ANALYTICAL CHEMISTRY
Walter J. Murphy, Editor

Industry Must Provide Postgraduate Fellowships

From several speakers at the recent analytical symposia at Louisiana State University and Pittsburgh and in conversations with a number of teachers of analytical chemistry and industrial analysts during these meetings we have received fresh confirmation of the fact that only a mere handful of men and women are taking the necessary graduate training to fit them to be top-flight analysts, the type that ultimately fits into responsible positions in industry.

All but a few students at the graduate level turn to organic, physical, or some other field because analytical is considered to be less glamorous. They also firmly believe that opportunities are fewer in the analytical field than elsewhere and the financial remuneration often below that paid researchers generally.

Whether the field of analytical chemistry is less glamorous than, let us say, organic synthesis is a debate that we intend to dodge, but we will say that analytical today presents a worthy challenge for our best chemical minds and only those who are highly endowed and especially well trained can hope to reach successful pinnacles either in teaching or industry. There is no shortage of routine workers and laboratory technicians, but there is evidence that industry is finding it increasingly more difficult to obtain personnel capable of initiating, directing, and interpreting research in analytical chemistry including, of course, physicochemical and purely physical methods.

Industry has a direct interest in this situation, but unfortunately does not seem to be aware of what is happening. Industrial progress cannot continue at its present rapid rate if we have a serious undersupply of highly trained analysts who can direct the technicians. Wonderful strides in instrumentation have been made in the past ten years, more especially in the past five, but this pace will slacken because we shall not have sufficient chemists who know how to employ these aids.

Even a selfish viewpoint should dictate to industry the wisdom of making certain that more highly trained analysts are developed in the next decade than have been turned out in the past ten years.

There must be at least 25, possibly 50 companies in this country which could well afford to establish postgraduate followships in the field of analytical chemistry, yet we know of only two, J. T. Baker Chemical Co. and Standard Oil of New Jersey, which have established such fellowships at our colleges and universities. Possibly there are more, but we cannot recall them at the moment.

We need more than just fellowships at the graduate level—we need several chairs of analytical chemistry endowed so that we can attract and hold top-notch teachers in the analytical field. How many of our colleges and universities emphasize to any worth-while degree the field of analytical chemistry? We know just one, Louisiana State, where particular emphasis is being given to the task of training analysts. The field has been most fortunate in that men like Furman, Kolthoff, Willard, Mellon, and a few others by their personal magnetism have kept alive an interest sufficient to attract a fair number of younger men who at personal sacrifices continue to teach, but the number is wholly inadequate to train properly the men and women industry will need in the next ten or twenty years. The older teachers and the younger ones have labored and continue to labor under discouraging conditions.

If industry fails to undertake at least support of such fellowships as we have suggested, it will find in the not too distant future that its operations will be severely handicapped. Quality cannot be maintained without the analyst and he is now or should be a part of all research teams working on new products. Industry has saved tremendous sums through the introduction of new analytical techniques. Additional savings will be made only if proper personnel is available.

Industry should also realize that by giving a sizable number of postgraduate fellowships in analytical chemistry it will in effect be saying to the young chemist trying to determine which field to enter, "We think analytical chemistry is important."

Today most young men and women who have the qualifications to become analysts of professional stature turn their backs on the opportunity because they doubt that industry yet has a proper conception of the value of analytical chemistry. We do have, we feel certain, a goodly number of companies which recognize the true worth of the analyst and will back up that understanding on their part by establishing fellowships. We stand ready to give credit where credit is due.

Chemical Assay for Crystalline Benzylpenicillin

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A rapid and accurate method is presented for the determination of benzylpenicillin (G) in commercial crystalline penicillin. The method as written is based upon samples of 50 to 75 mg. of penicillin. However, samples as low as 5 mg. and as high as 5 gram have been determined by a similar procedure.

IT HAS been shown that at least four penicillins exist (*2, 4*)—called G (benzylpenicillin), F, X, and K—that prior to 1944 commercial penicillin was predominantly the G variety (*3*), and that during 1944 the G variety decreased with a relative increase in the less stable penicillin K (*1, 6*). The accepted view is that the present-day fermentation produces penicillins G and K predominantly with possibly some F and very little, if any, penicillin X.

The commercial production and marketing of crystalline penicillin G were hampered by the lack of suitable means for the standardization of penicillin G. Partition chromatography (*8*) and countercurrent distribution (*5*) have been used to advantage in the detection and quantitative estimation of penicillin mixtures. These procedures are somewhat long and tedious and consequently do not lend themselves to control of penicillin manufacturing. A microbiological differential assay method, recently described (*9*), suffers from the inaccuracies inherent in most bioassays.

The method described in this paper was developed (*10*) in the laboratories of Merck & Co., Inc., and has been accepted by the Food and Drug Administration (*7*) as the official method for certification of crystalline penicillin G.

In actual practice the aqueous solution of a salt of penicillin is acidified, extracted with a suitable acetate, precipitated by formation of the sparingly soluble *N*-ethylpiperidine salt in acetate-acetone mixtures, and weighed.

The *N*-ethylpiperidine method is a rapid and accurate (standard deviation 1.9% in nine determinations on pure benzylpenicillin with an average of 99.6%) procedure for the determination of penicillin G. Penicillin K and F do not precipitate under the conditions outlined (Table I). Penicillin X forms an amine salt and precipitates with *N*-ethylpiperidine, although not quantitatively (about 50%), and consequently this method is applicable only to penicillins relatively free of penicillin X as determined by some suitable method such as ultraviolet absorption. Since commercial penicillin at the present time contains very little, if any, penicillin X, as determined on commercial samples available on the market, this does not present a serious problem.

Table I. Determination of Penicillin G

Synthetic Mixtures, % Penicillin Theory				*N*-Ethylpiperidine Salt Calculated as % Penicillin G	
G	K	F	X		Av.
100	...	...	...	99.0, 100.2	99.6
...	100	...	...	0.0, 0.1	0
...	...	100	...	0.0, 0.2	0
...	...	...	100	54.0, 53.1	53.5
74.2	25.8	...	...	73.8, 74.2	74.0
80.1	...	19.9	...	78.9, 79.7	79.3

REAGENTS

Amyl Acetate Solution. Merck's pure amyl acetate (suitable for penicillin analysis) (any amyl acetate which will give a 99 to 100% assay on a sample of crystalline penicillin that is 100% pure) is saturated with the *N*-ethylpiperidine salt of penicillin G by adding 200 mg. of the salt to 100 ml. of the solvent and shaking. This solution is cooled between 0° and 8° C. before use and is filtered when being withdrawn for use by wrapping a plug of cotton on the tip of the pipet.

Phosphoric Acid Solution, prepared by dissolving 2.0 ml. of reagent grade phosphoric acid (85%) in 8.0 ml. of water and cooled to 0° to 8° C. before use.

***N*-Ethylpiperidine Solution,** prepared by diluting 2.0 ml. of reagent grade *N*-ethylpiperidine with 8.0 ml. of pure amyl acetate. This solution is saturated with the *N*-ethylpiperidine salt of penicillin G at room temperature (about 40 mg. of salt per 10 ml. of solution). It is cooled below 8° C. for at least 30 minutes before use, and is filtered by means of a plug of cotton on the tip of the pipet.

Acetone Solution. Reagent grade acetone is saturated with the *N*-ethylpiperidine salt of penicillin G at room temperature (about 400 mg. of salt per 100 ml. of acetone). The solution is cooled below 8° C. and filtered before use.

Sodium Sulfate. Powdered anhydrous reagent grade sodium sulfate is used.

PROCEDURE

Preparation of Sample. The crystalline penicillin G is weighed directly into tared centrifuge tubes of 10-ml. capacity; between 50 and 75 mg., or 100,000 international units of samples are used for each determination.

Extraction and Precipitation. To the sample in the tared centrifuge tube are added 2 ml. of cold distilled water and the tube is shaken to put the sample into solution. From a calibrated 2-ml. pipet the cold amyl acetate solution saturated with amine salt is added. Finally 0.50 ml. of cold phosphoric acid solution is added. The mixture is well shaken until the insoluble penicillin acid disappears. The tube is centrifuged for approximately 15 seconds, or sufficiently to get a separation of the two layers.

The amyl acetate layer is removed by means of a hypodermic syringe with a long needle (about 1.7 to 1.8 ml. recovered), and dried by passing over about 0.1 gram of anhydrous sodium sulfate. To accomplish this, the sodium sulfate is placed in a micro filter funnel (medium porosity) and the amyl acetate is collected in a small test tube contained in a suction flask. As little suction is applied as possible to effect filtration, in order to prevent evaporation.

In a tared 15 × 50 mm. weighing bottle are placed 1 ml. of the cold amine salt–saturated acetone and 0.50 ml. of the cold amine salt–saturated *N*-ethylpiperidine solution. From a calibrated pipet 1.0 ml. of the dried amyl acetate filtrate is added.

Filtration. The weighing bottle containing the mixture is set in the refrigerator for 2 hours, after which the solution is filtered through a tared micro filter stick and the precipitate washed with a total of 1 ml. of cold amine salt–saturated acetone from a 1-ml. hypodermic syringe with a fine needle. The weighing bottle and filter stick are dried under vacuum at room temperature for at least one hour and weighed.

Calculation. The assay can be calculated as per cent sodium penicillin G in the sample according to the following formulas:

$$\% \text{ sodium penicillin G} = \frac{\text{mg. of } N\text{-ethylpiperidine G} \times 159.3}{\text{mg. of sample}}$$

where

$$159.3 = \frac{\text{M.W. of sodium penicillin G} \times 200}{\text{M.W. of } N\text{-ethylpiperidine G}}$$

The per cent potassium penicillin G may be calculated in the same manner, except that the factor 166.4 is used instead of 159.3.

NOTE. The solutions used should be kept in an ice bath at temperatures below 8° C. For accurate analysis the time elapsed from the point of acidification to the precipitation of the salt must be kept as short as possible, preferably less than 3 minutes. The *N*-ethylpiperidine should be stored in brown bottles and protected from carbon dioxide absorption by being kept in a refrigerator or in a desiccator containing potassium hydroxide.

Table II. Comparison of Solvents Used to Extract Penicillin

Solvent	% Sodium Penicillin G A[a]	B[b]
Pure amyl acetate		
No. 1	99.6	95.2
No. 2	99.6	95.8
No. 3	99.1	95.8
No. 4	97.1	94.8
Isoamyl acetate	97.0	95.4
n-Propyl acetate (dried)	..	97.4
Isopropyl acetate (dried and distilled)	..	99.7
n-Butyl acetate (dried)	..	95.7

[a] Supplied by Food and Drug Administration as pure sample of sodium benzylpenicillin.
[b] Commercial crystalline sodium penicillin G.

SOLVENT

Although numerous acetates were tried (Table II), best results were obtained with a commercial grade of amyl acetate (pure amyl acetate). Recovery of benzylpenicillin differed with individual lots of this grade of amyl acetate. Therefore, it is advisable first to test the acetate used by determining the recovery with a known sample of pure sodium or potassium benzylpenicillin. The propyl acetates were not considered good solvents because of their solubility in water, which would tend to give high results.

PURITY OF SALT

The salt lends itself to rigid characterization. The ultraviolet absorption (determined by A. A. Colon) in water shows an E_m of 273 (at 257 mu), and the optical rotation is $[\alpha]_D^{23°} = +240°$ (1% in water). Analysis, calculated for $C_{23}H_{33}O_4N_3S$: C, 61.71; H, 7.43; N, 9.39. Found: C, 61.55; H, 7.30; N, 9.51. Against *S. aureus* the biological activity is 1300 international units per milligram.

The salt precipitated from pure sodium benzylpenicillin, when carried through the assay, gives recoveries of better than 98.5%, the rotation being $[\alpha]_D^{23°} = +240°$ (1% in water). When the amine salt from precipitated amorphous penicillin is assayed, recoveries of 97.5% are obtained with a rotation of +238°. This salt carries down some of the yellow pigment present in crude penicillin.

PRECIPITATION TIME

Sixty minutes in the refrigerator were found to give a quantitative precipitation of the amine salt, 99% being precipitated in 30 minutes and 94.5% in 10 minutes. The 2 hours' precipitation time allowed in the method is more than sufficient.

SATURATED SOLUTION

The solubility of the amine salt of benzylpenicillin in the various solvents is small (Table III) but nevertheless in actual practice low values are obtained (about 92%) if the solutions are not saturated.

DECOMPOSITION

When the aqueous solution of a penicillin salt is acidified to pH 2 to 2.5, decomposition begins at the rate of approximately 1% per minute for the first 15 minutes. After extraction with amyl acetate and drying the solution, the benzylpenicillin is fairly stable, the decomposition progressing at the rate of about 1.5% per hour. It is therefore necessary to acidify the penicillin solution, extract with amyl acetate, and dry the solution in as short a time as possible.

Table III. Solubility of Amine Salt of Benzylpenicillin

Solvent	Solubility at 25° C. *Mg./ml.*
Acetone	2.3
Amyl acetate	0.6
Amine solution	2.0

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RECEIVED June 14, 1947.

Automatic Potentiometric Titrations

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THIS paper describes an automatic potentiometric titration apparatus which is applicable to all types of reactions and all types of electrode combinations; has accuracy and precision equal to those obtainable by the manual method in the same titrations; is capable of either recording the entire titration curve, or stopping the titration at the equivalence point, and of performing successive titrations of two or more substances in the same solution; and provides successive additions of small increments of the titrant near the equivalence point as demanded by the potential of the indicator electrode, which permits application with fairly slow reactions without overrunning.

The several ingenious devices for automatic potentiometric titration described in the literature (*1, 2, 4, 7, 11, 12, 14, 15, 16*) possess some of the foregoing characteristics, but none possesses them all. In particular, the tentative approach to the equivalence point, which is essential to highly accurate operation, is a unique feature of the present autotitrator.

The instrument utilizes a motor-driven syringe for delivery of the titrant, which not only is capable of greater precision than buret delivery but also enables the delivery rate to be varied conveniently to suit the characteristics of the particular titration reaction. The titration cell is connected to a recording potentiometer, which either records the entire titration curve or else actuates a switch that controls the syringe motor and stops delivery of the titrant at the equivalence point.

APPARATUS AND GENERAL TECHNIQUE

As shown in Figure 1, the autotitrator employs a 50-cc. hypodermic syringe driven via a screw by a reversible synchronous motor at constant speed. The gear train provides a slow speed for addition of the reagent during a titration, and a high reverse speed for rapid refilling of the syringe.

The shaft speed of the motor is 60 r.p.m., and the gear shift provides the lower pulley shaft with speeds of 6 and 100 r.p.m. The shift is made by sliding the lower shaft, which is held in

position in each speed and in neutral by a phosphor bronze spring wire which engages shallow grooves in the shaft. The pulley cones and belt drive permit the speed ratio to be varied further from approximately 0.5 to 2. Thus screw speeds of approximately 3, 6, and 12 r.p.m. in "low," and 50, 100, and 200 r.p.m. in "high," may be selected.

The screw was cut from 0.25-inch (6.25-mm.) brass rod with 40 threads per inch (16 per cm.) over a length of approximately 4 inches (10 cm.).

The traveling nut which drives the syringe plunger is a brass block 1.5 inch (38 mm.) high and 0.75 inch (19 mm.) thick. Since the nut engages 30 threads on the screw, the ratio of linear movement to screw revolutions is very constant over the whole screw length. The nut carries two spring arms which slide on parallel brass rods to hold it in a vertical position. A hemispherical peg on the nut bears against a flat glass plate cemented to the end of the syringe plunger exactly at 90° to the screw shaft; with this point-to-plane contact no linear displacement results from rotary movement of the plunger, or slight sidewise oscillation of the nut.

Since the plunger displays a tendency to creep ahead of the nut, two coil springs anchored to the frame of the instrument are attached to opposite sides of a brass collar on the end of the plunger to hold it back against the nut under slight tension. The syringe rests in U-shaped slots in the instrument frame, and is clamped in such a way that it may easily be removed.

The plunger fitted the syringe barrel so precisely that it could be used without a lubricant. Usually, however, a very light film of stopcock lubricant was applied to the rear half of the plunger.

Because the plunger is driven at constant speed, the volume dispensed can be related either to time or to revolutions of the screw shaft. For the latter purpose a 5-figure Veeder-Root revolution counter is geared to the screw shaft as shown in Figure 1, the gear ratio being such that one revolution of the screw produces 66.67 counts.

Calibration was accomplished by weighing the water dispensed over measured time intervals and counter readings. Each revolution of the screw shaft delivered 0.4088 cc., corresponding to 0.006132 cc. per count. The delivery was uniform to ±0.03% at all positions of the plunger. It was also found that a small (ca. 1-cc.) bubble of air in the syringe had no effect on the delivery rate, provided the rate was not greater than about 5 cc. per minute, and hence the accidental inclusion of a small amount of air when the syringe is filled causes no error.

The syringe is connected by a short section of rubber tubing to a three-way stopcock which connects it either to the 1-liter storage flask or to the delivery tip. The storage flask is closed by a standard-taper joint fitted with a stopcock, so that the reagent solution may be stored under an inert gas if necessary. The syringe is filled rapidly by turning the stopcock to connect it to the storage flask and operating the motor in reverse in high speed.

Limit switches (Microswitches actuated by the traveling nut, and indicated by S_3 and S_4 in Figure 2) are provided to stop the motor at each end of the travel, so that the filling and dispensing proceed to completion without attention. By adjustment of the positions of these switches the apparatus can be set to deliver accurately any desired volume when this function is desired.

Before the initial reading of the counter is taken at the beginning of a titration, the syringe is allowed to run for about a half minute to take up any slack in the drive mechanism. Titrations were usually made at delivery rates of 1.3, 2.4, or 4.1 cc. per minute. The maximum permissible delivery rate depends on the characteristics of the particular titration. Several examples follow.

The electrical circuit is shown schematically in Figure 2. The e.m.f. of the titration cell was recorded by the Brown Elektronik potentiometer recorder of a Sargent Model XX visible recording polarograph. The characteristics of this instrument have been described (*8*).

In the Model XX polarograph the Brown potentiometer recorder is connected as a current measuring device to record the small (0 to 5 mv.) potential drop across a variable resistance (10 to 10,000 ohms). For the present purpose a range of 0 to 1 or 0 to 1.5 volts is necessary. This was achieved, without any alteration of the circuit of the polarograph, by simply connecting the titration cell to the outlet leads of the polarograph in series with a high resistance (R in Figure 2), and setting the bridge of the polarograph to zero. In this way the instrument functions as a high resistance voltmeter, and a small current is drawn from the titration cell. A series resistance of either 100,000 or 215,000 ohms was used, and the desired voltage sensitivity of the recorder was obtained by adjustment of the sensitivity control of the polarograph.

The instrument was calibrated by impressing a known e.m.f. from a potentiometer. Usually a sensitivity of 20 cm. per volt was used, with a 215,000-ohm series resistance, corresponding to a current drain from the titration cell of only 4.65×10^{-6} ampere per volt. This current is so small that the "electrolysis error" at the indicator electrode is negligible; even if the cell e.m.f. is as large as 1 volt, the amount of electrolysis over a 30-minute period amounts to only $30 \times 60 \times 4.65 \times 10^{-6}/96500$ or 8.7×10^{-8} equivalent, corresponding to only 0.00087 cc. of 0.1 N reagent solution.

In order that the instrument could be used to stop the titration at the equivalence point, as well as to record the entire titration curve, an enclosed tip type of mercury switch (S_1 in Figure 2) actuated by the recording potentiometer, was placed in the syringe motor circuit. This switch was fastened by an adjustable clamp to the shaft of the drum which drives the recorder slide-wire contact. (Pamphlets published by the Brown Instrument Co. give detailed information concerning the use of mercury switches with the Brown recorder.) By simply adjusting the angular position of the switch to correspond with the equivalence point potential, the instrument can be set to

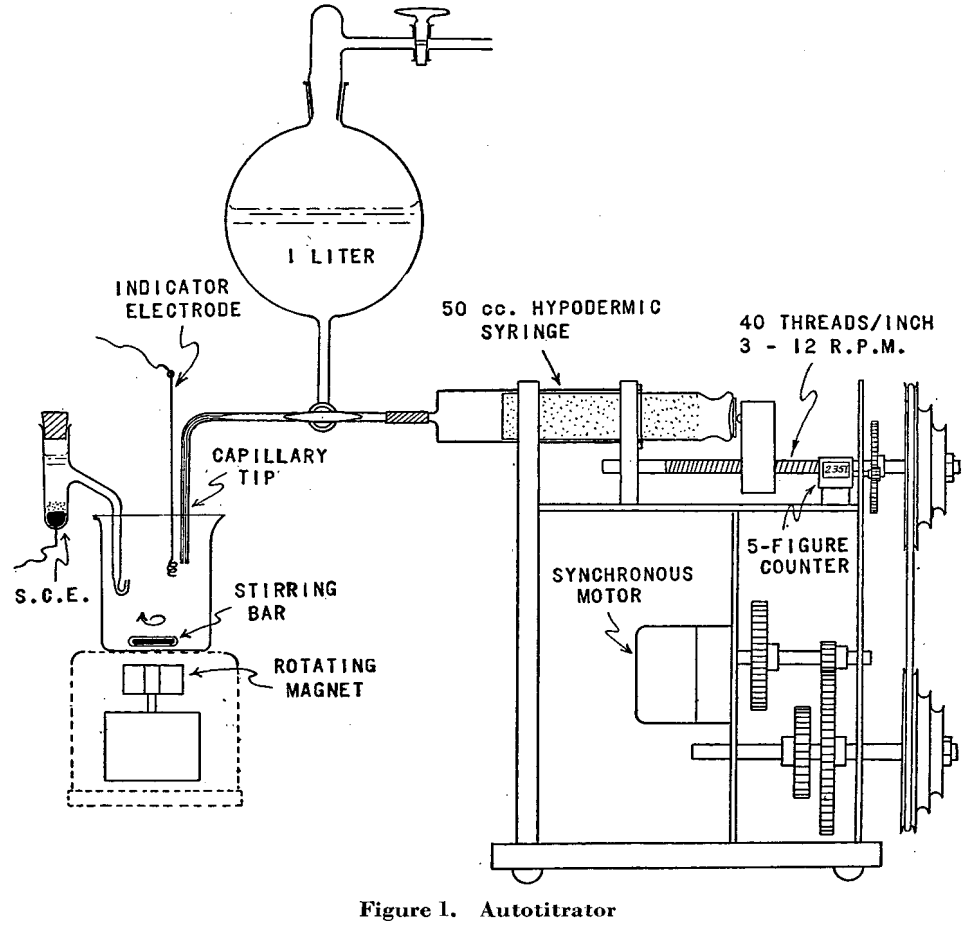

Figure 1. Autotitrator

An autotitrator is described which automatically performs potentiometric titrations with all types of reactions and all types of indicator electrodes, including the glass electrode. Precise delivery of the titrant is achieved by the use of a motor-driven hypodermic syringe. The titration cell is connected to a recording potentiometer which either records the entire curve or actuates a mercury switch in the motor circuit to stop delivery of the titrant at the equivalence point. The instrument adds the titrant in small increments as demanded by the potential change when the equivalence point is closely approached. In general, the accuracy and precision of the instrument are equal to manual methods with the same titrations. Using a platinum indicator electrode, automatic titrations of ferrous ion and vanadyl ion with ceric ion were precise and accurate to within ±0.1%, and equally good results were obtained in the titration of chloride ion with silver ion using a silver indicator electrode. Titrations of iodide ion with silver ion were accurate to 0.2%, the limiting factor being the great adsorptive properties of silver iodide. Mixtures of iodide and chloride ions were titrated with silver ion with the same accuracy (1 to 2%) as can be obtained by the manual method, the accuracy being limited by the coprecipitation of silver chloride with the silver iodide. Relatively dilute (ca. 0.006 *M*) sodium carbonate solutions were titrated with 0.1 *N* hydrochloric acid using the glass electrode. The bicarbonate end point was accurate and reproducible to ±0.4%, and the total titration to carbonic acid was accurate to ±0.1%, when volumes of titrant acid were 12 and 24 cc. in a total volume of ca. 200 cc.

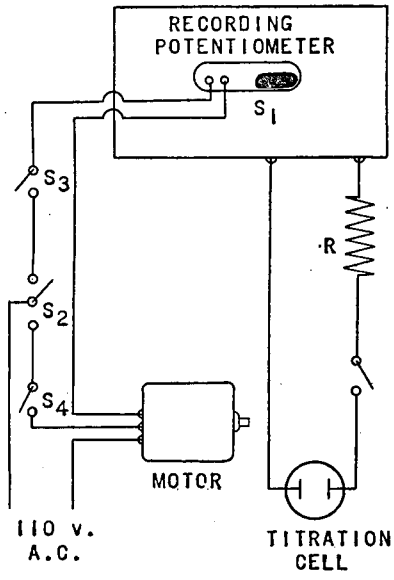

Figure 2. Schematic Electrical Circuit of Autotitrator

S_3 and S_4 are normally closed limit switches, and S_2 is a motor reversing switch. R should have a value of 100,000 ohms or more

open the motor circuit and stop the addition of the titrant at the equivalence point.

The angular dead zone of the switch is only 3°, corresponding to 1% of the recorder scale, but the cutoff point is reproducible to about ±0.3% of the scale length, or about ±0.005 volt when the recorder sensitivity is 20 cm. per volt.

When atmospheric gases are harmless an ordinary beaker serves as the titration vessel; in other cases a three-necked round-bottomed flask is used, and the solution is blanketed with an appropriate inert gas. The obvious requirement of very efficient stirring was satisfied by a glass-enclosed iron bar resting on the bottom of the vessel and rotated by a motor-driven magnet under the vessel as shown in Figure 1 (stirring apparatus of the Arthur H. Thomas Co., Philadelphia, Catalog No. 9235-R). Any other type of efficient stirrer would be equally satisfactory, although less convenient.

The indicator electrode is a stout wire of the appropriate metal (platinum, silver, etc.) whose lower end is wound as a small helix about 5 mm. high and 5 mm. in diameter. The usual type of saturated calomel reference electrode is employed. In titrations involving silver ion, and in other cases where chloride ion must be excluded, an agar bridge containing potassium nitrate was interposed between the saturated calomel electrode and the solution.

The syringe delivery tip, which is constructed of 0.5-mm. capillary tubing to minimize diffusion of the reagent into the solution, is immersed in the solution to provide continuous rather than dropwise addition of the titrant.

Overrunning is prevented by placing the indicator electrode close to the delivery tip, with the helix at the level of the tip and in front of it with respect to the direction of stirring (see Figure 1). Thus the potential of the electrode corresponds to the solution at a slightly more advanced stage of the titration than in the bulk of the vessel, which ensures that the titrator will properly anticipate the equivalence point. When the titration reaction proceeds very rapidly the indicator electrode is placed almost in contact with the delivery tip, but with slower reactions the electrode should be placed at some distance—i.e., 1 to 3 cm.—from the tip to allow sufficient time for the reaction to proceed as the solution travels from the tip to the electrode. The optimum separation of the electrode and delivery tip in any particular instance is easily established by trial. When the separation is optimum the potential will not drift significantly if the delivery is interrupted during the titration by stopping the syringe motor. If interruption of the delivery causes the potential to drift backward (away from the equivalence point potential), the electrode is too close to the tip, and if the potential drifts forward the electrode is too far from the tip.

Under proper conditions the instrument first stops momentarily slightly before the equivalence point is reached in the bulk of the solution. As soon as the syringe stops, stirring quickly equalizes the solution composition and, if the equivalence point has not been reached, the potential drifts rapidly backward and the resultant closing of the recorder switch causes the syringe to start delivery again. The potential then increases sharply, the recorder switch opens, and the delivery stops. This repetitive addition of small increments continues until the bulk of the solution attains the equivalence point potential, and the delivery then stops finally (see curve *b* in Figure 3). The instrument thus approaches the equivalence point tentatively and there is no tendency to overrun.

TITRATION OF FERROUS ION WITH CERIC ION

This titration was studied first because the reaction

$$Fe^{++} + Ce^{++++} = Fe^{+++} + Ce^{+++} \qquad (1)$$

is rapid, the indicator electrode functions reversibly, and the potential change at the equivalence point is very great. Hence it served as an optimum case to provide experience.

Weighed amounts of pure ferrous ammonium sulfate hexahydrate were dissolved in approximately 200 cc. of 1 *N* sulfuric acid and titrated with 0.1 *N* ceric ammonium nitrate at room temperature with a platinum indicator electrode and a saturated calomel reference electrode. Because Reaction 1 is very rapid, the indicator electrode was placed very close to the delivery tip.

Curve *a* in Figure 3 is a typical automatic recording of the entire titration of 0.5000 gram of ferrous ammonium sulfate, and curve *b* was obtained with the recorder switch set to cut off the syringe motor at +0.65 volt *vs.* the saturated calomel electrode. The delivery rate of the ceric solution was 1.3 cc. per minute. From curve *b* it is seen that sixteen tentative increments were

added before the apparatus stopped finally, and this action is characteristic of proper operating conditions. In curve *b*, Figure 3, 24 seconds elapsed between the addition of the next to the last and the last increment, and this last increment amounted to only 0.012 cc. or 0.1% of the total volume of ceric solution used.

