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Determination of Methylpropene

By Means of a Modified Denigès Reagent

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The determination of methylpropene by means of Denigès reagent is complicated by the solubility of the precipitate in nitric acid and the change in weight and composition of the precipitate on washing with water. The first source of error is eliminated by using a neutralized Denigès reagent. The second is avoided by using as the measure of methylpropene absorbed not the weight of the precipitate but the weight of mercury in the precipitate, which is constant under the conditions of the determination and amounts to seven atoms for each molecule of methylpropene used. Under these conditions the method is accurate and reasonably rapid.

TWO methods are at present available for the determination of methylpropene, using sulfuric acid (*4*) and hydrogen chloride (*3*), respectively.

Little attention appears to have been paid to the ability of methylpropene to form a precipitate with solutions of mercuric nitrate as originally reported by Denigès (*1*), possibly because the scanty information in the literature implies that the precipitate is of variable composition.

Denigès used a solution prepared by adding 20 grams of mercuric oxide to 100 ml. of water and 40 ml. of 75 per cent nitric acid, heating to dissolve the mercuric oxide, and then diluting with 400 ml. of water. After methylpropene was absorbed in this solution the product was boiled and an orange precipitate obtained which was stated to have the composition $HgNO_3.C_4H_8.Hg_2NO_3$, corresponding to a content of 76.96 per cent of mercury and 7.18 per cent of C_4H_8. Hurd and Goldsby (*2*), using the same solution, obtained 2.90 and 2.52 grams of precipitate from 0.13 and 0.115 gram of methylpropene, corresponding to a content of 4.48 and 4.56 per cent of methylpropene, respectively.

Experimental

In preliminary tests to examine the possibility of using the reaction with mercuric nitrate for the estimation of methylpropene, samples of the addition compound were prepared from the pure gas by the procedure specified by Denigès. In four determinations the results corresponded to 4.62, 3.65, 4.02, and 3.47 per cent by weight of methylpropene in the precipitate. It was observed, however, that the filtrates often deposited additional solid on standing or on dilution with water, and it was suspected that the variation in results was due to incomplete precipitation in the presence of nitric acid. When the precipitate was stirred with dilute nitric acid, no apparent decomposition occurred at room temperatures but opalescent solutions were formed from which solid could be reprecipitated by dilution or neutralization. The solubility of the addition compound in different strengths of nitric acid was determined by preparing suitable mixtures, filtering, and titrating mercury in the filtrates with standard potassium thiocyanate. The results are recorded in Figure 1.

Denigès reagent contains about 80 grams of nitric acid per liter and, as will be shown later, this concentration increases during reaction with methylpropene. Thus the reagent will retain in solution at least 0.11 gram of the precipitate per liter and, taking into consideration the nitric acid formed in the reaction, the amount retained will be dependent on the ratio of the volume of methylpropene to the volume of reagent used.

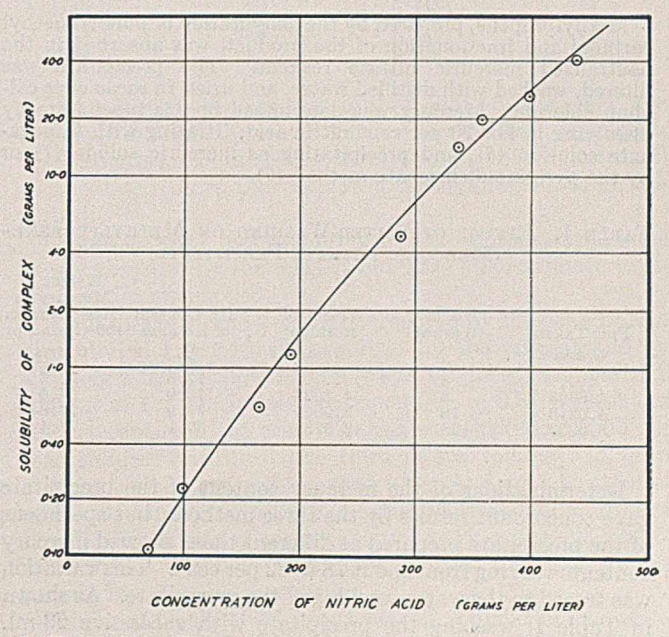

FIGURE 1. SOLUBILITY OF PRECIPITATE IN NITRIC ACID

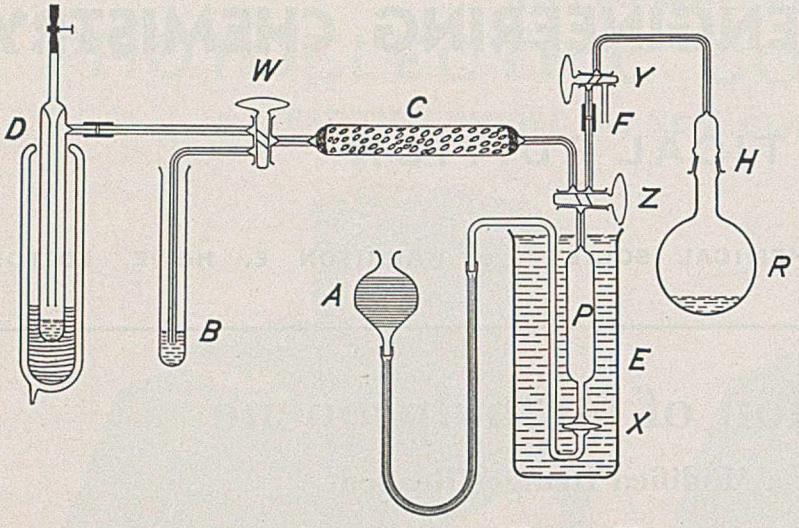

FIGURE 2. APPARATUS FOR DETERMINATION OF MERCURY-METHYLPROPENE RATIO

The authors believe that this circumstance accounts in part for the apparent differences in the composition of the precipitate as calculated from the results of previous workers.

In view of the above results the possibility of using a neutralized Denigès reagent, prepared by adding caustic soda solution to the acid reagent until basic mercuric nitrate began to precipitate, was investigated. This solution was found to absorb methylpropene rapidly but in contrast to the acid reagent no precipitate was formed in the cold. Precipitation took place on warming to temperatures above 70° C. or could be induced by adding a few drops of 75 per cent nitric acid to the cold solution and was accompanied by an appreciable fall in pH. No precipitation took place, even on boiling in a solution buffered to pH 5.4 with sodium acetate–acetic acid.

It appears that the reaction between methylpropene and mercuric nitrate solutions takes place in two stages. In the first stage methylpropene is absorbed with formation of a soluble complex and liberation of hydrogen ions. In the second stage, which does not take place above a critical pH value, the insoluble complex is formed.

Composition of Precipitate

Methylpropene, prepared by the dehydration of pure trimethyl carbinol and fractionation of the product, was absorbed in the neutralized mercuric nitrate reagent. The precipitate was filtered, washed with distilled water, and dried *in vacuo* over calcium chloride. Mercury was determined in this precipitate by dissolving in hot 70 per cent nitric acid, titrating with thiocyanate solution (*6*), and precipitating as mercuric sulfide (*7*) or by the pyridine-dichromate method (*5*).

TABLE I. EFFECT OF WATER-WASHING ON METHYLPROPENE–MERCURIC NITRATE PRECIPITATE

Ppt. Taken	No. of Washes	Weight of Residue	Hg Content of Residue	Weight of Mercury in Residue from 100-Gram Ppt.
Grams		*Grams*	*%*	*Grams*
....	3	....	77.6	77.6
4.8903	8	4.8023	78.9	77.5
4.0216	18	3.9050	79.9	77.6
3.0837	28	2.9789	80.1	77.4

Determinations of the mercury content of the precipitate gave concordant results by the three methods, but specimens of the precipitate prepared at different times showed mercury contents varying from about 78 to 82 per cent. This variation was traced to the water-washing of the precipitate. As shown in Table I, washing the precipitate with successive 20-ml. portions of water at room temperature (30° C.) decreased the weight of the precipitate but did not affect the weight of mercury in it. Nitrate could be detected in the wash water after all soluble mercury had been removed.

The change in weight appears to be due to a replacement of nitrate groups in the precipitate by hydroxyl or possibly water. It also appears that this replacement is reversible.

Combustion and gravimetric analysis showed that a product prepared, using the neutralized reagent with the minimum of water-washing and containing 78.3 per cent of mercury, had a composition corresponding closely to 1 C_4H_8 (3.13 per cent by weight), 7 Hg, 3 NO_3, 2 H, and by difference 9 O, the apparent molecular weight being 1793.

When a sample of the precipitate was boiled for 30 minutes with a large quantity of distilled water, a loss in weight of 7.8 per cent resulted and the recovered precipitate contained 80.8 per cent of mercury corresponding to 74.4 grams of mercury per 100 grams of original precipitate. This appreciable loss of mercury was accompanied by a change in color of the precipitate from orange to deep red, and unlike the products obtained by water-washing at room temperature, the color did not revert to light orange in contact with nitric acid.

It is concluded from these results that the total weight of precipitate obtained is not a satisfactory measure of the amount of methylpropene used. It appears, however, that the ratio of methylpropene used to mercury in the precipitate is constant.

TABLE II. RATIO OF MERCURY IN PRECIPITATE TO METHYLPROPENE USED

Volume of Methyl-propene	Temp.	Pressure	Weight of Methyl-propene	Weight of Mercury	Atoms of Hg per Mole of Methyl-propene
Ml.	*° C.*	*Mm. Hg*	*Gram*	*Gram*	
12.52	29.5	758.3	0.02823	0.7004	6.94
12.52	31.0	758.5	0.02811	0.6952	6.92
12.52	30.0	758.0	0.02818	0.7011	6.96

MERCURY-METHYLPROPENE RATIO. The apparatus shown in Figure 2 was used for determining the ratio of mercury to methylpropene in the precipitate.

A sample of pure methylpropene was passed from reservoir *D* through stopcock *W* and drying tube *C*, containing calcium chloride, into bulb *P*, fitted with mercury leveling bulb *A*. Its pressure was adjusted to atmospheric by escape of excess gas through *W* and water seal *B*. After its temperature and pressure had been recorded, the sample was transferred from *P* to reaction bulb *R*, which had previously been charged with a suitable volume of neutralized reagent and evacuated. *R* was detached at *F* and shaken vigorously for 10 minutes, air being admitted through *F* to sweep methylpropene from the capillary tubing. Finally, *R* was detached at *H*, and the solution was washed into a beaker, heated to boiling, cooled to room temperature, and filtered through a sintered-glass crucible. After being washed with cold distilled water until the filtrate gave a negative test for mercury with ammonium sulfide, the precipitate was dissolved in 70 per cent nitric acid, and the mercury was determined with thiocyanate.

The data for three such determinations given in Table II indicate that seven atoms of mercury are present for each molecule of methylpropene in the precipitate.

Reagents

MERCURIC NITRATE REAGENT. One hundred grams of pure mercuric oxide are mixed to a paste with about 100 ml. of distilled water and then dissolved in the minimum quantity of 70 per cent

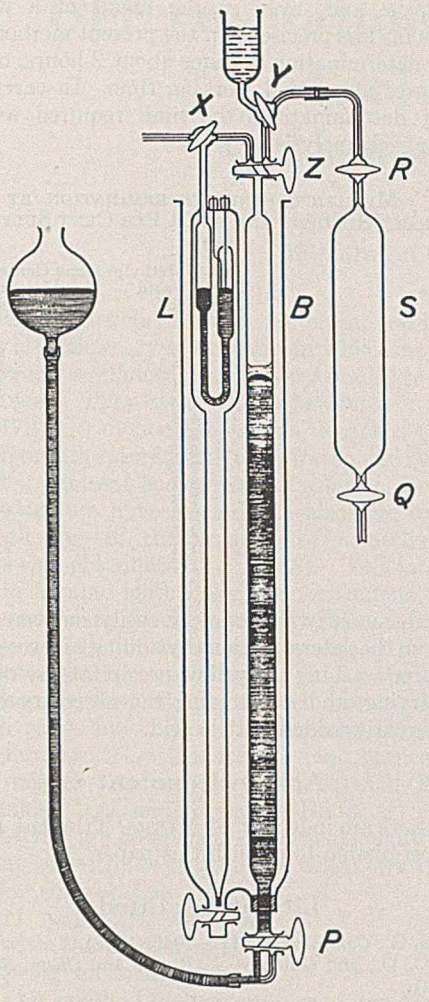

FIGURE 3. APPARATUS FOR ROUTINE DETERMINATION OF METHYLPROPENE IN GASES

nitric acid (about 90 ml.). About 10 grams of pure sodium hydroxide are dissolved in 15 ml. of water and the solution is added drop by drop to the mercuric nitrate solution until a faint but permanent white cloud of basic mercuric nitrate is produced. Great care must be taken not to add any appreciable excess of sodium hydroxide. The solution is diluted to 2 liters with distilled water, filtered, and stored in a dark, glass-stoppered bottle.

POTASSIUM THIOCYANATE. It is convenient to use a solution about 0.07 *N* in potassium thiocyanate, which is prepared by dissolving 15 grams of the pure salt in distilled water and diluting to 2 liters. The solution is standardized against 0.2 to 0.3 gram of pure mercury dissolved in 5 ml. of 70 per cent nitric acid.

Apparatus and Procedure

APPARATUS. A convenient form of apparatus for the routine determination of methylpropene in refinery gases is shown in Figure 3 and consists of a water-jacketed gas buret, *B*, a compensator, *L*, and an absorption tube, *S*.

PROCEDURE. About 1 ml. of water is drawn into the buret. The gas sample, varying from 35 ml. for 20 per cent methylpropene to 150 ml. for 1 per cent methylpropene, is drawn into the buret, and after standing for about 30 seconds its volume and pressure are recorded. Into the absorption tube, *S*, are drawn about 50 ml. of neutralized reagent and the tube is then evacuated by a good water pump, closed, and attached to the apparatus. The gas sample is transferred to the absorption tube, using the water to drive the gas through the connecting capillary tube, and taking care that no mercury is allowed to pass into the absorption tube. After closing the upper stopcock, *R*, the absorption tube is detached from the apparatus and shaken for 5 minutes and for a further 2 minutes after admitting air.

The liquid in the absorption tube is allowed to run into a 250-ml. beaker and the tube is washed out two or three times. The beaker is heated gently to 100° C. (but not boiled), and allowed to cool almost to room temperature, and the liquid is filtered through a No. 4 sintered-glass crucible. (A No. 3 crucible is more rapid and usually satisfactory but occasionally allows some precipitate to pass.) Washing of the beaker and the crucible is carried out with cold distilled water until the filtrate gives no precipitate with ammonium sulfide, after which the crucible is returned to the beaker and the precipitate dissolved by heating gently with about 10 ml. of 70 per cent nitric acid. When solution is complete, the crucible is washed and removed.

The solution in the beaker is oxidized to convert Hg^{+} to Hg^{++} by the dropwise addition of saturated permanganate until the pink color persists and the excess of permanganate is destroyed by the addition of a very slight excess of 10 per cent oxalic acid solution. After diluting to 100 to 150 ml., 2 ml. of saturated iron alum solution in 50 per cent nitric acid are added and the solution is titrated with standard potassium thiocyanate solution. One milliliter of 0.1 *N* potassium thiocyanate is equivalent to 0.01003 gram of mercury or 0.1601 ml. of methylpropene at 0° C. and 760 mm.

TIME REQUIRED FOR ABSORPTION. To determine the time required for the absorption of methylpropene, a number of analyses were carried out on a refinery C_4 fraction containing 0.67 per cent of methylpropene, using approximately the same volume of gas and the same volume (50 ml.) of reagent but different times of shaking. The results are given in Table III.

TABLE III. EFFECT OF TIME OF SHAKING ON ABSORPTION OF METHYLPROPENE

Volume of Dry Gas (S. T. P.)	Time of Shaking	Hg in Ppt.	Methylpropene Extracted
Ml.	*Min.*	*Gram*	%
165.1	7	0.0696	100
146.7	1	0.0608	98.2
161.4	0.5	0.0642	94.2
151.9	0.5[a]	0.0583	90.4

[a] Gas admitted to absorption vessel and then removed. No shaking.

All but the last traces of methylpropene are removed after shaking for 1 minute and 5 minutes are adequate for complete extraction.

VOLUME OF REAGENT. A refinery C_4 fraction containing 19.73 per cent of methylpropene was used in each of a number of determinations with different volumes of reagent and a constant shaking time of 5 minutes. The results are recorded in Table IV.

TABLE IV. EFFECT OF VOLUME OF REAGENT ON ABSORPTION OF METHYLPROPENE

Volume of Dry Gas (S. T. P.)	Volume of Reagent	Hg in Ppt.	Methylpropene Extracted
Ml.	*Ml.*	*Gram*	%
41.9	10	0.2070	40.0
39.6	25	0.4483	91.5
41.9	50	0.4981	96.4
40.4	100	0.4992	99.9
39.8	200	0.4927	100.0

The methylpropene present in the volume of gas taken was sufficient to combine with all the mercury in 10 ml. of reagent. Good results are obtained by using a volume of reagent equal to about ten times the volume of methylpropene to be absorbed.

OTHER GASES. Ethene, propene, and 1,3-butadiene were slowly absorbed by the reagent but gave no precipitate when the solution was boiled.

A mixture of 1- and 2-butenes prepared by the dehydration of *sec*-butanol was slowly absorbed by the reagent and on warming a faint yellow turbidity was produced, the mercury content of the precipitate corresponding to 0.37 per cent of methylpropene in the gas. The gas was shaken with 67 per cent sulfuric acid until one third of its volume had been absorbed, when the residue gave a barely detectable opalescence with mercuric nitrate reagent, corresponding to less than 0.006 per cent of methylpropene. After a second treatment with sulfuric acid the gas was slowly absorbed by the reagent but gave no precipitate.

Hurd and Goldsby (*2*) report that 2-methyl-2-butene gives no precipitate with the acid Denigès reagent. With the

neutralized reagent, the mixture of pentenes obtained by dehydrating 2-methyl-2-butanol gives a heavy precipitate under the conditions for methylpropene determination, as also does a C_5 cut fractionated from debutanized cracked gasoline. This is probably due to the presence of 2-methyl-1-butene in these two samples. The possibility of applying this method to the pentenes is being studied.

Quantitative tests of the effect of 1- and 2-butenes on the estimation of methylpropene were carried out by preparing synthetic mixtures and submitting them to the determination. The results are given in Table V.

Thus 1- and 2-butenes neither give a precipitate with the reagent nor interfere with the determination of methylpropene. The method is entirely satisfactory in presence of C_2, C_3, and C_4 olefins, but pentenes must be absent. To test the effect of air which is normally present during a determination, a mixture of methylpropene and air containing 5.90 per cent of the former constituent was analyzed and gave results of 5.96 and 5.93 per cent of methylpropene.

TABLE V. ANALYSIS OF SYNTHETIC MIXTURES OF METHYLPROPENE WITH 1- AND 2-BUTENES

Sample No.	Volume of Dry Gas (S. T. P.)	Hg in Ppt.	Methylpropene Content Calcd.	Found
	Ml.	*Gram*	%	%
1	9.3	0.5815	100.0	100.1
2	16.9	0.2257	21.3	21.3
	17.4	0.2345	21.3	21.4
3	27.0	0.0715	4.14	4.23
	33.3	0.0847	4.14	4.07

Table VI compares a number of determinations of methylpropene in refinery C_4 fractions by the present method and by a standardized form of the sulfuric absorption method. In the latter method the gas sample (100 ml.) is given two passes into a special bead-packed absorption pipet containing 64 per cent sulfuric acid and on each pass is left in contact with the acid for exactly 3 minutes.

The amount of 1- and 2-butenes present in the samples of Table VI ranges from about 28 per cent for the samples with 20 per cent of methylpropene to about 12 per cent for the samples with 1 per cent or less of methylpropene. The sulfuric acid method gives high results, the percentage error increasing with increase in the ratio of 1- and 2-butenes to methylpropene and, even in the hands of a well-trained operator, is far less precise than the present method.

A single determination requires about 2 hours, of which 40 to 45 minutes are actual working time. In carrying out a number of determinations the time required averages 40 minutes for each analysis.

TABLE VI. METHYLPROPENE DETERMINATION BY MERCURIC NITRATE REAGENT AND BY 64 PER CENT SULFURIC ACID

Sample No.	Methylpropene Content: Sulfuric acid method	Methylpropene Content: Mercuric nitrate method
	%	%
1	1.9	0.67
	1.6	0.67
2	3.1	2.72
	2.8	2.73
	4.4	
	3.3	
3	8.8	7.08
	7.2	
	8.7	
	7.4	
4	12.5	12.8
	12.5	
5	21.1	21.2
	21.2	21.2

If desired, mercury can be very easily recovered from the filtrates from the determinations by adding an excess of caustic soda solution, washing the yellow precipitate by decantation, filtering, drying, and redissolving the necessary quantity of the dry mercuric oxide in nitric acid.

Acknowledgment

The authors are indebted to Messrs. Trinidad Leaseholds, Ltd., for permission to publish this paper.

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Filter Aids

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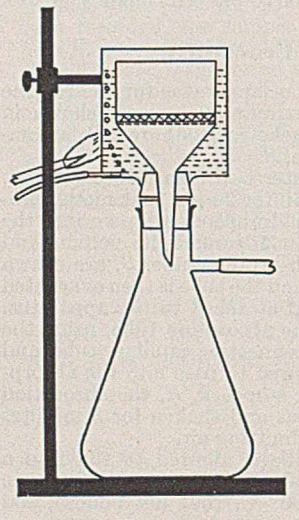
KAFIR fat at room temperatures is a pasty solid, and in recent investigations considerable difficulty was encountered in the usual procedure of purification. Even excessive amounts of hot solvents did little to relieve the difficulty because the mat of adsorbent, the small pores of the filter paper, and the small holes of the Büchner funnel prolonged the filtration time. Moreover, oily constituents separated from the fatty solids of higher melting point, so that the product of extraction was no longer representative.

Several different designs were tried before the following was chosen as most inexpensive and efficient. For a Büchner funnel 127 mm. in outside diameter, an ordinary 3.785-liter (1-gallon) bucket was cut to a 10-cm. (4-inch) wall and an air inlet and a dispensing outlet were soldered to the bottom. The former served to introduce air from the pressure line and bring about a more uniform temperature. The remaining parts of the device are self-explanatory. The weight of the unit rests securely on the suction flask and an attached buret clamp serves to stabilize the apparatus.



In actual operation, hot water is poured into the can, air is admitted, and a small flame is directed toward the side of the can. After 20 or 30 minutes the temperature of the Büchner funnel has reached working conditions and the treated fat-solvent mixture is poured through. Great quantities of the mixture may be filtered without difficulty.

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Determination of Cellulose in Fibrous Agricultural Wastes

A Rapid Method Using Monoethanolamine

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IN A RECENT article (*19*) Wise, Peterson, and Harlow described a preliminary experiment in which they obtained from beechwood a cellulose fraction very similar to Cross and Bevan cellulose. They extracted the sawdust for 5.25 hours with hot (170° C.) monoethanolamine, bleached the residue for 20 minutes with saturated chlorine water, and then treated it with hot 3 per cent sodium sulfite solution for 0.5 hour. Other investigators have used dilute alcoholic solutions of monoethanolamine in the isolation of holocellulose from woods, following chlorination (*5*, *17*, *18*). This laboratory is interested in a rapid method for the determination of cellulose. The procedure should cause but little degradation of the cellulose and should be suitable for use on fibrous agricultural wastes such as straws, stalks, etc., or the residue left after the bacterial fermentation of such wastes. Previously a critical investigation has been reported by this laboratory (*16*) on a number of such materials (and sprucewood), in which the results obtained by the Cross and Bevan (*3*), Norman-Jenkins (*14*), and Kürschner-Hoffer (*8*) methods of cellulose analysis are compared. Sufficient quantities of the original samples (with the exception of wheat straw) were still available for comparative analysis. The present paper is, therefore, concerned with the development of a method using monoethanolamine as a reagent for cellulose analysis and comparison of the results with those obtained by the methods of analysis mentioned above (*16*).

Approximately 300 cellulose determinations were made using monoethanolamine on the various materials, after which the resulting "crude celluloses" were analyzed for alpha-cellulose, pentosans, lignin, and ash. It was found from preliminary results that monoethanolamine is particularly suitable for the analysis of fibrous agricultural wastes. The cellulose fraction obtained is very similar to Cross and Bevan cellulose, except that the pentosan content is usually a little higher. The manipulation time is from 45 to 60 minutes per determination, or less than half of that required for the usual Cross and Bevan method. The cellulose analysis of 8 to 10 samples of straws or stalks may be completed (except for drying and weighing) in an 8-hour day.

Reagent

While technical monoethanolamine may be used for the determination of cellulose, the redistilled reagent was found to be more satisfactory for this purpose. One sample of the technical grade, which gave erratic results, was found to have corroded its metal container. The monoethanolamine was distilled, using a fractionating column, and that portion distilling between 167° and 172° C. was retained for use. The technical monoethanolamine costs about 30 cents per pound, amounting to about 5 cents for each cellulose determination. About two thirds of the used reagent may be recovered by distillation, thus reducing this cost materially.

Samples

The samples, with the exception of wheat straw, were those previously described (*16*) consisting of ground bagasse, rye straw, cornstalks of 40- to 60-mesh, and sprucewood of 40- to 60- and 60- to 80-mesh. These samples were homogeneous but not necessarily representative of the whole plant material from which they were prepared (*16*). Washed wheat straw was ground in a chopping mill and the portion passing a 40-mesh sieve but retained on 60-mesh was used for analysis. A sample of the same washed wheat straw was fermented and

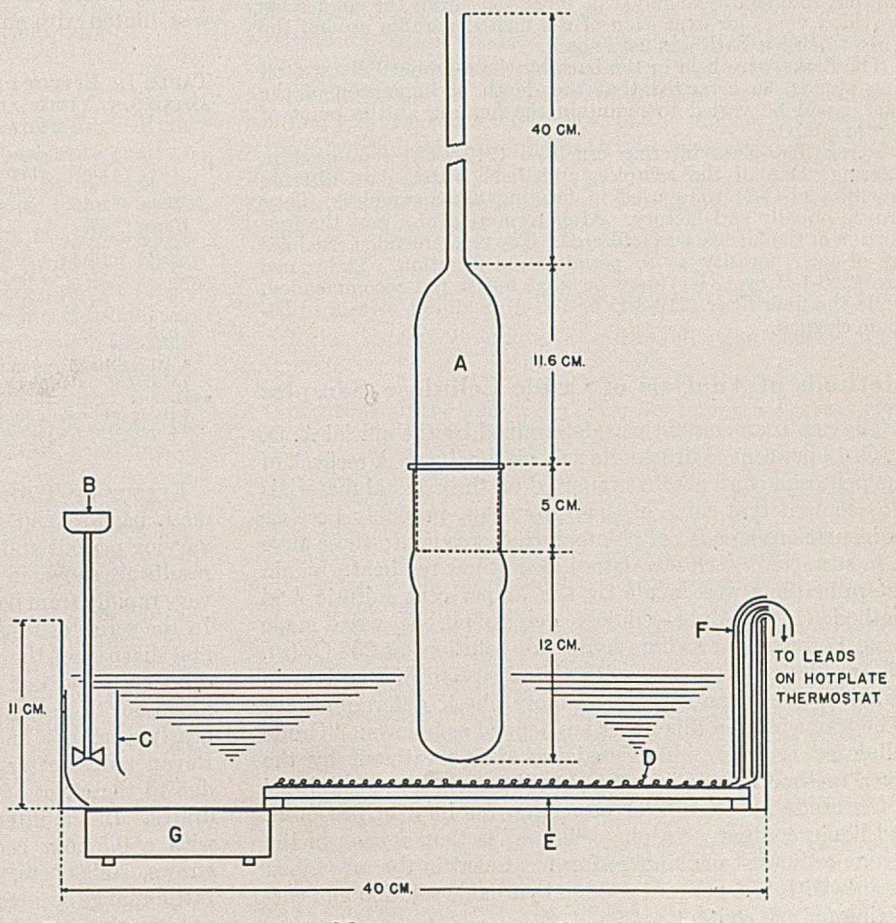

FIGURE 1. APPARATUS FOR MONOETHANOLAMINE CELLULOSE DETERMINATION

the residue, after washing and grinding, was also analyzed, as indicated in Table II.

Apparatus

The apparatus used in the determination is shown in Figure 1. The refluxing flask, *A*, of about 200-cc. capacity, was similar to that used for Kürschner-Hoffer cellulose determinations (*16*), and was made by sealing off the lower part of a 50/50 interchangeable ground-glass joint. The upper portion of the joint, about 12 cm. above the ground-glass portion, was sealed to a Pyrex tube (40 cm. long, 1 cm. in inside diameter). This large part in the upper portion served to condense most of the monoethanolamine and thus continuously to wash down the sides of the lower portion.

A paraffin oil bath was used to heat the samples. The bath, as shown in Figure 1 (40 cm. long, 9 cm. wide, and 11 cm. deep), was made from a single piece of thin sheet iron with welded corners (soldering was considered unsatisfactory), and was large enough for four to five simultaneous determinations. A small air-driven stirrer, *B*, at one end circulated the bath liquid through a pipe, *C*, which directed its flow over the heating coils. The bath was heated by a 600-watt Nichrome coil, *D*, mounted on and insulated by an asbestos board, *E*. Copper leads were brought to the surface through bent glass tubes, *F*. The coil must always be completely submerged in the oil to eliminate fire hazard.

The temperature was controlled thermostatically as follows: The leads to the heating element of a thermostatic hot plate, *G*, were removed and replaced by the leads of the Nichrome coil, *D*. When the surface of the hot plate was held against the bottom of the bath at a point directly below the stirrer, the thermostat, controlled by the plate surface temperature, served to keep the bath temperature within ±10° of the 190° C. desired, although there was some lag during the first half hour of operation.

A second 600-watt Nichrome coil (not shown in the drawing) was mounted independently on the asbestos board, *E*. This was connected directly to the power line and used only for auxiliary heating to bring the bath up to the desired temperature. Gas burners should not be used on account of fire hazard and the tendency of the concentrated heat to carbonize the paraffin. Several glass rods were placed vertically in the bath before the paraffin solidified. Removal of these rods from the solid mass provided vents for expansion of the melted paraffin around the wires when the bath was used again.

The flasks were held in the bath by three-pronged clamps, on ring stands, so arranged that the depth of immersion of the flask could be varied to maintain the heating at the point of gentle refluxing.

Pyrex fiber-glass filtering crucibles (210-2-C) were used in filtering some of the samples, and Jena fritted-glass filtering crucibles (1-G-2) were used in filtering other samples. They proved equally satisfactory. After formation of a mat the first portion of the filtrate was refiltered. The recommended crucibles are of such porosity as to permit rapid filtration. The coarse size (210-1-B or 1-G-1) may be used but is not recommended, while the finer filter (210-3-D or 1-G-3) is almost certain to become clogged.

Methods of Analysis of Crude Cellulose Samples

The pentosan content was determined by the official A. O. A. C. 12 per cent hydrochloric acid method (*1*). A recheck of the pentosan contents first reported on the original materials (*16*) showed that some of these were low, probably because an insufficient amount of phloroglucinol was used; these have been corrected. Ash was determined at low red heat. Lignin determinations were made by the 72 per cent sulfuric acid method (*15*). Alpha-cellulose determinations were made using 17.5 per cent sodium hydroxide solutions at 20° C. (*2*). The results reported are, with the exception of the data in Table I, the mean of two or more check determinations. (The terms used in this paper are defined as follows: "Crude cellulose" is the residue remaining after treatment by the given method for the isolation of cellulose. "Pure cellulose" is the crude cellulose after correction for its ash, pentosan, and lignin content. "Alpha-cellulose" is that portion of the "crude cellulose" remaining after treatment in the prescribed manner with 17.5 per cent sodium hydroxide solution and correcting for ash content.)

Preliminary Procedure. Preliminary runs were made for the purpose of determining approximately the optimum conditions. (These data are not included in this paper.) Samples were refluxed for different lengths of time in the apparatus described above. They were then allowed to cool for 30 minutes, filtered, washed, dried, and weighed, and the residues were analyzed for lignin. Some samples were bleached and the bleached residues analyzed for alpha-cellulose and pentosan contents.

Results with sprucewood were disappointing. The unbleached spruce sample retained 7.5 per cent of lignin even after 6.5 hours of refluxing with monoethanolamine. (Wise *et al.*, *19*, succeeded in reducing the lignin content of aspenwood to 2.62 per cent with 5.25 hours' refluxing.) On the other hand, monoethanolamine reduced the lignin content of bagasse to approximately 1 per cent in 2 hours. Furthermore, the sample was very light in color and required only a mild afterbleach to give a cellulose which compared favorably in yield and alpha-cellulose content with that obtained by the Cross and Bevan method. A careful examination was then made of the factors influencing the method, as well as of the effect of monoethanolamine on the plant constituents as detailed below.

Factors Influencing Method

Liquor-Solid Ratio. Identical yields of crude cellulose were obtained when 2-gram samples of bagasse were refluxed for 2 hours with 50-, 75-, and 100-cc. portions of monoethanolamine. Dilution with 50, 75, and 100 cc. of water, respectively, before filtration had no effect upon the yields but facilitated the filtration. As a compromise between ease of handling and economy of reagent, 75 cc. of reagent were used on approximately 2 grams of sample and, after a 30-minute cooling period (to avoid sudden effervescence), the mixture was diluted with an equal quantity of water before filtration.

Table I. Effect of Time of Refluxing with Monoethanolamine on Yield and Composition of Unbleached Pulps from Bagasse and Sprucewood[a]

	Bagasse 40- to 60-Mesh[b]				Spruce 40- to 60-Mesh[d]		
Time	Yield of pulp	Lignin in pulp	Lignin removed[c]	Pentosans in pulp	Yield of pulp	Lignin in pulp	Lignin removed[c]
Hours	%	%	%	%	%	%	%
0.5	70.7	2.03	94.2	28.9	70.5	25.9	36.5
1	69.4	1.49	95.8	28.2	66.6	23.0	46.9
2	67.3	0.83	97.5	30.0	61.2	17.3	63.5
3	66.7	0.57	98.3	29.2	56.0	12.1	76.4
4.5	66.1	0.30	99.2	29.5	55.8	8.4	83.7
6.5	..	..	..	..	55.6	7.5	85.4

[a] All results given on moisture-free basis.
[b] Original material contained 24.0 per cent lignin and 31.3 per cent pentosans.
[c] Basis original lignin.
[d] Original material contained 28.8 per cent lignin.

Effect of Time of Refluxing. Samples of 40- to 60-mesh bagasse and sprucewood were refluxed vigorously for varying periods and the lignin content was determined. The results are shown in Figure 2 and Table I. Lignin is extracted very rapidly from bagasse but rather slowly from sprucewood. In the series of runs on bagasse it was found that, after the first sharp drop, the yields of residue decreased slowly and this decrease could not be ascribed entirely to the reduction in lignin nor to loss of pentosan (Table I). The latter remained nearly constant and rather high (compared to Cross and Bevan pulps) after the first hour. The slight loss may be due to the effect of long-continued heating on the carbohydrates. In the interests of precision, a 2-hour reflux period, with a 0.5-hour cooling period, was selected for work on straws, stalks, etc. (A 3-hour reflux was used for wood.) Little change occurred in the sample after dilution and equally good results were obtained from samples filtered immediately,

with and without dilution, and from those filtered 30 to 90 minutes after dilution.

Effect of Moisture in the Sample. Two-gram samples of bagasse containing 0, 5, 33, and 72 per cent of moisture were refluxed in 75 cc. of monoethanolamine. The boiling points of the first two were approximately 170°, and for the latter two 160° and 146° C. The lignin contents of the unbleached residues were 1.2, 1.2, 1.9, and 2.3 per cent and the pentosan contents were 29.6, 30.5, 31.4, and 32.4 per cent, respectively. Therefore, either air-dry or oven-dry samples are more satisfactory than those containing a considerable amount of moisture.

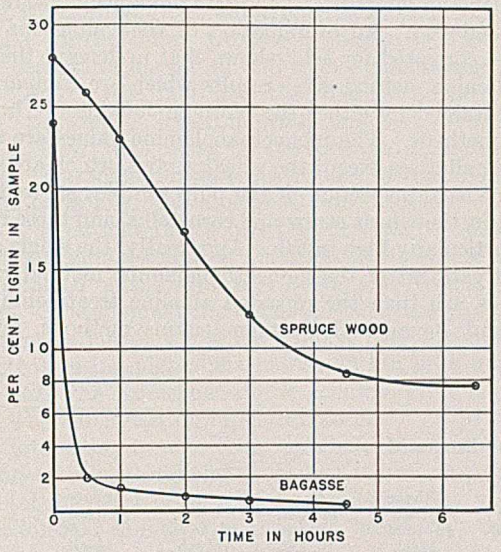

Figure 2. Effect of Time of Refluxing on Lignin Removal

Effect of Bleaching on Monoethanolamine Residues. Commercial 5 per cent sodium hypochlorite solution was used for bleaching the pulp samples (*16*). Removal of the small amount of lignin remaining in bagasse after 2 hours of refluxing (Table I) should be possible by mild bleaching. Therefore, bleaching tests were made using 1-, 5-, and 10-cc. portions of sodium hypochlorite in 100 cc. of mixture for 20 minutes at room temperature. Although the chlorine was not exhausted, color tests showed lignin remaining in the samples. Better lignin removal was obtained by holding the samples at 40° for 20 minutes, but the two stronger bleach solutions had a tendency to degrade the alpha-cellulose at this temperature. Bleaching with dilute acidified solutions of hypochlorite, followed by heating with 3 per cent sodium sulfite solution proved more satisfactory. Two cubic centimeters of the 5 per cent sodium hypochlorite solution plus 3 cc. of 10 per cent sulfuric acid in 100 cc. of mixture served to bleach bagasse to a good white in 5 minutes. For sprucewood a stronger bleach was necessary because about 12 per cent of lignin remained in the sample after 3 hours of refluxing. The best results on the 40- to 60-mesh spruce were obtained by using 10 cc. of sodium hypochlorite with 12 cc. of acid, followed, after boiling with sodium sulfite, by a second bleach of 5 cc. of sodium hypochlorite and 6 cc. of acid. The 60- to 80-mesh spruce was easier to bleach and two 5-cc. bleaches gave satisfactory results.

Effect of Monoethanolamine

On Alpha-Cellulose. It has been stated that cellulose suffers some decomposition when it is heated above 140° C., although there is some question as to whether this may be due entirely to oxidation (*4*, *9*). Therefore, a commercial sample of nitric acid high-alpha bagasse pulp (alpha-cellulose content, 96.6 per cent) was refluxed for 1-, 2-, and 4.5-hour periods with monoethanolamine (about 170°). The yields were 99.7, 99.7, and 99.4 per cent, respectively, and the corresponding alpha-cellulose contents were 96.0, 95.6, and 95.2 per cent. From these figures, it appears that boiling monoethanolamine (a reducing agent) has only a slight effect on alpha-cellulose.

On Lignin. Hot monoethanolamine does not merely dissolve lignin but also acts upon it to change its properties. The nearly complete removal of the lignin from the bagasse induced the hope that lignin might be recovered quantitatively from the filtrate. However, upon acidification of the filtrate no lignin was recovered. Six hours of refluxing with 150 cc. of monoethanolamine dissolved 25 grams of an isolated alkali corncob lignin. After removal of most of the monoethanolamine by steam distillation from alkaline solution and subsequent acidification of the residual liquor, a very small amount of material was recovered which was resistant to 72 per cent sulfuric acid. Although monoethanolamine, like most other strong alkalies, will dissolve isolated lignin in the cold and return a precipitate on acidification, refluxing at 170° renders at least part of the lignin soluble in both acid and alkaline solutions. This change is not due wholly to thermal decomposition, since the alkali lignin heated at 170° either alone or in an inert liquid (paraffin) did not become soluble in acid solution. The change might be due to the strong reducing action of monoethanolamine (*6*, *7*, *10–12*) or possibly to the addition of amino nitrogen to give an amphoteric compound.

Chloroform precipitated a gelatinous material from undiluted used monoethanolamine. An attempt to separate the lignin from the used monoethanolamine by diazotizing the latter gave a yellowish precipitate which may have been due to a decomposition product of lignin. Ferric salts as well as bromine water precipitated small amounts of substances from an acidified solution. When steam-distillation from alkaline solution was used to remove most of the monoethanolamine, a thick brown liquid residue was left and a small amount of an unidentified white solid was found in the distillate. The action of monoethanolamine on the lignin of agricultural wastes is being studied further.

Proposed Method of Analysis

On the basis of the above data, the following method was adopted:

Approximately 2 grams of dry or air-dry sample are accurately weighed into the flask (*A*, Figure 1) and 75 cc. of monoethanolamine are added. The flask is immersed in the oil bath, which is maintained at 190° C., to such a depth that the mixture will come to boiling in about 10 minutes. The mixture is refluxed gently for exactly 2 hours, removed from the bath, allowed to cool 0.5 hour, and is then diluted with approximately 75 cc. of water. About 100 cc. of the supernatant liquor are decanted into a 150-cc. beaker and the remainder of the mixture is poured rapidly into a 250-cc. beaker.

This thick mixture is filtered through a weighed glass filtering crucible with suction and the filtrate is refiltered through the mat. The decanted portion is then filtered through the crucible. (If the monoethanolamine is to be recovered by distillation, this filtrate is set aside without dilution by the subsequent washings.) The sample is then transferred completely to the crucible by means of water and the residue in the crucible is washed with about 200 cc. of hot water. The wash water is added in small portions and the crucible is sucked dry after each addition, but the mat must not be disturbed.

The sample is then transferred to the 250-cc. beaker by a narrow spatula and water from a wash bottle. (An excellent spatula for this use may be made from a Nichrome rod 0.4 cm. in diameter and 17 cm. long by hammering the end into a blade 0.9 cm. wide and 5 cm. long.) The mixture is made up to about 95 cc. with

water and acidified with 3 cc. of 10 per cent sulfuric acid, and then 2 cc. of 5 per cent sodium hypochlorite are added. The final volume should be approximately 100 cc. and is conveniently gaged by a mark previously made on the side of the beaker. The material is bleached for exactly 5 minutes at room temperature (about 25°).

After 5 minutes, the mixture is decanted as above, and filtered, forming a mat as before. The small beaker is rinsed with about 15 cc. of water into the 250-cc. beaker containing an equal volume of approximately 0.25 *N* sulfurous acid solution. The sample is washed with this 30 cc. of dilute sulfurous acid, followed by a little cold water and then with about 15 cc. of 3 per cent sodium sulfite solution. The sample is transferred to the 250-cc. beaker, as before, using not more than 50 cc. of wash water. To this, 50 cc. of 6 per cent sodium sulfite solution are added, so that the final concentration is 3 per cent sodium sulfite, the liquid level being gaged by the 100-cc. mark as before. The beaker is then covered with a watch glass and set in a boiling water bath for 20 minutes. The entire filtering, washing, and transference should take less than 5 minutes. It is therefore convenient to start each sample bleaching just before the preceding one is filtered.

The residue is then filtered as before and washed successively with 150 cc. of boiling water, a little cold water, about 25 cc. of cold 10 per cent acetic acid (rapidly), cold water, 150 cc. of boiling water, about 75 cc. of cold water containing 1 to 2 drops of concentrated ammonium hydroxide (to remove any trace of acid), and finally 150 to 200 cc. of boiling water. The bleached residue (designated "crude cellulose") is dried in the crucible at 105° and weighed in a weighing bottle.

With samples of sprucewood, the initial bleaching is carried out with 5 to 10 cc. of 5 per cent sodium hypochlorite solution and 6 to 12 cc. of 10 per cent sulfuric acid. After boiling with 3 per cent sulfite, the whole process is repeated, using 5 cc. of bleach and 6 cc. of acid.

If the conditions outlined in the proposed procedure are closely followed, the precision of the method is very good, duplicate samples checking within about 0.2 per cent cellulose for the above materials as compared to 0.1, 0.2 and 0.3 per cent for the Norman-Jenkins, Cross and Bevan, and Kürschner-Hoffer methods, respectively. Excessive refluxing of the sample may cause the precision to be less, a variation due principally to change in hemicellulose content.

Results and Discussion

The results of the analyses are given in Table II. For convenience in comparisons, the data previously obtained (*16*) are reproduced. An examination of the values for "crude cellulose corrected for ash" shows that in general the monoethanolamine method gives results which are comparable to those obtained by either the Norman-Jenkins or Cross and Bevan methods. The monoethanolamine values are slightly higher in all cases except the wheat straws, which are slightly lower. Visual inspection of the pulp samples showed grains of sand in the wheat straw and cornstalks, and these residues were particularly high in ash. Apparently, the single sodium sulfite treatment of the monoethanolamine method removes less silica ash than the repeated alkaline treatments of the Cross and Bevan and Norman-Jenkins methods, an effect

TABLE II. COMPARATIVE ANALYSES OF PLANT MATERIALS

(Monoethanolamine, Cross and Bevan, Norman-Jenkins, and Kürschner-Hoffer methods, respectively[a])

	Bagasse 40- to 60-Mesh %	Rye Straw 40- to 60-Mesh %	Corn-stalks 40- to 60-Mesh %	Wheat Straw 40- to 60-Mesh %	Fer-mented Wheat Straw %	Spruce-wood 40- to 60-Mesh %	Spruce-wood 60- to 80-Mesh %
Original Material							
Moisture	4.45	6.33	7.64	Nil[b]	Nil[b]	Nil[c]	Nil[c]
Pentosan	31.3	29.1	25.5	28.7	29.3	12.1	12.0
Ash	0.82	6.30	7.58	6.5	4.27	0.90	0.74
Lignin	24.0	13.3	15.7	20.7	24.2	28.0	27.7
Monoethanolamine Cellulose							
Crude cellulose	65.7	50.6	48.7	55.3	58.0	54.0	56.5
Ash in crude cellulose	0.62	2.14	8.82	4.4	1.30	0.96	0.97
Crude cellulose corrected for ash	65.3	49.5	44.4	52.9	57.2	53.5	56.0
Pentosan in crude cellulose	31.4	34.0	31.8	27.8	28.8	11.4	10.2
Lignin in crude cellulose	0.23	0.83	1.05	0.96	0.76	0.22	0.19
Pure cellulose in original material	44.4	31.9	28.4	37.0	40.1	47.2	50.1
Alpha-cellulose in crude cellulose	73.5	69.7	66.9	73.8	74.8	70.9	70.9
Alpha-cellulose calcd. on original material	48.3	35.3	32.6	40.8	43.4	38.3	40.0
Cross and Bevan Cellulose							
Crude cellulose	64.5	48.5	40.5	57.4	59.6	52.4	51.4
Ash in crude cellulose	1.30	1.83	2.20	1.70	1.51	1.80	1.28
Crude cellulose corrected for ash	63.7	47.6	39.6	56.4	58.7	51.5	50.8
Pentosan in crude cellulose	25.7	25.1	23.7	29.3	29.0	8.90	8.85
Lignin in crude cellulose	1.80	2.37	1.27	1.91	1.44	0.11	0.20
Pure cellulose in original material	47.0	34.3	29.5	38.5	40.6	46.8	46.1
Alpha-cellulose in crude cellulose	77.7	72.7	76.4	73.4	74.0	75.8	76.5
Alpha-cellulose calcd. on original material	50.1	35.3	30.9	41.4	44.1	39.7	39.3
Norman-Jenkins Cellulose							
Crude cellulose	63.6	49.7	41.8	59.8	62.6	50.6	51.6
Ash in crude cellulose	0.50	0.88	1.11	2.36	1.73	1.10	1.17
Crude cellulose corrected for ash	63.3	49.3	41.3	58.4	61.5	50.0	51.0
Pentosan in crude cellulose	26.9	31.5	28.1	30.2	29.4	7.84	7.92
Lignin in crude cellulose	4.06	1.66	6.62	1.80	1.11	0.07	0.13
Pure cellulose in original material	43.6	32.8	26.8	39.3	42.4	46.0	46.8
Alpha-cellulose in crude cellulose	76.6	74.5	72.1	70.2	71.1	72.6	71.6
Alpha-cellulose calcd. on original material	48.7	37.0	30.1	42.0	44.5	36.7	36.9
Kürschner-Hoffer Cellulose							
Crude cellulose	55.0	42.5	40.4	...	...	49.2[d]	49.0[d]
Ash in crude cellulose	0.80	2.80	9.50	...	...	0.20	0.23
Crude cellulose corrected for ash	54.6	41.3	36.6	...	...	49.1	48.9
Pentosan in crude cellulose	19.9	20.3	16.3	...	...	7.07	5.95
Lignin in crude cellulose	4.59	0.49	0.73	...	...	0.39	1.20
Pure cellulose in original material	41.1	32.5	29.7	...	...	45.4	45.4
Alpha-cellulose in crude cellulose	74.4	70.7	63.9	...	...	66.5	61.0
Alpha-cellulose calcd. on original material	40.9	30.0	25.8	...	...	32.7	29.9

[a] All results except moisture given on moisture-free basis.
[b] Oven-dried samples used.
[c] Previously extracted by alcohol and benzene.
[d] Faintly yellow.

which is reflected also in less change in weight of the glass filtering crucibles. The same effect had been noted with the Kürschner-Hoffer method. A residue with a uniformly lower lignin content is obtained by the monoethanolamine method than with either the Cross and Bevan or Norman-Jenkins methods, in the case of annual fibrous crops. The bleach requirements for obtaining a good white color with the monoethanolamine residues were very low.

