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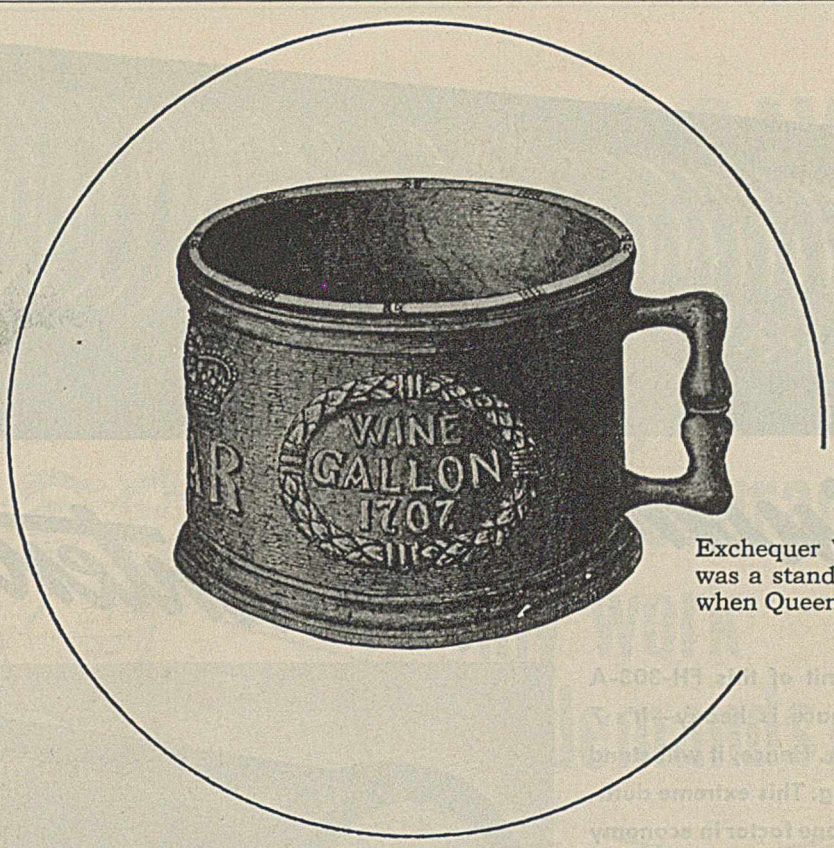

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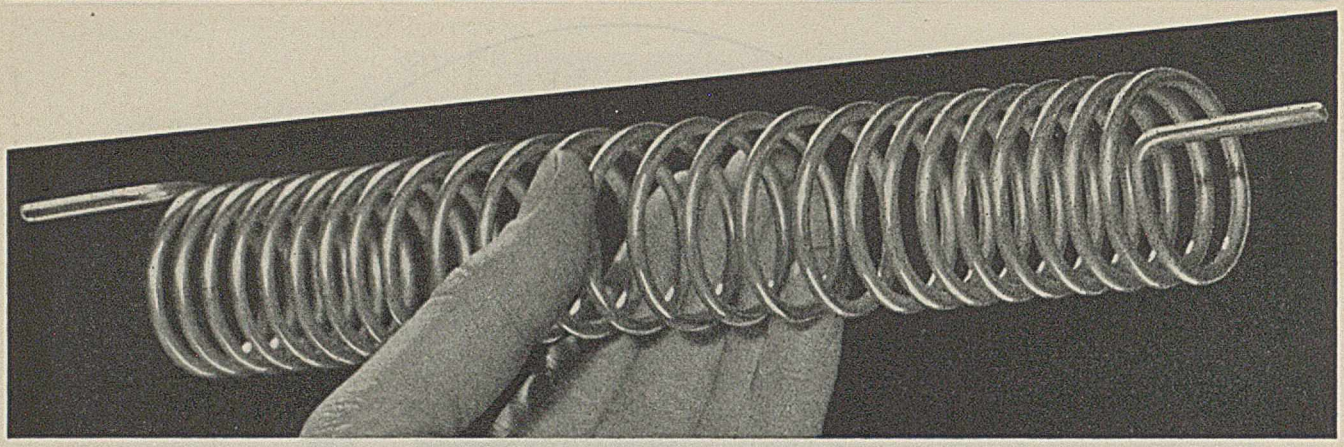

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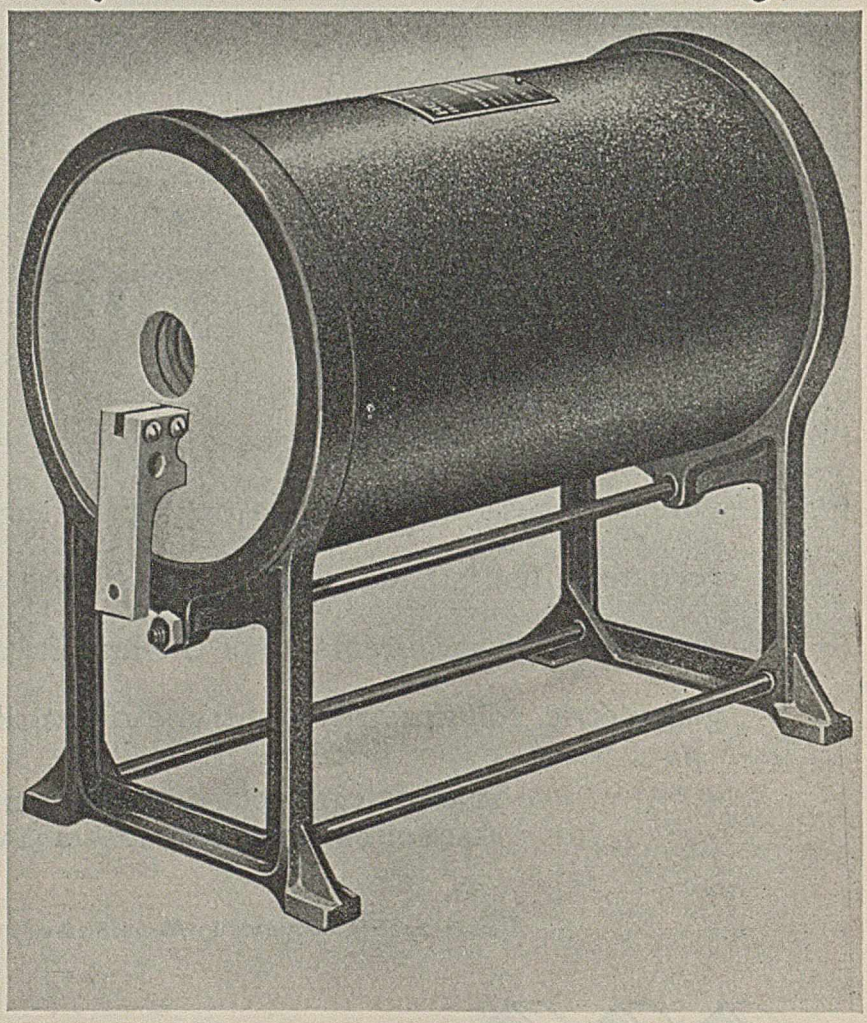

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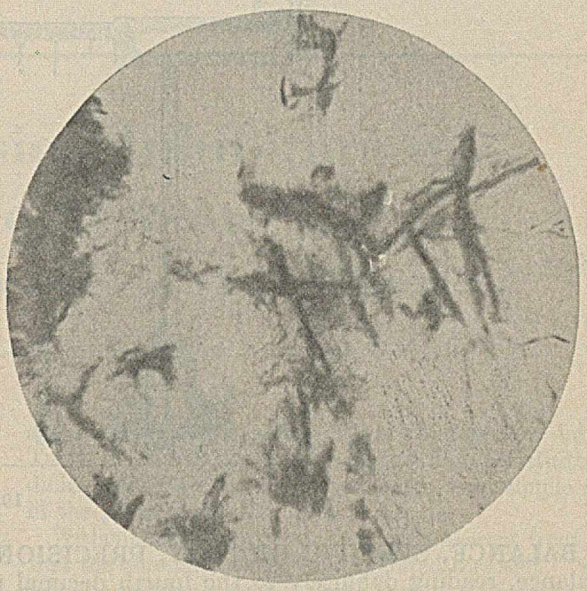

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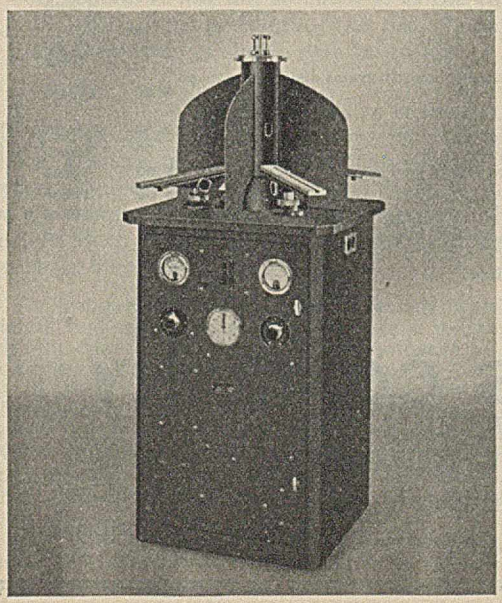

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INDUSTRIAL AND ENGINEERING CHEMISTRY

ANALYTICAL EDITION

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Chromate Corrosion Inhibitors in Bimetallic Systems

Compared by a Scoring Method Based on Visual Observations

MARC DARRIN, Mutual Chemical Company of America, Baltimore, Md.

Increasing amounts of water being used for air conditioning, washing, cooling, and condensing raise a problem in many localities in respect to water supply and sewage disposal. Present practice is tending toward recirculating systems to save the water and the use of corrosion inhibitors to save the systems. It is well known that the corrosion of many metals is greatly reduced by the presence of sodium chromate or sodium bichromate (*3*, *4*), but there are almost no data in the literature showing the relative efficiency of these chemicals for the retardation of bimetallic corrosion in ordinary recirculating water systems.

Comparisons are presented in this report for iron, galvanized iron, tinned iron, copper, brass, and aluminum, both alone and in contact with each other. The corrosion of these metals in various water systems was greatly retarded or completely inhibited by sodium chromate at the concentrations indicated. For some combinations sodium bichromate was equally satisfactory, but in general it was not so efficient as the chromate. In addition to prolonging the life of equipment, the formation of slimes may be avoided.

MANY corrosion processes may be followed by weight changes, provided precautions are taken to ensure that corrosion products either remain entirely on the specimens or are completely removed, without removal of uncorroded metal. Such data, however, may require interpretation by corrosion specialists.

For instance, iron may increase in weight because of formation of rust scales. Aluminum increases in weight, but the oxide film may be protective. In the presence of an oxidizing passifier such as sodium chromate, Hoar and Evans (*1*, *5*) have noted the formation of a dense inert protective film containing chromic oxide, Cr_2O_3. Others have noted the formation of protective films, such as zinc chromate on galvanized surfaces. Wilson and Groesbeck (*6*) have used salt-spray methods for testing corrosion inhibitors in air-conditioning equipment, and report that chromates and bichromates are the most efficient.

Corrosion tests conducted at this laboratory have revealed little relationship between weight changes in bimetallic systems and the practical value of chromate inhibitors. Table I gives some weight-change data illustrating how difficult they are to evaluate even for very simple systems in the presence of chromates.

Without visual inspection it is not apparent from Table I that the chromate completely inhibited corrosion. With bimetallic systems weight changes have been found to be even more complex and almost impossible to interpret without visual data. On the other hand it has been noted that the impression formed on visual examination corresponds in most instances to practical behavior. This does not apply to corrosion which produces concealed defects such as the intercrystalline corrosion of aluminum, the graphitizing of cast iron, or the caustic embrittlement of steel. Concealed corrosion can be evaluated best by physical measurements, such as loss of tensile strength, or by microscopic examination. Depth of penetration methods are also of practical value.

Various electrochemical methods that have been proposed provide fundamental assistance in studying the mechanism of corrosion, but require great skill and theoretical knowledge of the factors influencing corrosion. They are difficult because of innumerable local potential differences between different areas of the same metal, and between corrosion products. With dissimilar metals in contact in aqueous solutions—which is common from the practical viewpoint—the situation is particularly complex, resulting in electrochemical processes which may either intensify or retard corrosion.

Visual Scoring

This method is based on simple observations and the weighted scoring of characteristic corrosion criteria, which may be expressed numerically, and can be duplicated by different observers with a degree of precision satisfactory for practical purposes. The method may be varied to simulate

TABLE I. WEIGHT CHANGE AFTER 6 MONTHS AT 70° F.

	Aluminum	Iron	Galvanized Iron	Tinned Iron
Tap water	+0.123	−0.849	−0.564	−0.161
500 p. p. m. Na_2CrO_4	+0.021	+0.012	+0.017	+0.008
500 p. p. m. $Na_2Cr_2O_7$	+0.004	+0.007	−0.015	−0.020

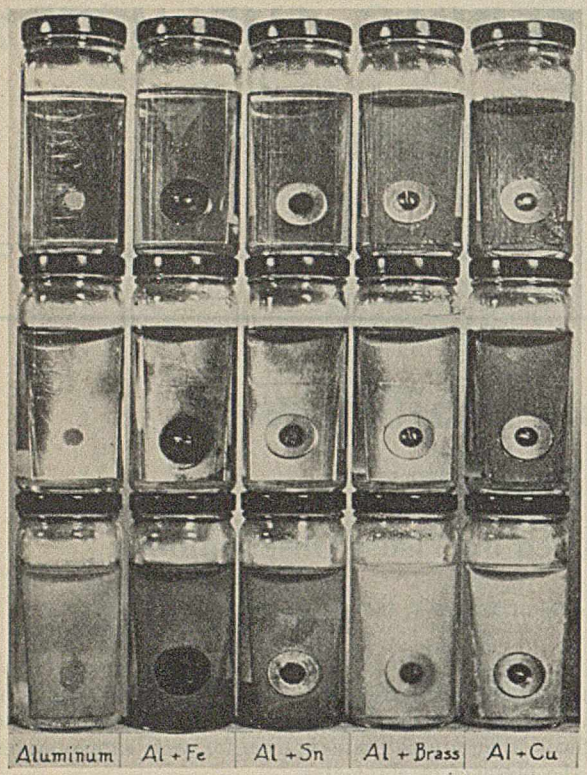

FIGURE 1. CORROSION TESTS

various practical conditions. Scoring may be reweighted to obtain comparisons for different applications.

Illustrative of a way in which corrosion criteria may be weighted for an air-conditioning system, cloudiness may be allowed 4 points out of a possible 100. Low scoring for cloudiness is indicated because it may not be harmful. If a precipitate settles, it may result in some harm and should be given a greater weight. If the precipitate is such that it may clog the system, a still greater weight is indicated. If the general appearance score is zero, the precipitate and cloudiness scores also will be close to zero. Other corrosion criteria may be selected and weighted in a similar manner.

The accompanying data further illustrate the scoring method. Forty points were allowed for the condition of each of the metals in contact; 20 points for the corrosion products in the liquid. The corrosion of each metal was scored on the basis of five types of observation: discoloration, roughening, pitting, depth of pits, and general corrosion. By general corrosion are meant the nature and distribution of corrosion, the general impression of the observer, and unclassified factors. Evidence of corrosion in the liquid was scored in a similar manner based on cloudiness, precipitation, and general appearance. Under general appearance of the liquid are included unclassified variables, the nature of the precipitate, and its probable harmful effect in a recirculating system.

The weightings employed in this report, shown in Table II, are arbitrary but logical and illustrate a practical application. Different weightings, however, are used for different applications. For instance, different weightings are indicated for an automobile cooling system than for an air washer. If discoloration or slimes are unimportant they may be disregarded. For some purposes the entire liquid score is disregarded. The scoring procedure in this laboratory is for the observers to check all of the descriptive terms on a report form. These data are assembled and weightings assigned according to the particular purpose of the corrosion project. The nature of the scoring method is such, however, that ordinary changes in weightings seldom result in important changes in the relative corrosion order. Of course the same weightings must be used in comparing corrosion scores.

A general idea of the protection efficiency may be obtained from the sum of the liquid and metal corrosion scores, but for many purposes it is best to consider them separately. In a bimetallic system, 100 represents perfect inhibition; with single metals, 60 indicates complete protection. A limitation of this method is that it does not afford a numerical comparison between monometallic systems and bimetallic systems, except under special conditions. There are technical difficulties in making comparisons between these systems due to differences in the nature of the initial corrosion centers or pits. The corrosion trend in monometallic systems is slower in developing. This was particularly evident with aluminum and to a lesser extent with zinc. For aluminum in tap water, the corrosion scores were better after 18 months than after 6 months. These changes appear to be due to the initial formation of many corrosion centers which are heavily weighted, because in most systems even small pits are an indication of the start of severe corrosion. It happens, however, that these initial pits, instead of deepening, either spread until they join to form a fairly uniform and resistive surface or change in light reflection so as to become invisible except under special illumination and low magnification. The procedure employed in this report is limited to corrosion criteria which can be seen by unaided visual inspection.

TABLE II. WEIGHTINGS

For Condition of Metal	
Discoloration	None (3), slight (2), moderate (1), considerable (0)
Roughening	None (4), slight (3), moderate (2), considerable (0)
Pitting	None (9), slight (6), moderate (3), considerable (0)
Depth of pits	None (12), shallow (9), moderate (6), deep (3), very deep (0)
General corrosion	None (12), slight (9), moderate (6), considerable (3), very bad (0)
For Condition of Liquid	
Cloudiness	None (4), slight (3), moderate (2), considerable (0)
Precipitate	None (8), slight (5), moderate (2), considerable (0)
General appearance	Good (8), fair (6), poor (4), bad (2), very bad (0)

It has also been found, when inhibitors are absent, that oxygen is so rapidly consumed as to cause corrosion to depend on the rate at which additional oxygen is obtained from the air, thus making numerical comparisons between some uninhibited systems meaningless—beyond the general observation that it is very severe as compared to inhibited systems. Irrespective of the explanation, a study of about a thousand corrosion record sheets indicates that technical difficulties in making numerical comparisons are very much less when inhibitors are present. The corrosion scores of all inhibited systems are given in Table III. Scores are also given for uninhibited bimetallic systems for purposes of general comparison, although they may be only approximately correct because of variations in oxygen deficiency. Corrosion scores have not been assigned to monometallic systems in tap water, but it can be seen from Figures 1, 2, and 3 that corrosion was severe in comparison with the inhibited systems. If numerical comparisons are desired between these systems a different procedure is indicated, involving aeration; but this is beyond the purpose of the present investigation which is to evaluate the protective efficiency of chromate inhibitors.

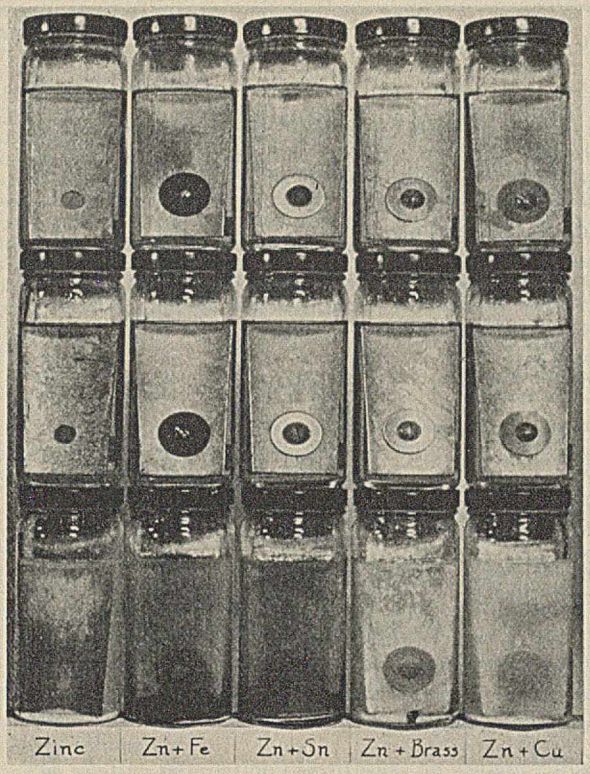

FIGURE 2. CORROSION TESTS

Experimental Data

Testing conditions which are not specified were identical for all specimens. "Tap water" refers to Baltimore city water. "Chromate" and "bichromate" refer to solutions of sodium chromate, Na_2CrO_4, and sodium bichromate, $Na_2Cr_2O_7.2H_2O$, at concentrations equivalent to 1000 parts of Na_2CrO_4 per million. All regular tests were run in closed 8-ounce screw-cap glass jars, without agitation or aeration. Some aerated tests also were run.

The aerated tests indicated that corrosion in the presence of sodium chromate is essentially the same in closed as in aerated systems. If no chromate is present the corrosion is very much more rapid when aerated. Since there are varying degrees of aeration in practical applications, and since aeration is often the rate-making factor when chromates are absent, the most conservative way of evaluating chromate corrosion inhibitors is without aeration. In other words, the conditions of test used in this investigation represent the minimum relative retardation of corrosion afforded by the chromate. There are many conditions of aeration where the merit of the chromate is considerably greater than that indicated. There was some access of air when the test samples were inspected, but all were handled in an identical manner, and the admission of air was less than under any ordinary condition of practical use. The black appearance of the corrosion products on the iron test panels in tap water (Figure 3) indicates insufficient oxygen.

The photographs (*2*), Figures 1, 2 and 3, show the general appearance of the corrosion tests after 6 months at room temperature.

The top rows contain sodium bichromate, the middle rows sodium chromate, and the bottom rows tap water. The bolts and washers show the effect of contact with a dissimilar metal. Markings on the photograph show the plate metal first, followed by the washer metal. In each test the bolts and nuts are the same metal as the washers. Zinc refers to heavily galvanized iron plates. Tin refers to heavily tinned iron plates. Both galvanizing and tinning were done by double-dipping after cutting and drilling. Brass refers to a commercial grade, analyzing about 60 per cent copper, 40 per cent zinc. Copper and aluminum refer to the commercially pure metals, analyzing about 99.9 per cent copper and 99.0 per cent aluminum. The iron panels were cut from a mild steel sheet known as type A tank (about 0.1 per cent carbon). All panels were uniformly polished, cleaned, and inspected for defects prior to testing.

The panels tested at 160° F. were placed in thermostatically controlled ovens with uniform heat distribution. No light was admitted to the ovens. The room-temperature test panels were shielded from direct sunlight and were inspected at frequent intervals up to 18 months.

The principal data on which this report is based are the composite observations of four men for the same panels shown in the photographs. Several hundred similar tests were run. There were slight variations between tests started at different times, but on the whole there were no discrepancies between other corrosion series and those reported here. There were some variations in details of scoring by different observers, but the total corrosion score of each observer for any particular condition was essentially the same, as was the composite score. The corrosion scores of the monometallic systems did not prove to be a measure of the relative merit of the inhibitors because either sodium chromate or sodium bichromate gave such perfect inhibition that comparison of their scores was almost meaningless.

The tabulated data for iron and copper in contact illustrate the scoring method. The data for the aluminum series, zinc series, and iron series present the corrosion scores for the more important metal combinations. The plates and washers may be considered separately or in combination, depending on the purpose of comparison.

HOT WATER TESTS. Corrosion inhibition in hot water roughly paralleled that at room temperature. As would be expected, corrosion is more rapid. At the end of 6 weeks at 160° F. the corrosion scores of most of the bimetallic test panels dropped to figures comparable to 6 months at 70° F. With combinations

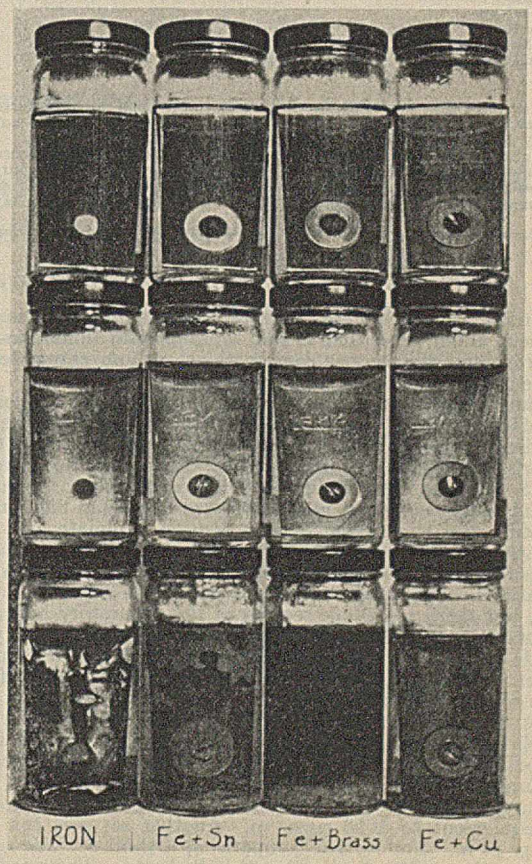

FIGURE 3. CORROSION TESTS

TABLE III. SCORING METHOD

	Tap Water	Chromate	Bichromate
Iron and Copper in Contact 6 Months at 70° F.			
Iron Plates			
Discoloration	Considerable	None	Slight
Roughening	Considerable	None	None
Pitting	Moderate	None	None
Depth of pits	Deep	None	None
General corrosion	Very bad	Slight	Slight
Copper Washers			
Discoloration	Slight	Slight	Slight
Roughening	None	None	None
Pitting	None	None	None
Depth of pits	None	None	None
General corrosion	Slight	None	None
Liquid			
Cloudiness	Considerable	None	Slight
Precipitate	Considerable	None	Slight
General appearance	Bad	Good	Fair
Plate score (40 = perfect)	6	37	36
Washer score (40 = perfect)	36	39	39
Liquid score (20 = perfect)	2	20	14
Protection efficiency (100 = perfect)	44	96	89
Aluminum Systems[a], 6 Months at 70° F.			
Aluminum Plates Alone			
Plate score	X	40	40
Liquid score	X	20	20
In Contact with Iron Washers			
Plate score	29	39	37
Washer score	20	38	25
Liquid score	5	20	14
In Contact with Copper Washers			
Plate score	10	19	14
Washer score	34	39	40
Liquid score	9	11	7
In Contact with Brass Washers			
Plate score	13	30	17
Washer score	34	39	33[b]
Liquid score	9	17	9
In Contact with Tin Washers			
Plate score	23	40	40
Washer score	15	40	39
Liquid score	9	20	20
Zinc Systems[a], 6 Months at 70° F.			
Galvanized Iron Plates Alone			
Plate score	X	40	39
Liquid score	X	20	20
In Contact with Plain Iron Washers			
Plate score	20	39	35
Washer score	34	37	37
Liquid score	4	17	14
In Contact with Copper Washers			
Plate score	9	36	28
Washer score	35	39	22[c]
Liquid score	4	14	14
In Contact with Brass Washers			
Plate score	20	40	29
Washer score	39	40	26[c]
Liquid score	8	20	16
In Contact with Tinned Washers			
Plate score	11	40	39
Washer score	14	39	39
Liquid score	7	20	17
Iron Systems[a], 6 Months at 70° F.			
Iron Plates Alone			
Plate score	X	40	37
Liquid score	X	20	20
In Contact with Copper Washers			
Plate score	6	37	36
Washer score	36	39	39
Liquid score	2	20	14
In Contact with Brass Washers			
Plate score	18	37	36
Washer score	39	39	36[b]
Liquid score	2	20	17
In Contact with Tinned Washers			
Plate score	21	37	37
Washer score	31	39	35
Liquid score	2	17	17

[a] X denotes that corrosion was severe in comparison with inhibited systems, as can be seen in photographs, but method employed does not give directly comparable figures for monometallic systems in tap water.

[b] Slightly lower score of brass washers in presence of sodium bichromate is due chiefly to discoloration, which may be disregarded for many purposes.

[c] Low score of copper washer in contact with zinc in presence of sodium bichromate is due to formation of a hard, rough, tightly-adhering layer of crystalline zinc compound over surface of copper, causing it to be reported as considerably roughened and pitted. Scraping off this deposit after 18 months revealed that copper had not actually been attacked. The scoring method is undoubtedly too severe in this instance, but shows that an undesirable condition exists when sodium bichromate is used in this system rather than sodium chromate. Somewhat similar conditions exist with brass washers.

of aluminum-copper and iron-copper, sodium chromate was more beneficial in hot water than in cold, whereas with aluminum-iron, zinc-iron, and zinc-copper combinations, sodium chromate was a little more effective at room temperature.

CHROMATE CONCENTRATION TESTS. Owing to accelerated corrosion at 160° F., a series of tests was run at this temperature, with iron plates in contact with copper washers, using triple proportions: 3000 parts per million. There was no corrosion after 8 weeks at 160° F. in the presence of sodium chromate, and only slight corrosion in the presence of sodium bichromate. Corrosion also was negligible with sodium chromate at a concentration of 1000 p. p. m. In other words, the protection afforded by sodium chromate was about the same at both concentrations. Lower concentrations were not included in this series, but there are ample records at this laboratory indicating that under most conditions concentrations of 200 to 500 p. p. m. provide good protection. Concentrations below 100 p. p. m. protect aluminum and zinc, but may cause stimulation of corrosion with iron systems.

REGULATED pH TESTS. A set of corrosion tests was run with iron plates in contact with copper washers, with the alkalinity of the sodium chromate adjusted to pH 7.0 by addition of sodium bichromate, the total parts per million of chromium being kept the same. Tests were for 8 weeks at 160° F. using a concentration equivalent to 1000 p. p. m. Another set was run with 3000 p. p. m. For further comparison, a third set was run at the same time without adjusting the alkalinity. These tests indicated that adjustment to pH 7.0 does not result in an impressive difference. If anything, sodium chromate without adjusting alkalinity gave slightly better protection.

AERATED TESTS. A set of aerated corrosion tests was run with the iron systems at 70° F. The air was drawn from outside the building by means of a water aspirator. It was well filtered, water-washed, and supplied at a low steady pressure. The air was bubbled through all test samples continuously at a slow uniform rate. All samples were tested at the same time and aerated in an identical manner. For comparison a closed set also was run at the same time without aeration but with other conditions identical. The corrosion of all the aerated test panels in tap water was very much faster and more severe. The behavior of the aerated samples in the presence of sodium chromate was the same as the corresponding nonaerated test panels.

EIGHTEEN-MONTH TESTS. The bimetallic systems retained essentially the same corrosion order, which was evident a few days after starting the tests, up to the time the photographs were taken 6 months later. Rescoring after 18 months showed essentially the same order as at 6 months. There were no signs that the sodium chromate had lost its effectiveness. In some cases the protection was better after 18 months than after 6 months.

PRACTICAL TRIALS. In trials with several small York air-conditioning units (34 × 37 inch washers), one Carrier (26 × 30 inch washer), and one large Sturtevant air-washer (60 × 60 × 84 inch spray chamber), weekly observations over a period of 18 months indicated that the use of sodium chromate not only prevented corrosion but also eliminated the formation of organic slimes. The nature of the microorganisms destroyed was not

investigated. Two new units without chromate accumulated a large amount of slime during the same period. One old unit which had accumulated slime and rust prior to the test completely cleared during the 18-month chromate treatment. Because of fluctuations in overflow the concentration of sodium chromate varied between 100 and 500 p. p. m. Each week it was adjusted to 500 p. p. m., on the basis of yellow color which was found satisfactory for practical purposes. Although chromate replacement is frequently due to mechanical loss through the water overflow, some consumption is due to the oxidation of organic material removed from the air. Many bacteria, molds, and accompanying odors are destroyed. Sodium bichromate is a little more reactive than sodium chromate, and consequently has a shorter life if much organic material is present. The foregoing results have been substantiated by other practical observations extending over 4 years, including several large air-conditioning units.

Summary

Visual scoring is adapted to the evaluation of chromate corrosion inhibitors. Results may be duplicated with an accuracy sufficient to be of practical value.

Sodium chromate is effective in retarding, or completely inhibiting, the corrosion of many bimetallic systems in water. Sodium chromate and bichromate are effective in retarding corrosion in most monometallic systems. Sodium bichromate also reduces corrosion in many bimetallic systems, but it is not so generally effective as sodium chromate.

Aluminum and copper, aluminum and brass, and zinc and copper combinations are difficult to inhibit. With these combinations, sodium chromate is very helpful but not completely inhibitive.

The relative retardation of corrosion by sodium chromate is essentially the same in hot water as in cold.

Neutralizing the alkalinity of sodium chromate to pH 7.0 does not result in any impressive differences. If anything, sodium chromate is more effective at its natural alkalinity of about pH 8.5.

Although the rate of corrosion of most systems is greatly accelerated by aeration, there is no important difference between aerated and closed systems when sodium chromate is present.

Increasing the concentration of sodium chromate slightly improves the protection, but this is not indicated except as a factor of safety when recirculating water is subject to dilution. No harm results from excess sodium chromate. Amounts less than about 100 p. p. m. should not be used for iron systems.

Sodium chromate retains its inhibitive properties until lost from the system, either mechanically or by chemical reduction. For many practical purposes its depletion may be judged by loss of color.

Acknowledgment

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Controlling Apparatus to Eliminate Waste of Water in Using the Ordinary Filter Pump

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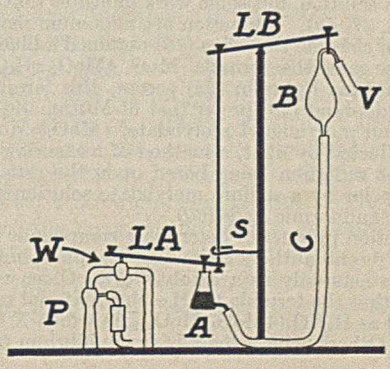
THE cost of operating the ordinary aspirator or suction pump is usually concealed in a single water bill which includes more legitimate uses as solvent, diluent, etc. The cost for a single aspirator may be on the order of thirty dollars per year, depending upon several obvious factors. For filtration, the removal of a supernatant fluid from a precipitate, the removal of used samples and reagents from such apparatus as the Van Slyke blood gas pipet, and many distillations under reduced pressure where rather large variations in pressure are not detrimental, a closed suction system with automatic control of the supply of water to the aspirator has proved satisfactory. The controlling apparatus shown in the accompanying figure has been found suitable for the purpose. It is highly reliable, having been in operation night and day for months during the past 3 years without attention or interruption.

P indicates the filter pump, connected to the vacuum line through a check valve (Bunsen valve), *W* is a whistle valve, and *V* is a rubber tube connecting flask *B* with the vacuum line. In operation, mercury in flask *A* depresses lever *LA*, opening the whistle valve and starting the aspirator. Increasing vacuum in the system "lifts" mercury from flask *A* to flask *B* until the shift



of weight is sufficient to depress *LB* and lift *LA*, shutting off the water. The two flasks and the rubber tubing connecting them, *C*, form a manometer in which the difference in mercury levels is a measure of the vacuum. The reduction in pressure in the system at the time of cutoff can be adjusted by raising or lowering the flasks in relation to the levers and to each other.

A controlling device for a suction system must have some lag in operation to avoid excessively frequent opening and closing and a tendency to chatter around the point of equilibrium. The lag in this apparatus is rather large, about 100 mm., but for many purposes this is not objectionable. If at any time the reduction below atmospheric pressure is not great enough, an inlet in the system may be opened to start operation of the aspirator. If uniform maximum suction is wanted, a pinchclamp may be placed on the connecting tube when flask *A* is filled with mercury, to prevent cutoff. When the suction system is not in use, the controlling apparatus may be left in the off position by placing the clamp on the connecting tube when flask *B* is full.

As shown, the entire control apparatus is accessible and in view for occasional inspection. It would be possible to use a similar device which had only one lever, the two flasks being hung from opposite ends. However, either one flask and the connecting tube would extend below the table or the piping and whistle valve would have to be elevated to several feet above it.

The material required is easily available, and construction is easy. The levers are of 5 × 16 mm. (0.187 × 0.625 inch) stock. The connecting rod between the levers is of 6-mm. (0.25-inch) cold-rolled steel. The support for the upper lever, *LB*, is 13-mm. (0.5-inch) pipe in a standard floor flange screwed to the table. All pivots (shown conventionally by black triangles) are 6-mm. (0.25-inch) bolt or 6-mm. rod, turning in drilled holes, except those above the flasks which are of iron wire hanging in V-shaped notches. The stop, *S*, is made of a piece of 6-mm. cold-rolled steel rod threaded and screwed into the support part of an ordinary ring-stand clamp. For connection with the heavy-walled rubber tubes, *C* and *V*, large capillary tubing (2 mm. in inside diameter) is fused to each of the 300-ml. flasks as shown. The capillary tubing is strong and tends to prevent violent surge in the mercury. The rubber connecting tube, *C*, must extend low enough to prevent mercury from being drawn below its lowest point and admitting air. A 13-mm. (0.5-inch) whistle valve is used. The upper lever, *LB*, extends 127 mm. (5 inches) to the left and 229 mm. (9 inches) to the right. The lower, *LA*, is 305 mm. (12 inches) long with 19 mm. (0.75 inch) between the pivot and the stem of the whistle valve. The support is 92 cm. (36 inches) high. It is desirable to place a trap in the line below *V* to prevent dirty liquid from getting into *B*, and the trap is essential to catch mercury, if the suction system is of copper tubing. A rubber stopper between *LA* and *S* cushions the blow when the lever hits the stop.

The Molybdenum Blue Reaction

A Spectrophotometric Study

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UNDER suitable conditions molybdates react with phosphates, arsenates, silicates, and certain other salts or acids to form heteropoly compounds, such as ammonium molybdiphosphate, $(NH_4)_3[P(Mo_3O_{10})_4]$, or molybdisilicic acid, $H_4[Si(Mo_3O_{10})_4]$. These complexes, after controlled reduction to give molybdenum blue, serve for the colorimetric determination of various reducing agents or of elements which function as central atom in the complex anion, such as phosphorus, arsenic, or silicon. The procedure must be carefully controlled, as the excess molybdate reagent itself may be reduced to molybdenum blue.

In view of the analytical uses of such reactions, it seemed of interest to make a spectrophotometric study of the processes in order to learn more about their nature, the effect of different variables, and the merits of the several procedures.

Previous Work

THE REDUCTION PROCESS. The nature and the composition of the material obtained on reducing simple molybdates are uncertain (*32*). Low concentrations of reactants yield what appears to be a solution, but some work indicates that the system is colloidal (*24*, *47*, *59*). By action of ammonium molybdate upon molybdenum chloride Berzelius (*10*) obtained a blue colored solid to which he gave the formula $MoO_2.4MoO_3.xH_2O$. For solid molybdenum blue Ephraim (*24*) accepts this formula, but Latimer and Hildebrand (*40*) prefer that of Muthmann (*48*) and call the compound molybdenyl molybdate, $(MoO_2)_2MoO_4$. The reduction is affected by light, a method of measuring the intensity of ultraviolet radiation being based upon the rate of formation of the blue color by a sodium molybdate solution acidified with hydrochloric and formic acids (*60*).

The reduction product of heteropoly compounds is also uncertain. Two recent authors claim that the phosphorus in molybdiphosphates acts only as a catalyst (*8*). Older work indicates (*17*, *20*, *22*) that the formula for the blue material separated may be expressed as $H_3XO_4(4MoO_3.MoO_2)_2$, in which X is phosphorus or arsenic. Others believe there is an equilibrium (*35*) such as

Molybdiphosphoric acid + chlorostannite ⇌ molybdenum blue + chlorostannate

From spectrophotometric data Gripenberg (*34*) assumed that six of the twelve molybdenum atoms in molybdiphosphate are reduced. On reduction with molybdenum chloride the final product is said to be $H_3PO_4(Mo_6O_{17}).xH_2O$, in which x is about 19 (*4*). Apparently a definite stage is reached in the reduction, as such a reaction is the basis of titrimetric methods for determining copper, iron, magnesium, and zinc (*30*). In a similar procedure for phosphate the reduced blue material, stated to be H_3PO_4-$(11MoO_3.MoO_2)_2$, is titrated with permanganate (*13*).

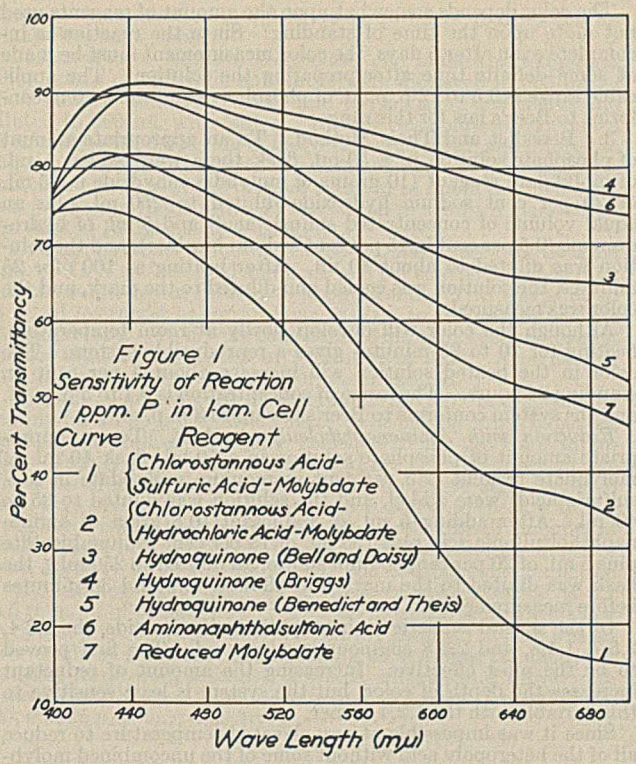
COLORIMETRIC APPLICATIONS. Since molybdenum blue arises from an interaction of oxidizing and reducing systems, there is a possibility of determining either reductants, through their action on some heteropoly compound, or heteropoly oxidants, through their action on suitable reductants. Thus, the cerous ion, in strongly basic solution, reduces molybdiphosphate (*38*); similarly, ascorbic acid may be determined as a reductant (*70*).

Several comparative visual or filter-photometric studies have been made on reductants as such (*1*, *3*, *35*). Specific compounds include chlorostannous acid (*50*), aminonaphtholsulfonic acids (*28*), benzidine (*26*), gallic acid (*52*), hydriodic acid (*72*), hydroquinone (*6*), *p*-methylaminophenol (*43*), sodium thiosulfate (*45*), and a molybdate reduced with some metal, usually molybdenum itself (*73*).

The determination of the central atom in heteropoly compounds has found most use.

Determination of Phosphorus. Most work has been done with phosphorus, presumably because of its wide distribution and biological importance. The principal points to be noted are the variety of reductants and the variations in their use.

Denigès (*19*) used chlorostannous acid under conditions to reduce only the heteropoly acid. Truog and Meyer (*64*) studied the effect of aluminum, calcium, iron, magnesium, manganese, and nitrate on the color, in addition to modifying the reagent and providing for interference of silica. Dickman and Bray (*23*) recommended hydrochloric acid in the reagent. Color studies have been made with the Leitz photometer (*5*, *54*) and the Lovibond tintometer (*36*). Color standards include indigo carmine (*29*), a reduced molybdate (*46*), and two mixtures of copper and



cobalt salts (*18*). Applications include the determination of phosphorus in argillaceous material (*68*), fertilizers (*55*), plants (*56*), soils (*16*), water (*25*), and tissues and blood (*11, 39*).

Bell and Doisy recommended hydroquinone as a reductant (*6*). Briggs (*14*) modified the reagent by making it acidic instead of alkaline, while Benedict and Theis (*7*) developed the color by heating. Denigès (*21*) and Rimington (*58*) pointed out certain errors in the method. This modification has been applied to blood (*57*), milk (*53*), soil extract (*2*), sugar (*15*), urine (*66*), and water (*61*).

Fiske and Subbarrow (*28*) used aminonaphtholsulfonic acid as a reductant. The best isomer has the amino and hydroxy groups para to each other (*65, 69*). Applications include orthophosphate in meta- and pyrophosphates (*12*) and phosphorus in muscle extract (*44*).

Zinzadze (*74, 76*) used a molybdate reduced with metallic molybdenum as reductant. By addition of bisulfite this reagent may be used to determine phosphorus in soils (*71*) and urine (*33*), in the presence of arsenates, iron, nitrates, and silicates. Copper (*21*) or a mixture of copper and lead (*42*) may be used in preparing the reagent.

Feigl (*26*) used benzidine in ammoniacal solution as a reductant. Tartaric acid prevents interference of arsenates (*27*). In this way phosphorus has been determined in rocks (*41*) and in water (*63*).

Determination of Silicon. By reduction of a molybdisilicate Isaacs (*37*) and Urbach (*67*) determined silicon. The method was criticized by Bertrand (*9*) but Foulger (*31*) refuted the criticism. Reducing agents include chlorostannous acid (*49*), hydroxylamine (*51*), sodium sulfite, sodium hyposulfite, and hydroquinone (*62*).

Determination of Arsenic. Zinzadze (*75*) and Truog and Meyer (*64*) determined arsenic by reducing the heteropoly molybdiarsenate. The reductants were reduced molybdate and chlorostannous acid, respectively.

Experimental Work

All color measurements were made with a General Electric photoelectric spectrophotometer, the absorption cells being 1.000 cm. thick and the spectral band 10 mμ wide. Distilled water was used in the blank cell, since the blank solution itself was slightly colored and the over-all color was desired.

Determination of Phosphorus. A standard phosphate solution containing 0.1 mg. of phosphorus per ml. was prepared by dissolving 0.4390 gram of recrystallized potassium dihydrogen phosphate in water, adding 15 ml. of 6 *N* sulfuric acid, and diluting to 1 liter. Dilutions of this stock solution were used.

Reduction with Chlorostannous Acid. A standard solution of chlorostannous acid reductant was prepared by dissolving about 100 grams of "stannous chloride" crystals in 100 ml. of concentrated hydrochloric acid and diluting to 4 liters. The solution was protected from the air by a layer of "Finol" 1 cm. thick, and the entrance to the bottle was trapped twice with alkaline pyrogallol. The original air in the bottle was removed by bubbling natural gas through the solution. The solution, found to be 0.1044 *M*, was oxidized about 2 per cent at the end of 14 weeks.

To prepare the reduced heteropoly compound, 10 ml. of a solution containing 0.01 mg. of phosphorus per ml. were measured into a 100-ml. volumetric flask and then 4 ml. of 2.5 per cent ammonium molybdate in 10 *N* sulfuric acid were added. After diluting to about 40 ml., 0.50 ml. of 0.1 *M* chlorostannous acid was added, and the solution was diluted to the mark. At the end of 7 minutes the spectrophotometric curve was determined. Spectral transmission curves, determined under different conditions for 1 p. p. m. of phosphorus, are shown in Figure 1.

The relative amounts of acid and molybdate in the reagent are important since, if the acid/molybdate ratio is too low, some of the molybdate is reduced along with the heteropoly acid; if too high, there is a marked decrease in the intensity of the color. The optimum ratio is that in which a blank would give no blue color. Tests showed that a 2.5 per cent solution of ammonium molybdate in 10 *N* sulfuric acid, or one 1.5 per cent in ammonium molybdate in 3.5 *N* hydrochloric acid, is the best reagent. Sufficient reagent should be used to make the final acidity about 0.4 *N* in sulfuric acid or 0.7 *N* in hydrochloric acid. Any residual acidity in the solution to be measured is neutralized to a slight yellow, using α-dinitrophenol as indicator, before adding the molybdate reagent. The amount of reductant need not be so carefully controlled as that of the molybdate reagent. Enough should be added to react with all the heteropoly acid, but too much leads to turbidity on standing. About 0.5 ml. of 0.1 *M* solution is sufficient for 100 ml.

Using 1-cm. cells, the range of concentration is 0.05 to 2.5 p. p. m. of phosphorus. A graph of the transmittancy on a logarithmic scale against concentration shows that the ammonium molybdate–sulfuric acid solutions conform to Beer's law up to 1 p. p. m., above which there is a negative deviation. The hydrochloric acid solutions conform up to 2.5 p. p. m.

Time of standing must be rather closely controlled. The full color develops in 3 to 4 minutes and begins to fade in about 10 minutes. The color measurement must be made, therefore, within this interval.

In studying the effect of diverse ions the ammonium molybdate–sulfuric acid reagent was used. The general procedure was the same as already described, except for the addition of the solution containing the diverse ion immediately preceding the ammonium molybdate reagent.

Of the 31 cations studied, aluminum, ammonium, beryllium, calcium, cerous, lithium, magnesium, manganese, potassium, sodium, and thorium in concentrations of 500 p. p. m. cause an error of less than 2 per cent of the phosphorus present. Barium, lead, mercuric, mercurous, silver, strontium, and zirconyl ions interfere by giving precipitates. Antimonous, bismuthyl, cadmium, chromic, cupric, ferric, and zinc ions form soluble complexes and thus bleach the color. Ferrous ions interfere, possibly through oxidation to the ferric state. Ceric ions interfere by oxidation. Cobaltous, nickelous, and uranyl ions interfere with their own hue. A summary of the effects of these cations is given in Table I.

Of the 32 anions studied acetate, benzoate, carbonate, formate, lactate, nitrate, perchlorate, salicylate, sulfate, sulfite, and tetraborate ions do not interfere in concentrations of 500 p. p. m. Oxidizing or reducing ions which interfere include chlorate, chlorostannate, cyanide, dichromate, nitrite, thiocyanate, thiosulfate, and vanadate. All the halogens interfere, the effect of fluoride being greatest. They decrease the intensity of the color, as do citrate, oxalate, and tartrate. Tungstate is reduced to form tungsten blue and must be absent. Arsenate, pyrophosphate, and silicate form heteropoly acids, which may be reduced and thus must be absent. Ar-

TABLE I. EFFECT OF DIVERSE IONS

Ion	Added as	Amount P. p. m.	Error %	Permissible Amount[a] P. p. m.
Ag^+	$AgNO_3$	..	Ppts.	0
AsO_3^{---}	$NaAsO_2$	10	13	1
AsO_4^{---}	Na_3AsO_4	1	25	0
Ba^{++}	$Ba(NO_3)_2$	..	Ppts.	0
Bi^{+++}	$Bi(NO_3)_3$	50	8	10
Br^-	NaBr	500	4	250
Cd^{++}	$Cd(NO_3)_2$	500	2	500
Ce^{++++}	$Ce(SO_4)_2$	100	22	10
Cl^-	KCl	500	4	250
ClO_3^-	$KClO_3$	100	11	10
Co^{++}	$Co(NO_3)_2$	500	8	50
Cr^{+++}	$Cr_2(SO_4)_3$	25	New hue	25
$Cr_2O_7^{--}$	$K_2Cr_2O_7$	10 Cr	5	0
CN^-	KCN	100	0	100
Cu^{++}	$Cu(NO_3)_2$	25	14	3
$C_2O_4^{--}$	$(NH_4)_2C_2O_4$	25	8	5
$C_4H_4O_6^{--}$	$(NH_4)_2C_4H_4O_6$	100	2	100
$C_6H_5O_7^{---}$	$H_3C_6H_5O_7$	100	5	30
F^-	NaF	25	8	5
Fe^{++}	$FeSO_4.(NH_4)_2SO_4$	100	2	100
Fe^{+++}	$Fe(NO_3)_3$	25	2	10
Hg^+	$HgNO_3$	..	Ppts.	0
Hg^{++}	$HgCl_2$	..	Ppts.	0
I^-	KI	500	12	100
MoO_4^{--}	$(NH_4)_2MoO_4$	100 Mo	17	0
Ni^{++}	$Ni(NO_3)_2$	100	New hue	100
Pb^{++}	$Pb(NO_3)_2$	..	Ppts.	0
$P_2O_7^{--}$	$Na_4P_2O_7$	5	9	0
Sb^{+++}	$SbCl_3$	50	27	5
SCN^-	KSCN	100	4	50
$S_2O_3^{--}$	$Na_2S_2O_3$	25	4	10
SiO_3^{--}	Na_2SiO_3	50 Si	10	10
$SnCl_6^{--}$	H_2SnCl_6	25 Sn	22	0
Sr^{++}	$Sr(NO_3)_2$	50	Ppts.	50
UO_2^{++}	$UO_2(C_2H_3O_2)_2$	500	2	500
VO_3^-	$NaVO_3$	10	0	10
WO_4^{--}	Na_2WO_4	100	New hue	100
Zn^{++}	$Zn(NO_3)_2$	500	2	500
ZrO^{++}	$ZrO(NO_3)_2$	..	Ppts.	0

[a] To give error within 2 per cent phosphorus.

senite interferes, possibly by oxidation to arsenate. Table I summarizes these effects.

Typical curves for the reduced molybdate (*46*) and indigo carmine (*29*) color standards are shown in Figure 2. Visually these standards look somewhat like the reduced heteropoly solutions, but there is no spectrophotometric match. In addition, the reduced molybdate solutions are not much more stable than the solutions to be measured. The copper standards (*18*) did not even resemble visually the solutions they are supposed to match.

Reduction with Hydroquinone. In the use of hydroquinone as a reductant three variations in procedure were followed.

1. Bell and Doisy Method. In this procedure the appropriate amount of phosphate solution, usually 0.25 mg. of phosphorus, was transferred to a 100-ml. flask. To this were added 5 ml. of a 5 per cent molybdate solution (in 1 *N* sulfuric acid) and 5 ml. of 2 per cent hydroquinone (in 0.03 *N* sulfuric acid), and the solution allowed to stand 5 minutes. Then 25 ml. of sulfite solution (200 grams of sodium carbonate plus 37.5 grams of sodium sulfite per liter) were added, the solution was diluted to the mark, and the color was measured immediately.

Since increased basicity makes the solution more blue and also increases the intensity of the color, the amount of sulfite solution must be controlled rather closely. Small changes in the concentrations of molybdate and hydroquinone do not greatly affect the color. There is little change in color on allowing the solution to stand 1 to 25 minutes after adding the hydroquinone, providing the measurement is made soon after addition of the sulfite reagent. The color begins to fade as soon as the sulfite is added, but this is not serious within 10 to 15 minutes. The range of concentration in a 1-cm. cell is 0.1 to 10 p. p. m. of phosphorus, and the system conforms to Beer's law over this range.

2. Briggs Method. To an appropriate amount of phosphate solution in a 100-ml. flask there were added 5 ml. of molybdate reagent (25 grams of ammonium molybdate in 300 ml. of water plus 200 ml. of a solution containing 75 ml. of concentrated sulfuric acid), 4 ml. of hydroquinone (0.5 per cent solution in 0.01 *N* sulfuric acid), and 1 ml. of 20 per cent sodium sulfite solution. After diluting to the mark and allowing to stand for 30 minutes, the color was measured.

The color depends somewhat upon the amount of reagents used but more upon the time of standing. Since the reaction is incomplete even after 5 days, the color measurement must be made at some definite time after preparing the solution. The applicable range is 0.5 to 12 p. p. m. of phosphorus and the system conforms to Beer's law for this range.

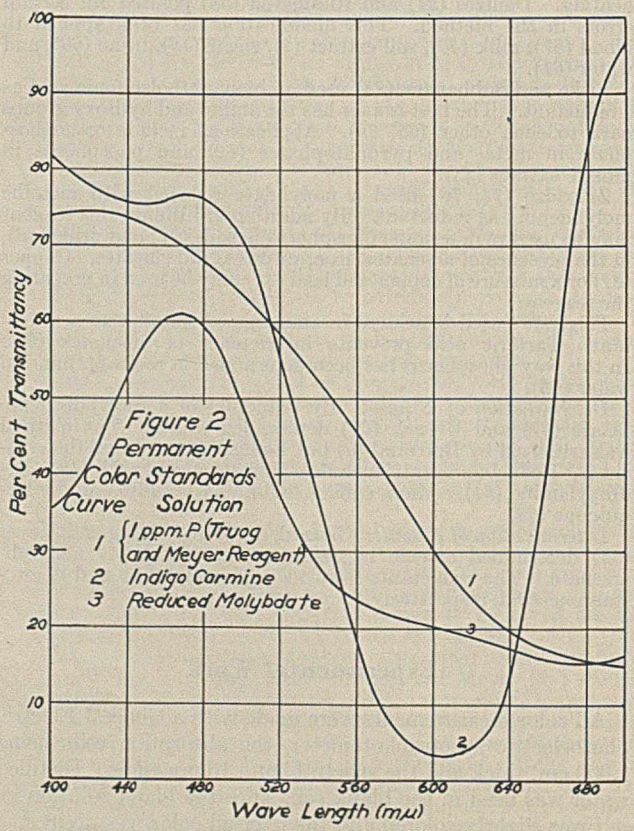
3. Benedict and Theis Method. To an appropriate amount of phosphate solution in a 50-ml. flask there were added 5 ml. of molybdate reagent (10 grams of molybdic anhydride in 25 ml. of 20 per cent sodium hydroxide diluted to 250 ml. plus an equal volume of concentrated sulfuric acid) and 5 ml. of hydroquinone (0.5 per cent in 15 per cent sodium bisulfite), and the solution was diluted to about 40 ml. After heating at 100° for 25 minutes, the solution was cooled and diluted to the mark, and the color was measured.

Although the color will develop slowly at room temperature, heating for 20 to 25 minutes gives a reproducible system. The color in the heated solution will increase about 4 per cent on standing 24 hours. The range of concentration is 0.1 to 5 p. p. m. and the system conforms to Beer's law up to 3 p. p. m.

Reduction with Aminonaphtholsulfonic Acid. To an appropriate amount of phosphate solution in a 50-ml. flask 10 ml. of molybdate reagent (2.5 per cent ammonium molybdate in 5 *N* sulfuric acid) were added, and the solution was diluted to 35 to 40 ml. After adding 5 ml. of reductant (0.5 gram of aminonaphtholsulfonic acid plus 195 ml. of 15 per cent sodium bisulfite plus 5 ml. of 20 per cent sodium sulfite, all diluted to 250 ml.), the flask was diluted to the mark and allowed to stand 30 minutes before measurement.

Of the several isomeric aminonaphtholsulfonic acids, the 1,2,4, 2,5,7, 1,4,8, and 2,6,8 compounds were used. The first proved to be the most effective. Increasing the amount of reductant increases the depth of color, but the system is least sensitive to this variable with the 1,2,4 isomer.

Since it was impossible at any elevated temperature to reduce all of the heteropoly acid without some of the uncombined molybdate, heating was impractical. Therefore the color was measured 30 minutes after the solutions were prepared. Low acidity leads to reduction of the molybdate and too much acid causes the color to develop more slowly. Over the range 0.6 to 1.3 *N* there is little difference in color if the solution is allowed to stand for only 30 minutes. The amount of molybdate reagent produces little effect on the color as long as there is sufficient to react. The

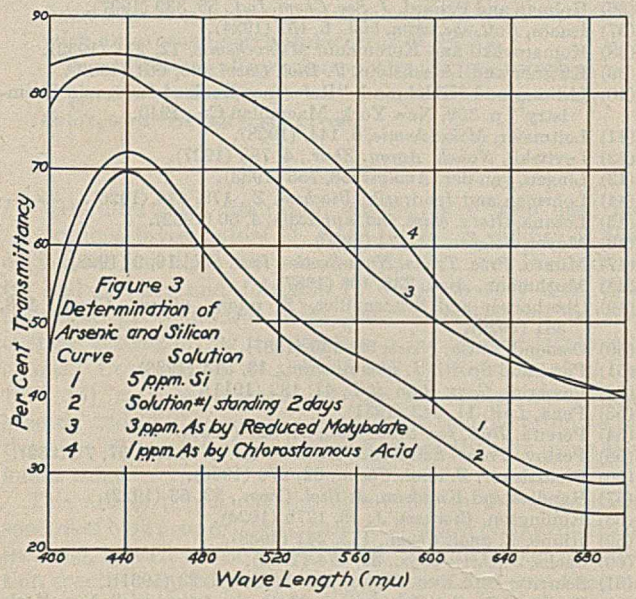


range of concentration is 0.2 to 10 p. p. m. of phosphorus for which Beer's law applies.

Reduction with Reduced Molybdate. To make the reductant, 20.06 grams of molybdic anhydride were dissolved in 505 ml. of 25 *N* sulfuric acid by heating, and the solution was cooled and diluted to 500 ml. with water. Half of this solution was boiled 15 minutes with 0.89 gram of molybdenum, cooled, and diluted to 250 ml. This solution was standardized against permanganate and then mixed with the original solution to make the final solution 0.1 *N* as a reductant. It was stored in a brown glass bottle and diluted 10 times before using.

To the phosphate solution in a 50-ml. flask there were added 5 ml. of 0.1 *N* sulfuric acid and 5 ml. of 8 per cent sodium bisulfite, and the solution was diluted to about 35 ml. This was allowed to stand overnight or for an hour at 100° to reduce any arsenic or iron present. To it were then added 5 ml. of molybdate reagent and the solution was heated for 30 minutes at 100°. After cooling and diluting to the mark, the color was measured.

The full color develops in 25 minutes at 100° and continued heating for an hour has little effect. The acidity must be rather carefully controlled, as higher values decrease the color intensity. Usually 5 ml. of *N* sulfuric acid are added to prevent the formation of molybdisilicic acid which would give high results if silica were present. Provided enough is added to react with the phosphorus excess molybdate has little effect. Small amounts of bisulfite have little effect, but large amounts increase the depth of color, possibly by increasing the pH and causing some reduction of uncombined molybdate. The range of concentration is 0.1 to 5 p. p. m. of phosphorus. The system conforms to Beer's law up to 4 p. p. m. and then deviates sharply from it. There was an error of about 5 per cent due to fading on standing 22 hours. A study of ferric iron, arsenate, arsenite, silicate, and tetraborate showed that the first four interfere and should not exceed 2, 25, 100, and 25 p. p. m., respectively.



DETERMINATION OF SILICON. Since molybdates may react with silicates to form molybdisilicic acid, the molybdenum blue reaction can be used to determine silicon.

The standard silica solution was made by dissolving 1.255 grams of sodium silicate monahydrate, $Na_2SiO_3.9H_2O$, in water containing 1 gram of sodium hydroxide and diluting to 250 ml. This solution, containing 1 mg. of silica per ml., was diluted tenfold for use.

To an appropriate amount of this silicate solution in a 50-ml. flask there were added 20 ml. of water, 6 ml. of 10 per cent acetic acid, and 10 ml. of 10 per cent ammonium molybdate. The solution was heated on a boiling water bath for 5 minutes, and 4 ml. of saturated sodium sulfite solution were added, after which the solution was cooled, diluted to the mark, and measured.

TABLE II. REDUCTANTS FOR DETERMINING PHOSPHORUS

Item Compared	Chlorostannous Acid	1-Amino-2-naphthol-4-sulfonic Acid	Reduced Molybdate
P concn. at 50% transmission[a], p. p. m. phosphorus	0.75	2.21	0.90
Working range, 1-cm. cell, p. p. m. phosphorus	0.02–2.0	0.2–10	0.1–5
Time and temperature, to develop color	3 min., 20–25°	30 min., 20–25°	30 min., 100°
Stability	15 min.	Changes[b]	10 hours
Effect of excess reagent	Slight decrease	None	Slight decrease
Effect of excess reductant	Slight increase	Slight increase	Slight increase
Conformity to Beer's law, p. p. m. phosphorus	0–1	0–10	0–4

	Hydroquinone		
	Bell and Doisy	Briggs	Benedict and Theis
P concn. at 50% transmission[a], p. p. m. phosphorus	1.65	2.62	1.06
Working range, 1-cm. cell, p. p. m. phosphorus	0.1–10	0.1–15	0.1–5
Time and temperature to develop color	5 min., 20–25°	30 min., 20–25°	25 min., 100°
Stability	15 min.	Changes[b]	10 hours
Effect of excess reagent	Slight decrease	.....	.....
Effect of excess reductant	Slight increase	Increase	.....
Conformity to Beer's law, p. p. m. phosphorus	0–10	0–12	0–3

[a] For 1-cm. cell at 700 mu.
[b] Measurement must be made after definite time.

The range of concentration is somewhat higher than for phosphorus, being 1 to 15 p. p. m. of silicon. If the color is measured a short time after preparing the solutions, they do not conform to Beer's law; but if they stand 2 days, the law applies. At high concentrations of silica the reduction seems to proceed more slowly than at lower values. A typical curve for the blue system is shown in Figure 3.

DETERMINATION OF ARSENIC. A heteropoly molybdiarsenate, formed from molybdate and arsenate, gives the molybdenum blue reaction and thus may serve for the determination of arsenic.

A standard arsenic solution was prepared by dissolving 0.950 gram of arsenic acid in water to which were added 100 ml. of *N* sulfuric acid and dilute permanganate to make the solution just pink. This solution, diluted to 1 liter, contains 0.5 mg. of arsenic per ml. It was diluted tenfold for use.

Using the reduced molybdate solution as reductant, the same procedure was followed in preparing the blue systems as for the determination of phosphorus, except that the addition of acid and bisulfite was omitted. The system conforms to Beer's law up to 8 p. p. m. of arsenic and then deviates sharply from it. The solutions are much more stable than the corresponding phosphorus solutions. On standing 5 days those containing less than 8 p. p. m. did not fade, but those of higher concentration changed appreciably. The chlorostannous acid reagent was tried also, using the same procedure as for phosphorus. This system conforms to Beer's law up to 2 p. p. m. Turbidity developed on standing. Selected curves are shown in Figure 3.

Discussion

Since the molybdenum blue method is based upon the selective reduction of the molybdenum in a heteropoly acid in presence of excess molybdate, there are several variables which must be rather closely controlled. These include ratio of molybdate to acid, acidity, amount of molybdate, time to develop the color, temperature at which the color develops, and the presence of certain diverse ions in the solution.

The optimum ratio of molybdate to acid is that which, in a given time, would give maximum reduction of the heteropoly

acid with no reduction of the uncombined molybdate. This ratio, once established, should be kept constant. A convenient means is to prepare the molybdate reagent in a given concentration of acid. Ordinarily the amount of reagent and of reductant will not greatly affect the color, provided, of course, there is sufficient present to effect the desired reaction. A general study of the effect of diverse ions, made with solutions using chlorostannous acid as reductant, showed many interferences.

A comparison of some of the characteristics of the different modifications for determining phosphorus, shown in Table II, indicates that the chlorostannous acid procedure is the most sensitive and requires the least time to prepare the color for measurement. The main disadvantages are the tendency to develop turbidity, especially with low concentrations of phosphorus, the relative instability of the color, and the difficulty of preserving a chlorostannous acid solution. The reduced molybdate reagent rivals it in sensitivity, conforms to Beer's law up to 4 p. p. m. of phosphorus, has greater stability, and excess reagent has very little effect. Its main disadvantage is the necessity of heating 30 minutes at 100° to develop the color. The reagent itself must be carefully prepared, but then it is reported to be stable for years. Silicate and arsenate interfere less than in the chlorostannous acid method. Hydroquinone and aminonaphtholsulfonic acid seem to have no advantages over the other two reductants. They are less sensitive and require time to develop the color, and in some cases a definite temperature must be maintained for a definite time.

Apparently each process of reducing the heteropoly acid to molybdenum blue produces a system of somewhat different light-absorptive characteristics. Thus in Figure 1 the spectral transmission curves are all for the same concentration of phosphorus but different reductants. It is obvious that no single permanent standard would serve. In addition, Figure 2 shows the failure of the proposed standards to match the system for which they were proposed.

Figure 3 shows curves for the application of the method to the determination of arsenic and silicon. Solutions for arsenic have nearly the same color as those for phosphorus. The reduced molybdate-arsenate solutions are more stable than the corresponding phosphorus compounds. In using acetic acid and sodium sulfite to reduce the silicon complex the process proceeds slowly with high concentrations of silicon. Freshly prepared solutions deviate markedly from Beer's law, but after two days the divergence nearly disappears.

Summary

A spectrophotometric study has been made of the application of the molybdenum blue method to the colorimetric determination of phosphorus, arsenic, and silicon. This included the range of the methods, conformity of the blue system to Beer's law, and the effects of variables such as time, temperature, acidity, and amounts of reactants upon the color developed. In addition, there was determined for phosphorus the effect of 63 diverse ions, the relative merits of chlorostannous acid, hydroquinone, aminonaphtholsulfonic acid, and a reduced molybdate solution as reductants, and the inadequacy of several proposed permanent standards.

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ABSTRACTED from a thesis submitted to the Graduate School of Purdue University by J. T. Woods in partial fulfillment of the requirements for the degree of doctor of philosophy.

Ultraviolet Absorption Spectra of Linseed Oil

Determination of Bodied-*in-Vacuo* and Blown Linseed Oil in Mixtures with Raw Linseed Oil

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An examination of the ultraviolet absorption spectra of a series of linseed oils bodied by blowing at 220° F. and by heating *in vacuo* at 585° F. shows that the absorption spectrum of a mixture of either type of oil with raw linseed oil may be used for the analysis of the bodied constituent.

Both types have maxima at 2320 Å., but the presence of a band with a maximum near 2700 Å. in the oxidized series distinguishes these oils from the oils bodied *in vacuo*. In the bodied-*in-vacuo* series the band at 2320 Å. has been attributed to the formation of conjugated octadecadienates as a step in the polymerization process.

Making use of the 2700 Å. band for the qualitative examinations and of the 2320 Å. maximum for the quantitative determinations, the amount and kind of bodied oil in mixtures with raw linseed oil have been measured. The amounts of bodied oil ranged from 5 to 50 per cent, and the analyses were made with an average percentage error of ±2.12.

In addition to absorption spectra, iodine number, refractive index, acid number, specific gravity, color, and viscosity are given.

THE ultraviolet absorption spectra have been used by various investigators (*1*, *2*, *8*, *12*, *16*) in studies of the drying oils and their fatty acids. Bradley and Richardson (*2*) and Bradley and Johnston (*1*) have recently used this technique in connection with their investigations of the changes accompanying the polymerization by heat treatment of oils. Conjugation, as pointed out by Crymble *et al.* in 1911 (*5*) may be detected by the ultraviolet absorption spectra, and by this means a much more reliable estimate of this type of unsaturation may be obtained than by using the iodine value.

This article points out the differences in the absorption spectra of blown, or oxidized, linseed oil and of oil polymerized *in vacuo*, as well as the use to which the ultraviolet absorption spectra may be put in the qualitative and quantitative determination of the amounts of these oils which have been added to untreated, or raw, linseed oil.

Instrument and Methods

All the absorption data were obtained from measurements made with a photoelectric spectrophotometer (*11*) employing a Hilger double monochromator with crystal quartz optics. As a source of ultraviolet light, a Munch (*18*) hydrogen discharge tube with a fused quartz window was used. Solutions of the oils for the curves presented in Figure 1 were made in diethyl ether, purified for optical use (*4*), and dilutions were made, if necessary, to maintain the values of $\log_{10}\frac{I_0}{I}$ between 0.200 and 0.800. Absorption cells 1 cm. in length with matched crystalline quartz end plates were used.

Slit widths of 0.25 mm. were used at 2320 Å. The spectral region isolated (*11*) at this wave length is 5.4 Å. for a slit width of 0.25 mm. However, when slit widths of 0.20 to 1.0 mm. were used at this wave length with the same solution the value of $\log_{10}\frac{I_0}{I}$ remained constant.

At 2680 Å. a slit width of 0.15 mm., isolating a region of 5.6 Å., was used. A constant value for $\log_{10}\frac{I_0}{I}$ was obtained at this wave length for the same solution when slit widths of 0.12 to 0.60 mm. were used.

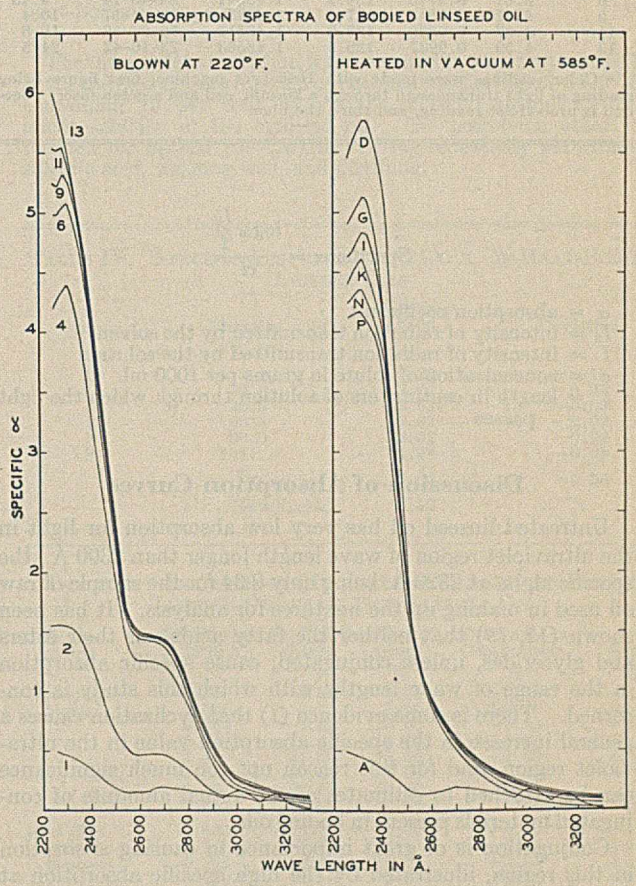

FIGURE 1

The effectiveness of the monochromator in the elimination of stray light at these wave lengths is shown by the fact that with the slits open to 1.0 mm., a 72-watt Mazda lamp, used as a light source in the visible spectrum, caused no galvanometer deflection. In the visible region with this same source, slits approximately 0.06 mm. wide are customarily used.

Lambert's law was demonstrated for the heat-bodied oils when cell lengths of 1 and 4 cm. were used.

The absorption curves are plotted, using the value of the specific absorption coefficient on the ordinate and the wave length in Ångstrom units on the abscissa.

TABLE I. LINSEED OIL BODIED IN MONEL AT 585° F.

Designation	Acid No.	Specific Gravity	Iodine No.	Refractive Index	Color[a] (20 Mm.)	Viscosity *Poises*
A	0.25	0.9310	178.2	1.47855	88-63-73	0.40
D	3.85	0.9510	139.3	1.48412	80-56-67	3.82
G	5.36	0.9608	131.2	1.48681	78-40-57	13.8
I	5.76	0.9652	128.5	1.48793	63-29-48	25.5
K	6.28	0.9664	127.5	1.48888	56-20-41	45.3
N	6.85	0.9688	126.4	1.48971	47-13-28	80.0
P	6.94	0.9703	126.1	1.48986	43-11-26	101.

[a] Color readings were made with Hess-Ives machine, first figure being reading of light transmission through a 20-mm. cell and green filter. Second is blue-violet reading, and third the blue.

TABLE II. LINSEED OIL BLOWN IN 100-BARREL IRON TANK AT 220° F.

