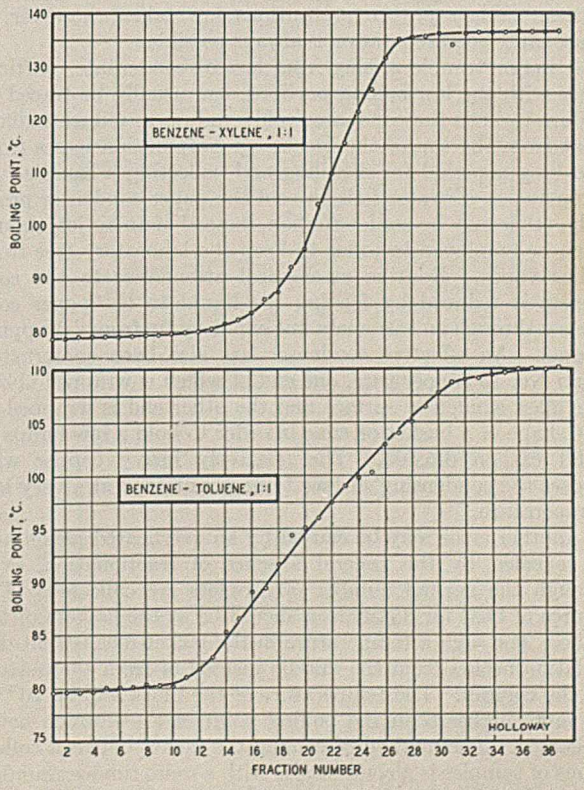
An operator should not attempt fractionation of an unknown mixture until he has first demonstrated his ability to distill and collect satisfactory samples from the fractionating capillary, using a drop with a single component such as benzene; and secondly, perfected his skill by practicing on a drop of known composition. These two preliminary trials should employ the technique to be used for the unknown sample.

The fractionating capillary is first weighed on an ordinary analytical balance. A single drop of liquid is pipetted into the tube, which is then centrifuged until the drop is forced through the constriction to the bottom of the capillary. The tube is weighed again in order to determine the weight of the sample. The paper condenser is next attached, the insulating jacket put in place, and the fractionating capillary inserted in the block.

Heat is applied gradually. The filter paper is wet and a fine stream of air directed against it to provide further cooling. When the first tiny droplet appears above the constriction, heating is discontinued, the asbestos cover pulled aside, and the fractionating capillary removed and centrifuged. An electrically operated centrifuge is preferred because the operator can continue to centrifuge while using this period to shut off the heat and cool the insulating jacket. Cooling is effected by a stream of air or by removal of the insulating jacket. In the latter case, the advantage of a fractionating capillary of the second type is apparent, for the glass or asbestos tube comes out with the capillary. After centrifuging, the capillary is replaced inside the jacket in the block. These operations need not be hurried. Three or four minutes of centrifuging may elapse before the fractionating capillary is replaced. The temperature of the block should also have fallen 4° to 5° during this period.

The whole sequence of operations, consisting of heating until a droplet is condensed, removing capillary, shutting off heat, centrifuging, cooling the jacket, and heating again is repeated three or four times. In each case care is taken to find the lowest temperature at which enough liquid for a determination will condense in the constriction within 1 or 1.5 minutes.

A fraction is next collected by touching the tip of the capillary boiling tube to the droplet. Heating is then reduced or discontinued and the fractionating capillary removed and put in the centrifuge. The small tip of the capillary boiling point tube is sealed. The boiling point can now be determined at once.



FIGURES 3 (*Above*) AND 4 (*Below*). BOILING POINTS OF BENZENE-XYLENE AND BENZENE-TOLUENE MIXTURES

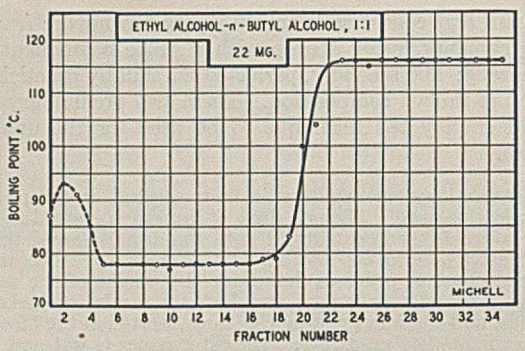


FIGURE 5. FRACTIONATION OF ETHYL ALCOHOL–BUTYL ALCOHOL DROP

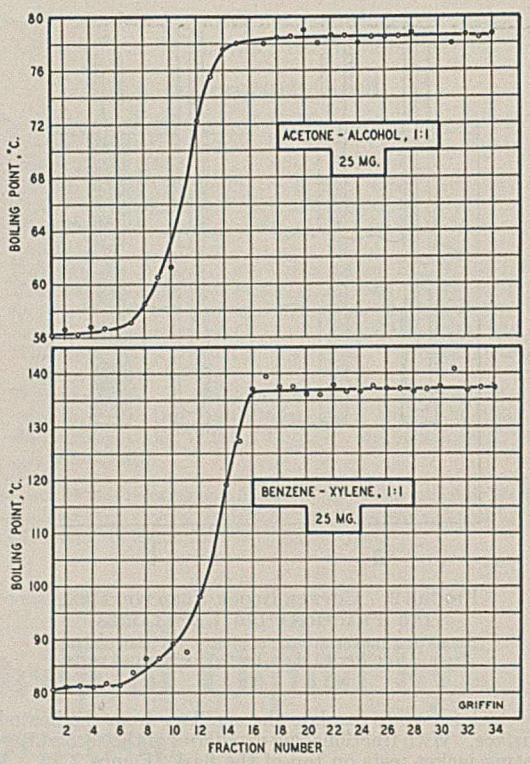
While making the necessary observations the insulating jacket is cooled. After observing the boiling point the fractionating capillary is taken from the centrifuge and replaced in the insulated jacket of the copper heating block preliminary to distilling the next sample. The block should not have cooled more than 2°. With some care all operations can be synchronized (not necessarily in the above order), so that the boiling point of one droplet can be determined before collection of the next. Such perfection of operations is ensured by having all apparatus assembled so compactly that minimum movement is required and by never permitting the temperature of the bath (used in determining boiling points) to fall more than 2° C. below the boiling temperature of the sample, so that little time is lost in this determination. After collecting the first two or three droplets, the process should be in smooth operation.

The number of fractions obtained varies with the individual. As many as seventy droplets[1] have been collected from a single drop weighing approximately 25 mg.; the usual number is thirty to thirty-five. The time required to fractionate and to observe all boiling points is 4 or 5 hours. Here also the time needed to perform rapid yet satisfactory work varies. When proper precautions are taken, however, a reasonably good separation is always possible.

A major trouble arising from loss of condensate, particularly with the low-boiling portions, can usually be traced to failure to keep the condenser cold; failure to reduce radiant heat from the top of the block; or evaporation losses from too long a period for distilling and collecting a sample. If evaporation of water fails to cool the paper condenser properly, ether may be used. Suction, instead of an air jet, should then be employed in order to keep the laboratory free from ether vapor. Routine mechanical attendance to the condenser is reduced by feeding the liquid to the paper condenser through an extremely fine capillary tip from a dropping funnel. An effective condenser has also been constructed from No. 13 copper wire, one end of which is wrapped about the glass condenser surface and the other end is wrapped in the shape of a basket or cone in order to hold a few lumps of solid carbon dioxide. The relatively heavy copper wire causes the condensing surface to be maintained at a very low temperature.

Another error may be caused by an overheated jacket and is revealed by the general absence of fractionation, even though the normal number of droplets are collected. The source of heat for distillation should be at the bottom of the tube. Too high a temperature in the jacket means that distillation occurs from the middle instead of from the bottom of the column. The temperature of the block will be 10° C. or more higher than the boiling point of the fraction being collected. The method of cooling the jacket between collections of samples is a compromise with a more time-consuming

[1] Since completion of the manuscript one of the students, Mr. Towle, has succeeded in obtaining 106 fractions from a drop weighing 23 mg.



FIGURES 6 (*Above*) AND 7 (*Below*). BOILING POINTS OF ACETONE-ALCOHOL AND BENZENE-XYLENE MIXTURES

operation and complicated apparatus required if the jacket temperature were accurately controlled. If the time for collecting a fraction is prolonged much beyond the minute or minute and a half specified, the jacket becomes too hot.

Lack of patience and care contributes considerably to poor results. Leisurely but steady work is better than a hurried pace.

Some additional refinements are possible. When it is apparent that the temperature is rising to a higher boiling fraction, greater care can be exercised to get minimum temperature for distillation, even if it is necessary to remove the tube, centrifuge, etc., a second time before collecting the next sample. It is also possible to keep better account of the amount collected by weighing each boiling point tube as the droplet is removed. Such operation becomes very tedious. With skill in making boiling point capillaries of uniform size and some practice in manipulation, it is possible to collect droplets of reasonably uniform volume. Boiling points of

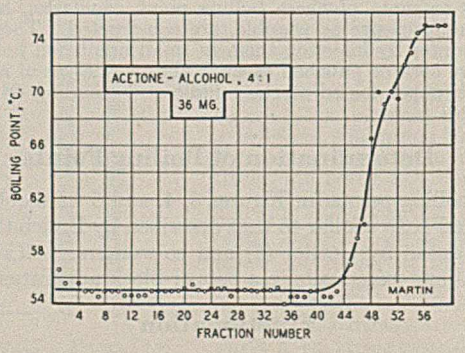


FIGURE 8. FRACTIONATION OF ACETONE-ALCOHOL MIXTURE

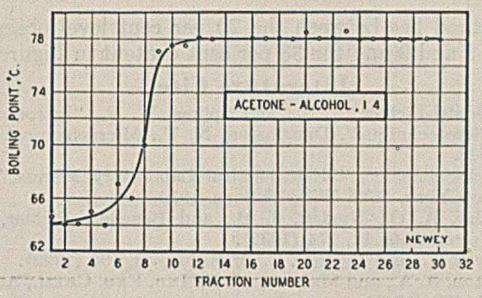


FIGURE 9. FRACTIONATION OF ACETONE-ALCOHOL MIXTURE

each fraction may then be plotted against the fraction number.

Greater accuracy can also be had by taking a larger sample with corresponding lengthening in time. When the quantity of one component falls to 20 per cent or lower, a larger sample (two drops, for example) is necessary if the boiling point of the smaller component is desired. Similarly, two drops would be necessary if a three-component mixture were being fractionated.

Results

Figures 3 to 12, inclusive, exhibit typical results of first-class work. Fractionation of unknowns in all instances was preceded by tests upon a known mixture of equal parts of benzene and xylene. Three of these preliminary trials are included (Figures 3, 7, and 9) because they show, by comparison with the adjacent graph obtained by the same experimenter for the unknown, the consistency with which an operator can reproduce his results.

Figures 3 and 4 form such a pair, the first on the trial mixture, the second on the more difficult benzene-toluene combination. The boiling points in each instance were observed in the conventional liquid bath. Assuming that fraction 22, half-way between the boiling point levels, in Figure 3 represents the mid-point, the composition would be 56 per cent benzene. If fraction 20 is the mid-point of the benzene-toluene pair, the composition would be 51 per cent benzene. No correction is made in either case for holdup in the column.

Figure 5 pictures the fractionation as an unknown of a drop containing equal parts of ethyl alcohol and *n*-butyl alcohol. The irregularity of the first four points is caused either by failure to heat, centrifuge, and reheat the microcolumn often enough to ensure constancy of operating conditions or by the slower rate at which the operations are necessarily performed during the first period. A similar effect on a smaller scale can be seen in Figure 9. Estimated composition on basis of a mid-point at the twentieth drop is 57 per cent ethyl alcohol with no correction for an assumed holdup.

Figures 6 and 7 show another pair of practice and unknown tests. The fractionations are particularly good as far as correctness of boiling point and sharpness of cuts are concerned. The percentage compositions are consistent with each other, although incorrect in absolute values. The difficulty is due to one of three possible causes: loss of some of the lower boiling component by ineffective condensing; collection of larger fractions at the beginning than were collected at the end of fractionation; or a personal factor on the part of the operator.

Figures 8 and 9 show extremes on two acetone-alcohol mixtures with different compositions. In both cases it proved impossible to get a correct value for the boiling point of the component whose concentration was as low as 20 per cent. The percentage compositions, however, were estimated with considerable accuracy, being 81 per cent acetone with the first mixture and 73 per cent alcohol with the second. No correction for holdup was applied in either case.

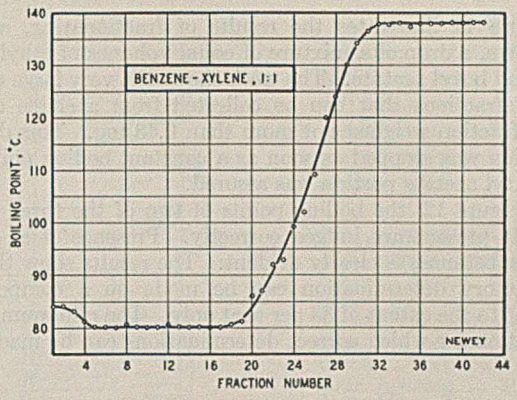


FIGURE 10. FRACTIONATION OF BENZENE-XYLENE MIXTURE

Figure 10 portrays the trial mixture which accompanies the results in Figure 9. Using the data in this practice test to make a correction for holdup in Figure 9, the results would be 80 per cent alcohol content, in agreement with the proportion used in the mixture. Such accuracy is not claimed in behalf of this process. The value of 73 per cent (uncorrected) on a mixture having 80 per cent of one component is, in fact, good work when the many variables in manipulation of this type are considered. Figure 10 also shows by comparison with Figure 3 the satisfactory results obtained in making determinations of boiling points in a copper block (*5*) instead of in a liquid bath.