Differences in yield of cellulose are due primarily to the difference in pentosan content. With the exception of the wheat straws the pentosan content of the monoethanolamine cellulose fraction is higher than that obtained by the other methods. Apparently the action of the monoethanolamine is similar to that of the agents used in the alkaline pulping process, which produce a pulp with a higher pentosan content than the corresponding Cross and Bevan cellulose. The higher pentosan content of the monoethanolamine residue may be due to the fact that the "tightly bound" or "resistant" pentosan is less completely removed. The data in Table I show that most of the pentosan removed from bagasse is extracted during the first hour of refluxing.

A comparison of the alpha-cellulose contents, calculated on the original material, shows that the results obtained on monoethanolamine cellulose are approximately the same as those obtained on the Cross and Bevan and Norman-Jenkins residues. The monoethanolamine method, therefore, causes little degradation of the alpha-cellulose which confirms the experiments using high-alpha pulp. As has been previously shown (*16*), the Kürschner-Hoffer cellulose gives anomalous results when analyzed for alpha-cellulose.

It is interesting to note that, in the case of fibrous wastes, the monoethanolamine alpha-cellulose values are uniformly higher (about 8 per cent) than the corresponding values for pure cellulose. A similar relationship holds with the Cross and Bevan and Norman-Jenkins values. However, in the case of sprucewood, the pure cellulose figures are 18 to 27 per cent higher than the alpha-cellulose values. This fact indicates that the hemicellulose part of the crude cellulose of the fibrous farm wastes differs considerably in composition from that of the wood. It is probable that the crude wood cellulose contains a greater percentage of hexosans other than cellulose.

The outstanding advantage of the monoethanolamine method is the saving in time and effort required to conduct a cellulose analysis. Manipulation time is approximately 0.75 to 1 hour per determination. Although the saving in time is not much greater than with the Kürschner-Hoffer method, the composition of the monoethanolamine residue, particularly with regard to alpha-cellulose content, shows that the monoethanolamine method is greatly superior.

This method was particularly successful when applied to fermented wheat straw (Table II). The low lignin content of the treated samples indicates that the ligno-protein complexes normally present (*13*) are probably dissolved by the monoethanolamine. Samples of this type are normally difficult to handle during analysis, even though a centrifuge is used. Since the monoethanolamine method involves only 3 filtrations (or centrifugings) as compared to at least 20 by the usual methods, this alone effects a great saving of time.

Summary and Conclusions

The use of hot monoethanolamine as a reagent for the determination of cellulose has been investigated and found to be very satisfactory, particularly for fibrous farm wastes.

Two hours of refluxing in the monoethanolamine decomposed the major part of the lignin in straws and stalks, removed the less resistant pentosans, but had little effect upon the alpha-cellulose either in the isolated form or in the plant material. The extraction with monoethanolamine was less effective upon sprucewood, the lignin content being reduced to 7.5 per cent after 6.5 hours as compared to about 1 per cent in bagasse after 2 hours. A mild bleach was used to remove the residual lignin.

A procedure for the determination of cellulose was applied to bagasse, rye straw, cornstalks, wheat straw, and fermented wheat straw ground to 40- to 60-mesh size, and to two samples of ground sprucewood, 40- to 60- and 60- to 80-mesh size. The resulting crude cellulose was analyzed for pentosans, ash, lignin, and alpha-cellulose. The results were calculated as pure cellulose and alpha-cellulose in the original material. The data are tabulated and compared with results previously obtained (*16*) on the same materials (except for the wheat straws) by the Cross and Bevan, Norman-Jenkins, and Kürschner-Hoffer methods of cellulose analysis.

The crude cellulose fractions, corrected for ash, compared favorably with those obtained by the Cross and Bevan and Norman-Jenkins methods. Variations were due principally to differences in pentosan contents which were usually a little higher in the monoethanolamine fractions. The monoethanolamine lignin contents were usually lower and the alpha-cellulose values compared very favorably with those of the other methods. The precision of the method was equal to that of the Cross and Bevan method.

The monoethanolamine method of cellulose analysis has an advantage over the other methods because of the saving of time and effort. It is possible to average more than one determination per hour when a large number of analyses are being carried out. The analysis of a typical fermented straw sample by this method was much simpler and quicker than when the same sample was analyzed by the usual methods. Although the monoethanolamine is not so effective in removing lignin from sprucewood as from representative fibrous farm wastes, it may be used if a more extensive bleaching procedure is carried out.

The monoethanolamine method of cellulose analysis is recommended particularly for the analysis of fibrous agricultural wastes, especially if the determination of alpha-cellulose on the residue is contemplated.

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Quantitative Spectrochemical Analysis of Dilute Solutions

A Safe Alternating Current High-Voltage Arc Circuit

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A safe and convenient alternating current high-voltage arc circuit together with its tie-in with the ordinary direct current arc and condensed spark circuits is described. The alternating current arc is a reproducible source for determining barium, strontium, tellurium, and phosphorus in dilute solutions. A precision of 50 to 100 parts per thousand was obtained using the comparison standard method.

SINCE the early days of chemical analysis with the spectrograph the greatest difficulty has been to obtain reproducible spectrograms on identical samples. Meggers (*5*) introduced the practice of photographing the spectra of a graded series of standards on the same plate with the sample and thus reduced to a minimum the errors due to photography. Gerlach's (*3*) concept of homologous pairs was another step forward, in that it reduced errors due to variable excitation. These workers, however, used spark excitation, which is not sufficiently sensitive to meet the demands of many modern analyses.

The direct current arc has been used in much recent work

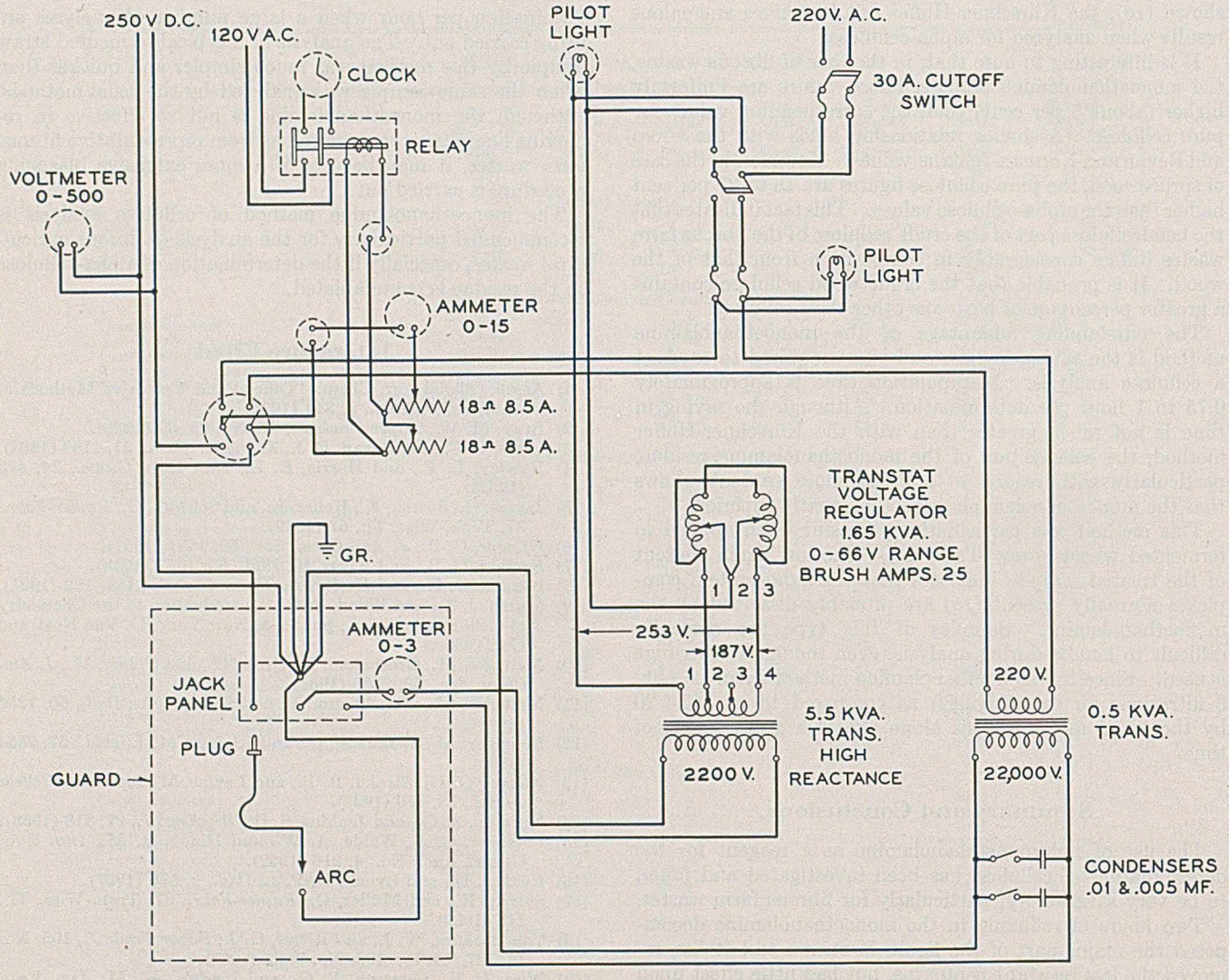

FIGURE 1. WIRING DIAGRAM FOR ALTERNATING AND DIRECT CURRENT ARCS AND CONDENSED SPARK

for both "comparison standard" and "internal standard" methods. While highly sensitive, the direct current arc is somewhat unreliable for comparison standard methods, showing an average error of ±10 to 20 per cent of element determined. When internal standardization is employed this error can be reduced, especially when only the straight-line portion of the characteristic curve of the plate is used. For best results it is necessary to calibrate the response of each plate (*2*) in order to use only the straight-line portion of the curve or to correct for deviations from it. Thus the procedure becomes somewhat involved if high precision is to be attained for determination of the smaller amounts of the metallic elements.

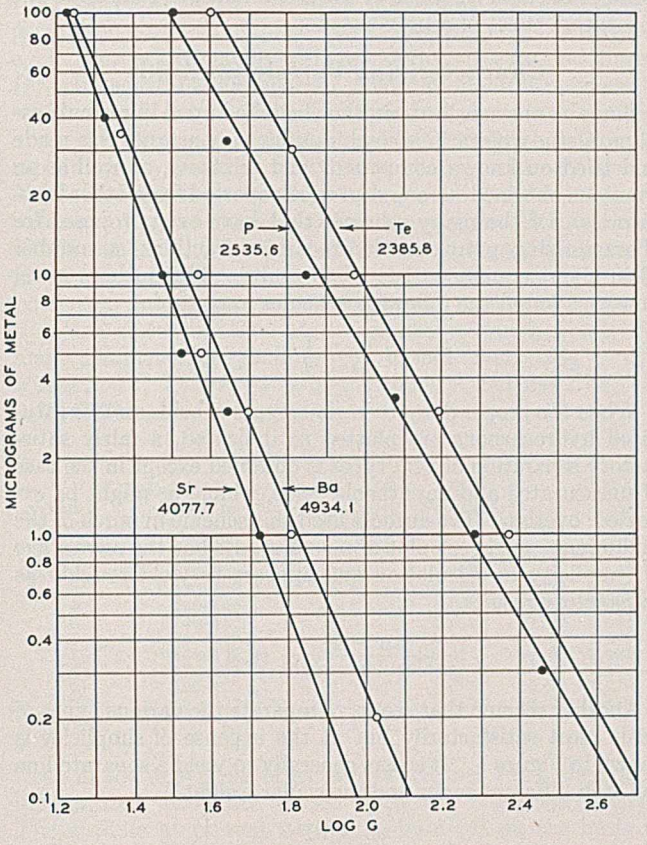

FIGURE 2. TYPICAL WORKING CURVES, COMPARISON STANDARD METHOD

Several workers have recently reported very high spectral sensitivity for metals in the 2000-volt alternating current arc. Duffendack and Wolfe (*1*) determined minute traces of various metals in caustic liquors, and Owens (*6*) reported greater sensitivity by a factor of ten than is shown by the direct current arc. The authors' experience indicates that this higher sensitivity is not universal but is evident only in certain cases. However the alternating current arc has an equally important advantage, in that it is much more reproducible than the direct current arc. It combines some of the precision of the condensed spark with the sensitivity of the direct current arc and is thus admirably suited to the analysis of dilute solutions.

Apparatus

Spectrographic equipment, densitometer, 5.5-kva. high-reactance transformer and Transtat voltage regulator (American Transformer Co.).

The high voltage alternating current arc is a considerably more dangerous apparatus than either the direct current arc or the condensed spark; consequently, extra precautions must be taken in making it safe for regular use. The authors' circuit and its tie-in with the direct current arc and the spark circuits also used in this laboratory are shown in Figure 1. Both transformers are caged in with a grounded expanded metal guard beneath the working table. The arc and spark stand is similarly caged. Entry is effected for changing electrodes, etc., through a door which mechanically obstructs the main alternating current switch when open. The direct current can be used with the door open. All secondary circuit controls in the alternating current lines are eliminated.

The spark is controlled by resistance in series with the primary. The alternating current arc is controlled for both voltage and current by a transtat in the primary line. The electrode separation can be adjusted while operating by means of an insulated flexible shaft on the ground side, the length of the gap being measured by an optical gage. The clock indicated is an inexpensive Telechron, the second hand of which times the direct current arc exposures by turning only when the arc current is passing.

Results

In Figure 2 are shown typical working curves for the comparison standard method using this source.

Aliquots of solutions of the concentration indicated were dried on flat-top graphite electrodes of 0.19 or 0.25 inch in diameter. Both upper and lower electrodes were first coated with a waterproofing material (collodion is suitable) and preheated to 100° C. before the solutions were transferred, in order to prevent their soaking into the electrodes. Exposures were for 120 seconds, during which time the lower electrode was rotated at 600 r. p. m. to steady the mean position of the arc column.

Densities were measured with a projection densitometer made by modifying a Moll instrument. In practice, the maximum swing of the galvanometer is set to a standard value by adjusting the area of the photocell surface illuminated, complete blackness corresponding to zero swing, so that the galvanometer reading, G, is proportional to the transmission.

As can be seen from the working curves, the precision of a comparison standard method with the alternating current arc is ±5 to 10 per cent, which is about as good as the authors have been able to secure on run-of-the-mill work with internal standardization and the direct current arc source (*4*). Undoubtedly some of the improvement is due to being able to measure density under the conditions of low background intensity characteristic of this source. Whether the alternating current arc will give better precision than the direct current arc when using internal standardization is still under investigation.

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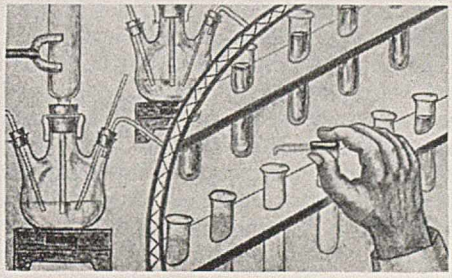

Separation and Characterization of Petroleum Acids

Some Texas Petroleum Acids

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Operations involved in various methods of fractionation of acidic material from Texas petroleum are described. The necessity for use of a combination of constants as an aid in characterizing the various cuts obtained is pointed out and several schemes are given.

Phenol, *n*-valeric, *n*-butyric, dimethylmaleic, *n*-octanoic, and solid hexahydro-*p*-toluic acids were isolated and identified.

WHEN a long-range program of research on petroleum acids was initiated in 1934, a survey showed that the only Texas petroleum material obtainable, including the boiling point range of C_6 to C_9 acids, consisted of alkali wash obtained in the refining of light burner oil at the Baytown Refinery of the Humble Oil and Refining Company. This material was obtained by washing a mixture of straight-run and cracking process products from Texas crudes. The offer of this company to furnish this material was gladly accepted, even though it was realized that the cracking process material would greatly complicate the mixture. It was felt from the first that physical methods should be employed much more extensively than in the past in the study of petroleum acids, and the success of such methods can be followed more readily on a complex mixture than on a simpler mixture consisting largely of members of a single homologous series.

Separation of types as well as of individuals among petroleum acids has become practically standardized in procedure involving the following steps:

1. Alkali neutralization of the acidic material
2. Liberation of acids from the sodium salt solution
3. More or less elaborate fractionation of the acids obtained
4. Esterification of the acids, leaving phenols and most tertiary acids unchanged but partially dissolved in the ester layer
5. Careful fractionation of the resulting esters by distillation
6. Saponification of each ester cut, followed by fractional distillation of the regenerated acids
7. Attempted isolation or identification of individual acids by formation of solid derivatives, various salts, amides, or degradation reactions

While under favorable conditions much has been accomplished considering the complexity of the raw material concerned, the use of other physical methods in addition to fractional distillation is clearly indicated, since separation by differences in vapor pressure alone would not be expected to succeed even when both the acids and their esters are fractionated. The authors developed and tried a number of methods, such as fractional esterification and saponification, fractional countercurrent neutralization and liberation, and fractional silver salt formation, but soon found that the development of these methods must go hand in hand with fast but reliable means of characterizing the various fractions, since these methods are not based on vapor pressure, neutral equivalent, density, or any other property alone; and therefore, no one of these can describe the fraction adequately. The combination and alternation of a number of different methods of fractionation lead soon either to an excessive number of small fractions or to recombinations that undo the separation effect of a previous step.

Characterizing Petroleum Acids

Since no one constant can then be relied upon in recombinations, a study of various combinations of constants was made and tried on known compounds and mixtures, as well as on fractions obtained here and in work reported from other laboratories. Of the many schemes that have been proposed for characterizing petroleum hydrocarbon fractions, a number yield about equally satisfactory results. The authors have, however, found the following schemes most useful.

$$\text{Plot of } \frac{n^2 - 1}{n^2 + 2} \text{ vs. } d_4^{20}$$

When the proper data for various types of acids and for saturated hydrocarbons are plotted as indicated, a fairly satisfactory separation of acid types is obtained except in the case of unsaturated and naphthenic acids, which, as might be expected, overlap. The authors used this scheme in much of the preliminary work. A plot of $n - 1$ *vs.* d_4^{20} has the advantage of simplicity in calculation and appears to yield roughly as satisfactory results.

$$\frac{n_D - 1}{\text{molecular weight}} \text{ vs. d}$$

Another scheme that seems to separate the various types of acids most satisfactorily but at the expense of simplicity is shown in Figure 1. It seems generally to yield a separate line for each different series and molecular weight.

$$n_D \times d_4^{20}$$

For fast routine work the authors have abandoned all of the more elaborate schemes for the simple purely empirical prod-

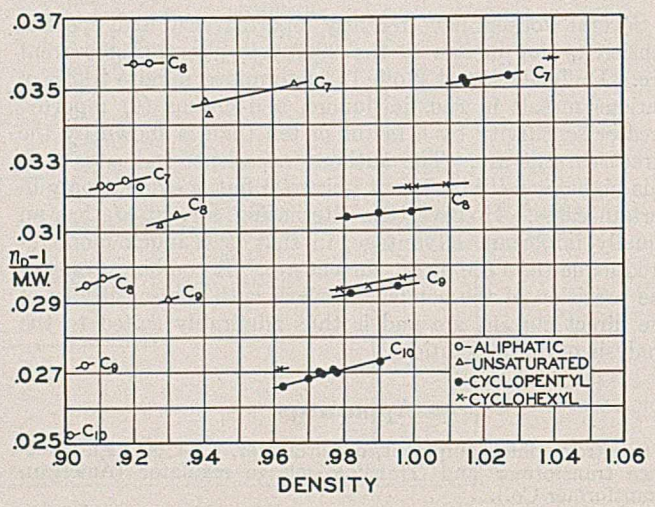

FIGURE 1. SEPARATION OF ACIDS

uct of $n_D^{20} \times d_4^{20}$. Calculation of this value for a large number of reported aliphatic, unsaturated, and naphthenic acids, phenols, and hydrocarbons with from 5 to 10 carbon atoms was undertaken. When the constants were reported at temperatures other than 20° ± 2° C., the 20° values were calculated, although in most cases the separation into acid type is so good that errors due to use of temperatures other than 20° do not shift the compound from one group to another.

The compiled data show that aliphatic acids have products ranging from 1.280 to 1.350 with most values between 1.300 and 1.310, the naphthenic acids range from 1.390 to 1.470 with most values between 1.410 and 1.440, while phenols have n·d products above 1.500 and hydrocarbon values below 1.300 and usually below 1.280.

The only exception for acids containing the cyclopentyl group—the typical naphthenic acids—was 1,2,3-trimethylcyclopentane carboxylic acid, reported by Noyes and Burke (7). Its constants as reported yield a product of only 1.313, which would list it with the typical aliphatic acids. The abnormally low reported density of 0.9008 led the authors to repeat their synthesis. (Correspondence with Professors Noyes and Burke shows that the original notes are not available, but they suspect that their density should have been 0.9908 instead of 0.9008.) The following constants were found on an acid finally purified by repeated treatments with potassium permanganate followed in each case by refractionation and determination of the constants which finally remained unchanged: boiling point, 745 mm., 244°; d_4^{20}, 0.9948; n_D^{20}, 1.4597; M_D, found 42.98; calculated 43.13; n·d 1.4573.

TABLE I. ACID CUTS

Fraction	Boiling Point, 35 Mm. ° C.	Approximate Volume Liters	n_D^{20} of Fraction	Acids %	n_D^{20} of Acids
1	100–110	3.8	1.4751	10.80	1.5279
2	110–120	9.5	1.4807	15.40	1.5275
3	120–130	45.4	1.4903	20.30	1.5258
4	130–140	34.1	1.4949	24.10	1.5230
5	140–150	26.5	1.5008	33.80	1.5159
6	150–160	34.1	1.5003	37.00	1.5095
7	160–170	26.5	1.4965	42.60	1.4935
8	170–180	11.35	1.4872	43.40	1.4795
9	180–190	7.6	1.4852	54.30	1.4765
10	190–200	1.9	1.4825	60.20	1.4753
Residue		15.2			

As in the case of all other simple schemes tried, the singly unsaturated acids are placed with the naphthenic acids. The authors at present recombine fractions on the basis of n·d, boiling point, and neutral equivalent.

Experimental

After a careful examination of various types of Texas acidic material available, one of the writers (Schutze) liberated the acids from 20 barrels of concentrated alkali wash from light burner oil as obtained at the Baytown Refinery of the Humble Oil and Refining Company. Three barrels of crude acids were obtained. This material was then distilled at the University of Texas Laboratory through a battery of twelve 2-liter Claisen flasks. The difficulty due to water in this material was best overcome by blowing air or natural gas through each charge as it was heated up until practically all of the moisture had been driven off. The acids were then distilled at water-pump vacuum to yield three cuts of 200 cc. each and a residue of about 1 liter from each charge of 1600 cc. This operation yielded 75.0 liters of material boiling at 110° to 135° C.; 75 liters boiling at 135° to 160°; and 75 liters boiling at 160° to 180°, all at 25 mm. The three cuts were then separately fractionated at 35 mm. from a 57-liter steel still with a 275 × 15 cm. unpacked column. Fractions were collected at 10° intervals to yield the cuts shown in Table I.

The high index of refraction of the first seven cuts indicates that they are predominantly phenolic in nature. In spite of this fact cut 3 with the largest volume was selected for detailed study because its boiling point range was the one desired.

Separation of Strong Organic Acids from Weak Acids, Phenols, and Hydrocarbons

Since large-size efficient fractionating apparatus was not available, it was decided to forego the advantage of "amplified distillation" or "carrier liquid" effect and to separate strong from weak acids before proceeding with separation by distillation.

TABLE II. SEPARATION BY SODIUM CARBONATE TREATMENT

Fraction	n_D^{20}	Volume Cc.
A1	1.4478	120
A2	1.4515	95
A3	1.4638	72
A4	1.4868	54
A5	1.5035	55
A6	1.5000	49

Steam-distillation of the sodium salts to remove material formed by hydrolysis and nonacidic compounds proved effective but required an excessive amount of steam and time, so only 7.5 liters (2 gallons) were treated in this manner.

The remainder of the acids were treated with successive 6-liter aliquots of 1 N potassium hydroxide. In this and in all subsequent fractional neutralization batch operations, prolonged and vigorous stirring with motor stirrer was employed to assure equilibrium neutralization. Each batch of potassium salts was then steam-distilled to remove hydrocarbons and other material carried down with the salt solution.

The strong organic acid fractions of the extracted acids were dissolved in an equal volume of petroleum ether and fractionally neutralized with 0.5 N sodium carbonate. Each alkali cut was acidified with sulfuric acid and saturated with sodium chloride to lower the solubility of any dissolved acids. The aqueous layers were extracted with several small batches of petroleum ether, since it has been found throughout this work that sodium salt solutions are much better solvents than water for phenols and hydrocarbons and further that hydrocarbons and phenols dissolve considerable amounts of sodium salts, so that double treatment as described above is always needed in concentrated solutions.

The degree of separation obtained with the sodium carbonate treatment just described is best shown by Table II.

While these 6 cuts represent only a small portion of the acidic material, the last two cuts obviously represent phenolic compounds which are being studied in a separate project, so no more fractions were obtained at this time.

TABLE III. CONSTANTS OF SELECTED CUTS

Cut	n_D^{20}	Boiling Point, 760 Mm. ° C.	Neutralization Equivalent	Density, 20/20	n·d
1	1.4100	161	99.80	0.9654	1.360
2	1.4358	167	131.5	0.9806	1.408
3	1.4444	173	143.3	0.9848	1.423
4	1.4520	176	153.3	0.9889	1.435
5	1.4490	178	147.0	0.9867	1.430
6	1.4395	181	133.2	0.9809	1.412
7	1.4338	182	126.0	0.9778	1.405
8	1.4309	184	119.0	0.9766	1.398
9	1.4293	185	118.6	0.9764	1.395
10	1.4311	188	118.3	0.9787	1.400
11	1.4419	191	131.8	0.9879	1.425
12	1.4478	194	138.6	....	...
13	1.4520	196	143.3	....	...
14	1.4561	199	148.0	....	...
15	1.4539	202	140.0	....	...
16	1.4515	203	134.0	1.0179	1.450
17	1.4539	207	129.9	1.0317	1.500
18	1.4635	213	139.2	1.0490	1.535
Residue					

Repetition of this procedure on the various nonphenolic fractions yielded a total of 365 cc. of strong organic acids from which, by similar operations, a 188-cc. fraction of the strongest acids was selected for more detailed study. This batch was next carefully fractionated into 10-cc. cuts. Some constants are presented in Table III.

During the fractionation it was observed that a solid separated in the condenser, especially from cuts 9 through 16. The solid obtained when cut 13 was cooled was recrystallized twice from petroleum ether; it now melted at 95.5° and boiled at 220° at 760 mm. Reprecipitation by adding hydrochloric acid to its solution in alkali, and resublimation did not change the compound. Since 3,6-dimethyl phthalic acid was suspected, it was treated with resorcinol and sulfuric acid and then neutralized to yield a dichroic solution, green by reflected and red by transmitted light. An analysis gave C, 57.12, and H, 4.58; calculated for $C_8H_8O_3$: C, 57.14, and H, 4.77 per cent. Since the compound was not changed by sublimation while the phthalic acid would yield the anhydride, dimethylmaleic acid anhydride with a reported melting point of 96° and boiling point of 223° appeared the most probable compound. This anhydride was synthesized according to Rach (*8*) and its melting and mixed melting point showed the acidic material to have been dimethylmaleic anhydride. This compound has since been isolated also from straight-run petroleum acids from Signal Hill, Calif., crudes (*4*).

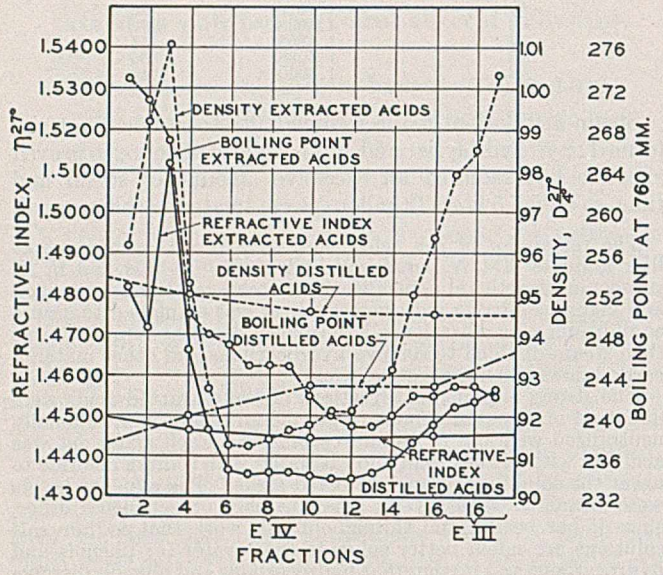

FIGURE 2

SEPARATION THROUGH SILVER SALTS. Cadmium, silver, and lead salts have been used in separating petroleum acids but the usual technique of fractional precipitation of the silver salts by adding silver nitrate with very thorough stirring to a solution of ammonium salts did not yield pure organic salts. Through experiments on known mixtures of isovaleric and *n*-caproic acids the following technique was developed and found very satisfactory.

The silver salts are fractionally precipitated in the usual manner and each cut is then dissolved in ammonium hydroxide. Normal nitric acid is added from a buret with a long tip extending to the bottom of the vessel, while the solution is stirred very vigorously by a motor stirrer until a fraction of silver salts of the desired size has been precipitated. After filtering off this fraction another is precipitated and this fractionation is continued until a slight excess of nitric acid has been added. The use of a long tip leading deep into the solution is made necessary by the fact that some of the silver salts tend to form a highly hydrophobic floating layer. As would be expected, only the least soluble salt can be obtained in a state of purity in one set of operations. This method will hereafter be termed the silver salt method.

When this technique was applied to 2 cc. of cut 5, Table III, the silver content (determined very easily by ignition of microsamples above and then in an electric crucible furnace) indicated that no pure salt resulted from one fractionation.

When the acids from the first fractions were liberated from the silver salts by mixing with an excess of phosphoric acid which had been dehydrated by heating to 180° at a pressure of 20 mm. and the organic acids then distilled off at water-pump vacuum with a capillary of sufficient capacity to help sweep the vapors out of the flask into the receiver, a main cut of dry colorless acids was readily obtained. This method of liberation will be referred to hereafter as the phosphoric acid method.

ISOLATION OF *n*-VALERIC ACID. Analysis of the *o*-phenylenediamine as well as the *p*-nitrobenzyl bromide derivatives showed that the heart cut of acid mentioned above was not pure enough to obtain a pure derivative, but a repetition of the silver salt method yielded a main fraction that gave an *o*-phenylenediamine derivative melting at 153°. Six recrystallizations failed to raise this melting point. A mixed melting point with a sample of derivative of known *n*-valeric acid also melted at 153°. Analyses for nitrogen: calculated for $C_{11}H_{14}N_2$, 16.08 per cent; found, 16.15 per cent. The analogous derivative of isovaleric acid melts at 189°. The presence of *n*-valeric acid is then definitely established.

ISOLATION OF PHENOL. When the final filtrate from the silver salt treatment was further acidified with hydrochloric acid and steam-distilled to recover the acids from soluble silver salts, the solution turned red and a yellow solid separated from the distillate. A melting and mixed melting point of 45° as well as analysis of the silver salt showed this to be *o*-nitrophenol. While the yield was less than 0.5 gram, the result is interesting because phenol has often been reported as missing in petroleum acids.

ISOLATION OF *n*-BUTYRIC ACID. Cut 1, Table III, was next fractionated by the silver salt method and this treatment repeated on the heart cut to yield 1.03 grams of acids recovered by the phosphoric acid technique. This fraction yielded an *o*-phenylenediamine derivative melting at 157.5°. The mixed melting point with a sample of derivative of known *n*-butyric acid melted at 158° while the known derivative melted at 158.5°. Analyses for nitrogen: calculated for $C_{10}H_{12}N_2$, 17.50 per cent; found, 17.50 per cent. The corresponding derivative of isobutyric acid melts at 234–235°.

Since the lower fractions so far studied seemed to be free of naphthenic acids, it was decided to examine fraction 6, Table I. Separation into strong and weak acids by fractional neutralization with potassium hydroxide soon led to very stable emulsions which were finally broken by saturation with carbon dioxide gas. This decreased the yield per cut but eliminated the long settling periods. Finally 3200 cc. of acidic material with *n*·d values less than 1.48 were selected for further study and were distilled through a 150-cm. (5-foot) marble-filled column with 10 to 1 or higher reflux ratio at 5-mm. pressure. This procedure was repeated four times with combination of similar cuts as indicated by density and refractive index.

Out of 16 cuts of 200 cc. each finally obtained, fractions 5, 6, and 7 were selected for detailed study. The constants of these and neighboring cuts are shown in Table IV.

TABLE IV. CONSTANTS OF FRACTIONS

Fraction	Boiling Point 5 Mm. °C.	Boiling Point 760 Mm. °C.	n_D^{27}	d_4^{27}	*n*·d	Neutralization Equivalent
4	94–95	236	1.4507	0.9555	1.390	176
5	95–98	240	1.4466	0.9499	1.373	164
6	98	243	1.4445	0.9455	1.365	159
7	98–103	245	1.4452	0.9450	1.365	159
8	103–105	248	1.4461	0.9450	1.367	161

The results obtained on cuts 5, 6, and 7 with a combination of fractional distillation and the rotary extraction column have already been reported. Figure 5 of the previous paper (*9*) is reproduced as Figure 2 to facilitate discussion of further results obtained on this material. *n*·d products for the 19 cuts indicate that Fraction E IV 3 is probably largely phenolic, that 4 and 5 represent mixtures, that 6 to 11 appear to be almost purely aliphatic, and 12 to 14 mixtures of types, while cuts E IV 15 and 16 and E III 15 to 17 seem to be naphthenic mixed with a new type of acid with abnormally high *n*·d values combined with high K_a value or solubility.

To check these predictions an exploratory study of this series of cuts was undertaken.

Study of Extraction Fractions E IV 1 to 16 and E III 15 to 17

FRACTIONS E IV 1 TO 4. Since the extraction should have concentrated the very weak acids in the first few cuts and since the density and index of refraction were high, it was expected that the first four cuts would be high in phenols. The total volume was only 16 cc. Qualitative tests for sulfur were positive for only 1 and 2. Three cubic centimeters of methyl esters were

obtained when the combined fractions 1 to 4 were refluxed with an excess of methanol–hydrochloric acid mixture. The unesterifiable portion was too weakly acidic to permit silver salt fractionation of possible tertiary acids present in this essentially phenolic mixture; neither could crystalline picrates of phenols be isolated.

Fraction E IV 5. Esterification, followed by fractionation of the unesterified 25 per cent of material through a 90 cm. (3-foot) × 10 mm. column with rotating steel band as filling (*6*) showed that this portion still contained large amounts of phenolic material. The esters were not studied further, since they were similar to those obtained from subsequent cuts.

Fractions E IV 6 to 8. The combined fractions were esterified and the esters fractionated through a 150 × 1.25 cm. (5 foot × 0.5 inch) Widmer column with rotating steel band as filling to yield six 14-cc. fractions and a 20-cc. residue. Cuts 4, 5, and 6 of this series had d_4^{33} of 0.8682, 0.8683, 0.8683, and n_D^{33} of 1.4179, 1.4180, and 1.4181; but in spite of this apparent purity neither *o*-phenylenediamine derivatives nor amides of acids from cut 5 assumed a constant melting point after six or more recrystallizations. The material must still be a mixture. The constants indicated that aliphatic acids predominate here, but no further attempt was made to isolate individuals.

Table V. Constants of Fraction

	Fraction 1	Caprylic Acid (*5*)
M. p.	15–16	16
B. p.	237.4	237.5
d_4^{20}	0.9116	0.911
n_D^{20}	1.4286	1.4272
$n{\cdot}d$	1.304	1.300
H, %	10.98	11.19 calcd.
C, %	66.68	66.55

Fractions E IV 10 to 12. Fraction E IV 9 as a transition cut was not studied, but 10 to 12 were combined, esterified, purified, and fractionated by the 90-cm. (3-foot) rotary column. Only 7 per cent (8 cc.) by volume of acids did not esterify. Five of seven 15-cc. fractions of esters were similar and were combined and saponified, and the acids liberated and fractionated. The acids of the first seven of thirteen 5-cc. cuts resulting were now fractionally precipitated by the silver salt method to yield thirteen crops of silver salts. This process was repeated starting with the first four cuts, adding ammonium hydroxide and aliquots of dilute nitric acid to get two cuts, then adding the next two cuts and repeating the fractional precipitation until finally all acids were again in the form of thirteen cuts. The acids of the dried salts were then liberated in groups of three and four cuts by the phosphoric acid method in vacuum. The first three fractions of acids now crystallized readily on cooling and consisted of almost pure caprylic acid, as shown by the constants of Table V.

The *p*-phenylphenacyl esters of fraction 1 and of a known caprylic acid were prepared according to Drake and Bronitsky (*2*) and gave the results shown in Table VI.

Table VI. Constants

	Fraction 1	Known Caprylic Acid	Mixed M. P.
Acid	15–16	14.6–15.8	14.8–16.0
p-Phenylphenacyl ester	66.6–67.3	66.7–67.4	66.7–67.4

Further silver salt fractionation of the remaining cuts of the series yielded only caprylic acid with a few cubic centimeters of a mixture of other acidic material. About 15 cc. of a mixture from which caprylic acid would not separate on cooling were now mixed with 100 cc. of a carefully purified mixture of neutral hydrocarbons and subjected to fractionation by Bailey's "amplified distillation" method (*1*). The acids extracted from the first four of eight cuts crystallized readily on cooling and proved to be pure caprylic acid and only the last cut of 8 cc. indicated by its constants that it was not practically purely aliphatic in nature.

Fractions E IV 13 to 16. Esterification of the combined cuts E IV 13 to 16 yielded the usual small amount of unesterifiable material and a good yield of methyl esters. Three systematic fractionations through the rotary Widmer column resulted now in two fairly clear cut series of cuts with n_D^{33} of 1.4179–1.4190 and 1.4285–1.4372, and d_4^{33} of 0.8788–0.8956 and 0.8983–0.9091. The first series is obviously aliphatic; the second contains acids with higher constants but not in high enough amount to warrant attempts to isolate individuals. Since cuts 15 and 16 should contain larger amounts of these acids, they were studied next.

Fractions E IV 15 and 16. Treatment of Fractions E IV 15 and 16 in exactly the same manner as 13 and 14 yielded a series of cuts with n_D^{29}, 1.4332–1.4430, and d_4^{29}, 0.9170–0.9284, which places all of these in the range of mixtures rich in esters with constants too high for aliphatic esters, but not yet purely naphthenic. If larger amounts had been available, further fractionation by extraction would probably have yielded acids rich enough in naphthenics to permit isolation of individuals.

Fractions III 15 to 17. Fractions III 15 to 17 were combined and fractionally esterified by adding 0.83 equivalent of methanol containing 2 per cent dry hydrogen chloride and refluxing overnight. The cold mixture was extracted twice with 6 per cent sodium hydroxide and finally with water. The acids were liberated, dried, and distilled while the esters were saponified, and fractionally esterified as before. This series of operations was repeated until four cuts of acids were obtained with properties shown in Table VII.

Table VII. Properties of Acid Cuts

Cut	Volume	n_D^{25}	$d_4^{27.5}$	$n{\cdot}d$	Neutralization Equivalent	Boiling Point, 760 Mm.
	Cc.					° *C.*
1	15	1.4625	1.0051	1.470	146.9	240
2	12	1.4583	0.9956	1.452	146.6	240
3	22	1.4538	0.9878	1.435	143.9	240.5
4	26	1.4523	0.9830	1.427	144.8	241.5

The $n{\cdot}d$ values indicate that all these cuts are well in the naphthenic acid range, although the density for the first cut at least is too high for ordinary naphthenic acids of this boiling range.

Cuts 3 and 4 were combined and fractionated through the small rotary column to yield eleven cuts of 4 cc. each with n_D^{33}, 1.4505–1.4578; d_4^{27}, 0.9788–0.9944; and $n{\cdot}d$, 1.420–1.450. The first six cuts were combined and fractionally precipitated by the silver salt method. The acids liberated from the silver salts had constants agreeing very closely; so the first four cuts were combined and converted to the acyl chlorides by thionyl chloride. The chlorides were then converted to the amides by pouring them into concentrated ammonium hydroxide. The amides were recrystallized from water and then from a petroleum ether–alcohol mixture. During this process a remarkable rise in melting point from 147° to 220° occurred during seven systematic recrystallizations. The only known 8-carbon atom amide melting as high as 220° appears to be that of solid *p*-hexahydrotoluic acid melting at 220–221°. Since only a few milligrams of the high-melting amide had been obtained, all of the cuts of the 3–4 series were recombined and converted to amides, which were then systematically recrystallized to obtain larger amounts of the 220–221° amide. In this process another amide melting at 155–157° (probably a meta isomer) and another melting at 151–152°, possibly a "cis" ortho isomer, appeared. While only 50 mg. of the 220° isomer were obtained, about 1 gram of each of the lower ones has been isolated but not yet in entirely pure form.

Attempts to prepare *p*-hexahydrotoluic acid by hydrogenation of *p*-toluic acid over Raney nickel at 275° to 350° C. and up to 5000 pounds per square inch failed; so the acid was prepared from 1,4-dimethylcyclohexanol by converting it to the chloride and this by the Grignard reaction to the acid. The yield from alcohol to chloride was 60 per cent and from chloride to acid was 90 per cent. The resulting acidic material was converted to the acid chloride and to the amide as before. Six recrystallizations of the amide yielded 1 gram of the pure amide melting at 219–220.8°. The isolated pure amide melted at 220–221° and the mixed melting point was 220–221°. Skraup and Binder (*10*) report 220–221°. Analysis for nitrogen: calculated for $C_8H_{15}ON$, 9.92 per cent; found, 10.03 per cent.

As a further check of identity a few milligrams of each of the amides were hydrolyzed in a sealed tube with 25 per cent sodium hydroxide, and the liberated acids recrystallized twice from water. The isolated, synthetic, and mixed acids all melted at 108–109°, whereas Gutt (*3*) reports 110–111° on a pure acid.

Finally a micro-Dumas determination was run on one of the amide fractions melting at 155–157° to determine whether the mixture had the same composition as the isolated isomer. Nitrogen determined was 10.10 per cent, while that calculated for amides of toluic acids is 9.92, so that the mixture is apparently one of the different isomers of toluic acid.

The naphthenic acids with unusually high $n{\cdot}d$ values turn out to be hexahydrotoluic acids, perhaps mixed with other cyclohexyl acids. While formerly naphthenic acids were thought to consist of cyclohexyl acids, the tendency in the last decade has been to assume that naphthenic acids are all cyclopentyl acids, since only very little indication of cyclohexyl acids had been obtained and none had been isolated. The amount of the pure solid isomer of *p*-hexahydrotoluic acid isolated herein is very small, but other isomers

appear to be present in larger amounts in this cut which boils below the boiling point of the pure acids, so that more may be present in somewhat higher boiling fractions. It should, of course, be remembered that the acids isolated were obtained from a mixture of straight-run and cracking process products, so that the cyclohexyl acids may have been formed during the cracking process.

Acknowledgment

The authors wish to thank the Humble Oil and Refining Company for donating the acids used and permitting them to isolate the crude acids at the refinery with their equipment. Thanks are also due Douglas Henson for cooperating in the early stages of this work.

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FROM theses submitted by Henry G. Schutze and Billie Shive in partial fulfillment of requirements for doctor of philosophy and master of arts degrees, respectively.

Determination of Sulfate by Tetrahydroxyquinone Method

Effect of Sodium Sulfite and Procedure for Its Elimination

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Sulfite, frequently used as a chemical deaerant in boiler water treatment, has some effect on the tetrahydroxyquinone method of sulfate determination, and a simple method has been established for its elimination. A rough correlation was found to exist between sulfite present and sulfite titrated. Restrictions and suggestions for the tetrahydroxyquinone method are presented.

IN PREVIOUS papers (*1–7*) describing the tetrahydroxyquinone method for sulfate and its modifications, the effects of certain ions have been discussed.

Since the appearance of the first paper, considerable research has been continually carried out to aid in the execution of this method.

The method has been found to be unaffected by silicate, tannin, magnesium, chloride, and calcium in concentrations occurring in natural and boiler waters (*3, 4*). Phosphate interfered if the titration was carried out at pH 8.3. An alternate method was devised for eliminating the effect of phosphate by adjusting the system to pH 4.0 before titration; by this method the tolerance of phosphate was raised above the normal excess soluble phosphate found in boiler waters (*4*). Aluminum, zinc, lead, copper, nickel, ferric and ferrous iron, etc., form insoluble compounds at pH 8.3 and should be filtered off before titration. The residual concentrations of these metals after filtration gave only slight interference, since their solubilities are very low (*3–6*).

Sodium sulfite, frequently used in boiler water treatment to remove oxygen, has been found to offer some interference and it is the purpose of this paper to define the extent of the interference and the simple method devised for its elimination.

Experimental

MATERIALS AND REAGENTS. Standard barium chloride solution: 1 cc. = 1 mg. of sulfate. Tetrahydroxyquinone indicator, manufactured by W. H. & L. D. Betz, Philadelphia, Penna., composed of disodium tetrahydroxyquinone dispersed in an organic medium. Standard sodium sulfate solutions, composed of 66 and 165 p. p. m. as sulfate. Ethyl alcohol, denatured ethyl alcohol No. 30 or 3A, or isopropyl alcohol. Anhydrous sodium sulfite, C. P., Baker's.