Designation	Acid No.	Specific Gravity	Iodine No.	Refractive Index	Color[a] (20 Mm.)	Viscosity *Poises*
1	0.24	0.9311	180.3	1.47858	83-64-72	0.40
2	0.92	0.9364	173.4	1.47914	88-72-87	0.60
4	2.87	0.9640	145.7	1.48221	89-54-66	2.25
6	3.67	0.9740	136.2	1.48341	84-46-74	4.45
9	4.31	0.9848	127.0	1.48453	78-34-57	10.4
11	4.57	0.9898	123.2	1.48517	76-24-50	16.8
13	4.59	0.9937	120.5	1.48563	73-16-42	24.5

[a] Color readings were made with Hess-Ives machine, first figure being reading of light transmission through a 20-mm. cell and a green filter. Second is blue-violet reading, and third the blue.

$$\text{Specific } \alpha = \frac{\log_{10} \frac{I_0}{I}}{cl}$$

α = absorption coefficient
I_0 = intensity of radiation transmitted by the solvent
I = intensity of radiation transmitted by the solution
c = concentration of solute in grams per 1000 ml.
l = length in centimeters of solution through which the light passes

Discussion of Absorption Curves

Untreated linseed oil has very low absorption for light in the ultraviolet region of wave length longer than 2300 Å., the specific alpha at 2320 Å. being only 0.34 for the sample of raw oil used in making up the mixtures for analysis. It has been shown (*12*, *19*) that neither the fatty acids nor their esters and glycerides, unless conjugated, cause specific absorption in the range of wave lengths with which this study is concerned. There is some evidence (*1*) that cyclization causes a general increase in the specific absorption value in the ultraviolet region, and for this reason not too much significance can be attached to estimates of the actual amounts of conjugated materials present in bodied oils.

Conjugation is of great importance in causing absorption in this region, illustrated by the high specific absorption at 2700 Å. found by Dingwall and Thomson (*8*) for α-eleostearic acid and the value of 120 found for 9,11-linoleic acid in the 2300 Å. region by Hulst (*12*). In this latter region 2,3-oleic acid, in which the double bond is conjugated with the carboxyl, also shows strong absorption, although no maximum could be observed by Hulst (*12*). Working with more simple compounds, Hausser (*10*) found the maxima for crotonaldehyde and crotonic acid in hexane to be, respectively, at 212 and 208 millimicrons, the double bond in crotonaldehyde being conjugated with an oxygen atom of the aldehyde group and in crotonic acid with that of the carboxyl group.

With both the blown and the heat-bodied oils (Figure 1) the development of a relatively high absorption band with a maximum between 2320 and 2325 Å. is characteristic. This band, in the case of esters and oils bodied in the absence of oxygen, has been attributed by Bradley (*2*) to the formation of conjugated octadecadienates by isomerization of the double bonds as a step in the polymerization process. This evidence is in support of Brod *et al.* (*3*) and of Scheiber (*20*) who presented similar hypotheses. Increased absorption of ultraviolet radiation in connection with prolonged heating on saponification has also been shown to occur (*6*, *7*, *9*, *13*, *17*). In this connection, the absence of any absorption band in the region of 2700 Å. in the bodied-*in-vacuo* oils must be taken as evidence that no systems of three conjugated double bonds are present. Kino (*14*) found that the polymerization of methyl linolenate takes place without a shift of the double linkages.

The oils bodied *in vacuo* and those bodied by blowing with air show, at first, a very rapid fall in the iodine number with very little corresponding increase in the viscosity (Tables I and II). In the heat-bodied series the iodine number drops from 178.2 for sample A, viscosity 0.40 poise, to 139.2 for sample D, viscosity 3.82. As the heat treatment is continued the viscosity rises rapidly without a comparable decrease in the iodine number.

The period during which the iodine number falls rapidly is also that in which the acid number, specific gravity, and refractive index rise sharply. Several investigators have noted that the first changes induced by the heat treatment seem to be due to intramolecular rather than to intermolecular reactions. During this period the marked change in the absorption spectrum is also noted. When the viscosity has reached 3.85 poises the specific alpha for the heat-bodied oil has a value of about 5.7 at 2320 Å. compared to 0.34 for the original oil. With further heating, however, the specific absorption at 2320 Å. falls gradually with the rise in viscosity and the fall in the iodine number (Figures 2 and 3).

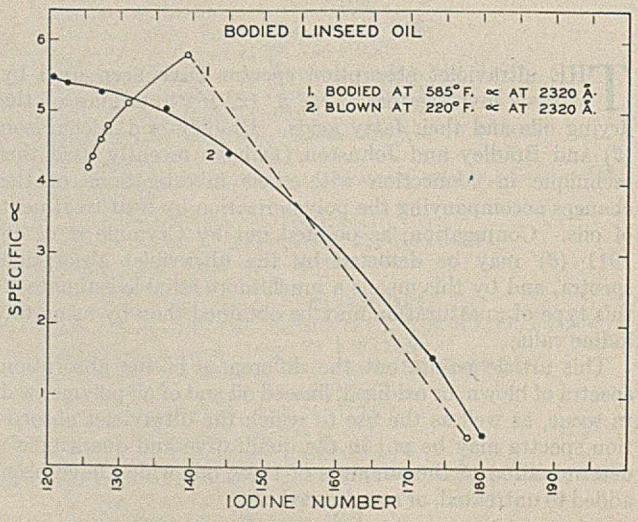

FIGURE 2. COMPARISON OF SPECIFIC ABSORPTION COEFFICIENTS AT 2320 Å. WITH IODINE NUMBERS OF OXIDIZED AND HEAT-BODIED LINSEED OILS

Based on the specific absorption coefficient of 120 found by Hulst (*12*) for 9,11-linoleic acid and neglecting other possible sources of absorption, the conjugated linoleic acid is estimated to be present to the extent of about 4.7 per cent in sample D, specific alpha 5.7, and about 3.5 per cent in sample P, specific alpha 4.2.

In the blown oils, however, the specific absorption coefficient rises gradually with the viscosity (Figure 3). This rise continues and in sample 13, viscosity 24.5 poises, the specific

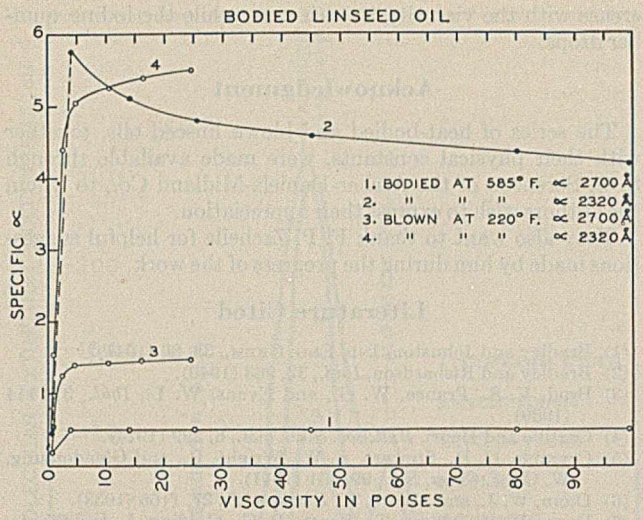

FIGURE 3. COMPARISON OF SPECIFIC ABSORPTION COEFFICIENTS AT 2320 Å. AND 2700 Å. WITH VISCOSITY OF OXIDIZED AND HEAT-BODIED LINSEED OILS

alpha is 5.7. In this sample no definite maximum was observed.

The small bands at 3000 and 3160 Å., present in the original oil, disappear very early during either type of treatment.

When the specific alpha at 2320 Å. is plotted against the iodine number (Figure 2), another difference between the two types of oils is apparent. In the oxidized series the specific alpha continues to increase with decreasing iodine value, while in the heat-bodied series the specific alpha first increases sharply and then falls with the decreasing iodine values.

TABLE III. SPECIFIC ABSORPTION AT 2700 Å. OF BLOWN LINSEED OILS

Sample	Viscosity *Poises*	Specific α at 2700 Å.	Deviation
6	4.45	1.39	−0.04
9	10.4	1.43	0.00
11	16.8	1.44	+0.01
13	24.5	1.45	+0.02
	Av.	1.43	±0.017
	Raw linseed	0.057	

Use of Absorption Curves for Analysis

The distinguishing characteristic between the spectra of the oxidized and the bodied-*in-vacuo* oils is the appearance of a second band between 2600 and 2700 Å. in the oxidized series. This band (Figure 1) continues to increase in height until a viscosity of about 4.45 poises and an iodine number of 136 is reached. Blowing continued after this condition is obtained produces no appreciable change in the specific alpha between these wave lengths, although the viscosity continues to increase. Table III, showing the average value of the specific alpha of blown samples having a viscosity of 4.45 poises and above to be 1.43 ± 0.017 at 2700 Å., illustrates this fact. Thus it is possible to use the value 1.43 at 2700 Å. to determine the amount of blown linseed oil of this series, from a viscosity of 4.5 to 25 poises, in a mixture with raw linseed oil.

The specific absorption coefficients of the bands in the oxidized series remain in the same relative order at 2700 as at 2320 Å. It is not possible, in the light of the present investigations, to identify the oxidized product which causes this band.

In the heat-bodied series the relative order of the curves becomes reversed, and the highest one at 2700 Å. is the lowest at 2320 Å. It may be observed in Figure 1 that the curves for this series cross near 2500 Å. Table IV shows the value for the specific alpha at 2500 Å. of the samples from viscosity 3.82 to 101 poises to be 2.04 ± 0.05. Thus, analyses for the amounts of heat-bodied oils of this series in mixtures with raw linseed may be made at 2500 Å. using the value 2.04 for the specific alpha of the bodied constituent.

The procedures described above will not give as accurate results as will the use of the maximum at 2320 Å. of the added bodied oil. However, when the absorption curve of the added oxidized or heat-bodied constituent cannot be obtained, these methods are capable of giving a good estimate of the kind and amount of bodied linseed oil in mixtures with raw linseed oil.

In Table V are given the analyses of mixtures of raw and bodied oils ranging from 5 to 50 per cent of the oxidized or heat-bodied component.

The ultraviolet absorption was measured in a solvent consisting of the purified hexane fraction of petroleum ether (65.5–66.5° C.) containing 10 per cent by volume of ethyl alcohol to bring about solution of the oxidized oil. The petroleum ether was purified for optical use (*15*) by stirring repeatedly with fuming sulfuric acid, washing well, and distilling.

TABLE IV. SPECIFIC ABSORPTION AT 2500 Å. OF HEAT-BODIED LINSEED OILS

Sample	Viscosity *Poises*	Specific α at 2500 Å.	Deviation
D	3.82	2.20	+0.16
G	13.8	2.03	−0.01
I	25.5	2.01	−0.03
K	45.3	2.02	−0.02
N	80.0	2.02	−0.02
P	101.	1.96	−0.08
	Av.	2.04	±0.05
	Raw linseed	0.10	

TABLE V. ANALYSES OF MIXTURES OF BLOWN AND BODIED LINSEED OILS WITH RAW LINSEED OIL

Designation	Specific α at 2320 Å.	Bodied Constituent Found %	Bodied Constituent Added %	Difference in Composition %	Error %
Raw plus blown	0.548	4.88	5.00	−0.12	−2.40
	0.666	7.79	7.50	+0.29	+3.86
	0.756	9.87	10.00	−0.13	−1.30
	0.874	12.85	12.50	+0.35	+2.80
	1.19	21.20	20.00	+1.20	+6.00
	1.50	29.20	30.00	−0.80	−2.66
	1.95	40.00	40.00	0.00	0.00
	2.35	50.00	50.00	0.00	0.00
Raw plus bodied-*in-vacuo*	0.600	5.10	5.00	+0.10	+2.00
	0.730	7.65	7.50	+0.15	+2.00
	0.866	10.35	10.00	+0.35	+3.50
	0.950	12.15	12.50	−0.35	−2.80
	1.31	20.00	20.00	0.00	0.00
	1.80	29.90	30.00	−0.10	−0.30
	2.28	39.40	40.00	−0.60	−1.50
	2.71	48.60	50.00	−1.40	−2.80
Raw plus Blown Bodied-*in-vacuo*	0.34	.....	.....		Av. ±2.12
	4.35	.....	.....		
	5.23	.....	.....		

The raw oil used in these mixtures had a viscosity of 0.40 poise, that of the blown was 1.40 poises, and that of the heat-bodied oil was 16.5 poises. Standard curves were obtained for the raw, oxidized, and heat-bodied oils. The portion of the absorption curves between 2550 and 2900 Å. was used to determine qualitatively the presence or absence of oxidized oil in the mixtures. Quantitative measurements of the blown or heat-bodied oil were then made by using the relatively high absorption maxima at 2320 Å.

The curves presented in Figure 1 relate only to linseed oil produced for this study and were bodied *in vacuo* at 307° C. (585° F.) and blown at 104.4° C. (220° F.). They are not intended for use as standard or reference curves in the quantitative analyses for bodied or blown oils produced under other conditions; it seems likely that the absorption curves for oils bodied or blown at other temperatures, for example, may not be the same. This point requires further study.

Summary

The ultraviolet absorption curves for two series of linseed oils, one heat-bodied at 585° F. and the other blown at 220° F., are presented and discussed.

A distinguishing characteristic between these two series is the development, in the blown series, of an absorption band with a maximum between 2600 and 2700 Å. The specific absorption coefficient of this band increases with the viscosity, on blowing with air, until a viscosity of about 4.5 poises is attained. After this stage of the process, continued blowing causes practically no change in the specific absorption coefficient at 2700 Å., although the viscosity continues to increase. Another absorption band with a maximum at 2320 Å. develops in the blown series. The specific absorption coefficient of this band continues to increase with the viscosity.

The development in the heat-bodied series of a strong absorption band with a maximum at 2320 Å., attributed to the formation of conjugated octadecadienates, takes place with relatively little change in the viscosity. On further heating the specific absorption coefficient of this band decreases gradually as the viscosity increases.

The presence of an absorption band at 2600 to 2700 Å. may be used to distinguish between linseed oil polymerized *in vacuo* and oxidized linseed oil in mixtures with raw linseed oil.

By use of the specific absorption coefficient at 2320 Å., quantitative analyses of oxidized or heat-bodied linseed oil, ranging from 5 to 50 per cent, in mixtures with raw linseed oil have been made with an average percentage error of ±2.12.

The physical constants for each of the samples are given. The acid number, specific gravity, and refractive index increase with the viscosity in each case, while the iodine number drops.

Acknowledgment

The series of heat-bodied and blown linseed oils, together with their physical constants, were made available through S. O. Sorenson of the Archer-Daniels-Midland Co., to whom the authors wish to express their appreciation.

They also want to thank F. P. Zscheile for helpful suggestions made by him during the progress of the work.

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Estimation of Aliphatic C—H Content of Toluene through Its Infrared Absorption

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WHEN toluene is subjected to such reactions as nitration, both the quality of the product and the hazards during the manufacturing operations are dependent upon the purity of the toluene used as the raw material.

The usual physical tests for purity—boiling point, distillation range, refractive index, and density—are not particularly sensitive to small amounts of impurities, and the chemical tests, such as the determination of the bromine number and the sulfonation test, while useful and specific for the impurities encountered, are time-consuming. These tests are conducted in order to estimate the quantities of paraffins, naphthenes, and olefins present in the samples of toluene. These impurities are the substances which exert the greatest influence upon the quality of the nitration products and upon the manufacturing operations. Any method for their estimation which was more sensitive or less time-consuming than the usual tests would be of value.

The use of infrared absorption spectra is not offered as a complete single test for all the impurities found in toluene but only to show that the method has possibilities. The speed, ease, and sensitivity of spectroscopic methods are well known.

First, let us consider the aliphatic nature of the paraffins, naphthenes, and olefins, which are related, in that all the compounds in each class have aliphatic carbon-hydrogen groups present in their structures. Several investigators (*1*, *3*, *4*, *6*) have observed that aromatic C—H vibrations occur in the 3.2μ region of the infrared spectrum while aliphatic C—H vibrations occur in the 3.4μ region. It has also been found that the extinction coefficient per C—H bond is greater in the case of the aliphatic vibration. These differences in the behavior

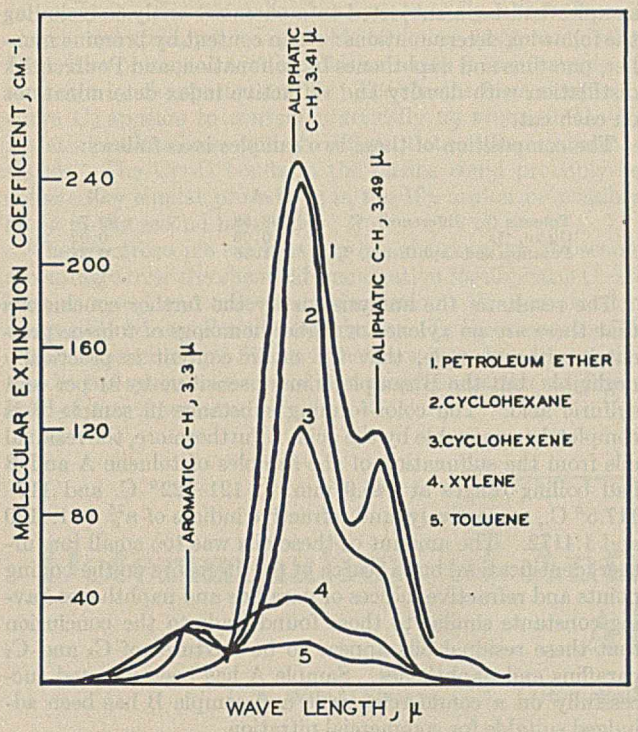

FIGURE 1. ABSORPTION SPECTRA

of the aromatic and aliphatic C—H vibrations should make it possible to detect rather small quantities of aliphatic material in the presence of aromatic substances. Inasmuch as toluene itself has an aliphatic CH_3 group, it is necessary to measure the absorption of the aliphatic C—H over and above that already present in the toluene molecule. This offers no great difficulty, as solutions of toluene can be used as standard absorbents and the presence of aliphatic C—H other than that present in pure toluene will then increase the absorption in the aliphatic region above that normally present in toluene.

Aliphatic C—H may be present in compounds of sulfur such as the sulfides and mercaptans, which may occur in quantities equivalent to 0.1 per cent of sulfur, depending upon the source of the toluene.

The quantity of —SH-bearing compounds may be estimated by determining the absorption in the 3.85μ region where the —SH frequency appears. The C=S frequency occurs between 6.5 and 6.8μ. Compounds containing this group may be studied in the 6.5 to 6.8μ region or possibly in the region of the second harmonic which would occur between 3.25 and 3.4μ. A similar estimation of —OH-bearing compounds may readily be obtained by studying the absorption in the 2.75μ region, while C=C may be estimated by studying the 6.1μ region.

Inasmuch as all these compounds have a number of aliphatic CH groups as well as the various other groups, a relatively simple test, such as the determination of the aliphatic C—H content, should give an indication of the purity of the toluene sample.

Experimental

The authors have compared the infrared absorption spectra of various samples of toluene with toluene purified by the method of Schwalbe (*5*) which is taken as a standard. Samples have been obtained from the stocks possessed by this laboratory and from the Atlas Research Laboratory of the Atlas Powder Company, which kindly permitted the authors to use the results of their analyses of two samples.

The authors have also studied the effect upon the 3.1 to 3.5μ infrared absorption spectrum of toluene of the presence of several molecules, including cyclohexane, cyclohexene, petroleum ether, and *m*-xylene. The first two substances were chosen because the authors were informed that naphthenes and unsaturates cause considerable trouble during the nitration of toluene and these materials were available in a reasonable degree of purity. The petroleum ether was used as a source of aliphatic C—H and because of its boiling range its constituents could be present in some samples of toluene. *m*-Xylene might possibly be a contaminant of toluene and it represents a type of impurity.

The toluene, which was purified by the method of Schwalbe (*5*), had a boiling point of 110.5° C. The cyclohexane was from the Eastman Kodak Company and after several fractionations had a freezing range from 6.3° to 6.5° C. The cyclohexene was obtained from the organic division of this laboratory and boiled between 82.1° and 83.3° C. The petroleum ether was the commercial fraction boiling at 90–110° C. The *m*-xylene was the commercial product which boiled at 138–139° C. after several fractionations.

The absorption spectra were obtained by the technique described previously (*2*, *3*). Carbon tetrachloride solutions of the materials were used and the absorption spectra were obtained by means of the Illinois rock-salt prism spectrometer.

Results

In Figure 1 are shown the absorption spectra of pure toluene, cyclohexane, cyclohexene, petroleum ether, and *m*-xylene. The petroleum ether was assumed to be a mixture of equal quantities of C_7H_{16} and C_8H_{18}. The intense aliphatic C—H absorption of petroleum ether, cyclohexane, and cyclohexene is clearly shown by these curves. It is this high molecular extinction coefficient of the aliphatic 3.4μ C—H, as compared with the relatively weak aromatic C—H in the 3.3μ region, that makes possible the detection and estimation of the quantity of C—H-bearing material in the samples of toluene.

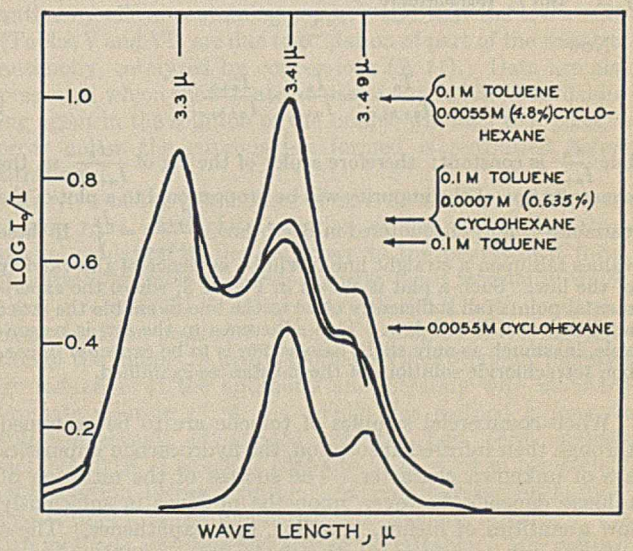

FIGURE 2. ABSORPTION SPECTRA

In Figure 2 curves are shown for different concentrations of cyclohexane added to a 0.1 molar solution of pure toluene. The relative heights of the peaks in the 3.4μ region indicate that the effect of the addition of various concentrations of cyclohexane is what might be expected if Beer's law were applicable.

To test the applicability of Beer's law cyclohexane was added to 0.1 molar toluene solutions in quantities sufficient to produce solutions 0.00012, 0.0006, 0.0012, and 0.0024 molar with respect to the cyclohexane. The logarithms of the ratios of the intensi-

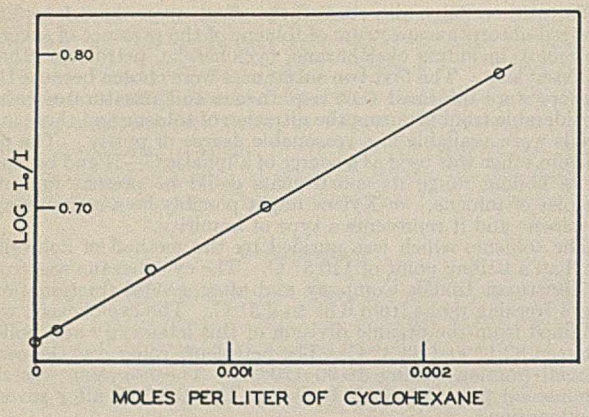

FIGURE 3

ties at the 3.4μ absorption peak of these solutions were determined, using a constant cell thickness. For strict adherence to the customary meanings of the symbols in Beer's law as written, $k = \frac{1}{c}\left(\log \frac{I_0}{I}\right)$, it would be necessary to obtain values for the intensity of radiation through a cell containing carbon tetrachloride plus toluene plus impurity (cyclohexane in this case). This intensity can be represented by I_{a+b+c} using a, b, and c for carbon tetrachloride, toluene, and the impurity, respectively. It is also necessary to measure the intensity of radiation through a cell containing carbon tetrachloride and toluene, which can be represented by I_{a+b}. With these two values of the intensity of radiation it is possible to formulate a statement of Beer's law in the usual form.

For convenience, however, the authors have determined the intensity of radiation through a carbon tetrachloride solution of toluene plus the impurity and through a cell containing only carbon tetrachloride. These values may be represented by I_{a+b+c} and I_a, respectively.

Now

$$\frac{I_a}{I_{a+b+c}} = \frac{I_a}{I_{a+b}} \times \frac{I_{a+b}}{I_{a+b+c}}$$

but $\frac{I_a}{I_{a+b}}$ is constant; therefore a plot of the log of $\frac{I_a}{I_{a+b+c}}$ *vs.* the concentration of the impurity will be proportional to a plot of the variables usually encountered in Beer's law, $\frac{I_{a+b}}{I_{a+b+c}} = \frac{I_0}{I}$. If these values fall upon a straight line it will be evidence of the validity of the law. Such a plot is shown in Figure 3, where the experimental points fall sufficiently close to the line to enable the strict application of Beer's law. This adherence to the law is reasonable, inasmuch as only slight association is to be expected in carbon tetrachloride solutions of the substances examined.

When commercial samples of toluene are to be examined through their infrared absorption, the hydrocarbon impurities are of unknown character. The success of the nitration of toluene depends, however, upon the presence in sufficiently low quantities of olefins, paraffins, and naphthenes. These substances have a strong infrared absorption at 3.4μ due to aliphatic C—H vibrations, and a comparison of the height of this absorption peak with the height of the peak exhibited by pure toluene may be used as an additional aid in the estimation of the suitability of toluene for nitration. The absorption exhibited by the unknown hydrocarbons can be calculated as equivalent to the absorption exhibited by known quantities of a definite compound. The authors have used the absorption shown by cyclohexane as their standard, both because it is a naphthene and naphthenes cause trouble in nitration and because the cyclohexane was of high purity.

Toluene samples A and B and their analyses were furnished through R. Max Goepp, Jr., and Sol Soltzberg of the Atlas Research Laboratory of the Atlas Powder Company. These samples had been subjected to a chemical analysis including the following determinations: olefin content by bromine number, paraffins and naphthenes by sulfonation, and Podbielniak distillation with density and refractive index determinations on each cut.

The composition of these two samples is as follows:

	A	B
Toluene (by difference), %	98.58	99.70
Olefin, %	0.06	0.02
Paraffins and naphthenes, %	1.36	0.28

The results of the analysis justify the further conclusions that there are no xylenes or higher homologs of toluene present in either sample, that the olefin content is practically negligible, but the B sample is more sensitive to 91 per cent sulfuric acid. The color-forming substance in sample B is completely removable by the acid. Furthermore, the residual oils from the sulfonation of the samples of toluene A and B had boiling ranges at 745.9 mm. of 121–122° C. and 117–117.5° C., respectively, and refractive indices of $n_D^{25} = 1.4150$ and 1.4172. The amount of these oils was too small for further identification, but a search of the literature on the boiling points and refractive indices of paraffins and naphthenes having constants similar to those found leads to the conclusion that these residual oils appear to be mixtures of C_8 and C_9 paraffins and naphthenes. Sample A has been nitrated successfully on a commercial scale and sample B has been adjudged suitable for commercial nitration.

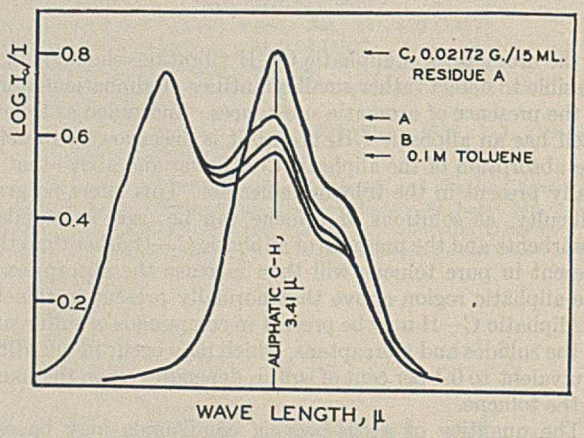

FIGURE 4. ABSORPTION SPECTRA

The absorption spectra of samples A and B and the residue from sample A are shown in curves A, B, and C, respectively, in Figure 4. These spectra were obtained after the spectrometer, a research instrument, had been disassembled and reassembled for an overhauling. The differences in the values of $\log \frac{I_0}{I}$ for pure toluene and those shown in Figure 2 are due to inescapable changes in the resolution of the instrument which develop during this major operation. To estimate the quantity of aliphatics present in the samples after such instrumental changes necessitates the redetermination of a calibration curve. A new curve is not shown, as it would be similar to that in Figure 3.

However, the absorption spectra show that curve A represents a sample of toluene containing aliphatic C—H equivalent to 0.00147 molar (1.44 per cent) cyclohexane and curve B represents a sample containing aliphatic C—H equivalent to 0.0007 molar (0.62 per cent) cyclohexane. These values are of the same order of magnitude as the results of the chemi-

cal analysis. Sample A has been successfully nitrated on a commercial scale, yet it is possible to detect spectroscopically impurities present in even smaller amounts than in sample A, as shown by the spectrum of B. The residue from sample A, curve C, appears to contain practically no aromatic C—H. No attempt has been made to differentiate olefins and naphthenes. The C=C bonds in the olefins could probably be detected by similar procedures in the 10μ region or possibly at 5μ in the second harmonic.

The spectroscopic examination has the usual spectroscopic advantages over the chemical examination for aliphatic C—H such as speed and ease of determination. An examination can readily be completed in 30 minutes. In the concentration region represented by Figure 3 the error introduced by variation in the spectrometer readings could be in the neighborhood of 1 or 2 per cent.

Acknowledgment

The authors wish to express their thanks to A. M. Buswell and W. H. Rodebush of the University of Illinois and R. Max Goepp, Jr., and Sol Soltzberg of the Atlas Laboratory for their interest and help, and to the Atlas Powder Company for permission to use the results of their analysis.

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Determination of Antimony in Lead-Antimony Alloys

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A new bromate method for the determination of antimony in lead-antimony alloys is described and compared with older methods. The method is rapid, convenient, and especially recommended for use on lead alloys that do not dissolve readily in sulfuric acid. The effect of the presence of copper on the antimony determination has been investigated. Under certain conditions copper can act as an oxidation catalyst and thus cause the results of an oxidimetric determination of antimony to be too low; under different circumstances copper can cause high results.

THE method most commonly used for the rapid determination of antimony in lead-antimony alloys is that of Low (*4*) in which the sample is dissolved in concentrated sulfuric acid and potassium bisulfate and, after addition of hydrochloric acid, the antimony is titrated at 10° C. with permanganate ion in the presence of the lead chloride-sulfate precipitate. This method has been applied to the analysis of 1 per cent antimony alloys and difficulty in obtaining complete sulfation of certain alloys is reported (*9*). In agreement with this finding the authors have also encountered alloys which do not dissolve completely, even though a preheated solution of sulfuric acid and potassium bisulfate is employed (*9*). Further work has revealed that alloys containing appreciable quantities of copper—e. g., 0.05 per cent—are always more difficult to sulfate than copper-free alloys; and that the metallic residue obtained in such cases is relatively rich in antimony and copper, suggesting the presence of an antimony-copper compound, probably Cu_2Sb.

The problem of obtaining complete solution of the sample has led analysts to increase the time and temperature of heating during the sulfation step, or to add an oxidizing agent such as cupric ion. In subjecting the sample to such treatments the assumption has been made that the subsequent titration of the trivalent antimony is unaffected. In the experimental section of this paper data are presented which show that prolonged heating at approximately 320° C. during sulfation results in low values for antimony in lead-antimony alloys containing copper; and that the low results (Tables V and VI) are due to oxidation of part of the trivalent antimony, catalyzed by copper ions (*8*, *11*). Data are also presented which show that the use of cupric ion as an oxidizing agent in the solution of the sample will lead to a positive error unless the cuprous ion formed is reoxidized before titration.

In view of the possibilities of error in the Low method resulting from incomplete sulfation of the sample and from partial oxidation of the antimony to the pentavalent form, it seemed desirable to develop a new, rapid method in which these errors would be closely controlled. The time element eliminated the possibility of using a method involving solution of the sample in hydrochloric acid and bromine, followed by reduction of the antimony and titration with standard bromate solution (*1*, *2*, *7*). While this method is long, it still has several advantages over the Low permanganate method, chief of which is that the alloy is taken completely into solution before titration. It occurred to the authors that it might be possible to combine the best features of the two methods, and this has proved practicable. The method developed involves the following simple operations:

Solution of the alloy in sulfuric acid and potassium bisulfate.
Addition of sufficient potassium chloride and hydrochloric acid to effect complete solution of all metal and metal salts by conversion to chlorides.
Titration of the antimony at 80° to 90° C. with bromate ion.

The method (henceforth called the bromate method) eliminates errors resulting from incomplete sulfation and occlusion of antimony, by taking the sample completely into solution. [Somewhat similar methods have been described by Pugh (*5*), Robinson (*6*), Shreider (*10*), and Vasil'ev and Stutzer (*11*).] The use of air-saturated distilled water in the dilution step was found to provide sufficient oxygen to effect

TABLE I. COMPARISON OF BROMATE AND PERMANGANATE METHODS

Alloy No.	Antimony		Copper
	Bromate	Permanganate	
	%	%	%
1	1.00	1.00	0.5
2	0.78	0.78	0.05
3	1.02	1.03	0.05
4	0.92	0.92	0.05
5	1.00	0.99	< 0.002
6	1.02	1.02	< 0.002
7	1.00	1.00	< 0.002
8	0.91	0.91	< 0.002
9	0.98	0.98	< 0.002

solution of all metal and to reoxidize any cuprous ion formed. The possibility of oxidation of trivalent antimony during sulfation is recognized and the error minimized by limiting the time of heating during sulfation to 7 minutes, a period found by experiment to be sufficient to expel the sulfur dioxide formed in the solution of the sample. The method is precise, convenient, and considerably more rapid than any of the modifications of the Low permanganate method known to the authors. Duplicate determinations may be completed in 20 minutes as compared to 45 to 60 minutes by the modified Low permanganate method.

TABLE II. CONTROL METHOD APPLIED TO SYNTHETIC SAMPLES OF KNOWN COMPOSITION

Antimony Present	Antimony Found	Error
Mg.	*Mg.*	*Mg.*
5.0	5.0	0.0
3.9	4.0	+0.1
3.0	3.1	+0.1
8.5	8.5	0.0
5.6	5.7	+0.1
3.4	3.4	0.0

Bromate Method

REAGENTS. *Methyl Orange Solution.* Dissolve 0.1 gram of methyl orange in 100 ml. of distilled water.

Potassium Bromate Solution (0.05 N). Dry a sample of finely ground, pure, bromide-free potassium bromate at 180° C. for an hour (*3, 12*). Dissolve 2.7837 grams of the dried salt in distilled water and dilute to 2 liters in a volumetric flask.

As further check, the solution may be standardized against pure arsenious oxide (Bureau of Standards Sample 83).

PROCEDURE (suitable as written for the determination of antimony in 1 per cent antimony–lead alloys). Weigh out 2 grams of the milled alloy and transfer to a 500-ml. Erlenmeyer flask. Add 10 ml. of sulfuric acid and 5 grams of fused potassium bisulfate, place on a hot plate, and heat for 7 minutes. (The temperature of the solution at the end of this heating period must be 320° ± 5° C.) Remove flask from hot plate and cool.

Add 230 ml. of cold distilled water (air-saturated), washing down the sides of the flask in the process. Add 15 grams of potassium chloride and 20 ml. of hydrochloric acid, heat just to boiling to dissolve all metal and metal salts, and boil vigorously for 1 minute, shaking (by rotation) the contents of the flask once or twice during the boiling. [If for any reason a black metallic residue is obtained which does not dissolve readily in the shaken, boiled chloride mixture, proceed as follows: To the hot solution add 2 ml. of copper sulfate solution (50 grams of copper sulfate pentahydrate per liter of water). Boil and shake until all the metal dissolves. Dilute to 350-ml. volume with hot water, heat to 90° C., bubble oxygen through the solution for 5 minutes, and titrate at 85° to 90° C.]

Add 100 ml. of air-saturated distilled water. Titrate slowly at 85° to 90° C. with standard bromate solution (0.05 *N*) using not more than 3 drops of methyl orange indicator.

Ml. of bromate solution × 0.1522 = per cent of antimony in sample.

Experimental

In order to compare the new bromate method with the permanganate method the authors have analyzed several representative 1 per cent antimony–lead alloys by the two methods. The permanganate method used was that of Shaw, Whittemore, and Westby (*9*), except that the heating period during sulfation of the sample was reduced from 30 to 7 minutes to minimize oxidation of trivalent antimony.

Table I gives a comparison of the antimony contents of nine alloys, obtained with the two methods. The alloys contain copper together with traces of other metallic impurities such as silver, bismuth, nickel, and iron. Arsenic and other interfering elements were not present in significant quantities. Owing to the high copper content of alloy 1, oxygen was passed through the solution in this case for 5 minutes immediately before titration. The data show that the two methods give almost identical results when approximately the same sulfation conditions are used.

Neither the new bromate nor the modified permanganate method can be tested on synthetic solutions. It was therefore necessary to develop a control method of known precision and to calibrate the two rapid methods against this method by the analysis of a common sample.

CONTROL METHOD (suitable as written for the analysis of antimony in 1 per cent antimony–lead alloys).

Weigh out 300 mg. of the milled alloy and transfer to a 500-ml. Erlenmeyer flask, add 15 ml. of sulfuric acid, heat to boiling and boil gently until the sample is completely decomposed. Heat vigorously on a flame to dissolve all traces of metal and lead sulfate. Add 25 mg. of hydrazine sulfate to the solution and heat vigorously on a flame until the solution is reduced to a volume of approximately 10 ml. Cool.

Add 220 ml. of distilled water, 20 ml. of hydrochloric acid, and a few glass beads, heat to boiling, and boil vigorously for 1 minute, shaking (by rotation) the contents of the flask once or twice during the boiling. Titrate the antimony with potassium bromate solution (0.01 *N*) using methyl orange as indicator. Determine blank on the reagents.

[Ml. of $KBrO_3$ − blank] × 0.2028 = per cent of antimony in sample

TABLE III. REPRODUCIBILITY OF PERMANGANATE, BROMATE, AND CONTROL METHODS

(Applied to alloy 9)

	Permanganate Method		Bromate Method		Control Method	
	Antimony found	Deviation	Antimony found	Deviation	Antimony found	Deviation
	%	%	%	%	%	%
	0.98	0.00	0.97	−0.01		
					0.972	−0.003
	0.97	−0.01	0.99	+0.01		
					0.970	−0.005
	0.97	−0.01	0.97	−0.01		
					0.973	−0.002
	0.97	−0.01	0.98	0.00		
					0.985	+0.010
	0.98	0.00	0.97	−0.01		
	0.99	+0.01	0.97	−0.01	...	.....
	0.96	−0.02	0.97	−0.01	...	.....
	0.98	0.00	0.98	−0.00	...	.....
Mean	0.98	±0.007	0.98	±0.008	0.975	±0.005

TABLE IV. EFFECT OF TEMPERATURE AND TIME OF HEATING DURING SULFATION

(Upon apparent antimony content of alloy 9 by the bromate method)

Antimony Found		Antimony Found	
Heating period, 10 minutes	Temperature	Heating period, 7 minutes	Temperature
%	° C.	%	° C.
0.97	325	0.98	325
0.96	325	0.98	325
0.97	325	0.97	325
0.97	325	1.00	300
0.97	325	0.99	300
0.97	325	0.99	290
0.98	325	1.02	290
		1.02	290

The data in Table II were obtained by the control method on synthetic mixtures of known composition closely approximating the composition of the lead-antimony alloys to be analyzed.

Table III shows a comparison of the reproducibility of the bromate, permangante, and control methods on alloy 9. The agreement is satisfactory.

In Table IV data on the effect of temperature and time of heating during sulfation upon the results obtained by the bromate method on alloy 9 are shown. At temperatures below 325° C. there is evidence of incomplete removal of sulfur dioxide, leading to high results. There is a slight trend to lower results when the time of heating is increased from 7 to 10 minutes.

Table V shows the effect of copper as a catalyst in the oxidation of antimony during sulfation of the sample in the permanganate method. The heating periods were timed from the moment the sample was dropped into the hot acid solution.

TABLE V. EFFECT OF COPPER AND TIME OF HEATING

(Permanganate method)

Alloy No.	Cu	Antimony Found: Heating period, 7 minutes	Antimony Found: Heating period, 45 minutes	Antimony Lost
	%	%	%	%
1	0.5	1.00	0.91	0.09
2	0.05	0.78	0.72[a]	0.06
3	0.05	1.03	0.95	0.08
4	0.05	0.92	0.85	0.07
5	< 0.002	0.99	0.97	0.02
6	< 0.002	1.02	0.99	0.03
7	< 0.002	1.00	0.97[a]	0.03
8	< 0.002	0.91	0.89	0.02

[a] After titration these samples were treated with sulfur dioxide, boiled vigorously for 15 minutes,, and retitrated at 10° C. Values found were: 2 = 0.79% Sb, 7 = 1.00% Sb.

TABLE VI. EFFECT OF PRESENCE OF COPPER ON OXIDATION OF ANTIMONY IN SULFURIC ACID SOLUTION

H_2SO_4 Present	Time of Heating	Copper Present	Antimony Present	Antimony Found	Error
Ml.	*Min.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
15	10	10	5.0	3.9	−1.1
25	30	10	5.0	3.5	−1.5
25	30	10	5.0	4.8[a]	−0.2
25	30	10	5.0	4.7[a]	−0.3
15	10	1	5.0	4.7	−0.3
25	30	1	5.0	4.3	−0.7
15	10	0	5.0	5.0	±0.0
25	30	0	5.0	5.0	±0.0

[a] After heating for 30 minutes the samples were treated with 100 mg. of hydrazine sulfate, heated on a flame to expel approximately 5 ml. of sulfuric acid, cooled, diluted, and titrated as usual.

In order to investigate further the effect of copper ions on the oxidation of antimony in sulfate solution the following experiment was carried out.

Five milligrams of antimony were dissolved in a solution of 15 or 25 ml. of sulfuric acid and 5 grams of potassium bisulfate in a 500-ml. Erlenmeyer flask. Weighed amounts of copper (as copper sulfate) were added. The samples were heated at 320° C. for 10 to 30 minutes, then cooled, diluted to 300-ml. volume with water, treated with 20 ml. of hydrochloric acid, and titrated at 90° C. with potassium bromate solution (0.01 *N*), using methyl orange as an indicator.

The results, corrected for a titration blank, are shown in Table VI.

To determine whether trivalent antimony in hot chloride solution is readily subject to air oxidation, a series of low-copper and high-copper alloys was analyzed by the bromate method as recorded above, except that oxygen was bubbled through the solution for 5 minutes just before titration. The results, contained in Table VII, show no significant difference

TABLE VII. EFFECT OF OXYGEN ON TRIVALENT ANTIMONY IN HOT HYDROCHLORIC ACID SOLUTION

Alloy No.	Copper	Antimony: With oxygen	Antimony: Without oxygen
	%	%	%
2	0.05	0.78	0.78
3	0.05	1.01	1.02
4	0.05	0.92	0.92
5	< 0.002	1.00	1.00
6	< 0.002	1.02	1.02
8	< 0.002	0.91	0.91

TABLE VIII. EFFECT OF CUPROUS ION IN BROMATE METHOD

Antimony Found: With oxygen	Antimony Found: Without oxygen
%	%
0.93	1.12
0.92	1.20
0.92	0.95
0.92	1.01

in comparison with those obtained by the method as normally used—i. e., without the use of oxygen. This is contrary to the finding of Zintl and Wattenberg (*12*).

To show that an error can result from the use of cupric ion in the solution of a sample, alloy 4 was analyzed as recorded in the bromate method, except that after dilution of the sample with water and chloride, 2 ml. of copper sulfate solution (50 grams of copper sulfate pentahydrate per liter) were added and the solution was boiled and shaken until all metal dissolved. The sample was then diluted to 350-ml. volume with hot water and (1) saturated with oxygen for 5 minutes before titration; or (2) titrated without the use of oxygen. The results, contained in Table VIII, show that precautions must be taken to reoxidize any cuprous ion formed during solution of the sample.

Summary

A new bromate method for the determination of antimony in lead-antimony alloys of low antimony content has been developed. For most alloys it is no more precise than the permanganate method (*9*), but it is considerably more rapid and convenient. The new method is especially suitable for the analysis of alloys that do not dissolve readily during sulfation.

The effect of copper in the antimony determination has been investigated. Under certain conditions copper can act as an oxidation catalyst and thus cause the results of an oxidimetric determination of antimony to be too low; under different circumstances copper can cause high results. Precautions which will eliminate or minimize these errors are specified.

With slight modifications in procedure the new bromate method can be used for the determination of antimony in lead alloys of high antimony content and in tin and lead-tin alloys.

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Photometry in Spectrochemical Analysis

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SINCE the photographic plate is the most widely used medium for the measurement of radiant energy, the relation between intensity and the photographic effect produced has been very extensively studied (*4*). Despite this work, however, the spectrochemical analyst frequently must reinvestigate the procedures, in order to establish their applicability for his work. In this paper are described the investigations made by the authors and the conclusions reached.

Definitions

The following definitions and symbols are used in this discussion:

i is the intensity of radiant energy which strikes the photographic plate. Strictly speaking, the plate does not measure intensity (energy per unit time) but rather integrated intensity or total energy (*10*). In most analytical work, however, an internal standard is employed to permit correction for variations in the exposure conditions, and all energies are stated in terms of the relative intensities of analysis and internal standard lines. Thus it makes no difference whether the effect is given in terms of energy per unit time or total energy. The absolute value of i is never determined in analytical work. All intensity values are stated in relative terms based upon the empirical scale given by a plate calibration curve.

I and I_o refer to the intensity of light transmitted by a developed photographic plate, as measured by a microphotometer. I refers to the photometer reading for the light transmitted by an exposed portion of the plate, usually a spectrum line, and I_o to the photometer reading for a clear unexposed portion of the same plate. In all measurements the ratio of the two readings is needed to express the photographic effect. The ratio is expressed in various ways, as transmission, opacity, or density.

Transmission, T, is defined by the expression

$$T = \frac{I}{I_o}$$

Opacity, O, is the reciprocal of transmission.

Density, D, is defined by the relation

$$D = \log_{10} \frac{I_o}{I} = \log_{10} \text{opacity}$$

Density is a more fundamental concept for expressing photographic response than opacity, because density is a linear function of visual response. Furthermore, density is proportional to the number of developed silver grains in the emulsion (*5*).

The relation between density and light intensity is usually expressed by an H. & D. curve (*5*), which is a plot of density *vs.* log relative intensity or relative exposure. Examples of such curves are shown in Figures 2 and 5. The basis for this method of plotting plate response lies in the fact that the curve obtained has a long linear portion which for some plates may extend from a density of above 0.5 to above 2.0. Furthermore, this type of plot is convenient when one wishes to obtain the intensity ratio of two lines, since the difference along the log exposure axis gives directly the logarithm of the intensity ratio of the two lines. Another useful type of plate response curve is shown in Figure 1, where density is plotted *vs.* intensity (or exposure). The advantage of this method is that any density value can be converted directly into the corresponding intensity value. A disadvantage is, of course, that the curve has no linear portion.

Plate Calibration

Photographic intensity measurements are made by comparison of the density of the line to be measured with the density produced by a line of known relative intensity. Since the effect produced by a given number of light quanta depends upon the properties of the plate, the wave length of the light, the developer used, the temperature and time of development, and many other factors, it is necessary to have for every plate a calibration curve or H. & D. curve. Such a curve is constructed by placing on the plate a series of exposures of known relative intensity, measuring the densities of the resulting lines, and plotting the densities as a function of the intensity.

The comparison of intensities by means of the photographic effects produced is subject to several kinds of errors (*4*). Strictly speaking, such a comparison is accurate only when the exposures are made simultaneously and nonintermittently; the wave lengths of the compared radiations are equal; the comparison is made between lines of equal density; and the comparison is made between lines lying on adjacent portions of the emulsion.

It is not possible to fulfill these conditions in routine analytical work. The plate calibration exposures are often intermittent and cannot be made simultaneously with the analysis exposures. Lines to be compared with one another are often widely separated on the plate. Usually it is necessary to make comparisons between lines of widely different densities. It is necessary, therefore, to determine to what extent the departure from ideal conditions will affect the accuracy of intensity measurements.

The first point to be investigated is the method for making exposures of known relative intensity for the construction of the calibration curve. There are three general methods for doing this.

STEPPED SECTOR. A rotating sector with stepped openings of known relative values is placed before the slit, which is uniformly illuminated from top to bottom. The spectrum obtained has steps of known relative exposure times. A source giving either continuous light or lines may be used for illumination.

STEPPED WEAKENER. A neutral weakener, made by sputtering a quartz plate with some reflecting or absorbing material in steps of known relative transmission, is placed before the slit. Either continuous or monochromatic radiation may be used.

STEPPED SLIT. The spectrograph slit is opened wide and is replaced by a slit with a series of stepped openings of known widths. A source of continuous radiation must be used for illumination, in order that the number of quanta striking unit area of the plate may be proportional to the width of the slit in each step.

Each of these methods has advantages and disadvantages. The stepped sector is easiest to construct and use, and it gives the same relative transmission in the various steps regardless of the wave length. The sector has long been mistrusted, however, because it gives intermittent exposures and because of possible reciprocity failures due to the unequal time of illumination for the various steps. The stepped weakener is theoretically the soundest method, but it is a research job to prepare and calibrate the absorbing layers and to ensure that the relative transmissions of the steps are the same for all wave lengths (*1*). The stepped slit is difficult to make accurately, since the smallest opening must be not over 0.125 mm. in width if one is to obtain five steps which have a transmission ratio of two. A further disadvantage is the difficulty of obtaining a suitable source of continuous radiation in the ultraviolet region commonly employed for spectrochemical analyses. The source must have a fairly uniform intensity over the whole wave-length region of interest, since the spectral purity of the image is a function of the slit width in the various steps; in a region of abrupt change of intensity the image given by the widest step will be affected to a greater extent than the image from the narrow steps.

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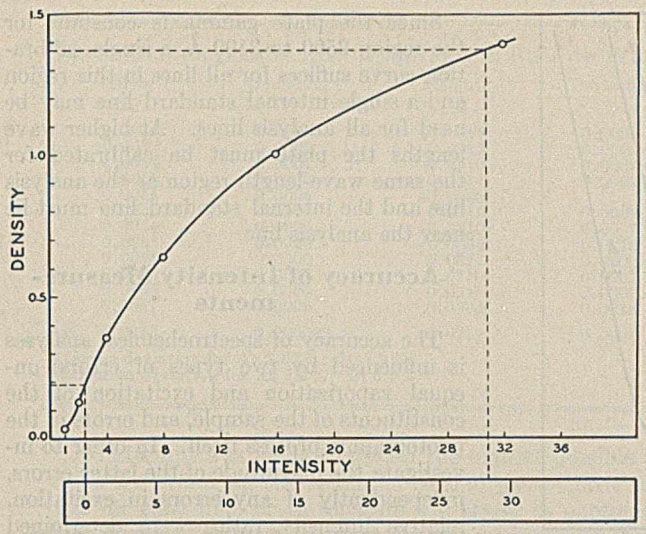

FIGURE 1. DENSITY-INTENSITY PLOT AS USED FOR BACKGROUND CORRECTION

Recently the step-sector method has grown in favor, since Webb (*14*) has shown that reciprocity failures disappear when illumination is made by intermittent exposures of high interruption frequency. His results may be summarized in the statement (*4*): "If the frequency of interruption is sufficiently high, an intermittent time-scale exposure becomes identical with an intensity-scale exposure. The critical frequency is a rate of interruption sufficiently high that each individual silver halide grain receives on the average approximately one quantum per flash." Experiments were performed to test whether, under the conditions employed in analysis, this equivalence between intermittent and uninterrupted light exists. In these experiments a comparison was made between plate calibrations obtained by use of the stepped slit and by the stepped sector.

A five-step slit was constructed to fit in the wedge guides of the spectrograph. Each step was 2 mm. in height; the widths of the steps were made approximately 0.125, 0.25, 0.50, 1.0, and 2.0 mm. The exact widths were determined by measurement with a filar micrometer at various positions and an average value was taken for each step. Illumination was obtained from an underwater spark between tungsten electrodes (*11*) with a Tesla coil discharge. This source gave continuous radiation extending down to a wave length near 2000 Å., with a fairly uniform distribution of intensity over the wave-length range which was used in this study, 2500 to 3400 Å. The source was placed about 2 meters from the slit of the Littrow spectrograph used with no intervening lens, so that uniform slit illumination was obtained from top to bottom. Exposure times of 5 to 8 minutes gave spectra of suitable density for measurement. Process plates were used, developed for 2 minutes at 18° C. with Eastman D9 developer. The densities of the various steps were measured at a wave length of about 3000 A. and H. & D. curves were constructed by plotting densities *vs.* $\log_2$ relative exposures. This log scale was chosen because the slit was made on this basis, the log values for the transmissions of the various steps being 0, 1, 2, 3, and 4.

A seven-step rotating sector was constructed from a brass disk. The stepped openings gave relative transmissions of 1, 2, 4, 8, 16, 32, and 73 (the last due to an error in cutting). Each step was 1.5 mm. in height. The sector was mounted on the shaft of a small motor (operated at 1800 r. p. m.) and the whole assembly was so placed on the optical bench that the sector stood at a distance of 1 cm. from the slit. Separate exposures were made with the continuous source described above and with a mercury vapor lamp operated on direct current. H. & D. plots of density *vs.* $\log_2$ relative exposure were made as in the stepped-slit experiments, at a wave length of about 3000 Å. for the continuous source and for the mercury 2967 A. line for the spectrum made from the arc.

The three H. & D. curves obtained, respectively, for the stepped slit, the stepped sector with continuous radiation, and the stepped sector with monochromatic radiation, were found to be within experimental error exactly equivalent; all three had the same slope in the linear portion and were very nearly parallel to one another in the toe portion. In further experiments, using the mercury vapor lamp as source, interruption frequencies as low as 100 per minute were found to give curves identical with those obtained at high frequencies. This result is in agreement with Malpica (*7*), who reports that he finds no difference in plate gamma for sector speeds varying from 1 to 3600 interruptions per minute.

From the results of these experiments, it appears that the stepped sector may be trusted as a calibration source; there seem to be no errors of reciprocity failure introduced by its use in the wave-length region studied, provided the total light intensity is low. Therefore, since it is easy to construct and use the sector, this method is recommended for plate calibrations. The following conditions have been found to give good results:

A suitable source of line radiation is mounted at a distance of 1.5 to 3 meters from the slit, so that uniform illumination is assured. No lens is used between the source and the slit. The source is operated at such intensity that an exposure of 3 to 6 minutes gives lines of suitable density for measurement. The sector is mounted on the shaft of a small induction motor and permanently left in position just before the slit; during the calibration exposure it is rotated, but for analytical exposures the sector is left in the open position. The source is mounted on a pivoted arm, so that it can be moved back and forth during the exposure, in order that the light beam may travel completely across the collimator lens. With the source in a fixed position only a narrow area of the lens and prism is utilized and the lines obtained are not flat (a fixed source may be used if a long-focus lens is placed just before the slit, so that the light is diffused to cover the collimator lens).

The source must give well-separated lines in the desired analytical region and must give a spectrum which is entirely free of background adjacent to the lines. A mercury vapor lamp, operated on direct current, was first used in this work, with excellent results. Later, it was found that equally good results were obtained from a 5-ampere arc between brass electrodes. Massive electrodes (diameter 15 to 20 mm.) must be used, so that heating of the anode to incandescence is avoided.

It has been found convenient to mount the calibration source permanently in a position at right angles to the external optical path and to use a right-angle quartz prism for bringing the light into the slit for the calibration exposures. This arrangement permits the operator to leave all equipment on the optical bench in place during the calibration exposure and only the right-angle prism need be removable.

It has been found, from the results with many plates, that calibration of every plate is unnecessary; in general it is sufficient to calibrate one plate from a box or from a lot, provided that a standardized procedure is used for development. Exact control of both the developer temperature and the agitation is important. Good results have been obtained by a rapid rocking procedure and also by brushing the plate during development. In the latter method the tray is left motionless and the emulsion is brushed by long sweeps of a camel-hair brush, so that each area receives approximately the same number of strokes.

It is not necessary to use a seven-step sector, as described above. For most purposes it is sufficient to use a sector with four steps, provided an uninterrupted portion of the beam is allowed to pass above the sector, so as to make a fifth step. The exposure should be so timed that the desired line has a density of 0.04 to 0.06 in the lowest exposure step. The density of the highest exposure step will then fall on the linear portion of the H. & D. curve, which can be extrapolated by

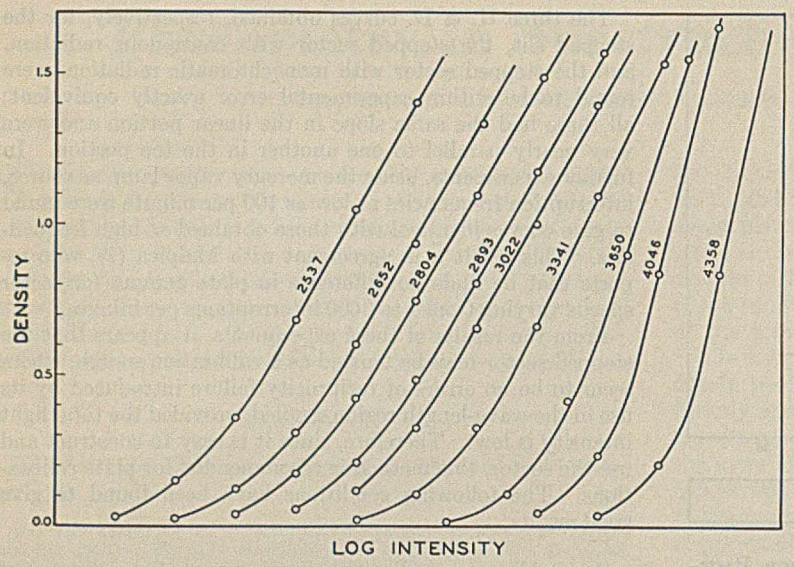

FIGURE 2. VARIATION OF GAMMA WITH WAVE LENGTH FOR PROCESS PLATE

prolonging the linear portion up to a density of 2 or above. The validity of the extrapolation can, at any time, be confirmed by construction of a curve for a stronger line of the same spectrum and plotting these data on the same scale, so as to extend the curve to higher densities.

The observation that the plate gamma (slope of the H. & D. curve) is the same for monochromatic and continuous light does not agree with results obtained by Strock (*12*). In experiments made with the copper 5782 Å. line and with continuous light of the same wave-length region, he found the slope of the density–log exposure curve to be considerably greater for the line than for the continuous radiation. His explanation is that penetration of the developer is different for the spectrum line and for the extended band of continuum, the narrow line receiving more developer than an equal area of the continuum. The reason for the discrepancy between Strock's results and those given above is not clear; a possible explanation is that his work was done on sensitized plates, the present work on unsensitized plates. Doubtless the response of the two types of plates is different, as related to speed of penetration of the developer. Certainly no evidence for the existence of the Eberhard "nachbar" effect was noted in the present experiments; the Process plate gives the same response per quantum of light (at a given wave length in the ultraviolet region) regardless of whether the light be monochromatic or continuous. This observation is important in connection with the question of background corrections, for it has been generally assumed, on the basis of Strock's results, that the response is different for background and line radiation. This belief has retarded the successful application of background corrections in spectrochemical analyses.

Variation of Gamma with Wave Length

It is well recognized that plate gamma is a function of wave length, but unfortunately most of the data published about this effect are for wave lengths above 3500 Å. Consequently it was found necessary to investigate the magnitude of the effect in the analytical region 2500 to 3400 Å. This was done for Eastman Process and for Spectrum Analysis No. 1 plates. Results for Process plates are shown in Figure 2. The H. & D. curves are strictly parallel to one another throughout the region 2500 to 3100 Å., but above 3100 Å. the slope of the curves increases with increasing wave length. Similar results were found for Spectrum Analysis No. 1 plates, except that the gamma value is greater in all regions than for Process plates.

Since the plate gamma is constant for the region 2500 to 3100 Å. a single calibration curve suffices for all lines in this region and a single internal standard line may be used for all analysis lines. At higher wave lengths the plate must be calibrated for the same wave-length region as the analysis line and the internal standard line must lie near the analysis line.

Accuracy of Intensity Measurements

The accuracy of spectrochemical analyses is influenced by two types of errors: unequal vaporization and excitation of the constituents of the sample, and errors in the photographic process itself. In order to investigate the magnitude of the latter errors, independently of any errors in excitation, relative intensity ratios were determined for several steps of line pairs in the same spectrum, photographed by means of the stepped sector used for calibration. Obviously, the actual intensity ratio for a given line pair is the same for all steps and any variations found can be attributed to errors in the photographic process. The following experiment was performed:

The spectrum of a mercury vapor lamp was recorded by means of a seven-step sector, in the manner described for plate calibration. A plate calibration curve was constructed for the 2894 Å. line. By means of this calibration curve relative intensity values were determined for all measurable steps of the 2652, 2654, and 2655 Å. lines; intensity ratios were then computed for corresponding steps (Table I). In further tests intensity ratios were determined for the same line pairs from several spectra, taken on different plates. The data are summarized in Table II.

TABLE I. INTENSITY RATIOS

Step	Intensity 2652/2654	Intensity 2652/2655	Intensity 2654/2655
1	2.14	3.47	1.62
2	2.07	3.35	1.62
3	1.97	3.26	1.66
4	2.10	3.36	1.65
5	2.14		
Av.	2.08	3.36	1.64
Av. % deviation	2.4	1.6	1.05

TABLE II. INTENSITY RATIOS

Intensity 2652/2654	Intensity 2652/2655	Intensity 2654/2655
2.14	3.47	1.62
2.07	3.35	1.62
1.97	3.26	1.66
2.10	3.36	1.65
2.14	3.54	1.65
2.14	3.32	1.57
2.14	3.40	1.61
2.14	3.27	1.57
2.10	3.46	1.60
2.10	3.32	1.61
2.07	3.40	1.61
2.13	3.37	1.61
2.09	3.49	1.59
2.19	3.22	1.59
2.04	3.25	1.62
2.03	3.03	1.49
2.07	3.24	1.63
2.00	3.24	1.62
2.00	3.36	1.62
1.93	3.36	1.65
2.14	3.47	1.65
2.05	3.36	1.66
2.08	3.42	1.66
2.14	3.24	1.56
Av. 2.08	3.34	1.61
Av. % deviation 2.4	2.7	1.8

TABLE III. DETERMINATION OF SILVER AND BISMUTH IN LEAD

	Silver %	Deviation %	Bismuth %	Deviation %
	0.0056	0.0001	0.0315	0.0002
	0.0057	0.0002	0.0315	0.0002
	0.0054	0.0001	0.0310	0.0007
	0.0055	0.0000	0.0315	0.0002
	0.0051	0.0004	(0.0280)	..
	0.0055	0.0000	0.0315	0.0002
	0.0055	0.0000	0.0310	0.0007
	0.0055	0.0000	0.0335	0.0018
	0.0055	0.0000	0.0315	0.0002
	0.0056	0.0001	0.0310	0.0007
	0.0055	0.0000	0.0315	0.0002
	0.0057	0.0002	0.0335	0.0018
Av.	0.0055	0.000092	0.0317	0.0006

Average deviation for Ag 1.67%; Bi 1.9%.
Maximum deviation for Ag 7.3%; Bi 5.7%

No attempt was made to obtain the highest possible precision by averaging the results of several photometric readings for each line, or to separate the errors in photometry from those inherent in the photographic process. Rather, it was sought to maintain the conditions of a routine analysis, so that the results may be taken as typical of ordinary work. In the wide density range covered, 0.1 to 1.6, it was found that average deviations of 2 to 3 per cent may be expected, with occasional maximum deviations as great as 5 to 7 per cent.

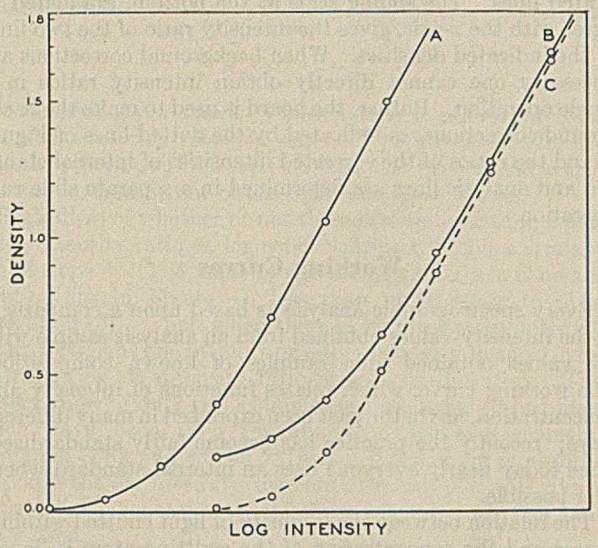

FIGURE 3. EFFECT OF BACKGROUND ON PLATE CALIBRATION

The precision obtained in the analysis of homogeneous metal samples is comparable to that of the preceding experiment. This is shown by the data of Table III, obtained by Tlapa (*13*) for silver and bismuth in lead. The excitation source was a high-voltage spark. Sawyer and Vincent (*9*) obtain results of about the same precision in the analysis of sheet steel, using an alternating current arc source. Numerous other results of comparable precision might be cited. It would seem, therefore, that under favorable excitation conditions the limiting factor in the accuracy of spectrochemical analyses is the photographic process. This is not true, however, for most of the determinations that are made by means of a carbon supporting electrode. There a mean deviation of 5 per cent in a series of duplicate determinations is good and often it is not possible to obtain precision better than 10 per cent. In such analyses the limiting factor appears to be unequal vaporization and excitation of the constituents of the sample, and only a small portion of the error may be attributed to the errors of the photographic process.

Effect of Background

When a spectrum line lies in a region of heavy background the density value obtained is high, and a correction must be applied for the effect of the background. This correction cannot be obtained by subtraction of the background density from the total density or by the equivalent procedure of making the I_o reading in the background region. Rather, it is necessary to subtract the background intensity from the total intensity. The method for doing this is shown in Figure 1, by the dashed lines. A total density of 1.37 corresponds to a relative intensity of 30.8. The background density of 0.19 corresponds to a relative intensity of 2.4. Subtraction gives the corrected line intensity of 28.4. If for the same values the correction is made by subtraction of densities, a corrected line intensity value of 22 is obtained, which is too low by about 25 per cent.

The validity of the method for making background corrections was tested in the following experiment:

Two seven-step mercury arc spectra were photographed on a plate. On one of the spectra was superimposed a heavy background by re-exposing that portion of the plate to the spectrum from a carbon arc. Intensity ratios were determined for the various steps of the 2652–2654 Å. lines for the spectrum without background, for the uncorrected spectrum with background, and for the corrected spectrum with background. The results are shown in Table IV. After correction the intensity ratios for the various steps are reasonably good, but if the uncorrected ratios are used the values for the lowest exposure steps are in error by as much as 40 to 50 per cent. Data for the same plate are shown in the H. & D. curves of Figure 3. Curve A is for the spectrum which has no background, curve B for the one with background. At high intensities the effect of background is not great, but in the lower exposure steps the measured density is chiefly that of the background. Curve C shows corrected line densities, and is in good agreement with curve A.

TABLE IV. EFFECT OF BACKGROUND

	Intensity Ratio		
Step	Without background	With background	Corrected
1	2.14	1.85	2.00
2	2.07	1.84	1.86
3	2.06	1.61	1.98
4	2.08	1.40	1.87
5	2.03	1.22	1.83
6	..	1.12	1.94

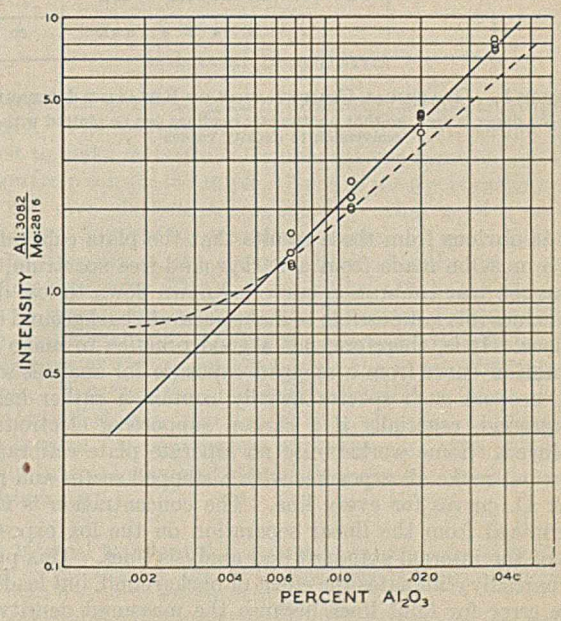

FIGURE 4. EFFECT OF BACKGROUND ON ALUMINUM WORKING CURVE

The effect of background in analytical work is shown in the working curves of Figure 4. The heavy line gives a working curve for the aluminum 3082 Å. line, made with the alternating current arc. The sample was a 3.6 *M* solution of sodium hydroxide to which known amounts of aluminum had been added. (All concentrations are based on the weight of dry sodium hydroxide.) Molybdenum 2816 Å. was used as internal standard line. The circles and the straight line give the intensity ratios corrected for background and for the residual amount of aluminum in the matrix. The dashed curve gives the intensity ratios obtained if the background correction is not applied; the concentration values have been corrected for the residuum. At high concentrations the two curves are nearly parallel to one another, but at low concentrations the uncorrected values indicate concentrations several hundred per cent in error. In fact, the curvature of the uncorrected line is like that which one obtains when the matrix holds a considerable residuum of the material sought in the analysis, and were the background correction not applied one might overcorrect for the amount of residuum.

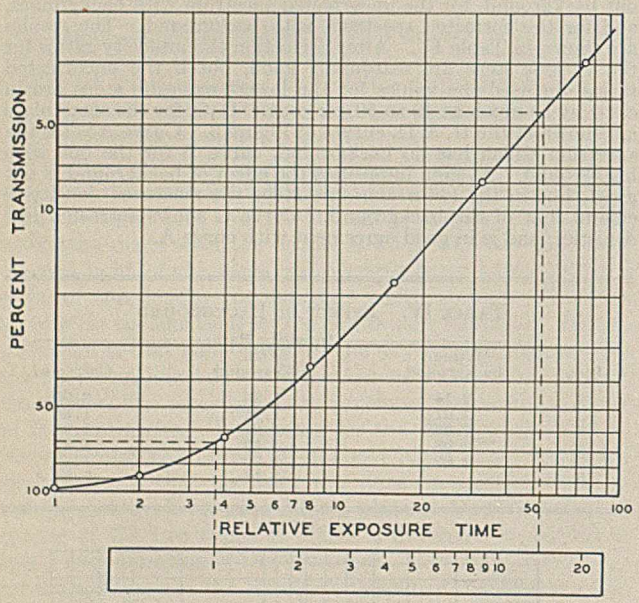

FIGURE 5. GRAPHICAL COMPUTATION OF RELATIVE INTENSITY
Log-log paper is used, so that photometer readings can be plotted without conversion to density values

It is obvious from these results that the plate calibration curve must be made from a background-free spectrum, for unless the true calibration curve is known down to low density values it is not possible to make accurate background corrections. It is, therefore, not a good practice to make the calibration curve from a stepped exposure for analysis samples, because such spectra usually contain a rather heavy background, especially if a carbon supporting electrode is employed. Some workers use no separate plate calibration curve but make all exposures with a stepped sector and plot H. & D. curves for every line. The concentration is then determined from the linear separation on the log exposure axis of the internal standard and analysis lines. This practice partially eliminates the effect of background, but leads to some error for faint lines because the measured density in such cases is largely that of the background, as can be seen from curve B of Figure 3.

Treatment of Experimental Data

The microphotometer data obtained from spectra are in the form of galvanometer deflections for clear plate and for spectral lines. From these readings one obtains intensity ratios for internal standard and analysis lines. The labor of this computation may be materially lightened by the proper choice of method.

It is generally advantageous to plot the plate calibration curve on a density–log intensity basis, when background corrections are not needed, because the curve obtained has a long linear portion. It is not necessary, however, actually to convert galvanometer readings into densities. If the photometer light intensity is so adjusted that the reading for the clear plate is 100, the readings obtained for lines are the percentage transmission values. These may be plotted *vs.* relative intensities by use of log-log paper, as shown in Figure 5. When background corrections are needed, however, it is advantageous to plot density *vs.* intensity. Here semilog paper can be used, with galvanometer readings on the log scale as shown in Figure 5 but with intensity values on a linear scale as in Figure 1.

Calculating boards of the type described by Owens (*8*) are advantageously used with either type of plot. The method for using the board to obtain intensity ratios when background corrections are not necessary is shown in Figure 5 by the dotted lines. The sliding scale at the bottom, graduated to agree with the x-axis, gives the intensity ratio of the two lines of the indicated densities. When background corrections are necessary one cannot directly obtain intensity ratios in a single operation. Rather, the board is used to make the background corrections, as indicated by the dotted lines of Figure 1, and the ratios of the corrected intensities of internal standard and analysis lines are determined in a separate slide rule operation.

Working Curves

Every spectrographic analysis is based upon a comparison of the intensity values obtained from an analysis sample with the values obtained from samples of known composition. The working curve, which relates functions of intensity and concentration, has in the past been expressed in many different ways; recently the practice has become fairly standardized, since today nearly everyone uses an internal standard whenever possible.

The relation between the intensity of light emitted within a source and the concentration of the emitting atom is linear. That is,

$$i = kC$$

or if an internal standard is used,

$$i_A/i_S = kC_A$$

where i_A and i_S are, respectively, the intensities of analysis and internal standard lines and C_A is the concentration of the constituent to be determined.

