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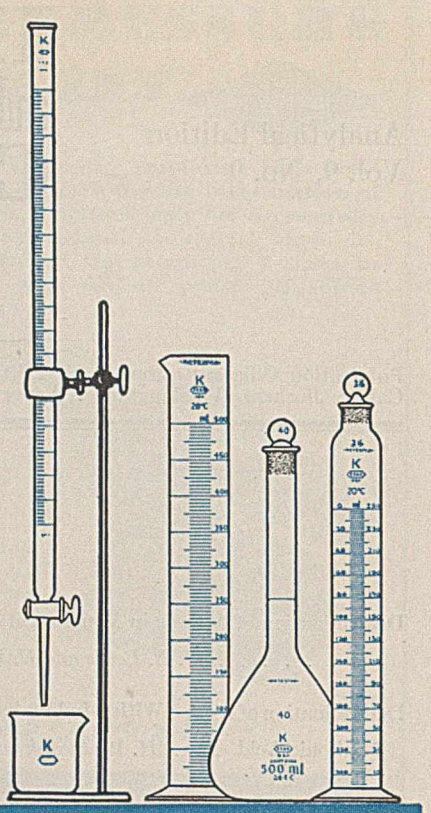

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ANALYTICAL EDITION

INDUSTRIAL and ENGINEERING CHEMISTRY

Harrison E. Howe, Editor

Determination of Arsenic in Mineral Oil Solutions

J. B. LEWIS AND E. L. BALDESCHWIELER, Standard Oil Development Company, Linden, N. J.

IN THE COURSE of a study on the solubility of arsenicals in mineral oils it was necessary to obtain with accuracy the arsenic content of the solutions under examination; the amounts of arsenic to be determined varied between 0.05 and 0.20 per cent. The problem was to destroy the mineral oil and convert the arsenic into a compound suitable for quantitative determination. All the procedures found in the literature gave low results, mostly because of loss of arsenic during the process of destroying the excessive amount of organic matter. This was true, for example, of the Kohn-Abrest procedure (*8*) involving calcination with magnesium nitrate, and of combustion with oxygen in a calorimeter bomb as recommended by Garelli and Carli (*5*). Combustion in a current of oxygen in a tube and bubbling the resultant gases in a caustic soda solution gave low results in the presence of combustion accelerators (PbO_2, Pb_3O_4, CuO), while incomplete combustion was obtained in their absence. Destruction of the organic matter with sulfuric and nitric acids as recommended by Baldeschwieler (*1*) was unsuccessful, probably owing to the volatilization of some of the arsenical.

Oxidation procedures found unsatisfactory, mainly because of the difficulty in completely oxidizing the mineral oils, included those of Rupp and Lehmann (*14*) using sulfuric acid, potassium permanganate, and hydrogen peroxide, or Ewins' modification (*4*) with potassium sulfate; Rogers (*13*) and Maillard (*10*) using ammonium persulfate and sulfuric acid; Poggi and Polverini (*11*) using potassium persulfate; Stollé and Fechtig (*16*) using potassium nitrate, ammonium sulfate, and sulfuric acid; Stettbacher (*15*) using sulfuric acid and hydrogen peroxide; Tabern and Shelberg (*17*) using fuming sulfuric acid and hydrogen peroxide; Leulier and Dreyfuss (*9*) using sulfuric perchloric and nitric acids; and Kahane (*7*).

The procedure adopted consisted of digesting the sample with sulfuric acid, nitric acid, and potassium sulfate in a Kjeldahl flask, as suggested by Robertson (*12*), Glycart (*6*), Delaville and Belin (*3*), and Cislak and Hamilton (*2*). A number of precautions were introduced in order to avoid losses by volatilization, either of the unchanged arsenical or of arsenious compounds which may be formed through reduction during the first stage of the process when charring occurs.

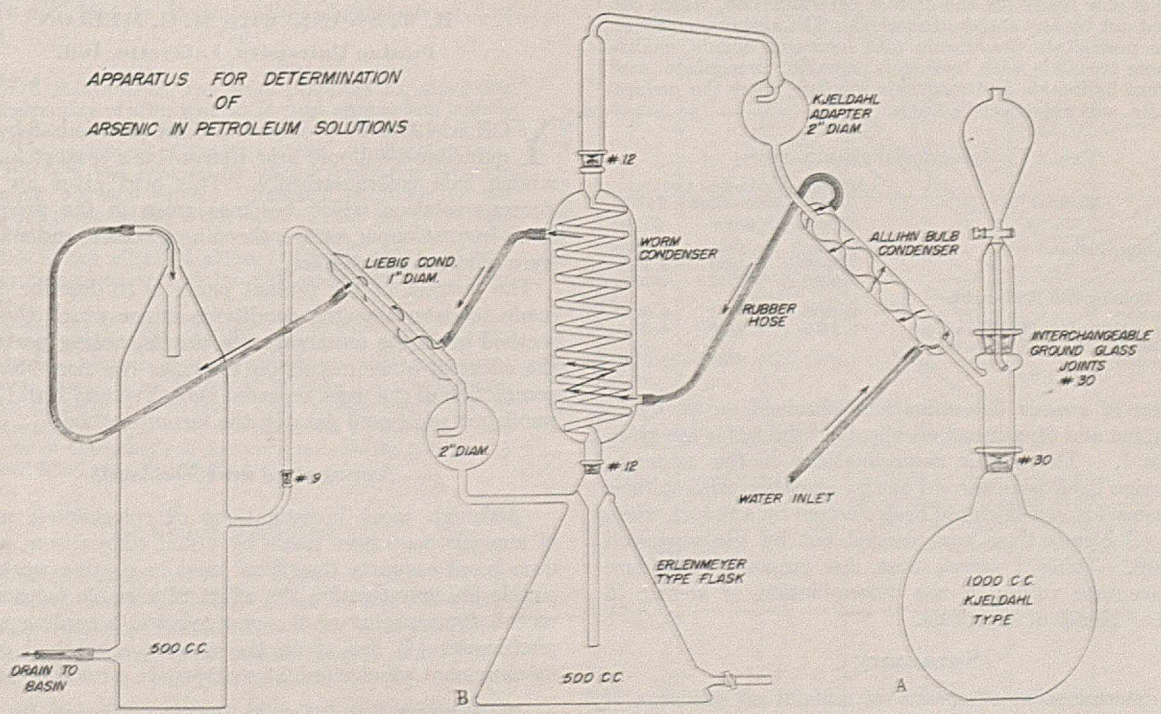

FIGURE 1

The development of a suitable apparatus required considerable experimenting. For this work the arsenic compound chosen was phenyldichloroarsine, $C_6H_5AsCl_2$, which is a typical and rather volatile arsenical. The determination of arsenic in the compound itself requires no elaborate setup, an 800-cc. Kjeldahl flask covered with a funnel with a bent stem being sufficient for the purpose. However, determination in mineral oil blends necessitates the use of a complicated setup, inasmuch as the more drastic treatment necessary to ensure complete decomposition of the additional organic matter greatly favors loss of arsenic by volatilization. In fact it was found that volatilization of some of the arsenic could not be altogether prevented; for this reason, the apparatus finally developed included provisions for recovering volatilized arsenic.

Apparatus

A sketch of the apparatus is shown in Figure 1. Pyrex glass was used throughout. The elaborate condensing system is necessary in order to recover the last traces of arsenic. A simpler apparatus consisting of a condenser surmounting a Kjeldahl flask was found unsatisfactory because of loss of arsenic and difficulties in the digestion procedure.

Method

A 3- to 5-gram sample of the mineral oil arsenical solution together with 10 grams of anhydrous potassium sulfate is placed in flask *A*, and the apparatus is assembled. About 20 cc. of concentrated sulfuric acid are then added through the separatory funnel, and the reaction is allowed to proceed until charring occurs. At this point 20 cc. of concentrated nitric acid are added and heat is applied with a very low flame. When the reaction subsides, more nitric acid is added and the heating is continued to gentle boiling, this process being repeated until the solution is clear and of a straw-yellow color. This should take about 2 hours. In general the heating should be so regulated that no undecomposed hydrocarbons pass out of the first condenser.

The apparatus is then disconnected. Both the washings from the condensers and the contents of receiving flask *B* are transferred to a large beaker. The solution is concentrated on a steam bath to a low volume and added to the solution in flask *A*. It is then heated over a wire gauze to fumes of sulfur trioxide; a few drops of nitric acid are added, and the solution is again heated to fuming, cooled in ice, and diluted with water. The solution is now ready for the arsenic determination, which can be carried out by any standard method. The authors, however, prefer to precipitate the arsenic with hydrogen sulfide, oxidize the arsenic trisulfide with hydrogen peroxide, precipitate with ammonium hydroxide and magnesia mixture, filter the precipitate, ignite, and weigh as $Mg_2As_2O_7$.

TABLE I. ARSENIC DETERMINATIONS

Material	Samples Used	Arsenic Content Theoretical	Found
	Grams	*Gram*	*Gram*
Phenyldichloroarsine	0.5000	0.1681	0.1670
	0.5900	0.1983	0.1983
	0.6000	0.2017	0.2022
Gas oil containing 0.75 % phenyldichloroarsine	8.3685	0.0212	0.0212
Same with 0.97% phenyldichloroarsine	4.2470	0.0138	0.0131
Lubricating oil with 1.09% phenyldichloroarsine	5.0072	0.0184	0.0182

Results of arsenic determinations obtained on phenyldichloroarsine and on mineral oil blends of the latter are given in Table I. The arsenic determinations on the diphenylchloroarsine itself were carried out by oxidation with sulfuric and nitric acids in a Kjeldahl flask covered by a funnel, while on the oil blends they were carried out by the suggested procedure. Table I shows that the proposed procedure yields accurate values in the determination of arsenic in mineral oil blends of arsenicals.

Summary

The determination of arsenic in mineral oil solutions of arsenicals is carried out in a Kjeldahl flask, provision being made for condensing and collecting volatile products in order to avoid loss of arsenic by volatilization, during the process of oxidizing the mineral oil. While admittedly long and tedious, the method gives accurate results.

Acknowledgment

The authors wish to thank R. G. Sloane of these laboratories for his counsel and criticisms.

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Determination of Iron

With 7-Iodo-8-hydroxyquinoline-5-sulfonic Acid

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Purdue University, Lafayette, Ind.

YOE in 1932 (*6*) proposed the use of 7-iodo-8-hydroxyquinoline-5-sulfonic acid (ferron) as a reagent for determining iron colorimetrically. This acid gives an orange aqueous solution which becomes green in the presence of ferric iron. Copper, nickel, chromium, cobalt, and aluminum were reported to interfere.

The purpose of the present paper is to describe a study made to determine the conditions under which the ferron method gives the most accurate results, noting particularly the effect of other metals on the color reaction. Since the completion of the work reported here, Yoe and Hall (*7*) have published additional data on the ferron method.

Apparatus and Methods

Although most investigations of colorimetric methods of analysis have been made by visual comparison, a procedure more accurate than that used in routine work is desirable for investigating the effect of variable factors. The recent development of the photoelectric recording spectrophotometer (*5*) has given the analyst a method of color measurement which eliminates subjective error and is capable

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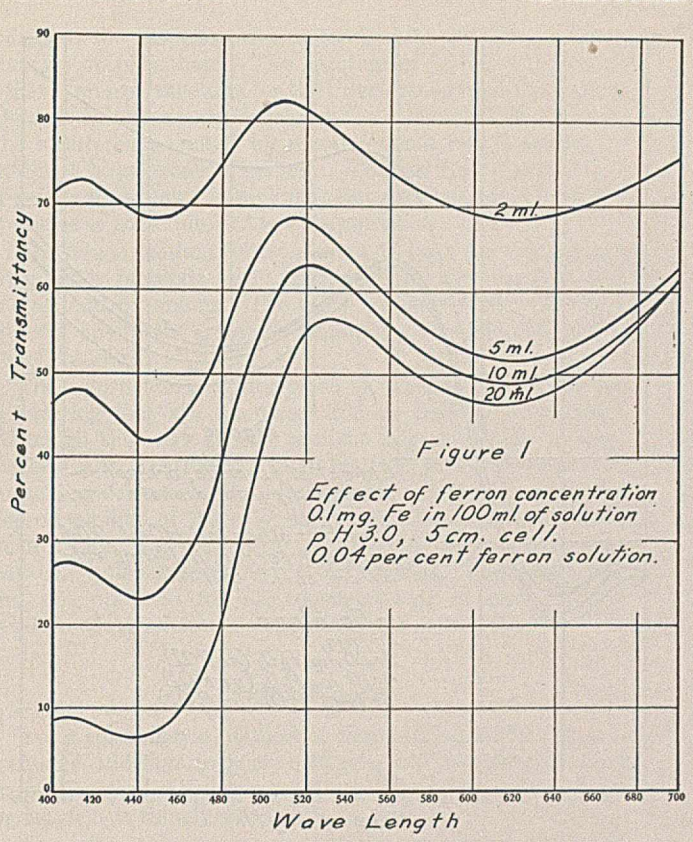


of high accuracy and precision. By means of the modified General Electric spectrophotometer used in this study, very small differences in color intensity can be detected with certainty.

The ratio of concentrations can be calculated from the transmittancy curves by means of Beer's law. If the color is not proportional to concentration, such calculations must be restricted to a small range. Only a few spectrophotometric curves are presented, but they are sufficient to indicate the advantages of this method.

Standard solutions of iron were prepared by dissolving weighed amounts of iron wire or ferrous ammonium sulfate in dilute sulfuric or hydrochloric acid. Perchloric acid was used in the presence of lead or silver. Complete oxidation of the iron was ensured by adding hydrogen peroxide and boiling out the excess. Standard solutions of the metals were prepared from the sulfate, chloride, or nitrate salts. Sodium, potassium, or ammonium salts were used for studies of the effect of anions. Clark's (*2*) and Britton's (*1*) directions served for the preparation of buffer mixtures.

Ten milliliters of 0.04 per cent aqueous ferron solution and 0.1 mg. of iron in 100 ml. of solution were used for all the curves shown in this paper, as this concentration gives an optimum intensity of color for comparisons through a depth of 5 to 10 cm. The transmittancy curves were determined for a solution thickness of 5.0 cm. The absorption of the glass cell was compensated by means of a similar cell filled with distilled water.

The Color Reaction

The transmittancy curves in Figure 1 show the extent to which the color is dependent on the concentration of the reagent. Similar curves for a fixed concentration of ferron and variable amounts of iron prove that the color is not proportional to the concentration of the iron. These data are summarized in Table I.

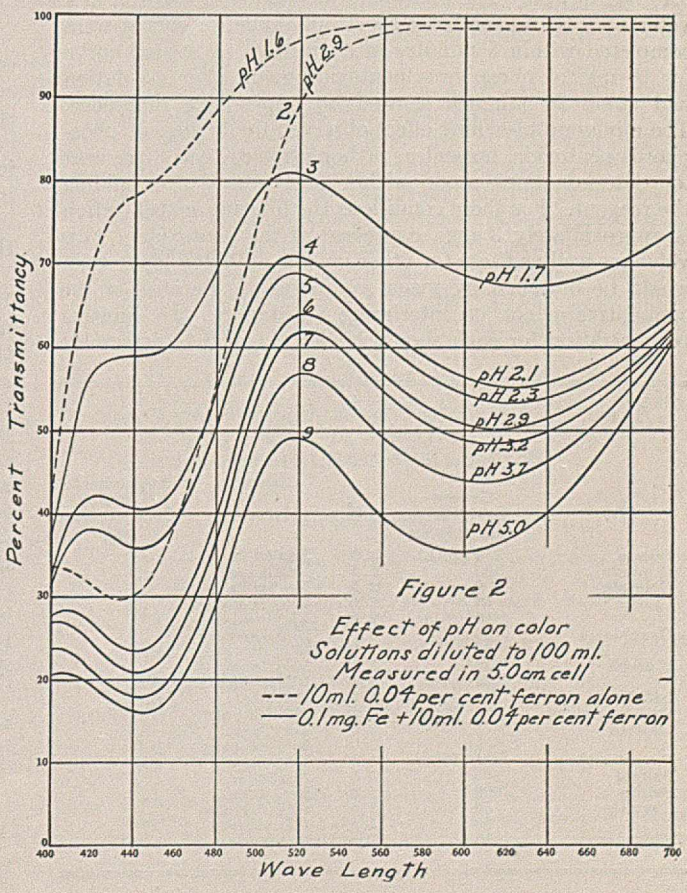
Table I. Validity of Beer's Law for a Fixed Concentration of Ferron and Varying Amounts of Iron

(pH 3.0; 5.0-cm. cell; 10 ml. of 0.04 per cent ferron reagent)

Iron	Transmittancy at 610 Mμ Observed	Calculated
Mg./100 ml.	%	%
0.02	84.5	...
0.05	69.0	65.5
0.10	46.5	43.0
0.20	25.5	18.5
0.50	12.5	1.5

The transmittancy value for 0.02 mg. of iron was taken as the starting point for the calculations. It is evident that as the iron concentration increases, the transmittancy does not decrease to the extent calculated from Beer's law. Yoe and Hall (*7*) reported that the color intensity does follow Beer's law, but their experiments were made with a constant ratio of ferron to iron, a condition that is not encountered in actual analytical work where the iron content is unknown.

Below a pH of 7 ferron functions as a hydrogen-ion indicator, the yellow or orange hue gradually decreasing with a decrease in pH (curves 1 and 2, Figure 2). The green hue produced by iron is equally affected by pH, as shown by curves 3 to 9. The shift in color is less pronounced between pH 2 and 3, but even then it is great enough to require holding the pH constant to within 0.2 unit if an error greater than 2 per cent is to be avoided. This conclusion is not in close agreement with that of Clark and Sieling (*3*) who concluded that the color does not change between pH 2.7 and 3.2. However, the curves show a change in color in-



tensity equivalent to a 5 per cent variation in iron content for a range in pH of 2.9 to 3.2 alone.

This sensitivity of the color reaction to slight changes in pH makes the use of a buffer imperative for accurate work. Potassium acid phthalate (*4*), glycine, or sodium acetate buffers are suitable, although each affects the color slightly. It is necessary to maintain the same concentration and type of buffer in both the standard and unknown. Buffers containing citrate or phosphate compounds are not suitable. The pH must be adjusted very nearly to that desired before adding the buffer mixture.

Tests over a period of 9 days showed no fading of the color of solutions exposed to diffuse daylight, confirming Yoe's statement concerning the stability of the color.

The Effect of Anions

Many of the common anions prevent the full development of the color. A typical example is given in Figure 3, curves 1 and 2, for the effect of 1.0 mg. of fluoride ion, and similar effects are caused by pyrophosphate, orthophosphate, cyanide, citrate, tartrate, and oxalate ions.

Pyrophosphate ion interferes to such an extent that it must be practically absent from solution (curve 5, Figure 3). The effect of orthophosphate is less pronounced and somewhat variable. Yoe and Hall (*7*) state that equilibrium is not reached immediately for the iron-ferron complex in the presence of orthophosphate ion, but that 5 minutes to 3 hours are necessary to reach the maximum intensity. Curves 3 and 4 show the effect of 20 and 10 mg. of phosphorus pentoxide, as sodium dihydrogen phosphate, on the color with 0.10 mg. of iron. The phosphate was added to the iron in a volume of 50 ml. and the spectrophotometric curves were completed within 5 minutes after mixing. A second curve for 10 mg. of phosphorus pentoxide made after a solution had stood 20 minutes showed no appreciable difference. The minimum bleaching effect observed for 10 mg. of phosphorus pentoxide, present as orthophosphate, was 8 per cent for determinations made within 10 minutes after adding the reagent. For these conditions the limiting concentration is approximately 3 mg., expressed as the pentoxide. This value, as well as those for other ions, is probably lower than would be obtained by visual comparison. Because of the cumulative effect of interfering substances, the limiting concentration for each should be set below that detectable by the eye. The effect of other anions is summarized in Table II.

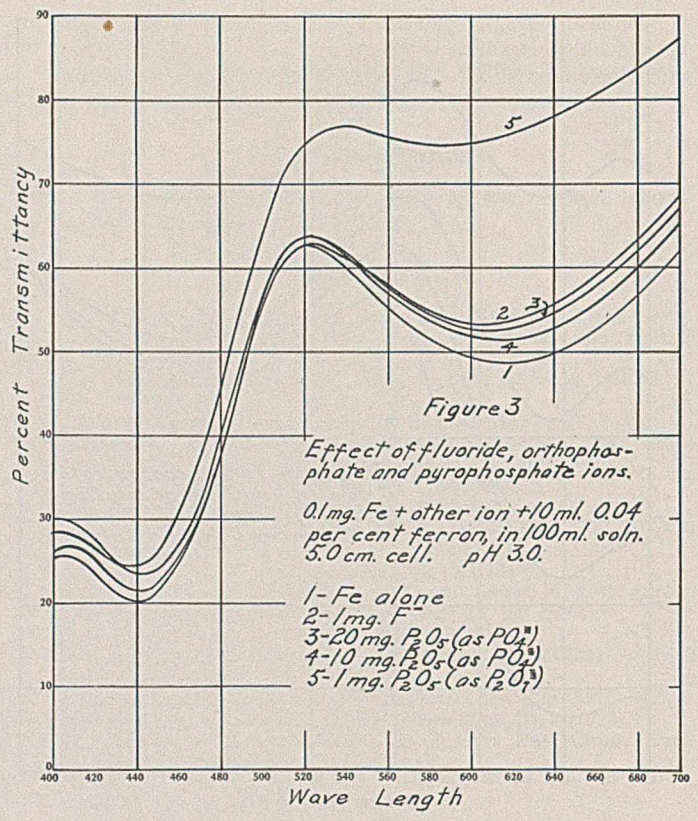


Table II. Effect of Common Anions on the Color Developed by Iron

(0.1 mg. of iron in 100 ml. of solution)

Ion	Concentration	pH	Change in Color Intensity	Approximate Limiting Concentration
	Mg./100 ml.			*Mg.*
Borate	150 (B_2O_3)	3.0	Negligible	...
Bromide	200	2.5	Negligible	...
Chlorate	200	2.5	Negligible	...
Citrate	50	2.0	30% decrease	...
	10	2.0	5% decrease	5
Cyanide	10	3.0	16% decrease	...
	2	3.0	10% decrease	0.2
Fluoride	10	3.0	Very large	...
	1	3.0	10% decrease	0.2
Iodide		2.5	Variable	0.0
Orthophosphate	10 (P_2O_5)	2.5	8% decrease	2 (P_2O_5)
Oxalate	2	2.0	Very large	...
	1	3.0	50% decrease	0.0
Pyrophosphate	1 (P_2O_5)	3.0	Very large	0.0
Sulfate	500	3.0	Negligible	...
Sulfite	200	2.5	Negligible	...
Tartrate	150	2.5	20% decrease	...
	50	2.5	4% decrease	25
Thiocyanate	50	2.5	Negligible	...

The Effect of Cations

Yoe reported that no cation other than iron forms a strongly colored complex with ferron and some workers have incorrectly inferred that other metals do not interfere. A spectrophotometric study shows that there is very little correlation between the absence of color and the degree of interference. The color is a function of the ferron as well as iron concentration; consequently, any ion which reacts with ferron, regardless of whether a color is produced, will affect the color produced by iron.

The effect of copper on ferron alone is evident from curves 1 and 2, Figure 4. Curve 4 shows the appreciable change in the transmittancy curve caused by 0.1 mg. of copper in the presence of an equal amount of iron. Cobalt and nickel react with ferron in a similar manner. With nickel, at least, the degree of interference varies with pH, being greater at pH 3.0 than at 2.0; with either nickel or cobalt, the limiting concentration is reached before the color of the ions becomes appreciable. Chromium shows no evidence of reacting with ferron. Aluminum combines with ferron to give a nearly colorless complex with a bleaching effect on the color with iron. The extent of the interference varies several fold for a change in pH from 2.0 to 3.0, with a more pronounced bleaching action at the higher value. Curve 5, Figure 4, indicates the extent of the change in the transmittancy curve produced by 2 mg. of aluminum for a pH of 3.0.

Table III indicates the effects of different amounts of aluminum as well as other metals which show little or no interference.

Discussion

The interference of anions is best interpreted as being due to the relative instability of the iron-ferron complex, com-

pared to the tendency of iron to combine with ions such as fluoride or phosphate. The reaction of ferron with metals other than iron accounts for the interference from this source. Because the color is dependent on the concentration of ferron, the interference caused by metals cannot be eliminated by adding a large excess of reagent. Yoe and Hall (7) state that a yellow filter helps in making color matches visually in the presence of some interfering substances.

The ferron method for iron seems to have few advantages over older methods with respect to interference by other metals and by anions. The great stability of the color is a distinct advantage from the standpoint of preservation of a set of standards.

The approximate limits given in Tables II and III for interfering ions are calculated for an error of 2 per cent. Although this may at first thought appear too low in comparison with an error of 5 to 10 per cent which may result in matching colors visually, each factor which influences the reproducibility of the color itself must be controlled within such a limit if a total error considerably greater than that caused by visual matching is to be avoided. The same limits are not obtained for all concentrations of iron, but the relative effect remains substantially the same.

Conclusions

Yoe's colorimetric method of determining ferric iron is a valuable addition to such methods, but necessitates more careful control of experimental conditions than use of a reagent such as thioglycollic acid.

The color is dependent on both the reagent concentration and the pH. A pH of 2.0 to 3.0 is suitable, but for a given series of comparisons the value must be held constant to 0.2 pH unit if the error from this source is not to exceed 2 per cent.

Potassium acid phthalate, glycine, or sodium acetate buffers are satisfactory for securing a constant pH. Citrate or phosphate buffers interfere with the development of the color.

The color is permanent for at least a week, even for solutions exposed to diffuse daylight.

Ferron is specific for ferric iron in the sense that no other ion tested gives a green color with it, but not in the sense that it is free from interference by other metals. Some common ions interfere enough to make a preliminary separation of iron necessary for the analysis of many products. The extent of interference for some metals is a function of pH. The effect of a metallic ion cannot be determined by noting whether a color is produced with ferron.

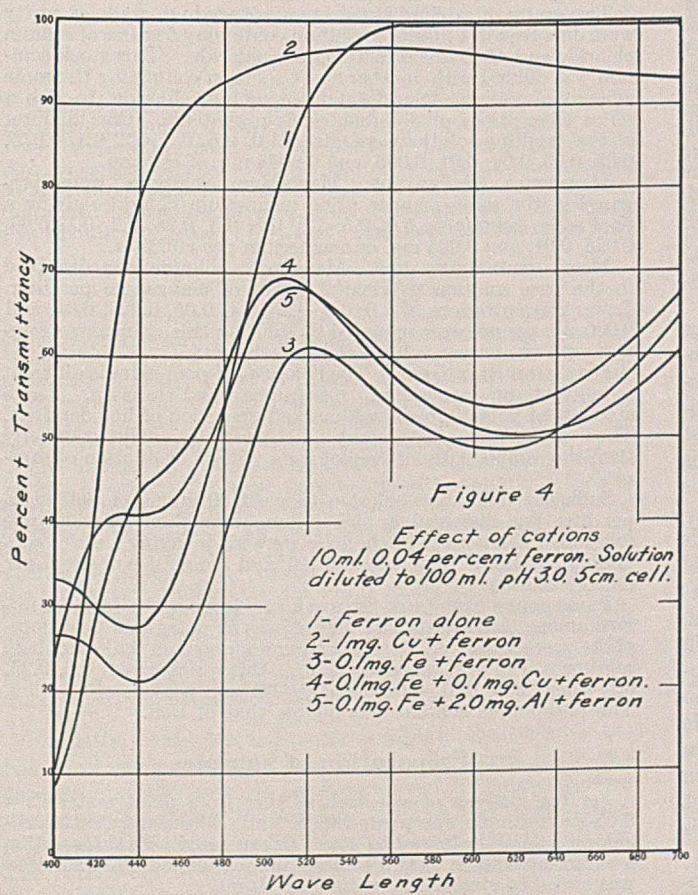
The color of the reagent and the lack of proportionality between the color of the ferron-iron complex and the concentration of the iron require the use of a constant-depth method of color comparison.

Table III. Effect of Common Cations on the Color Developed by Iron

(0.1 mg. of iron in 100 ml. of solution)

Ion	Concentration *Mg./100 ml.*	pH	Change in Color Intensity	Approximate Limiting Concentration *Mg.*
Aluminum	10	2.0	6% decrease	4
	2	2.5	5% decrease	1
	10	2.5	18% decrease	...
	2	3.0	13% decrease	0.3
	10	3.0	33% decrease	...
Ammonium	200	3.0	Negligible	...
Antimonous	2	2.5	Negligible	...
Arsenic	50	2.5	Negligible	...
Arsenious	50	2.5	Negligible	...
Barium	100	2.6	Negligible	...
Beryllium	50	2.0	Negligible	...
Bismuth	0.5	2.0	Negligible	...
Cadmium	250	2.5	Negligible	...
Calcium	150	2.0	Negligible	...
Chloroplatinate	7.5 (Pt)	3.0	4% increase	4
Chromic	2	3.0	15% increase	0.5
Cobaltous	10	3.0	16% decrease	...
	2	3.0	4% decrease	1
Cupric	0.1	3.0	Decrease	0.02
Lead	10	2.6	2% decrease	10
Lithium	150	3.0	Negligible	...
Magnesium	100	2.0	Negligible	...
Manganous	50	2.0	Negligible	...
Mercuric	50	2.6	Negligible	...
Nickelous	2	2.0	2% decrease	2
	0.2	2.6	4% decrease	0.1
Potassium	200	3.0	Negligible	...
Silver	60	2.7	Negligible	...
Sodium	200	3.0	Negligible	...
Stannic[a]	...	...	.....	...
Strontium	150	2.0	Negligible	...
Thorium[b]	...	...	.....	3
Uranyl	10	2.5	2% decrease	10
Zinc	50	2.0	Negligible	...

[a] Hydrolyzes.
[b] Bluish hue.



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Received April 23, 1937. Abstracted from a portion of a thesis submitted by H. W. Swank to the Graduate School of Purdue University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Spectroscopic Determination of Metals in Small Samples

Calcium, Magnesium, Potassium, Manganese, Iron, and Phosphorus

D. T. EWING, MABEL F. WILSON, AND R. P. HIBBARD, Michigan State College, East Lansing, Mich.

THE spectrograph is particularly adapted to two types of analysis: (1) the determination of traces of metals in alloys and compounds when large samples are available, such as manganese in steel; (2) the analysis of samples when only very small amounts are available, such as biological ash. This article will be confined to the second type, and especially to the determination of calcium, magnesium, manganese, potassium, phosphorus, and iron in samples too small to be analyzed quickly by ordinary wet methods. Plant ash has been rapidly analyzed for these six elements when the total sample available was less than 10 mg.

The method employed is similar to that used by Nitchie (*2*), exposing the spectra of the samples between those of a series of standard solutions containing varying known amounts of the substance being determined. From the blackness of the spectral lines working curves were formulated according to the method of Twyman and Hitchen (*3*), and from these standard curves unknowns were directly determined.

Apparatus

A Bausch and Lomb quartz spectrograph having a dispersion varying from 7 Å. per mm. at 2500 Å. to 21 Å. per mm. at 3500 Å. was used in this investigation. Excitation of the samples was obtained by arcing graphite electrodes with direct current furnished by a 15-ampere 300-volt motor generator. A revolving sector was used to regulate the fraction of incident light reaching the slit. Line blackness (plate blackening) was determined by means of a Bausch and Lomb density comparator.

PREPARATION OF ELECTRODES. Acheson graphite rods 8 mm. (0.31 inch) in diameter were cut in convenient lengths for arcing. One end of each was drilled approximately 7 mm. deep, 0.1 ml. of the solution was placed in the crater, and the electrodes were dried at 110° C. and used as lower electrodes (the anode). The cathode consisted of similar lengths broken from the same rod. Special graphite rods very free of metallic impurities were used in the calcium determinations.

Preparation of Standards

SELECTION OF BASE MATERIAL. The first problem to be considered in the preparation of standards was the material for use as a base. This substance serves two purposes: as a "filler," giving a greater amount of solid substance in the electrode; and as a means of holding back most of the carbon spectra until volatilization of the sample is complete. More even exposures and lighter backgrounds were obtained by employing the proper base.

It is the authors' experience that the chlorides of the metals reported in these investigations give rise to sharper spectral lines of the metals than do other negative radicals. All determinations were carried out in hydrochloric acid solution, in which the samples were found to be readily soluble. In the search for a suitable matrix material many metallic chlorides were tried. Zinc chloride was found to vaporize too readily, lead chloride was too insoluble, and sodium chloride in large amounts caused the arc to flare giving very uneven exposures. Very satisfactory arcing conditions and even exposures resulted from the use of ammonium chloride, but the spectral lines of the substance desired for use in analysis were not sharp and clear.