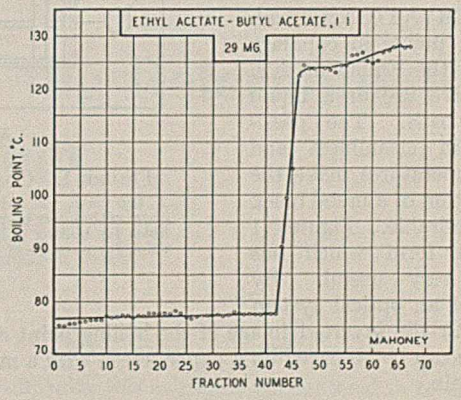


FIGURE 11. FRACTIONATION OF ETHYL ACETATE–BUTYL ACETATE MIXTURE

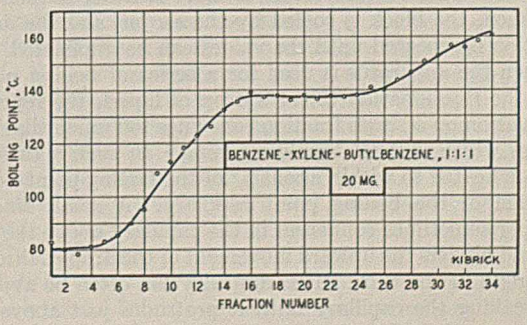


FIGURE 12. FRACTIONATION OF THREE-COMPONENT MIXTURE

Figure 11 illustrates the results of fractionating, as an unknown, a drop of a mixture of equal volumes of ethyl acetate and butyl acetate. The work shows the very large number of fractions that can be collected from a single drop. Each fraction weighed not more than 0.43 mg. This determination was stopped as soon as a constant boiling point of the butyl acetate portion was assured.

In Figure 12, the boiling points of two of the three components present are judged correctly. Presence of at least three substances is clearly evident. The results show that a satisfactory determination can be made on a component present to the extent of 33 per cent only. The minimum concentration for which correct determinations can be made by this method lies between the 20 per cent level observed in Figures 8 and 9 and the 33 per cent content in Figure 12.

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CONTRIBUTION No. 244 from the Research Laboratory of Organic Chemistry, Massachusetts Institute of Technology.

Copper Blocks and Optical System

For Determining Boiling Points (Emich Method) and Melting Points

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OBSERVATIONS of boiling points in capillary tubes by the Emich method (*1*) involve use of a liquid heating bath. The possibilities in cleanliness and high temperatures make the construction of a metal block very desirable. Figure 1 shows a form which has proved very useful. By means of an optical system (Figure 2) the inverted image of the boiling point capillary and drop can be observed on a paper screen with a minimum of eyestrain.

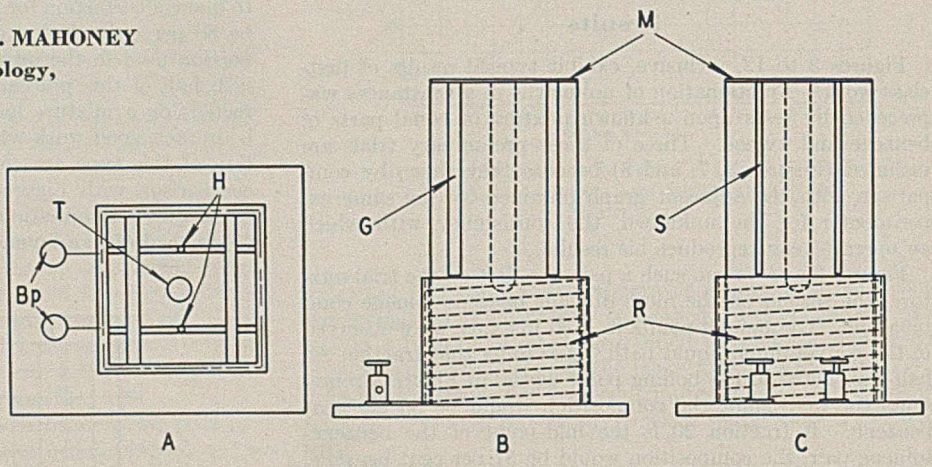

FIGURE 1. CONSTRUCTION OF COPPER BLOCK FOR USE WITH BOILING POINT CAPILLARIES

A, top view. *B*, side view parallel to light beam. *C*, side view at right angles to (facing) light beam. Visual observation can be made through slits *S* if a strong light is placed on the far side. *T*, thermometer hole. *H*, holes for boiling point capillaries in mica cover plate. *M*, mica cover plate. *G*, Pyrex glass plates 3.5 mm. thick for passage of light through block. *R*, resistance wire (40 feet of No. 30). *Bp*, binding posts. Block is 3.8 cm. across and 10.2 cm. high.

The determination of the boiling point in this apparatus need not vary from the usual practice in a liquid bath. The block is heated by the resistance wire until the droplet appears above the surface of the metal. Heating is then discontinued, the block is cooled by the air jet, and the determination is repeated until the values can be duplicated.

When the apparatus is used for a series of boiling points as in the fractionation (*2*) of a drop of liquid, the repeated observation on a single fraction consumes too much time in a process which is itself time-consuming. In such a case the block is heated to within about 5° of the boiling point before insertion of the boiling point capillary. A small amount of vapor sometimes condenses in the capillary above the surface and hinders the upward movement of the droplet through the length of the tube. This difficulty can often be avoided by breaking the capillary until it protrudes just above the surface, so that the vapor escapes. Such treatment occasionally results in a failure to get any value for one or two of

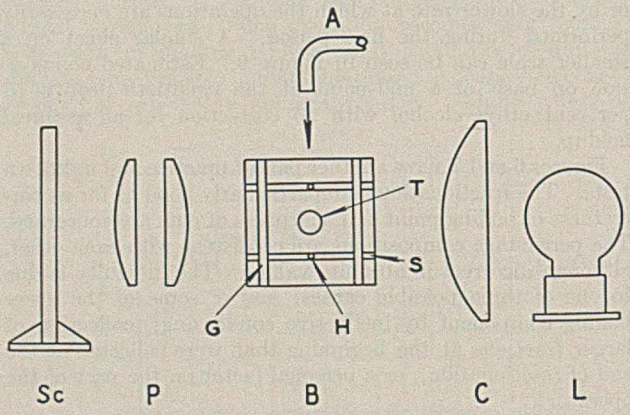

FIGURE 2. OPTICAL SYSTEM FOR BOILING POINT DETERMINATION

Sc, screen. *P*, projection lens. *B*, top view of copper block for boiling point determination. *A*, air blast directed against side of block. *T*, *S*, *H*, and *G*, as in Figure 1. *C*, condenser lens, 8.5 inches focal length. *L*, 100 watt lamp.

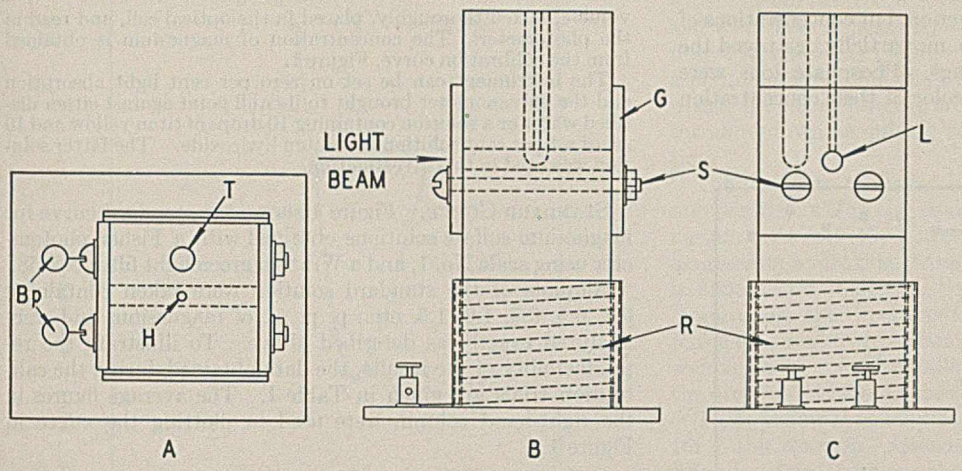

FIGURE 3. MELTING POINT BLOCK FOR USE WITH OPTICAL SYSTEM

A, top view looking down upon mica cover glass showing thermometer with cross slit and holes for capillary melting point tube. *B*, side view facing beam of light. *C*, side view parallel to light beam. *H*, 1.5-mm. hole for melting point tube. *L*, 5-mm. hole for light. *G*, Pyrex glass plates fastened to copper block by cross bolts, *S*. *R*, resistance wire (40 feet of No. 30). *Bp*, binding posts. Blocks are 3.8 cm. across and 10.2 cm. high.

the fractions, but the loss is not serious where so many determinations are made and a curve is plotted. Each separate determination in a series can usually be made more accurately than is possible in an isolated case, for the operator soon acquires a perfection in every detail of operation that is difficult to attain in individual cases.

This apparatus was used in most of the observations recorded by the authors (*2*).

MELTING POINT BLOCK. The authors constructed a copper block from 1.5 inches (38 mm. square) of copper for use with the same optical system (Figure 3). The relatively large opening in the side needed for the use of the optical system is protected by two Pyrex glass plates fastened closely to the side of the block by bolts as illustrated.

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CONTRIBUTION from the Research Laboratory of Organic Chemistry, Massachusetts Institute of Technology, No. 245.

A Photometric Method for the Determination of Magnesium

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IN A recent investigation involving the analysis of several soil extracts and fertilizers, it was necessary to determine fairly small quantities of magnesium. As a rapid and reliable method was desired, it was concluded that a colorimetric method, if sufficiently sensitive, might afford the most suitable procedure.

Several organic compounds which give color reactions with magnesium were studied, but none yielded as sensitive a test for this ion as did titan yellow. In an alkaline solution magnesium ions react with this compound to produce a red or pink color, which can be readily matched with standards or read in a photoelectric colorimeter. The use of titan yellow for the determination of magnesium was first suggested by Kolthoff (*2*), who stated that it was capable of detecting 0.2 part of magnesium per million of solution. He suggested using it in the analysis of tap water but reported only one determination.

In this investigation the method has been adapted to soil and fertilizer analysis and to a photoelectric colorimeter.

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Preliminary Investigation

Some difficulty was originally encountered in retaining the pink color developed after the addition of sodium hydroxide. In many instances the color faded rapidly and consistent readings could not be obtained; a small amount of hydroxylamine hydrochloride effectively stopped the fading.

In order to determine the stability of the color developed, various solutions of known concentration were read at intervals in the photometer over a period of several hours. The instrument was set on zero per cent light absorption and if necessary, adjustments were made to bring the galvanometer to its null point after each reading. The results obtained were very consistent—for example, one solution containing 1 p. p. m. of magnesium read 31, and when read at 20-minute intervals for the next 3 hours gave 31.0, 31.0, 31.0, 30.8, 30.8, 30.8, 30.5, and 30.5.

Solutions containing 4 p. p. m. of magnesium were found to be stable for 1.5 hours, whereas solutions containing 3 p. p. m. of magnesium were stable for 4 hours, and the more dilute solutions were stable for over 12 hours.

Ammonium ions up to a maximum concentration of 500 to

600 p. p. m. did not disturb the reaction, but concentrations of aluminum and tin as low as 4 p. p. m. partially destroyed the color and resulted in low readings. Phosphate ions were found effective in destroying the color, if their concentration was in excess of 100 p. p. m.

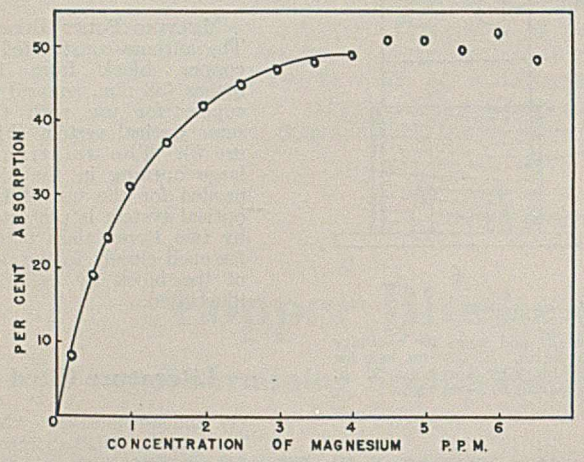

FIGURE 1. CALIBRATION CURVE FOR MAGNESIUM SULFATE

The curve in Figure 1 was originally calibrated in the presence of sucrose. It was believed this would aid in minimizing the effect of the calcium ion, and it was determined that calcium, in the presence of sucrose, up to a maximum concentration of 800 p. p. m. did not affect the readings—that is, 1 p. p. m. of magnesium could be determined in the presence of 800 p. p. m. of calcium. However, to obtain the greatest accuracy it is advisable to keep the concentration of calcium below 500 p. p. m. With no sucrose present calcium did not interfere until the concentration reached 500 p. p. m.

For accurate analysis 3 p. p. m. of magnesium is the maximum concentration that can be present in the solution being read in the colorimeter.

The light filter used was a Wratten No. 58, and its spectral absorption curve was determined by means of a spectrophotometer (curve 1, Figure 2). Since maximum transmission of light occurs at 530 millimicrons and maximum absorption of light by the colored complex occurs in approximately the same region (curve 2, Figure 2) it is apparent that the filter used was suitable for this particular color complex.

Method

REAGENTS. Titan yellow solution consisting of 0.15 gram of the dye dissolved in 75 cc. of 95 per cent ethanol and 25 cc. of distilled water.

Standard solution of magnesium sulfate, containing 100 p. p. m. of magnesium.

Four per cent solution of hydroxylamine hydrochloride, 5 per cent solution of sucrose, and 4 per cent solution of sodium hydroxide.