In determining the effect of residual sulfite, a 25-cc. sample of known sulfate concentration was treated with a weighed amount of solid sodium sulfite and adjusted to pH 8.3. Twenty-five cubic centimeters of the alcohol were added and the system was titrated with standard barium chloride solution until the indicator changed from yellow to red. Because of the difficulty in maintaining sulfite in solution, solid sodium sulfite was added to the test system in preference to a solution of sodium sulfite. In each case the test system was treated with 5000 p. p. m. of mannitol to help preserve the sulfite until the titration was complete.

TABLE I. EFFECT OF SULFITE ON SULFATE TITRATION BY TETRAHYDROXYQUINONE METHOD

Sulfate Present	Sulfate Found	Difference	Sulfite[a] Introduced	Sulfite Titrated
P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m. SO_4*	%
66	64	−2	...	...
165	168	+3	...	...
165	164	−1	...	...
66	80	+14	18	77.7
66	110	+44	55	80.0
66	140	+74	92	80.4
66	204	+138	168	82.1
165	182	+17	19	89.5
165	200	+35	39	89.7
165	232	+67	78	85.9
165	296	+131	157	83.4

[a] Error in weighing and purity of sodium sulfite did not exceed 2 p. p. m. as SO_4.

The results of these experiments, presented in Table I, show that residual sulfite offers some interference with the tetrahydroxyquinone method. Approximately 80 to 90 per cent of the sulfite introduced into the system was titrated. The effect of the sulfite is also brought out in Figure 1, which shows it to be linear throughout the range investigated.

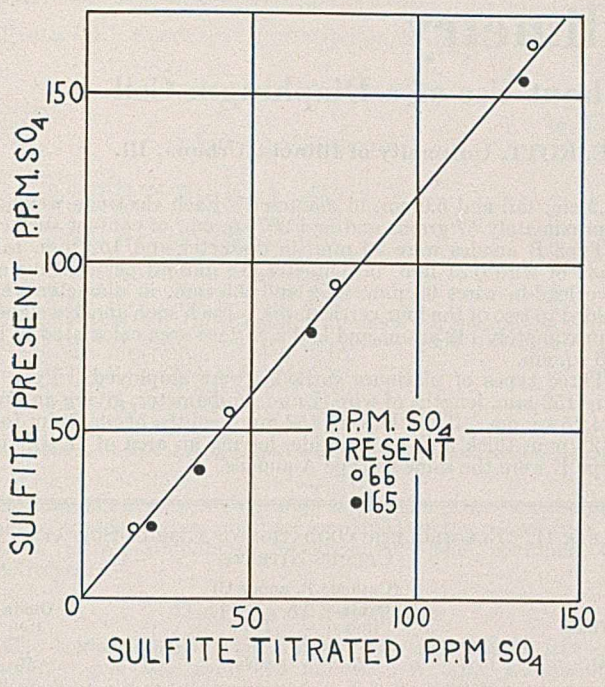

FIGURE 1

This curve can be used to give a rough correction to the sulfate analysis, if the sulfite concentration is known, by subtracting the sulfate equivalent to the sulfite from the total sulfate concentration as determined by the tetrahydroxyquinone method. However, more accurate and rapid results can be obtained by using the procedure for sulfite elimination described below.

No correlation was found to exist between the sulfite concentration remaining after the tetrahydroxyquinone titration and (1) the sulfite titrated or (2) the sulfite untitrated by the tetrahydroxyquinone method. The residual sulfite after the completion of the titration was analyzed by iodometry. These analyses exhibited wide variance, making results inconclusive. This work was carried out in order to throw some light on the mechanism by which the sulfite interfered with the sulfate titration. It is believed that two mechanisms occur simultaneously: (1) precipitation of barium sulfite, and (2) the oxidation of sulfite to sulfate, followed by precipitation as barium sulfate. Both mechanisms proceed in the direction to give high results, which is in accord with the experimental data presented.

TABLE II. RESULTS OF SULFITE ELIMINATION METHOD

Before Acid Treatment		After Acid Treatment	
Sulfate present	Sulfite present	Sulfate found	Difference
P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*
165	149	172	+7
165	37	168	+3
165	92	170	+5
165	244	180	+15

The following procedure was found satisfactory for eliminating the effect of the residual sulfite.

Procedure for Sulfite Elimination

A 25-cc. (or 25-ml.) sample is treated with 1.0 cc. of approximately 0.5 *N* hydrochloric acid, boiled 2 minutes, cooled, neutralized by sodium hydroxide just to the acid side of phenolphthalein, treated with 25 cc. (or ml.) of ethyl or isopropyl alcohol and 1 dipper of tetrahydroxyquinone indicator, and titrated in the usual manner. The hydrochloric acid treatment liberates the sulfur dioxide, which escapes during boiling. The evaporation is so small during this short time of boiling that no volume adjustment is necessary.

The experimental results are presented in Table II and show that the method was successful in eliminating even very high amounts of sulfite. The first and last experiments gave high sulfate results, owing to the small conversion of sulfite to sulfate before the acid completely liberated the sulfur dioxide. Mannitol was used to keep the sulfite from reverting to sulfate, but, although very helpful, is not able totally to preserve the sulfite. Where residual sulfite was present in concentrations which are normally carried in boiler waters—namely, 30 to 50 p. p. m.—there was less chance for the sulfite to revert to sulfate before elimination, and normal results were obtained.

Suggestions

TITRATION ASSEMBLY. A small table with a white surface should be used and the titration flask illuminated by a 50-watt bulb from either side or behind.

SHARPENING OF END POINT. The end point is sharpened by adding small amounts of silver nitrate: 1 cc. of 0.1 *N* (approximate) solution when chlorides are low in concentration, or 2 to 3 cc. (or ml.) when chlorides are high in concentration. The chlorides present must always have greater equivalent concentration than the silver nitrate added; otherwise a silver salt of tetrahydroxyquinone is formed which has an intense cherry color. When this develops, the titration must be repeated with less silver nitrate.

TITRATION LIMITS OF BARIUM CHLORIDE. The most distinct end point is obtained when the volume of barium chloride is restricted to a maximum of 10 cc. Titrations requiring more barium chloride should be carried out with a smaller sample diluted to 25 cc. with distilled water. A strength of barium chloride between 1 and 4 mg. of sulfate per cc. gives the best end points.

NEUTRALIZATION OF SAMPLE. In order to maintain as nearly as possible the 1 to 1 alcohol-water ratio, it is suggested that extremely alkaline boiler waters be roughly neutralized with 1 *N* hydrochloric acid, followed by 0.02 *N* hydrochloric acid or sodium hydroxide to the desired pH.

ELIMINATION OF EFFECT OF PHOSPHATE. Phosphate tolerance can be raised to 150 p. p. m. as phosphate by diluting a 10-cc. sample to 25 cc., followed by neutralization to the yellow range of bromocresol green indicator.

SULFATE RANGE. The most satisfactory results with this method are obtained with a 25-cc. sample containing 0.2 to 25 mg. of sulfate (8 to 1000 p. p. m.). The sample should be diluted or concentrated to this range. Higher sulfate content can be determined, but the end point of the titration is not so sharp because of increase of barium sulfate by precipitation in the system.

Conclusions

The presence of residual sulfite in feed and boiler waters interferes with the tetrahydroxyquinone method for the determination of sulfate. Approximately 80 to 90 per cent of the sulfite present titrates, giving high sulfate results. Sulfite may be simply and quickly eliminated by the method here presented.

Acknowledgment

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Cerate Oxidimetry

Electrolytic Oxidation of Cerium without Use of a Diaphragm Cell

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PREVIOUS contributions on cerate oxidimetry (*2*, *3*) have pointed out means through which the applications of tetravalent cerium in volumetric analysis can be greatly extended. It has been shown that the formal oxidation potentials of the ceric-cerous systems in hydrochloric, sulfuric, nitric, and perchloric acid media increase pronouncedly over the range 1.28, 1.44, 1.61, and 1.70 volts, respectively. In order to take advantage of the increased oxidation potential in the latter two cases, a simple procedure is desired for making available the solutions of tetravalent cerium required. It is the purpose of the present work to describe such a method.

Theoretical Considerations

The electrolytic oxidation of trivalent to tetravalent cerium has been described by Hengstenberger (*1*). Following this procedure, a diaphragm cell using a platinum anode and cathode was employed. The use of a diaphragm cell is a prerequisite if tetravalent cerium is assumed to exist in solution as a cation. However, with the knowledge that tetravalent cerium exists in solution primarily as an anion (*2*), a number of new conditions might be logically expected to prevail during electrolytic oxidation:

1. That a diaphragm cell would not be required. This would naturally result from the fact that the cathode would be expected to repel anions. (Hydrochloric acid solutions of cerous chloride are excluded.)
2. That the current efficiency and degree of completion of the oxidation would depend upon the extent to which a stable complex cerate ion is formed. The higher the complexity of tetravalent cerium as the $Ce(SO_4)_3^{--}$, $Ce(NO_3)_6^{--}$, and $Ce(ClO_4)_6^{--}$ anions, the higher the current efficiency and the completion of the oxidation would be.
3. That the ratio in surface area of the anode with respect to the cathode should be large to favor increased efficiency and completion of oxidation.
4. That side reactions might be predicted at the cathode in the oxidation of cerous nitrate to the nitrato cerate anion. This effect might be expected because of the possibility of reduction of the nitrate ion to the ammonium ion.
5. That the influence of the acid concentration on the completeness of oxidation should be small except in the oxidation of cerous perchlorate. This would be predicted from the findings of Smith and Getz (*2*).

In the following work these assumptions are shown to be justified by experiment.

Apparatus Employed

The apparatus employed is not described or illustrated because an electrolytic cell without diaphragm is simple in construction. The electrolyte in all cases was 2 to 3 liters in volume in 3- or 4-liter beakers.

Electrodes

Two types of anodes were employed, plain surface sheet platinum (Type A) and cylindrical platinum gauze (Type B). The former were 10.2 cm. square, the lower half of foil 0.127 mm. thick, and the upper half of sheet 0.25 mm. thick. Welded to the top of each such anode was a lead-in band 12.7 mm. wide, 50 mm. long, and 0.75 mm. thick. Two electrodes were bent to form half cylinders and mounted side by side to form a complete cylinder 10.2 cm. tall and 6.0 cm. in diameter. Each electrode weighed approximately 57 grams and had 207 sq. cm. of contact surface.

Type B anodes were 76 mm. in diameter and 152 mm. tall, made of wire 0.21 mm. in diameter, 18 meshes per centimeter. Two lead-in wires 90 mm. long and 2.5 mm. in diameter were welded to two of the four vertical ribs. Each such anode weighed approximately 142 grams and had a surface area calculated to be 935 sq. cm.

Three types of platinum cathodes were employed. Type C were 152-mm. lengths of wire, 1 mm. in diameter, giving an area of 4.75 sq. cm. Type D were 152-mm. lengths of platinum foil, 0.127 mm. thick and 8 mm. wide, having an area of 12 sq. cm. Type E were the same as type A anodes.

TABLE I. PLATINUM ELECTRODE SPECIFICATIONS

Electrode designation	Anode A	Anode B	Cathode C	Cathode D	Cathode E
Electrode type	Sheet	Gauze	Wire	Foil	Sheet
Surface, sq. cm.	207	935	4.75	12	207

TABLE II. ELECTROLYTIC OXIDATION OF CEROUS SULFATE AND CEROUS NITRATE

(Cathode B, anode C)

Time	Coulombs × 10^{-3}	Oxidation	Time	Coulombs × 10^{-3}	Oxidation
Min.		%	*Min.*		%
0.2525 mole of $Ce_2(SO_4)_3.8H_2O$ suspended in 2000 ml. of 1.5 molar H_2SO_4, at 3.5 to 3.2 volts and 10 amperes					
15	9	9.7	135	81	84.9
30	18	20.7	150	90	92.9
45	27	29.3	165	99	97.0
60	36	38.5	180	108	98.6
75	45	48.9	200	120	99.3
90	54	58.5	215	129	99.5
106	63.6	68.3	230	138	99.9
120	72	76.4	250	150	100.2
0.5116 mole of $Ce(NO_3)_3$ in 2000 ml. of 2.0 molar HNO_3, at 3.5 to 3.2 volts and 9 amperes					
15	8.1	7.3	150	81.0	72.1
30	16.2	12.8	165	89.1	75.6
45	24.3	21.2	180	97.2	86.5
60	32.4	29.0	195	105.3	93.4
75	40.5	35.1	210	113.4	97.8
90	48.6	42.7	225	121.5	98.8
105	56.7	50.0	240	129.6	99.2
120	64.8	57.0	270	137.7	99.5
135	72.9	64.7	287	155	99.7

Preparation of Solutions

Ceric oxide free from thorium and containing approximately 40 per cent CeO_2 was converted to hexanitrato ammonium cerate, $(NH_4)_2Ce(NO_3)_6$, by the process described by Smith, Sullivan, and Frank (*4*) except that the final crystallization was omitted. This resulted in the preparation of a starting material of 98 to 99.5 per cent purity, the remainder consisting of rare earths of the cerium group except thorium. A gram molecule of the complex nitrate was dissolved in dilute nitric acid and reduced, using a slight excess of 100-volume hydrogen peroxide. Hydrochloric acid was then added, as well as nitric acid, followed by gentle boiling until ammonium salts were decomposed. Finally the mixture was evaporated with excess nitric acid to give cerous nitrate containing but small amounts of excess nitric acid. For the oxidation of cerous nitrate, $Ce(NO_3)_3$, to nitrato ceric acid, $H_2Ce(NO_3)_6$, a definite amount of nitric acid was added with dilution to the proper volume.

Cerous perchlorate was prepared from known amounts of cerous nitrate, obtained as described above. After the addition of known amounts of 72 per cent perchloric acid and evaporation to strong fumes to remove nitric acid, the resulting product was ready for dilution to volume and electrolytic oxidation.

Cerous sulfate octahydrate, $Ce_2(SO_4)_3.8H_2O$, was prepared from cerous nitrate obtained as described above. An excess of sulfuric acid was added to a hot solution of cerous nitrate in water and the resulting product was filtered, using a fritted-glass filtering funnel. The product thus obtained was washed with hot water and dried at 110° C. Weighed quantities of this product were suspended in sulfuric acid of known strength and were oxidized electrolytically.

Cerous sulfate solutions were also made by dissolving anhydrous ceric sulfate in a known amount of sulfuric acid which had been diluted to approximately the desired volume, and adding 100-volume hydrogen peroxide to reduce the cerium, followed by dilution to the proper volume for electrolytic oxidation.

TABLE III. ELECTROLYTIC OXIDATION OF CEROUS SULFATE, NITRATE, AND PERCHLORATE IN SULFURIC, NITRIC, AND PERCHLORIC ACID SOLUTION

(Cell terminal voltage 3.5 to 3.2 volts)

Oxidation No.	Composition of Electrolyte	Type of Electrode		Current Density		Final Oxidation, % Complete	Final Norm. Ce^{++++}	Required for 80% Oxidation	
		Anode	Cathode	Anode	Cathode			Theoretical	Found
				Amps./sq. inch				*Coulombs*	
1	0.0457 *M* $Ce_2(SO_4)_3$ in 1 *M* H_2SO_4	A	E	0.312	0.312	60.6	0.0593	...	...
2	0.0508 *M* $Ce_2(SO_4)_3$ in 2 *M* H_2SO_4	A	E	0.312	0.312	68.5	0.0696	...	...
3	0.0522 *M* $Ce_2(SO_4)_3$ in 3 *M* H_2SO_4	A	E	0.312	0.312	59.8	0.0623	...	...
4	0.0484 *M* $Ce_2(SO_4)_3$ in 2 *M* H_2SO_4	B	D	0.069	2.66	99.4	0.0963	217,500	242,500
5	0.306 *M* $Ce_2(SO_4)_3$ in 0.882 *M* H_2SO_4	B	D	0.069	2.66	97.4	0.5970	156,000	163,000
6	0.2526 *M* $Ce_2(SO_4)_3$ in 1.5 *M* H_2SO_4	B	C	0.069	6.67	100.0	0.5051	76,000	76,500
7	0.1216 *M* $Ce(ClO_4)_3$ in 1.0 *M* $HClO_4$	A	E	0.312	0.312	36.9	0.0448	...	...
8	0.361 *M* $Ce(ClO_4)_3$ in 1.0 *M* $HClO_4$	B	D	0.069	2.66	89.8	0.3241	50,000	70,000
9	0.1238 *M* $Ce(ClO_4)_3$ in 1.0 *M* $HClO_4$ and 5.0 *M* $NaClO_4$	A	E	0.312	0.312	91.4	0.1131	17,500	35,500
10	0.1238 *M* $Ce(ClO_4)_3$ in 0.5 *M* $HClO_4$ and 5.5 *M* $NaClO_4$	A	E	0.312	0.312	99.2	0.1228	...	...
11	0.406 *M* $Ce(ClO_4)_3$ in 1.0 *M* $HClO_4$	B	C	0.069	6.67	87.5	0.3555	53,500	95,000
12	0.522 *M* $Ce(ClO_4)_3$ in 6.0 *M* $HClO_4$	B	C	0.069	6.67	98.9	0.5087	78,500	81,000
13	0.1036 *M* $Ce(NO_3)_3$ in 2.0 *M* HNO_3	A	E	0.312	0.312	60.7	0.0629	...	...
14	0.510 *M* $Ce(NO_3)_3$ in 2.0 *M* HNO_3	B	C	0.069	6.67	99.7	0.5087	76,000	90,000
15	0.0475 *M* $Ce_2(SO_4)_3$ in 2.0 *M* H_2SO_4	A	E	0.312	0.312	70.8	0.0672	...	...

If ammonium salts are not objectionable, a procedure comparable to that last given may be employed in the preparation of cerous nitrate–ammonium nitrate–nitric acid solutions for electrolysis.

Analysis of Solutions

The solutions during electrolysis were sampled at frequent intervals, generally every 15 minutes. A 5-ml. sample was withdrawn with a pipet, transferred to a 250-ml. beaker, and diluted to 100 ml. with 1 to 10 sulfuric acid. These samples were titrated, using 0.1 *N* ferrous sulfate with ferroin as indicator. The total concentration of cerium present was determined following complete oxidation by ammonium persulfate, using the method of Willard and Young (*5*). The total volume thus withdrawn from the cell (50 to 100 ml.) was small in comparison to the total volume present (2000 to 3000 ml.). Half the volume of sampling solution was deducted from the total starting volume of solution oxidized in the calculation of the theoretical coulombs required.

Substantiation of Preliminary Assumptions

Of the five conditions governing the control of experimentally variable factors, the first—that a diaphragm cell would not be required—was easily demonstrated in the case of the oxidation of cerous sulfate, nitrate, and perchlorate in their corresponding acid solutions. A hydrochloric acid solution of cerous chloride, however, was not correspondingly oxidized at an acid concentration 1 molar or above. Referring to the work of Smith and Getz (*2*), it was thought that the second condition would be most strikingly substantiated by studying the oxidation under similar conditions of cerous sulfate, nitrate, and perchlorate in their corresponding 2 *M* acid solutions. This was accomplished as shown by the results given for oxidations 1, 7, and 9 of Table III. The third and fourth conditions were substantiated by the results of oxidations 13 and 14. In these cases gassing at the cathode or anode was slight or absent entirely. The finished solutions gave a conclusive test for the presence of the ammonium ion. In experiment 12 at the end of the oxidation a considerable evolution of ozone was evident at the anode. The fifth condition was substantiated in experiments 9, 10, and 12.

Tabulation of Experimental Results

The results of typical oxidations are given in Table II in the case of cerous sulfate and cerous nitrate. The other results are omitted in order to conserve space. The remaining data are given in Table III.

Substitutes for Platinum as Electrode Materials

Graphite may be substituted for platinum as electrode material. Lead dioxide electrodes may be used as anodes and lead may be used as cathodes, if removed when oxidation is complete to prevent their solution in nitric or perchloric acid solutions.

Results Attained

The most important conclusion of this investigation is that the former study of electrode potentials of the cerate-cerous system (*2*) was correctly interpreted in the establishment of a cerate-ion concept. A convenient laboratory method for preparation of the various complex cerates has been developed. A satisfactory procedure for preparation of hexaperchlorato ceric acid, $H_2Ce(ClO_4)_6$, has been provided for the first time, as well as a convenient procedure for the regeneration of spent solutions of cerous salts. The current efficiency is nearly theoretical under the most favorable conditions up to 80 per cent total oxidation. The logarithmic variables $[Ce(SO_4)_3^{--}]/[Ce^{+++}][SO_4^{--}]^3$, $[Ce(NO_3)_6^{--}]/[Ce^{+++}][NO_3^-]^6$, and $[Ce(ClO_4)_6^{--}]/[Ce^{+++}][ClO_4^-]^6$, proposed by Smith and Getz (*2*) for application to the Nernst expression for calculating electrode potentials when applied to these systems, have been shown to be valid, at least in a qualitative sense.

The conditions under which cerous sulfate, nitrate, and perchlorate may be oxidized electrolytically without the use of a diaphragm cell to form sulfato, nitrato, and perchlorato ceric acids, $H_2Ce(SO_4)_3$, $H_2Ce(NO_3)_6$, and $H_2Ce(ClO_4)_6$, have been described.

Anode materials may be either platinum, lead dioxide, or graphite, and cathodes may be of platinum or lead.

A study has been made of the effect of variations in anode and cathode current densities and the potential employed upon the current efficiency and the completion of the oxidation.

The current efficiency for complete oxidation of cerous sulfate in sulfuric acid solution was found to be 74.1 per cent; for 80 per cent oxidation, 99.35 per cent.

The current efficiency in the oxidation of nitric and perchloric acid solutions of cerous nitrate and perchlorate for 80 per cent oxidation was found to be 84.4 and 56.3 per cent, respectively, in 2.0 molar nitric and 1.0 molar perchloric acid solution. Cerous perchlorate in 6.0 molar perchloric acid can be oxidized to 80 per cent completion with a current efficiency of 96.9 per cent.

The cerate-ion concept previously proposed (*3*) has been further substantiated and all predictions based upon this concept which govern electrolytic oxidations of cerium have been experimentally realized in the present work.

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Silicomolybdate Method for Silica

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THE present research represents an effort to standardize a colorimetric procedure for the rapid determination of small quantities and the detection of minute changes in the concentration of dissolved silica in natural waters of very low phosphate content.

A preliminary study indicated that two general procedures were available. The method of Dienert and Wandenbulcke (*4*) takes advantage of the yellow silicomolybdate color produced when ammonium molybdate reacts in acid medium with dissolved silica. Isaacs' method (*7*) attempts to extend the sensitivity of this reaction by reducing the molybdenum in the silicomolybdate complex to the relatively intense molybdenum blue color.

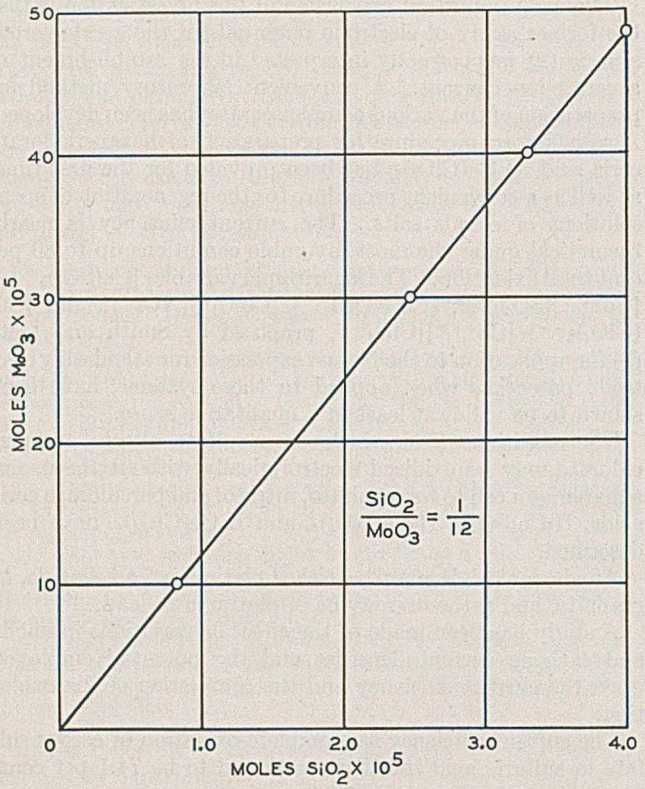

FIGURE 1

Phosphate interferes with both methods (*12*) but to a greater extent in the reduction method. Other serious objections to the reduction method are interference due to iron or other reducing agents and the instability of the blue color (*3*). Although modifications of Isaacs' procedure have been proposed to eliminate interference due to phosphates (*1*, *5*), the other objections remain.

The procedure of Dienert and Wandenbulcke was selected for this investigation because it was simple and rapid. The authors hoped to increase its sensitivity as well as its precision by a critical examination of the conditions surrounding the development of the color and by an improvement in the method of measuring the color.

Equipment

The following special equipment was used: a pH electrometer, a Duboscq colorimeter, matched Nessler tubes, a Cenco-Sanford-Sheard photelometer, an Evelyn photoelectric colorimeter, and a specially designed thermopile-type spectrophotometer employing a continuous light source (*8*).

Reaction

That the ratio of silica to molybdenum trioxide is 1 to 12 in the simple reaction between ammonium molybdate and sodium silicate has been established for some time. However, it was necessary to show that this relationship is constant over the entire range of concentrations studied. Known amounts of ammonium molybdate were added to solutions containing an excess of dissolved silica and after acidification and development of maximum color, measurement of the intensity of the color produced showed the amount of silica which reacted. Data for several concentrations are plotted in Figure 1. A straight line is obtained which extrapolates to the origin. The slope of the line is almost precisely $^1/_{12}$, indicating that the mole ratio is 1 SiO_2/12 MoO_3 for the concentrations studied.

Effect of Molybdate Concentration, Acidity, and Time

MOLYBDATE CONCENTRATION. It was recognized early in this investigation that although ammonium molybdate in molecular concentration twelve times that of the silica would suffice for the color reaction, a certain excess is desirable in order to reduce materially the time required for maximum color development.

Using the photelometer to detect intensity of color and allowing no more than 10 minutes for color development, the following study was made.

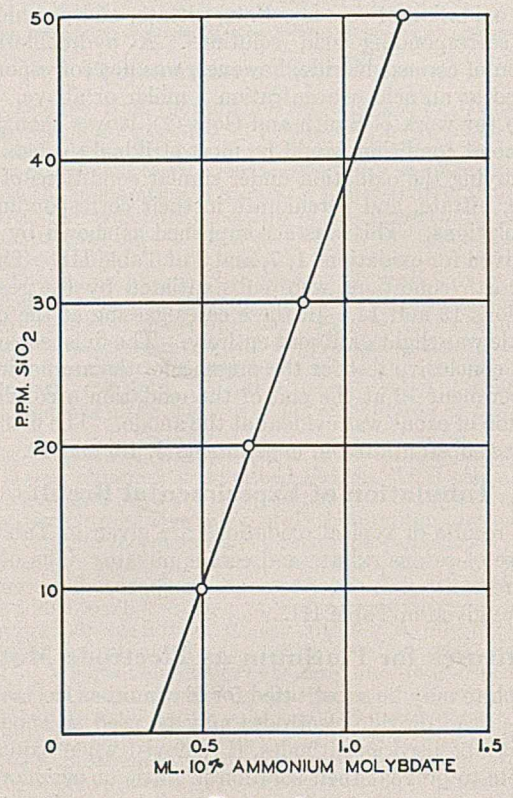

FIGURE 2

Six or more samples of the same concentration of dissolved silica were poured into sample tubes and a known amount of exactly 10 per cent ammonium molybdate solution was added to each. Ten minutes after acidification and dilution to 100 ml. the intensity of color of each sample was measured with the photelometer and the minimum concentration of molybdate necessary for full color development within the 10-minute period was recorded. This procedure was repeated for several different concentrations of dissolved silica.

The data are plotted in Figure 2, where the minimum molybdate concentration required for full color development within 10 minutes is plotted against the concentration of dissolved silica. It is apparent that the amount of molybdate required is a straight-line function of the silica concentration. By extrapolating the curve to 0 p. p. m. of silica, one can determine the excess of molybdate that is required for the maximum color development within 10 minutes.

This excess is found to be 0.32 ml. of 10 per cent ammonium molybdate solution in 100 ml. of sample, or 0.0036 mole per liter of molybdenum trioxide. A greater excess does not appreciably shorten the time required for full color development. A large excess is to be avoided because the color is less stable. In actual practice, 2 ml. of 10 per cent ammonium molybdate solution per 100 ml. of sample were found most satisfactory for concentrations of silica as high as 50 p. p. m.

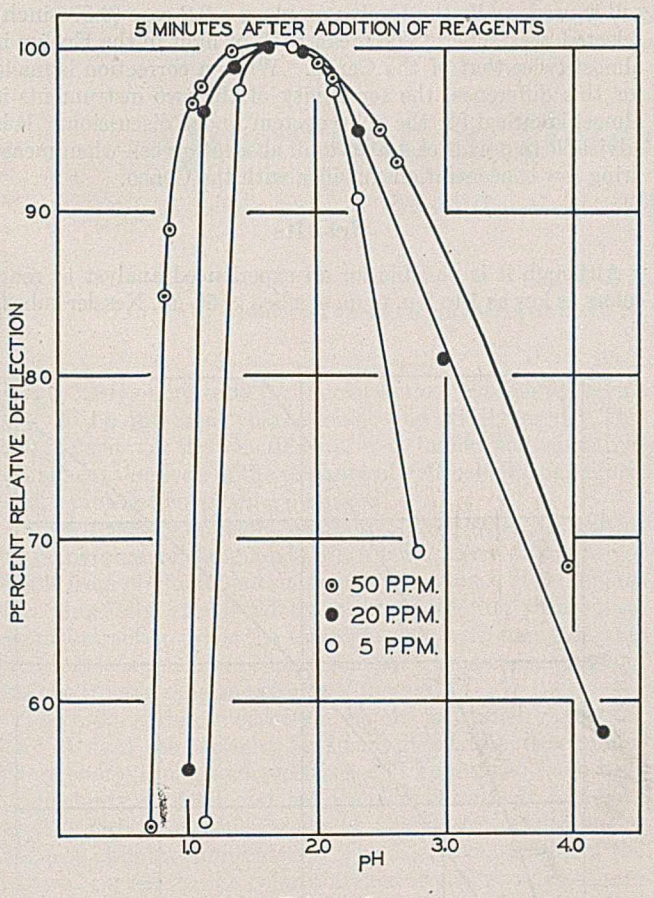

FIGURE 3

ACIDITY. Previous authors have reported that acidity has an important influence on color development (*6, 12, 15*), not only with respect to the intensity of the color but also the rate of color development and color decay.

Three concentrations of dissolved silica solutions were selected for this study: 50, 20, and 5 p. p. m. A series of 100-ml. sample tubes was filled with silica samples, all of the same concentration, and treated with 2 ml. of 10 per cent molybdate solution. Varying amounts of acid were added to give a series of solutions of decreasing pH. At 5 and 10 minutes after the acidification, the color development was noted on the photelometer. Such a series was repeated several times for each of the three concentrations of silica mentioned above.

The results are illustrated graphically in Figures 3 and 4, 5 and 10 minutes, respectively, being allowed for color development. In each figure the per cent relative deflection of the photelometer is plotted against pH, which is equivalent to plotting the per cent of theoretical color development against pH. Figures 3 and 4 indicate that the pH limit for maximum color development for small amounts of silica is 1.6 to 2.0.

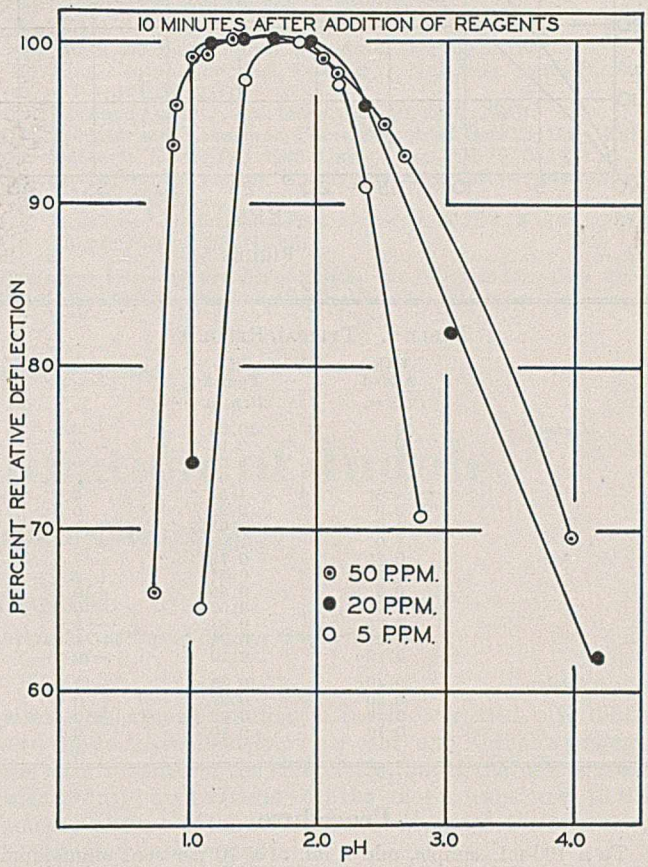

FIGURE 4

The effect of increased time for color development is to broaden the pH range for higher concentrations of silica, but for 5 p. p. m. of silica this effect is hardly noticed. It is evident that great precautions must be taken to ensure proper adjustment of pH for measurements of small amounts of silica. Whereas the adjustment is less critical for higher concentrations of silica, the color development falls off rapidly on either side of the optimum pH range and care should be taken to operate within the proper limits. Several buffer systems were investigated, but in general it was found more convenient and reliable to add a predetermined amount of free acid to the sample in order to produce the proper pH.

Instead of using several drops of concentrated acid as recommended in many procedures (*4, 9, 10, 11, 13, 15*), it is suggested that a larger volume of dilute acid be used. One milliliter of 4 *N* sulfuric acid gave the desired pH for samples of natural waters in this investigation.

TIME. Under the proposed procedure and within the limits of concentration used, it was found that the maximum color developed within 10 minutes and remained unchanged for at least 0.5 hour.

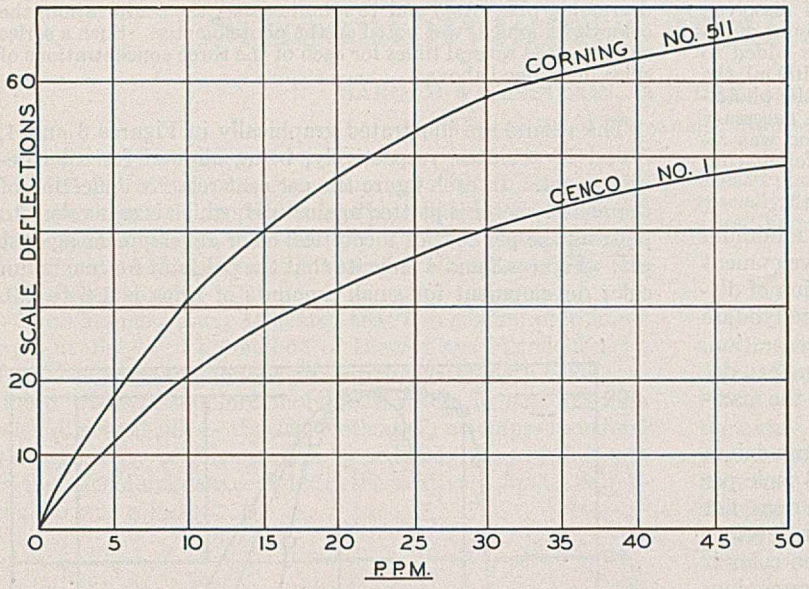

FIGURE 5

TABLE I. TYPICAL RESULTS

	SiO_2 Added P. p. m.	SiO_2 Found P. p. m.	Error %
Cenco	10	10.05	+ 0.5
	10	9.93	− 0.7
	8	8.00	0.0
	6	5.95	− 0.8
	4.5	4.5	0.0
	2.0	2.0	0.0
	1.0	1.03	+ 3.0
	1.0	1.01	+ 1.0
	0.75	0.72	− 4.0
	0.5	0.51	+ 2.0
	0.5	0.55	+10.0
	0.5	0.50	0.0
	0.25	0.23	− 8.0
	0.25	0.24	− 4.0
	0.12	0.10	−20.0
Evelyn	0.40	0.35	−12.0
	0.20	0.20	0.0
	0.20	0.22	+10.0
	0.10	0.10	0.0

Procedure

To a 100-ml. sample, add 2 ml. of a 10 per cent ammonium molybdate solution. Mix and immediately acidify to a pH of 1.6 to 2.0 (the authors used 1 ml. of 4 *N* sulfuric acid). The amount of acid needed should be predetermined from pH measurements of the original sample. (It is important that the acid be added immediately following the addition of ammonium molybdate. If there is more than a minute's delay, the time required for full color development is appreciably increased.) After 10 minutes, compare with standards or read in a photometer from which a standard calibration has been made.

Standards

Picric acid (*9–11*), potassium chromate (*2*), and buffered potassium chromate (*14*) have been suggested as permanent standards for the silicomolybdate procedure. Spectrometric studies made in this laboratory support the conclusions of Swank and Mellon. The authors strongly recommend the use of buffered chromate according to their procedure.

Photoelectric Measurements

Both the Cenco photelometer and the Evelyn photoelectric colorimeter were used in this study. The important feature was the selection of the proper filters. In general, it is not desirable to select a filter by rule of thumb. A spectrophotometric study of the color in question should be made and a filter selected which transmits a maximum amount of light in the spectral region where the colored solution is absorbing the maximum. Dilute solutions of silicomolybdate absorb strongly only at wave lengths shorter than 4500 Å. The Cenco No. 1 blue filter transmits light of considerably longer wave length and is not particularly satisfactory for dilute solutions, though it is satisfactory for high concentrations of silica. The Corning No. 511 blue filter has a maximum transmission at about 4100 Å. and accommodates the silicomolybdate color very well. Figure 5 shows the calibration for these two filters in the Cenco photelometer. The increased sensitivity of the Corning No. 511 ranges from 40 to 50 per cent when calculated from the slopes of the curves.

The Evelyn colorimeter is equipped with the Evelyn No. 420 blue filter, which has almost the same transmission characteristics as the Corning No. 511. Thus an interesting comparison was made between the two instruments. Figure 6 shows the calibration curves. Whereas the Evelyn appears more sensitive (more units deflection per p. p. m. of silica), the path lengths of light in the two instruments differ. In the Cenco a 10-mm. cell is used, while the Evelyn employs a 2.2-cm. (0.875-inch) selected test tube. The mean path of light in the Evelyn is almost twice that of the Cenco. When a correction is made for this difference, the sensitivity of the two instruments is almost identical for the color system under discussion. It is advisable to use at least a 20-mm. absorption cell when measuring low concentrations of silica with the Cenco.

Results

Although it is possible for an experienced analyst to read colors as low as 1 to 2 p. p. m. of silica in 50-ml. Nessler tubes,

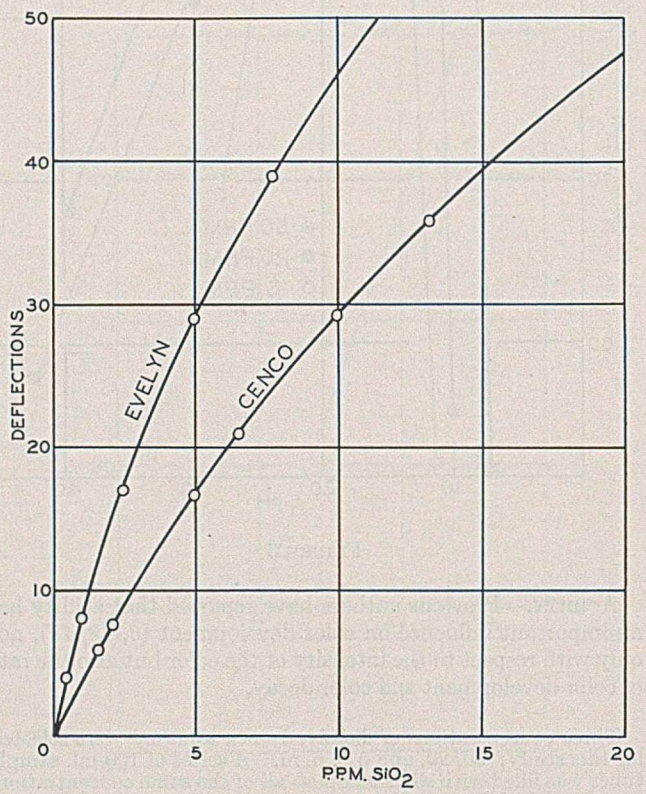

FIGURE 6

the error is comparatively high. Table I shows typical results using the Cenco and Evelyn photoelectric colorimeters. In concentrations as low as 1 p. p. m. using the Cenco instrument the per cent error is of the order of 1 per cent. In concentrations less than 1 p. p. m., the error increases, ranging to 10 per cent for concentrations as low as 0.25 p. p. m. Using a thicker cell such as the Evelyn provides, or a special 20-mm. cell for the Cenco, it is possible to make readings as low as 0.10 p. p. m. with an error of about 10 per cent.

Conclusion

Many natural waters in northern Wisconsin show very low silica concentrations. It is important not only to measure these low concentrations but also to follow small variations in silica concentration from time to time.

A more careful control of the Dienert and Wandenbulcke procedure for the colorimetric estimation of silica is described. By control of pH and use of photoelectric colorimeters for the measurement of color, the procedure has been made more precise. The sensitivities of the Cenco and the Evelyn photoelectric colorimeters were compared for this system and found to be nearly identical when cells of the same thickness were used. The careful selection of filters by means of a spectrophotometer is recommended.

Acknowledgment

The authors wish to thank the J. T. Baker Chemical Company for the grant which facilitated this research and also express appreciation to the J. T. Brittingham Fund for the special spectrophotometer which was used in this work.

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Quantitative Determination of Indole

Modification of Ehrlich's Reaction

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OF THE many tests for indole, that of Ehrlich (*4*) seems to be the most characteristic and widely used. This test depends on the reaction between indole and dimethylaminobenzaldehyde in the presence of hydrochloric or sulfuric acid, a pink-red color being produced.

Since a variety of colors may be produced with this reagent by many organic compounds (*6*), a preliminary separation of indole from products containing it is necessary. This separation is usually attempted by steam-distillation with subsequent extraction from the distillate, but with many organic mixtures this process is cumbersome and time-consuming and may be troublesome on account of foaming.

The method described herein, involving Ehrlich's reaction, may obviate the necessity for steam-distillation, is comparatively short, rapid, and sensitive, and appears to have wide applicability to food and biological material. It has been applied with reasonable success by Clarke *et al.* (*1*) to butter and to distillates from butter. The direct comparison of the spectral curves for the color obtained from butter distillates with that for the color obtained from pure indole furnished strong presumptive evidence that the results obtained in this case were due to a mixture of indole and a substance or substances very closely related to indole. Clarke *et al.* checked the accuracy and were able to determine by visual comparison as little as 1 microgram of indole per 50 grams of butter by direct application and by steam distillation. By means of a simple neutral-wedge photometer developed by Clifford (*2*) the amount of indole under favorable circumstances may be read to hundredths of a microgram.

This method is based primarily on the observation that when a chloroform solution of indole is treated with dilute acid (up to approximately 12 per cent) and Ehrlich's reagent, the color remains in the chloroform, but if the test is made with stronger hydrochloric acid the color is transferred to the aqueous phase. If the acid is too concentrated, the color may be inhibited or destroyed.

In the method described herein sirupy phosphoric acid is used instead of hydrochloric acid because, being of heavier density, it may be easily separated with the indole and reagent from a mixture with chloroform, forming the lower layer, which can be easily tapped off. Furthermore, being a weaker acid than hydrochloric acid, some reaction with other possible interfering substances (when chloroform extracts of biological material are made) may be avoided.

The addition of acetic acid increases the sharpness of separation and clarifies both layers. Almost all of the acetic acid remains dissolved in the chloroform. After separating the phosphoric layer, the characteristic color of the reaction is developed by adding acetic acid to it.

Method

REAGENTS.

A. Dissolve 0.2 gram of purified dimethylaminobenzaldehyde (*3*) in 100 cc. of 85 per cent phosphoric acid.

B. Phosphoric acid, 85 per cent

C. Glacial acetic acid, freshly distilled and free from formaldehyde

D. Chloroform, washed free of alcohol

To 50 cc. of chloroform containing from 1 to 10 micrograms of indole in a separatory funnel, add 5 cc. of reagent A. Shake 2 minutes and then add 25 cc. of acetic acid. Shake again for a moment and allow the mixture to stand until the layers separate

completely (a few minutes). With pure reagents both layers will be colorless or nearly so. Carefully tap off the lower phosphoric acid layer into a 50-cc. Nessler tube. Wash the chloroform layer with 2.5 cc. of phosphoric acid and add it to the acid in the tube. Make up to volume with acetic acid.

The characteristic purple-red color develops upon the addition of the acetic acid (*5*), the intensity being in direct proportion to the amount of indole present. By varying the amounts of indole, the color intensities produced may be used as standards for comparison, the strength of the color decreasing only slightly after 1 or 2 days. The comparison may also be made photometrically in a neutral-wedge (extinction) photometer such as that devised by Clifford. The spectral curve shows a maximum absorption in the neighborhood of 570 mu. By means of the photometer as little as 0.5 microgram of indole may be detected.

CAUTION. The reaction may be obtained by first adding the dimethylaminobenzaldehyde to the chloroform solution of indole and then shaking with phosphoric acid, but if added in reverse order, the indole is apparently polymerized by the acid and will not react with the benzaldehyde.

Discussion

Indole may be extracted by chloroform from either slightly alkaline or acid aqueous solution. Since interest in the determination of indole is almost entirely with reference to biological material which may contain many interfering substances of acidic or alkaline nature, it is suggested that such material be first made slightly alkaline with dilute sodium hydroxide and shaken vigorously with a measured amount of chloroform. After separation, the chloroform solution may be washed with a small amount of dilute hydrochloric acid, and an aliquot may then be treated with Ehrlich's reagent as above described. If emulsions form during the alkaline extraction, they may be broken usually by stirring in or shaking with sufficient powdered ammonium sulfate to cause separation of a clear liquid, after which the mixture may be centrifuged or decanted and filtered.

(The treatment with ammonium sulfate in alkaline solution, as indicated, also removes formaldehyde, which, if present, may prevent the color formation.)

Sometimes the separation of the chloroform and phosphoric acid layers may be difficult. The addition of 5 or 10 cc. more of acetic acid and somewhat longer standing (about 30 minutes) may obviate this difficulty. It is important that this separation be clean, as otherwise a turbidity may develop in the final solution when made to volume with acetic acid.

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Pressure Regulator for Vacuum Distillation

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THE regulation of pressure during vacuum distillation is usually accomplished with the aid of a manostat which keeps the pressure constant by causing a pump to operate intermittently or by controlling an air leak (*1*). Such devices require a good deal of time, labor, and materials before they can be placed in operation. A pressure regulator which can be quickly assembled from apparatus and materials available in any laboratory is shown in Figure 1.