Of the various mixtures of sodium and ammonium chlorides tried, the one consisting of 10 per cent sodium chloride and 90 per cent ammonium chloride was found to give satisfactory results both for arcing and sharpness of lines. The base solution finally selected consists of 5 grams of sodium chloride, 45 grams of ammonium chloride, and 4.5 grams of hydrochloric acid in 1 liter of solution.

TABLE I. CONDITIONS OF EXPOSURE

Substance	Exposure	Light, Sector Transmission
	Seconds	%
Potassium		
In 9% acid	40	5
In NH-Na base	40	5
In Ca-Mg base	60	6.25
Iron and phosphorus		
In NH-Na base	60	5
In Ca-Mg base	90	6.25
Iron (alone)	60	2
Magnesium	40	3.125
Manganese	40	2
Calcium	40	1.625

CALCIUM STANDARDS. No two elements under identical arcing conditions gave the same variation in line blackness for an equal change in concentration. Thus it was necessary to try various concentrations until a series of dilutions was obtained which gave a gradation of line densities suitable for analysis.

Two grams of calcium as nitrate of a very high grade of purity were dissolved in 1 liter of a solution containing 5 grams of sodium chloride and 45 grams of ammonium chloride. This stock solution was diluted with another stock solution containing the same amount of sodium chloride and ammonium chloride, to give a series of solutions of different calcium contents. One milliliter of the resulting solutions contained 1.0, 0.5, 0.3, 0.2, 0.1, 0.075, 0.05, 0.03, 0.02, 0.01, 0.005, and 0.0025 mg. of calcium.

MAGNESIUM STANDARDS. Magnesium solutions were prepared in the same manner using magnesium chloride, giving a final series containing 5.0, 2.5, 1.0, 0.5, 0.3, 0.25, 0.2, 0.1, 0.05, 0.025, 0.01, and 0.005 mg. of magnesium per milliliter.

MANGANESE STANDARDS. Manganese chloride was dissolved in the base solution to yield 2 grams of manganese per liter. Lower concentrations of 1.0, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01, and 0.005 mg. per ml. were prepared by diluting this manganese solution with the proper volume of base solution.

POTASSIUM STANDARDS. The behavior of potassium is different from that of magnesium, manganese, and calcium. Large amounts of potassium show a marked gradation of line densities. Thus higher percentages may be successfully analyzed directly from the sample without employing a "filler" or dilution of samples.

Sufficient potassium chloride to yield 30 grams of potassium per liter was dissolved in the sodium-ammonium chloride base solution. Dilutions with the base were made giving a series containing 30, 25, 20, 17.5, 15, 12.5, 10, and 5 mg. of potassium per ml. of solution.

PHOSPHORUS AND IRON STANDARDS. Iron in the form of ferrous ammonium sulfate and phosphorus as potassium acid phosphate were dissolved in the sodium-ammonium chloride base solution. The iron concentration for this series was 1, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01, 0.005, and 0.0025 mg. per ml. of solution. The phosphorus content was double that of iron.

Preparation of Samples

For the analysis of ash such as that from plant materials a 0.05-gram sample was treated with 1 ml. of concentrated hydrochloric acid and allowed to stand for an hour. To this solution a sufficient volume of the sodium-ammonium chloride solution was added to give a total volume of 10 ml.

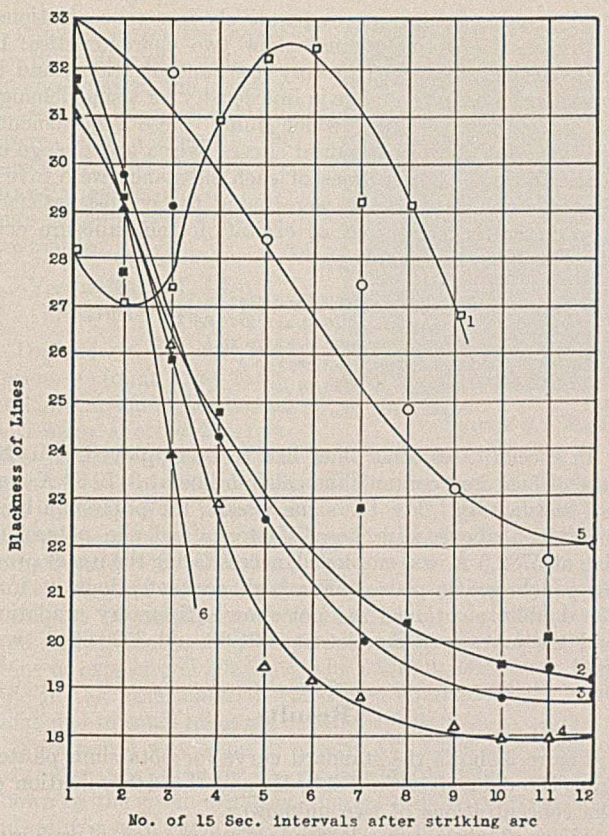

FIGURE 1. BEHAVIOR UPON VOLATILIZATION

1. Phosphorus, 2536.38 Å.
2. Magnesium, 2776.71 Å.
3. Iron, 3020.65 Å.
4. Manganese, 2576.12 Å.
5. Calcium, 3158.87 Å.
6. Potassium, 3447.38 Å.

Samples for potassium analysis were similarly prepared, except that they contained 0.200 mg. of ash per 10 ml. of solution.

A synthetic ash solution was prepared for check analysis by dissolving the salts in a 4.5 per cent hydrochloric acid solution, so that the resulting concentrations were 0.7 gram of calcium, 0.4 gram of magnesium, 0.01 gram of manganese, 0.15 gram of phosphorus, 0.05 gram of iron, and 14 grams of potassium per liter.

Conditions of Exposure

Eastman "33" commercial plates were used for all analyses except those in which iron interfered with potassium. Wratten-Wainwright process panchromatic plates gave satisfactory results in this case. The "33" plates were chosen because their characteristics were suitable for the particular conditions required for these analyses. Both types of plates were developed in Eastman developer formula D-11 for 5 minutes at 18° C.

The method of "continuous exposure" extensively employed by this laboratory for some time was used to determine the behavior of the six substances upon volatilization. The plate was placed in readiness for exposure, the slit was opened, and the electrodes were adjusted. Each 15 seconds from the instant of arcing, the plate was moved rapidly to a new position, allowing the arc to burn continuously for 4 or 5 minutes. In this manner the comparative amounts of each substance volatilized in any 15-second interval could be determined, as well as the time required for the volatilization of each to proceed to a point where no further change in effect was shown on the photographic plate under the conditions of exposure employed.

From the data obtained on such a plate the time for exposure of each substance was found. Sufficient light was used so that the heaviest lines employed for analysis were well under maximum blackening. In this way a gradation of line densities was obtained for the lower concentrations. The percentage of light reaching the slit was regulated by the adjustable revolving sector. Table I shows the conditions of exposure used for each substance when a current of 10 amperes was carefully maintained.

Slight variations in time or percentage of light were made when only the higher or lower concentrations of a series were used.

Evaluation of Blackness of Spectral Lines

The blackness of the proper lines from the various analyses was read on the density comparator. Only plates free of fogging were used; any which were slightly fogged or not entirely clear were discarded. A reading for blackness when no light entered the slit was taken first. Each line was read independently until an accurate check was obtained, the plate was then moved to a clear background in the same region, and this reading was recorded as the "background." The differences between the blackness of the line and absolute blackness and between the background and absolute blackness were calculated. The ratios of these differences were plotted against the logarithms of concentrations to obtain the working curve or "standard curve," according to the method of Twyman and Hitchen (*3*). Each plate held a set of spectra of standards from which a working curve was formulated and the spectra of a number of unknowns which were evaluated from this curve. Duplicate plates were made to check results.

Continuous Exposure

The behavior of each of the six substances upon volatilization was characteristically different.

Potassium, although present in the largest amount, volatilized most rapidly and practically disappeared in 45 seconds.

Phosphorus also volatilized to such an extent that no effect could be observed on a photographic plate. It was unique in behavior, volatilization beginning slowly, gradually increasing to a maximum, and then slowly decreasing. About 2 minutes were required, although the bulk of it vaporized in 60 seconds, the time of exposure used for analysis.

Manganese volatilized very rapidly at first, most of it disappearing in 45 seconds. A faint trace remained for 45 to 60 seconds longer.

Magnesium did not volatilize completely. As in the case of manganese, 45 seconds were sufficient for the greater portion to burn out. After that time the amount volatilized in each interval gradually decreased and finally reached a constant.

Calcium acted somewhat like magnesium, except that it behaved very erratically when only a trace remained.

Iron was found to be the most persistent of any of the six substances under investigation. However, very consistent results were obtained when 60-second exposures were used.

Figure 1 shows graphically how the substances compare in speed of volatilization and in the amounts of each vola-

TABLE II. RESULTS OF SPECTROGRAPHIC ANALYSIS

Substance	Found Spectrographically *Mg./ml.*	Present in Solution *Mg./ml.*	Error %
Magnesium	0.400	0.400	0
	0.390	0.400	2.5
Manganese	0.0103	0.0100	3.0
	0.0099	0.0100	6.0
Phosphorus	0.148	0.150	1.3
	0.148	0.150	1.3
	0.153	0.150	2.0
Potassium	14.25	14.00	1.7
	13.00	14.00	6.7
	14.00	14.00	0
Iron	0.048	0.05	4.0
	0.052	0.05	6.0
Calcium	0.68	0.7	3.0
	0.68	0.7	3.0

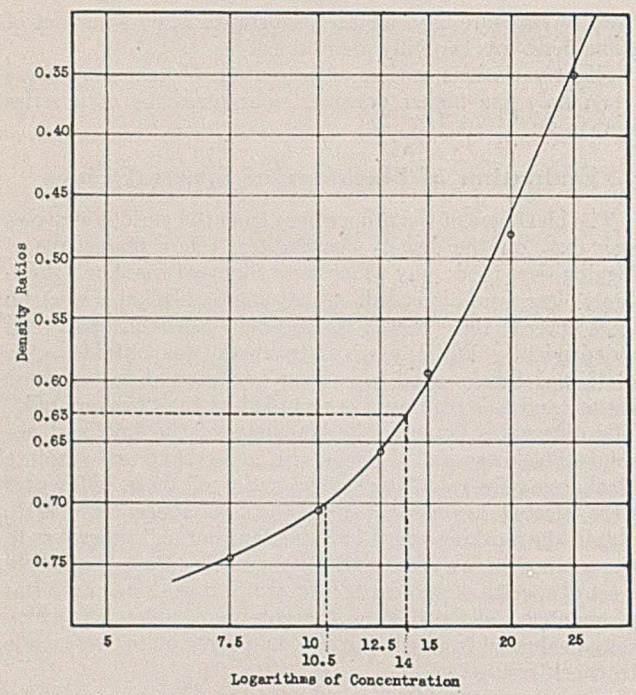

FIGURE 2. STANDARD CURVE FOR POTASSIUM, 3446.37 Å.

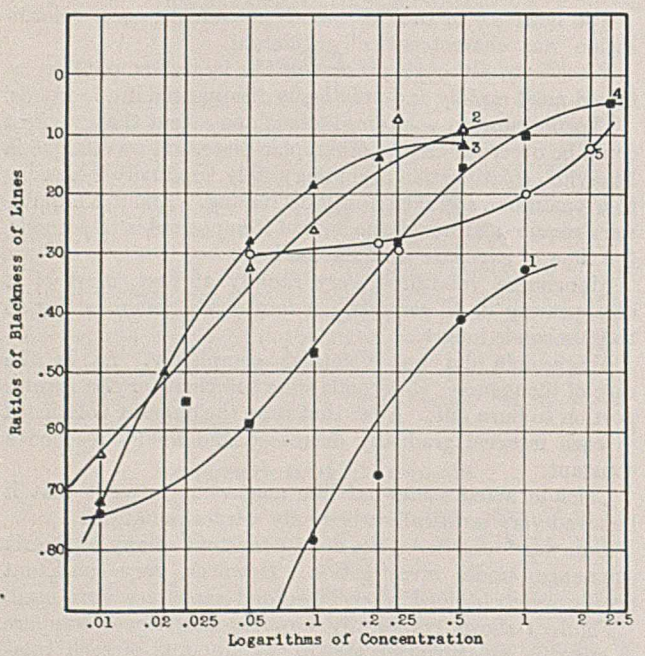

FIGURE 3. STANDARD CURVES

1. Phosphorus, 2536.38 Å.
2. Iron, 2599.40 Å.
3. Manganese, 2605.69 Å.
4. Magnesium, 2779.85 Å.

tilized in any time interval. Line blacknesses are plotted as ordinates against time intervals as abscissas. Potassium has the greatest slope; magnesium, manganese, and iron behave somewhat alike; calcium shows a slower rate of volatilization; and the erratic behavior of phosphorus is very evident.

Suitable Lines for Analysis

Very few *raies ultimes* are suitable for use in quantitative analysis using the method of arc excitation, as they show maximum blackening in comparatively low concentrations. A line used effectively must show two characteristics: It must be persistent even in very low concentrations, and it must show a definite change in line density for a small change in concentration. Lines which fulfill these requirements give very satisfactory standard curves over a short range of concentrations. Many lines of each substance were investigated and those selected were found to be most satisfactory under the conditions of excitation and exposure employed. They are as follows:

Potassium, 3446.37 and 3447.38 Å.
Magnesium, 2776.71 and 2779.85 Å.
Calcium, 3158.87 and 3179.33 Å.
Iron, 2698.08 and 2599.40 Å.
Phosphorus, 2536.38 Å.
Manganese, 2567.12 and 2605.69 Å.

In special cases other lines had to be employed. In the case of high iron content, the calcium line at 3179.33 Å. was not satisfactory. For the same reason, the potassium lines mentioned above were unsatisfactory, and the potassium line at 5782.6 Å. was employed, necessitating the use of process panchromatic plates. For high concentrations of iron the doublet mentioned did not show satisfactory gradation and the unresolved doublet at 3021.08 and 3020.65 Å. was used.

Results

Figure 2 shows the standard curve for potassium plotted from the data given in Table II, and the determination of the concentrations of two unknowns.

Figure 3 gives typical standard curves for phosphorus, iron, manganese, magnesium, and calcium, all plotted from experimental data.

Table II gives the results of a spectrographic analysis of a solution typical of that suitable for plant ash. One milliliter of this synthetic solution contains 0.400 mg. of magnesium, 0.010 mg. of manganese, 0.150 mg. of phosphorus, 0.050 mg. of iron, 0.70 mg. of calcium, and 14 mg. of potassium.

Figure 4 is taken from an analysis for phosphorus and iron. The phosphorus line 2536.38 Å. and the iron doublet 2598.08 and 2599.40 Å. are shown dotted. The gradations of both lines are very apparent to the eye.

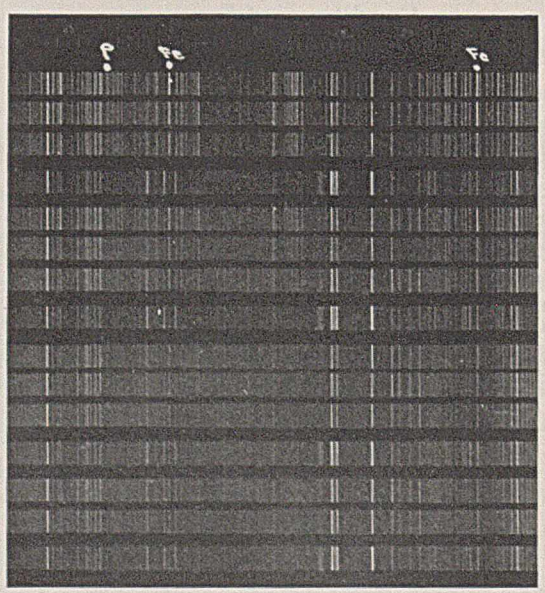

FIGURE 4. PHOSPHORUS AND IRON

Figure 5 shows two analyses of a sample, one for magnesium and one for manganese. The magnesium group of five lines in the region of 2780 Å. and the manganese group of three lines in the region of 2600 Å. are marked.

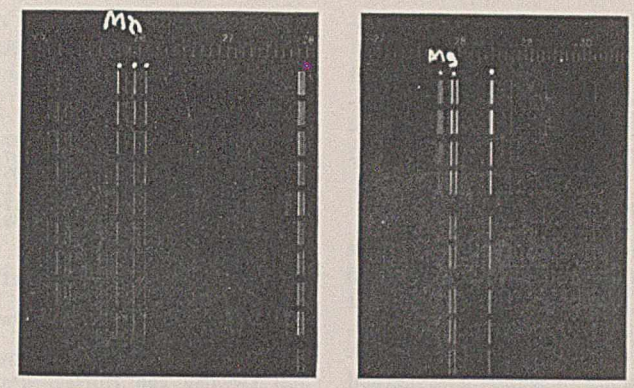

FIGURE 5. MANGANESE AND MAGNESIUM

Variation of Line Densities

Duffendack, Wylie, and Owens (*1*) found that magnesium, calcium, sodium, and potassium affected the relative intensity of the spectral lines of each of the other elements with regard to the spectral intensity of an internal standard, and that the addition of 2 per cent of sodium had the effect of eliminating further variations due to the other elements.

The effect of calcium, potassium, and sodium on the line blackness of magnesium was measured and found to be well within the range of experimental error for the conditions of exposure employed. The addition of sodium chloride in the base material would also eliminate slight variations due to small amounts of other substances.

No attempt was made to remove any substance from the ash before analyzing for the six substances determined. As shown by the graph resulting from a continuous exposure, all substances were vaporized concurrently.

PHOSPHORUS. The rate of volatilization of phosphorus differed markedly from that of the other five elements. Slow at first to volatilize, the rate would suddenly increase to a maximum and then gradually decrease. This behavior did not interfere with the accuracy of the determination (less than 2 per cent) when sufficient time was given to arcing the sample. The range within which phosphorus can be determined is limited. No attempt to show it in less than 0.01 mg. per ml. was made in this investigation. In cases where the phosphorus was not at first shown to be present, a concentration of the ash solution yielded a positive phosphorus determination. The solutions for phosphorus yielded a very satisfactory standard curve between the limits of 0.01 and 10 mg. per ml., making determinations of unknowns with a high degree of accuracy. There were no lines of any substances used or impurities occurring in the region of the phosphorus line, 2536.38 Å., and no background to interfere. The line was easily read on the density comparator and gave a smooth curve.

IRON. Iron had the advantage of having a number of lines which showed a definite gradation of line blackness, yielding satisfactory standard curves. The unresolved doublet at 3020.65 and 3020.08 Å. gave a workable curve for concentrations of iron from 0.05 up to 20 mg. per ml. This doublet would be more effective on an instrument with higher resolving power. The pair at 2598.38 and 2599.40 Å. is the most satisfactory over a long range. From a range of 2 mg. per ml. down to as low as 0.0005 mg. per ml., a standard curve can be made, which has less change in density for a small change in concentration than does the doublet at about 3020 Å. When only iron is being determined, the doublet at 3020 Å. is to be preferred except in extremely low concentrations. In conjunction with phosphorus, the pair at 2598.38 and 2599.40 Å. was most extensively used. They occur in a region clear of background, free of interfering lines, and are brought out satisfactorily under the same conditions used for phosphorus. An accuracy of 4 to 6 per cent was found in the case of iron in a concentration of 0.05 mg. per ml.

MANGANESE. Two lines, 2576.12 and 2605.69 Å., give standard curves for manganese over a range of 0.001 to 5.0 mg. per ml. by varying the amount of light. In the case of any analyzed synthetic ash containing 0.01 mg. per ml. the error was estimated at 5 to 10 per cent. As the manganese was found to contain a small amount of iron, the error was not extreme. The curve for manganese was very smooth, giving both the shoulder and toe of the curve as well as the straight portion. Determinations of higher concentrations are more easily made, being on a straight part of the curve.

MAGNESIUM. Magnesium was the easiest to analyze of the six elements. The curves obtained from the lines at 2776.71 and 2779.85 Å. more nearly resembled Hurter and Driffield curves than any of the others. Over the comparatively short range of 0.01 to 1.0 mg. per ml. a complete curve was obtained by proper exposure. Results on magnesium showed, also, the smallest percentage of error. In a group of five analyses, the average error was 3 per cent. Magnesium may be determined very satisfactorily in a range of 5.0 down to 0.001 mg. per ml. Its determination is probably the most accurate of the six analyses.

POTASSIUM. Analysis of potassium in ash proved to be the most difficult. In the first place, the range of concentration is very limited. Gradation of line blackness may be obtained best between concentrations of 1.0 and 50 mg. per ml., and only with difficulty under 5 mg. per ml. The sample should be heavy in potassium to be accurately analyzed.

Iron in any concentration greater than 0.1 mg. per ml. interferes with the unresolved doublet at 3446.37 and 3447.38 Å., making the employment of these lines for analysis impossible. An attempt to lessen the time of exposure and use the pair at 4044.16 and 4047.22 Å. was only partially successful. Another line obtainable on special red-sensitive plates, 5782.6 Å., can be used between limits for approximations but its spectrogram possesses continuous background to be used for formulating a standard curve.

The standard curve obtained for potassium using 3447.38 Å. in a range of concentrations from 5 to 30 mg. per ml. is very easily obtained and satisfactory for concentrations within that range when iron is absent or present in an amount less than 0.05 mg. per ml. A spectrograph giving a greater resolution of the doublet, thus freeing it from interference by iron, would yield very satisfactory results.

Results obtained on four samples of potassium containing less than 0.05 gram of iron per liter, and known to contain 14 grams of potassium, gave 15.0, 14.25, 13.0, and 14.0 grams per liter, an average of about 5 per cent error.

CALCIUM. Calcium is present in an appreciable amount in most graphite rods ordinarily used for spectrographic analysis, and considerable care is needed to select electrodes sufficiently free of calcium.

A series of standard solutions gives a smooth curve for calcium with very little change in density for a large change in concentration. Thus the determination of unknowns is much less accurate for calcium than, for example, magnesium. Two lines of calcium were found to yield satisfactory standard curves: 3158.87 and 3179.33 Å. Between the concentrations of 0.001 and 5 mg. per ml., smooth standard curves were obtained and successful determinations made. However, the percentage error was higher for calcium than for magnesium,

manganese, iron, or phosphorus, particularly when the concentration of the unknown was very low.

Many analyses supplied by the Botanical Department (by R. P. H.) have been made by the procedure herein discussed and the results will be recorded in a forthcoming publication.

Summary

The employment of a base solution composed of 5 grams of sodium chloride, 4.5 grams of hydrochloric acid, and 45 grams of ammonium chloride per liter of solution as a solvent for calcium, magnesium, manganese, potassium, and phosphorus makes it possible to determine by use of the spectrograph the amounts of these elements present. The method is especially suitable for the analysis of plant ash materials when even a very limited amount of the substance is available. The conditions of arc excitation, exposure, etc., have been worked out to give the maximum gradation of density for particular spectral lines selected as most satisfactory for the analysis of each substance. Under these conditions certain limits of concentration were found to exist:

	Å.	*Mg./ml.*
Calcium	3158.87 and 3179.33	0.001 to 5
Potassium	3446.37 and 3447.38	1 to 50
Iron	2598.08 and 2599.40	0.0005 to 2
Magnesium	2776.71 and 2779.85	0.001 to 5
Manganese	2576.12 and 2605.69	0.001 to 5
Phosphorus	2536.38	0.01 to 10

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(1) Duffendack, Wylie, and Owens, IND. ENG. CHEM., Anal. Ed., 7, 410–13 (1935).
(2) Nitchie, *Ibid.*, 1, 1–7 (1929).
(3) Twyman and Hitchen, *Proc. Roy. Soc.* (*London*) A133, 72 (1931).

RECEIVED April 5, 1937. A portion of a Ph.D. thesis presented to the Graduate Faculty of the Michigan State College, June, 1937, by Mrs. Mabel F. Wilson.

A Physical Method for Drying Liquefied Hydrocarbons

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THE author had occasion to dry thoroughly certain low-boiling olefin and diene hydrocarbons; these substances had been highly purified by Kistiakowsky and collaborators (*3–6*) and showed, in general, boiling point ranges of the order of 0.01° C. (The samples had been stored at −80° C. and each time one was opened, water vapor from the atmosphere condensed to such an extent that not only was a saturated solution of water in hydrocarbon formed but excess ice was present.)

Not a great deal is known concerning the factors influencing polymer formation and isomeric shift for some of these compounds; we are here concerned with reactions that might possibly form products to the extent of even 0.1 per cent, the approximate upper limit of impurities present. Taking this into consideration, it was decided to use no chemical treatment in the drying process after the distillation. A physical method was devised which proved so efficacious that it merits description.

Distillation in a vacuum line from a temperature of −60° C. to a condensing tube maintained at −80° (or −188° C. in some cases) was first tried; however, the product from such a distillation always showed more or less turbidity at −80°, which disappeared as the liquid was warmed to 0° C. Moreover, such a vacuum distillation is slow and becomes worse in this respect as higher boiling compounds are distilled.

It was suspected that chilling, followed by visual examination of the liquid hydrocarbon, constituted a delicate test for the presence of water. To establish this, an experiment was performed by adding to 100 grams of crude, water-free 2-methylpropene (this sample showed no detectable trace of turbidity at −135° C.), 0.7 milligram of water contained in a very small capillary tube open at both ends. Shaking the 2-methylpropene tube at room temperature displaced part of the water in the capillary. Upon cooling the tube to −80° C. a distinct turbidity developed throughout the liquid, but no diminution could be noticed in the 0.7 milligram of water.

Apparatus

An apparatus was then arranged to filter the liquid hydrocarbon under its own vapor pressure at low temperatures. Coffin and Maass (*1*) have described a somewhat similar apparatus but used no filler in the filter tube; their method of forcing the liquid through the filter tube is open to objection and they gave no data on the products. Hicks (*2*) has described the use of vacuum-jacketed funnels containing a porous plate, but they would not be applicable to the present task in the form shown. The essential details of the author's apparatus are presented in Figure 1.

The bottom fifth of the filter tube, *D* (ca. 150-cc. capacity), is packed rather loosely with glass wool before the top tube is sealed in place, providing an effective filter mat for the ice particles. The hydrocarbon is condensed in *B* (ca. 150-cc. capacity) and freed from air, the complete line being evacuated at this time by means of a mercury diffusion pump. A bath of solid carbon dioxide in acetone (this bath must not be colder than the freezing point of the compound undergoing drying) is placed about filter tube *D*. The contents of tube *B* are then brought slowly to room temperature by judicious cooling of the contents at intervals, so that evaporation takes place smoothly. This operation causes liquefaction to take place in tube *D;* upon observing this liquefaction, liquid air is placed about tube *F* (ca. 100-cc. capacity) in which liquefaction and subsequent solidification (for most substances) occur.

The resultant lowering in the vapor pressure in *F* and *J* gives rise to a pressure differential on the liquid in the filter tube, the liquid is thereby forced over into container *F* via tube *E*, and a steady flow of liquid is maintained, tube *E* becoming covered with frost. This delivery tube could be vacuum-jacketed, but it would unduly complicate the apparatus. After this delivery tube has cooled, the liquid air may be removed from about *F* and the filling of the final container(s) started by immersing *J* in a bath of solid carbon dioxide in acetone during the first two-thirds of the filtering. By not externally cooling *F* (it is internally cooled by the liquid dropping in) after the flow has once been started, distillation takes place from *F* and *J;* however, during the later stages of the filtration *J* must be immersed in a bath at a lower temperature than the one about *D*.

It is a good plan to attempt to empty *D* as rapidly as possible toward the end, for as soon as all the liquid has vaporized from *B*, liquid still remaining in *D*, there is no longer a pressure differential on the filter tube and the delivery to *F* practically ceases. Thereafter, distillation from the very small surface through the long, narrow (2.5-mm. bore) delivery tube is exceedingly slow and is not desirable.

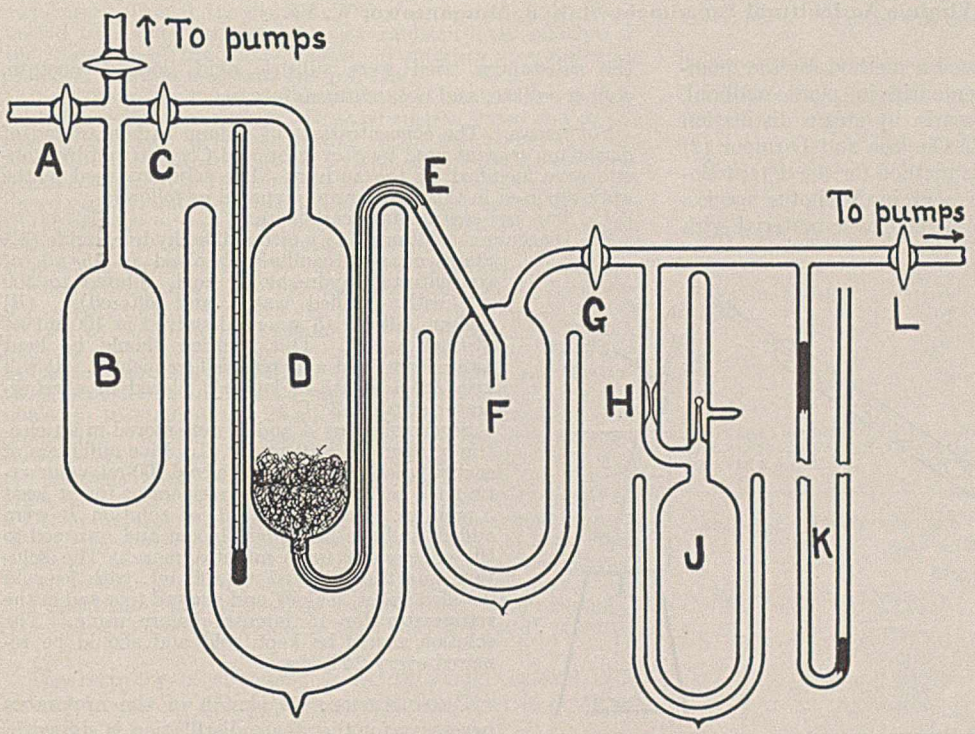

FIGURE 1. DIAGRAM OF APPARATUS

This means that at the end a small amount of liquid must be left in *D* or else a slow stream of an inert gas (not necessarily dry) admitted through cock *A* to force over the remnants. When the final container is filled, liquid air is placed about tubes *D*, *F*, and *J*, cock *L* is opened to the pumps, and the container is sealed off (the technic of sealing off under a vacuum is discussed by Scott, Cook, and Brickwedde, 7) at constriction *H* under a vacuum.

The containers, *J*, of the apparatus now in use are from 30- to 100-cc. capacity, varied according to the size of the sample to be filtered. The average amount filtered by the author was 75 cc., although an apparatus has been used in which the filter tube was 550 cc. in size and 400-cc. samples were run through satisfactorily.

TABLE I. DATA ON COMPOUNDS

[Compound[a]	Normal Boiling Point	Freezing Point	Filtration Temperature	Reference Number
	°C.	°C.	°C.	
Propadiene	−34.2	−136	−80	(6)
2-Methylpropene	− 7.0	−141	−135 to −130	(4, 8)
1-Butene	− 6.4	Glass	−80	(4, 8)
trans-2-Butene	+ 1.0	−106	−100 to −90	(4, 8)
cis-2-Butene	3.7	−139	−135 to −130	(4, 8)
1,4-Pentadiene[b]	26.1	−148	−80	(6)
2-Methyl-2-butene	38.6	−134	−80	(5)

[a] All the substances listed were prepared by Professor Kistiakowsky and his collaborators, of these laboratories, for their study of the heats of hydrogenation. For details of preparation, distillation, and some physical constants, see (3–6).

[b] This filtration was performed with the author's apparatus by W. R. Smith of these laboratories.

Discussion

In three cases shown in Table I, filtration was conducted at a much lower temperature than −80° C. This is more of a task, for baths must be prepared with the aid of liquid air, which will keep tube *D* at the proper temperature. Such good results were obtained at −80° C. that only in exceptional cases should it be necessary to go to lower temperatures.

In all cases, the product from the filtration showed no trace of turbidity at the temperature of filtration and when the products were examined visually at a lower temperature than that at which filtration was performed, the liquid hydrocarbons were absolutely clear. In view of the known low solubility of water in hydrocarbons at room temperature the author believes that the water content has been lowered to a negligible value.

From observations on the filtration of 2-methyl-2-butene, it would appear that liquids boiling above 50° C. would be difficult to filter in the manner described, since their vapor pressure at room temperature is not sufficiently high to give the proper pressure differential on the filter tube. This could be readily overcome by using a stream of inert gas to force over the liquid.