PROCEDURE. Iron, aluminum, and phosphorus are removed by precipitation as iron and aluminum phosphates (*4*). Calcium is removed by precipitation as the oxalate.

The filtrate from the above separations is evaporated to dryness, the ammonium salts are destroyed by ignition, and the residue is taken up in water. If the residue dissolves with difficulty a few drops of acid are added.

The solution is then made to volume and an aliquot is pipetted into a 100-cc. volumetric flask. Exactly 10 cc. of a 5 per cent sucrose solution and 2 cc. of a 4 per cent solution of hydroxylamine hydrochloride are added by means of pipets. Ten drops of the titan yellow solution are added and the contents of the flask are diluted to approximately 70 cc. with distilled water. Finally, 10 cc. of a 4 per cent solution of sodium hydroxide are pipetted into the flask while the contents are gently agitated. The color develops immediately and the solution is then made to volume, mixed thoroughly, placed in the optical cell, and read in the photometer. The concentration of magnesium is obtained from the calibration curve, Figure 1.

The instrument can be set on zero per cent light absorption and the galvanometer brought to its null point against either distilled water or a solution containing 10 drops of titan yellow and 10 cc. of a 4 per cent solution of sodium hydroxide. The latter solution was used in this investigation.

STANDARD CURVE. Figure 1 shows the standard curve for magnesium sulfate solutions obtained with a Fisher photometer using scale No. 1, and a Wratten green light filter No. 58.

Aliquots of the standard solution were taken containing 0.2, 0.5, 0.7, 1.0, 1.5, etc., p. p. m. of magnesium and were analyzed exactly as described above. To illustrate the reproducibility of the results, the data obtained during the calibration trials are given in Table I. The average figures in the right-hand column were used in plotting the curve in Figure 1.

TABLE I. PHOTOMETER READINGS CORRESPONDING TO DIFFERENT CONCENTRATIONS OF MAGNESIUM

Magnesium Concentration	Photometer Readings								
	1	2	3	4	5	6	7	8	Av.
P. p. m.									
0.2	8	9	8	7	7	7	..	..	8
0.5	19	18	19	19	19	18	18	..	19
0.7	24	24	25	24	23	24	..	..	24
1.0	30	31	31	31	31	31	32	31	31
1.5	37	37	37	37	..	..	..	..	37
2.0	42	41	41	43	43	42	40	42	42
2.5	45	45	45	45	45	..	..	..	45
3.0	46	46	47	47	47	..	..	..	47
3.5	49	49	49	47	46	48	..	..	48
4.0	49	49	48	49	48	48	47	..	49

Analytical Results

The method was used in the analysis of several commercial fertilizers, water, and six soil extracts, and the results are listed in Tables II and III. The magnesium was extracted from the 2-12-6 fertilizers by means of concentrated acids, and the phosphorus, aluminum, and calcium were removed as directed above.

Very excellent agreement between duplicates was obtained (Table II). With three samples results were identical, whereas with samples 1, 4, and 6 a deviation of 2.2, 3.8, and 6.6 per cent, respectively, was noted. With four of the samples different sized aliquots were taken and these results were almost identical with the other determinations. The analyses are therefore independent of the size of the aliquot.

Similarly, very excellent agreement among the triplicates run on tap water was obtained. This method is especially suited to water analysis, since it is very sensitive and rapid, and interfering ions are not ordinarily present in sufficient quantities to necessitate their removal.

TABLE II. DETERMINATION OF MAGNESIUM

Fertilizer No.	Magnesium Found		
	1	2	3
	P. p. m.	*P. p. m.*	*P. p. m.*
	Oven-Dry 2-12-6 Fertilizers		
1	525	533	538[a]
2	2000	2000	...
3	125	125[a]	...
4	313	325[a]	320
5	875	875[a]	...
6	75	80	80
Sample	Water		
1	12.50	12.50	12.75
2	5.75	5.70	5.70
3	3.00	3.00	3.00

[a] Different sized aliquots were used in these samples.

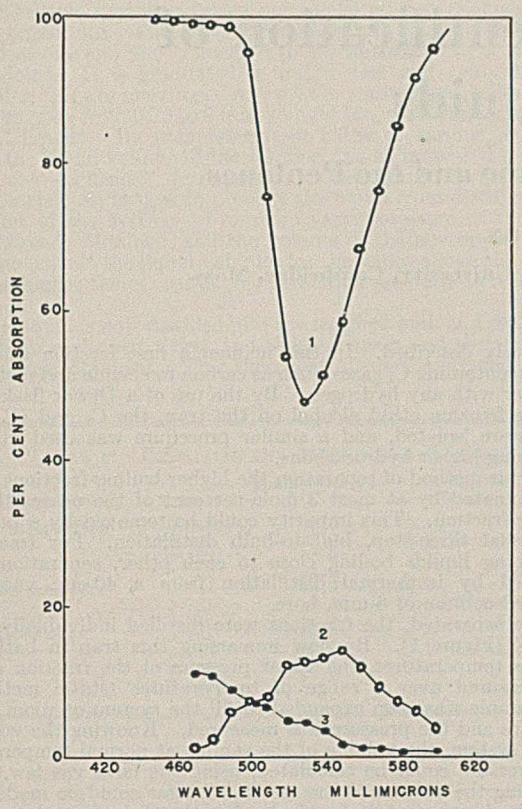

FIGURE 2. SPECTRAL ABSORPTION CURVES

1. Wratten green light filter 58
2. Color complex
3. Titan yellow

Exchangeable magnesium was determined on several soil samples by both gravimetric and colorimetric methods. The magnesium was displaced by leaching the soil with a neutral solution of normal ammonium acetate, the leachates were made to volume, and a 50-cc. aliquot was taken for the colorimetric analysis. Magnesium was determined gravimetrically on the remaining solution by precipitation as magnesium hydroxyquinolate (*3*). The results (Table III) show good agreement between the two methods. The maximum difference, about 8.5 per cent, occurs in sample 6, whereas the error for the other samples runs around 1 per cent or less.

TABLE III. DETERMINATION OF EXCHANGEABLE MAGNESIUM IN SOIL EXTRACTS

Sample	Colorimetric 1	Colorimetric 2	Colorimetric 3	Gravimetric[a]
1	7.00	7.00	7.00	7.03
2	7.00	7.00	6.50	6.90
3	5.50	5.50	5.80	...
4	11.60	11.40	11.20	11.53
5	10.00	9.50	9.80	9.68
6	8.50	8.20	8.50	7.68

[a] Acknowledgment is due Kirk Lawton, who determined the magnesium gravimetrically as magnesium hydroxyquinolate.

Discussion

The curve in Figure 1 flattens out in the neighborhood of 4 p. p. m. and for this reason the upper limit of concentration is set at 3.0 p. p. m. of magnesium. This flattening of the curve might appear as a possible weakness in the method, because on the level portion one could not judge whether he was dealing with concentrations of magnesium in the neighborhood of 4 p. p. m. or greater than 6.5 p. p. m. However, the point at which the curve levels off is easily detected, since a red, flocculent color lake appears almost immediately if the concentration of magnesium exceeds 4 p. p. m. Thus, if a solution gave a photometer reading of 48 or 49 a precipitate would appear with solutions containing around 6.5 p. p. m. Consequently, the aliquot can be quickly adjusted to the proper concentration.

The solubility of magnesium hydroxide is approximately 9 mg. per liter, which corresponds roughly to 4 p. p. m. of magnesium. At pH 13, however, the theoretical solubility of magnesium hydroxide (from solubility product calculations) is about 10^{-5} milligram per liter. The solubility may be larger under the experimental conditions because of the buffering action of hydroxylamine, or the possible formation of a complex with the titan yellow. The ultramicroscope showed the presence of colloidal particles in all the solutions examined.

Titan yellow is usually considered as an adsorption indicator in this reaction. Ginsberg (*1*) found that a state of equilibrium existed between the dyestuff and the so-called color lake, and from his equilibrium data concluded that a definite lake was not formed, since the lake formation follows the simple laws of adsorption.

As the maximum concentration of magnesium that can be present in any aliquot is 3 p. p. m., this method might appear applicable only over a very narrow range in concentration—namely, 0.5 to 3.0 p. p. m. of magnesium. However, as the size of the aliquot may be varied from 75 to 1 cc., the concentration range of the method actually lies between 0.5 and 300 p. p. m. of magnesium.

The procedure involved in this method is simple, and many samples can be analyzed in a very short time. It is much more sensitive and rapid than any gravimetric or colorimetric method in common use. It has given consistent results in this laboratory, but other materials should be investigated and further comparisons with other methods should be made.

Summary

A colorimetric method, based on the use of titan yellow and a photoelectric colorimeter, affords a rapid and reliable means for determining magnesium in quantities ranging from 0.5 to 300 p. p. m. in fertilizers and soil extracts. The colored complex is stabilized by hydroxylamine hydrochloride.

The procedure does not involve the separation and purification of any magnesium precipitate and thus eliminates several steps.

Calcium, up to a maximum concentration of 800 p. p. m., does not interfere with the determination of 1 p. p. m. of magnesium, but should be kept below 500 p. p. m. Similarly, ammonium and phosphate ions should be kept below 600 to 700 and 100 p. p. m., respectively, while aluminum and tin must be absent.

The spectral absorption curves for the colored complex and titan yellow were determined by means of a spectrophotometer. In the analysis of several commercial fertilizers, tap water, and soil extracts good agreement between duplicate determinations was obtained.

Results were found to agree well with those obtained by the hydroxyquinolate gravimetric method.

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CONTRIBUTION from the Soils Section, Michigan Agricultural Experiment Station. Authorized by the Director for publication as journal article No. 467 n. s.

Micromethod for Identification of Volatile Liquids

Vapor Pressures of Cyclopentane and the Pentenes

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IN THE course of a research on the photochemical decomposition of cyclic ketones, it became necessary to separate and identify very small quantities of volatile hydrocarbons. In this paper is described an apparatus for measuring the molecular weight, vapor pressure over a range of temperatures, and density of a liquid. As little as 5 mg. can be used and identified from the above properties. This method, together with the microanalysis technique of Blacet *et al.* (*1–5*) for gases, now constitutes a complete system for the identification of small amounts of products in kinetic reactions.

Apparatus

The unknown mixture of products was first fractionated by pumping it through a series of traps cooled to the appropriate temperatures. Where the mixture had a negligible vapor pressure in liquid air, the pumping was accomplished by using liquid air on the last trap. Where it contained permanent gases such as carbon monoxide or hydrogen, these gases were removed by pumping the sample through liquid air with a Toepler pump. Once they were removed, the separation could be carried on as previously described. In this manner a first fraction was obtained containing C_1 gases, such as carbon monoxide and methane together with any hydrogen. By the use of a Dewar flask containing freezing ethyl alcohol on the trap, the C_2 and C_3 fractions were isolated, and a similar procedure was used for the remaining higher hydrocarbons.

By this method of separation the higher boiling fractions were contaminated by at most 3 mole-per cent of the nearest lower boiling fraction. This impurity could be removed by a further isothermal three-step, bulb-to-bulb distillation. For fractions containing liquids boiling close to each other, separation was achieved by isothermal distillation from a 40-cm. vacuum-jacketed column of 3-mm. bore.

Once separated, the fractions were distilled individually into trap *T* (Figure 1). By now immersing this trap in baths at various temperatures, the vapor pressure of the fraction could be measured over a range of temperatures (static method). The sample was then expanded to fill the system at room temperature and the pressure was measured. Knowing the volume of the system, the volume of the sample at normal temperature and pressure could be calculated, using the ideal gas law. By adjusting the volume of the system, the gas could be made sufficiently dilute so as to be ideal. For the C_5 hydrocarbons, the pressures were below 30 mm. of mercury.

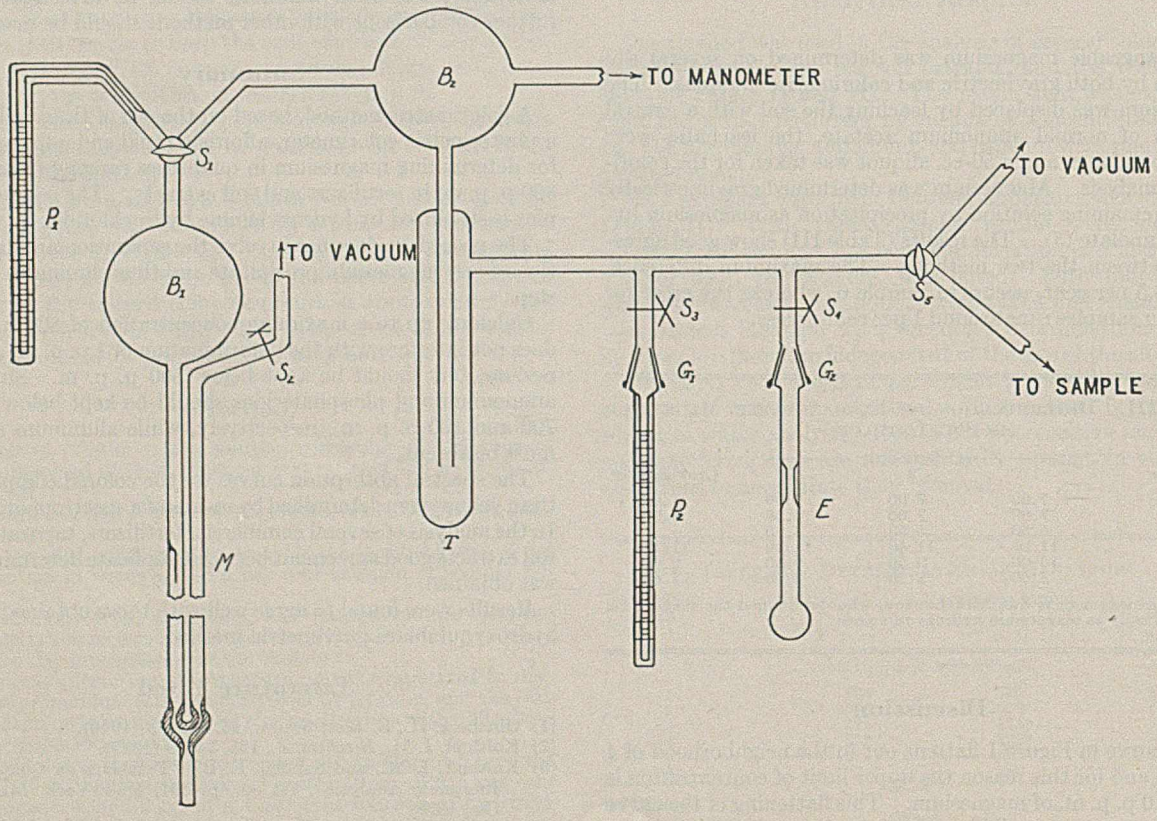

FIGURE 1. APPARATUS FOR MEASUREMENT OF PHYSICAL CONSTANTS

B.	100-cc. bulbs	*M*.	Manometer
B_1.	Toepler pump	*P*.	Micropycnometers
E.	Weighed bulb	*S*.	Stopcocks
G.	Ground-glass joints	*T*.	Trap

The sample was then frozen out in the micropycnometer, P_2, with liquid air. This micropycnometer is a capillary tube, sealed at one end and connected to the apparatus by a ground-glass joint. It is graduated in millimeters and calibrated by weighing drops of mercury. A few were constructed, with diameters varying from 0.75 to 3 mm. to hold larger or smaller quantities of liquids. By immersing it in baths at various temperatures the liquid volume of the fraction can be measured together with the coefficient of expansion. The stopcock above the pycnometer, S_4, is shut to prevent the liquid from distilling into the rest of the system. From the vapor pressure of the fraction, already obtained, and the volume of the micropycnometer one can correct the liquid volumes for the vapor above the liquid. At the higher temperatures this correction amounts to a few per cent.