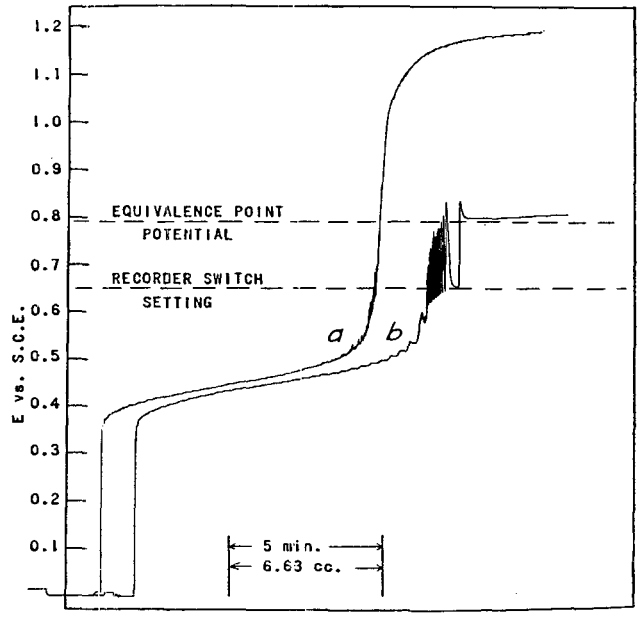

Figure 3. Automatic Titration of Ferrous Ion with Ceric Ion in 1 *N* Sulfuric Acid

The recorder switch should be set so that when the titrator stops finally the potential of the indicator electrode will coincide with the equivalence point potential of the particular titration. In a titration like the present one, where $\Delta E/\Delta V$ is very large, this condition is satisfied by adjusting the recorder switch to open at a value somewhat smaller than the equivalence point potential. From manual potentiometric titrations under the same conditions the equivalence point potential of the ferrous-ceric reaction was found to be +0.79 volt *vs.* the saturated calomel electrode. The optimum setting of the recorder switch for the automatic titrations at a delivery rate of 1.3 or 2.4 cc. per minute was found to be +0.65 to +0.70 volt, and when so set the final potential after the titrator stopped was always very close to the equivalence point potential (see Figure 3).

In four automatic titrations of 1.0000-gram samples of ferrous ammonium sulfate hexahydrate, at a delivery rate of 1.3 cc. per minute, the observed counter readings were 4145, 4135, 4142, and 4144, or an average of 4140 ± 4, corresponding to 25.39 ± 0.02 cc. of the ceric solution. At a delivery rate of 2.4 cc. per minute, three titrations yielded counter readings of 4143, 4139, and 4143, the average being 4142 ± 2, or 25.40 ± 0.01 cc. When 1-gram samples of the same ferrous ammonium sulfate were titrated by the ordinary manual method using *o*-phenanthroline ferrous ion indicator, the equivalent volume of the ceric solution was 25.40 cc. Thus the average of the automatic titrations agreed exactly with the ordinary manual method, and the precision was considerably better than ±0.1%.

Automatic titrations of two 0.5000-gram samples of the Mohr salt gave counter readings of 2068 and 2072, corresponding to 12.68 and 12.70 cc., compared to 12.70 cc. by the ordinary manual method.

As an additional check on the automatic titrations, 0.1 cc. of 0.025 *M* *o*-phenanthroline ferrous ion indicator was added after the titrator stopped. In four cases tested the indicator showed a pale orange color, and manual addition of only 1, 1.5, 2 and 1.5 counts (average 0.009 cc.) of the ceric solution was sufficient to discharge the reduced color of the indicator.

TITRATION OF VANADYL ION WITH CERIC ION

The reaction

$$VO^{++} + Ce^{++++} + H_2O = VO_2^{+} + Ce^{+++} + 2H^{+} \qquad (2)$$

proceeds rather slowly in dilute sulfuric acid solution. Furthermore, at 1 *M* hydrogen-ion concentration, the standard potential of the VO_2^{+}-VO^{++} half-reaction is rather large (+1.00 volt *vs.* the standard hydrogen electrode), so the potential change at the equivalence point is considerably smaller than in the ferrous-ceric titration. Thus the reaction provides a good test of the autotitrator under adverse circumstances.

The characteristics of the titration by the manual potentiometric method are well known from the studies of Furman (*3*) and Willard and Young (*17*). These investigators concluded that the titration was impractical in dilute sulfuric acid at room temperature because of the slowness of Reaction 2, but they obtained satisfactory results when the titration was performed at a temperature of 60° to 80° C. In agreement with these conclusions the writer found in manual titrations at 80° in 1 *N* sulfuric acid that the potential becomes constant 1 to 2 minutes after each increment of ceric solution is added. The equivalence point potential under these conditions was found to be +0.975 volt *vs.* the saturated calomel electrode.

Figure 4 shows automatically recorded titration curves under different conditions. The dashed curve is a plot of data obtained by the ordinary manual method in a titration at 80°. In all cases, 10-cc. portions of a 0.0958 *N* vanadyl sulfate solution were diluted to approximately 200 cc. and 5.6 cc. of concentrated sulfuric acid were added to provide a sulfuric acid concentration of 1 *N*. The solutions were titrated with 0.1002 *N* ceric ammonium nitrate solution at a delivery rate of 1.3 cc. per minute.

Curve 1 resulted when the titration was made at room temperature with the platinum indicator electrode almost touching the outflow tip of the syringe. Because of the slowness of the reaction and the close proximity of the electrode and delivery tip, the recorded potential under these conditions is actually greater than the equivalence point potential right from the start of the titration, and only a very slight inflection is observable at the equivalence point. Between points *a* and *b* the delivery was interrupted by stopping the syringe motor, and it is seen that the potential drifted rapidly backward nearly 0.4 volt before equilibrium was attained.

Curve 2 was obtained when the titration was performed at 80°, with the indicator electrode almost touching the outflow tip. Some improvement in the recorded potential is observed, but it is still about 0.2 volt too large, as the interrupted portion *a* to *b* shows.

Since the large backward potential drift in curves 1 and 2 when the delivery was interrupted indicated that the electrode was too close to the delivery tip with respect to the rate of reaction, curve 3 was recorded at 80° after placing the platinum indicator electrode about 2 cm. in front of the delivery tip to allow a short time for the reaction to proceed between the tip and the electrode. Under these conditions the curve is normal, and its shape conforms closely to the dashed curve obtained by manual titration. When the delivery was interrupted (points *a* to *b*) the potential change was very slight, showing that the electrode and delivery tip were properly positioned.

The oscillations, which are especially pronounced in curve 2 of Figure 4, apparently were caused by the cavitation produced by the rapid stirring, which imparts a small oscillatory vertical motion to the solution as it rotates and apparently causes variations in the conditions of flow of the solution between the delivery tip and indicator electrode. Such oscillations are not objectionable, provided they are no larger than in Figure 4.

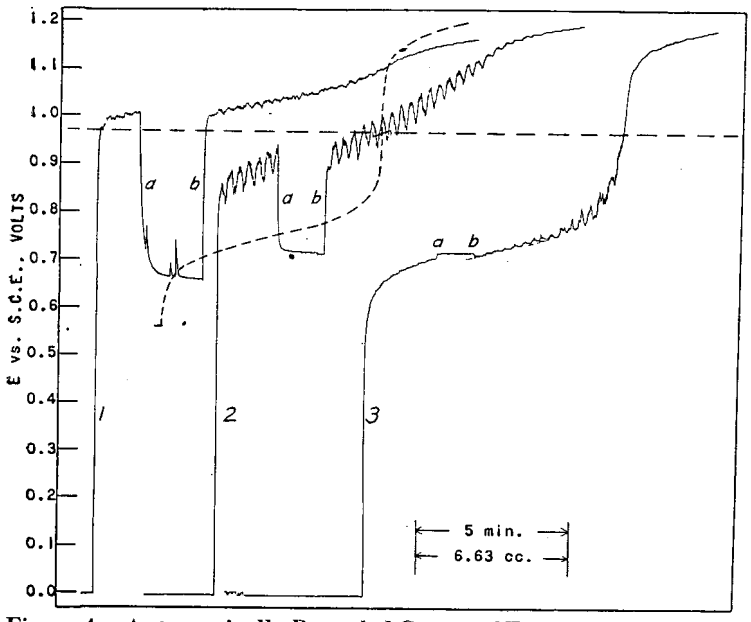

Figure 4. Automatically Recorded Curves of Titrations of Vanadyl Ion with Ceric Ion in 1 *N* Sulfuric Acid

Dashed curve is a plot of a manual titration at 80° C.

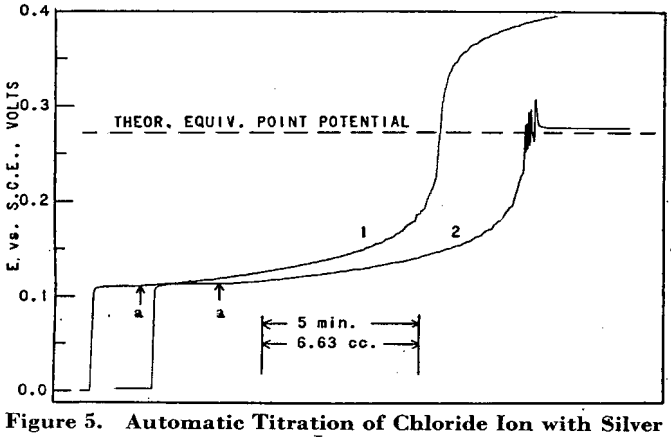

Figure 5. Automatic Titration of Chloride Ion with Silver Ion

Delivery begun at points *a*

In three titrations of 24.98-cc. samples of the vanadyl solution under the conditions of curve 3 of Figure 4, with the recorder switch set at +0.95 volt, counter readings of 3898, 3896, and 3888 were obtained, the average being 3894 ± 4 or 23.88 ± 0.02 cc. The equivalent volume of the ceric solution determined by manual potentiometric titrations was 23.87 cc. Thus in spite of the unfavorable characteristics of this titration, the titrator yields results that are precise and accurate to within 0.1% when the conditions are chosen properly.

TITRATION OF CHLORIDE ION WITH SILVER ION

This is a good example of a titration involving a rapid reaction and reversible behavior of the indicator electrode, but a relatively small potential change at the equivalence point.

A standard 0.1040 *N* silver nitrate solution was prepared determinately from pure silver and it contained a slight excess of nitric acid (ca. 0.05 *N*). A standard 0.05000 *N* sodium chloride solution was prepared determinately from pure sodium chloride. The concentrations of both solutions were known to better than 0.1%.

Measured volumes of the sodium chloride solution were diluted to approximately 200 cc. and titrated at room temperature with the silver solution; a silver wire was used as indicator electrode. A glass U-tube filled with 2 *N* potassium nitrate in 3% agar served as salt bridge between the saturated calomel reference electrode and the solution. The silver indicator electrode was placed virtually in contact with the outflow tip.

As the entire potential change is no more than about 0.3 volt during the complete course of a chloride-silver titration, the recorder was adjusted to a sensitivity of 40 cm. per volt (0.7 volt full scale) with a 100,000-ohm resistance in series with the titration cell.

Curve 1 in Figure 5 is a recording of a complete titration. Curve 2 was obtained with the recorder switch set at +0.270 volt *vs.* the saturated calomel electrode, the latter value being the theoretical equivalence point potential calculated from the standard silver-silver ion potential and the solubility product of silver chloride. Since the potential change in this titration is not very large, the recorder switch should be set exactly at the equivalence point potential, rather than at a smaller value as in cases where $\Delta E/\Delta V$ is large.

In six automatic titrations of 24.98-cc. portions of the 0.05000 *N* sodium chloride solution at a delivery rate of the silver solution of 1.3 cc. per minute the observed counter readings were 1957, 1958, 1957, 1952, 1957, and 1955, and the average 1956 ± 1.7 or 11.99 ± 0.01 cc. This is in excellent agreement with the theoretical 12.00 cc.

Even at the rapid delivery rate of 4.1 cc. per minute, very good results were obtained. In three titrations the observed counter readings were 1959, 1961, and 1953, and the average was 1958 ± 3, or 12.00 ± 0.02 cc., in exact agreement with theory.

It is evident that the automatic titrations are fully equal in both precision and accuracy to the manual methods of titrating chloride ion with silver ion.

TITRATION OF IODIDE ION WITH SILVER ION

Since the solubility product of silver iodide is very much smaller than that of silver chloride, the potential change at the equivalence point is much larger in the titration of iodide ion than in the titration of chloride ion. However, as a result of the great adsorption of silver and iodide ions by silver iodide, the titration of iodide ion actually is not so accurate as the chloride titration when ordinary concentrations are involved. Kolthoff and Lingane (*6*) have shown that the isoelectric point (zero adsorption of lattice ions) of silver iodide is at pAg = 6.0 instead of pAg = 8.0 (the equivalence point), that at the equivalence point the precipitate retains a very appreciable amount of adsorbed iodide ion, and that it is not easy to obtain an accuracy of better than 0.05% in the manual potentiometric titration of iodide ion with silver ion. To achieve this degree of accuracy it is necessary to wait a long time for the establishment of adsorption equilibrium and the attainment of steady potentials after each increment of silver solution is added. It was therefore of considerable interest to investigate the behavior of the autotitrator in this case.

A 0.05000 *N* potassium iodide solution was prepared from reagent quality potassium iodide which had been dried at 150°. Measured volumes of this solution were diluted to about 200 cc. and titrated at room temperature with 0.1040 *N* silver nitrate solution. The titration conditions were the same as in the titration of chloride ion, except that the recorder sensitivity was 20 instead of 40 cm. per volt. The silver indicator electrode was placed as close as possible to the outflow tip.

Curve 1 in Figure 6 is a recording of a complete titration of 40 cc. of 0.05 *N* potassium iodide solution in 200 cc. with 0.1040 *N* silver nitrate solution, and curve 2 was obtained with the recorder switch set at +0.08 volt *vs.* the saturated calomel

electrode. The delivery rate of the silver solution was 2.4 cc. per minute.

The equivalence point potential is +0.093 volt *vs.* S.C.E. (*6*). Since the potential change at the equivalence point is large, the recorder switch was set at the slightly smaller value +0.08 volt.

In three titrations of 49.97-cc. portions of the 0.05000 *N* potassium iodide solution, at a delivery rate of 2.4 cc. per minute, the observed counter readings were 3926, 3924, and 3930, the average being 3927 ± 3, or 24.08 ± 0.02 cc. This is 0.25% greater than the theoretical 24.02 cc.

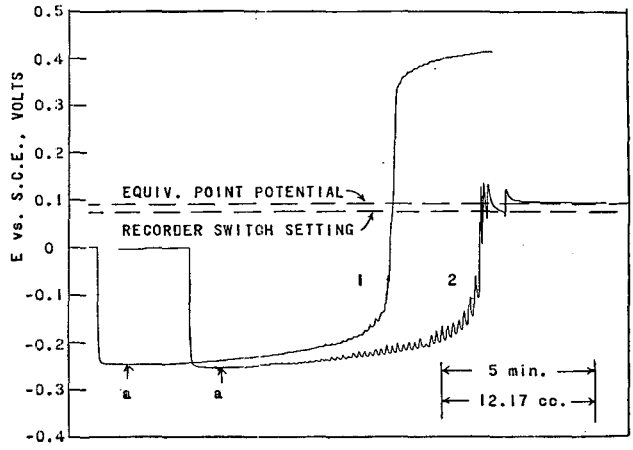

Figure 6. Automatic Titration of Iodide Ion with Silver Ion

Delivery started at points *a*

Three titrations of 24.98-cc. portions of the 0.05000 *N* potassium iodide solution yielded counter readings of 1963, 1963, and 1962; the average was 1963 ± 1, or 12.033 ± 0.006 cc., compared to the theoretical 12.003 cc.

Since the addition of salts like barium nitrate tends to decrease coprecipitation and improve results in the manual iodide-silver titration (*5, 10*), three 49.97-cc. portions of the 0.05000 *N* potassium iodide solution were titrated automatically after addition of 10 grams of pure barium nitrate per 200 cc. The observed counter readings were 3926, 3920, and 3927; the average was 3924 ± 3, or 24.06 ± 0.02 cc., compared to the theoretical 24.02 cc. Thus in the presence of barium nitrate the titration error is reduced from +0.25 to +0.17%.

In view of the difficulties encountered in this titration by the manual method, the results of the automatic titrations are satisfactory. By setting the recorder switch at a somewhat smaller value there is no doubt that the small titration error could be eliminated completely.

SUCCESSIVE TITRATIONS OF IODIDE AND CHLORIDE IONS WITH SILVER ION

It is well known (*5, 10*) that in the manual argentimetric titration of iodide-chloride mixtures the iodide value tends to be too large, and the chloride value correspondingly too small, because chloride ion coprecipitates with the silver iodide before the iodide equivalence point is reached. It was therefore of interest to determine whether the conditions extant in automatic titrations would lead to better or worse results than in the manual method.

Figure 7 shows a recording of the automatic titration of 200 cc. of a solution containing 40 cc. of 0.05 *N* potassium iodide and 25 cc. of 0.05 *N* sodium chloride with 0.1 *N* silver nitrate. The titration conditions were the same as in the separate halide titrations described above. The delivery rate was 2.4 cc. per minute. The change in slope of the curve near the middle of the iodide inflection point reflects coprecipitation of chloride ion with the silver iodide. If there were no coprecipitation, the precipitation of chloride from the 0.005 *M* concentration present would not have begun until the potential had reached +0.12 volt, and the curve would have shown a sharp bend at that potential as indicated by the dashed section.

Figure 8 shows curves obtained in three titrations under different conditions of mixtures of 24.98 cc. each of 0.05000 *N* potassium iodide and 0.05000 *N* sodium chloride. In each case the initial volume was approximately 200 cc., and the delivery rate of the 0.1040 *N* silver nitrate solution was 2.4 cc. per minute. The recorder switch was first set for the iodide end point, and after this had been reached and the counter reading noted, the recorder switch was reset to +0.270 volt for the chloride titration.

Curves 1 and 2 were obtained with the recorder switch set at +0.05 and 0.00 volt, respectively, for the iodide titrations. It is clear that a setting of +0.05 volt is too large, and that 0.00 volt is more nearly the optimum value. With the recorder switch set at +0.05 volt the counter reading for the iodide titration corresponded to 12.38 cc., which is 3.1% larger than the theoretical 12.00 cc., and the reading for the chloride titration was 11.57 cc. or 3.6% smaller than the theoretical value. With the recorder switch set at 0.00 volt (curve 2), the corresponding readings were 12.23 cc. (error, +1.9%) and 11.78 cc. (error, −1.8%), a marked improvement.

Curve 3 was obtained in the presence of 10 grams of pure barium nitrate per 200 cc., and with the recorder switch set at 0.00 volt for the iodide titration. The characteristics of this curve are better than in the absence of barium nitrate. The counter reading for the iodide titration was 12.13 cc. (error, +1.1%) and for the chloride titration it was 11.85 cc. (error, −1.2%).

As in manual titrations, addition of barium nitrate decreases but does not entirely eliminate the error due to coprecipitation. The errors in the automatic titrations are of the same order of magnitude as in the manual method (*5*).

ACID-BASE TITRATIONS

Acid-base titrations with low resistance electrodes (hydrogen, quinhydrone, antimony) may be carried out without any modification of the autotitrator. For titrations with the glass electrode a linear electronic amplifier must be interposed between the titration cell and the potentiometer recorder. The line-operated, direct-reading Macbeth pH Meter (Macbeth Corp., New York, N. Y.) was easily adapted to this purpose. This instrument is amply accurate (±0.05 pH unit) for titrations, and its operation is exceptionally simple and convenient.

The Sargent Model XX polarograph was simply connected in series with the indicating microammeter of the Macbeth

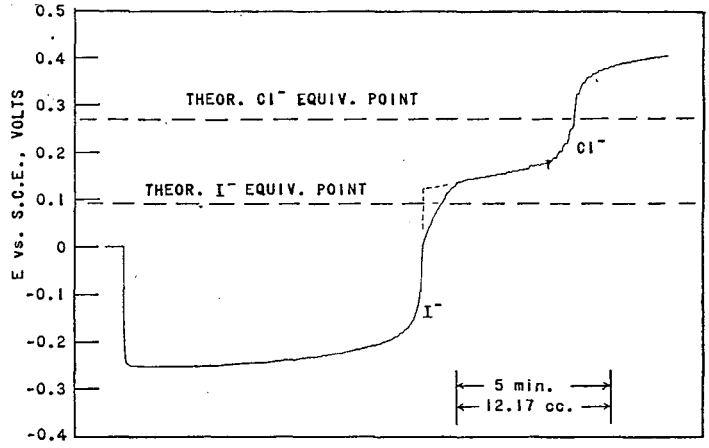

Figure 7. Automatically Recorded Curve of Titration of a Mixture of Iodide and Chloride Ions with Silver Ion

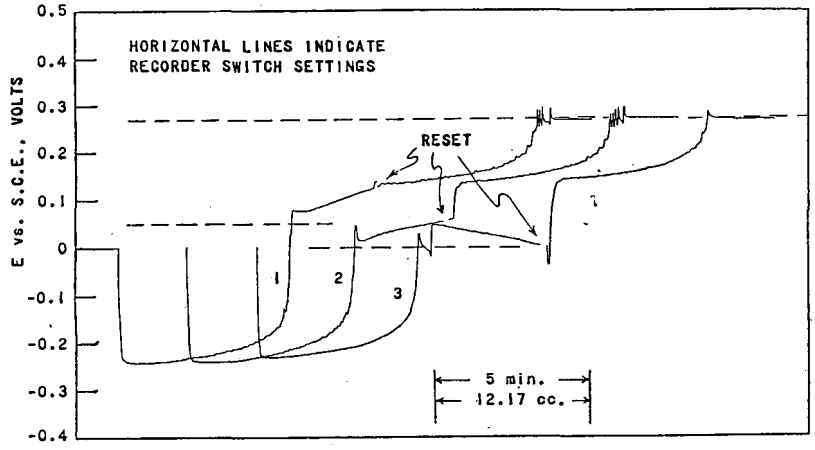

Figure 8. Automatic Titration of Iodide-Chloride Mixtures with Silver Ion

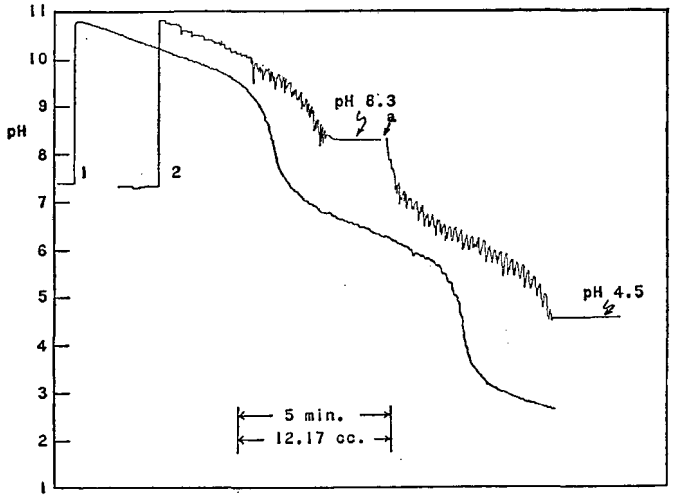

Figure 9. Automatic Titration of Sodium Carbonate with Hydrochloric Acid Using Glass Electrode

instrument, and the recorder sensitivity was adjusted so that full scale (28 cm.) corresponded to 14 pH units. Two binding posts were attached to the case of the Macbeth instrument in series with the meter circuit for convenient connection of the recorder leads. By merely connecting a copper wire jumper across these terminals the instrument could be returned to ordinary use. The introduction of the relatively low resistance (96 ohms) of the polarograph circuit did not alter the characteristics of the Macbeth instrument; hence the operational technique was exactly as in the ordinary use of the instrument.

The only difficulty encountered was that operation of the switches in the motor circuit of the titrator generated a transient signal which was picked up by the Macbeth meter and recorder, and this was eliminated by placing a capacitor-resistance combination (0.2 microfarad and 300 ohms) across the switch terminals.

The titration of carbonate ion with hydrogen ion was selected to test the autotitrator under circumstances which are sufficiently unfavorable to constitute a convincing test of its performance. The pH changes at the two equivalence points in this titration are so small that it is difficult to obtain precise results by the manual titration methods (*5*, *10*).

A standard 0.02570 *M* sodium carbonate solution was prepared determinately from reagent quality sodium carbonate which had been dried at 300°, and a standard 0.1069 *N* hydrochloric acid solution was prepared from the constant-boiling acid. Measured volumes (usually 49.97 cc.) of the sodium carbonate solution were diluted to approximately 200 cc. in a 250-cc. beaker and titrated with the standard hydrochloric acid at a delivery rate of 2.4 cc. per minute. The initial concentration of carbonate ion was only about 0.006 *M*.

Curve 1 in Figure 9 is an automatic recording of the complete titration. In this titration the glass electrode was placed about 1 mm. in front of the delivery tip.

From the dissociation constants of carbonic acid, 4.3×10^{-7} and 4.7×10^{-11} (*9*, *13*), the calculated pH at the bicarbonate equivalence point is 8.35, and at the carbonic acid point it is 4.30 for 0.006 *M* carbonic acid. The two inflection points in curve 1 agree very well with these values.

Curve 2 in Figure 9 is a typical record of an automatic titration in which the recorder switch was set first at pH 8.5 for the bicarbonate point, and then reset at point *a* to pH 4.6 for the final titration to carbonic acid. These settings of the recorder switch were established by trial; that they are optimum is demonstrated by the fact that the final values (8.3 and 4.5) after the titrator stopped at each end point agree with the theoretical values.

Curve 2 was recorded with the glass electrode placed at an angle and with the bulb in direct contact with the delivery tip; the fluctuations in the curve result from small variations in the stirring rate. Because of its relatively large size (area approximately 5 sq. cm.) the bulb of the glass electrode scans a large area of the solution, and hence it must be placed as close as possible to the delivery tip.

Four titrations of 49.97-cc. portions of the sodium carbonate solution were performed under the same conditions as curve 2 of Figure 9. The observed counter readings for the bicarbonate point were 1946, 1962, 1942, and 1956, the average being 1952 ± 8, or 11.97 ± 0.05 cc. The theoretical equivalent volume is 12.01 cc. In these same titrations the counter readings for the total titration to carbonic acid were 3918, 3914, 3918, and 3924, the average being 3919 ± 3, or 24.03 ± 0.02 cc., which agrees to better than 0.1% with the theoretical 24.02 cc. The precision and accuracy of these results are fully equal to what could be obtained in manual titrations with the glass electrode, and are considerably better than could be achieved in titrations with indicators in similarly dilute solutions.

It is obvious that titrations of strong acids and bases will yield even better precision and accuracy.

Titrations with sodium hydroxide solutions may, of course, also be performed. To prevent excessive attack of the glass strongly alkaline solutions should not be allowed to stand in the syringe for a long time, and it is advisable in such cases to use syringes of Pyrex.

CONCLUSIONS

The foregoing experiments demonstrate that the accuracy of the autotitrator is fully as good as manual potentiometric methods in all types of titrations. Since delivery from a syringe is inherently more precise than from a buret of equal volume, it should be possible to refine the instrument so that its precision would greatly excel that of manual titrations with a buret. By using a smaller syringe—e.g., 1 or 5 cc.—the instrument can be adapted to titrations on a semimicro scale without any significant decrease in precision.

Tentative approach to the equivalence point is essential if precise results are to be obtained in an automatic titration, and measurement of the equivalent volume from the inflection point of a complete automatically recorded curve cannot be recommended. To make the inflection point of such a curve—e.g., curve *a* in Figure 3—correspond to the true equivalent volume requires a much too critical empirical matching of the stirring rate and distance between the outflow tip and indicator electrode to the particular reaction rate.

Although accuracy rather than speed has been of primary concern in this investigation, automatic titrations with the present instrument absorb less operator time than the same titrations by the manual method. It is not feasible to increase the delivery rate much above 4 cc. per minute in most titrations, because overrunning is inevitable if the equivalence point is approached too rapidly. If greater speed is desired it should be obtained by adding most of the titrant at a rapid rate and then finishing the titration more slowly as in manual methods. This could be achieved relatively simply by employing a syringe-drive motor whose speed can be varied over a three- or five-fold range by a series resistance. A permanent resistor could be placed in series with the motor to establish the slow speed, and a second mercury switch operated like switch S_1 by the recording potentiometer would be connected across this resistor in the normally closed position to short out the resistor for high-speed delivery. This second switch would be set to open slightly before the equivalence point, so that most of the titrant would be delivered rapidly and the equivalence point would finally be approached at a slower speed just as in manual titrations.

The use of an expensive recording potentiometer is, of course, not necessary, since the titration curve does not have to be recorded once its characteristics are known. Any commercial potentiometer controller should be satisfactory, provided it has rapid response, proper range, and input characteristics (high impedance) suitable for electrode systems. A relatively inexpensive electronic trigger circuit has been developed in this laboratory to replace the potentiometer recorder, and this instrument will be described in a following paper.

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RECEIVED July 12, 1947.

Improvements in Rubber Testing in the Government Synthetic Rubber Program

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In the government synthetic rubber program, the precision and accuracy of testing rubber have been increased severalfold. The values of the standard deviations obtained at the beginning of the program in 1943 and 1944 were two to fifteen times as large as those obtained at present. The improvement in testing resulted from standardization and improvement of technique, refinement of existing methods of test, and development of improved methods of test. Each of these approaches is reviewed. The results which have been realized in this program indicate that a high degree of precision can be obtained if care is taken to control test conditions and proper technique is employed. Such precision has been attained without a significant increase in man-hours or cost of equipment.