This type of filtration should be applicable to any liquid in which water does not dissolve to an appreciable extent. If the liquid has a relatively high freezing point, it may not be possible to free it from water as thoroughly as was done in the cases under consideration.

Summary

A method of filtering is described which is applicable to liquids at low temperatures. The essential features are the manner of constructing the filter tube, the filtration being conducted under the vapor pressure of the substance, and the utilization of the vapor pressure to force the liquid through the filter tube. The method is applied to seven hydrocarbons and it is demonstrated that the product from the filtration contains a negligibly small amount of water.

Acknowledgment

The author wishes to thank Professor Kistiakowsky for allowing him to participate in the preparation of some of these compounds and for gifts of others, and to thank Professor Lamb for facilities placed at his disposal.

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Determination of Coumarin in Vanilla Extract

By a Modification of the Steam-Distillation Method

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IN 1934 the authors (*3*) proposed a method for the quantitative determination of coumarin in plant material, based upon the removal of coumarin by steam distillation under reduced pressure. In 1935 Clayton and Larmour (*2*) published a tentative colorimetric method for the determination of coumarin and melilotic acid in Melilotus species. This consisted in extracting the sweet clover material with 95 per cent alcohol and determining the coumarin and melilotic acid colorimetrically in aliquot portions of the extract. In 1936 Stevenson and Clayton (*5*) reported results obtained with sweet clover, extracting the samples with 50 per cent methyl alcohol instead of 95 per cent ethyl alcohol. The color is produced when solutions containing coumarin are treated with sodium carbonate and heated, and a solution of diazotized *p*-nitroaniline is added. The depth of color was shown to be in direct ratio to the amount of coumarin present. Roberts (*4*) modified Stevenson and Clayton's procedure by grinding the samples in a mortar with sand and extracting with 50 per cent methyl alcohol at room temperature.

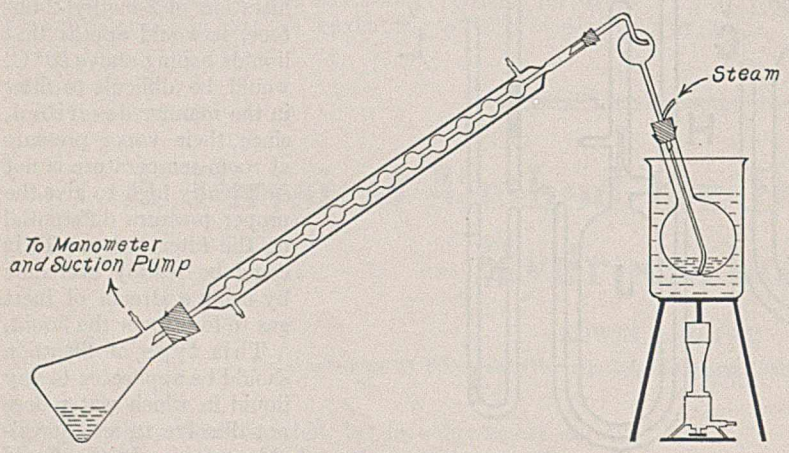

FIGURE 1. STEAM-DISTILLATION APPARATUS

The work done on the removal of coumarin from plant material by the steam-distillation method made probable its removal from vanilla extract by the same general procedure. Once separated from the vanilla extract, it was thought that coumarin in the distillate could be determined quantitatively by the colorimetric method. The results of this investigation are reported here.

Experimental

Preliminary trials indicated that coumarin can be removed from vanilla extract by the steam-distillation method; however, the possible interference of small amounts of vanillin which distill over along with the coumarin needed investigation. Tests showed that relatively concentrated solutions of vanillin react with the diazonium reagent and produce a red color similar to that given with dilute solutions of coumarin. Attempts were made to block the vanillin by adding a small amount of sulfuric acid or certain salts to the distillation flask and thus prevent it from distilling over. Among the substances tried were sulfuric acid, sodium acetate, copper sulfate, and potassium sulfate.

SOLUTIONS. The concentrations of sodium carbonate and of diazonium reagent used by Stevenson and Clayton and by Roberts were modified by the authors. The solutions used in the work reported in this paper were prepared as follows:

1. Two per cent sodium carbonate.
2. Diazonium solution: (*A*) *p*-nitroaniline hydrochloride (3.5 grams of *p*-nitroaniline dissolved in 45 ml. of concentrated hydrochloric acid, diluted to 500 ml. with distilled water, and filtered). (*B*) Sodium nitrite (5 grams dissolved in 100 ml. of distilled water. This solution should be kept away from light and renewed frequently. It will keep for a month or longer if placed in a refrigerator at 0° to 3° C.)

Stock solutions *A* and *B* were stored in an electric refrigerator at 0° to 3° C. Five milliliters of each solution were placed in a cold 100-ml. volumetric flask and kept in the refrigerator for at least 5 minutes. To this, 10 ml. of solution *B* were added, and the flask was shaken and returned to the refrigerator for 5 minutes more. The solution was then diluted to 100 ml. with ice-cold distilled water, shaken, and allowed to stand in the refrigerator for 15 minutes before using. The solution should be kept cold and should be renewed every 24 hours.

PROCEDURE. A sketch of the apparatus necessary for the steam distillation is shown in Figure 1.

Approximately 12.5 ml. of the vanilla extract together with 0.2 ml. of concentrated sulfuric acid or 0.5 gram of one of

TABLE I. RECOVERY OF COUMARIN FROM VANILLA EXTRACT[a]

(In the presence of sulfuric acid or certain salts by the steam-distillation procedure)

Reagent Used	Coumarin Added	Coumarin Recovered[b]	
	Gram	*Gram*	*%*
Sodium acetate, 0.5 gram	0.0300	0.0301	100.3
	0.0750	0.0742	98.9
	0.0750	0.0750	100.0
	0.0500	0.0498	99.6
	0.0500	0.0501	100.2
	0.0500	0.0502	100.4
	0.0500	0.0496	99.2
Concentrated sulfuric acid, 0.2 ml.	0.0279	0.0281	100.7
	0.0315	0.0308	97.8
	0.0459	0.0453	98.7
	0.0114	0.0115	100.9
	0.0273	0.0280	102.6
Potassium sulfate, 0.5 gram	0.0459	0.0457	99.6
	0.0250	0.0253	101.2
Copper sulfate, 0.5 gram	0.0140	0.0140	100.0
	0.0279	0.0281	100.7
		Mean	100.1

[a] Tincture No. 90, Vanilla, N. F., containing 40 per cent of alcohol, prepared by Eli Lilly and Co. This preparation is coumarin-free.

[b] To determine the precision of the method, 0.2500 gram of coumarin was weighed accurately and dissolved in 250 ml. of solution. Six aliquots of this solution (25.0, 25.1, 25.2, 25.3, 25.4, and 25.5 ml.) were measured from a precision buret and made up to 1-liter volume. Three 20-ml. aliquots were taken from each liter flask with a precision pipet and the color was developed according to the method described. This gave a total of 18 solutions. Precaution was taken to avoid psychological influence by withholding from the operator of the colorimeter knowledge of the concentration of the solutions being read and of the standard used for comparison. Ten readings were made on each solution and the mean was taken for calculation of the coumarin content. The triplicate values thus obtained then were averaged for comparison with the known values. The results were as follows:

Colorimetric reading, av.	20.24	20.13	20.11	20.03	19.98	20.02
Found, gram	0.0249	0.0250	0.0251	0.0252	0.0252	0.0252
Present, gram	0.0250	0.0251	0.0252	0.0253	0.0254	0.0255

the salts were placed in the 1-liter, long-necked, round-bottomed, Pyrex distillation flask. A definite amount of pure coumarin was added and the volume was diluted to approximately 100 ml. with distilled water. The flask was then fitted with a Kjeldahl distillation bulb and a steam inlet tube which extended almost to the bottom of the flask. The distilling flask was joined through the connecting bulb to a 63-cm. 12-bulb Allihn condenser and immersed in a boiling water bath. A 4-liter Pyrex beaker served as a transparent bath through which the steam distillation was observed and controlled. A 1-liter suction flask fitted to the condenser collected the distillate. The entire system was connected to a water suction pump by rubber tubing attached to the side tube of the suction flask. A mercury manometer inserted in the line measured the pressure.

It is convenient to run the distillations in duplicate by attaching two sets of apparatus to the same suction pump and to the same steam jet. A stream of dry steam was passed into the flask until the contents reached a vigorous boil and the distillate began to drip from the condenser. The pressure was then reduced rapidly, but at such a rate as to prevent boiling over, until the pressure in the system was 140 mm. of mercury. This was conveniently controlled by placing a screw clamp on a rubber tube which was connected to the system by means of a Y-tube.

The distillation was continued at this rate until the flask was dry, after which it was removed from the bath. An additional 100-ml. portion of water was added to the contents of the flask and the distillation procedure was repeated, after which the condenser was rinsed down into the receiving flask and the distillate transferred to a 1-liter volumetric flask. The flask was made up to volume and 20-ml. aliquots of the distillate were pipetted into 50-ml. volumetric flasks. Five milliliters of 2 per cent sodium carbonate solution were added to each flask and the contents were heated in a boiling water bath for 5 to 10 minutes or, if more convenient, heated in a water bath at 80° C. for 15 minutes. The flasks were cooled to room temperature, 5 ml. of cold diazonium solution were added, and the flasks were made up to volume. A series of standards containing from 0.0001 to 0.001 gram of coumarin was prepared at the same time and in the same way as the unknown solutions. Color comparisons were made in a colorimeter after standing for 15 minutes.

The recovery of coumarin added to vanilla extract in a series of trials by the procedure just described is given in Table I.

These data show that coumarin present in varying amount can be recovered quantitatively from vanilla extract by the steam-distillation procedure when a small amount of sulfuric acid, sodium acetate, potassium sulfate, or copper sulfate is added to the sample in the distillation flask. Some odor of vanillin could be detected in the distillates, but it was not in sufficient concentration to interfere. After the distillates had stood for 24 to 48 hours those distilled from sodium acetate and from copper sulfate gave somewhat higher results. Just what caused these higher values was not apparent, but it was thought that the small amount of vanillin or perhaps some other constituent which distilled over changed in some manner so that it reacted with the diazonium solution on long standing, thus giving solutions of higher color. Other trials in which zinc sulfate and potassium acid sulfate were used gave distillates which reacted like those just described, but the distillates from sulfuric acid or from potassium sulfate remained unchanged for periods up to 48 to 72 hours. These distillates were not tested over longer periods of time.

It was concluded, therefore, that either sulfuric acid or potassium sulfate might be used in so far as theoretical recovery of coumarin is concerned. However, because the sulfuric acid caused considerable charring of the organic material in the extract when the flasks were distilled to dryness, the flasks were rather difficult to clean after the distillation was completed, and for this reason potassium sulfate was preferred.

The next points to be considered were the effects of using other preparations of vanilla extract and of increasing the vanillin content. For this work two additional coumarin-free high-grade extracts were obtained (a Parke-Davis preparation containing 30 per cent of alcohol and a sample of bulk material used by the University of West Virginia Dairy Department). In these trials varying amounts of coumarin and vanillin and 0.5 gram of potassium sulfate were added to 12.5-ml. portions of the extract. The results of the trials are given in Table II, and show that coumarin added to each of the three vanilla extracts was recovered quantitatively by the steam-distillation procedure. Samples reinforced with relatively large amounts of vanillin behaved like those to which no vanillin was added.

TABLE II. RECOVERY OF COUMARIN ADDED TO VANILLA EXTRACTS[a]

(With and without added vanillin)

Vanilla Extract Used	Vanillin Added	Coumarin Added	Coumarin Recovered	
	Gram	*Gram*	*Gram*	%
Parke-Davis	None	0.0250	0.0251	100.4
	None	0.0250	0.0251	100.4
	0.25	0.0250	0.0247	98.8
	0.25	0.0250	0.0250	100.0
W. Va. Univ. Dairy	None	0.0250	0.0250	100.0
	None	0.0250	0.0250	100.0
	0.25	0.0250	0.0250	100.0
	0.25	0.0250	0.0249	99.6
Eli Lilly	None	0.0459	0.0457	99.6
	0.25	0.0250	0.0248	99.2
	0.25	0.0250	0.0253	101.2
			Mean	99.9

[a] Blank determinations for coumarin were made on all three extracts. All were found to be free from the characteristic color produced when coumarin is present.

TABLE III. RECOVERY OF COUMARIN ADDED TO 50-ML. SAMPLES OF VANILLA EXTRACT

(Official method)

Sample No.	Coumarin Added	Coumarin Recovered	
	Gram	*Gram*	%
1	0.0750	0.0678	90.4
2	0.1000	0.0952	95.2
3	0.1125	0.1064	94.6
4	0.1500	0.1336	89.1
		Mean	92.3

TABLE IV. DETERMINATION OF LOSS OF COUMARIN ADDED TO VANILLA EXTRACT DURING EVAPORATION PROCESS

Sample No.	Coumarin Added	Coumarin Recovered	
	Gram	*Gram*	%
1	0.1000	0.0949	94.9
2	0.1500	0.1409	93.9
		Mean	94.4

The final point to be considered was a comparison of the steam-distillation method with the official method (*1*) for the determination of coumarin in vanilla extract. The Parke-Davis extract to which a definite amount of coumarin was added was used in these trials. The results are given in Table III, and show that the authors did not recover quantitatively the coumarin added to vanilla extract. It is probable that with practice, somewhat greater recovery might be obtained.

It was thought that much of the coumarin loss might have taken place during evaporation of the alcohol, most of which occurred at a bath temperature of 66° to 68°; at no time did this exceed 70° C. In order to determine whether loss does occur during evaporation, 50-ml. samples of vanilla extract, to which a known amount of coumarin was added, were evaporated as described in the official method. The samples were then diluted to 100-ml. volumes and the coumarin was determined in aliquot portions by the steam-distillation procedure. Table IV shows that there is an appreciable loss of coumarin during evaporation of the alcohol. This probably accounts, to a great extent, for the low values obtained by the official method as reported in Table III.

Discussion

The data presented in this paper were obtained on the determination of coumarin added to three different samples of pure vanilla extract. Since the coumarin was recovered quantitatively even when the samples were reinforced with considerable quantities of pure vanillin, the method presumably can be applied to the determination of coumarin in factitious vanilla extracts with equally good results. While two distillations are required for the complete removal of coumarin from 10- or 12.5-ml. samples, approximately 95 per cent is removed by the first distillation. Twenty-milliliter samples may require three distillations for the complete removal of coumarin. Table V shows the amounts recovered by an increasing number of distillations.

The authors found that by using more sodium carbonate and more *p*-nitroaniline than Stevenson and Clayton used in developing the color, the solutions come to equilibrium in a shorter time.

The color intensity produced with 5 ml. of 1.1 per cent sodium carbonate and 5 ml. of diazonium reagent, prepared according to Stevenson and Clayton, were compared to the intensity given with 5 ml. of 2 per cent sodium carbonate and 5 ml. of diazonium reagent prepared according to the authors. A constant quantity of coumarin (0.5 mg.) was used in each trial by measuring 5 ml. of solution from a 50-ml. precision buret. Each solution was compared with a standard containing 0.5 mg. of coumarin, and prepared according to the authors' modification. Readings were made at the end of 10 minutes, 30 minutes, and 1, 2, 4, 22, and 48 hours. Each series of readings was compared with a new standard freshly prepared and allowed to stand for 2 hours before using.

TABLE V. RECOVERY OF COUMARIN WITH SUCCESSIVE DISTILLATIONS

Sample No.	Volume of Extract	Coumarin Added	Coumarin Recovered: One distillation	Two distillations	Three distillations
	Ml.	*Gram*	%	%	%
1	12.5	0.030	95.4	100.0	...
2	12.5	0.030	94.8	100.4	...
3	12.5	0.030	95.4	100.4	...
4	12.5	0.030	93.6	99.8	...
5	12.5	0.030	95.3	98.4	...
		Mean	94.9	99.8	
6	20.0	0.075	...	99.0	99.5
7	20.0	0.075	...	99.9	100.1
8	20.0	0.075	...	96.5	100.3
9	20.0	0.075	...	98.1	99.3
			Mean	98.4	99.8

Table VI gives the results obtained, calculated to weight of coumarin found, and shows that the color intensity of the solutions prepared by the authors' modification of the Stevenson and Clayton method reached a constant value after standing for 2 hours, and remained constant for 20 hours. The solutions changed only very slightly after they had stood for 1 hour. The color intensity of the solution prepared by the Stevenson and Clayton procedure was not entirely constant after standing for 4 hours, but increased appreciably up to 22 hours. In both cases off-shades developed when the solutions had stood for a period of 48 hours, thus making accurate comparisons in a colorimeter difficult. In other trials not reported in Table VI it was shown that the color intensity of the standards and unknown solutions changes at the same rate. Therefore, when the standards and unknowns are prepared at the same time, color comparisons may be made any time after 10 or 15 minutes up to 22 hours. In case it is desired to determine the coumarin in a large number of samples over a period of several hours using only one set of standards, both the unknown solutions and the standards should stand for 2 hours before comparisons are made.

Color comparisons can be made accurately in the range of 0.1 to 1.0 mg. of coumarin in a 50-ml. volume. By dilution and choice of the size of aliquots used, concentrated coumarin solutions can be brought within this range. If an extract contains only a small amount of coumarin, it is desirable to use a larger sample or to dilute the distillate to 500 ml. rather than to 1 liter.

TABLE VI. COUMARIN CONTENT AT SUCCESSIVE INTERVALS

Determined by color intensity produced by 0.5 mg. of coumarin with different amounts of alkali and diazonium reagent

Method	Sample No.	Read After: 10 min.	30 min.	1 hr.	2 hr.	4 hr.	22 hr.	48 hr.
		Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Stevenson and Clayton	1	0.415	0.420	0.444	0.459	0.471	0.483	[a]
	2	0.427	0.426	0.444	0.457	0.471	0.482	[a]
	3	0.435	0.439	0.456	0.459	0.471	0.482	[a]
	Mean	0.426	0.428	0.448	0.458	0.471	0.482	
Authors	4	0.471	0.476	0.496	0.499	0.499	0.497	[a]
	5	0.475	0.476	0.496	0.500	0.498	0.501	[a]
	6	0.468	0.476	0.495	0.498	0.500	0.501	[a]
	Mean	0.471	0.476	0.496	0.499	0.499	0.500	

[a] Field difficult to match.

Summary and Conclusions

The steam-distillation method for the determination of coumarin in plant material can be utilized for the quantitative removal of coumarin from vanilla extracts in which it is contained. The addition of 0.5 gram of potassium sulfate to the sample prevents the interference of vanillin. Two distillations are necessary for the complete removal of coumarin from a 10- or 12.5-ml. sample. Coumarin in the distillate is determined colorimetrically by a modification of the Stevenson and Clayton method, involving a coupling reaction with *p*-nitroaniline after previous treatment with sodium carbonate and heating.

By a combination of these two procedures in a large number of trials in which coumarin was added to pure vanilla extract, the coumarin was recovered quantitatively with a maximum error of 2.6 per cent. In most cases the recovery was within 1 per cent of the true value. It is concluded, therefore, that coumarin can be determined quantitatively in factitious or adulterated extracts of vanilla by this method. In the opinion of the authors, the steam-distillation method has an advantage over the official method in both speed and accuracy, and the apparatus required is simple and easily manipulated.

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CORRECTION. In the footnotes to Table I in my article on "The Principle of the Suspended Level" [IND. ENG. CHEM., Anal. Ed., 9, 85 (1937)] a typographical error should be corrected. The reference letters *a* and *b* have been interchanged and hence the magnitude of the possible error of the correction figures is wrongly stated.

The corrections marked *a* in the table are accurate within 0.1 per cent and those marked *b*, within 0.2 per cent. This difference becomes significant in precise measurement of viscosity.

LEO UBBELOHDE

A Wood's Metal Reductor

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THE most frequently used metal reductor is the amalgamated zinc of the familiar Jones reductor, the preparation and use of which are discussed in many textbooks of analytical chemistry. A sulfuric acid solution of the material being reduced is preferred for use with this reductor. The Walden silver reductor is used in a manner almost identical to that of the Jones reductor, the silver being prepared in a spongelike state of aggregation (*4*). A solution of the material being reduced in hydrochloric acid is employed when using the silver reductor. Instead of granular amalgamated zinc, as in the Jones reductor, an amalgamated zinc wire spiral as described by Smith and Rich (*3*), is often used as a convenient substitute. Liquid amalgams were first employed as reductors by Nakazono (*2*), who used a 3 per cent liquid zinc amalgam as a substitute for the Jones reductor. This work was extended to the additional use of lead, bismuth, and cadmium liquid amalgams by Someya whose work is summarized by Hillebrand and Lundell (*1*). Other metal reductors such as aluminum wire or foil, have been employed but are not entirely satisfactory because of the presence of iron as an impurity or for other reasons. The elements commonly reduced for quantitative determination by the various metal reductors are iron, titanium, chromium, molybdenum, vanadium, and uranium.

Wood's metal is an alloy of bismuth, lead, tin, and cadmium. The alloy containing 50, 25, 12.5, and 12.5 per cent, respectively, of these constituents has a specific gravity of 9.7 and a melting point of 65.5° C. If fused Wood's metal, like mercury, has a high hydrogen overvoltage to prevent its rapid solution in dilute acid, as a metal reductor it should be comparable to the liquid amalgams of Someya (*1*). The present paper describes the practical application of Wood's metal as a reductor.

General Considerations

The most obvious advantage in the use of Wood's metal as compared to liquid amalgam reductors is in the removal of molten alloy when the reduction is complete; the cooled and solidified reductor metal is removed mechanically.

Wood's metal requires little attention and a number of determinations can be made simultaneously. It is easily available and requires no pretreatment. Hydrochloric acid solutions can be used and determinations of iron in the presence of titanium made by limiting the concentration of acid. Unlike liquid amalgam reductors it requires no special apparatus.

Wood's metal would not be expected to be more specific than the processes previously mentioned. A second disadvantage is that a minimum of metal surface is available for reactivity, and reduction is somewhat slow.

Procedure for Determination of Iron

A known volume of standard ferric chloride in hydrochloric acid was transferred to a 400-ml. beaker and diluted to 100 ml. with hydrochloric acid, the concentration of which was varied between 1.5 and 5 per cent by volume of the concentrated acid. Eighteen to 20 grams of Wood's metal were added, and the beakers were covered and boiled gently for 0.5 hour on an electric hot plate. At the end of this time the reduction was complete and the beakers were transferred to a cold-water bath and a 15-cm. (6-inch) length of fairly heavy platinum wire was placed in the molten alloy to withdraw it after solidification. The reduced solutions were further cooled by adding 100 ml. of cold water, and the solidified Wood's metal was withdrawn and washed, using a stream from the wash bottle. Twenty milliliters of dilute (1 to 1) sulfuric acid were added and the iron was determined by titration with standard ceric sulfate, using ferroin as indicator. The solution of ferric chloride in *N* hydrochloric acid was standardized, using a Walden silver reductor, followed by titration with standard ceric sulfate exactly as in the former analyses. The ceric sulfate was standardized in the usual way by comparison with pure sodium oxalate. The ferric chloride solution was found by the average from five closely agreeing results to contain 0.004296 gram of iron per ml. A series of results from this procedure is given in Table I.

TABLE I. DETERMINATION OF IRON

(After 30 minutes' reduction with Wood's metal in hydrochloric acid at approximately 100° C.)

Iron Taken	Volume of Concd. HCl	Reduction Loss in Weight	0.03548 *N* $Ce(SO_4)_2$	Iron Found	Error in Iron Determination
Gram	*Ml./100 ml.*	*Gram*	*Ml.*	*Gram*	*Mg.*
0.0429(7)	1.5	0.040	21.70	0.0430	0.0
0.0429(7)	1.5	0.025	21.70	0.0430	0.0
0.1074(2)	5.0	0.114	54.18	0.1073	−0.1
0.1074(2)	5.0	0.147	54.20	0.1074	0.0
0.0429(7)	1.5	0.049	21.68	0.0430	0.0
0.1074(2)	2.5	0.125	54.18	0.1073	−0.1
0.1074(2)	2.5	0.123	54.19	0.1073	−0.1

A series of samples of Norrie iron ore, known to contain 66.65 per cent of iron, was taken into solution in the usual manner with recovery of the iron from the insoluble matter. The solution of the ore in dilute hydrochloric acid was reduced under conditions comparable to those shown in Table I. The average of six consecutive analyses gave 66.58 per cent of iron. The maximum deviation from the average result was less than 2 parts in 1000. It was also found that iron could be quantitatively determined, using Wood's metal as reductor, in solutions containing 5 per cent by volume of 70 per cent perchloric acid or 5 per cent by volume of concentrated sulfuric acid.

Determination of Iron in Presence of Titanium

A solution of titanium sulfate in 3 *M* sulfuric acid was standardized, using a Jones reductor, followed by titration with a solution of ferric chloride using potassium thiocyanate as indicator. The reduced titanium solutions were kept out of contact with air by use of a stream of carbon dioxide gas. As the average of three determinations, 25.00 ml. of the titanium solution required 10.70 ml. of the ferric chloride. The same solution of titanic sulfate in dilute sulfuric acid (25.00 ml. plus 10 ml. of 1 to 1 sulfuric acid plus 15 ml. of water) was now allowed to react at the boiling point in contact with Wood's metal and in an atmosphere of carbon dioxide for 45 and 60 minutes. The reduction was 73.3 per cent complete at 45 minutes and 92.5 per cent complete at 60 minutes.

Because of the incomplete reaction in the time allowed and the necessity of a carbon dioxide atmosphere, attempts to determine titanium quantitatively were abandoned. Reduction of titanium in hydrochloric acid solution was not found to improve conditions materially.

An approximately 0.1 *N* solution of ferric iron was standardized, using a Walden silver reductor followed by titration with standard ceric sulfate. To known amounts of this solution 10 ml. of the titanium sulfate solution described above were added. The resulting solutions were diluted to 50 ml. and boiled gently in air for 30 minutes after addition of Wood's metal. Otherwise, conditions were the same as those described in Table I, except that after cooling, diluting, and removing the Wood's metal a slow stream of air was passed through the solution for 5 minutes. The color of the solutions did not indicate the reduction of appreciable amounts of titanium.

The results are given in Table II, which shows that in low concentrations of free acid (sulfuric or hydrochloric) iron in the presence of titanium may be determined if the practically

negligible amount of titanium reduced is reoxidized, using a slow stream of air prior to titration of iron. Experiments similar to those of Table II show that iron can be determined in the presence of vanadium by the same technic. Any vanadium reduced to the vanadous form is reoxidized by the passage of an air stream for 5 minutes, using a drop of copper sulfate solution as catalyst. The solution of vanadyl and ferrous ions is made 5 *M* in sulfuric acid and the iron alone is oxidized by ceric sulfate, using ferroin as indicator.

TABLE II. DETERMINATION OF IRON

(In the presence of titanium, using Wood's metal reductor)

Iron Present	0.03548 *N* $Ce(SO_4)_2$ Required	Iron Found	Difference
Gram	*Ml.*	*Gram*	*Mg.*
0.0558	28.18[a]	....	....
0.0558	28.30[b]	0.0561	+0.3
0.0558	28.16	0.0557(8)	−0.02
0.0558	28.20	0.0558(2)	+0.02
0.0558	28.15	0.0557(7)	−0.03
0.0558	28.18	0.0558	0.0
0.0558	28.15	0.0557(7)	−0.03
0.0558	28.16	0.0557(8)	−0.02

[a] Blank determination to evaluate iron solution using the silver reductor and $Ce(SO_4)_2$.
[b] No stream of air used after reduction and before titration of iron.

Besides reducing titanium and vanadium to titanous and vanadous ions, molybdenum is reduced to the trivalent condition and tungsten in phosphoric acid solution to the blue form. Hexavalent chromium in sulfuric acid solution is reduced to the trivalent form but not appreciably to the chromous ion. These determinations were not studied because of the slow reduction rate and the influence of atmospheric oxidation.

Summary

Fused Wood's metal was found to have properties similar to those of bismuth, cadmium, and lead liquid amalgams when in contact with dilute sulfuric, hydrochloric, or perchloric acid solution containing iron.

A Wood's metal reductor is more conveniently employed than liquid amalgams, as the excess of fused reductor metal can be removed more readily by allowing it to solidify, and no special equipment is required.

The new reductor was found to give accurate results in the quantitative determination of iron in iron ore. The influence of titanium or vanadium was offset by using air oxidation to remove titanous and vanadous ions.

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Determination of Gold and Platinum and Detection of Platinum Metals

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AMONG the numerous organic chemicals that have been employed successfully for the quantitative precipitation of palladium, dimethylglyoxime has probably been the most widely used. A single precipitation of palladium with dimethylglyoxime made in the presence of platinum is usually found to be contaminated with platinum. This may be partly due to occlusion by the flocculent yellow precipitate, but under certain conditions the high results are due in part at least to the formation of a platinum organic combination.

Cooper (*1*) discussed this contamination and described the material formed with platinum and dimethylglyoxime. He suggested its use for the quantitative precipitation of platinum, stating that the precipitate may be collected and dried in a Gooch crucible for weighing as $Pt{\cdot}C_8H_{14}N_4O_4$, but gave no data either to prove the elementary composition of the precipitate or to indicate that precipitation is complete. The crystals form readily from hot dilute acid solution, are easily filtered and washed, and it seemed worth while to determine their platinum factor and whether or not this method could be used efficiently for quantitative analysis.

A slightly acid solution of platinum chloride and dimethylglyoxime was heated on the steam bath for about 10 hours, then filtered and washed with water until no foreign substance appeared on microscopical examination. The material was then dried in an oven at 140° C. Cooper (*1*) stated that the crystals decompose somewhat violently on direct ignition. The authors attempted to burn the material by slow ignition, but the combustion took place suddenly with the emission of much carbon, and the results were extremely low, indicating loss of platinum. According to the procedure finally adopted, the weighed sample of crystals was fumed with sulfuric acid and fuming nitric acid to remove organic matter. Most of the platinum settled out after this treatment but a small amount was dissolved. The solution was neutralized to pH_6 and the platinum precipitated with sodium formate. The metal was filtered by means of a 3-cc. filtering crucible and washed with 1 per cent ammonium chloride solution. A blank was determined and weighed 0.029 mg. The results obtained are given in Table I.

TABLE I. PLATINUM IN CRYSTALS FORMED WITH PLATINIC CHLORIDE AND DIMETHYLGLYOXIME

Sample Used	Platinum Obtained	
Mg.	*Mg.*	%
51.02	23.37	45.81
58.15	26.60	45.74
49.09	22.47	45.77
46.15	21.16	45.85

The calculated percentage of platinum in $Pt{\cdot}C_8H_{14}N_4O_4$ is 45.88.

Precipitation of Platinum by Dimethylglyoxime

A solution of platinum was made up from the spectrographically pure metal and by analysis with formic acid was found to contain 33.00 mg. of platinum per 25.00 cc. Then 25.00-cc. portions of this solution were made up to 50 cc. with water, and 0.01 cc. of hydrochloric acid and 200 mg. of dimethylglyoxime in alcoholic solution were added. The liquid was heated on a steam

bath for 5 hours, filtered cold through 7-cm. No. 42 Whatman filter paper, and the precipitate was washed with only 5 cc. of cold water.

Two series of tests were made on the filtrates. In one the total filtrate was evaporated, the organic matter removed with sulfuric and nitric acids, and the platinum determined with sodium formate. The results obtained were 0.506, 0.596, and 0.504 mg. of platinum. In the second series the total filtrate of 55 cc. was evaporated to about 5 cc. and a second filtration made. The platinum was precipitated from this filtrate and weighed 0.125 and 0.132 mg.

Precipitations made from 1.2 *N* acid solutions indicate that excess of acid interferes to a still greater degree with the precipitation. Cooper (*1*) suggested the use of sodium formate to reduce the platinum to a bivalent condition; the authors did not find that the use of sodium formate increased the completeness or the rapidity of the precipitation. Although the results obtained indicate that, under the conditions stated above, the precipitation of platinum by dimethylglyoxime is not complete, nevertheless on many occasions the authors have found this method of very considerable value for the separation of platinum from rhodium, iridium, and base metals such as iron, copper, etc.

Precipitation of Gold by Dimethylglyoxime

It has generally been accepted that the presence of gold interferes with the precipitation of palladium by dimethylglyoxime. Wunder and Thuringer (*7*) stated that this reagent precipitates gold completely, but gave no facts to substantiate this statement and described no procedure.