The liquid is now distilled into the weighed bulb and sealed off. The bulb is cleaned, dried, and weighed again and the weight of the sample obtained. This then gives, from the previous measurements of the vapor volume at normal temperature and pressure and liquid volume, the molecular weight and the density of the fraction. In this particular research, the fractions were generally single-component systems, and the molecular weight was sufficient for identification. In some runs mixtures of cyclopentane and 2-pentene were obtained and the densities were used to determine the per cent composition, assuming ideal solutions. For confirmation the liquids were titrated with bromine to determine the olefin content. A discussion of these determinations will appear together with some further data in a later paper.

Table I. Densities and Molecular Weights

Compound	Density	Temperature, ° C.	Molecular Weight
Cyclopentane	0.847	− 98.0	70.1
	0.821	− 80.0	70.1 (theoretical)
	0.802	− 55.0	
	0.792	− 44.0	
2-Pentene	0.752	−105.0	70.2
	0.735	− 80.5	70.1 (theoretical)
	0.720	− 59.5	
1-Pentene	0.751	−105.0	68.6
	0.735	− 80.5	70.1 (theoretical)
	0.718	− 59.0	
	0.711	− 47.0	

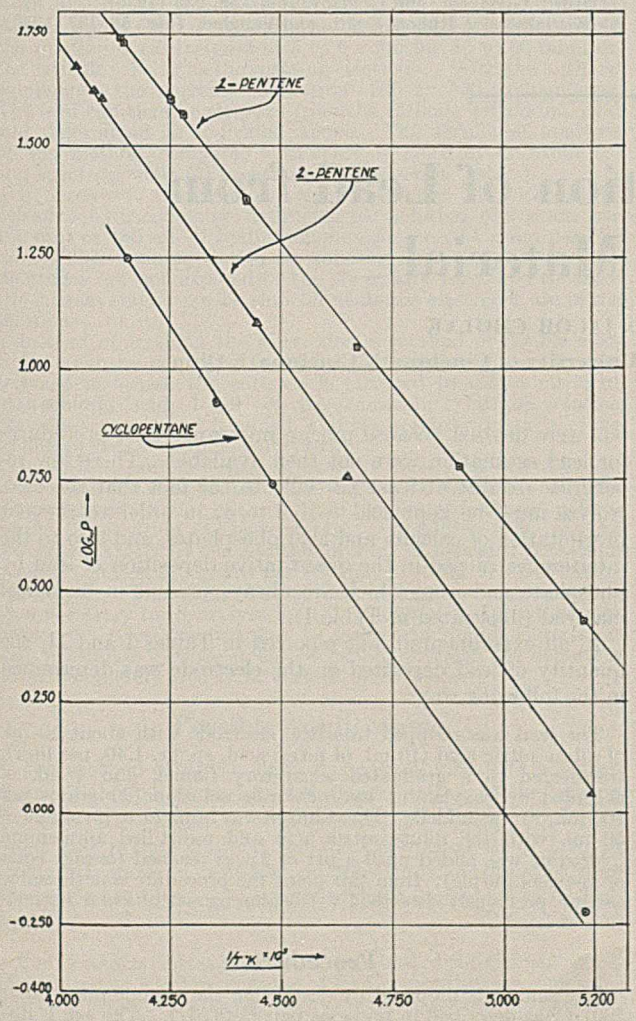

Figure 2. Vapor Pressures

The densities of 2-pentene and 1-pentene are too close to each other to use this property for identification, and in this case the vapor pressures were used to distinguish between them.

The Toepler pump and attached micropycnometer, P_1, are recent additions to enable the measurement of the densities of samples at or near room temperatures. At these temperatures, the vapor pressures are so high that the volume of the other micropycnometer, P_2, is too large and the material at these pressures begins to dissolve in the grease of the stopcock. The presence of stopcock grease in the system can be avoided and the volume of the system reduced to a few tenths of a cubic centimeter by using the Toepler pump as a mercury cutoff. These measurements at room temperature were found expedient, since they made possible the use of the densities noted in the literature. The extrapolation of these densities over a 100° range to the temperature of solid carbon dioxide is too inaccurate.

Materials

For a calibration of the apparatus, measurements were made on samples of cyclopentane, 1-pentene, and 2-pentene. The cyclopentane and 2-pentene were obtained from G. B. Kistiakowsky, who had used them in his calorimetric determinations of the heats of hydrogenation (*6*, *8*). The physical constants of the cyclopentane were: boiling point 49.1° C. at 760 mm. and n_D^{20} 1.4060. The 2-pentene had a boiling point of 36.5° C. at 760 mm. and n_D^{20} of 1.3798.

The 1-pentene was made by the method of Hurd (*7*) and was the middle fraction taken from the fractionation of the main product in a 90-cm. vacuum-jacketed still, packed with glass helices. It had a boiling point of 29.5–31.0° C. at 775 mm. and n_D^{20} of 1.3706.

All these samples were put through several bulb-to-bulb distillations before using them in the system.

Densities and Molecular Weights

The results of the measurements on the density and molecular weight of the hydrocarbons are given in Table I. The quantity of liquid used was in the neighborhood of 20 mg. and was determined to 0.1 mg. In a volume of the system (ca. 200 cc.) it had a pressure of about 30 mm. of mercury.

Table II. Vapor Pressures

P *Mm. Hg*	Log P	T ° K.	$1/T \times 10^3$
	Cyclopentane		
0.6	−0.222	193.0	5.181
5.5	0.740	223.0	4.484
8.4	0.924	230.0	4.348
17.7	1.248	240.5	4.158
	2-Pentene		
1.1	0.041	192.5	5.195
5.7	0.756	215.0	4.652
12.6	1.100	227.5	4.396
40.3	1.605	244.0	4.098
41.8	1.621	245.0	4.082
47.4	1.676	247.5	4.040
	1-Pentene		
2.7	0.431	193.0	5.181
6.0	0.778	204.0	4.902
11.2	1.049	214.0	4.673
23.9	1.378	226.0	4.425
37.1	1.569	233.5	4.282
40.2	1.604	235.0	4.255
54.0	1.733	241.0	4.149
54.9	1.740	241.5	4.141

The pressure was measured to 0.1 mm. of mercury on a U-tube mercury manometer, with a calibrated, glass, silver-backed scale. The liquid filled the micropycnometer to a height of about 25 mm. and this could be estimated to about 0.2 mm. The diameter of the capillary was about 1 mm. and its cross section about 8.5×10^{-4} cc. per mm. of length. Thus the calculated error on the measurements in Table I is about 0.5 to 1 per cent. This accuracy can readily be improved upon by using a smaller capillary and refining the other measurements—e. g., using a smaller system and weighing on a microbalance to 0.01 mg.

Vapor Pressures

The vapor pressures have been plotted in Figure 2 and the experimental points are recorded in Table II. Over the range of temperatures measured they fit very well the Clausius-Clapeyron equation:

$$\log P = -A/T + B$$

when P is in mm. of mercury at 25° C. and T is in degrees Absolute.

From the slope of the straight lines we can calculate the heats of vaporization in this range of temperature (Table III). In these measurements the pressures were read to 0.1 mm. of mercury and the temperatures to 0.5° C. Care was taken to ensure the establishment of equilibrium which was approached from both higher and lower temperatures. The calculated error in the constants recorded in Table III is about 0.5 to 1 per cent.

Table III. Heats of Vaporization and Clausius-Clapeyron Constants

Compound	A	B	$H_{vap.}$ Kcal./mole
Cyclopentane	1646.0	8.0880	7.524
2-Pentene	1512.5	7.7945	6.913
1-Pentene	1259.5	6.9570	5.757

The measurements of vapor pressures in the case of small samples have necessarily been limited to a narrow range of low pressures, because of the large size of the system. This limits the use of these vapor pressures in the determination of unknowns, since small amounts of volatile impurities introduce a rather sizable error in this range. Furthermore, the pressures are small and therefore not quite so accurate. An apparatus has recently been devised to measure vapor pressures on a micro scale up to the boiling point where small amounts of impurities do not create great errors and where the mole per cent can be determined. This will be fully described in a later paper.

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Electrolytic Deposition of Lead from Biological Material

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In an attempt to develop a polarographic method for the determination of microquantities of lead in biological material, it was found that the necessary separation and concentration of the metal could be accomplished electrolytically, provided certain requirements were fulfilled.

In most of the methods reported in the literature for this separation (*3*, *4*, *9*, *10*, *13*, *14*, *15*), lead is first precipitated as the sulfide or oxalate, in order to remove substances which would otherwise interfere with the electrolysis. Any procedure involving precipitation and filtration introduces opportunities for both loss and contamination when microquantities of lead are being handled and therefore a method was sought for the direct electrolysis of the prepared sample. Such a procedure would be even simpler than the one recommended by the Association of Official Agricultural Chemists (*1*), in which the lead is electrolyzed following its extraction with dithizone.

A few methods have been reported in which the lead in biological material is separated as lead dioxide by direct electrolysis of the prepared sample (*5*, *8*, *12*) or as metallic lead by electrolysis of fresh urine (*7*), but the authors were not able to secure quantitative separations by these procedures. It is possible that failure to obtain a complete recovery of traces of lead escaped notice at the time the methods were devised because precise microanalytical procedures for lead estimation were not then available. These low recoveries are due at least partially to the fact that the electrolyte must be kept acid (pH 3 to 4) in order to prevent precipitation of calcium and lead phosphates, and also to the interference caused in the quantitative deposition of lead by phosphate and iron, which are always present in biological material (illustrated in Table I).

In all experimental data reported in Tables I and II, the quantity of lead deposited on the electrode was determined in the following way:

The lead was stripped from the electrode with about 10 ml. of dilute nitric acid (10 ml. of nitric acid, sp. gr. 1.40, per liter), transferred to a graduated separatory funnel, and 1 ml. of deleaded hydroxylamine hydrochloride solution (20 grams per 100 ml., *2*) was added. The solution was brought to a volume of 50 ml. with the dilute nitric acid and redistilled ammonium hydroxide was added until a pH of 2 was reached (orange color of *m*-cresol purple); from this point the procedure was the same as that previously described (*2*) beginning with "Final Estimation of Lead".

Procedure

The sample is prepared by destroying the organic material at 500° C. as described in previous papers (*6*, *11*). The ash is dissolved in nitric and hydrochloric acids and double-distilled water.

Table I. Deposition of Lead

Sample	Pb Present	Lead Found: On anode, electrolyzed by A. O. A. C. method	Lead Found: On cathode, electrolyzed by new method (single)
	Micrograms	*Micrograms*	*Micrograms*
$Pb(NO_3)_2$ solution	50	46	..
$Pb(NO_3)_2$ solution + 0.1 gram of NaH_2PO_4	50	24	..
Same as above + 3 ml. of ammonium citrate solution	50	..	49.5
$Pb(NO_3)_2$ solution + 5 mg. of Fe as $FeCl_3$	50	17	..
Same as above + 3 ml. of ammonium citrate solution	50	..	49.0
Food 2786	22, determined by photometric dithizone method (*2*)	None	..
Same as above + 5 ml. of ammonium citrate solution			22.0

An aliquot or the entire sample, representing 10 to 25 grams of blood, 100 to 250 ml. of urine, 0.1 to 0.15 gram of fecal ash, or $^1/_{20}$ of a day's mixed food, is placed in a 20- to 100-ml. beaker. If the lead on the electrode is to be determined by dithizone, it is not necessary to prevent the deposition of iron, copper, or other substances which might interfere with the determination by other methods. Five to 10 ml. of deleaded ammonium citrate solution (40 grams of citric acid per 100 ml., *2*) are added to the sample, and the mixture is made alkaline to phenol red with distilled ammonium hydroxide, and diluted with 2 to 5 times its volume of double-distilled water.