THE purpose of this paper is to report the improvements in precision and accuracy of rubber testing achieved in the government synthetic rubber program during the past four years. These improvements have resulted largely from the activities of the Office of Rubber Reserve and its associated committees and synthetic rubber plants.

In 1943, the Office of Rubber Director, War Production Board, and the Rubber Reserve Company (now Office of Rubber Reserve, Reconstruction Finance Corporation) decided that to meet the rubber crisis the government plants should produce a single, general-purpose synthetic rubber of the butadiene-styrene type, which was designated GR-S. This decision necessitated that the production from all of the fifteen government GR-S plants be interchangeable and uniform in quality. To attain this goal, the Office of the Rubber Director and Rubber Reserve (*2*) jointly established a Committee on Specifications for Synthetic Rubbers, which held its first meeting on May 12, 1943. Besides representatives of these two agencies, representatives of the rubber compounders, the operators of the synthetic rubber plants, and the National Bureau of Standards were members of this committee.

The Committee on Specifications for Synthetic Rubbers recognized at its first meeting the poor reproducibility of testing then existing within and among the rubber laboratories. It also realized that a uniform product could not be achieved unless the precision and accuracy of testing were improved. Consequently, a Subcommittee on Physical Testing Methods was established, which in May 1944 became the Subcommittee on Test Methods (*2*) and thereafter embraced both chemical and physical testing. In order to reduce the variability in rubber testing, the activities of these committees and the Office of Rubber Reserve were directed toward:

1. Standardization and improvement of technique
2. Refinement of existing methods of test
3. Development of improved methods of test

STANDARDIZATION AND IMPROVEMENT OF TECHNIQUE

When the synthetic rubber plants began operation, only a few of the laboratory supervisory personnel had had any experience in the testing of rubber. Therefore, before the plants began production, the laboratory personnel were given several

Table I. Analysis of Blended GR-S Sample H by 17 Synthetic Rubber Laboratories

Plant	Acetone Extract	Fatty Acid	Soap	Ash	Viscosity GR-S ML.[b]	Viscosity Compound ML.[b]	Tensile[a] Strength	Ultimate Elongation[a]	Modulus at 300% Elongation 25-minute cure	50-minute cure	90-minute cure
	%	%	%	%			*Lb./sq. inch.*	%	*Pounds per square inch*		
0	7.7	4.8	0.15	1.00	46.5	57.0	3040	610	480	1080	1460
1	6.9	4.9	0.15	1.00	47.0	49.0	3000	625	490	1030	1525
2	6.7	4.5	0.20	1.15	50.0	65.0	3045	610	560	1015	1385
3	7.5	5.3	0.15	1.15	51.0	62.0	3025	600	550	1125	1600
4	7.3	4.7	0.10	1.00	49.0	60.0	2920	600	450	990	1380
5	7.1	4.8	0.10	1.00	52.0	55.0	3250	580	700	1210	1500
6	7.2	4.6	0.15	1.00	47.0	58.0	2940	625	510	925	1365
7	7.0	5.0	0.10	0.90	48.0	65.0	3020	640	550	1010	1290
8	7.0	4.6	0.10	1.05	50.0	61.0	3010	605	600	1115	1510
9	6.0	4.2	0.15	0.60	48.0	58.0	2890	610	465	1005	1400
10	6.6	4.8	0.10	1.05	50.0	66.0	2790	580	500	1065	1490
11	6.8	5.2	0.10	1.00	52.0	55.0	3070	625	455	985	1310
12	7.0	5.0	0.15	0.90	48.0	57.0	3110	575	500	1115	1440
13	6.6	4.7	0.10	1.05	49.0	62.0	3060	610	540	1015	1360
14	6.8	5.0	0.15	1.00	49.0	60.0	2980	615	510	1020	1320
15	7.0	4.9	0.10	1.05	55.0	66.0	2970	590	575	1040	1380
16	7.0	4.8	0.15	1.00	...	...	3075	600	590	1100	1410
High	7.7	5.3	0.20	1.15	55.0	66.0	3250	640	700	1210	1600
Low	6.0	4.5	0.10	0.60	46.5	49.0	2890	575	450	925	1290
Average	7.0	4.8	0.15	1.00	49.5	59.8	3010	605	530	1050	1420
Standard deviation	0.38	0.25	0.036	0.117	2.16	4.52	97	17	61	66	82

[a] 50-minute cure. All cures at 292° F.
[b] ML., Mooney viscometer with large rotor.

weeks' training to familiarize them with the various techniques. Nonetheless, it was a common experience to have extremely erratic results reported from a single laboratory. Likewise, marked variation was noted among the results of tests from the different laboratories on a single sample of blended rubber. The results obtained on one such sample are shown in Table I. These results, obtained after nearly one year of operation, made it apparent that testing variability had to be reduced if a uniform product interchangeable from plant to plant were ever to be realized.

One of the most important steps taken to standardize testing was the establishment of reference lots of rubbers for daily tests by each analyst. The primary requisite for a reference lot is uniformity, both from bale to bale and within each bale. To prepare such a lot of GR-S, a designated plant segregates during normal operation sufficient latex for 50,000 to 60,000 pounds of dry rubber. This latex is thoroughly agitated and then coagulated and dried on a single finishing line. Three hundred bales (20,000 to 25,000 pounds) from the central portion of the run are selected for the reference lot. Such a lot is uniform with respect to physical properties, but varies slightly in ash content and other chemical properties because of slight variations in the washing process. Consequently, for the chemical analyses, three of the bales are thoroughly blended on a large mill. This blended material becomes the reference lot in control testing for the chemical properties. Each plant receives a sufficient quantity of the blended material and enough bales of the unblended portion of the lot for a daily appraisal of testing for each property over a period of six months.

From each bale used by the plants, a 2-pound sample is taken at the time of production and sent to the National Bureau of Standards, together with a sample of the blended material. Values for the several chemical and physical properties are established from the results obtained by the National Bureau of Standards and the plants during the first month's testing of the reference lot. Reference lots of GR-I and GR-M are smaller in size, but the same care is taken to ensure uniformity.

By a comparison of the results of the daily tests made by each analyst with the established values the plant laboratories can determine whether improper techniques are being employed by a particular analyst or systematic errors are being introduced through faulty equipment. Although the reference lot serves admirably to indicate the quality of testing in a particular plant it is doubtful whether testing errors would have been eliminated without a simultaneous educational and coordination program (*3*). This program is under the supervision of the product-testing and quality-control group of the Office of Rubber Reserve. Members of this group, thoroughly familiar with the equipment and techniques involved in the several methods of test, frequently visit the plant laboratories to instruct and advise laboratory personnel. In addition, a field representative is assigned to each plant. These representatives assist in standardizing the testing techniques as well as verifying the quality of production.

As an illustration of such standardization, the experience with the determination of ash may be cited.

Although the method had been well established, the tendency of the plant laboratories in the early part of the program was to obtain low and erratic results for ash content. An examination of the techniques employed revealed that the temperatures of the muffle furnaces were variable. When the temperature exceeded the specification limits, sodium chloride and other salts were vaporized or decomposed. In order to obtain reproducible and accurate results, the temperature limits for ashing were changed from 550° ± 50° C. to 550° ± 25° C. and temperature controllers were installed on the muffle furnaces. As a further precaution, the use of aluminum crucibles was recommended. These crucibles distort and melt if the temperature is appreciably above that permitted by the specification. They also have other advantages over porcelain crucibles, such as lower cost, lighter and less variable weight, and more rapid heating and cooling. These improvements in technique have eliminated over 80 per cent of the testing error.

REFINEMENT OF EXISTING ANALYTICAL METHODS

In standardizing techniques in the government synthetic rubber plants, many instances were noted where the procedures were not sufficiently specific to yield reproducible results. When this occurred, the Subcommittee on Test Methods assigned to one or more laboratories the task of investigating the factors which were causing the erratic results. These studies led to a refinement of the methods in the specifications. Since the refinements made in the methods of test have been incorporated in the "Specifications for Government Synthetic Rubbers" (*4*), it will suffice to confine the discussion here to one typical case—extraction of GR-S.

In the early specifications, acetone was designated as the solvent to be used in the determination of extract and fatty acid in the GR-S. It was soon noted, however, that consistently high and erratic results for extract were obtained using acetone. On investigation it was found that the high value for extract resulted

from the polymerization of the acetone, owing to the presence of soap in the GR-S. The National Bureau of Standards investigated several solvents as a replacement of acetone, and finally recommended the use of an azeotrope of ethanol and toluene. This azeotrope swells the rubber, thereby permitting the solvent to penetrate and extract the sample more readily. This solvent also extracts the soap, and thus permits the determination of soap content on the same sample used to determine fatty acid content.

Some of the other refinements and improvements in testing procedures made during the past four years (*4*, *5*, *6*, *7*) are the following:

1. Improved fatty acid and soap titration by adoption of *m*-cresol purple as end-point indicator.

Table II. Improvement in Precision of Testing in a Typical Plant

(Summary of daily tests made on reference samples)

Property	August 1943 No. of tests	High	Low	Mean	Standard deviation	June 1947 No. of tests	High	Low	Mean	Standard deviation
Ash, %	30	1.61	0.36	0.94	0.24	23	0.50	0.34	0.40	0.04
Extract[a], %	30	9.08	5.71	7.58	0.72	23	7.42	7.21	7.26	0.05
Fatty acid, %	30	4.81	3.33	4.06	0.30	23	4.73	4.60	4.645	0.03
Soap, %	30	0.64	0.35	0.47	0.07	23	0.26	0.21	0.230	0.01
Stabilizer, %	..	.....	.....	.....	....	23	1.57	1.42	1.49	0.04
Bound styrene, %	..	.....	.....	.....	....	23	23.1	22.9	22.98	0.06
Viscosity, ML., 212° F. at 4 min.										
GR-S	30	57	53	55	1.06	23	51.0	50.0	50.65	0.34
Compound	30	73	60	66	2.86	23	59.0	57.0	58.10	0.52
Properties of vulcanizate at 82° F.										
Tensile strength[b], lb./sq. inch	30	3180	2525	2840	129	33	3540	3020	3233	118
Ultimate elongation[b], %	30	695	615	655	20	33	690	610	655	19
Stress at 300% elongation										
25-minute cure, lb./sq. inch	30	530	380	445	40	33	515	440	469	19
50-minute cure, lb./sq. inch	30	1030	800	915	65	33	1110	1030	1076	22
90-minute cure, lb./sq. inch	30	1375	1045	1230	71	33	1510	1420	1477	20

[a] Acetone used as solvent in 1943, ethanol-toluene azeotrope in 1947. [b] 50-minute cure. All cures at 292° F.

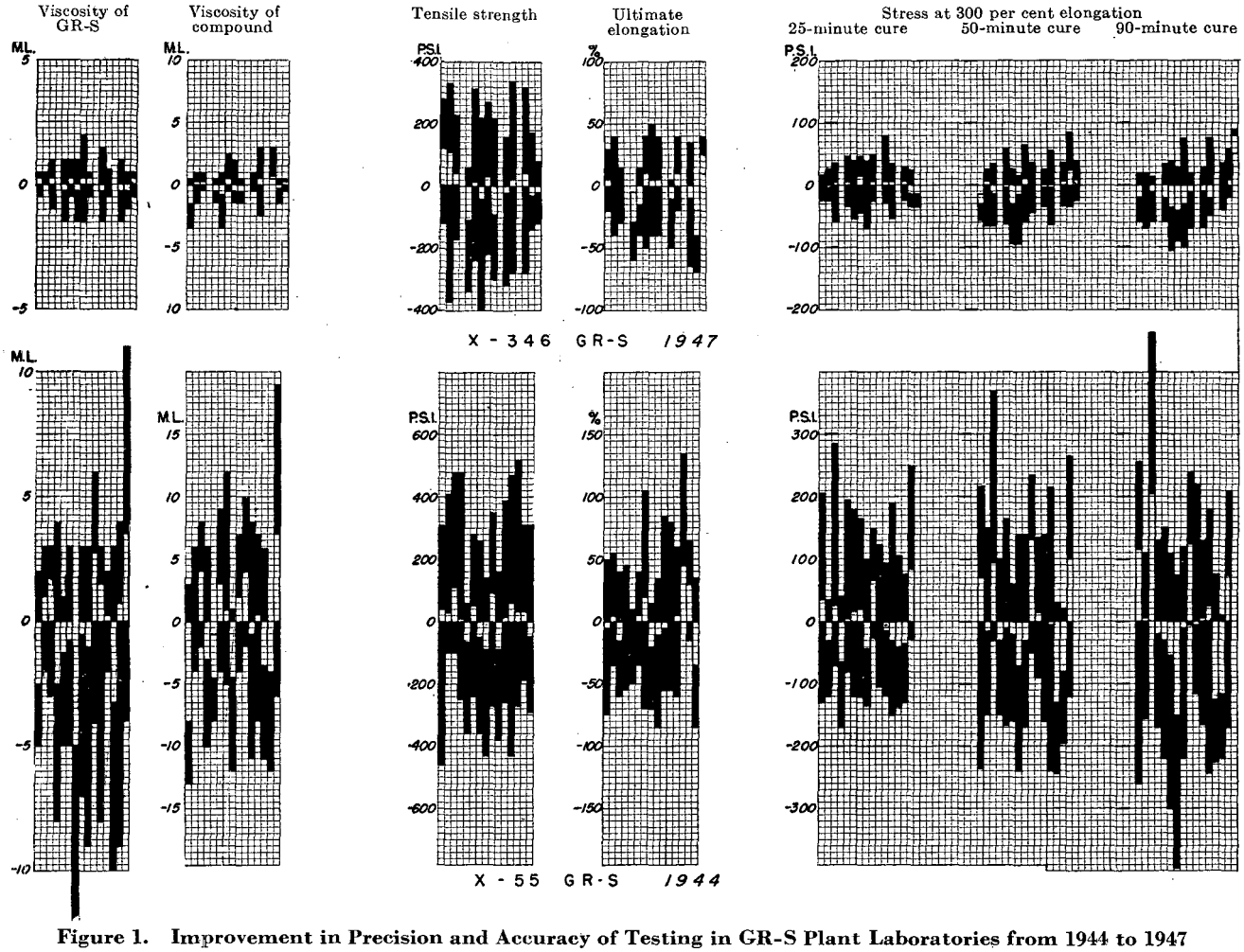

Figure 1. Improvement in Precision and Accuracy of Testing in GR-S Plant Laboratories from 1944 to 1947

The data on X-55 GR-S are the first obtained from daily tests of a reference lot and those on X-346 GR-S are the most recent available (June 1947). The performance of each plant laboratory is indicated by a bar, the length of which represents the range of individual values (approximately 90 values for the viscosity of GR-S and 25 for the other properties) and is a measure of the precision of testing. The length of the white segment in the bar represents the deviation of the mean value determined by the plant laboratory from the over-all average of results from all GR-S plants and is a measure of relative accuracy. The bars omitted in the figure correspond to the three plants not operating in June 1947 and the one plant which did not test X-55 GR-S

2. Modified ash procedure to permit rapid ashing without loss of precision and accuracy.
3. Improved design of Mooney viscometer and refined procedure for determining Mooney viscosity.
4. Established standard compounding ingredients.
5. Established detailed procedure for mixing and aging compound before curing.
6. Improved procedure for standardizing curing temperatures.
7. Improved specification for specimen die and procedure for preparation of test specimens.
8. Improved procedure for testing specimens and calibration of testing machines.

DEVELOPMENT OF IMPROVED ANALYTICAL METHODS

In many instances test methods for properties which are unique for synthetic rubber—e.g., bound styrene in GR-S—were required, and existing procedures sometimes were not satisfactory. In these cases, the Subcommittee on Test Methods undertook the development of new ones. Included among those developed are the following:

1. Mill method for volatile matter content.
2. Pyrolysis method for determination of carbon black in GR-S blacks.
3. Ultraviolet absorption method for determination of stabilizers.
4. Refractive-index method for determination of bound styrene.
5. Strain test for determination of stress-strain properties.

Since the first three methods are given in the "Specifications for Government Synthetic Rubbers" (*4*), only the last two methods will be discussed here.

Refractive Index Method for Determination of Bound Styrene. At the beginning of the government synthetic rubber program there was no suitable procedure available for the control testing of bound styrene in GR-S. To the National Bureau of Standards was assigned the task of developing a rapid and accurate test, and as a result the refractive-index method (*7*) was established. A relationship between bound styrene and refractive index was determined by carbon-hydrogen analyses and a conversion table was prepared. Briefly, the method consists of extracting nonhydrocarbon constituents from a small sample of GR-S, pressing the extracted sample into a thin sheet, placing this sheet on the prism of an Abbe-type refractometer, and measuring the index of refraction. By use of tables, the refractive index is converted to per cent of bound styrene.

Strain Test for Determination of Stress-Strain Properties. It was found early in the program that a significant part of the variability in testing was introduced by the testing equipment. Since the latter part of 1944, the National Bureau of Standards has been developing a strain test (*5*) to overcome this difficulty.

This test is based on the measurement of strain at a fixed stress instead of the measurement of stress at a specified elongation during the extension of the specimen. A semiautomatic tester (*1*) for this purpose has been constructed which reduces the man-hour requirements below those required in the usual tests. The variations introduced by this method of measurement are negligible in comparison with those introduced in the process of mixing and curing. An additional advantage of this test results from the observation that strain at a fixed stress decreases with time of cure, according to the laws of a second-order reaction. Thus, it is possible to calculate from strain test data three vulcanization parameters—scorch time, rate of cure, and structure rigidity.

DISCUSSION

The end result of these modes of attack can be seen in Table II and Figure 1. Table II shows the reduction in the variability of testing during the past four years within a single plant for each of the chemical and physical properties. Figure 1, which summarizes over 10,000 test values obtained in the laboratories of 16 GR-S plants during control testing of reference lot X-55 GR-S in 1944 and X-346 GR-S in 1947, shows for each of the physical properties the range of the individual values and the mean values with respect to the over-all average. The over-all length of the bars represents the range of the individual values or precision of testing. The white segment in the bar represents the deviation of the mean value found by the plant from the average of all values—that is, the accuracy of testing.

Table III. Precision of Testing at GR-S Plant Laboratory Operated by National Synthetic Rubber Corporation

(February 1947)

Property	Standard Deviation of Test Values[a]	Actual Range of Test Values[a]
Ash, %	0.02	0.06
E-T-A extract, %	0.04	0.15
Fatty acid, %	0.02	0.05
Soap, %	0.01	0.04
Stabilizer, %	0.02	0.08
Bound styrene, %	0.06	0.12
Viscosity of GR-S	0.37	1.5
Viscosity of compound	0.61	2.5
Tensile strength, lb./sq. inch	37	105
Ultimate elongation, lb./sq. inch	7	25
Stress at 300% elongation		
25-minute cure, lb./sq. inch	11	45
50-minute cure, lb./sq. inch	12	30
90-minute cure, lb./sq. inch	16	55

[a] Values obtained from 19 daily tests for each property.

Although there has been an improvement in precision of testing for all properties, the improvement is much more striking for some than for others. There has been an improvement in accuracy of testing for viscosity both before and after compounding and for stress at 300% elongation, but little or no improvement for tensile strength and ultimate elongation. Nonetheless the 1947 results represent a precision and accuracy of testing among plant laboratories which is probably the best performance ever experienced in the rubber industry.

The performance of the plant laboratory operated by the National Synthetic Rubber Corporation, Louisville, Ky., prior to the time it was placed in standby condition, surpassed even the best single-plant performance shown in Figure 1. The standard deviations and actual ranges of values obtained in daily tests of X-346 GR-S during February 1947 are shown in Table III. These results indicate that even the present precision of testing in the synthetic rubber plants can be improved. In physical testing, a large part of the error still remaining is caused by variations in atmospheric conditions of temperature and humidity during mixing and aging of the unvulcanized stock and variations in the testing machine. By use of the strain tester (*1*) recently developed and with air-conditioned laboratories, it should be possible to test rubber with a precision approaching that of testing simple chemical compounds. The pretext that rubber is variable in its properties can no longer be used to account for erratic test data.

The uniformity of current synthetic rubber production was made possible by improvements in the precision and accuracy of testing rubber. These improvements have been realized with little increase in cost of equipment or labor, since the man-hours required to follow prescribed procedures properly and carefully are no more than those required to test in a haphazard and careless manner. The little extra labor required to maintain testing equipment in good operating condition and to test daily a reference control sample for standardization purposes is well repaid in reliability of results. Most rubber manufacturers today accept the test data from the synthetic rubber plants without question.

CONCLUSIONS

The precision of testing in the synthetic rubber plants indicates that a rubber laboratory can readily attain results on re-

Table IV. Precision of Testing

Property	Standard Deviation	Total Range
Ash, %	0.02	0.10
Extract (E-T-A), %	0.12	0.60
Fatty acid, %	0.05	0.25
Soap, %	0.01	0.05
Stabilizer, %	0.02	0.10
Viscosity		
GR-S, ML., 212° F. at 4 min.	0.5	2.5
Compound, ML., 212° F. at 4 min.	0.8	4.0
Properties of vulcanizate		
Tensile strength, lb./sq. inch	150	750
Ultimate elongation, %	20	100
Stress at 300% elongation		
25-minute cure at 292° F. lb./sq. inch	20	100
50-minute cure at 292° F. lb./sq. inch	25	125
90-minute cure at 292° F. lb./sq. inch	25	125

peated daily tests of the same blend of GR-S using the same lots of compounding ingredients within limits given in Table IV.

The range is taken as five times the standard deviation, since 99 out of 100 values should fall within it. The average range for monthly plant data is approximately four times the standard deviation, which corresponds to a 95 out of 100 expectation.

The precision of testing natural and types of synthetic rubbers other than GR-S should be as good as or better than that of testing GR-S. On the other hand, the accuracy of testing any rubber cannot be determined conveniently by a single laboratory because of possible systematic errors in equipment or procedure. There should, therefore, be reference lots of rubbers available to the rubber industry for detecting systematic errors in the rubber laboratories. There should also be required standard lots of compounding ingredients for preparing test vulcanizates.

ACKNOWLEDGMENT

The accomplishments in testing reported herein were the result of the collaborative effort of groups in several laboratories. Although it is not possible to acknowledge separately each individual who made contributions, the authors wish to mention the following organizations, of which these individuals are members:

Canadian Synthetic Rubber, Ltd.
Copolymer Corporation
E. I. du Pont de Nemours & Co., Inc.
Firestone Tire and Rubber Company
General Tire and Rubber Company
The B. F. Goodrich Company
Goodyear Synthetic Rubber Corporation
Government Evaluation Laboratory
Humble Oil Company
National Bureau of Standards
National Synthetic Rubber Corporation
Navy Department, Bureau of Ships
Office of Rubber Reserve, RFC
Standard Oil Company of New Jersey
United Carbon Company
United States Rubber Company
University of Illinois

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RECEIVED September 18, 1947. Presented before the Division of Rubber Chemistry at the 112th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y.

Emission Spectroscopy in an Oil Laboratory

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The use of the spectrograph is different in the oil laboratory than in the metallurgical laboratory. It serves many functions not handled by any other analytical tool. In spite of the wide variety of materials handled, it enables the operator quickly to analyze most types of materials with an accuracy in most cases equal to that of the best chemical methods. It cannot be used indiscriminately, but usually methods can be developed for the elements it detects.

MANY articles during the past ten years have described methods of analysis using a spectrograph—direct current arc, alternating current arc, and alternating current spark methods, with many variations. Most have stressed the fact that the results are obtained more quickly, and with an equal or better accuracy than by other methods. Such procedures may be turned over to relatively untrained personnel without loss of either accuracy or speed. While these facts are true, sight has been lost of the need in industry for an analytical tool that is capable of giving information not otherwise obtainable, and of certain advantages that may sometimes be obtained by the use of a fairly long spectrographic procedure.

It is with the above situation in mind that this paper has been prepared. The methods presented are not the only solution to the problem under discussion, but are one satisfactory answer to a heterogeneous group of problems.

A Bausch & Lomb large Littrow spectrograph, A.R.L. Dietert source units, briquetting press, densitometer, and developing equipment make up the main facilities of the laboratory.

QUALITATIVE ANALYSIS

Direct Current Arc Techniques. It is possible to identify approximately 70 elements of the periodic table by means of an ordinary emission spectrograph. When there is interest in any of these 70 elements, most samples, regardless of physical state, are sent to the spectrographic laboratory for a qualitative analysis before any other work is performed on them. The working procedure is set up to give a semiquantitative as well as qualitative analysis.

Sampling is particularly important in view of the heterogeneity of many of the samples submitted. All oils, liquid samples, or organic residues are either evaporated to dryness or ignited and burned. The residue in the case of organic materials is heated to approxi-

mately 500° C. to remove most of the carbon. Volatile inorganic constituents—i.e., lead, mercury, and gallium—will be partially lost during this process and must be determined on a separate sample for most accurate results. The dried residues are then ground by a mullite mortar and pestle.

Three 10-mg. aliquots of each sample are weighed on a regular analytical balance to ±0.2 mg. Each portion is mixed with its own weight of pure graphite powder and loaded into a cup drilled in the end of highly purified graphite rods. These aliquots are burned to completion by means of a 10-ampere direct current arc source on the regular Bausch & Lomb arc stand; the range of the spectrograph is moved between samples. A complete sample is exposed over the range from 2000 to 8000 Å. in order to take in the principal lines of all elements possible under these conditions, including both arsenic and potassium. Spectra are photographed on Eastman 1L emulsions, which are developed in D-19 developer for 2 minutes at 70° C. and fixed in an acid fixing solution.

A supply of pure copper electrodes is maintained in order to detect elements whose most sensitive lines occur in the range of the cyanogen bands. The cyanogen bands occur as a result of the burning of the graphite electrodes. Copper electrodes are used to detect tungsten, the rare earths, and carbon.

The washed and dried plates are prepared for interpretation by projecting an image of the plate on a white wall or by placing the plate on a ground-glass viewing stand where it is examined with a Bausch & Lomb magnifier.

Interpretation of the plates is the most difficult step in the process, and is done by an experienced operator backed by a large number of chemical analyses. The position of the spectral lines indicates their identity and the blackness of the line is approximately proportional to the concentration of the element identified by the line. Table I is a typical qualitative analysis. Such an analysis will immediately indicate both major constituents and major and minor impurities. It does not indicate anything of the manner in which the elements are combined, nor does it necessarily reveal the identity of all the constituents present. For instance it will not indicate whether lead is present as oxide or phosphate or give information as to the presence of nitrate or chloride. However, such an analysis will reveal to the chemist which of the 70-odd elements are present, give him a rough idea of the quantities present, and indicate the absence of all elements detectable by the spectrograph but not reported.

In many types of processes such qualitative analyses are all that are needed. In so-called go–no go control, a specimen of a satisfactory catalyst with respect to sodium is compared qualitatively with other similar types of materials until the stage in the process is reached at which the sodium content of the catalyst materials becomes similar. Then, sodium can be determined quantitatively. This saves a lot of needless quantitative determinations, and is done quickly.

Table I. Engine Deposit

Major constituent	Pb	0.01 to 0.1%	Al
	Zn		Ba
	Ca		Sn
	P		Cr
1 to 10%	Fe	<0.01%	Sr
0.1 to 1%	Si		K
	Na		Mo
	Mn		B
	Cu		Ag
	Mg		Li

Small quantities of oils, sludges, or other material can be packed directly into the graphite cups and shot without ashing or weighing, where the sample is too small to give 30 mg. of ash. In these cases more uncertainty is introduced into the final percentage groupings.

From four to eight samples can be weighed, exposed, developed, and interpreted for the 70-odd elements in less than 2 hours. This type of service is not given by any other technique or instrument. Used oils, oil-field brines, oil additives, minerals, crude oils, corrosion products, steels, catalyst materials, and chemical residues are only a few of the types of materials sent into the laboratory for analysis.

Analysis of Surface Films. Frequently specimens are received in which the desired constituent is a thin film of metal or deposit on some other material. Such problems are not unique to an oil laboratory, nor is the solution that is used. If the base material is a conductor, it is possible to make a series of low-powered short-time alternating current spark discharges on the surface of the sample for one setting of the photographic plate. A graphite rod is used as the alter electrode. The result is a single exposure in which the surface of the material sparked is predominant. It is possible to detect on the order of 0.000001 gram of material, though this figure is influenced by the sensitivity of the element being excited. Platings on metal, lead on valves, copper on bearing surfaces, and solutions accidentally spilled on conducting surfaces have been identified. In all these cases the spectrograph meets a need not filled by other methods without destroying the materials involved.

QUANTITATIVE METHODS

In any analytical division, the question continually arises as to the best method or technique to use in order to accomplish a given analysis. In this laboratory sample types which do not recur fairly often are sent to the chemistry division for quantitative work, unless there is special interest in an element that is difficult to determine chemically. For example, low sodium determinations are made more quickly and accurately by the spectrograph than by chemical means; in this instance it is worth while to set up the method spectroscopically. However, iron contents of motor deposits are in general better determined chemically.