Unfortunately for the analyst, the intensity of light emitted from a source is not always a linear function of the concentration; some of the emitted light is often readsorbed in colder outer layers of the source. When such absorption occurs the relation between concentration and intensity is given by an empirical equation of the form

$$i_A/i_S = kC^n$$

The mechanism of light absorption is doubtless complicated, since the light traverses outer gaseous layers of various temperatures before it can be detected; a simple Beer's law absorption will not lead to the empirical intensity-concentra-

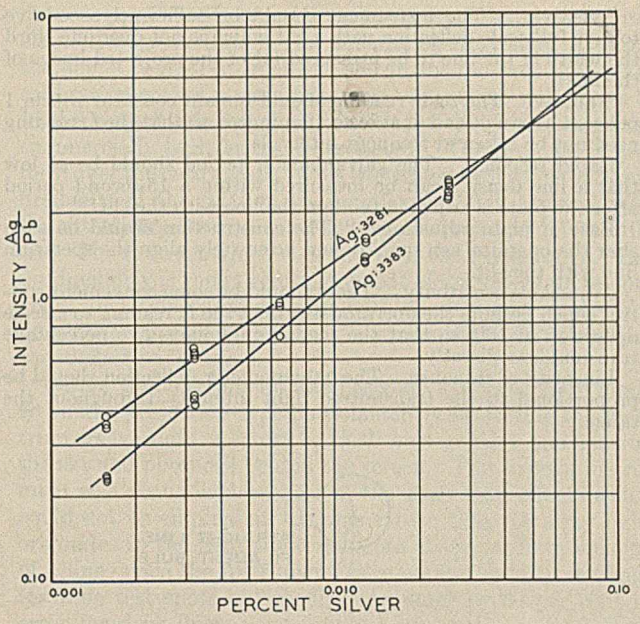

FIGURE 6. WORKING CURVES FOR SILVER 3281 AND 3383 Å. LINES

Illustrating effect of self-absorption on slope of intensity-concentration function

tion function given above. Consequently it cannot safely be assumed that this relation will hold in all cases. In the writers' experience, however, no case has been found which was not covered by one of the equations given above.

Regardless of whether or not there is self-absorption, a plot of log intensity ratio *vs.* log concentration will give a straight line of slope n. The value of n is unity when there is no absorption (as in Figure 4), less than unity when there is absorption (*2*). Thus, when there is no absorption one may obtain a straight line by plotting either intensity ratio *vs.* concentration or log intensity ratio *vs.* log concentration. The writers know of only two examples of such nonabsorbed lines which have been used for analytical purposes—namely, aluminum 3082 and silicon 2816 Å. When there is absorption only the log intensity–log concentration plot will give a straight line; consequently this form of plot is the one generally used.

The value of n varies, as one might expect, with different lines for the same element. An example of this is shown in Figure 6, which gives Tlapa's (*13*) results for the two silver lines 3281 and 3383 Å. At low concentrations the 3281 Å. line is the stronger, but at high concentrations this line is so highly absorbed that the 3383 Å. line appears to be the stronger. Because of this effect estimates of relative line intensities for a given element may be misleading because the apparent intensity may depend upon the concentration.

Working curves are usually prepared from spectral data for synthetic mixtures containing the matrix element and added amounts of the various constituents sought in the analysis. It often happens that the matrix material contains a residuum of unknown amount of some constituents sought in the analysis; in such cases all apparent concentration values are in error by a constant amount. When this happens one may from the spectral results make a determination of the amount of the residuum (*3*). This is done by a series of successive approximations.

First, a working curve plot is made of log intensity ratio *vs.* log apparent concentration. When a residuum is present the line will tend to become parallel to the concentration axis at low concentrations. A straight line is drawn through the points for the higher concentrations and the amount of the residuum is estimated by the magnitude of the departure of the lower concentration points from this line. Next, all concentrations are corrected by adding the estimated residual value, and the process is repeated. Usually two or three approximations will suffice to give a value which will bring all points onto a straight line. Finally, the correctness of the estimation is tested by using the working curve so obtained to determine spectrographically the amount of residuum in the pure matrix material.

The value so obtained should agree with the value obtained by means of the successive approximations. When this does not happen it is indication that some other factor, such as background, must be taken into account. For example, were one to attempt a residuum correction for the uncorrected aluminum working curve of Figure 4, the value obtained would be greatly in error because of the effect of the background.

Whenever one is fortunate enough to work with a nonabsorbed line, the determination of the residuum can be done in a single operation. A working curve is plotted as intensity ratio *vs.* apparent concentration, and a straight line is drawn through the points. Now, another parallel straight line is drawn through the origin; the displacement of the two lines on the concentration axis gives directly the amount of the residuum.

After a working curve has been accurately determined, the final form in which it is used depends upon whether background corrections are needed in the analytical spectra. No generalization can be made about this. When a carbon supporting electrode is employed it is usually advantageous to correct for background but in the analysis of metal samples by a spark source the background is often negligibly small. When background corrections are not essential it is advantageous to put concentration values directly on the slide of the calculating board instead of intensity ratios (this is facilitated by the linear relation between log intensity ratio and log concentration). When background corrections must be used it is, of course, impossible to obtain concentration values from photometer readings in a single operation, because of the necessity for subtraction of intensities. In this case the working curve is usually plotted as in Figure 4, and concentration values are determined from intensity ratios by means of this plot.

Internal Standards

Internal standards are used in practically all spectrographic analyses. The purpose of the standard is to permit the analyst to make corrections for unequal vaporization and excitation from sample to sample. None of the electrical sources used will give absolute reproducibility—that is, if a series of exposures is made for separate portions of a given sample, the line densities of the spectra obtained will vary over a considerable range. The chief reason for this lies in the fact that one cannot accurately control the rate of vaporization of the sample material. During the course of an exposure the temperature of the source changes as the vapor atmosphere changes. Therefore, the probability of excitation for a vaporized atom will change from time to time, since this probability depends upon the temperature of the source. The material used for internal standard should then possess the following characteristics:

The internal standard line should have the same excitation voltage as the analysis line, so that changes in temperature will affect both lines in the same manner.

The internal standard element should be vaporized at the same rate as the constituent sought in the analysis.

The internal standard should be present at such concentration that the line density is about the mean density found for analysis

lines in the concentration range investigated, so that the lines to be compared will be as nearly as possible of the same density.

The internal standard must be homogeneously mixed with the analysis sample.

In practice it is not always possible to attain all these conditions, particularly the first two, and the accuracy of the analysis depends upon the extent to which they are fulfilled. Best results are obtained in analyses made by spark excitation of homogeneous metal samples, where the vaporization rate and the source temperature may be held at a very constant level. Here almost any matrix element line of proper density and wave length can be used as standard. More difficulty is encountered in the analysis of solutions with the alternating current arc, because the choice of standard is often limited by the composition of the sample and by solubility in the sample solution. In some cases a line of the matrix material will serve as standard, but in others the standard must be an added constituent.

In selecting a standard for an analysis of this type, one first examines a spectrum to determine the impurity elements present, for no element present as impurity can be used as standard. Next, a wave-length table is examined for elements not present which give strong lines near the lines to be used for the analysis. From these, one selects those elements which have compounds soluble in the sample solution. Test spectra are made, with varying amounts of these elements added to the analysis sample, in order to select proper concentration ranges for the standard and to ascertain whether the spectrum of the standard has any lines which interfere with the analysis. This procedure will often limit the choice of standard to a few elements.

Among the most widely used internal standards are molybdenum and cobalt, because they are of infrequent occurrence, they provide many suitable lines in the ultraviolet region, and they vaporize from the source at an average rate. Zinc, cadmium, mercury, and other elements whose salts are very volatile are seldom used as standards because they do not vaporize at anything near the rates of those substances usually sought in analyses. In the analysis of powdered samples, for which the direct current arc must be used, it is often debatable whether the internal standard method offers any advantages. The source may change greatly in temperature during the arcing of a sample and unless the internal standard vaporizes uniformly with the sample it will not serve its purpose. Further, proper mixing of the internal standard with the sample entails laborious grinding. Because of these difficulties Slavin recommends (*10*) that the standard be omitted; he prefers to arc weighed portions of sample to complete vaporization.

Some have used background intensity in lieu of internal standard. This procedure is valid only in those cases where the background is excited concurrently with, but independently of, the analytical spectrum. An example of this is in Lundegardh's work (*6*) with flame excitation. Here the background comes from the flame itself and changes in excitation condition apparently are manifested by changes in background. In electrical excitation the background is due to cyanogen bands (when carbon electrodes are used), to incandescent particles of sample, to band spectra from sample material, and to nitrogen bands. All of these, except band spectra of the sample, may vary fortuitously from one sample to another.

Microphotometers

Nonrecording, photoelectric-type microphotometers are almost universally used in spectrochemical analytical work. A microphotometer of this type should have the following characteristics:

Freedom from stray light effects. If stray light reaches the photocell all transmission readings are too high and the error in line density measurements is increased.

Sensitivity. The instrument should be sufficiently sensitive to give full-scale deflection with a slit which is not over one third the width of the line to be measured (or of the projected image of the line).

Stability. The scale reading should remain constant within 1 per cent over a period of at least 10 minutes, so that the I_o reading need not be taken at frequent intervals.

Rapid response. The galvanometer period should be so low that a line density can be measured within a 15-second period after the plate is adjusted to align the line with the slit.

Ease of plate adjustment. The construction should be such that the operator can quickly and accurately align the spectrum line with the slit.

Adjustable sensitivity. There should be some convenient control which permits the operator to adjust the I_o reading to a scale deflection of 100, so that the readings for lines give percentage transmissions directly.

Linearity of response. The galvanometer deflection should be proportional to the transmitted light intensity throughout the range.

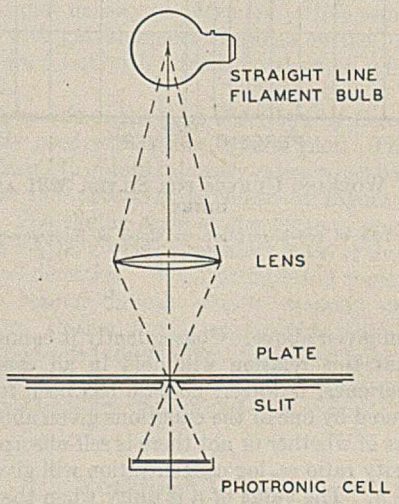

FIGURE 7. OPTICAL SYSTEM OF PHOTOMETER

An instrument which fulfills these criteria was constructed according to the design shown in Figure 7.

The essential features are a low-power light source that gives constant intensity and a slit arrangement that minimizes stray light. The plate is mounted on a screw-driven carriage, emulsion side down. The slit, usually measuring about 1.5 × 0.015 mm., is mounted beneath the plate, with a clearance of about 0.2 mm.

The source is a straight-line, coiled-filament headlight bulb operated at 6 volts and 5 amperes by a constant-power transformer. A reduced image of the filament is focused at the slit. A photovoltaic cell is used in conjunction with a sensitive galvanometer to measure light intensity. The maximum photoelectric current is between 10^{-5} and 10^{-6} ampere. Sensitivity is controlled by a resistance in series with the light, after a rough adjustment is made by varying the dimensions of the slit to bring the deflection to the approximate value desired.

The plate is aligned by means of a mark which runs perpendicular to the center of the slit opening, on the slit jaws. After the spectrum is aligned with the slit the operator slowly moves the carriage by a lead screw, so that the line is made to travel across the slit, while he observes the galvanometer deflection. With a little practice it is easy to coordinate the rate of movement with the galvanometer period, so that the point of maximum density is not overrun. The speed is somewhat better than a line per minute, including a reading of the background transmission.

In all respects except convenience of alignment and speed of operation this instrument compares favorably with the commercially built instruments which have been available for tests. It is very stable, the I_o value remaining constant for long periods after an initial warming up. Because of the low light intensities used and the isolation of the cell from the heat

of the lamp, the cell current is very steady and shows no appreciable drift with time. The amount of stray light entering the slit is negligibly small, as shown by the fact that plate calibration curves remain linear up to densities well above 2. In this respect this instrument is better than all but one of the commercially built instruments tested. The response is uniform over the entire scale. This was tested by a series of measurements of the relative transmissions of two screens, with the slit adjusted to give total deflections varying from 5 to 100 cm. The ratio remained constant over the whole range. Reproducibility is good; remeasurement of a whole plate seldom gives deviations greater than 0.01 density unit except for lines of density greater than 1.5, where the error in reading the deflection is large.

Before this instrument was designed attempts were made to build a projection-type photometer by projecting the spectrum image onto a screen at X10 enlargement and placing the slit and photocell behind the screen. This type of instrument was abandoned because of the stray light effects which could not be eliminated. It was shown that the stray light originates in the light scattered from the immediate vicinity of a line, since the instrument gave correct density readings for wide test spots but readings as much as 50 per cent in error for dark lines.

Summary

A study has been made of the photometric methods used in spectrochemical analyses and the procedures which have been found to give optimum results are listed. Included in this study are the methods for plate calibration, the variations in plate gamma with wave length, the effect of background and methods for correcting, and the accuracy obtained in photometric measurements of intensity ratios. Working curves and the factors governing the selection of an internal standard are discussed. Graphical computation methods are described. Criteria for evaluation of a photometer are given and the construction of a satisfactory instrument is described.

Acknowledgment

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Preparation of a Solution of *o*-Phthalaldehyde for Use as a Glycine Reagent

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GLYCINE is determined colorimetrically by condensing with *o*-phthalaldehyde according to the Patton (*3*) and Klein and Linser (*1*) modifications of Zimmermann's method (*6*). The aldehyde cannot be purchased and has generally been prepared from *o*-xylal bromide (*2*), which can no longer be obtained on the market. The authors attempted to prepare it by the bromination of *o*-xylene, but with very little success. Their efforts were then directed to the preparation of the *o*-phthalaldehyde reagent from *o*-xylene by modification of the method of Thiele (*4*, *5*).

A 1-liter three-necked balloon flask is fitted with a calcium chloride drying tube, a dropping funnel, and a stirrer. The flask is cooled in an ice bath while 150 ml. of acetic anhydride, 10 grams of *o*-xylene, and 8 ml. of concentrated sulfuric acid are introduced. Twenty-six grams of chromium trioxide are dissolved in a mixture of 50 ml. of acetic anhydride and 60 ml. of glacial acetic acid; this solution is added drop by drop from the funnel with stirring. The reaction flask is kept in the ice bath for 4 or 5 hours, during which time the mixture is continuously stirred. The contents are poured into a 1-liter beaker one fourth filled with cracked ice and placed in an icebox over night. A yellow oily scum collects on the surface. The whole is extracted several times with ether, and the combined ether extracts are washed with water to remove the last traces of chromous compounds and dried over anhydrous sodium sulfate. The ether is distilled off on a steam bath and the last of the ether is removed by a vacuum. All attempts to remove the acetic acid with carbonate at this stage resulted in a loss of the active compound.

The residue, consisting of acetic acid plus phthalaldehyde tetraacetate, is treated with 50 ml. of 10 per cent sulfuric acid and steam-distilled as long as the distillate gives a blue color upon adding a drop of ammonium hydroxide and acidifying with acetic acid. About 500 ml. of the glycine reagent result and should be stored in a dark bottle.

The reagent is now ready for use with either a protein hydrolyzate or a solution containing glycine (*2*), except that it should be brought to a pH of 7.4 to 7.8 immediately before use. Attempts to isolate the solid aldehyde resulted in unstable oily crystals; this phase was not pursued further.

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Woburn Iodine Absorption Method

A Measure of Total Unsaturation in the Presence of Conjugated Double Bonds

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The iodine reagents now in use react incompletely with substances containing conjugated double bonds. The methods recommended for determination of the total unsaturation of conjugated oils and fatty acids are complicated and have not found general use in industrial analysis.

Hanus solution, when used in large excess, measures the total unsaturation of dehydrated castor oil, but this procedure is unsuitable for other conjugated oils and fatty acids. By employing iodine bromide solutions of up to twice the concentration used in the Hanus method, a simple procedure is evolved for both conjugated and nonconjugated substances.

Fatty acids with conjugated double bonds, made by an isomerization process from natural fatty acids, give low iodine values with the standard methods. With the proposed method, their iodine values are identical with those of the nonconjugated fatty acids from which they were made. With dehydrated castor oil, constant values result over a wide range of excess of reagent.

With tung oil, a reproducible value of about 225 is obtained if temperature, excess, and time of contact are kept within certain limits. The effect of changes in working conditions on the value for the total unsaturation of tung oil somewhat resembles the effect of similar changes in determination of the Wijs iodine value of this oil.

Theoretical values are obtained with beta-eleostearic acid, 9,11-linoleic acid, and blends of the latter with nonconjugated fatty acids, if proper conditions are used. Values for oiticica oil and for nonconjugated oils and acids are listed.

NUMEROUS halogen-absorption methods are available for measuring the unsaturation of oils and fatty acids. The majority of the customary methods are satisfactory with oils containing only single ethylenic linkages and isolated double-bond systems. They are known to fail, however, with oils containing conjugated double bonds, such as tung oil and oiticica oil.

Gelber and Boeseken (*8*) found that the Wijs iodine reagent adds quickly to one of two conjugated double bonds in 9,11-linoleic acid, or two out of three conjugated double bonds in eleostearic acid, while the remaining double bond is saturated only after contact of 2 to 6 days. Ordinarily, the Wijs method will give a value with tung oil which is somewhat above the partial saturation point, where two out of three double bonds of eleostearic acid have taken up halogen. van Loon (*23*) termed the resulting value the "apparent iodine number". Various investigators have studied the effect of temperature, time, and excess of reagent on the apparent iodine value of tung oil and other oils with conjugated double bonds (*10*). As Gelber and Boeseken (*8*) and van Loon (*23, 24*) have shown, it is possible to determine the true total iodine value of conjugated oils with Wijs solution by extending the time of contact up to one week and applying up to 700 per cent excess of reagent. As a practical method of analysis such a procedure is inadequate because of its slowness.

The von Huebl iodine method, commonly used before introduction of the Wijs, Hanus, and other more recent methods, was applied to tung oil by Kreikenbaum (*20*), who found that it gave uniform results from 169 to 171. Kubelka and collaborators (*21*) showed that the methods of Margosches and Rosenmund-Kuhnhenn similarly give values for tung oil approximating the two-thirds or partial saturation point. None of these methods (and this is true of others as well) is a measure of the total unsaturation—i. e., all the double bonds present, individual, isolated, or conjugated.

With the Hanus method, the situation is somewhat different. The results obtained on tung oil vary even more than most of the others with time, temperature, and sample weight (*11, 32*). Kreikenbaum (*20*) found Hanus values on tung oil varying all the way from 188.9 to 210.8 and considered the Hanus method "inapplicable to Chinese wood oil". Other investigators had similar results. Wiernik (*43*) obtained a Hanus iodine value of 205 on a sample of tung oil, and the same sample when checked by a standard laboratory yielded values of from 139 to 153.7. Wiernik, therefore, concluded that sometimes 2 and sometimes 3 double bonds of eleostearic acid react with iodine bromide solution. The AMERICAN CHEMICAL SOCIETY and American Oil Chemists' Society Committee on Analysis of Commercial Fats and Oils found that the Hanus reagent is entirely unsuitable for tung oil, since extremely high and irregular results are obtained (*1*). Kubelka and co-workers (*21*) studied the effect of sample weight on the Hanus iodine value of tung oil and obtained a curve including values from 122.69 to 163.56 for sample weights of 0.1210 to 0.2542 gram. When the sample weight was kept constant, however, and the time of contact was varied from 15 minutes to 24 hours, values from 163.05 to 276.03 resulted. It was concluded that with Hanus reagent the true iodine value of tung oil was reached after 3 hours, when the absorption had attained a value of 243.42.

Ralls (*31*) modified the Hanus value to a microprocedure, in which iodine bromide solution of one half the strength ordinarily used is employed at ice-bath temperatures. While this procedure was apparently not tried on substances containing conjugated double bonds, it did give theoretical values on such substances as undecylenic acid and cholesterol, which ordinarily do not yield theoretical iodine values. However, this method gave erroneous values, in some cases almost 100 per cent too high, with castor oil, which does not afford difficulties with the common iodine procedures.

The method of Volmar and Samdahl (*42*) also was found applicable to substances with which other procedures fail, such as hexadecene and cinnamic acid, but no report on the use of this method with oils containing conjugated double bonds could be found. The method is slow, involves bromination at 0° to −10° C., isolation of the addition compound, and determination of the bromine by the Charpentier-Volhardt method.

Special Procedures for Compounds with Conjugated Double Bonds

There is, however, a group of halogen-absorption methods which is able to measure all double bonds, including those in the conjugated position.

Kaufmann (*16*) uses ultraviolet light to catalyze the reaction of bromine with the third double bond of eleostearic acid. With others, bromine vapor is allowed to act on a thin film of the substance to be tested. This procedure was first employed by Becker (*2*) and later improved by Rossmann (*35*). Modifications have been suggested by Sabalitschka and Dietrich (*36*), Toms (*39*), Boeseken and Pols (*4*), Brocklesby and Harding (*6*), and others, but have not found extensive use. The bromine absorption is determined gravimetrically and, because of the small sample weights used, a microbalance is required.

Some investigators have called attention to the difficulty of re-

moving the excess bromine vapor from the film (*4*). However, Rossmann (*33*) devised a procedure by which the excess bromine can be titrated, making superfluous the driving off of the excess bromine vapor from the film and the final weighing of the films to constant weight. He obtained theoretical values for alpha- and beta-eleostearic acids, 9,11-linoleic methyl ester, and various other substances with active and inactive double bonds. Later (*34*), however, he found that the volumetric determination fails in the presence of polymerized and oxidized oils, hydroxy acids, and certain other substances. Castor oil with an iodine value of 82 gave bromine-iodine values of 101 to 119 for 5 to 30 minutes' contact. Boeseken and Pols (*4*) believe the bromine-vapor methods to be unsuitable in the presence of hydroxyl groups and found that bromine attacks the hydroxyl radical in ricinoleic acid in addition to saturating the double bond present.

A different bromination procedure, in which bromine is formed in the presence of the fat to be analyzed by the action of potassium bromate on acidified potassium bromide solution, was proposed by Vaupel (*41*). By varying the excess of bromine and the time of reaction, Vaupel obtained so-called primary, secondary, and tertiary bromine values. These values have, however, no relationship to the total unsaturation of conjugated fatty acids and oils, as indicated by the fact that the tertiary bromine value of tung oil, 205, was found to be lower than that of linseed oil, 240 (*40*).

Among the methods not employing halogen, oxidation with peracetic acid was recommended by Smit (*37*) for oils with nonconjugated double bonds only; however, Knowles, Lawson, and McQuillen (*19*), using a similar reagent, claim better results for total unsaturation of conjugated systems than with the Wijs method.

Quantitative hydrogenation has been successfully applied to beta-eleostearin and tung oil by Jordan (*15*). This method is considered to yield accurate values for total unsaturation, as there is no possibility of substitution or other side reactions which frequently complicate the bromine-addition procedures.

Table I shows the iodine values for total unsaturation of tung oil obtained by various investigators, using the methods discussed.

None of these proposed methods for the determination of total unsaturation of oils and fatty acids with conjugated double bonds compares in simplicity with the ordinary volumetric iodine-absorption procedures, such as the Wijs or Hanus method. The modified Wijs procedure, used by Gelber and Boeseken or by van Loon, requires an undue length of time. The need of an ultraviolet lamp complicates the method of Kaufmann, and the use of this procedure has not frequently been reported. Hydrogenation requires special equipment and the same is true, although to a lesser degree, of the bromine vapor method. Bolton and Williams (*5*) say of the latter that it "requires very careful manipulation, particularly with regard to the weighing of the brominated compound".

Need for New Method

There is a steadily rising need for a simple method of determining total unsaturation, which is suitable for routine work, requires no special apparatus, and is applicable to both conjugated and nonconjugated substances. With tung oil, the apparent iodine value, determined by the present standard methods, is so little representative of its quality and performance that numerous practical tests, such as gelation tests, are commonly employed in its evaluation. The use of the partial rather than the apparent iodine value has been suggested (*5*, *27*). This is a true constant rather than an arbitrary value, and eliminates to a great extent the effect of varying sample weights and other details of operation.

The use of the true total iodine value of tung oil, on the other hand, would have at least three further practical advantages.

1. Tung oil, with the highest total unsaturation of all drying oils, would be moved to the top of the list where it belongs by virtue of its drying speed. Conjugated double bonds, which are the most effective for fast drying, will no more appear as a discount in the iodine value of the oil.
2. The common adulterants with iodine values near the apparent iodine number of tung oil will have a considerable effect on the total iodine value and become easier of detection. Similarly, changes in composition due to oxidation or polymerization may be observed by a decrease in total iodine value, whereas the partial and the apparent iodine values are little affected and may even increase during polymerization (*38*).
3. The total iodine value when determined in combination with the partial iodine value may serve as a measure of the amount of conjugated double bonds present.

Table I. Iodine Values for Total Unsaturation of Tung Oil Obtained by Various Authors

Date	Authors	Method	Iodine Values
1928	Toms (*39*)	Bromine-vapor	217.0–232.4
1929	Gelber, and Boeseken (*8*)	Modified Wijs	240–250
1930	van Loon (*24*)	Modified Wijs	220–227
1930	Bolton and Williams (*5*)	Bromine-vapor	223.8–229.0
1933	Lévy (*22*)	Bromine-vapor	228–234
1934	Jordan (*15*)	Hydrogenation	214–226
1935	Rossmann (*34*)	Bromine-vapor gravimetric	238–261
		Bromine-vapor volumetric	245–266
1935	Boeseken and Pols (*4*)	Bromine-vapor	244.4–249.5
1936	Brocklesby and Harding (*6*)	Bromine-vapor	227.7
		Hydrogenation	225.5 (corrected, 226.5)
1940	Knowles, Lawson, and McQuillen (*19*)	Oxidation	208

In the case of oiticica oil, it has not been possible to date to find any reproducible iodine value (*12*). Kaufmann and Baltes (*17*) ascribe the difficulties to the presence of the keto group which "interferes so much with the iodine test that the analyst finds it impossible to determine any iodine number at all". However, van Loon (*25*) reports that the Wijs iodine value of both oiticica and po-yoak oil with large excess of reagent became constant after one week, as in the case of tung oil. A practical method of determining the total unsaturation, if applicable to oiticica oil, may greatly facilitate the analysis and characterization of this oil.

The introduction of dehydrated castor oil in the United States during the last several years has increased the need for a practical method of determining total unsaturation. Priest and von Mikusch (*30*) discussed the inadequacy of using a partial or apparent iodine value in the comparison of either dehydrating processes or the resulting oils.

Finally, the shortage of tung oil and the search for new oils, which possess conjugated double bonds and the physical properties associated with them, has brought into existence other synthetic oils and fatty acids containing this particular double bond structure. In the development of these products, a method giving the total unsaturation has been indispensable, and such a method is also required for the proper analysis and characterization of the finished products. The manufacturing process of the products here referred to—for example, Conjusoy and Conjulin fatty acids (*44*)—includes the isomerization or shifting of the isolated double bonds of natural fatty acids. Since the ordinary iodine reagents measure all isolated but only a portion of the conjugated double bonds, the apparent iodine value drops during isomerization. On the other hand, the total unsaturation will stay the same if other reactions such as polymerization or oxidation are excluded. Where polymerization takes place simultaneously with isomerization, this will be shown by a decrease in the total iodine value. Similarly, in the finished product the total iodine value is of greatest significance; its use in combination with the partial iodine value for characterizing the composition of the fatty acid or oil will be discussed in another paper.

TABLE II. HANUS IODINE VALUES OF CONJUGATED LINSEED FATTY ACIDS

(Theoretical total iodine value, 185.9)

Weight of Sample	Excess of Halogen	Iodine Value Found	Weight of Sample	Excess of Halogen	Iodine Value Found
Gram	%		*Gram*	%	
A, 24 Ml. of Hanus Solution, 1 Hour			B, 49 Ml. of Hanus Solution, 2 Hours		
0.0565	550	164.8	0.0533	1182	173.6
0.0589	538	161.2	0.0603	1030	174.2
0.0829	340	159.9	0.0864	674	177.2
0.0842	336	157.9	0.1110	512	174.6
0.0912	310	155.2	0.1672	339	161.5
0.0990	290	150.4	0.2102	269	153.2

Tests with Hanus Solution

It was observed in this laboratory (*27*) a few years ago that Hanus' iodine bromide solution, used in an excess of more than 400 per cent, yields a reproducible value with dehydrated castor oil which agrees with its total unsaturation calculated from the decrease in acetyl value during dehydration. Moore and Cranmer (*28*), who used this special Hanus method with all commercially available dehydrated castor oils, found that it had a reproducibility of one unit.

In order to determine the usefulness of this procedure for other oils containing conjugated double bonds, possibly in greater proportion, a sample of conjugated linseed fatty acids (*44*) was tested with Hanus solution in varying excess. The resulting values were inconsistent and, unlike dehydrated castor oil, depended on sample weight even when more than 400 per cent of reagent was present (Table II, A).

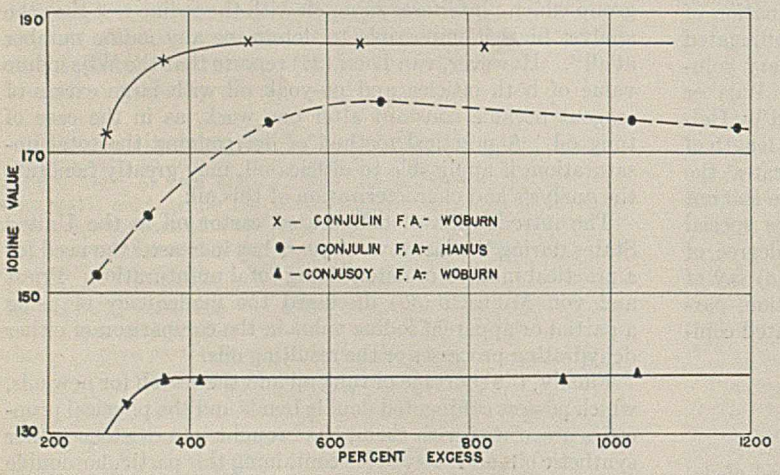

FIGURE 1. IODINE VALUES OF CONJUGATED FATTY ACIDS WITH HANUS AND WOBURN SOLUTIONS

In order to supply a still larger excess of reagent, another series of iodine values was determined with varying sample weights, using approximately twice the ordinary amount of Hanus solution—i. e., 49 instead of 24 ml. The time of contact in this series was 2 hours.

The results are listed in Table II, B, and plotted in Figure 1 (broken line). They show that Hanus iodine solution, even when employed in an excess of up to 1200 per cent, results in low values which vary with the sample weight. The theoretical total iodine value listed is the iodine value of the linseed fatty acids before isomerization.

It was, however, thought likely that by suitable changes an iodine bromide reagent could be caused to react with all double bonds present, since this reagent had previously given the highest iodine values with oils containing conjugated double bonds of any of the halogen solutions employed.

Among the factors which might be changed to make the reaction more complete with Hanus solution are temperature, light, and time. An increase in temperature was not believed advisable because of the known predominance of side reactions, such as substitution and others at higher temperatures (*7*). Similar considerations hold for exposure to irradiation. An excessive extension of the time of contact also was undesirable because of the need for speed in industrial analysis. A 2-hour period had been found insufficient (Table II, B). A further prolongation of contact could be considered only if all other possibilities should fail.

IODINE BROMIDE SOLUTIONS OF HIGHER CONCENTRATION. Changes in the excess of reagent already have been considered and found insufficient to ensure complete saturation. This left one other factor which might be modified—i. e., the concentration of halogen. A study of the extensive literature on iodine values reveals that this factor has not received much attention. Most investigations of the effect of concentration on the iodine number have been limited to increasing the volume of reagent used in a determination, or decreasing the sample weight. In the former case, the concentration of halogen, it is true, increases somewhat, provided the volume of solvent used with the sample is kept constant. In the latter case, for very small sample weight, the halogen concentration at the end of the reaction reaches a limiting value equal to its concentration at the beginning of the time of contact, while at the same time the concentration of the oil approaches zero. An increase in the concentration of both halogen and oil may be attained by reducing the volume of chloroform or carbon tetrachloride in which the sample is dissolved. The effect of increasing its volume in the Wijs method has been discussed by Keffler and Maiden (*18*) and tested in the analysis of tung oil in this laboratory (*27*). If the solvent for the sample is omitted altogether or reduced to a minimum, the concentration of reagent is increased in the ratio of 35 to 25, or by 40 per cent. This represents the maximum increase attainable for a given halogen solution.

In order to obtain a more substantial increase in the concentration of halogen, it is necessary to begin with a more concentrated reagent.

Hanus (*9*) prepared his solution by dissolving 20 grams of iodine bromide in 1 liter of acetic acid. His solution was, therefore, approximately 0.1934 *N* or 0.0967 molar. In the customary standard methods of preparing Hanus solution (*14*) this is made to contain 13.2 grams of iodine and the equivalent weight of bromine per liter and is, therefore, 0.208 *N* or 0.104 molar. The authors were unable to find references in the literature to the use of iodine bromide solutions of higher concentration, except in the method of Bellier (*3*), in which the dissolved sample is titrated directly with an iodine bromide solution containing mercuric chloride. One milliliter of Bellier's solution corresponds to 0.1 gram of iodine; the solution is, therefore, 0.788 *N*.

The data presented below show the action of 0.32 *N* to 0.40 *N* iodine bromide solutions in acetic acid. Directions for the preparation of the 0.32 *N* solution and details for carrying out the determinations are given at the end of this paper. The 0.40 *N* and other iodine bromide solutions were made in various ways; in principle they consisted of making a saturated solution of iodine in glacial acetic acid (empyreuma-free) with or without the aid of heat and adding, at room temperature, a solution of the equivalent amount of bromine in enough acetic acid to reduce the concentration to the desired normality. The solubility of iodine in acetic acid at 25° C. is 0.1025 mole of iodine, or 26.02 grams per liter (*13*). A saturated solution, therefore, contains 0.1 mole in 976 ml. and requires 24 ml. of bromine–acetic acid solution, containing 0.1 mole of bromine to make it 0.40 *N*.

TABLE III. TOTAL UNSATURATION OF CONJUGATED LINSEED FATTY ACIDS

(Theoretical iodine value 185.9, Wijs iodine value (400% excess) 152, concentration of IBr solution 0.40 *N*)

Weight of Sample	Excess of Halogen	Iodine Value Found
Gram	%	
0.0824	822	185.1
0.1016	648	185.9
0.1296	484	185.8
0.1653	363	183.5
0.2120	284	172.8

TABLE IV. TOTAL UNSATURATION OF CONJUGATED SOYBEAN FATTY ACIDS

(Theoretical total iodine value 138.0, Wijs iodine value (400% excess) 117, concentration of IBr solution 0.32 *N*)

Weight of Sample	Excess of Halogen	Iodine Value Found
Gram	%	
0.0643	1040	138.6
0.0713	934	137.9
0.1254	414	137.6
0.1588	365	137.9
0.1843	311	134.5

Isomerized Fatty Acids

The sample of conjugated linseed fatty acid which had given inconsistent and low results with Hanus solution (Table II) gave the values shown in Table III with the 0.4 *N* iodine bromide solution for 1 hour at 20° C.

The resulting values are independent of sample weight or excess of reagent if the latter is at least 450 per cent (Figure 1, top), and this value is identical with the iodine value of the fatty acids before isomerization.

A sample of conjugated soybean fatty acids, which in the unisomerized form had an iodine value of 138.0, was tested with 0.32 *N* solution (Woburn solution) with varying sample weights following Method A. The results again were constant over a large range of sample weights and corresponded to the theoretical value.

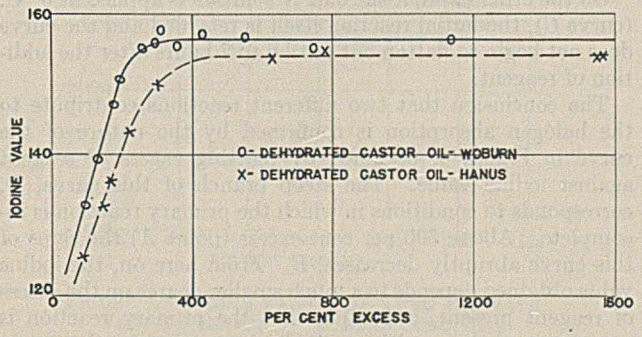

FIGURE 2. IODINE VALUES OF DEHYDRATED CASTOR OIL

DEHYDRATED CASTOR OIL. Samples of unbodied dehydrated castor oil (Isoline) of 1.4 poises viscosity (*F* on Gardner-Holdt scale) were tested by the new method, using varying sample weights. The theoretical total unsaturation of this oil is approximately 150 to 160, depending on the extent of dehydration (*30*). With a quantitative hydrogenation method, Muenzel (*29*) found that 1.5 grams of the oil take up 230 ml. of hydrogen at 15° C. and 740 mm. pressure, which corresponds to an iodine value of 160–161. A sample which had an apparent iodine value of 140 by the modified Wijs method with 400 per cent excess of reagent for 30 minutes at 20° C. gave constant values, within the experimental error averaging 156.4, with a 0.36 to 0.38 *N* iodine bromide solution applied in 300 to 1100 per cent excess (Table V).

Table V also shows the iodine values obtained with a similar solution on the same sample when the time of contact was varied from 5 to 60 minutes. These data indicate that over 90 per cent of the addition takes place in the first 5 minutes, over 95 per cent in the first 15 minutes, and that the constant region, corresponding to the horizontal section of the sample weight *vs.* iodine value curve (Figure 2, top) is reached between 45 and 60 minutes' contact. In this series the excess of reagent was at the lower limit required to obtain reproducible values in 1 hour. In the higher excess region constant values should be expected at shorter time of contact.

A different sample of unbodied dehydrated castor oil (Isoline), which had an apparent iodine value of 139.6 by the special Wijs method, gave Woburn values of 159.6 and 158.5 (average 159.1) with 0.4 *N* iodine bromide solution.

The Hanus iodine values which were found several years ago (*27*) on another sample of dehydrated castor oil have been plotted against excess of halogen in Figure 2 (broken line). Comparison of this curve with the new one shows that both solutions give reproducible values above a certain excess of reagent. However, with Hanus solution the minimum excess required is above 400 per cent, while the concentrated iodine bromide solution requires only 300 per cent. While in the modified Hanus method the effective range of sample weights is between 0.06 and 0.08 gram, samples weighing up to 0.16 gram are well within the region of constancy in the new method. The difference of 2 to 3 points between the constant portions of the two curves may be due to a difference in total unsaturation of the two oil samples.

TUNG OIL. Preliminary tests showed that the values obtained by Method A—i. e., 1 hour at 20° C.—could not be so well reproduced with tung oil as with the synthetic oils and fatty acids which contain both isolated and conjugated double bonds. Both excess of reagent and time of contact affected the results. When the time of contact was extended beyond 1 hour, the values increased, indicating that two reactions of different speeds were superimposed. In order to separate these reactions as much as possible, further determinations were made in an ice bath following the details of Method B, given below. The temperature of the reaction mixture under these conditions is 5° to 10° C. at the start but drops to 0° C. during the early stages of the reaction.

TABLE V[a]. TOTAL UNSATURATION OF DEHYDRATED CASTOR OIL

(Theoretica total iodine value 150–160, Wijs iodine value (400% excess) 140, concentration of IBr solution 0.36–0.38 *N*, temperature 20° C.)

Weight of Sample	Excess of Halogen	Iodine Value Found
Gram	%	
	(Time, 1 Hour)	
0.0627	1138	156.3
0.0903	747	155.3
0.1179	542	156.8
0.1476	412	157.0
0.1692	350	155.7
0.1857	307	157.2
0.1932	289	156.0
0.2107	258	155.4
0.2625	196	150.5
0.3008	164	147.2
0.3600	133	139.5
0.4479	97	133.0
	Excess of Halogen, 320 to 300 Per Cent)	
	(Time, Min.)	
0.2053	5	144.8
0.1917	15	152.5
0.2104	30	153.2
0.1924	45	154.9
0.1726	60	156.6

[a] Values determined by Victor Volk.

The following samples of tung oil, some characteristics of which are listed in Table VI, were used:

1. Old sample of unknown origin, stored without precaution against oxidation. Partial iodine value (*27*) 158.1.
2. Fresh drum lot of Chinese origin, analyzed immediately after sampling.
3. Florida tung oil, several years old, stored without precaution against oxidation.
4. Fresh sample of Chinese origin, through the courtesy of John R. Rea of New York.
5. Standard analyzed sample of American tung oil, cold-stored in well-filled containers; from National Paint, Varnish and Lacquer Association through the courtesy of H. A. Gardner. The characteristics supplied with this sample, other than those listed in Table VI, were:

Specific gravity at 15.5–15.0° C.	0.940
Saponification No.	193.0
Unsaponifiable matter, %	0.44
Refractive index at 25° C.	1.5170
Iodine No. (Wijs)	167.4

Table VII contains the values found on these five tung oil samples with varying conditions. The use of the ice bath as described in Method B is indicated by "0° C." in the column headed "temperature".

TABLE VI. CHARACTERISTICS OF TUNG OIL SAMPLES TESTED

Sample No.	Origin	Viscosity (Gardner-Holdt)	Color (Hellige)	Acid Value	Browne Heat Test, Min.
1	Unknown	K-L	4L-4	6.4	10.75
2	Chinese	I-J	2L	4.5	11.75
3	American	U	2L	0.93	6.25
4	Chinese	J	3L-3	3.5	10.25
5	American	I-J	1-2L	0.9	11.5

TABLE VII. IODINE VALUES OF TUNG OIL

Normality of IBr Solution	Time *Hours*	Temperature ° C.	Excess %	Iodine Value Found
		Sample 1		
0.36	3	20	689	213.4
0.36	3	20	585	213.2
0.36	3	20	289	202.8
		Sample 2		
0.352	1	20	496 ± 2	224.2 ± 0.3
		Sample 3		
0.362	1	20	670	230.0
0.362	1	20	550	225.8
0.362	1	20	300	230.5
		Sample 4		
0.324	1	0	458 ± 58	206.2
0.324	2.5	0		216.4
0.324	4.2	0		220.6
0.324	6	0	389 ± 53	215.8 ± 1.9
0.324	11.5	0		222.0 ± 0.6
0.324	18	0		225.1 ± 1.9
0.400	1	0	545 ± 30	219.9 ± 0.5
0.400	2.5	0		225.4
0.400	3	0		226.6
0.400	5	0		226.8 ± 0.7
0.400	18	0		253.8
0.393	1	0	442 ± 13	218.0 ± 1.9
0.393	1	0	778 ± 10	221.1 ± 1.1
0.385	3	0	1275	229.2
0.385	3	0	1148	228.8
0.385	3	0	683	225.2
0.385	3	0	590	224.2
0.385	3	0	402	212.5
0.385	3	0	256	203.8
0.400	1	20	490 ± 10	234.6 ± 0.5
0.325	1	20	465 ± 15	220.2
0.325	3.2	20		229.4
0.325	4	20		231.6
0.325	18.5	20	290 ± 10	260.1 ± 0.5
		Sample 5		
0.326	3	0	518 ± 52	214.0 ± 0.2
0.326	5	0		224.4 ± 0.8
0.326	7	0		224.2 ± 1.9
0.326	17	0		233.3 ± 1.6
0.408	3	0	833 ± 50	227.1 ± 1.0

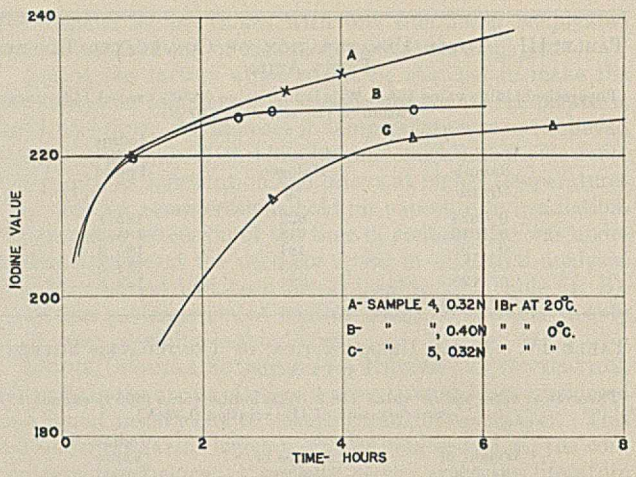

FIGURE 3. IODINE VALUES OF TUNG OIL WITH WOBURN SOLUTIONS

In order to facilitate the interpretation of these results, the iodine values of samples 4 and 5 have been plotted against time of contact (Figure 3). The shape of these curves indicates the presence of two distinct halogen-consuming reactions proceeding at different rates.

Curve *A* shows that, with 0.32 *N* iodine bromide solution at 20° C., a rapid absorption of halogen takes place during the first hour, corresponding to an iodine value of about 220. This is followed by a much slower absorption corresponding to 3 or 4 units per hour.

In comparison, a 0.40 *N* solution at 0° C. (curve *B*) results in an equally fast absorption during the first hour and the value continues to rise slightly during the second and third hours but stays almost constant thereafter for several hours. Thus, the use of the more concentrated solution at lower temperature retards the secondary reaction without decreasing the speed of the primary absorption. However, even in this case, halogen absorption continues and a value of 253.8 is obtained after 18 hours' contact (Table VII, sample 4).

On the other hand, if the 0.32 *N* solution is applied at 0° C. (curve *C*), the initial reaction itself is retarded and the curve does not begin to flatten out until 6 to 7 hours after the addition of reagent.

The conclusion that two different reactions contribute to the halogen absorption is confirmed by the nature of the curve in Figure 4, obtained by plotting excess of reagent against iodine value. The steep branch of this curve, *D*, corresponds to conditions in which the primary reaction is incomplete. Above 590 per cent excess (point *A*) the slope of this curve abruptly decreases, *E*. From here on, the iodine value obtained depends to a much smaller degree on the excess of reagent present, indicating that the primary reaction is complete and the resulting value is determined by the extent to which the secondary reaction participates. Thus, under the conditions used, 590 per cent excess of reagent is just sufficient to complete the first, speedy absorption, without interference by the secondary reaction.

These observations recall the influence of time, temperature, and excess reagent on the Wijs iodine value of tung oil (*10*). In that case the two reactions involved are known to be a rapid addition of halogen to two of the three conjugated double bonds of the eleostearic acid radical and a slow subsequent addition to the third double bond. The true partial iodine value of tung oil was found to coincide with the intersection of the two branches of the curves obtained by plotting iodine value against either excess reagent or sample weight (*27*).

By analogy it appears, therefore, that the value of 224.2 obtained with 590 per cent on tung oil sample 4 is an intrinsic iodine value of this oil, whereas the other points on the curves are apparent iodine values.

In view of the observation by previous investigators, who used hydrogenation methods for determining the unsaturation of tung oil (Table I), the authors believe the conclusion justified that this value of 224.2 represents the true total unsaturation of the sample examined. None of the hydrogenation-iodine values for tung oil found in the literature are higher than 227. This fact, together with the present observation of a break in halogen absorption in the neighborhood of this value, indicates that the total unsaturation of tung oil is attained at this point. The higher values found with bromine vapor or upon prolonged exposure with iodine bromide solutions are then due to such secondary reactions as substitution, addition to secondary valences (*34*), or new double bonds formed during the determination (*31*, *40*).

This conclusion is corroborated by data obtained with beta-eleostearic acid shown below.

On the basis of these observations, we may define the conditions necessary for the determination of the total unsaturation of tung oil. Several procedures are possible, according to accuracy and speed required.

If greatest accuracy is needed, several values should be determined with 0.38 to 0.40 *N* reagent in an ice bath, using varying sample weights and the values plotted against excess of reagent as in Figure 4. The intersection of the two branches of this curve represents the total unsaturation.

As a quicker and more convenient method, the iodine value is determined as described in Method B, with a sample weight which will leave 650 to 800 per cent excess of halogen at the end of the reaction. Figure 4 shows that within these limits the dependence of the iodine value on excess is less than two units.

Finally, where speed is most important, a 0.32 *N* reagent may be employed for 1 hour at 20° C., using sample weights below 0.08 gram. This method will give the total unsaturation within 3 to 4 points, a margin of error which is no greater than that encountered in most of the bromine vapor addition methods.

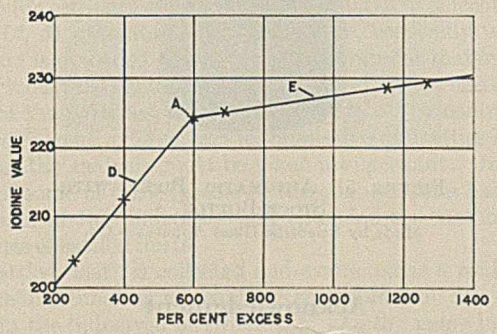

FIGURE 4. IODINE VALUES OF TUNG OIL WITH VARYING EXCESS OF WOBURN SOLUTIONS
3 hours at 0° C.

OTHER OILS AND FATTY ACIDS. Beta-eleostearic acid (melting point, 71.5° C.) was prepared by repeated recrystallization of irradiated tung oil fatty acids in petroleum ether, using carbon dioxide to prevent oxidation. The average values found in several determinations are listed in Table VIII, together with the conditions used in each case. Similarly, values obtained on 9,11-linoleic acid (melting point, 53.4° C.) prepared according to Mangold (*26*) and on a blend containing 50 parts of this acid and 50 parts of soybean fatty acids, sample 2 (Table IX), are listed. A sample of raw oiticica oil, several years old, gave the value listed in Table VIII as the average of three determinations agreeing within ±0.8.

TABLE VIII. IODINE VALUES OF CONJUGATED COMPOUNDS

Sample	Normality of IBr Solution	Excess of Halogen	Temperature	Time	Iodine Values Found[a]	Iodine Values Calcd.
		%	° C.	Hours		
Beta-eleostearic acid	0.32	550–600	20	1	272.7	273.7
	0.40	600–2000	0	3	273.8	...
9,11-Linoleic acid	0.32	400–500	20	1	183.3	181.2
	0.32	400–500	0	3	182.6	181.2
	0.32	400–500	0	5	183.7	181.2
50% 9,11-acid + 50% soybean fatty acid	0.40	600–700	0	3	163.7	159.2
	0.32	400–500	20	1	160.6	159.2
Oiticica oil	0.40	800–1000	0	3	203.9	...

[a] Averages of 2 or 3 determinations each.

TABLE IX. WIJS AND WOBURN IODINE VALUES

Oil or Fatty Acid	Wijs Iodine Value	Woburn Iodine Value	Normality of Woburn Solution
Soybean oil, sample 1	130.5	126.5	0.36
Soybean oil, sample 2	131.8	127.2	0.36
Soybean pentaerythritol ester	127.6	128.4 ± 0.1	0.40
Soybean fatty acids, sample 1	137.8	136.0 ± 0.6	0.40
Soybean fatty acids, sample 2	138.8	139.6 ± 1.1	0.39
	138.8	137.2 ± 0	0.32
Linseed oil, sample 1	186.8	185.5 ± 0.1	0.36
Linseed oil, sample 2	185.0	185.8 ± 0.5	0.40
Linseed fatty acids, sample 1	194.2	196.4 ± 0.4	0.40
Linseed fatty acids, sample 2	190.3	191.1 ± 0.2	0.40
Linseed fatty acids, sample 3	191.2	186.9	0.36
Sardine oil, winterized	196.9	189.2	0.36
Sardine fatty acids, winterized	205.0	196.2	0.36
Teaseed oil	87.7	88.8 ± 1	0.32
Tall oil, distilled	169.5	203.8 ± 1.1	0.40
Perilla fatty acids	198.9	202.3 ± 0.2	0.40
Walnut oil	155.2	156.0 ± 0.1	0.32
Castor oil	87.4	88.9	0.32

This is significant, in view of the fact that this oil cannot be successfully dealt with by any of the known iodine procedures (*12*, *17*).

The fact that the proposed method is applicable not only to substances containing conjugated double bonds, but to non-conjugated fatty acids and oils as well, is demonstrated by the values for total unsaturation listed in Table IX. The Woburn values listed in this table were determined according to Method A, except that the concentration of the reagent varied between 0.32 and 0.40 *N*.

Except in the case of tall oil and sardine oil, these values agree with the Wijs iodine value within 1 to 5 units. The discrepancy with tall oil is understandable, in view of the presence of large proportions of rosin acids, sterols, and possibly other compounds, which react incompletely with Wijs reagent. The difference of approximately 8 units in the case of sardine oil, and the smaller differences with some of the other oils and fatty acids, are comparable to the discrepancies which result when other halogen-absorption methods are compared with each other.

The Woburn iodine values obtained on castor oil show that the hydroxyl group of ricinoleic acid is not attacked to any extent by 0.32 *N* iodine bromide solution.

Further data will be presented in another paper, in which the use of the Woburn method for the determination of the diene value and the composition of conjugated oils will be demonstrated.

Preparation of Woburn Iodine Solution

Woburn iodine solution consists of a 0.32 *N* iodine bromide solution in glacial acetic acid. The concentration should not vary more than ±0.01 *N*.

MATERIALS. Empyreuma-free 99.5 per cent acetic acid (bichromate test = 0.5 hours) (supplied by J. T. Baker Chemical Company as acid acetic, C. P. special, Lever Brothers), resub-

limed (reagent) iodine, C. P. bromine, 0.17 *N* sodium thiosulfate solution, 15 per cent C. P. potassium iodide solution and starch solution.

To prepare approximately 1 liter of the solution, dissolve 20.5 grams of iodine in 925 ml. of the acetic acid. This is best done in a 1-liter flask with the aid of occasional stirring and moderate heat (hot plate or steam bath). When all is dissolved, allow to cool to room temperature, measure out 25 ml. with a pipet, and titrate with 0.17 *N* sodium thiosulfate solution, using starch as indicator in the usual manner (*14*). From the titration, calculate the number of additional milliliters, *Y*, required to dilute the solution to 0.32 *N*, as follows:

a = ml. of thiosulfate solution used
b = grams of iodine equivalent to 1 ml. of thiosulfate solution

$$Y = 1773\,ab - 900$$

Then calculate the weight of bromine, *X*, necessary to double the halogen equivalent, as follows:

$$X = 22.7\,ab$$

Tare a graduated cylinder, containing about 25 ml. of acetic acid, on a scale accurate to 0.1 gram, and add *X* grams of bromine by means of an eye dropper. Add acetic acid until the volume in the cylinder equals *Y* ml. After the bromine solution in the cylinder is added to the remaining 900 ml. of iodine solution, the reagent is ready for use. If kept in the dark or in diffused daylight, it is stable for several months without substantial change in concentration.

Other Required Reagents

In preparing the 0.17 *N* sodium thiosulfate solution, follow the customary precautions (*14*), such as using freshly boiled distilled water (carbon dioxide–free and sterile) and filtering before use; 42.2 grams of C. P. sodium thiosulfate ($Na_2S_2O_3.5H_2O$) are required per liter of solution. Standardize this solution with resublimed iodine in the usual manner and calculate the iodine equivalent of 1 ml.

Prepare the 15 per cent potassium iodide solution and the starch indicator in the customary way (*14*).

Determination of Woburn Iodine Value

METHOD A. Conditions: 0.32 ± 0.01 *N* iodine bromide solution, 1 hour at 20° C.

Weigh accurately 0.06 to 0.16 gram of fatty acid or oil, depending on the expected iodine value (the excess of halogen should be from 500 to 800 per cent of the quantity absorbed), into a clean and dry glass-stoppered 250-ml. (8-ounce) bottle. Add 10 ml. of chloroform and swirl gently until sample is dissolved. Add exactly 25 ml. of 0.32 *N* iodine bromide solution, preferably from an automatic buret or pipet (Figure 5). Swirl and place the bottle in the dark inside a water bath kept at 20° C. After 1 hour add 20 ml. of 15 per cent potassium iodide solution, washing down any iodine bromide which may have collected in the neck or on the walls of the bottle. Mix and titrate with 0.17 *N* sodium thiosulfate solution in the ordinary manner, using starch indicator when the mixture has become pale yellow.

Blank determinations with 10 ml. of chloroform are made in the same manner.

The iodine value is calculated as follows:

$$I.\,V. = \frac{100\,(A - B)F}{S}$$

A = ml. of sodium thiosulfate solution used for blank
B = ml. of sodium thiosulfate solution used for sample
F = grams of iodine equivalent to 1 ml. of sodium thiosulfate solution
S = weight of sample in grams

METHOD B. Conditions: 0.40 ± 0.01 *N* iodine bromide solution, 3 hours at 0° C.

This method is recommended for tung and oiticica oils. Ice is used to cool the reaction mixture and thin-walled Erlenmeyer flasks are required to assure proper cooling.

Weigh out 0.060 to 0.075 gram of oil in a thin-walled glass-stoppered Erlenmeyer flask, add 10 ml. of chloroform to dissolve the oil, and swirl the flask in a beaker containing crushed ice for 3 to 5 minutes. Continue swirling and deliver 25 ml. of 0.40 *N* iodine bromide solution. Note time of the addition of reagent. Wet the glass stopper with potassium iodide solution to prevent evaporation of the halogens, but do not allow any of this solution to come in contact with the reaction mixture. Continue to swirl in the ice bath for 2 to 3 more minutes, then immediately place the flask in a container almost filled with crushed ice in the dark, and allow it to remain there for 3 hours after the addition of reagent. After this period proceed as in Method A, but keeping the concentration of sodium thiosulfate preferably between 0.21 and 0.22 *N*.

NOTE. With some compounds, such as 9,11-linoleic acid, best results were obtained by following the details of Method B, but using 0.32 *N* rather than 0.40 *N* iodine bromide solution.

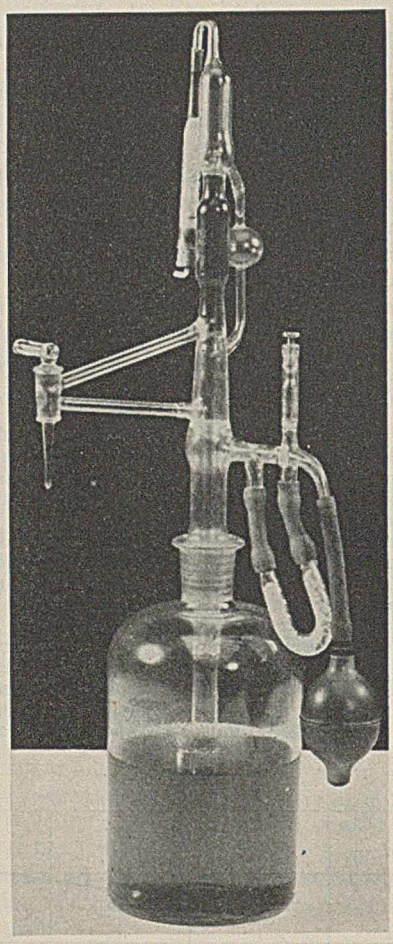

FIGURE 5. AUTOMATIC PIPET WITH STOCK BOTTLE
Made by Scientific Glass Apparatus Co.

Acknowledgment

The authors wish to thank A. G. H. Reimold and J. E. Good for encouragement of this investigation, G. W. Priest and J. D. Ransom for their constructive criticism, and Victor Volk for determination of the figures listed in Table V.

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Hydrofluosilicic Acid Method for Determination of Quartz

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HYDROFLUOSILICIC acid affords a differentiation between quartz and other silicon-containing compounds such as the silicates. This reagent dissolves most silicates very rapidly, while its action on quartz is exceedingly slow. However, in spite of many attractive features, its use as a reagent for estimating the amount of quartz in siliceous materials (*2*, *5*) presents certain difficulties, especially when the method is extended to finely divided granular materials such as rock dusts. Factors such as temperature, the size distribution of the particles, and the magnitude of the control blank on the reagent, plus certain manipulative difficulties in carrying out the analysis, must be taken into account. Investigation of some of these factors in these laboratories has cleared up some of the difficulties and extended the method to the analysis of settled dusts.

"Settled dust" is collected and examined as a routine procedure in estimating atmospheric contamination due to dustiness in the industrial environment. As the name implies, it is the dust which has settled on rafters, window ledges, roofs of buildings, and various other sites, relating to the industrial environment.

A line must be drawn somewhat in defining certain material as settled dust, which certainly does not include pebbles, match sticks, bits of rubbish, etc. It has been the practice of this laboratory, therefore, to sieve all samples through a 200-mesh sieve and to analyze this sieved material as "settled dust". The average diameter of such granular material is usually about 6 to 8 microns. However, if samples ground to pass a 200-mesh sieve are again sieved through a 325-mesh sieve, the average diameter of the particles retained on the 325-mesh sieve may vary from 48 to 90 microns (*3*).

According to the hydrofluosilicic acid procedure as described by Knopf, the loss of quartz from a pure quartz sample ground to pass a 150-mesh screen is 1.4 per cent per 48 hours after 14 days of treatment. The rate of decomposition was found to differ considerably in different silicates. Certain refractory silicates required a week or more to decompose (*5*).

Moke (*6*) in a study of foundry dusts (*4*) used Knopf's hydrofluosilicic acid procedure and determined the solubility of quartz on particles of 10 microns and less. The loss was from 13.6 to 48.5 per cent in 48 hours. In this connection Moke says that if sufficient time and acid were employed, all the quartz would undoubtedly go into solution. The rate of solubility increases greatly with decrease in grain size.

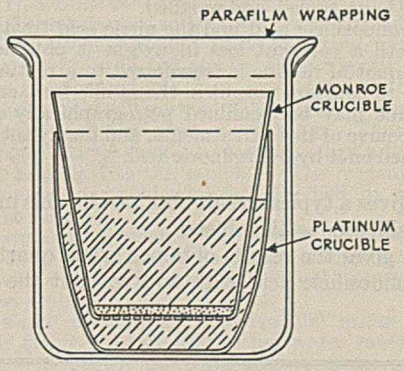

FIGURE 1

Care must be exercised (*5*) not to raise the temperature during the hydrofluosilicic acid treatment, because hydrofluosilicic acid (H_2SiF_6) decomposes on heating into silicon tetrafluoride (SiF_4) and hydrofluoric acid (HF) which will readily attack free silica.

Material and Reagents

Samples of settled dust were employed in this investigation. The quartz sample used as a control was Brazilian quartz, sieved through a 200-mesh screen.

Three grams of silica gel were added to 500 grams of a commercial grade of hydrofluosilicic acid.

A 1 to 1 nitric acid solution was used.

Procedure

A sample of dust weighing 0.2 to 0.5 gram is taken for analysis. It is ignited in a platinum crucible and an aliquot of the residue is transferred to a Munroe crucible. (This is similar to a Gooch crucible but is of platinum and contains a platinum mat. Such crucibles may be obtained through dealers in platinum laboratory ware.) The Munroe crucible is set inside an ordinary platinum crucible. Ten milliliters of hydrofluosilicic acid reagent are added to the sample in the Munroe crucible and the crucibles are set inside a 100-ml. beaker (Figure 1), which is covered with Parafilm (a gas-tight material obtained from the Fisher Scientific Company), placed in the electric refrigerator at 10° C., and allowed to remain there for 48 hours.

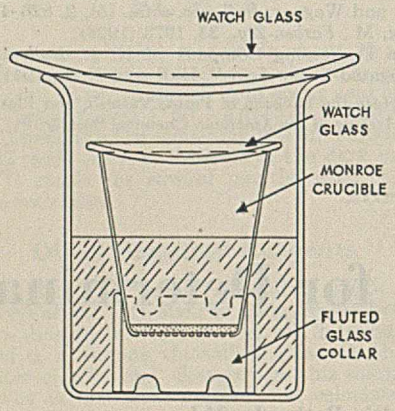

FIGURE 2

The Munroe crucible is now transferred to the filter funnel and the reagent is drawn off by suction. The filter flask used for this purpose is partly filled with water to prevent etching. The filter is washed with cold water, the filter flask is emptied, and the filter is finally washed with nitric acid. The Munroe crucible is now set in a 150-ml. beaker on a glass collar which is fluted at both ends (Figure 2). The crucible is covered with a small watch glass, nitric acid is added, and the beaker is covered over with a watch glass. The contents are warmed at about 70° C. for 0.5 hour. The nitric acid is filtered off by suction, the filter is washed with water, and the crucible and contents are dried at 120° for one hour, cooled, and weighed.

The hydrofluosilicic acid and the nitric acid treatment is now repeated until a constant loss in weight is obtained. At this point an aliquot of residue is transferred to a platinum crucible and ignited to constant weight in the muffle furnace.

The residue may be examined petrographically at any time during the course of these treatments, and finally total silica may be determined on it by hydrofluoric acid.

Table I gives a typical result obtained by this method when applied to siliceous settled dusts.

Table II gives the results obtained when quartz is treated with hydrofluosilicic acid with and without the addition of silica gel.

TABLE I. DETERMINATION OF QUARTZ

(Settled Dust 686, sample 0.3143 gram, 6 treatments with H_2SiF_6)

Treatment	Loss in weight
	Gram
1	0.0863
2	0.0216
3	0.0115
4	0.0082
5	0.0061
6	0.0045
Total	0.1382

0.1665 gram of residue treated with H_2F_2 gave a loss of 0.1588 gram. Quartz (uncorrected), 44%

TABLE II. DETERMINATION OF QUARTZ

Sample 0.2380 gram, 4 treatments with H_2SiF_6 (SiO_2 added)

Treatment	Loss in Weight
Hours	*Gram*
48	0.0057
72	0.0033
24	0.0008
24	0.0016

Average loss for 48 hours, 1.4%

Sample 0.2140 gram, 2 treatments with H_2SiF_6 (no SiO_2 added)

48	0.0120
48	0.0182

Average loss for 48 hours, 7.1%

Sample 0.1789 gram, 5 treatments with H_2SiF_6 (SiO_2 added)

48	0.0004
48	0.0003
48	0.0008
48	0.0002
48	0.0003
Total	0.0020

Loss after ignition, 0.0024. Blank on empty platinum crucible. Loss, 0.5 mg. or 0.1 mg. per 48 hours. Loss for quartz (corrected), 0.38 mg. per 48 hours or 2.1% per 48 hours.

Discussion of Method

The solution of quartz by the reagent is greatly reduced by employing a low constant temperature (10° instead of room temperature). This temperature is purely arbitrary. The electric refrigerator was used because of its availability, convenience, and the relative constancy of the temperature compared to that which obtains in the laboratory. The procedure described here can be employed with advantage even at room temperatures, if the sample is not too finely divided. However, the magnitude of the quartz blank in the case of a dust (13.6 to 48.5 per cent in 48 hours) is so great as to preclude the use of the method at ordinary room temperatures.

The addition of silica gel to the hydrofluosilicic acid makes for a low constant blank, probably by removing any excess hydrofluoric acid which may be present. Quartz control samples weighing 0.2 gram lost from 6 to 9 per cent by weight when untreated hydrofluosilicic acid was used. With the treated hydrofluosilicic acid the quartz samples lost only 0.4 mg. in 48 hours. This compares with 2.8 mg. in 48 hours found by Knopf using 150-mesh quartz (*5*). Different lots of hydrofluosilicic acid gave concordant results.

The Munroe crucible must be tested for cracks before each complete analysis. It should be able to retain freshly precipitated calcium oxalate. Cracks are easily mended (*1*).

The use of nitric instead of hydrochloric acid prevents the formation of cracks and solution of the platinum. Controls with nitric acid showed losses of only 0.1 mg. per 48 hours in the course of a complete analysis; 48-hour treatments were found most convenient and almost as efficient as 24-hour treatments and allow the analysis to be carried on over week ends.

The initial ignition of the sample is to be avoided in certain cases—for example, in limestones containing small amounts of quartz, when a preliminary hydrochloric acid treatment is to be preferred. The use of the Munroe crucible avoids the repeated transfer of sample onto a filter paper and therefore avoids the errors inherent in such operations. The time required for manipulative detail is cut down to about 30 minutes for each 48-hour period; this enables samples to be removed and started again within the same day.

Summary

A method for the analysis of the quartz content of granular material using hydrofluosilicic acid has been extended to include "settled dusts". Mechanical losses and losses due to the solution of quartz by the reagent have been greatly reduced. There is a substantial saving of time in an analysis which may take as long as 2 weeks to complete.

Acknowledgment

The author wishes to thank R. W. Schayer for his assistance in carrying out most of the experimental detail.

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Determination of Traces of Tin in Malt Beverages

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THE presence of traces of tin in beer, due to solution from tin surfaces in brewery and dispensing equipment, has long been known to produce haziness and turbidity in the finished beverage. The relatively high intensity of the colloidal haze produced seems out of all proportion to the minute quantities of tin involved. As little as 0.1 part per million will affect the clarity of the beverage.

Goob (*3*) in 1912, writing on tin turbidities, noted the unsatisfactory state of the quantitative methods for the chemical determination of traces of this metal. A search of the literature failed to reveal any specific methods or improvements of methods for the chemical determination of these traces of tin in beer since that time. There are many published chemical methods for determining small amounts of tin in food and related products, but these, without exception, are for quantities greatly in excess of those which may cause trouble in beer. Some of these methods might be adapted to determining traces of tin by using extremely large volumes of sample, but this is not a completely satisfactory expedient—for instance, it would be necessary to examine 4 liters of a beer containing 0.25 part per million of tin in order to recover 1 mg. of tin. Large charges necessitate increased volumes of reagents and solutions and the effective increase in sensitivity thus obtained is not great. In most cases, the minute traces of tin involved would be lost in the elaborate separations used.

The development of spectrographic analysis (*4*) has brought a new tool which may be applied to this problem, but this method requires special training and expensive apparatus and is beyond the means of the average analyst.

Nearly all the methods in the literature for determining tin in biological materials utilize a tedious wet digestion for destroying the organic matter prior to separating and measuring the tin and avoid the more convenient ashing. During ashing, the tin changes to tin oxide, which is extremely refractory and resists solution in all common acids. The widespread use of the wet digestion has probably been due to a desire to avoid the formation of this refractory tin oxide.

Tin oxide readily becomes soluble when fused with a number of different substances. A method for determining tin in foods, which utilizes an ashing and a fusion of the ash, was described (*2*) in 1928. This method is not sufficiently sensitive for determining tin in beer, but the treatment of the sample prior to actual analysis is of interest.

Until recently, there has been a notable lack of good specific sensitive organic reagents for detecting tin. The older tests utilized, in one way or another, the reducing properties of the stannous ion or the oxidizing properties of the stannic ion. In 1936, Clark (*1*) recommended the dimercaptobenzenes as sensitive and specific reagents for tin. Tin combines with these organic reagents to give red precipitates.

By combining the ashing and fusion technique for the preliminary preparation of the sample with the new organic reagent, it was possible to develop a simple, rapid, and accurate method for the determination of traces of tin in beer. This quantitative determination also serves as a qualitative test, thus ensuring that the measured factor is actually tin. The availability of the reagent (1-methyl-3,4-dimercaptobenzene, obtainable from the Organic Products Company, 17 Thompson St., New York, N. Y., or the British Drug House through the Eastman Kodak Company, Rochester, N. Y.) and the perfection of a stable solution add further to its convenience.

Reagents

FUSION MIXTURE. Quickly grind (to avoid local evolution of hydrocyanic acid by atmospheric carbon dioxide) with a mortar and pestle 12.5 grams of sodium cyanide with 37.5 grams of anhydrous sodium carbonate to give a homogeneous powder.

HYDROCHLORIC ACID. Dilute 1 volume of concentrated hydrochloric acid with 1 volume of water.

1-METHYL-3,4-DIMERCAPTOBENZENE (DITHIOL) SOLUTION. Warm the dithiol slightly so that it liquefies, dissolve 0.25 ml. in 10 ml. of thioglycolic acid, and dilute to 200 ml. with 95 per cent ethyl alcohol. Store in full tightly corked small bottles in the dark. The reagent is stable if protected from air, but in a bottle that has been repeatedly opened will frequently become oxidized and no longer give the reaction with tin. Thus, the reagent from an opened bottle should be tested against a known tin solution before adding it to the sample. (The reagent has a strong sulfidic odor and may be deodorized, if spilled, by washing with a dilute iodine solution.)

TIN STANDARD. Dissolve 1.90 grams of stannous chloride dihydrate in 20 ml. of the hydrochloric acid solution and dilute to 1 liter with water. This solution should be prepared fresh, as it is not very stable.

GUM ARABIC SOLUTION. Dissolve 100 grams of powdered gum arabic (U. S. P.) in 1 liter of hot water containing 100 ml. of 0.1 per cent phenyl mercuric acetate solution. When dissolved, filter through paper in a hot water funnel. This solution is stable and will not get moldy.

Method

Char and then ignite (preferably in a muffle at about 550° C.) 100 to 200 ml. of well-mixed, degassed beer in a silica dish to give a white fluffy ash. Avoid too high a temperature during ashing to prevent fusion of the ash. Transfer the ash by brushing to a No. 00 Coors high-form porcelain crucible, tamp down ash in the crucible, and cover with 1 gram of the fusion mixture. Fuse over a Méker burner for about 15 seconds, holding the crucible with a pair of tongs and swirling, so that the melt is given

TABLE I. RECOVERY OF ADDED TIN

Sample Taken for Analysis	Tin Added		Tin Found	
Ml.	*Mg. per charge*	*P. p. m.*	*Mg.*	*P. p. m.*
200	Nil	Nil	Nil	Nil
200	0.05	0.25	0.05	0.25
200	0.03	0.15	0.03	0.15
200	0.03	0.15	0.04	0.20
100	0.04	0.40	0.04	0.40
100	0.075	0.75	0.075	0.75
100	Nil	Nil	Nil	Nil
100	0.05	0.5	0.05	0.5
100	0.05	0.5	0.04	0.4
100	Nil	Nil	Nil	Nil
100	0.01	0.1	Nil	Nil
200	0.02	0.1	0.01	0.05
100	0.015	0.15	Nil	Nil
200	0.03	0.15	0.015	0.08
100	0.025	0.25	0.025	0.25
100	0.05	0.5	0.04	0.4
100	0.05	0.5	0.04	0.4

a rotating motion. Do not overheat or fuse too long. Cool and place the crucible upright in a 50-ml. Pyrex beaker which is covered with a watch glass. All further operations, because of the poisonous character of the liberated hydrocyanic acid gas, must be conducted in a well-ventilated hood.

Put 5 ml. of the hydrochloric acid solution directly into the crucible by means of a pipet introduced through the space between the watch glass and the beaker spout. Allow it to react until the evolution of gas ceases. Remove the watch glass and wash down its undersurface with water, permitting this wash water to run into the crucible. With a thin stirring rod overturn the crucible and heat. This will generally cause further evolution of gas from the crucible. Continue heating and stirring until no further evolution of gas takes place, pick up the crucible by means of the stirring rod, and wash with water. Cover the beaker with the original watch glass and boil down the combined solution and washings to a volume of less than 10 ml. Transfer and wash this solution into a 15-ml. centrifuge tube graduated at 10 ml., make up volume to 10 ml., mix, and centrifuge at high speed. Transfer the clear supernatant liquid to a test tube, add 0.5 ml. of the tin reagent, mix, and heat for 1 minute in a slowly boiling water bath. Cool, add 2 ml. of the gum arabic solution, cork, and shake thoroughly. Compare the resulting colored turbidity with standards by reflection, using daylight.