An electrolytic apparatus similar to that described by the Association of Official Agricultural Chemists (*1*) is employed and the solution is electrolyzed at 5 to 6 volts for 30 to 60 minutes, the cathode serving as the rotating electrode. With the authors' equipment, the current varies from 200 to 500 milliamperes. The lead can then be stripped from the cathode with dilute acid and determined as indicated above. The results of analyses by this method, compared with those obtained by dithizone (*2*) and spectrographic (*6*) procedures, are given in Table II.

If necessary, the electrodes can be washed with 100 ml. of deleaded hydroxylamine hydrochloride solution (0.2 gram per 100 ml.) or 100 ml. of hydroquinone solution (0.1 gram per 100 ml.). This washing should be done by siphoning, so that the electrodes are not exposed to the air at any time, and the electric current should not be shut off while the electrodes are in the solution.

Instead of washing the electrodes, a method of double electrolysis can be employed in which the sample solution is electrolyzed as usual, the cathode is stripped in dilute nitric or hydrochloric acid, 1 ml. of the ammonium citrate solution previously mentioned is added, and the solution (made alkaline to phenol red) is again electrolyzed.

In certain samples, such as those of feces and mixed foods, the copper and iron content is so high as to interfere with subsequent determination of the lead on the electrode by the polarograph. Deposition of those metals, particularly iron, can be prevented largely by the addition of 2 ml. of deleaded potassium cyanide solution (10 grams per 100 ml., *2*) to the solution before electrolysis. This delays the plating action, however, and it is then necessary to electrolyze for 1 to 2 hours. If desired, greater purity of the lead deposit may be secured by double electrolysis with potassium cyanide in each solution, although the deposit is never completely free from certain other metals.

Possible Applications

The method of direct electrolysis, combined with a photometric dithizone procedure for determination of the lead on the electrode similar to that referred to above, provides a rapid and precise method for the determination of lead in biological material. A few modifications of the dithizone procedure would be necessary to make it applicable to routine work.

For instance, instead of three standard dithizone solutions, each suited to a different range of lead concentrations, one solution only could be employed, and varying quantities used according to the amount of lead present. The strength of the solution could be so adjusted that 5 ml. would be equivalent to 10 micrograms of lead; if a 5-ml. portion of the solution turned red after the lead-containing solution was shaken with it, additional quantities of the standard could be used, in 10-ml. portions, until an excess of dithizone was present; the dithizone solution would not be drained out between successive additions, and the color of the final mixture could then be read photometrically. While such a method would not be more accurate than the better dithizone extraction procedures, it would probably be much more rapid, provided a number of electrolytic units were available and could be used simultaneously.

A polarographic method of determining the lead deposited on the cathode will be described in a later paper (*5a*). It is possible that other ways of determining the lead on the electrode, such as titration or colorimetric methods, can also be worked out, particularly in view of the fact that the lead deposit is often very pure.

Summary

In a new method for the electrolytic deposition of traces of lead from biological material, the solution of the ashed sample is electrolyzed directly, without previous separation of the lead. The electrodes can be washed and other metals can be complexed, so that the lead deposit on the cathode is often almost entirely free from other substances. The lead can then be determined by the use of dithizone, the polarograph, or by other methods.

Table II. Deposition of Lead

Sample	Sample Electrolyzed	Lead Found: By electrolysis	Lead Found: By dithizone extraction (*2*)	Lead Found: By spectrograph (*6*)
	Micrograms	*Micrograms*	*Mg./l.*	*Mg./l.*
$Pb(NO_3)_2$	10	9.6[a]	..	..
$Pb(NO_3)_2$	10	9.8[b]	..	..
	Ml.	*Mg./l.*		
Urine 2919	92	0.077	0.077	..
Urine 2919	9.2	0.087	..	..
Urine 3948	66	0.160[a]	0.17	0.17
	Grams	*Mg./100 g.*	*Mg./100 g.*	*Mg./100 g.*
Blood 4397	8	0.067[a]	0.07	0.065
Blood 4011	8.5	0.065[b]	0.055	0.06
Blood 3687	17.5	0.04[b]		0.045
		Mg.	*Mg.*	*Mg.*
Feces 3555	0.1–0.15 ash	0.29[c]	0.30	0.285
Feces 3499	0.1–0.15 ash	0.70[d]	0.71	0.696
Feces 3499	0.1–0.15 ash	0.71[e]	..	..
Feces 3226	0.1–0.15 ash	0.31[d]	0.315	..
Feces 3226	0.1–0.15 ash	0.31[e]	..	..
Food 2786	1/50 day's food	0.022	0.022	..
		Mg./100 g.		*Mg./100 g.*
Tissue (suprarenal) 3894	10	0.057	..	0.045

[a] Electrodes washed with hydroxylamine HCl solution.
[b] Double electrolysis, no KCN present.
[c] KCN present, electrodes washed with hydroxylamine HCl solution.
[d] KCN present, double electrolysis. [e] Single electrolysis, KCN present.

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Microanalytical Determination of Sulfur

A Modified Bomb Method

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THE Elek-Hill microbomb method for the quantitative determination of sulfur in organic compounds (*1*) requires the use of a Neubauer or Gooch type of crucible for the precipitate. The quantitative transfer of microquantities of barium sulfate from a glass dish to a crucible is apt to be attended with some difficulty, owing to the tenacity with which the precipitate sticks to glass. It was thought that if this excellent method of combustion could be modified to permit its use in conjunction with the automatic filtration technique (*6*) a decided advantage would be gained. This change, herein described, is in the nature of a reduction in the amount of fusion mixture, so that the resulting sulfate solution can be evaporated to a small volume and transferred to a filter-stick crucible.

After considerable experimentation this reduction was established, but only after substituting potassium chlorate for the sugar-nitrate mixture used by Elek. The use of potassium chlorate in this connection was also observed by another worker (*3*). The elimination of potassium nitrate reduces the well-known coprecipitation of nitrates, which has been quantitatively studied in a recent paper dealing with macroamounts (*2*). The lowered concentration of salt solution from which the sulfate is precipitated is an added advantage (*4*).

With a mixture of 0.06 gram of potassium chlorate and 0.35 gram of sodium peroxide as the charge, most compounds were quantitatively oxidized. Some substances, particularly sulfones, required treatment with 3 to 4 drops of elementary bromine after the combustion. The bromine treatment is now used as a matter of routine. For this treatment and also to facilitate subsequent transfer of the solution, a 125-ml. "iodine flask" (with a lip for pouring) has been found most convenient.

The procedure is about the same as given by Elek except for the oxidizing mixture given above. The bomb must be heated in a roaring flame for approximately 60 seconds, and in so doing, its lead washer must be protected from excessive heat. This can be accomplished by placing the bomb with its holder in a hole cut out of an ordinary asbestos pad, mounted on a tripod, so that only the cup portion of the bomb is in direct contact with the flame. To acidify the alkaline solution resulting from the combustion 1.5 ml. of concentrated hydrochloric acid are necessary. After the bromine is added to the acidified solution in the flask, the solution is concentrated to about 5 to 7 ml. and transferred to a weighed filter-stick crucible with appropriate rinsings (*5*). The precipitate should be washed thoroughly with a total of about 20 to 30 ml. of 1 to 200 hydrochloric acid and water.

TABLE I. TYPICAL ANALYSES

Substance	Sulfur Found %	Sulfur Calcd. %
Cystine	26.74 26.68	26.69
Thiourea	42.01 42.07	42.12
Thiocarbanilide	13.97 14.20	14.05
Sulfanilic acid	18.50 18.57	18.50
Trithioformaldehyde	69.58 69.81	69.59
Sodium β-naphthoquinone sulfonate	12.18 12.39	12.31
Diphenyl sulfone	14.53 14.66	14.68
Diaminophenoxathiin	14.06 14.00	13.92
Glutathione	10.60 10.53	10.42
Sulfonal	27.95 28.09 28.10	28.09
Ethyl-4-methylthiazole-5-acetate HBr	11.95 11.96	12.05
Methionine	21.35 21.43	21.49
P-Br Benzylcysteine	11.12 11.01	11.00
S-Benzyl-*N*-formyl cysteine	13.20 13.36	13.39
Thiamine HCl	9.50 9.46	9.51
Phenyl isothiocyanate (liquid)	23.79 23.68	23.71

Table I gives the results of some typical analyses by the proposed method. The average sample weight was 6.1 mg.; the largest and smallest samples were 9.8 and 3.2 mg., respectively. The average amount of barium sulfate weighed was 8.6 mg.; the largest and smallest precipitates weighed 23.1 and 3.6 mg., respectively.

Since the over-all agreement of these 33 individual analyses with the calculated per cent of sulfur is better than 1 part per 1000 (assuming all the compounds to be pure), it is clear that the effect of coprecipitation is negligible. To check this further, all but one of the precipitates of barium sulfate corresponding to the analyses in Table I were washed again after the initial weighing, reignited, and weighed again (*5*). The average loss in weight was 0.031 mg., distributed as follows:

Loss in Weight *Mg.*	No. of Determinations	Loss in Weight *Mg.*	No. of Determinations
0	1	0.03	12
0.01	1	0.04	9
0.02	6	0.05	3

Since this average loss in weight is less than 4 parts per 1000 of the average weight of barium sulfate obtained in this series, it would appear that coprecipitation does not seriously affect the results.

The reduced amount of fusion mixture obviates the necessity of using reagents of special purity. The regular C. P. grade of sodium peroxide used for macroprocedures comes within the tolerance for this procedure. As Table I indicates, the accuracy of this method is comparable to the other existing determinations. This procedure also has the advantage of speed, especially in multiple runs.

Summary

The reduction of the fusion mixture for the determination of sulfur by the bomb method which, at the same time, permits the use of the filter-stick method for filtration is the basis for the proposed method. The sodium peroxide is reduced from 1.5 to 0.35 gram and 0.06 gram of potassium chlorate is substituted for the 0.30 gram of sugar–potassium nitrate mixture, giving a total of approximately one fifth of the quantity used in the original method.

Acknowledgment

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Modification of Pregl's Apparatus for Electrodeposition

Determination of Copper and Nickel in German Silver

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A modification of Pregl's apparatus for the quantitative microdetermination of metals by electrodeposition permits quantitative collection of electrolyte and washings in an efficient and simple manner. The apparatus was applied to the separation of copper and nickel previous to their determination in German silver. Reasonably satisfactory results were obtained when 5 grams of the alloy were dissolved, and the microanalyses were performed on small portions of the solution. The alloy was not sufficiently homogeneous to give representative samples of 5- to 10-mg. weight when single small shavings were taken for analysis.

PREGL'S apparatus (*7*) was designed for the quantitative microdetermination of copper in preserved foods by electrodeposition and weighing of the metal. No provision had to be made for the quantitative collection of electrolyte and washings. Thus, whenever the electrolyte was needed for further quantitative determinations, a change of design was required (*3*). The simple modification of Pregl's apparatus, which is proposed here, makes use of a siphon (*2*, *5*) for collection of the electrolyte.

Apparatus

The electrolytic cell and the cathode are the same as used by Pregl. The anode consists of a platinum wire approximately 0.5 mm. in diameter, which is wound in the form of a helix around the ascending arm of the siphon (Figure 1). The siphon is made of glass tubing of 2-mm. bore, and two pairs of glass horns are fused to the ascending arm, to hold the anode in the proper position. A small funnel slides up and down on the ascending arm of the siphon and rests on the opening of the cell during electrolysis. The wide test tube with side arm contains a large microbeaker in which the electrolyte and washings are to be collected. The suction tube may be connected with either the vacuum line or a supply of inert gas.

Separation and Determination of Copper and Nickel

The apparatus was applied to the microanalysis of copper-nickel alloys obtained in the form of turnings or shavings for use as students' unknowns. All weighings were performed with an analytical balance, for which the average deviation of a single weighing had been determined as equal to ±0.012 mg.

Approximately 7 mg. of German silver were weighed on a 2.5-cm. (1-inch) watch glass and then transferred into the test tube serving as electrolytic cell. The metal was covered with 50 cu. mm. of distilled water, and a pear-shaped glass bulb, shown in the upper right corner of Figure 1, was placed on the opening of the test tube. The bulb was slightly lifted, and 20 cu. mm. of 16 molar nitric acid were added slowly from a pipet. If necessary, solution was hastened by gently heating with a small flame so that the evolution of nitrogen oxide never became violent. Finally, 10 cu. mm. of 18 molar sulfuric acid were added and heating was continued until solution of the alloy was complete. When the evolution of gases had ceased, bulb and test tube were rinsed down with 0.5 ml. of water, and the bulb was removed. The solution was heated for 5 minutes on a steam bath, and air was blown from time to time into the test tube to remove the liberated nitrogen oxide.

The test tube was now clamped into the electrolytic stand, and the wire gauze cathode was inserted. One drop of ethyl alcohol was added, and then sufficient 1 molar sulfuric acid to bring the meniscus of the electrolyte up to the top of the wire gauze electrode. A slow current of hydrogen was sent through the suction tube and the siphon, and then the siphon with the anode was inserted in the electrolytic cell. The rate of gas flow was immediately adjusted to obtain approximately 1 bubble in 3 seconds emerging from the tip of the siphon touching the bottom of the electrolytic cell.

After making the electric connections (*3*), the electrolyte was heated to approximately 70° C., the flame removed, and the electrolysis started with 2.7 to 3.1 volts across the electrodes. The small funnel shown in Figure 1 must rest on the opening of the cell during the electrolysis. The space between siphon and stem of the funnel was sealed with a drop of water. After 5 minutes of electrolysis the color of the electrolyte indicated that most of the copper had been precipitated. Thus, the electrolyte was heated to boiling and the ring of condensate was driven up close to the opening of the test tube. The flame was then removed, and test tube as well as funnel was rinsed down with a few drops of water. The electrolysis was continued for 10 more minutes, and during the second half of this period the electrolyte was cooled to room temperature, using tap water (*7*). The e. m. f. across the electrodes was never permitted to exceed 3.1 volts.