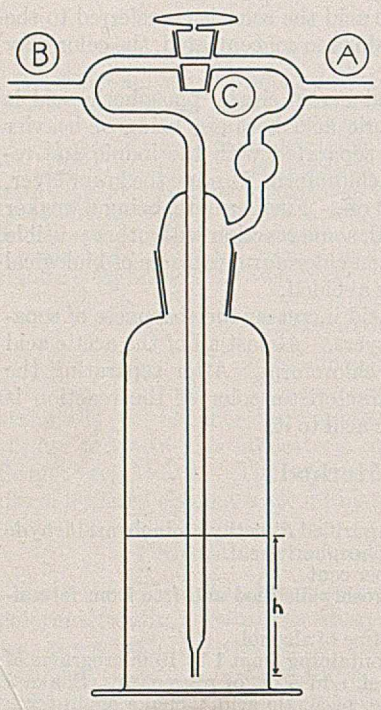

FIGURE 1

The principle upon which this regulator is based is simple. The gas in the system whose pressure is to be controlled must overcome the pressure of a column of liquid before it can be pumped out. In principle all that is required is a gas washing bottle containing a quantity of a liquid having an insignificant vapor pressure at room temperature. In practice the two refinements shown in Figure 1 make operation easier.

When the pump, connected at *A*, is first started the bulk of gas in the system, connected at *B*, is removed through the open stopcock, *C*. When the pressure has almost reached the desired pressure *C* is closed, forcing the remaining gas to be pumped through the head of liquid, *h*. The end of the gas inlet tube is constricted, so that when the system has come to equilibrium the constant leaks therein (including the distillation capillary) cause a slow steady stream of bubbles instead of the more intermittent larger bubbles that result if no constriction is made. The inside diameter of the constriction should be about 1 to 1.5 mm. Before admitting air when the distillation has been completed, *C* is opened in order to avoid violent splashing of the liquid in the bottle.

Although the author has used this device for regulating pressure for only a few months, it has proved to be extremely efficient. After allowing about 15 minutes for the entire system to come to equilibrium, the pressure, as read on the usual type of mercury-filled manometer, has remained constant during distillations requiring as long as 10 hours. A pressure range of 1.5 to 16 mm. is conveniently covered by the ordinary gas washing bottle. Theoretically taller wash bottles or a series of short ones would allow for a wider pressure range. The author has used ethyl phthalate as a suitable liquid, but undoubtedly other liquids could be substituted.

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Solution Method for Spectrographic Analysis

Utilizing a Dropping Electrode

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THERE often arises in spectrographic analysis a need for a simple, yet reliable, method for the quantitative determination of substances in solution. Procedures for the preparation of standard powder samples for spectrographic work and their subsequent analysis are generally more laborious and time-consuming than those utilizing solutions. Also, the homogeneity of standard solutions is much more certain and their preparation is simpler than those for either powders or metallurgical specimens.

Several methods for solution analysis have been described in the literature. Each has its individual merits and disadvantages as to ease of manipulation, complexity of design of container for the solution, adaptability to available electrode stand design, reproducibility of excitation conditions, and quantity of solution needed for an analysis. The forms of apparatus described by Gerlach and Schweitzer (*1*), Twyman and Hitchens (*5*), and Jolibois and Bossuet (*2*) employ special electrode equipment and relatively large quantities of solution. Of several types of electrodes described in the literature, one attributed to Necke is mentioned by Löwe (*3*) and it has some similarity to the method proposed in the present paper. However, no data are given by Löwe to show the reliability of the apparatus in quantitative work, and the original communication could not be located.

After completion of the work of this paper, it was found that Lundegardh (*4*) also refers to the work of Necke and has suggested changes in procedure analogous to those employed here. The technique, which in the present work was designed for use with a cold, high-potential spark, involves a slow and regulated addition of the solution through a hollow upper electrode.

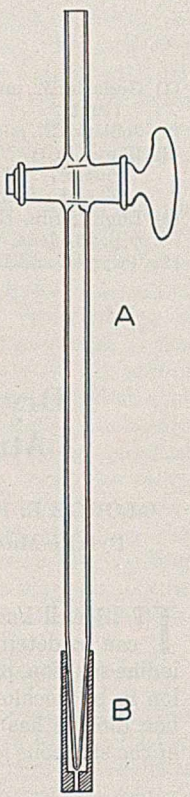

FIGURE 1. APPARATUS

Electrode System

The lower electrode is an ordinary spectroscopic carbon rod with a flat end surface exposed to the spark. The upper carbon electrode is constructed, as shown in *B* (Figure 1), from a piece of 0.25-inch (6.35-mm.) spectroscopic carbon rod 1.25 inches (31.8 mm.) long. One end of the electrode is drilled to a depth of 1 inch (25.4 mm.) with a 0.11-inch (2.78-mm.) drill. (The size and depth of drill were chosen to fit the particular size to which the glass capillary end was drawn.) The remaining 0.25 inch of the electrode is drilled with a 0.08-inch (1.98-mm.) drill. The smaller hole is necessary to effect a steady flow of solution into the spark and to prevent large drops from playing upon the spark and varying excitation conditions.

The device for introducing the solution, a "dropping electrode", is constructed from a piece of glass tubing 0.25 inch (6.35 mm.) in diameter, 7 inches (17.8 cm.) long, and fitted with a stopcock at one end, as shown in *A* (Figure 1). The other end is softened in a flame and drawn to a pointed capillary. The capillary tip is then fire-polished until the orifice will permit a flow of approximately 0.9 cc. per minute, when the stopcock is adjusted to the maximum opening. (These specifications are for use only when the tube is filled to a point below the stopcock level—i. e., in use, the glass tube is never filled to include liquid in the stopcock. Filling the tube past the stopcock should be avoided, since the rate of flow cannot be easily duplicated.) If larger volumes of solution are needed for excitation, the tube should be made of greater length or greater diameter.

Since corrosive liquids are sometimes employed, it is necessary to shield the electrode stand, etc., from the spray which the spark produces during excitation. A most satisfactory and inexpensive form of shield can be made from a piece of absorbing paper (Bluebird lining paper), cut and folded into a box, with an open end and top, and of such dimensions that it can be fitted between the electrode holders. A small slot is cut in the bottom of the shield to allow the lower electrode to protrude.

Operation

After the spectrograph has been adjusted and the plate holder loaded, the carbon electrodes are placed in the electrode stands, with the usual precautions as to electrode heights and spark gap distances. The drilled carbon serves as the top electrode, while the flat-surface carbon serves as the bottom electrode. The dropping electrode is then inserted firmly into the top carbon electrode (no other support being necessary to hold it), the absorbing paper splash-shield is placed about the electrodes, the stopcock is opened, and the spark is started immediately. Observation of the time is noted the instant the spark is excited, and a preliminary sparking of 15 seconds is given the electrodes (solution flowing) before the shutter of the spectrograph is opened. After the 15 seconds' preliminary sparking, the shutter is opened, and the exposure is continued for 60 seconds. (The spark is not interrupted between the preliminary sparking and the time the shutter is opened.) The shutter is then closed, the spark discontinued, and the stopcock closed in the order given.

The dropping electrode is removed, drained, cleaned, rinsed with the next solution to be run, and finally filled. New or repurified carbon electrodes are placed in the stand, and the procedure is repeated until all desired solutions have been examined. To facilitate draining of the tube when cleaning and refilling, the capillary tube is inserted in a suction flask, equipped with a one-hole rubber stopper. After the spectra have been taken, the plate is developed, fixed, washed, and dried, and the desired line densities are determined. Here, as in all quantitative spectrographic analysis, standardization and duplication of technique are of utmost importance.

The dropping electrode is easily cleaned and filled by attaching a small piece of rubber tubing to the upper end and connecting to a vacuum line. The solution to be analyzed is sucked into the tube to a mark below the stopcock, the vacuum line is removed, the stopcock is closed, and the tube is removed from stock solution and wiped dry.

The rate of flow from the dropping electrode is governed by the size of the capillary orifice. This arrangement allows the solution to flow continuously and evenly into the spark, where a fresh surface is constantly being formed, and the excess solution is expelled from the electrodes by the force of the spark. The high-potential (cold) spark must be used for excitation, since the direct current arc develops too high a temperature, which affects the delivery of the solution through the capillary orifice and at times would be sufficiently hot to melt the glass.

Experimental

Standard solutions of manganese were prepared of the following manganese concentrations: 0.5, 0.25, 0.10, 0.05, 0.025, and 0.01 mg. per ml.

The solvent used was 4.5 per cent hydrochloric acid. Copper was chosen as the internal standard, and a solution of cupric sulfate was prepared, of such concentration as to give satisfactory line densities for the copper line at 2369.8 Å. The solutions actually used in the analysis were prepared by mixing equal volumes of manganese and cupric sulfate solutions.

TABLE I. DENSITOMETER READINGS OF MANGANESE AND COPPER LINES

Mn Concn.	Plate 1 Mn	Plate 1 Cu	Plate 2 Mn	Plate 2 Cu	Plate 3 Mn	Plate 3 Cu	Plate 4 Mn	Plate 4 Cu	Plate 5 Mn	Plate 5 Cu	Plate 6 Mn	Plate 6 Cu
Mg./cc.												
0.5	46.9	23.6	46.7	23.1	42.2	21.1	42.2	20.0	42.0	20.8	35.7	17.1
0.25	39.6	22.0	41.8	24.1	37.0	21.2	36.2	20.0	36.1	21.1	37.7	21.7
0.10	30.4	23.6	30.3	23.7	28.1	22.1	26.5	20.3	27.5	21.3	28.5	20.0
0.05	23.1	24.9	23.6	23.0	19.8	20.3	19.3	21.2	18.6	20.4	22.1	22.9
0.025	15.5	23.6	15.9	23.2	12.6	21.0	13.1	21.2	13.7	21.8	15.6	22.8
0.01	8.1	24.0	6.7	23.2	6.2	21.6	6.5	22.5	6.5	21.5	8.1	24.0

TABLE II. RATIO OF DENSITIES OF MANGANESE LINE TO COPPER LINE

Mn Concn.	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Average	Mean Deviation	Av. Deviation of Mean
Mg./cc.									
0.5	1.99	2.02	2.00	2.11	2.02	2.08	2.03	0.036	0.014
0.25	1.80	1.73	1.74	1.81	1.71	1.74	1.75	0.031	0.012
0.10	1.29	1.28	1.27	1.31	1.29	1.42	1.31	0.036	0.014
0.05	0.93	1.02	0.98	0.91	0.91	0.96	0.96	0.035	0.014
0.025	0.66	0.68	0.60	0.62	0.63	0.68	0.64	0.030	0.012
0.01	0.34	0.29	0.29	0.29	0.30	0.34	0.31	0.021	0.0086

The electrodes were placed at a distance of 60 cm. from the slit of the spectrograph. The spark gap was adjusted to 6 mm., and duplication of the gap distance was accomplished by the use of a glass spacer 6 mm. thick. No external condensing lens was used.

A Bausch & Lomb large Littrow (quartz) spectrograph was employed, using a slit width of 70 microns and 4-mm. height. Prism position 7, covering the range 2300 to 2900 Å., was used to register the section of spectrum which contained the manganese and the copper lines selected.

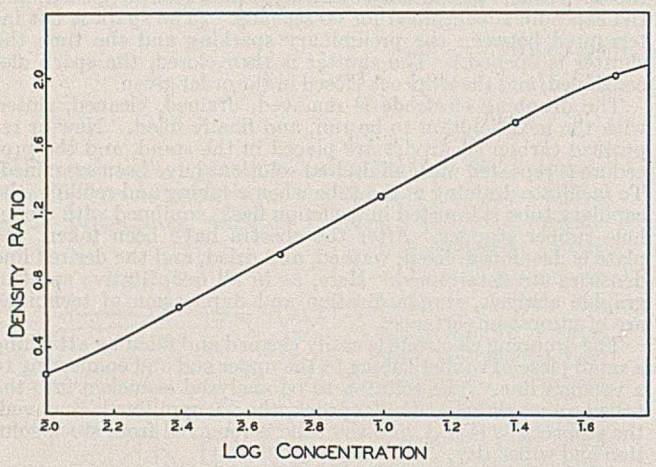

FIGURE 2

Excitation of the sample was obtained by a high-potential spark supplied from a 220-volt source, with sufficient resistance to produce a current of 17.5 amperes in the primary of a 1000 to 1 5-kw. transformer. The secondary circuit consisted of 8 condensers (in parallel) of 815×10^{-4} microfarad each. No inductance coil was used in the secondary circuit.

The solutions were analyzed by the technique already described, and the spectra were recorded on Eastman D. C. (double-coated) orthochromatic plates. The plates were processed using Eastman standard x-ray developer and fixer, and were developed for 5 minutes at 18° C. After development, they were fixed for about 20 minutes, washed in running water for at least one hour, rinsed in distilled water, and dried in air.

The Gaertner visual microdensitometer, having a scale range of 0 to 100 divisions corresponding to density units of 0 to 4, was used to evaluate line densities.

The lines chosen were free of any background, so that no correction was necessary. The ratio of the density of the manganese line to the copper line was plotted against log concentration.

Table I gives densitometer readings of the manganese line at 2576.12 Å. and the copper line at 2369.8 A. for different manganese concentrations. Six separate plates were exposed, using the same standard manganese solutions.

Table II gives the ratio of the densities of the manganese line to the copper line, the average ratio value, mean deviation, and average deviation of the mean.

When the average ratio values are plotted against the log-concentration (mg. of manganese per cc.), a curve is obtained, as shown in Figure 2.

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Dry Ice as a Preventive of Atmospheric Oxidation

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IT IS well known that tin in commercial tin-lead solders can be determined accurately by titration with standard iodine solution, provided that it is present entirely as stannous ion in hydrochloric acid solution (*1*). The use of solid carbon dioxide has been found helpful in preventing oxidation of the stannous ion previous to the titration.

After a sample of solder filings has been prepared and dissolved in hydrochloric acid in the usual way, and any tin in the stannic form has been entirely reduced to the stannous form, immediately place a small piece of solid carbon dioxide in the solution and place the flask in a cooling bath of ice and water. Add more small pieces of dry ice and maintain a vigorous evolution of gaseous carbon dioxide within the Erlenmeyer flask until the solution has been cooled to room temperature and is ready for titration. Just preceding the titration, wash down the wall of the Erlenmeyer flask with distilled water containing a small piece of dry ice, add 5 ml. of starch solution as indicator, and titrate the solution with 0.10 *N* iodine solution.

The use of solid carbon dioxide prevents the formation of stannic ion due to atmospheric oxygen, makes it unnecessary to employ a bicarbonate of soda solution as a wash water, and eliminates the necessity of setting up apparatus for passing gaseous carbon dioxide or any other gas through the solution during cooling. Dry ice may serve equally effectively in other determinations where protection of the solution from atmospheric oxidation is important.

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Gaging Adsorption Power of Colloidal Ferric Oxide by Dye Adsorption

Development of a Standard Test

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THE dye adsorption test described in this paper was developed to permit a study of the colloidal properties of iron oxide purifying masses with regard to their ability to remove hydrogen sulfide from gas. Results obtained by its use have been correlated with the activity and capacity for the primary sulfiding reaction and succeeding sulfiding reactions. A suggested method for rating iron oxide purifying materials has been proposed and from the fundamental information thus obtained an explanation has been developed for various phenomena in oxide purification, hitherto unexplained. For a more complete discussion of this work the reader is referred elsewhere (*2*).

In rotary drilling procedures for petroleum and natural gas the drilling mud walls off the hole, lubricates the drilling bit, carries the cuttings to the surface, deposits them, and is once more returned to the hole, completing the cycle. The use of high-colloidal muds has made possible the rapid drilling of soft formations; in addition, a number of properties of the mud, important in practice, are dependent upon the colloid content. As no simple means of determining the colloid content of mud is available, any simple test developed for rapidly measuring the colloid content of a material whose physical structure is closely related to drilling mud, should be of interest to the chemical and petroleum production engineer. This test presented here has been used to determine the colloid content of commercial iron oxides used in weighting drilling mud (*7*), and with some alterations may possibly be used to determine the colloid content of other drilling masses.

Determination of Surface and Colloid Content

Dyes are important in determining certain properties of a colloidal oxide, because the adsorption of the dye by the oxide under certain conditions depends upon the surface properties of the oxide. Technically a great deal of attention has been paid to the relations in question because many dyes are so weakly adsorbed by wool, silk, and cotton that they are of little value to the practical dyer unless used in connection with certain oxides or other materials which serve as mordants. The term "mordant", meaning to bite or corrode, was first used by the French to describe metallic salts which were supposed to give color access to the fiber by opening passages in the fiber. In the early work, the effectiveness of alum in fixing certain dyes was believed to be due to the solvent or sulfuric acid; however, it is now known that the real mordant is the hydrous oxide and not the acid derived from the salt.

In dyeing mordanted cloth, in most cases, the mordant rather than the fiber takes up the dye. When a dye is taken up by a mordant in the absence of a fiber, the complex is known as a color lake. In general, the most common color lakes are those formed with dyes and hydrous oxide.

Dye-adsorption methods have been used to determine the colloid content of various substances. Paneth and Radu (*5*) measured the surface of powdered diamond, microscopically and by the adsorption of methylene blue, assuming that the surface was covered 1 molecule thick. As the results obtained by the two methods were confirmatory, they assumed that the same rule holds true for the adsorption of dyes by other forms of carbon and used this method to determine the specific surfaces of various charcoals. Rideal and Wright (*6*) also used the dye-adsorption method to determine the surface of charcoals prepared from nitrogenous sources which possess, in general, greater specific surface than charcoals produced from nitrogen-free compounds.

Development of Test

Malachite green was examined because it had been used by Moore and others to determine the colloid properties of clay, and because this test may be a practical and rapid means for determining the properties of colloid surfaces. However, it was necessary to modify this test to overcome or at least minimize the seeming disadvantages or possible errors inherent in previous methods with respect to the calcium-ion effect upon the dye and the nonuniform adsorption due to varying pH of the solution.

Ashley's (*1*) work on the colloidal properties of clay showed that calcium and alkaline salts affect the adsorption of malachite green. He therefore limited the use of this test to high grades of clay which were not contaminated by lime or alkaline ingredients.

Moore (*4*) and others, in their work on the determination of the colloidal content of clay with malachite green, appear to have overcome the effect of calcium by precipitating it in the form of the insoluble calcium oxalate by the addition of sodium oxalate. They showed also that an excess of sodium oxalate did not affect the malachite green. The present authors agree that sodium oxalate does not affect the dye but have shown experimentally that the shift of the pH of the solution, which may be brought about by the addition of alkaline salts, will cause a colloidal substance to adsorb a greater quantity of dye at a higher pH of the solution.

Weiser (*8*) and Gordon (*3*) conducted researches which indicate that the adsorption of a dye by a hydrous oxide is dependent upon the pH of the solution. Weiser showed also in his work with alizarin dyes that the hydrous oxides adsorb the sulfate and oxalate radical but that the dye is adsorbed in preference to the sulfate or oxalate when both are in the bath, except in very acidic baths in which the sulfate and oxalate replace the dye.

After a study of the foregoing researches, it was decided to work with a buffer solution containing so much acid that the addition of the basic dye gave a solution which was still acidic. A buffer solution of 1 *N* oxalic acid and 1 *N* potassium oxalate was used, mixed in a proportion of 4 parts of salt to 1 part of acid, giving a solution whose pH was approximately 5. Using this buffer, it was believed that the following conditions would prevail:

1. The oxalate radical would precipitate any calcium present, in the form of the insoluble calcium oxalate, thereby eliminating the calcium effect upon the dye.
2. The capacity of the buffer would tend to hold the pH of the solutions approximately constant, eliminating the possibility of nonindicative dye adsorption due to varying pH of the solution.

Effect of Varying pH and Excess Oxalic Acid

In order to study the adsorption of malachite green in varying pH baths, sample 3, an oxide high in colloid content, was treated in the following manner:

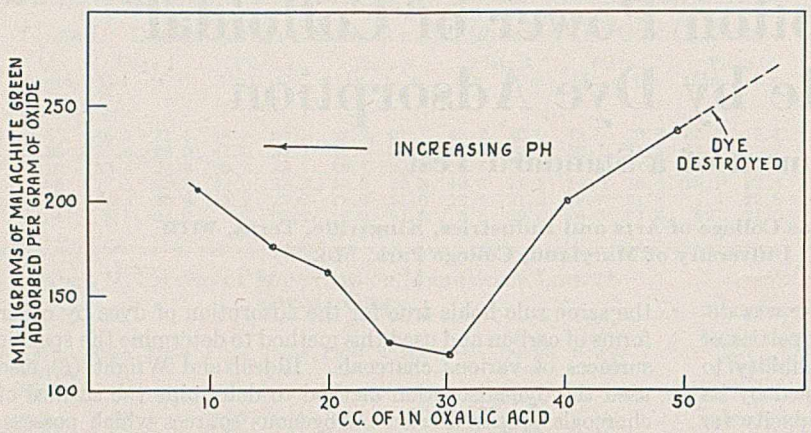

FIGURE 1. EFFECT OF CHANGE IN pH AND EXCESS OXALIC ACID ON ADSORPTION OF MALACHITE GREEN

To 1-gram samples of the oxide were added 50 cc. of buffer solution and various portions of 1 *N* oxalic acid and the samples were shaken for 1 hour. Fifty cubic centimeters of standard dye were then added (300 mg. of malachite green) and the whole was again shaken for 1 hour and allowed to stand overnight. The next day the adsorption was determined. (All samples were heated at 100° C. for 1 hour and ground to pass a 60-mesh sieve.)

Table I shows the adsorption of malachite green with a given amount of colloid (sample 3) in varying pH baths. These results are plotted in Figure 1. The ordinates give the milligrams of malachite green adsorbed per gram of oxide and the abscissas the cubic centimeters of 1 *N* oxalic acid which were added to the 50 cc. of buffer solution.

TABLE I. ADSORPTION BY SAMPLE 3

(Malachite green 50 cc., buffer solution 50 cc., water 50 cc. Adsorption calculated as explained in standard procedure)

Dilution Factor	1 *N* Oxalic Acid	Total Solution	Dye Adsorbed
Cc.	*Cc.*	*Cc.*	*Mg.*
21	10	160	201
25	15	165	176
27	20	170	163
33	25	175	126
34	30	180	117
18	40	190	199
10	50	200	239
[a]	60	210	[a]

[a] Dye destroyed.

It will be noted that the adsorption decreases with increasing acidity until a minimum adsorption of 117 mg. of dye per gram of oxide is reached with the addition of 30 cc. of 1 *N* oxalic acid. Further addition of acid gives an increase in adsorption, until the dye is apparently destroyed when 60 cc. of 1 *N* oxalic acid have been added.

This series of tests appears to confirm the conclusion of Moore and others that an excess of oxalic acid destroys the structure of malachite green.

Effect of Dye Remaining in Solution after Adsorption

Moore and others observed in their work that the size of the samples used, with a constant initial amount of dye, showed that a distribution effect was playing a part in determining the amount of dye adsorbed. They apparently overcame this difficulty by keeping a constant amount of dye in solution after adsorption.

In the work represented by this problem it was observed that practically all the present-day iron oxide gas-purifying materials generally accepted as good adsorbed from 300 to 600 mg. of dye per gram of oxide. Keeping a minimum of 150 mg. of dye in solution after adsorption prevented error due to distribution effects.

In studying the distribution effects the following experiments were conducted. One-gram samples of Nos. 23, 12, 24, and 25 were treated with a constant initial amount of dye with varying portions of buffer solution and the adsorption was determined. The results of these tests are tabulated in Table II.

The adsorptions in test X were obtained by using 25 cc. of standard buffer solution, 25 cc. of distilled water, and 125 cc. of standard dye solution. The resulting pH of this solution was dependent upon the proportions of buffer and dye solutions, and this solution had the highest pH in this series of tests. Test Y was obtained by using 50 cc. of buffer and 125 cc. of standard dye solution (standard test), the resulting pH being lower than that of test X. Test Z consisted of 100 cc. of buffer and 125 cc. of standard dye solution, the pH of this solution being lower than either test X or test Y.

The results of these tests are plotted in Figure 2. Although the amount of dye remaining in the solution after adsorption varies, there is no definite distortion of the curves due to distribution effects. This also appears to be confirmed by the approximate parallel relationship between the curves of the different oxides.

TABLE II. EFFECT OF VARYING BUFFER SOLUTION

(125 cc. of malachite green used. Adsorption calculated as explained in standard procedure)

Test	Oxide No.	Buffer Solution	Water	Dilution Factor	Total Solution	Dye Adsorbed
		Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*
X	23	25	25	22	175	635
	24	25	25	33	175	577
	12	25	25	48	175	498
	25	25	25	73	175	376
Y	23	50	..	32	175	582
	24	50	..	41	175	534
	12	50	..	59	175	440
	25	50	..	85	175	307
Z	23	100	..	36	225	500
	24	100	..	50	225	419
	12	100	..	66	225	335
	25	100	..	83	225	190

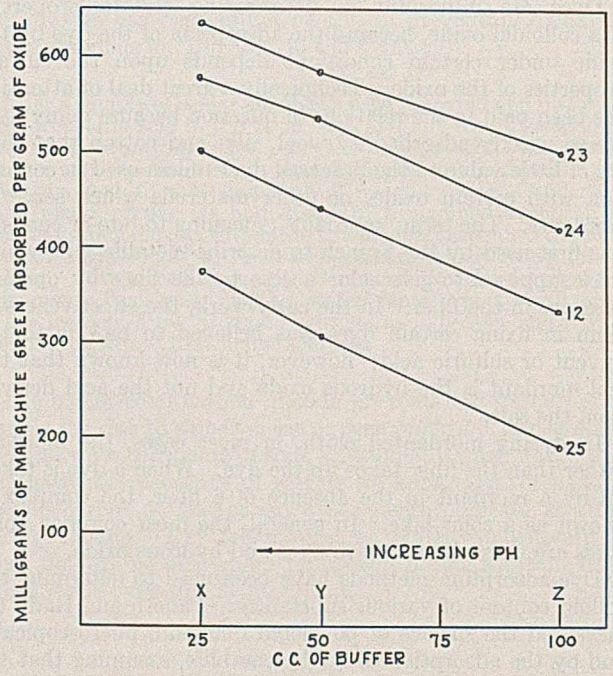

FIGURE 2. EFFECT OF VARYING QUANTITIES OF MALACHITE GREEN REMAINING IN SOLUTION AFTER ADSORPTION

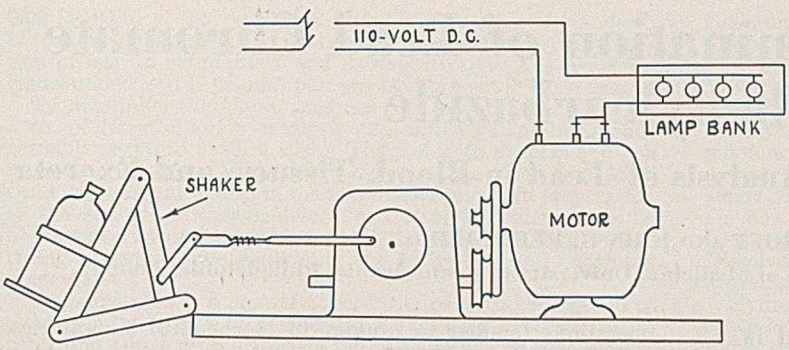

FIGURE 3. SHAKER

From these tests it appears that errors due to distribution effects may be eliminated by maintaining the correct amount of dye in solution after adsorption and that a minimum of 150 mg. of standard dye in 175 cc. of solution is sufficient.

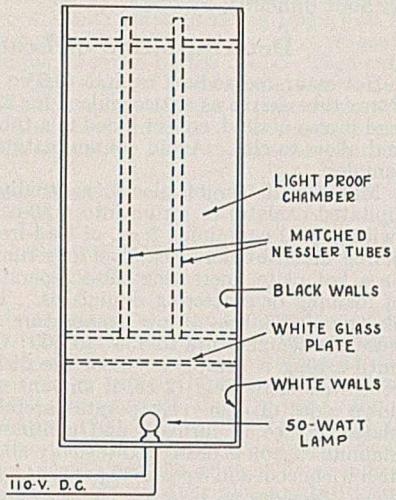

FIGURE 4. COLORIMETER

Effect of Calcium upon Dye

To test the efficiency of the buffer solution in removing the calcium ion from solution, 0.05, 0.10, and 0.15 gram of lime [$Ca(OH)_2$] were treated with 50 cc. of buffer solution and shaken for 1 hour. Malachite green was then added (125 cc.), the solution was again shaken for 1 hour, and the adsorption was determined. In all tests there was no dye removed or destroyed in the solutions.

Standard Test for Gaging Adsorption Power of Colloidal Ferric Oxide by Malachite Green

In a 200-cc. wide-mouthed bottle provided with a rubber stopper 50 cc. of buffer solution (1 part of 1 *N* oxalic acid to 4 parts of 1 *N* potassium oxalate) were placed, and 1 gram of the sample was added. The solution was shaken at once by hand to prevent clotting and then placed in a shaker (Figure 3), and shaken for 1 hour to precipitate any calcium present. Then 125 cc. of standard dye solution (6 grams of malachite green oxalate in 1000 cc. of distilled water) were added and the whole was again shaken for 1 hour. The sample was allowed to settle overnight and on the following day a convenient amount of the supernatant liquid was taken out by a 10-cc. pipet, the end of which was inserted to approximately one fourth the depth of the liquid; this sample was placed in a test tube and corked until the adsorption was determined by color comparison.

It was found that 1 cc. of the standard solution diluted to 200 cc. gave the best color comparison when a beam of white light was passed up through exactly 10 cc. of this solution in one of a pair of matched Nessler tubes, as shown in Figure 4. Exactly 1 cc. of the sample removed from the test bottle was transferred to a 100-cc. graduate and diluted until the color matched perfectly the standard color obtained by diluting the standard solution.

For example, a match was obtained when a 1-cc. sample of No. 23 was diluted to 32 cc. As 1 cc. of the standard contained 0.006 gram of dye, the 200 cc. of test solution also contained 0.006 gram of dye. Where x is the amount of dye remaining in solution and 175 cc. is the total solution, 0.006 gram : 200 cc. : : x : (32 cc. $\times$ 175 cc.) or x = 0.168 gram.

The original solution (125 cc.) contained 0.006 gram per cc., or a total of 0.750 gram; the adsorption was 0.750 gram − 0.168 gram, 0.582 gram or 582 mg. of dye. In this proportion all terms were constant but the dilution factor of 32 cc.; therefore, the remainder of the proportion was represented by a constant calculated to be 0.00525 (which applies only where the total solution is 175 cc.). This constant when multiplied by the dilution factor gives the grams of dye remaining in solution.

It was found most efficient to determine the approximate dilution factor first, and then repeat the operation using this approximate figure as a guide. Water was added 1 cc. at a time and the volume added was recorded for the final value of the dilution factor when further addition of water gave a color lighter than the standard.

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THE experimental material presented in this paper forms a portion of the dissertation submitted by Frank H. Dotterweich to the Board of University Studies, Johns Hopkins University, in partial fulfillment of the requirements for the degree of doctor of philosophy.

Removal of Phosphates from Solutions of Hydrogen Peroxide

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MANY solutions of hydrogen peroxide contain phosphoric acid as a stabilizing agent. Since hydrogen peroxide is used in many oxidations in which phosphate is subsequently determined, the removal of phosphate is a necessary step in the procedure. It is possible to remove it by distillation at reduced pressure (*2*), but since this technique is comparatively expensive in routine analysis it was thought advisable to develop a less complex, more rapid method of removing phosphates from solutions of 100-volume hydrogen peroxide.

Place 100 ml. of peroxide in a 250-ml. beaker. Add 10 ml. of 2 per cent ferric chloride, stir the solution, and then add about 5 grams of calcium carbonate. Stir again for a moment and filter immediately by suction through a Büchner funnel prepared previously. The filtrate should be clear and almost colorless. Add 0.5 ml. of concentrated hydrochloric acid to the purified peroxide and store in a black bottle. Prepare a week's supply at a time.

Five milliliters of the peroxide were evaporated to dryness and on analysis by a colorimetric method (*1*) contained 0.2 p. p. m. of phosphorus. The peroxide loses very little activity by this treatment.

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PUBLISHED with the approval of the Director of the Illinois Agricultural Experiment Station.

Colorimetric Determination of Lead Chromate by Diphenylcarbazide

Application of a New Method to Analysis of Lead in Blood, Tissues, and Excreta

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After ashing biological material, lead is precipitated as lead potassium chromate by addition of potassium chromate to a solution of the ash containing chloride, citrate, acetate, and ammonium ions at pH 6.6 to 7.4. The precipitation is accomplished in a centrifuge tube and the double chromate is separated and washed by centrifuging. Lead is determined colorimetrically by means of the red color formed by diphenylcarbazide with chromate. Preliminary separation of lead is omitted. Absence of interference by other metals has been demonstrated.

CONCLUSIVE diagnosis of chronic lead poisoning usually requires determination of lead in body fluids and excreta. Work in progress (*16*) suggests that chronic lead poisoning occurs more frequently than is realized, that evidence of exposure often is lacking, that symptoms often are atypical or obscure, that harmful effects in the body are widespread, and that the condition often is unrecognized in the clinic or at necropsy. The use of chemical evidence for diagnosis of lead poisoning has been reviewed recently by Smith, Rathmell, and Marcil (*18*) and Kaplan and McDonald (*11*), who point out the superiority of blood and serum analysis for this purpose. However, chemical analysis is applied less frequently than is desirable and often is omitted.

Measurement of the minute amounts of lead in blood has been accomplished up to the present time chiefly by extraction of lead with dithizone or by spectrographic analysis. Although the introduction of dithizone constituted a major advance in lead microanalysis, existing techniques require training and skill of an order not available to many laboratories. The cost of dependable apparatus for spectrographic analysis of lead restricts its use to a few institutions. The authors' purpose is to describe a new method for determination of lead in biological material that may prove more convenient than existing methods for certain types of analysis. Previous experience with *sym*-diphenylcarbazide as a quantitative reagent for chromate in connection with development of a colorimetric micromethod for chloride (*15*) indicated that this substance would be satisfactory for the determination of lead chromate as well. Jones (*10*) has found diphenylcarbazide to be suitable for this purpose. However, the use of diphenylcarbazide as a reagent for lead analysis has been criticized adversely by Fairhall (*6*), Kehoe, Thamann, and Cholak (*13*), and Cholak, Hubbard, McNary, and Story (*3*). It seemed possible that the unsatisfactory experience of these workers was to be attributed not to the use of diphenylcarbazide itself but to other details of their procedures.

Investigation along these lines has enabled development of a new method for the determination of lead by means of *sym*-diphenylcarbazide. The new method is simple, utilizes operations familiar to workers in biochemical laboratories, and presents a distinct improvement in sensitivity and accuracy over existing methods employing diphenylcarbazide. About 16 determinations can be completed within 24 hours without difficulty by one worker.

Determination of Lead in Blood

Hot saturated sodium oxalate (0.6 cc., low in lead) dried in a Pyrex tube serves as anticoagulant for 22 cc. of blood. If serum lead is also desired, collect blood in a tube without anticoagulant and allow to clot. Avoid contamination with lead in collecting samples.

Measure 10 cc. of blood (suspending thoroughly any precipitated oxalate) or serum into a 30-cc. silica evaporating dish (with spout) containing 5 cc. of lead-free water for whole blood or 5 cc. of ferric chloride catalyst for serum. Evaporate to dryness on a hot plate, increasing the temperature gradually as danger of foaming or spattering diminishes. Transfer the dish to an electric muffle, heat at low temperature until material is charred, raise the temperature to 450° to 500° C., and continue heating until ashing is complete. Cool the dish and add 1 cc. of concentrated nitric acid (2 cc. if amount of ash is large), washing down sides of dish. Evaporate carefully to dryness on a hot plate, replace in furnace, and continue heating until any remaining carbon is destroyed (usually about 30 minutes). To the dish when cool add 5 cc. of 20 per cent hydrochloric acid, washing down the sides as before. Evaporate cautiously to about one-third volume on the asbestos-covered hot plate, avoiding evaporation to dryness, cool, add 2 cc. of 20 per cent sodium citrate solution (again washing down the sides of the dish) and 1 drop of phenol red, and while stirring (with Pyrex rod) add concentrated ammonium hydroxide until the indicator becomes just pink. Filter through a 4.25-cm. (Whatman No. 44) paper, washed as directed below, into a 15-cc. centrifuge tube which has been selected for ability to retain precipitate. Wash dish and paper four times with 1 cc. of 0.1 *N* ammonium hydroxide. Add 25 per cent acetic acid sufficient to change indicator to orange-yellow (avoid excess acid; usually 1 to 3 drops suffice). Using a calibrated pipet, add precisely 1 cc. of standard lead acetate solution containing 0.01 mg. of lead and follow with 1 cc. of 40 per cent ammonium acetate solution, added so as to wash down any material on the walls of the tube. Add 1 cc. of 30 per cent potassium chromate solution, and mix completely by stirring but do not scratch or rub sides of tube. Avoid splashing of the concentrated chromate solution. Cover and allow to stand overnight.

Remove stirring rod and wash adherent material into the centrifuge tube with about 3 cc. of 0.4 per cent ammonium acetate. Centrifuge 10 minutes at about 2400 r. p. m., decant supernatant fluid, invert tube, and drain 5 minutes. Wipe any remaining fluid from the mouth of the tube. Wash with 10 cc of 0.4 per cent ammonium acetate, taking care to wash all portions of the inner surface of the tube, suspend the precipitate by stirring gently, and wash stirring rod as before. Centrifuge for 10 minutes, decant, and drain 5 minutes. Repeat the washing with another 10-cc. portion of ammonium acetate solution. Again centrifuge, and after draining 5 minutes wipe the mouth of the tube, replace the stirring rod, and add 3 cc. of 10 per cent hydrochloric acid, washing stirring rod and walls of the tube. Stir until the precipitate dissolves, add 10 cc. of diphenylcarbazide solution, remove stirring rod, stopper, and mix by inversion. Allow 10 minutes for color development.

Measure color in a photoelectric colorimeter (green filter, maximal transmission 540 mu), or compare in colorimeter with standard lead chromate solutions treated with diphenylcarbazide as follows: Measure 3 cc. of each of the lead chromate working standards into test tubes, add 10 cc. of diphenylcarbazide solution, mix, and allow 10 minutes for color development. The color is stable for 1 hour or more. Mg. of lead per 100 cc. $= \left[\frac{S}{U} \times\right.$

0.03 (or 0.06) × 0.5] −(blank), where S = reading of standard, U reading of unknown, and 0.03 and 0.06 represent the concentration of lead in standards 1 and 2, respectively. The blank includes added lead (0.01 mg.) plus any lead present in the reagents.

To determine the blank, measure 5 cc. of lead-free water, or 5 cc. of ferric chloride catalyst, and anticoagulant in amount actually used into a silica dish. Evaporate to dryness and ash in electric furnace. When cool, proceed as described for blood. Once the amount of lead in the reagents employed in preparation of the sample is known, determination of the blank may be abbreviated by omitting this part of the procedure, and substituting a control tube containing the added lead and remaining reagents.

To prepare such a control tube, included with each group of unknowns, measure 5 cc. of lead-free water into a 15-cc. centrifuge tube, and add 1 drop of phenol red, 2 cc. of 20 per cent sodium citrate, 1 drop of 25 per cent acetic acid, exactly 1 cc. of standard lead acetate solution, 1 cc. of 40 per cent ammonium acetate solution, and 1 cc. of 30 per cent potassium chromate solution. Treat according to the directions outlined for unknown samples. To the result representing the lead taken (0.01 mg.) plus lead in the reagents, add the amount of lead (if any) found in reagents used in preparing the sample, as given by the blank. Then subtract this value from the total amount of lead found in sample and reagents. Citric acid should replace sodium citrate when the former is required in the analytical procedure. In this case neutralization with ammonia is necessary. Cool to room temperature before proceeding.

CEREBROSPINAL FLUID. Treat 10 cc. as directed for blood.

Determination of Lead in Excreta, Tissues, and Foods

URINE. Collect a 24-hour sample of urine in an acid-washed (preferably Pyrex) container using toluene as a preservative. Add 5 cc. of 20 per cent hydrochloric acid and, making certain that all sediment is dissolved or uniformly suspended, remove 15-, 25-, 35-, or 50-cc. samples, respectively, if the 24-hour volume is below 600, between 600 and 1200, between 1200 and 1500, or over 1500 cc. The volumes specified for samples ordinarily avoid unwieldy amounts of ash. Evaporate the urine to dryness in a silica dish. Ash and proceed as described for blood, substituting 2 cc. of 50 per cent citric acid for sodium citrate. Results are best expressed on the basis of 24-hour excretions. Analysis of less than 24-hour collections, or calculation on a per liter basis, may lead to gross misinterpretations.

FECES. Ash a 0.2-gram aliquot of a well-mixed sample of dried pulverized feces. Proceed as described for blood, substituting 2 cc. of 50 per cent citric acid for sodium citrate. If desired, larger samples can be ashed and dissolved with the aid of proportionately larger volumes of reagents, diluted to volume, and aliquots equivalent to the amount specified taken for analysis.

TISSUES. Ash a 0.1- to 0.3-gram portion of well-mixed minced tissue after heating at low heat until dry. Analyze as described for blood.

BONE. Ash a 0.1-gram sample and proceed as described for blood. Substitute 2 cc. of 50 per cent citric acid for sodium citrate. Treat vascular portions of bone as directed for tissues.

FOODS, ETC. Ash a 1- to 2-gram sample of well-mixed dried ground material prepared for sampling by an approved procedure (*1*) with precautions to avoid contamination. Substitute 2 cc. of 50 per cent citric acid for sodium citrate. Larger samples can be taken, if desired, as described for feces.

WATER. Evaporate 1 liter or more of water and ash the residue. Use 50 per cent citric acid in place of sodium citrate.

Reagents

Distilled water for preparation of reagents and for use in the method is freed from lead by redistillation in a Pyrex still after addition of a few drops of phosphoric acid.

Acetic acid, 25 and 1 per cent, glacial acetic acid diluted with water.

Ammonium acetate, 40 per cent in water, filtered. Ammonium acetate, 0.4 per cent, prepared from the preceding, and stored in cold.

Ammonium hydroxide, concentrated reagent quality. Ammonium hydroxide, approximately 0.1 N, 0.7 cc. of concentrated ammonium hydroxide diluted to 100 cc. with water.

Citric acid, 50 per cent, lead-free crystals, dissolved in water and filtered.

Diphenylcarbazide, 0.02 per cent. Transfer 0.100 gram of pulverized *sym*-diphenylcarbazide to a 1000-cc. beaker. Add 500 cc. of ammonia-free distilled water, cover with a watch glass, and dissolve completely by boiling several minutes, stirring if necessary. Cool, dilute to 500 cc., and store in a brown bottle. It will keep 2 months at ordinary room temperature.

Ferric chloride catalyst for ashing of serum, approximately 0.04 per cent ferric chloride. Dissolve 10 grams of ferric chloride hexahydrate in 1000 cc. of distilled water. Add 34 cc. of ammonium hydroxide with stirring, allow precipitate to settle, decant, collect precipitate on a Büchner funnel, and wash with about 3000 cc. of distilled water, then twice with lead-free water. Do not allow precipitate to dry before washing is completed. Remove the precipitate, dry at 105°, and powder in mortar. Dissolve 0.1 gram in 10 cc. of 20 per cent hydrochloric acid by warming. Dilute to 250 cc. with lead-free water.

Hydrochloric acid, 20 per cent. Distill equal parts of concentrated hydrochloric acid and distilled water in an all-glass Pyrex still.

Hydrochloric acid, 10 per cent. Dilute 23 cc. of concentrated hydrochloric acid to 100 cc. with water.

Nitric acid, special lead-free, concentrated.

Potassium chromate, 30 per cent. Dissolve 60 grams of potassium chromate in distilled water and dilute to 200 cc. If possible, allow to stand 14 days or more before filtering through Whatman No. 44 paper which has been washed with chromate solution just before use. Solutions filtered before this time should be refiltered after 14 days.

Phenol red, 0.03 per cent aqueous.

Sodium citrate, 20 per cent, lead-free salt, dissolved in water and filtered.

Standard lead acetate solution. Dissolve 0.183 gram of lead acetate trihydrate in 1 per cent acetic acid and dilute accurately to 100 cc. 1 cc. = 1 mg. of lead. Dilute standard lead acetate solution. Dilute 1 cc. of the preceding to 100 cc. with 1 per cent acetic acid. 1 cc. = 0.01 mg. of lead.

Standard lead chromate solution. Dissolve 39 mg. of lead chromate in 10 per cent hydrochloric acid and dilute to 100 cc. in a volumetric flask. 1 cc. = 0.25 mg. of lead. It will be stable 3 months or more if stored in refrigerator. For working standards 1 and 2, dilute 2 and 4 cc., respectively, to 50 cc. with 10 per cent hydrochloric acid, thus obtaining solutions equivalent to 0.01 and 0.02 mg. of lead in 1 cc. It will be stable in cold at least 3 weeks.

Filtration when required implies use of ash-free paper washed with lead-free water. Glassware must be of Pyrex or silica. Silica dishes are stored in concentrated hydrochloric acid, and centrifuge tubes are stored in cleaning solution, after thorough brushing with soap suds. Before using, dishes and tubes are washed with running tap water, distilled water, and lead-free distilled water. Occasionally silica ware contains lead. New dishes should be ignited and treated with hot concentrated hydrochloric acid before use.

Discussion of Method

Dry-ashing was adopted because it could be adapted readily to the requirements for precipitation in a centrifuge tube. The addition of 5 cc. of lead-free water to blood and of the ferric chloride catalyst to serum or plasma decreases the time required for ashing. Rapid combustion, overheating, or prolonged heating during ashing causes serious loss of lead. The importance of proper ashing technique has been emphasized recently by Fairhall (*4*). In the present method, ashing and preparation of the ash solution for analysis follow in a general way the procedure described by Horwitt and Cowgill (*9*). Filtration of the ash solution removes insoluble matter which may interfere with proper packing of the precipitated chromate. The amount of such material is minute and no lead is lost.

Provision is made to overcome the interfering effect of phosphate in lead analysis by the use of small samples, known quantities of lead being added to accelerate precipitate formation and to secure sufficient bulk for centrifuging. The presence of citrate likewise hinders interference by phosphate. Preliminary separation of lead as sulfide or sulfate has been omitted. Instead, lead is precipitated directly as chromate from a solution of the ash of the material being analyzed. The precipitated chromate is separated and washed by centrifuging rather than by filtration. Separation of the chromate precipitate by centrifuging depends for success upon the use of suitable centrifuge tubes.

TABLE I. INFLUENCE OF pH ON PRECIPITATION OF LEAD POTASSIUM CHROMATE AND LEAD CHROMATE

Acetic Acid Concentration[a]	pH	Lead Added	Lead Found Calculated as $PbCrO_4$	Lead Found Calculated as $PbK_2(CrO_4)_2$
%		*Mg.*	*Mg.*	*Mg.*
0	7.4	0.02	0.0406	0.0203
0.1	..	0.02	0.0404	0.0202
0.3	6.75	0.02	0.0406	0.0203
0.4	..	0.02	0.0406	0.0203
0.5	6.65	0.02	0.0402	0.0201
0.6	..	0.02	0.0391	0.0196
0.2	7.0	0.02	0.0408	0.0204
0.5	6.65	0.02	0.0408	0.0204
1.0	6.1	0.02	0.0336	0.0168
1.5	5.7	0.02	0.0324	0.0162
1.8	..	0.02	0.0306	0.0153
2.0	..	0.02	0.0222	0.0111
2.2	5.4	0.02	0.0206	0.0103

[a] Calculated in terms of acetic acid in relation to final volume and disregarding neutralization or acetate added as ammonium acetate.