PREPARATION OF STANDARDS. Dilute 20 ml. of fresh tin standard solution and 10 ml. of hydrochloric acid to 200 ml. with water. Pipet the following suggested quantities of this standard solution into a series of test tubes: 0.0, 0.1, 0.25, 0.50, 0.75, and 1.0 ml. (equivalent to 0.0, 0.01, 0.025, 0.05, 0.075, and 0.1 mg. of tin, respectively). Add 1 ml. of hydrochloric acid solution and make volume up to 10 ml. with water. Add 0.5 ml. of tin reagent, mix, and heat in a slowly boiling water bath for 1 minute. Cool, add 2 ml. of gum arabic solution, cork, and shake thoroughly. Standards thus prepared are stable for a month or more if kept corked and in the dark. After standing, they should be vigorously shaken before using.

Recovery of Added Tin

Since there is no other reliable method available against which the accuracy of the method could be checked, recourse was had to a determination of added tin. Beers that were brilliant on chilling are found to give a negative test for tin by this method. Such beers were assumed to be tin-free and tin was added in varying amounts. After standing some time, they were analyzed and the results are given in Table I.

Interferences

As shown by Clark, numerous heavy metals give varicolored precipitates with this reagent, but the red color of tin is characteristic; the only metal which approaches this color is bismuth, which exhibits a completely different shade of red. Other metals, if present to excess, can interfere in the method by masking the color of the tin precipitate. Iron, the only metal that is liable to be encountered in excess in beers, will not interfere in the test because during the cyanide fusion it is converted to compounds which, on later acidification, form the insoluble Prussian blue. The Prussian blue is then removed along with the other insoluble salts.

Table II shows the effect of the presence of 10 parts per million of interfering heavy metals on the determination of tin in a beer containing 0.5 part per million of tin.

Except for iron, the results in Table II are of no practical interest. Excess iron is removed in the course of the analysis and the copper content of beer is effectively limited by the normal removal of excess copper by the yeast. None of the other metals is present in beer in quantities sufficient to cause interference.

In the case of a rare sample in which large amounts of the interfering metals may be present, they may be dissolved out of the ash by dilute acids, leaving the insoluble tin oxide behind. This purified precipitate containing the tin oxide, after filtration, washing, and re-ashing, can then be treated as in the regular method. This procedure tends to reduce the sensitivity of the method and great care is needed to prevent the fine tin oxide precipitate from mechanically passing into the acid-soluble filtrate. In this laboratory, where many different samples representing all types of beers and ales have been examined, as yet no sample has been encountered containing a sufficient quantity of interfering metal to necessitate the use of this longer and less sensitive modified procedure.

This method has been tried on a number of different types of materials including several foods, water, and other beverages and the indications are that it may readily be adapted to materials other than beer. It is especially useful where only small amounts of sample are available.

TABLE II. EFFECT OF PRESENCE OF HEAVY METALS ON DETERMINATION OF TIN

(Beer containing 0.5 p. p. m. of tin)

Metal Added (10 P. P. M.)	Results
None	0.5 p. p. m. tin, red turbidity
Bismuth	No tin, yellow precipitate
Cobalt	No tin, dirty yellow precipitate
Copper	No tin, black precipitate
Iron	0.5 p. p. m. tin, no interference
Lead	0.5 p. p. m. tin, no interference
Manganese	0.4 p. p. m. tin, orange turbidity
Nickel	0.1 p. p. m. tin
Zinc	0.25 p. p. m. tin, orange turbidity

Summary

Minute traces of tin, much below the practical limit of sensitivity of existing chemical analytical methods, produce haziness in beers and ales. A method is described for determining such traces of tin, wherein the beer is ashed and the ash fused with a sodium carbonate–sodium cyanide mixture. After solution in acid, the tin is determined by the intensity of the color of the red precipitate produced by a solution of the specific organic reagent 1-methyl-3,4-dimercaptobenzene (dithiol). Recoveries and interferences are noted. This method may be adapted to the determination of traces of tin in other foodstuffs, water, and biological materials.

Acknowledgment

The writer wishes to thank Roy W. Seaholm for conducting many of the determinations reported here.

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Rapid Photometric Determination of Ascorbic Acid in Plant Materials

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THE method described here is an adaptation and modification of the photometric determination of ascorbic acid in blood serum as reported by Mindlin and Butler (*7*) and modified by Bessey (*1*) to include colored or turbid solutions and plant tissue extracts. The new features reported here involve chiefly the methods of extraction, filtering instead of centrifuging, and various changes in concentration of reagents to permit measurement of a wider range—1 to 14 micrograms—of ascorbic acid in the final aliquots. These modifications were developed especially to permit high-speed work on large numbers of plant samples daily, as in evaluating thousands of individuals among segregating populations incidental to breeding for high vitamin C content in vegetables. By these modifications, an analyst with two assistants can readily run 120 samples daily.

Since the photometric method has been discussed by others (*1*, *7*) and the use of the indophenol dye has been reviewed by King (*5*), the principles and the advantages of the basic method need not be reviewed here.

Apparatus and Reagents

The fresh plant tissues were first reduced to a fine pulp in the Waring blender as described by Davis (*3*), using two containers alternately to increase the output of the machine.

The following solutions were required: (1) 3 per cent metaphosphoric acid; (2) sodium citrate buffer of 211 grams of citric acid in 2 liters of 1 *N* sodium hydroxide; (3) a buffer at pH 3.6 (±0.1); mixture of 3200 ml. of solution 1 and 868 ml. of solution 2; and (4) a solution of 2,6-dichlorophenolindophenol containing 34.4 mg. (Eastman Kodak Co. preparation) in 1 liter of water. Solutions were used within 5 days and always stored in the ice chest overnight. Control of pH was accomplished by means of the McGinnes (*6*) glass electrode. The purity of the ascorbic acid used for preparing the calibration curve, and for the recovery experiments, was tested by titration with standard iodine solution as described by Bessey and King (*2*). Quantitative titration values were obtained within 2 per cent of theory.

The photoelectric colorimeter described by Evelyn (*4*) was used with green filter No. 520 (transmission limits 495 to 550 millimicrons). Six dozen 17.5 × 2.2 cm. (7 × 0.875 inch) absorption test tubes were selected which agreed to within 0.25 galvanometer unit.

Procedure for Calibrating Standard Curve

A fresh solution of ascorbic acid is prepared by dissolving 25 mg. in 250 ml. of buffer at pH 3.6 (solution No. 3). (After 2 days, at 4° C., this solution lost 5 per cent of its reducing power.) One- to 14-ml. amounts, by 1-ml. increments, are added to 100-ml. volumetric flasks, which are then made to volume with the buffer at pH 3.6. Five-milliliter portions of the dye solution are added to each of a series of colorimeter test tubes with a pipet reserved for this purpose, thus assuring as near the same quantity of dye as practicable in all experiments.

The colorimeter is set at 100 per cent transmission, using a tube containing 5 ml. of buffer (pH 3.6), 5 ml. of the dye, and a few crystals of ascorbic acid for complete decoloration; this is conducted in triplicate to assure a correct galvanometer setting. The center setting (without any tube in the instrument) is then recorded and maintained constant for the subsequent determinations: five milliliters of a sample solution are quickly delivered from an Ostwald pipet into 5 ml. of the dye and shaken vigorously about 5 seconds; a reading is taken 15 seconds after initial mixing and again at 30 seconds. After observing the values for all 14 samples, a blank reading in triplicate is obtained using 5 ml. of the buffer. The differences between the 15- and 30-second readings did not exceed 0.5 galvanometer unit and the average of the two was used.

Table I. Calibration Data for Photometric Determination of Ascorbic Acid[a]

Micrograms/Ml.[b]	Galvanometer Readings[c]	Photometric Density (2 − log10 G)[d]	Log G Sample − Log G Blank Observed	Log G Sample − Log G Blank Calculated[e]	Ratio[f] (Observed × 100 / Calcd.)
0 (blank)	20.50	0.6830	....	....	...
1.006	23.63	0.6220	0.0610	0.0581	105.0
2.012	26.13	0.5750	0.1080	0.1024	105.5
3.018	28.13	0.5435	0.1395	0.1467	95.0
4.024	31.63	0.4930	0.1900	0.1912	99.4
5.030	35.63	0.4425	0.2405	0.2355	102.1
6.036	39.00	0.4060	0.2770	0.2799	99.0
7.042	42.63	0.3680	0.3150	0.3243	97.2
8.048	47.38	0.3220	0.3610	0.3686	98.0
9.054	54.00	0.2656	0.4174	0.4130	96.1
10.060	60.50	0.2182	0.4648	0.4573	101.6
11.066	66.00	0.1805	0.5025	0.5017	100.3
12.072	74.25	0.1293	0.5537	0.5461	101.3
13.078	81.25	0.0901	0.5929	0.5904	100.3
14.084	87.75	0.0568	0.6262	0.6348	98.7

[a] 5 ml. of dye (34.44 mg. of 2,6-dichlorophenolindophenol per liter) mixed with 5 ml. of sample solutions containing 1 to 14 micrograms of ascorbic acid per ml.
[b] Ascorbic acid stock solution contained 25.18 mg. dissolved in 250 ml.
[c] Galvanometer was read to nearest quarter division and mean of 15- and 30-second readings used.
[d] Corrected for slight deviations from true linearity of relation between current and galvanometer deflection.
[e] Calculated from best line (derived by method of least squares) satisfying observed points.
[f] Coefficient of variation = 1.1%.

In Table I the data obtained for the calibration are presented. By the method of least squares, the equation for the line best satisfying the experimental points was found to be: $Y = 0.0441\ X + 0.0137$, where X = micrograms of ascorbic acid per ml. and Y = the log of the galvanometer readings for samples minus that for the blank (Figure 1). The coefficient of variation for the deviations from the line was only 1.1 per cent. A repetition of the calibration curve after a period of 5 weeks resulted in a similar series of points varying by only about 1 per cent from the original calibration.

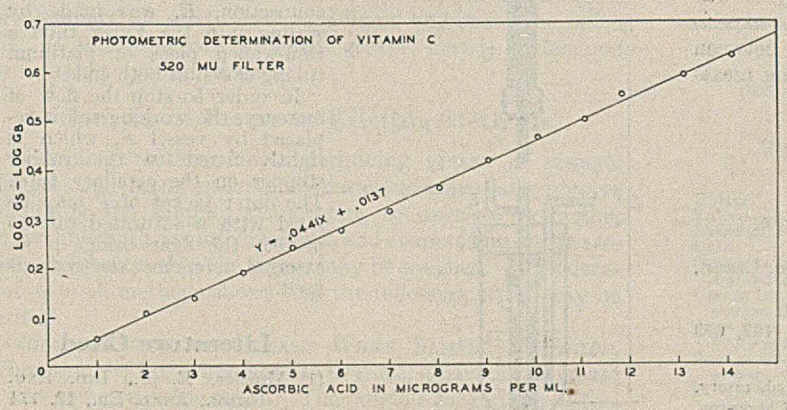

FIGURE 1

Procedure for Plant Materials

APPLICATION TO BEANS AND CABBAGES. From a dispensing buret 100 ml. of 3 per cent metaphosphoric acid are added to the container of the blender; 25 grams of fresh tissue, weighed to the nearest 0.1 gram, are added and mixed at high speed for 2 minutes. The fresh vegetable tissues are thus reduced to an extremely fine pulpy suspension, and a temperature rise of about 5° C. occurs. About half of the contents is then filtered through a dry fluted No. 12 Whatman filter paper. Since the filtrate comes through cloudy at the start, about 10 ml. are discarded. About 10 ml. of clear filtrate are collected in a dry Erlenmeyer flask.

The aliquot of filtrate taken for dilution will depend upon the approximate vitamin content of the sample. A volume is selected which is expected to contain between 200 and 600 micrograms, so that after dilution to 50 ml. the final concentration in the aliquots analyzed may conveniently lie between 4 and 12 micrograms per ml. For cabbage, which may contain about 40 to 90 mg. of ascorbic acid per 100 grams of fresh tissue, a 3-ml. aliquot is adequate, whereas for snap beans, containing about 20 to 40 mg. per 100 grams, a 5-ml. aliquot is taken.

Aliquots of the clear filtrates are transferred to 50-ml. volumetric flasks and a sufficient quantity of sodium citrate buffer (solution No. 2) is added to bring the pH to approximately 3.6; in the case of cabbages, beans, and several other vegetables studied, 0.25 ml. of buffer per ml. of aliquot was required. The mixture is then made to volume with the citrate-phosphate buffer (solution No. 3). The final pH should be 3.6 ± 0.1. When many samples of similar material are analyzed in groups of 24, a pH determination on occasional samples will permit adequate control.

In calculating the ascorbic acid content of plant tissue the water in the sample must be taken into account. In the present investigation the results obtained with the recovery experiments indicated that the vitamin is distributed in the liquid phase of the mix, with no measurable amount absorbed.

As a test of the accuracy of the method, the recovery of ascorbic acid was studied. Since a great deal of air is whipped into the extracting solution during the 2-minute stirring period, the possibility existed that oxidation might necessitate the use of an empirical correction factor. This was shown unnecessary, however, by quantitative recoveries when buffered solutions of ascorbic acid, at various concentrations, were carried through all the steps in the analysis. The possibility that ascorbic acid oxidase might partially destroy the vitamin during the analysis was also excluded by the quantitative recoveries obtained. Recovery experiments are complicated by the difficulty in obtaining uniform 25-gram samples of plant tissue. Four separate series, using individual cabbages, were conducted in the following manner:

The head was sliced along its polar axis and 25-gram sections were cut for analysis; five alternate sections were supplemented with different amounts of pure ascorbic acid and the average value for the unsupplemented five sections was used in the recovery calculations. A fresh solution containing 1 mg. of ascorbic acid per ml. was added—4, 6, 8, 10, and 12 ml., respectively—to each section before conducting the extraction.

The average recovery for the 20 separate analyses was slightly high, 103 ± 1.3 per cent. However, in these experiments a difference in total vitamin concentration, between supplemented and unsupplemented extracts, is being measured.

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A Constant Mercury Level for the Dropping Mercury Electrode

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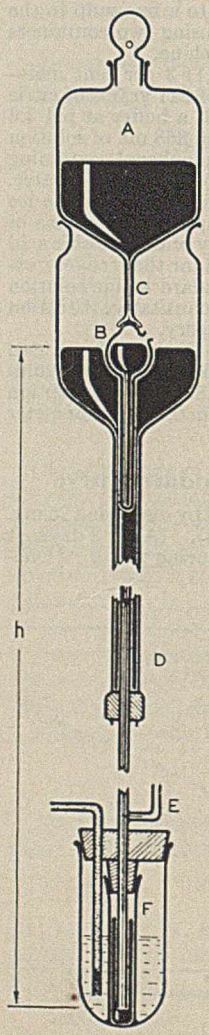
FROM the Ilkovic equation for the diffusion current and Poiseuille's equation for the flow of liquids through capillaries, it can be shown that the diffusion current is proportional to the square root of the pressure or to the square root of the mercury height—i. e., $i_d = k\sqrt{h}$—for constant concentration and temperature. In a single analysis, the amount of mercury used is small; thus if a large reservoir is used, the height is essentially unchanged during the analysis, but it becomes inconvenient to correct the height manually if a series of analyses is being run.

Mueller (*1*) described a simple device for maintaining a constant mercury pressure, using the principle of the Mariotte flask. In these laboratories a floating bulb valve device, described below, has been used satisfactorily for over a year.



A ball, *B*, attached to the centering tube floats on the surface of the mercury pool which is to be kept at constant level. The hemispherical ground end of the capillary, *C*, rests on a ground projection blown on the upper side of *B*. Capillary *C* is joined to the reservoir, *A*, which sits loosely on the lower one. There is a hole on the upper side of *B* for introducing mercury for ballast. If sufficient care is taken in grinding *C* to fit *B*, the lower level is kept constant to within 1 mm., which is sufficiently constant for polarographic purposes.

The lower container may be connected to the dropping capillary by means of rubber tubing. If there is danger of clogging the capillary by contamination from the rubber, the two concentric movable tubes, *D*, may be used. The head necessary to obtain the proper drop time can easily be adjusted and the mercury is in contact with only the very small area of rubber resulting from the seal. The electrical connection, *E*, was made by sealing into the Pyrex tube a thin-walled piece of platinum tubing closed at both ends.

In order to stop the flow of mercury, the working cell is replaced by vessel *F*, which is tightly fitted to the rubber stopper on the capillary tube. The outer jacket of *F* may be filled with a saturated salt to protect the agar bridge if an external reference electrode is used.

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Determination of Grease in Sewage, Sludge, and Industrial Wastes

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Prevailing methods for determining the grease content of sewages, sludges, and liquid industrial wastes permit many errors. Recognizing the need for a standard procedure which will give a reliable measure of the true content of "grease" the authors have devised a procedure which eliminates or greatly reduces most of the sources of error. Application to a variety of samples gave results with differences from the means averaging 2.0 per cent. Results of analyses are much less influenced by variations of technique and of solvent than is the case with older methods.

IT HAS long been recognized that certain errors are present in prevailing methods for determining grease in sewage and sludges. Because of the need for more accurate procedure, W. T. Knowlton, City of Los Angeles Bureau of Engineering, called together a committee to study the problem. This group consisted of Mr. Knowlton: V. W. Thews and A. A. Appel, Maintenance Division, Sewer Department of the City of Los Angeles; T. C. Wilson, Los Angeles City Bureau of Standards; and the authors, who conducted the research. The other members of the committee assisted by suggestions and by making many analyses. More than ordinary expressions of appreciation are due these men for their part in this work.

In considering the accuracy of methods for determination of grease, it is necessary to have an understanding of what is sought. From the viewpoint of the sanitary engineer, a grease analysis should include those materials which impart greasy characteristics to the waste when they are present in sufficient quantity. It is also desirable to include oil, since oil differs from grease only in melting point. The material separated from sewage in grease analyses always contains both solid and liquid substances. If the solid constituents are present in sufficient quantity, the mixture is solid at room temperature and may properly be called grease, but if the liquids predominate the mixture remains liquid and should be called oil. In this discussion the word "grease" may be understood as meaning "grease or oil".

Sources of Errors in Existing Methods

Prevailing methods for determining grease in sewage, sludges, and many other fluid materials essentially involve acidifying the sample, evaporating to dryness, extracting thoroughly with a suitable solvent, and evaporating the grease-laden solvent so that the grease may be weighed. Consideration of such methods shows that the following errors may be serious.

VOLATILIZATION OF GREASE WHEN SAMPLE IS DRIED. When samples are evaporated to dryness, a part of the grease is lost along with the steam. The possible magnitude of this error was demonstrated experimentally for several greases.

The greases were prepared for use by holding in a stoppered bottle in a melted condition for at least 24 hours, after which clarified grease was pipetted out, except in the cases of raw sludge and digested sludge greases, which were recovered from analyses.

Samples were weighed into tared conical flasks and distilled water was added. The flasks were placed on a hot plate, and as the water evaporated more was added until 500 ml. had been used. The grease was then dried and weighed.

Table I shows that the losses were variable, reaching 25 per cent for grease from sewage skimmings. It is not possible from these results to predict the exact magnitude of the losses in analyses, but it is evident that they are likely to be an important source of error.

REVERSION OF FATTY ACIDS TO INSOLUBLE SOAPS. Insoluble soaps are an important factor in the greasiness of sewage; hence they should be included in grease analyses. Since they can be dissolved only with difficulty, it is common practice to acidify the sample and thus liberate the fatty acids which constitute about 95 per cent of the soap.

When hydrochloric acid is used for this purpose, the reaction may be represented by the equation

$$Ca(RCOO)_2 + 2HCl \rightarrow CaCl_2 + 2\ RCOOH$$

If the sample is dried, the above reaction reverses, regenerating the insoluble soaps. To prove that the reverse action can occur, 117 mg. of fatty acids from sewer grease and 144 mg. of calcium chloride dihydrate were mixed with ether and water in a dish, and evaporated to dryness. Thirty-four per cent of the fatty acids were converted to insoluble soaps. More intimate contact and longer drying time, such as might prevail in a sludge analysis, might easily result in conversion of most of the fatty acids into insoluble soaps.

Theoretically, the difficulty can be overcome by using sulfuric acid, but one is faced with the task of adding exactly enough to convert all chlorides, carbonates, soaps, and other salts of weak or volatile acids into sulfates. Any excess beyond the required quantity will attack the organic matter when the water is removed.

PRODUCTION OF ETHER-SOLUBLE MATTER. Combined fatty acid radicals are present in many carbohydrates (*8*), in some proteins (*5*), and in the complex lipides, especially the

TABLE I. WEIGHT LOSSES

(500 ml. of water evaporated in presence of grease samples)

Grease	Weight Taken	Final Recovery	Gain or Loss
	Mg.	*Mg.*	%
Butter	96.1	96.8	+ 0.73
Tallow	84.8	84.8	0.00
Beeswax	90.8	90.5	− 0.33
Lard	85.2	81.8	− 3.99
Grease from raw sewage sludge	115.5	100.2	−13.25
Grease from digested sewage sludge	126.5	108.1	−14.55
Soap acids from Ivory soap	118.5	101.2	−14.60
Sewage skimmings grease	83.1	67.9	−18.29
Sewage skimmings grease	87.8	65.8	−25.06

TABLE II. INSOLUBILIZING OF GREASE BY HEATING 24 HOURS IN PRESENCE OF AIR

	Initial Hexane-Soluble	Loss by Vaporization	Insoluble Residue	Final Hexane-Soluble
	Per cent of initial weight			
Grease from raw sludge	100	26.92	7.29	65.79
Grease from digested sludge	100	15.27	1.06	83.67
Fish oil	100	1.51	61.72	36.77
Tallow	100	5.54	13.42	81.04

relatively abundant phospholipides. In such combinations they exhibit none of the characteristics of grease.

A notable example is egg yolk, which, according to nutritional tables, is about one-half water, one-third fat, and one-sixth protein, yet it is not greasy, as the fatty material is combined in the form of the phospholipide lecithin. In food analyses it is customary to subject samples to acid hydrolysis, for only in this way is it possible to separate all the material which is fat, as judged from the nutritional viewpoint (*3*). The sanitary engineer, however, is generally not concerned about these chemically combined fats, since they are not separable by physical means and do not impart greasy characteristics to the waste. Hence they should not be included in grease analyses.

If a sample of sewage or sludge is evaporated to dryness while in an acidified condition, the combined fatty acid radicals will be partially liberated, to an extent depending upon the acidity and other conditions controlling the intensity of hydrolysis. A sample of egg yolk yielded 28 per cent of grease (wet basis) when analyzed by the standard procedure for sludge (*2*).

INSOLUBILIZING OF OILS BY OXIDATION AND POLYMERIZATION. Evaporation of sewage samples to dryness may require 24 hours or more, depending upon the sample size and the method used. In this time there is ample chance for oxidation of fats, especially where they collect on the sides of the vessel above the receding water. The possible extent of this change was indicated by heating grease samples (about 100 mg. each) in dishes for 24 hours at 105° C.

Table II shows that considerable amounts of the greases became insoluble. It is evident that oxidation is potentially an important source of error.

EXTRACTION OF NONGREASES. Glaring evidence of the extraction of nongrease is afforded by the presence of ash in burned samples of grease, obtained by extraction of dry sludges. Knechtges, Peterson, and Strong (*7*) report from 6 to 21 per cent ash in extracts. Soaps could not account for more than 3 per cent of ash, in view of the figures given for content of soap acids.

Ether extraction, by the authors, of samples of digested sludge which had been acidified and dried yielded a grease with 8.0 per cent of ash, whereas samples of the same sludge which were washed with acid and water to remove soluble inorganic salts before ether extraction yielded grease with an ash content of only 0.24 per cent. It is evident that the ash in the grease was due to the dissolving of inorganic salts by ether.

If organic solvents can extract such considerable amounts of inorganic matter, it is even more to be expected that they will extract other nongreasy matter, such as water-soluble organic acids, amino acids (which may be formed by the hydrolysis of proteins), amine salts, sugars, gums, resins, rubber, hydrocarbons of the naphthalene type, solid chlorinated hydrocarbons, sulfur, etc. The reporting of 0.25 and 0.3 per cent of nitrogen in grease samples by Knechtges, Peterson, and Strong (*7*) suggests the extraction of amino compounds.

Errors due to extraction of nongreases have also been commented on by Gehm and Trubnick (*6*).

VARIATION OF RESULTS WITH VARIOUS SOLVENTS. "Standard Methods" (*2*) permits use of petroleum ether, ethyl ether, or chloroform (solvent to be stated). In sludge analyses chloroform gives results averaging 75 per cent higher than petroleum ether. Ethyl ether and most other solvents are ranged between these two in extractive power. Until a solvent is specified which can be shown to give a true measure of the content of grease in the appropriate sense of the word, there must remain a large element of uncertainty in the results.

ERRORS. When grease dissolved in the organic solvent is dried preparatory to weighing, three errors may arise: incomplete removal of solvent, oxygen absorption by the grease, and evaporation of grease. These errors are considered in detail in the next section.

Perfection of Procedure for Removing Solvent from Extracted Grease

In the course of research on the method of determining grease, it became evident that extraction methods could not be satisfactorily compared until accurate procedures were devised for drying and weighing the grease. Therefore, attention was directed first to that problem.

If considerable amounts of low-boiling oils are present and it is desired to include them with the oil or grease, extraction with a volatile solvent cannot be used. The American Petroleum Institute has adopted a procedure for oil-field waste water which involves partial distillation of the sample, collection of the distillate, separation of distilled oil by gravity, and volumetric measurement. A procedure of this sort must be used whenever volatile oils are of significance, since there is no practical way to remove solvent from such oils. But in analyses of most sewages and industrial wastes it is sufficient to determine those oils and greases which are heavy enough to permit their easy separation from volatile solvents. Since any grease will vaporize to some extent if heated for a long time, it was necessary to determine what technique would just remove the solvent with the minimum of vaporization or oxidation of the grease.

The grease may be dried and weighed in either flasks or dishes. Flasks have been chosen in this work because they permit recovery of solvent, are easier to manipulate, and occupy less space in the desiccator.

In order to determine the effect of time of heating on the weights of grease samples under conditions prevailing in analyses, a series of samples was added to 125-ml. conical flasks, which were then heated for various successive intervals of time at 105° C. The results, as given in Table III, show initial increases of weight in most cases, followed by decreases. The increases may be accounted for by absorption of oxygen, as was demonstrated by four series of tests, using lard and fish oil. Pairs of samples were weighed into flasks. One flask of each pair was kept filled with an atmosphere of inert gas, while the others were open to the air. The four samples exposed to air gained an average of 0.99 per cent when heated for one hour, while the greases protected by inert gas lost an average of 0.10 per cent.

A correct weight might be obtained at the time when the absorption of oxygen has just been offset by vaporization, but this time will vary with the nature of the grease, the size of the sample, the type of vessel, and other factors. It is better to minimize the heating period, so as to get a weighing before much oxygen has been absorbed.

In using a short heating time, removal of the solvent becomes critical. When flasks are heated on a steam bath until the solvent is apparently gone from the grease, the flask still remains filled with vapor, which will condense when cooled.

TABLE III. CHANGES IN WEIGHT OF GREASE AT 105° C.

Grease	Weight Taken	Gain or Loss after Total Heating Times as Shown						
		5 min.	15 min.	60 min.	3 hours	8 hours	24 hours	100 hours
	Mg.	%	%	%	%	%	%	%
Mackerel oil	333.5	Zero	+0.12	+0.63	+1.80	....	+0.45	− 2.85
Herring oil	312.6	−0.10	+0.10	+1.79	+3.20	....	+1.92	− 3.17
Sewage skim	311.1	−0.74	−1.60	−2.06	−2.76	....	−5.27	−11.60
Tallow	312.9	+0.16	+0.35	+0.38	+0.45	+0.89	+0.16	−13.77
Beeswax	307.8	+0.07	+0.29	+0.32	+0.26	+0.26	+0.36	−16.25
Lard	305.3	+0.10	+0.16	+0.72	+1.15	+1.64	+0.23	−16.43
Butter	316.2	Zero	+0.22	+0.38	+0.44	+0.98	+0.73	−18.05
Soap acids	325.8	+0.06	0.00	−0.55	−0.92	....	−7.77	−36.82

If the flask is transferred to the oven without removing this vapor, a heating period of an hour is required to ensure that the vapor will diffuse out of the flask. A more satisfactory procedure is to remove the vapor before transferring to the oven. This may be done by tipping the flask to allow the vapor to flow out, or by blowing dry air or gas through the flask while it is on the steam bath, using a volume of air about ten times the volume of the flask.

In order to determine completeness of removal of solvent, pairs of flasks containing weighed quantities of grease (around 100 mg.) were run in parallel. Hexane was added to one of each pair of flasks; these were then placed on the water bath to vaporize the hexane. (The word "hexane" is here used to refer to a petroleum fraction consisting of a mixture of isomeric hexanes.) The vapor was blown out with ten volumes of gas; the flasks were transferred to the oven for various times, cooled, and weighed. Flasks which did not receive hexane were given the same heating in the oven, then cooled and weighed. Results are shown in Table IV.

In the case of herring oil, lard, and butter, the results at 10 minutes show evidence that a trace of hexane remained with the grease. At 15 and 20 minutes, results are somewhat erratic, but show no significant evidence that any hexane remained. With the sewage greases and soap acids, all samples showed losses, and in the cases of skimmings and soap acids the losses tend to be greater in those samples which had received hexane.

There seems to be no simple procedure to remove the solvent without some loss of volatile constituents from the sewage greases. It seems best to choose a procedure which will eliminate the added solvent and give reproducible results, ignoring the small amounts of volatile oils which are inevitably lost.

The ideal heating time will depend upon the solvent used and upon the quantity and kind of grease. Any specified time will be arbitrary and somewhat a matter of opinion, and subject to change when the procedure is applied to different kinds of materials. When using a solvent of boiling point similar to hexane, in the analyses of sewage or sludge samples yielding up to 500 mg. of grease, it seems reasonable to recommend heating for 15 minutes.

When using dishes the rates of vaporization are higher, as may be noted by comparing corresponding data of Tables II and III. A few tests indicated that if a grease solution in a dish is evaporated on a steam bath and heated for 5 minutes after the solvent is no longer visibly present, no additional heating in the oven is required.

The time in the desiccator also influences the results, as grease weights generally increase with time. With grease from sewage skimmings the increases were around 1 per cent in 48 hours. Fish oil samples sometimes increased as much as 4 per cent overnight. It is recommended that the time in the desiccator be between 1 and 3 hours.

Extraction Procedures

GENERAL PRINCIPLES. Having determined a method for evaporating the solvent and weighing the grease, attention was directed to the extraction procedure. After trying a number of variations of the usual routine, it was concluded that the only hope of securing results of satisfactory accuracy lay in extracting the sample without evaporating the water. This plan is referred to as the "wet method", in contrast to the prevailing "dry methods". [Extraction in the wet state is an established method for analysis of oil-field waste water (*1*) and for milk (*4*), but as far as the authors are aware it has not previously been applied to analysis of sewage sludges and similar wastes.]

The wet method essentially involves the following steps:

Acidify the sample.

Shake thoroughly with successive quantities of solvent until removal of the grease is complete. Transfer the portions of solvent through a filter to a suitable receiving vessel.

Evaporate the solvent, heating just sufficiently to ensure complete removal of solvent. Cool in a desiccator and weigh after 1 to 3 hours.

Within the above outline, any variations of detail which seem reasonable to an experienced chemist and which are carried out with due attention to the requirements of quantitative analytical technique are permissible. The results of the wet method are not appreciably influenced by minor modifications of procedure.

Compliance with the general directions leads to certain difficulties, notably that of separating the solvent after shaking. Methods devised to accomplish this are set forth in the following suggested procedures. Procedure I is suitable for all types of sewage, sludges, and other fluid materials. No. II may be used with samples which do not form stubborn emulsions, and by proper manipulation, it may be used for sewage.

WET EXTRACTION, PROCEDURE I. (1). The amount of sample required for a test varies with the nature of the waste. It is generally desirable that the yield of grease be between 50 and 500 mg. Sewage samples of 800 to 2000 ml. have been found convenient,

TABLE IV. COMPLETENESS OF REMOVAL OF SOLVENT FROM GREASE

Grease (Approximately 100-Mg. Sample)	Gain or Loss in Weight of Grease					
	10 Minutes' Heating		15 Minutes' Heating		20 Minutes Heating	
	No hexane	Hexane added	No hexane	Hexane added	No hexane	Hexane added
	%	%	%	%	%	%
Herring oil	+0.66	+1.34	+0.30	+0.81	+0.95	+1.21
Lard	−3.24	+0.44	+1.13	+0.69	+0.97	+0.69
Butter	−0.36	+0.59	−0.07	+0.44	+0.07	+0.37
Grease from raw sludge	−2.42	−2.32	−2.42	−2.32	−3.00	−2.32
Soap acids	−0.74	−2.96	−0.66	−2.96	−0.17	−3.31
Grease from sewage skimmings	−1.77	−2.18	....	....	−3.60	−2.89

but where heterogeneity of the material makes large samples desirable, volumes as large as 4000 ml. may easily be analyzed by using vessels of suitable size. In the case of sewage sludges, samples containing 1.0 to 5.0 grams of dry solids are usually satisfactory, but samples with 18 grams of dry solids have been analyzed without difficulty.

It is generally desirable that the sample be delivered in a glass-stoppered bottle containing the amount of material suitable for one analysis, so that any grease adhering to the vessel may be washed into succeeding apparatus.

2. Acidify the sample, using 5 ml. of 1 to 1 sulfuric acid. (For samples larger than 1000 ml., or for very alkaline materials, appropriately larger amounts of acid should be used.) Transfer to a separatory funnel of suitable size having a standard-taper ground mouth. (In the case of samples too large for available

TABLE V. GREASE REMOVED BY SUCCESSIVE EXTRACTIONS OF SEWAGE AND RAW SLUDGE

Extraction No.	Grease Recovered: Weight	Grease Recovered: P. p. m.	Per cent of total recovery
	Mg.	*P. p. m.*	
Sewage (Procedure II), Hexane Solvent			
1	36.8	47.6	82.5
2	7.0	9.1	98.2
3	0.8	1.0	100.0
4	0.0	0.0	100.0
Raw Sludge (Procedure I), Hexane Solvent			
1	262.8	13,140	79.23
2	46.3	2,315	93.19
3	12.9	645	97.08
4	6.7	335	99.10
5	2.8	140	99.94
6	0.2	10	100.00
7	0.0	None	100.00
8	0.0	None	100.00
Isopropyl Ether Solvent[a]			
9	4.9	245	....
10	3.7	185	....
11	1.8	140	....
12	1.2	60	....
13	0.2	10	....
14	0.0	None	....
15	0.0	None	....

[a] Following the eighth extraction, isopropyl ether was substituted for hexane, and the extraction continued.

funnels, the extraction may be carried out in bottles or other glass-stoppered apparatus.) If the entire contents of the sample vessel are being analyzed, rinse the containers out with a little solvent, adding the rinsings to the funnel. Add to the funnel 75 to 150 ml. of solvent which has been distilled in all-glass apparatus. Use proportionally larger amounts for samples larger than 1000 ml.

3. Shake the funnel vigorously for 2 minutes. Thin very thick sludges with water to facilitate this extraction step.

4. Connect the mouth of the funnel to a vertical condenser having a standard-taper ground-glass connection, and lower the assembly into a water bath maintained at a temperature of 90° to 100° C. (Cork connections may be substituted for ground-glass joints with introduction of scarcely appreciable errors.)

5. Reflux briskly until the emulsoid mixture has separated into its component parts. This usually requires from 15 to 30 minutes. It is desirable that the ebullition be principally from the top of the water layer. If the boiling occurs from the bottom of the funnel, the emulsion may not break.

6. Disconnect the funnel and cool for 5 or 10 minutes, preferably with water, set up a filter over a weighed conical flask of 125- to 250-ml. capacity, and transfer the clear solvent solution to the filter, using a 25-ml. pipet fitted with a 30-ml. rubber bulb. In order to avoid spilling drops of the solvent solution, the mouth of the separatory funnel should be held over the filter. Solvent must not be drawn into the rubber bulb.

To facilitate complete removal of the solvent layer, the water may be withdrawn from the bottom of the funnel, and then returned to the funnel after the solvent layer has been removed.

7. Add 50 to 100 ml. of solvent to the funnel and repeat operations 3 to 6. If desired, all or part of the water may be dis-

TABLE VI. DEMONSTRATION OF WET METHOD

Laboratory	Procedure	Solvent	Grease Content *P. p. m.*	Average Difference from Mean *P. p. m.*	Average Difference from Mean *%*
Sewage 1 (Screened)					
A	II	Isopropyl ether	189		
B	II		191		
B	II		188	1.6	0.84
B	II		192		
B	II		192		
			Av. 190		
A	II	Benzene	196	...	...
A	II	Chloroform	194	...	...
Sewage 2 (Screened)					
A	II	Hexane	29		
B	I		31		
B	I		29		
B	I		33		
B	I		32		
B	I		31	0.91	2.9
B	I		30		
B	I		31		
B	I		31		
B	I		32		
B	I		30		
			Av. 31		
A	II	Isopropyl ether	40	...	...
Sewage 3 (Unscreened)					
B	II	Hexane	133	3.5	2.7
B	II		126		
			Av. 130		
A	A. P. H. A.		105		
A	A. P. H. A.		104	...	...
Sewage 4 (Unscreened)					
A	II	Hexane	67		
A	II		75		
A	II		69		
A	II		70		
A	II		72	2.4	3.4
B	I		67		
B	I		73		
B	I		73		
B	I		73		
			Av. 71		
Sewage 5 (Unscreened)					
A	I	Hexane	197	...	...
A	A. P. H. A.		135		
Sewage 6 (Unscreened)					
A	I	Hexane	127	...	...
A	A. P. H. A.		108		
Sewage 7 (Unscreened)					
A	I	Hexane	82	...	...
A	A. P. H. A.		59		
Sewage 8 (Unscreened)					
A	I	Hexane	92	...	...
A	A. P. H. A.		57		
Sewage 9 (Unscreened)					
A	I	Hexane	127	...	...
A	A. P. H. A.		101		
Sewage 10 (Unscreened)					
A	I	Hexane	48	...	...
A	A. P. H. A.		32		
Raw Sewage Sludge 1 (Screened)					
A	I	Isopropyl ether	17,560		
B	I		16,930	310	1.8
B	I	Ethyl ether	16,800		
			Av. 17,100		
A	I	Hexane	16,480		
A	I		16,590		
B	I		16,040	306	1.9
B	I		15,620		
B	I		15,950		
B	I		15,760		
			Av. 16,070		
A	I	Chloroform	16,790	270	1.6
B	I		16,250		
			Av. 16,520		
A	A. P. H. A.	Hexane	16,060		
Raw Sewage Sludge 2 (Unscreened)					
A	I	Hexane	14,110	55	0.39
A	I		14,220		
			Av. 14,165		
Raw Sewage Sludge 3 (Unscreened), 7.62% Solids					
A	I	Hexane	18,150	105	0.58
A	I		17,940		
			Av. 18,045		
A	A. P. H. A.		17,250	1060	...
A	A. P. H. A.		15,130		
			Av. 16,190		
Digested Sewage Sludge 1 (Unscreened), 8.6% Solids					
A	I	Isopropyl ether	8650	115	1.3
B	II		8420		
			Av. 8535		
B	II	Benzene	8600	...	...
B	II	Petroleum ether	7530	...	...
Digested Sewage Sludge 2 (Unscreened)					
A	I	Hexane	4420	40	0.90
A	I		4460		
			Av. 4440		

carded after the second extraction, since the remaining grease will be almost entirely in sludge solids and residual solvent. Make as many subsequent extractions as required to accomplish practically complete removal of the grease. For sewage, three or four extractions are sufficient, but sludges may require five or six extractions.

8. After all solvent layers have been transferred to the flask, wash the filter thoroughly with fresh solvent.

9. Separate the solvent from the fatty matter by a source of heat which cannot rise above 100° C. Either a water or steam bath is convenient. The flask may be connected to a condenser for recovery of the solvent, using ground-glass connections.

10. When no more liquid solvent is visibly present, introduce a jet of dry air or gas into the flask while it is still on the heating bath. This serves to displace the heavy vapor. The amount of air or gas used should be about ten times the volume of the flask. Use of larger volumes increases errors due to vaporization of grease.

11. Place the flask in a drying oven at 100° to 105° C. for 20 minutes, transfer to a desiccator, and weigh when cool. In order to minimize absorption of oxygen, the time in the desiccator should not exceed 3 hours. As a check on the completeness of the drying of the grease, reheat the flask for 10 minutes and reweigh. If the grease does not lose more than 2 per cent of its weight as a result of this reheating, the first weighing should be considered correct.

PROCEDURE II. (1) Prepare the sample as in Procedure I, steps 1 to 3. For samples such as sewages, which tend to form emulsions, the shaking must not be too vigorous.

2. Allow the mixture to stand for 30 minutes, then draw off the lower layer of turbid water, which should separate into a second separatory funnel. Add 50 ml. of solvent to this second funnel and shake as before.

3. Returning to the first funnel, a layer composed of an intimate mixture of water, solvent, and solids will be found, which may be covered with a clear grease-bearing layer of solvent. Shake the mixture vigorously. This will generally cause solvent to separate and the residue to become granular. If no considerable separation occurs, add 50 to 100 ml. more of solvent and again shake well. If at this stage a resistant emulsion remains, continue the test by Procedure I.

4. When the solvent layer has clarified sufficiently, transfer it to a tared conical flask through a filter, as in Procedure I.

5. When the second separatory funnel has stood for a suitable time, withdraw the aqueous layer and discard it. Transfer solvent and emulsoid layer to the first funnel, rinsing with a few milliliters of fresh solvent. Shake the combined mixture again and allow it to stand until clear solvent can be transferred to the filter.

6. Make subsequent extractions of the residue in the separatory funnel by adding quantities of solvent at least twice the volume of the emulsoid mixture and shaking as before. Withdraw progressively any water which separates and discard after each shaking. The number of extractions will vary with the volume of the residue and the nature of the sample. In the case of sewage, a total of three or four extractions is sufficient.

7. Conduct the remaining operations as in Procedure I, steps 9 to 12.

Demonstration of the Method

Belief in the accuracy of the wet method rests in part upon experiments and in part upon theoretical considerations.

TABLE VI. DEMONSTRATION OF WET METHOD (*Cont'd*)

Laboratory	Procedure	Solvent	Grease Content	Average Difference from Mean	
			P. p. m.	*P. p. m.*	*%*
Digested Sewage Sludge 3 (Unscreened), 5.90% Solids					
A	I	Hexane	6920	40	0.58
A	I		6960		
			Av. 6940		
A	A. P. H. A.		6790		
A	A. P. H. A.		6220	285	...
			Av. 6505		
Meat Packing Waste Water (Screened)					
A	I	Petroleum ether	120		
A	I		117		
A	I	Hexane	117		
A	I		109	4.4	5.36
B	I		116		
B	I		117		
B	I		105		
			Av. 114		
A	I	Isopropyl ether	137	1.0	0.74
B	I		135		
			Av. 136		
B	I	Chloroform	137	...	...
Oil-Field Waste Water					
A	II	Benzene	71	2.0	2.9
B	II		67	...	...
			Av. 69		
A	II	Hexane	89	...	...
A	II	CCl_4 + ethyl ether			
Milk Sample 1					
A	II	Isopropyl ether	4093	35	0.86
A	II		4024		
			Av. 4058		
A	II	Chloroform	393	...	...
Milk Sample 2					
A	II	Isopropyl ether	3734		
A	II		3708	14	0.38
A	II	Ethyl ether	3723		
A	II		3694		
			Av. 3715		
A	II	Chloroform	3599	...	...
Fish Cannery Waste 1 (Screened)					
A	I	Isopropyl ether	274		
A	I		273	4.3	1.6
B	I		264		
			Av. 270		
A	I	Chloroform	281		
A	I		276	7.7	2.8
B	I		261		
			Av. 273		

Laboratory	Procedure	Solvent	Grease Content	Average Difference from Mean	
			P. p. m.	*P. p. m.*	*%*
Fish Cannery Waste 1 (Screened) (*Cont'd*)					
A	I	Petroleum ether	240		
A	I		255		
B	I	Hexane	236	11.4	4.9
B	I		211		
B	I		219		
			Av. 234		
Fish Cannery Waste 2 (Screened 20-Mesh)					
A	I	Hexane	896		
A	I		893		
A	I		888		
A	I		851		
A	I		864	11.3	1.28
A	I		874		
A	I		884		
A	I		884		
A	I		893		
A	I		879		
			Av. 881		
Fish Cannery Waste 3 (Screened 50-Mesh)					
A	I	Hexane	815		
A	I		815		
A	I		817		
A	I		815		
A	I		816	0.9	0.11
A	I		814		
A	I		816		
A	I		814		
A	I		817		
A	I		816		
			Av. 816		
Fish Cannery Waste 4 (Unscreened)					
A	I	Hexane	641		
A	I		699		
A	I		654	18.8	2.83
A	I		650		
A	I		676		
			Av. 664		
A	A. P. H. A.		777		
A	A. P. H. A.		252	154	...
A	A. P. H. A.		527		
A	A. P. H. A.		434		
			Av. 498		
			Weighted average		2.00

Experimental proof of a method would desirably include analyses of mixtures of known grease content. It is not practical to prepare such mixtures which could be considered identical in behavior with sewage or sludge, but a combination of substances was tried which may in some respects represent sewage. The preparation contained 1000 p. p. m. of diatomaceous earth, 1600 p. p. m. of shredded filter paper, 171.0 p. p. m. of lard absorbed on the filter paper, 113.1 p. p. m. of soap acids saponified with sodium hydroxide, and 350 p. p. m. of calcium hydroxide. Five analyses of this mixture by the wet method, Procedure I, gave results ranging from 280 to 286, averaging 283.2 or 99.67 per cent of the grease added.

The extent to which the grease recovered in analyses may be contaminated by inorganic substances may be judged by ashing. Three samples of grease separated by the wet method from sewage, raw sludge, and digested sludge showed, respectively, 0.43, 0.49, and 0.42 per cent of ash. (Hexane was used as a solvent.)

To be reliable, a method of analysis depending upon extractions must come to a sharp end point. By weighing separately the grease obtained in successive extractions, it is found that the wet method does exhibit a sharp end point. In fact, it appears that the grease remaining after each extraction is roughly proportional to the amount of solvent remaining. Table V shows tests of this sort for sewage and sludge. (In the analysis of sewage by Procedure II, the free water is discarded after the second extraction. The conclusion that all true grease is completely extracted from the water is based chiefly upon the fact that results obtained in duplicate analyses with and without discarding the water after two extractions differed by insignificant amounts.)

In sludge analyses, the amount of solvent remaining entrained in the sludge is variable, and generally greater than in sewage analyses; hence the extraction approaches completion irregularly and more slowly. Yet here also definite end points are indicated, as shown in Table V.

The importance of acid for decomposing the insoluble soaps was shown by analyzing raw sludge with and without acid. Results were, respectively, 6180 and 3280 p. p. m.

The possibility of acid hydrolysis of compounds such as lecithin was investigated by analyzing egg yolk. No grease was obtained by extracting in the cold, nor was any significant amount obtained after short periods of heating, but refluxing for 30 hours yielded 17 per cent of grease. Dry extraction by the A. P. H. A. method (*2*) gave 28 per cent of grease.

Data bearing upon the important questions of reproducibility of results, effect of choice of solvent, comparison of Procedures I and II, and comparison with the standard A. P. H. A. dry procedures, are presented in Table VI.

In many cases samples were analyzed independently in the laboratories of the authors (A and B, Table VI). Some samples, as indicated, were screened, the purpose being to facilitate division into representative subsamples.

The following observations can be made from the data of Table VI:

Reproducibility is satisfactory, the averages of the differences from the means being 2.0 per cent. (By standardizing all details of the procedure, as was done in the case of fish cannery waste No. 3, remarkably close agreement can be attained.)

There is a slight tendency toward higher results by Procedure I than by Procedure II, but the difference is usually of little importance.

Ethyl ether, isopropyl ether, chloroform, and benzene give results which differ by scarcely significant amounts but are considerably higher than results obtained with alkanes (petroleum ether, hexane). The differences, however, are not so great as have been reported in the case of dry methods.

In the analysis of sewage, using hexane as a solvent, the wet method gives results consistently higher than the A. P. H. A. method. With sludges the differences are in the same direction, but are smaller. In the case of fish cannery waste, it is impossible to secure reproducible results by dry methods. (The data given for fish cannery waste No. 4, A. P. H. A. method, are typical of several attempts to apply dry methods to this material.)

Since the variation of results with type of solvent is greater by the dry method than by the wet method, the observed relationship between results by the two procedures might be reversed if a solvent other than an alkane were used. Sewages and sludges from other origins might also show different relationships.

Considering again the sources of error in prevailing dry methods, as enumerated at the beginning of this article, it is clear that the change from a dry to a wet method has eliminated or reduced errors due to volatilization of grease when the sample is dried; resaponification of fatty acids from insoluble soaps; liberation of fats by hydrolysis of phospholipides, etc.; insolubilizing of oils by oxidation; and extraction of nongrease.

As to the inexactitudes which might be more serious in the wet than in the dry method, there are two worthy of mention: Acetic acid, and other lower members of the fatty acid series, if present, may be extracted to a considerable extent if ethers are used and to a slight extent with alkane solvents. The ease of extraction progressively increases with the higher homologs, so that butyric acid probably would be largely extracted even by alkanes. In analyses of sewage and raw sludge, this effect undoubtedly causes errors, which, however, are not likely to be important.

In some cases, the solvent may fail to contact and dissolve greasy particles which are surrounded by other material. Samples containing many large particles of solid matter may be broken up in some apparatus such as a food "liquefier" to reduce this source of error. That such inaccuracy is not likely to be serious in ordinary work is indicated by the following consideration:

Prolonging the time of shaking beyond that recommended in the procedures does not influence the test, and even the much more thorough contact between sludge particles and solvent in Procedure I, as compared with Procedure II, does not give significantly higher results.

The consistency of the results suggests that the extraction is not greatly dependent upon the variable chance of contact between solvent and grease particles.

Grease particles must be effectively surrounded by hard material which is preferentially wet by water rather than hexane in order to escape solution by the hexane.

In spite of these considerations, some grease does escape extraction, as, for example, on the inside of seeds. However, such sequestered grease is of relatively little concern to the sanitary engineer, for as long as the encasement remains unruptured, it does not impart greasy characteristics to the waste.

In judging the merits of methods, time requirements are important. Results by the wet method can be obtained in less than 8 hours, which is considerably under the time required for the A. P. H. A. dry method, but perhaps twice as much of the chemist's time is required. With adequate equipment, one chemist can conveniently analyze five samples per day by the wet method.

Choice of Solvent

A liquid of ideal solvent action should dissolve all substances having the sanitary significance of grease, including such water-insoluble materials as oils, fats, insoluble soaps (or the corresponding soap acids), waxes, and soft tars. Unfortunately, any solvent which will completely dissolve these substances will also dissolve some nongreases. Thus hexane, which gives lowest results of any of the liquids used, will dissolve such nongreaselike compounds as naphthalene, *p*-di-

chlorobenzene, *o*-toluidine, benzoic acid, etc., yet there is a soft brown tar in oil-field waste water which is insoluble in hexane.

As far as is known, chloroform and benzene will dissolve all materials which could be classed as grease, but these solvents also attack resins, dried paint, rubber, hard gums (such as chicle), etc. As noted previously, the results of grease analyses obtained with these solvents are higher than with alkanes.

When the material extracted by chloroform from sewage sludge was treated with hexane, there remained a black, powdery solid. This was resoluble in chloroform, had a density greater than unity, and was only slightly softened in boiling water. Clearly, such material is not grease. Meat-packing waste yielded a similar substance. Thus it appears, at least in the cases of sewage and meat-packing wastes, that the higher results obtained by chloroform were due chiefly to extraction of nongreases, rather than to more effective removal of greases. The same conclusion probably applies to benzene and the ethers.

The authors are of the opinion that alkanes should be used for analyses of sewage, sewage sludges, and most industrial wastes. A petroleum fraction approximating the hexanes in properties (or pure isohexane or *n*-hexane) is preferred to petroleum ether, since the latter product is variable in properties and has a boiling point undesirably low.

Oil-field water and possibly some other wastes may require a different solvent, but the choice should be based upon a study of the materials dissolved, rather than upon the assumption that the solvent giving highest results is best.

Summary

Many errors are inherent in those grease analysis methods which call for evaporating the water present in the sample. Procedures have been devised whereby the extraction is effected without this preliminary drying. Application of these procedures to a variety of sewages, sludges, and industrial wastes gave results with differences from the means averaging 2.0 per cent. Results are much less influenced by variations of technique and of solvent and more accurately represent the true content of "grease" than is the case in "dry" methods.

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A Rapid Procedure for Determination of Carbonate

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THE simplest procedure for the determination of carbonate consists in addition of acid to the sample, removal of carbon dioxide by boiling, and titration of the excess acid. This procedure has very limited application, as it may be used only when the carbonate is all present as either carbonate or bicarbonate and when other acidic or basic substances are absent.

The modification of this procedure here described extends its use, so that it may be used more generally.

The total carbon dioxide is quantitatively precipitated as strontium carbonate and the solution is adjusted to neutral to phenolphthalein. A known amount of acid is added to liberate the carbon dioxide, which is then aerated from the solution, and the excess acid is titrated with alkali. If organic acids or bases are present they are either wholly or partially neutralized in the preliminary pH adjustment; after the addition of acid they are brought back to this same state of neutralization. Phosphate is precipitated as strontium phosphate, and after the addition of acid, reprecipitated in the back-titration. Iron is precipitated as its oxide and is titrated back to this form. When present in small amounts ammonia does not interfere; in large amounts it interferes with the phenolphthalein end point. Magnesium is precipitated as the hydroxide when the solution is first made alkaline and it dissolves slowly when the solution is neutralized. It interferes in so far as it is not dissolved during this neutralization.

This procedure is somewhat faster than others, especially when a number of samples are run at the same time, and it may be used when other procedures based on the formation of insoluble salts are not applicable. Thus, the procedure based on titration in the presence and absence of barium (*1*) is limited to alkaline solutions which do not contain phosphate or calcium. For the quantitative precipitation of carbonate an alkaline solution of strontium chloride is preferred to the more generally used barium hydroxide (*3*, *4*) because strontium carbonate is less soluble than barium carbonate and because the large excess of strontium decreases this solubility still further. The procedure (*2*) in which carbonate is precipitated as calcium carbonate, filtered off, and titrated cannot be used in the presence of other ions which form insoluble calcium salts.

Method

In each of four large 35 × 200 mm. test tubes place 10 cc. of carbonate-free 0.03 *N* sodium hydroxide and a few drops of phenolphthalein. [Carbonate-free alkali may most easily be prepared by measuring saturated sodium hydroxide (19 *N*) into slightly acidified boiled distilled water. To prepare the saturated sodium hydroxide, dissolve 100 grams in 100 cc. of water and allow the solution to cool and settle.] Add carbonate solution to two of the tubes, delivering the sample underneath the alkali to prevent loss of any free carbon dioxide. Add 10 cc. of 20 per cent strontium chloride solution to each tube. Mix the contents of the tubes, stopper, and allow them to stand for several minutes. The

TABLE I. DETERMINATION OF CARBONATE

Expt. No.	Composition of Unknown Solution[a] Total volume	0.1 *M* carbon dioxide	Other 0.1 *M* constituents	0.1 *M* Carbon Dioxide Recovered	
	Cc.	*Cc.*	*Cc.*	*Cc.*	%
1	80	0.30	None	0.291	97
2	15	1.50	None	1.50	100.0
3	25	25.00	None	25.04	100.2
4	5	4.00	None	4.02	100.5
5	5	4.00	0.3 KH_2PO_4	3.96	99.0
6	7	4.00	2.0 KH_2PO_4	3.96	99.0
7	6	4.00	1.0 $FeCl_3$	4.03	100.8
8	6	4.00	1.0 $MgCl_2$	3.96	99.0
9	10	4.00	5.0 $MgCl_2$	4.33	108.0
10	6	4.00	1.0 NH_4Cl	3.99	99.8
11	10	4.00	5.0 NH_4Cl	4.00 ± 0.2	100.0 ± 5
12	15	4.00	10.0 NH_4Cl	4.00 ± 0.4	100.0 ± 10
13	9	4.00	2.0 $MgCl_2$ 2.0 KH_2PO_4	3.96	99.0
14	7	4.00	1.0 $FeCl_3$ 1.0 KH_2PO_4	4.25	106.0
15	5	0.56	5 cc. urine	0.46	82
16	5	3.70	5 cc. urine	3.60	97
17	11	0.21	1 cc. blood	0.17	81
18	5	0.30	3 cc. 1.5% albumin	0.303	101

[a] In experiments 1 to 15 and 18, known amounts of sodium bicarbonate and other constituents were dissolved in water. Urine and blood samples (experiments 15 to 18) were analyzed by Van Slyke manometric technique.

solutions must remain alkaline. Adjust the reaction to just pink to phenolphthalein by slowly adding 0.1 *N* hydrochloric acid from a small-tipped buret. In order to avoid the accumulation of acid in any part of the solution, with consequent loss of carbon dioxide, stir each solution with a thin glass rod, bent and flattened on one end. Avoid breaking the surface of the liquid with the stirring rod and the addition of an excess of the acid.

Add 5 cc. of 0.1 *N* acid to each tube to dissolve the precipitated strontium carbonate. Aerate the contents of each tube rapidly for 4 minutes, using a Folin aeration tube (a tube perforated with several small holes). Titrate the excess acid with 0.03 *N* alkali. The difference between the titration values of the sample and the blank gives the amount of carbon dioxide in the sample. Since carbonate acts as a divalent acid, 1 mole of alkali is equivalent to 0.5 mole of carbon dioxide.

The amounts and concentrations of reagents used should be adjusted to the amount of carbonate and other buffering substances present in the sample. The values given in the above description are suitable for samples which contain from about 0.75 to 3.00 cc. of 0.1 *M* carbon dioxide, when little other buffering substance is present (experiment 2, Table I). When very little carbonate is present (experiment 1), use smaller amounts of reagents, a larger unknown sample, run blanks on an equal volume of carbon dioxide-free water, allow the reaction mixture to stand for 15 minutes before neutralization, and aerate with carbon dioxide-free air. With urine samples (experiments 15 and 16), it is convenient to measure 5 cc. of urine into 5 cc. of 0.3 *N* alkali, add strontium chloride, neutralize, add 10 cc. of 0.3 *N* acid in excess, and titrate with 0.1 *N* sodium hydroxide.

Discussion

This procedure can be used under widely different conditions, provided a number of precautions are observed. At no time previous to the aeration may the sample become acidic. When strontium chloride is added it reacts with bicarbonate or phosphate to form hydrochloric acid. The reaction mixture must contain sufficient alkali to neutralize this liberated acid.

When the excess alkali is neutralized, acid must be added slowly and the solution must be mixed continuously to avoid loss of carbon dioxide from the acidified surface of the solution. The alkaline tubes must be kept stoppered to prevent absorption of carbon dioxide from the air. The amount of absorption, even in stoppered tubes, depends on the amount the tubes are shaken. They may be kept under nitrogen, but nitrogen should not be run into the tubes while excess alkali is being neutralized because of danger of carrying off carbon dioxide liberated at the surface of the solution.

When phosphate is present it is sometimes reprecipitated somewhat slowly when the excess acid is titrated, resulting in a fading end point. This continues for only a minute or two and then gives rise to a stable end point. Neither iron nor phosphate interferes when present singly (experiments 5, 6, and 7), although they indirectly decrease the precision by requiring the use of larger amounts of reagents. When present together in the same solution (experiment 14) they may be precipitated as ferric phosphate in the preliminary neutralization and as ferric hydroxide and strontium phosphate after the excess acid is titrated. Since these forms contain different amounts of alkali, these substances may interfere when present together.

If a relatively large amount of magnesium is present (experiments 8, 9, and 13) some is precipitated as hydroxide when the solution stands in excess alkali. When the alkali is neutralized this dissolves slowly. It is sometimes necessary to readjust the solution to neutral several times over a period of about a half hour before a permanent end point is obtained. Although quantitative recovery may be obtained when magnesium is present, it increases the time required to run the determination to such an extent that it should be considered an interfering substance.

When applied to acid urine samples which contain very small amounts of carbonate, the procedure is not accurate. This is at least partly due to the fact that the carbonate is so small a fraction of the total buffers present. With more alkaline urine samples which contain more carbonate (experiment 18), the results are within 4 per cent. When the method was used for the determination of carbon dioxide in blood serum, irregular results were obtained which were lower than values obtained by gasometric procedures. Egg albumin at about the same concentration as the proteins of blood did not interfere with the determination.

TABLE II. DETERMINATION OF CARBONATE IN LIMESTONE[a]

Sample No.	Composition of Limestone R_2O_3	$MgCO_3$	CO_2	Carbon Dioxide Determined Titrimetrically	
	%	%	%	%	%
2	5.83	40.15	42.78	42.88	42.95
10	4.9	34.7	42.89	42.95	.....
42	41.8	16.53	25.95	25.93	25.87
44	48.3	8.87	22.65	22.71	22.50
x			21.63	21.81	21.86

[a] Analyzed samples kindly supplied by H. V. Moyer, Chemistry Department, Ohio State University.

This general procedure may also be applied to the determination of carbon dioxide in solid materials. The data in Table II give analyses for limestones.

Weighed samples (0.28 gram) were treated with 1 cc. of 0.1 *N* sodium hydroxide, 5 cc. of 20 per cent strontium chloride, and a few drops of phenolphthalein. The mixtures were allowed to stand for a few minutes, so that any bicarbonate which might be present would be changed to carbonate. The reaction mixtures were then neutralized and treated with an excess of 25 cc. of 0.3 *N* hydrochloric acid. The mixtures were heated at 80° C. in a water bath and shaken at 5-minute intervals over a period of 30 minutes in order to dissolve the carbonate in the limestone. After they had cooled the tubes were aerated for a few minutes and the excess acid was titrated with 0.1 *N* alkali. The difference between this titration and a blank titration represents the amount of carbon dioxide in the sample.

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Proximate Analysis of the Heartwood and Sapwood of Some American Hardwoods

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SEVENTEEN years have elapsed since any intensive analyses of American woods have been undertaken. Since that time many of the methods have been revised, and new ones introduced, notably a reasonably rapid method for the evaluation of the total carbohydrate fraction in wood (holocellulose). It was therefore deemed advisable to select the best of these methods and incorporate them into a new scheme of wood analysis which in reality is a modification of the original U. S. Forest Products Laboratory procedure as proposed by Schorger *et al.*

In contrast to the work of earlier wood analysts (*14*), the authenticity of the wood samples in all instances has been verified by means of microscopic identification. The leaves and fruit of each tree have been collected, identified, and are now on file at the Arnold Arboretum, Boston, Mass. Furthermore, the silvicultural characteristics, collection date, age, and height have been recorded for reference purposes.

Analytical Methods

While the methods generally have been described fully in the literature, they are outlined briefly in the interest of better interpretation of the results.

All wood samples were taken from the bole of each tree at a point approximately 20 feet above the ground. This particular section was chosen because strength tests at the U. S. Forest Products Laboratory (*19*) have shown this portion to be most representative of the specimen as a whole. The wood was then chipped and reduced to sawdust in a Wiley mill. Material which passed a 60-mesh sieve but was retained on an 80-mesh screen was chosen as the optimum particle size for analysis.

The determination of ash content and solubility in water, alcohol-benzene, and ether conformed with standardized procedures (*19*). Acetyl was estimated by a slightly modified procedure of Freudenberg's (*4*) method, which depends upon the transesterification in alcohol of the acetyl constituents by *p*-toluenesulfonic acid. This was followed by titration, after saponification of the ethyl acetate removed by distillation. Estimation of the methoxyl content was accomplished according to the well-known method of Zeisel (*21*). The lignin content was determined by the method of Klason as modified by Ritter, Seborg, and Mitchell (*17*). Inasmuch as the accuracy of the results obtained compares favorably with those of other well-known methods, and is most acceptable where many determinations are to be made, it was thought advisable to adopt this procedure for the present study.

The pentosan and cellulosan content of the wood was determined by the volumetric potassium bromate-bromide method of Powell and Whittaker (*12*). Kline and Acree (*9*) as well as Iddles and Robbins (*7*) have reported that this procedure consistently gives accurate results which compare favorably with the well-known gravimetric methods. For this reason and because of the rapidity with which results may be obtained, the method was selected for use in the present study.

For clarification of Table I, it should be pointed out that the term "cellulosan" is used to designate the pentosan associated with the cellulose. It was first proposed by Hawley and Norman (*6*), and has been generally accepted by workers in the field of wood chemistry.

Cellulose was estimated by the Cross and Bevan method as modified by Ritter and Mitchell (*16*). The quantitative determination of the total carbohydrate content of the wood, termed holocellulose, formulated by Ritter and Kurth (*10*, *15*) depends on the isolation of this fraction by alternate treatment of extractive-free wood with chlorine gas and alcohol-pyridine solution until the weight loss equals that of the lignin present in the original wood. Since it was felt that bleaching might possibly alter some of the constituent groups in this residue all analyses were made on unbleached holocellulose.

The analytical results presented in Table I represent the average of three determinations.

Discussion

HOLOCELLULOSE DETERMINATION. During the course of the present investigation approximately 75 different holo-

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TABLE I. ANALYSIS OF AMERICAN WOODS

[Summary of analytical results in percentages of oven-dry (105° C.) samples]

Species	Ash	Solubility: Cold water	Solubility: Hot water	Solubility: Alcohol-benzene	Ether	Acetyl	Methoxyl	Pentosan	Lignin[a]	Cellulose	Cellulosan	Holocellulose	In Holocellulose: Acetyl	In Holocellulose: Methoxyl	In Holocellulose: Pentosan
													(*Per cent on original wood*)		
Big tooth aspen															
Sapwood	0.26	2.70	3.13	2.41	1.02	5.48	5.27	23.33	16.33	62.67	14.45	78.01	5.17	0.65	22.51
Heartwood	0.33	1.36	0.99	2.13	1.03	6.07	5.35	23.75	16.92	64.42	15.78	80.00	5.05	0.72	22.98
Beech															
Sapwood	0.31	2.33	2.17	1.37	0.20	7.13	6.28	25.55	20.61	60.83	17.88	76.20	5.15	0.95	24.46
Heartwood	0.57	0.23	0.43	0.96	0.57	6.05	6.44	24.49	22.26	60.71	18.33	76.85	4.76	1.24	23.52
Sugar maple															
Sapwood	0.32	0.81	2.08	1.31	>0.10	6.56	6.34	22.78	20.33	60.39	16.02	72.26	5.12	0.83	22.67
Heartwood	0.84	0.52	1.20	1.22	0.89	4.94	6.50	22.98	21.79	60.27	14.25	75.96	4.05	0.97	22.57
Fire cherry															
Sapwood	0.17	1.96	4.03	2.97	0.72	7.54	4.61	26.16	12.27	59.98	14.32	80.96	7.51	1.06	25.01
Heartwood	0.41	0.67	2.48	1.18	0.47	6.77	4.77	27.32	14.82	58.26	14.93	81.58	5.23	1.16	25.65
Paper birch															
Sapwood	0.24	1.28	2.39	3.31	0.79	7.12	6.10	28.77	17.56	55.58	17.98	76.61	6.01	0.97	28.45
Heartwood	0.21	1.12	2.15	6.44	2.19	7.60	5.75	28.64	19.61	50.96	13.97	70.85	5.29	0.95	26.93
Yellow birch															
Sapwood	0.11	1.20	1.30	0.97	0.36	8.79	6.01	26.89	18.56	59.36	19.27	79.50	6.13	1.31	26.49
Heartwood	0.50	0.93	1.29	1.89	0.30	6.11	6.04	26.87	20.19	58.37	16.99	76.45	5.81	0.93	24.94

[a] Ash-free.

TABLE II. SUMMATIVE ANALYSIS OF AMERICAN WOODS[a]

Species	Ash	Solubility: Hot water	Solubility: Alcohol-benzene	Lignin	Holocellulose	Total
	%	%	%	%	%	%
Big toothed aspen						
Sapwood	0.26	3.13	2.41	16.33	78.01	100.14
Heartwood	0.33	0.99	2.13	16.92	80.00	100.37
Beach						
Sapwood	0.31	2.17	1.37	20.61	76.20	100.66
Heartwood	0.57	0.43	0.96	22.26	76.85	101.07
Yellow birch						
Sapwood	0.11	1.30	0.97	18.56	79.50	100.44
Heartwood	0.50	1.29	1.89	20.19	76.45	100.32
Paper birch						
Sapwood	0.24	2.39	3.31	17.56	76.61	100.11
Heartwood	0.21	2.15	6.44	19.61	70.85	99.26
Fire cherry						
Sapwood	0.17	4.03	2.97	12.27	80.96	100.40
Heartwood	0.41	2.48	1.18	14.82	81.58	100.47
Sugar maple						
Sapwood	0.32	2.08	1.31	20.33	76.26	100.30
Heartwood	0.84	1.20	1.22	21.79	75.96	101.01

[a] Results in percentages of oven-dry (105° C.) wood.

cellulose residues were isolated. The outstanding objection to the holocellulose determination is the lack of a definite end point indicating complete removal of all lignin. At present the results obtained are entirely dependent upon the accuracy of the lignin determination, and since in the latter case there is no one method in use today which has received the unanimous endorsement of all workers in this field, the empiricism of the holocellulose procedure may be seriously questioned. It would be highly advantageous, therefore, to modify the determination in such a manner that it would be independent of other procedures, thus increasing both its efficiency and accuracy[2].

SUMMATION OF PROXIMATE GROUPS. As a means of accounting for the total wood substance analyzed a résumé of the analytical data is given in Table II. In this summary it has been assumed that the total mineral content of the wood is included under "ash", whereas any waxes, fats, etc., are accounted for under "alcohol-benzene solubility". Certain of the simpler carbohydrates and water-soluble dyes will be removed by the hot-water extraction. The remaining residue is thus composed of a noncarbohydrate fraction, designated as lignin; and a carbohydrate fraction, designated as holocellulose. As shown in Table II, the summation equals 100 per cent (±1 per cent) for each species analyzed.

RELATION OF SAPWOOD AND HEARTWOOD CONSTITUENTS. Ritter and Fleck (*14*) have reported that in the analysis of softwoods certain definite conclusions may be drawn concerning the relationship between the various constituents of the sapwood and heartwood, although they add that it is difficult to generalize in the case of the hardwoods. These authors also observe that the acetic acid which results from the hydrolysis with mineral acid is consistently higher in the sapwood than in the heartwood of both softwoods and hardwoods.

The results of the present investigation fail to confirm the conclusions of these investigators. That the relationship suggested by Ritter and Fleck fails to exist is not unusual when it is considered that certain vital factors such as age, growing conditions, and habitat are responsible for the amount and distribution of these constituents.

The data of the present study, however, do permit certain generalizations to be drawn which must be interpreted strictly as such, and not as conclusions.

With but one exception (paper birch) the percentage of ash is considerably lower in the sapwood than in the heartwood fraction.

In every case the hot- and cold-water solubility is greater in the sapwood than in the heartwood.

With the exception of the two birches, alcohol-benzene solubility is also higher in the sapwood of the remaining four species.

Of the woods investigated no generalizations can be drawn relative to a specific distribution of ether-soluble residue, pentosans, or holocellulose.

The acetyl content of the sapwood is greater than that of the heartwood in four of the six species. Aspen and paper birch are exceptions.

With the exception of paper birch, the methoxyl residue is greater in the heartwood fraction.

Inasmuch as it is generally agreed that methoxyl is for the most part associated with lignin, it is not surprising that the heartwood of every species contains more lignin than the sapwood.

In contrast, with one exception (aspen), the cellulose content of the sapwood of all species is higher than that of the heartwood.

Any generalization as to the distribution of the cellulosan fraction between sapwood and heartwood must be made with extreme caution, because of the possible formation of small amounts of furfural-yielding degradation products during isolation of the cellulose. With such reservations in mind, it is observed that in four of the woods studied the cellulosan content is higher in the sapwood than in the heartwood. Aspen and beech are exceptions.

DISTRIBUTION OF PENTOSAN, METHOXYL, AND ACETYL GROUPS. The results of the present investigation provide some interesting observations regarding the distribution of pentosan, methoxyl, and acetyl groups between the carbohydrate (holocellulose) and noncarbohydrate (lignin) portions of the wood. By calculation it was found that 98 per cent of the pentosan residues present in the original wood could be accounted for in the holocellulose fraction of each species. This figure is in excellent agreement with the results of Ritter and Kurth (*15*), who report 96.6 per cent of the total pentosans to be present in the holocellulose isolated from maple wood.

The results given in Table I also show that an average of 16 per cent of the total methoxyl content of the wood may be accounted for in the carbohydrate fraction.

Earlier investigators, Benedikt and Bamberger (*1*) and Dore (*3*), have stated that methoxyl is entirely associated with the lignin. In contrast, Ritter and Kurth (*15*) report that 15.6 per cent of the total methoxyl is present in the holocellulose residue; and Hägglund and Sandelin (*5*) have shown that 12 per cent of the methoxyl content of wood is present in the carbohydrate portion. Added confirmation is supplied by the work of O'Dwyer (*11*) and Schmidt *et al.* (*18*). Thus, the bulk of experimental evidence at present disproves the former conception that all of the methoxyl residues in wood are associated with the lignin.

Cross and Bevan (*2*), Pringsheim and Magnus (*13*), and Klason (*8*) claim that all of the acetyl residues are associated with the lignin. Dore (*3*), however, believes they are joined with the cellulose. Ritter and Kurth (*15*) also claim to disprove the earlier belief by reporting that the total acetyl groups of the original wood of sugar maple may be accounted for in the holocellulose fraction. However, Hägglund (*5*) reports a reduction of 40 per cent of the acetyl content of holocellulose as compared to that in the original wood. Kurth and Ritter have raised the objection that Hägglund's use of alkaline calcium hypochlorite bleach may possibly have resulted in the removal of some of the acetyl residues, thus accounting for the low results reported.

The results of the present study, which covers a total of twelve separate sets of analyses of six different hardwoods including sugar maple, indicate that an average of 81 per cent of the total acetyl is accounted for in the holocellulose. Un-

[2] Since the completion of this study, Van Beckum and Ritter (*20*) have published their chlorine–alcoholic ethanolamine method, which is a better procedure than the use of chlorine-alcohol-pyridine in that a sharp end point is said to be obtained upon removal of the last traces of lignin.

fortunately, these results cannot be interpreted as substantiating Hägglund, because examination of Table I will show that in the cases of aspen sapwood, fire cherry sapwood, and yellow birch heartwood the acetyl content of the holocellulose represents 94, 99, and 98 per cent, respectively, of that in the original woods. Therefore, in three instances the results confirm those of Ritter and Kurth (*15*). Because of these contradictory results no clear-cut conclusion can be drawn regarding the location of the acetyl group. Possibly a portion of the acetyl groups in the other woods have been destroyed during the isolation of the holocellulose, owing to partial oxidation during chlorination and removal of the decomposition products during the pyridine-alcohol extraction.