Without interruption of the current, the electrolyte was completely siphoned off by applying slight suction. The suction was interrupted for washing, and cell and funnel were rinsed with distilled water until the cathode was again completely immersed. The hydrogen current was started and electrolysis allowed to continue for 2 minutes, then the wash liquid was completely siphoned off without interrupting the electric current. The washing was repeated twice in exactly the same manner. Finally, the suction was interrupted, the cathode was again covered with water, and siphon with anode and then the cathode were withdrawn from the cell in the order mentioned. The cathode was prepared for weighing as usual (*7*).

For the determination of nickel, the electrolyte and washings were concentrated on the steam bath to a volume of 1 to 2 ml., and then quantitatively transferred to a microbeaker which previously had been tared together with a filter stick (*4*). The transfer was accomplished by means of a capillary siphon operated by suction. In the microbeaker the solution was treated with 50 cu. mm. of 6 molar hydrochloric acid and 1 mg. of solid ammonium citrate, and mixed by imparting a rotary motion to the contents of the beaker. After addition of a small drop of alcoholic solution of methyl red, air laden with ammonia gas was blown on the surface of the solution in the microbeaker until the color changed to yellow. For this purpose, pure air of low pressure was passed through a gas wash bottle containing dilute ammonia solution (*1*); the contents of the microbeaker were mixed from time to time by a rotary motion with the hand holding the beaker.

The ammoniacal solution remained clear in every instance and was immediately diluted with water to a volume of approximately 4 ml., heated on the steam bath, and treated with a slight excess of alcoholic dimethylglyoxime reagent, which was added in very small drops by means of a reagent pipet with a fine capillary tip. The precipitate collected at the bottom of the

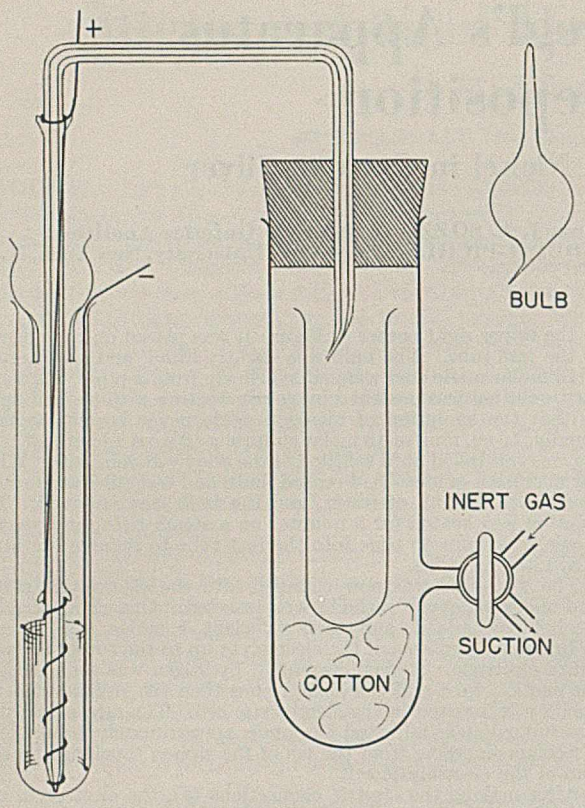

FIGURE 1. DIAGRAM OF APPARATUS

beaker, and it was easy to recognize when the precipitation was complete. Addition of 2 or 3 more droplets of the reagent provided a sufficient excess. The beaker with the precipitate was left for 10 minutes on the steam bath, then placed under a bell jar and allowed to stand for 30 minutes. Filtration (*4*) was followed by washing with four 1-ml. portions of hot water. Each time the walls of the beaker were washed down with hot water from the wash bottle. Beaker and contents were then heated for 1 minute on the steam bath, and the hot liquid was withdrawn through the filter stick. Drying at 120° C. required 10 minutes with the use of the apparatus of Benedetti-Pichler (*4*).

The results presented in Table I show very poor precision, while the averages of the series agree closely with the results of macrodeterminations. To exclude the influence of heterogeneity of the metal shavings, it was decided to repeat the microdeterminations on small aliquots of a solution of a large amount of the alloy.

Thus, 5.007 grams of the German silver were treated in a covered Erlenmeyer flask with 50 ml. of water, 20 ml. of 16 molar nitric acid, and 10 ml. of 18 molar sulfuric acid. Solution of the alloy was effected without heating. The solution was boiled to remove nitrogen oxide, diluted, treated with 1 gram of urea, and boiled again. After cooling, the solution was transferred to a volumetric flask and diluted to a volume of 250.0 ml. After careful mixing, 0.500-ml. portions were transferred to the electrolytic cell by means of a wash-out pipet (*7*). After insertion of the cathode, addition of a drop of alcohol, and dilution with distilled water to immerse the wire gauze electrode, the outline given above was followed without modification.

The results listed in Table II show a considerable improvement of the precision, which is now solely determined by the shortcomings of the chemical procedure, the microtechnique, and the balance used.

TABLE I. DETERMINATIONS ON MILLIGRAM SAMPLES OF GERMAN SILVER[a]

No.	Alloy Taken	Copper Obtained	Nickel Dimethylglyoxime Obtained	Copper	Nickel
	Mg.	*Mg.*	*Mg.*	%	%
1	5.03	3.19	3.91	63.4	15.8
2	6.12	4.10	5.11	67.0	16.9
3	4.76	2.95	..	62.0	....
4	4.62	2.87	4.01	62.1	17.5
5	4.16	2.44	3.31	58.7	16.2
6	4.19	2.59	3.31	61.8	16.1
7	9.94	4.46	7.52	62.2	15.4
8	8.74	5.37	6.82	61.4	15.9
			Arithmetical mean	62.3	16.2
			a_s	±1.4	±0.55
			Macroanalysis[a]	62.58	16.20

[a] Supplied by The Fales Chemical Co., Inc.

The spray produced by the liberation of gases from solutions constitutes a very serious source of loss in microprocedures. One must, therefore, carefully guard against such losses during the process of solution. Addition of alcohol to the electrolyte serves to reduce the stability of the very fine spray obtained during electrolysis; nevertheless, part of this spray would be carried out of the electrolytic cell, if too rapid a current of inert gas were passed through the electrolyte. The current of inert gas is necessary to prevent electrolyte from entering the siphon before precipitation of copper is complete. The stirring effect (*6*) must not be overrated, since the gas current has to be slow, for the reasons mentioned.

TABLE II. DETERMINATIONS ON 0.500-ML. PORTIONS OF A SOLUTION OF 5.007 GRAMS OF GERMAN SILVER IN 250.0 ML.

No.	Copper Obtained	Nickel Dimethylglyoxime Obtained	Copper	Nickel
	Mg.	*Mg.*	%	%
1	6.24	7.75	62.21	15.6
2	6.25	7.83	62.31	15.9
3	6.29	7.72	62.71	15.6
4	6.27	7.82	62.51	15.8
5	6.25	8.04	62.31	16.3
6	6.25	8.03	62.31	16.3
7	6.26	8.04	62.46	16.3
		Arithmetical mean	62.40	16.0
		a_s	±0.14	±0.26
		Macroanalysis[a]	62.58	16.20

[a] Supplied by The Fales Chemical Co., Inc.

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Bromo Complexes for the Identification of Metals and Alkaloids

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Conditions are described for the use of bromometallic salts of alkaloids, sparingly soluble in bromide solution, as microanalytical tests for the metals of the mercury, cadmium, bismuth, antimony, lead, and tin group, or as tests for alkaloids of certain tertiary types. Such salts are often beautifully crystalline and characteristic in appearance. Likely sources of interference are discussed, and concentration limits are given for alkaloids and for metals. Several reactions of special significance are indicated. Application of the tests to mixtures has not been studied.

THE formation and properties of salts of alkaloids with metallic bromo complexes, salts which are sparingly soluble in bromide solutions, have been described (*4*). The composition of selected salts is there shown to conform to the formulas B_xMBr_4 or B_xMBr_5, where B represents an alkaloid ion and M a metallic ion, divalent in the first formula and trivalent in the second. Of the common metals only the bromo complexes of mercury, cadmium, bismuth, antimony, lead, and tin give difficultly soluble salts with alkaloids. The ability of alkaloids to give sparingly soluble salts of this kind depends upon the type of nitrogen in the organic base, and it has been demonstrated that only certain types of tertiary bases possess this property. Thus, tests with metallic bromo complexes may be of use in elucidating the type of nitrogen, and the behavior of alkaloids of known constitution may be predicted within certain limits.

The present paper deals with the use of bromo complexes in microchemical tests for alkaloids and for the above-mentioned small group of metals. The test for cadmium based on the separation of the brucine salt of the bromo complex was introduced by Meurice (*3*) and later used as a slide test by Martini (*1*), who (*2*) also employed the bromo complex of lead for the microchemical detection of cocaine and other alkaloids. Recently the use of the bromo complexes of cadmium, mercury, gold, and platinum for the detection of alkaloids has been described by Whitmore and Wood (*5*), but the work reported below had been completed without knowledge of their paper. Certain discrepancies between their results and those of the writer have been checked carefully. They appear to be caused by differences in the techniques and concentrations.

Tests for Metals

One drop, 0.015 ml., of the solution of the metal is placed in the depression of a hollow-ground slide, and to it are added 2 drops of a 40 per cent solution of alkaloid in either water or 2 per cent acid. Small amounts of acid, up to 10 per cent in the test drop, have little effect on the sensitivity of the tests. The test is observed under the low-power microscope for 20 minutes. This time suffices for the crystallization of amorphous precipitates and for the separation of crystals from very dilute solutions.

The exact shape and arrangement of crystals are reproducible over a large range of concentrations. Some typical precipitates are shown in photomicrographs (Figures 1 and 2) of bromometallic salts of alkaloids formed by 1 drop of 1 per cent metal solution, 2 drops of 40 per cent potassium bromide solution, and 1 drop of 1 per cent alkaloid solution. No attempt is made to describe the appearance of precipitates, since in practice it will be best to compare the forms with those obtained from known solutions of approximately identical concentration. Complications may arise from the separation of difficultly soluble metal bromides, alkaloid hydrobromides, and products of the hydrolysis of metallic ions. Silver bromide is practically insoluble in potassium bromide solution. If much lead is present, the 2 drops of bromide solution are insufficient for the complete solution of the lead salt. The formation of crystalline hydrobromides of certain alkaloids is discussed below. The oxyhalides of bismuth and antimony may be dissolved by adding more acid which, of course, causes some reduction of the sensitivity of the tests. The statements in Table I concerning the crystalline or amorphous appearance of the precipitates apply to the use of a 1 per cent solution of the metallic ion. Use of the following alkaloids may be generally recommended:

COCAINE. Presence of lead causes the separation of needles (Figure 2, 11) which are readily detectable in the presence of large amounts of amorphous precipitate produced by mercury, cadmium, antimony, or bismuth. The concentrations of tin normally met with are not liable to interfere with this very sensitive test for lead.

TROPINE. Only lead gives a precipitate (Figure 2, 12) not specially sensitive.

BRUCINE. All six metals mentioned above are precipitated even in dilute solution. In spite of the high limiting concentration for tin, brucine may be used as a group reagent to prove the absence or presence of mercury, cadmium, antimony, bismuth, lead, and tin. The separation of small amounts of brucine hydrobromide, the crystals of which resemble those obtained with mercury or cadmium, must be prevented by adding a small amount of dilute acid.

The crystals formed with cadmium (Figure 1, 1) and mercury (Figure 1, 2) are similar in appearance, and so are those with bismuth (Figure 1, 3), and antimony (Figure 1, 4). Lead produces an amorphous precipitate. Since tin does not give a precipitate

TABLE I. LIMITING CONCENTRATIONS FOR METALS

	Hg	Cd	Sb	Bi	Pb	Sn
Narcotine	3000 A[a]	2000 A	700 A	700 A	300 A	..
Narceine	900 A	100 A	...	200 A	...	..
Cotarnine	3000 C	4000 C	200 A	200 A	300 C	..
Hydrastinine	800 C	800 C	100 A	100 A	50 C	..
Morphine	40 A	...	...	...	...	..
Codeine	1000 C	200 C	...	...	...	..
Dionine	20 A	20 A	...	20 A	...	..
Apomorphine	100 A	...	100 A	...	...	..
Quinine	300 C	100 C	80 A	30 A	...	..
Quinidine	....	....	....	....	...	..
Cinchonine	1100 C	700 C	100 A	100 A	...	..
Cinchonidine	....	...	...	...	...	..
Sparteine	300 C	100 C	....	....	....	..
Brucine	4000 C	3500 C	11000 C	30000 C	700 A	10 A
Yohimbine	350 A	100 A	100 A	100 A	...	..
Aconitine	800 A	150 A	...	...	...	..
Tropine	....	....	....	....	600 C	..
Cocaine	800 A	300 A	100 A	500 A	25000 C	50 C
Tropacocaine	6000 C	9000 C	150 A	2000 A	1000 C	..
Atropine	...	...	...	...	30 C	..

[a] A, amorphous; C, crystalline.