TABLE II. SPECIFICITY OF DIPHENYLCARBAZIDE METHOD

	Lead *Mg./100 cc.*
Blood	0.067
Blood plus 1 mg. of Cu as $CuSO_4$	0.068
Blood plus 1 mg. of Zn as $Zn(C_2H_3O_2)_2$	0.069
Blood plus 1 mg. of Bi as $Bi_2O_3.CO_3$	0.068
Blood plus 1 mg. of Ag as $AgNO_3$	0.069

The authors have used 15-ml. graduated Pyrex centrifuge tubes (Corning Glass Works Catalog No. 8080) selected to conform to the following measurements: capacity 16 to 17 ml., uniform straight taper to an inside diameter of 3 to 3.5 mm. at bottom with curvature of 1.5- to 1.75-mm. radius inside. The most important criteria are that the tube retain unbroken the pad of precipitate when inverted during draining, and that it drain cleanly. The suitability of tubes can be established by a test run with a known amount of standard lead solution. Tubes failing to give quantitative recovery are rejected. Proper cleaning, as directed, likewise is essential. Tubes meeting these requirements can be obtained on special order from the Corning Glass Works.

Silica dishes obtained from the Thermal Syndicate, Ltd., have been found satisfactory.

The chromate precipitate is washed twice with ammonium acetate solution. A single thorough washing and draining leaves less than 1 microgram of lead. However, a second washing has been included to assure complete removal of soluble chromate.

The reagent used for development of color consists of dilute, aqueous *sym*-diphenylcarbazide rather than a stronger solution in acetic acid used by Cazeneuve (*2*) and other workers. This modification of the conditions for color development stabilizes the color for hours. Results reported have been obtained by comparison in a visual colorimeter and by means of a photoelectric colorimeter (*17*).

Precipitation of a Double Chromate of Lead

The chromate precipitated under the conditions described differs from ordinary lead chromate in that two equivalents of chromate are present for each equivalent of lead. For this reason the factor 0.5 is introduced into the calculation.

The formation of a chromate differing from ordinary lead chromate was not anticipated and it was only when results consistently twice those expected were obtained that an irregularity was suspected. Lead chromate was then prepared on a larger scale but under conditions similar to those established in the method, except that the precipitate was filtered on a Büchner funnel, washed 10 times by suspending in water, and dried at 110° C. overnight. Titration with thiosulfate established with presence of two equivalents of chromate for each one of lead. The concentration of color per equivalent of lead formed on treating with diphenylcarbazide was exactly twice that given by a standard solution of lead chromate. The low solubility of the compound, together with the practically neutral reaction at which the precipitate formed, excluded lead dichromate. It seemed probable, therefore, that a double chromate of lead and potassium had formed, like that obtained by Lachaud and LePierre (*14*) and by Gröger (*8*) under different conditions. Analysis of 0.0125 gram of the chromate precipitate for potassium by the chloroplatinate method gave 0.00162 gram of potassium as compared with a theoretical value of 0.0018 gram required for $PbCrO_4.K_2CrO_4$. The evidence obtained therefore indicates that the latter compound is formed.

The double chromate forms only within a limited pH range extending from pH 7.4 to 6.6 (Table I) as determined by the glass electrode. When the pH is further decreased, the chromate content of the precipitate decreases until at pH 5.4, or less, lead chromate forms. In selecting conditions for the precipitation of lead as chromate, it was not without hesitation that dependence was placed upon an unfamiliar compound rather than one that has been so thoroughly studied as lead chromate. The doubled sensitivity gained by use of the double chromate, together with the consistent results obtained by analysis of known solutions of lead salts, determined the choice. However, it is entirely feasible by altering the pH of precipitation to carry out the method based upon separation of ordinary lead chromate. Actually this was done for several months while the technique was being developed and before the existence of the double chromate was known and the advantage offered by its use was appreciated. The results were satisfactory. The double chromate is sufficiently stable to withstand washing with ammonium acetate solution. It is not decomposed by cold or hot water.

Previous methods for determination of lead by precipitation as chromate have stressed the importance of separating lead from other metals before proceeding with the precipitation of lead chromate. However, Funk and Schormüller (*7*) found that lead could be precipitated quantitatively from solutions containing copper, mercurous, or mercuric ions. Karaoglanov and Michov (*12*) state that lead can be separated as chromate from copper, silver, nickel, calcium, barium, strontium, manganese, zinc, cadmium, aluminum, and iron. For this reason the authors anticipated no difficulty because of the presence in biological material of other metals listed as having insoluble chromates. It seemed necessary, however, to establish specificity of a method based on the formation of a double chromate. The results of experiments designed to test possible interference from other metals found in biological material are shown in Table II. Each metal was added to blood samples in amounts greatly exceeding the highest possible concentration in this or other tissues and in excreta other than feces. The results demonstrate that interference from these sources is avoided and that omission of preliminary isolation of lead is permissible.

TABLE III. ANALYSIS OF SOLUTIONS OF LEAD ACETATE

Lead Present *Mg.*	Lead Found *Mg.*	Lead Present *Mg.*	Lead Found *Mg.*
0.0050	0.0048	0.0200	0.0200
0.0050	0.0051	0.0300	0.0299
0.0050	0.0050	0.0300	0.0301
0.0100	0.0098	0.0300	0.0300
0.0100	0.0099	0.0300	0.0298
0.0100	0.0100	0.0300	0.0303
0.0100	0.0103	0.0400	0.0398
0.0100	0.0098	0.0400	0.0400
0.0100	0.0101	0.0400	0.0402
0.0100	0.0100	0.0400	0.0405
0.0100	0.0102	0.0400	0.0399
0.0200	0.0201	0.0500	0.0498
0.0200	0.0198	0.0500	0.0502
0.0200	0.0202	0.0500	0.0501
0.0200	0.0204	0.0600	0.0603
0.0200	0.0200	0.0600	0.0600
0.0200	0.0201		

TABLE IV. RECOVERY OF LEAD ADDED TO BLOOD

No.	Blood Lead[a]	Lead Added to Blood	Lead Added to Solution of Ash[b]	Added Lead Recovered	Difference between Added and Recovered Lead
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
1	0.0060	0.0011	0.01	0.0107	−0.0004
2	0.0060	0.0024	0.01	0.0133	0.0009
3	0.0060	0.0036	0.01	0.0140	0.0004
4	0.0060	0.0042	0.01	0.0155	0.0013
5	0.0064	0.0060	0.01	0.0154	−0.0006
6	0.0028	0.0100	..	0.0099	−0.0001
7	0.0028	0.0100	..	0.0100	0
8	0.0028	0.0100	..	0.0100	0
9	0.0028	0.0100	..	0.0101	0.0001
10	0.0028	0.0100	..	0.0100	0
11	0.0028	0.0100	..	0.0098	−0.0002
12	0.0044	0.0100	..	0.0102	0.0002
13	0.0064	0.0120	..	0.0115	−0.0005
14	0.0064	0.0160	..	0.0148	−0.0012
15	0.0028	0.0200	..	0.0200	0
16	0.0044	0.0200	..	0.0204	0.0004
17	0.0064	0.0250	..	0.0270	0.0020
18	0.0028	0.0300	..	0.0300	0
19	0.0044	0.0300	..	0.0304	0.0004
20	0.0044	0.0400	..	0.0403	0.0003

[a] Figures in second and sixth columns can be converted to mg. of lead per 100 cc. by multiplying by 10.
[b] As described in procedure.

TABLE V. COMPARISON OF METHODS FOR ANALYSIS OF BLOOD

Type of Blood	No.	Lead Recovery Diphenylcarbazide	Lead Recovery Dithizone
		Mg. per 100 cc.	
Normal	5258	0.015	0.020
	5128	0.027	0.019
	1189a	0.028	0.045
	5238	0.029	0.049
	888a	0.031	0.028
	928	0.036	0.045
	858	0.048	0.038
	558	0.049	0.051
	4138	0.140[a]	0.037[a]
Lead poisoning	888b	0.080	0.067
	2149	0.103	0.092
	1239	0.110	0.089
	2189	0.117	0.105
	1189b	0.124	0.085
	2119a	0.136	0.115
	1179	0.145	0.145
	2119b	0.150	0.128
	1279	0.167	0.224
		Av. 0.082	0.079

[a] Omitted from calculation of average.

Analysis of Biological Material

Table III demonstrates the suitability of the method for determination of lead in quantities found in samples of clinical material of a size readily obtainable. There is no significant deviation from the theoretical at any concentration of lead tested. It is apparent that addition of 0.01 mg. of lead as directed could be omitted when the lead present exceeds this amount. The authors have found it convenient, however, to make this addition to all unknowns.

It is apparent from Table IV that lead added to blood can be determined with sufficient accuracy to enable the new diphenylcarbazide method to be used with assurance. Lead was added in amounts to include a wide range of values encountered in body fluids.

Table V shows a series of analyses made for the purpose of comparing the results of the diphenylcarbazide method with the dithizone method of Horwitt and Cowgill (*9*). Satisfactory agreement is shown, with one exception, in bloods of both low and high lead concentrations. The difference between the averaged results is not significant. However, at high lead concentrations the diphenylcarbazide method gives slightly higher results than the Horwitt and Cowgill method. This is attributed to somewhat low recoveries given by the latter in this range.

Table VI shows lead concentrations found by means of the new method in blood of normal subjects and of patients who had had no known exposure to lead. The average normal value, 0.03 mg. per 100 cc., is close to the averages found in larger groups by Kaplan and McDonald (*11*), Willoughby and Wilkins (*19*), and Smith, Rathmell, and Marcil (*18*) who made analyses by the dithizone method. The table includes also, in the right-hand column, typical results obtained in the presence of chronic lead intoxication.

Table VII shows slightly higher results by the diphenylcarbazide method than by Fairhall's titrimetric method (*5*) for determination of lead in urine. However, it is the experience of the authors as well as of others that the Fairhall method tends to give low results, and the differences found do not exceed errors encountered by them in the past in applying the method to analysis of urine. The upper limit of normal urinary lead excretion has been established tentatively at 0.08 mg. per 24 hours. The new method has been used extensively also for analysis of tissues and in Table VIII are shown typical results for kidney. Because case histories and other evidence bearing on diagnoses of certain of the patients included are incomplete, it is not intended that the table be used as a guide to post-mortem diagnosis. Instead, it is presented to demonstrate that tissues containing lead in amounts that vary over wide limits can be analyzed without difficulty. Among other tissues that have been examined by means of the new method are brain, liver, spleen, bone, fingernails, hair, and pituitary and pineal glands. Lead was found in all except fingernails and hair.

While no data are shown, the diphenylcarbazide method has been applied to the analysis of a variety of foods. Analysis of feces by the new method gave results in close agreement with those given by the dithizone method.

The method has been used for about 2 years for routine lead analysis in Philadelphia General Hospital, and results have correlated well with clinical findings. Where unusual or unexpected results were obtained, verification by means

TABLE VI. BLOOD LEAD CONCENTRATIONS MEASURED BY NEW DIPHENYLCARBAZIDE METHOD

Lead in Normal Blood No.	*Mg./100 cc.*	Lead Poisoning No.	*Mg./100 cc.*
1	0.009	22	0.060
2	0.010	23	0.064
3	0.014	24	0.080
4	0.014	25	0.080
5	0.015	26	0.091
6	0.015	27	0.095
7	0.024	28	0.095
8	0.027	29	0.098
9	0.028	30	0.099
10	0.029	31	0.103
11	0.030	32	0.110
12	0.030	33	0.110
13	0.031	34	0.111
14	0.035	35	0.117
15	0.036	36	0.124
16	0.038	37	0.134
17	0.040	38	0.136
18	0.048	39	0.136
19	0.048	40	0.140
20	0.049	41	0.146
21	0.050	42	0.150
47	0.028	43	0.167
		44	0.189
		45	0.235
		46	0.080
Mean	0.0295 ± 0.0029 (standard error)		

TABLE VII. COMPARISON OF METHODS FOR ANALYSIS OF URINE

No.	Diphenylcarbazide Method	Fairhall Method	Difference
	Mg. of lead per 24 hours		
1	0.025	0.038	−0.013
2	0.036	0.040	−0.004
3	0.059	0.050	0.009
4	0.088	0.100	−0.012
5	0.140	0.110	0.030
6	0.203	0.210	−0.007
7	0.210	0.207	0.003
8	0.250	0.200	0.050
9	0.310	0.240	0.070
10	0.447	0.375	0.072

TABLE VIII. ANALYSIS OF KIDNEY FOR LEAD BY DIPHENYLCARBAZIDE METHOD

No.	Normal or Unexposed[a] Mg./100 g.[b]	No.	Lead Poisoning Proved or Suspected Mg./100 g.[b]
1	0.54	15	1.50
2	0.55	16	1.62
3	0.58	17	1.65
4	0.58	18	1.70
5	0.58	19	2.22
6	0.58	20	2.27
7	0.63	21	4.10
8	0.68	22	4.51
9	0.74	23	4.60
10	0.74	24	5.00
11	0.90	25	5.33
12	1.10	26	5.80
13	1.10	27	7.69
14	1.13	28	14.00

[a] No recorded symptoms attributed to lead and no known recent exposure.
[b] Mg. in 100 grams of whole dry kidney, medial slice, including cortex and medulla.

of the dithizone method has steadily increased confidence in the new procedure. Experience indicates that the method is suitable for use as a diagnostic aid in establishing the presence of lead poisoning or absorption of lead.

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Mortar and Pestle for Powdering Glass

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AN INEXPENSIVE, convenient, and safe mortar and pestle for powdering glass have been devised in this laboratory. The mortar consists of 5 inches of 1-inch iron pipe (nipple), on one end of which is screwed a pipe cap, loosely fitted in order to facilitate removal of the powdered glass. The pestle is a 16-inch length of 0.75-inch iron rod, threaded at one end and fitted with a large iron nut having a diameter a little greater than 1 inch. The nut is tightly adjusted to the rod and trimmed by a silicon carbide wheel to a size that will permit it to be inserted with ease into the mortar. The nut and rod are ground at the end until a flat pounding surface is attained.

Identification of 2-Aminoethanol

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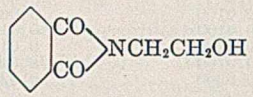
IN AQUEOUS solution 2-aminoethanol (ethanolamine, monoethanolamine) can be identified readily by reacting with phthalic acid and confirming the melting point, 127° C., of the resulting 2-hydroxyethylphthalimide:



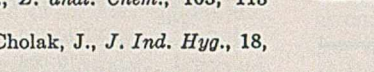
PROCEDURE. Mix equimolar proportions of phthalic acid and the 2-aminoethanol—for example, dissolve 0.166 gram of phthalic acid in hot water and add it to the aqueous solution containing 0.061 gram of 2-aminoethanol. The concentration of 2-aminoethanol in the sample must be assumed or be determined by some means such as titration with hydrochloric acid. One cubic centimeter of 1 *N* hydrochloric acid ≈ 0.061 gram of 2-aminoethanol.

Evaporate the solution to dryness and heat the residue at 210° C. for about 5 minutes. 2-Hydroxyethylphthalimide (*2*) will be formed in quantitative yield. Recrystallize from water, or, if inorganic impurities such as potassium chloride or sulfate are present, from an anhydrous solvent such as benzene or absolute alcohol. The resulting diamond-shaped plates melt at 127° C.

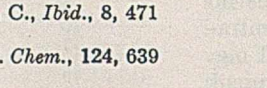
Conversely, this reaction can be used for the identification of phthalic acid.

Identification by Oxalic Acid

2-Aminoethanol can also be identified in aqueous solution by reacting with oxalic acid and confirming the melting point, 199–200° C. uncorrected (correction, +3.8° C.), of the resulting salt formed by the oxalic acid and the amine



In addition, this salt may be converted to N,N′-bis-(2-hydroxyethyl)-oxamide, which has a melting point of 168° C. uncorrected (correction, +2.4° C.).



PROCEDURE. Mix equimolar proportions of oxalic acid and 2-aminoethanol—for example, dissolve 0.090 gram of oxalic acid in water and add it to the aqueous solution containing 0.061 gram of the 2-aminoethanol. Evaporate the solution to dryness and heat the residue at 110° C. for 5 minutes. Recrystallize from 70 per cent alcohol. The resulting elongated hexagonal plates melt with decomposition at 199–200° C., uncorrected. The salt is very soluble in water, and insoluble in absolute alcohol or glacial acetic acid.

Analysis for $C_6H_{16}O_6N_2$: calculated, N 13.20, oxalic acid 42.5; found, N 12.65, oxalic acid 44.0 per cent.

If heated past the melting point to about 222° C., brisk boiling occurs with formation in good yield of N,N′-bis-(2-hydroxyethyl)-oxamide, melting point 168° C., uncorrected. This material, prepared in a different manner, has been previously described (*1*).

This reaction may also be used for the identification of oxalic acid.

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An Improved Mobilometer

For Measuring Consistency of Fluid and Semifluid Greases

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The Gardner mobilometer, which is a special type of falling-weight viscometer, is reasonably satisfactory for determining the consistency of fluid and semifluid greases, but has a number of defects which have been corrected in a new and improved instrument, designated as the S. I. L. mobilometer.

A NEED has long been felt for an instrument or a method for the determination and expression of the consistency of fluid and semifluid greases that are too mobile to be tested with the A. S. T. M. penetrometer (*1*). The latter gives satisfactory results for the solid or heavier types of greases but with soft greases having penetrations higher than 360 the results are usually erratic. Efflux viscometers such as the Saybolt are not suitable for testing these products, which are not true liquids and which sometimes change their characteristics when heated.

Torsion viscometers such as the MacMichael (*5*) or Stormer (*2*, *4*, *8*) have been used for testing fluid or semifluid greases but are not usually regarded as entirely practical for routine use in the petroleum industry. The Gardner mobilometer (*3*, *6*, *7*), which is a special type of falling-weight viscometer, has been considered preferable to the torsion type of instrument, and is rather extensively used.

The Gardner instrument is supplied with a set of three perforated disks and may be operated with a variety of loads, so that it is applicable to practically the entire range of fluid and semifluid greases. As used in the authors' laboratory, however, it was found to have a number of defects, and the work undertaken to remedy them resulted in the development of a new instrument, which differs from the Gardner in so many respects that it has been designated as the S. I. L. (Standard Inspection Laboratory) mobilometer.

The characteristic features of the S. I. L. mobilometer are: (1) the use of a perforated cone instead of a perforated plate as the part that moves through the oil or grease under test; (2) the use of a positive but almost frictionless guide bearing for the rod that carries the cone and weights; and (3) a general design that permits rugged, accurate construction, resulting in maximum ease and convenience in operation.

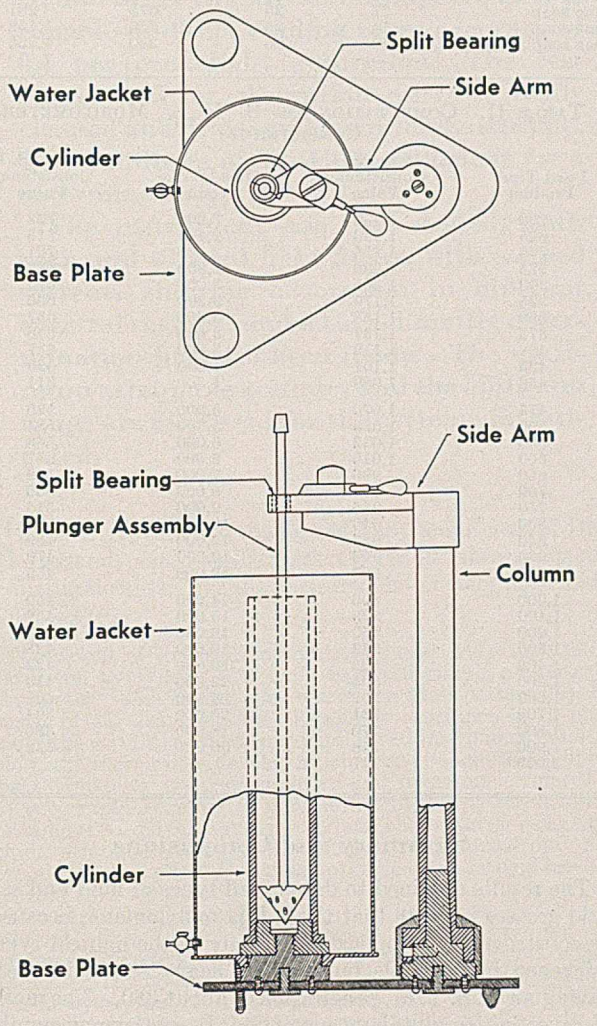

FIGURE 1. ASSEMBLY OF MOBILOMETER

Apparatus

Figure 1 shows diagrammatically the assembly of the instrument. It consists essentially of (1) a base plate with a column and side arm to carry the split bearing, (2) a water jacket to control temperatures, (3) a cylinder to hold the sample under test, and (4) a plunger assembly consisting of a shaft having a carefully designed perforated cone mounted on the lower end and a weight holder on the upper end. An accessory device shown in Figure 2 is a "worker", consisting of a perforated disk mounted on a rugged shaft. Figure 2 shows details of the perforated cone, the weight holder, and the weights.

The cylinder, which screws into the base plate, is patterned after the Gardner mobilometer cylinder and is fitted with a removable bottom to facilitate cleaning. It is made of metal sufficiently heavy to permit machining to a uniform diameter of 3.9 cm. throughout its length of 23 cm. A filling mark is inscribed on the inside of the cylinder, 20 cm. from the bottom. The cylinders are interchangeable and, if desired, a number of them may be used with one instrument.

The base plate, equipped with leveling adjusters, supports a vertical rod and side arm which in turn holds a split bearing situated directly over and 5 cm. (2 inches) above the cylinder. The vertical support moves on a pivot, allowing the arm to swing away from the cylinder. The split bearing serves as a guide for the plunger and permits the removal of the plunger assembly for cleaning before and after tests.

The plunger assembly consists of a light (15 grams) or heavy (90 grams) shaft having a weight holder attached to one end and a removable cone attached to the other end. The shafts are made of stainless steel 30.5 cm. long and 6.35 mm. in diameter, with two marks 10 cm. apart inscribed thereon. The cone is made from an aluminum shell having an angle of 75°. It is 2.54 cm. in height, 3.8 cm. in diameter at the base, and has a center shank for attaching to the shaft. Two rows of seven equally spaced holes are drilled parallel to the axis of the cone and seven equally spaced semicircular recesses or notches are located in the outer rim. The holes of the outer row are staggered in relation to the notches

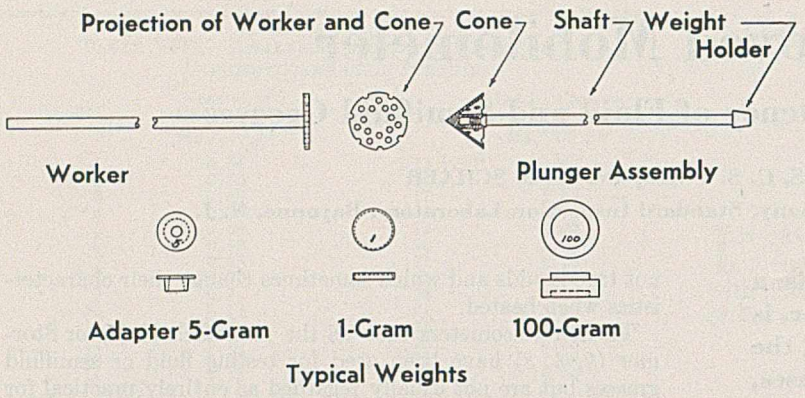

FIGURE 2. DETAILS OF PLUNGER ASSEMBLY, WORKER, AND WEIGHTS

and the holes in the inner row. The diameters of the holes and the recesses are 3.15 and 4.15 mm., respectively. The diameters of the circles passing through the centers of the holes in the inner and outer rows are, respectively, 2.937 and 4.604 cm. The total weight of the plunger assembly is 25 grams when the light shaft is used and 100 grams when the heavy shaft is used.

The weights, ranging from 1 to 300 grams, are interlocking and are recessed to fit the weight holder on the plunger assembly and to fit each other. They are so designed that the load will be applied directly over the center of the plunger, thus preventing side pressure which would occur with an unbalanced load.

The worker consists of a rod and perforated disk. The perforations are drilled to correspond with the holes and notches in the cone and are of the same size.

The bath is of 1.9-liter (2-quart) capacity and has a spigot located at the bottom for drainage purposes. It screws into the base plate and is recessed to allow the cylinder to be screwed into it.

Procedure

The cylinder is filled to the 20-cm. mark with the grease to be examined, and the cylinder is placed in the bath and brought to the test temperature of 77° F. (25° C.). The worker is passed through the grease with ten up and ten down strokes. The time per single stroke should not be less than about 3 seconds and the worker must be almost, but not completely, withdrawn from the grease between strokes, as this may cause the inclusion of air bubbles. The 25-gram plunger assembly, without added weights, is fitted into the split bearing and the point of the cone is brought to the surface of the grease.

The assembly is released and the time for the two marks on the shaft to pass a reference point is recorded by means of a stop watch. The marks on the shaft are so placed that the timing period indicates the passage of the cone through the center portion of the sample being tested. If this time is 32 seconds or less, three runs are made and the consistency number is calculated from the formula, using the average time. If the time of passage exceeds 32 seconds, weights are added to the plunger assembly until the time of passage is reduced to between 30 and 32 seconds. Two additional determinations are made with these added weights. The average time of the three determinations is used in calculating the consistency number.

The consistency number of the sample is calculated from the following formula:

$$\text{Consistency number} = \frac{5000}{\sqrt[4]{L \times T}}$$

L = load, or weight of plunger assembly + grams added
T = time, in seconds

Data

RANGE OF INSTRUMENT. By the use of the above formula arbitrary values have been calculated for products ranging in consistency from an approximate A. S. T. M. penetration of 380 to approximately 300 seconds' Saybolt viscosity at 100° F. From these values, greases may be designated as having consistency numbers from 380 to 1330 as determined by the S. I. L. mobilometer, the lighter products having the higher consistency numbers.

PRECISION OF INSTRUMENT. The times of tests obtained with this instrument are reproducible to within a fraction of a second if the bath temperature is maintained to ±0.1° F. and if the material tested is homogeneous.

RESULTS. Table I shows results of tests of typical samples of greases ranging from the semisolid to the most fluid type and between the two limits of the S. I. L. mobilometer.

Table II shows consistency numbers corresponding to a series of load-time figures and eliminates the necessity of making computations unless an unusual degree of precision is necessary. In case it is preferred to use a chart instead of a table to avoid computations, one can be prepared by plotting two points from Table II on log-log paper and drawing a straight line through them.

TABLE I. S. I. L. CONSISTENCIES OF TYPICAL SAMPLES

	Time *Sec.*	Load *Grams*	Load-Time Product	Arbitrary S. I. L. Consistency Numbers at 77° F.
Lime soap	31	510	15,810	445
Soda soap	32	400	12,800	471
Soda and lime soap	31	240	7,440	540
Soda soap	30	225	6,750	553
Aluminum soap	31	160	4,960	597
Lime soap	31	155	4,805	601
Soda and lime soap	30	125	3,750	640
Lime soap	31	100	3,100	670
Soda and lime soap	32	85	2,720	691
Aluminum soap	31	75	2,325	720
Lime soap	32	45	1,440	814
Lime soap	30	32	960	900
Lime soap	27	25	675	981
Lime soap	14	25	350	1,158
Lime soap (very fluid)	10	25	250	1,255

TABLE II. COMPUTATION OF S. I. L. MOBILOMETER CONSISTENCY

Load-Time Product	Arbitrary S. I. L. Consistency Value	Load-Time Product	Arbitrary S. I. L. Consistency Value
200	1,330	2,200	732
225	1,290	2,400	715
250	1,255	2,600	701
275	1,230	2,800	688
300	1,205	3,000	675
325	1,179	3,200	666
350	1,158	3,400	657
375	1,137	3,600	646
400	1,119	3,800	637
425	1,101	4,000	629
450	1,089	4,500	611
475	1,070	5,000	595
500	1,059	5,500	581
550	1,032	6,000	568
600	1,010	6,500	557
650	990	7,000	549
700	973	8,000	530
750	955	9,000	515
800	940	10,000	500
850	926	11,000	489
900	912	12,000	479
950	900	13,000	469
1,000	890	14,000	460
1,100	870	15,000	452
1,200	850	16,000	445
1,300	833	18,000	428
1,400	820	20,000	422
1,500	805	22,000	410
1,600	792	24,000	401
1,700	781	26,000	394
1,800	770	28,000	386
1,900	758	30,000	379
2,000	747		

Summary and Conclusions

The results obtained to date on all types of fluid and semifluid greases indicate that the S. I. L. mobilometer, because of its construction, is applicable not only to the lightest type of thickened mineral oils but also to heavy semifluid greases having an A. S. T. M. penetration of about 380. The method of reporting results in terms of S. I. L. consistency numbers

should be helpful in understanding the relative fluidity of those greases having consistencies which cannot be determined with the A. S. T. M. penetrometer.

The authors are also of the opinion that this instrument might be applied successfully to other fluid and semifluid products, such as paints, varnishes, emulsions, etc.

Acknowledgment

The authors extend thanks to E. W. Dean and C. A. Neusbaum for their assistance and advice in the development of the instrument. Acknowledgment is extended to A. Beerbower, of the Esso Laboratories, for preparing the consistency table.

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Thermoelectric Absorptiometer for Analytical Work

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An improved thermoelectric absorptiometer employing thermopiles has been constructed. The thermopiles are steady, sensitive, and rapid, and are used in a balanced circuit that compensates for slight fluctuations in source intensity. The instrument is rugged and compact, and is capable of direct reading to an accuracy of 0.1 per cent light absorption. The test tubes used for absorption cells are easily cleaned and dried, and are interchangeable. Ten milliliters of liquid are sufficient for a determination.

The instrument has been tested, both with and without light filters, with colored systems showing absorption in different spectral regions, and a turbidimetric determination has also been made. The maximum attainable accuracy and the optimum range are discussed for the systems investigated.

SEVERAL years ago the firm of Kipp and Zonen of Delft, Holland, placed on the market the Moll "absorptiometer" or "extinctiometer" for the measurement of color or turbidity of liquids (*8*).

The circuit of the Moll instrument is essentially the same as that given by Wilcox (*17*) for a balanced circuit photoelectric colorimeter. The thermopiles are of the Moll pattern (*9*), and consist of 17 elements; the thermopile resistance is 10 ohms. Under the conditions used in the improved instrument described below, the thermopiles develop a potential of about 25 millivolts. The thermopiles are very sensitive and free from errors; they are very rapid—coming to equilibrium in 2 or 3 seconds—and hence have an advantage over certain photoelectric cells which exhibit a fatigue effect (*16*, *21*) which may disappear only after several minutes, although a claim of little or no fatigue has been reported (*4*).

The Moll absorptiometer appears to have been put to very little use. Van Tussenbroek (*14*) made a comparative study of various colorimeters; he used the Krüss colorimeter, Lovibond tintometer, Hilger-Nutting spectrophotometer, Keuffel and Esser color analyzer, and Moll absorptiometer, and concluded that the Moll instrument gave the most reproducible results. He also studied the use of glass filters for taking readings with different wave lengths of light (*15*).

In spite of the fact that the thermopile absorptiometer is rapid and sensitive, and therefore capable of serving as a useful analytical tool, the instrument as put on the market has many serious defects. The lamp, thermopiles, and cell holders are mounted on standards on an optical bench; each of the five units has horizontal, vertical, and rotational adjustments, and the task of keeping the units in permanent optical alignment during use is no small problem. The cell holders are not provided with stops to ensure that the absorption cells are placed always in the same lateral position; a slight change in lateral position was shown by the authors to influence the reading. Furthermore, different absorption cells are not of the same optical characteristics, and are therefore not interchangeable. The lamp provided has a long filament and the lenses do not provide for bringing all of the light beam onto the thermopiles, so that considerable radiation is not being utilized. The divided shunt can give direct reading only to the nearest 1 per cent; greater accuracy requires interpolation from galvanometer readings, which is inconvenient. The complete assembly suffers from lack of compactness and stability.

Construction of the Instrument

The essential features of construction of the new instrument are shown in Figure 1.

W is a 6-volt 50-candle-power small filament lamp; L, lens systems; S, shutters, operated from front panel of instrument box; and F, light filters. A_1 and A_2 are absorption cells, consisting of 20 × 85 mm. Pyrex test tubes which fit snugly into brass holders; T_1 and T_2, Moll thermopiles; P, circular slide-wire of approximately 140 ohms, scale graduated from 0 to 100 and capable of being read (not estimated) to 0.1 per cent; G, pointer-type panel-mounting galvanometer (G-M Laboratories, No. 2561-D); and R, 20-ohm variable resistance for balancing slight inequalities in making the zero adjustment. The lamp, cell holders, and thermopiles, in fixed positions for alignment in the optical axis of the system, are mounted on units movable on a track and are locked in position after adjustment for optical balance. The circuit used has automatic compensation for slight fluctuations in source intensity (*10*).

Figure 2 is a photograph of the new instrument. The case measures 37 × 24 × 12 cm. The lamp housing and the top and bottom of the case are provided with ventilating holes; compressed air introduced through the bottom of the case aids in conducting away the heat generated by the lamp. Tests showed that the reading on a stable colored solution was the same whether the instrument was cool or hot; however, air-cooling would undoubtedly be advantageous when measuring unstable systems.

In regard to cost of construction, the largest single item for parts is the cost of the thermopiles. In October, 1939, Moll small-surface thermopiles No. E2 were quoted by Kipp and Zonen at 45 Dutch guilders (approximately $24) each, net; import duty is assessed at 40 per cent. A duplicate of the instrument

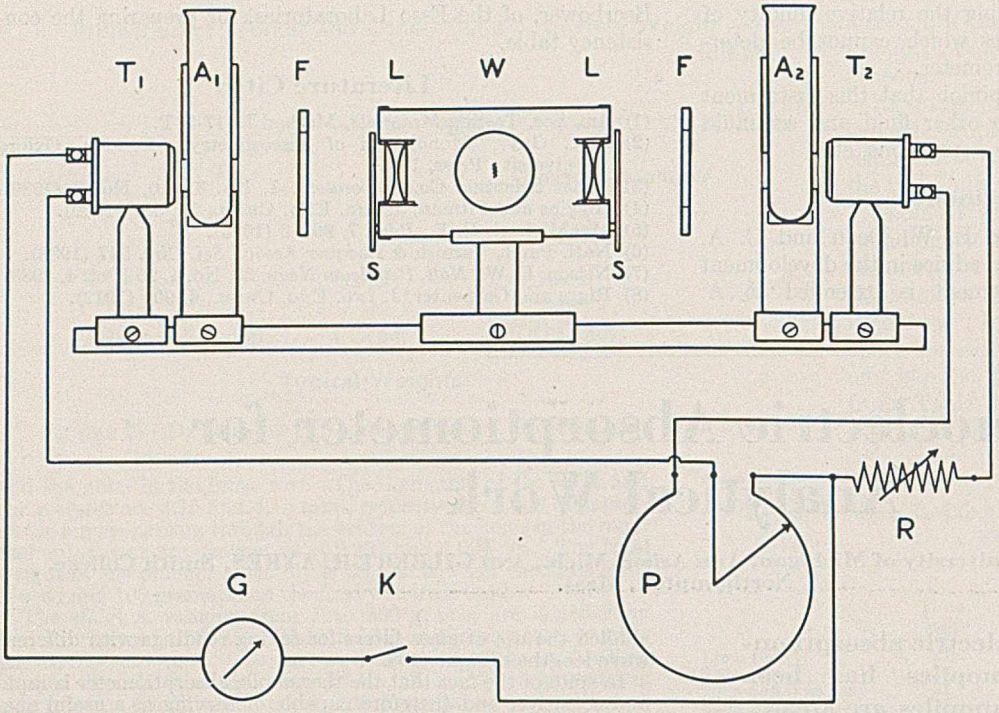

FIGURE 1. DIAGRAM OF IMPROVED THERMOELECTRIC ABSORPTIOMETER

described above is being constructed at a total cost, for parts only, of $140 to $170 depending on the type of galvanometer selected.

Method of Operation

The test tubes used for absorption cells are carefully selected for diameter to fit the holders, and for freedom from imperfections in the glass. For the purpose of matching the absorption tubes, a tube containing liquid—for example, water, or better, the blank solution to be used in a determination—is placed in each cell holder. The optical system having been brought previously to approximate balance by adjustment of the units on the track, the electrical circuit is now brought to balance by means of the variable resistance. The tube on the right is then slowly rotated, while the galvanometer circuit is closed, until a position is found at which the galvanometer shows little deflection on slight rotation of the tube. This tube is then marked with a fine scratch to coincide with a mark on the end of the cell holder. The tube on the left is then rotated to locate on it a similar position, and one at which the galvanometer stands at zero; slight adjustment of the variable resistance may be necessary to obtain the latter condition. Additional tubes may be marked in the same way against the right tube serving as a "standard". By careful selection and matching, the various working cells are interchangeable against the standard tube.

As in most objective methods, this instrument requires a blank solution which contains all components, in proper concentration, except the one being determined. The authors' experience has been that absorption tubes which match when filled with one liquid do not necessarily match when filled with another. It is necessary, therefore, to readjust the variable resistance to bring the system into balance, when changing from one series of measurements to another. In fact, the zero setting is frequently checked during a series of measurements to be sure that the instrument is in proper adjustment.

The instrument is calibrated for a determination by measuring the absorption of a series of solutions of known concentrations, from which a calibration curve is constructed. When connected as shown in Figure 1, the reading of the slide-wire scale gives the percentage absorption by the liquid in A_1; by reversal of the connections to the slide-wire, the scale would read percentage light transmission. The former is considered more simple in practice, since low concentrations give low scale readings.

Use of Light Filters

In all objective colorimetric methods, greater accuracy and sensitivity are obtained by utilizing only that light which is most strongly absorbed by the system being measured. In order to approach this condition, suitable filters can be used; for very accurate work, their selection must be made with care (*10*). For use with the instrument described above, several Corning glass filters were available; each filter was 1 mm. thick, and four identical glasses of each kind were provided. The filter slot of the instrument accommodates two filter frames, so that the glasses can be used singly, in pairs of the same glass for greater filter density, or in pairs of different filters for better spectral isolation. Transmission curves for the filters are given by the manufacturer (*5*); however, the energy distribution of the light source influences the effective filtering action. In Table I the absorption regions indicated were determined by direct visual observation with a spectroscope, using a Mazda lamp source.

TABLE I. CHARACTERISTICS OF LIGHT FILTERS

Corning No.	Description	Principal Absorption Regions
227	Gold ruby	Some blue, considerable green
246	Lighthouse red	Violet through green, some yellow
346	Amber shade A	Some far violet
396	Light shade Aklo	Far red, essentially a heat filter. Given by maker, for 2-mm. thickness and Mazda source: transmits 75% of visible radiation, 21% of total radiation
401	Sextant green	Violet and red, some blue and orange
430	Dark shade blue-green	Red, some orange and violet
556	Signal blue	Absorption bands in red, yellow, green
585	Blue purple ultra	Absorption bands in red, orange, green; high transmission in violet, blue, and far red. Makers indicate high transmission for infrared also

The thermopile is a nonselective radiometer (*7*); hence it develops a potential which is proportional to the total energy absorbed by the receiver. As the radiation from an incandescent tungsten filament consists mostly of the longer wave lengths, absorption measurements made without filters indicate the total absorption by the solution. By the use of infrared filters, the light incident upon both thermopiles lies

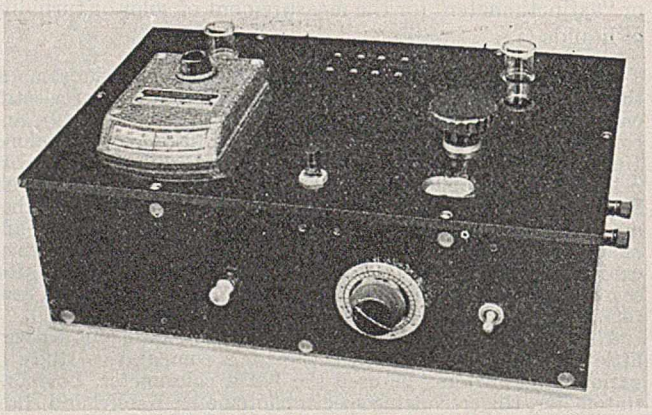

FIGURE 2. THERMOELECTRIC ABSORPTIOMETER

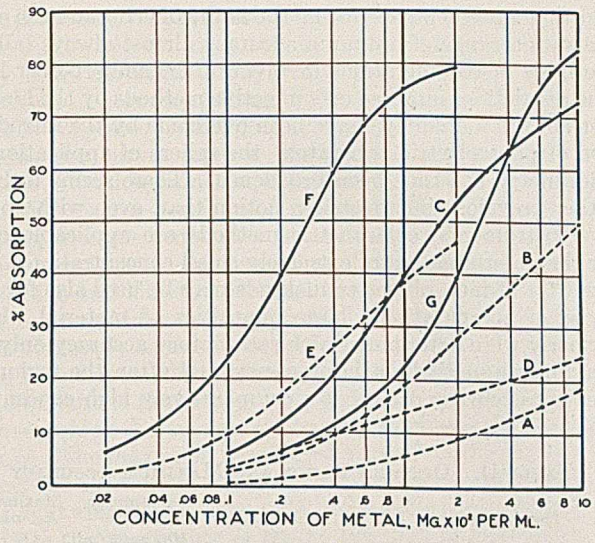

FIGURE 3. CALIBRATION CURVES

A. Manganese as permanganate, no filter
B. Manganese as permanganate, 396 filter
C. Manganese as permanganate, 430 filter
D. Iron as ferric thiocyanate, no filter
E. Iron as ferric thiocyanate, 396 filter
F. Iron as ferric thiocyanate, 430 filter
G. Zinc with ferrocyanide (turbidimetric)

essentially in the visible region, and although this is a small fraction of the total radiation from the source, the light reaching the thermopiles after traversing the colored solution and blank solution, respectively, shows a greater differential than when no filters are used (see, however, the exception noted in the next paragraph), so that a higher slide-wire setting is necessary to obtain electrical balance of the system. Hence, for a given colored substance in solution, a steeper calibration curve is obtained by the use of heat filters than without them; the colorimetric determination therefore becomes more accurate, provided the instrument can be balanced with the same accuracy. When the use of filters reduces the total radiation reaching the thermopiles to a considerable extent, the galvanometer current becomes less for a given unbalance in the circuit; it is then necessary to use a more sensitive galvanometer—for example, G-M No. 2564-D—in order to obtain an accurate balancing by the slide-wire. Similar considerations apply not only to infrared filters, but also to filters for isolation of spectral regions in which the solution shows maximum absorption.

When the system being measured itself shows considerable infrared absorption, the above considerations regarding increased accuracy by the use of infrared filters do not apply; in fact, the use of such filters would decrease the accuracy. The data on copper chloride and on ferrous sulfate solutions clearly illustrate this point; for these systems, compared with the curves obtained without filter, the No. 396 filter gave flatter curves, whereas steeper curves were obtained by a filter such as No. 585 which has a high red and infrared transmission. In all other cases thus far measured, the use of No. 396 filter gave higher readings, for a given concentration, than when no filter was used, although the No. 396 filter was not necessarily the best one to use from the standpoint of increased sensitivity.

In the determinations outlined below, the indicated absorption by the solutions was determined by visual observation with a spectroscope using a Mazda lamp source. Although a filter selection could usually be made by comparing the absorption by the solution with the observed transmission of the filters, the final selection of a filter was made by actually measuring with the instrument the absorption of a solution by the use of various filters or filter combinations; the filter or combination giving the highest instrument reading was chosen as being the most suitable.

Results

MANGANESE AS PERMANGANATE. Permanganate standards were made in solution containing sulfuric acid, phosphoric acid, and potassium periodate, so as to simulate the conditions used in the Willard and Greathouse method for manganese (*19*). The solutions show absorption of violet through orange if concentrated, and absorption bands in green if dilute. The data obtained are shown in curves *A*, *B*, and *C* of Figure 3. A permanganate calibration curve has been utilized in checking the concentration of solutions of potassium permanganate, and also in determining the solubility of tetraphenyl arsonium permanganate in sodium chloride solutions of various concentrations (*18*).

IRON AS FERRIC THIOCYANATE. The method followed was essentially that given by Snell and Snell (*13*); the standard solution consisted of ferric alum in 0.5 per cent sulfuric acid. Concentrated solutions absorb all but the red wave lengths; as the concentration decreases, absorption of orange, green, and blue disappears. As the solutions show considerable fading, colorimetric readings were made at a definite time interval following development of the color. The data are represented by curves *D*, *E*, and *F* of Figure 3.

COPPER AS CUPRIC CHLORIDE IN 28 PER CENT HYDROCHLORIC ACID. The method of Hüttner (*6*), slightly modified, was used. In spite of an indicated inadvisability of trying to keep permanent standards of cupric chloride in concentrated hydrochloric acid (*12*), the standards in 28 per cent hydrochloric acid did not change in 5 months. The solutions are greenish yellow in color and show absorption in violet, blue, and far red, as well as infrared. The results are shown in Figure 4, curves *A*, *B*, and *C*.

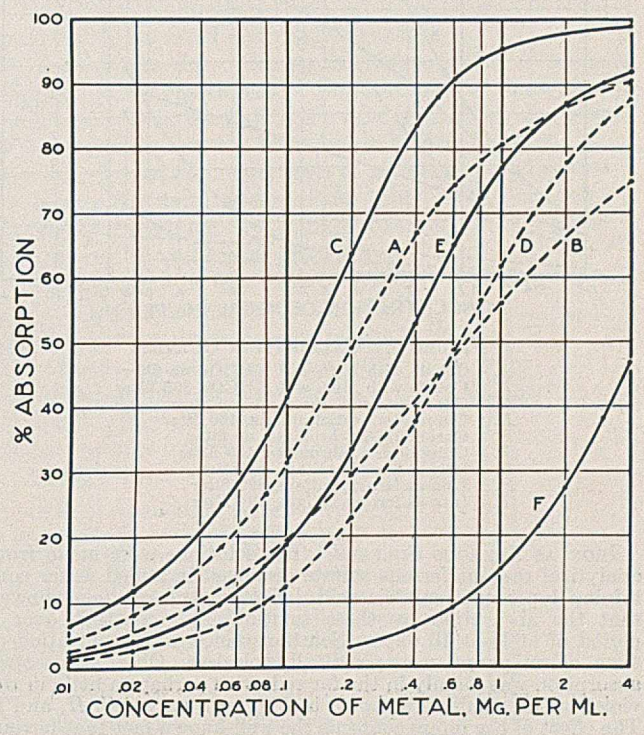

FIGURE 4. CALIBRATION CURVES

A. Copper as $CuCl_2$ in 28 per cent HCl, no filter
B. Copper as $CuCl_2$ in 28 per cent HCl, 396 filter
C. Copper as $CuCl_2$ in 28 per cent HCl, 556 and 585 filters
D. Copper as cupric-ammonia complex, no filter
E. Copper as cupric-ammonia complex, 396 filter
F. Nickel as nickel-ammonia complex, no filter

COPPER AS CUPRIC-AMMONIA COMPLEX ION. The stock standard solution was cupric nitrate prepared from metallic copper. Each solution of the standard series contained 20 per cent by volume of concentrated ammonia. The deep blue solutions show absorption in the red, orange, and yellow regions. Data are shown as curves *D* and *E* of Figure 4.