Analysis for Alkalies. Many samples originate in the catalyst development section, in which the interest is in the alkali content. Extreme precision is not necessary (±10% is satisfactory), but a reasonable degree of speed is desirable. The method described is for sodium only, though it could be adapted to any or all of the alkalies. An alternating current spark method of analyzing the samples was chosen, not because it was the only procedure that would work but because the author had had experience with such methods and was able to predict results somewhat better than by other procedures. Burdett and Jones have obtained good results using a multisource power unit (*1*) and Helz has obtained satisfactory results using a direct current arc source (*6*).

PREPARATION OF STANDARDS. In the preliminary steps of this investigation it was found that both the sodium and internal standard lines were very noticeably affected by a change in the composition of the sample. This made it desirable to run a qualitative analysis (assuming the composition was not known) before attempting the quantitative analysis. In addition, it necessitated setting up standards to approximate the composition of the sample. This was not unexpected, but was disappointing in view of the desired universality of the method.

One type of material consisted of 80 to 90% silica and 10 to 20% alumina in the form of an aluminum silicate. Increasing additions of sodium chloride were made to aliquots of a large batch of this catalyst that was low in sodium content. It was determined that the form in which sodium occurred did not appreciably affect its analysis, so it was convenient to use sodium chloride. These weighed mixtures of sodium chloride and catalyst were thoroughly mixed. Individual standards were then prepared by mixing 1 gram of catalyst and sodium chloride, 2.2 grams of purified natural graphite (National Carbon Co. SA1), and 50 mg. of lithium carbonate, to act as the internal standard. Lithium was picked as the internal standard because it has an ionization potential similar to that of sodium or the other alkalies, and because its spectral lines occur close to the sodium lines. These mixtures were briquetted in the form of 1.25-cm. (0.5-inch) pellets at as low a pressure as they would stay together—about 800 pounds gage pressure on an A.R.L. Dietert briquetting press. The low pressure was chosen so that the spark would be able to penetrate the briquet, and as the sample "scaled" or "eroded" away a new surface would be presented to the spark. This results in using more of the sample and makes the mixing process less critical.

Table II. Working Conditions

Spectrograph	Bausch & Lomb large Littrow
Range	2
Power	4/3 kva.
Inductance	0.365 mh.
Primary voltage	75 volts
Prespark	5 seconds
Exposure	25 to 35 seconds
Distance from slit	18 inches
Slit width	30 microns
Upper electrode	0.5-inch briquet
Lower electrode	Hemispherical cone, 0.25-inch graphite rod
Development	2 minutes, D-19
Analysis lines	Na 5889.95/Li 4971.9
	Na 5895.92/Li 4971.9

Table III. Reproducibility of Sodium in Catalyst Materials

Sample No.	Chemical Analyses	Spectroscopic Analyses Run 1	Run 2	Run 3	Run 4	Run 5
106K	0.11	0.10	0.12	0.11	0.10	0.10
RN-23-25	0.33	0.32	0.35	0.32	0.34	0.35
RN-18-96A	..	0.14	0.16	0.13	0.16	0.15
RN-18-96B	0.18	0.19	0.16	0.16	0.20	0.17
RN-18-96C	..	0.20	0.23	0.21	0.22	0.23

Table IV. Comparison Analyses of Brines

Sample 3, Mg./L.			% Deviation from Chemical Values	Sample 3, Mg./L.			% Deviation from Chemical Values
Na	Spect.	8,607	0.7	Si	Spect.	21	12
Na + K	Chem.	8,663			Chem.	24	
Ca	Spect.	1,160	12.6	B	Spect.	24	
	Chem.	1,030		Total dissolved solids		27,476	
Mg	Spect.	294	26				
	Chem.	398					
K	Spect.	134		Na, K, + Li	Spect.	8,749	0.9
Li	Spect.	8			Chem.	8,663	
Sr	Spect.	220		Ca + Mg	Spect.	1,454	1.9
Fe	Spect.	5	33		Chem.	1,428	
Al	Spect.	3					
Al + Fe	Chem.	12					

PREPARATION OF SAMPLES. Samples are received from the catalyst units and are assumed to be representative of the batch material. The entire sample received is ground to pass a 100-mesh screen and is quartered to approximately 2 grams. This representative aliquot is then ignited at about 800° C. in order to drive off any moisture present and to make all analyses on material of the same basis. Exactly 1 gram of the ignited sample is mixed with 2.2 grams of graphite and 0.05 gram of lithium carbonate and is briquetted.

PROCEDURE AND RESULTS. Standards can be run on each plate, although working curves can be set up so that only one or two standards are needed. The briquets, prepared as described above, are sparked in a special briquet holder similar to that described by Churchill and Russell (*3*). The complete working conditions are given in Table II. In view of the possibility of error in a method having as much weighing and mixing as are here involved, two pellets are shot on both sides and four results averaged for the final analysis. Table III gives typical results from this procedure. Two persons working together can analyze from 20 to 30 samples in an 8-hour day. They could as easily and quickly determine more elements in the same samples if this were desirable. The results, particularly in low percentages (below 0.2%), are more accurate than routine chemical determinations, and a distinction is automatically made between the various alkali metals that might be present. Because the standards must approximate the composition of the samples, this method is uneconomical for the miscellaneous specimen.

Analysis of Brines. Oil-field brines are often sent in for analysis. A spectroscopic method was desirable because it would distinguish between the various alkalies present, and would provide a quick and convenient procedure for quantitatively determining boron. The net result is that a relatively accurate procedure has been developed for the analysis of brines including the major constituents, sodium, calcium, and magnesium.

The complete details and a technical discussion of the method will be the subject of a paper to appear in the near future. The procedure consists of a combination internal standard and so-called "mutual standard" technique (*4*) using an alternating current spark form of excitation. Table IV gives results obtained using this procedure.

The percentage deviation is calculated, assuming the chemical results to be accurate. The net result is that on the basis of the sample analyzed, the spectroscopic answer is as bad as it could possibly be, and yet is a reasonably good analysis. This method is capable of handling 10 to 20 samples per day without undue strain on the personnel.

Analysis of Oil Additives. A unique method gives promise of being widely used where numerous samples of similar type are sent in for analysis. This method, by Calkins and White (*2*), uses the oil directly for a quantitative analysis of the additive in it. The purified graphite electrode is heated to red heat and dropped directly into the oil to be analyzed. The pores of the electrode soak up the oil and the spectra are obtained by sparking the oil-filled electrodes. The background near the analysis lines is used as the internal standard.

There are at least two possible objections to the method for general use. The details of the spark source are so given that the source conditions appear critical for the operation of the method. If this is true, the usefulness of the method will be seriously diminished. If any power source can be adopted to the procedure, it may have extended use. The second point arises in extending the method to used oils, an important group of oils in the nonroutine laboratory. Such a procedure of sampling would not be applicable to a used oil containing sludge and other insoluble particles. Regardless of these objections, the procedure is a definite advance in the analysis of unused oil additives and may open new fields to spectrographic analysis.

Analysis of Small Quantities of Solutions. The analysis of residues of various kinds from oils, waters, or sludges is difficult because of the smallness of many of the samples. A procedure published by Fred, Nachtrieb, and Tomkins (*5*) is useful for such samples, if a high degree of accuracy is not necessary.

The method consists in evaporating given volumes of solutions on the top of pure copper electrodes. These electrodes are then sparked in such a fashion that the evaporated film on the electrode is excited. Such a procedure would be useful for the above types of samples, as all that would be necessary would be to get them into solution. The procedure is capable of detecting on the order of 0.001 mg. of an element with an error of ±10%. The one drawback of this method is its sensitivity. Very small samples can be handled, but this very sensitivity is bad from the point of view of contamination. Extreme care must be taken in handling the electrodes and for many of the more common elements an air-conditioned dust free laboratory is necessary.

ACKNOWLEDGMENT

The author wishes to express his appreciation to Paul D. Foote, executive vice president of the Gulf Research and Development Company, for permission to publish this paper.

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RECEIVED August 15, 1947. Presented before the Pittsburgh Section of the AMERICAN CHEMICAL SOCIETY at the Second Analytical Symposium, March 8, 1947.

Spectrographic Determination of Sodium in a Silica-Alumina Catalyst

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A spectrographic method for the determination of sodium in a silica-alumina cracking catalyst is described. The procedure involves the use of a copper counter electrode to provide reference lines and is especially suited for analyses in which an internal standard is not available. Determinations of the sodium content in the range 0.001 to 3.0% sodium oxide are obtained in approximately 40 minutes for routine industrial control.

THE most generally accepted procedure for the chemical determination of sodium in silica-alumina catalysts is the gravimetric method, which employs zinc uranyl acetate reagent (*1*, *7*). The sodium is precipitated as sodium zinc uranyl acetate, $NaZn(UO_2)_3(C_2H_3O_2)_9.6H_2O$, over a 45-minute interval, washed with 95% ethyl alcohol saturated with sodium zinc uranyl acetate, dried, and weighed.

This chemical procedure requires a great deal of sample preparation and considerable time, particularly for samples that contain interfering cations or only traces of sodium (*2*), and it has been the author's experience that the results obtained by semiskilled analysts are frequently inaccurate.

Several alternate procedures have the advantages of speed and a higher accuracy than is ordinarily obtained in routine chemical analysis. The increase in accuracy is derived from averaging large numbers of chemical analyses for sodium in order to calibrate these procedures, thus averaging the deviation in the chemical results. Briefly, the procedures consist of the use of the flame photometer (*2*), the nephelometer (*6*), and the spectrograph. The first two methods are recommended for the analysis of solutions. The spectrograph seems to be the best available means of analysis when the sodium is contained in an insoluble and inert material, as the time and labor required to prepare solutions from the analytical samples would exclude the photometer and the nephelometer.

The silica-alumina cracking catalysts, as used in the oil industry, belong to this latter category of inert and insoluble materials. For the specific catalyst analyzed, the silicon content was approximately 85% silica with a maximum content of sodium of 0.02% as sodium oxide.

APPARATUS

A Bausch & Lomb medium quartz spectrograph was used. This instrument covered the region 2200 to 7000 Å. with one 25-cm. (10-inch) exposure, thereby making a large portion of the ultraviolet and all of the visible spectrum simultaneously available. However, with this type of spectrograph the reduction in dispersion in the range 5000 to 7000 Å. is very pronounced and a small grating instrument with high efficiency in the first-order spectrum might be more suitable.

Auxiliary equipment consisted of a Leeds & Northrup recording microphotometer, an electrode cutter, a plate-developing machine, and a plate dryer. Dow high purity graphite electrodes were employed.

PHOTOGRAPHY

Eastman panchromatic process plates were used to photograph both the ultraviolet and visible spectrum. These plates were processed for 5 minutes in D-8 developer at 70° F., washed for 3 minutes in water, rinsed in ethyl alcohol to remove the red emulsion dye, and fixed in standard sodium thiosulfate solution for 2 minutes. An additional 5 minutes was utilized for washing the finished plate, although, when time permitted, a longer period was used, since prolonged washing further reduces the red tint remaining from the emulsion-sensitizing dye.

PRELIMINARY INVESTIGATION

The excitation source was a 2300-volt, 2.5-ampere, alternating current arc. In order to reduce the hazard to the operator a safety circuit was designed, which consisted of a remote control switch that automatically remained open unless the operator maintained pressure on it. This switch was so positioned that it was impossible to hold it closed and touch the electrodes, although the operator was able to view the focus of the arc relative to the slit. Three signal lamps were inserted in the safety circuit: one directly in front of the operator, one over the door, and the third conspicuously located in the spectrographic laboratory.

A survey of the most sensitive available sodium lines is given in Table I (*3*, *5*).

Table I. Sodium Lines Available for Analysis

Wave Length, Å.	Sensitivity
5895	2
5890	1
3303	4
3302	3
2852	5

Preliminary investigation indicated that for the range of sodium concentration in the finished product (0.02% or less) the D lines at 5890 and 5895 Å. were the only ones showing in the exposures. This was unfortunate from the standpoint of the spectrograph employed, as the reduction in dispersion in the range 5000 to 7000 Å. did not permit resolving the D lines.

The preliminary investigation also disclosed that the internal standard available was silicon, an element emitting comparatively few lines. The only available silicon lines in the vicinity of the sodium D lines were at 4100 Å. and, thus, not homologous from the standpoint of wave length (*4*). In addition, the intensity of these lines was entirely too high.

Instead of silicon, a substitute internal standard was utilized. Copper offered convenient homologous lines, as well as ease of preparation. Pure copper, obtained by precipitation from C.P. copper sulfate solution by addition of C.P. zinc, was mixed in 1 to 1 proportions with the material to be checked for sodium content. The mixture of copper and sample was placed in the crater of a high purity graphite electrode, obtained from the Dow Chemical Co., and volatilized in the alternating current arc.

The main objection to the above method was sodium contamination. Blank determinations very frequently produced sodium percentages of the same order of magnitude as the routine analyses. The correlation between chemical and spectrographic results was very poor.

In an effort to reduce contamination, a copper electrode was substituted for the graphite counter electrode and powdered cop-

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Table II. Range of Sodium Concentrations, Line Pairs, and Exposure Times

Range	Low	Medium	High
Concentration, % Na_2O	0.001 to 0.03	0.03 to 1.0	1.0 to 3.0
Line pairs	Na 5890–95 / Cu 5700	Na 5890–95 / Cu 5218	Na 3302–3 / Cu 2961
Pre-exposure time, sec.	5	5	0
Exposure time, sec.	20	10	30

per was abandoned as a standard. The copper electrodes were cut from 0.6-cm. (0.25-inch) pure copper bar stock. After use, these electrodes were cleaned with emery cloth, dipped in concentrated nitric acid, and washed with distilled water.

The use of copper electrodes had several advantages. The sodium contamination, which originated in the plant dust that seeped into the laboratory was greatly reduced. The sensitivity of the analysis was doubled, owing to the discarding of the practice of diluting the sample 1 to 1 with the powdered copper. The elimination of time-consuming procedures was an added advantage.

The only apparent objection to the use of copper electrodes as a source of reference lines is the possibility of noncorrelation between the intensities of the copper lines and the sodium lines. The probability of this noncorrelation also exists to a lesser extent when powdered copper is used as an internal standard. To overcome this objectionable feature each sample was analyzed three or four times and the final result was obtained as the average.

ANALYTICAL PROCEDURE

In setting up spectrographic calibration curves for sodium the lack of correlation between chemical and spectrographic results provided a problem, which was solved by assuming spectrographic correlation and by securing a large number of chemical determinations. Approximately twenty to thirty results of chemical analyses for each range of concentration required were plotted against the corresponding averaged spectrographic line intensity ratios, using log log graph paper. Linear curves were drawn, averaging the points for each range. The average correlation was found to be positive; this indicated the validity of the method. The slopes of the calibration curves increased in going from a lower to a higher concentration. This increase seems in agreement with theory and previous observation. Some spectroscopists are of the opinion that the slopes of calibration curves should approximate 45°, which is the optimum sensitivity compromise. This is a desirable condition, but it has been the author's experience that a 45° slope is obtainable only in a limited range of concentration.

The spectrographic calibration curves for sodium were set up in three ranges, low, medium, and high, as given in Table II. The calibration curves obtained are linear. They are based on plant samples secured at various stages of the purification process in which sodium is removed. The low range was specifically designed to cover the finished production of the catalyst, while the medium and high ranges covered the intermediate plant process.

In order to check the calibration curves, an independent outside source of analyses for sodium was obtained (Table III). Samples of catalyst were secured that were analyzed in the laboratory of the M. W. Kellogg Co. by trained chemists highly experienced in the determination of the sodium content of silica alumina catalysts.

The average deviation in the range of specification (0.02% Na_2O or less) is 0.002% sodium oxide. Improvements in the technique discussed in the summary in all probability would improve the accuracy. However, the spectrographic results, as is, are satisfactory for routine control, particularly as analyses by the chemical laboratory on the Kellogg samples produced errors that were uniformly greater than those produced in spectrographic analysis.

The good agreement noted between the Kellogg chemical analyses and the spectrographic analyses indicates that the technique of averaging large numbers of chemical determinations in order to obtain calibration curves is valid, that the method of chemical analysis contains no systematic errors, and that the large deviations found are the result of inexperience and the difficulty of the method. Undoubtedly in the hands of an experienced chemist the zinc uranyl acetate method produces very accurate results. However, the spectrographic technique offers a far more rapid and fairly accurate determination of sodium content and does not require a high degree of training or long practice.

SUMMARY AND DISCUSSION

An easy and rapid method for the spectrographic determination of sodium involves the use of a copper counter electrode as a standard to furnish reference lines. The material to be analyzed is placed in the crater of a graphite electrode and volatilized in an alternating current arc. This procedure is repeated three or four times and the analytical results obtained from the processed plate are averaged. The accuracy is adequate for industrial control and superior to the average chemical methods in the low percentage range. Analyses are obtained in about 30 to 50 minutes, much quicker than by chemical means.

Several modifications in the technique may improve the accuracy of the method. The use of a graphite electrode with a center post undoubtedly would localize the arc, provide steadier volatilization of the sample, and lead to greater reproducibility. It may be possible to correct the experimental data by providing a curve based on a carbon-to-copper line pair. The magnitude of the ratio of this line pair would indicate relative volatilization of the sample and graphite electrode and a positive or negative correction would be applied to the percentage of sodium oxide, depending on whether the carbon-to-copper ratio was greater or less than the average for the specific percentage of sodium oxide. However, this correction has been considered only from a theoretical standpoint and remains to be checked by experiment.

Table III. Analyses of Kellogg Samples

Sample	Chemical Analysis, %	Spectrographic Analysis, %
1	0.005	0.009
		0.005
		0.005
		0.005
		0.007
		0.006
		0.008
2	0.018	0.021
		0.026
		0.019
		0.017
		0.015
		0.018
3	0.04	0.03
		0.04
4	0.33	0.31

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RECEIVED February 5, 1947.

Estimation of Thiophene in Gasoline

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A colorimetric method for the direct estimation of thiophene in gasoline, without interference from other sulfur compounds present, has been worked out. The test is based on the blue color produced by the indophenine reaction between thiophene and isatin, in the presence of sulfuric acid. While extreme accuracy is not possible, the method is much quicker and easier than the conventional type of thiophene determination. Two variations are possible: an approximate method in which colors are matched by visual observation, and a more precise method utilizing a spectrophotometer or other photoelectric colorimeter.

THE method of thiophene estimation presented here was developed in conjunction with a study of thiophene removal from petroleum fractions, particularly gasoline. The conventional methods of sulfur analysis found in the literature involve the removal of the various sulfur constituents by means of selective solvents and precipitating agents, and the determination of total remaining sulfur by the lamp method after each removal (Ball, *1*, and Cross, *2*). While this type of analysis is accurate, it is rather long and involved, particularly when thiophene is to be determined. Since thiophene is usually the last sulfur constituent to be determined, it is necessary to remove all other sulfur constituents (generally stepwise) before making the thiophene determination. However, the authors found that the indophenine reaction can be used as a basis for thiophene determination, making it possible to determine thiophene directly without interference from other sulfur compounds which may be present in the gasoline, since they have no effect on the reaction producing the blue color. The indophenine reaction was used by Schwalbe (*5*) to determine thiophene in benzene in 1905, but no evidence of recent work of this type was found in the literature.

The proposed method seemed to work satisfactorily with various kinds of gasoline fractions. The presence of aromatic compounds from crude oil occasionally caused some difficulty which necessitated the use of special precautions, but did not prevent accurate analyses. Concentrations as low as 0.005% thiophene sulfur (0.013% thiophene) by weight have been readily determined, and it is possible that lower concentrations could be detected with little or no decrease in accuracy.

Many factors, some of them difficult to control, affected the intensity of color produced, and caused slight variations of the color intensity as measured by a photoelectric colorimeter. Consequently, the results obtained were not always accurate to within less than 15 to 20% of the amount of thiophene sulfur present, although better accuracy was obtained in many cases. When the colors were matched by visual observation, the results were usually accurate to within 25 or 30% of the amount of thiophene sulfur present, although this naturally depended to a large extent upon the individual operator. Experience greatly increased accuracy in this determination.

While it may seem that this degree of accuracy is not very precise, thiophene concentrations were very low in many of the samples analyzed, and this permitted more precision in measuring the absolute quantity of thiophene present. For example, an accuracy of within 20% of the amount of thiophene sulfur present, in analyzing a sample containing 0.05% thiophene sulfur, would give a maximum error of only 0.01% thiophene sulfur. The larger errors were obtained in analyzing samples of higher thiophene concentration (0.50 to 1.00% thiophene sulfur).

The proposed method has been used primarily to aid in the study of various methods of removing added thiophene from straight-run gasoline. In this work, 1% thiophene sulfur (by weight) was added to a substantially thiophene-free gasoline, and the resulting mixture subjected to various reactions designed to remove the thiophene. Samples were analyzed according to the procedure presented here.

The proposed method can also be applied to cracked gasolines and gasolines containing some aromatics, by observing certain precautions as explained below.

PRECAUTIONS

One factor which caused some difficulty was a secondary reaction that took place between the gasoline and the isatin-acid reagent. This reaction proceeded over a period of hours or days, and produced a red color. This secondary reaction was not studied completely, but seemed to be more evident in the presence of compounds of aromatic or cyclo- structure than in the presence of aliphatic compounds.

When a blank solution of thiophene-free gasoline was used for comparison, the same color change took place in the blank during the same period of time. Therefore, no error in analysis was caused by this secondary reaction, since the analysis is based on a comparison of the intensity of monochromatic light passed through the blank and unknown solutions. It was important to use a blank solution containing similar gasoline and the same amount of gasoline as the solution being tested whenever possible.

However, it was not always possible to use a blank solution containing gasoline identical to the sample, since the reactions used in attempting to remove thiophene sometimes changed the gasoline by removing some aromatics. In the case of high-temperature reactions, there was occasionally some evidence of cracking or other change in the molecular constitution of the gasoline. These changes (particularly the cracking) sometimes caused the secondary reaction to be more noticeable with the sample being tested than with the blank solution prepared from the original gasoline. In no case was this secondary reaction noticeable when the test solutions were first prepared, but appeared some time later, usually in from 2 to 24 hours.

Therefore, the best criterion for judging the effect of this secondary reaction seemed to be the factor of time. The original reaction producing the indophenine blue color appeared to be complete within 20 to 30 seconds. As long as no additional color changes took place after this period, no errors in analysis were noted in any case, although test solutions were occasionally analyzed after standing for several hours.

Errors due to the reaction between gasoline and isatin-acid reagent were avoided by observing the following precautions: (1) A blank solution containing gasoline similar to that being tested was used whenever possible, and both solutions were prepared in the same way at the same time. (2) If this was impossible, all matching and checking of colors were completed as soon as possible after the solutions were made up.

No samples of gasoline were encountered in which this secondary reaction proceeded rapidly enough to make it impossible to complete a thiophene analysis before the reaction became evident. However, such gasolines might be encountered in

Table I. Data for Spectral Transmittance Curve

[Wave length *vs.* per cent transmittance for *A* 0.80%, *B* 0.50%, *C* 0.30% and *D* 0.15% thiophene sulfur]

Wave Length, mμ	Per Cent Transmittance			
	0.80%	0.50%	0.30%	0.15%
400	44	56	64	76
425	26	33	46	60
450	11	21	30	49
475	5	15	27	48
500	3.1	12	24	47.8
525	1.8	7.5	19	44
530	1.75	6.5	18	42.5
540	1.5	5.5	16	41.8
550	1.35	5.0	16	41.2
560	1.40	5.0	15.5	42.1
570	1.5	5.1	15.2	43.4
585	1.5	5.5	17	47
600	1.8	7.8	21	51.7
625	2.7	15	30	61.7
650	3.08	19.5	35	68
675	3.95	18.7	38.5	72
700	1.32	18.7	36.7	75.3
725	4.8	38	56.3	84
750	29	63	75.3	91
775	56	75	83.5	94
800	66	80	87.8	96
825	71	84	91	98

working with cracked gasolines or those high in aromatic content. If such a gasoline were encountered, the proposed method of thiophene analysis could not be used.

According to the suggestion of Wray (*6*), one drop of concentrated nitric acid was added to the isatin-acid reagent to act as an oxidizing agent and to aid in the formation of the characteristic blue color. However, if more than one drop was added, the reaction was inhibited and results were inaccurate. When the nitric acid was added to isatin-acid reagent, the dark brown color of the latter was changed to a lighter shade upon agitation.

REAGENTS

Isatin Acid. Isatin (0.400 gram) was dissolved in enough concentrated sulfuric acid (sp. gr.>1.84) to make 1000 ml. of solution. If the concentration of isatin was too small, the resulting solutions showed irregular color characteristics and accurate analysis was impossible. If the concentration of isatin was too great, the total range of color change took place within a very narrow range of thiophene concentration. The amount specified above seemed to be the best average between these two factors.

Nitric Acid, concentrated, C.P.

Thiophene-Gasoline. Solutions of C.P. thiophene in gasoline were used as a standard. These were made up volumetrically and the thiophene concentration was converted to a weight basis, using the density of the gasoline as determined by a pycnometer, and the data of Fawcett and Rasmussen (*3*) concerning the density of thiophene. All concentrations were expressed as per cent by weight of thiophene sulfur.

PROCEDURE

In making up test solutions, 50 ml. of isatin-acid reagent were measured by pipet and placed in a 200-ml. flask or bottle. One drop of concentrated nitric acid was added, and the mixture was shaken. The required amount of thiophene-gasoline solution (1 ml. in most cases) was added with a graduated pipet. Upon shaking, the characteristic blue color appeared almost immediately, and the reaction appeared to be complete within 20 to 30 seconds.

PHOTOELECTRIC METHOD

Because of interference between the brown isatin color and the blue indophenine color, visual matching of colors was sometimes difficult for an inexperienced observer. Better results were obtained by measuring the color with a photoelectric instrument, using monochromatic light. The tests described here were made with a Beckman quartz spectrophotometer, after the method of Mellon (*4*).

To determine the spectral characteristics of the solutions, spectral transmittance curves were prepared for four concentrations, using 1 ml. of thiophene-gasoline solution (Figure 1). As can be seen, the minimum transmittance occurred at 550 mμ wave length. Partial minima occurred at 475 and 710 mμ, but these were not considered in detail.

For analytical work, the transmittance at 540, 550, and 560 mμ was determined, and curves of concentration *vs.* transmittance were prepared. The curve for 550 mμ is shown in Figure 2. For good results, only readings of transmittance between 5 and 95% were recorded. With solutions containing 1 ml. of thiophene-gasoline, this included concentrations between approximately 0.50 and 0.025% thiophene sulfur. Best results were obtained by considering only that portion of the curve which showed the greatest change in transmittance in proportion to concentration change—namely, from 50 to 95%. The irregularities which appeared from time to time also seemed to be in the darker solutions where transmission was less than 50%.

Tables I and II give the data used in preparing the curves of Figures 1 and 2.

It is suggested that calibration curves be prepared for the particular instrument to be used, before attempting analytical work. It will then be possible to analyze unknown solutions by measuring the per cent transmittance against a blank solution containing thiophene-free gasoline, and referring to the calibration curves to find the concentration of thiophene sulfur in the solution being analyzed. The usual method was to take readings at 540, 550, and 560 mμ, and average the results obtained from calibration curves at these wave lengths.

If the concentration of thiophene sulfur was less than 0.025%, 2 ml. of the gasoline being tested were used instead of 1 ml. The result as read from the calibration curve was then divided by 2 to obtain the actual concentration present in the sample. By such a process, amounts of thiophene-gasoline solution up to 5 ml. have been used with no decrease in accuracy. At 96%

Table II. Data for Calibration Curve for 550 mμ

(Concentration, % thiophene sulfur *vs.* per cent transmittance for 1-ml. sample)

Concentration	% Transmittance
0.02	98.5
0.04	88.8
0.07	75.0
0.10	64.5
0.15	41.2
0.20	35.7
0.30	15.5
0.40	5.5
0.50	5.0

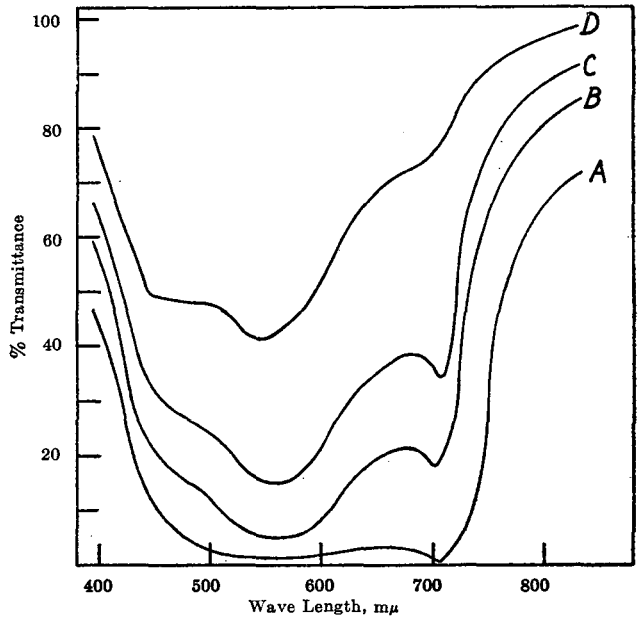

Figure 1. Spectral Transmittance Curve

Thiophene sulfur

A. 0.80% *B.* 0.50% *C.* 0.30% *D.* 0.15%

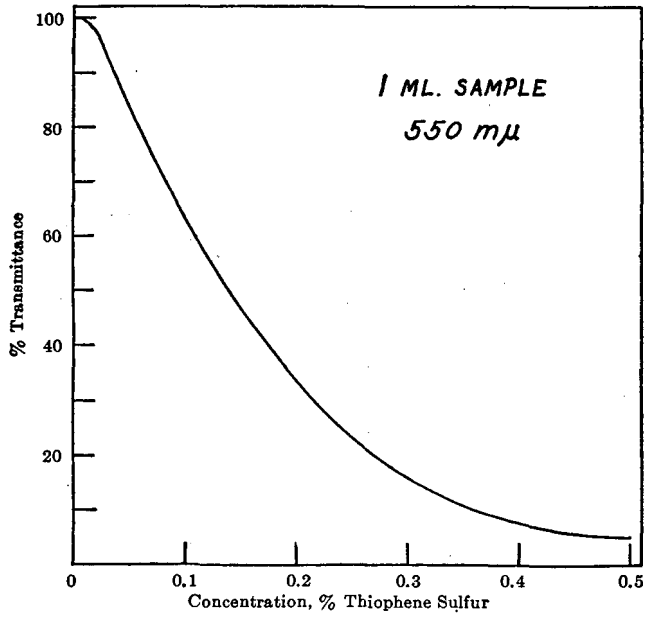

Figure 2. Calibration Curve of % Thiophene Sulfur *vs.* % Transmittance

transmittance, this corresponded to a concentration of $^1/_5 \times 0.025$, or 0.005% thiophene sulfur.