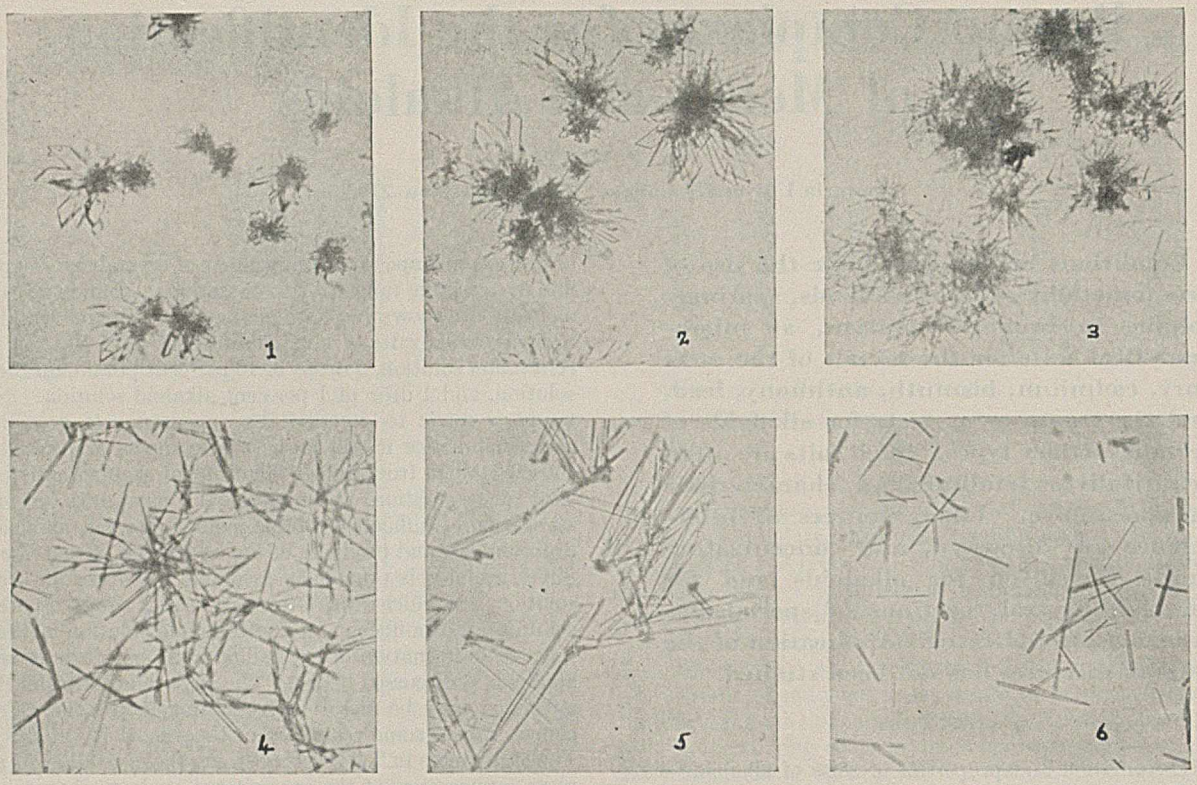

FIGURE 1. BROMOMETALLIC SALTS OF ALKALOIDS (× 100)

1. Cadmium brucine bromide
2. Mercury brucine bromide
3. Bismuth brucine bromide
4. Antimony brucine bromide
5. Cadmium cotarnine bromide
6. Mercury cotarnine bromide

at moderate dilution, brucine may be used for the detection of antimony in a not too strongly acid solution of Group II B of the analytical scheme. The brucine reagent is not recommended as a test for cadmium in the presence of copper, since other reagents of better selectivity and higher sensitivity are available for this purpose.

COTARNINE. Crystals separate with cadmium, mercury, lead, and bismuth (Figures 1 and 2). Antimony gives an amorphous precipitate.

Tests for Alkaloids

The tests are carried out in the same manner as for metals. One per cent solutions of the metals are first treated with twice the volume of bromide solution, and the solution of alkaloid is then added to the mixture. The alkaloids are dissolved in water or 2 per cent acid, so as to obtain solutions of the concentration range between 1 in 50 and 1 in 100. This concentration range is necessary, since with dilute solutions of the alkaloid often no precipitates are obtained, as may be seen from Table II.

TABLE II. LIMITING CONCENTRATIONS FOR ALKALOIDS

	Hg	Cd	Sb	Bi	Pb
Narcotine	300 A	200 A	200 A	300 A	100 A
Narceine	300 A	100 A	...	200 A	...
Cotarnine	800 C	900 C	200 A	200 C[a]	400 C
Hydrastinine	400 C	300 C	200 A	200 A	400 C
Morphine[b]	30 A	...	...	...	...
Codeine	300 C	300 C	...	...	...
Dionine	20 C	20 A	...	20 C	...
Apomorphine	100 A	...	200 A	...	...
Quinine	150 C	100 C	80 A	30 A	...
Quinidine	40 A	30 A	40 A	80 A	...
Cinchonine	300 C	250 C	20 A	100 A	...
Cinchonidine	30 A	30 A	20 A	30 A	...
Sparteine	200 C	100 C	...	...	...
Brucine	450 C	350 C	2000 C	3000 C	150 A
Yohimbine	250 A	...	...	...	...
Veratrine	200 A	200 A	100 A	100 A	100 C
Aconitine	250 A	200 A	...	...	...
Tropine	...	...	...	...	300 C
Cocaine	150 A	100 A	100 A	150 A	550 C
Tropacocaine	2500 C	1000 C	1200 C	750 A	1000 C

[a] Very small C.
[b] Gelatinous, some aggregation to an ordered arrangement.

The notations "crystalline" and "amorphous" (C and A, Table II) are based on the above-described conditions of the test. The effect of the concentration on the appearance of the precipitate becomes significant with either very concentrated or very dilute solutions. From concentrated solutions precipitates described as crystalline may occasionally separate in an amorphous state and crystallize slowly. Some amorphous precipitates may be crystallized from hot water. The appearance of the precipitates should be compared under the microscope with that of precipitates obtained from known solutions of approximately identical concentrations. The possibility of a separation of oxybromides and of alkaloid hydrobromides must be continuously kept in mind.

With berberine, emetine, harmine, and strychnine the hydrobromides precipitate profusely under the conditions of the test, using 1 per cent solutions of the alkaloid. Other alkaloids, like brucine, codeine, and tropacocaine, give only a few crystals of hydrobromide under these conditions. The trace of brucine hydrobromide is similar in appearance to the crystals produced by the cadmium or mercury reagent in dilute solutions of the base. The interference of the hydrobromide is negligible, however, if the brucine is applied in 5 per cent acid solution. With codeine the separation of the hydrobromide affects the identification of the bromo salts, especially when the conditions of the test approach the low concentrations. Limiting concentrations given in Table II are those at which the bromo salts can be detected readily in the presence of hydrobromide. The hydrobromide of tropacocaine separates in thin plates exhibiting interference colors. By

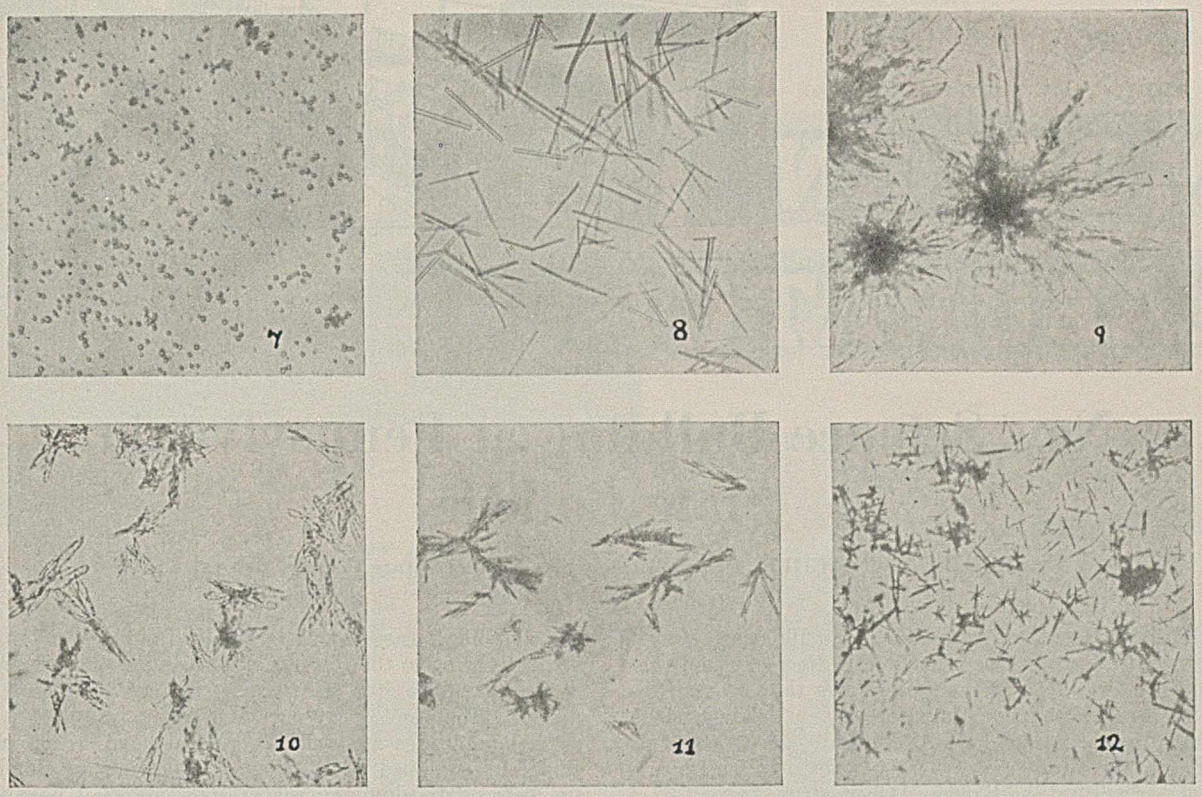

FIGURE 2. BROMOMETALLIC SALTS OF ALKALOIDS (× 100)

7. Bismuth cotarnine bromide
8. Lead cotarnine bromide
9. Cadmium cinchonine bromide
10. Lead tropacocaine bromide
11. Lead cocaine bromide
12. Lead tropine bromide

using a 10 per cent solution of potassium bromide, the separation of the hydrobromide can be delayed for a long time and the bromo salts can be identified readily. No difficulty should be experienced with certain other alkaloids giving hydrobromides crystallizing sparingly only when 5 per cent solutions of the base are employed: aconitine, dionine, narceine, narcotine, and yohimbine. Concentrated solutions of cinchonidine and quinidine produce oily drops which crystallize on adding acid.

A few organic bases give precipitates of different structure from the bromo salts, soluble in an excess of bromide solution. Papaverine and ethylenediamine are examples (*4*). Some of the precipitates listed in Table II are slightly soluble in bromide excess, and the limiting concentrations can be improved in such instances by the use of less potassium bromide. This holds for dionine among others.

The bromo complex of tin is not suited as an alkaloid reagent, tin giving no precipitates in 1 per cent solution (Table I). Using the bromo complexes of mercury, cadmium, antimony, bismuth, and lead, the following scheme is suggested for the identification of alkaloids.

Identification of Alkaloids

I. Sparingly soluble hydrobromides
   - A. A large precipitate is given by 1 per cent solutions of the alkaloid: berberine C, emetine A, harmine C, strychnine C
   - B. Large precipitates form only from more concentrated solutions of the alkaloids: aconitine C, brucine C, codeine C, narceine C, narcotine C, tropacocaine C, yohimbine A
   - C. Oily globules separate which crystallize on adding acid: cinchonidine, quinidine

II. Number of bromo complexes giving precipitates
   - Five. All A: narcotine
     Lead C, others A: cocaine, veratrine
     Lead, mercury, cadmium C, others A: hydrastinine, tropacocaine
     Lead A, others C: brucine
     Antimony A, others C: cotarnine
   - Four. Mercury, cadmium, bismuth, antimony A: cinchonidine, quinidine
     Mercury, cadmium C, bismuth, antimony A: cinchonine (Figure 2, 9), quinine
   - Three. Mercury, cadmium, bismuth A: narceine
   - Two. Mercury, cadmium C: codeine, sparteine
     Mercury C, cadmium A: dionine
     Mercury, cadmium A: aconitine, apomorphine
   - One. Mercury A: morphine, yohimbine
     Lead C: tropine
   - None (up to 5 per cent solutions of the alkaloid). Adrenaline, arecoline, atropine, caffeine, colchicine, coniine, cytisine, ephedrine, eserine (physostigmine), homatropine, hyoscyamine, hyoscine (scopolamine), *d*-lupanine, nicotine, taxine, and theobromine

III. Bromo complexes of mercury and bismuth
   - Both C: brucine (Figure 1, 2 and 3), cotarnine (Figures 1 and 2, 6 and 7), dionine
   - Mercury C, bismuth A: cinchonine, hydrastinine, quinine, tropacocaine

IV. One bromo complex
   - Cadmium, no precipitate: apomorphine, morphine, tropine, yohimbine
   - Mercury, no precipitate: tropine
   - Bismuth C: brucine (Figure 1, 3), cotarnine (Figure 2, 7), dionine
   - Antimony C: brucine (Figure 1, 4)
   - Lead C: cocaine (Figure 2, 11), cotarnine (Figure 2, 8), hydrastinine, tropacocaine (Figure 2, 10), tropine (Figure 2, 12), veratrine
   - Lead A: brucine, narcotine

Literature Cited

(1) Martini, A., *Mikrochemie*, 6, 1 (1928).
(2) *Ibid.*, 12, 111 (1932).
(3) Meurice, R., *Ann. chim. anal. (II)*, 8, 130 (1926).
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(5) Whitmore, W. F., and Wood, C. A., *Mikrochemie*, 27, 249 (1939).