NICKEL AS NICKEL-AMMONIA COMPLEX ION. The method of Ayres and Smith (*1*) was used. Measurements cannot be extended above a nickel concentration of about 4 mg. per ml. on account of presence of a small amount of precipitate. The solu-

tions are blue with a tinge of violet; they show some absorption in yellow and yellow-green regions, and also a little in the violet. Curve *F* of Figure 4 shows the results, readings being taken without light filter. No filter of those listed in Table I materially changed the slope of the curve.

COBALT BY HYDROGEN PEROXIDE AND POTASSIUM BICARBONATE. By the method of Blanchetière and Pirlot (*2*), cobalt produces a green color attributed to a hydrocarbonate. Measurements cannot be extended above a cobalt concentration of 4 mg. per ml. on account of the development of turbidity. The solutions exhibit absorption for all wave lengths except yellow and green. Curves *A* and *B* of Figure 5 represent the results.

CHROMIUM AS DICHROMATE. Standard series solutions consisted of potassium dichromate in 1 per cent acetic acid. The system shows absorption of violet, blue, and some green. In Figure 5, curves *C* and *D* indicate that the method is unsatisfactory unless a more suitable light filter is used.

CHROMIUM AS CHROMATE. The solutions consisted of potassium chromate in solution with 0.1 per cent potassium carbonate. The solutions absorb violet and blue. Reference to Figure 5, curve *E*, indicates that the system is not capable of accurate measurement by the instrument without light filters. The best filter available was No. 430; even with this filter (see curve *F*) the determination of chromate is inaccurate.

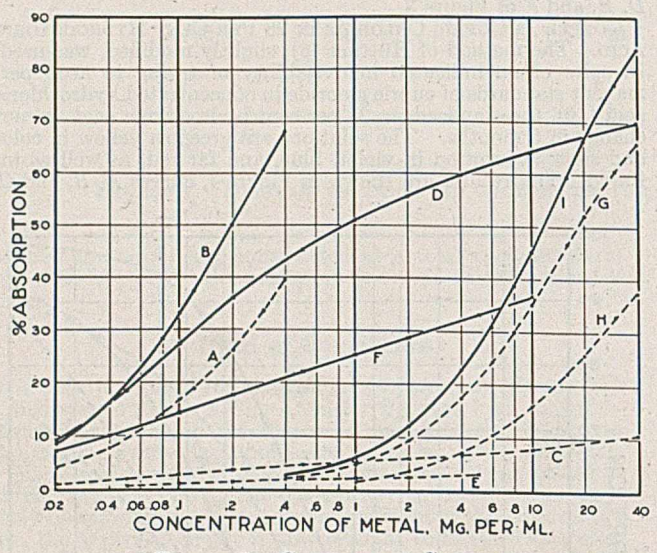

FIGURE 5. CALIBRATION CURVES

A. Cobalt with H_2O_2 and $KHCO_3$, no filter
B. Cobalt with H_2O_2 and $KHCO_3$, 396 filter
C. Chromium as dichromate, no filter
D. Chromium as dichromate, 430 filter
E. Chromium as chromate, no filter
F. Chromium as chromate, 430 filter
G. Iron as ferrous sulfate, no filter
H. Iron as ferrous sulfate, 396 filter
I. Iron as ferrous sulfate, 585 filter

IRON AS FERROUS SULFATE. The solutions were made from analytical reagent ferrous sulfate dissolved in boiled water containing 1 per cent sulfuric acid. Photometric readings showed that the absorption by these solutions was constant over a period of at least 10 days. Solutions below a concentration of 4 mg. of iron per ml. were practically colorless. Observed spectral absorption was mainly in the far red, with perhaps a little in the violet. The results are shown in Figure 5, curves *G*, *H*, and *I*. The effect of the filters confirms the well-known fact that ferrous sulfate solutions absorb in infrared. This case presents an interesting application of the absorptiometer in measuring a solution which has little visible color; although the concentration range of best accuracy is rather high, the curve with filter No. 585 is one of the steepest thus far obtained.

ZINC WITH POTASSIUM FERROCYANIDE. This determination was made in order to show the applicability of the instrument to turbidimetric methods. The procedure was essentially that of Bodansky (*3*) as concerns the development of the turbidity. The calibration curve shown in Figure 3, *G*, was used with satisfactory results in the determination of zinc after separation from iron (*20*).

Discussion

Recently Ringbom (*11*) has called attention to the fact that the accuracy which in the literature has been ascribed to colorimetric methods has been very arbitrarily established, and that statements regarding the limits of error and the concentration range of optimum accuracy almost always fail on the basis of considerations involving the Lambert-Beer law. The restrictions imposed on subjective methods by the limitations of the human eye have been overcome by the introduction of photoelectric apparatus; the sphere of application of colorimetry has thus been broadened. There seems to be a rather prevalent but mistaken notion that, even with objective instruments, colorimetric methods are applicable only for the determination of extremely small concentrations. As a matter of fact, objective methods may be used also for analysis of comparatively large quantities of material which formerly were determined with satisfactory accuracy only by gravimetric or titrimetric processes, and often the region of greatest accuracy occurs at a comparatively high concentration.

TABLE II. OPTIMUM RANGE AND MAXIMUM ACCURACY

Determination	Filter	Optimum Range	Maximum Accuracy
		Mg. metal/ml.	%
Manganese as permanganate	396	0.01–>0.1	0.7
	430	0.004–0.03	0.5
Iron as ferric thiocyanate	None	0.001–>0.1	2.3
	396	0.001–0.02	0.8
	430	0.0015–0.006	0.4
Copper as $CuCl_2$ in 28% HCl	None	0.1–0.5	0.4
	396	0.2–0.8	0.6
	556 + 585	0.08–0.5	0.3
Copper as cupric-ammonia	None	0.3–1.5	0.4
	396	0.2–1.0	0.3
Nickel as nickel-ammonia	None	2–4	0.3
Cobalt with H_2O_2 + $KHCO_3$	None	0.15–0.4	0.5
	396	0.1–0.4	0.4
Chromium as dichromate	430	0.02–0.3	0.8
Chromium as chromate	430	<0.01–>10	1.9
Iron as ferrous sulfate	None	8–30	0.4
	396	15–>40	0.6
	585	6–30	0.3
Zinc with ferrocyanide (turbidimetric)	None	0.015–0.05	0.3

The accuracy with which a given determination can be made is dependent upon the particular concentration range selected for measurement, and upon the use of a suitable light filter. From a simple calibration curve, in which light absorption (or transmission) is plotted against concentration, one can determine empirically the region of greatest accuracy by noting in what portion of the curve a given error of measurement produces the least percentage error on the concentration represented. Ringbom has shown that in objective colorimetry the analysis error is at a minimum when the light absorption of the system is 63.2 per cent, although the error is not much greater at absorptions between 40 and 80 per cent. These figures are based upon considerations which assume the validity of the Lambert-Beer law. If a sufficient concentration range has been investigated, a plot of light absorption against the logarithm of the concentration will always give a curve showing an inflection; the point at which this inflection occurs represents the maximum accuracy, since at this point the curve has the greatest slope. The inflection always occurs at 63.2 per cent light absorption if the Lambert-Beer law holds, but may appear at a lower or higher light absorption for systems or conditions under which the law does not apply. The analysis error is determined by the formula:

$$\frac{\%\ \text{analysis error}}{1\%\ \text{absorption}} = \frac{230}{\dfrac{dI}{d\log c}}$$

The quotient $\dfrac{dI}{d\log c}$ is determined in per cent light absorption per logarithmic unit, and the formula then gives the analysis error at any point on the curve; as mentioned above, the error is a minimum at the point where the curve

shows an inflection. If one knows the accuracy with which the light absorption can be measured, the maximum attainable accuracy in the determination is immediately available.

The curves in Figures 3, 4, and 5 are plotted according to the method suggested by Ringbom. It will be noted that in most cases the curves have a considerable linear portion around the inflection point, so that the maximum accuracy is attainable over a considerable concentration range. In some cases, measurements were not followed to sufficiently high concentrations to indicate the inflection point. The curve for the turbidimetric determination of zinc indicates that the same considerations which apply to the colorimetric methods also apply to this turbidimetric method.

Table II indicates the maximum accuracy obtainable in the determinations previously described; the concentration region over which the maximum accuracy applies is approximate only. The maximum accuracy is figured on the knowledge that the instrument can be read to an accuracy of 0.1 per cent light absorption. The data in the table are sufficient to illustrate the importance of proper selection of light filter and concentration range if maximum accuracy is to be obtained.

The results obtained with ferrous sulfate indicate the possibility of the application of the thermoelectric absorptiometer in the determination of substances which have little or no visible color, if they show absorption in the infrared. Organic liquids seem to offer good possibilities along this line. Preliminary tests on 45 organic liquids have shown characteristic differences between various types of organic compounds, and slight differences between compounds in a given homologous series.

Summary

An improved thermoelectric absorptiometer using a balanced circuit has been constructed. It is rugged and compact and therefore portable, and is capable of giving a direct reading to an accuracy of 0.1 per cent light absorption. The thermopiles are sensitive and rapid. The ease and speed with which the test tubes used for absorption cells can be cleaned and dried, aid in reducing the time required in making measurements. Ten milliliters of liquid are sufficient for a determination.

The instrument has been tested with nine systems showing a wide range of visible color and spectral absorption, and a turbidimetric determination has also been made. There seems to be no reason why it could not be applied to any colorimetric or turbidimetric measurement, as well as to measurements of colorless systems showing infrared absorption. Although thus far the instrument has been used only in series measurements, it would seem possible to apply it in comparison methods involving duplication or dilution.

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Modified Rohrig Extraction Tube

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THE Roese-Gottlieb method, using a Rohrig extraction tube, is standard for estimation of fat in milk and milk products (*1*). However, as described by Bigelow and Fitzgerald (*2*), it has been criticized because of frequent failure to give duplicable results and a tendency to give low fat values when compared with other methods. The authors, whose experience with the Roese-Gottlieb technique has been principally in the determination of fat in evaporated milk, have found that this method as executed by an experienced technician will give rapid and reliable results.

Rohrig tubes are closed during extraction of the samples by a stopper or by the thumb of the operator; in the latter case, the vent hole in the wall of the tube is closed by the first finger. Occasional low results may be explained either by persistent adherence of fat particles to the stopper, or by the loss of sample through the end of the tube or the vent hole, if the thumb or forefinger slips during extraction or during the blowing down of the tube after extraction. Loss of sample may be due to failure of the operator's hand to conform to the size of the Rohrig tube, or to fatigue of the thumb or forefinger resulting from a large number of consecutive determinations.

These difficulties may be largely obviated by use of a modified form of tube shown in Figure 1.

A section of a test tube, *A*, is sealed to a glass stopcock, *B*, and this assembly is in turn sealed to the top of the Rohrig tube, *C*. The top of the Rohrig tube must be drawn out somewhat to eliminate the hole in the wall of the tube and to accommodate the diameter of the stopcock. The test tube has a capacity of about 20 ml. after sealing; the bore of the stopcock is 3 mm.

This modified type of tube has been used successfully at this laboratory for the past 6 months. It permits more thorough mixing and shaking of the sample and also allows control of gas pressure developed within the tube. By careful manipulation of the stopcock, the pressure may be slowly released and the tube blown down without loss of the sample.

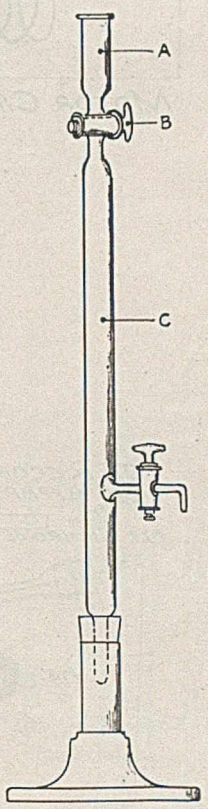

FIGURE 1

Literature Cited

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An Automatic Constant Flow Regulator for Low Gas Flows

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THE regulator described was designed to maintain a constant flow of 0.5 liter per minute through gas recorders for low concentrations of hydrogen sulfide and carbon bisulfide, but it can also be used for other gases and vapors. The regulator will automatically compensate for increasing or decreasing line resistance and will also take care of variations in voltage or water pressure, depending upon the suction source. (This apparatus was built in conjunction with the development of recorders by Philip Drinker for the American Viscose Corporation.)

The apparatus is shown in Figure 1. It consists of an X-type inclined manometer connected across a capillary flowmeter and a by-pass valve operated by an electric relay. The manometer is made of 7-mm. (4-mm. inside diameter) glass tubing with the ends flared for 00 rubber stoppers. A piece of platinum wire is fused into the lower part of the manometer for a contact and the Nichrome wires shown form the other two contacts needed. The flowmeter is calibrated directly using a wet gas meter. Only readings on the rising leg need be taken. Using the ratio 1 to 8—that is, the sine of the angle is $^1/_8$—it was found that a 1-mm. capillary, 95 mm. long, gave a rising leg deflection of about 15 mm. on the inclined scale. With the desired flow found, contacts B and C are set to within 1 mm. of the mercury meniscus.

Motor Conversion

A universal motor is easily converted into a reversible motor. An old Victrola motor was used here, but a laboratory slow-speed stirrer or mixer can be used. The brush leads are disconnected, the wires are brought outside the housing, and two leads are fastened to the brushes. A shunt-wound motor can be reversed in a like manner. This gives four connections, as shown in the upper left of Figure 1. When F_1 is connected to A_1 and F_2 to A_2, the motor will run in one direction, and when the field leads are interchanged—that is, F_1 to A_2, and F_2 to A_1—it will run in the opposite direction. These leads are connected to the relays, as shown in Figure 1. The relays operate in pairs, essentially the same as double-pole relays. When connection AB is made, the left-hand pair closes and the others close when connection AC is made.

The handle from a brass Hoke needle valve, similar to the control valve shown, was removed and a piece of 0.94-cm. (0.375-inch) brass tubing of 0.31-cm. (0.125-inch wall) was soldered to it. This valve was then coupled directly to the motor-worm wheel shaft by using a piece of vacuum hose and two light cotter pins. This gave a flexible coupling, so that the motor shaft and valve

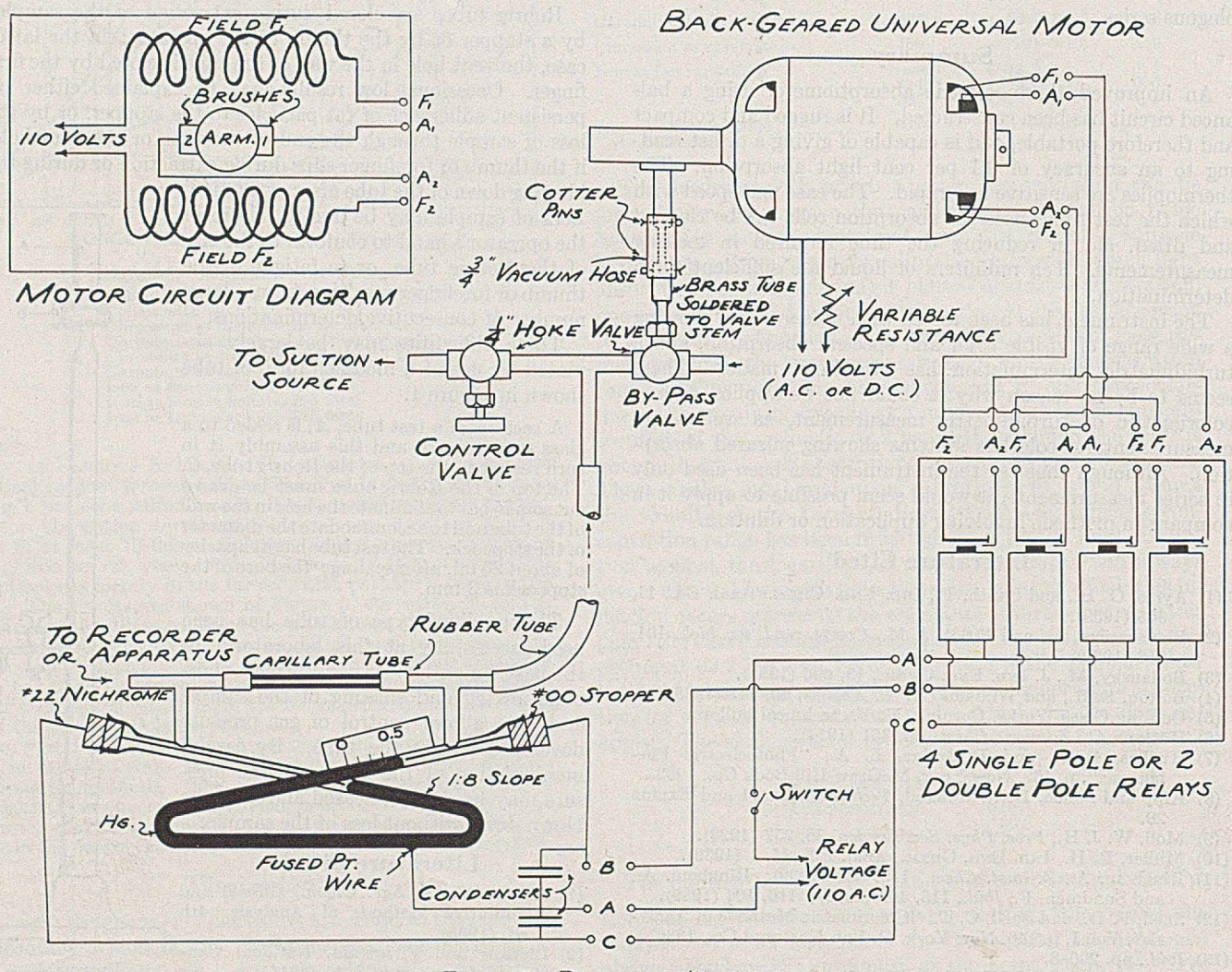

FIGURE 1. DIAGRAM OF APPARATUS

stem did not have to be aligned exactly. If the valve sticks, the cotter pins will shear off and prevent damage to the motor. The valve base was mounted rigidly, so that it would not turn with the stem. In other designs, it should be possible to couple the motor directly to the rheostat and thus get current, rather than airflow regulation.

Operation

In operation the desired flow is set by the control valve and the by-pass valve is set approximately halfway open. The by-pass will then take care of either high or low deviations from the desired rate.

By using the variable resistance, the correct motor speed can readily be determined. Too high a motor speed will cause continued oscillation of the manometer; too low a speed will cause sluggish response.

The relays used were obtained from an old piece of electrical equipment and operate directly from a 110-volt source. Hence, condensers were placed across the contacts to reduce arcing; 0.25-mfd., 110-volt, is adequate capacity. The relay switch should be opened when the apparatus is not in use. A small bottle can be placed in the line after the flowmeter to trap the mercury if any relay contacts fail to open.

Laboratory Electric Stirring Motor

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A WIDE variety of stirring motors is on the market, and yet few possess the characteristics desired in the organic laboratory. Small electric motors develop but little power at low speeds and direct drive is usually unsatisfactory for any but the lightest and most constant loads. If the character of the mix changes rapidly, or if the load is released suddenly, the motor may speed up to a point where breakage occurs. For this reason laboratory stirring motors should incorporate some type of speed-reduction mechanism which allows increased torque at low speed.

The method of mechanical speed reduction is usually the determining factor in the cost, marketability, and life of a stirring motor. The simple belt and pulley drive device has been neglected in favor of more exclusive and complicated designs with possible patent features. The mediocre results experienced with a number of these over a period of several years have led to the development of the motor and pulley assembly with electrical speed control described below. This unit has been found to be very satisfactory for driving wire stirrers (*1*).

The series-wound motor is particularly suited for this purpose because of its favorable speed-torque characteristics. Sparking at the brushes has not been found to be a serious fire hazard and the ordinary organic laboratory precautions suffice. Shaded-pole induction motors are admittedly safer, but their essentially constant speed characteristics make them useful only in connection with a step pulley where a

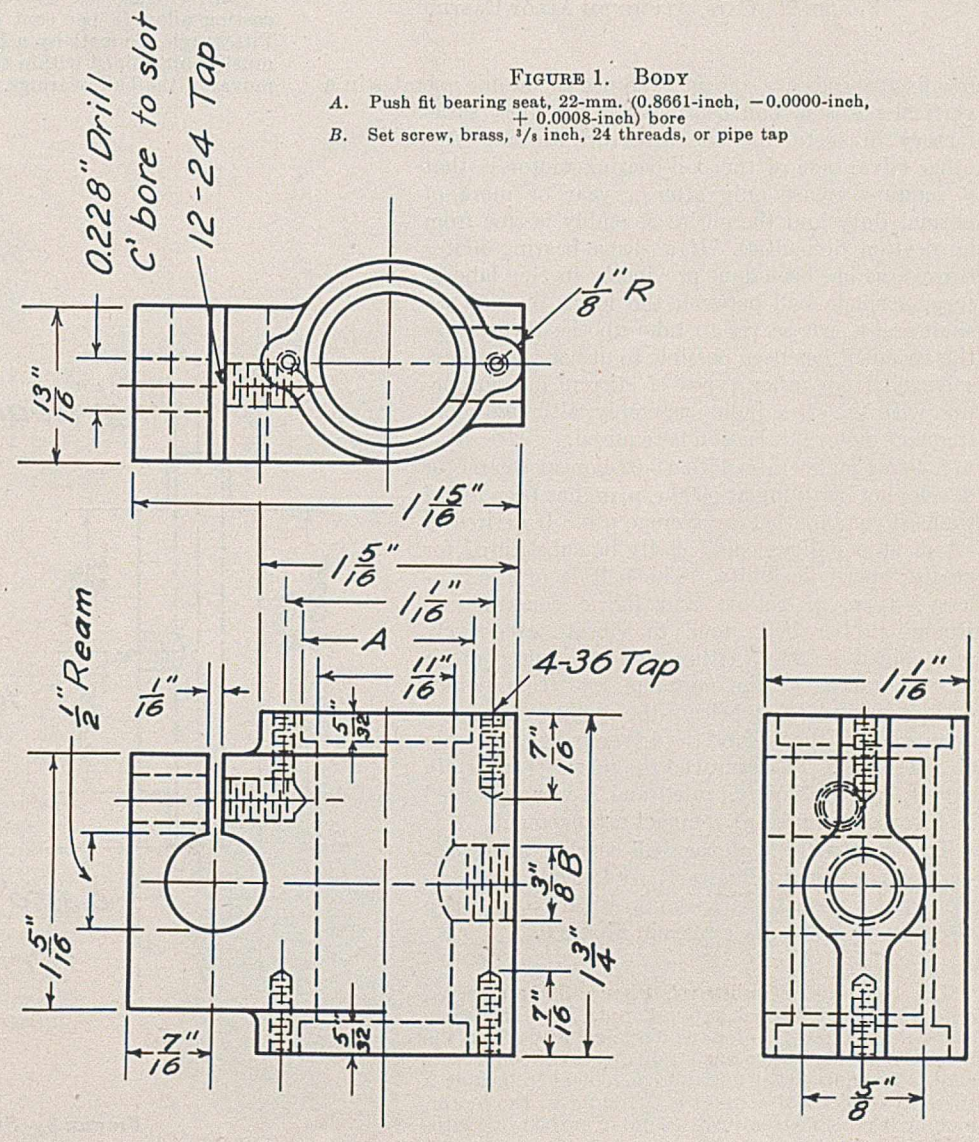

FIGURE 1. BODY

A. Push fit bearing seat, 22-mm. (0.8661-inch, −0.0000-inch, + 0.0008-inch) bore

B. Set screw, brass, 3/8 inch, 24 threads, or pipe tap

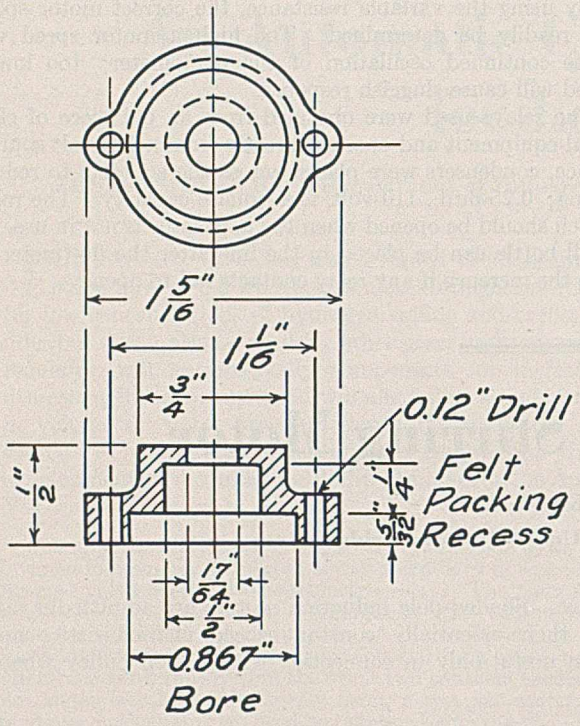

FIGURE 2. CAPS, ALUMINUM ALLOY CASTING

few fixed speeds are sufficient. Since the motor operates in a vertical position, ball bearings are the most satisfactory in sustaining the armature thrust. Another advantage of the ball-bearing motor is that it requires grease only after a year or more of normal duty and the entire assembly is free from lubrication difficulties. If a sleeve-bearing motor is used, as has been done previously in this laboratory, a single ball between the lower end of the shaft and a seat serves to take up the thrust. By this means it has been possible to utilize the motors salvaged from other types of stirrers in conjunction with the same pulley assembly, although more frequent motor lubrication is required.

A 4-to-1 pulley ratio with a 0.02-horsepower motor developing its rating at 5000 r. p. m. has been found the best combination for average use. If desired, a set of step pulleys could easily be substituted for greater speed flexibility. The belt is of the endless V-type, of rubber and fabric construction. Round rubber belts tend to vibrate excessively and joined leather belting cannot be used on so short a radius as the motor pulley. In previous designs using leather belting the motor was placed on one end of the rod and the driven pulley assembly on the other. This removed the motor to a certain extent, but the unit vibrated and it was discarded in favor of the present compact arrangement.

The construction of the ball-bearing pulley assembly is given in Figures 1 to 4, while the complete motor assembly is shown in Figure 5, and two alternate rheostat assemblies in Figure 6.

The ball-bearing pulley, *C*, Figure 5, is clamped onto the stainless-steel support rod, *D*. The same screw holds the clamp for the electrical leads from the motor, relieving it from any strain due to the connecting cord. The author has found it convenient to include a twist-lock electrical connector, *E*, close to the motor, making it possible to adjust the unit over the work without a tangle of wire. The locking feature then prevents accidental disengagement. A heavy-duty clamp, *F*, necessary to support the weight at the furthest extension of the rod, gives adjustment in two planes. As a rule the shaft is connected to the stirrer with a piece of heavy-walled rubber tubing, but provision has been made for the use of a chuck which may be purchased standard for a 0.61-cm. (0.25-inch) shaft. In the author's experience the first-named method is preferable with ball-bearing glass stirrers (*1*).

Both rheostat assemblies shown in Figure 6 have been used with equal success. That on the left has a switch permitting stoppage without disturbing the rheostat setting, which is an advantage in inspecting the progress of a reaction. The rheostat on the right incorporates an off position, so that the circuit is broken when the dial is turned counterclockwise to the stop. Each is mounted between two pieces of hard asbestos board and forms a unit detachable from the motor, a feature which is helpful when adjusting either assembly. Other electrical control apparatus, such as autotransformers, are convenient but relatively too expensive at the present time.

The following specifications provide for the construction of a unit of the highest quality material, and in some cases alternate sources of equal reliability have been given. An exhaustive investigation has not been made and other reliable makes should prove satisfactory.

The construction of these units has been made possible by the close coöperation of A. H. Gedies, machinist of the Mallinckrodt Chemical Laboratory at Harvard.

Assembly and Specifications

BODY (Figure 1). The casting was made of No. 12 aluminum casting alloy (8 per cent copper, Aluminum Co. of America, Pittsburgh, Penna.) by a local foundry. Both bearing recesses must be machined within the tolerances given to permit easy removal of the ball bearings.

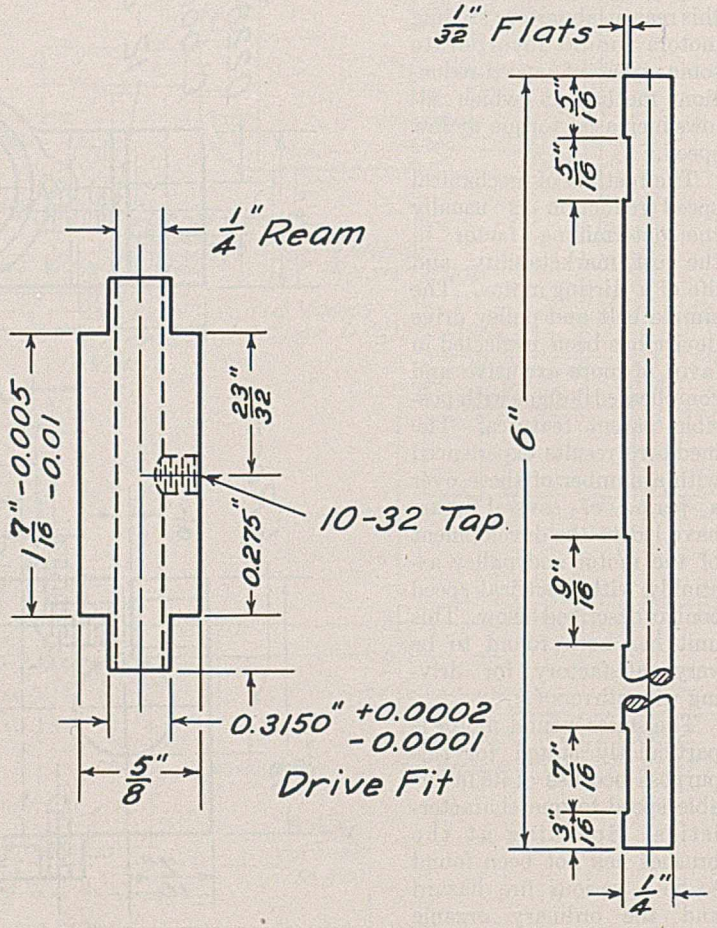

FIGURE 3. SLEEVE AND SHAFT

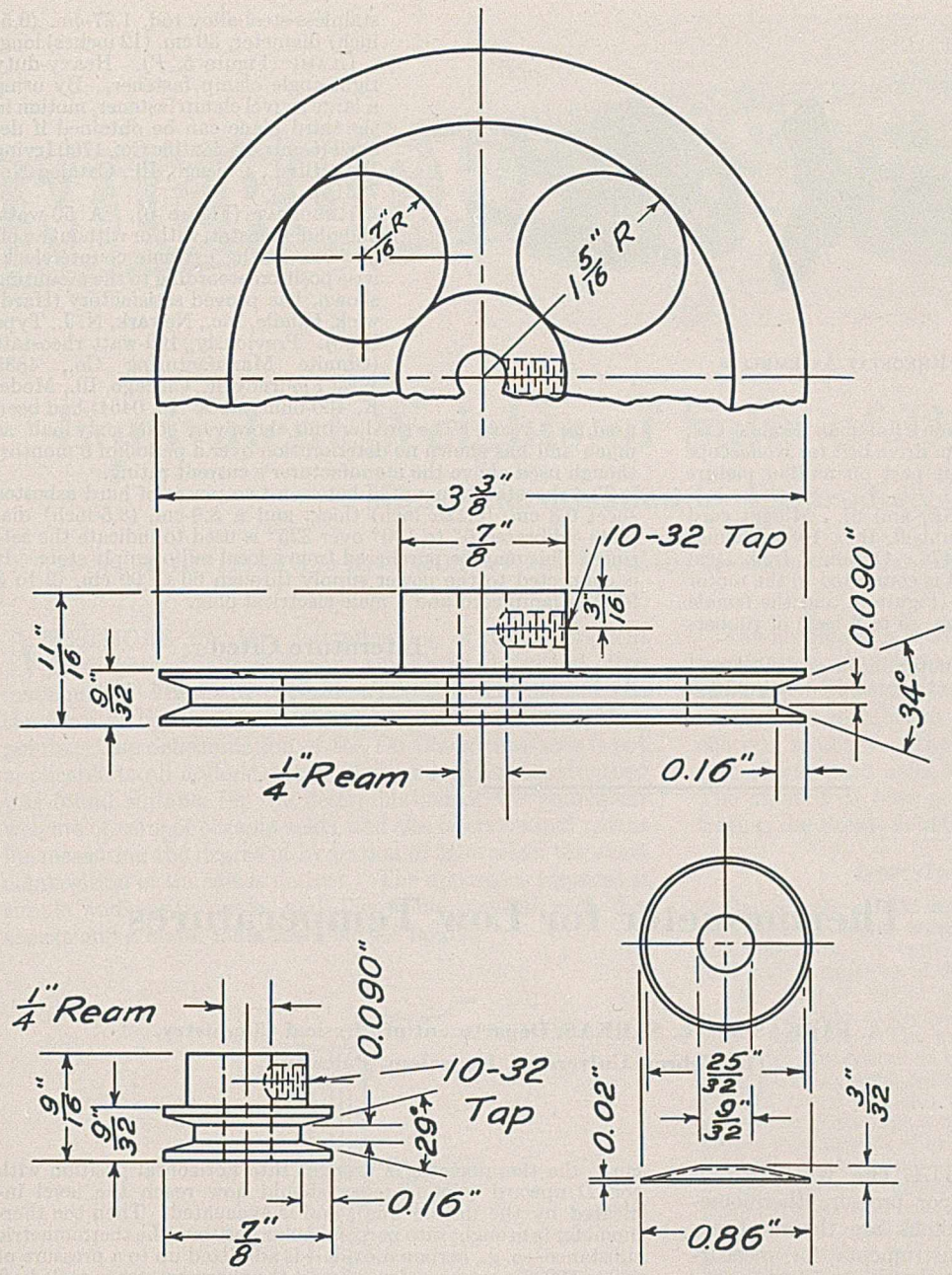

FIGURE 4. PULLEYS AND SPRING BRONZE COMPRESSION WASHER

Upper pulley, driven pulley machined from aluminum alloy casting
Lower, motor pulley machined from round stock

the bearing, holding it firmly against the body, and at the same time compresses the felt packing washers in the upper recess. Each cap is held in place by two 1.27-cm. (0.5-inch) screws.

BALL BEARINGS. Two single-row ball bearings, 8-mm. bore, 22-mm. outside diameter, 7 mm. wide (extra-small type, single row, deep groove, bearing No. 38, SKF Industries, Inc., 440 East 34th St., New York, N. Y., obtained from local hardware distributor).

SLEEVE (Figure 3). This is machined from round brass stock and must conform to the tolerances given to permit the ready removal of the bearings without damage to bearings.

SHAFT (Figure 3). Cut from 0.61-cm. (0.25-inch) ground polished finish, 18–8 stainless steel alloy rod with three flats filed in it for the large pulley, sleeve, and chuck.

MOTOR (Figure 5). Series-wound, varying speed, universal motor, 0.02 horsepower, 5000 r. p. m., 110 volts, ball-bearing (General Electric Co., Schenectady, N. Y., Model 5P35CA1A, type P, frame 35 C. If a more powerful motor is desired, the 0.033-horsepower motor, frame 36, may be substituted. This will require a 100-watt rheostat).

V-BELT (Figure 5). Inside diameter 8.9 cm. (3.5 inches), outside diameter 9.53 cm. (3.75 inches), width at outside diameter 0.396 cm. (0.156

Access to the setscrew of the sleeve (Figure 3) is through hole *B* (Figure 1), and this is closed with a brass screw plug. A tight fit is obtained by threading only a part of the hole or by using a pipe tap.

CAPS (Figure 2). Both caps are identical and are machined from castings of the same alloy as the body. The large end is faced and in this case the bearing recess is made slightly larger than that in the body, in order that the caps may be removed by hand. The compression washer shown in Figure 4 fits in this recess above the bearing and below the felt packing. When the cap is screwed down this exerts a positive pressure against

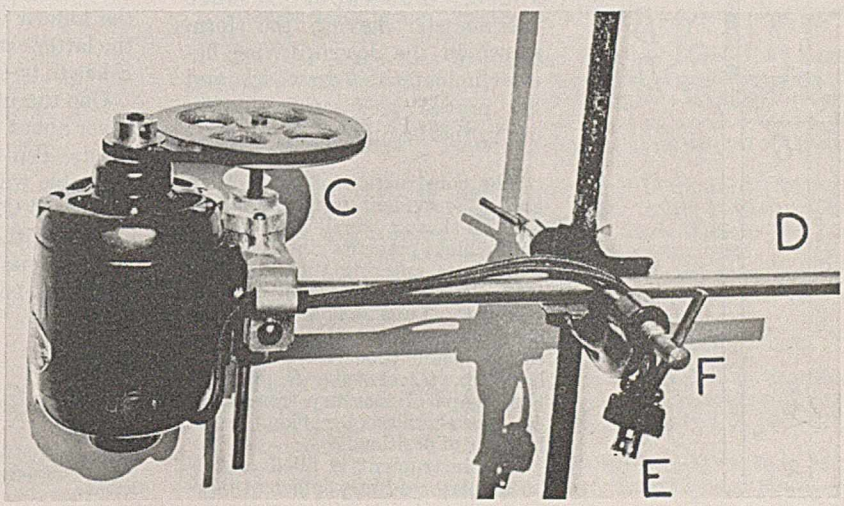

FIGURE 5. COMPLETE MOTOR ASSEMBLY

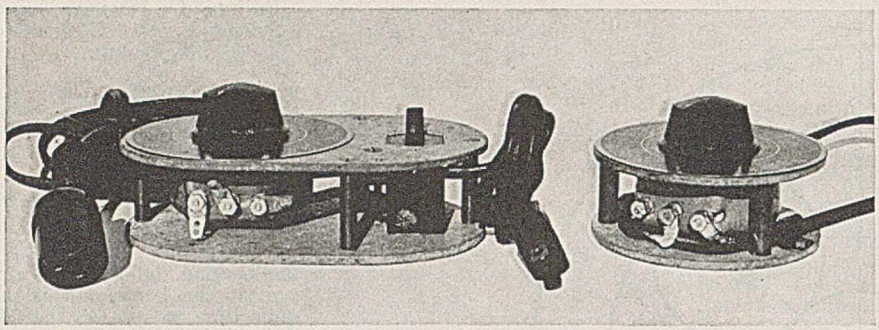

FIGURE 6. RHEOSTAT ASSEMBLIES

inch). Rubber and fabric construction (Eastman Kodak Co., Rochester, N. Y., No. 57012 mechanism drive belt for Kodascope E or EE). This belt is a replacement part on motion picture projectors.

ELECTRICAL CONNECTOR (Figures 5*E*, and 6). Midget cord connector with cord grip (Harvey Hubbell, Inc., 1930 Thomas St., Bridgeport, Conn., catalog No. 7478. Obtained from local electrical supply shop). The male end is connected to the motor leads which are clamped to the body (Figure 5) and the female end to the rheostat with 150 to 180 cm. (5 to 6 feet) of rubber-covered lamp cord.

SUPPORT ROD (Figure 5*D*). The motor block is drilled and tapped (0.25-inch pipe size) at the center to accommodate the 18–8 stainless-steel alloy rod, 1.27-cm. (0.5-inch) diameter, 30 cm. (12 inches) long.

CLAMP (Figure 5, *F*). Heavy-duty right-angle clamp fastener. By using a large swivel clamp fastener, motion in the third plane can be obtained if desired (Central Scientific Co., 1700 Irving Park Blvd., Chicago, Ill., Catalog No. 72315).

RHEOSTAT (Figure 6). A 50-watt, 400-ohm rheostat, with or without an off position in the extreme counterclockwise position according to the mounting shown, has proved satisfactory (Hardwick, Hindle, Inc., Newark, N. J., Type B-50). Previously, 100-watt rheostats (Ohmite Manufacturing Co., 4835 West Flournoy St., Chicago, Ill., Model K, 400-ohm, stock No. 0454) had been used for 3 years. The smaller unit, however, costs only half as much and has shown no deterioration over a period of 6 months, though used above the manufacturer's current rating.

The rheostat is mounted between two pieces of hard asbestos sheet 0.3 cm. (0.125 inch) thick, and a 8.9-cm. (3.5-inch) dial plate calibrated 0° to 100° over 325° is used to indicate the setting. This may be purchased from a local radio supply store. It is connected to the power supply through 60 to 90 cm. (2 to 3 feet) of lamp cord and a male electrical plug.

Literature Cited

(1) Hershberg, IND. ENG. CHEM., Anal. Ed., 8, 313 (1936).

Thermometer for Low Temperatures

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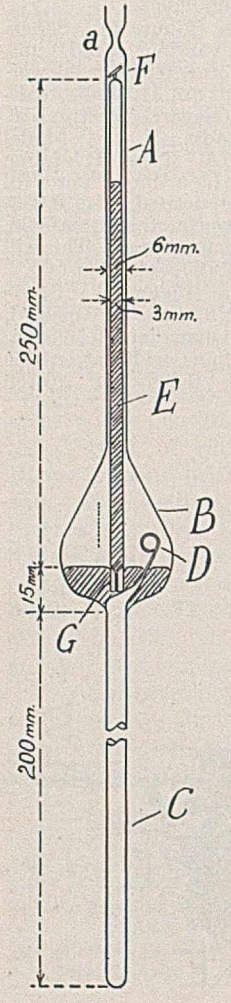
The Hebrew University, Jerusalem, Palestine



WHILE the well-known vapor pressure thermometers of Stock are the most accurate instruments for measuring low temperatures, they appear unhandy if the measurements are to be performed in a limited space. The vapor pressure thermometer having the form shown in the accompanying figure eliminates this drawback, and has proved very satisfactory in use in this laboratory.

The construction of parts *A*, *B*, and *C* is evident from the figure. Coil *D* has a small hole in its end which prevents the mercury in *B* from entering tube *C*, while it ensures communication between *B* and *C*. Tube *E* is sealed to the short glass rod, *F*, which in turn is sealed inside tube *A*. The capillary tip, *G*, of tube *E* prevents the escape of mercury from *E* in case the thermometer is kept in the horizontal position.

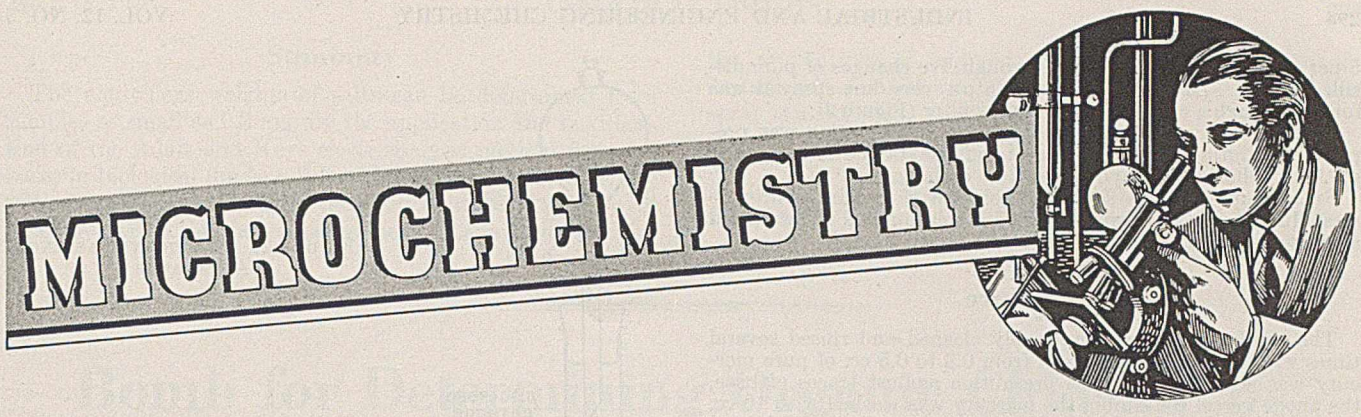
The instrument is filled as follows: After adding sufficient mercury, the thermometer is brought into horizontal position with coil *D* upward (the mercury should now reach the level indicated by the dotted line), and is evacuated. Then the thermometer is brought into perpendicular position, the thermometric substance—e. g., carbon dioxide—is admitted up to a pressure of 400 to 500 mm. and condensed, and the thermometer is sealed off at *a*.

The thermometer can be calibrated empirically or by using the known vapor pressures of the thermometric agents. In the latter case the capillary depression of the mercury in tube *E* has to be allowed for.

One thermometer of the dimensions given in the figure will cover about 15° to 25°, depending on the gas with which it is filled. Ten thermometers filled with the substances listed in the table will cover nearly every range of temperature down to −215° C.

Once a thermometer has been filled and sealed, it can be handled just like any ordinary mercury thermometer. Thus it will not be affected if it is kept in the horizontal position.

Substance	Temperature Range °C.	Substance	Temperature Range °C.
Pentane	+ 5 to − 35	Ethylene	−122 to −150
Butane	− 35 to − 73	Nitric oxide	−159 to −175
Propane	− 71 to −100	Methane	−175 to −188
Carbon dioxide	− 93 to −112	Oxygen	−193 to −205
Ethane	−112 to −140	Nitrogen	−205 to −215



Equivalent Weights of Salts of Organic Acids

Microdetermination by Electrodialysis

KARL H. DITTMER AND R. G. GUSTAVSON, University of Colorado Chemistry Department, Boulder, Colo.

METHODS for the determination of the equivalent weights of salts of organic acids are tedious and often inadequate. Frequently organic acids must be identified in the form of their salts, which seldom have definite melting points. The determination of the Du Claux constants is not applicable to all organic salts. The method herein described was found suitable for the determination of the equivalent weights of salts of organic acids, and also offers a rapid means for measuring the degree of hydration of salts when the exact composition of the salt is desired. The apparatus required is simple and easily made, and the whole method with five checks and a blank takes less than 1.5 hours.

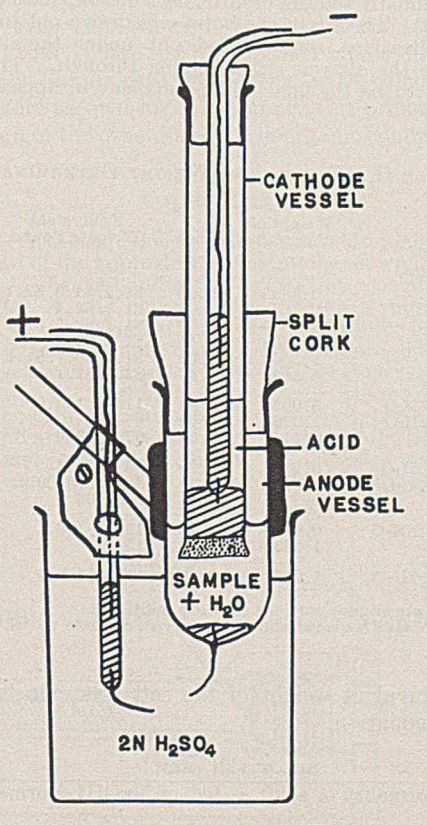


FIGURE 1. ASSEMBLY OF ELECTRODIALYZING UNIT
Crosshatching is mercury

The method is a modification of that proposed for the determination of total bases in biological fluids by Adair and Keys (*1*). The basic ions are electrodialyzed through a sintered-glass membrane, above which stands negatively charged mercury. The base amalgam reacts with an excess of standard acid which is placed above the mercury. The amount of base present in the sample is found by titrating the excess standard acid with standard base.