Application of Results

In one instance the results of the present investigation have brought to light information which otherwise might have escaped attention. It was noticed that the lignin content of fire cherry was exceptionally low, thus predicting a high carbohydrate content. Evaluation of the cellulose content yielded an average figure of only 60 per cent. The difference between the cellulose and holocellulose contents suggested the presence of a considerable quantity of hemicellulose which was isolated and is now under investigation.

Acknowledgment

The authors are indebted to H. P. Brown, New York State College of Forestry, Syracuse, N. Y., for collection and identification of the woods analyzed.

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A Laboratory-Scale Flow Regulator

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EXPERIMENTS conducted in this laboratory involving the slow transport of gases have made necessary the use of a device for regulating their flow.

Although a few designs intended for laboratory use are reported in the literature (*1–4*), all possess certain limitations in regard to expense, simplicity, reproducibility, and personal supervision. To meet all these factors, the conditions of which were imposed by a problem under investigation, it was necessary to design a regulator.

The apparatus (Figure 1) is fashioned from the escapement wheel, *W*, and the lever, *L*, of a pendulum clock. A perforated strip of metal, *S*, 30 inches long and 0.75 inch wide, is welded to the end of the escapement lever. A weight, *M*, is then attached to the lower end of the metal strip, producing a typical pendulum with a relatively long period of vibration.

A drum, *D*, is fastened on the axis of the escapement wheel, and is then wound with a stout cord or wire.

A leveling bulb containing mercury or some other liquid is now suspended from the drum by the cord. The weight of the leveling bulb thus acts on the escapement wheel and provides the force necessary to keep the pendulum in motion. As the wheel turns and the drum unwinds, the leveling bulb is lowered slowly, reducing the pressure within a bottle to which the leveling bulb is connected and causing gas to flow into the bottle.

The purpose of the pendulum is to maintain a constant lowering of the leveling bulb. The weight of the bulb increases as it fills with liquid, but the effect on the pendulum is one of increased amplitude of vibration rather than shortening of the period.

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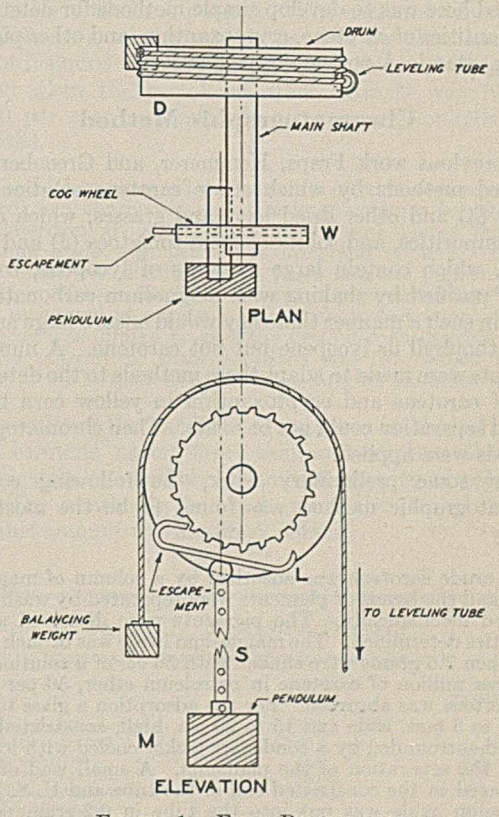

FIGURE 1. FLOW REGULATOR

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Determination of Carotene and Cryptoxanthin in Yellow Corn

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YELLOW corn is an important source of vitamin A in feeds, even though the amount it contains is low when compared to good alfalfa and other hays of good quality. Such large quantities of yellow corn are usually fed that it may afford a considerable portion of the vitamin A potency an animal receives. Kuhn and Grundmann (*10*) in 1934 and Buxton (*2*) later reported that as much as 90 per cent of the active pigments in yellow corn may consist of cryptoxanthin. According to Kuhn and Grundmann (*9*) cryptoxanthin has one half the vitamin A potency of beta-carotene. Therefore, in order to determine by chemical methods the vitamin A potency of yellow corn and commercial feeds which contain it, it is necessary to determine both cryptoxanthin and beta-carotene.

Chromatographic methods have been used extensively to separate and identify carotinoid pigments in plant materials and have been applied to the determination of carotene and cryptoxanthin in yellow corn (*2*, *10*). The methods heretofore described are slow and complicated, and losses of pigments in the column have sometimes been so high that the final results obtained are doubtful. The purpose of the work reported here was to develop simple methods for determining the quantities of carotene, cryptoxanthin, and other pigments having vitamin A potency in yellow corn.

Chromatographic Method

In previous work Fraps, Kemmerer, and Greenberg have reported methods by which crude carotene solutions from alfalfa (*4*) and other dried hays and grasses, which contain some impurities, and solutions from tomatoes (*5*) and watermelon, which contain large amounts of lycopene, could be highly purified by shaking with magnesium carbonates activated in such a manner that they would adsorb large amounts of xanthophyll or lycopene but not carotene. A number of attempts were made to adapt these methods to the determination of carotene and cryptoxanthin in yellow corn but the desired separation could not be made. Then chromatographic methods were applied.

After some preliminary work, the following complete chromatographic method was found to be the most satisfactory:

The crude carotene was adsorbed by a column of magnesium oxide, and the bands of pigments were separated by washing and removed mechanically. The pigments were dissolved and the quantities determined. The magnesium oxide was of such quality that when 2.5 grams were shaken with 50 cc. of a solution of 2.0 parts per million of carotene in petroleum ether, 50 per cent of the carotene was absorbed. For the adsorption a glass tube was used 5 to 8 mm. wide and 15 to 20 cm. high, constricted at one end and surrounded by a condenser jacket cooled with ice water during the separation of the pigments. A small wad of cotton was placed in the constricted end of the tube and U. S. P. light magnesium oxide was put into the tube in 0.2-gram portions. Suction was applied and each portion of magnesium oxide was packed gently but firmly by means of a cork with a smooth surface, attached to a glass rod. Portions of magnesium oxide were added and packed until a column about 10 cm. long was obtained.

For the determination 25 grams of finely ground yellow corn were refluxed with 12 per cent alcoholic potassium hydroxide and the crude carotene was obtained in petroleum ether (Skellysolve F, boiling point 30° to 76.7° C.) as described in the A. O. A. C. method for carotene (*1*). The crude carotene solution was diluted to 200 cc. in a graduated flask and the amount of pigment, expressed as carotene, was determined with a photoelectric colorimeter. Then the solution was concentrated to about 25 cc. and placed on the column described above. The column was washed with petroleum ether until the bands of pigment had separated, which usually took about 4 hours. Each band of pigment was scraped from the column into a beaker, with a long wire flattened at one end, and was always kept covered with petroleum ether. The bands were separately extracted with petroleum ether containing 2 per cent ethanol, the extracts were washed with water to remove the alcohol, and if the solutions were cloudy they were washed once with dilute hydrochloric acid (1 to 100). The solutions were dried over anhydrous sodium sulfate and diluted to suitable volume in graduated flasks, and the amount of pigment (expressed as carotene) was determined with the photoelectric colorimeter.

Analyses by Complete Chromatographic Method

Seven bands of pigment were frequently formed in the chromatographic column.

(1) At the top of the column, for a few samples, was a small narrow band of orange pigment, which the authors termed "impurity". (2) Next was a wide orange band of cryptoxanthin, and then (3) a band of what Zechmeister and Tuzson (*13*) call neocryptoxanthin. Very close to and below the neocryptoxanthin band were two extremely fine bands (4), one red and the other orange. It was impossible to separate these bands quantitatively from the neocryptoxanthin by the mechanical technique used. In the work reported here they were included with the neocryptoxanthin. Next (5) was a light yellow band, which, because of its position in the column and because it was later found to possess vitamin A potency, the authors call K carotene. Next (6) was a sharply separated orange band of beta-carotene, and finally (7) a small orange band of alpha-carotene.

The alpha- and beta-carotene bands in the column were identified by means of the mixed chromatographic technique

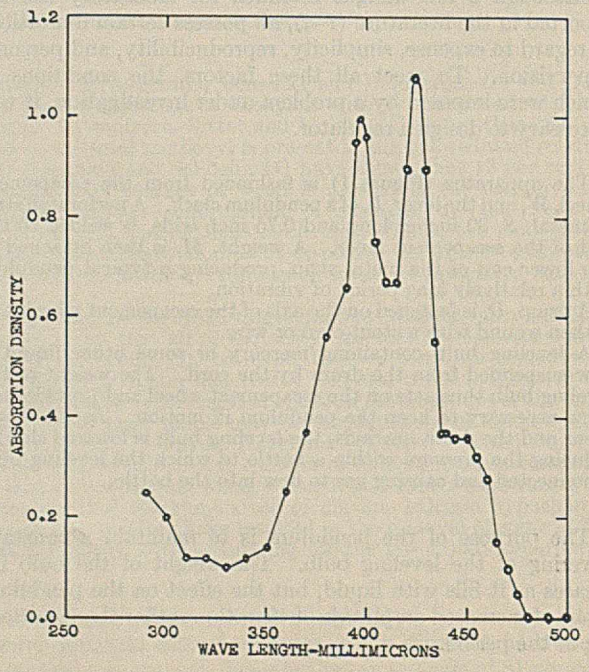

FIGURE 1. ABSORPTION CURVE OF K CAROTENE

TABLE I. CONSTITUENTS OF CRUDE CAROTENE FROM YELLOW CORN

Laboratory No.	Variety	Crude Carotene	Composition of Crude Carotene: Beta-carotene	Alpha-carotene	K carotene	Cryptoxanthin	Neocryptoxanthin	Impurity[a]
		P. p. m.	%	%	%	%	%	%
55,710	Golden June	5.0	30.3	1.4	3.7	50.4	14.1	0.1
55,711	Ferguson Yellow Dent	6.4	30.9	1.7	5.6	44.2	17.5	0.1
55,712	Jarvis Golden Prolific	8.0	26.7	2.5	5.7	47.0	15.5	2.6
55,713	Texas Golden Prolific	6.0	35.6	4.1	7.4	37.7	15.2	0
55,718	Mickle's Yellow Dent	4.0	24.8	0.8	7.9	48.4	18.1	0
54,547	Yellow corn meal	5.3	30.4	5.3	6.5	39.5	18.3	0
54,551	Yellow Surecropper	3.7	28.9	6.1	4.4	41.1	19.4	0.1
54,552	Corn Belt Hybrid, Pfester	1.3	31.0	7.1	3.5	46.1	12.2	0.1
55,847	Johnson's Yellow Dent	6.4	29.9	5.1	5.2	44.5	15.3	0
55,848	Pluger's Mammoth Yellow	6.4	28.3	5.4	5.6	42.4	18.3	0
55,849	Good's Golden Prolific	4.1	33.5	4.2	7.9	41.8	12.6	0
55,719	Reese Yellow Squaw	4.4	32.5	6.1	5.7	40.3	15.4	0
55,720	Yellow Tuxpan	5.0	33.9	1.5	5.3	53.3	5.9	0.1
55,721	Reese Yellow Drought Resister	5.4	29.1	4.5	4.5	36.9	18.5	6.5
55,722	Yellow Paymaster	6.2	34.7	2.9	6.8	38.1	12.6	4.9
55,723	Yellow Surecropper	3.4	23.9	4.0	5.3	38.8	19.4	8.6
55,850	Leudtke's Yellow Sure	4.6	31.6	4.9	5.5	33.3	20.2	4.5
58,941	Yellow Paymaster	4.1	35.8	5.1	6.2	35.5	17.6	0
	Average, Group 1 (18)	5.0	30.7	4.0	5.7	42.2	15.8	1.5
55,714	Golden Thomas	7.3	22.6	3.1	5.6	39.7	29.0	0
55,715	Hastings Golden Dent	7.4	16.8	0.9	6.9	50.0	21.2	4.2
55,716	Hill's Yellow Dent	6.0	18.4	0.2	3.4	53.2	24.7	0.1
55,717	Reese Giant Yellow Dent	3.7	21.8	0	5.2	51.8	21.1	0.1
	Average, Group 2 (4)	6.1	19.9	1.1	5.3	48.7	24.0	1.1
	Average of Groups 1 and 2 (22)	5.2	28.7	3.5	5.6	43.4	17.3	1.5

[a] When less than 1%, impurity is determined by difference.

crude carotene of the entire group ranges from 0.0 to 7.1 with an average of 3.5 per cent, that of the K carotene from 3.4 to 7.9 with an average of 5.6, that of the cryptoxanthin from 33.3 to 53.3 with an average of 43.4, the neocryptoxanthin from 5.9 to 29.0 with an average of 17.3, and the impurity from 0 to 8.6 with an average of 1.5.

The average pigment lost in the column was about 3 per cent, but in 27 determinations there were three losses of more than 10 per cent, one of which was as high as 18 per cent. In nine of the determinations there was a slight gain of pigment, probably due to error in the colorimetric determinations, and in one determination there was a gain of 14 per cent. When the loss or gain is high, the results should be discarded.

(*12*) and by spectroscopic examination. The pigment called K carotene was at first thought to be gamma-carotene, but spectroscopic examination showed it to have absorption maxima at 425 and 397 millimicrons. This is very different from gamma-carotene, which, according to Zechmeister (*11*), has maxima in hexane at 494, 462, and 431. The absorption curve of K carotene in petroleum ether is given in Figure 1. It is unlikely that the K carotene was formed from carotene during the chromatographic separation or because of solution. Solutions of a mixture of alpha- and beta-carotene have been chromatographically separated many times in this laboratory and the band of K carotene was never observed to form from them. A survey of the literature indicated that K carotene has not heretofore been reported. It comes nearest to being B-dehydro carotene (*8*) but differs from it in having vitamin A potency.

Zechmeister and Tuzson (*13*) and Carter and Gillam (*3*) have shown that carotinoid pigments isomerize in solution, and according to Zechmeister and Tuzson (*13*) part of the cryptoxanthin in solution easily isomerizes into neocryptoxanthin. Both these groups of workers have shown that the isomerizations are due to solution and not to changes in the column of adsorbent. In order to check these findings, cryptoxanthin was removed from a column, dissolved in petroleum ether, and passed into another column. A band of cryptoxanthin and a band of neocryptoxanthin were always formed. When neocryptoxanthin was eluted and passed into a column, bands of cryptoxanthin and of neocryptoxanthin were formed. Cryptoxanthin and neocryptoxanthin appear to be isomers of each other and in solution either pigment can form the other. Consequently, in the chemical determination of vitamin A potency of yellow corn the neocryptoxanthin may be included with the cryptoxanthin.

The results of the chromatographic analyses for 22 samples of corn are given in Table I, in percentage of total carotinoids recovered from the chromatograph column, so that any loss or gain was prorated. The analyses fall into two groups as regards beta-carotene content and cryptoxanthin. In the last 4 varieties listed, the crude carotene contains 16.8 to 22.6 per cent of beta-carotene with an average of 19.9, while in the group of 18 samples the percentage is 23.9 to 35.8 with an average of 30.7. The cryptoxanthin plus neocryptoxanthin averages 72.7 per cent in the first group and 58.0 per cent in the second. The percentage of alpha-carotene in the

Biological Activity of Pigments

The K carotene, alpha-carotene, cryptoxanthin, and neocryptoxanthin from two samples of yellow corn were tested with rats for vitamin A potency. The extremely fine bands just below the neocryptoxanthin band in the chromatograph were not included with the neocryptoxanthin. The pigments removed when the petroleum ether extracts were washed with 90 per cent methanol, to remove xanthophylls, were also tested.

For the biological tests large amounts of corn were saponified, extracted, and chromatographed in large columns by the technique described above. The pigments were eluted from the magnesium oxide with petroleum ether and ethanol. The petroleum ether was distilled off *in vacuo* and the pigments were taken up in Wesson oil. The amount of pigment in the Wesson oil was estimated as carotene with a photoelectric colorimeter, and the oil was diluted to give the desired concentration. The biological potency of these solutions was determined by the modified U. S. P. method used in the authors' laboratory (*6*).

The results of the biological tests, given in Table II, show that K carotene, neocryptoxanthin, and cryptoxanthin have approximately one half the vitamin A potency of beta-carotene. The xanthophylls which were also tested had no detectable amount of vitamin A potency.

TABLE II. VITAMIN A POTENCY

(International units per microgram of pigment)

	Corn 57,948	Corn 58,929
Standard carotene in oil	1.7	...
Beta-carotene	1.1	...
K carotene	0.5	0.6
Cryptoxanthin	0.6	0.6
Neocryptoxanthin	0.4	0.4
Xanthophylls	...	0.0

Abridged Chromatographic Method

When separation of all the pigments in the crude carotene extracts of yellow corn is desired, the chromatographic method just described can be used. This method is tedious, and when

Table III. Carotene and Cryptoxanthin by Chromatographic and Abridged Chromatographic Methods

Laboratory No.	Chromatographic Method			Abridged Chromatographic Method		
	Carotene	Cryptoxanthin	Impurity	Carotene	Cryptoxanthin	Impurity
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
58,941	1.9	2.0	0.2	1.7	1.6	0.3
58,942	1.7	1.9	0.1	1.7	1.6	0.3
58,943	0.5	0.4	0.1	0.3	0.3	0.0
58,945	2.6	4.7	0.2	2.8	4.6	0.3
58,946	1.4	2.2	0.1	1.7	2.0	0.3
58,947	0.4	0.5	0.1	0.5	0.5	0.1
58,949	2.6	4.4	0.4	3.0	4.2	0.3
58,950	1.3	2.3	0.1	1.5	2.2	0.1
58,951	0.7	1.5	0.1	0.7	1.3	0.2
58,953	3.7	4.0	0.1	4.4	3.3	0.4
58,954	2.2	3.2	0.1	2.5	2.8	0.2
58,955	1.1	0.8	0.1	1.3	0.7	0.1

analyses are to be run on large numbers of corn samples, a quicker and simpler method is needed. For this purpose an abridged chromatographic method was devised in which only the pure carotene, the cryptoxanthin, and the impurities are determined.

The U. S. P. light magnesium carbonate should be tested by placing approximately 1 gram in a tube as described below and passing a solution of purified carotene (1.0 to 1.5 parts per million) through it, washing with petroleum ether, and determining the carotene in the filtrate. The carotene should be washed through within about half an hour without loss of over 5 per cent. If the magnesium carbonate is too retentive of carotene, another lot should be tried.

An adsorbent column was prepared by placing approximately 1 gram of magnesium carbonate in a glass tube 5 to 8 mm. wide and about 15 cm. tall, constricted at one end and plugged with a wad of cotton. Suction was then applied and the magnesium carbonate was packed firmly but not too tightly with a cork with a smooth surface attached to a glass rod.

For the determination 10 grams of corn meal were saponified, as in the A. O. A. C. method, and the crude carotene extract was diluted to exactly 100 cc. The amount of crude carotene was determined with a photoelectric colorimeter and a 50-cc. aliquot concentrated *in vacuo* to about 15 to 20 cc. A few cubic centimeters of petroleum ether were placed on the column of magnesium carbonate, suction was applied and, before the petroleum ether was drawn in, the crude carotene solution was put in the tube. The magnesium carbonate was then washed with petroleum ether. The pure carotene did not form a band, but passed through the column. This solution was made up to volume and the pure carotene determined. The pure carotene includes alpha-carotene, beta-carotene, and K carotene.

An impurity formed a small band near the bottom of the column, and was washed out after the carotene had been removed. A slight band of impurity was sometimes found at the top of the column. If formed, this was scraped out, eluted with petroleum ether containing 2 per cent of ethanol, and after the ethanol had been washed out with water, it was combined with the impurity from the bottom of the column and diluted to volume and the quantity was determined. The average quantity of impurity is so small that it can be disregarded.

Bands of cryptoxanthin and of neocryptoxanthin were formed above the impurity at the bottom of the tube. They were scraped out together and eluted with petroleum ether and alcohol, the alcohol washed out with water, the solution made up to volume, and the quantity determined.

Samples of corn meal were analyzed by this method and by the complete chromatographic method (Table III). The results by the complete chromatographic method were added to give the corresponding constituents as by the abridged method. Examination of Table III shows that satisfactory agreement was secured, considering the small quantities present.

The method was also tested by putting known amounts of pure carotene through the column. Recovery of 100 per cent was obtained. The recoveries of corn pigments were 95 to 105 per cent.

The principal disadvantages of this method are that alpha-, beta-, and K carotene are not separated and every lot of magnesium carbonate must be tested before it is used.

Calculating Beta-Carotene Equivalent and Vitamin A Potency from Analyses

For some purposes it may be sufficient to calculate the carotene and cryptoxanthin content of yellow corn from the crude carotene. Table I shows that on an average the crude carotene in yellow corn contains 3.5 per cent of alpha-carotene, 28.7 per cent of beta-carotene, 5.6 per cent of K carotene, and 60.7 per cent of cryptoxanthin and neocryptoxanthin combined. Since the alpha-carotene and K carotene have the same vitamin A potency as the cryptoxanthin and only half that of the beta-carotene, they should be included with the cryptoxanthin rather than with the beta-carotene.

The crude carotene is multiplied by 0.29 to get the beta-carotene and by 0.70 to get the sum of the alpha-carotene, K carotene, and cryptoxanthin.

Carotene in feeds is usually expressed as beta-carotene. If the equivalent in beta-carotene is desired, the crude carotene is multiplied by 0.64 ($0.70 \times 0.5 + 0.29$). If the vitamin A potency in U. S. P. units is desired, the crude carotene is multiplied by 1.1 (0.64 divided by 0.6).

Although this method is only approximate, the results are probably more accurate than those of a biological assay. The figure 1.1, which is the number of International Units of vitamin A potency per microgram of crude carotene in yellow corn, agrees fairly well with 1.4, the figure found by Fraps, Treichler, and Kemmerer (7). The beta-carotene equivalent of the 22 samples in Table I was calculated both from the crude carotene only and from the results of the chromatographic analyses in Table I. The average difference of the 22 samples was 0.15 part per million, and the standard deviation was 0.06. If the variety of corn is known, the calculation should be more exact if the average composition of the group to which this variety belongs is used (Table I) instead of the average of all the samples of corn.

Summary

The alpha-carotene, beta-carotene, K carotene, cryptoxanthin, neocryptoxanthin, and impurity in yellow corn can be determined by a chromatographic method. An abridged method may be used for determining the carotenes, cryptoxanthin, and impurities. The approximate quantities of these constituents can be calculated from the crude carotene and the beta-carotene equivalent and U. S. P. vitamin units can be calculated from the crude carotene with a fair degree of accuracy.

Twenty-two samples of yellow corn differing in genetic strain were analyzed by the chromatograph method. Two groups of corn were observed, one containing on an average 19.9 per cent beta-carotene and 72.7 per cent cryptoxanthin in the crude carotene, the other and larger group containing 30.7 per cent beta-carotene and 58.0 per cent cryptoxanthin in the crude carotene. The average percentages found were beta-carotene 28.7, alpha-carotene 3.5, K carotene 5.6, cryptoxanthin 43.4, and neocryptoxanthin 17.3. Neocryptoxanthin was formed when cryptoxanthin was dissolved, and cryptoxanthin was formed when neocryptoxanthin was dissolved, so that cryptoxanthin may be considered to be the sum of the two.

A new form of carotene, termed K carotene, is found in yellow corn. It has a biological potency equal to that of alpha-carotene.

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Fluorescent Tests for Beryllium and Thorium

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FLUORESCENT tests for both beryllium and thorium with morin and cochineal have been described by Goto (*1*), but in neither case is the reaction specific, since these reagents produce a fluorescence with many other cations. Recently morin as a reagent for beryllium has been thoroughly studied by Sandell (*2*). The response of beryllium and thorium to a common reagent is also shown by 1,2,5,8-tetrahydroxyanthraquinone (quinalizarin), which gives the well-known cornflower blue color with beryllium. Willard and Winter (*3*) have noted that thorium also gives a characteristic color reaction with this material.

In a search for reagents to give specific fluorescent tests with the cations, the idea of testing the hydroxyanthraquinones for beryllium and thorium presented itself. Several of these were tried and it was found that 1-amino-4-hydroxyanthraquinone gives an intense fluorescence with beryllium in alkaline solution and with thorium in acid solution.

Characteristics of Reagent

The reagent, 1-amino-4-hydroxyanthraquinone, is a purplish-red powder, insoluble in water but soluble in alcohol. Water solution of alkalies, acids of concentration greater than 0.5 *N*, and alcohol solutions of over 50 per cent dissolve the compound. The color in alcohol and acids is red and in bases is purple. The alcohol and acid solutions fluoresce red, but the alkaline solutions do not fluoresce. If an alcohol solution of the material is added to a slightly acid solution of almost any cation other than thorium, a curious mode of precipitation takes place. A sacklike membrane seems to form on the walls of the test tube. This gradually contracts and the film of the same shape as the tube becomes smaller and smaller until it forms a flocculent suspension on the surface of the solution. Th^{++++} is the only ion that will keep this high-colored reagent dispersed in dilute acid solution. Oxidizing agents destroy the reagent; however, it seems to be stable in the presence of air. No changes in alcohol solutions are noticed after a period of several months.

For testing purposes an approximately 0.1 per cent solution was made by dissolving 0.1 gram of the reagent in 100 ml. of 95 per cent ethyl alcohol. It is sometimes necessary to warm the alcohol for about 15 minutes to effect complete solution.

Apparatus

The source of the ultraviolet rays used in these experiments was the 100-watt, type 4, red-purple bulb, mercury lamp of the General Electric Vapor Lamp Company, Hoboken, N. J., which gives radiations between 3100 and 4000 Å. with a maximum at 3650 Å. Observations were made in a partially darkened room and ordinary soft-glass test tubes were used as containers.

Beryllium Test

The test solutions used contained 0.1 gram of beryllium per liter. In making the test 0.1 ml. of this was placed in a test tube and 1 ml. of 2.5 *N* sodium hydroxide was added. This was diluted to 10 ml. and 0.5 ml. of 0.1 per cent alcohol solution of 1-amino-4-hydroxyanthraquinone was added. This solution under the ultraviolet lamp gave a red fluorescence extending from about 6300 to 6800 Å. In visible light the beryllium solution had the same purple color as an alkaline solution of the reagent. The test is excellent to the extent of one part of beryllium in 10^6 parts of water and can be observed in a dilution of 1 in 4×10^7 parts. One or 2 ml. of solution can be easily observed, but it is better to use over 5 if that quantity is available. At this concentration the test is much more definite than the familiar one with quinalizarin.

If such a quantity of beryllium salt is used that the hydroxide does not dissolve in 1 ml. of 2.5 *N* sodium hydroxide, a 10 per cent solution of sodium potassium tartrate is added drop by drop until the precipitate dissolves. An excess of sodium hydroxide must be avoided, since over 0.3 *N* alkali causes an appreciable diminution in the fluorescence. Mixtures containing beryllium were run through the usual procedure of analysis and the beryllium was easily detected in the presence of the aluminum by dissolving the hydroxide precipitate in sodium hydroxide and adding the reagent.

INTERFERING IONS. Reasonable quantities of cations and anions, except lithium, have no effect on this test. It requires 20 grams of sodium chloride in 100 ml. of solution to destroy the fluorescence. Lithium in concentrations of 0.007 gram per 10 ml. or greater gives a fluorescence like that of beryllium. This similarity in action of lithium and beryllium was also noted by Sandell (*2*) when using morin as a reagent. Colored ions such as Cr^{+++} in small quantities cause no difficulty, but a deep green solution will mask the fluorescent color. The other cations which remain unprecipitated in 0.2 *N* alkali seem to have no effect. Calcium ions in saturated calcium hydroxide produce a faint fluorescence, but the amount remaining after addition of 0.2 *N* sodium hydroxide has no effect. Small amounts of iron causing a brownish color need not be precipitated but may be rendered noninterfering by the addition of tartrate. The cations listed below under thorium were examined in this test.

The common anions, such as chloride, nitrate, sulfate, borate, and fluoride, have no effect. Ions which precipitate beryllium in alkaline solution, such as phosphate, arsenate, molybdate, tungstate, and uranate, may be nullified by having tartrate present. Tartrate decreases slightly the intensity of the beryllium fluorescence, but this is not serious unless exceedingly large concentrations of tartrate are used with small concentrations of beryllium. It requires 0.5 gram of sodium potassium tartrate tetrahydrate to destroy the fluorescence of 1 microgram of beryllium. Chromate oxidizes the reagent and destroys the test. If ammonium ions are present in the solution, care must be taken to make the solution decidedly

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alkaline, since these ions will neutralize an equal quantity of sodium hydroxide.

Thorium Test

Thorium salts in weakly acid solutions with 1-amino-4-hydroxyanthraquinone produce a purple colloid which gives a red fluorescence of the same wave-length range as that given by the beryllium in alkaline solution. With this reagent thorium gives a fluorescence only in acid solution and beryllium only in alkaline solution.

The thorium solution to be tested must be adjusted to a pH of about 2 without too large a quantity of salt present. This is best accomplished by using thymol blue as an indicator and adding acid or alkali until the yellow point representing a pH of 1.75 is reached. Neutralizing to the phenolphthalein end point precipitates the thorium, which is rather difficult to redissolve. In addition, the quantity of salt produced in this operation is likely to precipitate the thorium colloid formed. It is important not to have the solution too acid, since the reagent itself dissolves to a sufficient extent in 0.5 *N* acid to give an intense fluorescence. The purple color of the thorium complex in visible light is given in the acid solution by no other element except zirconium, and it is only to distinguish this that the fluorescence need be used. The test is not apparent for less than 40 micrograms of thorium in a dilution of 1 to 125,000.

Thorium nitrate or chloride forms the most convenient test solution, which is made to contain 1 gram of thorium ion per liter. The acidity of this concentration is satisfactory for the test without further adjustment. One milliliter of this solution is mixed with 10 ml. of water and 0.5 ml. of 0.1 per cent 1-amino-4-hydroxy anthraquinone is added. The mixture is examined either directly or under the ultraviolet lamp.

In testing for thorium from highly acidic mixtures such as are obtained from the separation of monazite sand, it is necessary to precipitate the thorium as a hydroxide, iodate, or oxalate, filter, and redissolve in concentrated hydrochloric acid or aqua regia. The latter is used in the case of the iodate and oxalate and hydrochloric acid is added to this until all the nitrate is removed. The material is then evaporated just to dryness to remove the excess hydrochloric acid, and the crystals are dissolved in water and adjusted to the proper pH with thymol blue.

Interfering Ions. The only cations, other than thorium, found to give the slightest fluorescence under the above conditions were gallium and praseodymium, which give a weaker fluorescence of a little darker shade than that of thorium. If a trace of thorium is mixed with these elements, the intensity is much greater and there seems to be little chance of confusion. Both gallium and praseodymium produce red solutions in contrast to the purple of thorium and, on standing a few minutes, they form red curdy precipitates, whereas the thorium is permanently stable. It requires 1.5 mg. of gallium and 10.0 mg. of praseodymium to produce the same fluorescence as 0.1 mg. of thorium. Zirconium and ferric ions do not cause a fluorescence but decrease the intensity of thorium. Oxidizing agents of the order of Ce^{++++}, Ag^{+}, Au^{+++}, Hg^{+}, and the ions of the platinum metals destroy the reagent.

In addition to those already indicated, solutions of the following cations were examined and found to have no effect: lithium, sodium, potassium, rubidium, cesium, copper, beryllium, magnesium, calcium, barium, strontium, zinc, cadmium, aluminum, lanthanum, cerous cerium, neodymium, a mixture of the rare earths as taken from monazite, indium, thallium, zirconium, hafnium, tin, lead, bismuth, antimony, chromium, manganese, cobalt, and nickel. The influence of some of the anions seems to be the effect of charged ions on a sensitive colloid, and in other cases precipitation of the thorium takes place. It required 4 grams of sodium chloride in 10 ml. of solution to destroy the colloid, and hence the fluorescence, produced by 2×10^{-3} gram of thorium. Phosphates, fluorides, and sulfates present in quantities expressed in grams per liter half as large as the thorium destroy the fluorescence. Iodates, arsenates, oxalates, molybdates, tungstates, and uranates precipitate the thorium.

Results with Other Hydroxyanthraquinones

With 1,8-dihydroxyanthraquinone, beryllium in alkaline solution gives a red fluorescence which is not so intense as that described above but can be used to detect beryllium in dilutions as low as 1 in 10^6. The smallest quantity detected was 1×10^{-5} gram. The other ions soluble in sodium hydroxide do not interfere. Neither beryllium nor thorium fluoresces in acid solution with this reagent.

In alkaline solution 1,5-dihydroxyanthraquinone gives a slight fluorescence with beryllium but is not of sufficient intensity to serve as a qualitative test. Neither beryllium nor thorium affects this reagent in acid solution.

No fluorescence is given by 1,2,5,8-tetrahydroxyanthraquinone with either beryllium or thorium. In slightly acid solution this reagent with aluminum gives a beautiful orange-red fluorescence (6100 to 6500 Å.) which is destroyed by the addition of thorium; 2×10^{-5} gram of thorium will destroy the fluorescence of 1×10^{-3} gram of aluminum in 10 ml. of solution. The reagent is sensitive to only 1×10^{-4} gram of aluminum in 10 ml. and this is destroyed by many other ions and by addition of acid; hence it cannot be considered a good test reagent for either aluminum or thorium.

4,8-Diamino-1,5-dihydroxy-2-sulfonic acid anthraquinone does not fluoresce with either thorium or beryllium; 1-amino-5-hydroxyanthraquinone gives a slight fluorescence with beryllium in alkaline solution but none with thorium in acid solution.

Summary

The reagent 1-amino-4-hydroxyanthraquinone serves well for detecting beryllium in alkaline solutions and thorium in acid solution. In the case of beryllium, the test is less sensitive but more specific than morin, and is much more definite than with quinalizarin.

In application to thorium the sensitivity is not so great as might be desired, but is sufficient for many practical purposes and provides a vivid color reaction for the identification of this element. Several hydroxyanthraquinones were tested with these elements and the only other one found to present analytical possibilities was 1,8-dihydroxyanthraquinone.

While all possibilities have not been tried, it seems obvious that tests with metallic ions may assist in identifying the location of groups in the anthraquinones.

Acknowledgment

The authors wish to express their appreciation to W. Reeve and W. H. Power of the Organic Department of the university for preparing the 1-amino-4-hydroxyanthraquinone and to J. Lander for preparing the 1-amino-5-hydroxyanthraquinone.

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

Correction. In the article entitled "A Fluorescent Method for Aluminum" [Ind. Eng. Chem., Anal. Ed., 9, 430 (1937)], we find it an improvement to make up the Pontachrome Blue Black R in 95 per cent ethyl alcohol and to heat the test solution to about 80° C. before adding this reagent. This gives a permanently stable reagent, and using the higher temperature gives an immediate result with low concentrations of aluminum which would require several hours to develop at room temperature.

C. E. White

A Continuous Calcium Carbonate Saturation Balance Indicator

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INTERNAL protection of most pipe lines carrying water supplies depends mainly upon the formation and maintenance of a thin protective scale high in calcium carbonate. The tendency of a water either to corrode metal or to lay a protective film may be determined experimentally by the magnitude and direction of its changes in alkalinity and pH when contacted with calcium carbonate, employing the so-called marble test (*3*). Until Enslow (*1*) devised a continuous flow tube packed with calcium carbonate, experimental technique consisted in mechanically agitating batch samples in various types of containers with calcium carbonate powder.

The assembly described below is an amplification of the Enslow tube with additional provisions which include (1) sufficient length of packed column to ensure equilibrium for the water undergoing test, (2) an internal filter to prevent powder carry-over, (3) a companion unpacked column, or synchronizing tube, to preserve proper time relationship between the original water and its stabilized counterpart, especially applicable for rapidly changing supplies, and (4) continuous e. m. f. indication by means of an integral concentration cell. As a safeguard against gas release from supplies high in carbon dioxide, water is made to flow within the apparatus against a positive head. Effects of momentary aeration, ordinarily permitted when using external electrodes, are completely avoided by making measurements within the closed path of water travel. With suitable arrangements for heating and insulation, measurements may be made at elevated temperatures. As a single operating requirement, this apparatus (Figure 1) uses a large volume of relatively clear water under pressure.

The logical locales in which this device is expected to serve most effectively in guiding plant operation to maintain effluents near calcium carbonate balance are:

Plants employing simple anticorrosion treatment, such as aeration or limestone beds. Mildly corrosive effluents are known to diminish pipe-line carrying capacity, even though red water is not evident; hence the value of a sensitive automatic indicator for this type of supply.

Plants controlling corrosion by means of alkali. The same point applies as above, with the added requirement that the treatment be not overstepped; otherwise excessive scaling is induced in hot-water fixtures.

Softening plants employing recarbonation. Proper control of the extent of recarbonation is essential, as the calcium carbonate balance for softened waters of this type may be sensitive.

Zeolite installations having a low alkalinity in the effluent. With a water of this character, which is deprived of the protective effect of both calcium and bicarbonate ions, a continuous indicator may be helpful in controlling blends with a small proportion of raw water.

For untreated aggressively corrosive waters the use of this apparatus is unnecessary, as the appearance of the effluent is sufficient evidence of corrosive attack on pipe-line metal.

Description and Operating Notes

Influent under pressure is divided equally, by means of petcocks *G* and *H* at the bottom tee connection, between the powdered calcium carbonate column, *C*, and the empty synchronizing tube, *B*, whose volume is made to equal the void space in *C*. Tube *B* thus provides a detention period equal to that of column *C* and in this way furnishes a continuous reference sample of the original water for comparison with its corresponding stabilized effluent from *C*. When leaving *C*, effluent in equilibrium with the calcium carbonate solid phase (equilibrate) passes through a coarse sintered-glass crucible or filtering tube which prevents carry-over. The packing in *C* rests on a layer of pea gravel sandwiched between retaining pads of glass wool. A vent is provided at the top for releasing air that accumulates above the crucible level when *C* is first placed in service.

Effluents from *B* and *C* flow through Gooch crucible holders *N* and *M*, respectively, which contain an antimony or glass electrode pair to form a simple concentration cell that connects with a vacuum tube potentiometer, preferably of the recording type. *X*, a potassium chloride–agar bridge, completes the circuit in which liquid junction errors cancel by symmetry. No calomel reference electrode is needed, as the differential in pH is the quantity to be measured and its value is read directly from the potentiometer. Cell effluents flow to waste continuously at the high points, W_E and W_T, after first filling the 250-ml. Erlenmeyer flasks, S_E and S_T, to furnish a continually available set of current samples for alkalinity titration.

The author's calcium carbonate column, measuring 4.8 cm. (1.875 inches) in diameter by 112 cm. (44 inches) high, allows adequate contact for the slightly scaling local water. A flow rate of from 4 to 6 ml. per minute is satisfactory; faster rates cause excessive back pressure at the bottom of column *C*, while slower rates permit an extended time lag and so may fail to represent prevailing plant conditions. The friction head ordinarily is about 137 cm. (4.5 feet) of water, although this quantity varies with the rate of flow and the degree of compaction of the powder. At the bottom of *C* the rubber stopper must be strapped securely with

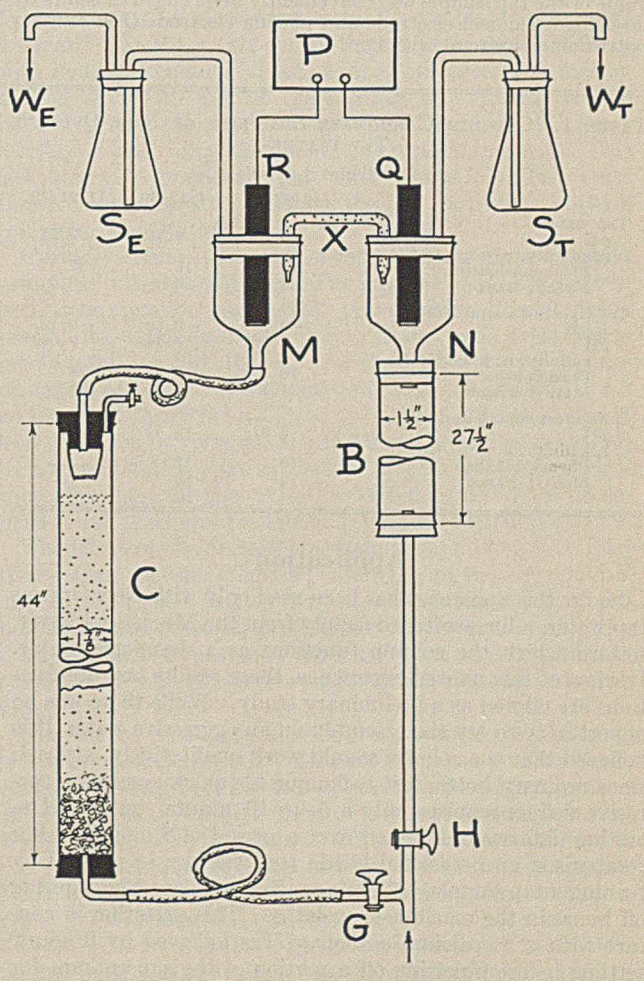

FIGURE 1. ASSEMBLY

adhesive tape to prevent a blowout. In order to expand the powder and thereby reduce the loss of head, direction of flow is upward through the column. During operation the powder settles to a level several centimeters below the crucible, so that generally only slightly turbid equilibrate reaches the filter, as the packing acts as its own upflow filter; the powder also separates at times somewhere near the middle of the column by a water space of variable length between the lower and upper portions. Through this lower portion the ascending water tends to channel against the smooth glass wall but, beyond the central liquid partition level, penetrates upward through the remainder of the packing by percolation. A number of deflecting devices placed in the bottom of *C* failed to prevent channeling and were abandoned.

It is evident that short tubes fail to guarantee complete equilibrium because of both insufficient length and lack of intimate contact. Connections to *C* are made with liberal lengths of rubber tubing to allow shaking and overturning every few weeks to break up lumps and reduce the loss of head through the powder. Gravel bottoms were found superior to simple glass wool layers in preventing powder from falling through the bottom of *C* into the inlet connection. Glass wool padding was inserted as indicated to keep the powder and the gravel in place during the overturning action.

Coated filter sand and two varieties of crushed marble were tried as packing material but with lime-softened water caused too low a phenolphthalein reading in the effluent, apparently converting some of the normal carbonates into bicarbonate alkalinity. Precipitated calcium carbonate, C. P. grade, proved to be the most reliable contact medium after the assembly was aged to leach out soluble impurities. For the internal filter, a Berkefeld candle, porosity N, was tried for a month, but did not age completely and an Alundum crucible gave indifferent results. However, no type of filter showed any tendency to clog when filtering equilibrated water.

Electrodes were checked occasionally by comparing their e. m. f. in the same solution. This was done externally rather than by adding a by-pass between vessels *M* and *N*. The pH value of the tap sample was conveniently determined in cell *N* by inserting a calomel electrode and placing electrode *Q* in another potentiometer circuit with it.

Table I. Calcium Carbonate Stability of New Orleans Tap Water

(Preliminary data)

	6/22/39	7/13/39	11/21/39
Tap water			
pH	9.88	9.79	9.81
Alkalinity, p. p. m. $CaCO_3$			
Phenolphthalein	17	15	27
Methyl orange	33	30	53.5
$CaCO_3$ column equilibrate			
Δ pH	−0.29	−0.30	−0.12
pH	9.59	9.49	9.69
Alkalinity, p. p. m. $CaCO_3$			
Phenolphthalein	10	7	18
Methyl orange	21.5	15	38
Bottle test supernatant			
pH	9.61	9.55	....
Alkalinity, p. p. m. $CaCO_3$			
Phenolphthalein	10	11	....
Methyl orange	19	22	....

Application

So far this apparatus has been used only with New Orleans tap water, a lime-softened supply from the Mississippi River, toward which the column functions as a desupersaturator. In view of this limited experience, these results and observations are offered as a preliminary study. While there was no opportunity to try the assembly on an aggressive water, it is believed that the column should work qualitatively, at least, since ordinary bottle test technique for quick results on corrosive waters requires only a 5- to 10-minute contact. Operating data were obtained over a period of 6 months. For comparison an occasional bottle test was run in parallel by turning a tap sample, held in a special cage, overnight during off hours in the machine-shop lathe. This agitation in contact with C. P. calcium carbonate was followed by 8 hours' settling before pipetting off a portion of the supernatant for titration. Data typical of results obtained are given in Table I.

Almost without a single exception, more complete stabilization was obtained in the column than in bottle tests, even though bottle agitation was extended to 60 hours, as evidenced by lower pH values and alkalinities by the column method. Throughout the period of use alkalinity values in the column effluent approached the theoretical solubility for calcium carbonate, indicating equilibrium in this regard. However, passage through the calcium carbonate tower usually caused a loss of about 1 part per million of phenolphthalein alkalinity beyond that reduced by stabilization, this result causing an apparent gain in bicarbonates ranging from 0 to 3 p. p. m.

In searching for the cause for this slight drop in the phenolphthalein alkalinity of the equilibrate, other properties than calcium carbonate quantities were determined and some were found to change on percolation through the column. Chlorine residuals of about 0.7 p. p. m. in the tap, due to chloramines, disappeared entirely in the equilibrate; instead of the usual light green residual a strong nitrite interference color, characteristically brown, formed slowly with *o*-tolidine. Ordinary bottle tests, on the contrary, always retained a strong chlorine residual. Production of nitrites within the packing from free ammonia and that added for chloramine formation was evident, and it was found that nitrite increased from 0.10 p. p. m. in the tap to 1.68 p. p. m. in the column effluent, whereas the bottle test supernatant was raised only to 0.115 p. p. m. Presence of Nitrosomonas, suggested by the elevation in nitrites, was demonstrated in the packing (*2*), although both tap water and column effluent were sterile on A. P. H. A. nutrient agar. These effects, by releasing small amounts of nitrous and hydrochloric acids which transformed normal carbonates into bicarbonates, were probably responsible for the slight depression in phenolphthalein alkalinity and in pH of the stabilized effluent from the apparatus.

Nitrate nitrogen increased very slightly from 0.10 p. p. m. in the tap to 0.20 p. p. m. in the tube effluent but the nitrate content in the bottle test remained unaffected. Passage through the column caused a drop of 1 p. p. m. in dissolved oxygen but oxygen consumed from potassium permanganate showed practically no change.

Gravimetric calcium was determined in the column effluent on several occasions in order to calculate pH_s in Langelier's formula (*4*). The calculated value for pH_s was from 0.3 to 0.6 pH unit less than the pH value of the equilibrated effluent.

Compared with the bottle test this column is decidedly superior, as it destroys supersaturation usually from 2 to 7 p. p. m. of calcium carbonate more completely and at the same time provides automatic, continuous, and reasonably current information on plant production. In waters containing free ammonia or chloramines the equilibrium pH value is slightly depressed by nitrite formation and hydrochloric acid release, but the small error of less than 0.05 pH lies on the side of safety in the case of the local water and the differential obtained with this assembly is an index of the tendency to deposit scale.

Acknowledgment

Most of the experimental work was performed in the New Orleans Water Purification Plant Laboratory. All glass electrodes and a Coleman potentiometer were borrowed from sources in the vicinity.

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Separation and Determination of Lead with Salicylaldoxime

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SALICYLALDOXIME has been used as a reagent for the gravimetric determination of copper (*3*), nickel (*9*), palladium (*5*), lead (*6*), bismuth (*4*), and zinc (*4*). Separations of copper from nickel (*1*, *2*, *9*), copper from lead (*7*), bismuth from zinc (*4*), and bismuth from silver (*4*) have been carried out by control of pH or the use of ammonia complex formation. A careful study has been made of the effect of pH on the precipitation of copper salicylaldoximate and nickel salicylaldoximate (*1*). It seemed desirable to study lead in a similar manner, inasmuch as the data obtained could be used as the basis for separating lead from other metals.

Determination of Optimum pH for Precipitation

SOLUTIONS. Standard lead nitrate solutions were prepared from twice-recrystallized reagent grade lead nitrate, dissolved in water containing approximately 0.02 mole of redistilled nitric acid per liter. Standard lead acetate solutions were prepared from twice-recrystallized analytical reagent lead acetate dissolved in 0.02 molar acetic acid solution. The lead solutions were standardized by precipitation of the lead as lead sulfate.

The salicylaldoxime was obtained from the Eastman Kodak Company. A 1 per cent solution was prepared according to Ephraim (*3*) by slowly pouring a solution of 1 gram of salicylaldoxime in 5 ml. of 95 per cent ethyl alcohol into 95 ml. of water heated to 80° C. The solution was cooled and filtered before using. It was freshly prepared the same day as used.

PRECIPITATION TECHNIQUE. A 10-ml. portion of a 1 per cent salicylaldoxime solution was added to 25-ml. portions of the standard lead solutions. Ammonia solution was added in amounts estimated to give pH values over the desired range, and in each case water was added to give a total volume of 65 ml. The resulting precipitates and solutions were each stirred for 1 hour, and the precipitates were washed by decantation and filtered with suction through No. 4 Jena glass filtering crucibles. The precipitates were washed with water until free from salicylaldoxime as shown by the absence of coloration of the filtrate upon addition of ferric chloride solution, dried at 105° C. for 1 hour, and weighed as $PbC_7H_5O_2N$.

MEASUREMENT OF pH. The hydrogen-ion concentration was measured with a glass electrode pH meter (*8*) calibrated with Clark and Lubs buffer solutions. The measurement was taken upon the first filtrate decanted from the precipitate, and before any washings had been added. The pH values were reproducible to ±0.02 pH unit.

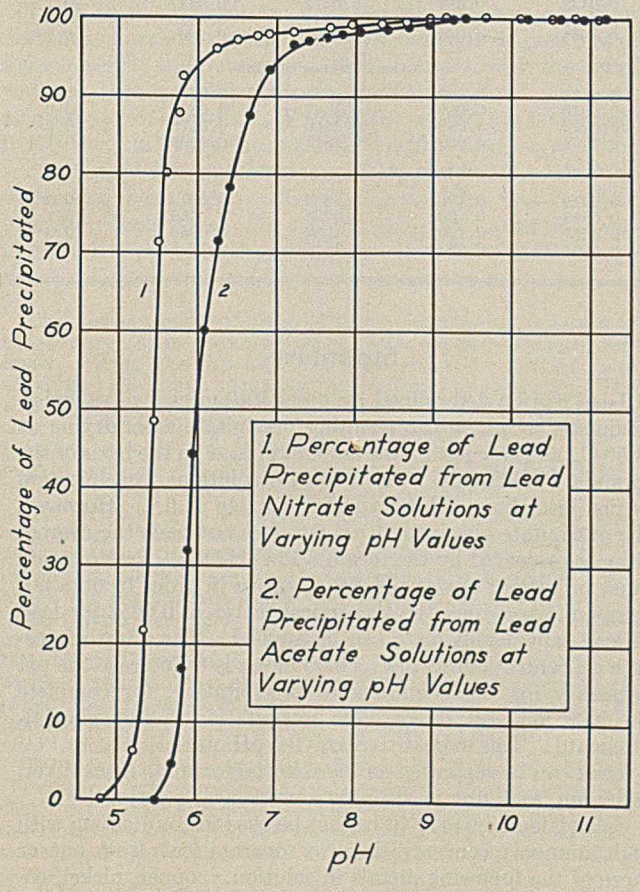

FIGURE 1. PRECIPITATION OF LEAD WITH SALICYLALDOXIME

pH RANGE FOR PRECIPITATION. The results of the precipitation of lead from lead acetate solutions are shown by curve 2, Figure 1. The amount of lead present in each determination was 0.1095 gram. The weight of precipitate should be 0.1809 gram if precipitation was complete and if the formula $PbC_7H_5O_2N$ (Pb = 0.6053) was applicable to the compound precipitated. The results show that precipitation begins at about pH 5.5, but that the pH must be equal to or greater than 9.4 before lead can be determined as lead salicylaldoximate using the theoretical factor 0.6053. Precipitation of the lead was actually complete at a much lower pH than 9.4, as shown by the saturation of the filtrate with hydrogen sulfide. There was no evidence of formation of lead sulfide in any of the filtrates with pH above 7.3, indicating that the precipitates formed up to pH 9.4 are a mixture of lead salicylaldoximate and a basic lead salt. This is contrary to previously published results (*6*), which state that it is only necessary to have the pH above 6.5 to obtain complete precipitation of lead as $PbC_7H_5O_2N$.

Curve 1, Figure 1, is a plot of representative data obtained using lead nitrate solutions. The amount of lead present in each determination was 0.0971 gram, which should give a precipitate weighing 0.1604 gram on the basis of complete precipitation as $PbC_7H_5O_2N$. As shown by the curve, precipitation of lead salicylaldoximate begins just above pH 4.8 and is complete as $PbC_7H_5O_2N$ above pH 8.9. Precipitation of lead was actually complete at pH 6.9 and above, as evidenced by no precipitation upon saturation of the filtrate with hydrogen sulfide, but the theoretical factor 0.6053 could not be applied to the precipitate obtained in the pH range of 6.9 to 8.9.

The effect of the acetate ion, even though present in relatively small concentration, is to shift the curve above 0.5 pH unit to the right. Higher acetate-ion concentration would probably necessitate an even higher pH to obtain complete precipitation. It has been reported (*4*) that complete precipitation of lead can be prevented by use of ammonium acetate.

The curves become nearly horizontal during precipitation of the last few percentages of lead. This flat portion of the curve, extending over about 2 pH units, represents the interval during which precipitation of the lead is complete, not solely as lead salicylaldoximate, but probably as a mixture of the salicylaldoxime complex and a basic acetate or nitrate. Thus, if it is desired only to separate the lead from solution, a pH of 6.9 for the nitrate solution and 7.3 for the acetate solution will suffice; but to weigh the precipitate as $PbC_7H_5O_2N$, the pH must be above 8.9 for the nitrate solution, and above 9.3 for the acetate solution.

TABLE I. AMMONIA REQUIRED TO DISSOLVE SALICYLALDOXIMATES

Metal Salicylaldoxime	Volume of Concentrated NH_3 Added	NH_3 Concentration of Resulting Solution
	Ml.	*Molar*
Silver	0.05	0.01
Zinc	0.4	0.1
Cadmium	1.2	0.3
Nickel	6.6	1.5
Cobalt	6.7	1.5
Copper	50.0	7.0

Separation of Lead

These data show that lead can be quantitatively precipitated from strongly ammoniacal solution. It seemed likely that the separation of lead from other metals which form insoluble complexes with salicylaldoxime might be effected if precipitations were carried out in solutions containing a high concentration of ammonia.

SOLUBILITY OF COMPLEXES IN AMMONIA. Solutions of Bi^{+++}, Cd^{++}, Co^{++}, Cu^{++}, Fe^{++}, Mg^{++}, Mn^{++}, Hg^{++}, Ni^{++}, Ag^{+}, and Zn^{++} ions were prepared from reagent grade salts to contain 4.0 grams of the metal per liter of solution. To 10 ml. of each of these solutions were added 20 ml. of a 1 per cent salicylaldoxime solution. The solutions were diluted to 65 ml. with water. One drop of 4 molar ammonia solution was added to each to produce precipitation of the metal salicylaldoximate, except that the addition of ammonia was not necessary to produce precipitation with copper or cobalt. Concentrated ammonia solution was then added dropwise, with stirring, to determine which of the complexes were soluble in ammonia solution, and the relative ease with which they dissolved.

It was found that bismuth, ferrous, magnesium, manganous, and mercuric salicylaldoximates did not dissolve in ammonia solution in concentrations up to 8 molar. The salicylaldoximates of the metals which form ammonia complexes dissolved upon addition of varying amounts of ammonia, as shown in Table I. These results do not represent equilibrium conditions; the precipitates undoubtedly would have dissolved with less ammonia if a longer time had been allowed between addition of successive increments. However, from an analytical standpoint, the data as given are more valuable than would be the data for equilibrium conditions.

SEPARATION TECHNIQUE. Solutions of copper, cobalt, zinc, and cadmium were prepared by dissolving reagent grade acetate salts in 0.02 *M* acetic acid. Nickel and silver solutions were prepared from reagent grade nitrates dissolved in 0.02 *M* nitric acid. Each solution contained 4.0 grams of metal per liter.

Fifteen-milliliter portions of 1 per cent salicylaldoxime solution were added to mixtures of lead acetate solutions, prepared and standardized as described above, and solutions of the various metals from which precipitation was to be attempted. Concentrated ammonia solution was then added in amount sufficient to precipitate lead salicylaldoximate and to prevent precipitation, if possible, of the salicylaldoximate of the other metal. Water was added to make 65 ml. The precipitate and solution were stirred for 1 hour, and the precipitate was then permitted to settle. The supernatant liquid was decanted through a No. 4 Jena glass filtering crucible. The precipitate was washed, by decantation, with a solution of salicylaldoxime and ammonia of the same concentration as that in which precipitation had occurred. Finally, the precipitate was washed with 20 per cent ethyl alcohol solution, by decantation and also after transfer to the filtering crucible, until it was free from salicylaldoxime. It was dried for 1 hour at 105° C., cooled, and weighed as $PbC_7H_5O_2N$.

Copper. Attempts to separate lead from various mixtures of copper solution and lead acetate solution were unsuccessful. Copper salicylaldoximate precipitated with lead salicylaldoximate from solutions with ammonia concentrations as high as 8 *M*.

Cobalt. Portions of the cobalt solution were mixed with lead acetate solution and separations were attempted employing the technique outlined above. The data in Table I indicate that cobalt salicylaldoximate would not precipitate in 1.5 molar ammonia solution, but coprecipitation of the cobalt complex with the lead actually occurred in ammonia concentrations up to 8 *M*. The precipitate obtained in all cases was a mixture of cobalt and lead salicylaldoximates.

Nickel. The results obtained were very similar to those with cobalt. Coprecipitation of nickel salicylaldoximate was not so pronounced as was the coprecipitation of cobalt salicylaldoximate, but was sufficient to make separations impossible.

Silver. Separations of lead from mixtures containing portions of the silver solution and of the lead acetate solution were accomplished, following the procedure given above. The results are listed in Table II. A volume of 2.5 ml. of concentrated ammonia solution was used in a total volume of 65 ml. of mixture. The data in Table I suggest that a much lower concentration of ammonia would prevent precipitation of silver salicylaldoximate.

Zinc. It was possible to precipitate lead salicylaldoximate uncontaminated by the zinc complex from mixtures of the zinc solution and lead acetate solution (Table II). Although zinc salicylaldoximate does not precipitate from a zinc solution containing less than 0.5 ml. of concentrated ammonia solution in a total volume of 65 ml., it was necessary to have 12.5 ml. of concentrated ammonia solution in a total volume of 65 ml. to prevent coprecipitation of zinc salicylaldoximate with lead salicylaldoximate.

Cadmium. The experiments on the separation of lead from cadmium gave results similar to those with zinc (Table II). A volume of 12.5 ml. of concentrated ammonia solution was used in a total volume of 65 ml.

Metals Not Forming Ammonia Complexes. Since the solubility experiments showed that bismuth, ferrous, manganous, magnesium, and mercuric salicylaldoximates do not appreciably dissolve in ammonia solutions, separations of lead from these ions by salicylaldoxime and high ammonia concentrations are impossible.

TABLE II. DETERMINATION OF LEAD

Lead Present	Metal Present	Precipitate Found	Lead Found	Error
Gram	*Gram*	*Gram*	*Gram*	*Mg.*
		In the Presence of Silver		
0.0219	0.100	0.0363	0.0220	+0.1
0.0438	0.080	0.0722	0.0437	−0.1
0.0876	0.080	0.1447	0.0876	0.0
0.1095	0.060	0.1808	0.1094	−0.1
		In the Presence of Zinc		
0.0438	0.100	0.0725	0.0439	+0.1
0.0876	0.080	0.1447	0.0876	0.0
0.0876	0.080	0.1450	0.0878	+0.2
0.1095	0.060	0.1811	0.1096	+0.1
		In the Presence of Cadmium		
0.0219	0.100	0.0362	0.0219	0.0
0.0438	0.100	0.0725	0.0439	+0.1
0.0657	0.100	0.1082	0.0655	−0.2
0.1095	0.060	0.1810	0.1096	+0.1

Summary

Lead can be determined by precipitation as lead salicylaldoximate, weighing the resulting precipitate after drying at 105° C., and calculating the amount of lead on the basis of the formula $PbC_7H_5O_2N$. From nitrate solutions, precipitation begins just above pH 4.8 and is complete at 6.9. However, the precipitate obtained at pH 6.9 does not have the composition represented by the formula $PbC_7H_5O_2N$. Precipitation must be carried out at pH 8.9 or above in order to obtain a compound to which the theoretical factor of 0.6053 for lead in lead salicylaldoximate can be applied. The effect of acetate concentrations as low as 0.05 molar is to increase the pH necessary for the beginning of precipitation, for complete precipitation, and for complete precipitation as lead salicylaldoximate. This increase is about 0.5 pH unit.

Lead can be separated as the salicylaldoximate from silver, cadmium, and zinc in strongly ammoniacal solutions.

Salicylaldoxime reagent cannot be used in conjunction with high ammonia concentrations to separate lead from one or more of the following metals in solution: copper, nickel, cobalt, bismuth, iron, magnesium, manganese, and mercury.

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Determination of Starch by the A. O. A. C. Malt-Diastase Method

Effect of Pretreatment of Samples

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IN THE production of starch from sweet potatoes at the Laurel Starch Plant, Laurel, Miss., it was found impossible to obtain a starch balance based on the starch content of the raw materials entering the factory and on that contained in the final products—commercial starch, pulp, and waste waters—as determined by the official A. O. A. C. malt-diastase method (*1*). The unknown loss was much greater than could be ascribed to faulty sampling or to any losses which were not being estimated.

In order to demonstrate whether this loss was actual or fictitious, starch was mechanically extracted from potatoes of known weight and determined starch content, with the aid of water and other solutions used at the Laurel plant: sulfurous acid (approximately 0.015 *N*), alkaline sulfite (approximately 0.04 *N* sodium hydroxide and 0.02 *N* sulfur dioxide), and lime water (approximately 0.02 *N* calcium hydroxide). As lime water had been used exclusively during the previous two operating seasons at the plant, particular attention was given in this study to this solution and its effect on the analysis of sweet potatoes and the products derived therefrom.

The mechanical extraction of starch from sweet potatoes in the factory (*5*) is accomplished by grinding the potatoes very finely, mixing the ground mass with water or solution, and separating the starch suspended in the liquid by screening. The washing and screening operations are repeated several times on a countercurrent principle. In these laboratory tests 500 grams of potatoes, ground with a sugar-beet sampling rasp, were thoroughly agitated with 2500 cc. of water or one of the solutions mentioned above, and the starch milk was separated from the pulp with a 200-mesh screen. This operation was repeated twice, employing a fresh portion of liquid in each cycle. The starch water obtained from the three extractions was combined and allowed to settle in a rather shallow layer for about 4 hours, after which the supernatant liquid was siphoned off and allowed to settle further overnight. The first crop of starch was collected in a Büchner funnel and washed, then transferred to a drying tray, where it was dried at about 45° C. The dried starch was weighed and analyzed. The second settlings were simply collected and the entire amount was subjected to analysis. The residual pulp was dewatered by filtering in a Büchner funnel, after which it was weighed and analyzed.

These products were analyzed by the A. O. A. C. malt-diastase (*1*) method, with very slight modifications. As it was thought that the potatoes and pulp were sufficiently disintegrated for the determination of starch, the samples were simply weighed out, transferred to Gooch crucibles, and washed with water until sugar-free. Ordinarily from 100 to 150 cc. of water were used in this step. The contents of the crucibles were transferred to beakers, the starch was gelatinized by boiling for 15 minutes on a hot plate, and 25 cc. of malt diastase, prepared by digesting 10 grams of freshly ground barley malt grain with 150 cc. of water for at least 1.5 hours were added and allowed to act for 1 hour at 55° C. The boiling and malt treatment were repeated as specified. After the malt conversion, the solutions were acidified with 5 to 8 drops of glacial acetic acid to aid clarification, cooled, made up to 250 cc., and filtered; 200 cc. of the filtrate were digested with hydrochloric acid for 2.5 hours on a steam bath, cooled, neutralized, and made to a final volume of 500 cc. Reducing sugars were determined in the resulting solutions by the Lane-Eynon titration method (*3*), employing 10 cc. of Fehling's solution. For determination of the blank, a double portion of the malt extract—100 cc.—was digested with hydrochloric acid and made up to a final volume of 500 cc. It was later found that this procedure for determining the blank, although specified by the A. O. A. C. method, introduced a slight error, which, however, did not alter the conclusions based upon these data. The error in the blank arises from the fact that heating the malt extract, acidifying, and filtering apparently remove from solution something which possesses a slight reducing action after acid digestion.

Applying these extraction and analytical procedures to several lots of sweet potatoes, an average unaccountable loss of 4.2 per cent of the starch in the potato was obtained with water extraction, 7.3 per cent with lime water, 7.1 per cent with alkaline sulfite, and 3.1 per cent with sulfurous acid. With the exercise of every possible precaution to prevent appreciable loss of starch, the only logical conclusion was that the methods of analysis were faulty; otherwise a rather constant unknown loss would be expected, regardless of the solution used in aiding the extraction of starch from potatoes. It would not seem logical to obtain a progressively larger unknown loss of starch in changing the reaction of the liquid phase from acid to alkaline. It was certain that the alkaline solutions did not dissolve any starch and there was no evidence that the actual loss of starch in the alkaline waste waters was any greater than in the acid waters.

In view of such consistent results, it was decided to pretreat duplicate samples of potatoes taken for analysis, in the last two extraction experiments, with the reagents used for the extractions—water, 0.02 *N* calcium hydroxide, and alkaline sulfite (0.04 *N* sodium hydroxide–0.02 *N* sulfur dioxide). This treatment was made after samples were weighed out and prior to washing them in the Gooch crucible. The starch values obtained were rather surprising. The samples given

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only a water treatment indicated 19.54 per cent starch, while those treated with lime gave 18.12 per cent and with the alkaline sulfite, 18.33 per cent.

When these data were used in making the starch credit for the individual extractions, the unaccountable losses became negligible in case of lime water and alkaline sulfite but with water amounted to 3.8 per cent in comparison with an average of 4.2 per cent for the complete series of eight experiments employing water.

Table I. Average Starch Value of Pretreated Sweet Potato Samples

Treatment	Starch Value	Deviation from Starch Value after Water Treatment
	%	%
Water	22.99	...
$Ca(OH)_2$, 0.02 *N*	21.98	−4.4
$Ca(OH)_2$, 0.04 *N*	21.83	−5.0
NaOH, 0.02 *N*	22.05	−4.1
NaOH, 0.04 *N*	21.98	−4.4
Alkaline sulfite { 0.04 *N* NaOH, 0.02 *N* SO_2	22.01	−4.3
Alkaline sulfite { 0.08 *N* NaOH, 0.04 *N* SO_2	22.13	−3.8
Sulfurous acid, 0.015 *N*	23.02	+0.1
Sulfurous acid, 0.03 *N*	22.99	0
Alcohol (80%)-ether	23.06	+0.3

These results suggested a series of experiments in which duplicate 5-gram samples of rasped potatoes were treated with 100 cc. of two concentrations each of calcium hydroxide, sodium hydroxide, alkaline sulfite, and sulfur dioxide for 1 hour and were compared with duplicates of the same samples treated with water and with those preserved with 80 per cent alcohol and set aside for some time before analysis. All the samples, except the last, were washed with 100 cc. of water after being transferred to Gooch crucibles and finally with a portion of alcohol. The samples preserved with alcohol were transferred to a Gooch crucible and washed with 80 per cent alcohol and finally with ether. The procedure of gelatinization and conversion with malt and acid was the same as described.

The average starch values obtained with six lots of sweet potatoes (four different varieties) analyzed in this manner are presented in Table I.

For all practical purposes, sulfur dioxide and alcohol gave results identical with water, while the alkaline solutions may be grouped together, with calcium hydroxide having slightly the greatest effect. In case of calcium hydroxide, the action responsible for the differences in starch value from that of the water-treated samples appears to be a function of its concentration, increasing as the concentration of the alkali is increased. The maximum effect with 5-gram samples of potatoes was obtained when they were treated with 100 cc. of about 0.04 *N* calcium hydroxide solution. In later tests where barium hydroxide was substituted for calcium hydroxide, similar results were obtained, but increasing the concentration of the alkali above 0.04 *N* had no additional effect. Increasing the time of the action of 0.04 *N* calcium hydroxide from a few minutes to 2 hours, either at room temperature or at 55° C., did not alter the results beyond experimental errors.

These findings are of primary importance to all analysts making starch determinations and raise the questions of how much dependence can be placed on any methods for determining starch in plant materials, what causes these differences, and just what criteria should be selected to determine the accuracy of these methods. It is obvious that many values can be obtained on a given sample with a single analytical procedure, depending upon treatments to which the sample was subjected prior to analysis.

Reasons for Deviations

In attempting to explain the reasons underlying this effect of calcium and other compounds on the determination of starch in sweet potatoes, a number of possibilities come to mind.

1. Compounds of calcium and other alkaline earths affect the reducing-sugar determination with Fehling's solution, but since sodium hydroxide exhibited an effect similar to calcium hydroxide, the amount of the latter which remained unwashed from the plant tissue could not have an appreciable effect upon the determination of dextrose itself. In fact, as much as 5 cc. of 0.04 *N* calcium hydroxide added to 100 cc. of malt extract did not change its starch value. It had long been observed that pretreatment of sweet potatoes, or pulp, with an alkali had a clarifying action on the malt-digested solutions. Such solutions filtered easily and the filtrates became sparkling clear, while water- or acid-treated samples always remained more or less opalescent and slow to filter. It has not yet been determined, however, what significance to ascribe to this behavior and appearance of the samples at this stage of the analytical procedure.

2. There might be some constituent soluble in alkaline solutions and thus removed from the potato sample which, if not eliminated, would be converted into reducing sugars and thus estimated as starch. Examination of extracts prepared by treating sugar-free samples of potatoes with weak solutions of calcium or sodium hydroxide, however, failed to reveal the presence of any substance having appreciable reducing action either before or after conversion with hydrochloric acid.

3. Since enzymes are sometimes easily affected by certain chemicals, pretreatment of the samples with calcium or sodium hydroxide might prevent the normal action of malt diastase on starch. However, starches (commercial) alone are unaffected by a pretreatment and no evidence has been found that malt diastase is unable to convert starch of the sweet potato if it is available under the existing conditions. Tissues which have been treated with calcium hydroxide do appear, however, to disintegrate with greater difficulty on boiling, so that a few unconverted starch granules are always found in the residue, when examined microscopically, even after a

Table II. Effect of Pretreatment[a] with Chemicals on Starch Values of Sweet Potatoes

Salts (0.04 *N*)	Starch Value	Deviation from Value with Water	Alkalies (0.04 *N*)	Starch Value	Deviation from Value with Water
	%	%		%	%
(Water)	23.02	...	(Water)	22.73	...
NaCl	22.65	−1.6	NH_4OH	22.34	−1.7
$BaCl_2$	22.33	−3.0	NaOH	22.06	−2.9
$NaCl_2$	22.41	−2.5	KOH	21.90	−3.6
Na_2SO_4	22.57	−2.0	$Ba(OH)_2$	21.67	−4.7
$CaSO_4$	22.41	−2.5	$Ca(OH)_2$	21.83	−4.0
$Al_2(SO_4)_3$	22.49	−2.3			
$NaHCO_3$	22.49	−2.3	Acids		
Na_2CO_3	22.49	−2.3	(0.02 *N*)		
(Water)	22.60	...	(Water)	22.60	...
KH_2PO_4	22.60	0	HCl	22.60	0
NaOCl (0.025 gram available Cl_2 per 100 cc.)	22.11	−2.1	H_2SO_3	22.60	0
			H_3PO_4	22.52	−0.5
			Acetic	22.60	0
			Oxalic	22.68	+0.5
			Citric	22.45	0.6

[a] Pretreatment consisted of adding 100 cc. of solution to 5-gram samples and allowing to stand 1 hour at room temperature before transferring to Gooch crucibles and washing with water.

TABLE III. STARCH VALUES OF SWEET POTATOES AND OF PULP AFTER REMOVAL OF STARCH WITH WATER

Expt. No.	Product	Starch Value after Treatment: H_2O	Starch Value after Treatment: 0.04 N $Ca(OH)_2$	Difference	Deviation from Value with Water
		%	%	%	%
1	Sweet potato	24.19	23.16	1.03	−4.3
	Sweet potato pulp	11.90	8.94	2.96	−25.8
2	Sweet potato	25.99	24.65	1.34	−5.2
	Sweet potato pulp	9.90	6.71	3.19	−32.2
3	Sweet potato	22.14	21.16	0.98	−4.4
	Sweet potato pulp (1st extraction)	14.51	11.43	3.08	−21.2
	Sweet potato pulp (2nd extraction)	6.77	4.27	2.50	−36.9
	Sweet potato pulp (3rd extraction)	5.10	2.77	2.33	−45.7
4	Sweet potato pulp[a] (agitated about 45 min.)	4.26	2.42	1.84	−43.1 (moisture 86.01%)
5	Sweet potato pulp[a]	4.02	2.61	1.41	−35.1 (moisture 80.64%)

[a] Coarse particles removed by 20-mesh screen.

double malt treatment. Since sodium hydroxide has an effect similar to calcium hydroxide in the determination of starch but an opposite effect on the physical properties of the pulp, there must be some other explanation for their action. There is not sufficient unconverted starch in the samples pretreated with an alkaline solution to account for the large reduction in their starch value; if present, it could be detected by simple staining with iodine or by microscopic examination, if necessary, for with potatoes the average difference corresponds to 35 mg. of starch per 5-gram sample, while with pulp it corresponds to about twice this amount.

To furnish further proof that unconverted starch was not a factor, samples of sweet potatoes were analyzed with and without further disintegration by grinding with sand. No significant increase in starch values was obtained, indicating that the samples were ground finely enough by the rasp to yield consistent results with potatoes, regardless of pretreatment with calcium hydroxide. In case of pulps resulting from the extraction of starch with water, grinding with sand did seem to increase the starch value of those samples pretreated with lime but not with water. However, this increase probably does not represent liberated starch.