A solution of spectrographically pure gold was analyzed by means of hydroquinone. Four samples were measured containing, respectively, 24.92, 24.92, 10.01, and 10.01 mg. of gold. The solution was made up to 50 cc. and acidified with about 0.5 cc. of concentrated hydrochloric acid, 100 mg. of dimethylglyoxime in alcoholic solution (1 per cent) were added, and the yellow precipitate was allowed to form. The mixture was then boiled for 30 minutes and the precipitated gold filtered by means of a filtering crucible and washed with water. Finally the residue was burned and weighed as gold. The results obtained were 24.90, 24.87, 9.99, and 9.99 mg. of gold.

Detection of Platinum Metals

While developing procedures for the analysis of platinum metals and gold, the authors were constantly faced with the necessity of determining the presence or absence of each of the above metals, silver, and certain base metals when many or all were present together. A very large number of reasonably specific tests for palladium and gold have been recorded in the scientific literature but there are very few tests of any kind for rhodium and iridium. Many chemicals will give tests for platinum, but most of these are by no means specific. Whitmore and Schneider (*6*) have developed a considerable variety of crystallographic tests for each of the platinum metals, but in many cases it is difficult to produce crystals from 0.1 or 0.2 cc., which contain as little as 0.01 mg. of the metal ion. The tests listed below have been thoroughly investigated and are recommended as fairly reliable in experienced hands.

Platinum. Stannous chloride, 40 per cent by weight in hydrochloric acid of specific gravity 1.19, gives an orange or yellow color with solutions containing platinum, a faint yellow color being obtained if the solution contains 0.01 mg. of platinum per cc. Palladium and gold produce interfering colors and must first be removed. Silver, of course, will yield the chloride.

A test for platinum, by means of thallium nitrate and stannous chloride, has been described by Tananaev and Dolgov (*5*). With spot paper 0.1 mg. of platinum per cc. of solution can be detected with assurance. Although this test is not very sensitive, small quantities of gold, palladium, iridium, and rhodium do not interfere.

If a drop of 10 per cent potassium iodide solution is added to a hydrochloric acid solution of platinum, a red-brown color is produced. A definite test can be secured with 0.002 mg. of platinum per cc., but this test is not specific, as traces of palladium, gold, and many base metals produce interfering colors. Also, because it is a strong reducing reagent, a large number of oxidizing materials will produce the iodine color. If the test is used, blanks must be very carefully made.

The most reliable, although not very sensitive, test for platinum is the formation of crystals with dimethylglyoxime. This is best made with very low acid concentration and the solution must be taken to a volume of about 0.2 cc. The crystals appear either bronze or blue, apparently depending upon the conditions under which they are produced. Under the microscope, they are prismatic and distinctly pleochroic. In reflected light the crystals are yellow to bronze when the vibration direction of the light is at right angles to the long axis of the crystal, and dark blue to bluish purple when the vibration direction of the light is parallel to the long axis. Its extinction is parallel.

Palladium. Feigl (*2*) described a sensitive test for palladium using dimethylaminobenzilidene rhodanine, a definite color being obtained with 0.0001 mg. of palladium per cc. The other platinum metals do not interfere, but both gold and silver give much the same color as palladium, and strong mineral acid solutions also interfere with the test.

Feigl (*2*) described a test for palladium with mercuric cyanide and stannous chloride which will detect 0.05 mg. of palladium per cc. Gold and platinum do not interfere, although the sensitivity is reduced if much platinum is present.

The stannous chloride reagent described above gives a brownish color, and 0.05 mg. per cc. can be detected. If platinum or gold is present it must first be removed, and in such cases the authors use dimethylglyoxime to remove the platinum. Potassium iodide solution will detect 0.005 mg. of palladium per cc. of slightly acid solution. The limitations of this test have been described.

Iridium. The well-known test with sulfuric acid and fuming nitric acid was found the most satisfactory. The blue color can be detected definitely in 0.4 cc. of sulfuric acid containing 0.1 mg. of iridium. A slightly greenish color appears with 0.05 mg. of iridium in 0.4 cc. of solution. The other platinum metals, gold, and silver do not produce this color. Because of the amber-colored solution, large amounts of palladium or rhodium will interfere.

Rhodium. In Ivanov's (*3*) test for rhodium, stannous chloride in hydrochloric acid is added to the small volume of the solution to be tested and the liquid is heated. A yellow to orange color will appear with 0.006 mg. of rhodium per cc., and on standing this solution will eventually become somewhat red. Palladium and platinum will interfere because they produce yellow to brown colors in the cold. With a little experience it is possible to detect very small amounts of rhodium in the presence of traces of both palladium and platinum, but it is necessary to make blank tests by adding traces of these metals to dilute solutions of rhodium in order to become familiar with the various shades of color produced. The authors have used this test under these conditions and secured dependable results.

Osmium. The well-known thiourea test for osmium, referred to by Singleton (*4*), is perhaps the most satisfactory. The osmium must be in the form of osmate and 0.01 mg. of osmium per cc. of solution will produce a definite pink color. None of the other platinum metals gives this rose color. Palladium with thiourea turns yellow and ruthenium produces a blue color on heating the solution.

The authors have found that an aqueous solution of pyrogallol produces a blue color with a solution of sodium osmate. If a drop of hydrochloric acid is added to the test solution followed by a drop of concentrated pyrogallol solution, a definite color is obtained if the test solution contains 0.01 mg. of osmium per cc. Rhodium and iridium do not interfere but palladium and platinum give a brown, and gold gives a purple color.

If a drop of a saturated aqueous solution of ephedrine hydrochloride is added to a caustic solution of sodium osmate, an orange color is obtained. The sensitivity of the test is increased by shaking the colored solution with carbon tetrachloride, giving an amber-yellow color. If this liquid is allowed to stand, 0.01 mg. of osmium per cc. will produce a faint color. Platinum and rhodium produce no color reaction. Palladium and gold give a very faint yellow color, but the test is not sensitive for these metals nor for iridium, which gives a very faint green under the same conditions.

Singleton (*4*) records a test for osmium with thiocarbanilide which gives a distinct color if the test solution contains 0.1 mg. of osmium per cc. If the solution is shaken with ether, the ether

layer takes on a deeper red color. By this means it is possible to detect 0.01 mg. of osmium per cc. of solution.

Summary

The percentage of platinum in the crystals formed with solutions of dimethylglyoxime and platinic chloride has been determined.

Gold, but not platinum, can be quantitatively determined by means of dimethylglyoxime.

Solutions of pyrogallol and ephedrine hydrochloride can be used as qualitative tests for osmium.

The efficiency and adaptability of various reagents for the detection of the platinum metals and gold are discussed.

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Determination of Nitrogen and Carbon in the Same Sample

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A METHOD for determining carbon and nitrogen in the same sample, which is in brief the conversion of the Kjeldahl (*5*) method for nitrogen into a wet method for carbon at the same time, has been used in this laboratory for several months. After the apparatus is set up, the only additional work required to make the carbon determination is the weighing of a carbon dioxide absorption bulb. Because the method is applicable to solids, liquids, and liquids containing dissolved gases, it may prove of value to other workers.

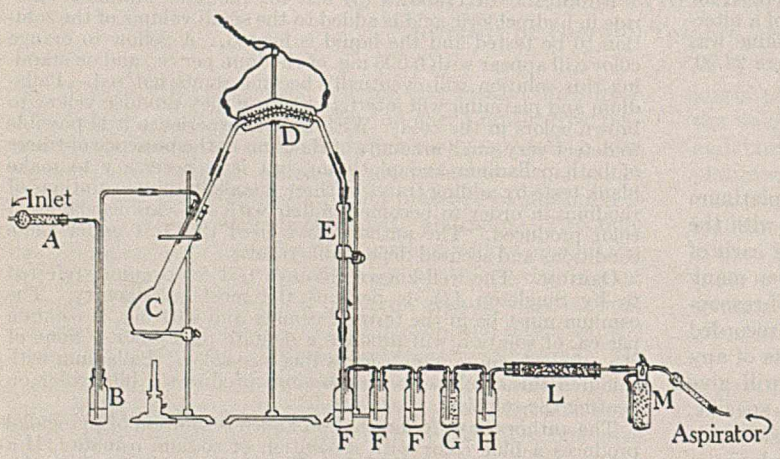

FIGURE 1. DIAGRAM OF APPARATUS

As far back as 1880 Warrington and Peake (*8*) showed that the wet chromic-sulfuric oxidation method gave lower results than combustion methods. Believing the losses to be due to incomplete combustion, Gortner (*3*) introduced a small furnace into the train to complete the combustion. Nicloux (*6*) seems to have accomplished the same thing by the use of silver chromate as a catalyst in the acid digestion mixture. This method has had extended use. Gailhat (*2*) used hydrated manganese dioxide in place of chromic acid, and made a nitrogen determination on the residue. However, the method of determining the nitrogen is rather involved, compared to simple distilling over as ammonia. The author believes that the combined method for nitrogen and carbon, given here, has some advantages over previous methods.

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Apparatus

The gas apparatus consists of a tube, *A*, Figure 1, filled with carbon dioxide absorbent to purify the oxygen, which is then passed through a 60-ml. (2-ounce) wash bottle, *B*, containing a little water. This serves as a trap to prevent any oxygen once entering the Kjeldahl flask, *C*, from getting back to tube *A*, in case of rapid expansion or evolution of gases. The wash bottle is fitted with a high inlet tube, so that any back pressure in the train may be noted by the water rising in this tube.

The acid digestion is carried out in the 300-ml. Kjeldahl flask, *C*, and the reagent used is 10 cc. of concentrated sulfuric acid with the addition of 0.1 to 0.2 gram of copper sulfate (*4*). The gases from the digestion flask are drawn through *D*, essentially a Pyrex tube containing platinum gauze, and placed in a small elbow-shaped electric furnace for heating. The combustion of the gases from the oxidation of the sample in the acid digestion is here completed.

The small condenser, *E*, cools the gases coming from the furnace and condenses steam from liquid samples such as urine, digested in *C*. The first 60-ml. (2-ounce) wash bottles, *F*, *F*, *F*, contain a solution of 6 per cent potassium dichromate and 10 per cent sulfuric acid, and absorb all the sulfur dioxide and trioxide fumes from the digestion flask, as well as all but traces of other acid fumes from the salts in the sample. These are followed by *G*, containing granular zinc to absorb any traces of volatile acids, as the halogen acids, that may be aspirated this far.

From *G* the oxygen, now containing nothing but moisture and carbon dioxide, is passed through *H*, containing concentrated sulfuric acid, and *L*, containing Dehydrite. Finally the dried gases are drawn through the absorption bulb, *M*, where the carbon dioxide is absorbed.

ELBOW ELECTRIC FURNACE. The housing of the furnace is made of a piece of 5-cm. (2-inch) copper tubing 15 cm. (6 inches) long. The tubing is notched in the center and one half is bent down 30° on the other. The tube is then cut lengthwise into two symmetrical halves, which are hinged together at one end, and a latch is provided to keep the two sections closed together. A device for clamping to a stand is riveted to the side-center of one section.

The furnace tube is made of 12-mm. outside diameter Pyrex tubing 37.5 cm. (15 inches) long, bent to an angle of 30° in the center; two other 30° bends are made each 7.5 cm. (3 inches) from the center. The cross section shown in Figure 2 indicates the manner of assembly. The center of the Pyrex tube is filled with platinum gauze, 52-mesh, 0.1-mm. (0.004-inch) wire. A piece 1 cm. wide and 15 cm. long, bent about each 1 cm. to form a wavy strip, is sufficient.

A wet strip of asbestos paper about 3 cm. wide is wound

spirally on the Pyrex tube for about 9 cm. on each side of the center, and dried. For a 15-volt supply, a piece of No. 20 Nichrome wire 183 cm. long is wound over this asbestos paper, turns being about 4 mm. apart. The winding is covered with wet strips of asbestos paper until this core of the furnace fits the housing. It is then placed in the housing, the protruding ends are pressed into neat and compact shape, and the assembly is dried.

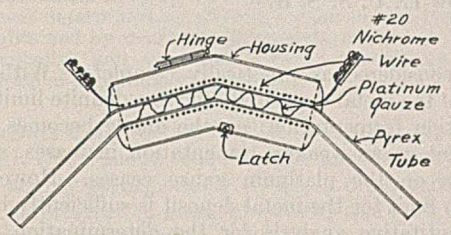

FIGURE 2. FURNACE TUBE ASSEMBLY

As shown in Figure 1, this furnace is mounted so that its center is also the highest point in the carbon train. Any water that may condense in the cooler parts of the tube runs away from the hot part, preventing damage.

Operation

After the furnace tube has been brought to a dull red heat, visible at the ends of the furnace only in very subdued light, and the weighed carbon dioxide absorption bulb has been placed, the Kjeldahl flask is charged and properly connected. A sample that will give a weight of 100 to 300 mg. of carbon dioxide is placed in the flask, and 0.1 to 0.2 gram of copper sulfate is added, followed by 10 cc. of concentrated sulfuric acid. When a material is readily decomposed, it is better to pour the 10 cc. of concentrated sulfuric acid into 3 or 4 cc. of water, cool, and then add to the flask. Danger of loss of gases before the flask can be connected into the train is thus reduced to a minimum.

Oxygen is drawn through the train at the rate of 5 to 8 bubbles per second, and the heating of the Kjeldahl flask is begun gently to avoid excessive back pressure. Heat is gradually increased to boil off water, which collects mostly in the first bottle, *F*. It is necessary to put new reagent in this bottle each fourth or fifth determination.

The sample is digested for some time after the acid digestion bath has become colorless except for the color made by the copper sulfate, the flame is removed, the flow of oxygen is stopped, and the absorption bulb is weighed. The nitrogen determination is completed by the usual distillation.

Discussion

Copper oxide, wire form, has been used in the furnace tube, held in place by plugs of platinum gauze, and occupying only the hottest part of the tube. The furnace must then be operated above the decomposition point of copper sulfate, and at this temperature the life of the furnace tube is short.

A convenient carbon dioxide solid absorbent (7) may be made by heating together in an iron pan over a gas stove 35 grams of sodium hydroxide, 5 grams of hydrated lime, and 15 grams of commercial asbestos fiber. The mixture is stirred to granulate and cooled, the lumps are crushed, and the fine is sifted out on a screen, 16 or 20 meshes to the inch, moistened by dropping 3 cc. of water evenly over the granules, and stored in a tight bottle.

The small error in the method, caused by the slight solubility of carbon dioxide in the wash bottle solutions, is reduced to practically nothing after the solutions become saturated by the first passage of carbon dioxide. A little carbon dioxide evolved in the Kjeldahl flask from a carbonate and acid, and drawn through the train, will bring the solutions to the equilibrium point.

Irregularities in the gas flow caused by condensed vapor flowing back to the hot acid digestion mixture are not serious if the inlet tube does not extend too far into the Kjeldahl flask, and if the flask is inclined.

While scarcely a trace of hydrochloric acid, from the reaction of sulfuric acid with chlorides in the sample, or of chlorine has been found to pass the potassium dichromate wash bottles, the granular zinc is used at this point as an extra precaution. A silver sulfate solution has also been used in this bottle for the absorption of any halogen acids.

To prevent bumping, both in the acid digestion and in the distillation of the nitrogen, the end of a spatula of pure calcium hydroxide is added to the Kjeldahl flask after its contents have been diluted and neutralized, ready for distillation. Any sodium carbonate present is precipitated, and the precipitated carbonate and the undissolved calcium hydroxide form myriad points for the dispersal of heat. A thin layer of calcium sulfate forms on the bottom of the flask, further preventing bumping. Some of this tight film is not removed on washing, and when the flask is again used in the carbon train the concentrated acid may be boiled without bumping.

This carbon train may be used to determine the volatile carbon compound in a mixture or in a solution containing both volatile and nonvolatile carbon compounds. This is accomplished by heating the mixture in the Kjeldahl flask, without addition of acid, to 103° C. in an oil bath, drawing the vapor through the elbow furnace, purifying, absorbing, and weighing.

TABLE I. CARBON DETERMINATION

	Theoretical %	Found %
Sugar solution	2	1.99 2.04 2.015 2.004
Urea	20	20.05 20.01 19.96 19.95
Uric acid	35.714	35.63 35.696 35.75 35.63 35.71 35.74 35.61 35.67
Urine sample	...	0.773, 0.782 0.689, 0.687 0.573, 0.572 0.807, 0.813 0.841, 0.823 0.771, 0.787

The method is reasonably accurate, as indicated by the check results on knowns and unknowns given in Table I.

Summary and Conclusions

A wet method is given for determining carbon in liquids or solids while a Kjeldahl nitrogen digestion is being made on the sample. The only additional work involved is that of weighing the carbon dioxide absorption bulb. The elbow furnace gives a means for distilling off much water from a sample without the loss of carbon from volatile compounds. This device makes it possible to determine carbon in a very dilute solution, whereas if the chromic-sulfuric-argentic oxidation mixture is diluted with such a sample oxidation may be incomplete (*1*).

The method is well adapted to the accurate determination of the C:N quotient in urine and in blood serum.

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Internal Electrolysis without Diaphragms

Determination of Copper and Bismuth in Lead-Zinc Ores

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THE electroanalytical determination of metals by depositing them inside a galvanic cell, using the electromotive force of the cell itself, was first proposed by Hollard as early as 1903 (*3*) for the determination of nickel in zinc salts. In 1919 it was advanced again by François (*2*) for the deposition of precious metals, but it was not adopted in the analytical laboratory until 1930, when the papers of Sand (*7*) and Collin (*1*) were published. All these authors used in their experiments rather complicated apparatus in which the anode and cathode spaces were separated by a semipermeable diaphragm.

In a series of experiments the present writers (*4*, *5*, *6*) showed that the diaphragm could be successfully dispensed with, and that the results obtained without it would be no less accurate than in apparatus provided with a diaphragm. The diaphragm increases the internal resistance of the cell and involves the use of a mixer, which greatly complicates the apparatus. The authors' apparatus, having no diaphragm, is very simple: It consists only of an ordinary platinum gauze for electrolysis, upon which is put a metallic plate, bent at right angles. To secure a better contact, the platinum gauze and the metallic plate are bound by copper wire. Such an apparatus can be afforded by any laboratory. Its internal resistance is very low, which makes possible (as will be shown in a later paper) deposition of metals with a very small potential difference.

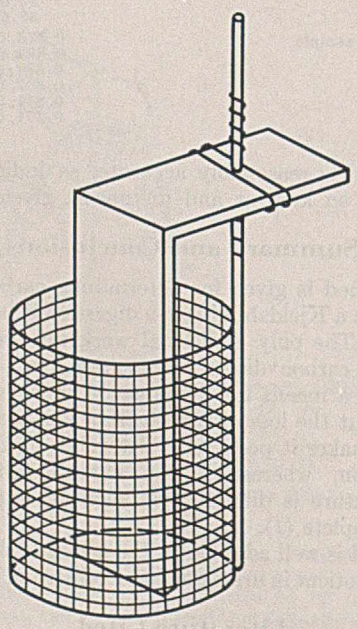

FIGURE 1

When using this apparatus, one should first consider the possibility of direct deposition of the metal to be determined on the metallic plate and not on the platinum gauze. The authors' experiments have shown that with a small quantity of metal deposited, the process takes place only in the desired direction; the metal is deposited on the anode (a metallic plate) in negligible amounts, and deposition on the platinum may be considered as practically complete. With a large amount of the metal deposited (above a definite limit), direct precipitation (cementation) on the anode becomes marked, the current grows weaker, cementation increases, and soon deposition on the platinum gauze ceases. However, the maximum limit for the metal deposit is sufficiently high, and in a quantitative analysis for the determination of small amounts of admixtures the diaphragm could well be dispensed with.

TABLE I. DETERMINATION OF COPPER

No.	Introduced: Copper	Salts	Acetic acid	Copper Found	Note
	Gram	*Gram*	*Ml.*	*Gram*	
1	0.0049	.....	5	0.0048	
2	0.0098	.....	5	0.0096	
3	0.0147	.....	5	0.0147	
4	0.0147	.....	5	0.0148	
5	0.0149	.....	10	0.0148	
6	0.0098	.....	10	0.0099	
7	0.0147	.....	10	0.0146	
8	0.0147	.....	10	0.0147	
9	0.0147	.....	10	0.0149	
10	0.0196	.....	10	0.0196	
11	0.0245	.....	10	0.0238	Cementation of copper on the anode was observed.
12	0.0294	.....	10	0.0270	
13	0.0147	NH_4NO_3, 5	10	0.0148	
14	0.0049	$Pb(C_2H_3O_2)_2$, 5	10	0.0048	
15	0.0049	$Pb(C_2H_3O_2)_2$, 2	10	0.0048	

The phenomenon of cementation on the anode is favored by too large a potential difference, as shown by Lurie and Troitzkaja (*6*); when copper was deposited with the aid of a nickel anode, cementation on the anode occurred, and when a lead anode was used the recovery of copper on the platinum gauze was complete.

In all experiments with the simple apparatus without diaphragm, it is most important to maintain a close contact between the electrodes and the copper wire. The places of contact must always be well cleaned with emery paper before proceeding to work.

Apparatus

The authors' apparatus is shown in Figure 1. The cathode is a Fischer's platinum gauze; the anode, a plate of refined lead. The length to the bend is 10 cm.; the length of the arm, 3 cm.; the width of the plate, 1.8 cm.; the thickness, 1.5 mm. In the arm a hole is made so near the bend that the plate when put on the gauze is just in the center. To secure a full contact the electrodes are firmly bound together with a copper wire.

Deposition of Copper and Bismuth

Copper and bismuth nitrate solutions precisely measured with a buret were placed in a 400-ml. beaker, the mixture was neutralized with ammonia and acidulated with concentrated acetic acid, 5 to 10 ml. in excess were added, and the solution was diluted with hot water to 200 ml. and heated to 85° to 90° C.

The electrodes above described, with the cathode previously weighed, were placed in the heated solution and left on an electric plate, maintaining this temperature for 30 to 40 minutes until the metal had completely deposited. Then the electrodes were removed by hand (rubber-gloved) from the electric bath and quickly placed for 5 minutes in a beaker containing 250 ml. of hot water slightly acidulated with acetic acid. The electrodes were then removed and disconnected, the cathode was washed with pure alcohol, and the electrodes were dried in an oven at 100° to 150° C. and weighed. The increase in the weight of the platinum gauze showed the sum of the weights of copper and bismuth.

For a determination of bismuth, the weighed cathode was placed in a 150-ml. beaker, 40 ml. of diluted nitric acid (1 to 4) were added, the contents of the beaker were heated until the metals had dissolved, and the cathode was removed and carefully washed with water over the beaker. To the solution obtained 0.2 gram of alum was added, and aluminum together with bismuth was precipitated with ammonia by the usual method.

The filtered and washed precipitate was dissolved in 20 ml. of hot 20 per cent sulfuric acid, and bismuth was determined by the usual colorimetric method with potassium iodide. The copper content was determined by difference (the sum of bismuth plus copper, obtained by weighing the cathode, minus bismuth, found colorimetrically) and colorimetrically in the filtrate after the precipitation of aluminum and bismuth.

The results of the determination of copper only are given in Table I, which shows that the determination may be made with an acidity corresponding to 2.5 to 5 per cent of acetic acid. The presence of ammonium salts and lead salts does not affect the determination.

Experiments 11 and 12 showed the phenomenon of cementation of copper on the lead plate mentioned above. It is evident that the copper content should not exceed 20 mg. to 200 ml. of solution. The lead plate must be cleaned with emery paper before each determination. Care should be taken that the contacts should be close at all places; with contacts insufficiently close, cementation on the anode will also take place.

The results of the determination of copper and bismuth in the mixture of their nitrates are given in Table II.

TABLE II. DETERMINATION OF COPPER AND BISMUTH[a]

No.	Introduced		Found		
	Copper	Bismuth	Copper plus bismuth	Bismuth	Copper by difference
	Gram	*Gram*	*Gram*	*Gram*	*Gram*
1	0.0050	0.0010	0.0062	0.0010	0.0052
2	0.0048	0.0010	0.0059	0.0009	0.0050
3	0.0050	0.0005	0.0056	0.0005	0.0051
4	0.0096	0.0005	0.0102	0.0004	0.0098
5	0.0096	0.0010	0.0107	0.0009	0.0098
6	0.0100	0.0010	0.0110	0.0009	0.0101
7	0.0100	0.0010	0.0110	0.0009	0.0101

[a] Conditions of experiments: volume, 200 ml.; $t°$, 80–90° C.; free acetic acid, 10 ml.; time of electrolysis, 35 min.

Because the lead-zinc ores contain considerable amounts of iron (10 to 30 per cent), the authors also had to determine the possibility of precipitating copper and bismuth in the presence of large amounts of iron.

The experiments were carried out under the same conditions but with addition of a ferric salt, and with an acidity of 10 ml. of 80 per cent acetic acid to 200 ml. of solution. With a lower acidity and the high temperature required (85° to 90°), a basic ferric acetate is precipitated. The first experiments showed that neither copper nor bismuth was precipitated under these conditions, in spite of the very low voltage of the current as compared to the usual electrolysis. To eliminate the oxidizing action of ferric iron, hydrazine sulfate was added to the solution heated to 80° (previous to the electrolysis) until the solution was discolored (a total of about 1 gram). The experimental results are given in Table III.

The introduction of hydrazine sulfate leads to the formation of a small amount of lead sulfate, mechanically contaminating the cathode during the electrolysis. To remove this deposit the authors used an additional washing. The bound electrodes removed from the electrolyte bath were submerged for 5 to 7 minutes in a 5 per cent sodium acetate solution heated to 85° C. and acidulated with acetic acid; then they were transferred to a beaker containing hot water acidulated with acetic acid and finally disconnected, the cathode being washed with strong alcohol.

In addition to iron, which has a dissolving effect upon the deposit of copper and bismuth, the elements having a more positive electrode potential than lead might interfere with

TABLE III. DETERMINATION IN PRESENCE OF IRON

No.	Introduced			Found	
	Copper	Bismuth	Iron	Copper	Bismuth
	Gram	*Gram*	*Gram*	*Gram*	*Gram*
1	....	0.0002	0.10	....	0.0002
2	....	0.0003	0.10	....	0.0002
3	....	0.0005	0.10	....	0.0004
4	....	0.0010	0.10	....	0.0009
5	....	0.0010	0.20	....	0.0010
6	....	0.0015	0.20	....	0.0014
7	0.0030	0.0005	0.10	0.0030	0.0005
8	0.0030	0.0005	0.10	0.0030	0.0005
9	0.0050	0.0005	0.10	0.0048	0.0005
10	0.0050	0.0005	0.10	0.0048	0.0005
11	0.0050	0.0010	0.10	0.0048	0.0010

TABLE IV. DETERMINATION IN PRESENCE OF ARSENIC AND ANTIMONY

No.	Introduced				Cu Found	Note
	Cu	Fe	As	Sb		
	Gram	*Gram*	*Gram*	*Gram*	*Gram*	
1	0.0129	0.10	0.0020	....	0.0129	The copper deposited on the cathode was of a pure pink color
2	0.0129	0.10	....	0.0010	0.0130	
3	0.0129	0.10	0.0020	0.0010	0.0129	
4	0.0129	0.10	0.0020	0.0010	0.0129	

determination of copper and bismuth. Lead-zinc ores may contain only three elements of this group: silver, antimony, and arsenic.

The data obtained by previous authors show with sufficient clearness that arsenic and antimony, if they are at the beginning present in their higher valency state, are not deposited by lead under conditions of internal electrolysis. The authors made a number of experiments to determine copper in the presence of arsenic and antimony under conditions specified above. The results are given in Table IV.

An admixture of silver may be removed by adding a small amount of chloride or iodide ion. A small amount of chloride ion does not interfere with the electrolysis and does not affect the platinum (*4*).

Determination of Copper and Bismuth in Ores

Copper and bismuth were determined in ores, using the procedure described below. In dealing with the decomposition of the ore two modifications are given, one for the sulfide, the other for the oxidized ores, as the latter are difficultly decomposed by nitric acid, and the insoluble residue contains both copper and bismuth. Those ores are readily decomposed by hydrochloric acid.

PROCEDURE. *Decomposition of Sulfide Ores.* A 1- to 2-gram sample (according to the copper content) of the ore or concentrate is placed in a 250-ml. Erlenmeyer flask and moistened with 3 to 5 ml. of water; 15 to 30 ml. of nitric acid (d = 1.4) are added, and the contents of the flask are boiled for 10 minutes, diluted with 15 to 20 ml. of hot water, and filtered through a loose filter (black ribbon), collecting the solution in a 400-ml. beaker. Without washing, the residue is transferred with a stream of water back to the flask, and the boiling is repeated with 10 ml. of nitric acid (1.4). Then it is diluted again with 15 ml. of water, filtered through the same filter, and the residue is washed 4 or 5 times with hot 3 per cent nitric acid.

Decomposition of Oxidized Ores. A 1- to 2-gram sample (depending on the copper content) of ore is placed in a 250-ml. Erlenmeyer flask and moistened with 3 to 5 ml. of water, 15 to 25 ml. of hydrochloric acid (d = 1.19) are added, and the mixture is boiled for 5 to 10 minutes and evaporated to dryness to remove the hydrochloric acid. To the residue 20 ml. of dilute nitric acid (1 to 1) are added, and the mixture is boiled. Then the contents of the flask are diluted with 15 to 20 ml. of hot water, boiled again, and filtered through a filter of medium thickness (white ribbon). If the insoluble residue is bulky, the nitric acid treatment is repeated.

The nitric acid solution obtained as described above is neutralized with ammonia until ferric hydroxide appears, the precipitate is dissolved in acetic acid, and an excess of 10 ml. of 80 per cent acetic acid is added. The solution is diluted to 200 ml.,

TABLE V. DETERMINATION OF BISMUTH AND COPPER

No.	Added to Ore Free from Copper and Bismuth: Copper	Added to Ore Free from Copper and Bismuth: Bismuth	Found: Copper + bismuth	Found: Bismuth	Found: Copper
	Gram	*Gram*	*Gram*	*Gram*	*Gram*
1	0.0050	0.0010	0.0062	0.0010	0.0052
2	0.0050	0.0010	0.0059	0.0010	0.0049
3	0.0100	0.0010	0.0110	0.0009	0.0101
4	0.0100	0.0010	0.0110	0.0011	0.0099
5	0.0150	0.0010	0.0160	0.0009	0.0151
6	0.0200	0.0010	0.0206	0.0010	0.0196

TABLE VI. DETERMINATION OF COPPER AND BISMUTH

Ore	Sample	Found by Usual Method: Cu	Found by Usual Method: Bi	Found by Internal Electrolysis: Cu	Found by Internal Electrolysis: Bi
	Grams	%	%	%	%
Oxidized ore	1.0000	0.52	0.027	0.55	0.025
	2.0000	0.52	0.027	0.55	0.025
	2.0000	0.52	0.027	0.55	0.025
	2.0000	0.52	0.027	0.54	0.025
Sulfide ore	1.0000	1.30	0.007	1.30	0.008
	1.0000	1.30	0.007	1.30	0.007
	2.0000	1.30	0.007	1.30	0.007
	2.0000	1.30	0.007	1.29	0.006
Zinc concentrate	1.0000	2.11	None detected	2.12	None detected
	1.0000	2.11	None detected	2.12	None detected
	1.0000	1.55	None detected	1.54	None detected
	1.0000	1.55	None detected	1.56	None detected

heated on a plate to 85° C., and after removing from the plate hydrazine sulfate is added in portions until the solution is discolored (a total of about 1 gram).

The electrodes, bound as shown in Figure 1, with the cathode previously weighed, are placed in the solution and left on a plate for 30 to 40 minutes, the temperature being maintained at 85° to 90° (overheating should be avoided). Then the electrodes are removed by hand (rubber-gloved) and transferred to a 150-ml. beaker containing 100 ml. of a 5 per cent sodium acetate solution, acidulated with 3 ml. of concentrated acetic acid, and heated to 80° C. In 5 to 10 minutes the electrodes are removed in a similar manner and transferred to a beaker with hot distilled water acidulated with acetic acid. In 5 minutes the electrodes are removed and disconnected, the cathode is washed with alcohol, dried in an oven at 100° to 105°, and cooled, and the sum of copper and bismuth is found by weighing.

The weighed deposit is dissolved in 40 ml. of nitric acid (1 to 4) in a small beaker (120 to 200 ml.) with gentle heating. After dissolution, the cathode is washed over the beaker, 0.2 gram of alum, ammonia to a feeble odor, and 0.1 to 2.0 grams of ammonium carbonate are added, and the solution is heated to boiling.

When the precipitate has coagulated, it is filtered through a filter of medium density (white ribbon) and washed as usual with a hot 2 per cent ammonium chloride solution, containing some drops of ammonia. With a high copper content the precipitate is reprecipitated. The washed precipitate is dissolved in 20 ml. of hot 20 per cent sulfuric acid and bismuth is determined colorimetrically after an addition of 1.5 to 2 grams of potassium iodide and 1 ml. of a 5 per cent sodium bisulfite solution, or better, one drop of a stannous chloride solution.