In the same manner, concentrations of thiophene sulfur greater than 0.50% were measured by using 0.5 ml. of thiophene-gasoline solution and multiplying the result by 2, etc.

In each case, a blank solution was used containing the same amount of gasoline (thiophene-free) as the test solution, and both solutions were made up at approximately the same time. This eliminated inaccuracy due to the reaction between the gasoline and the isatin-acid reagent.

VISUAL OBSERVATION METHOD

If no photoelectric colorimeter was available, unknown solutions of thiophene in gasoline were analyzed by visual observation. This could have been accomplished by using a simple colorimeter in which the intensity of color is matched by looking through two samples, and diluting one to a measured extent, obtaining the same depth of color in each sample. However, in an attempt to simplify the analytical procedure as much as possible and to eliminate the necessity for any type of special equipment, most of the work was done by purely visual observation. With such a method, sufficient accuracy was obtained for the work being done.

Two solutions were used, each containing 50 ml. of isatin-acid reagent and one drop of concentrated nitric acid. To one solution was added 1 ml. of a standard thiophene-gasoline solution containing approximately the amount of thiophene expected in the unknown. This standard solution replaces the blank solution of thiophene-free gasoline that was used in the photoelectric method. To the other solution the unknown gasoline sample was added until the indophenine color was observed.

Both solutions were agitated and their intensity of color was compared. If the solution containing the unknown was not so dark as the one containing the standard thiophene-gasoline solution, more of the unknown sample was added. After agitation, the color was again checked against the standard solution. Final comparison was made under a strong light on a white background, after both solutions had stood for 4 or 5 minutes. This addition of the unknown sample can be made readily with a 1-ml. graduated pipet, and the quantity which is added can be measured to within 0.1 ml.

If too much of the unknown sample was added by accident, the standard solution was made darker by adding a measured amount of the thiophene-gasoline standard solution, to compare with the darker unknown.

When the two solutions appeared identical in color, the amount of thiophene sulfur present in the unknown was computed according to the formula:

$$C_1M_1 = C_2M_2$$

where C_1 = concentration of thiophene sulfur in unknown sample
M_1 = ml. of unknown sample used
C_2 = concentration of thiophene sulfur in standard solution
M_2 = ml. of standard solution used

For example, if a standard solution containing 1 ml. of a solution of 0.10% thiophene sulfur was used, and 1.2 ml. of unknown solution were required to produce the same shade of color, the thiophene concentration was found as follows:

$$C_1)\ (1.2) = (1.0)\ (0.10)$$

$$C_1 = \frac{(1.0)\ (0.10)}{1.2} = 0.083\%\ \text{thiophene sulfur in unknown}$$

The secondary reaction between gasoline and isatin-acid reagent is more noticeable as the relative amount of gasoline is increased, giving a more rapid color change in the solution containing the larger amount of gasoline. Therefore, it is advisable to have an approximately equal amount of gasoline in the standard and unknown solutions, and all checking of colors should be completed within 30 to 45 minutes of the time the solutions are first prepared.

CONCLUSIONS

While not extremely precise, the method of analysis presented here is very simple and easy of execution. After the necessary reagents and standard solutions are at hand, an unknown sample can be analyzed in a few minutes by either of the two methods described.

Another advantage of the proposed method is that only 1 or 2 ml. of the gasoline being tested are required to prepare a test solution; this enables one to work with very small amounts of gasoline if necessary and still be able to analyze the results properly.

In all, about 100 determinations of thiophene in gasoline have been made to date. The majority of the samples were analyzed by the visual observation method. The unknown concentrations varied from 1.00% thiophene sulfur down to a mere trace of thiophene, of the order of 0.002% thiophene sulfur. A detailed study of the data obtained indicated that the results were consistent in all cases within reasonable limits, as shown by the fact that smooth curves were obtained with the results of each series of tests. Such curves would obviously have been impossible without accurate analyses, regardless of the nature of the reactions being studied. Therefore it is seen that the proposed method of analysis is accurate enough for experimental work, although the accuracy is not equal to that obtained by other methods.

ACKNOWLEDGMENT

The writers wish to acknowledge the assistance of J. A. Shenk and R. J. Morris of the Chemistry Department, Ohio State University, in making the spectrophotometer tests. The writers also wish to express appreciation to Ralph G. Pearson, Chemistry Department, Northwestern University, whose preliminary suggestions aided in organizing the work.

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RECEIVED July 22, 1947.

Biological Assay of Vitamin A

Liver Storage Test of Guggenheim and Koch

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A biological assay procedure for vitamin A is presented which depends on the colorimetric estimation of the amount of this factor deposited in the livers of previously depleted weanling rats following administration of standard and sample doses. The method is recommended for its specificity, precision, and economy over the U.S.P. curative-growth method, with which it shows substantial agreement. Application of the method to the assay of a series of vitamin A oils shows, in confirmation of previously reported work, that nonbiological assay methods, with the possible exception of the colorimetric estimation on the unsaponifiable fraction, overestimate the sample potency in direct proportion to the divergence of the ultraviolet absorption curve from that of pure vitamin A. The nature of the feeding oil diluent influences the biological response, in relation to its composition and antioxidant content.

THE need for an improved bioassay for vitamin A has not been eliminated by the recent advances in our knowledge of this factor. From a purely analytical point of view, the more precise nonbiological methods are still based upon and derive what accuracy they possess from conversion factors obtained from bioassay data. At the same time, it is obvious that a study of the physiological properties of vitamin A cannot be dependent on nonbiological methods of assay. The widespread use of these latter methods at the present time is not due to a lack of appreciation of these facts, but rather to the lack of a bioassay method that is accurate and convenient in regard to cost, time, and labor. The curative-growth (U.S.P.) method now employed discourages extensive investigation of the problems involved in the physiology, utilization, and assay of vitamin A because it is laborious, costly, and inaccurate.

Guggenheim and Koch (*3*) have recently proposed a method which offers at least a partial solution to the bioassay problem. Their method depends on the well recognized fact that the amount of vitamin A stored in the liver varies directly with the dose fed.

ASSAY PROCEDURE

In repeating this work and applying it to a routine bioassay procedure, the authors have established the following scheme, the details of which, naturally, will vary from laboratory to laboratory depending on local conditions.

Weanling rats (18 to 22 days of age and 40 to 45 grams in weight) from a piebald strain reared in the authors' laboratory are arranged into groups of 12 for each test level, and given the usual U.S.P. vitamin A–free ration for a depletion period of 6 days. The animals are then dosed on 2 successive days with 0.1 ml. of oil containing from 150 to 300 units of vitamin A diluted from either the standard or a test sample. On the fourth day after the first dose the livers are removed and composited in groups of 4 (2 males and 2 females) for analysis by a modified Carr-Price procedure according to the method of Gallup and Hoefer (*1*).

In practically all cases, multiple-level assays have been employed. A typical protocol (Table I) illustrates the arrangement of data. It is seen that each assay group yields three analytical values from which separate calculations of the average number of units per liver are made. The potency of each test dilution is obtained graphically from the plot of the standard data. The known dilution factor then allows the potency of the original sample to be determined.

DISCUSSION OF METHOD

For the sake of clarity, certain points involved in the development of the method require mention. First, the U.S.P. vitamin A–free ration (*5*) as used by the authors contains corn oil (Mazola) as the fat component. This oil imparts appreciable quantities of tocopherol to the diet as well as other protective factors advantageous in the economy of vitamin A in vivo.

The depletion period was purposely made as short as possible, since it is desired to avoid any reduction of the vitamin A in the tissues or in circulation. Six days were found sufficient to reduce the liver vitamin to about the limiting level of the analytical method.

The number and size of the doses necessary to give an optimum storage response in the shortest time were determined experimentally. The idea of a single dose was discarded when it was found to result in erratic values, whereas two doses on successive days resulted in sufficiently uniform data for the purpose of the test.

Throughout the work, corn oil (Mazola) was used for the routine dilution of the standard and test samples. This is an important factor, since the diluent oil has been found to exert a marked influence on the absorption and storage of the vitamin. The relationship between the potency of the original sample and the percentage of diluent oil in the solution "as dosed" is demonstrated in Table II. This two-dose procedure is limited to samples having potencies greater than 3000 units per gram. While this limit may be lowered by altering the number or size of the doses, special consideration must be given to the assay of materials whose potencies fall below the range of 10,000 to 20,000 units per gram, because of the disproportionate amounts of diluent oil in the two dosing dilutions.

From the beginning of work with this method, it was realized that the confused state of affairs with regard to the vitamin A standards would have to be side-stepped. Following Gridgeman's suggestion (*2*), a "spectroscopic unit" was adopted and defined as the quantity of vitamin A necessary to give a value for $E^{1\%}_{1\ cm.}$ of 0.0005 at 3280 Å. Since this corresponds to a con-

Table I. Typical Data from a Liver Storage Assay

	Dose Level[a] per Rat per Day	Liver Response, Units per Liver Group	Liver Response, Units per Liver Av.	Equivalent Vitamin A, Units per Dose	Potency, Units per Gram
Standard	145 units	74			
		78			
		75	76	...	...
	290 units	198			
		204			
		190	197	...	...
Sample	2.66 mg.	57			
		60			
		68	62	129	48,400
	5.30 mg.	189			
		183			
		200	191	283	53,300
					Av. 50,900

[a] Each dosage level made up of 3 groups of 4 rats each.

version factor of 2000, the unit has the same spectrophotometric value as that which has been widely used by the industry. At the same time, the authors employed crystalline vitamin A acetate (Distillation Products, Inc.) as the reference standard for the bioassay. This combination of unit and standard permits the assay results to be calculated in terms of either the common (U.S.P.) unit or a definite weight of a pure, active vitamin A derivative.

Table II. Percentage of Corn Oil in Feeding Dilutions with Samples of Decreasing Potency

Sample Potency, Units per Gram	Percentage of Corn Oil	
	150-unit level	300-unit level
1,000,000	99.85	99.7
62,200	97.5	95
32,600	95	90
16,300	90	80
8,150	80	60
5,400	70	40
4,100	60	20
3,260	50	0

Table III. Range in Liver Response to Reference Standard

Assay No.	No. of Analytical Groups[a]	Dose[b], Units per Rat per Day	Av. Liver Response, Units per Liver	Range, % Response
12	2	75	19	26.3
	2	121	40	22.5
14	3	71	20	40.0
	3	114	55	16.4
15	3	71	19	42.1
	2	114	42	11.9
17	3	143	75	16.0
	3	286	219	1.4
18	3	143	61	16.4
	3	143	66	6.1
	3	143	61	14.8
	3	143	55	9.1
19	3	143	76	5.3
	3	286	197	7.1
20	3	139	73	45.2
	3	278	181	8.8
21	3	150	70	8.6
	3	300	171	37.4
23	3	143	67	6.0
	3	292	179	9.5
25	3	143	66	10.6
	3	143	75	29.4
26	3	146	67	16.4
	3	292	195	25.1
27	3	149	71	73.3
	3	298	130	56.2
28	3	149	63	15.9
	3	298	164	33.5
29	2	145	76	1.3
	3	291	180	5.6
30	3	145	64	25.0
	3	290	186	11.8
31	3	142	79	16.5
	3	284	196	13.3
32	2	145	63	3.2
	3	290	173	19.1
33	2	143	69	16.0
	3	285	182	15.4
34	2	143	66	33.4
	3	285	192	9.9
37	3	149	76	14.5
	3	299	198	29.3
38	3	149	74	23.0
	3	298	183	11.5
39	3	149	66	6.1
	3	298	166	17.5
40	3	151	48	20.8
	3	303	146	24.6
41	3	148	58	20.7
	3	296	172	29.0
				Av. 19.6

[a] Four animals per group.
[b] Dose per rat per day given for two consecutive days.

Since this was written, the United States Pharmacopoeia Committee of Revision has announced the adoption of a new reference standard for vitamin A which became official January 1, 1948. This new standard is a cottonseed oil solution containing 3.34 mg. of crystalline vitamin A acetate equivalent to 3.0 mg. of vitamin A alcohol per gram. The biological potency of the solution, fixed by definition, is 10,000 U.S.P. units per gram. Collaborative spectrophotometric data have established the conversion factor as 1894. Consequently, where units are given in this paper, the figures may be converted to U.S.P. units by multiplying by 1894/2000 or 0.947.

DISCUSSION OF RESULTS

The nature of the response curve obtained with crystalline vitamin A acetate is essentially linear over the dose range employed, with a slope equivalent to 0.84 unit of vitamin A stored per liver for each unit of dose per rat per day.

The precision of the Guggenheim and Koch method has been roughly estimated from a series of assays, since an individual assay does not lend itself conveniently to statistical treatment. Two indexes of precision have been selected: (1) the range in the analytical values for liver response to the reference standard, expressed as per cent of the average response (Table III), and (2) the range in potency between two assay dosage levels, expressed as per cent of the potency (Table IV). The average range in liver response to the standard was 19.6%, which, in view of the slope of the dose-response curve, is equivalent to a 13% range in potency. This compares well with a 9.4% range in estimated potency of assay samples calculated from the range in potency between sample levels.

A number of samples that have been assayed more than once by the liver storage method have been collected in Table V. In general, the agreement between assays appears to be as good as or better than that obtained in the curative-growth method.

In a few instances, the same samples have been assayed by

Table IV. Range in Potency between Dosage Levels in Individual Bioassays

Assay No.	Sample No.	Average Potency, Units per Gram	Range, % Av. Potency
17	1	870	18.4
20	2	1,400	1.3
28	3	10,600	17.9
26	4	14,000	6.4
23	5	14,100	5.0
30	6	14,100	11.3
23	7	16,500	8.5
21	8	16,800	1.8
26	9	17,100	12.9
33	10	26,000	4.2
34	11	32,900	6.4
29	12	34,800	7.2
15	13	36,800	18.5
28	14	42,300	7.1
19	15	50,900	9.6
29	16	136,000	15.5
26	17	244,000	13.1
26	18	261,000	21.0
21	19	273,000	10.6
30	20	289,000	14.5
23	21	316,000	10.1
29	22	362,000	9.7
23	23	371,000	12.9
28	24	384,000	4.4
33	25	484,000	3.3
28	26	783,000	3.2
29	27	860,000	6.5
19	28	882,000	1.4
			Av. 9.4

Table V. Variation in Replicate Bioassays

Sample	Individual Results	Av. Potency	Range	Range, % Av. Potency
	Units per gram			
a	32,900, 36,800	34,900	3,900	11.2
b	722,000, 731,000	727,000	9,000	1.2
c	289,000, 270,000	280,000	19,000	6.8
d	371,000, 362,000	367,000	9,000	2.5
e	1,400, 1,360	1,380	40	2.9
f[a]	1,080, 870, 800, 1,180	980	380	37.8

[a] Different bottles of U.S.P. reference oil 3.

both the U.S.P. curative-growth method and the Guggenheim-Koch liver storage test (Table VI). The authors feel that there is no indication of a major discrepancy between the two methods.

By comparison, the liver storage technique shows considerable advantages over the curative-growth method in the following respects:

Short Depletion Period. Time required is one third to one fourth that for the U.S.P. assay.

Very Short Dosing Period. Only about one fifteenth of the time required for U.S.P. assay, which minimizes not only labor cost but also chances for vitamin A loss in the dosing dilutions or in the original sample during the test.

Normal Assay Animals. Because of the short time involved, there is little danger of intercurrent infections or loss of animals from assay groups. There are no losses from overdepletion. The rats grow normally throughout the test.

Specific Criterion of Response. Graded accumulation of liver vitamin A in response to graded dosing is a definitely specific response, whereas growth rate may be influenced by a variety of uncontrolled factors. The fact that a nonbiological analytical method must be employed for the liver determination offers no theoretical objection to the validity of the assay. The use of a reliable reference standard nullifies any small, constant error in the colorimetric liver analysis.

The principal disadvantage of the liver storage procedure as employed lies in the high sample potency requirement. The time and labor required to analyze the livers is a minor drawback. One analyst can handle 16 to 18 liver samples in an 8-hour day and the livers may be deep-frozen and stored without appreciable loss of vitamin A.

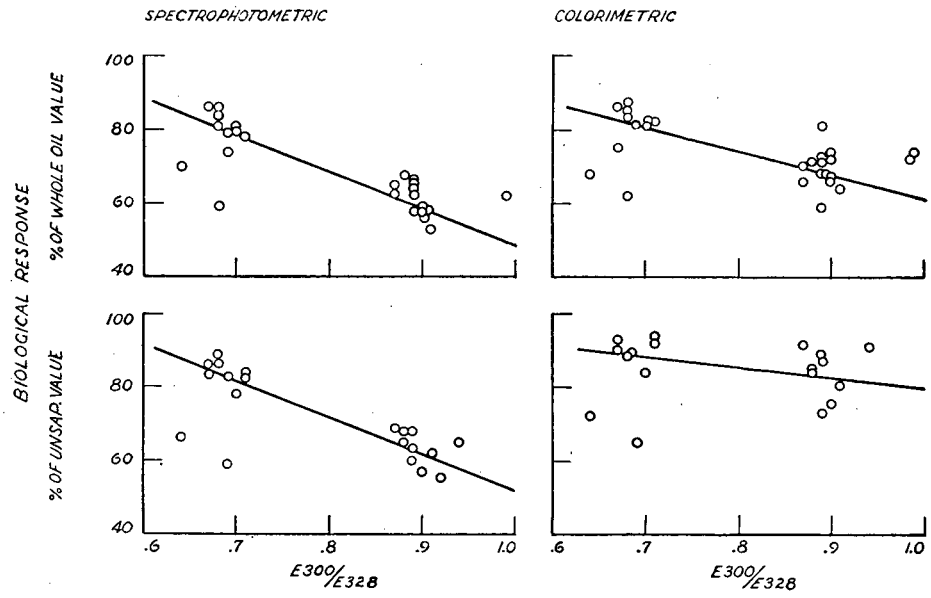

Figure 1. Relation between E_{300}/E_{328} Ratio and Biological Response

Expressed as percentage of nonbiological value for samples having potencies above 10,000 units per gram

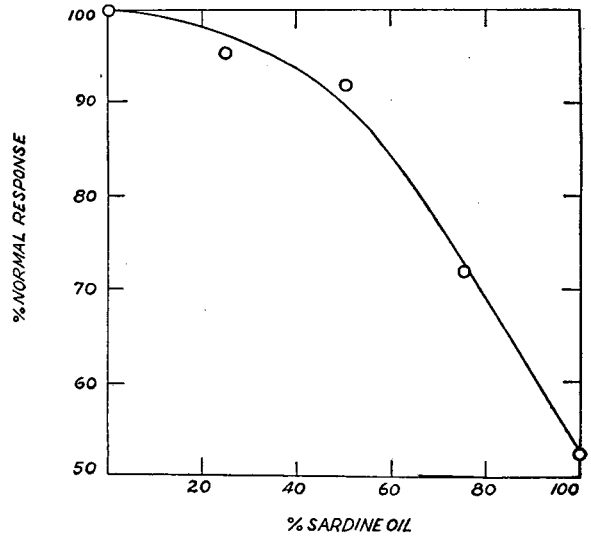

Figure 2. Decrease in Biological Response to Vitamin A Acetate with Increasing Proportions of Sardine Oil in Feeding Dilution

BIOLOGICAL *vs.* NONBIOLOGICAL ASSAYS

Throughout the course of this work, the bioassay results have been compared with those of the spectrophotometric and colorimetric determinations on both the whole oil and unsaponifiable extract basis. As has been previously reported by Oser *et al.* (*4*), the difference between the biological and nonbiological methods is related to the divergence of the ultraviolet absorption curve from that of pure vitamin A. This divergence is most conveniently expressed by the ratio of the E values at 3000 and 3280 Å. (R_{300}). When the biological values are expressed as percentages of the nonbiological potencies and plotted against the R_{300} figures for a series of vitamin A materials, apparently linear relationships such as those shown in Figure 1 are obtained.

It is plain that the spectrophotometric determination on the whole oil overestimates vitamin A potency, as the R_{300} values increase while the colorimetric determination on the unsaponifiable extract is almost independent of that factor. Among the nonbiological assays, therefore, the latter is the method of choice for materials of high R_{300} values, since the results parallel more closely those obtained by bioassay.

Table VI. Comparison between Curative-Growth and Liver Storage Assay Values

Sample	Potency by Curative-Growth Method	Potency by Liver Storage Method	% Av. Difference
	Units per gram		
Vitamin A concentrate	156,000	155,000	0.64
U.S.P. reference oil (No. 3)	1,370 1,230	1,180	9.7
β-Carotene	2,060,000[a] 1,820,000[a]	2,150,000[b]	10.2

[a] Sample of β-carotene furnished in collaborative U.S.P. assay.
[b] Sample of β-carotene from General Biochemicals, Inc.

Table VII. Effect of Feeding Oil Diluent on Biological Response to Vitamin A Acetate

Diluent	% Response
Corn oil	100 (by definition)
Sesame oil	101.1
Cottonseed oil	89.3
Olive oil	82.5
Mineral oil	71.3
Menhaden oil	52.7
Sardine oil	47.1

However, the foregoing statements hold more nearly true for oils having potencies in excess of 10,000 units per gram. Below this level the biological response averages 40 to 60% below the nonbiological value, with no correlation between the two. This is true even for comparisons with colorimetric assays on the unsaponifiable extract of oils with low E_{3000}/E_{3280} ratios. The low biological response of these oils appears to be due to the composition of the oils themselves apart from their vitamin A content. From the data of Table II it is apparent that when the potency of a material falls below 10,000 units per gram, the sample itself will constitute more than 50% of the feeding dilution and, therefore, be able to influence the biological response, not only by its vitamin A content, but also by its composition. This hypothesis was confirmed when identical dilutions of crystalline vitamin A acetate in sardine oil and corn oil were compared and it was found that the sardine oil dilution showed only half the activity of the corn oil dilution. The quantitative influence of increasing amounts of sardine oil on the biological response to crystalline vitamin A acetate was investigated and is illustrated in Figure 2. The effect is small with concentrations of sardine oil up to 50% of the feeding dilution, but above this the response falls off sharply.

Several oils were examined for their effect on the biological response to crystalline vitamin A acetate. The results, given in Table V, vary with the nature of the oil.

From previous work of Hickman and others it might be inferred that the observed variations in biological response could be explained by differences in the tocopherol content of the oils. The authors therefore investigated the effect of α-tocopherol on liver storage in several experiments. It was observed that α-tocopherol had no effect when crystalline vitamin A acetate was fed in a corn oil dilution, which was not surprising in view of the excellent protective characteristics of this oil. However, when vitamin A acetate was diluted in sardine oil, α-tocopherol was definitely effective, 4.6 mg. per dose increasing the response from 44.0 to 87.7% of the corresponding response of a corn oil dilution.

These data indicate that two types of factors are operative in influencing the biological response to vitamin A: (1) protective factors, such as the tocopherols, tending to increase the response, and (2) inhibitive factors, essentially uncharacterized, tending to lower the response. On this basis we can at least bring into line such discordant facts as the lowering of response with sardine oil, which is less apparent with mineral oil, and the ability of tocopherol to counteract partially, but only partially, this effect of sardine oil.

Obviously, the answers to many of the questions which have been raised must be left to further studies. The authors believe that the biological assay method outlined will be of substantial value in work of this nature.

ACKNOWLEDGMENT

The authors are indebted to Lawrence Rosner for advice regarding the colorimetric liver analyses and to Mildred Rose for technical assistance with the animal assays.

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RECEIVED July 17, 1947. Presented before the Division of Biological Chemistry at the 111th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Improved Steam-Distillation Apparatus

Application to Determination of Nicotine in Green and Dry Tobacco

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A new steam-distillation apparatus for determination of nicotine eliminates many of the disadvantages of older forms. The nicotine in a 0.1- to 2.0-gram portion of tobacco can be distilled in 5 to 7 minutes from a volume of 5 to 15 ml., the final distillate volume ranging from 75 to 250 ml. Portions for analysis are introduced and removed without disconnecting the apparatus; frothing causes no difficulty; the apparatus requires no attention during operation and is easy to clean. A new method for determining nicotine in green tobacco, with recovery of nicotine from an acetone extract as its basic feature, permits determination of the chloroplast pigments and gives more accurate values for green leaves than determinations made after drying them. Use of Selas rather than Gooch crucibles decreases the time involved in filtering, igniting, and weighing the precipitates without decreasing the accuracy of determination. In the presence of nornicotine, high results for nicotine are obtained; the error is directly correlated with the nornicotine-nicotine ratio and seldom exceeds 5% in ordinary tobacco. Reproducibility of the method is comparable with that of conventional methods.

THE steam-distillation apparatus and methods for nicotine determination described herein were developed when the apparatus and method of Avens and Pearce (*2*), formerly used in this laboratory, proved inadequate for the number of determinations to be made. The apparatus eliminates many of the objectionable features of previously described steam-distillation apparatus (*1, 2, 5*) and is much more rapid. The method of determining nicotine in dried samples is essentially that of Avens and Pearce (*2*) except for the use of Selas crucibles in filtering and igniting the precipitates. In determining the nicotine content of green leaves, the nicotine is distilled from a portion of an acetone extract of the leaves. The rest of the determination is the same as for dried samples.

APPARATUS

A sectional diagram of the distillation apparatus is given in Figure 1. The lower section of the distillation chamber should be turned at point 3 perpendicular to the plane of the paper. The three-dimensional features are shown in Figure 2, a photograph of two such stills assembled to operate from a common steam trap.

Construction of the electrical heating elements is shown in section *AA* of Figure 1. Each element is made by winding a single thickness of asbestos paper around the glass wall of the distillation chamber, winding 300 cm. (10 feet) of No. 28 Nichrome resistance wire (about 10 turns per cm.) over this, and working liquid asbestos No. 8 (B & F Manufacturing Company, Des Moines, Iowa) into the space between the wires. Two to three layers of asbestos paper and a layer of asbestos cord, wound on top of the wire, complete the heater. The heaters are connected in series with each other, a variable resistor with a maximum capacity of at least 80 ohms, and a switch. Current is from a 110-volt alternating current supply line. A 7.5- to 10-watt light bulb connected in parallel as shown in Figure 1 acts as a pilot light.

The steam before entering the distillation apparatus should be trapped to remove excess water. A satisfactory trap for the simultaneous operation of two stills is diagrammed in Figure 3. The trap is made from 8- and 44-mm. (outside diameter) Pyrex tubing. The drain tube from the trap is connected by pressure tubing to a 1-mm. capillary tube which extends to the bottom of a 1-liter suction flask. Water also enters this flask from the condenser overflow of one distillation apparatus and overflows to the drain through the arm of the suction flask. The water column in the flask causes slight pressure in the trap and is a means for getting rid of waste steam. Although steam is lost by this arrangement, it prevents excessive pressure in the trap when one of the stills is turned off. Rubber tubing is used to connect the trap with the steam source and with the distillation apparatus. The trap described is designed specifically for use with a high-pressure steam line with take-off through a needle valve, but may be used with any source of steam capable of producing a pressure of 3 cm. of mercury (0.58 pounds per square inch) in the trap when both stills are operating. Where steam must be obtained by boiling water in a flask, or from some such low-pressure source, more efficient use of steam may be obtained by substituting a stopcock for the 1-mm. capillary tubing and operating it while open just enough to permit trapped water to escape.

Operation. To start operation, water to the condensers is turned on with both stopcocks open and steam is fed into the trap until it emerges from the tip of the capillary in the suction flask. After distillation starts, the stopcock of one of the units is turned so that water in the distillation chamber is removed to the drain. The stopcock is then closed and magnesium oxide and the portion of sample analyzed are added through opening 1. The rubber stopper is replaced and the stopcock turned so that steam enters the distillation chamber. The heaters are turned on and the apparatus may be left until distillation is finished. While the first sample is distilling, the next sample and magnesium oxide are introduced into the other still.