Description of Apparatus

Figure 1 shows one unit of six cells which were connected in parallel with direct current, the voltage varying between 100 and 150 volts. A voltmeter and an ammeter were connected for rough indications of voltage and amperage.

The cathode vessel was made by sealing a sintered-glass membrane into a cut-off and slightly tapered 15 × 125 mm. Pyrex test tube. The membranes were prepared by a method similar to that described by Kirk *et al.* (*2*). The purified ground-glass particles were between 0.25 and 0.60 mm. in diameter—that is, those particles which would pass through a 30-mesh sieve but not through a 60-mesh sieve. The molds for the membranes were lengths of brass or nickel tubing cut according to the thickness of the membranes desired.

The rings were placed on a sheet of nickel, filled with the prepared glass, and baked for approximately 15 minutes at about 830° C. in a small electric furnace. When wet instead of dry glass was packed into the rings less shrinkage occurred in the thickness and a little more in the diameter, thus facilitating the removal of the finished membranes from the rings. The proper temperature for this process was found to correspond very nearly to the temperature at which a Pyrex glass tube placed horizontally in the furnace collapses. With a little experience, the proper temperature and the optimum length of baking time were easily determined.

After the membranes were sealed into the tubes, they were ground on a Carborundum stone to a thickness which allowed easy passage of water but retained the mercury. Each membrane was tested in actual practice for defects, using a salt solution of known concentration. Tubes which did not give total recovery were discarded.

The anode vessel was made from a 25 × 200 mm. Pyrex test tube with a piece of platinum wire sealed in the bottom. The Pyrex glass and platinum wire seal were made tight by the application of a small amount of de Khotinsky's cement.

The cathode vessel and the cathode electrode were held in position with split corks wrapped in lead foil.

Preparation and Purification of Reagents

WATER. All water used in preparing solutions or for washing purposes was redistilled from a glass still. To remove all ammonia, a few drops of phosphoric acid were added to each liter of water distilled.

MERCURY. Mercury for use as the anode and cathode electrodes was purified by spraying it three times through 80 cm. of

5 per cent nitric acid and then through five changes of pure distilled water. To get the mercury into a very fine spray it was forced through a coarse sintered-Pyrex filter (Figure 2).

STANDARD ACID. A standard sulfuric acid solution was prepared by diluting concentrated acid and was standardized against a 0.05 *N* solution of anhydrous sodium carbonate.

STANDARD BASE. A saturated solution of sodium hydroxide was diluted to an approximately 0.05 *N* solution and standardized against a standard hydrochloric acid.

Procedure

The anode vessel was thoroughly cleaned and rinsed several times with redistilled water and from 0.3 to 0.5 cc. of pure mercury was added. As a final precaution against traces of base, the anode vessel containing the mercury was washed with 10 cc. of redistilled water. This final wash water was removed from the anode vessel with a finely drawn-out glass siphon attached to an aspirator. Salt samples were added to five of the six anode vessels. The sixth one was taken as a blank.

Individual samples of 0.3 to 5.0 mg. of pure crystalline salt were weighed and placed in each anode vessel, or an aliquot portion of a dilute solution of the salt was measured into each vessel by means of a Rehberg buret or recalibrated pipets. The solution was prepared of such concentration that 1 to 5 cc. contained between 0.3 and 5.0 mg. of the salt. The samples which the authors analyzed were all between 0.3 and 5.0 mg. The volume of solution in the anode vessel was kept between 4 and 8 cc., regardless of the amount of sample used.

TABLE I. EFFECT OF TIME AND SIZE OF SAMPLE ON TOTAL RECOVERY

Salt Analyzed	Time Dialyzed	Analyzed	Recovered[a]	Error	Error
	Min.	*Milliequivalent*			%
Na acetate	30	0.0109	0.0093	−0.0016	14.7
	60	0.0109	0.0105	−0.0004	3.6
	45	0.0043	0.0041	−0.0002	4.6
	60	0.0043	0.0043	0.0000	0.0
	90	0.0651	0.0651	0.0000	0.0
Na citrate	80	0.0042	0.0042	0.0000	0.0
Ca acetate	60	0.0040	0.0040	0.0000	0.0
Ca lactate	60	0.0040	0.0041	+0.0001	2.5
Zn acetate	80	0.0040	0.0037	−0.0003	7.5
	120	0.0040	0.0041	+0.0001	2.5

[a] Milliequivalent recovered is average of all values, not best checks of each determination.

Each cathode vessel was cleaned between successive determinations by allowing it to stand in aqua regia until any dark coloration in the sintered glass had been removed. The cathode vessels were put into aqua regia immediately after a titration and were ready to be washed with water when the next determination was begun. The membranes were then washed by forcing tap water through them by connecting them directly to the tap with a piece of rubber tubing. This washing was generally continued for from 3 to 5 minutes and was followed by three more washings with redistilled water, rinsing inside and out. The upper part of the cathode tube was wiped dry with a towel, but the lower part and the membrane were never touched with anything but redistilled water.

One to 1.5 cc. of pure mercury were added from a dispensing buret to each cathode vessel. A membrane which held 1.5 cc. of mercury above it when sodium ions were dialyzed did not always retain the same amount of mercury when calcium ions were being dialyzed. This difficulty, which may have been due to a lower surface tension for calcium amalgam than that of other base amalgams, was overcome by using 0.5 to 0.75 cc. of mercury above the membranes.

An excess of standard sulfuric acid, delivered from a Rehberg buret, was placed above the mercury. The amount of sulfuric acid varied from 0.100 to 1.500 cc., depending upon the amount of the sample to be analyzed. The tip of the buret was rinsed with a small amount of water into the cathode tube. The lower, outside part of the tube and the membrane were again rinsed with redistilled water, and the tube was immersed about 1 cm. in the solution of the anode vessel to be analyzed. The negative electrode was rinsed with water and placed so that the platinum wire was just below the surface of the cathode mercury.

The current was switched on as soon as the first cell was prepared. The other cells were successively prepared, each preparation requiring about 5 minutes; this is the same length of time required for the titration of the excess acid in each cathode vessel, making the total time of dialysis the same for each cell.

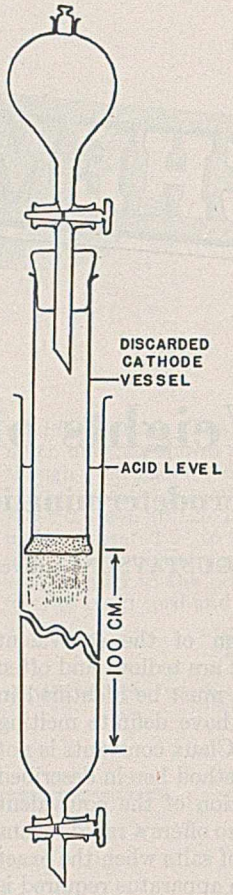


FIGURE 2. MERCURY WASHING APPARATUS

The rate at which bubbles formed at the electrodes in the 2 *N* sulfuric acid indicated the rate of dialysis. The entire unit was gently shaken every 5 or 10 minutes to produce faster and more complete dialysis and also to help decompose the base amalgam. Each unit was dialyzed until no more bubbles formed in the 2 *N* sulfuric acid, usually one hour. The time for complete dialysis varied with the amount of sample to be analyzed, the kind of base ion present, and the type of salts analyzed (Table I).

After complete dialysis, the cathode vessel was raised above the anode solution, thus breaking the circuit, and the cathode electrode was rinsed with 1 to 2 cc. of water into the excess acid. The base amalgam was decomposed by bubbling a current of air or dry oxygen through the mercury for 1 to 2 minutes. One drop of methyl red was added and the excess acid titrated with a standard sodium hydroxide solution, measured from a microburet. The tip of the buret was drawn out to a fine point and the titrations were carried out under the surface of the acid, while the air was still bubbling through. The end point was found to be the final and permanent disappearance of all red color.

TABLE II. EQUIVALENT WEIGHT DETERMINATIONS

Salt Analyzed	Weight of Sample[a]	Acid[b]	Equivalent Weights Found	Theoretical Equivalent Weight
	Mg.	*Cc.*		
$NaC_2H_3O_2$	0.3566	0.30	82.1, 84.5, 82.1 81.1, 82.1	82.02
	0.8915	0.30	84.1, 82.5, 84.1	82.02
	1.7830	0.45	81.8, 82.1	82.02
	3.5660	1.00	81.1, 80.7, 81.1	82.02
	5.3490	1.40	82.1, 82.1	82.02
$Na_2C_4H_4O_6.2H_2O$	5.0020	0.90	115.3, 115.0	115.03
$Na_3C_6H_5O_7.2H_2O$	2.0706	0.60	98.1	98.02
	0.4141	0.30	97.4, 98.6	98.02
$Ca(C_2H_3O_2)_2.H_2O$	0.8808	0.30	84.7, 87.5	88.07
$Ca(C_2H_3O_2)_2.H_2O$	0.3525	0.30	88.08, 86.00 88.08	88.07
$Ca(C_2H_3O_2)_2.5H_2O$	0.6165	0.30	154.1, 158.1	154.12
	1.2330	0.30	154.1	154.12
$Zn(C_2H_3O_2)_2.2H_2O$	1.0974	0.30	110.3, 109.7	109.73

[a] Samples are aliquot portions of dilute solutions.
[b] 0.0555 *N* H_2SO_4 placed above mercury in cathode vessel.

The equivalent weight of the salt was calculated by the following equation:

$$\text{Equivalent weight} = \frac{\text{mg. of salt sample}}{(\text{cc. of } H_2SO_4)(\text{normality of acid}) - (\text{cc. of NaOH})(\text{normality of base})}$$

Representative values obtained for the equivalent weights of salts tested are given in Table II.

Summary

The equivalent weights of salts can be determined from samples as small as 0.3 mg. by the application and modification of the Adair and Keys electrodialysis method for total bases in biological fluids. When the procedure was carefully followed, values obtained for the equivalents were rarely over 3 per cent from the theoretical values. A simple method for the preparation of sintered-glass membranes is described. An efficient method for dividing mercury into a very fine spray is mentioned.

Literature Cited

(1) Keys, A. B., *J. Biol. Chem.*, **114**, 449–59 (1936).
(2) Kirk, P. L., Craig, R., and Rosenfels, R. S., IND. ENG. CHEM., Anal. Ed., **6**, 154–5 (1934).

Bomb for Determining Organic Chlorine by Lime-Fusion Method

WILLIAM M. MACNEVIN AND WILLIAM H. BAXLEY, Ohio State University, Columbus, Ohio

THIS paper describes the construction of a simple closed metal tube or bomb and its use in a semimicro modification of the classical, but little used, lime-fusion method (*2*) for the determination of organic chlorine. The investigation was undertaken to find a method for determining organic chlorine which would require only simple and easily attainable apparatus and at the same time would be as rapid as any of the new methods now in use.

Investigation of the literature (*2*) on the various methods for determining organic chlorine revealed that the chief limitation of the classical lime-fusion method which was always conducted in an open glass tube, was that easily volatile samples escaped before reacting with the calcium oxide. The results were therefore often low. This, together with the requirement of a large amount of calcium oxide in each determination, is considered to be the principal reason for lack of general popularity of the classical method.

Recently it has been shown in this laboratory (*1*) that if a closed metal tube is used, easily volatile organic fluorides are quantitatively decomposed by heating with calcium oxide. It has therefore seemed logical to apply this principle to the determination of the other halogens in organic combination.

Apparatus

The bomb consists of a hollow cylinder of cold-rolled steel open at one end and of the approximate dimensions shown in Figure 1. The open end is flanged and may be closed by a combination of threaded collar, *A*, and plug, *B*. (*A* and *B* are standard brass fittings used in the refrigeration industry and may be obtained from any refrigerator supply house. The hollow cylinder can be turned from a bar of cold-rolled steel. Exact dimensions do not appear important. Most laboratory supply houses are also able to provide the complete assembly.) A soft copper disk placed between plug *B* and the inner flange of the neck of the bomb serves as a washer and provides a tight fit. (Cold-rolled sheet copper may be softened by heating to near redness and quenching in water.)

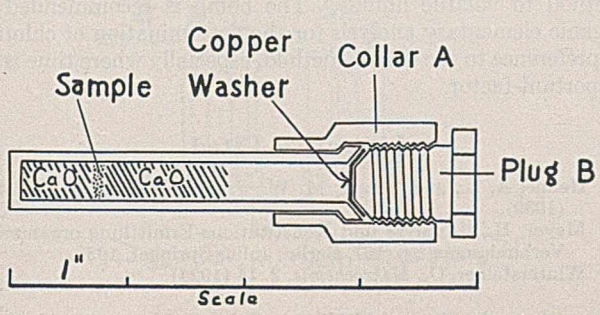


FIGURE 1. HIGH-TEMPERATURE CALCIUM OXIDE BOMB

The bomb is sealed by turning plug *B* into collar *A* and is conveniently done by clamping *A* in a vise and using a wrench to tighten *B*. The bomb is clamped to a ring stand in a horizontal position and is heated with a Pittsburgh or Méker burner. A metal screen is placed between the operator and the bomb because of a remote possibility of an explosion. However, on no occasion has there been any evidence of dangerous pressure during heating. Observation of the bomb while heating is accomplished by the use of a small mirror standing at the back of the desk or hood.

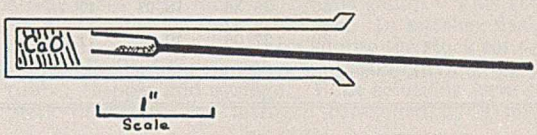


FIGURE 2. INTRODUCTION OF SOLID SAMPLE INTO BOMB

A long-stemmed weighing tube of the approximate dimensions indicated in Figure 2 is required for introducing solid samples into the bomb. Liquid samples are drawn up in previously weighed thin-walled glass bulbs (Figure 3) which are then sealed off.

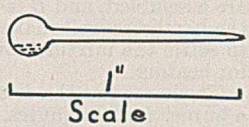


FIGURE 3. SAMPLE BULB FOR LIQUIDS

Porcelain Gooch microcrucibles with asbestos mats have been used for filtering the silver chloride. They have sufficient capacity for amounts of precipitate up to 130 mg. When used with the Wintersteiner filtering microapparatus (*3*), the process of filtering and drying the precipitate requires not more than 25 minutes.

The balance used in this work was an Ainsworth of semimicro type, with a sensitivity of 0.05 mg. per division of the pointer scale. The weights were calibrated to the nearest 0.01 mg. All weighings were made to the nearest 0.01 mg.

Reagents

Calcium oxide was prepared by heating analytical reagent quality calcium carbonate at 900° C. for 2 hours, and after cooling was stored in a desiccator containing Drierite. The diameters of most of the particles ranged from 5 to 10 μ. About 1.5 grams are required for each determination.

The nitric acid, silver nitrate, and potassium nitrate were of analytical reagent quality.

Distilled water free from chloride was used for preparing all solutions. Several blank determinations with usual amounts of all reagents failed to show any trace of chloride as indicated by turbidity after 24 hours' standing.

Procedure

The bomb is cleaned by washing with water and acetone and drying in a blast of air. Calcium oxide is added until the bomb is about one-third full (Figure 1). Enough sample to give not less than 8 mg. of silver chloride is introduced by means of the

TABLE I. DETERMINATION OF ORGANIC CHLORINE

Sample	Combustion Period	Weight of Sample	Weight of AgCl	Cl Calcd.	Cl Theoretical
	Min.	*Mg.*	*Mg.*	%	%
Monochloroacetic acid, $C_2H_3O_2Cl$	5	12.38	18.40	36.77	37.53
	10	14.38	21.80	37.50	
	15	13.49	20.55	37.68	
	20	8.38	12.66	37.37	
	20	35.43	53.58	37.41	
β-Hydroxytrichlorobutyric acid, $C_4H_5O_3Cl_3$	20	27.69	57.55	51.41	51.26
	20	18.44	38.53	51.69	
	20	20.39	42.57	51.65	
Chloroacetophenone, $C_6H_5COCH_2Cl$	20	12.09	11.12	22.75	22.95
	20	8.78	8.13	22.91	
p-Chloroacetanilide, C_8H_8ONCl	10	8.85	7.03	19.65	20.90
	15	11.80	10.01	20.99	
	20	11.92	10.04	20.84	
	20	9.25	7.75	20.73	
p-Nitrobenzyl chloride, $C_7H_6O_2NCl$	20	28.14	23.18	20.38	20.66
	20	9.77	8.23	20.84	
p-Chloronitrobenzene, $C_6H_4O_2NCl$	20	14.54	13.15	22.37	22.51
	20	9.07	8.26	22.53	
Carbon tetrachloride, CCl_4	20	13.93	52.21	92.71	92.20
	20	35.33	131.31	91.94	
Tetrafluorotetrachloropropane, $C_3Cl_4F_4$	20	9.05	20.63	56.39	55.87
	20	13.82	31.38	56.17	
Tertiary butyl chloride, C_4H_9Cl	20	5.98	9.25	38.26	38.30
	20	7.45	11.51	38.22	
α-Chloronaphthalene, $C_{10}H_7Cl$	20	16.93	14.79	21.61	21.80
	20	13.17	11.61	21.81	
p-Dichlorobenzene, $C_6H_4Cl_2$	20	22.51	42.21	46.39	48.24
	35	20.78	39.52	47.05	
	20	24.38	47.65	48.35[a]	
	20	15.35	30.06	48.44[a]	
Hexachlorobenzene, C_6Cl_6	20	10.68	32.34	74.91[a]	74.71
	20	12.25	37.05	74.82[a]	
Research compounds					
$C_{18}H_{25}O_{12}Cl$	20	44.65	13.49	7.47[a]	7.56
	20	40.79	12.23	7.42[a]	
$C_{19}H_{13}O_3Cl$	20	12.10	5.28	10.79	10.93
	20	18.96	8.27	10.79	
$C_{24}H_{14}O_2Cl_2$	20	19.61	13.80	17.41	17.50
	30	26.36	18.91	17.75	
	40	30.39	21.54	17.53	
	20	27.06	19.21	17.56[a]	

[a] 100 mg. of KOH_3 were added to calcium oxide.

weighing tube. More calcium oxide is added until after gentle tapping on its bottom end, the bomb is about two-thirds full. The copper washer is put in place, the various parts of the bomb are assembled, and *B* is turned tightly into *A*. The contents of the bomb are well shaken, after which the bomb is again tapped to settle the mixture toward the bottom end. It is then ready for heating.

The lower two thirds of the bomb is heated to dull redness over a burner for 20 minutes. After cooling under the water tap, the bomb is opened and inverted over a 250-ml. beaker containing 50 ml. of water, and the contents are removed by gentle tapping. Traces of calcium oxide which adhere to the walls of the bomb are removed by rinsing several times with small amounts of hot water.

The covered beaker with contents is kept cool in a pan of water and concentrated nitric acid is added until the solution is acid; usually 3 to 5 ml. are sufficient. The solution is filtered through filter paper to remove carbon and the filter is well washed with hot water. The volume at this point should not exceed 125 ml. One milliliter of 5 per cent silver nitrate is added slowly with stirring and the precipitate is coagulated by heating nearly to boiling. A test for complete precipitation is now made by adding another drop of silver nitrate solution.

After cooling the solution in an ice-water bath to about room temperature, the precipitate is filtered on a weighed Gooch micro- or semimicro crucible with an asbestos mat. Fifty milliliters of 0.5 per cent nitric acid solution are used in small portions for washing. A policeman is used to remove traces of adhering precipitate. The precipitate is dried to constant weight at 225° to 250° C. If microcrucibles are used, 10 minutes' heating is sufficient.

For liquid samples, the sealed bulb containing the unknown is placed between the two layers of calcium oxide. Shaking is of no value in this case, since the sample can escape only after the bulb has burst on heating.

Discussion

The results of the analysis of fifteen organic compounds of widely different nature are given in Table I. All samples used were tested for purity by observing some indicative property such as melting or boiling point. In general the accuracy obtained is sufficient for the organic chemist, who is usually interested in determining the number of chlorine atoms in a molecule.

The results with monochloroacetic acid and with *p*-chloroacetanilide show that less than 20 minutes' heating time is insufficient.

Three nitrogen compounds were included in the group of compounds analyzed in order to see whether cyanide formation might lead to appreciable errors. Apparently it does not.

The results for the four volatile liquids are in excellent agreement with the theoretical and indicate the advantage of using the closed tube rather than the open tube of the earlier workers. The technique of handling liquid samples is considered important. By sealing the bulb containing the sample, the escape of vapor is prevented until the calcium oxide has reached a fairly high temperature. Only then is enough pressure generated within the bulb to burst it.

Some unburned carbon is always left and has to be filtered from the nitric acid solution of the oxide. In most cases this carbon does not retain any chlorine. However, the authors' results indicate that whenever more than one chlorine atom is attached directly to a benzene ring, it is difficult to remove all of the chlorine without the use of an oxidizing agent. The addition of about 100 mg. of potassium nitrate to the bomb has been found to oxidize the carbon sufficiently so that satisfactory chlorine values are obtained. Consequently, addition of potassium nitrate is recommended in every case where the condition of the halogen is unknown or where the ratio of carbon to chlorine is high.

The outstanding difference between this method and that of Carius is in the comparatively short time required to obtain a result. For a single determination about 1.25 hours are required. If two bombs are available and several determinations are made, the average time may be cut to 50 minutes.

When this method is compared with that using the Parr bomb, the principal differences are: (1) the bomb may be assembled at a relatively small cost; (2) decomposition is attained by using a higher temperature with no, or at most a trace of, oxidizing agent; (3) the reagents are comparatively easier to keep and are less dangerous to handle; (4) less pressure is generated than in the Parr bomb, and the bomb is therefore easier to seal.

The application of this high-temperature calcium oxide bomb to other determinations is in progress in this laboratory.

Summary

A simple bomb has been constructed for carrying out the decomposition of organic chlorides by the lime-fusion method. The satisfactory determination, on a semimicro scale, of chlorine in fifteen organic compounds including four liquids is described.

The use of a closed metal tube instead of an open glass tube as formerly used extends the application of the lime-fusion method to volatile liquids. The bomb is recommended in organic elementary analysis for the determination of chlorine in preference to the Carius method, especially where time is an important factor.

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Microgravimetric Determination of Active Hydrogen by the Grignard Reagent

Application to Analysis of Impregnated Paper Insulating Tapes

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THE application of the Grignard reagent to the determination of active hydrogen in compounds is frequently referred to as the Zerewitinoff (*3*) method. Recently the use of the Grignard reagent for the study of the rate of formation of oil deterioration products has been reported by Larsen (*4*), Balsbaugh and Oncley (*2*), and Assaf and Gladding (*1*). These authors employ a volumetric method and determine the excess of reagent at the end of the test in order to compute the quantity of reagent entering into an addition reaction. An excellent review and description of the volumetric procedure are given by Niederl and Niederl (*6*).

The method described in this paper employs a more precise weight procedure in which the evolved methane is burned to carbon dioxide and water, which are absorbed in microchemical absorption tubes. By taking advantage of the fact that a Grignard reagent such as *n*-butylmagnesium halide yields four times the weight of carbon dioxide per unit volume of hydrocarbon compared with methyl magnesium halide, a considerable increase in precision may be obtained. The weights of carbon dioxide and water formed on combustion prove the composition of the hydrocarbon evolved from the Grignard reagent and thus avoid the uncertainty of other unknown gases which may be included in the volume measurement.

The apparatus is the same as that employed in the determination of water in impregnated paper insulating tapes (*3*), except that cell *E* is replaced by the two cells shown in Figure 1. The novel features are the introduction of the reagent into the reaction chamber through a serum rubber stopper commonly employed in the medical laboratory, by means of a syringe fitted with a stainless steel needle, and the use of two reaction cells in series, permitting siphoning the reagent onto the sample without air contamination. The siphoning is accomplished by momentarily releasing the pressure in the train by turning the three-way stopcock in the nitrogen line. The reaction cells were heated by air baths described by Master (*5*). The rate of gas flow was approximately 1 liter per hour for the combined gases at the exit end of the train.

Two complete trains were used in the experimental work to be described. It was thus possible to carry out a blank determination under duplicating conditions in experiments which required the trains to be opened momentarily to the air.

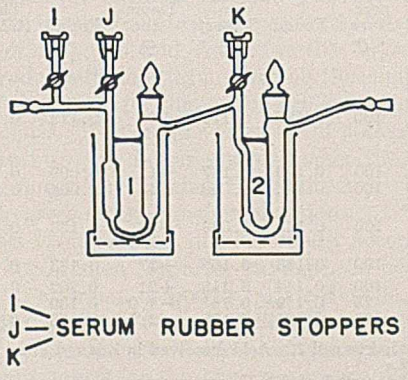

FIGURE 1. ALTERNATE CELL FOR GRIGNARD REACTION

Water and a dilute aqueous solution of phosphoric acid stored in an atmosphere of nitrogen were used in different experiments to determine the excess reagent.

Most of the preliminary experiments with known compounds were carried out at elevated temperatures in accordance with general practice. In some cases confusing side reactions occurred at 100° C. and the desired reaction was obtainable only at a reduced temperature.

Preparation of Grignard Reagent

The reagent was made by distilling at reduced pressure isoamyl ether from methyl magnesium iodide into a flask to which some magnesium turnings had been added. Nitrogen after passage over hot reduced copper oxide, Ascarite, and Dehydrite was admitted to the flask. By means of the serum rubber stopper and syringe, a weighed amount of distilled methyl iodide was slowly added and the reaction was controlled by immersion in an ice-water bath. The solution was refluxed *in vacuo* as the hydrocarbon gases were removed by the pump. This solution served as a concentrated stock solution. In another flask, the more dilute reagent was prepared by adding the stock solution to isoamyl ether distilled from methyl magnesium iodide, using the serum rubber stopper and syringe. Both solutions were stored in the dark under purified nitrogen controlled by a mercury piston.

TABLE I. STABILITY OF GRIGNARD REAGENT

(Temperature 100° C.)

Nitrogen *Liters*	H_2O *Mg.*	CO_2 *Mg.*	H_2O/CO_2	Remarks
			Experiment A	
1	0.09	0.14	0.6	814 mg. of solution of reagent in isoamyl ether to cell 2 by syringe
1	4.37	5.37	0.818	Added 607 mg. of solution of palmitic acid in isoamyl ether to cell 2 by syringe
1	0.08	0.12	0.7	
1	2.42	2.98	0.812	Added 1 ml. of H_2O
			Experiment B	
0.75	0.16	0.25	0.6	835 mg. of solution of reagent in isoamyl ether to cell 2 by syringe
0.50	0.06	0.09	0.7	
1.50	0.16	0.21	0.8	Added 774 mg. of solution of benzophenone in isoamyl ether to cell 2 by syringe
0.50	0.03	0.05	0.6	
1.0	9.38	11.55	0.812	Added 1 ml. of H_2O
Theoretical for CH_4			0.819	

Stability of Grignard Reagent

In Table I, two typical experiments illustrate the test procedure as well as the extent of decomposition of the reagent. The ratio of water to carbon dioxide during the periods before and after the addition of the sample often deviates from the theoretical value for methane. This deviation may be due to the passage of traces of isoamyl ether through the liquid air trap or to a slight decomposition of the reagent. In actual magnitude, the weights of carbon dioxide and water obtained were small, but greater than the blank on the train when the reagent was absent. This effect and its bearing on the reagent added are receiving further study. Experiments in which the water-carbon dioxide ratio did not correspond closely to the theoretical value for methane were rejected.

TABLE II. ACTION OF GRIGNARD REAGENT ON WATER

Experiment	Nitrogen	Temperature	CH_4 Evolved Found	CH_4 Evolved Theory	Deviation from 1 Mole
	Liters	° *C.*	*Millimole*		%
1[a]	2	100	0.131	0.110	+19
2[b]	2	100	0.101	0.075	+35
3[a]	2	100	0.117	0.090	+30
4[c]	1	25	0.049	0.060	−18
	2	25	0.059	0.060	−1
	3	25	0.064	0.060	+7
	21	100	0.113	0.060	+88
5[d]	1	25	0.114	0.058[e]	+97

[a] Reagent added by syringe to cell 2. Water added in capillary to cell 1. Reagent siphoned back.
[b] Reagent added by syringe to cell 2. Water added in capillary to cell 1. Water distilled from cell 1 to cell 2.
[c] Reagent added by syringe to cell 2. Water added in capillary to cell 2 through bore of stopcock *K* (Figure 1).
[d] Reagent added by syringe to cells 1 and 2. Isoamyl ether containing known amount of water added by syringe to cell 1 through modified stopper.
[e] Concentration of water in isoamyl ether was determined independently by weight method (*3*), correction being applied for water due to combustion of ether.

Action of Grignard Reagent

ON WATER. One of the first substances to be tried out was water. In Table II, the experimental results indicate that the second hydrogen in the water molecule is active toward the Grignard reagent, as illustrated by the following equation:

$$CH_3Mg\,I \xrightarrow{H_2O} CH_4\uparrow + Mg\begin{matrix}OH\\ I\end{matrix}$$

$$Mg\begin{matrix}OH\\ I\end{matrix} \xrightarrow{CH_3Mg\,I} CH_4\uparrow + MgO\,.\,MgI_2$$

In experiment 4 (Table II), since a solid hygroscopic product is formed in the reaction, the total added water does not have intimate contact with the Grignard reagent which is periodically forced around the bend in cell 2 by the carrier gas, nitrogen. Experiment 5 (Table II) was designed to ensure an excess of reagent at the zone of reaction. A dilute solution of water in isoamyl ether was added by syringe to cell 1 through the stopper, modified so that it was equipped with a serum rubber stopper and stopcock. In this way the water sample was dropped directly into the bulk of the Grignard reagent and the reaction went to completion in 1 hour at room temperature.

ON UNIMPREGNATED PAPERS. Preliminary experiments were carried out on sulfate cable paper tapes, 5 mils in thickness. The results in Table III substantiate the work of Wood (*7*). The water by weight was determined independently on the alternate combustion train as previously reported (*3*). The data show clearly that the water when removed from the paper behaved toward the Grignard reagent as recorded in Table II but that when the water was combined with the paper it behaved as if only one hydrogen were active—i. e., as an alcohol. Although the amount of methane evolved is small, calculation of the free hydroxyl per unit of $C_6H_{10}O_5$ from the first experiment indicates that one hydroxyl in 200 is active, whereas in the third experiment the ratio is 1 to 1000. No correlation between the reagent added and other groups in the cellulose molecule was attempted.

ON ORGANIC ACIDS. The normal reaction of the Grignard reagent on acids consists of the evolution of one mole of methane and the addition of an equivalent mole of methane as indicated in the following equation:

$$-C\begin{matrix}O\\ OH\end{matrix} + 2\,CH_3Mg\,I \longrightarrow -C\begin{matrix}O\,MgI\\ CH_3\\ O\,MgI\end{matrix} + CH_4\uparrow$$

The results in Table IV bring out that at elevated temperatures an acid irrespective of type gave high results at 100° C. for the methane evolved, but that at 25° C. results corresponding to theory may be obtained. This phenomenon was entirely distinct from that described previously in the behavior of water toward the Grignard reagent, since a blank determination following the addition of the acid sample invariably yielded negligible quantities of carbon dioxide (cf. Table I). A trace of peroxide was found to be present in the abietic acid recrystallized from *n*-pentane, but this fact could not account for the similar results with the National Bureau of Standards benzoic acid. The addition reaction gave extremely erratic results which did not correspond to theory in any case. The effect of time, temperature, and steric hindrance may be expected to influence the yield of ketones synthesized from the interaction of acid and Grignard compound.

ON MISCELLANEOUS COMPOUNDS. The reaction of methyl magnesium iodide was carried out on a single member of the following classes of organic compounds: alcohol, ester, ketone, and peroxide. The results listed in Table V indicated that the state of purity of the compound was the chief factor of uncertainty in obtaining theoretical results except in the case of peroxides. The benzoyl peroxide type of the latter class of compounds gave results which could be explained only by a deep-seated decomposition of the Grignard reagent or oxidation of the benzene nucleus to form a phenol with the subsequent evolution of methane. It would be extremely unsafe, however, to generalize on the application of the reagent as an analytical tool based on the results of the tests on the selected compounds in Table V.

TABLE III. ACTION OF GRIGNARD REAGENT ON UNIMPREGNATED CABLE PAPER

(Equivalent millimoles of CH_4. Temperature 100° C.)

Nitrogen	Weight of Sample	Grignard Evolved, Found	Water by Weight	Deviation	Remarks
Liters	*Mg.*			%	
2.5	105	0.458	0.448	+2.2	Reagent in cell 2, 50% humidity, paper cell 1, siphoned reagent from cell 2 immediately after addition of paper
2.5	102	0.625	0.425	+47	Reagent cell 2, 50% humidity, paper cell 1, water distilled from cell 1 to cell 2
3	282	0.005[a]	..	..	Paper dried 18 hours at 105° C. in cell 1. Added reagent to cell 2 and siphoned back to cell 1.

[a] 0.02 mole added to paper sample in this experiment.

TABLE IV. ACTION OF GRIGNARD REAGENT ON ACIDS

(Equivalent millimoles of CH_4)

Substance	Temperature	Grignard Evolved Found	Grignard Evolved Theory	Grignard Evolved Deviation	Grignard Added Found	Grignard Added Theory (1 Mole)	Grignard Added Deviation
	° *C.*			%			%
Cyclohexane[a] carboxylic acid as received	100	0.127	0.116	+9	0.112	0.116	−3.5
Abietic acid[b] crystallized from *n*-pentane	100	0.196	0.177	+11	0.009	0.177	−97
	100	0.102	0.090	+13	0.004	0.090	−96
Palmitic acid[a] as received	100	0.119	0.093	+28	0.159	0.093	+71
Acetic acid[a] crystallized	100	0.133	0.105	+27	0.133	0.105	+27
Benzoic acid[b] fused	100	0.144	0.119	+21	0.202	0.119	+70
	25	0.159	0.167	−4.0	0.130	0.167	−22
	25	0.125	0.128	−2.5	0.096	0.128	−25

[a] Reagent added to cell 2. Acid dissolved in isoamyl ether and added by syringe to cell 2.
[b] Acid added to cell 1 at 25° C. and reagent added to cell 2. Siphoned reagent from cell 2 to cell 1.

TABLE V. MISCELLANEOUS COMPOUNDS

(Equivalent millimoles of CH_4. Temperature 100° C.)

Substance	Grignard Evolved Found	Grignard Evolved Theory	Grignard Added Found	Grignard Added Theory
n-Butanol over[a] CaO	0.100	0.096	0.008	.....
n-Butanol over[b] activated Al_2O_3	0.173	0.172	Nil	.....
Benzophenone[b] as received	0.006	.....	0.114	0.115
Benzophenone[c] recrystallized from pentane	Nil	.....	0.126	0.123
Ethyl benzoate[b] distilled	0.004	.....	0.162	0.162 (2 moles)
Benzoyl peroxide[c]	0.060	0.083 (1 mole)	0.287	0.249 (3 moles)

[a] Added in capillary to cell 1 and distilled into reagent in cell 2.
[b] Substance dissolved in isoamyl ether and added by syringe to reagent in cell 2.
[c] Added to cell 1 and siphoned back reagent from cell 2.

Conclusion

A microgravimetric Zerewitinoff method is described which has numerous advantages over the volumetric procedure. The preliminary tests show that theoretical results may be obtained for the active hydrogen content of a typical organic compound at 25° C. in the absence of certain types of peroxides. It appears unlikely, however, that arbitrary conditions of time and temperature can be set to include all compounds containing active hydrogen. The presence of acids renders the measurement of the quantity of reacting reagent without evolution of methane extremely inaccurate. It was concluded that the method showed promise for the determination of Grignard evolved of oil and oil-impregnated paper samples but that no correlation between known groups with the Grignard added could be expected.

Acknowledgment

The authors wish to express their appreciation of the interest taken in this work by W. F. Davidson, director of research.

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A Semimicro-Dumas Method for Difficult Compounds

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A procedure is given for the determination of nitrogen in carbon compounds which form methane and tars upon pyrolysis. A special nitrometer for use in this procedure is described.

ORGANIC compounds which form tars, graphitic carbon, or methane upon pyrolysis, present difficulties when analysis is attempted by the standard Dumas method. The following modification of the method previously published by the author (*5*) has given satisfactory results on this class of compounds for the past 2 years, in the hands of both the author and others.

Spies and Harris (*6*) and Hayman and Adler (*3*) have proposed methods for burning tar- and graphite-forming compounds. The large amounts of copper acetate required by the procedure of Hayman and Adler for the larger semimicro-sample introduces difficulties in burning the sample at a slow and uniform rate, and for this reason preference is given to the method of Spies. The greater quantity of potassium chlorate necessary to burn the larger sample makes the use of boats out of the question. Mixing the potassium chlorate with cupric oxide gives satisfactory combustions.

Precipitated manganese dioxide made according to the method of ter Meulen (*7*), after having been heated to 500° to 600° C. in a stream of purest carbon dioxide, will quantitatively burn methane to carbon dioxide and water at 500° to 600° C. Although ter Meulen uses manganese dioxide in place of copper oxide for the Dumas nitrogen, he seems to be unaware of the oxidizing power of manganese dioxide toward methane at a higher temperature than he recommends (400° to 450° C.).

The combustion tube is made of Supremax glass. Figure 1 shows the arrangement of the burners and the dimensions of the various fillings. Sections *a*, *h*, and *l* are copper spirals, and *b* is a section of iodine pentoxide (60-mesh). Section *c* is 10-mesh pumice, which serves to insulate the hot copper oxide (60-mesh), *d*, from the iodine pentoxide. Section *e* is 40-mesh copper reduced by hydrogen, which is followed by section *f*, platinized asbestos, and section *g*, manganese dioxide pretreated in the following manner: The freshly precipitated and washed manganese dioxide is allowed to dry in Petri dishes, the lumps are broken up with a razor blade and screened, and the product, >20- to <40-mesh, is saved.

A Supremax combustion tube 40 cm. long is filled with this hydrated manganese dioxide and heated to 500° to 600° C. while a steady stream of purest carbon dioxide flows through the tube at a rate of 2 or 3 bubbles per second. This treatment is continued for 4 to 6 hours. At the end of this time the tube is allowed to cool and is emptied. This manganese dioxide is then used to fill the combustion tube. [ter Meulen states (*7*) that manganese dioxide decomposes at 450° C. During the heating, quantities of oxygen are given off, but the oxide remains a deep chocolate brown. The appearance of a pale brown oxide indicates that too high a temperature has been used and the product is worthless.] The carbon dioxide used was obtained from a Kipp generator equipped with a mercury valve. Treated marble chips and dilute hydrochloric acid were used to generate the gas.

Section *i* is 40-mesh copper reduced by hydrogen and is renewed for each analysis as specified by Spies and Harris (*6*). Section *j* consists of sample and fine copper oxide mixed in the usual manner. Section *k* is a mixture of fine copper oxide and 0.1 to 0.15 gram of potassium chlorate. The part indicated as *m* is heated by a hollow mortar containing boiling xylene as described in a previous paper (*5*). The long burner, *p*, is placed as shown, while *r* and *s* are movable burners. The various sections should be separated from each other by small wads of asbestos (not shown).

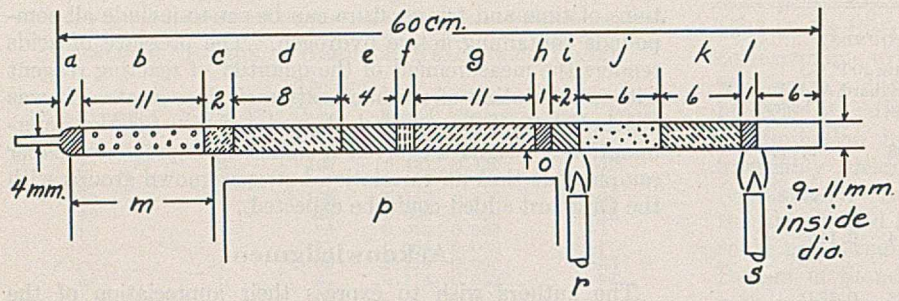

FIGURE 1. COMBUSTION TUBE

In carrying out the analysis, the reduced copper, *i*, the sample, *j*, the potassium chlorate–copper oxide mixture, *k*, and the copper spiral, *l*, are introduced in the order given. After the tube has been freed of air in the usual manner, the mortar burner and the long burner are ignited. A Pyrex beaker is slipped over the end of the long burner as far as *o* to prevent premature burning of the sample. When the tube has come to full heat and microbubbles appear in the nitrometer, burner *r* is ignited, the beaker is removed, and the sample is burned by gradually moving *r* away from the long burner.

When the sample has been burned, burner *s* is ignited. Burners *r* and *s* are then simultaneously moved toward the long burner at such a rate as to generate the oxygen evenly. A rate of one bubble per second is usually sufficient. When gases cease to be evolved, the tube is swept free of nitrogen at a rate of two bubbles per second. The nitrogen collected in *a* (see Figure 2) is transferred to *c* and allowed to stand over the yellow phosphorus until no more fog forms. It is then transferred to *d* and the volume read after a lapse of 2 to 3 minutes. This stage of the procedure follows that of Parker (*4*) and Foxwell (*2*), who used it to determine nitrogen in coke.

In a few instances the results were low in spite of the greatest care in carrying out the combustion. The solution of this difficulty is as follows:

The tube is cooled, and the copper oxide–potassium chlorate section and the cupric oxide sample section are emptied into different evaporating dishes. Into each dish 0.1 to 0.15 gram of finely powdered potassium chlorate is put, and each mixture is ground thoroughly by means of a small glass pestle. The mixtures are again placed in the tube and the combustion is carried out as if a new analysis were being made. The nitrogen from the two combustions is collected and the volume measured.

One exceedingly difficult compound, belonging to the above-mentioned class, required four such treatments for complete combustion. Such compounds are rare, however, and one procedure as described will give satisfactory results with the majority of compounds.

TABLE I. ANALYSES OF PYRIMIDINES

(Analyses performed by L. P. Ferris, II. Weight of samples, 9 to 20 mg.)

	Nitrogen Determined			
Compound	Dumas (Pregl, I_2O_5 type) %	Spies-Harris %	MnO_2 method %	Theory %
2-Methyl-5-*n*-butyl-4,6-dihydroxypyrimidine	15.14	...	15.32	15.38
	15.10	...	15.49	...
	12.40	...	...	...
	14.00	...	...	...
	15.02	...	...	...
2-Methyl-5-*n*-amyl-4,6-dihydroxypyrimidine	...	14.66	14.32	14.29
	...	14.65	14.33	...
2-Methyl-5-ethyl-4,6-dihydroxypyrimidine	...	18.60	18.09	18.19
	...	...	18.28	...

No definite directions can be given as to the rate of burning. In general, the more methane a compound forms during the analysis, the slower must be the burning of the sample. The manganese dioxide filling gradually loses its reactivity toward methane, and should be renewed after about 15 to 20 analyses. More time is required to remove the last traces of nitrogen than is necessary with a standard filled tube. The analysis will require about 1.5 hours for a single combustion procedure.

At times during the analysis a sudden evolution of gas may occur, and may carry small quantities of oxygen past the reduced copper. Also, some compounds require several potassium chlorate combustions to be burned completely. For these reasons the nitrometer described in Figure 2 is recommended for this procedure.

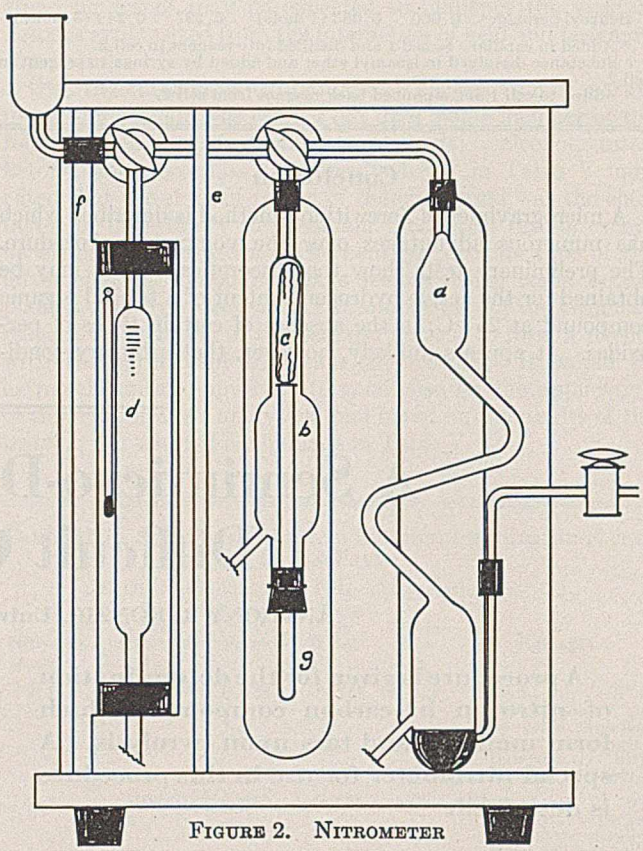

FIGURE 2. NITROMETER

Parts *a*, *b*, and *d* are the nitrometer, phosphorus tube, and gas buret. The stick of yellow phosphorus, *c*, is in buret *b*, and is pushed up by glass rod *g*, as it is consumed. The iron rods, *e* and *f*, are used to support the leveling bulbs for the gas buret and the phosphorus tube. The solution used to fill nitrometer *a* is 50 per cent potassium hydroxide. The confining liquids in the phosphorus tube, *b*, and the gas buret, *d*, are distilled water. Care should be taken in the transfer of gases that no potassium hydroxide be allowed to flow into *b*, as this would cause the formation of phosphine. The potassium hydroxide reservoir for *a* is of the desk-top type.

The results obtained on several members of a series of pyrimidines which could not be successfully analyzed by the author's previously published method are shown in Table I. Some of the compounds are tar-forming and also give methane on pyrolysis. The compounds mentioned by Craig (*1*) were also analyzed by this procedure.

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Systematic Qualitative Organic Microanalysis

Determination of the Refractive Index of Liquids

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THE determination of the refractive indices of liquid and solid compounds is very important in any systematic scheme of qualitative organic analysis—e. g., the microanalytical scheme described by Alber (*1*). Various procedures for measuring the refractive index by means of the microscope are available for minute amounts of material (*6, 7*). For the purposes of qualitative organic microanalysis a satisfactory method in the authors' opinion should meet the following requirements: (1) An accuracy within ±0.001 is all that is usually necessary in the method of identification of an unknown organic compound, since small amounts of impurities or changes of 1° C. in the temperature of the laboratory will influence the fourth decimal place to a considerable extent. (2) Special emphasis should be placed on liquid samples in view of the dearth of data on the refractive index of organic solids. (3) The amount of liquid used for one determination must not exceed 10 cu. mm. and should, at least to a large extent, be recoverable unchanged after the determination. (4) Manipulation should be easy, allowing quick determinations; a time limit of 10 minutes appears reasonable if one considers the small amounts to be measured. (5) The total range of refractive indices should extend from that of water to at least 2.00.