The results of analyses made by this method are given in Tables V and VI.

Conclusions

The deposition of copper and bismuth by internal electrolysis may be carried out with great accuracy, using a simple apparatus without diaphragm and mixer.

The interfering influence of ferric iron may be eliminated by the addition of hydrazine.

If the copper content of the solution does not exceed 20 mg. to 200 ml. of solution, the lead plate has a smooth surface and the contacts at the places of junction of the metals are complete, the cementation of copper and bismuth on the lead plate is practically equal to zero.

A simple, accurate, and relatively rapid method for the determination of copper and bismuth in lead-zinc ores has been developed, which has the following advantages over the usual method for copper determination by external electrolysis: no preliminary deposition of copper (by thiosulfate, hydrogen sulfide, thiocyanate, etc.) is required; the method is accessible for laboratories having no source of direct current (field laboratories); and less than 1 mg. of copper, which may be determined by external electrolysis only with difficulty, is easily deposited by the internal hydrolysis method, covering the cathode with an exceedingly even and beautiful coating. Bismuth may be determined by internal electrolysis with a considerable saving of time, eliminating the numerous and tedious separations.

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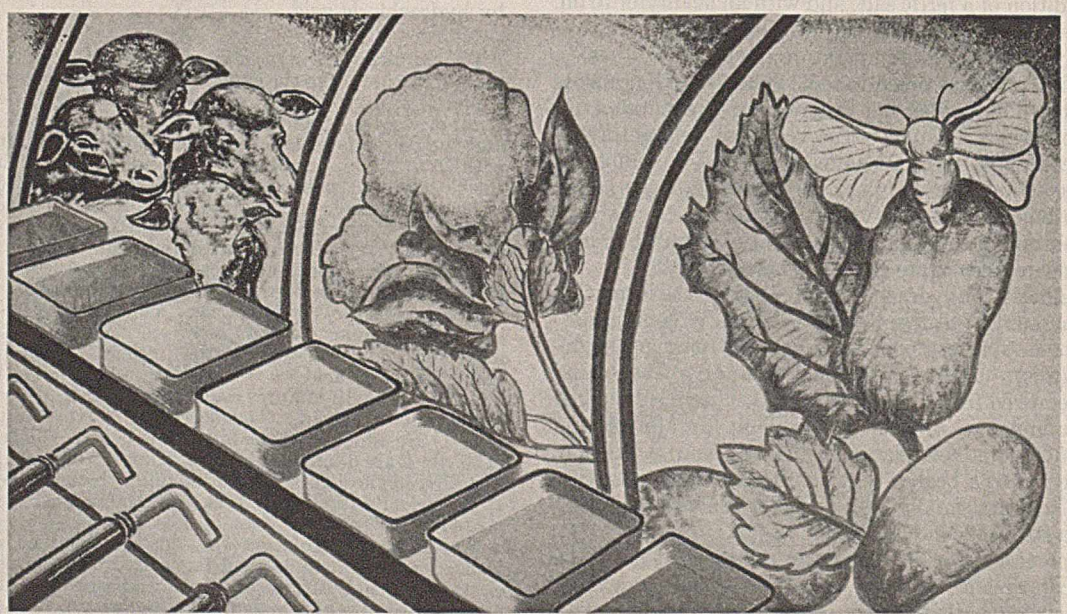

Biological Assay of Vitamin E

Application to Wheat Germ and Wheat Germ Oil

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EVANS and Burr (*1*) have adequately described the general procedure which must be followed in order to demonstrate vitamin E. Birth of young following administration of a known quantity of test substance to female rats of proved sterility because of vitamin E deficiency is the basis of Evans' (*3*) rat unit of vitamin E.

The smallest quantity of test substance which results in birth of young, when given in a single dose prior to the fifth day of gestation, is regarded as containing one rat unit. The α-tocopherol described by Evans, Emerson, and Emerson (*2*), which produced this effect in 2.5-mg. dose, thus contained 400 rat units of vitamin E per gram of substance. Olcott and Mattill (*8*) and Olcott (*7*) had previously prepared concentrates of vitamin E having essentially the same potency. Suggestions have been made to reduce the size of the unit (and thus increase the number of calculated units per gram of substance) by dividing the effective single dose by the number of days of gestation in rats. Evans and Burr (*1*) have presented a few data in support of this by showing that a single dose of wheat germ oil has the same effect as when aliquots one twenty-first as great are given daily throughout gestation. However, the quantitative relationships thus indicated cannot be regarded as very convincing when it is considered that the quantity of vitamin E needed daily must increase greatly with advance in gestation if the vitamin is required primarily for cell nuclear function of the fetal and placental tissues, as suggested by the work of Mason (*6*) on testicular degeneration in E deficiency. Furthermore, it is difficult to visualize the processes by which oral doses of vitamin-containing product consumed during the last few days of gestation are digested and metabolized and the vitamin is effectively used by the cells which need it, when the animal is assumed to be receiving the minimum dose. Indeed, it has not yet been determined what is the latest day in the gestation period when a potent vitamin E product can be given by mouth with assurance that the vitamin will exert a physiological effect in that gestation.

The conclusion seems justified that the single dose procedure or its essential equivalent is the only logical one to employ in a biological assay of vitamin E until more study has been given to questions raised in the foregoing discussion.

A survey of the published vitamin E assays based on litters produced, from which one may calculate the units of E present per gram of test substance, shows only a general quantitative relation between different doses of the same substance and the percentage of animals littering. Data regarding the number of young born (both living and dead) do not improve the quantitative character of the test. In view of the fact that vitamin E seems to function exclusively during gestation in connection with the nourishment of the fetus at the site of implantation, observations regarding the percentage of implantations which give rise to living young in a properly conducted assay suggested possibilities for improving the expected quantitative relations between dose and effect. This was one of the principal reasons for the present study. Although the outcome was disappointing, the results have a certain qualitative value and may assist other workers to evaluate their results properly.

At the outset it seemed desirable to bring together into one comprehensive statement the details of conducting a vitamin E assay. The published procedures lack standardization in certain particulars in which uniformity seems desirable. Certain details of procedure were adopted arbitrarily without supporting data to justify them, provided they seemed theoretically important and reasonable. The data secured were intended to determine primarily the "live implant efficiency" (L. I. E., percentage of placental implants which resulted in the birth of live young). The results, however, also made it possible to calculate other efficiency values—for example, a (T. I. E., "total implant efficiency") value involving both live and still-born young. In addition, littering efficiency values could be calculated, such as "live-litter efficiency" (L. L. E., percentage of pregnancies which result in litters containing living young) and "total litter efficiency" (T. L. E., percentage of pregnancies which result in live and still-born litters). The theoretical basis for recording individual still births and litters consisting exclusively of still-born young is the assumption that still-born young indicate some, although insufficient, vitamin E.

Procedure for Vitamin E Assay of Oils and Other Products

BASAL RATION. The following basal ration, which is a slight modification of the Evans (*3*) ration, is used throughout. Batches of the ration are kept in the refrigerator until used.

Ingredient	%
Commercial casein	27
Autoclaved tapioca (dextrin)	35
Commercial bulk lard	22
Dried brewer's yeast	10
Hawk and Oser (*4*) salt mixture	4
Cod liver oil, U. S. P., mixed in ration at weekly intervals	2

PRELIMINARY PERIOD. Young female rats exactly 21 days old are placed in cages having raised screen floors and given the basal ration *ad libitum*. A number of rats of approximately the same age are kept in the same cage. At about 90 to 100 days of age, daily observations are begun of the sexual cycle, using the vaginal smear technic of Long and Evans (*5*). As soon as an approaching heat stage is noticed by the presence of large numbers of nucleated epithelial cells, the animal is mated with a normal male in a separate cage having a raised screen floor. If successful copulation is determined to have occurred through the presence of sperm and/or plug in vagina or in tray of cage at the next daily examination, further examinations are omitted until the fifth and again on the tenth succeeding day. If no indication of sexual cycle is obtained it is assumed that the animal is pregnant. (Pseudo-pregnancies occur rather frequently. In this case the animal will sometimes be in heat again on the ninth to eleventh day but more frequently on the twelfth to fourteenth day. In these cases the animal must be carried through another resorption before being used for assay.) The animal is kept isolated throughout in a single cage having a raised screen floor.

Beginning the tenth day after impregnation the daily vaginal examination is resumed until the "erythrocyte sign" is secured, usually on the thirteenth to fifteenth day. (The "erythrocyte sign" is the term employed by Evans and Burr, *1*, to "designate the appearance in the vaginal canal on the thirteenth, fourteenth, and fifteenth days of gestation of recognizable numbers of red blood corpuscles.") If this is obtained, pregnancy is assured. Beginning the day the erythrocyte sign is obtained, the animal is weighed daily without further vaginal examination. If the weight increases and then decreases suddenly without evidence of any young on the twenty-first or twenty-second day and if manual examination at time of weighing also indicates the presence of fetuses which are never born or which disappear, or if weight remains essentially stationary (due to the presence of only one or two fetuses) without young being born, the conclusion is drawn that the animal is suffering from vitamin E deficiency. This

animal is now ready for use in a vitamin E assay. An animal which fails to show resorption in the first gestation may be carried through a second gestation or even a third, if necessary, to deplete it of body stores of vitamin E. After a third failure to show resorption the animal is discarded.

CONDUCTING THE ASSAY. Six female rats proved to be suffering from vitamin E deficiency which have been kept continuously on the basal ration are examined daily for sexual cycle and bred to normal males in separate cages at the next estrus. When impregnation is established as in the preliminary period, the animals are isolated and kept in separate cages. The vaginal examinations are omitted until the fifth and tenth days, after which they are resumed daily until the erythrocyte sign is obtained. Cages having raised screen floors are used throughout.

In the meantime the standard or experimental dose of material to be tested is administered to the animal in a single dose in a separate nonscattering feed cup between the first and fourth day of gestation, while withholding the basal ration. Oils are given, mixed carefully with sufficient dry yeast to make a friable paste, or mixed with a few grams of basal ration from which the fats have been omitted; other solid material to be tested is offered in weighed amount also in a separate nonscattering feed cup, the basal ration being withheld for that day.

When the erythrocyte sign is obtained, the test animals are weighed daily and the presence of fetuses is established as soon as possible by increase in live weight and by manual examination. Fetuses can usually be established by manual examination on the fourteenth day. Daily weighing is continued until parturition should occur. Animals which are about to cast a litter, as shown by manual examination and weight increase, are placed on a larger screen, 1-inch mesh, which permits the young to fall through to tray of cage. Resorptions can frequently be detected in advance by vaginal bleeding at some time subsequent to the erythrocyte sign and prior to the day of parturition. However, all rats in a test are carried through the twenty-second day of gestation, regardless of evidence of complete resorption of the fetuses. All animals are autopsied after parturition, or after the twenty-second day if no young are born, to determine the number of implantations and resorptions.

CRITERION OF VITAMIN E POTENCY. If five of the six animals become pregnant at the mating immediately preceding the administration of the test substance and four of these five animals to which the standard or experimental dose of test substance has been administered give birth to normal living young corresponding to 10 per cent or more of the implantations, the vitamin E potency of the test substance is established. Record is kept of number and weight of young born, number of live and still births, and total number of implantations.

If an assay is started with a rat which proves not to have been pregnant at the time the test material was administered the rat is continued in the assay if successfully bred at the next normal heat, but if this breeding fails the animal is discarded.

THE STANDARD DOSE OF TEST MATERIAL. This is the minimum amount of test material administered in a single dose which, when allowing for a margin of variation from natural causes, will produce normal living young corresponding to 10 per cent or more of the implantations in the uterus of a vitamin E–deficient female rat and thus permit the statement to be made that the product contains a measurable amount of vitamin E in such dose.

USE OF LESS THAN FIVE RATS PER TEST. The standard number of female rats to be placed on a single test for a standard assay is six. Conclusions may be drawn if five of the six animals are successfully bred at the mating immediately preceding the administration of the test substance. For exploratory research or in the tentative assay of an unknown at various doses it is permissible to use three rats per dose, provided they are all successfully bred. It is expected that at least two out of the three animals in such a test will give positive evidence of vitamin E potency in a potent test product.

PERIOD OF RETAINING VITAMIN E–DEFICIENT RATS BEFORE TEST. It is assumed that a vitamin E–deficient animal is suitable for test purposes any time within 3 months after its E deficiency has been established, provided it is kept on the basal ration.

QUALITY OF INGREDIENTS IN BASAL RATION. The only ingredient whose quality is of special importance is the cod liver oil, which must be U. S. P. grade. Each ingredient should represent a single lot of substance for any given series of tests, in order to avoid introduction of vitamin E into the basal ration of an animal after establishing its deficiency and its use for assay purposes.

DISCUSSION. In the author's experience with assays conducted according to this procedure, the chief cause of initial fertilities in female rats reared on the basal ration has been starting the rats on the ration when more than 21 days old. The most annoying difficulty has been a fairly high percentage (10 to 20 per cent) of pseudo-pregnancies after administration of test substance in spite of definitely successful matings with normal males.

TABLE I. REPRODUCTION EFFICIENCIES IN 54 VITAMIN E ASSAYS

Kind of Efficiency	Mean	Standard Deviation
Total litter efficiency[a]	85.59	21.37
Total implant efficiency[b]	71.96	23.35
Live litter efficiency[c]	70.98	26.28
Live implant efficiency[d]	55.59	27.08
Efficiencies Correlated	r	P. E. (r)
L. L. E.:T. I. E.	+0.7935	0.034
L. L. E.:L. I. E.	+0.6069	0.058
T. L. E.:T. I. E.	+0.5430	0.065
T. L. E.:L. I. E.	+0.3206	0.082

[a] Percentage of pregnancies resulting in live and still-born litters.
[b] Percentage of placental implantations resulting in live and still-born young.
[c] Percentage of pregnancies resulting in litters containing living young.
[d] Percentage of placental implantations resulting in live young.

Experimental Results

The mean values, their standard deviation, and the correlation coefficients between the different reproduction efficiencies previously indicated are shown in Table I for 54 vitamin E assays in which 217 rats gave evidence of the vitamin E potency of 39 different samples of material of unknown vitamin E value. (The products tested were furnished by General Mills, Inc., Research Laboratories, Minneapolis, Minn.)

The highest efficiency calculation is determined by counting both live and dead litters in relation to the number of pregnancies, the next highest when counting both live and still births in relation to placental implantations, the third

TABLE II. RELATIONSHIPS BETWEEN RELATIVE DOSES OF VITAMIN E AND RELATIVE EFFICIENCIES OF REPRODUCTION

Sample No.	Total No. Rats Used	Relative Dose	Relative T. I. E.[a]	Relative T. L. E.[b]	Relative L. I. E.[c]	Relative L. L. E.[d]
1	6	75	240	150	280	150
2	6	67	19	67[e]	19	67[e]
3	6	67	72[e]	33	58	33
4	6	67	108	67[e]	105	67[e]
5	6	73	82	100	89	100
6	4	50	59	100	41	100
7	6	50	108	100	83	33
8	20	67	82	64[e]	100	73[e]
9	22	67	58	142	44	150
	Mean	64.8	92	91.4	91	85.9

Values Correlated	r	P. E. (r)
Relative dose:relative T. I. E.	+0.455	0.178
Relative dose:relative L. L. E.	+0.406	0.188
Relative dose:relative L. I. E.	+0.321	0.202
Relative dose:relative T. L. E.	+0.084	0.223

[a] Percentage of placental implantations resulting in live and still-born young.
[b] Percentage of pregnancies resulting in live and still-born litters.
[c] Percentage of implantations resulting in live young.
[d] Percentage of pregnancies resulting in litters containing living young.
[e] Relative efficiency within 10% of the relative dose.

highest when counting litters containing living young in relation to pregnancies, and the lowest efficiency when counting only living young in relation to placental implantations. The method giving the highest values evidently gives the most liberal interpretation of vitamin E potency and the method giving the lowest values the strictest interpretation.

Although there seems to be little choice between the placental implant efficiency which considers both live and still births and the live litter efficiency, as judged by the mean values and standard deviations, the highest correlation coefficient is between the live litter efficiency and the live implant efficiency. From a biological standpoint the latter correlation seems reasonable even if the statistics do not, but biologically one would also expect a highly significant correlation between the total litter efficiency (live and dead litters) and the total implant efficiency. Instead, this correlation coefficient is barely significant from a statistical standpoint. The probable reason is that too few rats were used in the single tests, the result being that a high percentage of the total litter efficiency values (63 per cent) were 100. Only one-third of the living litter efficiency values were 100.

Although these results may give some basis for choosing the most desirable data to secure in a vitamin E assay, they give no information as to the validity of such data in relation to the quantity of vitamin administered.

TABLE III. COMPARATIVE VITAMIN E ASSAYS OF WHEAT GERM AND EXPELLER PROCESS: WHEAT GERM OIL

Sample	Dose	No. of Rats	Efficiency Values T. L. E.[a]	T. I. E.[b]	L. L. E.[c]	L. I. E.[d]
	Grams			%	%	%
Fresh raw germ I	5.60	3	100	100	100	100
Fresh oil from germ I	0.75	3	100	88	100	75
Fresh raw germ II	5.50	6	100	91	75	61
Fresh processed germ (Embo) from II	5.50	6	100	100	100	75
Fresh processed germ (Embo) III	5.50	3	100	89	100	82
Stored processed germ (Embo) IV	5.50	6	100	78	83	56

[a] Total litter efficiency.
[b] Placental implant efficiency (live and still-born).
[c] Live litter efficiency.
[d] Placental implant efficiency (live births).

TABLE IV. STABILITY OF VITAMIN E IN EXPELLER-PROCESS WHEAT GERM OIL UNDER STORAGE

Sample	Age	Storage Conditions	Dose	No. of Rats	Efficiency Values T. L. E.[a]	T. I. E.[b]	L. L. E.[c]	L. I. E.[d]
	Mos.		*Milligrams*		%	%	%	%
A	3	Sealed tin	750	3	100	88	100	75
A	6	Sealed tin	750	6	83	83	83	72
B	1	Sealed tin	750	6	83	65	67	47
B	8	Sealed tin	750	6	100	64	83	48
C	1	Sealed tin	500	3	67	17	67	17
C	12	Sealed tin	500	11	64	28	27	8
D_1	5	Sealed tin[e]	750	3	100	83	67	79
D_2	5	Sealed glass[f]	750	3	100	92	67	67

[a] Total litter efficiency.
[b] Placental implant efficiency (live and still-born).
[c] Live litter efficiency.
[d] Placental implant efficiency (live births).
[e] Kept at −20° F.
[f] Kept at 32° F.

Table II shows the results of the relation between each type of efficiency calculation and the dosage of vitamin obtained from a limited number of tests in which several products were administered at two different levels. The data shown are the relative levels and the corresponding relative efficiency values of each type, and the correlation coefficients. Possibly the samples are too few in number or an insufficient number of rats were employed in each test to warrant final conclusions; nevertheless the correlations are not significant. One is therefore forced to the conclusion that the assay of vitamin E thus conducted is at best merely a general quantitative assay and does not warrant very rigid interpretation in terms of units. Assuming that the relative efficiency should be within ±10 per cent of the relative dose, it is seen that this occurred in only seven of the 36 cases, and only two of these occurred even when the number of rats employed was 20 or more. A reference standard, if available, probably would not make the results now obtainable more quantitative, primarily because the assay of the standard would be subject to a large error. This may be too pessimistic a view.

The data obtained in this study gave information with reference to various vitamin E–containing products from wheat germ and may be assumed to have definite qualitative value, at least. Table III shows comparative vitamin E assays of raw wheat germ and of the oil expelled from it, raw wheat germ and a processed wheat germ (Embo) made from it, and fresh processed wheat germ and a sample of the same kind of product after standing for one year at room temperature. The samples in the latter comparison were taken for assay from a sealed evacuated tin in which the product is merchandised.

It is evident that the oil expelled from fresh raw wheat germ had definite vitamin E potency, that the type of processing employed in the case of the wheat germ samples tested did not injure the potency, and that vitamin E was retained in the processed germ to a considerable degree for a long period in a sealed evacuated tin kept at room temperature. The latter conclusion is based on comparisons with the other samples of processed germ tested, no assays having been made of the original product when fresh.

Table IV shows comparative vitamin E assays of several samples of wheat germ oil (expeller process) when fresh and after storage at refrigeration temperatures, in sealed tin or glass containers. It is evident that the vitamin E was retained in these samples for a period of at least 8 months in cold storage in sealed tin containers, and that no better preservation occurred in glass; only in sample C, stored for 12 months, was there evidence of some loss of vitamin E.

Conclusions

Biological assays of vitamin E, using oral administration to sterile, vitamin E–deficient rats in the earliest stages of pregnancy using a standardized procedure, have only a general quantitative value. However, results of such assays may be used for a comparison of potent vitamin E–containing products. When thus applied in this study to wheat germ and wheat germ oils it was found that there was (1) a close correlation between the vitamin E in the raw or processed wheat germ (Embo) tested and the pure oil expressed from the fresh germ; (2) a high retention of vitamin E in the processed (Embo) wheat germ kept for one year at room temperature in a sealed, evacuated can; and (3) a high stability of vitamin E in the expressed wheat germ oil for several months at refrigeration temperature in sealed containers, either glass or tin.

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A New Fluorescent Test for Aluminum

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FLUORESCENT tests have not found much application in general qualitative analysis because of the inconvenience and expense of the necessary source of ultraviolet radiation. Within the past year low-priced mercury vapor lamps have come on the market and the possibility of using argon bulbs for this purpose is also apparent. The work reported here is the first of a series of experiments begun with the thought of developing specific fluorescent tests which may be applied in routine analysis.

A review of the literature shows no method of detecting aluminum in the presence of beryllium. Aluminon (*4*), the most widely recommended reagent for the qualitative detection of aluminum, will respond to beryllium. Alizarin (red) S (*1*) forms a red lake with beryllium similar to that formed with aluminum. Morin, which gives a green fluorescence in the presence of aluminum, also fluoresces with beryllium (*3*).

Investigation of various organic materials, of structure similar to morin, as possible reagents for the detection of aluminum, revealed a specific fluorescent reaction between Pontachrome Blue Black R and aluminum ion.

Solutions

PONTACHROME BLUE BLACK R. The zinc salt of 4-sulfo-2,2′-dihydroxy azonaphthalene was obtained from du Pont laboratories. Titration with titanous chloride indicated that the dye contained 62.4 per cent of the zinc salt.

ALUMINUM SOLUTION. Baker's analyzed potassium aluminum sulfate was used as a source of aluminum.

BERYLLIUM SOLUTION. Beryllium nitrate of C. P. quality from the Brush Beryllium Company was used in making beryllium solutions. The absence of aluminum in this reagent was indicated when no precipitate was obtained with 8-hydroxyquinoline.

Apparatus

A Hanovia quartz mercury vapor lamp equipped with "Wood's glass" (*2*) as a filter served as a source of ultraviolet light. A number of the fluorescing solutions were also observed under ultraviolet light produced by a battery of three argon electric light bulbs, to determine whether such a source of ultraviolet radiation could be used for this purpose. Observations were made in a dark room. Containers were ordinary soft-glass test tubes. Quartz containers were found to have no advantage over soft glass.

Experimental

A series of solutions containing 10 ml. of aluminum solution and increasing amounts of the dye solution in intervals of 0.05 ml. was prepared and examined for fluorescence. A bright orange-red fluorescence was obtained in each case. As observed through a spectroscope, the color emitted corresponded to a red band lying between wave lengths 6975 Å. and 6365 Å. The intensity of the fluorescence appeared to increase until the solution containing 0.30 ml. of dye solution was reached. From this point to the solution containing 0.50 ml. of dye solution no difference in intensity could be distinguished.

Addition of 1 ml. of 1 *N* alkali to 10 ml. of a fluorescing solution destroyed the red color of the dye and also the fluorescence, the resulting mixture appearing a dull blue under the lamp. Nitric acid destroyed the dye. Addition of 1 ml. of normal sulfuric, hydrochloric, or phosphoric acid to 10 ml. of a fluorescing solution destroyed the fluorescence. One milliliter of 6 *N* acetic acid had no effect on the fluorescence, other than to change the color slightly to a lighter red.

A series of solutions having decreasing amounts of aluminum, each containing one-tenth as much aluminum as the preceding solution, was prepared. Solutions as dilute as one part per 100,000 showed a fluorescence immediately. After standing about half an hour fluorescence could be observed in all concentrations down to a solution of one part per million. The solutions were allowed to stand overnight before making a final observation for fluorescence. One part of aluminum in 5,000,000 parts of water could be detected in this way. Using argon bulbs as the source of ultraviolet radiation, one part of aluminum, in 100,000 parts of water was readily detected but smaller concentrations did not give positive results.

The following ions did not interfere with the detection of aluminum: silver, mercury, lead, bismuth, cadmium, arsenic, antimony, tin, zinc, manganese, gallium, indium, thallium, calcium, strontium, barium, magnesium, sodium, potassium, ammonium, lithium, rare earth, chloride, nitrate, sulfate, phosphate, and tartrate. The presence of the highly colored ions, copper, chromium, iron, nickel, and cobalt masks the fluorescence, but after precipitating with sodium hydroxide in excess and acidifying with acetic acid fluorescence is evident in each case. Hexavalent chromium interferes but may be removed by ordinary procedures of qualitative analysis. Fluorides interfere but may be removed satisfactorily by precipitating with calcium chloride.

Beryllium gives no fluorescence with the dye itself nor does it in any way interfere with aluminum fluorescing.

Analysis of the fluorescing material indicates that an aluminum salt of the dye is formed.

This analysis (Table I) corresponds to one atom of aluminum, one atom of zinc, and five dye radicals, the samples appearing to be mixtures of the zinc and aluminum salts of the dye. Although these data are not conclusive proof that an aluminum salt is formed, they are at least a fair indication.

TABLE I. ANALYSIS OF FLUORESCENT MATERIAL

	Aluminum %	Zinc %	Dye Radical %
Sample prepared from 16 grams of alum to 1 gram of dye	1.35	3.33	95.8
Sample prepared from 13 grams of alum to 1 gram of dye	1.36	3.42	95.5

Procedure

The solution should be made alkaline with sodium hydroxide, any precipitates forming being filtered off. Hexavalent chromium must be reduced and fluorides must be removed. The solution is then made acid with acetic acid and 0.5 ml. of a 0.1 per cent solution of Pontachrome Blue Black R is added. Where solutions as dilute as 1 part per million of aluminum are being tested, a half hour should be allowed for the fluorescence to develop.

Summary

The orange-red fluorescence produced under ultraviolet light by Pontachrome Blue Black R with aluminum ion is sensitive to one part in 5,000,000 parts of water, and can be used in the qualitative detection of aluminum in the presence of beryllium and other elements with which it is commonly

found. With argon bulbs, a cheap and convenient source of ultraviolet radiation, concentrations as low as one part of aluminum in 100,000 parts of water may readily be detected.

The intensity of the fluorescence appears to vary with the aluminum content within a limited range.

Analysis indicates that an aluminum salt of the dye is formed.

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Volumetric Determination of Selenium

A Critical Study of the Norris and Fay Method

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THE accuracy of the Norris and Fay method (*7*) for the determination of selenious acid has long been accepted on the basis of rather limited evidence (*1, 4, 6, 8, 11*). This method depends on the following reaction:

$$4Na_2S_2O_3 + H_2SeO_3 + 4HCl \longrightarrow Na_2SeS_4O_6 + Na_2S_4O_6 + 4NaCl + 3H_2O$$

An excess of a standard thiosulfate solution is titrated with a standard iodine solution, the titration being carried on near 0° C. presumably to prevent decomposition of the thiosulfate. It occurred to the authors, after the revision of the atomic weight of selenium from 79.2 to 78.96 (*3*), that a reëxamination of this method using pure selenium as a reference material was desirable. In the course of the investigation some simplification of technic was introduced and the conditions for the accurate application of the method were established.

Preparation of Materials

SELENIUM. A technical grade of selenium (supplied through the courtesy of the United States Metals Refining Co.) was dissolved in pure sodium cyanide and air was bubbled through the solution for several hours to precipitate any tellurium, which was then removed by filtration. The filtrate was poured into an excess of dilute hydrochloric acid to precipitate the selenium.

This purification procedure was repeated twice and the final product oxidized to selenious acid with concentrated nitric acid. Upon evaporation to dryness, the selenium dioxide was subjected to three sublimations and the pure white oxide thus obtained was dissolved in water. After making the solution 5 *N* with hydrochloric acid, the selenium was precipitated with hydroxylamine hydrochloride and thoroughly washed with water and then with alcohol and ether. It was dried, carefully melted, and poured into water to form small pellets.

SODIUM CYANIDE. A pure grade, free of ferricyanide and reducing agents, was used.

POTASSIUM BROMATE, POTASSIUM IODATE, POTASSIUM DICHROMATE. Baker's analyzed salts were twice recrystallized from water with centrifuging. These substances were used for standardizing the thiosulfate solution.

Experimental

The following procedure was employed for preparing the solution of the pure selenium for the Norris and Fay titration:

From 0.35 to 0.45 gram of the element was weighed into a 250-ml. flask, 2 ml. of 1.42 nitric acid were added, and the flask was covered and warmed carefully until solution was complete. After cooling, 5 ml. of 1.84 sulfuric acid were added, the solution was again cooled and 3 grams of urea were added to discharge the excess of nitric acid. (Bruckner, *2*, states that solutions of selenious, nitric, and sulfuric acids can be evaporated to fuming without loss of selenium. This suggested means of eliminating nitric acid before using the Norris and Fay titration. In the authors' experience, however, repeated experiments with known amounts of selenious acid invariably resulted in low values on subsequent titration.)

After the vigorous evolution of gases ceased the solution was digested for 15 minutes on the hot plate, diluted to above 75 ml., transferred to a volumetric flask, and diluted to exactly 250 ml. For each titration 50-ml. aliquots were used, diluting to make the final volume 150 ml. All titrations were made at 20° C. The excess thiosulfate solution was controlled by a method described below. The normality of the thiosulfate solution was checked independently by three primary standards: potassium bromate, potassium iodate, and potassium dichromate.

The results in Table I refer the Norris and Fay method directly to pure selenium and their average shows that the method can be depended on to an accuracy of less than 2 parts in 1000. It seemed advisable to support the above data by using another method to oxidize purified selenium to selenious acid. Standard potentials for the following couples are available (*5, 9*):

1. $Se + 3H_2O \longrightarrow H_2SeO_3 + 4H^+ + 4e \qquad E = -0.74$ (black)
2. $Br^- \longrightarrow {}^1/_2Br + e \qquad E = -1.064$

From these data it seems that the oxidation of selenium with bromine in 1 *N* hydrobromic acid should be quantitative. The formation of selenium monobromide made it impossible to oxidize the black modification smoothly, but this difficulty was avoided by getting the selenium into the red form and keeping it in a finely divided state. The equilibrium constant for the reaction

$$H_2SeO_3 + Br_2 + H_2O \longrightarrow HSeO_4^- + 2Br^- + 3H^+$$

is 0.88 (*10*). The velocity of the reaction for a 1 *N* hydrobromic acid solution for the oxidation of selenious to selenic acid should not be significant if the excess bromine is carefully controlled.

PROCEDURE. From 0.35 to 0.45 gram of selenium and 5 ml. of 10 per cent sodium cyanide solution were digested on a hot plate in a 250-ml. flask until the selenium completely dissolved. The solution of sodium selenocyanate in excess sodium cyanide was diluted to 250 ml. and 50-ml. aliquots were evaporated to about 20 ml. Then 2 ml. of a filtered 1 per cent gum arabic solution and 5 ml. of 5 *N* hydrobromic acid were added. This caused the decomposition of the selenocyanate with the liberation of red selenium which was kept colloidally dispersed by the protective agent.

A rapid stream of air was bubbled through the solution for 15 minutes, which removed hydrogen cyanide sufficiently so that the formation of cyanogen bromide was minimized.

Most of the selenium was oxidized by adding dropwise a slight excess of a saturated solution of potassium bromate. Then the

[1] Deceased.