When distillation is complete, the heaters are turned off, the stopcock is turned so as to wash the spent sample to the drain, stopper 2 is removed, and the upper part of the chamber is washed by directing a stream of water from a wash bottle around the walls of the chamber. A wash bottle similar to that described by Batson (*3*) is better for this purpose than the conventional type. Stopper 2 is then replaced and stopper 1 removed. The lower part of the distillation chamber is washed and when the still is completely drained, the stopcock is closed. Sample and magnesium oxide are then introduced and distillation is started. Removal of sample, cleaning the chamber, and addition of new sample can be accomplished in less than 1 minute. The rate of distillation can be adjusted so that the two stills keep the operator constantly at work.

A minimum pressure of 3 cm. of mercury in the trap is necessary to prevent the materials in the distillation chamber from entering the trap. Pressure in the trap may be varied from about 3 to 45 cm. of mercury (as measured by an open-end mercury manometer placed between the trap and the steam source), depending upon the experience of the operator and the volume of distillate necessary for complete distillation. The maximum speed thus far obtained by experienced operators has been an average of 2 to 3 minutes per sample when a number of samples are distilled. This is very nearly the maximum rate, since introduction and removal of materials and tests for completeness of distillation are the limiting factors. At this rate, volumes of distillate ranging from 75 to 225 ml. have been obtained, depending somewhat on the nicotine content of the tobacco. The minimum distillate volume, required for complete removal of nicotine with the majority of tobacco samples, is less than 100 ml. However, in a group of low-nicotine tobacco samples tested, the average volume

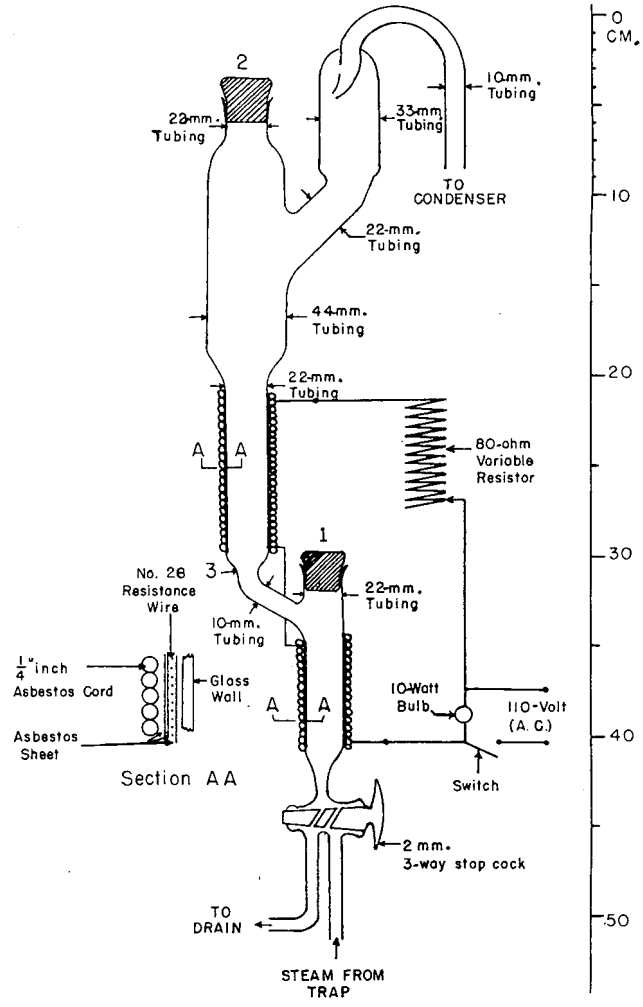

Figure 1. Diagram of Distillation Apparatus

was about 250 ml. The average time of distilling such widely different samples remains essentially the same, since the distillate volume can be controlled by varying the pressure in the trap. At a pressure of 45 cm. of mercury, the distillate volume is about 45 ml. per minute and a very efficient condenser is required. A 0.6-cm. (0.25-inch) copper tube 40 cm. long, surrounded by a glass shell as shown in Figure 2, has proved satisfactory for handling this rate of distillation. No differences in the final volume of distillate for a given sample have been found, even when the pressure has been varied between the maximum limits given above.

In work with a series of dry samples where the initial volume is normally low, the variable resistance should be adjusted during distillation until the volume remains constant. An outside resistance of 56 ohms for each apparatus giving 50-watt heaters (25 watts for each heating element) has proved satisfactory for this type of sample. In distillation from liquid samples where the initial volume is high, if the outside resistance is decreased, thereby increasing the wattage of the heaters and decreasing the sample volume, greater speed and efficiency can be obtained. It is also possible to control the heating by adjusting the voltage with a Variac or Powerstat transformer. When this method is used, the variable resistance is removed from the circuit and the heaters are connected through the transformer to the 110-volt input.

PROCEDURE

Dried Samples. Except for the stills, the procedure for determining nicotine in dried samples is essentially the one reported

by Avens and Pearce (*2*). However, several modifications are time-saving and necessary with the new apparatus.

The receiver, containing 3 ml. of hydrochloric acid (1 to 4) is placed beneath the condenser. About 0.5 gram of magnesium oxide and a sample containing 5 to 10 mg. of nicotine are put into the chamber and distillation is started. After 75 to 100 ml. of distillate are collected, additional 10-ml. portions are collected and tested with silicotungstic acid until complete distillation is assured. The nicotine is precipitated by adding 1 ml. of 12% silicotungstic acid and heating on a steam bath for 30 minutes. It is then cooled to room temperature, tested with silicotungstic acid for completeness of precipitation, and placed overnight in a refrigerator at 7° C.

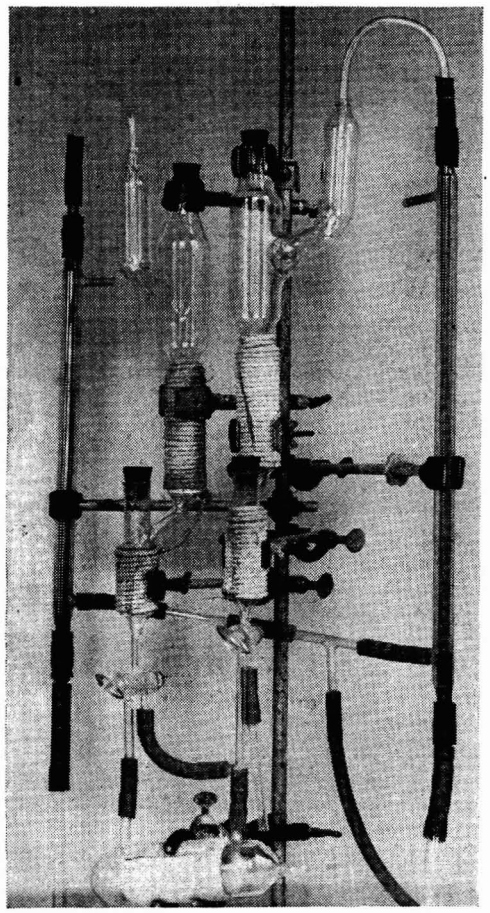

Figure 2. Two Stills Assembled to Operate from a Common Trap

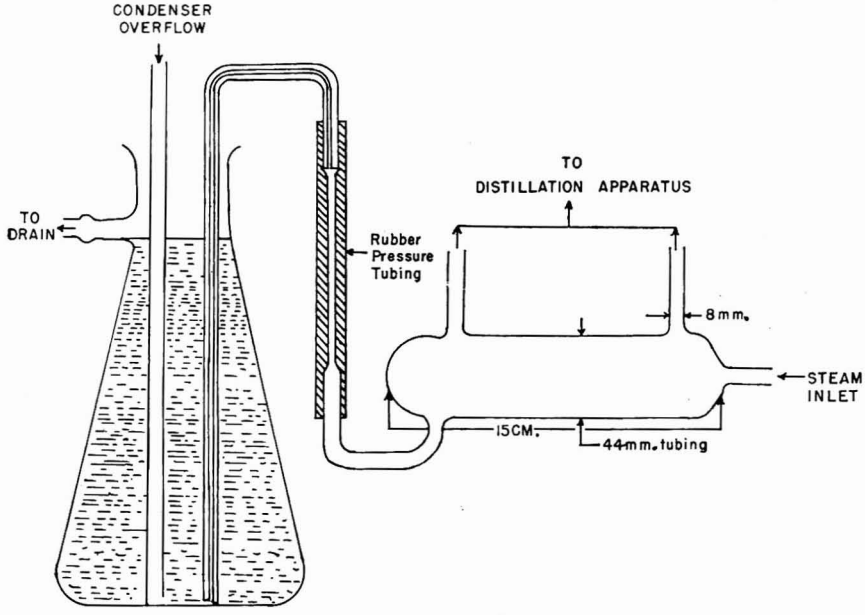

Figure 3. Diagram of Steam Trap

The precipitate is then filtered through a tared medium-porosity Selas crucible. Diluted (1 to 2000) hydrochloric acid in a wash bottle is used in making the transfer and in washing the precipitate free from silicotungstic acid. The precipitate is then ignited in a muffle furnace at 650° ± 10° C. for one hour. After being cooled to room temperature in a desiccator, the crucible is weighed and the weight of nicotine calculated by multiplying the weight of precipitate by the factor 0.114. Additional precipitates may be filtered through the same crucible, using the previous final weight as the initial weight.

The dried tobacco samples should be ground to pass a 60-mesh screen to facilitate removal through the 2-mm. stopcock after extraction and must be thoroughly mixed. The most rapid and convenient method of obtaining the weight of the analyzed portion is to place the samples in weighing bottles, introduce the portion to be analyzed into the chamber with a measuring spoon, and obtain its weight by difference. With practice, it is possible to approximate the weight very closely with the spoon and to change the weight of the duplicate portion according to the amount of nicotine estimated in the first run. Any particles remaining on the spoon are washed into the chamber with a little water from the wash bottle. The size of the analyzed portion is varied to contain 5 to 10 mg. of nicotine for accuracy.

In the Avens-Pearce procedure (*2*), the tobacco is adjusted to slightly alkaline reaction, using a strong sodium hydroxide solution and phenolphthalein as the indicator. Since great care must be taken in adjusting the reaction, if accurate, reproducible results are to be obtained, use of powdered magnesium oxide is preferred. The quantity recommended above ensures an excess with a resulting constant pH. It may be quickly added to the still with a measuring spoon, and consistent results are obtained. In experiments involving nicotine and nornicotine recovery from pure and mixed solutions with magnesium oxide as the alkali, 100% of the nicotine and 35 to 40% of the nornicotine were obtained in the distillate. Using the A.O.A.C. method and apparatus (*1*) 10 to 30% of the nornicotine was obtained. Although nornicotine is distilled under the conditions recommended in this procedure, the error involved will seldom exceed 3 to 5%, since the nornicotine content of most tobacco is less than 10% of the total steam-volatile alkaloids. Use of magnesium oxide tends to increase the speed and precision but decreases the accuracy in comparison with the A.O.A.C. and Avens-Pearce methods. It would seem that the accuracy of these methods and the method herein described is a function of the nicotine-nornicotine ratio.

Other alkalies may be substituted for those recommended without changing the described procedures in any other way. However, the alkali used will affect the results obtained and the above data are presented to show the accuracy of nicotine determinations when magnesium oxide is used. If an excess of sodium hydroxide and sodium chloride as recommended by Bowen (*5*) is used, the results obtained should be recognized as total steam-volatile alkaloids rather than nicotine, since other alkaloids such as nornicotine will be determined if present in the sample.

With the improved distillation apparatus, filtration, ignition, and weighing the precipitates are the limiting factors with respect to time. Use of Selas crucibles rather than the conventional kinds decreases the time re-

quired for these steps. Filtration by suction, ignition without the preliminary charring of filter paper, and use of only one weight per sample add to the speed without decreasing accuracy. Most of these advantages are obtained with the use of Gooch crucibles as recommended by Bowen (*4*), but Selas crucibles require less care in use. After 10 to 15 samples have been run through a crucible, the quantity of oxides becomes excessive and the crucible should be emptied, cleaned, and reignited. Even with constant daily use, the crucibles will give accurate results for 2 to 3 years.

Green Tobacco. Twenty-five to 100 grams of green tobacco leaves are extracted with acetone in a Waring Blendor for 5 to 10 minutes. Any leaf particles on the side of the Blendor cup are washed down once during the extraction, with pure acetone in a wash bottle. The volume of acetone originally added to the cup will vary with the size of sample and should be chosen so that the acetone concentration is at least 80% by volume with the quantity of water in the tissue considered. This is not a critical concentration and can be roughly approximated, since the pure acetone used in washing the sides and the residue will be sufficient to ensure complete extraction. The resulting solution is removed from the residue by filtration through a Büchner funnel. The residue in the funnel is washed with pure acetone until washings are colorless. The volume of acetone extract is measured in a graduated cylinder or made to a convenient volume in a volumetric flask. A 5- to 25-ml. portion of the extract (depending on nicotine content of the tissue) is pipetted into a 25-ml. separatory funnel and 1 or 2 drops of concentrated sulfuric acid are added. The funnel stem (inserted through a No. 3 rubber stopper) is then placed in opening 2 (Figure 1) and the extract is run into the distillation chamber. The separatory funnel stopcock is then closed and the distillation started by admitting steam to the chamber as previously described. When the acetone is distilled over, it is discarded, a receiver containing 3 ml. of 1 to 4 hydrochloric acid is placed beneath the condenser, and 5 ml. of 40% sodium hydroxide are added to the chamber through the separatory funnel without stopping the distillation. The rest of the determination is the same as for dry samples.

Sodium hydroxide is used in this case instead of magnesium oxide because it can be added to the chamber without stopping distillation, and in all tests thus far made, no differences have been obtained in the analytical results. Magnesium oxide can be used if distillation is stopped after the acetone has been distilled off, the magnesium oxide is added through opening 2, and the apparatus is closed as quickly as possible to prevent loss of nicotine. Since acetone interferes with precipitation of nicotine as the silicotungstate, it is necessary to remove all acetone before distilling the nicotine.

Table I. Nicotine Content of a Pure Solution

Method	Mg. of Nicotine/Ml. of Solution
Direct precipitation	3.70
	3.66
	3.65
	3.64
	Av. 3.66
Distillation	3.65
	3.65
	3.67
	3.67
	Av. 3.66

TESTS OF THE PROCEDURES

To test the completeness of nicotine recovery from the distillation apparatus, a nicotine solution was prepared by distilling 1 ml. of nicotine (approximately 90% free nicotine) into hydrochloric acid and making to volume in a 250-ml. volumetric flask. Each of four 3-ml. portions of this solution was diluted to about 100 ml. and the nicotine precipitated with silicotungstic acid. Four other 3-ml. portions of the solution were distilled in the apparatus by the same procedure. The results of the eight determinations given in Table I show that complete recovery was obtained.

Table II. Interlaboratory Comparison of Nicotine Determinations on Four Tobacco Samples

Sample	Nicotine Content, Per Cent: Using new apparatus	Reported by Tobacco By-Products and Chemical Corp.
1. Bright stems	(*a*) 0.529	0.521
	(*b*) 0.527	
	(*c*) 0.492[a]	
	Av. 0.528	
2. Burley stems	(*a*) 0.740	0.700
	(*b*) 0.717	
	(*c*) 0.723	
	Av. 0.727	
3. Seed-leaf stems	(*a*) 0.975	0.987
	(*b*) 0.977	
	(*c*) 0.926[a]	
	Av. 0.976	
4. Dried One Sucker tobacco	(*a*) 3.512	3.50
	(*b*) 3.528	
	(*c*) 3.491	
	Av. 3.510	

[a] Not included in average, since value is obviously low.

For testing the dry-sample method, determinations were made on four samples of tobacco obtained from the Tobacco By-Products and Chemical Corporation of Louisville, Ky., and Richmond, Va. In making the determinations, 0.4- to 1.6-gram portions (depending on the nicotine content of the sample) and the above procedure were used. The average distillation time for each determination was 7 minutes and the distillate volume ranged from 75 to 100 ml. Results of three separate determinations with the new apparatus and procedure are compared in Table II with the values reported by the Tobacco By-Products and Chemical Corporation analysts. Their analyses were made by the A.O.A.C. method (*1*) except for the use of magnesium oxide as the alkali. Their values are averages of three determinations by two analysts.

The (*c*) determinations of samples 1 and 3 are obviously low and are not included in the averages, since in routine work a sample with comparable variation in duplicate values would be rerun. The agreement in average values obtained by the two laboratories compares favorably with comparisons reported in the literature (*4*), even when similar apparatus and methods are used.

To test the acetone-extraction method, two tobacco leaves were selected from the greenhouse and the midribs were removed. Each half leaf was weighed immediately. One half of each leaf was extracted with acetone and the nicotine determined as described above. The other half was chopped up and placed in a 500-ml. Kjeldahl flask equipped for steam distillation. An excess of magnesium oxide and about 50 ml. of water were added to each and distillation was continued until 1 liter of distillate was collected. During distillation the volume was maintained at 50 ml. When 1 liter had been collected, negative tests for nicotine were obtained and the distillate was made to volume. The nicotine in a 100-ml. portion was precipitated and determined in the usual way.

The results, which are typical of other results obtained, are given in Table III. The value for direct distillation of leaf 1 was 5.0% lower than that of the acetone extract, while for leaf 2 it was about 5.6% higher. Such differences can readily be attributed to sampling error, since differences of 5% are common with the half-leaf method of analysis as used here.

Table III. Comparison between Direct Distillation of Nicotine from Green Tobacco Leaves and Distillation of Nicotine from Acetone Extract

Leaf	Method of Distillation	Nicotine (Green Weight Basis), %
1	Direct	0.170
	Acetone extract	0.179
2	Direct	0.246
	Acetone extract	0.233

DISCUSSION

The A.O.A.C. method (*1*) of determining nicotine has disadvantages, as pointed out by Avens and Pearce (*2*), who described an apparatus and method for eliminating some of them. Although the Avens-Pearce method is faster than the A.O.A.C. method, it is too slow for rapid routine analysis of many samples. The apparatus requires too much attention during operation to permit the simultaneous use of several units. Bowen and Barthel (*5*) describe an apparatus well suited to the use of several in a bank; however, there is considerable increase of sample volume during distillation and a great tendency for frothing, and the distillation time is about the same as with the Avens-Pearce apparatus. The conventional steam-distillation apparatus as used in the A.O.A.C., Avens and Pearce, and Bowen and Barthel methods must be disassembled for sample introduction, removal, and cleaning.

The apparatus described herein is far more rapid; there is no tendency to froth, and sample introduction, removal, and cleaning are merely a matter of turning a stopcock and removing rubber stoppers. The apparatus requires a minimum of desk space and because of the speed of distillation the need of a bank of stills is eliminated.

The method for determining nicotine in green samples eliminates drying and grinding the sample and gives a more accurate value for the nicotine content of green tissue, since 10 to 30% of the nicotine is lost in the common methods of drying. Although drying at low temperature prevents this loss, such drying methods are slow and require equipment not available in most laboratories. This method also permits the simultaneous determination of nicotine and chloroplast pigments as described by Griffith and Jeffrey. (*6*). The acetone-extraction method yields quantitative results if the nicotine is distilled immediately, but nicotine is lost if the acetone extract is allowed to stand. This loss occurs even at temperature as low as 7° C. but can be prevented by the use of concentrated sulfuric acid.

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RECEIVED June 2, 1947. The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

Determination of Lithium Aluminum Hydride in Solution

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A rapid method for determination of the concentration of lithium aluminum hydride solution is based on the liberation of hydrogen by the hydrolysis of this reagent. An apparatus is described in which this is accomplished at constant temperature and the evolved hydrogen is measured by change in pressure.

SOON after the discovery of lithium aluminum hydride by Finholt, Schlesinger *et al.* (*1*, *2*) it was found that this reagent is of great value as a synthetic tool for the reduction of many organic compounds (*3*). In using this reagent at this laboratory a rapid quantitative method for its determination was needed. Because the lithium aluminum hydride was prepared and used in ether solution, a method was sought for the determination of its concentration without the removal of the solvent.

It has been shown (*1*) that lithium aluminum hydride is decomposed by water to liberate hydrogen quantitatively according to the equation

$$LiAlH_4 + 4H_2O \longrightarrow LiOH + Al(OH)_3 + 4H_2$$

The present method of analysis is based on this reaction and an apparatus was designed and constructed in which a known volume of solution is hydrolyzed and the evolved hydrogen is measured by change in pressure.

Inasmuch as the hydrogen is determined by pressure change, the error due to variation in the vapor pressure of ether with temperature may be considerable. This error is practically eliminated by maintaining the decomposition flask at 0° C. with crushed ice and water. Although it would be desirable to keep the entire apparatus at 0° C., it was found that by reducing the volume of the exposed portion to a minimum, sufficiently satisfactory results are obtained. Use of the apparatus with the flask maintained at a higher temperature led to erratic results.

APPARATUS

The apparatus used is shown in Figure 1. The decomposition flask is a 2-liter round-bottomed flask with a 35/20 spherical socket joint. A 10-ml. buret having a pressure-equalizing bypass and take-off arm is attached to the flask. The buret is closed by a tightly fitting rubber stopper. A standard ball-and-socket locking clamp is used to hold the joint between the flask and buret in order to prevent leakage. The take-off arm on the buret is connected through a T-tube to a manometer by means of

Table I. Analyses of Lithium Aluminum Hydride Solutions

Soln. No.	Vol. of Sample *Ml.*	Net Free Volume *Ml.*	Pressure Increase *Mm. Hg*	Molarity Found	Molarity by Al Method	Difference
1	8.35	1992	210	0.735	0.732	
	8.05	1984	204.5	0.740	0.714	
	10.00	1974	252.5	0.732	0.723	
				Av. 0.736	0.723	0.013
2	9.83	1990	236	0.701	0.710	
	9.90	1980	238	0.699	0.707	
	9.67	1971	231.5	0.693	0.710	
	8.32	1962	199.5	0.691		
				Av. 0.696	0.709	0.013
3	10.20	1990	162	0.464	0.474	
	10.55	1979	170	0.468	0.479	
	10.15	1969	169	0.481		
	10.40	1995	168.5	0.474		
	10.80	1984	173.5	0.468		
				Av. 0.471	0.477	0.006
4	11.40	1989	122	0.312	0.314	
	10.15	1978	108	0.309	0.319	
	10.50	1968	112	0.308	0.315	
	10.85	1957	117.5	0.311	0.312	
	10.55	1946	114	0.309		
	10.70	1936	116.5	0.309		
				Av. 0.310	0.315	0.005

small-bore tubing. A drying tube is attached to the T-tube with a short piece of rubber tubing which may be closed by a pinchclamp. Before use, the volume of the entire system is measured to within a few milliliters and the volume of the ungraduated lower portion of the buret above the stopcock is determined accurately.

PROCEDURE

The decomposition flask is clamped into place and surrounded with crushed ice and ice water. A mixture of 160 ml. of cold 10% sulfuric acid and 40 ml. of cold ether is placed in the flask and the remainder of the apparatus is assembled. The apparatus is allowed to stand for 5 minutes with the pinchclamp closed. If a change of pressure is observed, the pinchclamp is opened momentarily and the process repeated until equilibrium is reached.

Approximately 10 ml. of the lithium aluminum hydride–ether solution are added to the buret and its volume is estimated to 0.01 to 0.02 ml. The system is closed and allowed to stand for a few minutes to ensure that it is at equilibrium. The solution is run into the flask slowly until the buret is drained. After equilibrium is reached (usually 5 to 10 minutes), the increase in pressure is read to 0.5 mm. The system is readied for a subsequent analysis by opening the pinchclamp and re-equilibrating to atmospheric pressure. The concentration of hydride is calculated from the equation:

$$\text{Molarity} = \frac{\text{pressure increase (mm. of Hg)} \times \text{net free volume (ml.)}}{\text{volume of sample (ml.)} \times 68{,}100}$$

The net free volume is the total volume of the system less the volume of all solutions added. The factor 68,100 combines R, T, and the fact that 4 moles of hydrogen are liberated per mole of lithium aluminum hydride.

RESULTS

Solutions at four different concentrations of lithium aluminum hydride in ether were analyzed by this procedure. As a check method, samples of these same solutions were decomposed by 1 N hydrochloric acid and the aluminum was determined by the method of Snyder (*4*). The results of these determinations, given in Table I, indicate that the method has good reproducibility and for most purposes is sufficiently accurate for the quantitative determination of lithium aluminum hydride in ether.

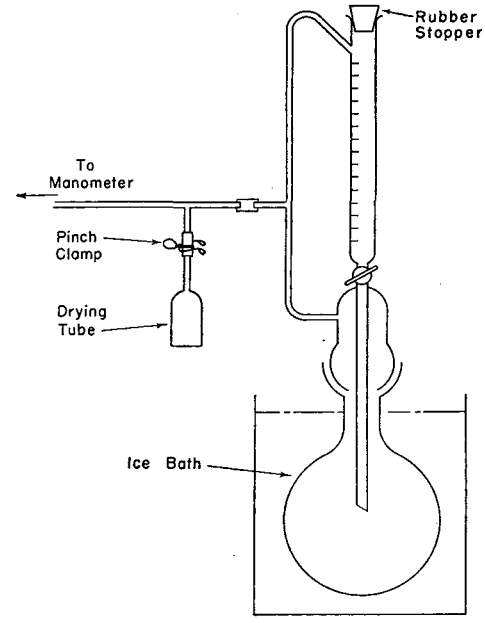

Figure 1. Apparatus for Analysis of Lithium Aluminum Hydride

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Received July 17, 1947.

Determination of Monomer in Polystyrene

Spectrophotometric and Solubility Methods

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A KNOWLEDGE of the amount of monomer left after polymerization is of importance in the manufacture of polystyrene. The conventional method of determining the monomer by ascertaining the methanol-soluble fraction of the product gives satisfactory results, but the time involved is lengthy, a matter of several hours. The fact that a spectrophotometric procedure will reduce the time required for an analysis to approximately half an hour is of interest for process control purposes. This paper describes a spectrophotometric procedure and also one dependent on solubility in methanol, as well as a comparison of data obtained by the two methods.

RESIDUAL MONOMER STYRENE BY SPECTROPHOTOMETRIC METHOD

An examination of the absorption spectra of monomeric and polymeric styrene revealed that, while the former possessed bands at about 282 and 291 millimicrons, the latter showed only slight general absorption in these regions (Figure 1). Owens (*3*) had utilized this fact to analyze partially polymerized styrene samples with a medium quartz spectrograph. The authors have adapted the method to a spectrophotometer and further studied the variables affecting the analysis. The elimination of photographic detection greatly simplified the labor involved and reduced to about 30 minutes the total time required for a single determination.

The presence of dimer styrene and other polymers of low molecular weight would tend to give high results for the monomer analysis, inasmuch as their spectra overlap that of the monomer (Figure 1). However, after the polymerization reaction, it is believed that little material of such low molecular weight remains. As regards higher polymers, it was thought worth while to investigate the dependence of the monomer absorption upon the molecular weight of the accompanying polymer. Accordingly, the absorption of 1 and 5% monomer styrene solutions, also containing 99 and 95%, respectively, of polymer fractions of varying molecular weights, was measured near 282 and 291 millimicrons. Six samples were investigated which contained polymer whose specific viscosities (*1*) divided by the concentration ranged from 0.5 to 5.4. Within the experimental error, constant absorption at each said wave length was observed (Figure 2). These results are in agreement with the work of Smakula (*4*) and Meehan (*2*), who found that polystyrenes of varying molecular weights showed

An ultraviolet spectrophotometric procedure has been developed for the rapid and accurate determination of monomer in polystyrene. For the same analysis a method dependent upon the solubility of the sample in methanol has also been devised. Results of the two methods are compared. It is shown that a large reproducible difference in the answers obtained by the two procedures can be taken as indication of the presence of an additive in the polystyrene.

the same absorption per unit weight at 262 millimicrons. This fact allowed an analytical calibration curve constructed with a particular polystyrene to be generally applicable for all polystyrenes.

The routine spectrophotometric analysis for monomer in polystyrene was carried out as follows. Calibration was effected by measuring the optical densities (read directly from a Beckman spectrophotometer Model DU) of known monomer-polymer mixtures dissolved in chloroform, at 282 and 291 millimicrons. Although the use of only one wave length would have been sufficient for the analysis, the use of both wave lengths afforded a convenient check on the determination. Plotting the quotients obtained by dividing the readings by the total sample concentrations against the percentage of monomer in the particular mixture gave

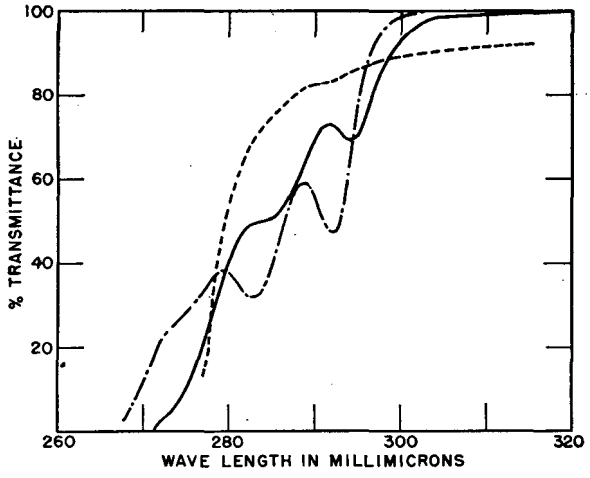

Figure 1. Ultraviolet Spectra of Various Styrenes

Chloroform solutions, 10-mm. cells. —·— Monomer, 0.057 g./l. ———Dimer, 0.058 g./l. — — — Polymer (twice precipitated), 10.15 g./l.