4. The more plausible explanation of the effect of pretreatment upon the starch value of sweet potatoes would seem to be the presence of constituents capable of being converted into reducing compounds, probably sugars, when in their natural condition, by heating or malt diastase and acid digestion; after treatment by free alkalies or by salts, this characteristic is altered to a greater or less extent, depending upon the degree of combination which occurs and the inertness of the resulting compounds. The data in Table II, presented in support of this conception, show that acids apparently have no effect; that alkalies have the greatest effect; and that salts are intermediate in action. The effects of concentration of the various solutions were not established except with calcium and barium hydroxides. Concentrations of the reagents were kept low, to be certain that raw starch itself was not affected by them.

Because those constituents of the sweet potato, other than starch, which contribute to its starch value, appear to be fairly insoluble in cold water or the reagents used in this study, one would expect to find much greater effects caused by pretreatment in the analysis of the residual pulp after extraction of starch with the aid of water. Examination of the data given in Table III reveals this to be the case.

The extraction experiments indicate a loss of substances which interfere with starch determination in the waste waters; otherwise an appreciable unknown loss would not have been obtained when either water alone or a solution of sulfurous acid was used to remove starch from the ground potato. Treating samples for an hour or longer with water at 55° C. caused a reduction in the starch value of both potatoes and pulp, aside from any likely conversion and loss of starch caused by the naturally occurring diastase.

Pretreatment of samples with alkaline solutions prevents to a very slight degree complete conversion of starch with malt, apparently because starch from such treated material is not so readily freed by boiling. Although grinding potato samples with sand after pretreatment did not increase starch values beyond experimental errors, the effect of additional grinding of pulp samples was variable, depending upon whether the grinding was done before or after treatment with lime. The data indicate that the combination of calcium hydroxide with these compounds is not always completed by the simple treatment usually given the samples, and that increase in the starch value is largely due to the liberation of some of the material in an uncombined condition in which it is available for extraction or conversion by malt diastase, rather than to liberating additional starch. The reaction between calcium and these constituents seems to be easily affected—for instance, neutralization of the excess alkalinity with acetic acid just prior to transferring and washing the samples in the Gooch crucible appreciably raised the starch value and neutralization was less effective if the samples were treated with calcium hydroxide after being more finely ground

TABLE IV. STARCH VALUES OF SWEET POTATOES (OBTAINED BY DIFFERENT DIASTASE PREPARATIONS WITH A. O. A. C. PROCEDURE)

Expt. No.	Enzyme Preparation	Enzyme Used per sample	Starch Value after Treatment with: Water	Starch Value after Treatment with: 0.04 N $Ca(OH)_2$	Deviation from Value with Water	Iodine Reaction of Solution after Enzyme Conversion
		Grams	%	%	%	
1	Malt + Amylase	≈3.3 grams grain + 0.1	22.14	21.43	−3.2	
	Malt + Taka-diastase	≈3.3 grams grain + 0.1	21.77	21.34	−2.4	
2	Malt + Amylase	≈3.3 grams grain + 0.05	23.54	22.72	−3.5	
3	Malt	≈3.3 grams grain	23.52	22.33	−5.1	Water and $Ca(OH)_2$ samples, both yellow
	Amylase	0.005	...	21.03	...	$Ca(OH)_2$ sample, blue
	Amylase	0.010	...	20.90	...	$Ca(OH)_2$ sample, blue
	Amylase	0.020	...	21.76	...	$Ca(OH)_2$ sample, blue
	Amylase	0.050	23.36	22.19	−5.0	Water sample, yellow; $Ca(OH)_2$ sample, reddish purple
	Amylase	0.100	...	22.19	...	$Ca(OH)_2$ sample, red

with sand; the use of a large dosage of malt extract slightly increased the starch values of pretreated pulp.

In order to obtain further proof that some constituents of sweet potatoes other than starch contribute to the starch value determined by malt diastase, amylase (Wallerstein's) and taka-diastase were substituted for it, but otherwise the same procedure was used. Comparisons were made on the assumption that these preparations are mixtures of enzymes in varying proportions and that different results would be obtained after the same pretreatment if the variation in apparent starch value is due to the converting action of the enzymes. On commercial starch these enzymes gave identical values, within analytical errors, but in the analysis of sweet potatoes gave somewhat variable results (Table IV).

The low starch values obtained with less than 0.05 gram of amylase were apparently due to incomplete conversion of starch into a fully soluble form. In all these samples calcium hydroxide reduced the degree of conversion by amylase, judging from the iodine reaction. This is doubtless caused, to a certain extent, by an increase in pH above the optimum; these solutions were not buffered but the mere presence of calcium seems to possess a slight retarding action. In the case of malt diastase there was either sufficient buffering action or sufficient enzyme concentration to overcome any appreciable retarding action of the calcium compound, for the reaction of the converted starch solution was always yellow toward iodine. The pH of the lime-treated samples after conversion with malt diastase was somewhat higher than that of the untreated samples—for example, in case of potatoes, the pH was approximately 7.5 instead of 7.0. As salts themselves affect the starch determination, it is not possible to employ buffer solutions on potatoes and differentiate precisely between their effect and that of hydrogen ions, although it would be extremely desirable to do so.

Limited tests by the official A. O. A. C. method (*2*) have indicated the presence of pentosans, aside from those derived from the malt extract, in the solution obtained from fleshy plant materials after boiling with water and treatment with malt extract at 55° C. It is obvious that the pentosans were derived from the plant material, but no attempt was made to determine whether these became soluble as a result of enzymatic action of the malt extract or as a result of degradation caused by boiling the sample prior to malting. Probably the latter is partially responsible, judging by the relatively high solubility of pectinous material in hot and relatively weak salt solutions—for example, sodium or ammonium oxalate and sodium phosphate–citric acid buffer solutions whose reaction is around neutrality—which, of course, would give pentosan reactions. If such pentosans are present, they will be determined as dextrose and calculated as starch. There are probably other constituents which give pentosan reactions which also contribute to the errors of starch analysis of fleshy plant materials.

The solubility of these constituents is doubtless altered by the treatment accorded the sample prior to digestion. A relatively large proportion appears to be acidic in nature, as they seem to react with bases or salts to form compounds having a lower solubility in water or inactivity toward the enzymes of the malt extract (or both). Treatment of such fleshy plant materials as sugar-beet pulp, carrots, and rutabagas, which contain no starch or at most very little, with lime water prior to analysis reduces their apparent starch content from 58 to 100 per cent below the original value determined by the official malt-diastase method. In cases where pentosan determinations were made, pretreatment of the sample with lime water reduced the apparent pentosan content of the solution after malt treatment from 65 to 75 per cent below the original value. These results (Table V) indicate that pretreatment of vegetable matter with alkalies, particularly calcium (or barium or strontium), materially reduces the error resulting from solubilizing certain constituents which exhibit reducing power after malt and acid conversion and hence are calculated as starch.

Errors of a similar order were also evident in the recently developed polariscopic method (*4*) in which dispersion of the starch is accomplished by small amounts of sodium hypochlorite at boiling temperatures. Pretreatment of the samples with lime water seemed to correct the polarization of the starch solution prepared with the hypochlorite more effectively than it did the reducing power after malt and acid digestion. Further work should be conducted, however, to determine the accuracy of this belief, possibly on starch-free fleshy vegetable matter such as carrots to which pure starch can be added in varying proportions to simulate starchy vegetables.

TABLE V. EFFECT OF PRETREATMENT OF FLESHY PLANT MATERIALS WITH CALCIUM HYDROXIDE

Plant Material	No. of Samples Analyzed	Starch Value after Pretreatment: Water	Starch Value after Pretreatment: 0.04 *N* $Ca(OH)_2$	Difference	Deviation from Value with Water
		%	%	%	%
Sweet potatoes	51	22.25	21.55	0.70	−3.1
Sweet potato pulp	33	7.34	5.89	1.45	−19.8
White potatoes	5	13.04	12.54	0.50	−3.8
White potato pulp	2	7.21	3.97	3.14	−44.9
Dasheen	1	26.91	26.20	0.71	−2.6
Rutabagas	1	0.45	0.15	0.30	−66.7
Carrots	1	0.68	0.00	0.68	−100.0
	1	1.04	0.44	0.60	−57.7
Sugar-beet pulp	1	8.74	0.64	8.10	−92.7
	1[a]	3.19	1.35	1.84	−57.7

	Pretreatment	Pentosan Content of Solution after Malt Digestion
		%
Beet pulp[a]	H_2O	2.75
	$Ca(OH)_2$	0.44
Sweet potato pulp	H_2O	0.43
	$Ca(OH)_2$	0.11

[a] Same sample.

Summary

The data on sweet potatoes indicate that a considerable error in starch value arises with the malt-diastase method when the usual procedure is followed. Pretreatment with calcium or barium hydroxide solution prevents, to a very great extent, the action of malt diastase on certain nonstarchy constituents usually determined as starch, and for the most accurate determination of starch in sweet potatoes such pretreatment is very essential.

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Effect of Soft Glass on Melting Point of Rotenone

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JONES and Wood (*1*) have recently shown that the melting point of α-toxicarol is markedly lower in soft-glass than in hard-glass capillary tubes; one sample that melted at 230–231° C. in Pyrex melted at 205–206° C. in ordinary soft glass. The effect was shown to be due to the greater alkalinity of the soft glass. Rotenone has also been found to exhibit a less marked but definite depression of melting point in soft glass. Pure rotenone melted about 3° lower in ordinary soft glass than in Pyrex and about 5° lower in a more alkaline soft glass (Corning electrode glass No. 015) than in ordinary soft glass.

Table I. Melting Points and Degrees of Purity of Rotenone in Capillary Glass Tubes

Rotenone Sample	In Pyrex Glass		In Ordinary Soft Glass		In Corning Electrode Glass	
	Melting point	Degree of purity	Melting point	Degree of purity	Melting point	Degree of purity
	° C. (*cor.*)	%	° C. (*cor.*)	%	° C. (*cor.*)	%
Pure	163–164	100	159.5–160.5	99.4	155–156	98.6
Impure, sample A	161–162	99.8	157.5–159	99.0	154.5–155.5	98.4
Impure, sample B	151.5–153.5	97.8	149–151	97.2	146.5–148.5	96.6

This effect is important in the analysis of derris and cube roots for their rotenone content. Many workers use the melting point as a qualitative indication of the purity of the rotenone obtained. Furthermore, in the quantitative method of Meijer and Koolhaas (*3*) the melting point of the separated rotenone is used as one means of calculating the purity, and from this the content, of the sample.

The melting points of pure rotenone and of two samples of impure rotenone in Pyrex and in two soft glasses are given in Table I. The glass was cleaned, and melting points were determined by the methods used in the work on α-toxicarol (*1*). From the qualitative standpoint a melting point of 155–156°, the value obtained for pure rotenone in Corning electrode glass, would not be considered to indicate a high degree of purity. Furthermore, the melting points in soft glass are not only lower but less sharp than in Pyrex and thus give every indication of material of lower purity.

By use of a graph given by Koolhaas (*2*), the degree of purity indicated by these melting points was determined, on the assumption that the graph held for the samples used here, and these values are also recorded in Table I. Meijer and Koolhaas (*3*) state that for calculation of purity by this method the melting point should not be less than 140°; hence, samples with lower values have not been included in this work. The maximum difference in apparent purity of any one sample as calculated from the melting points in Pyrex and Corning electrode glass is shown to be 1.4 per cent. This would introduce an error of only about 0.1 per cent in the rotenone content of a 5 per cent rotenone sample and about 0.2 per cent in that of a 10 per cent sample. Although the discrepancy in melting point between Pyrex and soft glass does not introduce a very serious error into results by this method of calculation, it is a recognized source of error which is easily eliminated and should be avoided.

To specify that all melting points should be taken in the same type of soft glass is not sufficient, since the lowering effect, as stated for α-toxicarol, must depend on the degree of contact with the glass, and hence on the size of the crystals and other factors. In all analytical and investigative work dealing with rotenone and related compounds, melting points determined by capillary tube should be made in a glass of low alkalinity such as Pyrex.

It is not intended to imply that the melting-point method of determining rotenone purity is used or recommended by this writer. He merely wishes to point out to those who employ it that use of soft glass for melting point constitutes a possible source of error. As a matter of fact it is his opinion that other sources of error are also present in this method and that purity of rotenone may be more accurately determined by other means.

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Stability of the Permanganate-Periodate Color System

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IN MAKING a spectrophotometric study of the Willard and Greathouse periodate method (*2*) for the colorimetric determination of manganese the writer (*1*) found no evidence whatever of fading or other change in the color of the system over a period of two months.

The solutions which had been used in this test, containing 1.25, 2.50, 3.75, and 5.0 mg. of manganese with 10 ml. of concentrated sulfuric acid and 0.3 gram of potassium periodate per 250 ml., were allowed to stand in glass-stoppered Pyrex bottles in diffuse light for an additional 23 months. Spectral transmission curves were then made and compared with the curves given by the corresponding freshly prepared solutions. From the transmittancy at 522 mμ the percentage error in the apparent concentration of manganese was calculated (*1*) by use of the special color slide rule.

Table I. Stability of Color

Concentration of Manganese	Transmittancy at 522 mμ Fresh solution	Old solution	Apparent Change in Concentration of Manganese
Mg./l.	%	%	%
5	39.5	39.8	−0.7
10	15.2	16.6	−4.6
15	5.9	6.0	−0.5
20	2.0	2.7	−7.5

The results, given in Table I, prove that the color of the permanganate-periodate system is exceptionally stable.

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The Signer Method for Determining Molecular Weights

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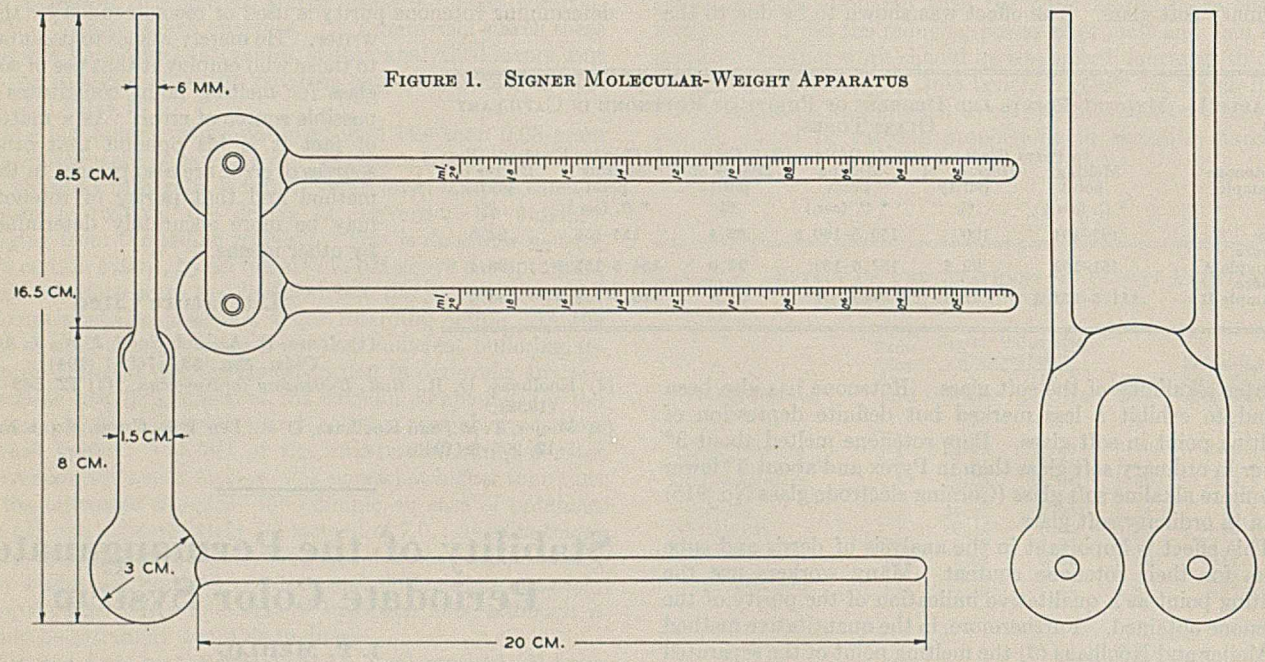

FIGURE 1. SIGNER MOLECULAR-WEIGHT APPARATUS

THE purpose of this communication is to call attention to and elaborate upon an extremely accurate but little known method for determining molecular weights. The procedure published by Signer (*1*) involves the principle of isothermal distillation and has been shown experimentally to be practical. However, the original directions are such that more specific information is desirable. For this reason a workable outline for making the determination is presented.

The experiment consists in permitting two solutions in an evacuated system, with solvent vapors in contact, to arrive at vapor-pressure equilibrium by isothermal distillation. Arrangements must be available for determining the volumes of each solution. The apparatus used to realize this is shown in Figure 1. It is smaller than the original, to make possible accurate measurements of 1.5 to 1.7 cc. of liquid. The solutions usually employed are approximately 0.1 molar, from which it follows that the quantity of substance necessary for a determination is small.

The samples of standard and unknown material, in the form of pellets, are weighed and dropped through the open side arms of the apparatus, so that one bulb receives the standard and the other the unknown. The filling tubes are then constricted near their bases to facilitate subsequent sealing. As soon as the glass cools, 2 cc. of solvent are added to each bulb, after which one tube is sealed at its constriction. As this seal is made, a very gentle stream of dry air should be blown through the tube to prevent vapors of the solvent from coming in contact with the hot glass. The system is then evacuated from a line in which is interposed 1 meter of 1-mm. capillary tubing, and in this manner approximately 0.3 cc. of solvent is distilled from each bulb. While the distillation continues, the constricted part of the connecting tube is sealed with a soft gas-oxygen flame. The closed evacuated system then contains two solutions containing definite quantities of standard and unknown material arranged as outlined above. Therefore, if the entire apparatus is isothermally insulated, solvent will distill from the solution of greater vapor pressure to the one of less, until equilibrium is established. When this occurs the volumes of the two solutions will be constant and equimolar. These volumes may then be read by tilting the apparatus and draining the solutions into the graduated side arms. Five minutes are arbitrarily taken for this purpose.

With the data thus available, it follows from Raoult's law that

$$M_1 = \frac{G_1 M V}{G V_1}$$

where M, V, and G are, respectively, the molecular weight, volume of solution, and weight of the standard, and M_1, V_1, and G_1 are the corresponding values of the unknown. In practice, the volumes are read every 1 to 3 days, depending upon the solvent used, until they become constant. The results thus obtained may be plotted (volume against time) and, if the experiment is progressing normally, smooth typical curves are obtained as presented in Figures 2 and 3.

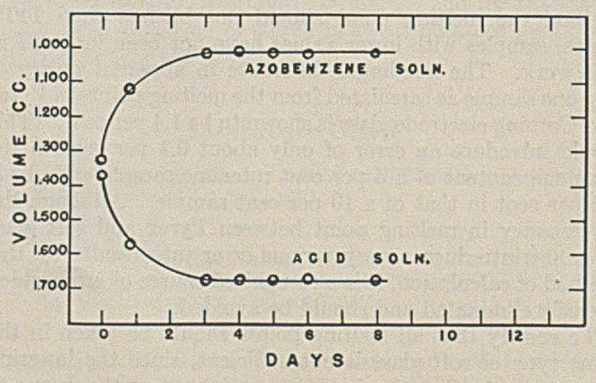

FIGURE 2. AZOBENZENE–*o*-CHLOROBENZOIC ACID

Solvent, ether; solutions 0.1416 molar at equilibrium. Molecular weight of *o*-chlorobenzoic acid, 156.5; found, 158

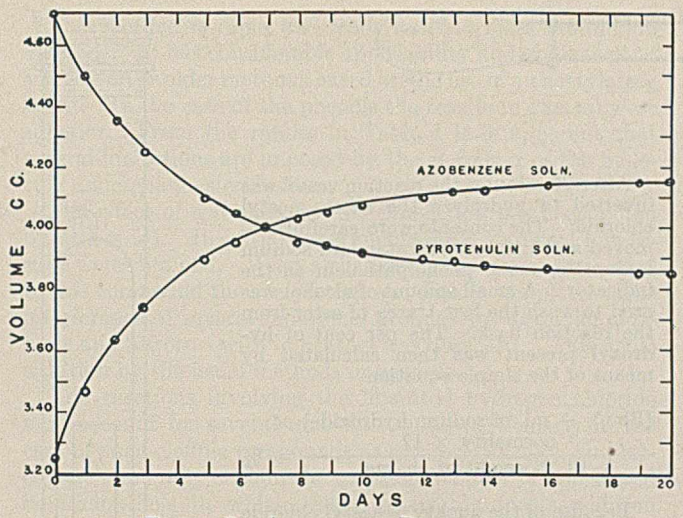

FIGURE 3. AZOBENZENE-PYROTENULIN
Solvent, chloroform; solutions 0.1367 molar at equilibrium. Molecular weight of pyrotenulin, 288.3; found, 288.4

The essential experimental factor in this determination is the maintenance of the apparatus in an isothermal condition. A very simple way to do this is to conduct the experiment at room temperature in a heavy metal container, such as an aluminum pressure cooker, which has a high thermal conductivity. Under these conditions the time necessary for a pair of solutions to reach equilibrium is greater than at elevated temperatures, but the simplicity of the procedure and the accuracy of the results warrant its use. The duration of the experiment is also dependent upon the concentration of the solutions, their relative molarity when prepared, and the solvent used. The best solvents are those with high vapor pressures. Ether, acetone, ethyl bromide, and chloroform have been employed, but others would undoubtedly be as good. These factors can be judged by a study of the curves in Figures 2 and 3, where ether and chloroform are the respective solvents. Azobenzene is an excellent standard where organic solvents are used. It is easily purified, is permanent in the air, and is readily soluble in most solvents, and the color of its solution distinguishes it from the unknown.

TABLE I. MOLECULAR WEIGHTS DETERMINED BY METHOD DESCRIBED

Substance	Molecular Weight Found	Molecular Weight Calculated
α-Nitronaphthalene, $C_{10}H_7NO_2$	173.7	173.1
Rotenone, $C_{23}H_{22}O_6$	393.3	394.3
2-(3-Chlorobenzoyl)-benzoic acid, $C_{14}H_9O_3Cl$	260.7	255
o-Chlorobenzoic acid, $C_7H_5O_2Cl$	156.5	157.4
Pyrotenulin, $C_{17}H_{20}O_4$	288.3	288.4
Isotenulin, $C_{17}H_{22}O_5$	304	306.4
Ferulic acid, $C_{10}H_{10}O_4$	195.5	198.1
p,p'-Dibromodiphenyl, $C_{12}H_8Br_2$	313	312

The procedure as outlined has given uniformly good results with all classes of compounds studied and appears to be generally applicable. It is thus one of the best methods available for the determination of molecular weights. Several results obtained by it are presented in Table I.

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Determination of Hydroxyl Content of Organic Compounds

Acetyl Chloride as a Reagent

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THE simplest methods for the determination of the hydroxyl radical in organic compounds are those based on esterification procedures. Acetic anhydride with and without pyridine has been widely used in this connection (*2*, *3*, *4*, *6*), but although acetyl chloride is a much more active acetylating agent, little attention has been given to the possibility of using it in quantitative work (*5*). It is difficult to measure and handle small amounts of this reagent.

Smith and Bryant (*5*) were the first to recognize its possibilities and employ it for analytical purposes. These workers avoided the problem of volatility and activity by employing it in the form of an acetyl pyridinium chloride suspension in toluene; in other respects their method was similar in principle to the acetic anhydride–pyridine procedures. In this indirect use of acetyl chloride it is probable that ketene (*8*) was the acetylating agent.

Much would be gained if pure acetyl chloride rather than a suspension of acetyl pyridinium chloride in toluene were used as the esterifying agent. This would eliminate the annoying pyridine vapors, the diluent and solubility effects of the toluene, and the use of a solid acetylating agent.

In order to use pure acetyl chloride a way must be found to measure and control it. Acetyl chloride reacts very slowly at low temperatures even with methanol; consequently solid carbon dioxide offers a convenient means of controlling its reaction rate. Linderstrom-Lang and Holter (*1*) have described an ingenious pipet for the precise measurement of small volumes. Using a modified form of this instrument, a simple technique has been developed for using pure acetyl chloride in determining the hydroxyl content of organic compounds.

Experimental

REAGENTS. Acetyl chloride, Eastman (practical), sodium hydroxide, 0.3 *N* (carbonate-free), phenolphthalein indicator, and dry ice.

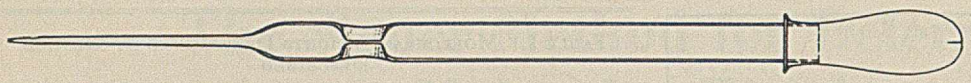

FIGURE 1. DIAGRAM OF PIPET

APPARATUS. The pipet illustrated diagrammatically in Figure 1 was constructed from 10-mm. Pyrex tubing, was 21 cm. over-all, and delivered 0.5 ml. of liquid. Its two important features were the 0.1-mm. constriction and the split rubber cap. By pressure parallel to the slit, the cap functioned as a medicine dropper. When a slight pressure was exerted in the opposite direction, the slit opened and the contents were then free to drain.

The pipet was filled in the manner of a medicine dropper, the slit was opened, and the contents were drained to the constriction. The acetyl chloride could now be transferred to the reaction vessel. In delivery, by opening the slit, the contents of the pipet were allowed to drain into the lower capillary and then a slight pressure was exerted on the closed rubber cap, slowly forcing all the liquid out of the pipet.

The reaction vessel (Figure 2) was constructed from a 20-cm. (8-inch) and a 10-cm. (4-inch) test tube. An inverted 15-cm. (6-inch) test tube fitted with a glass hook served to seal the water trap.

ANALYTICAL PROCEDURE. Samples of 0.1 to 0.750 gram (depending on the compound) were weighed in a 10-cm. (4-inch) test tube which in case of volatile materials was stoppered during the weighing. The test tube was then immersed in dry ice. After the charge was sufficiently chilled, a measured charge of acetyl chloride was added with the pipet and the small test tube was placed in a 20-cm. (8-inch) test tube containing 5 to 10 ml. of water. As indicated in Figure 2, a 15-cm. (6-inch) test tube inverted over the smaller test tube served as a seal. The tube was then stoppered and placed in a bath at 40° C. for 20 minutes. In many cases this was unnecessary, since reaction took place immediately on coming to room temperature.

After 20 minutes the reaction vessel was inverted to hydrolyze the excess acetyl chloride. The contents were carefully removed and titrated with standard sodium hydroxide, using phenolphthalein as the indicator. A small amount of alcohol was used to wash the last traces of ester from the reaction flask. The per cent of hydroxyl present was then calculated by means of the simple equation

$$\frac{(\text{Blank} - \text{ml. of sodium hydroxide}) \times \text{normality} \times 17}{10 \times \text{weight of charge}} = \%$$

Because of the quality of acetyl chloride used and because its titer may change with temperature, it is necessary to make blank determinations simultaneously. These blank runs also serve as a good check on the analyst's precision. Whenever fading of the end point occurred, the base was added in small increments (0.03 ml.) until the color persisted for 30 seconds.

Results and Discussion

The results obtained with this procedure are given in Table I.

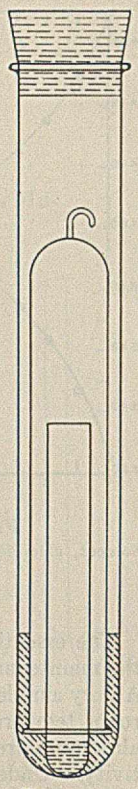

FIGURE 2

TABLE I. ACETYLATION OF ALCOHOLS AND PHENOLS

	No. of Detns.	Per Cent of Theoretical Hydroxyl Content	Average Deviation
Primary and secondary alcohols			
Methanol	4	95.4	0.3
Ethanol	5	95.7	0.2
Propanol-1	4	95.4	0.3
Propanol-2	4	95.4	0.1
Butanol-1	5	98.5	0.5
2-Methylpropanol-1	5	98.9	0.3
Butanol-2	3	98.2	0.1
Pentanol-1	5	96.1	0.3
3-Methylbutanol-1	5	100.1	1.0
Pentanol-3	5	99.0	0.2
Hexanol-1	5	93.9	0.2
Cyclohexanol	5	99.7	0.2
2-Ethylhexanol-1	5	100.0	0.1
Octanol-2	5	98.6	0.2
Lauryl alcohol	4	97.2	0.8
Benzyl alcohol	5	101.0	1.0
Furfuryl alcohol	5	[a]	
Cinnamyl alcohol	5	99.0[a]	1.2
Tertiary alcohols			
2-Methylpropanol-2	5	49.0[b]	1.9
2-Methylbutanol-2	5	88.7[b]	2.4
Benzopinacol	1	3.5[c]	
Polyhydric alcohols			
Glycerol	4	98.8	0.4
Ethylene glycol	4	99.1	0.3
Propylene glycol	4	99.0	0.4
Diethylene glycol	5	99.0	0.4
Mannitol	3	93.4[d]	3.2
Substituted alcohols			
Ethylene chlorohydrin	5	98.6	0.2
1,3-Dichloropropanol-2	4	100.3	0.3
Terpenes			
Borneol	5	100.9	0.1
Menthol	5	100.7	0.3
Geraniol	5	140.4[c]	4.5
Linaloöl	5	131.0[c]	1.5

Interfering factor:
[a] Poor end point, unable to titrate.
[b] Reacts with evolved hydrogen chloride.
[c] Rearrangement.

	No. of Detns.	Per Cent of Theoretical Hydroxyl Content	Average Deviation
Miscellaneous			
Lithium lactate	3	102.2	1.5
Rochelle salt	3	65.8[d]	1.7
Citric acid	3	38.3[d]	3.1
Benzoin	2	124.1[f]	3.2
Phenols			
Phenol	5	99.4	0.2
o-Cresol	5	100.6	1.1
m-Cresol	4	99.9	0.2
p-Cresol	5	101.0	0.5
p-*tert*-amyl phenol	2	101.2	0.1
α-Naphthol	2	107.7[c]	4.5
β-Naphthol	2	110.6[c]	5.5
Thymol	5	99.5	0.7
Xylenol	2	99.3	0.3
Catechol	4	100.1	0.5
Hydroquinone	2	98.2	0.3
Resorcinol	2	98.7	0.3
Orcinol	2	98.0	1.4
Phloroglucinol	5	[a]	
Pyrogallol	2	98.7	0.8
Substituted phenols			
Guaiacol	5	99.7	0.5
Isoeugenol	5	137.2[c]	4.1
Vanillin	5	[a]	
p-Chlorophenol	3	101.0	0.4
o-Chlorophenol	5	99.2	0.5
2,4-Dichlorophenol	2	96.5	0.1
3-Bromo-4-phenylphenol	2	100.6	1.3
2,4,6-Triiodophenol	1	8.1	
o-Nitrophenol	2	100.9	1.1
m-Nitrophenol	2	103.1	2.1
p-Nitrophenol	2	100.0	0.9
2,4-Dinitrophenol	2	9.5	0.5
2,4,6-Trinitrophenol	5	1.6	
3,5-Dinitro-*o*-cresol	2	[a]	
Salicylic acid	2	3.3	
m-Hydroxyl benzoic acid	2	61.9	4.3
p-Hydroxyl benzoic acid	2	89.8	6.5
Methyl salicylate	5	14.8	3.1
α-Nitroso-β-naphthol	2	[a]	

[d] Solubility.
[c] Possibly addition with evolved hydrogen chloride.
[f] Enolization.

This method is rapid and lends itself to mass production methods. It has considerable applicability and gives as good a precision as older methods based on the use of an acetylating agent. In the case of the phenols the results in general were superior. From the results in Table I it is apparent that several limitations are imposed by the character of the molecule undergoing esterification.

The effect of solubility in this connection has been noted by others (*5*). In initial experiments with mannitol low results were obtained. When a small amount of water was added to this compound the acetylation was almost complete. The interference of several functional groups—for example, aldehyde and nitroso—with indicators was observed. This made titration by the usual methods impossible.

Side reactions involving the liberated hydrogen chloride may account for several unusual results, particularly in the case of such olefinic compounds as geraniol, linaloöl, and isoeugenol, and may explain the unusual behavior of alpha- and beta-naphthols in which one ring readily forms addition products (*7*). The data obtained with tertiary alcohols can also be explained on this basis, since such alcohols are known to react readily with hydrogen chloride (*7*).

Interesting observations were noted in the studies of the substituted phenols. As indicated in Table I, the more acidic phenols such as picric acid were not acetylated. This was to be expected, as the hydroxyl group is more acidic than phenolic in character and therefore behaves as an acid (*7*).

The failure to acetylate salicylic acid and its isomers cannot be adequately explained on this basis; perhaps it is more a question of chelation. It is evident that such factors as solubility, chelation, unsaturation, enolization, and rearrangement are important in determining the hydroxyl content of organic compounds by the use of acetyl chloride.

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A Method of Installing Tube-Wall Thermocouples

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THE determination of heat transfer coefficients through liquid films requires a method of measuring tube-wall temperatures.

McAdams (*4*) gives an excellent discussion of the measurement of surface temperature, citing the literature on the subject. Colburn and Hougen (*3*) also discuss the errors resulting when thermocouple junctions are installed directly on the surface or when the leads are brought out through a medium either hotter or colder than the junction. All workers agree that it is advisable to bring the leads from the thermocouple junctions through a substantially isothermal zone (as through the metal wall itself rather than through the fluid stream), but McAdams states that the difficulty of construction has probably prevented wide use of this method.

Rietschel (*6*) installed the thermocouple junctions in grooves in the wall of a pipe or tube and brought the leads out in grooves covered over with litharge and glycerol cement. Reiher (*5*) and Colburn and Hougen (*3*) also used a groove installation and depended on litharge and glycerol cement for insulation, although the leads were finally brought out through the hot vapors.

Baker and Mueller (*2*) used an installation similar to that of Colburn and Hougen, in that a groove was cut three quarters of the distance around the tube and the junction was installed in a drilled hole at the end of the groove. The leads were sealed into the groove and finally carried out through the hot vapors. Various methods of sealing the lead wires into the groove were tried by Baker and Mueller who state that "pure Bakelite varnish plus a filler had a tendency to shrink and crack in service. Litharge and glycerol cement disintegrated. Litharge and glycerol cement with a covering of the Bakelite varnish also failed." This was probably due to the fact that they condensed vapors of organic compounds as well as steam. In the method finally adopted, they enclosed the leads in small brass tubes and sealed them into the grooves with solder which was polished until flush with the surface of the heat transfer tube. However, the leads were brought out of the condenser shell through the hot vapors.

Akin and McAdams (*1*) installed thermocouple junctions in holes drilled tangentially in the wall of the heat transfer tube and insulated the lead wires with Pyrex capillary tubing.

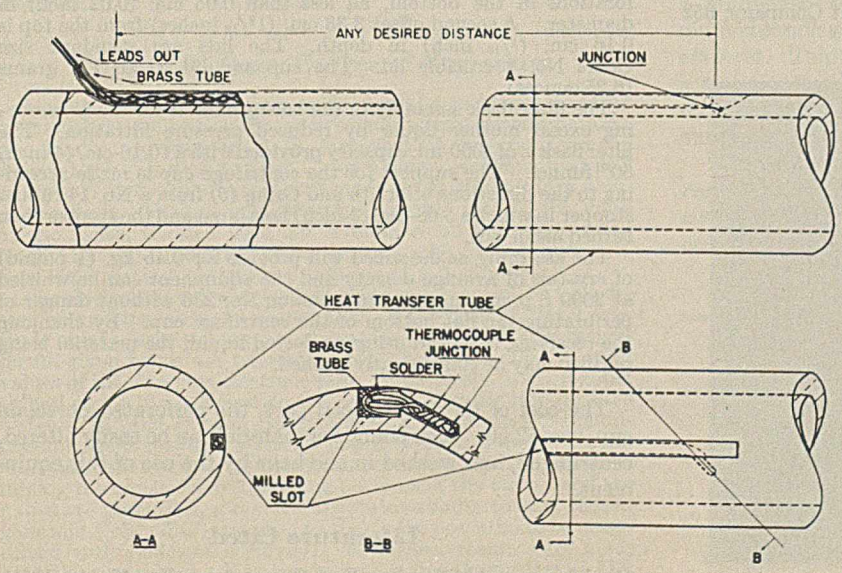

FIGURE 1. DETAILS OF INSTALLATION

In investigating the heat transfer coefficients for the condensation of mixed vapors of turpentine and water on a single horizontal tube, the authors sought a method of tube-wall thermocouple installation which would meet the following stipulations: (1) lead wires to be brought out entirely through a substantially isothermal zone; (2) no insulation to be exposed to turpentine, which readily attacks and softens litharge-glycerol cement and similar materials; (3) con-

struction to be sufficiently simple to be performed in the average machine shop at moderate cost.

The method which was finally worked out apparently satisfies these conditions.

Details of the installation appear in Figure 1, which shows only the tube and the method of installing the thermocouple junctions in the tube wall. This tube could be that of a single-tube test condenser, liquid-to-liquid heat exchanger, evaporator, or other piece of experimental equipment where the measurement of tube-wall temperature is necessary. The tube used in this work was 1-inch, extra-heavy copper pipe.

A slot is milled in the outside wall of the tube, parallel to the longitudinal axis, from the point at which the junction is to be installed to a point where the leads may be brought out without influencing the transfer of heat or the flow of fluids. This operation is easily performed in a well-equipped machine shop. The junction is installed in a 0.062-inch hole drilled 0.25-inch into the tube wall at an angle from the slot. The junction is inserted in this hole and soldered in place. The leads are enclosed in a small brass tube and laid in the slot. The brass tube is bent slightly at the end and butted against the wall of the slot, in such a way that it completely covers the leads. The slot is then filled in with solder and is polished down to the original contour of the tube. The size of the slot depends on the size of the brass tubing necessary to enclose the leads. For a pair of No. 24 enameled, silk-covered wires, a brass tube 0.093 inch in outside diameter and 0.071 inch in inside diameter will be found satisfactory. This tube will fit nicely in a milled slot about 0.10 inch wide and 0.10 inch deep. By making the slot wider, two or three sets of leads may be brought out from each end of the tube, and if more than four to six couple installations are desired in one tube wall, the number of slots may be increased.

This method of installation may be used to locate a thermocouple junction at any point in the wall of the tube and the leads may be carried to any desired point through a substantially isothermal zone. The possibility exists that the solder, having surface characteristics different from those of the tube metal, might influence the flow of condensate over the surface of the tube. This effect could be eliminated by plating the surface of the tube after the thermocouple installation. If the heat flux is high, a correction should be made for the temperature drop between the tube surface and the point of junction installation. This can easily be done when the heat flux and the thermal conductivity of the tube metal are known.

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Small-Scale Centrifuge Accessories for Use with Corrosive Materials

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THE purification of 0.45 to 2.27 kg. (1 to 5 pounds) of reagent chemicals by recrystallization from strong acid or alkaline solutions presents a special problem in apparatus assembly. Where the liquids involved are not particularly corrosive Johnson and Miller (*1*) suggest the use of centrifuge cups and receivers machined from Lucite. The present description involves an improvement, since standard parts are involved and possible interaction of equipment and reagents is eliminated.

The apparatus is shown in Figure 1, mounted on an International centrifuge size 2 (International Equipment Company, 352 Western Ave., Boston, Mass.). In the center foreground is a half section which illustrates the assembly of trunnion cup No. 373 with rubber cushion insert No. 582 and bottle No. 558, cut into two parts and the top discarded. The special centrifuge cups were made by the Coors Porcelain Co.

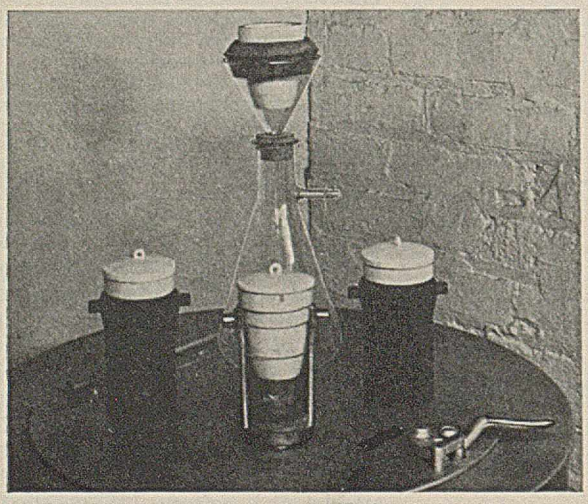

FIGURE 1. APPARATUS

The centrifuge cups are 8.57 cm. (3.375 inches) tall with an outside diameter at the base of 4.13 cm. (1.625 inches) and at the top of 5.72 cm. (2.25 inches). The inside diameter at the top is 5.4 cm. (2.125 inches) and the upper flange is 1.75 cm. ($^{11}/_{16}$ inch) wide and is offset 0.32 cm. (0.125 inch) to provide a shoulder support on top of the trunnion cup. The inside depth is 8.26 cm. (3.25 inches) and the capacity is 170 ml. The cups are glazed inside and outside except for the bottoms. There are 190 to 200 perforations in the bottom, all less than 0.05 cm. (0.02 inch) in diameter. A second offset 3.33 cm. ($1^{5}/_{16}$ inches) from the top is 0.16 cm. ($^{1}/_{16}$ inch) in depth. The lids are standard size Coors No. 4 crucible lid. The cup and lid weigh 177 grams (6.25 ounces).

The filter flask assembly in the background is for use in removing excess mother liquor by reduced pressure filtration. The filter flask is of 4000-ml. capacity provided with a 10.16-cm. (4-inch) 60° funnel. The support for the centrifuge cup is made according to the directions of Smith and Gring (*2*) from a No. 14 rubber stopper in which a 5.08-cm. (2-inch) hole is cut and the stopper then turned inside out.

The assembly as described will provide for 0.45 kg. (1 pound) of crystals of average density and the equipment can be whirled at 2000 r. p. m. supported in trunnion No. 236 without danger of perforating the flat bottom of the centrifuge cup. By changing the receiving cups and using a selected liquid, the material being purified may be conveniently washed.

The cost of the only special part, the perforated porcelain cup, was $2.50. Ten pounds of material can be easily filtered, centrifuged, and washed in one hour by the use of this equipment.

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Europium and Ytterbium in Rare Earth Mixtures

Polarographic Determination

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IT IS well known that europium and ytterbium differ from the rest of the rare earth metals in their relative ease of reduction from the trivalent to the divalent state. Bruckl and Noddack (*1*, *12*) have determined the reduction potentials of various rare earth sulfates in 0.01 *M* solution, in the absence of indifferent electrolyte, using the dropping mercury electrode. In each case, two polarographic waves were obtained, indicating a two-step reduction, first to the divalent ion and then to the metal. Their results suggested the possibility of determining either europium or ytterbium, or both simultaneously, in rare earth mixtures.

Holleck (*3*) has proposed an indirect polarographic method for the determination of europium, involving addition of a known concentration of zinc to the rare earth solution and calculation of the europium concentration from the relative wave heights obtained for europium and zinc. Such a method tacitly assumes that the zinc ion and the europic ion have equal diffusion coefficients. Owing to the larger size of the hydrated europic ion and its higher charge (*3*), its diffusion coefficient would be expected to be lower than that of the zinc ion. It is shown below that Holleck's method is only approximate.

No polarographic methods have previously been suggested for the determination of ytterbium.

It was first necessary to determine the current-voltage curves of pure europium and ytterbium in the presence of an excess of suitable indifferent electrolyte to establish a basis for the detection and determination of these elements. Ammonium chloride was chosen as the indifferent electrolyte, because it provides a sufficiently acid medium to prevent the hydrolysis of dilute rare earth chlorides without causing interference with the ytterbium wave due to hydrogen evolution. Moreover, ammonium chloride is very easily removed from the samples, allowing a simple recovery of the rare earth material.

Current-Voltage Curves of Europium

A sample of pure europium oxide, one of a series prepared by fractional crystallization for use in atomic weight determinations, was kindly provided by B. S. Hopkins. A 0.1-gram sample, weighed after ignition to 700° C. for 5 hours, was dissolved in 2 ml. of 6 *N* hydrochloric acid and made up to 100 ml. in a volumetric flask. Various portions were pipetted into 50-ml. volumetric flasks, titrated with 0.5 *N* ammonium hydroxide using methyl red as an indicator, and adjusted to the desired concentration of ammonium chloride by addition of 1 *N* solution.

The electrolysis cell and dropping electrode were of the type described by Lingane and Laitinen (*9*). A saturated calomel electrode of large area was used as an anode. All potential measurements given below are referred to this electrode. The *m* and *t* values of the dropping electrode were, respectively, 1.931 mg. per second and 4.10 seconds with the capillary dipping into distilled water at 25° and an open electrical circuit. For most of the diffusion current readings, a Fischer Electropode with the galvanometer scale calibrated to read microamperes was used. For determining the equations of the rising portions of the curves and the half-wave potentials, a manual apparatus similar to that of Lingane and Kolthoff (*8*) was used to enable the potential to be determined with sufficient accuracy. All measurements were made with the electrolysis cell immersed in a water thermostat at 25°, regulated to ±0.02°.

Typical current-voltage curves for various concentrations of trivalent europium in 0.1 *N* ammonium chloride solution are shown in Figure 1. The values of the diffusion current, i_d, at −0.9 volt are given in Table I, together with the values corrected for the residual current. A satisfactory proportionality between the diffusion current and the europium concentration was observed.

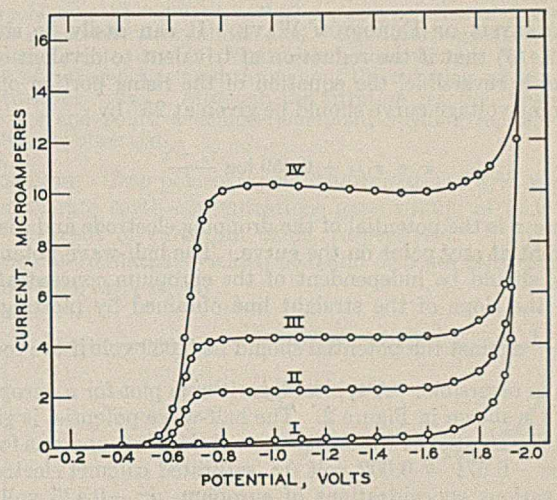

FIGURE 1. REDUCTION OF EUROPIUM IN 0.1 *N* AMMONIUM CHLORIDE

A millimolar solution of europium yielded a diffusion current of 2.88 microamperes. With the same dropping electrode, a millimolar solution of zinc chloride in 0.1 *N* ammonium chloride gave a corrected diffusion current of 6.34 microamperes. Taking into account the fact that the reduction of a zinc ion requires two electrons, it is evident that a 10 per cent correction is necessary if zinc is used as a standard in europium determinations.

It is apparent from Figure 1 that the diffusion current regions of europium, particularly with the highest europium concentration (curve IV), show a downward trend with increasing negative potential. This behavior, although de-

TABLE I. DIFFUSION CURRENT OF EUROPIUM IN 0.1 *N* AMMONIUM CHLORIDE

C	i_d Observed	i_d Corrected	i_d/C
Millimoles/liter	*Microamperes*		*Microampere/millimole/liter*
0.693	2.20	2.01	2.90
1.386	4.19	4.00	2.89
3.466	10.25	10.06	2.90
6.932[a]	19.75	19.56	2.83
			Av. 2.88

[a] In 0.2 *N* NH_4Cl.

scribed as anomalous by Holleck (*4*), is to be expected from the Ilkovic (*5*) equation for the diffusion current

$$i_d = 0.63\ nFCD^{1/2}m^{2/3}t^{1/6} \qquad (1)$$

The decreasing value of the drop time, t, with increasing negative potential causes a decreasing diffusion current. The total observed current is the sum of the residual current (curve I) and the true diffusion current. Because of the upward slope of the residual current line, the observed current increases continually with very low concentrations of europium but decreases with increasing negative potential at higher concentrations. The following data show that the effect is quantitatively predicted by Equation 1.

At a potential of −0.9 volt, the diffusion current, corrected for residual current, is 10.06 microamperes, while at a potential of −1.6 volts it is 9.53 microamperes. In this interval of potential the drop time changed from 4.06 to 2.98 seconds, while m remained practically constant. Multiplying the current of 9.53 microamperes by the factor $(4.06/2.98)^{1/6}$, we have 10.04 microamperes, which is in excellent agreement with the observed value at −0.9 volt.

Analysis of Europium Wave. It can easily be shown (*2*, *7*, *13*) that if the reduction of trivalent to divalent europium is reversible, the equation of the rising portion of the current-voltage curve should be given at 25° by

$$\pi = \pi_{1/2} + 0.059 \log \frac{i_d - i}{i} \qquad (2)$$

where π is the potential of the dropping electrode and i is the current at any point on the curve. The half-wave potential, $\pi_{1/2}$, should be independent of the europium concentration, and the slope of the straight line obtained by plotting log $\frac{i_d - i}{i}$ against the potential should be 0.059 volt if the reduction is reversible. A typical logarithmic plot for a europium wave is shown in Figure 2. The half-wave potential is given by the potential at which log $(i_d - i)/i$ is zero, and was found to be −0.671 ± 0.002 volt (*vs.* saturated calomel electrode) for various concentrations of europium or −0.425 volt referred to the normal hydrogen electrode.

The average slope of the logarithmic plot was found to be 0.079 volt rather than 0.059 volt as given by Equation 2, indicating that the dropping electrode does not behave as a strictly

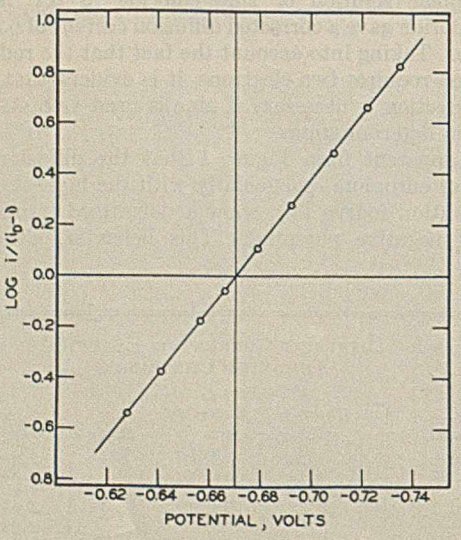

Figure 2. Analysis of Europium Reduction Curve

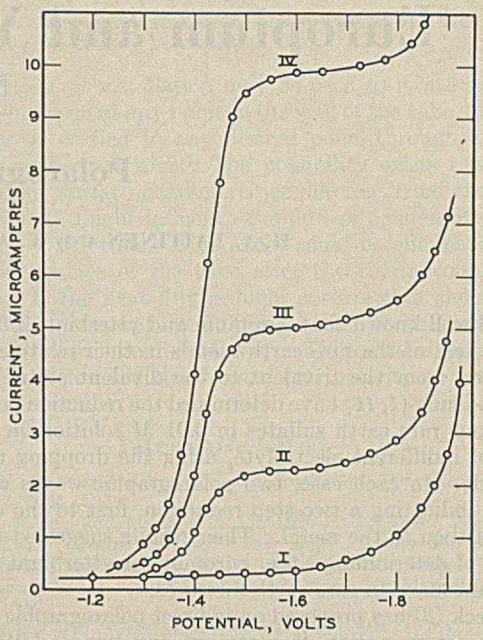

Figure 3. Reduction of Ytterbium in 0.1 *N* Ammonium Chloride

reversible europic-europous ion electrode. However, the deviation from reversible behavior is only small, as can be seen by a comparison of the half-wave potential with the normal potential of the europic-europous ion electrode.

It can easily be shown that the half-wave potential and the normal potential, π°, are related by Equation 3 (assuming reversible electrode behavior).

$$\pi_{1/2} = \pi^\circ + RT/F\ ln\ \frac{\gamma_{III}D^{1/2}_{II}}{\gamma_{II}D^{1/2}_{III}} \qquad (3)$$

γ_{III} and D_{III} are, respectively, the activity coefficient and diffusion coefficient of the trivalent ion and γ_{II} and D_{II} are the same quantities for the divalent ion. Although the values of γ and D for these ions are not known, it is reasonable to assume that $\gamma_{II} > \gamma_{III}$. Also $D_{II} > D_{III}$ because of the higher hydration of the trivalent ion. Thus the last term of Equation 3 cannot be large, and the half-wave potential should closely approach the normal potential if the electrode behavior is reversible. Actually the value of −0.425 volt for the half-wave potential is in close agreement with the value −0.43 volt for the normal potential (based on concentrations rather than activities) reported by McCoy (*11*).

Current-Voltage Curves of Ytterbium

A sample of ytterbium oxide prepared by a threefold electrolytic reduction of a rare earth fraction high in ytterbium content was available. Spectroscopic analysis showed traces of thulium and lutecium. The sample was dissolved in hydrochloric acid and treated in the same way as the europium sample.

Typical current-voltage curves for various concentrations of ytterbium in 0.1 *N* ammonium chloride are shown in Figure 3. The diffusion current values at −1.6 volts are given in Table II. By logarithmic plots similar to that shown in Figure 2, the half-wave potential was found to be −1.415 ± 0.003 volts (*vs.* saturated calomel electrode) or −1.169 volts (*vs.* normal hydrogen electrode). The average slope of the logarithmic plots was found to be 0.066 volt.

TABLE II. DIFFUSION CURRENT OF YTTERBIUM IN 0.1 *N* AMMONIUM CHLORIDE

C Yb^{+++}	i_d Observed	i_d Corrected	i_d/C
Millimoles/liter	*Microamperes*		*Microamperes/millimole/liter*
0.623	2.32	1.93	3.10
1.559	5.21	4.82	3.09
2.595	8.32	7.93	3.06
3.110	9.89	9.50	3.06
		Av.	3.08

Comparison of Diffusion Currents of Europium and Ytterbium

Owing to the closely similar structure of the various trivalent rare earth ions, and particularly the nearly identical conductance values of rare earth salt solutions (*6*), a much closer agreement would be expected between the diffusion current constants of europium and ytterbium than is shown by Tables I and II. In order to make an exact comparison it is necessary to correct the observed values to the same value of the capillary constant, $m^{2/3}\,t^{1/6}$. Correcting the observed value of 2.88 microamperes per millimole per liter for europium to the diffusion current region of ytterbium at -1.6 volts, we have 2.74 microamperes per millimole per liter. If the observed difference between europium and ytterbium were entirely due to a difference in the diffusion coefficient, a simple calculation from Equation 1 shows that the diffusion coefficients of ytterbium and europium would be in the ratio of 1.26 to 1.

It is probable, however, that the diffusion coefficients of europium and ytterbium ions are nearly equal and that another explanation accounts for the inequality of the diffusion currents. It is well known that ytterbous ions have a pronounced tendency to react with hydrogen ions according to the equation

$$2Yb^{++} + 2H^+ \rightarrow 2Yb^{+++} + H_2 \qquad (4)$$

while europous ions have a much smaller tendency to undergo a similar reaction in a medium of the same pH. Thus the diffusion current value of ytterbium would be increased because of the increased supply of ytterbic ions at the electrode surface due to the reaction represented by Equation 4. If this view is correct, a slightly increased diffusion current of europium would be expected in a very strongly acid medium, in which the rate of oxidation of europous ions by hydrogen ions is increased. Actually, in 1.2 *N* hydrochloric acid solution a 5 per cent increase in the diffusion current of europium was observed. At higher acid concentrations no further increase was obtained, but a shift of the half-wave potential of europium to more negative values indicated a formation of complexes of europium with a large excess of chloride, with a consequent change of the diffusion coefficient. A similar effect could not be directly determined in the case of ytterbium because of interfering hydrogen-ion discharge even in very slightly acid medium. However, even in the presence of 10^{-4} *N* hydrochloric acid added to 0.1 *N* ammonium chloride, the observed diffusion current of 1.5×10^{-3} *M* ytterbium increased 7 per cent, partly because of hydrogen-ion discharge. Therefore great care must be taken to neutralize the excess acid used in dissolving ytterbium samples.

Determination of Europium

Since europium occurs only in very small concentrations in natural rare earth minerals, the usual analytical problem involves its determination in rare earth mixtures in which the europium concentration has been greatly increased by partial separation. In the present study, a synthetic mixture of europium and ytterbium oxides was analyzed for europium by the polarographic method and by titration after reduction with the Jones reductor.

PROCEDURE. For the polarographic analysis, a weighed sample was dissolved in hydrochloric acid and treated as described above. The final electrolysis was carried out in a medium of 0.1 *N* ammonium chloride. From the diffusion current value at a potential of -0.9 volt, the percentage of europium was calculated, using the calibration value from Table I. A current voltage curve of 25 ml. of solution containing 0.00995 gram of rare earth as oxide is shown in Figure 4.

The volumetric determination used was a modification of the method of McCoy (*11*), in which the reduced europium solution is passed into a standard iodine solution and back-titrated with standard thiosulfate. Difficulties due to volatilization of iodine were encountered, with the result that the determinations were not uniformly reproducible. However, the most closely checking values by the method of McCoy were in agreement with those given below. Substitutions of stannic chloride for iodine removed the possibility of error due to volatilization and yielded the advantage of a direct titration over a back-titration. Forty-five milliliters of a solution containing 0.3498 gram of rare earth as oxide and 0.2 *N* in hydrochloric acid were passed through a Jones reductor into 30 ml. of 0.5 *N* stannic chloride solution. The reducing column was washed with 100 ml. of 0.06 *N* hydrochloric acid. Each 100 ml. of solution was treated with 20 ml. of concentrated hydrochloric acid and 2 ml. of 0.02 per cent solution of diphenylamine in concentrated sulfuric acid. The resulting solution was titrated with standard 0.1 *N* potassium dichromate. An atmosphere of nitrogen was used throughout the reducing column and the titrating vessel. Although the titration reaction was slow near the end point, a definite end point yielding reproducible results was observed.

RESULTS. Two polarographic determinations with widely differing rare earth concentrations gave results of 55.0 and 54.7 per cent of europium oxide, respectively. Two volumetric determinations yielded 56.6 and 56.5 per cent of europium oxide, respectively.

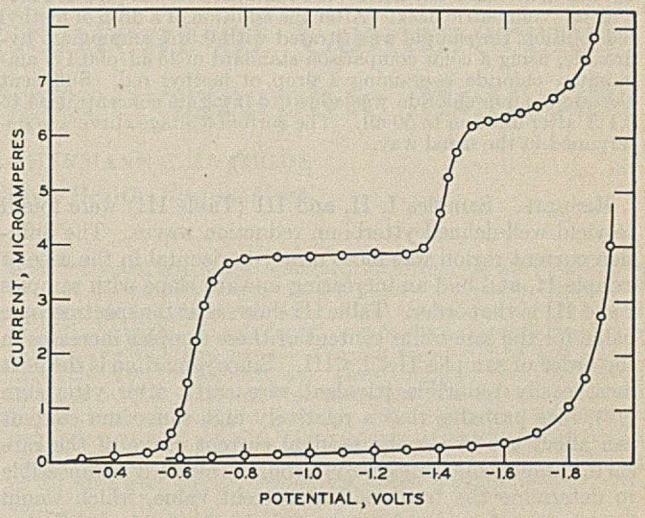

FIGURE 4. CURRENT-VOLTAGE CURVE OF EUROPIUM-YTTERBIUM MIXTURE

The results obtained by the two methods agree to an accuracy of 3 per cent. The polarographic procedure has the advantages of greater speed and simplicity and is applicable to samples containing only traces of europium. The volumetric method is probably more accurate with samples of high europium content, but requires much larger samples and involves a much more difficult recovery procedure for the rare earth material.

Determination of Ytterbium

The successful polarographic determination of ytterbium in rare earth mixtures depends upon obtaining well-defined

TABLE III. COMPOSITION OF ORES

Spectroscopic Composition, %	I Euxenite	II Ferguson-ite	III Samar-skite	IV Xenotime	V Cerite
Y	29.0	25.0	21.8	10.25	2.0
Nd	7.4	7.4	6.8	19.5	20.00
Sm	4.55	3.3	4.9	6.0	4.25
Gd	4.1	3.4	4.2	4.0	1.75
Dy	3.3	2.4	6.4	3.3	1.0
Yb	6.05	6.2	1.5	1.7	1.0
La	...	..	..	10.5	9.9
Polarographic Percentage					
Yb	5.3	7.0	0.5	...	...

reduction waves of ytterbium in the presence of relatively large concentrations of the other rare earths. Preliminary experiments showed that if the ytterbium concentration was 25 per cent or more of the total rare earth content, well-defined waves having the appearance of those shown in Figure 3 were obtained.

In order to determine its limits of applicability, the polarographic method was applied to the analysis of mixed rare earth oxides obtained from various representative classes of naturally occurring minerals. Oxide samples which had been prepared and analyzed spectroscopically by McCarty, Scribner, Lawrenz, and Hopkins (*10*) were available. The composition of the various ores, as determined spectroscopically, is given in Table III, together with the polarographic values for ytterbium.

PROCEDURE. Approximately 2 grams of the oxide mixture were dissolved in 15 ml. of 6 *N* hydrochloric acid and made up to 100 ml. in a volumetric flask. A 10-ml. portion was pipetted into a 50-ml. volumetric flask. After the addition of a drop of methyl red solution, the sample was titrated with 0.5 *N* ammonium hydroxide, using a color comparison standard of 25 ml. of 0.1 *N* ammonium chloride containing a drop of methyl red. Sufficient 1 *N* ammonium chloride was added to bring its concentration to 0.1 *N* after dilution to 50 ml. The current-voltage curve was determined in the usual way.

RESULTS. Samples I, II, and III (Table III) were found to yield well-defined ytterbium reduction waves. The diffusion current region was most nearly horizontal in the case of sample II, and had an increasing upward slope with samples I and III in that order. Table III shows that the spectroscopic value for the samarium content of these samples increases in the order of samples II < I < III. Since samarium is the next most easily reducible trivalent rare earth after ytterbium (*12*), it is probable that a relatively high samarium content can affect the imaginary residual current curve of the rare earth sample containing no ytterbium; hence it is impossible to determine the true residual current value, which would have to be subtracted from the measured ytterbium diffusion current in order to calculate the percentage of ytterbium. The usual polarographic procedure of determining the diffusion current by the vertical distance between parallel tangents to the current-voltage curve was therefore used. In the case of sample II, the tangent method yielded the same result as was obtained by subtracting the residual current of the 0.1 *N* ammonium chloride medium, while lower results were obtained by the tangent method than by the subtraction method for samples I and III where the diffusion current showed an abnormal upward slope.

The polarographic and spectroscopic values for samples I and II were found to check within the limits of accuracy of the latter method, which were stated to be ±15 per cent (*10*). For sample III, a lower value was obtained by the polarographic method.

To check the possibility of interference of other constituents of the sample, known amounts of ytterbium were added to solutions of samples II and III, and the increase in the diffusion current of ytterbium at a potential of −1.6 volts was determined.

Table IV gives the results of adding ytterbium, in the form of a solution made from a mixture of rare earth oxides containing 75.5 per cent ytterbium ozide, to 25 ml. of a solution of sample II containing 0.1160 gram of rare earth oxides. The amount of added ytterbium found was calculated from the increase in the diffusion current, allowing for dilution.

Table V gives the results of similar additions of ytterbium to 25 ml. of a solution of sample III, containing 0.0886 gram of rare earth oxides.

Tables IV and V indicate that no interfering substances are contained in the fergusonite sample, but that some constituent of the samarskite sample causes interference with the polarographic determination. The nature of the interfering substances is unknown because complete analyses of the mixtures were not available.

Samples IV and V showed no ytterbium reduction waves, because the final unlimited current rise began sooner than in the above cases. Apparently there is interference by some other constituents of the oxide mixture in these cases. The first three samples are yttrium earth ores, while the last two are relatively low in yttrium and high in cerium group content.

It is concluded that reliable polarographic determinations of ytterbium can be made in rare earth mixtures if the ytterbium reduction wave is well defined with a reasonably constant diffusion current region. This condition is favored by an increasing ytterbium content and decreasing samarium content, and appears to be fulfilled in the case of yttrium earth ores, but not in ores high in cerium group content. The polarographic method should be useful in following changes in ytterbium content in making rare earth separations.

TABLE IV. ADDITION OF YTTERBIUM TO FERGUSONITE

Increase in *id* Microamperes	Yb_2O_3 Added Mg.	Added Yb_2O_3 Found Mg.
0.53	0.79	0.88
1.33	2.37	2.39
2.08	3.95	3.99

TABLE V. ADDITION OF YTTERBIUM TO SAMARSKITE

Increase in *id* Microamperes	Yb_2O_3 Added Mg.	Added Yb_2O_3 Found Mg.
0.35	0.79	0.59
1.07	2.37	1.92
1.71	3.95	3.30
2.58	6.32	5.48

Simultaneous Determination of Europium and Ytterbium

Since the reduction potentials of europium and ytterbium are widely separated, no difficulty is encountered in making a simultaneous determination of the two elements. However, in determining the diffusion current of ytterbium in such a mixture, care must be taken to correct for the decreasing diffusion current of europium with increasing negative potential (Figure 1).

The recommended procedure may be described by reference to Figure 4. The total diffusion current of ytterbium and europium at −1.6 volts is 6.00 microamperes after subtracting the residual current. The observed diffusion current of europium at −0.9 volt is 3.58 microamperes. Multiplying by

the factor $\left(\frac{2.98}{4.06}\right)^{1/6}$ as above, we find 3.40 microamperes as the diffusion current of europium at a potential of −1.6 volts. Thus the true diffusion current of ytterbium is 6.00 − 3.40 or 2.60 microamperes.

The results obtained for ytterbium, in the mixture of europium oxide and ytterbium oxide described above, were 42.0 and 43.1 per cent, respectively, in two determinations with different concentrations of rare earth. The summations of europium oxide and ytterbium oxide were 97.0 and 97.8 per cent, respectively. Since the sample was known to contain only small amounts of other rare earths, it is believed that the results for ytterbium are essentially correct. If no correction had been made for the effect of decreasing drop time on the europium diffusion currents, the summations would have been 94.1 and 94.7 per cent, respectively. The tangent method of measuring wave heights would likewise lead to erroneously low results for ytterbium because of the unsymmetrical nature of the second wave. The magnitude of the correction due to changes of drop time will, of course, increase with an increasing ratio of europium to ytterbium.

Summary

Ammonium chloride is a suitable indifferent electrolyte for the determination of europium and ytterbium. In 0.1 *N* ammonium chloride the half-wave potentials of europium and ytterbium were found to be −0.671 and −1.415 volts with reference to the saturated calomel electrode.

The polarographic method for europium gives results agreeing within 3 per cent with the volumetric method using the Jones reductor.

Conditions favoring the successful polarographic determination of ytterbium are discussed. Reliable results can be obtained if well-defined reduction waves resembling those of pure ytterbium are obtained. Satisfactory results were obtained with yttrium earth ores containing about 5 per cent ytterbium. Ores of low ytterbium content, particularly those high in cerium group content, were found to yield low results by the polarographic method.

In the simultaneous determination of europium and ytterbium it is necessary to correct for the effect of decreasing drop time with increasing negative potential.

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Determination of Lead Content of Commercial Ciders and Vinegars by Spectrographic Methods

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BECAUSE of the necessary spraying of vegetation subsequently to be used for food there results a certain amount of residual lead which often cannot be removed, particularly in the by-products of the apple crop. Excessive spray residue is often removed from apples which are sold for consumption as such, but not necessarily from apples intended for use in the manufacture of cider and vinegar. This investigation was undertaken to devise rapid and accurate spectrographic methods for the determination of lead in cider and vinegar, as well as to determine the range of lead content of these products as they appear on the market.

All apparatus used was freed from lead by rinsing with hot nitric acid and lead-free water redistilled in a Pyrex still. The bismuth chloride was freed from lead by precipitating bismuth oxychloride with lead-free water, decanting the supernatant liquid, dissolving the precipitate in double-distilled hydrochloric acid, and repeating the process until the salt was spectroscopically free from lead. The calcium acetate was freed from lead by precipitating with hydrogen sulfide, using copper as a coprecipitant.

Analysis of Cider. Twenty-five cubic centimeters of cider were placed in a platinum dish, to which were added 0.5 mg. of bismuth as bismuth chloride and 0.04 gram of calcium acetate. The addition of calcium was found to enhance both the lead and the bismuth lines. The mixture was evaporated nearly to dryness on the steam bath and charred over a small flame, being allowed to take fire when charring was almost complete. The char was ground to a fine powder and three approximately equal portions of such size, experimentally determined, as would give lines of convenient length on the plate were placed in cupped graphite electrodes—about one sixth of the total char was placed in each electrode. It was found unnecessary to weigh these portions, as considerable variation in the amount used had no appreciable effect on the analysis. These samples were then burned in an arc of 220 volts and 9 amperes, the lead being determined by the internal standard method, using a rotating logarithmic sector in front of the slit. The lengths of the bismuth line at 2898 Å. and the lead line at 2833 Å. were compared. The lengths of the lines from the divided samples were averaged before plotting.

Table I. Lead Content of Cider and of Vinegar

	Cider	Cider Vinegar	Distilled or Spirit Vinegar	Malt Vinegar	Malt and Spirit Vinegar
No. of samples	13[a]	49	5	1	1
	Parts per million				
Lowest	0.10	0.18	0.00	..	..
Lower quartile	0.18	0.37	..	..	..
Median	0.27	0.51	..	..	..
Average	0.43	0.55[b]	0.26	0.03	0.08
Geometric mean	0.32	0.50[b]	..	..	..
Upper quartile	0.54	0.78	..	..	..
Highest	1.50	11.80[c]	1.20	..	..

[a] Not including 5 samples containing less than 1 part lead per million.
[b] Exclusive of highest sample. Including highest sample: average = 0.78 geometric mean = 0.53.
[c] Duplicate determination by dithizone extraction method gave 8.0 parts of lead per million.

In a series of seven analyses in the range of from 0.14 to 3.4 parts per million of lead the average error was 1.5 per cent and the maximum error was 6.0 per cent, using cider containing known added amounts of lead. In this case six separate differences in line lengths were averaged to give each of the seven points on the working curve. Eighteen samples of commercial cider were examined, and all contained minute amounts of lead (Table I). Five samples which were examined only to an accuracy of less than 1 p. p. m. of lead are not included in the table.

Analysis of Vinegar. Twenty-five cubic centimeters of vinegar were evaporated to dryness in a platinum dish, and the residue was dissolved in 0.5 cc. of double-distilled hydrochloric acid containing 1 mg. of bismuth per cc. The well-mixed solution of the vinegar residue was divided equally among three cupped graphite electrodes which had previously been treated with one drop of lead-free kerosene to prevent the solution from soaking into the electrodes. The determination was then carried out as in the cider method.

In one series of twelve analyses, using vinegar containing known added amounts of lead in the range of from 0.4 to 1.2 p. p. m., the average error was 7 per cent and the maximum error was 20 per cent. In another series of six analyses in the range of from 0.08 to 0.4 p. p. m. of lead the average error was 9 per cent and the maximum error was 25 per cent.

Fifty-six samples of commercial vinegar were examined, and all but two, both distilled and spirit vinegar, contained lead (Table I). The geometric mean is nearer the median than is the arithmetic mean, indicating the geometric nature of the series.

The lead tolerance adopted by the United States Department of Agriculture under the old food and drug law was 0.025 grain per pound, which is equivalent to 3.5 p. p. m. Only one of these samples exceeded that figure. The next lower result was 1.8 p. p. m. or approximately one half of the tolerance.

The lead content of the cider was on the average less than that of the cider vinegar. There are two possible explanations. The cider was made from New England apples which do not require so much spraying as do western apples, but the vinegar was not exclusively a Massachusetts product. It is customary in the manufacture of sweet cider prior to placing it on the market to filter the apple juice through sand or some other type of filter which may remove some of the lead, but in the manufacture of vinegar this filtration is not always carried out. Furthermore, vinegar is a good solvent for lead in paint, metals, etc., with which it may come in contact during processing.

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A Method for the Identification of Nitriles

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SEVERAL methods have been proposed for the identification of nitriles (*1*, *2*, *3*). The authors have found that adaptation of the well-known method of reduction of nitriles to primary amines by sodium and absolute alcohol gives a practical method for the identification of aliphatic nitriles. Aromatic nitriles do not give such good results, but this class can usually be readily determined by hydrolysis to the corresponding amide or acid.

Procedure

A solution of 1 cc. (0.8 to 1.0 gram) of the nitrile in 20 cc. of absolute alcohol is placed in a 200-cc. round-bottomed flask fitted with a reflux condenser. (It is essential that the alcohol be absolute, otherwise considerably less derivative is obtained.) The flask is immersed to the neck in a water bath heated to 50° to 60° C. Fresh, finely cut sodium (1.5 grams) is added gradually through the top of the condenser as rapidly as possible without allowing the reaction to become too vigorous. When all the sodium has reacted (10 to 15 minutes) the reduction is complete.

The mixture is cooled to 20°, and 10 cc. of concentrated hydrochloric acid are added in small portions through the top of the condenser. Care is necessary on account of the spattering which takes place when the acid strikes the strongly alkaline mixture.

The reflux condenser is disconnected, the system is set up for ordinary distillation, and 20 cc. of alcohol are distilled into a graduated cylinder. The residue in the flask is cooled to 20° and a solution of 6 grams of sodium hydroxide in 6 cc. of water is cautiously added. The reaction at this point is violent, and care is necessary to avoid loss of amine by volatilization. The flask is swirled to ensure mixture of the ingredients and then rapidly reconnected to the condenser. Using a smoky flame the flask is heated until the contents are nearly dry, catching the distillate in a 50-cc. Erlenmeyer flask containing 3 cc. of water. The condenser should be fitted with an adapter dipping beneath the surface of the water in the flask.

If the original substance was a nitrile, the distillate will be alkaline at this point.

Phenylisothiocyanate (0.5 to 1.0 cc.) is then added to the distillate, and the mixture vigorously shaken for 2 or 3 minutes. If no derivative forms on shaking, scratching the walls of the Erlenmeyer and cooling under a tap or in an ice bath will bring down the precipitate. Aliphatic derivatives as a rule respond to shaking; aromatic compounds require cooling in an ice bath. The crude derivative is filtered, washed with 50 per cent alcohol and recrystallized from dilute alcohol in the usual manner.

Because reduction in the case of aromatic nitriles is less smooth, an initial sample of 2 cc. or 2 grams is recommended.

In Table I are given the results obtained with ten aliphatic and four aromatic nitriles. The product was in most cases recrystallized from dilute alcohol, two recrystallizations usually being sufficient to yield a pure product. The method is applicable only to those aliphatic nitriles which form a volatile amine upon reduction. In the aromatic series the method works less well, probably because of the lack of volatility of the amine and the fact that reduction in the aromatic series is accompanied by side reactions.