TABLE I. DETERMINATION OF SELENIUM

0.09195 N $Na_2S_2O_3$		Selenium	
Corrected, after titration	Excess	Present	Found
Ml.	*Ml.*	*Gram*	*Gram*
42.06	3.09	0.07632	0.07642
39.23	0.79	0.07119	0.07128
40.30	0.55	0.07315	0.07322
39.88	0.65	0.07234	0.07246
47.12	0.56	0.08553	0.08561
35.16	0.36	0.06379	0.06388
46.03	0.73	0.08352	0.08362
43.73	0.61	0.07933	0.07945
41.89	0.62	0.07602	0.07611
41.84	0.43	0.07591	0.07602

TABLE II. DETERMINATION OF SELENIUM

0.09195 N $Na_2S_2O_3$		Selenium	
Corrected, after titration	Excess	Present	Found
Ml.	*Ml.*	*Gram*	*Gram*
38.44	0.18	0.06981	0.06984
38.09	0.17	0.06923	0.06921
41.14	0.41	0.07480	0.07475
37.04	0.16	0.06738	0.06730
46.36	0.62	0.08433	0.08424
46.61	0.74	0.07752	0.07742
39.57	0.50	0.07193	0.07189
44.66	0.57	0.08118	0.08114
40.65	0.37	0.07393	0.07385
38.18	0.50	0.06937	0.06934

oxidation was carefully continued with 0.1 N bromate until the discharge of the red selenium marked the completion of the reaction. A few drops of a saturated alcoholic solution of acetanilide were added to discharge the excess bromine. The flask was covered and heated just to boiling.

After diluting the sample to make the final volume 150 ml., starch was added and the solution was cooled to 20° C. and titrated.

The average of the results of a number of analyses, given in Table II, shows an accuracy for the Norris and Fay method of less than 1 part per thousand when pure selenium is analyzed. A small excess of thiosulfate solution was used, as shown in Tables I and II. It is evident that pure selenium can be used as a primary standard for thiosulfate solutions which are to be used for the determination of selenious acid.

The Norris and Fay method called for the use of an excess of sodium thiosulfate solution in an acid solution and a low temperature in order to suppress the decomposition of the thiosulfate. Only a limited amount of information is available on the effect of temperature and the amount of excess thiosulfate on these titrations. To study these effects, solutions of selenious acid were carefully standardized by the method of Lenher and Kao (*4*).

Measured volumes of selenious acid solution were pipetted into a 250-ml. Erlenmeyer flask. After adding starch solution and 5 ml. of 6 N hydrochloric acid, the solution was diluted to make the final volume 150 ml. In a series of twenty analyses the excess of thiosulfate was varied from a few tenths of a milliliter to 15 ml. and the titrations were made at 0° and 20° C. Results obtained indicate that so long as the excess of thiosulfate is no greater than 5 ml. of 0.1 N solution, an accuracy of 1 part in 1000 is obtainable regardless of whether the titration is carried out at 0° or 20° C. A greater excess should not be used, since unsatisfactory values are obtained both at 0° and 20° C. Many selenium determinations by the Norris and Fay method have been made in this laboratory at room temperature in an entirely satisfactory manner when a small excess of thiosulfate is used. It is unnecessary to cool the solutions to 0° C.

In any critical examination of this method, the effect of potassium iodide on the starch-iodide end point should be considered.

The procedure employed was the same as that previously described. Titrations were made at 20° C. and when potassium iodide was used the concentration was made 2 per cent of the final volume by adding a 25 per cent solution after the addition of an excess of thiosulfate. Thiosulfate solutions of three normalities were used. Six analyses were carried out with each normality, three with and three without the presence of potassium iodide.

When using 0.1 N solutions, results equally satisfactory are obtained in the presence or absence of potassium iodide. With 0.01 N solutions, potassium iodide should be employed and its use is imperative when the solutions are 0.001 N.

It is obvious that a method for limiting the excess of thiosulfate to 5 ml. or less is desirable. This can be done by adding potassium iodide solution (2.5 per cent) dropwise, 1 to 3 drops producing a local starch-iodide blue which fades on stirring.

$$SeO_2 + 4HI \longrightarrow Se + 2H_2O + 2I_2$$

Thiosulfate is added in 5-ml. portions until upon the addition of potassium iodide, the starch-iodide blue is no longer produced. Since the stoichiometrical relations are the same for the hydriodic acid reduction of selenious acid as for the thiosulfate, no correction is necessary for the addition of the iodide. When 0.01 and 0.001 N solutions of thiosulfate are employed, 25 per cent potassium iodide should be used for the indicator. This method for keeping the excess of thiosulfate to less than 5 ml. is rapid and reliable, and is important, since with the use of a larger excess the Norris and Fay titration is less accurate.

In the Norris and Fay procedure the titration is carried on in the presence of hydrochloric acid. The authors found that hydrobromic, sulfuric, and perchloric acids were equally satisfactory at both 0° and 20° C., with thiosulfate solutions 0.1 N, 0.01 N, and 0.001 N. The weak acids, acetic and phosphoric, were found to be unsatisfactory, as was nitric acid.

Summary

Using pure selenium as a primary standard, the accuracy of the Norris and Fay method was found to be between 1 and 2 parts per 1000.

This titration can be carried out at room temperature if a small excess of thiosulfate solution is used. A rapid method for controlling this excess is described. This modification results in a considerable saving of time in the use of this method. For accuracy only a small excess of thiosulfate solution should be used.

Hydrobromic, sulfuric, perchloric, and hydrochloric acids can be used in the Norris and Fay procedure.

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Improved Extractor for Monoamino Acids

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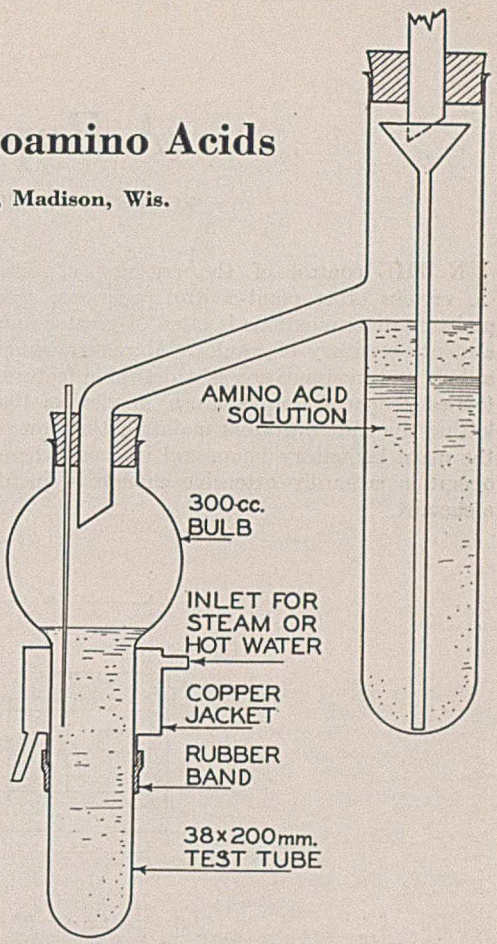
THE extraction of monoamino, monocarboxy amino acids from neutral aqueous solution with butyl alcohol (*1*) has been shown to be useful in the isolation of these compounds. One difficulty has been that because of the relatively high temperature required to boil the butyl alcohol, some of the amino acids are converted into their anhydrides. While this difficulty has been partly overcome by reducing the pressure in the extractor, the amino acids which precipitate in the boiling flask adhere to the walls and consequently are still subjected to rather elevated temperatures.

The apparatus shown in the diagram eliminates this difficulty, for as the amino acids crystallize in the modified boiling flask, they settle to the bottom of the tubular part where they remain at room temperature. Since the capillary extends only to the bottom of the heated zone, the lower layer of liquid is not stirred and the extract remains quiescent. Suction from a water pump is applied at the top of the condenser and is regulated by adjusting a screw clamp on a piece of rubber tubing attached to a side arm at the top of the condenser. The temperature of the boiling butanol is about 50° C. With this extractor no amino acid anhydrides could be detected in the extract. While this apparatus was developed primarily for the extraction of amino acids, it undoubtedly would be useful wherever it is necessary to extract heat-labile substances from solution with an immiscible solvent.



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Reaction of Alcohol-Gasoline Blends to the Doctor Test

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ONE of the common qualitative methods for detecting the presence of mercaptans in motor fuel is the doctor test. The chemistry of this test has been reviewed by Wendt and Driggs (*4*) who summarize the reactions as follows:

$$2RSH + Na_2PbO_2 \longrightarrow 2NaOH + Pb(SR)_2$$
$$Pb(SR)_2 + S \longrightarrow PbS + (RS)_2$$

That other reactions occur was recognized by Hallett and Sowers (*3*). Peroxides are known to affect the reaction (*2, 3*).

The commercial distribution of alcohol-gasoline blends led to reports that these fuels give a sour reaction in this test very similar to that obtained with mercaptans or peroxides. In view of these reports a series of tests was made on various substances which are commonly used as denaturants for ethyl alcohol, ethyl alcohol itself, and ethyl alcohol in blends with gasoline. The results of these tests are given in Table I. All blends of gasoline with 40 per cent or more ethyl alcohol show a sour reaction.

In view of these results it is recommended that the A. S. T. M. methods be used for the determination of sulfur in alcohol-gasoline blends. If a qualitative test for mercaptans is necessary, the presence of these interfering substances must be taken into account. The authors have not studied these reactions further, since the test is not quantitative and is not generally accepted (*1*).

TABLE I

Denaturants:	
Gasoline	Sweet
Benzene	Sweet
Acetone U. S. P.	Sour
n-Butanol C. P.	Sour
Mixed isonitriles	Sour
Wood alcohol	Sour
Pontol K	Sour
Ethyl alcohol C. P.	Sour
Alcohol-gasoline blends:	
Blend 1. Gasoline 95%, ethyl alcohol 5%	Sweet
Blend 2. Gasoline 90%, ethyl alcohol 10%	Sweet
Blend 3. Gasoline 80%, ethyl alcohol 20%	Sweet
Blend 4. Gasoline 70%, ethyl alcohol 30%	Sweet
Blend 5. Gasoline 60%, ethyl alcohol 40%	Sour

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A Rapid Moisture Tester

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IN THE control of the crushing of oilseeds and in various other plant-control programs, speed in making moisture determinations is often of greater importance than absolute accuracy of results. Moreover, simplicity of procedure and manipulation is a desirable feature of a test intended primarily for plant use, in view of the necessity of letting the shift operators maintain their own control after the usual laboratory hours and in cases where the scale of operation is hardly extensive enough to justify employing a chemist.

limited. Moreover, the accuracy obtained in both methods is not all that one might desire.

The tester described here follows the conventional drying oven principle and is a modification of an English-made device, the Carter-Simon tester, for which the writer has found only one domestic source of supply (The Grain Reporting Bureau, Chicago). The accuracy is about the same as obtained in the conventional oven. The present design possesses the following advantages over its prototype: (1) Twice as many samples may be put through in one run.

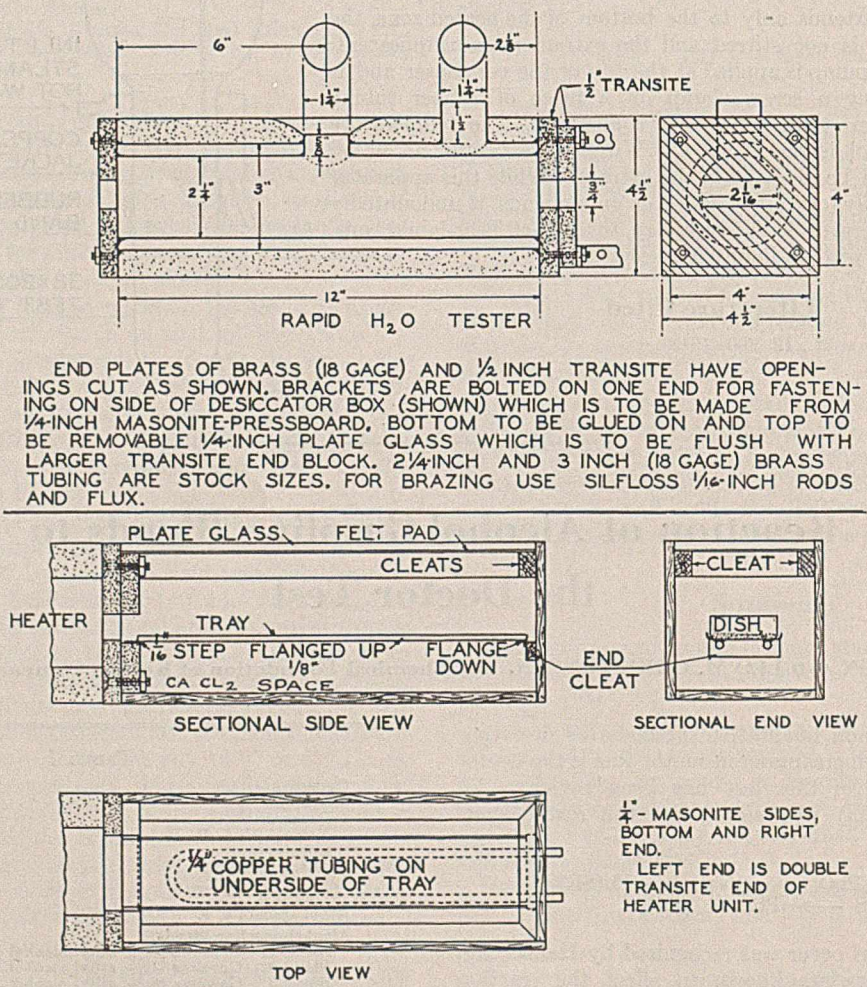

FIGURE 1

Most of the rapid moisture-testing equipment in common use suffers from one objection or another. The Tag-Heppenstall resistance meter is extremely useful in handling large numbers of grain samples with a minimum of delay and a maximum of ease, but the first cost is high and it is not adaptable to ground materials, nor to those of relatively low moisture content. The Brown-Duval tester is bulky and elaborate; it requires the handling of considerable oil, and the number of samples which can be run at one time is

(2) A built-on water-cooled desiccator saves about 15 minutes by reducing the cooling period. (3) When fitted with a driving mechanism, six samples may be run through automatically and left in the desiccated cooling chamber until the operator can make the final weighings.

The usual objection to the use of a high temperature for shortening the time of drying results from the loss, through volatilization or decomposition, of matter not water. This apparatus overcomes that objection, at least in part, by

ensuring the rapid passage of such a quantity of hot, dry air over the material that the moisture is removed before the material itself has time otherwise to lose weight. This action is accomplished by the simple and ancient means of providing an adequate stack to induce a strong draft. (See Figures 1 and 2.)

Apparatus

The heating space consists of a 5.7-cm. (2.25-inch) brass tube, 30.5 cm. (12 inches) long, brazed at each end to brass plates 11.4 cm. (4.5 inches) square, into which are cut openings just large enough to pass easily the official American Oil Chemist Society's aluminum moisture dishes (2 × 0.75 inches, 5 × 1.9 cm.). Concentric with the air chamber tube, and also brazed to the end plates, is a 7.6-cm. (3-inch) brass tube forming, with the inner tube, a jacket in which the heating liquid is boiled. Through the center on the top passes a 3.7-cm. (1.5-inch) brass nipple for connecting the glass or metal stack, which also serves as a thermometer well. Another short nipple communicating only with the jacket carries a reflux condenser. Running longitudinally through the air chamber is a shelf of brass on which the moisture dishes rest.

Heat is furnished by a 250-watt, 30.5-cm. (12-inch) space heater wired on the bottom. The outside is lagged with 1.27 cm. (0.5 inch) of asbestos-magnesia, except on the end plates, where 1.27-cm. (0.5-inch) transite pieces are secured with bolts. On the outlet end, however, a second smaller square of transite is fastened to serve as the end of the desiccator chamber, which is simply a box 11.4 × 11.4 × 30.5 cm. (4.5 × 4.5 × 12 inches) in outside dimensions, made of 0.63-cm. (0.25-inch) masonite (or transite) and held together by angle brackets bolted on the inside. Down the length of this is a brass strip, flanged slightly on either side to keep the dishes centered. A loop of 0.63-cm. (0.25-inch) copper tubing is soldered to the under side of this strip, through which water may be passed to cool the strip and its burden of hot moisture dishes, thus saving several minutes. Calcium chloride is kept in the box to a level almost flush with the strip. The cover is a removable glass plate resting on all sides on cleats faced with felt.

Table I. Temperature Variations

Time *Min.*	No. Tests in Oven	Temperature at Center °C.
Start	Empty	142
1	2	142
4	2	142
6	4	127
9	4	127
11	6	124
14	6	128
16	4	128
19	4	136
21	2	138
24	2	141

Table II. Check Results

Material	1.5 Hours at 110° C. in Freas Oven %	15 Min. at 135–140° C. in Freyer Tester %
Linseed meal	8.28	8.36
	11.13	11.19
	10.72	10.54
	10.88	10.86
Castor beans	5.0	5.0
Fuller's earth	9.01	9.16–9.20[a]
Meal	9.1	9.1
	8.3	8.3
	9.5	9.55

[a] Same material put through second time.

The heating liquid should be chosen so that when it boils, the air temperature at the base of the stack is 136° to 142° C., and does not fall below 120° C. when the oven is loaded. The writer uses a petroleum fraction boiling at 240° to 250° C. Table I shows how the temperature varied during a full run.

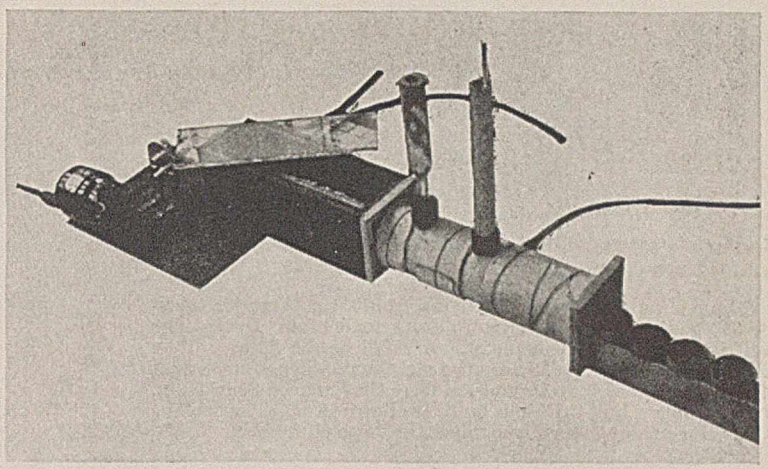

Figure 2

Operation

Two dishes containing the material being tested (5 grams each) are shoved into the open end, left for 5 minutes, and then moved into the center of the air space by shoving the next two tests in behind them. Five minutes later two more tests are introduced, each dish moving up two spaces. At the end of 15 minutes the first two will have been shoved into the desiccator; and at 5-minute intervals different pairs will pass out of the oven into the desiccator, each pair having been in the heater for 15 minutes; and each test will have occupied (relative to the ends and center) the same position as every other test, and will have been submitted to the same heating conditions.

In this respect the present method possesses an advantage over the conventional drying oven, in which, unless specially designed, temperature variation from one shelf to another may occur. The conditions chosen were such as to give the same results on soybean and linseed meals and cracked soybeans as are obtained in a Freas oven at 110° C., drying for 1.5 hours. How well the results check may be seen in Table II.

If desired, the tester may readily be equipped with a simple mechanism to draw the samples through the heater at the proper rate. The photograph shows a small motor with built-in double reduction gears driving a shaft, around which winds a piece of string drawing through a small sled-like strip of metal carrying the loaded moisture dishes. It is timed so that 30 minutes elapse during the passage of the first dish from the entrance of the tester to the end of the desiccator, or 61 cm. (24 inches). It would be very easy to rig a tripping device to shut the motor off when all dishes have passed through the heater. Ordinarily, an interval timer is used, and becomes almost a necessity if the motor drive is not employed, and if the operator's attention is required on other duties. An additional convenience is a small stick 45 cm. (18 inches) long and marked at 10-cm. (4-inch) intervals to use in shoving the pairs of tests through the oven. The marks serve to indicate the positions occupied by the tests when only a few are run.

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A Sensitive Glass Electrode of Durable Form

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THE fragility of the ordinary bulbular form of glass electrode is a matter of common experience. The electrode described here approaches the ideal combination of low resistance and rugged form. The steps in its construction are outlined in Figure 1.

A bulb of fairly heavy wall is blown from 1-cm. diameter Corning 015 glass. This bulb is blown at an angle of 45° to the tube and is about 2 cm. in diameter. A small needle flame is then directed against the bulb near the stem and, when a small spot of glass is hot, the bulb is removed from the flame and sucked in, thus forming a bulb within a bulb, as shown in *B*. The sucking is best done through a rubber tube in order to observe the formation of the inner bulb. The bulb is allowed to cool somewhat and a spot on the bottom, diametrically opposite the top opening, is heated with a tiny gas flame. A flame about 1 cm. long, burning on the end of a drawnout glass capillary, is suitable for this operation. A small bulb is sucked in until it touches the inner bulb and forms a flat membrane. Without further suction, the flame is directed at the center of this membrane until the glass melts through and the hole opens out.

It has not been found necessary to attempt to anneal these electrodes. Breakage from strain occurs principally in electrodes having too thick an outer bulb. Electrodes which are so thin as to lack durability can be eliminated by gently sucking or blowing in them. A membrane which shows any spots of color, or which clicks when blown in, is too thin. The thickness of the inner bulb can be regulated somewhat by the size of the area heated before the bulb is sucked in. Dependable electrodes having resistances of from 1 to 10 megohms are easily made.

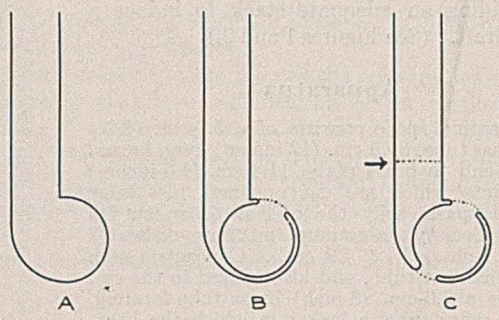

FIGURE 1

The electrodes are filled with a small amount of buffer to the level indicated by the arrow in *C*, and a suitable reference electrode is inserted. The electrode is immersed in the unknown solution to the depth indicated by the arrow.

Electrodes of this form are very sturdy, but it is obvious that a severe jolt will break them when they are filled with liquid. Because of the form of the sensitive membrane, they are not well adapted to measurements in liquids of high viscosity. Electrodes of this type have been used continually for 6 months in routine measurements, where they have replaced electrodes of the ordinary bulbular form which were broken on the average of once a week.

RECEIVED June 17, 1937.

A Buret-Filling Device for Portable Reagent Reservoirs

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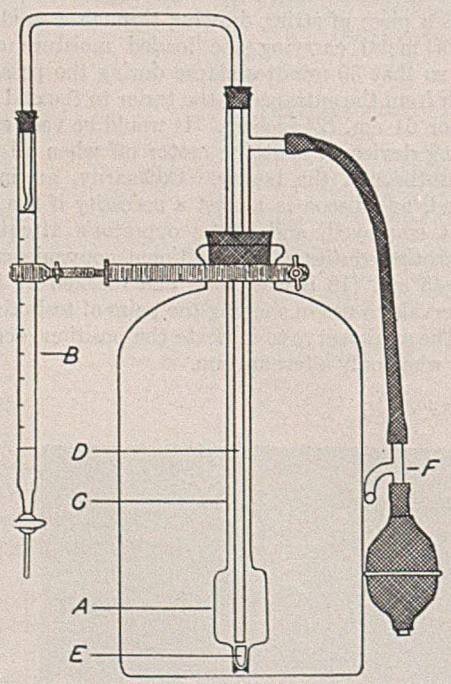


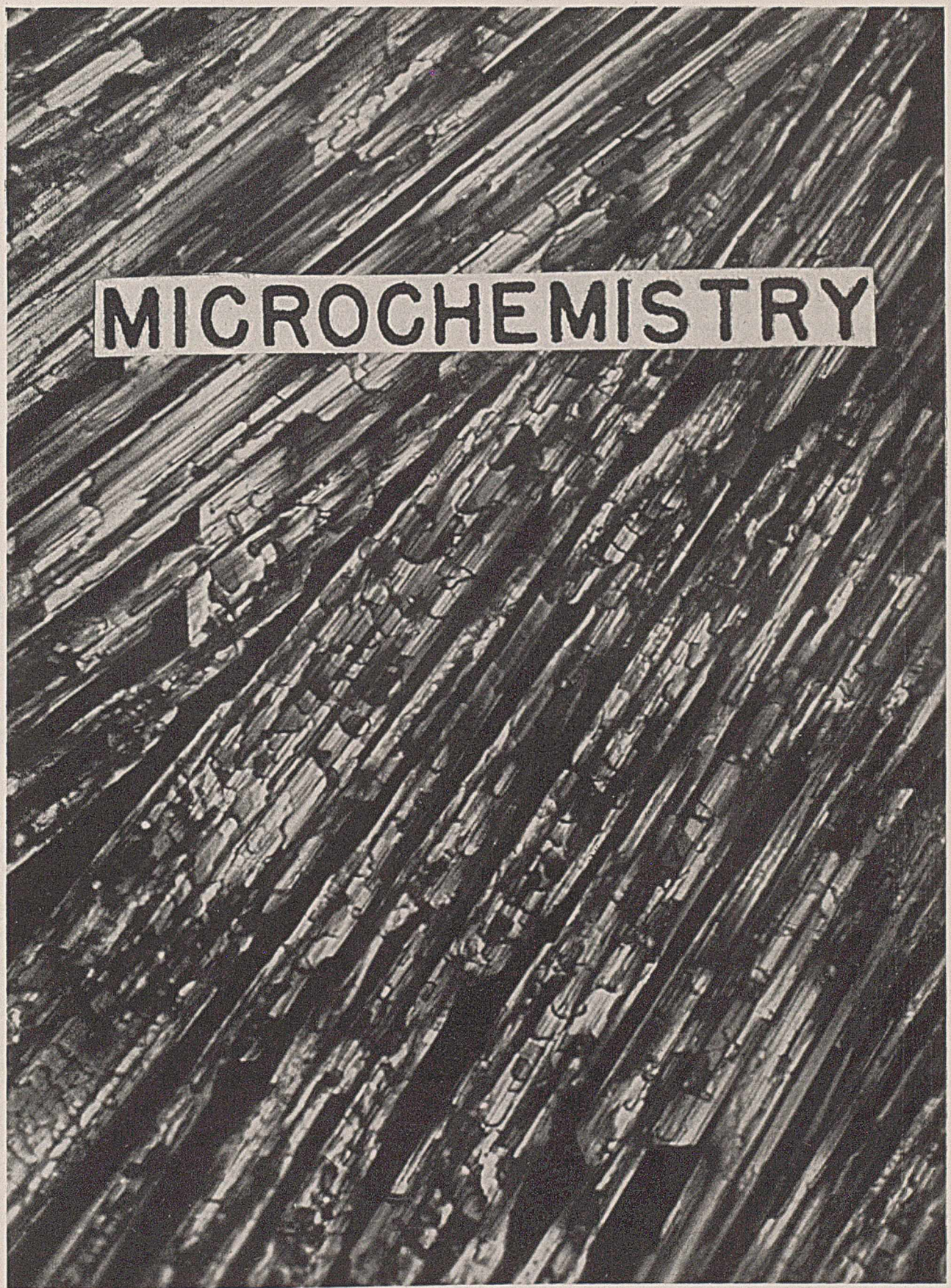
THE use of compressed air for filling burets with reagents from portable containers is limited to reservoirs of a capacity which requires frequent filling. The device presented has the advantage that it can be adapted to bottles of any size, and the buret can be filled by the use of a small amount of air affected only to a negligible extent by the solution level in the bottle.

The diagram shows the construction of the accessory which is used in this laboratory in 20-liter (5-gallon) bottles. The bulb, *A*, at the bottom of the bottle has a capacity slightly greater than that of the buret, *B*, and is of a diameter as large as the neck of the bottle will allow; this facilitates a more complete withdrawal of solution from the bottle. It is sealed to a tube, *C*, of a size which permits the entrance of the delivery tube, *D*, carrying the solution to the buret. The valve, *E*, is made by grinding a glass rod into a seat obtained by sealing 4-mm. tubing to the bulb. The ground portion of the glass rod is broken to a 6-mm. (0.25-inch) length and inserted into the ground seat. The delivery tube is adjusted so that it prevents the valve from rising too far and becoming unseated.

The small stopper in the buret and the large one in the bottle should each carry a small opening to permit the passage of air.

The side arm of the T-tube, *F*, immediately above the pressure bulb is bent both towards the bulb and forward to facilitate closing with the thumb when pressure is applied. One squeeze is sufficient to fill the buret.

RECEIVED August 7, 1937.



Courtesy, J. R. Rachele and J. T. Bryant

Hydroquinone in Polarized Light

Determination of Chloroform Extract of Beet Leafhopper

A Micromethod

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IN CONNECTION with certain nutritional studies of the beet leafhopper, *Eutettix tenellus* (Baker), it was desired to determine the chloroform-soluble components, or extractives, of the insect as an aid in judging the amount and nature of its reserve energy. Owing to the small size of the beet leafhopper (maximum weight 1.2 mg., females; minimum 0.7 mg., males) and to the necessity of working with small samples and sometimes with single insects, a micromethod was required.

The method devised is an empirical one and the results are of value for comparative purposes only. Any deviation in the technic described alters the results.

TABLE I. CHLOROFORM EXTRACTIVES OF BEET LEAFHOPPERS COLLECTED IN SOUTHERN IDAHO, 1931

Date	Locality	Host Plant	Chloroform Extractives %
May 20	Mountain Home, Elmore County	*Sophia parviflora*	34.9, 34.5, 34.7, 34.6
May 20	Glenns Ferry, Elmore County	*Sophia parviflora*	35.4, 35.5, 35.1, 35.4
July 8	Oakley, Cassia County	*Beta vulgaris*	42.4, 42.6, 42.1, 42.3
July 14	Shoshone, Lincoln County	*Salsola pestifer*	36.2, 36.4, 36.0, 36.3
July 14	Pocatello, Bannock County	*Salsola pestifer*	38.7, 39.0, 38.5, 38.8
July 14	Adelaide, Minidoka County	*Salsola pestifer*	39.4, 39.5, 39.2, 39.4
July 14	American Falls, Power County	*Salsola pestifer*	39.2, 38.8, 39.0, 39.3
October 8	Roseworth, Owyhee County	*Artemisia tridentata*	42.7, 42.6, 42.4, 42.5

Apparatus

The apparatus[1] (Figure 1) is a modification of the regular Wiley-Soxhlet extractor, reduced in size to permit extraction from individual and small numbers of insects and to eliminate excessive glass surface, which would hold film of oils difficult to remove by continuous extraction. It is made entirely of heat-resisting glass.

A standard-taper ground-glass joint is used in the construction of the apparatus to permit the use of several receivers with one condenser. The extraction thimble, *E*, is approximately 4 mm. in inside diameter and 15 mm. long, and has a small outlet in the bottom to permit the chloroform extract to pass to the receiver, *F*. A thin layer of long-fiber cotton is placed in the thimble to prevent the fine material from passing to the receiver. A small loop of glass is fused to the top of the thimble for suspension from the condenser. The condenser, *C*, consists of a water jacket sealed into the male portion of the ground-glass joint, surrounding a tube, *A*, through which the water enters. The water is forced directly to the lower end of the condenser. The returning water is removed through the side arm, *H*. The receiver, *F*, is made from the thin-walled tubing attached to the female portion of the ground-glass joint.

The extraction thimbles have a maximum weight of 1 gram, and the receivers a maximum weight of 8.5 grams. This load permits weighing on the microbalance with satisfactory precision. The microbalance used in this work was constant in sensitivity from 0 to 12 grams.

Counterpoises with approximately the same surface as the receiver are used to compensate for any humidity change during the extraction. It has been found that high humidities (above 50 per cent at 23.89° C., 75° F.) interfere with the determination because of the hygroscopic nature of the insect material. Low humidities that are usually found in the desert regions are ideal for the performance of the method.

The use of the apparatus may be best illustrated by a description of a typical extraction.

The thimble is first extracted for 1 hour to remove any loose fragments of cotton fiber. The thimble and receiver are then placed in the drying oven (48° C., 118.4° F.) for 5 hours to ensure complete removal of the water film. They are removed and placed near the microbalance and allowed to stand 15 minutes for the glass surface to reach equilibrium with the moisture of the air. In regions of high humidity the reservoir and thimble must first be cooled in a desiccator.