During recent years the authors systematically tested several methods with numerous compounds of varying constitution. Without doubt, the most convenient instrument for the determination of refractive indices is the Abbé refractometer, but it requires about 50 to 100 cu. mm. of liquid sample, and has a limited range. In some cases, the volume can be reduced to about 10 cu. mm. by distributing the liquid on a 15 × 10 mm. piece of fine tissue paper—e. g., Japanese lens paper—and placing it between the prisms. Under these conditions the recovery of the substance is rather difficult. The dipping refractometer of Zeiss can be equipped with an auxiliary prism designed by Pregl (*17*) and a diaphragm, allowing measurements on about 20 cu. mm. of the liquid sample with an accuracy of a few units in the fifth decimal place; unfortunately, this instrument is expensive.

The "*schlieren*" method of Emich and co-workers (*4, 6, 9, 19*) is a true microprocedure, since one is able to determine the refractive index on a few cubic millimeters of the liquid with a sensitivity of about 0.0001. This qualitative method has been adapted for quantitative measurements by Alber and von Renzenberg (*5*) and has been found by Mayrhofer (*15*) to be very useful in pharmaceutical analysis. With inexpensive apparatus results accurate to within ±0.0005 can be obtained, but the recovery of the sample, distributed in a larger amount of a reference liquid, involves too many difficulties for frequent use. Also, a considerable number of standard liquids with $\Delta n = 0.0250$ must be available to cover the range of refractive indices encountered in qualitative organic analysis.

Methods involving the use of a microscope possess certain advantages for the microanalyst. The Becke-line method (*6, 7*), the sensitivity and accuracy of which have been carefully studied by Saylor (*18*), can be applied to liquids if a few particles of a glass powder with known refractive index are immersed in about 5 cu. mm. of the unknown liquid. If the refractive indices of the glass and sample are close, an average accuracy of 0.003 can be obtained on unknown organic liquids under ordinary laboratory conditions. Unfortunately, it is rather difficult to obtain sets of glass powders with closely graded refractive indices. Kofler (*13, 14*) has recommended a set of 16 powders differing in refractive index by 0.02 to 0.03 and covering the range from 1.4339 to 1.8052. In testing, this procedure was found impractical with volatile and viscous substances, the difficulties being caused by the several additions and removals of glass particles necessary to bring the refractive indices to a match.

Wright (*20*) gave a splendid review of methods for single "drops" of liquids as used in connection with petrographic work, but the special apparatus required is not easily available. The method of Jelley (*10*) applies the smallest amounts of liquid of any known method, 0.1 cu. mm. being mentioned; apparently it is accurate to within ±0.001. Since the apparatus was not available at the time of the authors' investigations, the modified form suggested by Edwards and Otto (*8*) was tested, which gave results accurate to only ±0.004 on 10-cu. mm. liquid samples. Kirk and Gibson (*11*) came to the same conclusion, and then developed a method which correlates the change of depth of focus with a change in refractive index of the liquid. This method was published after completion of the authors' experimental work, and could not be included in this report; but it seems that the procedure is equivalent in accuracy and ease of manipulation to the one described below.

The procedure finally adopted was that of Nichols (*16*), who kindly cooperated in the construction of a microrefractometer with a capacity of from 6 to 7 cu. mm., which meets the above requirements and is easier to manipulate than the corresponding macroinstrument for 100 to 200 cu. mm. (According to a private communication, smaller cells with 5-cu. mm. capacity have since been constructed.)

Description of Microrefractometer

For details the reader is referred to the original article by Nichols (*16*). Two prisms, G and G_1, of the same refractive index, $N = N_1$, are cemented into a metal ring and covered with the unknown liquid, L, of refractive index N_2 to be determined (Figure 1). Light strikes the fine line Y', drawn across the base of the prisms, passes undeviated through the prisms, and, upon entering L, is deviated, depending upon the difference in the refractive indices. In the case of an assumed smaller refractive index of the liquid ($N_2 < N_1$ or N), the path of light will resemble the schematic drawing of Figure 1; lines Z and Z_1 are produced by passing through the surface of the liquid, formed by a cover glass. The angle between Z and Z_1 measures the double deviation of the light beam and is directly proportional to the Δn between glass prisms and liquid, the distance of Z from Z_1 being determined with a microscope at the virtual lines Z' and Z_1', best at points 1 and 2.

In the actual microrefractometer (obtainable through the Arthur H. Thomas Co., Philadelphia, Penna.) the two tiny prisms are mounted in a metal ring 5 mm. in diameter, the upper rim of which is above the top of the prisms. A slide, 40 × 80 × 3 mm., carries two independent cells, having prisms with refractive indices

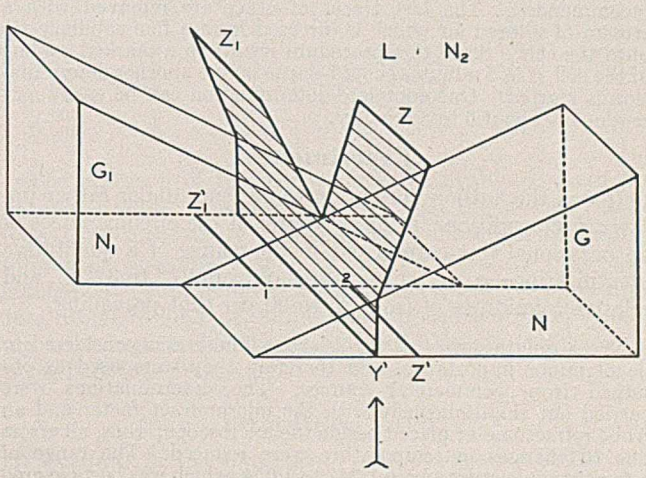

FIGURE 1. SCHEMATIC PRESENTATION OF PLANE OF LIGHT PASSING THROUGH CELL

L Liquid with lower refractive index than that of prisms G and G_1
$N_1 > N_2$, $N > N_2$, $N = N_1$

of 1.52 and 1.72, respectively. (The experiments in this study were carried out with a cell of $n = 1.75$; glass prisms of this refractive index are no longer obtainable.) Below the prisms is drawn a fine line which is viewed through the microscope. For greater accuracy or for use with very volatile liquids, the cells can be surrounded by a water jacket cemented to the glass slide.

Procedure

The instrument is best calibrated by means of several stable liquids of known refractive index—e. g., mixtures of paraffin and Halowax oils (standards 1 to 5 in Table I).

TABLE I. REFRACTIVE INDEX DETERMINATION OF LIQUIDS AT 28° C.

(For this series of experiments a cell marked 1.75 was used.)

Liquids Tested	Microrefractometer Readings in divisions	n found from graph or table	Abbé Refractometer Readings	Error in Units of $0.00x$
Standard 1 (1.4803, 21° C.)	19.0	1.479	1.4779	+1
Standard 2 (1.5198, 21° C.)	16.4	1.517	1.5174	...
Standard 3 (1.5508, 21° C.)	14.2	1.548	1.5482	...
Standard 4 (1.5972, 21° C.)	10.9	1.595	1.5952	...
Standard 5 (1.6348, 21° C.)	8.3	1.633	1.6333	...
Isobutyl alcohol	24.6	1.398	1.3968	+1
Diethyl oxalate	24.0	1.407	1.4059	+1
Oleic acid	20.4	1.458	1.4578	...
Cyclohexanol	20.1	1.462	1.4618	...
Cyclohexane	19.8	1.468	1.4681	...
Benzyl alcohol	15.0	1.536	1.5362	...
Nitrobenzene	14.3	1.547	1.5475	−1
Bromoform	11.5	1.587	1.5877	−1
α-Bromonaphthalene	6.6	1.657	1.6547	+2

In making a measurement, the liquid is transferred into the cell by means of a capillary pipet, so that the liquid forms a convex meniscus above the rim. A cover glass is slipped into place, care being taken that no bubbles are formed. The microscope should allow a magnification of from × 80 to 140. The ocular is provided with a scale, or, better, with a filar micrometer. The tube length of the microscope must be the same throughout all experiments. The microscope is sharply focused on the two lines and the distance between them measured on the micrometer scale. The number of divisions (or the corresponding millimeters) is entered as the abscissa, the corresponding refractive index as the ordinate, and the value plotted. By using at least four liquids of known refractive index a straight line is obtained, the slope of which depends upon applied constants—i. e., magnification, size of divisions in millimeters, and refractive index of the cell. Both cells are calibrated in this manner, so that the whole range from the refractive index of water to about 2.00 is available. Since the change in refractive index with temperature is known (see Table I), one can derive similar lines for other temperatures, or can interpolate all the values for increasing refractive indices and prepare a table, from which the refractive index can be read directly.

After the measurement most of the liquid is removed from the cell by means of a capillary pipet and the rest is washed out with a suitable solvent. As standard procedure washing the cover glass and cell with a stream of absolute alcohol from the one-piece wash bottle of Alber (*2*), and following this with ether, are recommended. The last traces of ether are removed with a stream of filtered air which is directed from a fine capillary tip onto the cell. Since this procedure results in a marked cooling of the cell, 3 to 5 minutes should elapse before another determination is started. One complete determination can be easily carried out in about 6 to 8 minutes.

Experimental

It was the authors' intention to measure under rather unfavorable conditions, so as to approach the circumstances of an occasional test carried out in the course of a qualitative identification—e. g., temperatures of from 27° to 31° C. and relative humidities of from 75 to 90 per cent prevailing.

The absolute purity of the selected liquids was considered to be of minor importance, and therefore they were used as obtained from commercial sources. The determinations were carried out simultaneously with the microrefractometer and an Abbé refractometer placed beside the microscope; thus, all errors due to changes in temperature were reduced. The range of refractive indices was from 1.347 to 1.654, which was best covered by using the cell marked 1.75. The microscope was a Leitz instrument, with objective No. 3 (× 10.75) and a Reichert eyepiece × 13, giving a magnification of about × 140. The microscope tube length was held constant at 170 mm. Under these conditions one division of the scale in the eyepiece micrometer corresponded to 0.019 mm., and 0.1 division could be estimated. Since the lines of the authors' refractometer as observed through the microscope had a width of about 0.3 division, it was necessary to take the readings on the corresponding edges of the lines. This uncertainty in the reading seems to be responsible for the major part of the error of this method. When exact focus has been attained there is no parallax and results can be easily duplicated.

Table I shows some of the results obtained. The mean deviation between the two methods was calculated from over 100 determinations to be −0.0002; the maximum deviation observed was ±0.002 in two cases only, one of which is reported in Table I. In general, an accuracy within ±0.001 in the determination of the refractive index can be safely assumed.

With white light no difficulties have been encountered with dispersion, especially if lower magnifications (× 80) were used (eyepiece × 8 and objective × 10 as recommended by Nichols, *16*). Only a few slightly colored liquids have been tested up to the present.

With controlled light and temperature conditions the accuracy can be increased to within ±0.0005, in comparison with the Abbé refractometer, the measurements of which are assumed to be accurate to ±0.0001. Sodium filters, Eastman Kodak Company, No. 64 or 73, are placed before the light source and give ample light of uniform wave length. The water jacket previously mentioned keeps the temperature constant to within 0.5° C.

The refractive index of the standard liquids was determined at three temperatures, 21.0°, 26.2°, and 28.0° C., the average deviation per 1° C. change in temperature being about 0.0003; the results of the determination at two temperatures are given in Table I. Thus it was possible to calculate and prepare graphs or tables for any desired temperature.

Determinations of the refractive index at elevated temperatures can be carried out with the help of Kofler's hot stage (*12*). However, it is not advisable to work above 55° to 60° C.

In order to prove that the accuracy was about the same in both the 1.52 and 1.75 cells, several liquids were tested at about the same time in each of the two cells and in the Abbé refractometer; the results of these experiments are reported in Table II. The cell marked 1.52 is recommended to be used for refractive indices below 1.40 and above 1.65, the cell marked 1.75 (in newer instruments 1.72) for refractive indices between 1.40 and 1.65 and from 1.85 to 2.00.

TABLE II. COMPARISON OF ACCURACY OF TWO CELLS AT 28° C.

Liquids Tested	Cell Marked 1.52: Readings in divisions	Cell Marked 1.52: n found from graph	Cell Marked 1.52: Error in $0.00x$	Abbé Refractometer	Cell Marked 1.75: Readings in divisions	Cell Marked 1.75: n found from graph	Cell Marked 1.75: Error in $0.00x$
Ethyl alcohol	14.9	1.360	+1	1.3584	27.3	1.359	+1
Heptaldehyde	10.1	1.413	−1	1.4143	23.3	1.416	+2
Ethylene glycol	8.8	1.427	−1	1.4285	22.6	1.427	−2
Di-*n*-butyl phthalate	3.2	1.489	...	1.4891	18.4	1.488	−1
β-Phenylate bromide	−2.6[a]	1.553	...	1.5532	13.9	1.553	...

[a] This indicates displacement of lines from their undeviated positions in direction opposite from that in the other cases (ordinarily designated with +).

In a special series of experiments, the capacity of the cells was determined as 6.2 and 7.0 cu. mm., after the cover glass had been slipped in place. Approximately 8 cu. mm. of the unknown liquid were needed, since a small amount was displaced by the cover glass. The recovery after the determination was about 5 cu. mm. Liquids of varying viscosities, vapor pressures, surface tensions, etc., were examined. The recovered samples were used later for specific gravity determinations by the milligram procedure of Alber (*1*), micro

boiling point determinations, quantitative elementary analysis, or the preparation of derivatives for further identification (*3*). No changes in the cement were noticed after these investigations, as established by photographic recording, but care should be taken that liquids with a marked solvent action do not remain in contact longer than absolutely necessary for the determination.

Useful application of the microrefractometer may be made in the determination of refractive indices of solid compounds. After carrying out the Becke-line test with consideration of the details stressed by Saylor (*18*), frequently only 10 to 20 cu. mm. of immersion liquid remained, which were best measured in the microrefractometer. Satisfactory results are assured, since the accuracies of both methods (±0.001) are equivalent.

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Improvement of Formaldoxime Colorimetric Method for Manganese

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THE author's formaldoxime colorimetric method for manganese (*1*) was found inadequate by Wiese and Johnson (*2*) and in more recent studies also by the author in biological materials high in phosphates. However, the interference of phosphates can be eliminated by precipitation with Pb^{++} in acetic acid solutions and the original method may be employed with very satisfactory results.

Method of Procedure

An aliquot of 5 ml. of the acidified (hydrochloric acid) solution of the ashed biological materials is titrated with *N* sodium hydroxide using methyl red as indicator (and the volume of sodium hydroxide required is recorded). This aliquot is discarded, because the indicator (methyl red) will interfere with the accuracy of the results of the colorimetric determination. A new aliquot, preferably 10 ml., is placed in a graduated 50-ml. centrifuge tube or other container and a calculated volume of *N* sodium hydroxide is added, based on the titration value obtained with methyl red. The sample is then acidified with 2 ml. of a 20 per cent solution of acetic acid.

The neutralization of hydrochloric acid with sodium hydroxide favors the formation of ferric and manganic phosphate precipitates. However, the addition of acetic acid to the mixture dissolves the manganic phosphate precipitate but not that of ferric phosphate. Excess phosphate, with plant tissues of a high phosphorus content, is removed by the addition of 0.5 ml. of a 5 per cent solution of lead acetate. The mixture is agitated, allowed to stand for 10 minutes, and then treated, to remove excess lead, with 1 ml. of a 20 per cent solution of sodium sulfate. After 30 minutes the mixture is either centrifuged or filtered. A 10-ml. aliquot of the centrifugate or filtrate is neutralized with 40 per cent sodium hydroxide, 3 to 4 drops of the formaldoxime reagent are added, and then more of the 40 per cent sodium hydroxide solution until the pigment develops (*1*). The addition of sodium cyanide as stated in the original publication (*1*) is not necessary. Ferric chloride need not be added to the standard if the removal of iron from the unknown was complete. The final volume of the unknown and of the standard is made to either 15 or 20 ml. in a graduated test tube or in a volumetric flask.

Reagents

A 5 per cent solution of lead acetate, $Pb(CH_3COO)_2.3H_2O$, and a 20 per cent solution of sodium sulfate, $Na_2SO_4.10H_2O$.

TABLE I. MANGANESE RECOVERED FROM SOLUTIONS CONTAINING KNOWN AMOUNTS OF MANGANESE AND PHOSPHATE

Mn Present	Treatment			Mn Found	Recovery	Difference
	PO_4	Pb	SO_4			
Mg.	*Mg.*	*Ml.*	*Ml.*	*Mg.*	%	%
0.01	0.100	0.5	1.0	0.0096	96.0	−4
0.01	0.100	0.5	1.0	0.0098	98.0	−2
0.01	0.050	0.5	1.0	0.0100	100.0	0
0.01	0.050	0.5	1.0	0.0098	98.0	−2
0.01	0.025	0.5	1.0	0.0100	100.0	0
0.01	0.025	0.5	1.0	0.0096	96.0	−4
0.01	0.010	0.5	1.0	0.0096	96.0	−4
0.01	0.010	0.5	1.0	0.0100	100.0	0
0.005	0.050	0.5	1.0	0.0050	100.0	0
0.005	0.050	0.5	1.0	0.0048	96.0	−4
0.01	0	0.5	1.0	0.0100	100.0	0
0.01	0	0.5	1.0	0.0096	96.0	−4
0.01	0	0.5	1.0	0.0096	96.0	−4
0.01	0	0	0	0.0100	100.0	0
0.01	0	0	0	0.0100	100.0	0
0.005	0	0	0	0.0050	100.0	0
0.005	0	0	0	0.0050	100.0	0

The precision of the method may be evaluated from the data reported in Table I, which show that the method can be used very successfully with biological materials containing interfering amounts of PO_4^{--} and Fe^{+++} after both substances have been removed according to the above procedure. In a 10-ml. volume of sample containing from 0.005 to 0.01 mg. of manganese in the presence of 0.010 to 0.100 mg. of phosphate the average error was 2 per cent.

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Microtitration of Selenium

As Applied to Abnormal Amounts of Selenium in Urine

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THE volumetric determination of very small amounts of selenium has been successfully carried out by Mathews, Curl, and Osborn (*8*), who used an iodine-thiosulfate method and determined the end point electrometrically. Later, Curl and Osborn (*3*) used starch to determine the end point with somewhat less precise results. The method reported here is simpler than either of these methods, and gives results of about the same precision as the electrometric method.

Method

The organic matter in a 100-ml. sample of urine was destroyed with concentrated sulfuric acid in the presence of mercury. The selenium was separated from the digestion mixture by distillation in the presence of hydrobromic acid and then precipitated (using sulfur dioxide and hydroxylamine hydrochloride), filtered, washed, and redissolved in a solution of hydrobromic acid and bromine. The bromine was destroyed and an excess of standard sodium thiosulfate was added along with a little potassium iodide. The unused sodium thiosulfate was titrated with standard potassium iodate using the dead-stop method (*2*, *6*, *7*) for determining the end point.

Reagents

SODIUM THIOSULFATE. A standard 0.1 *N* solution was made by dissolving 25.0 grams of the C. P. crystals in freshly boiled and cooled distilled water to make 1 liter of solution. This solution was standardized against C. P. potassium dichromate after it had stood for 24 hours. It was stored in a glass-stoppered bottle in a cool place and remained standard for several weeks without the addition of any preservative.

Standard 0.001 *N* sodium thiosulfate was made up daily by diluting exactly 10 ml. of 0.1 *N* solution to 1 liter with freshly boiled and cooled distilled water. It was found that this solution would remain standard for at least 36 hours. One milliliter of 0.001 *N* sodium thiosulfate equals 19.74 micrograms of selenium.

POTASSIUM IODATE. Potassium biniodate, C. P., was recrystallized twice from water and the crystals were dried in an electric oven at 105° C. A standard 0.1 *N* solution was made by dissolving 3.250 grams of the dry crystals in water to make 1 liter of solution. Standard 0.001 *N* solution was made by diluting exactly 10 ml. of 0.1 *N* solution to 1 liter. The dilute solution was standardized daily against freshly prepared 0.001 *N* sodium thiosulfate using the dead-stop method and the same amounts of all reagents as were used in titrating the selenium. The ratio of this solution to various 0.001 *N* sodium thiosulfate solutions made by diluting 0.1 *N* sodium thiosulfate remained constant for many days, showing that dilute solutions of potassium iodate do not deteriorate.

Potassium iodide (free from iodate), 5 per cent solution prepared fresh daily

Sodium sulfite, 5 per cent solution

Phenol, U. S. P., 5 per cent by volume in water

Hydrobromic acid–bromine solution, 30 per cent hydrobromic acid containing 1 per cent of liquid bromine by volume

Hydrobromic acid–bromine solution, 48 per cent hydrobromic acid containing 1 per cent of liquid bromine by volume

Sulfur dioxide, liquid, in a steel tube

Hydroxylamine hydrochloride, Eastman reagent No. 340

STANDARD SOLUTION OF SELENIUM. Selenium dioxide was prepared from C. P. selenium by dissolving the metal in concentrated nitric acid, evaporating the solution to dryness, and twice subliming the solid selenium dioxide. The calculated weight of dry selenium dioxide (0.1405 gram) was dissolved in water containing 5 ml. of 0.5 *N* sodium hydroxide and the whole diluted to make 1 liter of solution. One milliliter contained 100.0 micrograms of selenium.

Dead-Stop End Point

The apparatus (Figure 1) consisted of two platinum wire electrodes hooked up as shown in Figure 2. The electrodes were made by sealing small loops of platinum wire into short pieces of 4-mm. soft-glass tubing. It was found that the electrodes sometimes developed microscopic cracks around the seal which made them useless for detecting the end point. When the cracks were closed in a flame, the electrodes regained their original sensitivity. Erratic behavior was observed when the platinum was not completely immersed in the solution.

The dead-stop end-point apparatus was used as follows:

The solution of sodium thiosulfate, to be titrated, was made up to about 25 ml. in a 125-ml. beaker and 1 ml. of potassium iodide solution was added. The beaker was placed in the apparatus and, with mechanical stirring, the rheostat, *R*, was adjusted so that a small current was flowing through the electrodes as was indicated by the displacement of the galvanometer reading from its rest point by several scale divisions. The potential between the electrodes was usually about 100 millivolts. Standard 0.001 *N* potassium iodate was added steadily until the galvanometer began to show deflections. As the end point was approached, the galvanometer showed a temporary deflection after each drop of reagent was added. At the end point, one drop of reagent

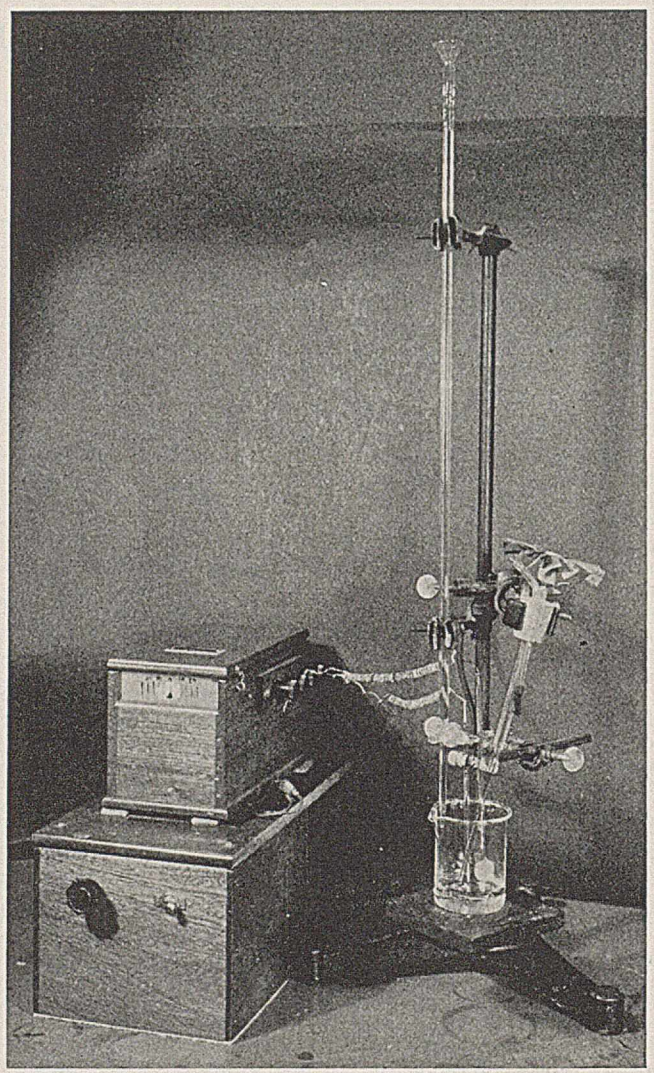

FIGURE 1. DEAD-STOP END-POINT APPARATUS

TABLE I. TITRATION OF SOLUTIONS OF SELENIUM

(1 ml. of 0.000994 N $Na_2S_2O_3$ = 19.62 micrograms of selenium)

$Na_2S_2O_3$ 0.000994 N Ml.	KIO_3 0.000966 N Ml.	KIO_3 0.001008 N Ml.	Net $Na_2S_2O_3$ Ml.	Se Found	Se Added	Difference
				Micrograms		
9.00	..	1.34	7.64	149.9	150.0	−0.1
9.00	1.50	..	7.54	147.9	150.0	−2.1
8.00	..	1.65	6.33	124.2	125.0	−0.8
7.00	..	2.00	4.97	97.5	100.0	−2.5
7.00	2.02	..	5.04	98.9	100.0	−1.1
5.00	..	1.14	3.84	75.3	75.0	+0.3
5.00	1.17	..	3.86	75.7	75.0	+0.7
4.00	..	1.43	2.55	50.0	50.0	0.0
4.00	1.50	..	2.54	49.8	50.0	−0.2
3.00	..	1.65	1.33	26.1	25.0	+1.1
3.00	1.75	..	1.30	25.5	25.0	+0.5
2.00	..	1.38	0.60	11.8	10.0	+1.8
2.00	1.52	..	0.52	10.2	10.0	+0.2
1.00	..	0.61	0.38	7.5	5.0	+2.5

TABLE II. PRECIPITATION AND TITRATION OF SELENIUM

(1 ml. of 0.000994 N $Na_2S_2O_3$ = 19.62 micrograms of selenium)

$Na_2S_2O_3$ 0.000994 N Ml.	KIO_3 0.000994 N Ml.	KIO_3 0.001008 N Ml.	Net $Na_2S_2O_3$ Ml.	Se Found	Se Added	Difference
				Micrograms		
9.00	1.45	..	7.55	148.1	150.0	−1.9
9.00	..	1.38	7.60	149.1	150.0	−0.9
7.06	1.98	..	5.08	99.7	100.0	−0.3
7.00	..	1.94	5.03	98.7	100.0	−1.3
5.00	1.15	..	3.85	75.5	75.0	+0.5
4.00	1.43	..	2.57	50.4	50.0	+0.4
4.00	..	1.47	2.51	49.2	50.0	−0.8
3.00	1.65	..	1.35	26.5	25.0	+1.5
2.00	..	1.50	0.48	9.4	10.0	−0.6
2.00	1.35	..	0.65	12.8	10.0	+2.8

caused a permanent galvanometer deflection of 3 to 5 scale divisions, and further addition of potassium iodate sent the galvanometer reading completely off the scale. The end point was sharp and reproducible, so that 0.05 ml. of excess 0.001 N potassium iodate solution could be detected in 50 ml. of titration mixture.

The dead-stop apparatus is simple to assemble and convenient to operate. Various authors have used it successfully (*9*, *10*), although one author (*1*) failed to get satisfactory results with it.

Experimental Procedure

The first series of experiments was made by measuring various amounts of standard selenium solution from a 10-ml. buret into 150-ml. beakers containing 3 ml. of 30 per cent hydrobromic acid–bromine solution and 15 ml. of water. Sodium sulfite was added, dropwise, until the color of bromine was almost gone. (If too much sodium sulfite was accidentally added, the bromine color was restored with a few drops of hydrobromic acid–bromine solution.) The last trace of bromine was removed with 2 to 3 drops of phenol solution.

Standard 0.001 N sodium thiosulfate was then added to the solution, so that 2 to 3 ml. were present in excess. After the addition of 1 ml. of potassium iodide solution, the excess sodium thiosulfate was immediately titrated with 0.001 N potassium iodate using the dead-stop method to determine the end point. The results of two series of experiments are given in Table I.

A second series of analyses was made in which various amounts of standard selenium solution were measured from a 10-ml. buret into 150-ml. beakers containing 40 ml. of 30 per cent hydrobromic acid–bromine solution. Gaseous sulfur dioxide was passed slowly through the solutions until the bromine was just destroyed, avoiding an excess. One-half gram of solid hydroxylamine hydrochloride was added to each beaker and the mixtures were warmed for 10 minutes on the steam bath. After the mixtures had cooled for 2 hours, the red selenium was filtered off through a No. 1 porous-bottomed porcelain crucible and washed thoroughly with distilled water. Three 1-ml. portions of hydrobromic acid–bromine solution were poured through the filter, which was then washed with six to eight 2-ml. portions of distilled water.

The filtrate and washings were collected in the original beaker, and treated first with sodium sulfite solution and then with phenol solution to destroy the bromine. The analyses were completed as described above. The results of two series of experiments are found in Table II.

The last series of analyses was made using 100-ml. samples of urine to which various amounts of standard selenium solution were added from a 10-ml. buret. Twenty-five milliliters of concentrated sulfuric acid were added to each sample (in 300-ml. Kjeldahl flasks) along with 0.7 gram of mercuric oxide and several glass beads. The flasks were heated over a gas flame until the water was evaporated and the organic matter completely destroyed (about 3 hours). The clear solutions were transferred to 300-ml. flat-bottomed flasks using 60 ml. of distilled water to wash out the Kjeldahl flasks. Twenty-five milliliters of concentrated sulfuric acid were added to each distilling flask along with 50 ml. of 48 per cent hydrobromic acid–bromine solution, and the ground joint was moistened with concentrated sulfuric acid. The flasks were then attached to small Liebig condensers similar to those used by Robinson *et al.* (*11*) and the mixtures distilled until 35 to 40 ml. of distillate collected. The distillate was treated by the same procedure as was used in the second series of analyses. The results are given in Table III.

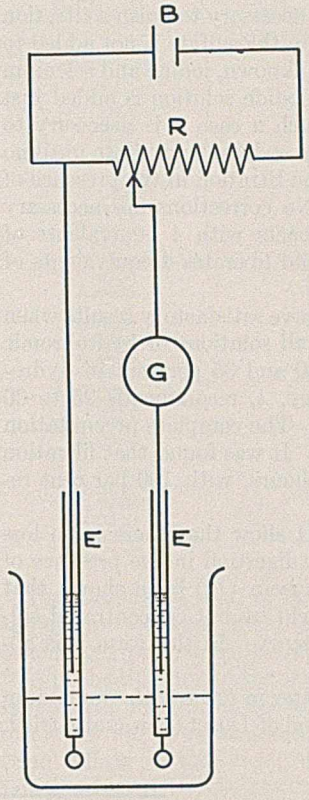

FIGURE 2. WIRING DIAGRAM

B. 1.5-volt dry cell
E. Platinum electrode
G. Leeds & Northrup galvanometer of enclosed lamp and scale type. Sensitivity, 0.025 microampere per scale division
R. Potentiometer-type rheostat of about 1500 ohms' resistance

Discussion

It is believed that this method is capable of giving results as precise and as accurate as any method reported in the literature. As little as 3 micrograms of selenium can be positively identified by its characteristic red color on the filter pad. The precision is about ±2 micrograms in the range 2 to 150 micrograms and the accuracy is within these limits. On the basis of a 100-ml. sample, the method is sensitive to 0.02 part per million, which is sufficient for the present purpose. It is believed that increased sensitivity may be attained by starting with a large sample, say 1 liter, rather than by using more dilute reagents.

The method has been used satisfactorily for determining up to 2000 micrograms by taking an aliquot portion of the hydrobromic acid–bromine distillate for the final titration.

Two alternate methods of making the titration were tried and abandoned in favor of the one used.

1. Potassium iodide solution was added to the selenite solution after the bromine had been destroyed with phenol, and the liberated iodine was titrated directly with standard sodium thio-

TABLE III. DETERMINATION OF SELENIUM IN URINE

(1 ml. of 0.001087 N $Na_2S_2O_3$ = 21.46 micrograms of selenium)

$Na_2S_2O_3$ 0.001087 N Ml.	KIO_3 0.001004 N Ml.	Net $Na_2S_2O_3$ Ml.	Se Found	Se Added	Difference
			Micrograms		
9.00	2.14	7.02	150.6	150.0	+0.6
8.00	2.44	5.75	123.4	125.0	−1.6
7.00	2.56	4.64	99.6	100.0	−0.4
5.00	1.67	3.46	74.3	75.0	−0.7
4.00	1.78	2.36	50.6	50.0	+0.6
3.00	1.93	1.22	26.2	25.0	+1.2
2.00	2.16	0.01	0.2	0.0	+0.2

sulfate solution. The dead-stop end point was not so sharp nor so reproducible when iodine was titrated with sodium thiosulfate as when sodium thiosulfate was titrated with potassium iodate in the presence of potassium iodide.

2. Potassium iodide solution was added to the selenite solution as above and the iodine was reduced with standard sodium thiosulfate solution. The excess sodium thiosulfate was then titrated with potassium iodate solution.

In these titrations, red selenium was precipitated and the results were not so precise as when selenium was not present.

However, it will sometimes be necessary to finish a titration by method 2. If sufficient sodium thiosulfate is not added to react with all the selenite in an unknown, iodine and selenium will be formed when potassium iodide solution is added just before the final titration. In such a case it is necessary to continue the addition of standard sodium thiosulfate until no iodine remains and then finish the titration in the presence of a small amount of selenium. No corrections are necessary because one mole of selenite reacts with 4 equivalents of sodium thiosulfate in one case and liberates 4 equivalents of iodine in the other.

The recommended procedure gave satisfactory results when selenium was precipitated from all solutions of hydrobromic acid which contained between 20 and 35 per cent of hydrobromic acid by weight. (Dudley, *4*, recommends 25 to 30 per cent of hydrobromic acid.) The complete precipitation of selenium required two hours. It was found that filtration may be delayed as long as 24 hours, with 100 per cent recovery of the selenium added.

The results given in Table III show that there is no loss of selenium during the Kjeldahl digestion in the presence of mercury. Sreenivasan and Sadasivan (*12*) have shown that selenium is oxidized to selenic acid by hot concentrated sulfuric acid in the presence of mercury. In this form it is apparently not volatile.

Destruction of the organic matter in urine with ammonium persulfate according to the method of Duret (*5*) was also tried. Selenium was oxidized to selenic acid during this digestion and 100 per cent of the selenium was recovered in every case. The method was abandoned, however, after several rather violent explosions. The explosions seemed to be spontaneous and in the gaseous phase throughout the reflux condenser. No glassware was ever shattered.

No mention has been found in the literature of the danger of explosions while using the persulfate in the wet way to destroy organic matter. It is quite possible that in this case volatile, unstable organic peroxy compounds were formed.

Summary

A new method for titrating very small amounts of selenium has been shown to give good results when applied to solutions containing known amounts of selenium, and when used to determine small amounts of selenium in urine.

Acknowledgment

The authors wish to thank Dr. J. H. Sterner and Miss Viola Lidfeldt for many suggestions during the course of this work.

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Determination of Minute Amounts of Potassium

Iodometric Evaluation of the Cobaltinitrite Precipitate, Using Ceric Sulfate

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SMALL amounts of potassium are usually determined titrimetrically by precipitation with sodium cobaltinitrite, followed by solution of the washed precipitate in a known excess of an oxidizing reagent. Potassium permanganate was originally used and continues to be used as the oxidant for dissolving the precipitate, its excess being determined by titration with standard sodium oxalate. More recently (*3*) the excess permanganate has been determined iodometrically, a procedure capable of detecting very small quantities of potassium.

Harris and others (*2*, *5*) have pointed out that the use of ceric sulfate instead of potassium permanganate is advantageous. When potassium permanganate is used, a precipitate of hydrated manganese dioxide is often formed, which necessitates the addition of an excess of sodium oxalate to effect its solution, after which the end point is reached by an additional titration with permanganate. This difficulty is, of course, not encountered when ceric sulfate is used. Ceric sulfate solutions are more stable and consequently require fewer standardizations.

Brown, Robinson, and Browning (*2*) have lately published a procedure using ceric sulfate for dissolving the precipitate of sodium potassium cobaltinitrite. The excess ceric sulfate is titrated with ferrous ammonium sulfate, using *o*-phenanthroline ferrous complex as indicator. Using this procedure, potassium in amounts ranging from 0.2 to 1.0 mg. could be detected with an average error of 2 per cent.

A variation of the ceric sulfate procedure applicable to very small amounts of potassium has been developed and used for over 2 years. An excess of potassium iodide is added to the excess ceric sulfate and the iodine liberated is titrated with standard sodium thiosulfate using starch as indicator. Be-

cause of the sharpness of the end point, very dilute solutions of sodium thiosulfate can be used for the back-titration and potassium in amounts as small as 0.02 mg. can be determined accurately. This method has all the advantages of others where ceric sulfate is employed, in addition to the sensitivity of iodometric titrations.

Reagents

Sodium Cobaltinitrite Reagent, prepared according to Kramer and Tisdall (*6*). Solution A. Cobalt nitrate crystals (25 grams) are dissolved in 50 cc. of water and to this solution are added 12.5 cc. of glacial acetic acid.

Solution B. Sodium nitrite (potassium-free, 120 grams) is dissolved in 180 cc. of water, giving a total volume of about 220 cc. To all of Solution A are added 210 cc. of Solution B. An evolution of nitric oxide gas occurs at once. Air is drawn through the solution until all the gas has passed off. The reagent is placed in an ice chest and filtered each time before using. It will keep at least a month, usually 2 or 3 months.

Ceric Sulfate. About 4.5 grams of anhydrous ceric sulfate are dissolved in 500 ml. of distilled water to which 100 ml. of concentrated sulfuric acid have been added. The solution is diluted to 1000 ml. This solution, which is approximately 0.01 *N*, should be standardized before use with standardized sodium thiosulfate.

Sodium Thiosulfate, 0.002 *N*, prepared by the dilution of a 0.1 *N* solution. The latter is prepared by dissolving 25 grams of hydrated sodium thiosulfate in 1 liter of freshly boiled and cooled water, and adding 0.1 gram of anhydrous sodium carbonate. This solution is diluted 500 times to prepare the 0.002 *N* solution, which should be standardized before use with 0.01 *N* potassium iodate.

Potassium Iodide Solution. An approximately 1 per cent solution is prepared and kept in a small glass-stoppered dropping bottle. It is discarded when the addition of starch solution shows it to contain free iodine.

Starch Solution, 0.2 per cent. Two grams of starch are dissolved in 1 liter of boiling water and about 10 mg. of mercuric iodide are added. When cool the solution is kept in a glass-stoppered bottle in a refrigerator.

Procedure

A 0.5-ml. aliquot of the potassium solution is placed in a 15-ml. centrifuge tube previously cleaned with cleaning mixture, 0.5 ml. of precipitating reagent is added dropwise and with shaking, and the contents of the tube are thoroughly mixed and allowed to stand for 1 hour at a temperature of from 20° to 25° C. The precipitate is firmly packed in the bottom of the tube by centrifuging for about 10 minutes at about 2000 r. p. m. The supernatant liquid is removed by aspiration and the sides of the tubes are washed with 5 ml. of distilled water, care being exercised not to disturb the precipitate. The tube is recentrifuged at the same speed for about 2 minutes and the supernatant liquid again aspirated. This procedure is repeated twice more (making 4 centrifugations in all).

One milliliter of the ceric sulfate reagent is added (more than this for amounts of potassium greater than 0.06 mg.) and the tube is heated in a water bath for 2 or 3 minutes, until the precipitate dissolves. The solution is cooled to room temperature, 1 drop of the potassium iodide reagent is added, and it is titrated with standardized sodium thiosulfate, a few drops of starch solution being added near the end point. The end point is very sharp and easily visible in daylight. At the same time 1 ml. of the ceric sulfate solution is titrated with the standardized sodium thiosulfate.

Calculation

Mg. of K = 6.95 NY

N = normality of $Na_2S_2O_3$

Y = difference in ml. of $Na_2S_2O_3$ required to titrate equal amounts of $Ce(SO_4)_2$ before and after addition of the sodium potassium cobaltinitrite precipitate

Discussion

The results of a number of recoveries of potassium from solutions of potassium chloride are given in Table I. In determinations involving more than 0.06 mg. of potassium, 2 ml. of ceric sulfate solution were used. A study of the table reveals that from 0.036 to 0.120 mg. of potassium can be recovered with an average error of +0.5 per cent. With amounts ranging from 0.030 to 0.02 mg., the average error is −3.5 per cent; with even smaller amounts the error increases, being −10 per cent between 0.020 and 0.010 mg. and −20 per cent for smaller amounts. This increase in error may be due to incompleteness of precipitation or, more likely, to the solubility of the precipitate in the wash water. The use of organic solvents alone and mixed with water as wash reagents did not improve the results.

Table I. Recovery of Potassium from Solutions of Potassium Chloride

Potassium Present	Potassium Recovered	
Mg.	*Mg.*	%
0.1200	0.1190	99
0.1200	0.1188	99
0.1100	0.1122	102
0.1100	0.1108	101
0.1000	0.0997	100
0.1000	0.1007	101
0.0900	0.0908	101
0.0900	0.0908	101
0.0800	0.0806	101
0.0800	0.0797	100
0.0700	0.0709	101
0.0700	0.0698	100
0.0600	0.0595	99
0.0600	0.0598	100
0.0500	0.0499	100
0.0500	0.0505	101
0.0400	0.0413	103
0.0400	0.0391	98
0.0360	0.0366	102
0.0360	0.0364	101
0.0300	0.0297	99
0.0300	0.0289	96
0.0240	0.0231	96
0.0240	0.0230	96
0.0200	0.0191	96
0.0200	0.0191	96

The factor 6.95 used in the calculation of the potassium in the precipitate is empirical. It was noted by Drushel (*4*) and others and proved by Brown, Robinson, and Browning (*2*) that in the titration of the nitrite in the precipitate, the cobalt is reduced, accounting for one equivalent of the nitrites, the other eleven being accounted for by the oxidizing reagent, ceric sulfate in this case. If the precipitate has the composition $K_2NaCO(NO_2)_6$ as determined by Adie and Wood (*1*), the stoichiometric factor is 7.10 when the action of the cobalt is considered. This theoretical factor, however, is very rarely obtained, owing to the variable composition of the precipitate. Temperature of precipitation, manner of precipitation—i. e., rate of addition of precipitating reagent, volume in which precipitation occurs, and length of time for precipitation—ratio of sodium to potassium in the solution analyzed, and the analyst's technique are all important in the determination of the proper factor to be used. It is advisable for each experimenter to ascertain his own factor after a number of recoveries have been run. Once determined, the factor has been found to remain remarkably constant.

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Correction. In the article on "Estimation of Submicroquantities of Calcium" [Ind. Eng. Chem., Anal. Ed., 12, 118 (1940)], first line on page 118 following the heading "Method" and in the fifth line on page 119 the words "bromophenol blue" should have been used instead of "bromothymol blue".

Albert E. Sobel
I. Allen Kaye

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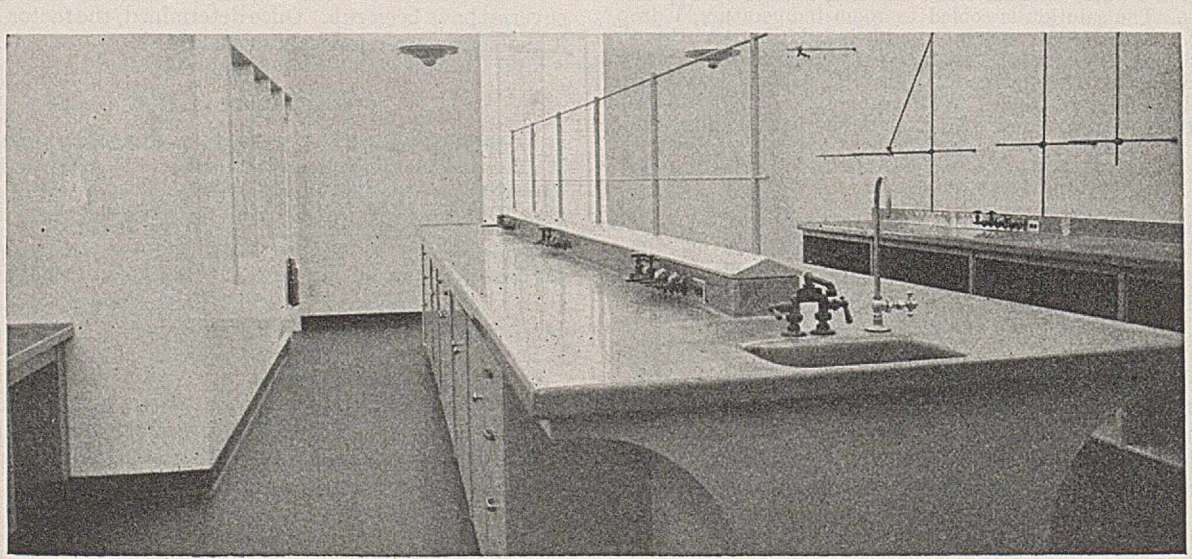

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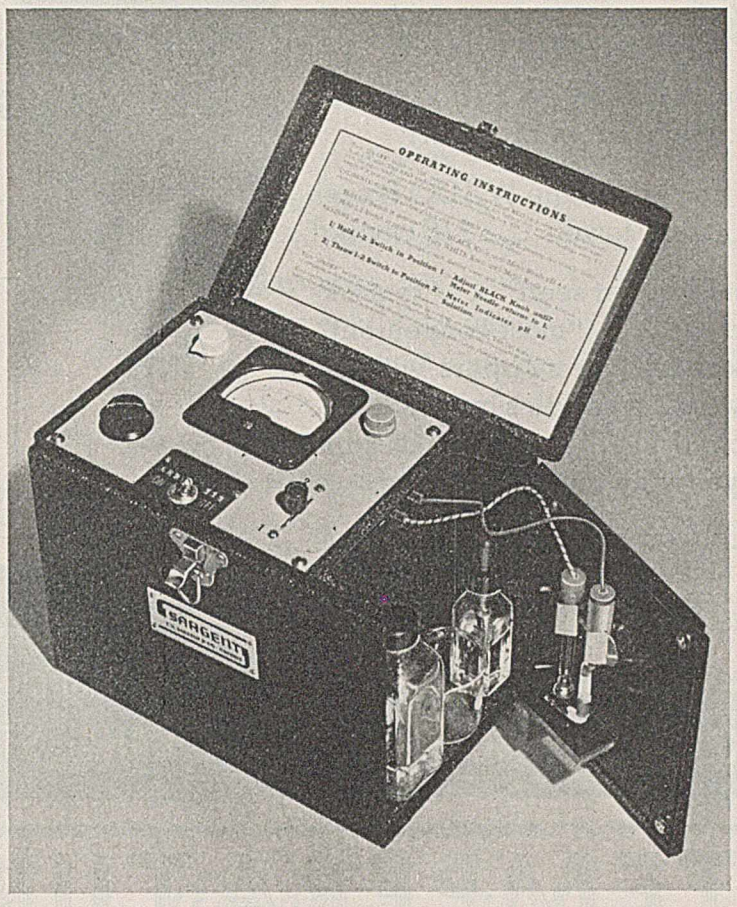

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