Table I. Identification of Nitriles

	Weight of Nitrile Used	No. of Crystallizations	M. P. of Phenyl Thiourea	Weight of Derivative Obtained	Nitrogen Calculated	Nitrogen Found
			° C.	Gram	%	%
Acetonitrile	0.8	2	106	0.8	...	...
Propionitrile	0.8	2	63	0.8	...	...
n-Butyronitrile	0.8	2	65	0.7	...	...
Isobutyronitrile	0.8	2	82	0.6	...	...
n-Valeronitrile	0.8	2	69	0.7	...	...
Isovaleronitrile	0.8	2	102	0.6	...	...
n-Capronitrile	0.8	2	77	0.3	...	...
Isocapronitrile[a]	0.8	2	112	0.25	11.85	11.97
Glutaronitrile[a]	0.8	2	148	0.9	15.04	15.00
Succinonitrile[a]	0.7	2	168	0.9	15.63	15.64
Benzonitrile[a]	2.0	2	147	0.3	11.56	11.62
p-Tolunitrile[a]	1.0	3	144	0.10	10.93	10.84
o-Tolunitrile[a]	3.0	4	179	0.3	10.93	11.35
β-Naphthonitrile[a]	3.0	5	140	0.33	9.58	9.69

[a] Derivatives not previously described. No satisfactory results could be obtained with *α*-naphthonitrile or with *m*-tolunitrile.

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Continuous-Reading Electronic Voltmeter

For Use with Glass and Other High-Resistance Electrode Systems

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Shell Development Company, Emeryville, Calif.

A full-range, sensitive, low-current-drain, continuous-reading electronic voltmeter is described. The meter has a range of ±2.11 volts and a sensitivity of ±0.001 volt over the entire range. It has been designed for the accurate determination of potentials of electrode systems that have a resistance of 5000 megohms or less.

THE increased use of durable glass electrodes and nonaqueous, high-resistance titration media in potentiometric titrations has emphasized the need for a versatile potentiometer, or millivoltmeter, that will satisfactorily measure the potential between electrodes of a cell having a resistance of 150 to 5000 megohms between the electrode terminals. Instead of the combination of potentiometer, thermionic galvanometer, and electrode system entirely surrounded by a large electrostatic shield through which the adjustments are made by remote control, the authors have found it advantageous to use a continuous direct-reading meter along with flexible shielded leads and electrodes. [The Beckman pH meter, Industrial Model M, and the Beckman Model O electronic voltmeter (National Technical Laboratories, Pasadena, Calif.) are commercial examples. The latter is a versatile meter with operating characteristics equivalent, except for somewhat lower sensitivity, to those of the meter described in this paper.] The continuous indicating meter not only requires less manipulation by the operator, but also enables him easily and definitely to ascertain when the potentials have reached equilibrium. For greatest usefulness and versatility, a modern meter should continuously indicate the applied e. m. f., operate on less than 10^{-12} ampere over the entire range of approximately 2 volts, be sensitive and accurate to ±0.001 volt in any part of the range, and provide a simple means of attaching the e. m. f. source to the meter by means of flexible shielded leads (*14*).

Since the first application of the thermionic electron tube by Goode in an electro-titration meter (*7*), recognition of the

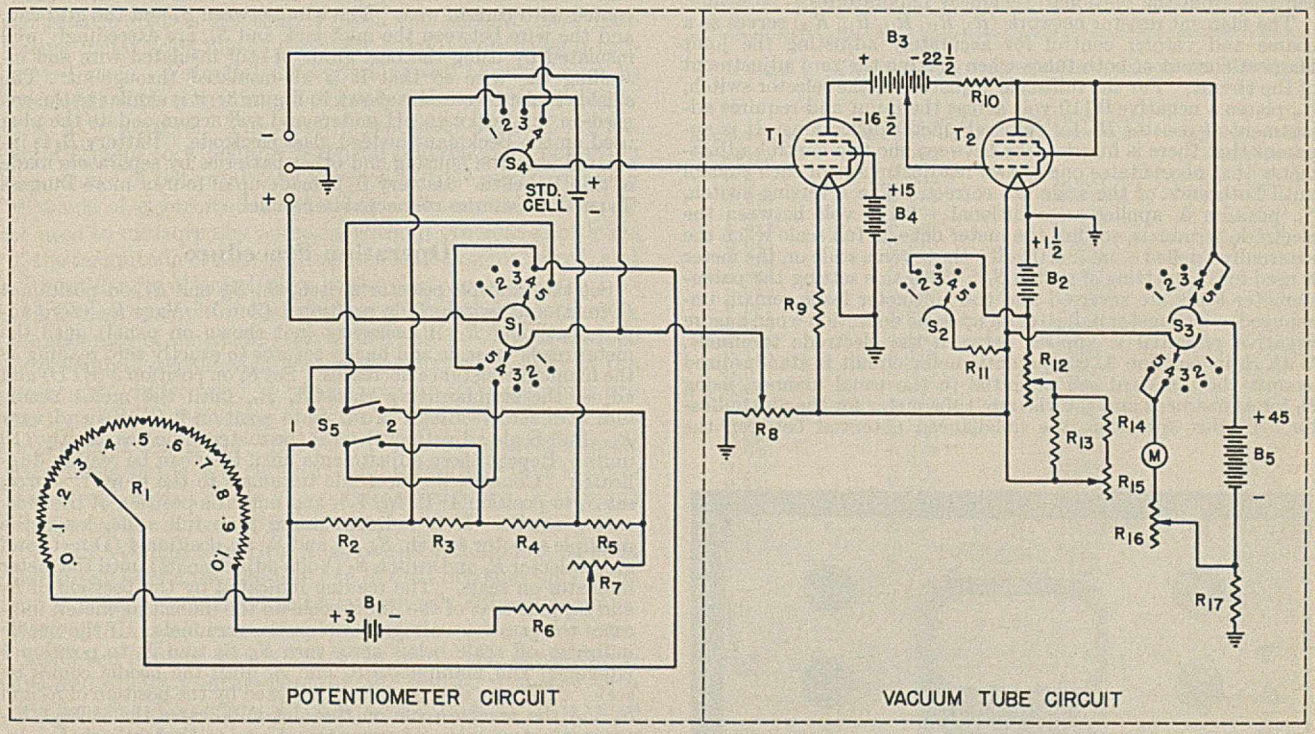

FIGURE 1. DIAGRAM OF CIRCUIT

M. Weston Model 801 0–0.20 direct current milliammeter (400 ohms)
R_1. General Radio Type 510, decade resistance 100 ohms per step
R_2. 110-ohm precision resistor
R_3. 890-ohm precision resistor
R_4. 19.0-ohm precision resistor
R_5. 91.0-ohm precision resistor
R_6. 400-ohm wire-wound
R_7. 600-ohm wire-wound variable
R_8. 1000-ohm wire-wound variable
R_9. 7.5 ohms
R_{10}. 10 megohms
R_{11}. 25 ohms
R_{12}. 6-ohm wire-wound with "off" position
R_{13}. 1-ohm wire-wound
R_{14}. 5-ohm wire-wound
R_{15}. 25-ohm wire-wound variable
R_{16}. 15,000-ohm wire-wound variable
R_{17}. 2000 ohms
S_1. 5-position 2-circuit Centralab selector switch
S_2. 5-position single-circuit shorting type Centralab
S_3. 5-position 2-circuit Centralab selector switch
S_1, S_2, and S_3 mounted on same shaft operated by a single control
S_4. Centralab Isolantite selector switch
S_5. Double-pole double-throw jack switch
Std. Cell. Eppley saturated 1.0190 volts
T_1. Type 32
T_2. Type 1A5G

importance of electronic millivoltmeters in analytical chemistry has progressed steadily (*1–13, 15–20*). These titrimeters require a constant filament current; for this reason battery-operated meters often have been preferred to line-operated alternating current equipment. Most of these meters have been found objectionable from the standpoint of application to high-resistance systems (100 to 2000 megohms), because of lack of sensitivity over their entire range, inadequate shielding of electrode leads, or the presence of a grid current greater than 10^{-11} ampere. The object in developing the electronic voltmeter described in this paper was to obtain a universal meter particularly suited for analytical laboratory use.

Electrical Circuit

Since it is practically impossible to meet the requirement of wide range with high sensitivity in a single indicating meter, a step potentiometer has been combined with an electronic voltmeter; the potentiometer has ten steps of 0.100 volt each and one step of 1.000 volt. The indicating meter has a range of 0.110 volt, so that the total range is ±2.110 volts. The smallest scale division is 0.002 volt, and it is easily possible to estimate the reading to 0.0005 volt.

The circuit is shown in Figure 1. The vacuum tube circuit is that of a two-stage direct-coupled amplifier using selected, aged, standard receiver type vacuum tubes. The first tube (Type 32) is used with reduced potentials on all elements to reduce primary and secondary emission from the elements, is covered with a coating of ceresin wax to decrease surface leakage, and is shielded from light to prevent photoemission from the grid. The tube must be tested for microphonic properties and grid current under operating conditions of mounting, etc. (Tested tubes are obtainable from the National Technical Laboratories.)

The filament resistor network (R_9, R_{12}, R_{13}, R_{14}, R_{15}) serves as a coarse and vernier control for accurately adjusting the joint filament current of both tubes when making the zero adjustment of the circuit. For the full-scale adjustment, the selector switch, S_4, places a negative 0.110 volt across the input and requires adjustment of resistor R_{16} for full-scale meter deflection. It is apparent that there is interlocking between the two control adjustments that necessitates one or two readjustments of each control until both ends of the scale are correct. The reversing switch, S_1, position 5, applies an additional −0.110 volt between the electrode terminals, so that the meter deflects full scale when the externally applied e. m. f. is 0 volt; the reverse scale on the meter is used for this setting of switch S_1. With this setting the potentiometer leads are reversed and the voltmeter leads remain unchanged. The meter indicates an up-scale deflection when a more negative potential is applied between the electrode terminals. With S_4 in position 3, the potentiometer circuit is standardized against the standard cell potential in the usual manner, using R_7 for adjustment and the vacuum tube meter for the null indicator. In this operation, the unbalanced potential between the standard cell voltage on the potentiometer and the actual voltage of the standard cell is placed across the terminals of the voltmeter. Adjustment R_8 is used to set the grid bias voltage to a point which gives the most linear calibration. In some instances, the linear response may be improved by varying the 15-volt screen potential and the load resistor, R_{10}.

By turning resistor R_{12} to off position and selector switch S_2 to position 2, the meter is on the standby position in which the filaments are operated at one-half operating current and the plate battery is disconnected. The meter is turned off by setting R_{12} on the off position and placing S_2 in position 1. In order to avoid erratic changes in the meter adjustment due to small changes in contact resistance of the switch, a separate switch is not used in the filament circuit. For the same reason, it is recommended that the two rheostats used in the filament circuit (R_{12} and R_{15}) should have a spiral "pigtail" connection between the rotor and the variable terminal.

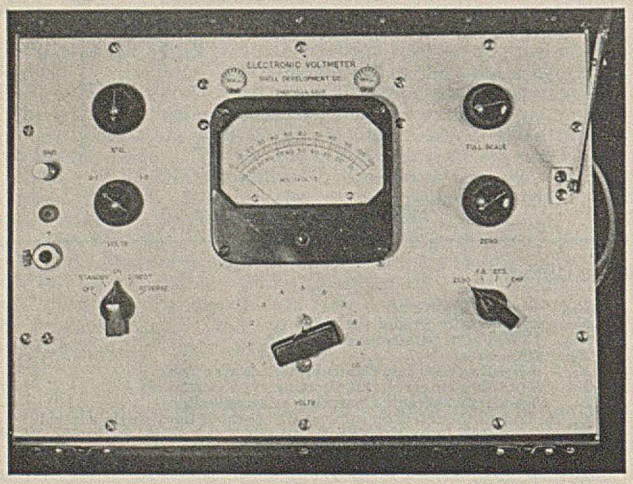

FIGURE 2. INSTRUMENT PANEL

Constructional Details

The unit is housed in a metal cabinet and a sloping control panel is provided to facilitate observation of the indicating meter and adjustment of the controls. It is essential to surround the entire unit with metal cabinet, or metal-lined cabinet, in order to avoid disturbing influence of stray external potentials. The control panel is arranged for convenience of operation; the arrangement of the control knobs is shown in Figure 2. Multipoint switches are used for direct and reverse electrode connections, plate battery and standby connections, and standardizing operations. Adjustable controls are provided for zero, full-scale, and potentiometer standardizing adjustments.

The rugged d'Arsonval-type indicating meter is calibrated linearly from 0 to 110 millivolts and from 110 to 0 millivolts. The "reverse" scale is provided so that it is not necessary to subtract the meter reading from the potentiometer setting when the electrode terminals are connected in the reverse position.

In order that the instrument may function on a high input resistance, good proved insulation is used throughout the grid circuit of the first tube. Switch S_4 is made of Isolantite and is coated with ceresin wax. The wire between S_4 and the grid cap, and the wire between the inlet jack and S_4, are exceedingly well insulated by using the best grade of stiff insulated wire and installing the wire so that it is air-insulated throughout. The shielded inlet terminal is shown in Figure 3; it is similar to the one used on the Beckman pH meters and will accommodate the plug used on the Beckman shielded glass electrode. Battery B_3 is insulated from the housing and other batteries by separators made of thin Bakelite. Battery B_2 is made up of four or more Burgess Type 4FJ batteries connected in parallel.

Operation Procedure

Set the multiple selector switch, S_1, S_2, and S_3, on position 4 (Direct) and switch S_4 on position 1 (Zero). Vary R_{15} (Zero adjustment) and R_{12} if necessary (not shown on panel) until the meter reads up scale and finally returns to exactly zero reading as the filament resistance decreases. Set S_4 on position 3 (STD) and adjust the standardizing rheostat, R_7, until the meter needle indicates zero reading. Turn S_4 to position 2 (F. S.) and vary R_{16} (Full-Scale adjustment) until the meter indicates exactly 110 units. Repeat these adjustments until they can be readily duplicated. Connect the electrode terminals to the e. m. f. source, set S_4 to position 4 (E. M. F.), and note the position of the indicating needle. If it indicates more than full scale, leave the multiple selector switch, S_1, S_2, and S_3, on position 4 (Direct) and vary selector R_1 and switch S_5 (Volts adjustments) until the meter indicates on scale. The reading indicated by the position of R_1 and S_5, plus that of the direct scale on the indicating meter, indicates the positive voltage between the terminals. If the needle indicates off scale below zero, turn S_1, S_2, and S_3 to position 5 (Reverse) and manipulate R_1 and S_5 until the needle comes on scale. The sum of the reading indicated by the position of R_1 and S_5 and the reverse scale on the meter indicates the voltage between the terminals (of opposite polarity to that indicated).

The meter, cell stand, motor frame, and other accessories are connected to a ground in order to avoid errors due to stray electrostatic potentials and alternating current leaks.

When the meter is used daily, the multiple selector switch, S_1, S_2, and S_3 is turned to position 3 (on) and S_4 is turned to position 1 (Zero) between determinations and overnight. For intermittent use, the filament adjusting rheostat, R_{12}, is turned to the off position and the multiple selector switch, S_1, S_2, and S_3, is placed on position 2 (Standby), and the meter is adjusted to operate approximately one hour before it is used. By maintaining the instrument in this condition, when it is not used regularly, the drift and battery consumption are reduced to a minimum. The me-

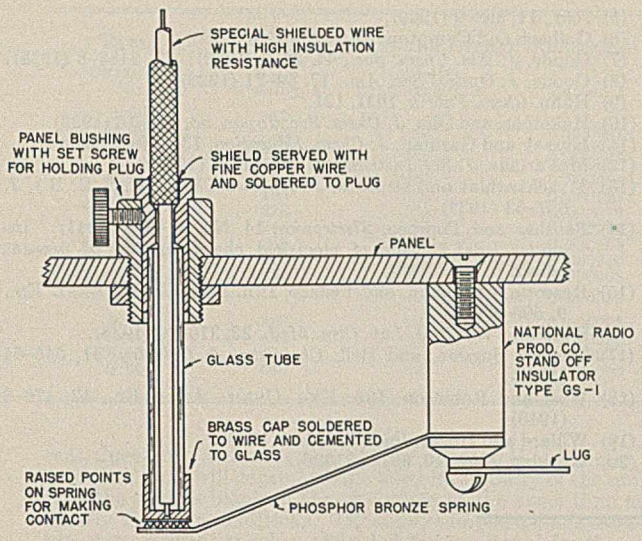

FIGURE 3. SHIELDED INLET TERMINAL JACK

ter is completely turned off by putting S_1, S_2, and S_3 on position 1 and turning the filament adjusting rheostat, R_{12}, to its off position.

Operating Characteristics

The meter is capable of continuously measuring the potential across electrodes of any system that has a resistance less than 5000 megohms with a precision of ±0.001 volt. The systematic error is less than ±0.005 volt using a linear indicating meter scale and is less than ±0.001 volt using a calibrated scale. Potential differences of ±2.11 volts may be measured with a maximum current drain of approximately 3×10^{-13} ampere. The meter is especially useful in adjusting a system to a definite potential difference, in observing the change of potential over a period of time, and in making all kinds of electrometric titrations. The meter may safely be used to measure any potential without previous knowledge of the magnitude or sign of the potential being measured, and without danger of polarization of the electrode system.

A typical example of the influence of the input resistance on the accuracy of the meter is given in Table I. The deviations at zero input resistance indicate the slight nonlinearity of the instrument under test; the deviations at 5000 megohms are hardly greater, indicating that the error introduced by this resistance is negligible. The error increases for greater input resistances, but is of such nature and magnitude that the meter operates satisfactorily as a titration meter even with input resistances as high as 100,000 megohms.

In any vacuum tube circuit capable of functioning on such high input resistance, the problem of drift becomes important and is usually considered to be exceedingly difficult to solve. While drift has not been completely eliminated in this circuit, it has been reduced to 0.002 volt per hour; this is not excessive and allows measurements of the desired accuracy with only occasional resetting of the zero adjustment. This value of drift is obtained only after the meter has been in operation for several hours. If the meter has been left on standby for some time, the drift is approximately 0.020 volt the first hour, after which it rapidly diminishes to the regular value. The potentiometer circuit and the full-scale adjustment do not drift more than 0.001 volt in 8 hours unless the batteries have been depleted.

The four filament supply batteries will operate the meter continuously for 4 months. The life of the 45-volt (B_5) battery is approximately 6 months; it must be replaced when its voltage falls below 40 volts. The remainder of the batteries have approximately "shelf life", as the current drains are negligible.

Discussion

The vacuum tube circuit is a modification of a similar previous circuit using Type 1B4 (tetrode) and 1F4 tubes in place of Type 32 and 1A5G, respectively. In the early circuit, 3-volt filament supply batteries and a 0 to 1.0-milliammeter were used along with the following changes: $B_4 = 13.5$ volts; $B_2 = 3$ volts; $R_{10} = 7$ megohms; $R_{16} = 600$ ohms; $R_9 = 34$ ohms; and $R_{12} = 10$ ohms. The earlier circuit operated with a grid current drain of less than 5×10^{-12} ampere, and for systems having a resistance less than 500 megohms was capable of exactly linear calibration and accuracy within 0.0005 volt. However, for input resistances over 1000 megohms, the modified circuit showed an advantage in accuracy; thus at 11,500 megohms it had about one quarter the error of the earlier circuit. Some trouble was experienced in selecting for the earlier circuit Type 1B4 tubes that were not subject to microphonic disturbances and were insulated well enough to give satisfactorily low grid current drain.

A possible improvement would be to enclose all the batteries in a separate compartment, leaving the tubes and other components in a practically gas-tight space. This arrangement would prevent the ammonia fumes and moisture released by the batteries from creating leakage paths over insulated surfaces and would allow the use of a dehydrator in the tube compartment. However, a properly installed tube and accessories will not exhibit troublesome surface leakage, provided the cabinet contains several small vents for air circulation.

TABLE I. INFLUENCE OF INPUT RESISTANCE ON METER READING

Applied E. M. F.	Meter Reading on Linear Scale	Deviation from Applied Value	Input Resistance
Volts	*Volts*	*Volts*	*Megohms*
0.0	0.000	0.000	0.0
0.012	0.010	0.002	0.0
0.022	0.020	0.002	0.0
0.033	0.030	0.003	0.0
0.043	0.040	0.003	0.0
0.052	0.050	0.002	0.0
0.062	0.060	0.002	0.0
0.072	0.070	0.002	0.0
0.081	0.080	0.001	0.0
0.090	0.090	0.000	0.0
0.100	0.100	0.000	0.0
0.110	0.110	0.000	0.0
0.0	0.000	0.000	5,000
0.053	0.050	0.003	5,000
0.101	0.100	0.001	5,000
0.111	0.110	0.001	5,000
0.003	0.000	0.003	11,500
0.055	0.050	0.005	11,500
0.102	0.100	0.002	11,500
0.115	0.110	0.005	11,500
0.014	0.000	0.014	50,000
0.066	0.050	0.016	50,000
0.116	0.100	0.016	50,000
0.128	0.110	0.018	50,000
0.024	0.000	0.024	110,000
0.081	0.050	0.031	110,000
0.133	0.100	0.033	110,000
0.146	0.110	0.036	110,000
1.146[a]	1.110[a]	0.036	110,000

[a] Higher e. m. f.'s (introduced by step potentiometer) cause no further error, as may be seen by comparison with figures above.

Uses

Two of these electronic voltmeters have been in daily use for two years in the analytical department of the Shell Development Company. The meter is an excellent substitute for a potentiometer in any system with a resistance less than 5000 megohms; it will function as a potentiometer with all the advantages of a continuous reading meter. It has been used satisfactorily to measure the e. m. f. between the electrodes in electrometric determinations involving the use of high-resistance glass, silver, platinum, antimony, and tung-

sten and the corresponding reference electrodes. It is applicable to potentiometric determinations in nonaqueous as well as aqueous solutions and it has no tendency to produce polarization of the electrodes. The meter is well suited for titrating to a definite potential difference, or to a maximum potential change per increment of reagent, and for determining points for plotting potential-volume curves. It is excellently suited for making pH determinations.

Acknowledgments

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Determination of Citral

By Means of the Photoelectric Colorimeter

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CITRAL is an important constituent of lemon flavors. It is present in oil of lemon, which in turn is present in lemon extract.

The federal standard provides that terpeneless extract of lemon shall contain not less than 0.2 per cent by weight of citral (*5*). The Illinois standard specifies the same minimum citral content for imitation and terpeneless lemon extracts and flavors (*2*). For regulatory purposes a quantitative determination of citral is necessary. The official A. O. A. C. or Hiltner method determines citral colorimetrically (*1*). With *m*-phenylenediamine hydrochloride citral forms an intense yellow colored solution, the intensity of which is proportional to the amount of citral present. The amount of citral is determined by comparing this color with that produced by a standard citral solution. In analyzing extracts made with lemon and orange oils, dark-colored solutions sometimes form which mask the resultant yellow color. Parker and Hiltner overcame this by adding some oxalic acid to the reagent, and this improvement is included in the official A. O. A. C. method (*3*).

The method works satisfactorily on unknown solutions that are clear and colorless, but the artificial yellow color of some imitation lemon extracts interferes with observation of the yellow color produced in the course of analysis. Still others are of an emulsion type and contain gums, resins, and starches, in addition to artificial color, which make it impossible to determine citral by comparing the color with that of a standard by the visual colorimeter.

To overcome this difficulty the photoelectric colorimeter was investigated. The one used was the Klett-Summerson (*4*), which is of the double photocell type, the cell current being measured with a potentiometer. The scale is calibrated logarithmically.

When solutions obey Beer's law the relationship between concentration and scale reading is logarithmic. When plotted on ordinary graph paper a logarithmic curve is obtained, while on logarithmic graph paper a straight line is produced. The same result can be obtained by using a logarithmic scale on the instrument; the concentration of solutions obeying Beer's law is directly proportional to the scale reading.

In the preliminary work different strengths of citral solutions were prepared. Aliquot portions were taken and mixed with the *m*-phenylenediamine reagent prepared by the official method. Readings were made on these solutions using various light filters. A blue filter of 420 millimicrons gave the largest difference in scale reading between the lower and higher citral concentrations, and was used satisfactorily in the subsequent work. It was necessary to change the official dilution ratio in order to adapt the A. O. A. C. method to this type of colorimeter.

Reagents

m-Phenylenediamine hydrochloride–oxalic acid solution. Dissolve 1 gram of *m*-phenylenediamine hydrochloride in about 45 cc. of 85 per cent alcohol, and 1 gram of oxalic acid crystals in about the same amount of alcohol. Pour the two solutions into a 100-cc. volumetric flask, add 2.5 grams of fuller's earth, dilute to the mark with 85 per cent alcohol, shake thoroughly, and filter, pouring the fuller's earth into the filter so as to form a filtering medium. Refilter the first 15 to 20 cc. of filtrate through the same filter. This reagent is stable for about 2 days, but after that it is not sufficiently reliable.

Oxalic acid solution. Dissolve 1 gram of oxalic acid crystals in about 90 cc. of 85 per cent alcohol and dilute to the 100-cc. mark with alcohol of the same strength.

Method

Weigh a 10-gram sample and with alcohol transfer to a 100-cc. volumetric flask. Use 95 per cent alcohol for extracts made with lemon oils and 50 to 95 per cent alcohol for terpeneless extracts. Add 10 cc. of *m*-phenylenediamine reagent with an accurate pipet. Complete the volume with alcohol, mix, and pour out a sufficient amount to read in the colorimeter.

Run a sample blank by taking a 10-gram sample in another 100-cc. flask and adding 10 cc. of oxalic acid solution. Pour out a sufficient amount to read in the colorimeter. On clear colorless liquid samples this is unnecessary, as the reading would be zero.

Run a reagent blank by pipetting 10 cc. of *m*-phenylenediamine reagent into a 100-cc. volumetric flask, complete the volume with 50 per cent alcohol, mix, and pour out a sufficient amount to read in the colorimeter.

Use the blue 420 millimicron light filter.

Samples of emulsion type are colloidal when diluted and would give absurd readings. Pour a sufficient quantity from the 100-cc. flask into a centrifuge tube of around 20-cc. capacity, and

TABLE I. ANALYSIS OF STANDARD CITRAL SOLUTIONS

Citral, y, %	Scale Reading, x	Net Scale Reading, $x - a$	Factor, f
0.00	15	...	...
0.06	78	63	0.000952
0.10	120	105	0.000952
0.13	151	136	0.000955
0.16	182	167	0.000957
0.18	204	189	0.000952
0.20	228	213	0.000939
0.22	246	231	0.000952
0.24	266	251	0.000956
0.27	300	285	0.000946
0.30	325	310	0.000968
			Av. 0.000953
0.34	360	345	0.000985
0.40	435	420	0.000953
0.50	530	515	0.000971
0.60	640	625	0.000960

centrifuge until a clear or almost clear solution is obtained. The speed and time will depend on the colloidal stability of the sample. Run the sample blank in the centrifuge at the same time under exactly the same conditions. On cloudy samples a blank should be run, even if without color. Pour off the top liquid for the colorimeter reading.

Calculate from the equation:

$$y = f[x - (a + b)]$$

in which y = per cent citral, f = factor, x = scale reading, a = reagent blank, and b = sample blank.

Note that the sample is diluted tenfold. If the unknown sample was 0.1 or 0.2 per cent solution it becomes 0.01 or 0.02 gram per 100 cc., respectively.

Standard Citral Solutions

Weigh accurately 1 gram of citral and with 95 per cent alcohol transfer to a 100-cc. volumetric flask. Dilute to the mark and mix thoroughly. Pipet 1 or 2 cc. into another 100-cc. volumetric flask, add 10 cc. of *m*-phenylenediamine reagent and dilute to the mark with 95 per cent alcohol, mix, and pour out a sufficient amount to read in the colorimeter. The solution will now contain 0.01 or 0.02 gram per 100 cc., respectively, equivalent to 0.1 or 0.2 per cent of an unknown diluted tenfold. Prepare a series of varying dilutions and with them run a reagent blank as above. Since the standard citral solution from which the set was prepared was colorless, the sample blank is not necessary, as it would be zero. Calculate for the factor, using the same equation: $y = f[x - (a + b)]$.

Once the average factor is determined from the series, it will be unnecessary to repeat the standards.

Experimental

A series of varying dilutions of standard citral solutions was prepared and analyzed on the Klett-Summerson photoelectric colorimeter. The citral used was obtained from the Eastman Kodak Company, Rochester, N. Y., and had a narrow boiling range of 114–115° C. at 25 mm. and a refractive index of 1.489 at 20° C. The average of a series of eight runs is given in Table I. Since a solution containing 0.01 or 0.02 gram of citral per 100 cc. would be equivalent to 0.1 or 0.2 per cent of an unknown diluted tenfold, the standard citral solution may be referred to the corresponding percentages. The 0 per cent citral is the reagent blank, and this reading subtracted from the scale reading for a definite strength of citral gives the net scale reading due to the contact of the *m*-phenylenediamine with the citral. In plotting these net scale readings, a straight line is obtained within the practical range (Figure 1); from the straight-line relationship it follows that this reaction obeys Beer's law. A factor for this relationship can be obtained by dividing the per cent citral by the corresponding net scale reading. The factors for the percentages of citral run (Table I) show a small variation over the range studied, and the average factor, 0.000953, was used in the succeeding calculations. Only one run was made on the 0.34 to 0.60 per cent citral and for that reason was not included in figuring the average factor.

No precautions were taken to assure aldehyde-free alcohol as in the A. O. A. C. method. An increase in reading due to this impurity affects the reagent blank reading by an amount equal to that produced in the citral solution reading, and since the difference of the two readings is used in the calculation, the results would not be affected. The reagent solution deepens in color with age and although still reliable causes variations in the reagent blank readings. This variation does not affect the results, because all readings are greater by the same amount.

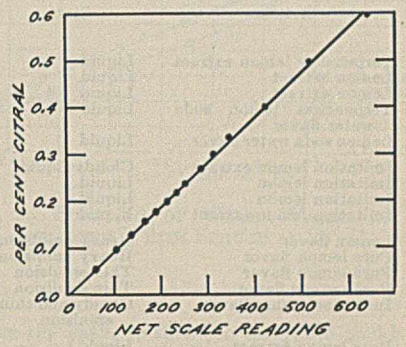

FIGURE 1. RELATION BETWEEN PER CENT CITRAL AND NET SCALE READING

As it was also necessary to run some artificially colored samples, 0.2 per cent citral solutions were prepared and colored with yellow and orange certified dyes. To determine the amount of scale reading due to the dye, the sample was run as before, using oxalic acid solution instead of the *m*-phenylenediamine reagent, since the same amount of oxalic acid was present in the reagent. This is the sample blank. The sum of the scale readings of the reagent blank and the sample blank, subtracted from the scale reading of the sample, will give the net scale reading due to the contact of the *m*-phenylenediamine with the citral. This difference will, when multiplied by the factor 0.000953 previously determined, give the 0.20 per cent citral. Table II shows that the intensity of the yellowness varied greatly, yet the deviation of error was −0.01 to +0.02 per cent and can be attributed to experimental error.

On the market there are so-called lemon creams and emulsions, which vary in color, consistency, and ingredients. Samples were invariably cloudy, and gave erroneous and inconsistent results on reading in the colorimeter. Filtering was not practicable because of the gelatinous nature, volume change due to the alcohol evaporation, and color adsorption by the filter paper. The samples with their blanks were prepared as before, and 15 to 20 cc. were poured into centrifuge tubes with conical bottoms and centrifuged together until

TABLE II. ANALYSIS OF COLORED STANDARD CITRAL SOLUTIONS

Dye Used	Reagent Blank, a	Sample Blank, b	Scale Reading, x	Citral Present %	Citral Found, y %
Colorless solution	10	1	216	0.20	0.20
Yellow 6 (Sunset Yellow)	10	58	268	0.20	0.19
	10	240	480	0.20	0.22
Yellow 2 (Naphthol Yellow S)	10	53	258	0.20	0.19
	10	185	415	0.20	0.21
Yellow 5 (Tartrazine)	10	70	288	0.20	0.20
	10	116	340	0.20	0.20
Yellow 3 (Yellow A B)	10	21	246	0.20	0.21
Yellow 4 (Yellow O B)	10	9	230	0.20	0.20
Orange 1 (Orange I)	10	75	280	0.20	0.19
Orange 2 (Orange SS)	10	31	260	0.20	0.21

TABLE III. ANALYSIS OF EMULSION-TYPE COLORED STANDARD CITRAL SOLUTIONS

Description	Citral in Original Sample	Citral in Final Sample	Citral Found
	%	%	%
Heavy yellow emulsion	0.042	0.260	0.229
Heavy yellow emulsion	0.080	0.264	0.251
Thin orange emulsion	0.0	0.182	0.179
Thin yellow emulsion	0.0	0.240	0.243

TABLE IV. ANALYSIS OF COMMERCIAL LEMON AND ORANGE FLAVORS

Sample No.	Article	State	Color	Citral
				%
CD 39	Terpeneless lemon extract	Liquid	Colorless	0.102
MO 180	Lemon extract	Liquid	Colorless	0.227
RS 196	Lemon extract	Liquid	Colorless	0.191
H 358	Terpeneless lemon soda water flavor	Liquid	Colorless	0.116
H 357	Lemon soda water flavor	Liquid	Colorless	0.123
NK 195	Imitation lemon extract	Cloudy liquid	Whitish	0.005
NE 109	Imitation lemon	Liquid	Light yellow	0.008
JJ 389	Imitation lemon	Liquid	Yellow tint	0.006
NE 33	Imitation lemon extract	Liquid	Light yellow	0.000
MC 63	Lemon flavor	Heavy emulsion	Deep yellow	0.042
MO 128	Pure lemon flavor	Heavy emulsion	Yellow	0.080
MO 135	Pure lemon flavor	Thin emulsion	Light yellow	0.000
MO 133	Pure orange flavor	Thin emulsion	Deep orange	0.000
BL 70	Imitation orange flavor	Cloudy and thin emulsion	Light yellow	0.026
HL 7	Pure lemon extract	Liquid	Colorless	0.318
CM 69	Pure lemon extract	Liquid	Colorless	0.281
MC 49	Pure lemon extract	Liquid	Colorless	0.400
HL 28	Pure lemon extract	Liquid	Yellow tint	0.524

clear or almost clear. The top liquors were then poured off for colorimeter readings and found to be low in citral. To the original part of these samples a known amount of citral was added, thoroughly stirred in, and again analyzed. The results are given in Table III. The error varied from −0.031 to +0.003 per cent citral. It is evident that the citral was separated from the gums, starches, etc., by the alcohol and that the yellow color produced by the citral and the reagent was not adsorbed and thrown down with the sediment during the centrifuging.

Application

Commercial samples picked up by inspectors were analyzed for citral (Table IV). These determinations were made to illustrate the application of this method to commercial products of varying compositions.

Summary

The Hiltner official A. O. A. C. method for the determination of citral was modified to make possible the analysis of flavors which contain color, emulsifying agents, or both, with the same degree of accuracy as colorless flavors. Color comparisons were read with the photoelectric colorimeter. Once a factor is determined for the particular photoelectric colorimeter used, standard solutions do not have to be repeated. This has the advantage of being quicker than the visual colorimeter.

These experiments on standard citral solutions show that the solution obeys Beer's law.

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A Convenient Six-Tube Vapor Sorption Apparatus

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THE Forest Products Laboratory has felt the need for a multiple-unit sorption apparatus that could be operated readily over a considerable range of temperatures and used for other vapors than water vapor. The apparatus of Seborg (*4*) in which sorption measurements are made under atmospheric conditions has furnished a great deal of valuable data (*3–6*). The apparatus, however, is not adapted to use over a considerable temperature range and can be used only for water vapor.

The apparatus described in this article was designed at the Forest Products Laboratory to meet these further requirements and to make the attainment of equilibrium as rapid as possible. Diffusion distances were made a minimum (about 40 cm.) and six sorption tubes were arranged so as to be equally accessible to the vapor source. It was considered important to avoid the adding or removing of definite volumes of vapor as many investigators have done because, under these conditions, equilibrium is approached under decreasing or increasing relative vapor pressure conditions which have been shown to affect the results (*7*). Maintaining a definite temperature difference between the vapor source and the samples is the only simple method of holding the equilibrium relative vapor pressure constant as sorption proceeds, that is suitable for various vapors. This method of vapor pressure control was adopted. Quartz spirals (*1*), which have proved their worth in so many different forms of sorption apparatus, were used for the weighing. Although the measurements could be made in this form of apparatus in the presence of air, the rate of attainment of sorption equilibrium was greatly increased by designing the apparatus so that the system could be evacuated and the vacuum maintained.

Apparatus

Figure 1 gives a horizontal plan and a vertical elevation of the apparatus which is mounted in the center of a thermostatically controlled water bath with an inside diameter of 50 cm. (20 inches) and a height of 50 cm. (20 inches). The bath has four vertical side windows, 40 × 10 cm. (16 × 4 inches). None of the control parts for the water bath is shown in the figure. Heating and cooling coils are on the bottom of the tank under the brass turntable gear wheel, *W*, which is supported about 5 cm. (2 inches) above the bottom of the tank. *W*, which supports all the apparatus, is rotated by gear wheel *G*, by turning handle *J*.

A wide-mouthed, unsilvered Dewar flask, *D* (17.5 × 10.5 cm., 7 × 5 inches inside), which serves as the inner bath, is centered on the turntable. This flask is partially filled with a strong aque-

ous glycol solution so as to stand temperatures of −20° C. without freezing and is sealed with a heavy rubber stopper, *Z*, which, for convenience of assembly, is made up of one stopper within another. The bulb, *B*, which serves as the vapor source is centered in the Dewar flask, and connects through a distributing head with six equally spaced tubes (12 mm.) connecting to the sorption tubes, *T*. Each tube is held by ball and socket clamps, *C*, attached to posts, *P*, which are shown only in the horizontal plan to avoid undue complication of the diagram. The six connecting tubes are made up with ground-in joints for convenience in assembly. The sorption tubes, *T*, are also made up with ground-on tops. All these ground joints and stopcocks 1 and 2 are platinum-plated to give better seals with less sticking.

B is equipped with a stirrer, *S*, operated by the electromagnet, *E*. The paddle stirrer, which has been described in detail by the authors (*8*), oscillates slowly back and forth in the liquid, so as to create a new surface continuously without throwing any liquid spray. A mercury manometer, *M*, connected between stopcocks 1 and 2, serves to indicate the vapor pressure of the liquid in *B* at the temperature of the outer bath. *H* is an electrical heating element for the inner bath, *D*. For convenience of varying the heat input, it is connected through a variable voltage transformer to a sensitive relay and an adjustable sealed mercury thermoregulator, *R*.

Y is a cooling coil connected to a refrigeration unit. An expansion valve just above the liquid level in the outer bath prevents undue thermal losses in the line. Since the cooling unit was made entirely of brass tubing, it was necessary to mount a flexible coil above the bath, so that the turntable could be rotated. This was done by bending the brass tubing back and forth in a single plane with about 5-cm. (2-inch) diameter bends and 25-cm. (10-inch) straight sections. The plane of the tubing was then bent around into a circle about 60 cm. (2 feet) in diameter and mounted above the bath with the center line passing vertically through the center of the bath. When the turntable was rotated in one direction the circle merely contracted; on reversing the rotation the circle expanded. The refrigerating unit was set to give a temperature within the inner bath slightly below the desired temperature. The desired temperature was maintained by the heating unit.

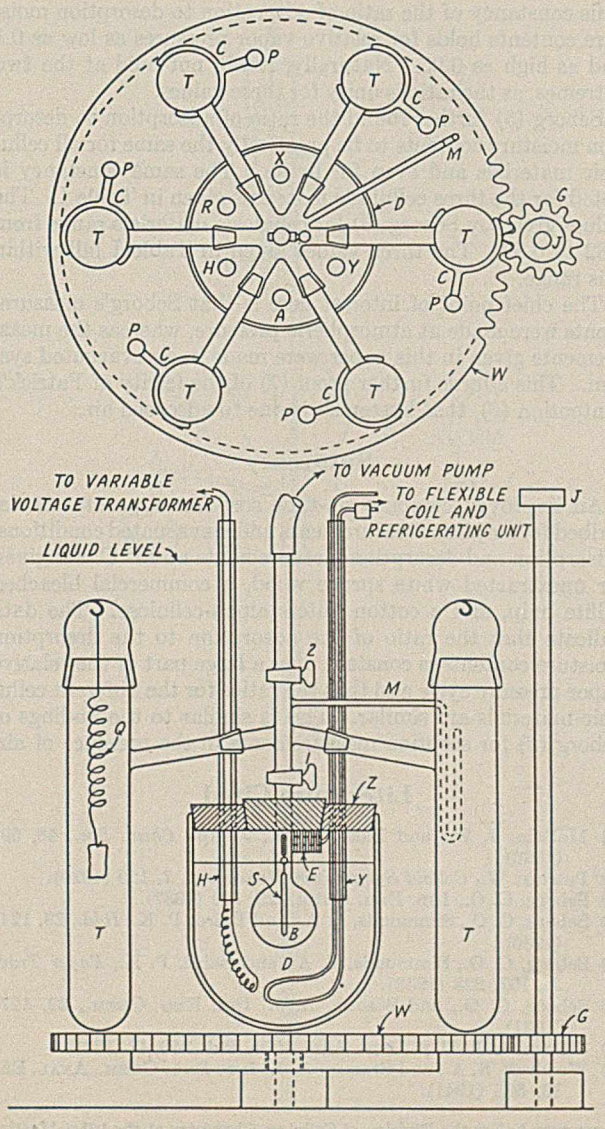

FIGURE 1. SORPTION APPARATUS

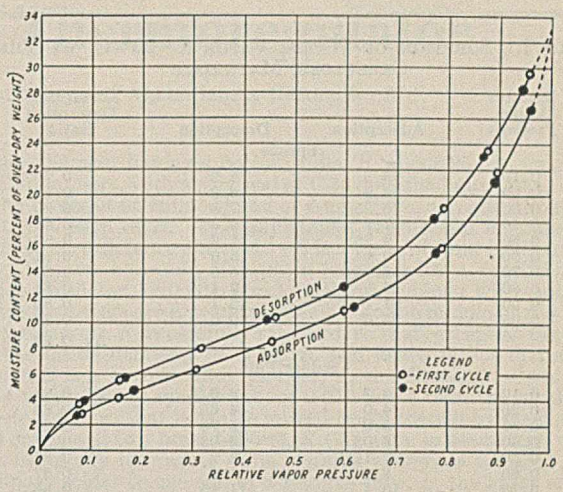

FIGURE 2. SORPTION OF WATER VAPOR BY UNEXTRACTED WHITE SPRUCE AT 20° C.

The small inner bath was adequately stirred by bubbling air through tube *A*. *X* represents a thermometer well for holding a standardized thermometer which could be read to 0.05° C. When all the controls were properly adjusted, the temperature of the inner bath could be held within an accuracy of at least 0.1° C. at temperatures as low as −8° C. up to the outer bath temperature.

The quartz spirals, *Q*, for following the weight of the samples were 1.2 cm. in diameter and had about 20 turns in a length, under their own weight, of 6 to 8 cm. Under a load of 100 mg. they extended from three to four times their unloaded length. The calibration curves were linear up to loads of at least 100 mg. Moist sample weights never exceeded this value. Tests showed that the extension of the spirals was not affected by changes in relative humidity, as was found by Seborg, Simmonds, and Baird (*5*). This may be due to the fact that a fresh stock of quartz rod was used for drawing out the quartz threads.

The extension of the quartz spirals was measured with a cathetometer permanently mounted in front of one of the outer bath windows. The turntable was rotated so as to bring each tube in succession and also the manometer in line with the cathetometer. Cathetometer readings could be readily checked within an accuracy of 0.005 cm. A 50-mg. load could thus be weighed within an accuracy of 0.05 mg. or 0.1 per cent.

Air-dry samples of the material to be tested (50 to 75 mg.) in a thin sheet form are suspended from the quartz spirals, *Q*. Bulb *B* is one-third filled with distilled water through a fine glass capillary. The apparatus is sealed, stopcock 1 is closed, stopcock 2 is opened, and then the apparatus is evacuated with a high vacuum pump protected with a vapor trap. The water in *B* is frozen, stopcock 1 is carefully opened for a few minutes and then closed. The ice in *B* is melted, stirred, and again frozen, followed by the opening of stopcock 1. After repeating this several times to remove all entrained air, stopcock 1 is kept closed and the outer system is evacuated for 48 hours to bring the samples to constant dry weight. After weighing the samples, *B* is brought to the lowest desired temperature. If this is below the freezing point, the inner stirrer cannot be operated, but at higher temperatures it should be continuously operated. Stopcock 2 is then closed and stopcock 1 opened. The sorption of moisture by the samples from *B*, under the relative vapor pressure determined by the temperatures in the inner and outer baths, was in practically all cases complete in less than 12 hours and in all cases was complete in 24 hours. After determining the equilibrium weights, the temperature in *B* is again raised and the sorption at the newly established higher relative vapor pressure is de-

TABLE I. SORPTION OF WATER VAPOR AT 20° C. BY THREE CELLULOSIC MATERIALS

Relative Vapor Pressure	Adsorption	Desorption	Adsorption-Desorption Ratio
	Spruce		
0.10	3.3	4.2	0.79
0.20	4.9	6.2	0.79
0.30	6.25	7.8	0.80
0.40	7.7	9.5	0.81
0.50	9.25	11.2	0.82
0.60	10.9	13.0	0.84
0.70	12.9	15.5	0.83
0.80	16.3	19.4	0.84
0.90	21.7	25.1	0.86
0.95	25.6	29.0	0.88
			Av. 0.83
	Sulfite Pulp		
0.10	2.7	3.2	0.84
0.20	4.0	4.65	0.86
0.30	5.0	5.75	0.87
0.40	6.05	6.95	0.87
0.50	7.15	8.2	0.87
0.60	8.4	9.6	0.87
0.70	10.1	11.45	0.88
0.80	12.4	14.3	0.87
0.90	16.5	19.1	0.87
0.95	20.9	23.6	0.88
			Av. 0.87
	Cotton Linters Alpha-Cellulose		
0.10	2.1	2.5	0.84
0.20	3.2	3.8	0.84
0.30	3.9	4.6	0.85
0.40	4.75	5.6	0.85
0.50	5.6	6.6	0.85
0.60	6.7	7.8	0.86
0.70	8.0	9.2	0.87
0.80	9.9	11.4	0.87
0.90	13.0	14.9	0.87
0.95	16.9	18.0	0.88
			Av. 0.86

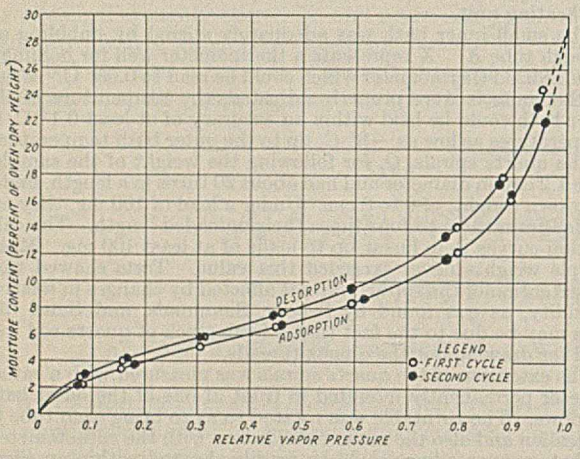

FIGURE 3. SORPTION OF WATER VAPOR BY A COMMERCIAL BLEACHED SULFITE PULP AT 20° C.

termined. The stepwise increases in the inner bath temperature are repeated until the inner bath temperature exceeds the outer bath temperature by a few tenths of a degree. This condition is maintained for 1 to 2 hours to ensure supersaturation of the sample, after which the desorption cycle is followed in a similar manner to the adsorption by decreasing stepwise the inner bath temperature.

Preliminary Sorption Data

Sorption measurements were simultaneously made at 20° C. on two samples of an unextracted white spruce wood two samples of a commercial bleached sulfite pulp, and two samples of a cotton linter alpha-cellulose. The materials were carried through two complete relative vapor pressure cycles. The data are graphically shown in Figures 2, 3, and 4. In all cases the deviations in sorption between the check samples

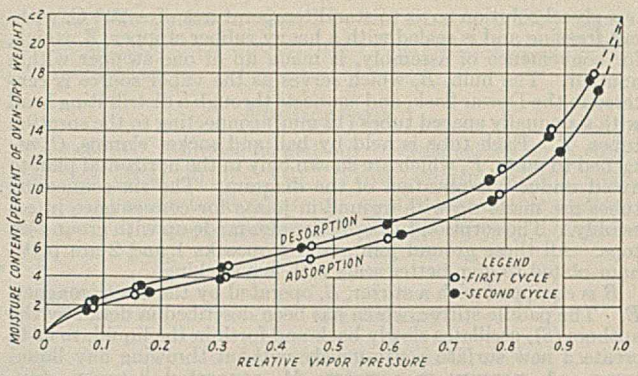

FIGURE 4. SORPTION OF WATER VAPOR BY COTTON LINTERS ALPHA-CELLULOSE AT 20° C.

were less than 0.3 per cent moisture content and averaged about 0.1 per cent; hence they could not be shown on the graphs.

Seborg (*3*) has shown that the ratio of the adsorption to the desorption moisture content is constant over a rather broad range of relative vapor pressures. This is also the case for the data of this investigation, as is shown in Table I. This constancy of the ratio of adsorption to desorption moisture contents holds for relative vapor pressures as low as 0.1 and as high as 0.95. Naturally, it will not hold at the two extremes, as the ratio is unity for these values.

Seborg (*3*) further found the ratio of adsorption to desorption moisture contents to be practically the same for all cellulosic materials and even for lignin. The same constancy is noted for the three cellulosic materials given in Table I. The values given by Seborg (*3*) for cellulosic materials range from 0.82 to 0.89. The three values given in Table I fall within this range.

The chief point of interest here is that Seborg's measurements were made at atmospheric pressure, whereas the measurements given in this paper were made in an evacuated system. This affords further proof (*7*) of the falsity of Patrick's contention (*2*), that hysteresis is due to adsorbed air.

Summary

An improved compact six-tube sorption apparatus is described for sorption measurements under evacuated conditions. Adsorption and desorption measurements at 20° C. are given for unextracted white spruce wood, a commercial bleached sulfite pulp, and a cotton linters alpha-cellulose. The data indicate that the ratio of the adsorption to the desorption moisture contents is constant over a large part of the relative vapor pressure cycle and that the ratios for the different cellulosic materials are similar. This is similar to the findings of Seborg (*3*) for sorption measurements in the presence of air.

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An Adjustable Vapor Thermoregulator

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GREEN and Loring (*1*) described a method for constructing an ether vapor thermostat and gave the advantages of using it for room temperature control. They pointed out that the regulator, as described by them, must be constructed at the place where it is to be used.

The modified ether vapor thermoregulator described in this paper has given very satisfactory results for about a year in regulating the temperature in a small room. With a proper relay and heating system, a temperature variation of ±0.02° C. at the thermoregulator can easily be maintained. Its design not only overcomes the objection pointed out by Green and Loring, but also makes it easily adjustable over a temperature range of 20° C. (20° to 40° C.). It can be readily made to cover a much wider temperature range, if desired, by simply lengthening the mercury column.

The vapor thermoregulator, shown in Figure 1, is made of Pyrex glass except for the adjustment mechanism. *A* is a 500-ml. vapor chamber having a bottom tube, *B*, about 65 cm. long and of 12-mm. bore, with a ring seal at *C* to hold the liquid ether in *A*. Tube *D*, about 95 cm. long and of 4-mm. bore, extends through *A* to the bottom of *B*. The adjustment mechanism is connected to *D* a short distance above *A*. *E* is a piece of Jena KPG tubing (of 8.04-mm. bore and 50 mm. in length) with a very uniform inside diameter (±0.001 mm.). (Pyrex tubings with uniform inside diameters are now obtainable.) To the middle of *E* a mercury reservoir, *F*, is carefully attached, so that, except at the seal, *G*, the inside diameter of *E* above and below is not disturbed. Inside *E* is a Fernico alloy plunger, *J*, the diameter of which is only very slightly smaller (about 0.015 mm.) than the inside diameter of *E*, so that it can be moved freely therein by means of a rod connected to a brass bellows, *K* (250 mm. in diameter), without using any lubricant. The metallic bellows is soldered with ordinary solder to one end of a Fernico tube, *L*, the lower end of which is sealed to the Pyrex tubing above *E*. The upper and lower solder joints of the bellows as well as the one to the Fernico tube should be gas-tight. Both the Fernico alloy and the KPG Jena tubing can be sealed directly to Pyrex glass. By turning the thumbscrew *T*, the bottom of the Fernico plunger in *E* can be raised above point *G* or lowered beyond that point. *G* should be about 15 mm. below contact point *H*, so that the mercury always exerts a little pressure against the bottom of the plunger.

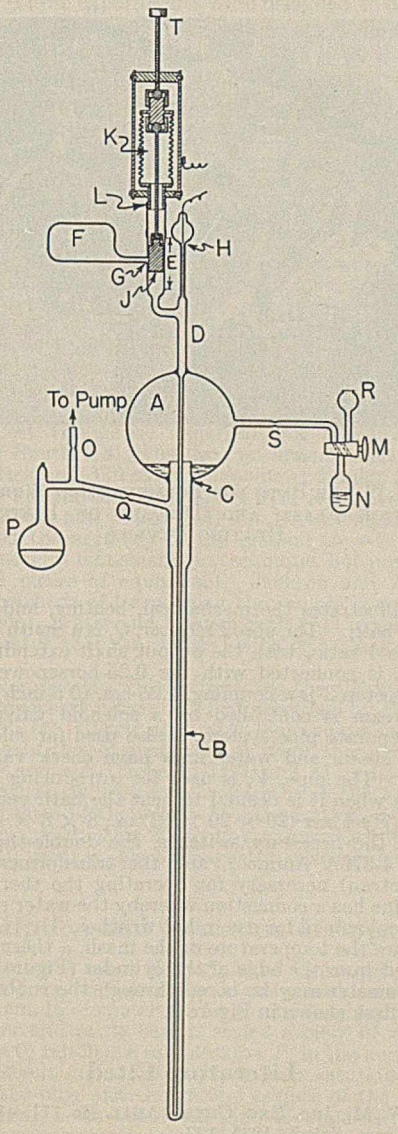

FIGURE 1

After the system has been thoroughly cleaned and dried, mercury and ethyl ether are introduced into the system in the following manner: With stopcock *M* opened to bulb *N*, the system is evacuated with a mercury pump and then sealed off at *O*. Before sealing at *Q*, enough mercury is distilled over from flask *P* to fill tube *B* to a point a little below *Q*. Now cup *R* is filled with dried ethyl ether, about 5 ml. of which are carefully introduced into bulb *N*. Then about 2 ml. of the liquid are distilled into *A* by slightly warming bulb *N*. Some of the ether condenses and remains in tube *B*. The ether in *A* is then cooled with dry ice to reduce the ether vapor pressure in the system before sealing off at *S*.

After removing the dry ice bath from *A*, the instrument is ready for use. Thumbscrew *T* is turned so that the bottom of the Fernico plunger in *E* is a little above *G*. As the thermoregulator begins to warm up to the desired temperature—30° C. for example—the vapor pressure of the ether in the system increases and forces the mercury in *B* to rise in *D*. Since there is enough mercury in this instrument to operate at 20° C., the excess mercury goes into reservoir *F*. When the room temperature is about 0.5° C. below the desired temperature, the plunger is lowered below *G* by turning the thumbscrew to cut off the mercury in the reservoir from the main column. In this respect, the plunger acts as a stopper. It also serves as the final adjustment for the temperature control, which is accomplished by gradually lowering the plunger still further in *E*, so as to force the mercury into the upper part of *D*, until it barely reaches contact point *H*, when the room temperature is exactly at 30° C. Then the distance between *H* and the mercury surface in tube *B* is 63.48 cm., which is the vapor pressure of ethyl ether at this temperature.

The room temperature can easily be raised from 30° to 35° C., by moving the plunger above *G*, to let more mercury into the reservoir *F*, as the temperature in the room is increased. As in the previous operation, the plunger is lowered below *G* again when the room temperature reaches about 0.5° C. below the desired temperature. The final adjustment of the temperature is carried out as before.

To lower the room temperature from 35° to 25° C., the plunger is again raised above point *G*, but this time the mercury in reservoir *F* is allowed to flow back into the system as the room is being cooled, until the room temperature reaches the desired point. The final adjustment is the same as before.

The thermoregulator is flexible. Although contact point *H* is sealed in an evacuated chamber, this thermoregulator (unlike most adjustable thermoregulators of this type) does not require setting in a bath of the desired temperature when changing from one temperature setting to another.

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A Laboratory Water-Bath for Cooking, Mashing, and Fermentation Studies

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THE water baths described in the recent literature (*1*, *2*, *3*) and those commercially available were too small for the authors' requirements in fermentation studies. A bath was desired in which the three stages of cooking, mashing, and fermentation could be conducted with volumes varying from only a few cubic centimeters up to 10 liters. The bath shown in Figures 1 and 2 was designed to satisfy these requirements.

The bath is large enough (300 liters) to accommodate two 12-liter flasks if desired. The over-all dimensions are: 116.2 cm., 46.5 inches (long) × 70 cm., 28 inches (wide) × 66.88 cm., 26.75 inches (high), not including the angle-iron stand. The inside width is 50 cm. (20 inches) and the length is 100 cm. (40 inches) at the widest points. The bath is insulated with at least 5 cm. (2 inches), at the narrowest points, of building insulation wall bats, 37.5 × 57 cm. (15 × 23 inches). The walls are of 0.62-cm. (0.25-inch) Transite with 17 standard aluminum angle 0.3 × 2.5 cm. (0.125 × 1 × 1 inch). The bottom is of white pine.

The inside of the oval bath is tinned copper. The bottom is false and has in the middle, crosswise, a ridge partly visible in Figure 1, *A*, directly under the stirrer. This ridge, 15 cm. (6 inches) higher than the lowest part of the bath, effectively eliminates dead water spots. The depth of the bath at the trough is 60 cm. (24 inches).

The larger glass apparatus is held by two types of baffles which are introduced into slots, *B*, soldered on the sides of the bath, 25 cm. (10 inches) from each end. One type, *C*, holds the tall cylindrical Pyrex jar (45 cm., 18 inches high and 15 cm., 6 inches, in inside diameter), *D*, in which the atmospheric cooking and mashing are carried out. The mash is then transferred for the fermentation to a 12-liter flask which is supported in the bath by a two-pronged baffle, *E* (Figure 2). The baffles are made of polished aluminum sheet, 0.3 cm. (0.125 inch) thick, reinforced at several places with additional aluminum strips. The baffle, *C*, is 23.5 cm. (9 inches) high and 49.67 cm. (19.87 inches) long. The slots attached to the wall are soldered at the lower end.

The top of the bath is covered with copper sheet and refrigerator rubber door gaskets are tacked about each opening upon which rest the aluminum covers, *F*, when the bath is in use. These covers are held in place by the screw fasteners, *G*, serve as another support for the 12-liter flasks, and cover the bath about the tall cylinders when the water is at the boiling point.

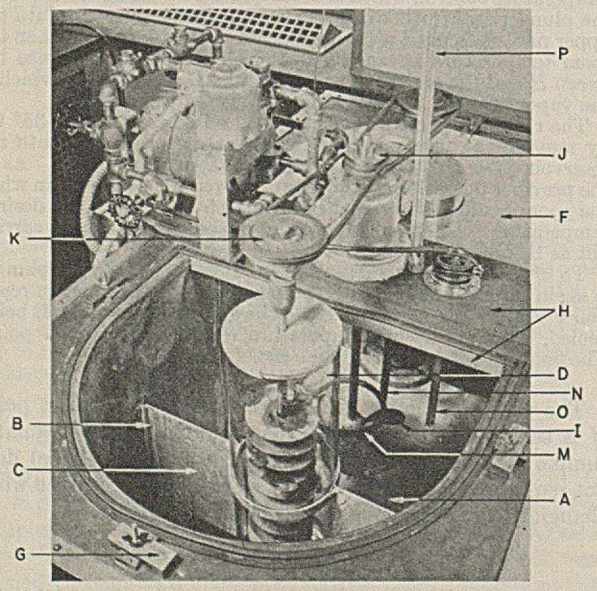

FIGURE 1. WATER BATH FOR FERMENTATION WORK SHOWING COOKING AND MASHING ARRANGEMENT

The bath is emptied by means of two 3.75-cm. (1.5-inch) drains (type K soft copper tubing) and there is also an overflow opening of the same size just underneath the crosspiece, *H*, which is made of a 0.9-cm. (0.375-inch) steel plate, 16.25 cm. (6.5 inches) in width, resting on aluminum angle. The water is stirred by a 20-cm. (8-inch) 3-blade L. H. aluminum propeller, *I*, mounted on a brass shaft. The jar contents, *D*, are stirred by a Monel screw-type stirrer. The brass center pulley, *J*, 5 cm. (2 inches) in diameter, has a speed of 254 r. p. m. The cast-iron end pulleys, *K*, have diameters of 9.4 cm. (3.75 inches) and are mounted on hinges, *L*, to facilitate removal of stirrers and flasks. The stirrers are held by No. 6 Millers Falls straight-shank drill chucks, 0.9-cm. (0.375-inch) cap, along with combination ball bearings and thrust ball bearings.

Figure 1 shows further the steam ring, *M*, directly above the stirrer, the water pipe, *N*, used for filling the bath, and the thermoregulator, *O*, which has a 15-cm. (6-inch) extension (Aminco). The thermometer, *P*, is a special length 101° C. instrument.

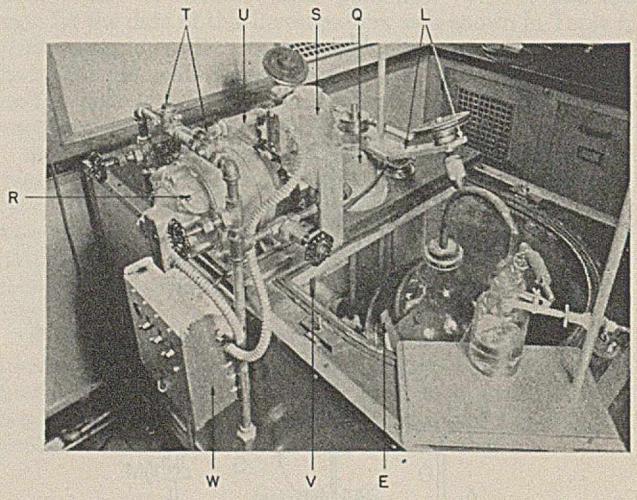

FIGURE 2. WATER BATH FOR FERMENTATION WORK, SHOWING FERMENTATION FLASK AND DETAILS OF ELECTRICAL AND HEATING DEVICES

Figure 2 illustrates the mechanical, heating, and cooling features of the bath. The speed reducer, *Q*, is a Smith No. 2 BV reducer, 7.5 to 1 ratio, with the output shaft extending from the bottom. It is connected with the 0.25-horsepower motor, *R*, through a Boston 3-jaw coupling, 1.25-cm. (0.5-inch) hole, FCN-12. The steam is controlled by a solenoid valve, *S*, which, through a separate pipe system, is also used for cold water control. Both steam and water lines have check valves, *T*, and strainer, *U*. The pipe, *V*, is used for introducing steam under full pressure when it is desired to heat the bath very quickly.

The sheet steel box (20 × 20 × 10 cm., 8 × 8 × 4 inches), *W*, contains all the necessary switches, the double-throw mercury relay (No. 4-376A Aminco), and the transformer (No. 4-460 Aminco Rectran) necessary for operating the thermoregulator. The water line has a connection whereby the water can be passed through a cooling coil for use in hot weather.

To measure the temperature of the mash, a thermometer may be suspended from the edge of the cylinder (Figure 1), or a hole for a thermometer may be bored through the rubber stopper of the 12-liter flask shown in Figure 2.

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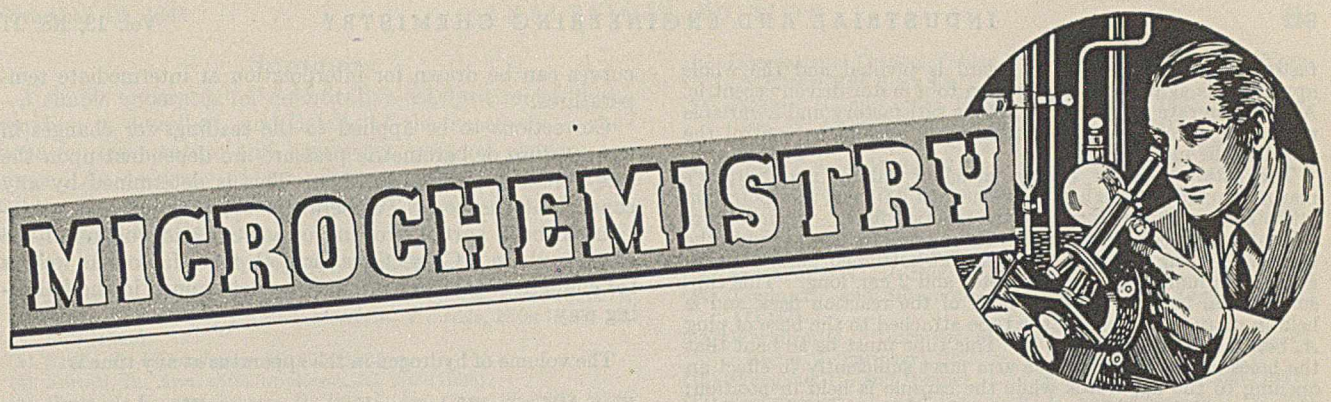

A Simplified Microhydrogenation Apparatus

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IN THE course of certain investigations in this laboratory on compounds of plant origin it was desired to carry out quantitative catalytic hydrogenations on small amounts of material. These experiments were designed to determine the number of reducible groupings such as ethylenic double bonds present in the compounds studied, with a view to obtaining structural information concerning them. The various kinds of apparatus previously described for such work have been based on one or the other of two principles: the measurement at constant volume of the decrease in pressure resulting from the consumption of hydrogen, and the volumetric measurement at constant pressure of the hydrogen absorbed.

Apparatus for the manometric procedures were modified from the original Warburg (*15*) manometers for metabolism studies. Hyde and Scherp (*5*) and Kuhn and Möller (*9*) designed apparatus of a complicated nature which gave results of ±2 and ±0.5 per cent accuracy, respectively. Tsuda and Sakamoto (*14*) reported a much simpler apparatus based on the same principle. Foresti (*4*) made use of a solenoid-activated stirring mechanism in an apparatus of high sensitivity.

The earliest volumetric apparatus for microhydrogenations was that of Smith (*13*), who used an arrangement with a compensation flask and differential manometer similar to that in the manometric apparatus and obtained with it an accuracy of ±2 per cent. Slotta and Blanke (*12*) and Bretschneider and Burger (*1*) increased the accuracy of the method to ±1 per cent and introduced several refinements in technique, but produced highly complicated pieces of equipment. Jackson and Jones (*7*) devised new methods of introducing the sample and agitating the reaction flask. Prater and Haagen-Smit (*11*) recently described a new apparatus that can be used with or without the compensation flask.

Simplified apparatus which eliminated the differential manometer and compensation flask was first described by Ebel, Brunner, and Mangelli (*2*). Their apparatus, however, was designed for semimicroquantities of material, as were those of Erdös (*3*), Zechmeister and von Cholnoky (*17*), and Mayeda (*10*). Kaufmann and Baltes (*8*) devised an apparatus on this same principle for determination of the degree of unsaturation of fats by catalytic hydrogenation, using 20- to 40-mg. samples. Weygand and Werner (*16*) used an electromagnetic stirring device in their apparatus.

Description and Operation of Apparatus

The apparatus is made throughout of Pyrex glass. The bulb of the reaction flask is 3.5 cm. in diameter and the neck and side arm are 1.1 cm. in diameter. The ground-in plug, *A*, in the side arm is hollow and to its base is sealed a piece of 2-mm. tubing which serves to retain the sample tube, *F*, in the neck of the flask until the solvent and catalyst have been saturated. Through the side of the plug and the ground surface of the side arm are bored matching holes about 0.5 mm. in diameter. At *B* is a ground-glass connection to a capillary of 1-mm. bore which leads to the measuring buret. This buret is made of tubing of uniform bore, 4 mm. in inside diameter, and about 43 cm. long; its capacity is therefore about 5.5 cc. It is calibrated in 0.1-cc. divisions which are sufficiently long to be read accurately to ±0.01 cc. At point *D*, the base of the buret, a side tube of the same diameter as the buret is sealed. This serves both as a manometer for accurate leveling of the buret liquid and as an inlet tube, when connected at *C* to the source of hydrogen. A leveling bulb is connected by rubber tubing to the apparatus at *E*.

The apparatus is clamped firmly to a 0.94-cm. (0.375-inch) board, about 13.8 × 50 cm. (5.5 × 20 inches), at the points shown in Figure 1. The capillary above *B* is bent out from the board a distance sufficient to accommodate the bulb of the reaction flask, and is supported at *B* by being clamped to a block of wood

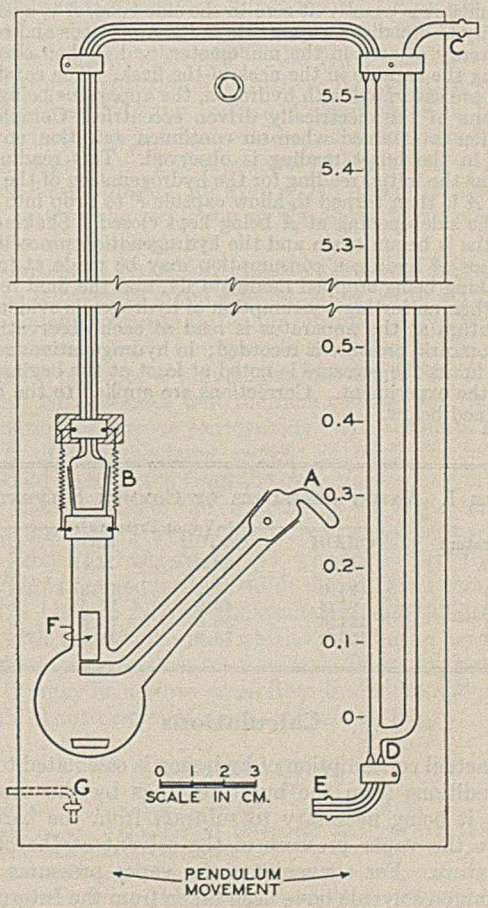

FIGURE 1. APPARATUS

fastened to the board. The board is pivoted and the whole apparatus is agitated by connection to a motor-driven eccentric. A vibration rate of 200 per minute is satisfactory and a variable resistance is connected in the electrical system to control the speed of the motor.

The catalyst, either Raney nickel or Adams and Shriner's platinum oxide, is weighed to the nearest 0.01 mg. in a porcelain microboat and introduced into the reaction flask. Then 5 cc. of solvent are added. The sample, 5 mg. or more, is weighed to the nearest 0.01 mg. in a thin-walled, flat-bottomed glass capsule, *F*, about 8 mm. in outside diameter and 2 cm. long. This capsule is then inserted into the neck of the reaction flask and is held in place by the end of the tube attached to the base of plug *A*, bent as shown in Figure 1. This tube must be so bent that the holes in the plug and side arm meet sufficiently to effect an opening to the apparatus while the capsule is held in position; it must also be possible to turn plug *A* sufficiently to close off the opening through the holes, still retaining the capsule in position. The side arm of the reaction flask must be blown outward above its connection to the flask, as shown in the figure, to allow withdrawal of plug *A*. With *A* adjusted at the open position, the flask is attached to the capillary manifold through the carefully greased connection at *B*, and is held firmly in position by springs. The liquid in the buret, preferably mercury, is lowered below *D* and hydrogen is swept through the apparatus for several minutes, in at *C* and out at *A*. This procedure is a distinct advantage over the method previously used, of alternately evacuating the apparatus and introducing hydrogen, repeating 6 to 10 times.

Before the hydrogen enters the apparatus it is bubbled through a tower containing alkaline stannite solution to remove traces of oxygen and then through a saturator containing the same solvent used in the reaction flask. This procedure reduces to a minimum the time necessary for saturation of the atmosphere in the apparatus. The hydrogen is led through a small-bore tube to the bottom of a side-arm test tube, 17.5 × 2.2 cm. (7 × 0.875 inches), which is filled with glass beads covered by the solvent. It is advisable to insert a Bunsen valve in the inlet tube from the hydrogen cylinder.

When the apparatus has been thoroughly swept out the source of hydrogen is disconnected, plug *A* is closed, still holding capsule *F* in place in the neck of the flask, and the apparatus is let stand until thermal equilibrium is reached. The mercury is then raised until it is exactly at zero in the side tube, *A* is opened momentarily to equalize the pressure in the apparatus and to adjust the mercury at zero in the manometer, and is then closed, still retaining the capsule in the neck of the flask. The catalyst and solvent are saturated with hydrogen, the apparatus being shaken by means of the electrically driven eccentric. Completion of saturation is attained when on continued agitation no further change in the buret reading is observed. This reading is recorded as the initial reading for the hydrogenation of the sample.

Plug *A* is then turned to allow capsule *F* to drop into the solvent, the side opening at *A* being kept closed. Shaking of the apparatus is begun again and the hydrogenation proceeds. Observations of hydrogen consumption may be made at intervals, the shaking being stopped momentarily, and the final reading is made when no further consumption of hydrogen is evident. The temperature at the apparatus is read at each observation, and the barometric pressure is recorded; in hydrogenations requiring several hours the pressure is noted at least at the beginning and end of the experiment. Corrections are applied to the readings as outlined below.

Table I. Vapor Pressures of Common Solvents (*6*)

Temperature	Vapor Pressure			
	CH_3OH	C_2H_5OH	H_2O	CH_3COOH
° C.	*Mm.*	*Mm.*	*Mm.*	*Mm.*
15	71.0	32.2	12.8	8.5
20	93.3	43.9	17.5	11.7
25	121.6	59.0	23.8	15.0
30	156.9	78.8	31.8	20.6

Calculations

The actual consumption of hydrogen is calculated to standard conditions from the buret readings by the usual procedure, it being necessary to subtract from the barometric pressure the vapor pressure of the solvent at the observed temperature. For convenience the vapor pressures of several common solvents have been taken from the International Critical Tables (*6*) and are presented in Table I. From them curves can be drawn for interpolation at intermediate temperatures.