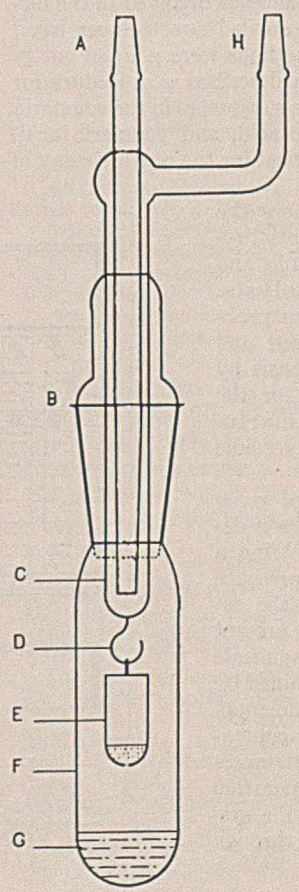

FIGURE 1. CONSTRUCTION OF MICROEXTRACTOR

A. Water inlet
B. Standard-taper ground-glass joint
C. Condenser
D. Condenser hook
E. Extraction thimble
F. Receiver
G. Solvent
H. Water outlet

Procedure

The weighing procedure is as follows:

The thimble is filled with the ground insect material and weighed. It is then attached to the condenser hook and 3 ml. of chloroform are put in the receiver. The apparatus is assembled, as shown in Figure 1, and placed on the steam or air bath. As

[1] Since the development of this method, two extractors have been described, which are applicable to biological problems (*1*, *2*).

soon as the vapors of chloroform begin to form, the joint is separated to allow the air to escape. When the small droplets of chloroform appear near the glass joint, it is closed for the duration of the extraction. After 16 hours, the receiver and thimble are removed from the condenser and again placed in the drying oven. At the end of 5 hours they are removed and allowed to cool 15 minutes before being weighed. The weight of the extracted insect plus the weight of the extract is used as a check on the original total weight of the insect.

Large numbers of beet leafhoppers were collected from localities in south-central Idaho with especial reference to host plant and its condition. The insects were killed immediately with a minimum dose of cyanide and placed between sheets of crepe paper to dry. This method of collection has been found to be the most satisfactory where samples must be transported over rough desert country. After their sex had been determined, the insects were dried (at 48° C., 118.4° F.) for 24 hours, as this period had been found to give constant weight of the insect material.

Table I shows a series of determinations made from eight localities in southern Idaho in which four different host plants were involved—namely, flixweed (*Sophia parviflora*), sugar beet (*Beta vulgaris*), Russian thistle (*Salsola pestifer*), and sagebrush (*Artemisia tridentata*). Five females were used for each analysis reported. All percentages are based on the dry weight of the insect.

Literature Cited

(1) Gorbach, G., *Mikrochemie*, 12, 161 (1932).
(2) Titus, L., and Meloche, V. W., IND. ENG. CHEM., Anal. Ed., 5, 286 (1933).

RECEIVED April 30, 1937.

Determination of Coumarin and Melilotic Acid

A Rapid Micromethod for Determination in Melilotus Seed and Green Tissue

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ATTEMPTS to improve the agricultural usefulness of common sweet clover (Melilotus) by selecting and breeding varieties low in coumarin have been impeded by the lack of suitable methods for the estimation of coumarin in seed and green tissue. [Coumarin and related substances are responsible for unpalatability (bitterness). It also appears that the same class of substances is involved in the formation of the toxic principle of spoiled sweet clover hay.] The limitations of the existing methods were pointed out in a previous publication (*1*) which described a procedure for the accurate measurement of three substances in the coumarin group—e. g., coumarin, melilotic acid, and coumaric acid. This method is, however, impractical for the routine assay of large plant populations.

In the method described here the authors have achieved a substantial reduction in the size of the sample and the time required for each analysis. They have also placed the entire problem of coumarin analysis of seed and green tissue on a much firmer basis by including an incubation period for the enzymatic release of bound coumarin. (The bound coumarin might be glycosidic or in other combinations.)

When the coumarin content of green sweet clover tissue or viable seeds is determined by any method involving a maceration of the tissue in the presence of water, opportunity exists for the enzymatic hydrolysis of coumarin present in the combined state. The magnitude of the coumarin figures is determined by two factors: the nature of the nonaqueous solvent used to extract the coumarin, and the length of time that lapses between the initial maceration operation and the inactivation of the enzyme system either by the solvent or through heating.

With viable seed tissue which usually contains only traces of free coumarin but substantial quantities of bound coumarin, the rate at which the enzyme system is inactivated is particularly significant. The authors have found that the errors incident to the analysis of seed tissue when an incubation period is not involved can be of a high order of magnitude. On the other hand, in succulent green tissue free coumarin usually predominates. Hence the coumarin figures reported on green tissue by methods that do not employ an incubation period (*1*) probably represent very close approximations of the actual coumarin content. The value of this finding (the necessity of the incubation period) to the geneticist attempting to select low coumarin-bearing plants through coumarin

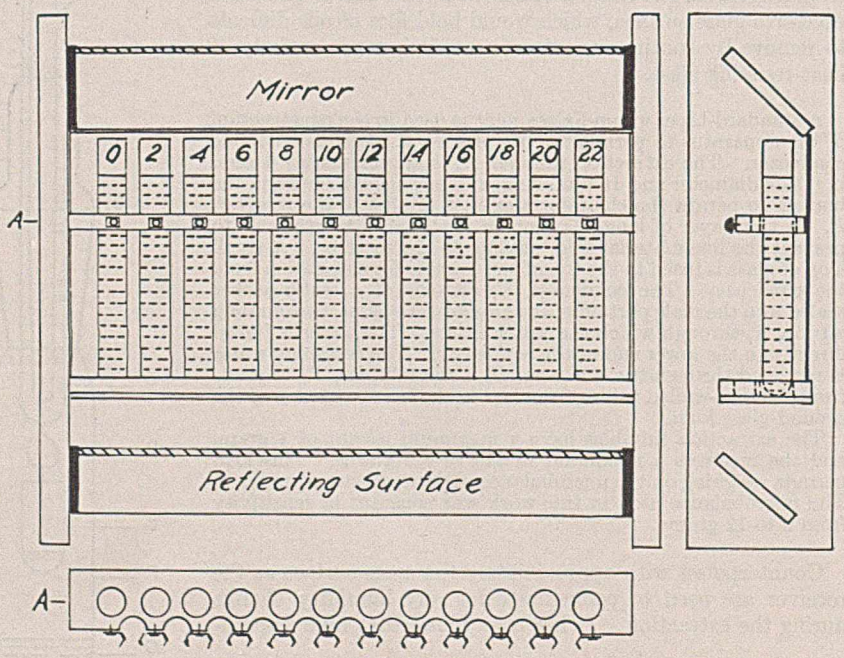

FIGURE 1. COLOR COMPARATOR

analyses on either the green tissue or viable seed tissue is obvious. Heretofore the possibility existed that seeds classified as low in coumarin (or free from coumarin) on the basis of the direct extraction methods might actually be high coumarin-bearing and thereby all attempts to gain the desired objective by breeding experiments might be vitiated.

The essential features of the microprocedure described below are as follows:

The finely ground seeds or disks of green tissue (representing 1 to 6 mg. on dry weight basis) are incubated in an aqueous medium for 1 hour at 40° C. Coumarin and related compounds are then extracted by grinding first with a small quantity of ethyl alcohol and then with ethyl ether (peroxide-free). The ether is evaporated from the filtered extract and the remaining alcoholic solution is made to volume with 0.05 *N* sulfuric acid. Heating to 85°, followed by cooling and filtering, yields a water-clear filtrate which is then ready for color development with the diazotized *p*-nitroaniline solution first introduced by Clayton and subsequently employed by Stevenson and Clayton (*2*) and by Roberts and Link (*1*). The melilotic acid is measured by treating an aliquot of the aforementioned solution with alkali at 85°. After cooling, acidification closes the coumarin ring and the development of the color upon adding the diazonium solution is due to melilotic acid. Total melilotic acid and coumarin are obtained by treating another aliquot with alkali at 85° C. In this case the coumarin ring is not closed, since there is no addition of acid and the color developed upon adding the diazonium reagent is due to melilotic acid and coumarin. Coumarin is then calculated by difference.

Experimental

Apparatus. Pyrex test tubes, 15 × 125 mm. marked for 10.0 ml. Color comparison tubes, matched 13 × 100 mm. Pyrex test tubes marked for 8.0 ml. Matched Nessler tubes of the same size would be superior.

Color comparator (Figure 1), 60 × 220 × 270 mm. The comparator accommodates 12 standard tubes and carries half circles fitted with clips for holding the unknown tubes during the matching process. The tubes rest on glass and are protected from horizontal light incident to the constant light source by a white cardboard background. The light is reflected through the length of the tubes by a movable white surface below the tubes. The colors are observed in an adjustable mirror above the tubes.

Filtration assembly (Figure 2, left). Side-arm test tubes, 18 × 150 mm., fitted with small long-stemmed glass Büchner funnels of 18-mm. diameter.

Incubation tubes, mortar and pestle assembly (Figure 2, right). Pyrex test tubes, 10 × 75 mm., fitted with corks carrying small pestles prepared from 4-mm. glass rod. The length of the pestles is 150 mm., the diameter of the grinding surface 7 mm.

Solvent. Ethyl ether free from peroxides (anesthesia grade).

Reagents. *p*-Nitroaniline hydrochloride (solution *A*). Dissolve 3.5 grams of *p*-nitroaniline in 45 ml. of 37 per cent hydrochloric acid, dilute to 500 ml. with distilled water, and filter. This solution keeps indefinitely if stoppered.

Sodium nitrite (solution *B*). Dissolve 5 grams of sodium nitrite in 100 ml. of distilled water. Keep this solution in a dark bottle away from light and renew it frequently.

Diazonium solution. Thoroughly chill a 100-ml. flask and solutions *A* and *B* in chipped ice. Pipet 3 ml. of solution *A* and 3 ml. of solution *B* into the flask, chill for 5 minutes, add 12 ml. of solution *B*, mix, chill for another 5 minutes, fill to the mark with ice-cold distilled water, mix, and place in chipped ice for 15 minutes before using. If kept in ice this solution remains stable for at least 24 hours.

Preparation of Color Standards. Add standard coumarin solution (an aqueous solution containing 4 gamma of coumarin per ml.) in 2-gamma increments to 12 color comparison tubes. The first tube contains 0 gamma and the last tube 22 gamma of coumarin. Next add exactly 1 ml. of 1 per cent sodium carbonate to each tube and sufficient distilled water to make a volume of about 6 ml. Mix each tube by stoppering with the finger and inverting; then place in a water bath at 85° C. for 5 minutes. Cool to room temperature and add 1 ml. of the diazonium solution drop by drop, mixing after each addition. Make to volume with distilled water, mix, and place in the color comparator. These standards should be discarded at the end of each day because of the development of a yellow color which darkens the original red shade.

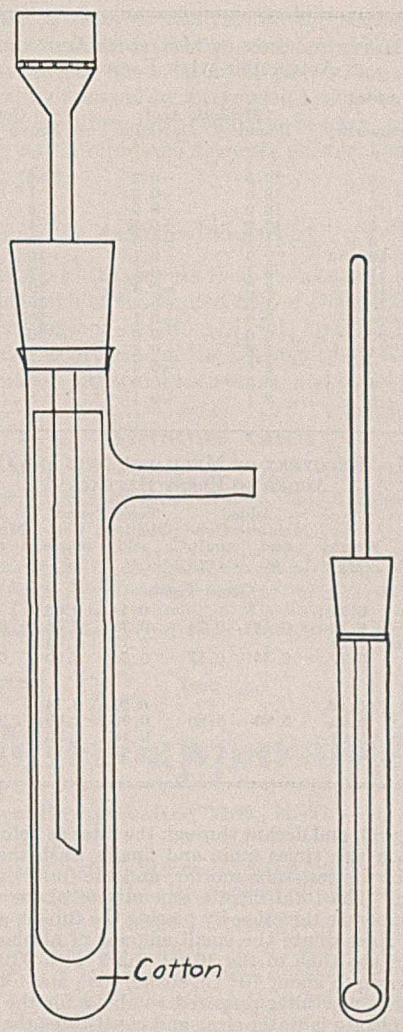

Figure 2. Filtration Assembly (Left) and Incubation Tube (Right)

Analytical Procedure. Seed sampling. Grind six or more seeds to a fine powder in a small mortar, weigh 4 mg. or less to ±0.01 mg., and transfer the weighed sample to an incubation tube containing about 0.1 gram of 70-mesh sharp-edged quartz sand. (Single seeds or portions of a seed weighing as little as 0.5 mg. may also be analyzed.) Moisture may be determined in the usual manner if the results are to be calculated to the moisture-free basis.

Green Tissue Sampling. The coumarin content varies with the stage of development of the plant and with the part of the plant from which the sample is taken. For attempts to correlate the coumarin content in green tissue with that in the seed from which the plant was grown, it is tentatively suggested that the plant should be sampled just before blossoming.

A sharp cork borer cutting 6- to 7-mm. disks can be used for the sampling. Cut single disks (eight in all) from the center of eight leaflets of fully developed leaves on the upper part of the plant. Transfer 4 disks to an incubation tube containing 0.1 gram of 70-mesh sand and thoroughly dry 4 other disks at 105° C. for the determination of the dry weight of the 4-disk sample.

Preparation of "Solution for Analysis." Add 0.1 ml. of water to the sample of seed or green tissue disks in the incubation tube, grind, and stopper by sliding the cork over the handle of the pestle. Incubate for 1 hour at 40° C., remove, and add 4 drops of alcohol. Grind, add about 1 ml. of ethyl ether, grind, and filter into the test tube marked for 10 ml. For filtration use the small glass Büchner funnel fitted with a disk of filter paper and a heavy pad of dry asbestos. Again add 4 drops of alcohol, grind,

TABLE I. DETERMINATION OF MELILOTIC ACID AND COUMARIN IN ADMIXTURE WITH EACH OTHER

Substance Added		Melilotic Acid		Coumarin	
Melilotic acid	Coumarin	Found	Deviation	Found	Deviation
γ	γ	γ	γ	γ	γ
20	0	19.8	−0.2	0	0
20	1	19.8	−0.2	1	0
18	2	18.0	0.0	2	0
16	4	16.2	+0.2	4	0
12	8	11.7	−0.3	9	+1
10	10	9.9	−0.1	10	0
8	12	8.1	+0.1	12	0
4	16	3.6	−0.4	16	0
2	18	1.8	−0.2	19	+1
1	20	0.9	−0.1	20	0
0	20	0.0	0.0	20	0
2	4	1.8	−0.2	4	0
4	2	3.6	−0.4	2	0
4	4	3.6	−0.4	5	+1
8	8	8.1	+0.1	8	0

TABLE II. RECOVERY OF MELILOTIC ACID AND COUMARIN ADDED TO PLANT EXTRACT

Sample	Weight	Added Melilotic acid	Added Coumarin	Total Found Melilotic acid	Total Found Coumarin	Recovered Melilotic acid	Recovered Coumarin
	Mg.	%	%	%	%	%	%
Green Tissue							
M. dentata 92	6.72	..	..	0.14	0.00	..	..
	6.72	0.14	0.74	0.28	0.78	0.14	0.78
Common white	5.77	..	..	0.17	1.04	..	..
	5.77	0.35	0.87	0.54	1.90	0.37	0.86
Seed							
M. dentata 89	5.43	..	..	0.21	0.14	..	..
	5.02	0.80	0.80	0.99	1.00	0.78	0.86
Common white	5.01	..	..	0.18	1.70	..	..
	2.52	1.19	1.58	1.47	3.15	1.29	1.45

add ether, grind, and decant through the filter as before. Repeat this operation two times more and finally wash the pestle, the outer edge of the test-tube mortar, and the funnel with a small jet of ether. The total filtrate amounts to about 4 ml. Now carefully evaporate the ether by placing the tube in a water bath at 45° C. Next dilute the small amount of alcohol and water remaining in the tube to the 10-ml. mark with 0.05 *N* sulfuric acid, mix, warm to about 70° C., shake, cool, and filter with suction through a new filter prepared as above for the ether filtration. The filtrate is water-clear and constitutes the solution for analysis.

A. Determination of Melilotic Acid. Pipet a 4-ml. aliquot of the solution for analysis to a color comparison tube, add 1 ml. of 0.5 *N* sodium hydroxide, mix, and heat for 5 minutes at 85° C. Cool to room temperature, add 1 ml. of 0.5 *N* sulfuric acid, mix, and let stand at least 10 minutes to ensure complete closure of the coumarin ring. Next add 1 ml. of diazonium solution, mix, and add 0.5 ml. of 5 *N* sodium hydroxide in small drops, mixing after the addition of each drop. Make to volume, mix, and compare immediately against the coumarin standards.

B. Determination of Melilotic Acid and Coumarin. Pipet a 1- to 4-ml. aliquot (depending upon the coumarin content) of the solution for analysis into a comparison tube and, if less than 4 ml. are used, add sufficient 0.05 *N* sulfuric acid to make 4 ml. Next add 1 ml. of 7 per cent sodium carbonate, mix, heat for 5 minutes at 85° C., and cool to room temperature. Now add 1 ml. of the diazonium solution drop by drop with mixing after each addition, make to volume, mix, and compare immediately against the coumarin standards.

CALCULATIONS. Melilotic acid = $A \times 0.9$; coumarin = $B - A$. Calculate per cent on the basis of the weight of the dried disks or the ground seed and the aliquots used.

AN APPROXIMATE RAPID METHOD FOR COUMARIN. For rapidly classifying large populations of seeds and plants into low-, medium-, and high-coumarin groups, the procedure outlined in this paper may be further shortened.

For this purpose use a 1-mg. sample of ground seed or a single disk of green tissue (the sampler is adjusted to cut disks weighing 1 mg. when dry) from the center of a fully developed terminal leaflet of a trifoliate near the top of the plant. Grind, incubate, and extract according to the regular procedure. After evaporation of the ether add exactly 3 ml. of 0.05 *N* sulfuric acid (instead of making to 10 ml. with the same reagent), heat, shake, and filter directly into a comparison tube. Wash with exactly 1 ml. of 0.05 *N* sulfuric acid. Next develop the color in the entire filtrate according to determination *B*. The gamma observed divided by 10 equals per cent of coumarin and melilotic acid.

By choosing similar leaves in plants of the same age the weight of the dry disks is remarkably constant, regardless of the slight variation in the moisture content. Results obtained in this manner on tissue disks will not vary over 0.2 per cent from the actual content, and 1-mg. samples of ground seed weighed against a 1-mg. counterpoise on a balance sensitive to 0.1 mg. show greater accuracy.

Results of Analyses

Table I shows that reliable results are realized when the method is applied to pure solutions of melilotic acid and coumarin. With a 4-ml. aliquot from a 5-mg. sample made to 10 ml., an error of 1 gamma in the color-matching process would amount to 0.05 per cent of melilotic acid or coumarin. The table shows, however, that an error of this magnitude is infrequent.

Table II shows good recoveries when pure melilotic acid and coumarin are added to aliquots of the solution for analysis.

Table III shows typical results obtained on seed and on green tissue produced in a hothouse. There is good agreement between duplicates. (The melilotic acid content of green tissue is often many fold that reported in this table, *1*.)

TABLE III. MELILOTIC ACID AND COUMARIN CONTENT OF GREEN TISSUE AND SEEDS OF MELILOTUS AND ALFALFA

Sample	Weight	Melilotic Acid	Coumarin
	Mg.	%	%
Green Tissue (Per Cent on Dry Basis)			
Alfalfa	4.12	0.18	0.08
M. dentata AC 92	6.72	0.14	0.00
Common white A 43	5.77	0.15	0.87
Common white A 190	5.64	0.16	1.06
Common white	5.59	0.23	1.44
Common yellow[a]	5.05	0.18	1.60
Seed (Per Cent on Basis of Entire Seed)			
Alfalfa	4.84	0.19	0.00
M. dentata annual	5.45	0.16	0.09
M. dentata AC 89	5.43	0.19	0.14
M. dentata AC 85	5.44	0.16	0.18
Common white A 43	5.02	0.27	1.10
Common white A 190	4.93	0.19	1.41
Common white	5.01	0.18	1.70
Common white	2.57	0.17	1.75
Common yellow	12.71	0.31	2.02
Common yellow	5.51	0.31	1.95
Common yellow	2.41	0.28	2.06

[a] This sample of green tissue was taken from a plant with very old leaves Most of the blossoms were gone. Earlier runs during the development of the method indicated a much higher coumarin content.

Table IV shows the course of the hydrolysis of bound coumarin, probably glycosidic. From the data it is apparent that the enzyme liberates coumarin rapidly at 40° C. Although the reaction was complete in 30 minutes at 40° C., 1 hour at this temperature was selected as a suitable incubation period.

From a consideration of the structure and properties of coumarin and possible coumarin glycosides, it appears likely that an equilibrium between coumarin and the glycoside is not set up. The hydrolysis apparently goes to completion because of closure of the coumarin ring as the reaction proceeds. Once closed it is unlikely that the ring would open again, under these conditions, to permit glycoside formation.

Summary

A rapid microcolorimetric procedure for the separate estimation of coumarin and melilotic acid in sweet clover (green and seed tissue) is presented. The procedure is based on the production of a red dye produced by coupling coumarin and melilotic acid with a diazotized solution of *p*-nitroaniline.

The method is suitable for routine assay work in genetical

TABLE IV. LIBERATION OF COUMARIN IN SWEET CLOVER SEED BY ENZYMATIC ACTION[a]

Weight of Sample	Temperature	Time	Melilotic Acid	Coumarin
Mg.	° *C.*	*Hours*	%	%
5.60[b]	..	..	0.15	0.02
5.01	25	12	0.32	1.55
5.51	25	24	0.31	1.98
4.64	25	48	0.32	1.90
5.60[b]	..	..	0.15	0.02
4.76	40	0.5	0.32	1.96
4.81	40	1.0	0.32	1.92
5.07	40	1.5	0.31	1.92
4.75	40	2.0	0.32	1.95
4.83	40	3.0	0.32	1.89
4.72	40	4.0	0.32	1.90

[a] Similar ether-extraction of intact green leaf tissue indicated 0.63 per cent of free coumarin. Total coumarin obtained by inserting an incubation period showed a coumarin content of 1.43 per cent. Bound coumarin therefore amounted to 0.80 per cent.

[b] The amount of free coumarin in the seed was determined by grinding a 5.60-mg. sample with sand and ether in such a way as to remove free coumarin if present (no incubation period was employed).

studies involving the examination of a large population of green or seed tissue and requires samples of only a few milligrams.

A significant feature of the method is the inclusion of a suitable incubation period (1 hour at 40°) for the enzymatic release of bound coumarin prior to the extraction.

All heretofore reported analyses on the coumarin content of green sweet clover or viable seed tissue are inaccurate, since allowance has not been made for the bound coumarin which is readily released by enzymatic action.

Geneticists seeking low coumarin-bearing sweet clover plants have now a rapid and accurate chemical assay method for this purpose.

Acknowledgment

The writers wish to express their thanks and appreciation to E. A. Hallowell of the United States Department of Agriculture, Washington, and R. A. Brink and W. K. Smith of the Genetics Department of the University of Wisconsin for their coöperation, financial assistance, and counsel.

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A Fractional-Distillation Microapparatus

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THE development of organic micromanipulation has been largely due to the necessity of using it in the study of rare natural products or very costly materials. It has certain advantages, such as speed, economy, etc., and also has limitations. A very serious limitation, as compared with the customary organic macroprocedures, is that of fractional distillation. Although several apparatus for microdistillation and in a degree for microfractionation (*1*, *2*) have been reported, no attempt has been made to reach the precision attained in macrowork by the use of carefully controlled packed columns, with the exception of Podbielniak's still (*4*) which requires a few cubic centimeters or more.

Since in the degradative study of certain natural products, the author has constantly been confronted with the need of more precise fractional distillation of small quantities, he has attempted to develop a more efficient apparatus. Results of the study at this point seem to justify a preliminary communication, although the work is still in progress.

Design of Apparatus

Although the principle is the same as that employed in macrocolumns, the requirements for proper execution are somewhat different. Obviously an extremely small holdup becomes necessary. This requirement, however, can be partially counterbalanced by the fact that the capacity of the microcolumn can be extremely small and proportionately small reflux is thus required. Bearing in mind these factors, the apparatus shown in Figure 1, designed for volumes of 0.5 to 2.0 gram of material, has been devised. When approximately 0.3 gram of distillate was collected per hour, it gave a separation of benzene–carbon tetrachloride mixture corresponding to approximately 8 theoretical plates according to the method of Fenske and co-workers (*3*). It has a holdup (weight of material in apparatus when distilling flask has just become dry) of approximately 0.1 gram for these liquids, the column being 10 cm. in length and approximately 7 mm. in diameter. The apparatus (Figure 1) is constructed as follows:

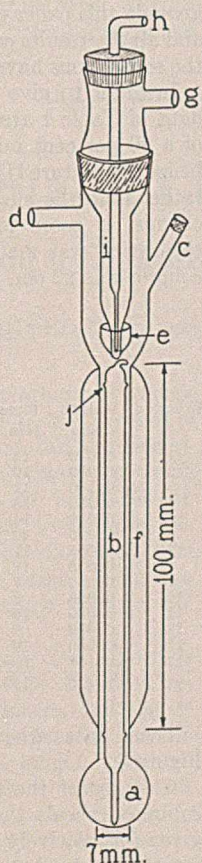

FIGURE 1

The distilling flask, *a*, is a round bulb of 4-cc. capacity. Into the flask is sealed a tube which is as straight as possible but very thin-walled, made by drawing out from a large tube so that it is about 7 mm. in diameter. Experiments with the standard Pyrex tubing, in which the walls are 1 to 1.5 mm. thick, have given poor results. A good explanation of this seems to be lacking and must be investigated further. This tube, which serves as the outside of the column, is surrounded by a sealed-on glass jacket, *f*, for protection and insulation. The effect of silvering and evacuating the jacket has not yet been studied. A tube 15 cm. wide and 50 mm. long is sealed to the upper end of the column and has a ground-glass joint at its upper end, through which extends the condenser, *i*. This tube has an outlet tube, *d*, for evacuating when reduced pressure is used, and another outlet tube, *c*, fitted with a small ground-glass stopper.

The condenser, *i*, carries a rubber stopper at its top, through which extends a glass tube, *h*, for the entrance of water into the condenser, the water leaving the condenser through the exit tube, *g*. The lower end of the condenser tube is drawn out into a rather narrow thin tip which has sealed on its end a cup, *e*, which holds approximately 0.2 cc. Liquid condensing on the upper part of the condenser will run down and be caught in the cup, where it can easily be withdrawn through tube *c*, when a fraction is desired, by means of a small pipet. The inside part of the column is represented by *b*, which is a very thin-walled tube, sealed at both ends, of shape represented. It is not attached to the rest of the apparatus and can be removed at will by a small

glass hook at its top. Its lower, drawn-out end rests on the bottom of flask *a* and the upper part is held away from the outside part of the column at a distance of about 1.5 mm. by six tiny glass nipples, 1.5 mm. long, which are sealed on the surface of tube *b*. The outside diameter of *b* must therefore be approximately 4 mm. in order to give the necessary 1.5-mm. clearance all around.

Heat is supplied to flask *a* by means of a small oil bath, which is kept constantly stirred mechanically with a small air-driven motor. A thermometer in the oil bath is the only measure of temperature possible for low-boiling liquids with this apparatus. Experiments with a tiny thermocouple at the top of the column were not promising.

The liquid to be fractionated is inserted with a pipet through the top of the apparatus before the inside part of column *b* is put in place. The temperature of the oil bath is then slowly raised until the ring of condensing liquid at the top of the vapor just reaches the tip of cup *e* or the top of the column. To do this, it is necessary to adjust the temperature of the oil bath to ± 0.5°. Material will then slowly diffuse from the ring of liquid through the vapor phase up to the upper part of condenser *i* and then run down to cup *e*. In this way approximately 0.3 gram of liquid can be obtained in an hour, and this has been found by experiment to be about the capacity of the still for maximum fractionation with carbon tetrachloride–benzene mixtures. When run more quickly than this, the efficiency of the still was rapidly affected.

When operated in the way described, the inside part of the column gets its reflux from material condensing on the outside of cup *e* and a thin film of liquid accordingly runs down over the entire surface of *b*. In the corresponding way the outside part of the column gets its reflux from the top, this being exposed to the air just above jacket *f*. A uniform flow of liquid is thus furnished to the entire inside surface of the outside part of the column.

In fractionating a mixture, arbitrary fractions are withdrawn by means of a capillary pipet through *e*, and the boiling point, refractive index, etc., are taken on each fraction by well-known micromethods.

For maximum operation there must be a small, constant temperature gradient between the inside part of the column and the immediate outside atmosphere. This gradient would be too large for high-boiling liquids, too much condensation would occur at the top of this column, and flooding would result. Too large a vapor velocity would be necessary to supply the reflux and the efficiency of the column would drop rapidly.

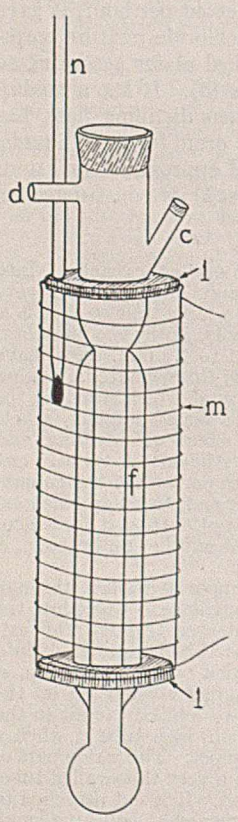

FIGURE 2

To overcome these difficulties, the apparatus is placed in a glass tube of approximately 3.5-cm. inside diameter which reaches from a point at the base of *c* to the lower part of jacket *f*, as shown in Figure 2. The glass tube is held in place on the apparatus by means of thin corks, 1, cut accordingly. The outside of the glass tube is wrapped with a suitable amount of Nichrome wire, *m*, so as to serve as a heating unit and is attached to a variable resistance. A thermometer, *n*, passes through the cork at the top of the glass tube and is thus suspended between jacket *f* and the glass tube. In this way the still can easily be maintained at a temperature best suited for its maximum performance.

Tests on Synthetic Mixtures

In order to be sure of the value of a fractionating column, actual tests on the separation of synthetic mixtures are necessary. Figure 3 represents graphically some of the many tests made. The first mixture (mixture I) was a 50 per cent by weight mixture of chlorobenzene and toluene. These two

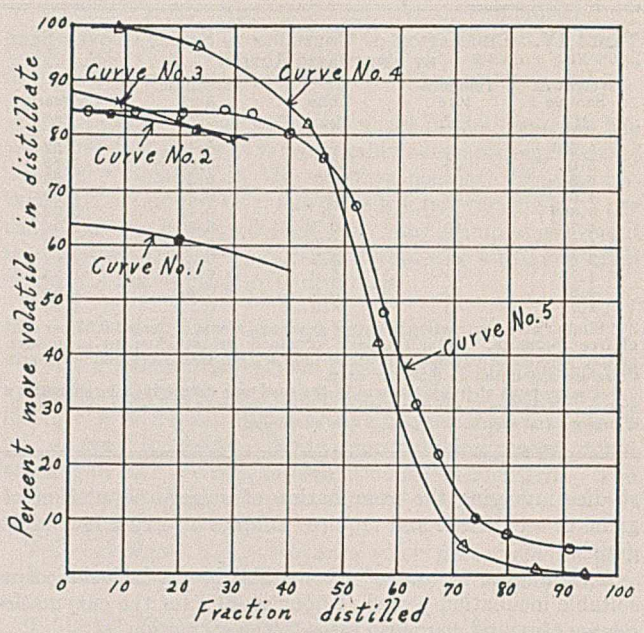

FIGURE 3. TESTS ON SYNTHETIC MIXTURES

1, 2, 3, 4. Mixture I
5. Mixture II

liquids boil 21° apart. An ordinary distilling flask gave the result represented by curve 1 (Figure 3). Curve 2 is the separation given by a Vigreux column 18 cm. in length. Curve 4 is the separation given by the distillation apparatus described and illustrated in Figure 1 (10-cm. column), and curve 3 is the separation given by this column when the center stick (*b*, Figure 1) is removed from this column. All analyses given in this paper were made by the refractive index method and all the results are easily reproducible. Check runs on all the separations have been made.

In order to give a complete picture of a separation, the data of Table I are presented. For this distillation 1 gram of a 50 per cent mixture by weight of carbon tetrachloride–benzene (mixture II) was used. Amounts are given by weight rather than the customary mole fraction because the organic chemist is more interested in the relative weights. The separation was made in a still as described, except that the column was 22 cm. in length.