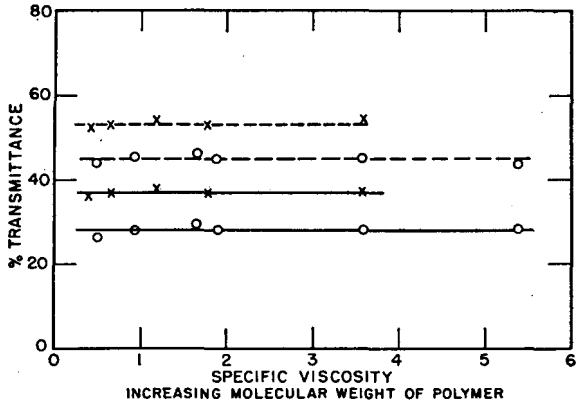

Figure 2. Independence of Monomer Absorption as Regards Molecular Weight of Accompanying Polystyrene

5% monomer, 95% polymer --x-- 291, —x— 282 millimicrons. 1% monomer, 99% polymer --o-- 291, —o— 282 millimicrons. Chloroform solutions; 1% and 5% mixtures at different dilutions

a calibration curve for that wave length. Straight lines were obtained for both wave lengths, the one for the lower wave length possessing the greater slope. Owing to a slight absorption by the polymer itself, the curves cut the absorption axis somewhat above its origin. The fact that the curves were straight lines permitted ready calibration with only two synthetics. An analysis was carried out by determining the optical densities of a chloroform solution of the sample at both wave lengths, dividing this quantity by the sample concentration, and then reading the percentage monomer from the appropriate calibration curve. The use of the quotients mentioned above instead of optical densities in the calibration procedure eliminated the necessity of using an exact predetermined sample concentration. Where the absorptions at both wave lengths were determined in an analysis, the results for percentage of styrene as obtained from the calibration curves agree to ±0.05 or better for low concentrations of monomer.

Although the use of the calibration curve is recommended for routine analyses, the monomer content can also be calculated from a knowledge of the specific absorption coefficients (100 ml. per gram cm.) for pure styrene and a polystyrene. With the aid of Beer's law, the monomer contents of five synthetic mixtures were calculated from their absorption data. As a first approximation the presence of the polymer was ignored. Then, using these approximate answers, a correction was applied to compensate for the presence of the polymer. The results of these calculations are set forth in Table I. For many purposes, it is plain that it would be sufficiently accurate to ignore the presence of the polymer.

The use of toluene as a solvent in this analysis has also been investigated. Although more desirable as regards volatility and price, its solvent action was slower than that of chloroform. In addition, the quality of the toluene as regards its spectral transparency differed markedly as its source varied, and its own absorption permitted the analysis to be carried out only at 291 millimicrons. Chloroform is recommended as a solvent, but satisfactory results can be obtained by the consistent use of a given quality of toluene. A comparison of data obtained in different laboratories, one using chloroform and the other a uniform

Table I. Spectrophotometric Determination of Monomer Styrene

Synthetic	% Monomer at 282 Millimicrons Uncorrected	Corrected	% Monomer at 291 Millimicrons Uncorrected	Corrected
66.6	67.2	...	66.8	...
50.2	51.0	50.8	51.2	50.9
20.2	20.6	20.4	20.4	20.3
11.2	11.4	11.2	11.3	11.2
2.46	2.65	2.44	2.62	2.46

Table II. Spectrophotometric Analyses of Polystyrene in Different Solvents

Sample No.	Per Cent Monomer Chloroform solution	Toluene solution
1	0.4	0.2
2	0.6	0.7
3	0.6	0.5
4	0.8	0.8
5	1.1	1.3
6	1.6	1.2
7	1.7	1.4
8	3.8	3.9

grade of toluene, is shown in Table II. These data represent the results obtained on standard commercial products and the values for each method were obtained with a single analysis.

RESIDUAL MONOMER STYRENE BY METHANOL SOLUBILITY PROCEDURE

Polystyrene is insoluble in methanol while the monomer, dimers, and trimers are soluble. But, as the amounts of dimers and trimers and impurities from the styrene present in polystyrene are relatively small, the methanol-soluble portion is principally styrene and can be taken as a measure of its presence. That this is the case is demonstrated by the data on the reproducibility of the following procedure and the agreement obtained between it and the aforementioned spectrophotometric analysis.

Ten grams of polystyrene were dissolved in 100 ml. of toluene. Complete solution was usually obtained by allowing the mixture to stand overnight, but rapid solution was possible if the polymer-toluene mixture was stirred. After solution had been completed, the mixture was slowly poured into an operating Waring Blendor which was two thirds filled with methanol (about 700 ml.). A piece of plate glass was used to cover the vessel to prevent splashing. The portion of the solution that could be added before replenishing the methanol depended upon the amount of polymer present. The methanol slurry of the fiberlike precipitate was filtered with suction, rinsed with methanol, and then dried at 65° C. to constant weight. The difference between this latter weight and the original gives the amount of monomer present in the sample.

In order to determine the reproducibility of the results, a series of tests was run on two different materials by two different operators, each making triplicate analyses. The findings are presented in Table III.

The difference between the mean values obtained in the two sets of analyses was 0.191 for sample A and 0.265 for sample B. From these two sets of data it appeared that, where three separate analyses were run on a given material, the mean value obtained was reproducible to ±0.10 if 2% monomer was present and to ±0.13 for materials containing about 9% monomer.

CHEMICALS USED

The monomer employed in the above tests was inhibited styrene obtained from Koppers Co., Inc. Its purity as determined by freezing point was 99.7% or better. The polymer used in the calibration was twice precipitated with methanol from toluene solution to remove monomer. The unfractionated polymer possessed specific viscosities varying from 1.5 to 2.0. The methanol was C.P. material obtained from various manufacturers. Nitration-grade toluene was utilized in the methanol solubility work. U.S.P. grade chloroform from various suppliers was the solvent in the spectroscopic measurements, and all the products employed appeared to be of suitable quality.

Table III. Precision of Methanol Solubility Procedure

Sample		Per Cent Monomer Operator 1	Operator 2
A		1.241	1.654
		1.323	1.385
		1.415	1.507
	Mean value	1.328	1.519
	Variation	0.087	0.134
B		7.976	7.592
		7.800	7.770
		7.702	7.389
	Mean value	7.839	7.574
	Variation	0.137	0.185

Table IV. Analysis of Styrene in Polystyrene

Sample	Per Cent Styrene Methanol solubility	Ultraviolet absorption	Difference between Methods
TP71	3.9	3.9	0.0
TP74	7.1	6.8	+0.3
TP75	4.4	4.5	−0.1
TP76	4.0	4.2	−0.2
TP84	9.8	9.9	−0.1
TP85	0.6	1.2	−0.6
TP86	1.9	2.1	−0.2
TP91	1.5	1.4	+0.1
TP97	2.4	2.2	+0.2
TP98	1.7	1.6	+0.1
TP104	0.3	0.6	−0.3
TP106	1.4	1.4	0.0
TP107	0.6	0.5	+0.1
TP108	1.6	1.1	+0.5
TP109	1.2	1.3	−0.1
398-4	10.7	10.9	−0.2
398-6	13.7	13.5	+0.2

DISCUSSION

Both methods of analysis were used on approximately 60 polystyrene samples taken at various times during the polymerization reaction. Some typical results are presented in Table IV. It is plain that the agreement between the two procedures is of the order of ±0.2 for monomer contents up to 13%, which is as good as the reproducibility of the methanol solubility method. As an absolute method for small percentages of monomer, the latter procedure is inherently less accurate than the spectrophotometric method because of its dependence upon the difference between two large quantities. There was no indication, however, that the spectrophotometric method gives higher or lower results than the solubility procedure on polymer made from pure styrene.

It is evident from these findings that variations in the molecular weight of the polystyrene do not affect the spectroscopic analysis. The results indicate that the spectrophotometric analysis offers a convenient, rapid, and suitably accurate method for determining monomeric styrene.

Table V. Indication of Additives in Polystyrene

Commercia Sample	Per Cent Monomer Methanol solubility	Spectrophotometric	Difference
1	1.7	0.6	1.1
2	3.4	1.4	2.0
3	3.1	0.7	2.4
4	2.0	0.2	1.8
5[a]	3.8	1.6	2.2
6[a]	3.4	1.0	2.4

[a] Polystyrene to which 2% modifier had been added.

INDICATION OF ADDITIVES IN POLYSTYRENE

Additives are frequently incorporated in polystyrene to change its physical characteristics. It is often desirable to know whether a given material is pure polystyrene or whether it has been modified. The methanol solubility procedure will indicate as monomer any material soluble in methanol. By comparison the spectrophotometric analysis is more specific. The presence of any additional material, as long as it does not intensely absorb radiation in the neighborhood of 282 and 291 millimicrons, will not produce high results. A reproducible difference in the answers obtained by the two methods can be taken as an indication of the presence of an additive. This view was substantiated by the fact that the analyses obtained by the two procedures agreed well on material polymerized from pure styrene, but definitely differed on various commercial samples (Table V). For the one sample which was further investigated subsequent chemical analysis showed the presence of an additive. For samples in which 2% modifier had been mixed, the expected differences in the results were obtained (Table V).

ACKNOWLEDGEMENT

The authors desire to record their appreciation to Anna Lee Welborn, Esther McCarriher, and Marion Allee, who assisted in the experimental work. They are also indebted to H. M. Hartong of Koppers Co., Inc., for some of the experimental data.

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RECEIVED August 11, 1947. Presented before the Division of Analytical and Micro Chemistry at the 111th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Helical Packing for Small Laboratory Distilling Columns

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A type of packing for small distilling columns is described. These packings, made of continuous wire helices wound about a center core, combine ease of fabrication, low operating hold-up, and high efficiency. The method of construction is described and the results of operational tests are presented.

IN THE organic chemistry laboratory there often arises the need for highly efficient, low-holdup distilling columns for fractionating small amounts of complex mixtures. Of the innumerable types of columns described in the literature, the two that seem best fitted for the purpose are the center tube column of Naragon and Lewis (*4*) and the Heli-Grid column of Podbielniak (*6*). Both columns have very low H.E.T.P.'s and very low holdup. The Heli-Grid type of packing seemed to offer the advantage of greater flexibility of throughput and take-off, but because it was expensive and difficult to construct, it was decided to investigate a simplified version of that general type. An efficient column packing evolved from this work, which can be easily constructed by anyone of ordinary mechanical skill and does not require special tools or much glass-blowing ability.

CONSTRUCTION

The helices were made of Chromel A wire, No. 30 AWG. A steel rod 1.57 mm. ($^1/_{16}$ inch) in diameter and 100 to 150 cm. long, with a small hole drilled in one end to engage the wire, served as a mandrel on which to form the helices. It was mounted in a lathe with the drilled end free. One centimeter of wire was threaded through the hole and then, while the rod was supported with one hand, the lathe was started and the wire was close-wound to form the helix.

The cores about which the finished helices were coiled were made of Pyrex. Some trouble was experienced with the 2-mm. cores' breaking while the helices were being coiled about them. It was found that less breakage occurred when heavy-walled 2-mm. tubing rather than solid rod was used. With the larger sized cores no breakage occurred. An eyelet of about 2-mm. diameter was made in each end of the core. After one end of each of the helices had been fastened to one of the eyelets, the helices were closely and evenly wound about the core with only enough tension to hold them in place, but not enough to stretch them. A paper clip or a short piece of stiff wire run through the free eyelet made a convenient handle with which to hold the core while coiling the helices. Figure 1 shows a packing of four helices in two layers in the process of being wound on the core.

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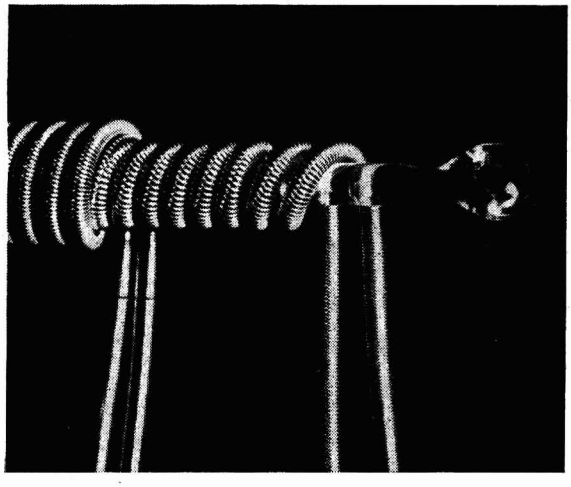

Figure 1. Packing in Process of Being Wound on Core

The selection of the column tube required some care. A piece of straight Pyrex tubing was selected which had a slight taper, no more than 0.2 mm. in a 60-cm. length, and was of such a diameter that the large end would easily admit the packing, but the small end would not pass it. Most Pyrex tubing, as received from the manufacturer, has a slight taper and about one piece in twenty selected at random has been found to have the desired characteristics. Then, after both packing and tube had been cleaned and oiled, the packing was carefully inserted in the large end of the tube and pushed in slowly until it jammed tight at the small end. The tube was cleaned with ether and dried and 12/30 standard-taper joints were sealed on as close to the packing as possible.

OPERATION

The columns were operated inside a silvered, evacuated jacket, which was in turn enclosed in a heated air jacket kept 1° or 2° below the temperature registered at the still head. A total condensation head was used with the columns. The boiler was heated electrically.

As is normal with this type of packing, the column required preflooding in order to attain the highest efficiency. Figure 2 shows a section of one of the columns before and after preflooding. The liquid seals formed between packing and tube are clearly visible. During a normal distillation the packing showed little tendency to dry out.

The efficiency of the columns was determined, using *n*-heptane–methylcyclohexane mixtures. The *n*-heptane was Phillips Petroleum pure grade, redistilled, n_D^{25} 1.3877, literature value 1.3877 (*1*). The methylcyclohexane was Eastman White Label, redistilled, n_D^{25} 1.4233, literature value 1.4230 (*2*). The tests were run under total reflux. After equilibrium had been established, samples of about 0.1 ml. were taken simultaneously from the head and boiler. Analyses of the samples were made by index of refraction and the curve of Lecky and Ewell (*3*) was used to determine the number of theoretical plates.

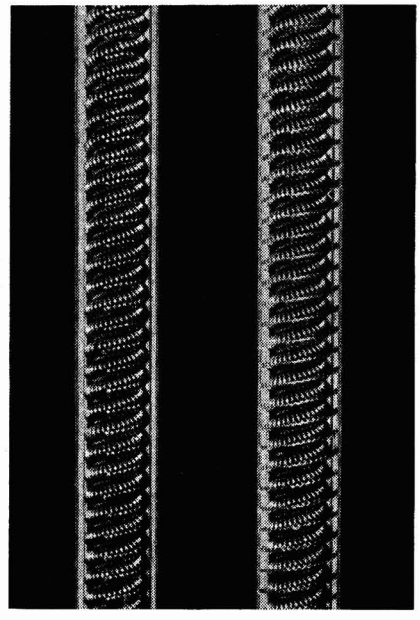

Figure 2. Packed Column before and after Preflooding

A vapor-sampling technique was used in collecting the boiler samples. This prevented any possible contamination of the sample by traces of the nonvolatile lubricant used on the ground joints of the still. A boiler was used with a side arm 6 mm. in diameter, which was closed with a rubber policeman. To minimize damage to the rubber by the hot organic vapors, the rubber tube was pinched shut with a clamp as near the end of the side arm as possible. A hypodermic syringe fitted with a 3-inch 22-gage needle was used to withdraw the sample. After the clamp was removed, the needle was inserted through the wall of the rubber policeman near its closed end and pushed down inside the side arm until the tip

entered the top portion of the boiler but remained above the liquid. By slowly withdrawing the plunger of the syringe, vapor was drawn into the needle and condensed by the cold metal. It entered the body of the syringe as a liquid.

Holdup was determined gravimetrically. While a hydrocarbon mixture was refluxing through the column at the desired rate, an empty boiler was quickly substituted for the full one. After cooling, both column and boiler were weighed. Their weight while dry having been previously determined, the increase in weight was the weight of the liquid holdup. The accuracy of this method is about ±10%. This is considered sufficient for the present purpose.

Pressure drop was measured by means of an open-ended U-tube manometer filled with the boiler mixture and attached to the side arm of the boiler.

Throughput was determined by counting drops from calibrated drippers at the bottom of the columns.

Tests were run on columns A, B, C, and D throughout their useful range of throughputs. The minimum was the rate below which the efficiency failed to increase, or in the case of column A the rate below which temperature readings and take-off could not be made in the head employed. The maximum rates shown for columns A and B are the incipient flooding points for the columns and for C and D the flood points for the head. Columns E and F were tested over only a small portion of their useful range because their relatively low efficiencies made further testing unprofitable.

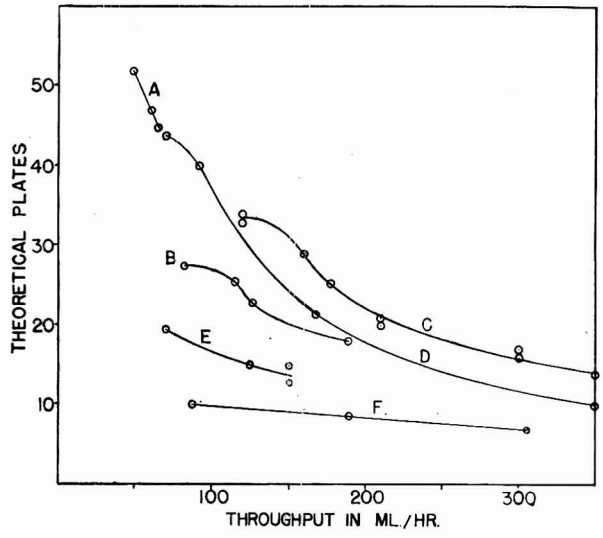

Figure 3. Efficiency of Packings at Total Reflux

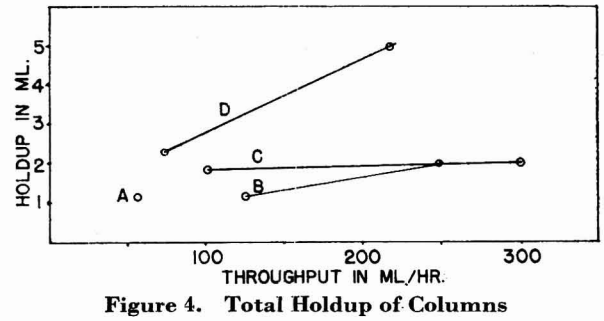

Figure 4. Total Holdup of Columns

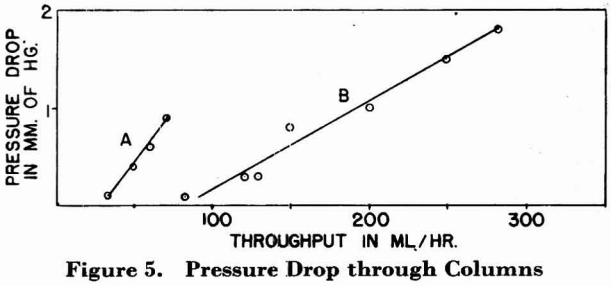

Figure 5. Pressure Drop through Columns

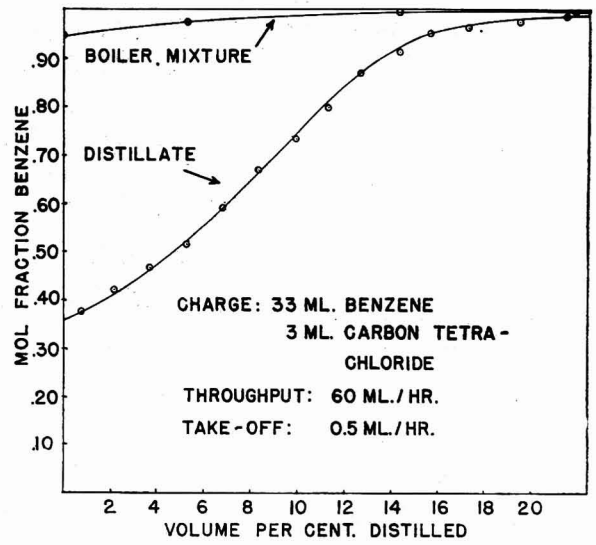

Figure 6. Distillation of Carbon Tetrachloride and Benzene through Column A

Table I. Description of Packings

Column	Packing Diameter, Mm.	Core Diameter, Mm.	Layers of Packing	Coils per Layer	I.D. of Helices, Mm.
A	6.5	2	1	2	2.0
B	6.5	2	1	4	2.0
C	11.0	6.5	1	6	2.0
D	11.0	3.5	2	4	2.0
E[a]	11.0	3.5	2	4	2.0
F	11.0	4	1	3	3.3

[a] Identical with D, except helices of outer layer stretched to give twice normal spacing between turns of wire.

CHARACTERISTICS

Six columns were constructed with various combinations of core diameter, number of layers, and number and size of helices. All the packings were 40 ± 1 cm. long. A description of the various packings is given in Table I.

The operational characteristics of the packings are shown in Figures 3, 4, and 5. Packing D (double layer of close-wound helices) showed good efficiency and flexibility of throughput, but its holdup is much greater than the single-layer types (see Figure 4). Indeed, the holdup is practically the same as that of a comparable column random-packed with single-turn wire helices of the same size (7). However, the sharpness of separation and the size of intermediate fractions depend considerably on the holdup (5). For this reason, attention was focused on the single-layer types. Of these, A and C are clearly the best; type A for fractionations of small amounts of material, 5 to 20 ml., because of its extremely small holdup and high efficiency, and type C for larger amounts of material.

Test distillations of known mixtures at various reflux ratios showed about the same variation in fractionating efficiency as other wet-wall packed columns. An elimination curve of carbon tetrachloride from benzene is shown in Figure 6.

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RECEIVED February 24, 1947. Contribution 1121 from the Gates and Crellin Laboratories, California Institute of Technology.

Determining Vitamins D_2 by Two Physical Chemical Methods

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Two methods for determining the vitamin D_2 content of oil solutions of irradiated ergosterol are described. In a spectrophotometric method the nonsaponifiable fraction of the oil is chromatographed, using a column of Superfiltrol and a solvent composed of a mixture of 50 parts of hexane, 10 parts of ether, and 1 part of alcohol. Interfering substances are adsorbed on the Superfiltrol while the vitamin D_2 portion passes through the column. The vitamin D_2 content is evaluated by measuring its extinction at 265 mμ. A colorimetric method is proposed in which the nonsaponifiable fraction of the sample is treated directly with the antimony trichloride reagent as prepared by Nield, Russell, and Zimmerli and the potency of the original sample is calculated from the extinction at 500 mμ of the treated sample. Potency values for 49 irradiated ergosterols in corn oil and five irradiated ergosterols in fish liver oil, as determined by the chromatographic method, are given with the corresponding bioassay values. Results obtained for 51 irradiated ergosterols in corn oil, when using the colorimetric method, are compared with their bioassay values.

VARIOUS methods have been proposed for determining the vitamin D_2 content of irradiated provitamins D solutions and of fish liver oils. An extensive review of these methods has been given (*5*).

Nield, Russell, and Zimmerli (*7, 12*) found that by adding acetyl chloride to the antimony trichloride reagent of Brockmann and Chen (*3*), its sensitivity could be increased and the presence of zinc, tin, or antimony had a tendency to stabilize the color reaction. They also observed that sterols having double bonds in the side chain did not affect the reaction and those having double bonds in their ring structures gave the following absorption coefficients:

1 double bond	$E^{1\%}_{1\,cm.}$	2.2 at 500 mμ
2 double bonds	$E^{1\%}_{1\,cm.}$	7 at 515 mμ
D_2 and D_3	$E^{1\%}_{1\,cm.}$	1800 at 500 mμ

Thus, the effect of sterols upon the reaction with the modified reagent appears to be small.

Shantz (*10*) found that to obtain reproducible results by the antimony trichloride reaction, temperature, light, and concentration must be rigidly controlled.

In these laboratories, the two-step chromatographic procedure, which was used successfully by Ewing, Kingsley, Brown, and Emmett (*5*) for determining the vitamins D content of fish liver oils, has been found by several investigators (*2, 6, 11*) to be inapplicable to irradiated solutions of ergosterol. Hage (*6*) modified the second chromatographic step of this procedure for assaying fish liver oils by swirling the benzene-Skellysolve solution of vitamin D_2 and sterols with Superfiltrol instead of using a column of the adsorbent to separate the sterols from the vitamins D fraction. For assaying irradiated erogsterols in volatile solvents, Powell (*9*) further modified this method by completely eliminating the second chromatographic step. He found that by using a longer column of the adsorbent and prewashing with the solvent, vitamin D_2 could be separated, quantitatively, from crude irradiated ergosterols in volatile solvents and the chromatographed material gave an ultraviolet absorption curve similar to that of a standard calciferol.

DeWitt and Sullivan (*4*) also separated vitamins D from both fish liver oils and irradiated ergosterols by chromatographic procedure. A column made up of a 1 to 1 mixture of magnesia and diatomaceous earth was used as the adsorbent and petroleum ether as the solvent. Separation was achieved by eluting, separately, the fluorescent zones observed on the column when exposed to ultraviolet light and determining the vitamins D content of the eluates colorimetrically, using as the reagent antimony trichloride dissolved in ethylene chloride.

From this review it is evident that in an accurate physical chemical method for determining the vitamin D_2 content of oil solutions of irradiated ergosterol, either all interfering materials must be removed or a suitable reagent must be used which, although it may not be specific for vitamins D_2, will not introduce an appreciable error due to its reaction with the interfering substances.

With this in mind two methods are proposed by the authors:

In the first method the interfering materials are removed from the nonsaponifiable fraction of oil solutions of irradiated ergosterol by a chromatographic procedure which is a modification of the method used by Ewing *et al.* (*5*). The vitamin D_2 content of the purified solution is then evaluated from its extinction at 265 mμ.

The second method is a colorimetric procedure using the modified antimony trichloride reagent of Nield, Russell, and Zimmerli (*7*). The nonsaponifiable fraction of the sample is treated directly with this reagent and the potency of the original sample is calculated from the measured extinction at 500 mμ of the treated sample. The effect of interfering substances is considered negligible.

EQUIPMENT AND REAGENTS

Adsorption Columns. A 9-cm. column of Superfiltrol is used except for the fish liver oils, when the length is 11 cm. It is prepared by the method of Ewing *et al.* (*5*). The packed column must be thoroughly washed with the chromatographic developing solution before the sample is added. Various lots of Superfiltrol, with which the authors have worked, give different results in the chromatographic separation. This effect, probably due to a difference in activity, seems to be associated with the color of the material; the whiter grades of Superfiltrol give a better chromatographic separation than the gray colored grades.

Spectrophotometers. For ultraviolet measurements a Beckman spectrophotometer (quartz) equipped with a hydrogen discharge tube is used. Either a Bausch & Lomb visual spectrophotometer equipped with a Martin's polarizing unit and 1-cm. glass cells or a Beckman spectrophotometer is employed for making measurements in the visual range.

Ethyl Alcohol (absolute). A C.P. grade of absolute alcohol is purified by distillation, after being decanted from a silver oxide precipitate, prepared by adding 3 grams of potassium hydroxide and 1.5 grams of silver nitrate per liter of alcohol. Further purification is obtained by adding activated aluminum amalgam, decanting, and redistilling. The purified solvent should transmit to 215 mμ.

Alcoholic Potassium Hydroxide. Fourteen grams of high-grade potassium hydroxide are dissolved in 500 ml. of 95% ethyl alcohol which has been purified in the manner described for absolute ethyl alcohol.

Ethyl Ether. Anhydrous ether (C.P.) is used as obtained. It should transmit to 219.5 mμ and be free of peroxides.

Hexane. A commercial grade of hexane is redistilled to remove any residue. The distilled product should transmit to 210 mμ.

Chromatographic Developing Solution. This solution is made up from the purified reagents described above by taking 50 parts of hexane, 10 parts of anhydrous ethyl ether, and 1 part of absolute ethyl alcohol.

Chloroform. Small amounts of alcohol are removed from C.P. chloroform by washing seven times with an equal volume of distilled water. It is then dried over anhydrous sodium sulfate, decanted, and distilled; the first and last 10% of the distillate are discarded.

Antimony Trichloride Reagent. This reagent is prepared fresh for each day's run. Eighteen grams of C.P. antimony trichloride are dissolved in 100 ml. of purified chloroform. After this solution is filtered, 2 ml. of redistilled acetyl chloride are added.

PROCEDURE

Preparation of Sample. SAMPLES IN OIL. Oil samples are weighed into small glass capsules and then placed in 125-ml. Erlenmeyer flasks containing 10 ml. of alcoholic potassium hydroxide. (When the chromatographic-ultraviolet absorption curve method was used 1-gram samples containing 100,000 to 1,500,000 units per gram were successfully run. For the antimony trichloride method a large enough sample to contain about 20,000 units is most convenient, but samples containing only 500 units can be determined accurately, as shown by Table IV.)