Corrections to be applied to the readings for changes in temperature or barometric pressure are dependent upon the free volume of the apparatus. This is determined by any convenient method. Temperature changes produce variations in both partial pressure of solvent and volume of the gaseous phase. Corrections for both these factors as well as for changes of temperature may be determined in the following way:

The volume of hydrogen in the apparatus at any time is

$$V_{H_2} = [V_{total} - V_{solvent} - V_{(boat\ +\ capsule)} - V_{buret}] \times \frac{273}{T} \times \frac{P - vp}{760}$$

where P = barometric pressure and vp = vapor pressure of the solvent at temperature T. This volume, V_{H_2}, is calculated at the time the hydrogenation of the sample is started and again at the end. The difference between the two values is the volume of hydrogen, at standard conditions, used by the sample.

Example. Fumaric acid, hydrogen number, 116. Sample, 5.302 mg. Catalyst, PtO_2, 3.112 mg. Solvent, alcohol, 5 cc. Total volume, 35.3 cc. Volume of boat and capsule, 0.4 cc.

Hydrogenation of catalyst: Initial readings, $V_{buret} = 0$; $P = 741.0$; $t = 28.0°$. Final readings, $V_{buret} = 0.69$; $P = 741.3$; $t = 28.5°$.

Hydrogenation of sample: Initial readings, $V_{buret} = 0.69$; $P = 741.3$; $t = 28.5°$. Final readings, $V_{buret} = 2.06$; $P = 741.3$; $t = 28.3°$.

Vapor pressure of alcohol, 70.2 mm. at 28°, 71.5 mm. at 28.3°, 72.5 mm. at 28.5°

$$\text{Initial } V_{H_2} = (35.3 - 5.0 - 0.4) \times \frac{273}{301} \times \frac{741 - 70.2}{760} = 23.94 \text{ cc.}$$

After hydrogenation of catalyst

$$V_{H_2} = (35.3 - 5.0 - 0.4 - 0.69) \times \frac{273}{301.5} \times \frac{741.3 - 72.5}{760} = 23.27 \text{ cc.}$$

$$\text{Observed } V_{H_2} \text{ used by catalyst} = 23.94 - 23.27 = 0.67 \text{ cc.}$$

$$\text{Calculated } V_{H_2} \text{ used by catalyst} = \frac{3.112}{227} \times 2 \times 22.4 = 0.61 \text{ cc.}$$

After hydrogenation of sample

$$V_{H_2} = (35.3 - 5.0 - 0.4 - 2.06) \times \frac{273}{301.3} \times \frac{741.3 - 71.5}{760} = 22.23 \text{ cc.}$$

$$V_{H_2} \text{ used by sample} = 23.27 - 22.23 = 1.04 \text{ cc.}$$

$$\text{Hydrogen number} = \frac{5.302}{1.04} \times 22.4 = 114$$

It is, of course, unnecessary to calculate the volume of hydrogen used by the catalyst. The calculation is put in here to show the close agreement between the observed volume and the volume calculated from the weight of platinum oxide employed. This agreement makes it obvious that catalyst and sample can be hydrogenated simultaneously, correcting the total hydrogen uptake by subtracting the calculated volume of hydrogen used by the catalyst. While the accuracy to be expected by this method is only ±6 per cent, the vigor of the catalyst is considerably greater and the time required for the hydrogenation shorter. Where extreme precision is not necessary the method has some advantages. When using the platinum on zirconium oxide and palladium on zirconium oxide catalysts prepared by the American Platinum Works, the volume of hydrogen absorbed is so small that it can be neglected except where highest precision is required.

Summary

A simple apparatus for quantitative catalytic microhydrogenation is described, and details of the necessary corrections for temperature and barometric pressure changes are given. An accuracy of ±2 per cent, sufficient for determining the number of hydrogenated groupings in organic compounds, is attainable.

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Detection of Carbon Dioxide and Sulfur Dioxide from Mixtures of Carbonates and Sulfites

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IF A GAS containing small quantities of sulfur dioxide and carbon dioxide is passed through a few drops of an acidic solution of ferric ferricyanide, the sulfur dioxide will be oxidized to the nonvolatile bisulfate ion, the brown ferric ferricyanide will be reduced to Turnbull's blue, and the carbon dioxide will pass through the liquid and can be detected with barium hydroxide solution without interference. The simple apparatus (Figure 1) has been developed to carry out the test on a semimicro scale.

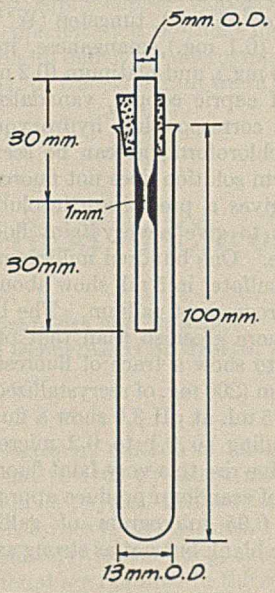

FIGURE 1

Place a drop of a saturated solution of barium hydroxide in the top of the tube and work the drop down into the constricted part. Then put in the lower end of the tube enough of a freshly prepared ferric ferricyanide solution, made by mixing one drop of 0.17 *M* potassium ferricyanide, one drop of 0.3 *M* ferric nitrate, and one drop of 5 *N* hydrochloric acid, to form a thin layer of solution. One or two strands of glass wool may be inserted in the tube to aid in retaining the solution, although they are not necessary.

Carefully remove any liquid on the outside of the tube with filter paper. Put the solid or liquid neutral sample containing about 1 mg. of sulfite and carbonate ion in the test tube, add 4 drops of 5 *N* acetic acid, assemble the apparatus, and place it in a water bath maintained at 70° to 80° for 2 minutes.

If as little as 0.005 mg. of sulfite ion is present the brown solution of ferric ferricyanide will turn blue within a minute. A white precipitate will form in the lower meniscus of the drop of barium hydroxide if as little as 0.025 mg. of the carbonate is present. In the absence of a carbonate, no precipitate forms in the barium hydroxide when as much as 3 mg. of sulfite ion is present. The tube must be cleaned scrupulously after each determination, for even a thin film of ferrous ferricyanide hastens the reduction of the ferric ferricyanide, although the mechanism is not clear.

The solution of ferric ferricyanide should be freshly prepared, for it becomes green after standing 15 to 20 minutes and will no longer oxidize the sulfur dioxide. A similarly prepared solution of ferric ferricyanide was used by Noyes (*2*) to detect the presence of reducing anions in suitably prepared solutions. Noyes states that the blue color is due chiefly to the formation of ferrous ferricyanide. He accounts for the formation of this substance instead of ferric ferrocyanide, which would be expected from the oxidation-reduction potentials, by the slowness of the reduction of the ferricyanide ion by sulfur dioxide. This conclusion is in agreement with the work of Eibner and Gerstacker (*1*), who found that the ferricyanide ion is not reduced by sulfur dioxide even though the solution is boiled. Nitrites, thiosulfates, and sulfides when treated with acids form gases which are reducing agents and will interfere with the test for a sulfite. However, strontium acetate (*3*) may be used to precipitate the sulfite and carbonate ions without precipitating the interfering ions.

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Detection of Gallium

By a Fluorescence Reaction with 8-Hydroxyquinoline

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MINUTE amounts of gallium can be detected by adding 8-hydroxyquinoline to a solution having a pH of 2.6 to 3 and shaking with chloroform. In the presence of gallium, the chloroform layer shows a yellowish fluorescence in ultraviolet light which is due to the extracted gallium hydroxyquinolate. Indium reacts slightly at pH 3 but not at pH 2.6. Other metals do not give an appreciable fluorescence when present in low or moderate concentrations, but a few metals prevent the reaction of gallium.

Procedure

In the absence of ferric iron, cupric copper, vanadate, and molybdate the test may be carried out as follows:

The pH of the solution to be tested is adjusted to 2.5 to 2.6 in any convenient way if indium is present, or to 3.0 if indium is absent (less than 0.05 mg.). The reaction is slightly more sensitive at pH 3.0 than at 2.6. Adjustment of the pH to 3 can be made very simply by acidifying 5 to 10 ml. of the test solution with a drop or two of hydrochloric acid, adding a drop of methyl orange and then a dilute (1 *M*) solution of sodium acetate until the indicator shows the first deviation from its full acid color. One-fourth milliliter of 0.1 per cent 8-hydroxyquinoline solution (obtained by warming 0.10 gram of 8-hydroxyquinoline with 1 ml. of 4 *M* acetic acid until dissolved and then diluting to 100 ml. with water) is added, and the solution is mixed and shaken vigorously with 1 ml. of analytical reagent chloroform in a 1.8 × 15 cm. glass-stoppered flat-bottomed tube. The chloroform is allowed to settle and the layer is viewed transversely while the tube is held vertically over a source of ultraviolet radiation in a dark room. (In the present work a Westinghouse type G-5 ultraviolet lamp was used.) A yellow fluorescence with a tinge of green indicates gallium. When minimal amounts (less than 0.5 microgram) of gallium are to be tested for, the test solution should be compared against a blank, because the chloroform itself fluoresces slightly.

Iron if present in the ferric state must be reduced to the ferrous condition before the gallium test can be applied. This may be done as follows:

One-half gram of hydroxylamine hydrochloride is dissolved in 5 ml. of the slightly acidified solution which may contain as much as 30 to 40 mg. of iron. Sodium acetate solution is added until a brown color appears, and the solution is allowed to stand for 10 minutes (it should become practically colorless in a few minutes). A drop of methyl orange is added, the pH is adjusted to 3.0 (or to 2.6 if appreciable amounts of indium may be present), 0.25 ml. of 0.1 per cent 8-hydroxyquinoline solution is added, and the mixture is shaken with 1 ml. of chloroform. The reduction of iron may not be complete and the chloroform may show a slight brownish color in daylight due to a trace of ferric hydroxyquinolate. So long as the chloroform is only slightly darkened, no harm is done. Prolonged shaking of the solution should be avoided because this leads to reoxidation of the iron.

Vanadium as vanadate can be reduced by hydroxylamine hydrochloride in much the same way as iron. The slightly acidified test solution containing dissolved hydroxylamine hydrochloride is treated with sodium acetate until a greenish color appears, and the solution is allowed to stand until it has become practically colorless. A drop of methyl orange is added, the pH is adjusted to 2.6 to 3, and the solution is then tested as described above.

When copper is present it is precipitated as cuprous thiocyanate by adding potassium thiocyanate and sodium sulfite to the slightly acid solution. Gallium may then be tested for in the filtrate after adjustment of the acidity.

Molybdenum as molybdate can be precipitated with a slight excess of lead nitrate in acetic acid solution, and the filtrate tested in the usual way.

Sensitivity

At pH 3.0 under the conditions described above, 0.1 microgram of gallium in 5 or 10 ml. of solution gives a faint fluorescence that is distinctly stronger than that given by the blank. This corresponds to a concentration of 1 to 10^8. Doubtless gallium can be detected in concentrations less than 10^{-8}, provided the absolute amount is at least 0.1 microgram. The sensitivity at pH 4 is but slightly greater than at pH 3.0. At pH 2.6, in a biphthalate buffer, 0.2 microgram of gallium can be detected with certainty when a blank is used for comparison. The ratio of intensity of fluorescence at pH 2.6 and pH 3.0 is approximately 0.6.

Effect of Other Elements

The following metals give no fluorescence with 8-hydroxyquinoline at pH 3.0 under the conditions already described (25 to 50 mg. of metal in 5 ml. of solution unless otherwise indicated): sodium, potassium, rubidium, copper (Cu^{II}, 10 mg.), silver (nitric acid solution), gold (0.5 mg.), calcium, strontium, barium, magnesium, zinc, cadmium, mercury (Hg^{I} and Hg^{II}, 10 mg.), yttrium (3 mg.), lanthanum (2 mg.), aluminum, thallium (Tl^{I}, nitric acid solution), titanium (Ti^{IV}, 1 mg.), zirconium (1 mg.), cerium (Ce^{III}, 10 mg.), thorium (2 mg.), germanium (0.3 mg.), tin (Sn^{II} and Sn^{IV}), lead, vanadium (V^{V}, 5 mg.), columbium (1 mg. as columbate), tantalum (5 mg. K_2TaF_7), arsenic (As^{V}), antimony (Sb^{III}, 10 mg.), bismuth (10 mg.), chromium (Cr^{III}), molybdenum (Mo^{VI}, 5 mg.), tungsten (W^{VI}, 5 mg.), uranium (U^{VI}), tellurium (0.1 mg.), manganese, iron (Fe^{III}), cobalt (5 mg.), nickel (5 mg.), and platinum (0.2 mg.).

In the case of cupric copper, vanadate, molybdate, and ferric iron, the corresponding hydroxyquinolates are extracted by the chloroform, as can be seen from the color, but the chloroform solution does not fluoresce. Titanium in small amounts gives a precipitate insoluble in chloroform. Lithium appears to give a very faint fluorescence with 8-hydroxyquinoline. One hundred milligrams of lithium (0.75 gram of lithium sulfate) in 5 ml. show about as much fluorescence as 0.1 microgram of gallium. The hue of the lithium fluorescence is more greenish than that of gallium. Beryllium also seems to show a trace of fluorescence. Ten milligrams of beryllium (200 mg. of recrystallized beryllium sulfate tetrahydrate) in 5 ml. at pH 3.0 show a fluorescence (yellow-green) corresponding to 0.1 to 0.2 microgram of gallium. Scandium may give rise to a very faint fluorescence at pH 3.0. Two milligrams of scandium produce approximately as much fluorescence as 0.05 microgram of gallium (fluorescence stronger than the blank but not as strong as 0.1 microgram of gallium).

At pH 3.0, 1 mg. of indium shows about as much fluorescence as 2 micrograms of gallium. The fluorescence of indium is more yellow than that of gallium, the trace of green present in the gallium fluorescence not being evident. The extractability of indium hydroxyquinolate increases rapidly as the pH of the solution is increased above 3. At pH 2.6 (biphthalate buffer) small amounts of indium show no fluorescence. Thus 1 mg. of indium in 5 ml. of solution containing 0.25 ml. of 0.1 per cent of 8-hydroxyquinoline shows a doubt-

TABLE I. FLUOROMETRIC DETERMINATION OF GALLIUM

Addition	Gallium Present	Gallium Found
Mg.	*Microgram*	*Microgram*
30 Al	0.0	0.0
30 Al	0.1	0.1
30 Al	0.5	0.55
30 Al	1.0	1.0
50 Al	0.5	0.4
10 Fe^{III}	1.0	0.9

ful fluorescence after shaking with 1 ml. of chloroform, a blank being used for comparison; the fluorescence is less than that of 0.1 microgram of gallium.

The detection of 0.5 microgram of gallium offers no difficulty in the presence of the following metals (25 to 50 mg. present except as indicated): alkalies, alkaline earths, silver, beryllium (10 mg.), magnesium, zinc, cadmium, mercury (Hg^{I} and Hg^{II}, 10 mg.), scandium (2 mg.), yttrium (3 mg.), lanthanum (2 mg.), aluminum, indium (1 mg.), thallium (Tl^{I}), zirconium (1 mg.), cerium (Ce^{III}, 10 mg.), thorium (2 mg.), lead, arsenic (As^{V}), chromium (Cr^{III}), tungsten (W^{VI}, 5 mg.), uranium, manganese, cobalt (5 mg.), nickel (5 mg.), platinum (0.2 mg.), and palladium (0.2 mg.). It is believed that less than 0.5 microgram of gallium can be detected in the presence of most of these metals even when they are present in amounts greater than those specified. In the case of tin, antimony, and bismuth, which yield heavy precipitates by hydrolysis, 1 to 2 micrograms of gallium are required for a definite reaction when 10 mg. of one of these metals are present. The precipitate in the chloroform makes it difficult to recognize a fluorescence, and these elements are best separated before applying the test. Titanium, columbium, tantalum, and tellurium also should be separated unless present in very small amounts.

The metals interfering seriously with the detection of gallium are those already mentioned as forming hydroxyquinolates soluble in chloroform under the conditions of the test—namely, ferric iron, cupric copper, vanadate, and molybdate. By reduction or precipitation of these metals as described above, it is possible to detect 0.5 microgram of gallium in the presence of 30 mg. of iron, 5 mg. or more of vanadium, and 10 mg. of copper. One microgram of gallium can be detected in the presence of 5 mg. of molybdenum.

Fluoride reduces the sensitivity of the gallium reaction enormously. In 5 ml. of solution of pH 3.0 containing 10 mg. of sodium fluoride, 2 micrograms of gallium are barely detectable. However, if aluminum is present in sufficient amount, the sensitivity is not reduced. Citrate also inhibits the reaction. Phosphate reduces the sensitivity only slightly. One-half microgram of gallium in 5-ml. solution of pH 3 containing 100 mg. of ammonium monohydrogen phosphate still gives a fluorescence, but a weaker one than in the absence of phosphate. With 10 mg. of ammonium monohydrogen phosphate in 5 ml., 1.0 microgram of gallium gives approximately the same intensity of fluorescence as 0.9 microgram in the absence of phosphate.

Quantitative Application

Preliminary experiments indicate that the reaction described may be applied to the determination of small quantities of gallium in the presence of relatively large amounts of such elements as aluminum and iron. The results given in Table I were obtained by fluorometric titration.

A dilute standard solution of gallium was added to a comparison solution having the same volume and pH as the unknown solution, and containing the same amount of 8-hydroxyquinoline and chloroform, with shaking after each addition until the chloroform layers in both solutions showed the same intensity of fluorescence. The solutions were adjusted to pH 3.0 and ferric iron was reduced with hydroxylamine hydrochloride. The comparison solution did not contain aluminum or iron.

There appears to be but little diminution in the fluorescence intensity of gallium in the presence of moderate amounts of aluminum or iron. Zinc, however, reduces the intensity of the gallium fluorescence markedly. Thus, 1.0 microgram of gallium in the presence of 20 mg. of zinc (5-ml. volume, pH 3.0) gave approximately as much fluorescence as 0.5 microgram of gallium in a zinc-free solution. Therefore, if gallium is to be determined in the presence of zinc without making a separation, approximately an equal amount of zinc must be present in the comparison solution.

Micromethod of Chromatographic Analysis

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THE chromatographic method of analysis originated by Tswett (*9*) and revived by Kuhn and associates (*5*, *6*) has proved a valuable physical method for the isolation and purification of substances from mixtures.

Considerable difficulty is sometimes encountered and large quantities of material are required in the selection of suitable solvents, adsorbents, and elutriants for the substance to be investigated. A microchromatographic method that requires only a few drops of material has been developed, and has been used in this laboratory for the past two years for rapid preliminary survey before filtering solutions through columns in preparation for spectroscopic examinations.

A number of adsorbents are placed in the cups of a "spot plate" or cupped porcelain dish such as water colorists use. A very small

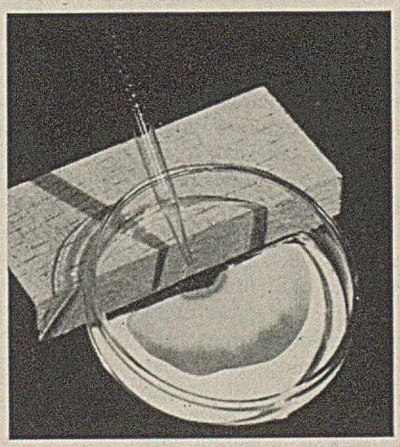

FIGURE 1. TOP VIEW OF PETRI DISH, SHOWING ZONE FORMATION IN ADSORBENT MATERIAL

quantity (0.25 teaspoon) of the adsorbent in the powdered form is placed in the cup and moistened with various solvents; 2 or 3 drops of a prepared creamy mixture of adsorbent and solvent may be used. A drop or two of the solution to be investigated is then placed at the rim of the cup and allowed to flow into the adsorbent; the combination that is most suitable for the particular substance to be studied is determined.

Then a glass Petri dish about one quarter full of the chosen adsorbent is gently shaken in a tilted position, so that the adsorbent settles in the form of a wedge that is very thin at the upper edge and a few millimeters thick at the lower edge. The solution that is to be analyzed is dropped from a 1-ml. pipet into the center of the Petri dish, which is held in a tilted position (Figure 1) so that the solution may flow gently towards the thin edge of the wedge and then filter slowly downwards through the adsorbent. The solvent is added drop by drop to form broad zones of separated material. Experience will determine in each case whether it is better to allow the solution to flow into the adsorbent in the dry state or when moistened slightly with the solvent.

If material that contains fluorescing substances is used, these spread out into broad semicircular fluorescing zones in the light of a Hanovia analytic quartz lamp. This method is very useful in the analysis of biologic materials when only very small quantities are available, and has been found more satisfactory than other micromethods (*1–4, 7, 8, 10*) in the analysis of certain biologic substances.

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Modified Electrometric Determination of Metallic Silver

By a Dead-Stop End-Point Procedure

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NO CHEMICAL method is known for determination of metallic silver in suspensions, such as one would have in exposed photographic emulsions, if silver is present to the order of 1 to 10 $\times$ 10^{-9} mole per liter. A volumetric method can now be reported for estimating such small amounts of silver which makes use of the polarized-electrode method of Foulk and Bawden (*1*). This method measures metallic silver only and is not affected by the presence of silver ions. The reactions involved in the determination are:

$$2Ag^{\circ} + I_2 \rightarrow 2AgI \quad (1)$$

$$I_2 + Na_3AsO_3 + H_2O \rightarrow Na_3AsO_4 + 2HI \quad (2)$$

The silver iodide formed is dissolved by the excess potassium iodide and any hydrogen iodide is neutralized by the sodium bicarbonate. At such low concentrations of silver, special precautions to reduce errors are necessary. A method of following the titration was developed which allows greater precision to the end point. This paper is an account of these factors.

Titration of iodine using the dead-stop end point may be divided into four categories—addition of an iodine solution to a reducing agent in an acid or in an alkaline medium; and addition of a reducing agent to an iodine solution which is acid or alkaline. Wernimont and Hopkinson (*2*) report that titration of iodine with sodium thiosulfate in an acid medium is not so reproducible as when the reverse titration is carried out. The authors studied the titration of iodine and arsenite solutions from both directions and found both end points repeatable.

Experimental Procedure

All chemicals used were of reagent quality. The alkalinity of solutions to be titrated was adjusted to pH 8.3 with 0.02 *N* sodium bicarbonate. Solutions were made with freshly distilled conductivity water and stored in Pyrex bottles. The iodine

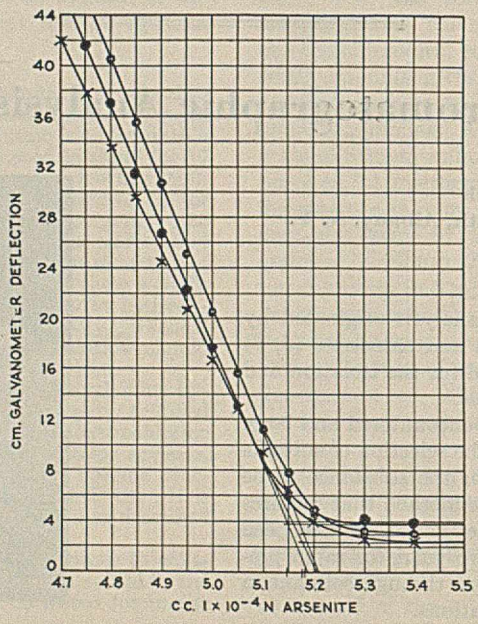

FIGURE 1. TITRATION OF IODINE WITH ARSENITE
30 per cent KI, 0.02 *M* $NaHCO_3$
E. m. f., 100 millivolts

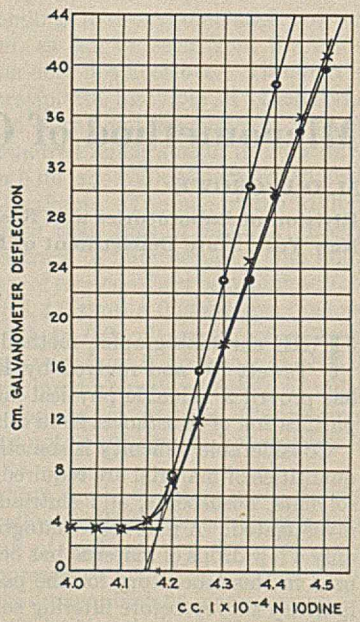

FIGURE 2. TITRATION OF ARSENITE WITH IODINE
30 per cent KI, 0.02 *M* $NaHCO_3$
E. m. f., 100 millivolts

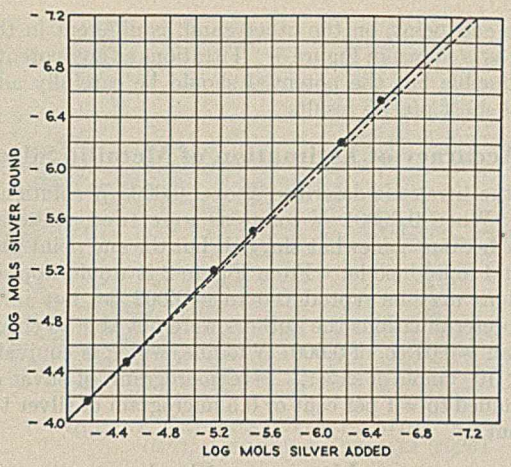

FIGURE 3. DETERMINATION OF SILVER

0.02 *M* $NaHCO_3$, 30 per cent KI
Dashed line indicates no shortage in silver found

solutions contained 40 grams of potassium iodide per liter to increase their stability.

The apparatus was similar to that of Wernimont and Hopkinson (*2*) with the addition of a variable shunt across the galvanometer in order to adjust its sensitivity suitably over a wide range of concentrations. The galvanometer used was the Leeds & Northrup type P, of 1171 ohms' resistance and sensitivity of 8×10^{-10} amperes per mm. A precision dial was attached to a type 371 (General Radio Company) rheostat-potentiometer of 900 ohms' resistance for adjusting the voltage supplied by three dry cells connected in parallel to the electrodes. By calibrating the dial, voltages across the electrodes were ascertained.

It was found necessary to maintain an atmosphere of nitrogen over the solution in the titration vessel and to bubble nitrogen through silver bromide sols during exposure (when silver was obtained from this source). The nitrogen was purified by passing it through a train of chromous sulfate and acid silver nitrate containing suspended silver bromide. Oxygen was removed by the chromous sulfate, sulfur by silver nitrate, and the silver bromide effected the removal of some agent which was able to reduce silver bromide even in the absence of light. The treatment of the nitrogen must be very efficient, since traces of oxygen cause oxidation of potassium iodide and the resulting iodine gives a spurious end point.

The dial is adjusted to the desired voltage and either iodine or arsenite solution is placed in the titration vessel. In the first case, a large deflection of the galvanometer is immediately observed. Addition of small increments of arsenite causes the galvanometer to move towards its rest point (to some extent affected by the voltage across the electrodes) by definite amounts. In the second case, a momentary large deflection of the galvanometer takes place but it quickly returns to a position near zero deflection (not appreciably influenced by voltage). When the end point is reached, small increments of iodine cause the galvanometer to move away from its rest point by definite amounts. A record is made of cubic centimeters of solution added and of galvanometer deflection.

Graphical Treatment of Data

In Figures 1 and 2 curves of such data are shown. Experiments were in triplicate. It is apparent that a linear relationship exists, over a wide range of titration, between amount of titrant added and galvanometer deflection. If this straight-line portion is extrapolated to the base line which represents the minimum galvanometer deflection, the intersection read off on the volume axis of the graph is the end point.

A significant difference is observed in the shape of the toe of the curves in the two sets of data. Whereas, when arsenite is added to iodine (Figure 1) a very broad curvature obtains, the reverse titration (Figure 2) shows a very sharp curvature. This makes it apparent that with the former a sharp end point would be hard to obtain by merely watching the galvanometer come to rest, while the latter shows a permanent deflection of the galvanometer only where the end point is very nearly reached. Even with this graphic method the data show that titration of arsenite with iodine is relatively more reproducible than the reverse procedure. No attempt was made to adjust the iodine solution equivalent to the arsenite. In fact, the end points for such equivalent solutions would not be the same for the reverse titrations. Thus, iodine must first be standardized against arsenite, and in an analysis the titration must be carried out in the direction used for standardizing.

Reliability of Silver Determination

A colloidal silver sol, carefully freed of silver oxide and other possible sources of silver ions, was prepared and its silver content was determined by solution in nitric acid and potentiometric titration of the resulting silver ions with potassium iodide. The stock silver sol was diluted in various amounts with conductivity water carefully freed from oxygen and the silver content of the diluted sols was measured, using the method described in this paper. The data are shown

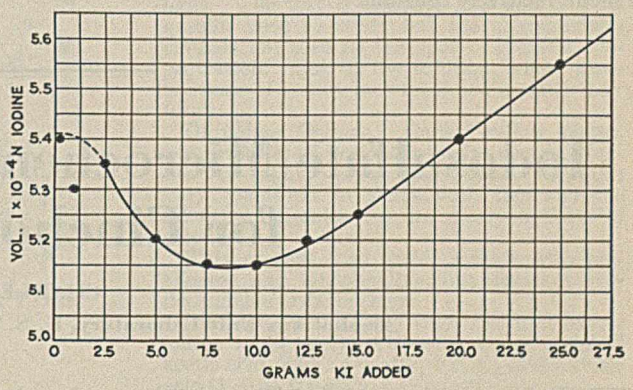

FIGURE 4. EFFECT OF POTASSIUM IODIDE CONCENTRATION ON END POINT

0.02 *M* $NaCO_3$, solutions 1×10^{-4} *N*
E. m. f., 100 millivolts

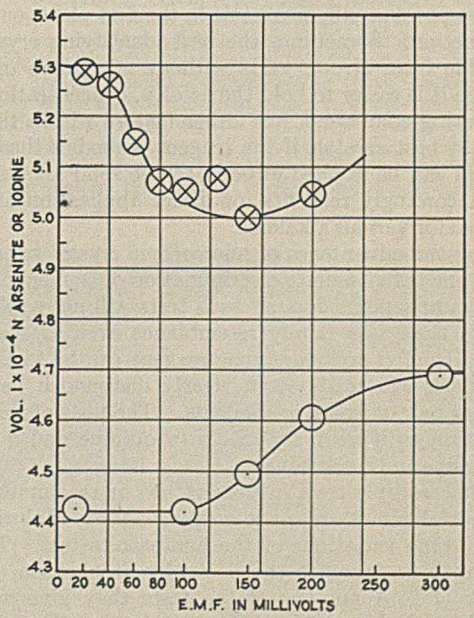

FIGURE 5. EFFECT OF E. M. F. ON END POINT

0.02 *M* $NaHCO_3$, 30 per cent KI
⊙. Iodine titrated with arsenite
⊗. Arsenite titrated with iodine

graphically in Figure 3 and indicate that the new method is reliable over a considerable range of silver concentrations. The slight deviation from the theoretical line may be due to traces of dissolved oxygen in the water used for dilution.

Influence of Potassium Iodide Concentration

In order to determine photolytic silver in silver halide sols free from gelatin it is necessary to dissolve the sol in nearly 30 per cent potassium iodide. This factor was studied from 0 to nearly 50 per cent of iodide. The result is shown in Figure 4. The data at low iodide concentration are somewhat erratic and this appears to be caused by nonuniform sampling of the solid iodide. Use of a stock solution eliminates the difficulty. Above 5 per cent, however, samples are uniform and the solid salt may be used.

Effect of Voltage across the Electrodes

The voltage across the electrodes affects both the end point and the slope of the straight-line portion of the titration curve. The slopes for titration from either direction increase as the voltage increases up to about 100 millivolts, after which they remain relatively constant.

The end point, on the other hand, is different in the two cases, as is shown in Figure 5. Titrations at any potential are reproducible but the potential should be carefully adjusted for the most precise results.

Accuracy of Estimation of Metallic Silver

In the titrations described above, 0.0001 N solutions were used. When 0.00001 N solutions were tried, the electrode action became somewhat sluggish but the end point was definite and reproducible. From Figure 2 it would appear that titrations may be reproduced to ± 0.002 cc. but in actual silver determinations the authors were not able to check better than ± 0.01 cc. of 0.0001 N iodine, which is equivalent to $\pm 1 \times 10^{-9}$ mole of silver. Five micrograms of silver can be determined to ± 1 per cent or 0.5 microgram of silver to ± 10 per cent.

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COMMUNICATION No. 801 from the Kodak Research Laboratories.

Iodosulfate Microchemical Identification Tests for Cinchona Alkaloids

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THE identification of alkaloids and other amines by microscopical observation of their crystalline precipitates has been developed to a high degree. In the past these tests have been made almost invariably on aqueous solutions, although usually the dry substance is available, either submitted as powder, pill, or capsule in the first place, or isolated by extraction. Sometimes the best identifying crystals are obtainable from strong acids without admixture of water; and often it is easier to hold the acidity, concentration of the precipitating compound, and other factors within the limits giving the best crystals if the reagent embodies these conditions and can be applied directly to the solid alkaloid or its salt. Accordingly reagents for direct application are being developed for various alkaloids.

Among the advantages of microscopic crystal tests an important one is the definite discrimination of isomers or closely related compounds. Not all such tests will accomplish this, for often there is a family resemblance even in the crystal forms. Usually, however, some reagent can be found or devised which will readily and clearly distinguish between a particular pair of isomeric alkaloids. The tests of this article yield entirely different crystals with quinine and quinidine, cinchonidine and cinchonine.

The microscopic tests in present use or recommended for these four alkaloids are made on their aqueous solutions, except for some variations of the herapathite test. The best known may be found in the books by Stephenson (*14*) and Amelink (*1*). A number of the tests they give were due originally to Behrens (*3*) or Grutterink (*8*). These may be supplemented by certain suggestions of the writer: chloromercuric acid with 15 volumes per cent of hydrochloric acid for quinine (*6*), bromoplatinic acid for cinchonine (*6*), dilute (0.2 per cent) picric acid for cinchonine (*7*), and ferrocyanide with phosphoric acid for cinchonine and cinchonidine (*7*). Recent studies, with new recommendations, have been made by Whitmore and Wood (*16*) and Martini (*11*). There does not appear to be as yet any general agreement as to the best test for any one of the four alkaloids.

The crystals of quinine iodosulfate, or herapathite, so notable and well known for their extreme dichroism, provide certain identification of quinine when they can be obtained. Even without a microscope they are characterized by a dark beetle-green metallic luster or iridescence. Numerous variations of this quinine test have been given since its discovery by Herapath (*1, 2, 4, 9, 10, 12, 15*). Mulliken (*13*) and Fuller (*5*) state that cinchonidine in this test yields microscopic needles without metallic luster, and that quinidine also yields an iodosulfate precipitate, which according to Fuller is reddish brown and much more soluble than that of quinine, so that it often takes a considerable time to form. Quinidine may not give the test, depending on the variation used. When quinidine iodosulfate crystals are obtained they do not resemble herapathite in optical properties.

In the writer's experience none of the previous versions of the herapathite test has been very satisfactory, even for quinine alone, on the small scale used for other microcrystal tests for alkaloids. His purpose at first was to perfect this quinine test for microchemical use. It was found that excellent iodosulfate crystals could also be obtained with quinidine, cinchonine, and cinchonidine.

Quinidine, the stereoisomer of quinine, gives canary-yellow, nondichroic crystals. Cinchonine gives plate crystals varying in depth of color but also nondichroic, as observed with polarized light. Of the isomers cinchonine and cin-

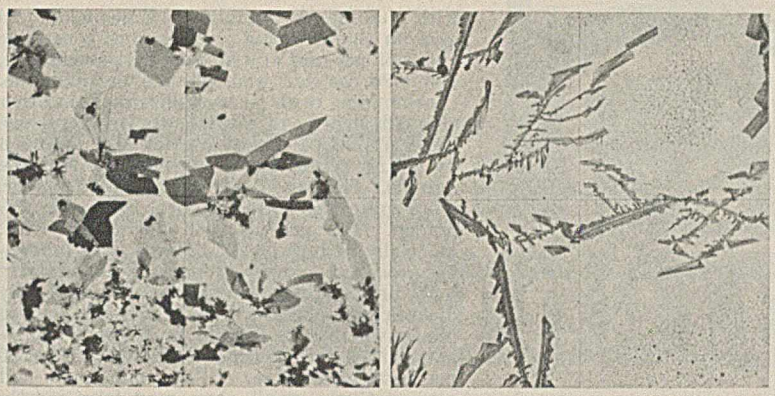

FIGURE 1. QUININE CRYSTALS (HERAPATHITE), WITH POLARIZED LIGHT (*left*); QUINIDINE CRYSTALS (*right*)

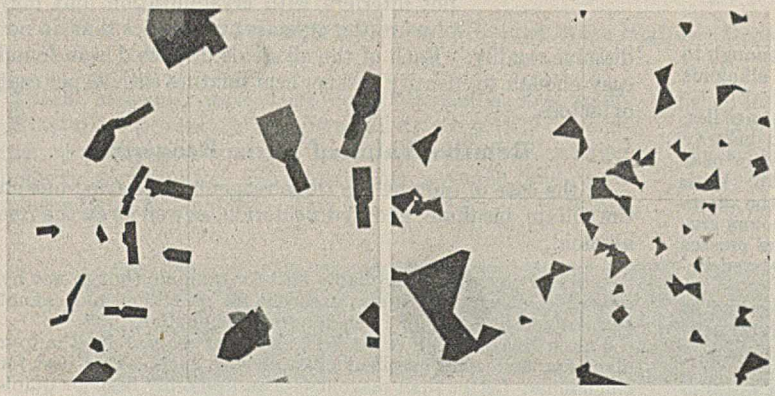

FIGURE 2. CINCHONINE CRYSTALS, RECTANGULAR AND PENTAGONAL TYPES (*left*); CINCHONINE CRYSTALS, TRIANGULAR TYPE (*right*)

chonidine, cinchonidine corresponds the more closely to quinine, cinchonine to quinidine. Cinchonidine gives two distinct kinds of crystals, under different conditions, needles and plates, the latter showing dichroism as extreme as that of herapathite. This dichroism is an almost complete absorption of light vibrating in one direction in the crystal, with transparancy to light vibrating at right angles. All the crystals are highly birefringent, and appear bright with crossed nicols.

Method

Put a small amount of the dry, powdered alkaloid or its salt in a little heap on the microscope slide, add a drop of reagent, and apply a cover glass. The tests are sensitive, but the characteristic crystals are formed at a fairly high ratio of alkaloid to reagent, at least for quinine and cinchonidine; hence the amount used is put in a small heap, not scattered over the slide. Crystallization takes place immediately with quinine, quickly (in most cases) with the others. Examine the crystals under a microscope, with a magnification of 50 to 100, making examination by polarized light if possible (using only the polarizer or only the analyzer) in order to observe the dichroism. However, the crystals are characteristic and can be used for identification with ordinary light.

Solutions for Making Reagents

WAGNER'S NO. 1. Iodine, 1 gram; potassium iodide 1 gram; water, 100 cc. Dissolve the iodine and potassium iodide completely in 1 to 2 cc. of the water, add a few excess iodine crystals, and dilute with the rest of the water.

WAGNER'S NO. 2. Iodine, 1 gram; potassium iodide, 1.75 grams; water, 100 cc. Dissolve the iodine and potassium iodide in 2 to 3 cc. of the water, then dilute with the rest of the water.

IODINE IN (2 + 1) ACETIC ACID. Dissolve 0.5 gram of iodine in 50 cc. of glacial acetic acid (this may take several days, with occasional shaking); then dilute with 25 cc. of water. Leave in contact with excess iodine crystals.

Glacial acetic acid, water, and diluted sulfuric acid (1 + 3): one part by volume of concentrated sulfuric acid plus 3 parts by volume of water.

Reagents and Crystals

IODINE REAGENT Q. Wagner's No. 2, 3.0 cc.; glacial acetic acid, 1.5 cc.; sulfuric acid (1 + 3), 1.5 cc.

Quinine Crystals. Plates, mostly of a pale olivaceous green tint by ordinary light, red where they overlap. By polarized light they are variously colored; practically colorless, yellowish, greenish, olive-green, gray-green, pink, red, and black. Rotation of the stage or of the nicol discloses the pleochroism, colorless or greenish to dark red or black. The photomicrograph (Figure 1, left) is by polarized light. The test is sensitive and only a little quinine need be used; but the characteristic crystals are seen at the spot of greatest concentration. Out in the solution, where there is excess of iodine, brown colored crystals may form.

Quinidine Crystals. Canary-yellow plates, not dichroic, of various irregular shapes. Figure 1 (right) shows one type. Crystals vary somewhat in shape with this and the following reagents, but are nearly uniform in color.

This reagent may yield crystals with cinchonine and cinchonidine, but does not give dependable tests for them.

IODINE REAGENT C-1. Wagner's No. 1, 4.4 cc.; iodine in (2 + 1) acetic acid, 3.1 cc.; glacial acetic acid, 2.1 cc.; water, 1.8 cc.; and sulfuric acid (1 + 3), 0.6 cc.

Quinine Crystals. As with the preceding reagent, but usually smaller and more densely matted together.

Quinidine Crystals. As with the preceding reagent, but generally thinner and more branched and irregular in shape.

Cinchonine Crystals. Plates, commonly red-brown in color, varying to red, brown, black, and yellow; scarcely if at all dichroic. They are of several different but definite shapes, the rectangular and pentagonal generally predominating with this reagent (Figure 2, left), sometimes the triangular (Figure 2, right), the latter usually predominating with reagent C-2. Reagent C-1 is the best of the three reagents for cinchonine. The test is sensitive and usually gives numerous crystals in various areas under the cover glass; but occasional difficulty in readily obtaining crystallization has been experienced. Submicroscopic seeding, even just by the reuse of slides, washed and then wiped

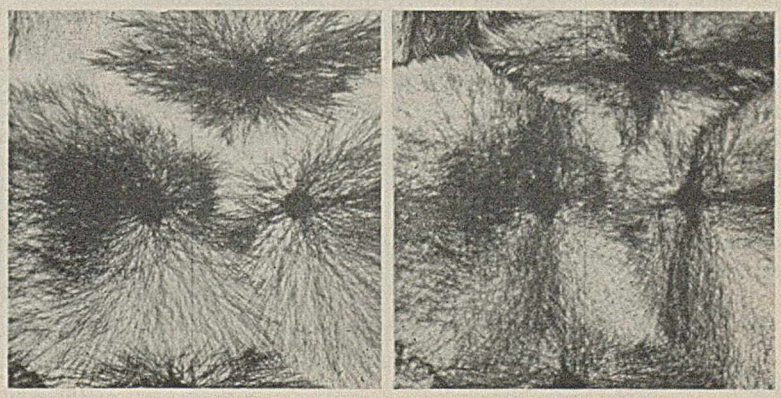

FIGURE 3. CINCHONIDINE NEEDLE CRYSTALS, REAGENT C-1 (*left*); CINCHONIDINE NEEDLE CRYSTALS WITH CROSSED NICOLS, SHOWING POLARIZATION CROSSES (*right*)

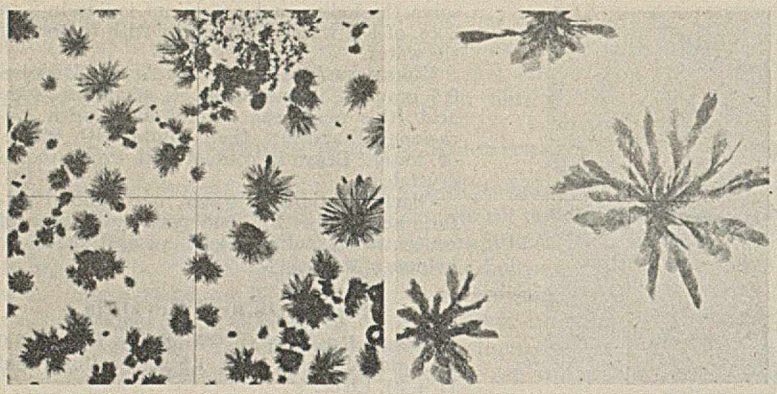

FIGURE 4. CINCHONIDINE PLATE CRYSTALS, WITH ORDINARY LIGHT, REAGENT C-2 (*left*); CINCHONINE-CINCHONIDINE MIXED CRYSTALS, REAGENT C-1 (*right*)

dry, on which crystals have been obtained, seems to be enough to obviate this difficulty. Crystals of one of the other alkaloids can be used for this seeding.

Cinchonidine Crystals. Rosettes of light brownish needles, showing with polarized light a dichroism in tint, purplish one way and yellowish at right angles. These form gradually, at or near spots of highest alkaloidal concentration (Figure 3, left). With crossed nicols they show marked polarization crosses, the directions in a rosette of the purplish and brightest yellow colors giving the dark arms of the cross (Figure 3, right). The crosses revolve like the spokes of a wheel when the stage is rotated between crossed nicols.

IODINE REAGENT C-2. Wagner's No. 1, 3.3 cc.; iodine in (2 + 1) acetic acid, 4.4 cc.; water, 0.5 cc.; and sulfuric acid (1 + 3), 3.8 cc.

Quinine Crystals. Similar to the description given above.

Quinidine Crystals. As previously described, but not forming as readily, and tending toward more regular forms of slightly deeper color, sometimes orange-yellow prisms.

Cinchonine Crystals. See description under the preceding reagent, and Figure 4 (right). In a few tests bunches of yellowish-brownish branching threads, and some splinter crystals, also formed.

Cinchonidine Crystals. Dense black rosettes of pointed plates. Individually (as seen at the edges of the rosettes or with the occasional separate plates, which are little pointed ellipses) the crystals are pale gray or yellowish gray in ordinary light. With polarized light the separate crystals vary, and with rotation change from virtually colorless and almost invisible to black and perfectly opaque. The photomicrograph (Figure 4, left) shows better developed crystals than most, and was taken by ordinary light to show the structure of the rosettes.

Mixtures

The tests, like others of their kind, are primarily intended to make identification absolutely certain, not to provide for detection in mixtures. Nevertheless, as herapathite is very insoluble and yet crystallizes readily, quinine, even in small proportion by weight, can be identified in many mixtures without separation, whenever it predominates in the precipitation. In general, the presence of some other alkaloid or amine that precipitates with the reagent will cause interference, and in any considerable amount may ruin the tests for the other cinchona alkaloids. Numerous other alkaloids precipitate and yield crystals, the reagents then acting like iodine–hydriodic acid rather than iodosulfate reagents. Other crystals, so far as known, do not resemble those described.

In the presence of one another the cinchona alkaloids give their characteristic crystals as follows:

Quinine, from a mixture containing up to twice as much quinidine, three times as much cinchonine, four or five times as much cinchonidine. Quinidine, from a mixture with an equal amount of quinine, two and one-half times as much cinchonine, or three times as much cinchonidine. Cinchonine, when constituting 50 per cent or more of mixture with cinchonidine, or 70 per cent with quinine or quinidine. Cinchonidine to give its characteristic crystals must constitute about 65 per cent of a mixture with cinchonine, about 80 per cent with quinine or quinidine. Cinchonidine tends to crystallize with the other alkaloids instead of yielding its own crystals. The herapathite crystals are actually improved by a large admixture of cinchonidine with the quinine. The quinidine crystals become smaller, more grainlike, and a deeper color, brown-orange. The mixture with cinchonine yields, especially with reagent C-1, fairly large branching groups of square-cut plate crystals, brown by ordinary light, noticeably but not extremely dichroic by polarized light, brownish yellow to red-brown (Figure 4, right). These predominate with 40 to 80 per cent cinchonidine, 60 to 20 per cent cinchonine. When numerous and comparatively small they may form mostly as roundish plates.

An inert substance such as lactose does not interfere with crystallization, but may obscure the crystals by particles that do not dissolve readily. Each of the alkaloids discussed was found easy enough to identify in 5 per cent mixture with 95 per cent of lactose.

Relative Value of Three Reagents

In the case of quinine the three reagents were tried out on the sulfate, bisulfate, and hydrochloride, as well as on the free alkaloid.

Iodine Reagent Q is probably best for quinine, though not by any wide margin. It is also excellent for quinidine, but cannot be satisfactorily used for cinchonine or cinchonidine.

Iodine Reagent C-1 is the best all-round reagent for the four alkaloids, and gives excellent crystals with each. It is best for cinchonine; better than reagent C-2 and possibly best for quinidine; and better than reagent Q and possibly best of the three for cinchonidine. It is also best for the mixed crystals of cinchonidine and cinchonine.

Iodine Reagent C-2 is chiefly of interest and value for its cinchonidine plate crystals which show extreme dichroism. It gives excellent quinine crystals and can be used for cinchonine and quinidine.

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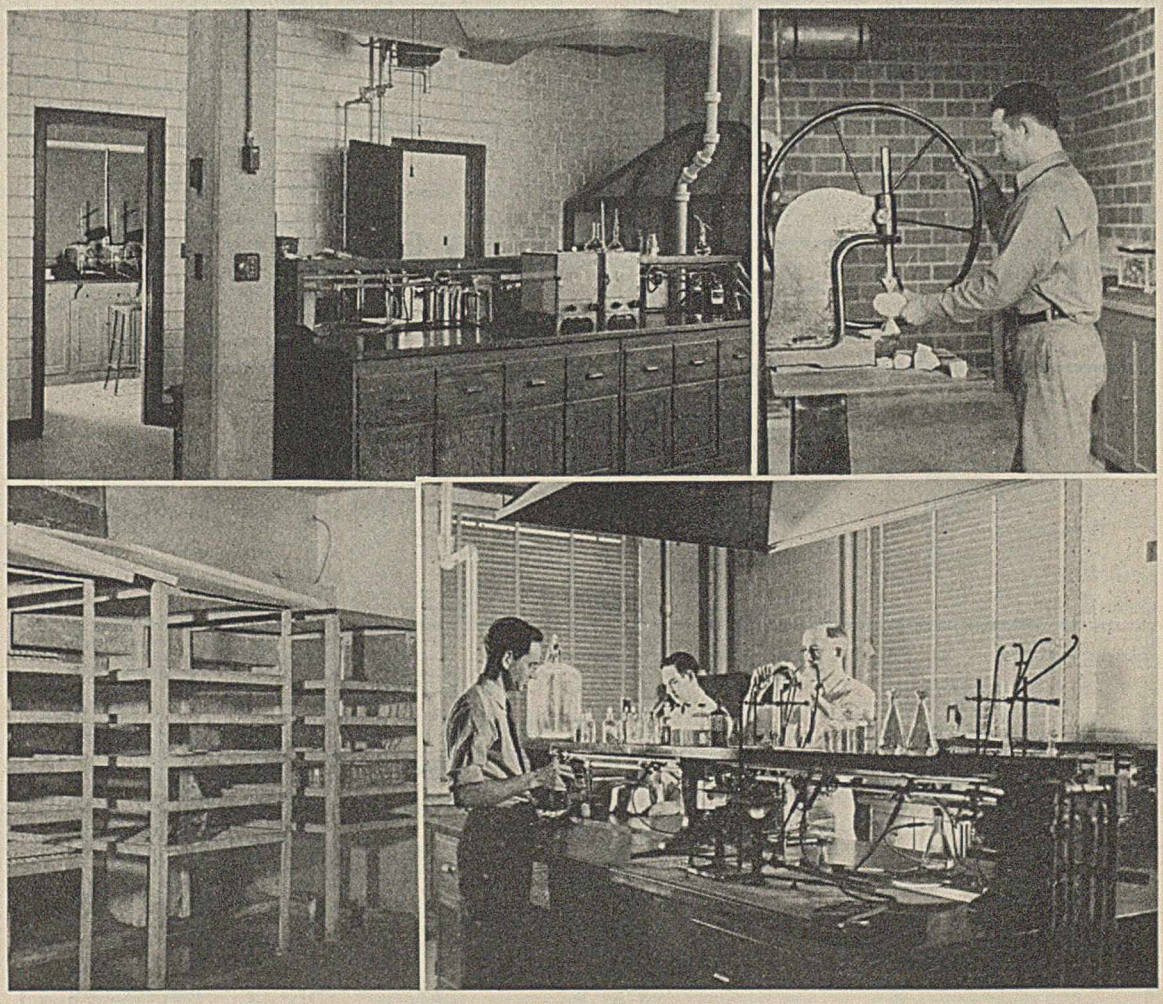
PHOTOMICROGRAPHS by John B. Dalton, Police Department, Saint Paul, Minn.

MODERN LABORATORIES

The New Missouri Highway Laboratory Building

VICTOR H. LYON, Missouri State Highway Department, Jefferson City, Mo.

THE Missouri State Highway Commission has maintained a laboratory in Jefferson City since 1922 for conducting tests to control the quality of materials entering highway construction and conducting research and work of investigational nature. The first laboratory was established in the basement of the State Capitol. Later, in 1928, it was transferred to the basement of the Highway Building which had just been completed. These quarters, considered ample at the time, eventually proved inadequate, owing to the increased demands for testing and research in connec-



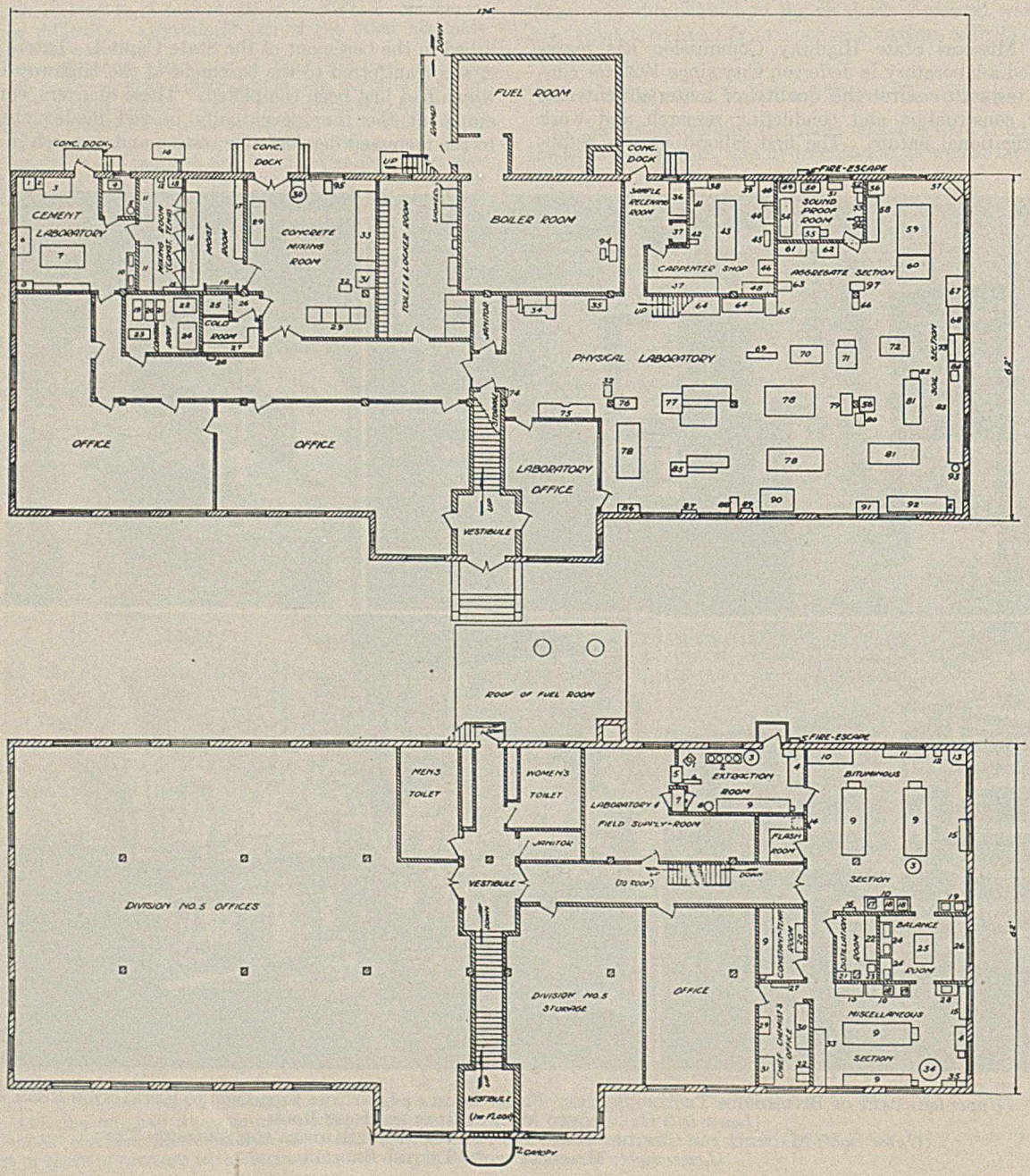
(*Upper left*) Part of Bituminous Testing Section, Flash Room at Left, and Entrance to Extraction Room
(*Lower left*) Galvanized Metal Racks in Moist Room
(*Upper right*) Machine for Cutting Cubes from Ledge Stone Samples for Abrasion Test
(*Lower right*) Miscellaneous Testing Section

tion with continued development of the highway system and advancement of construction practice. To provide for the increased needs of the laboratory, the Highway Commission authorized the construction of a building specifically designed for laboratory use. This building, financed in part by a PWA grant, has been completed and is now occupied.

The new laboratory building is a two-story structure, 62 feet wide by 174 feet long, and is of steel and concrete construction with brick walls and partitions. The exterior walls are buff-colored face brick trimmed with limestone. Large steel-sash windows provide ample light and ventilation. The building is completely equipped for the physical and chemical testing of all highway materials and for the continuance of research and investigational work.

The chemical laboratory, in which chemical analyses on various materials are conducted, is located on the second floor. It is partitioned into rooms, each of which is equipped for testing specific types of materials. One large room, approximately 30 feet square, is utilized for testing bituminous materials. A smaller room, used for testing miscellaneous materials, connects with the bituminous section by means of an intermediate room equipped with balances and a titration desk. This arrangement makes the analytical balances and titration desk readily accessible to the operators in both sections. The three analytical balances are indirectly lighted and both natural and artificial light are available on the titration desk. Water-shower sprays with pull chains are installed in the two larger rooms as a safety feature in case the clothing of any operator becomes ignited.

There are separate rooms for gasoline testing, flash and fire determinations, and extractions, and a constant-tempera-



ture room with controlled humidity. The constant temperature in this room is maintained with a Fairbanks-Morse 2-horsepower Model FQ 200W compressor which is located in the control room on the first floor. A rheostat is provided for the lights in the flash room, so that they may be dimmed to determine flash points more accurately.

The fumes from the various hoods in the chemical laboratory are exhausted by three blowers located in a penthouse on the roof. To compensate for this exhausted air during the winter months, two Nesbit Syncretized air heaters are provided to pump outside air into the rooms from grilled exterior openings, conditioning it to room temperature.

Chemical desks are supplied with all utilities, including natural gas, 110- and 220-volt electricity, compressed air, and steam. All drains are constructed of acid-resistant metal.

The cement laboratory, in which the physical tests of cement are made, is located on the east side of the first floor. It consists of a general room, a constant-temperature room with controlled humidity, and a small room with heat-exhaust facilities for housing the steam pat boiler and autoclave. The general room contains the cement briquet-testing machines and Wagner turbidimeter and is equipped with tables, balances, and the necessary equipment for weighing the batches which are transferred to the constant-temperature room for mixing.

The constant-temperature room conditions are maintained by a Fairbanks-Morse unit, using a 3-horsepower Model FQ 300W compressor located in the control room. A series of cabinet doors opens from the constant-temperature room to the moist room, which is provided with galvanized metal racks for holding test specimens.

The moist room measures 10.5 by 19 feet and is insulated with 2 inches of cork on the walls and ceiling. It is designed with an entrance on the opposite side from the constant-temperature cement-mixing room and thus serves the double purpose of curing concrete as well as cement paste and mortar specimens. The room is conditioned by pumping air charged with moisture from a conditioning unit located in the controll room into a copper duct fitted with three grille openings inside the moist room. Extra water sprays are installed in the duct to promote additional humidity. Temperature balance within the moist air-conditioning unit is provided by hot water and a Fairbanks-Morse 3-horsepower Model FQ 300W compressor. The temperature in the moist room is maintained at 70° F. and the relative humidity at 96+ per cent continuously. A TAG recording psychrometer is used to keep a constant record of the conditions in the room.

A cold room is provided for conducting freezing tests on aggregates, concrete, and other materials. This room measures 9 by 9 feet, including a vestibule and brine specimen tank. It is insulated with 8 inches of cork on the walls, ceiling, and floor with 4 inches of concrete laid on the floor insulation. The room is designed with copper ceiling coils and steel rack coils on which specimens may be placed for freezing.

The brine specimen tank is insulated from the room and is reached from the end of the vestibule. This tank measures 2 feet 6 inches by 3 feet 4 inches by 1 foot 10 inches in height, inside dimensions. The brine is refrigerated by means of coils in an overhead 200-gallon reserve tank and flows to the specimen tank by gravity with a safety shut-off valve to stop the flow when the specimen tank is filled. A motor-driven centrifugal pump, which is operated by means of an automatic temperature controller, returns the brine to the reserve tank, making the flow continuous when the temperature of the brine in the specimen tank rises above the controller setting.

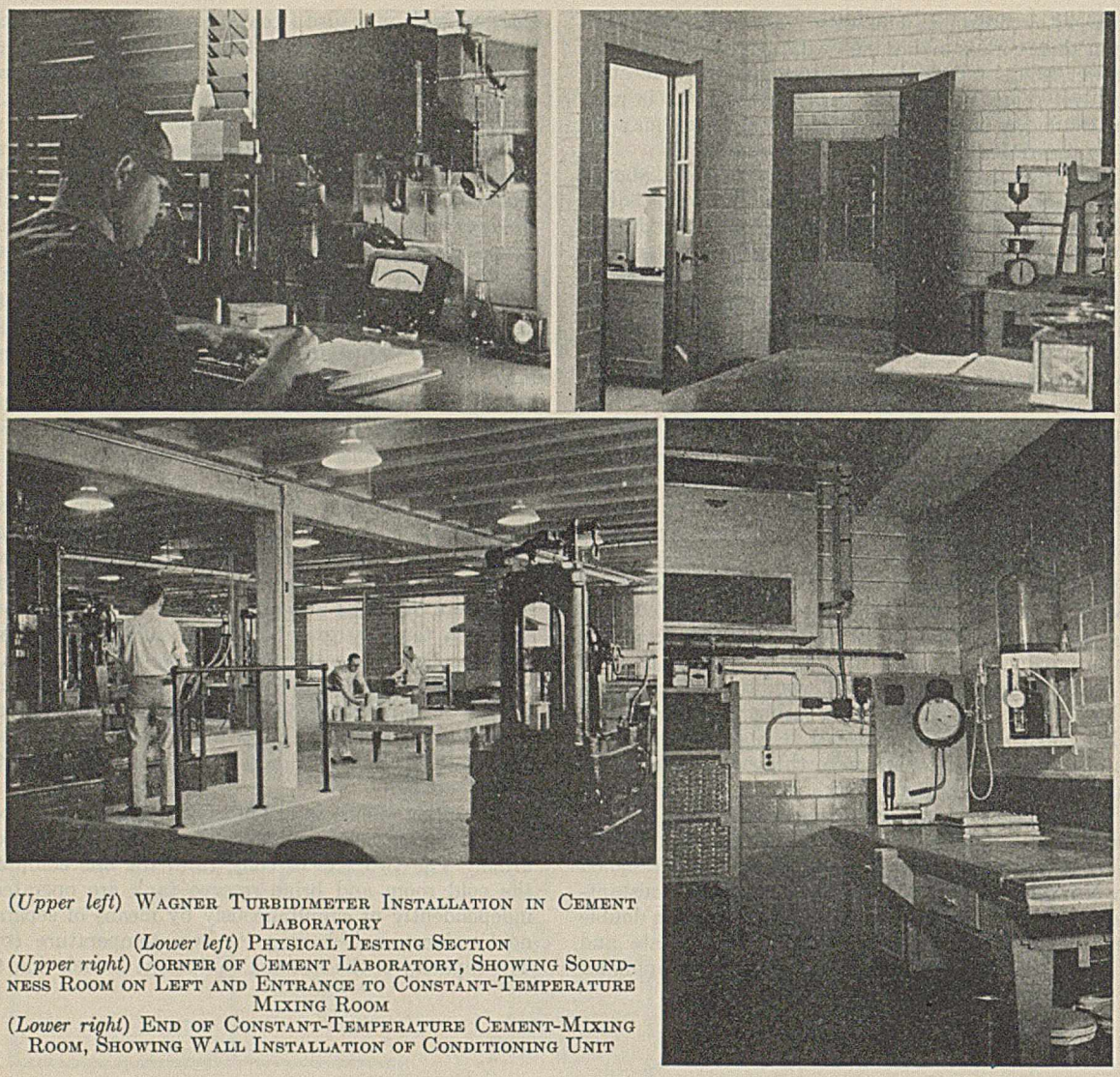
A Fairbanks-Morse 10-horsepower 2-speed Model FV 1000W compressor is used for refrigerating the cold room and brine. The two sets of coils, from the one compressor, to the cold room and brine reserve tank are operated either independently or simultaneously by means of separate solenoid valves, each having its own temperature controller. The capacity of the freezing equipment is such that the tem-

FIRST FLOOR PLAN (*Opposite Page, Above*)

1. Kerosene supply
2. File cabinet
3. Desk
4. Pat boiler
5. Autoclave
6. Turbidimeter
7. Sample preparation table
8. Storage cabinet
9. Sample shelves
10. Briquet machines
11. Mixing table
12. Recording psychrometer
13. Sink
14. Water-cooling tower
15. Mold rack
16. Briquet rack
17. Specimen rack
18. Concrete cylinder rack
19. 2nd floor constant temperature room compressor
20. Cement constant temperature room compressor
21. Moist room compressor
22. Moist room conditioning unit
23. Water pump
24. Cold room compressor
25. Brine tank
26. Cold room vestibule
27. Specimen rack coils
28. 10-Point temperature recorder
29. Thawing tanks
30. Concrete mixer
31. Sonic apparatus
32. 250-lb. platform scales
33. Cylinder capping table
34. Carborundum saw
35. Steel bending machine
36. Sample table
37. Freight elevator
38. Paint cabinet
39. Tool grinder
40. Supply cabinet
41. Wall tool cabinet
42. Drill press
43. Carpenter bench
44. Tool chest
45. Grind stone
46. Utility table
47. Lumber rack
48. Waste material
49. Ball mill
50. Rotap
51. Grinding lap
52. Jaw crusher
53. Burr mill
54. DeVal abrasion machine
55. Los Angeles abrasion machine
56. Drying oven
57. Dorry hardness machine
58. Sample cabinet
59. Aggregate screening table
60. Soil preparation table
61. Sink
62. Sand preparation table
63. Diamond drill
64. General storage cabinet
65. 500-lb. platform scales
66. Page impact machine
67. Sink
68. Desk
69. Bituminous compaction machine
70. Equipment cabinet
71. Freezing cabinet
72. Soaking tanks
73. Water bath for hydrometer test
74. First-aid cabinet
75. Brinell hardness machine
76. Testing machine accessory cabinet
77. 200,000-lb. Universal testing machine
78. General purpose table
79. Hot-plate table
80. Asphalt stability machine
81. Soils table
82. Soil compacting block
83. Soils bench
84. Stoneware sink
85. 100,000-lb. Universal testing machine
86. Testing machine accessory cabinet
87. Wall tool box
88. Strip tester accessory cabinet
89. Scott strip tester
90. Drafting table
91. Calculator table
92. Three-section desk
93. Centrifuge
94. Air compressors
95. Concrete saw
96. Soil pulverizer
97. Stone-cutting machine

SECOND FLOOR PLAN (*Opposite Page, Below*)

1. Rotarex
2. Bituminous extractors
3. Waste container
4. Wash bench
5. Service door and shelf
6. Culvert metal shears
7. Freight elevator
8. Centrifuge
9. Chemical desk
10. Hood
11. Ductility machine
12. Penetrometer
13. Constant temperature bath
14. Wall safe
15. Nesbit Syncretized air heater
16. Sample rack
17. Muffle
18. Hot plate
19. Loss ovens
20. Paint drying rack
21. Steel sample cabinet
22. Steel work table
23. Stone sink
24. Balances
25. Utility cabinet
26. Titration table
27. Book case
28. Drying oven
29. Calculator table
30. Three-section desk
31. Desk
32. File cabinets
33. Chemical supply cabinet
34. Rotating filtration table
35. Water still



(*Upper left*) Wagner Turbidimeter Installation in Cement Laboratory
(*Lower left*) Physical Testing Section
(*Upper right*) Corner of Cement Laboratory, Showing Soundness Room on Left and Entrance to Constant-Temperature Mixing Room
(*Lower right*) End of Constant-Temperature Cement-Mixing Room, Showing Wall Installation of Conditioning Unit

perature of 30 cubic feet of concrete can be lowered from 80° to −10° F. in air in 20 hours and held indefinitely at this temperature, or 6 cubic feet of concrete can be lowered from 80° to −20° F. in 8 hours in the brine and held indefinitely at this temperature. Both conditions can be met simultaneously, using 24 cubic feet in air and 6 cubic feet in the brine or a total charge of 30 cubic feet.

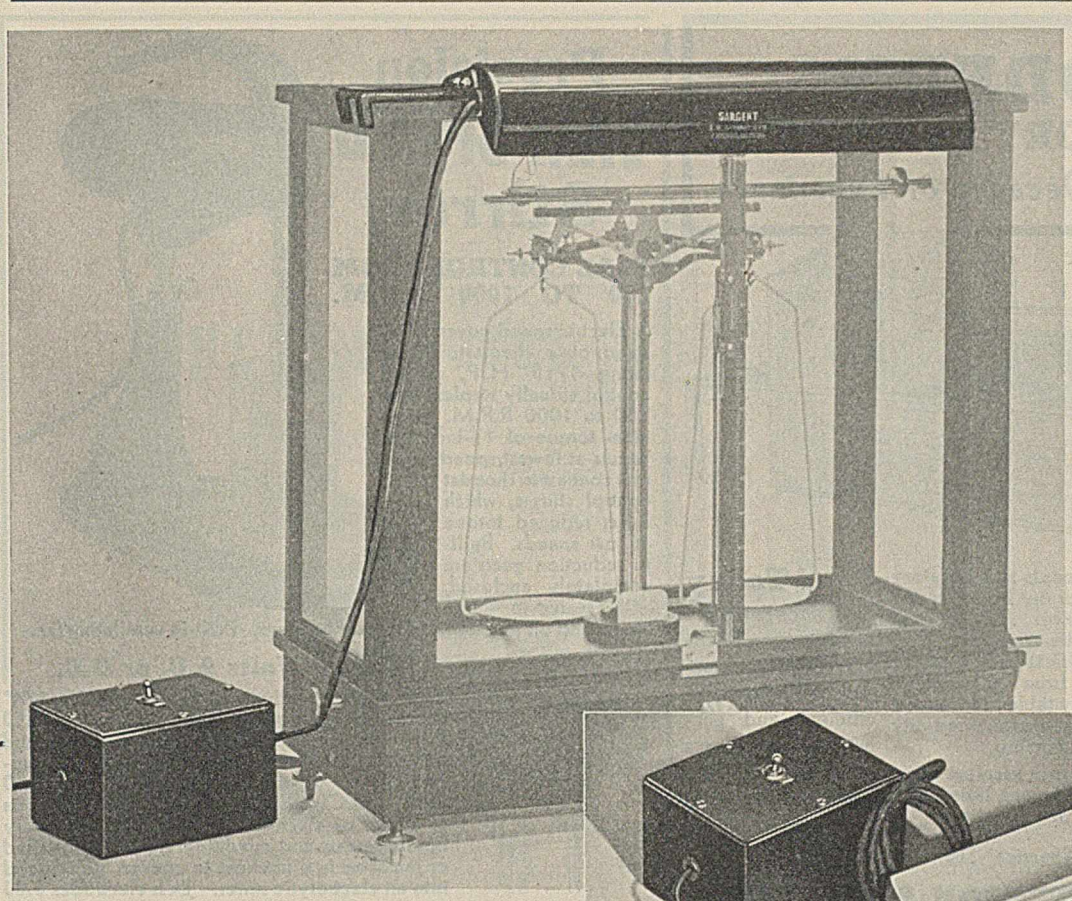
The concrete-mixing room is used principally for proportioning, mixing, and molding concrete test specimens for investigational purposes. A Model SW Lancaster laboratory mixer is available, as well as a series of mechanically agitated metal thawing tanks. Apparatus for the sonic measurement of modulus of elasticity and a high-speed Felker Di-Met concrete saw are located in this room.

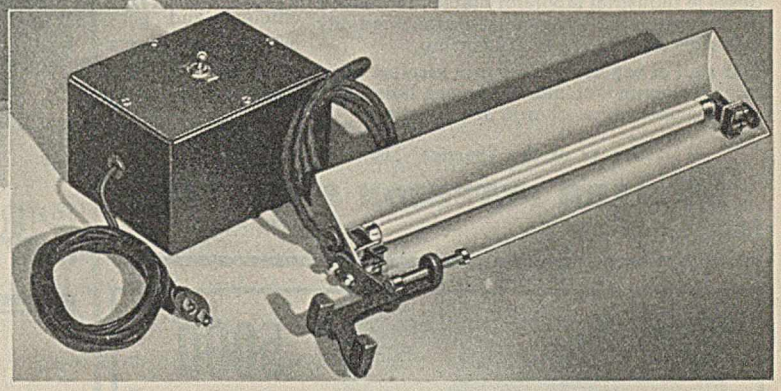
The front half of the east wing on the first floor is partitioned into offices which are occupied by several departments of the Materials Bureau, as is another office on the second floor adjacent to the chief chemist's office. The laboratory office is located just west of the front entrance on the first floor.

The entire west wing of the first floor comprises the physical laboratory, which includes the soils and aggregate sections. Two Riehle Universal testing machines are located in this section, with other items of equipment for the physical testing of materials. Two large gas-fired drying ovens are provided for drying soil and aggregate samples, and the fumes and heat from these ovens and other drying equipment are exhausted by forced draft. A soundproof room houses the equipment which necessarily is noisy in operation, such as the rattlers, grinding lap, rotap, ball mill, crusher, and grinder.

A carpenter shop provides the necessary facilities incident to laboratory needs. No machine shop was included in the new laboratory building, as the Highway Department maintains a fully equipped shop in the headquarters garage which is available to the laboratory.

Adjoining the main building is a one-story maintenance building of similar exterior design, a section of which is provided for the laboratory. This space is partitioned to make three separate rooms, in one of which an octane machine, for determining the antiknock characteristics of gasoline, is located. This machine, a Waukesha ASTM-CFR engine, was located here, rather than in the main building, because of the noise and vibration during operation. The other two rooms are used for chemical storage and as a garage for the laboratory truck.





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