TABLE I. DISTILLATION DATA

Fraction	Weight	Time	Temperature of Oil Bath	Temperature of Column Jacket	Refractive Index	Per Cent More Volatile
	Gram	*Min.*	*° C.*	*° C.*		
1	0.070	14	86–7	42.5	1.4678	84.5
2	0.080	10	86.5–87.5	42.5	1.4678	84.5
3	0.100	12	87–87.5	42.5	1.4680	84.0
4	0.060	15	86.5–7	42.0	1.4676	85.0
5	0.050	14	87.5–8	42.0	1.4681	84.0
6	0.075	10	89–90	43.0	1.4706	80.0
7	0.055	12	90	43.0	1.4726	76.0
8	0.055	12	90–3	43.5	1.4767	67.5
9	0.060	7	93	44.0	1.4851	48.0
10	0.050	8	93–93.5	44.0	1.4910	31.0
11	0.050	10	93.5–94	44.0	1.4937	22.0
12	0.070	9	94–6	44.0	1.4962	10.5
13	0.050	10	96–8	44.0	1.4968	7.5
14	0.150	Residue in still		....	1.4974	5.0
	0.975	143				

These data, when represented graphically, give curve 5, Figure 3.

A study of the literature concerning macrofractionating columns reveals the fact that efficiency curves are always given for relatively small molecules and at atmospheric pressures. Most substances encountered by the organic chemist in laboratory work are larger molecules where it is necessary

to use reduced pressure. With this in mind, a study of the behavior of this type of column under greatly reduced pressure is under way. Part of the data will be presented here.

The first mixture studied was methyl laurate–diphenyl ether (mixture III). With the column as described (22 cm. in length), very poor separations were obtained and the liquid did not appear to wet the glass surfaces of the column but collected in globules. When, however, the walls of the column and of the outside surface of the inside column were lined with a coil of No. 35 Nichrome wire similar to the column of Podbielniak (*4*), approximately 6 turns per cm., a uniform film of descending liquid was spread over the surface and curve 1, Figure 4, was obtained when the distillation was done under 0.20-mm. pressure and 1 gram of the mixture was used. An ordinary distilling flask gave with this mixture and under this pressure a distillate containing 67 per cent of the more volatile. Without the center part of the column (*b*, Figure 1), curve 2 was obtained, thus again confirming the desirability of the center part (*b*, Figure 1) of the column. The results obtained with this mixture were duplicated by the results obtained with a 50 per cent mixture of α-chloronaphthalene and methyl laurate.

Curve 3, Figure 4, was obtained by distilling 1 gram of a mixture of 50 per cent α-chloronaphthalene and diphenyl ether (mixture IV) under 0.2-mm. pressure. This mixture, when distilled from a distilling flask under 0.2-mm. pressure, gave a distillate containing 52 per cent of the more volatile and the two components appear to form a constant-boiling mixture similar to carbon tetrachloride–benzene at approximately 95 per cent of the more volatile, as the column refused to give further separation when a mixture of this composition was distilled. With this curve and with the curve for mixture II the more efficient separation was obtained with the last part of the distillation—the bottoms.

Curve 4 represents the separation obtained when mixture IV was fractionated at 60-mm. pressure. An ordinary distilling flask gave, under 60-mm. pressure, a distillate containing not more than 50.3 per cent of the more volatile.

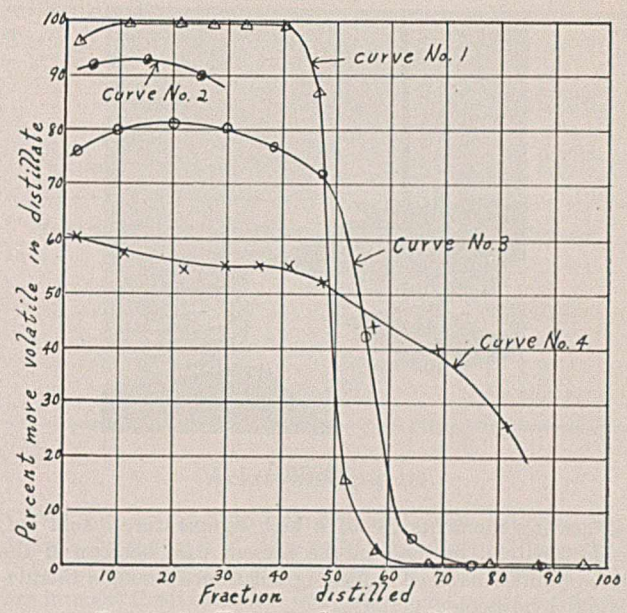

FIGURE 4. TESTS ON SYNTHETIC MIXTURES
1, 2. Mixture III
3, 4. Mixture IV

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Removal of Static Charges from Glassware

Using a High-Frequency Discharge

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MOST analysts have at some time or other had to cope with disturbances in weighing due to the presence of static charges on glassware. The present authors experienced such difficulties when it became necessary to weigh previously wiped glass-stoppered flasks of about 30-ml. capacity. Although the wiping always left a charge on the flask, it was only occasionally found objectionable. The usual 10-minute period of waiting before weighing was apparently sufficient on ordinary days to allow most of the charge to leak off.

Marked disturbances were noted on certain clear, dry days when the humidity was low. On these days the flask retained a high charge for as long as 35 minutes, and the irregular excursions of the balance pointer indicated that the flask was either increasing or decreasing in weight. The results reported below show that the authors' flask, when charged, sometimes gained approximately 1 mg. in weight; this was gradually lost as the charge leaked off. On the other hand, there were times when the presence of a charge caused the vessel to weigh less than normally. Change in temperature of the vessel, due to rubbing, cannot be held to account for the change in weight since, before weighing, the flask was always permitted to stand at least for 10 minutes just outside the partly open balance door.

The methods suggested in the literature for removing charges from objects to be weighed are relatively few and all but two were proved unsatisfactory under the authors' conditions. These two methods were not tested, since they involve (1) introducing a radioactive material (*3*) into the balance case and (2) humidifying the laboratory air (*1*). The first procedure seemed to involve the risk of having an active deposit form on parts of the balance; the second required expensive apparatus or considerable attention. Gamma radiations, from radioactive substances enclosed in capsules, were also not tried because of the danger involved in the continued exposure to uncontrolled amounts of radiation. The authors believe, however, that controlled methods involving radioactive substances can be satisfactorily employed, although no details appear in the literature. One well-known method of removing charges from objects, that

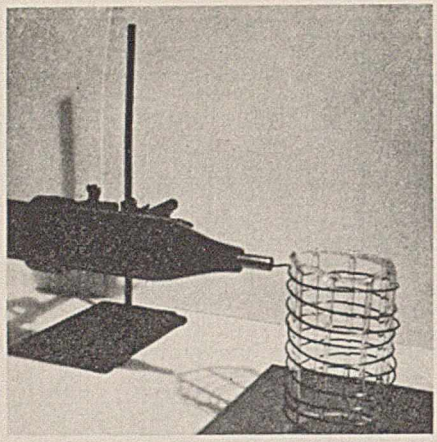

FIGURE 1

of heating momentarily to a high temperature, could not, unfortunately, be used in the present case because of the uncertainties involved in heating a flask containing a thermolabile hygroscopic material.

High-Frequency Discharge Apparatus

Since the known methods seemed unsuitable, a new procedure for discharging glass and similar nonconducting vessels was devised. The charged vessel is held in a region of silent discharge for a fraction of a minute, during which it becomes completely discharged. In the case of the flasks used in the authors' analyses, a helix of copper wire (Figure 1) of diameter sufficient to accommodate the flask was found a satisfactory means of setting up a zone of high-frequency discharge. The helix was set up on a frame of glass rods with its axis normal to a grounded metal plate. The most effective shape and size of the discharging zone will necessarily be determined to some extent by the shape of the vessel to be discharged. Thus an absorption tube of the type used in microanalysis required only to be held between two conducting strips, 4 × 20 cm., separated by about 2.5 cm. of air space. The one strip was connected to the high-frequency coil, the other to the ground. High-frequency currents were obtained from a compact device often employed in the laboratory as a leak detector in high-vacuum lines, and listed in recent apparatus catalogs as a "high-frequency vacuum tester." A silent discharge due merely to high tension—as, for example, that given by an induction coil capable of producing a 2.5-cm. spark between points in air—does not remove static charges from glassware nearly as effectively as the high-frequency discharge. The latter discharge has no significant effect upon the temperature of a glass object. A precision thermometer, kept in the discharge for 1 minute, altered in temperature by less than 0.01°.

Macroweighings

Table I epitomizes the results of a number of attempts to weigh the same flask in both the uncharged and discharged (after charging) condition and indicates clearly that the method of discharging is completely satisfactory. Before each successive weighing in the latter part of the table, the flask was charged by rubbing, and then the charge was completely removed from the vessel, yet no additional systematic or accidental errors were introduced.

In using the proposed method for discharging glass or other vessels, it is advisable to have on hand a small pith ball (3 mm. in diameter) suspended by a silk thread. This, or simpler, a frayed-out piece of silk thread, will act as a charge detector. Every portion of the "discharged" vessel should be brought near the pith or silk fibers. If any attraction is noted, the vessel should be subjected to the silent discharge for perhaps 15 seconds longer, this time in such a position that the part still charged will be in the most intense zone of the discharge. Uneven or irregular steps in the excursions of the balance pointer are the best indications (provided the vessel is in temperature equilibrium) that the vessel is too highly charged to permit accurate weighing. A vessel may be charged to a slight extent, as indicated by pith balls or silk fibers, and still permit accurate weighing. Twelve experiments carried out on six different days showed that a slightly charged flask allows regular swinging of the pointer and gives a weight which agrees within the limits of experimental error (0.00005 gram) with the weight of the uncharged flask. The latter is one that has stood in the laboratory until it has come to equilibrium so far as temperature and charge are concerned. It produces no movement of a pith ball or a silk fiber when brought within a distance of 1 mm.

TABLE I. ACTUAL WEIGHTS OF FLASK BEFORE CHARGING AND AFTER DISCHARGING

Condition of Flask	Date	No. of Successive Weighings	Average Weight	Average Deviation
			Grams	*Gram*
Uncharged	3/12	4	16.22879	0.000020
	3/13	4	16.22882	0.000035
	3/18	4	16.22882	0.000025
	3/23	1	16.22887	.....
Discharged (after charging)	3/12	4	16.22874	0.000050
	3/16	4	16.22909	0.00015
	3/17	4	16.22888	0.000050
	3/25	4	16.22894	0.000028
	3/15	1	16.22888	.....
	3/23	1	16.22899	.....

Microweighings

Disturbances due to electrostatic charges on glassware seem to be relatively greater on the microbalance than on the ordinary analytical balance. In order to test the effectiveness of the high-frequency method of charge removal, the authors selected a standard piece of glassware—the ordinary C—H absorption tube of the Pregl type—known to give occasional difficulties because of accumulated static charge. A Kuhlmann balance of the older type, with ordinary reading glass, was used. The technic of handling and wiping the absorption tube was practically the same as suggested by Hayman (*1*), the tube being kept for 10 minutes outside the balance case and 5 minutes inside the case before weighing. As has been reported a number of times, it was occasionally impossible to make satisfactory weighings under these conditions because of the electrostatic attraction or repulsion between the glass tube and the balance parts, particularly the pan. In these instances it was found that holding the charged tube in a high-frequency discharge for 15 seconds was sufficient to remove all trace of charge and permit satisfactory weighing.

Table II compares a series of weighings of an absorption tube that has stood in the laboratory overnight with another series for the same tube after wiping (and thus charging) and discharging as above. As usual, the "weight" is given in comparison to a counterpoise. The gain in weight is to be attributed to temperature change and perhaps to other factors not known. Small temperature fluctuations (<1°) may be responsible for changes of this magnitude, as has been shown by the thoroughgoing experiments of Schwarz-Bergkampf (*2*).

Table III gives the results of another series of repeated weighings of an absorption tube that had acquired charge due to wiping and was then discharged by holding in the high-frequency field for 30 seconds.

The weights (against tare) were reproducible in each case to less than 2 gamma, showing that little change was taking place while weighings were carried out. Larger changes were noticed from run to run and these are attributed primarily to fluctuations in laboratory temperature (±2°), even though correction was made for changes in zero point of the balance. The variations in weight are sufficiently low to make the method of handling, wiping, and discharging satisfactory for many routine organic microanalytical procedures. The authors believe that a more careful control of temperature will reduce these variations, their own experience having indicated that, using a newer type of Kuhlmann balance (with telescope attachment) and with temperature control of ±0.5° in the room and balance case, it is possible to reduce the average deviation of repeated weighings of the same object to 0.5 gamma and, when wiping and waiting intervened, to 2.0 gamma.

Table II. Effect of Discharge on Microabsorption Tube

Treatment	No. of Weighings	Average Weight of Tube	Average Deviation
		Mg.	*Mg.*
None	4	1.453	0.0026
Wiping and discharging	4	1.469	0.0012

Table III. Reproducibility of Weighings of Vessel after Discharge

Series	No. of Weighings	Average Weight of Tube	Average Deviation
		Mg.	*Mg.*
1	4	1.234	0.0015
2	4	1.247	0.0017
3	4	1.244	0.0017

Summary

A generally applicable method is proposed for removing electrostatic charges from glassware prior to weighing. It employs a high-frequency discharge which is easily obtainable in most laboratories. By modifying the size and shape of the electrodes a zone of discharge may be obtained that will accommodate and, in less than a minute, completely discharge ordinary glass vessels such as those used in macro- and microanalysis.

Acknowledgment

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Colorimetric Microdetermination of Manganese

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The amount of manganese in soil or plant extracts or in the ashes of plant tissues is determined colorimetrically with the formaldoxime reagent of Denigès. The presence of iron interferes with the determination, forming a purple coloration. This is eliminated by the addition of sodium cyanide, with which iron produces a faint greenish yellow coloration. The addition to the standard of approximately the same amounts of iron as in the unknown masks the greenish yellow color, and highly accurate determinations of manganese can be made.

DENIGÈS (*1*) described in 1932 a highly sensitive reagent for the qualitative detection of metals of the iron and manganese groups, but did not carry his studies to the point where the reagent could be adopted for the quantitative determination of either one or both elements. The reagent, a formaldoxime, is prepared by mixing trioxymethylene with hydroxylamine. When it is added to an alkalinized solution containing iron a purple coloration develops gradually. The color obtained in alkalinized solutions with manganese is wine red and develops instantaneously.

Studies on the conditions influencing the development of color with iron salts have shown that atmospheric oxygen and time are essential factors. The development of color with iron salts proceeds with time from the surface to the bottom of the solution in the test tubes, suggesting an oxidation-reduction reaction. The rate of color development can be speeded by agitating the solution vigorously. Similar tests with manganese salts have indicated that, in the course of 8 hours, either time or oxidation factors were of little or no influence in the development of color.

Various tests have indicated that the speed of iron reactivity with the reagent for the production of color can be blocked or partly inhibited by sodium cyanide, by ammonium tartrate, and by any other such substances with which iron forms double salts. The addition of either sodium cyanide or ammonium tartrate to the solution of iron salts causes the formation of a faint yellowish green pigment, characteristic of the double salts of iron, which interferes with the determination. Attempts to eliminate this color from the solution were fruitless. The difficulty was overcome by adjusting to equal values the amounts of iron in the unknown and in standard samples. This was accomplished by adding ferric iron to the standard solution in amounts equal to those obtained after chemical analysis of the unknowns with the *o*-phenanthroline method of Saywell and Cunningham (*2*). Using this technic, thoroughly satisfactory results were obtained.

Determination of Manganese

The procedure for the determination of manganese in plant tissues or soil extracts in the presence of iron is as follows:

A quantity of plant tissues containing 0.010 to 0.050 mg. or more of manganese is ashed, taking care that iron is completely oxidized during ashing to Fe^{+++}. The ash is dissolved in 2 to 5 ml. of 0.077 *N* hydrochloric acid. The solution is filtered, the filter paper is washed with water, and the solution is then made to 25-ml. volume.

The rest of the procedure varies with the amounts of manganese and iron in the solution. If the solution contains more than 0.020 mg. per ml. of manganese, further dilution is necessary. Before proceeding with the determination of manganese the amounts of iron in the unknown are estimated, using the *o*-phenanthroline (*2*) or any other reliable and convenient method.

The rest of the procedure is as follows:

To 10 cc. of the unknown 0.25 ml. (about 5 drops) of a solution of 40 per cent potassium hydroxide is added and stirred with a glass rod. Then 0.50 ml. of a solution of 20 per cent sodium cyanide is added to the mixture. After mixing again, 3 drops of the formaldoxime reagent are added and the contents are agitated. A wine-red color develops immediately with an intensity directly proportional to the amount of manganese in the solution. Standard solutions employing three different concentrations of manganese—namely, 0.002, 0.008 and 0.012 mg. per ml.—must be on hand and prepared in the same way as the unknowns. Such standard solutions must contain amounts of iron approximately equal to the unknown, which are added to the standards from a stock solution containing 0.100 mg. of Fe^{+++} per ml. A 5 per cent solution of gum Ghatti (0.5 ml.) is added to the standard and unknown solutions to prevent adsorption of the pigment to the colloidal suspensions formed after the addition of potassium hydroxide.

After adding all the reagents, both the standard and unknown solutions are allowed to stand for about 20 minutes and then the color is read in the colorimeter.

Results

The results obtained from a series of determinations of solutions with different amounts of manganese, employing different concentrations of manganese standards, are reported in Table I.

Table I. Controls for Colorimetric Determination of Manganese

(With the use of Denigès reagent in solutions free from iron. The standard solutions contained the following quantities of manganese per ml.: A, 0.010 mg.; B, 0.005 mg.; C, 0.001 mg.)

Manganese Taken, γ	Manganese Found: Standard A γ	Standard A No. of detns.	Standard A Error, %	Standard B γ	Standard B No. of detns.	Standard B Error, %	Standard C γ	Standard C No. of detns.	Standard C Error, %
20	20	1	0	..	...	..	..	...	..
15	15	1	0	..	...	..	..	...	..
10	10	1	0	11.5	4	11.5	..	...	..
8	..	...	..	8.0	2	0	..	...	..
7.5	7.5	1	0	7.5	3	0	..	...	..
6.0	..	...	..	6.2	2	3	..	...	..
5.0	4.6	1	8	5.0	4	0	6.5	2	13
4.0	3.6	1	10	4.0	2	0	4.0	3	0
3.0	2.7	1	10	2.9	3	8	3.0	3	0
2.0	1.7	1	15	2.0	2	0	2.0	3	0
1.0	..	...	..	0.9	3	10	1.0	3	0
0.5	..	...	..	..	...	..	0.5	3	0
0.25	..	...	..	..	...	..	0.25	2	0

In accordance with the data in Table I, very accurate results can be obtained only when the concentration of the standard is neither appreciably greater nor smaller than that of the unknown solution. In view of this condition, three to four manganese standard solutions with concentrations of from 1 to 20 gamma of manganese must be employed to cover the entire range of concentrations of manganese of the unknowns. Variations in the iron content between manganese standards and samples of unknowns must be reduced to a minimum, as the greenish yellow color of the ferricyanide salt interferes with the accuracy of manganese determinations. With a proper adjustment of the amounts of iron in the standard and unknown solutions, the results obtained are very accurate, as shown in Table II. The magnitude of the error, which may result in the determination of different amounts of manganese between solutions containing 5 and 20 gamma of iron, respectively, ranges from 5 to 15 per cent, as Table III shows. This error is mainly due to a greater intensity of the greenish yellow color of the ferricyanide salt and not to that of the wine-red color of the manganese complex pigment. However, such errors can be easily avoided if the instructions are properly observed.

Table II. Controls for Colorimetric Determination of Manganese

(With the use of Denigès reagent in solutions containing iron. The standard solutions contained the following quantities of manganese per ml.: A, 0.010 mg.; B, 0.005 mg.; C, 0.001 mg.)

Manganese Taken, γ	Manganese Found: Standard A[a] γ	Standard A Error, %	Standard B[a] γ	Standard B Error, %	Standard B[b] γ	Standard B Error, %	Standard C[a] γ	Standard C Error, %
20	20	0	..	..	..	..	..	..
15	15	0	16.5	10	16.5	10	..	..
10	10	0	10.0	0	10.0	0	..	..
7.5	7.5	0	7.5	0	7.5	0	10.0	25
5.0	4.6	8	5.0	0	5.0	0	5.0	0
4.0	3.7	8	4.0	0	4.0	0	4.0	0
3.0	2.8	7	3.0	0	3.0	0	3.0	0
2.0	1.7	15	2.0	0	2.0	0	2.0	0
1.0	..	..	0.8	20	0.8	20	1.0	0
0.5	..	..	..	..	..	..	0.54	8

[a] 20 gamma of iron per ml.
[b] 5 gamma of iron per ml.

Table III. Ratio of Light Absorptions between Solutions of Equal Concentrations of Manganese But with Different Amounts of Iron (Fe^{+++})

Manganese, γ	$\frac{\text{Fe } 20\gamma}{5\gamma}$	Error, %
20	1.05	5
15	1.05	5
10	1.10	10
7.5	1.10	10
5.0	1.10	10
4.0	1.15	15
3.0	1.15	15
2.0	1.15	15
1.0	1.15	15

Reagents

Formaldoxime. Dissolve by boiling in 100 ml. of distilled water 20 grams of trioxymethylene and 47 grams of hydroxylamine sulfate.

Sodium Cyanide. Dissolve 20 grams of sodium cyanide in 100 ml. of distilled water and filter.

Potassium Hydroxide. Dissolve 40 grams of potassium hydroxide in 100 ml. of carbon dioxide–free distilled water.

Manganese Standard. Dissolve 0.4061 gram of $MnSO_4 \cdot 4H_2O$ in 1 liter of distilled water and add 1 ml. of concentrated sulfuric acid. The solution contains 0.100 mg. of Mn^{++} per ml. and standards of lower concentrations are prepared by dilution with water.

Ferric Chloride. Dissolve 0.4876 gram of $FeCl_3 \cdot 6H_2O$ in 1 liter of distilled water and add 5 ml. of 0.077 *N* hydrochloric acid. The solution contains 0.100 mg. of Fe^{+++} per ml. Addition of this solution to solutions of manganese standards or of unknowns must be made with a microburet.

Gum Ghatti. Dissolve by boiling in 100 ml. of distilled water 5 grams of gum Ghatti. Filter through cotton while warm and then store in a flask with 1 ml. of toluene.

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Quantitative Determination of Arsenic in Small Amounts in Biological Materials

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THE fact that there are between four- and five-score methods in the literature for the determination of arsenic is convincing proof that they are not applicable in the hands of all individuals to the determination of arsenic in all forms and in all materials.

All these methods are divided into two parts: (1) The material containing the arsenic is either "wet" or "dry"-ashed. The "dry"-ashing procedure has as yet not proved satisfactory against loss. (2) The arsenic is determined in the ashed material by one of four general procedures—namely, the Berzelius-Marsh method (*1*, *4*, *9*, *11*), the Gutzeit method (*6*, *8*), conversion to arsenic trichloride and subsequent titration (*13*), or the colorimetric method, in which the arsenic forms a colored complex compound in acid-molybdate solution (*2*, *3*, *7*, *10*, *12*, *14*). The last is the basis of the method which the authors have been using for 18 months with satisfaction. The method is simple, accurate, relatively rapid, very sensitive, and quantitatively applicable to very minute as well as relatively large amounts of arsenic. Of all the methods tried, those of Maechling and Flinn (*10*) and of Truog and Meyer (*12*) are the best but are not as satisfactory in the authors' hands as the one proposed here.

Reagents

The reagents used were: concentrated nitric and sulfuric acids, 70 per cent perchloric acid, 20-mesh zinc, a 75 per cent solution of potassium iodide, commercial hydrogen, approximately 6 *N* hydrochloric acid, acid-molybdate solution [1 gram of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ per 100 cc. of 5 *N* sulfuric acid solution], and hydrazine sulfate (0.15 gram of $N_2H_4\cdot H_2SO_4$ per 100 cc. of aqueous solution).

Preparation of Sample

If dry, the sample is accurately weighed and transferred to a three-neck, liter, round-bottomed Pyrex flask (the necks are all ground for standard taper connections). One neck is connected by means of glass connections to a condenser for downward distillation and a flask used to catch the distillate. (The distillate, as definitely shown by Kahane and Pourtoy, *5*, and confirmed by the authors, sometimes contains arsenic, especially if the content is high and at some stage the solution has turned brown. It can be regenerated and added to the original digest.) The other two necks are fitted with funnels carrying standard taper connections for addition of acids.

A mixture of nitric and sulfuric acids is carefully added (for a 10-gram sample of animal tissue or food the authors add 20 to 30 cc. of a one-to-one mixture of concentrated nitric and sulfuric acids). The mixture is then heated to foaming and concentrated nitric acid is added from one funnel in a relatively rapid dropwise manner during the concentration period (25 to 50 cc. additional of nitric acid are usually required). After there is complete solution of the sample, perchloric acid (4 to 6 cc.) is added (perchloric acid is a dangerous explosive in the presence of large amounts of organic matter), also dropwise, relatively rapidly from the second funnel while continuing the addition of nitric acid from the other funnel. The guide for the rate of addition of nitric acid is the color of the solution, which must not be allowed to turn brown. This usually begins at the point of contact of the flame with the flask and is easily observed.

The digestion is continued with addition of nitric acid until the solution is water-clear. The addition of nitric acid is then stopped and the heating continued for 0.5 to 1 hour longer for complete destruction of all organic nitrogen compounds and complete removal of all traces of nitric acid, both of which may interfere with the subsequent determination of arsenic. This ashing procedure may be used in the determination of many metals in foods and biological materials. The time required is usually about 1.5 hours, and two or more may be run at once. The digest is quantitatively removed from the flask and made up to a convenient volume.

Separation of Arsenic from Digestion Mixture

The arsenic must be separated from interfering substances, such as phosphorus, in the digestion mixture.

APPARATUS FOR ISOLATION OF ARSENIC. A 50-cc. Erlenmeyer flask is fitted with a two-hole rubber stopper, one hole of which carries a glass tube with the lower end extending almost to the bottom of the flask. The other end leads to a stopcock attached to the bottom of a 15-cc. reservoir. The second hole of the rubber stopper carries a 10-cm. (4-inch) water-cooled upright condenser, the upper part of which is curved at a right angle to the position of the condenser. A scrubber is attached to this by means of thick-walled rubber tubing, so that there is glass-to-glass contact. The scrubber consists of a tube approximately 1 cm. in diameter and constricted at each end to approximately 6-mm. outside diameter.

The tube is filled with ignited, washed sand, which is held in place by a small amount of glass wool at each end of the large portion of the tube. Before the apparatus is put in use, the sand is moistened with a 20 per cent solution of lead acetate by adding a few drops from a pipet and then drying in an oven. The decomposition of the arsine is carried out in a quartz tube consisting of two parts. One part is 6 mm. in outside diameter and 20 to 30 cm. in length. The other part consists of a capillary approximately 10 cm. in length and 1 mm. in inside diameter. The large end of the tube is attached by means of thick-walled rubber tubing to the scrubber, so that the quartz and glass are in actual contact.

The large end of the quartz tube extends through a 10-cm. electric furnace which maintains a temperature of approximately 800° C. For cooling, a moist cloth wick about 6 mm (0.25 inch) in diameter with the end in water is used around the capillary part of the tube about 6 or 7 cm. from the furnace, to aid in condensing the arsenic. The authors use six of these units set up side by side; they may be run simultaneously or in pairs.

THE SEPARATION. Five grams of 20-mesh zinc and 1 cc. of a 75 per cent solution of potassium iodide are added to the Erlenmeyer flask. The apparatus is then assembled, the Erlenmeyer flask is immersed to its neck in a water bath kept at about 85° by means of a hot plate, and the whole system is flushed with commercial hydrogen from a cylinder. The hydrogen is conducted through the apparatus by means of the reservoir, which is temporarily fitted with a one-hole rubber stopper carrying a piece of glass tubing. After the system is completely filled with hydrogen, as determined by testing the issuing gas for explosive properties, the hydrogen flow is discontinued. (Each unit is flushed separately with hydrogen. The issuing hydrogen is tested in the usual manner by collection in a test tube and removal to a Bunsen burner where it is ignited. If it ignites and burns long enough to return to the apparatus for ignition of the hydrogen at the end of the quartz tube, there is no danger of explosion.) The furnaces heat up very rapidly, hence they should not be connected until the system is filled with hydrogen, but should be hot before the sample is added.

The sample in a volume of 10 cc. or less is then added, followed by hydrochloric acid in small quantities sufficient to maintain a slow, uniform rate of evolution of hydrogen, usually 15 cc. of 6 *N* acid over a period of 45 minutes. Tin, which is usually used, has been found of no value in this method. The acid is added to the reservoir in three 5-cc. portions, in order to ensure quantitative removal of the sample.

The arsenic distills over as arsine and is set free as metallic arsenic by the heat from the furnace and is deposited in the capillary portion of the quartz tube just outside the furnace.

The tubes are removed by disconnecting them from the scrubber and drawing them through the furnace, holding the capillary end. They are immediately cooled under a running jet of cold

water. Glass tubes cannot be used for this reason. The arsenic mirror is dissolved in 0.2 cc. of concentrated nitric acid, and quantitatively washed into a 25-cc. arsenic-free Pyrex Erlenmeyer flask with a small quantity (approximately 5 cc.) of distilled water. This solution is then evaporated to dryness on the steam bath and the flask heated for an hour in an oven at 120° to 125°, to remove all traces of oxidizing agents, since they interfere with the subsequent color development.

Development of Color

The acid-molybdate solution and hydrazine sulfate reagent are mixed in the proportions of 10 cc. of the acid molybdate and 1 cc. of the hydrazine sulfate for each 100 cc. of total solution. The 10 cc. of acid molybdate are diluted to approximately 90 cc. with distilled water before the 1 cc. of hydrazine sulfate reagent is added. The final volume is then made to 100 cc. with distilled water. After thorough mixing, 10 cc. of the above solution are added to each 25-cc. Erlenmeyer flask containing the arsenic pentoxide to be determined. How much arsenic could be determined by this amount of reagent the authors do not know, but they do know that the amount of the reagent is in excess of that required in the ranges which they have worked.

The flasks fitted with glass bulbs are heated for 10 minutes in boiling water, allowed to cool, and the colors read in a spectrophotometer. (Although the authors use a spectrophotometer and make all readings at 6100 millimicrons in a tube 10 cm. in length, of small bore, because of the straight-line absorption of the color a photometer with a filter may be used. The readings are higher with the longer wave lengths; hence the reason for reading in this range. Because the color is not very intense with small quantities, it is impossible to use a colorimeter with accuracy.) From the readings the quantity of arsenic is determined from a standard curve obtained by treating known amounts of arsenic in exactly the same manner as that described for the unknown digestion mixture above. The authors have shown that antimony does not interfere in this determination.

Discussion and Summary

During the course of the work, several different means of color development were investigated. One that gave good results was the use of ammonium molybdate, stannous chloride, and sulfuric acid as reagents (*3*). The second was the method described by Truog and Meyer (*12*), which made use of the same reagents, but in different concentrations. In the third, ammonium molybdate, hydrazine sulfate, and sulfuric acid were used, and in the development of the color was similar to the method of Maechling and Flinn (*10*).

The objections to the first method are the slightly yellow color of the blank, the instability of the stannous chloride solutions, and the variability in the intensity of color with time of heating. The objection to the second method is that the color is not stable and noticeable fading may be detected soon after the color is developed. The first method has no advantage over the other two. The second method has the advantage of developing color at room temperature and a more intense color for a given quantity of arsenic. These are outweighed by the disadvantage already mentioned. The third method offers several advantages: Hydrazine sulfate is stable in solution, the blank on the color reagents is water-clear, the intensity of the color for a given quantity of arsenic is independent of the time of heating the solution, provided the heating is long enough to develop the maximum color (10 minutes in the volumes used), and finally, the mixed reagents as described may be used.

TABLE I. DETERMINATION OF ARSENIC

Substance Analyzed	Arsenic Added	Arsenic Recovered	Percentage Recovery
	Micrograms	*Micrograms*	
Control	1[a]	0.95	95.0
	5[a]	5.00	100.0
	10[a]	10.00	100.0
	15[a]	14.50	96.6
	20[a]	19.00	95.0
Urine, 200 cc.	None	9.50	...
	50 as As_2O_3	60.50	102.0
Diet A, 10 grams	None	None	...
	500 as As_2O_3	484	96.8
Diet A, 5 grams	1140 as $Ca_3(AsO_4)_2$	1130	99.2
	1140 as $Ca_3(AsO_4)_2$	1150	100.9
Diet A, 10 grams	2280 as $Ca_3(AsO_4)_2$	2300	100.9
	2280 as $Ca_3(AsO_4)_2$	2280	100.0
Diet A, 20 grams	4560 as $Ca_3(AsO_4)_2$	4360	95.3
	4560 as $Ca_3(AsO_4)_2$	4400	96.6

[a] Six determinations were made at each level with a variation from 94 to 101 per cent recovery. This represents recovery of arsenic after distillation compared with known quantities not distilled.

Results with known quantities of arsenic are reported in Table I. The authors have made many determinations on biological materials, but the results of these findings are being withheld until the work is completed.

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