MODERN LABORATORIES

New Science Building at Pennsylvania State College

R. ADAMS DUTCHER, Pennsylvania State College, State College, Penna.

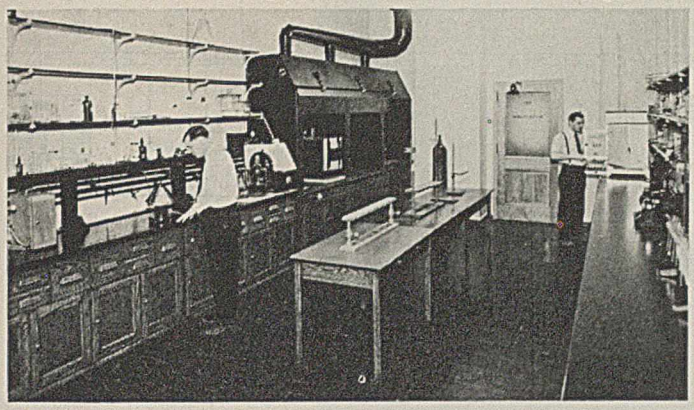
THE Departments of Agricultural and Biological Chemistry and Zoology and Economic Entomology of Pennsylvania State College are now occupying the new Agricultural Science Building, one of the several new buildings made available by the General State Authority in 1940. This brief description deals solely with the facilities of the Department of Agricultural and Biological Chemistry.

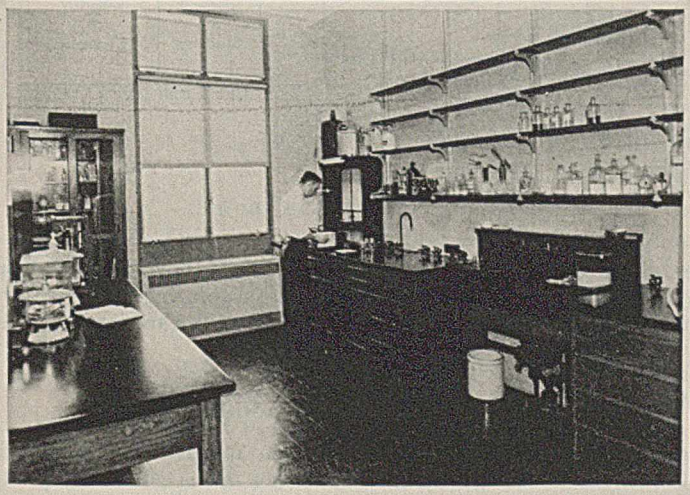
The building has been named Frear Laboratories in honor of the late William Frear, nationally known agricultural chemist, who devoted 37 years to Pennsylvania agriculture and national pure food problems. It is constructed of cream-colored Roman brick, trimmed with Indiana limestone. The building measures 205 by 70 feet and consists of four floors. Owing to the contour of the campus three floors are above ground level on the front, while four floors are above ground at the rear.

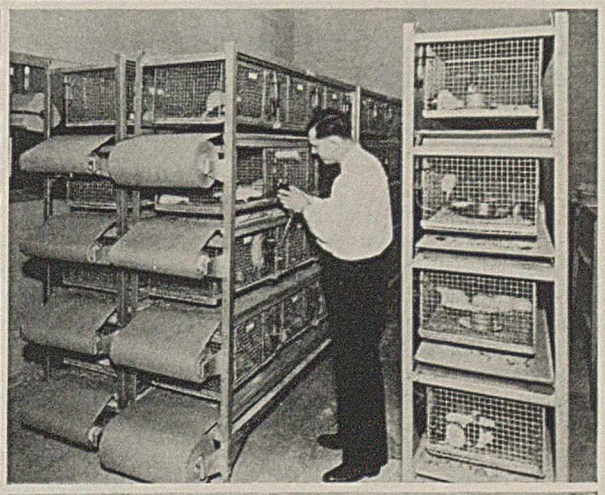
All laboratories are well lighted and have cream-colored tile walls equipped with adjustable shelving on aluminum brackets set in aluminum slots. This makes it possible to have shelves of any desired width or height without mutilating the walls. Acidproof plumbing is standard equipment throughout and all plumbing is exposed (overhead) for convenience in making repairs.

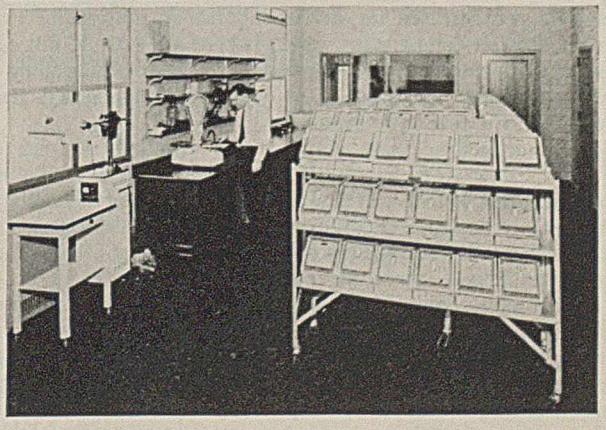
Floors, with the exception of a few special rooms, consist

Upper Left. Corner of Protein Research Laboratory
Lower Left. Graduate Student Laboratory, Accommodating Eight Students
Upper Right. Typical Student Laboratory
Lower Right. Private Laboratory for Tobacco Research



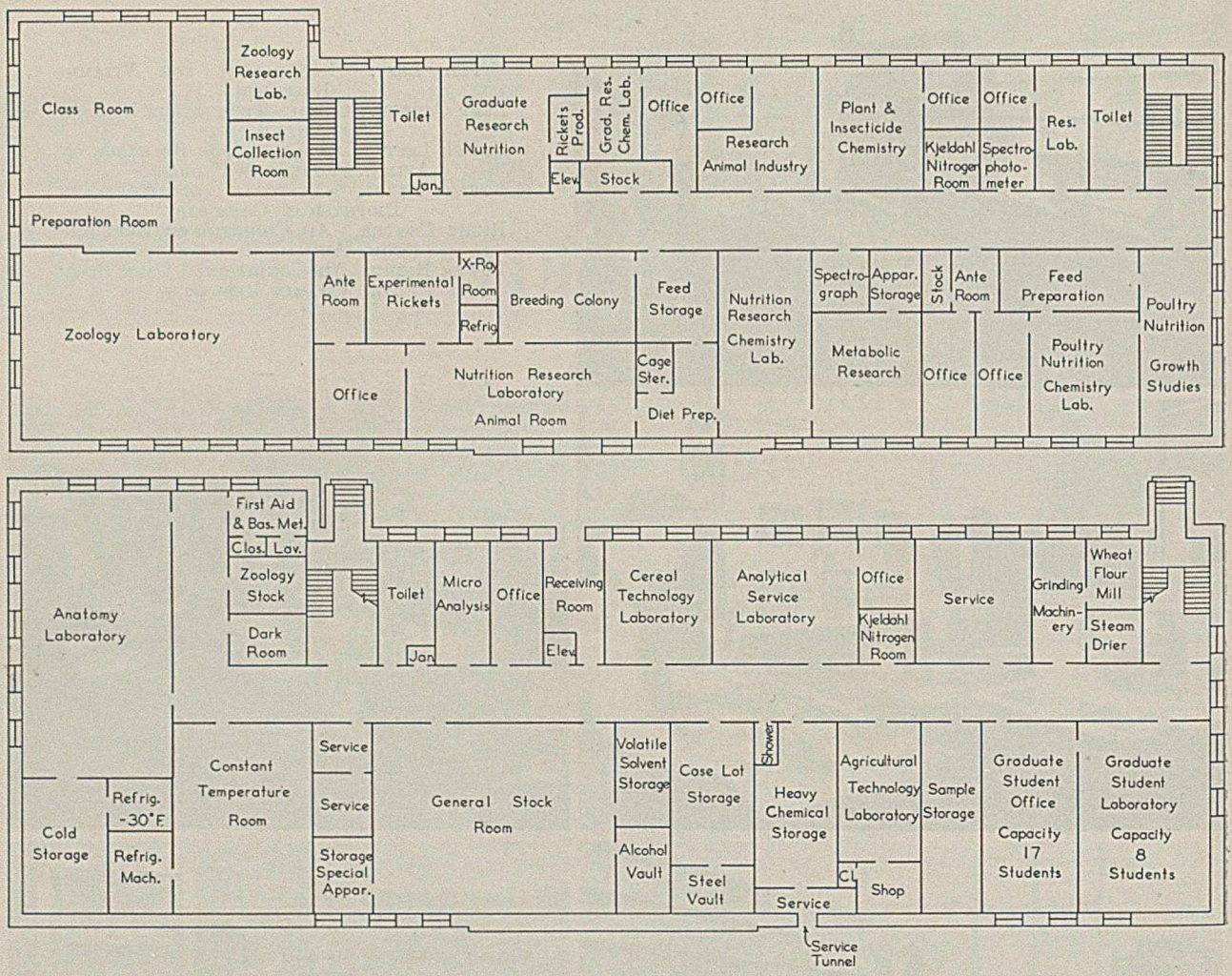






UPPER LEFT. LABORATORY FOR VITAMIN RESEARCH
LEFT CENTER. CEREAL TECHNOLOGY LABORATORY
LOWER LEFT. LABORATORY FOR RESEARCH ON ANIMAL PRODUCTION PROBLEMS
UPPER RIGHT. POSTGRADUATE WORK IN BIOPHYSICAL CHEMISTRY
RIGHT CENTER. AIR-CONDITIONED BREEDING ROOM, ANIMAL LABORATORY
LOWER RIGHT. AIR-CONDITIONED CAGE ROOM FOR VITAMIN RESEARCH

of black linoleum tile. All laboratory desks are of wood construction with tops made of chemically treated laminated pressed wood, covered with a chemical-proof plastic finish. Desks in all laboratories are equipped with gas, cold and hot water, compressed air, vacuum lines, steam, and alternating and direct current Distilled water is conducted in aluminum tubing to laboratories and corridors from reservoirs in the attic. Forced ventilation is available for all classrooms and laboratories and all fume hoods are equipped with individual motors. Four large student laboratories, each of which will accommodate from 192 to 224 students, are designed for the various service courses in biochemistry. Balance, Kjeldahl, fat-extraction, and instrument rooms are sepa-



ABOVE, THIRD FLOOR; BELOW, BASEMENT

rate from but easily accessible to the large student laboratories, as are three small classrooms, with a seating capacity of from 45 to 60. Two laboratories are set aside for graduate student research and a large office accommodates from 15 to 20 graduate students. Other graduate students have offices adjacent to private laboratories of professors with whom they are working on special problems.

Two cold rooms, maintained at 32° F., are available for quantity storage, while all laboratories are equipped with electric refrigerators for active laboratory materials. One room, for low-temperature work, is kept at −20° F. but can be used at temperatures as low as −32° F. A laboratory is also designed for pilot-plant and semicommercial-scale research in agricultural technology.

Milling facilities are available for grinding samples of every type, from rocks and soils to foods and feeds. An experimental flour mill is available for work in cereal technology. A specially designed steam dryer, made of stainless steel, has a capacity of 200 to 400 pounds for rapid drying of such materials as silage, tobacco, and agricultural crops.

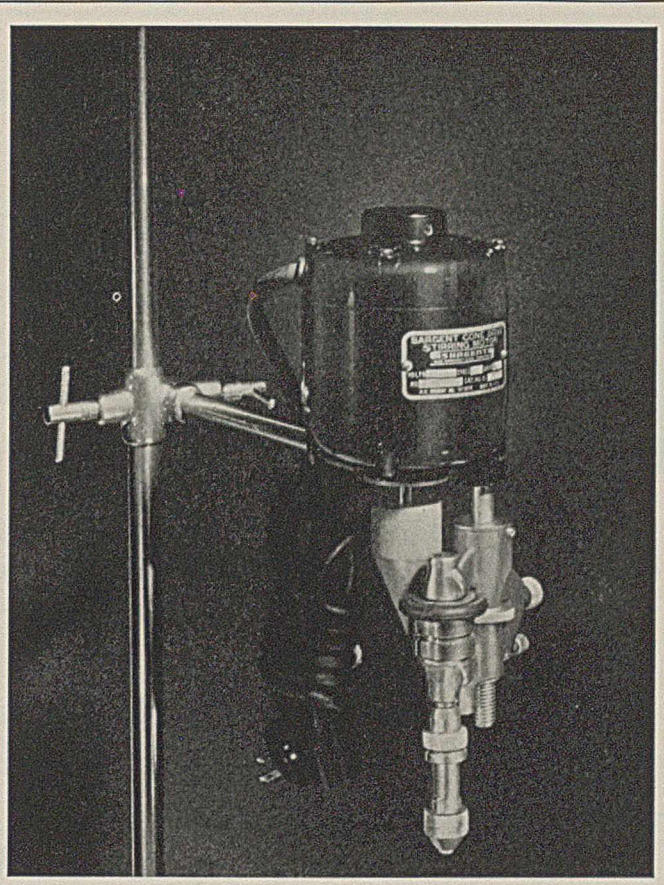
A complete baking laboratory has been designed for student instruction and research. This laboratory has a baking oven with rotary hearth, fermentation cabinet (automatically controlled for temperature and humidity), dough mixers, sheeting roll, molder, and complete equipment for measuring gluten strength of flours, loaf volume, etc.

All principal storage rooms are on the basement floor. Small subsidiary stockrooms (for dispensing of stock) are located on each laboratory floor. Supplies are carried to the subsidiary stockrooms by elevator.

Experiment Station Projects

The top floor is devoted solely to research work on experiment station projects. Instrument rooms containing quartz spectrograph, spectrophotometer, fluorometers, polariscopes, and colorimeters, and an x-ray room and a darkroom for photographic work are available for all workers. Air-conditioned laboratories have been designed for animal nutrition studies and facilities are available for sterilizing all cage equipment. Battery cages are used for poultry nutrition studies and separate laboratories are available for student research in nutrition. Observation windows have been constructed so that the public can see the animals in the breeding colony and in the nutrition laboratory without entering the air-conditioned rooms and interfering with the regular research work.

The Department of Agricultural and Biological Chemistry has 108 undergraduate students majoring in the department and 33 candidates are now working for advanced degrees. The department offers service courses to approximately 1200 students from other departments and schools on the campus.



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