A short-stemmed funnel is then placed in the neck of the flask and the sample is saponified in a water bath at 70° C. from 0.5 to 1 hour or until saponification is complete. Then 20 ml. of water are added to the saponified solution and the nonsaponifiable fraction is extracted in a separatory funnel, using five 20-ml. portions of ether. The combined ether extracts are washed with at least six 50-ml. portions of water or until the ether interface is clear and the water layer is not alkaline to phenolphthalein. The first three washings are made without shaking, to prevent the formation of an emulsion.

The washed ether extract is then filtered through an anhydrous sodium sulfate filter pad into a 125-ml. Erlenmeyer flask and, after the separatory funnel is rinsed and the filter pad washed with 25 ml. of ether, the combined ether portions are evaporated to dryness, using gentle suction and a water bath at about 50° C.

SAMPLES IN VOLATILE SOLVENTS. If the sample is an irradiated ergosterol in a volatile solvent, all the above procedure can be eliminated and only the solvent needs to be removed by evaporating to dryness, using suction and a hot water bath. The dry residue from the above procedure can then be treated by either of the two following methods:

Chromatographic Ultraviolet Absorption Curve Method. The residue is taken up in 10 ml. of the chromatographic developing solution. This is allowed to percolate through the prepared 9- or 11-cm. column of Superfiltrol which has been previously washed with 40 to 60 ml. of the developer and has not been allowed to become dry. A 10-cm. differential in pressure is maintained throughout the whole procedure.

The flask is rinsed with 3 to 5 ml. of the developing solution, which is added at once to the column. By means of a short-stemmed separatory funnel which is fitted to the tube, the developing solution is added to the column drop by drop until the vitamin D_2 has passed through the adsorbent column. In the authors' experience this separation is complete when the lowest visible band reaches the bottom of the column.

The filtrate from the column is then evaporated to dryness, using suction and a hot water bath (about 50° C.). The residue is taken up in absolute alcohol and the extinction at 265 mμ is measured on the Beckman quartz spectrophotometer. The complete absorption curve of this alcoholic solution should have a maximum at 265 mμ and be similar to that exhibited by pure calciferol (Figure 1).

Table I. Extinction Ratios of Crystalline Calciferol and Chromatographed Sample in Ethanol

Wave Length, mμ	Extinction Ratios (265 mμ) Test material	Calciferol
240	0.76	0.67
250	0.89	0.85
260	0.98	0.98
270	0.95	0.98
280	0.75	0.73
290	0.47	0.43
300	0.20	0.23

Comparison of the absorption curves of ethanol solutions of crystalline calciferol and of the chromatographed sample is well shown in Table I. The extinction ratios for given wave lengths are tabulated according to the method of Oser, Melnick, and Pader (*8*).

The potency of the original oil in vitamin D_2 units per gram is then determined by calculating the $E^{1\%}_{1\,cm.}$ of the sample from the extinction obtained at 265 mμ and multiplying it by 86,960. This factor is obtained by dividing the number of vitamin D_2 units per gram of the standard calciferol (40,000,000) by the $E^{1\%}_{1\,cm.}$ at 265 mμ, which is 460. Since this work was done, Arnold (*1*) has indicated that calciferol contains 49,000,000 units per gram. Further studies are being made in this laboratory on the conversion factor.

Antimony Trichloride Colorimetric Method. The residue from the ether extract is taken up in 10 ml. of the purified chloroform. To 1 ml. of this solution, 10 ml. of the antimony trichloride reagent are added. After 30 seconds' swirling, a 1-cm. cell is filled and the extinction at 500 mμ is measured in exactly 3 minutes from the time the reagent was first added, using the Bausch & Lomb visual spectrophotometer.

The potency of the original oil in vitamin D_2 units per gram is then determined by calculating the $E^{1\%}_{1\,cm.}$ from the extinction at 500 mμ and multiplying by the factor 19,300 as determined by Ewing *et al.* (*5*). For the purpose of this publication this conversion factor is used, although it is being made the subject of further investigation.

RESULTS

The first part of Table II gives the potencies of various irradiated ergosterols in corn oil as determined by the chromatographic ultraviolet absorption curve method. In every case the absorption curves obtained are similar to that of pure calciferol, exhibiting a maximum at 265 mμ, and the respective potencies, calculated from these curves, agree fairly well with the bioassay values, when the U.S.P. procedure is used. Animal tests

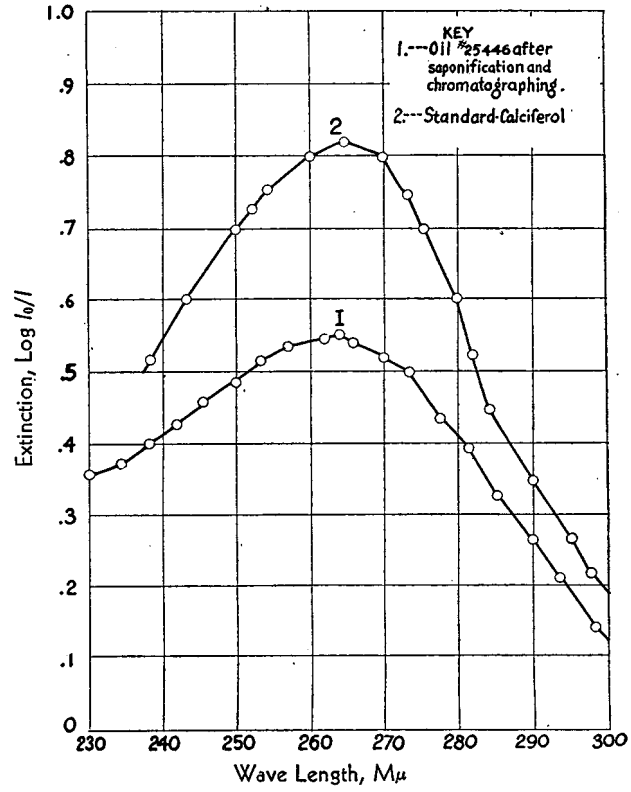

Figure 1. Absorption Curve of Oil in Alcohol after Saponification and Chromatographing

Table II. Potencies of Irradiated Ergosterols as Determined by Chromatographic Ultraviolet Absorption Curve at 265 Mμ

Sample No.	$E^{1\%}_{1\text{ cm.}}$, 265 mμ	Calculated, D_2 Units/G.	Bioassay, U.S.P. D_2 Units/G.
	High-Potency Samples in Corn Oil		
97,195	16.77	1,458,000	1,200,000
87,114	17.89	1,555,000	1,200,000
3,155	15.18	1,318,000	1,200,000
7,305	13.39	1,164,000	1,200,000
1,015	14.07	1,221,000	1,000,000
1,735	11.89	1,033,000	1,000,000
4,985	5.43	472,000	600,000
25,446	5.51	479,000	525,000
2,915	4.68	407,000	525,000
3,265	4.95	430,000	525,000
0,145	5.04	437,500	525,000
89,025	4.27	371,000	525,000
91,285	4.26	370,000	525,000
92,345	4.11	357,000	525,000
94,435	5.16	448,000	525,000
97,025	6.09	529,000	525,000
98,655	5.70	495,000	525,000
21,226	5.52	479,000	525,000
21,976	4.47	389,000	525,000
21,986	5.86	509,000	525,000
24,646	5.04	438,000	525,000
23,196	4.64	404,000	525,000
6,105	5.44	473,500	500,000
7,705	5.13	446,500	600,000
0,555	4.68	407,000	475,000
18,546	5.05	439,000	400,000
99,775	5.48	476,000	450,000
97,775	5.11	444,000	450,000
3,005	5.91	513,000	400,000
19,196	4.58	398,000	450,000
24,876	4.54	395,000	450,000
19,196	5.26	456,000	450,000
78,174	3.22	280,000	330,000
84,163	3.09	268,500	330,000
80,434	2.75	239,000	300,000
79,404	3.35	281,000	275,000
83,024	2.96	257,000	275,000
64,184	2.69	234,000	250,000
64,824	2.60	226,000	250,000
65,104	2.74	238,000	250,000
67,784	2.28	198,000	250,000
68,564	2.25	195,500	250,000
69,934	2.60	226,000	250,000
5,305	2.43	211,000	250,000
20,826	1.81	157,500	250,000
80,904	2.52	219,000	204,500
84,174	2.60	226,000	204,500
65,464	2.48	216,000	200,000
70,514	2.46	214,000	200,000
	High-Potency Irradiated Ergosterols in Fish Liver Oil		
74,254	2.57	223,000	250,000
66,844	2.42	210,500	200,000
71,064	2.32	202,000	200,000
20,206	2.97	258,300	300,000
25,646	2.02	175,800	200,000

were run at two or three levels, 15 to 20% apart, and the U.S.P. reference oil was used as the standard. As no attempt was made to interpolate between the bioassay levels, some of the discrepancies between the physical chemical and the biological data may be due to the 15 to 20% range at which the samples were tested.

The values obtained from the animal tests represent the highest biological potency that could be obtained from the levels at which the samples were tested. For example, the data of a sample tested at 1,200,000 and 1,000,000 units per gram might indicate the material to be slightly less than 1,200,000 units per gram but above 1,000,000 units per gram. In this case the assay would be reported at 1,000,000 units per gram, although the material might actually contain 1,100,000 or 1,150,000 units per gram.

Of 49 irradiated ergosterols in corn oil assayed by the chromatographic ultraviolet absorption curve method, only 8 oils differed from the bioassay values by more than 25%. The maximum per cent difference from the bioassay figures was 36.9, and the average variation of all the oils run by this method was 14.5%.

The results obtained when using the chromatographic method for five high-potency irradiated ergosterols, in fish liver oil containing vitamin A, are tabulated in the second part of Table II. The close agreement of these results with the bioassay values indicated that the chromatographic method might also be applicable to fish liver oils fortified with irradiated ergosterol. However, not enough oils of this type were tested to recommend using this method for them.

Table III. Potencies of Irradiated Ergosterols as Determined by Antimony Trichloride Colorimetric Method

Sample No.	Average $E^{1\%}_{1\text{ cm.}}$, 500 mμ	Calculated, D_2 Units/G.	Bioassay, U.S.P. D_2 Units/G
	High-Potency Samples in Corn Oil		
87,114	71.28	1,376,000	1,200,000
88,875	58.08	1,121,000	1,200,000
97,195	70.10	1,359,000	1,200,000
1,015	56.76	1,095,000	1,000,000
1,735	52.80	1,019,000	1,000,000
7,305	50.49	974,000	1,200,000
89,025	16.72	323,000	600,000
94,435	23.76	459,500	600,000
25,446	25.45	492,000	525,000
92,345	18.53	376,000	525,000
25,476	24.86	480,000	515,000
25,456	25.82	498,000	500,000
6,105	23.10	446,000	500,000
4,985	22.22	429,000	600,000
7,705	20.57	397,000	600,000
19,656	22.60	436,000	525,000
21,226	23.75	459,000	525,000
21,976	23.10	446,000	525,000
21,986	26.10	504,000	525,000
23,196	25.10	484,000	525,000
91,095	23.76	458,500	400,000
25,496	21.34	412,000	450,000
8,645	15.40	297,500	325,000
78,174	14.11	275,000	330,000
84,614	11.22	216,500	330,000
77,424	9.46	182,000	300,000
80,434	12.98	251,500	300,000
79,404	13.97	270,000	275,000
83,024	13.42	259,000	275,000
64,184	13.64	263,500	250,000
64,824	10.12	195,500	250,000
65,104	12.32	238,000	250 000
67,784	9.46	182,500	250,000
68,564	9.68	187,000	250,000
69,934	10.01	193,000	250,000
84,944	11.00	212,500	250,000
5,305	10.23	197,500	250,000
80,904	9.35	180,500	204,500
84,174	10.34	199,500	204,500
65,464	10.18	198,500	200,000
70,514	10.56	204,000	200,000
74,294	10.85	209,500	200,000
73,534	10.56	204,000	180,000
42,943	6.09	117,000	160,000
38,133	5.19	100,000	125,000
75,584	8.14	157,000	100,000
18,546	22.70	438,000	400,000
19,196	20.45	395,000	450,000
20,826	8.03	154,800	250,000
24,646	23.10	446,000	450,000
24,876	22.45	433,000	450,000
	Low-Potency Samples in Corn Oil		
0,855	0.594	11,500	14,000
0,865	0.550	11,000	14,000
2,985	0.605	12,000	14,000
0,905	0.418	8,000	10,000
74,334	0.450	9,000	10,000
89,545	0.594	11,500	10,000

Table III gives the potencies of various irradiated ergosterols in corn oil as determined by the antimony trichloride colorimetric method. The first part of Table III is made up of values obtained for high-potency irradiated ergosterols in corn oil, and the second part consists of values for low-potency samples in corn oil or around 10,000 units per gram. These were obtained on the open market.

Out of the 51 high-potency oils assayed by the antimony trichloride colorimetric method, 10 oils showed a difference from the bioassay of more than 25%. The maximum per cent difference shown by this method was 57.0, while the average variation of all the oils tested was 15.8%. The maximum variation shown when testing six low-potency oils of about 10,000 vitamin D_2 units per gram by this method was 21.4%. The average per cent difference was 16.4.

The potency values of an oil determined by each of the two methods agree very closely and usually fall on the same side of the bioassay value. In almost every case where the difference from the bioassay value was appreciable, the two physical-chemical values agreed with each other very well.

Table IV. Reproducibility for Irradiated Ergosterols in Corn Oil

$E^{1\%}_{1\ cm.}$	Calculated, D_2 Units/G.	Bioassay D_2 Units/G.
Chromatographic Ultraviolet Absorption Curve Method, Oil 65,464		
2.28	187,000	200,000
2.09	182,000	
2.12	184,500	
2.02	176,000	
2.10	183,000	
	Av. 182,500	
Antimony Trichloride Colorimetric Method, Oil 25,456		
25.52	492,500	500,000
25.30	488,500	
24.42	471,500	
25.96	501,000	
25.96	501,000	
26.18	505,500	
26.40	509,500	
25.74	497,000	
25.96	501,000	
26.40	509,500	
	Av. 497,700	

Table V. Effect of Amount of Sample upon Accuracy of Antimony Trichloride Colorimetric Method

Wt. of Sample, G.	Calculated D_2 Units in Sample (Based on Bioassay)	$E^{1\%}_{1\ cm.}$ 500 mμ	Calculated Potency, D_2 Units/G.
1.000	14,000	0.606	11,690
0.4992	7,000	0.473	9,130
0.2514	3,520	0.542	10,480
0.1262	1,769	0.634	12,220
0.0640	897	0.676	13,030
0.0372	521	0.537	10,370
0.0144	202	0.389	7,510

Results obtained by both physical chemical methods can be reproduced very easily, as shown in Table IV. Five separate samples of oil 65,464 were run by the chromatographic ultraviolet absorption curve method and the maximum deviation from the average value was 8.7%. Ten different determinations for oil 25,456 by the antimony trichloride colorimetric method showed a maximum deviation from the average of 1.6%.

Reliable determinations were obtained by the antimony trichloride colorimetric method with oils containing as low as 10,000 vitamin D_2 units per gram and the indications are that oils of much lower potency can be evaluated successfully.

In order to determine how small an amount of sample may be employed and still an accurate potency determination be obtained by the antimony trichloride colorimetric method, various amounts of an oil containing about 10,000 vitamin D_2 units per gram were put through the procedure. The results, as shown in Table V, indicate that samples containing as low as 500 vitamin D_2 units can be assayed with a fair degree of reliability. However, when working with very small amounts of the sample, it is necessary to add the reagent directly to the ether extract residue. Ordinarily, in a large sample, the residue is taken up in chloroform and a 1-ml. aliquot of this solution is mixed with 10 ml. of reagent. Thus, the concentration of the reagent in the solution cell is slightly more dilute than that in the solvent cell. This difference cannot be detected on the visual spectrophotometer at 500 mμ, however, and no appreciable error is introduced by adding the reagent directly to the dry residue.

Although very small amounts of vitamin D_2 may be measured as indicated by Table V, the antimony trichloride colorimetric method is not recommended for oils containing below 10,000 vitamin D_2 units per gram. Corn oil alone also exhibits, to a slight extent, the same color reaction with the antimony trichloride reagent as vitamin D_2, thus introducing another error in the determination. At the present time more work is in progress to determine the extent and possible ways of correcting for the error due to a high concentration of corn oil.

DISCUSSION

For testing oil solutions containing less than 50,000 vitamin D_2 units per gram the antimony trichloride colorimetric method is preferred, since the residue in the solvents which have been used as developers of the Superfiltrol column may show a considerable amount of absorption in the ultraviolet region.

The absorption, due to a residue picked up from the Superfiltrol, may be caused by dissolved impurities. This can, however, be reduced to a minimum by first washing the column with 40 to 60 ml. of the developer. The last portion of the wash solution will have an extinction of about 0.01 when referred to the original developing solution. This small amount of absorption, however, will introduce a considerable error in the determination of vitamin D_2 oils containing less than 50,000 units per gram when 1-gram samples are used. Taking larger samples will increase the accuracy when working with low-potency oils.

The method of packing and making the chromatogram is very similar to that used by Ewing *et al.* (*5*). The main differences include an essential prewashing of the column, the use of a longer column, and the substitution of hexane for Skellysolve in the developing solution. The Skellysolve used by Ewing *et al.* (*5*) cannot be employed successfully in this method because of its absorption in the ultraviolet. Redistilled hexane does not have this absorption and is very satisfactory.

CONCLUSIONS

Two physical-chemical methods for determining the vitamin D_2 content of samples of irradiated ergosterol in corn oil have been developed.

One method, using the ultraviolet absorption curve of the nonsaponifiable fraction of the oil sample which had first been chromatographed to separate the impurities, gave an average variation from the bioassay value of 14.5% when 49 different oils were tested. This method is recommended for oils containing 50,000 or more vitamin D_2 units per gram, when 1-gram samples are used.

A colorimetric method, using the color reaction of vitamin D_2 obtained by adding an antimony trichloride reagent to the nonsaponifiable fraction of the oil sample, gave an average variation of 15.8% from the bioassay values of 51 high-potency oils and an average difference of 16.4% from the bioassay values of 6 low-potency oils. This method is recommended for oil solutions of irradiated ergosterol, having a potency of 10,000 vitamin D_2 units or more per gram.

Applications of these two methods to oil solutions of lower potencies than recommended may be possible if interfering materials introduced by the chromatographic procedure and those present in corn oil are eliminated. Further study is in progress.

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RECEIVED April 8, 1947. Presented before the Division of Biological Chemistry at the 110th Meeting of the AMERICAN CHEMICAL SOCIETY, Chicago, Ill.

Double Freezing-Point Method for Determination of Styrene Purity

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A method is described for determining the purity of recycle and blended styrene monomer, in which the purity is from 87 to 98% and the principal impurities are ethylbenzene, 4-vinylcyclohexene, and 1,3-butadiene. The data required are the freezing points of the original sample and of a portion from which the volatile impurities have been removed. The separation of low-boiling hydrocarbons is accomplished by complete evaporation of a portion of the sample under low pressure and condensation of the styrene and heavy impurities as solid in a trap at −65° C. The probable error of the method is estimated at ±0.15 weight % of styrene in samples of lower purity.

THE measurement of the freezing-point depression is one of the most convenient ways of determining the purity of hydrocarbons in general. The method presented here demonstrates what may be accomplished by the use of liquid-in-glass thermometers for routine work in industrial laboratories where the time, personnel, and equipment may not be available for the more accurate technique using platinum resistance thermometers. The method also includes a simple and effective procedure for separating very volatile impurities when they are present in amounts up to a few per cent.

The method was developed for determination of the purity of recycle and blended styrene in the synthetic rubber plants under the control of the Office of Rubber Reserve of the Reconstruction Finance Corporation.

THEORETICAL

If a solution which is predominantly styrene is ideal with respect to all the constituents, and if the impurities remain in the liquid phase while the styrene is being frozen, we may write (*4*, *5*):

$$-\ln N_1 = A\,\Delta t(1 + B\,\Delta t \ldots) \qquad (1)$$

where N_1 is the mole fraction of styrene, $\Delta t = t_0 - t_f$, the difference between the freezing points of pure styrene and solution,

$$A = \frac{\Delta H_0}{RT_0^2} \qquad (2)$$

and

$$B = \frac{1}{T_0} - \frac{\Delta C_{p_0}}{2\,\Delta H_0} \qquad (3)$$

T_0, ΔH_0, and ΔC_{p_0} represent, respectively, the freezing point (degrees Kelvin), molar heat of fusion, and change of molar heat capacity on fusion, of pure styrene. Terms of higher order than those indicated in the parentheses in Equation 1 may be neglected.

Since in plant practice it is required that the purity of the sample be given in weight per cent, it is necessary to have an evaluation of the number, nature, and relative amounts of the impurities, in addition to the conditions imposed in Equation 1. The principal impurities in recycle styrene are ethylbenzene, 4-vinylcyclohexene, and 1,3-butadiene (*1*, *2*); it has been assumed that others are present in negligible amounts. If it is further assumed that the ratio of ethylbenzene to 4-vinylcyclohexene is approximately constant, only two independent measurements are needed to fix the weight per cent of styrene. The last assumption is justified because the molecular weights of the two C_8 impurities are so close together that for a given number of total moles of ethylbenzene and 4-vinylcyclohexene, even large variations in their molecular ratio will cause only small differences in their total weight per cent actually present in the sample and also calculated from freezing-point depression if, again, the solution is ideal.

The two independent measurements chosen are the freezing points of the sample before and after separation of the light hydrocarbons from it. It is obviously of practical advantage to have a single procedure for both measurements.

By applying Equation 1 to both freezing points, equations were developed for the weight per cent of C_8 impurity in the C_4-free sample, the weight per cent

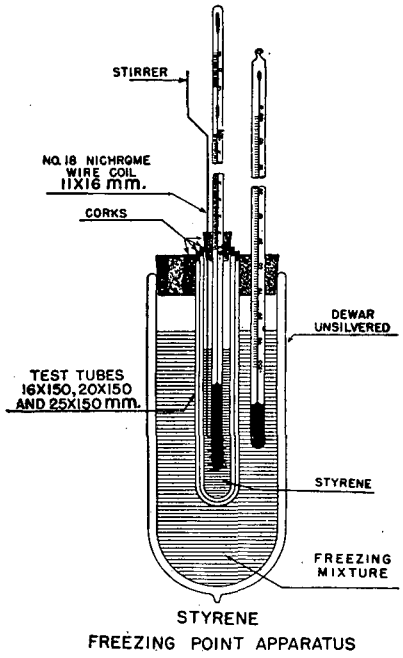

Figure 1. Styrene Freezing Point Apparatus

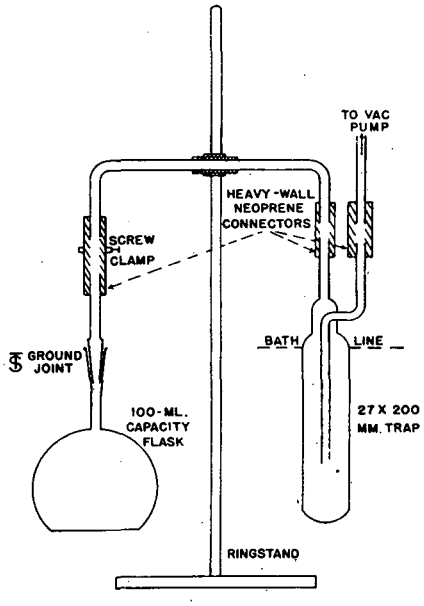

Figure 2. Apparatus for Separation of Low-Boiling Impurities

of butadiene, and the weight per cent of styrene. The assumption of ideality, on which these equations were based, is invalid, however, for solutions of ethylbenzene and butadiene in styrene. Since it was found that an error of as much as 0.7%, in 90% styrene solutions, can be introduced by the use of the theoretical equations, they were discarded in favor of empirical relations obtained from experimental data, as described below.

APPARATUS AND MATERIALS

The freezing-point apparatus (Figure 1) consists of a 665-ml. unsilvered Dewar vacuum flask of Pyrex, a nest of three test tubes, and a stirrer operated by a reciprocating vacuum-type stirrer motor. (This apparatus is substantially the same as that designed by Irving Madorsky, Rubber Section, National Bureau of Standards, for freezing-point purity of fresh styrene.) The temperature of the styrene is measured with a mercury-thallium-filled thermometer, designed in this laboratory especially for freezing points of recycle styrene. This thermometer is graduated from −42° to −29° C. in 0.05° with an auxiliary scale from −0.5° to +0.5° C., and has a total length of 470 to 480 mm., a bulb length of 50 to 60 mm., and a diameter of 6 to 7 mm.

The apparatus for separation of low-boiling impurities (Figure 2) consists of an evaporating flask and a cold trap, connected as shown by glass tubing (8 to 10 mm. in outside diameter) and heavy-walled neoprene connectors. The bath surrounding the cold trap is contained in a Dewar flask, preferably silvered. The vacuum pump used with this apparatus is capable of reducing the pressure to less than 0.5 mm. of mercury and has a capacity sufficient to maintain the pressure below 10 mm. of mercury when pumping on the impure styrene solution.

The materials necessary for an analysis are dry ice and a freezing mixture made of equal parts by volume of chloroform and carbon tetrachloride.

The following materials were used in making up the solutions for investigation of the method:

Styrene. Two lots of styrene monomer were specially purified by the Dow Chemical Company. The purities of the two lots, by freezing point, were 99.80 and 99.90 weight %.

Ethylbenzene. A sample purified by the Petroleum Laboratory of the Pennsylvania State College, at least 99.5% pure by melting point, was used.

4-Vinylcyclohexene. The sample used was purified by fractional distillation by the Koppers Company. From refractive index and specific gravity measurements, its purity was judged to be better than 95%.

1,3-Butadiene. Phillips Petroleum Company's Pure Grade butadiene, 99% pure by freezing point, was used without further purification.

PROCEDURE

The following procedure, although describing operations on plant samples, is also applicable to the synthetic mixtures discussed later. The first step in an analysis is to determine the freezing point, t_1, of a portion of the original sample. The next step is to remove the light hydrocarbon impurities from another portion of the sample and determine its freezing point, t_2. From t_2 is computed the weight per cent of heavy impurity, p_1, in this sample from which the light hydrocarbons have been removed. Finally the weight per cent of light impurity, p_2, in the original material is computed from the difference in the two freezing points, $t_2 - t_1$.

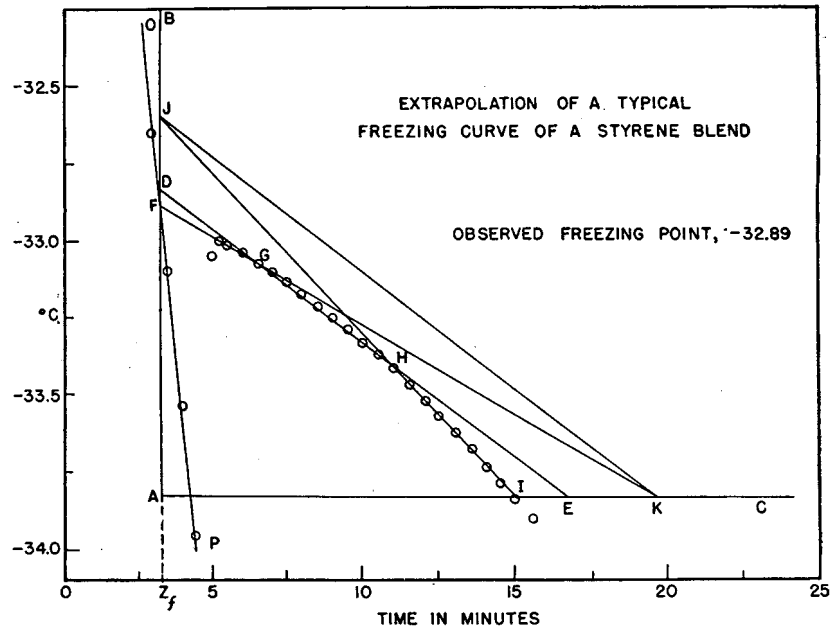

Figure 3. Freezing Curve of Styrene Blend

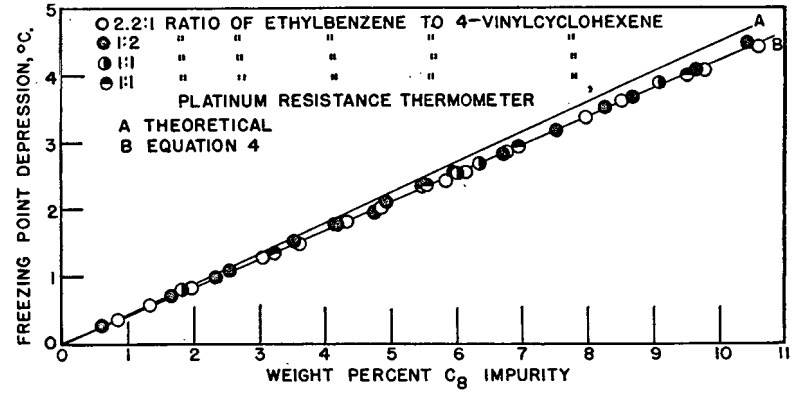

Figure 4. Experimental Freezing Point Depression of Styrene by Ethylbenzene and 4-Vinylcyclohexene

Samples of recycle and blend styrene to be analyzed are obtained in clean screw-cap bottles of about 1-pint capacity. These samples are chilled to 40° F. (4.4° C.) before analysis in order to minimize the loss of light hydrocarbons by evaporation.

Determination of Freezing Points. The freezing mixture in the Dewar flask is cooled with dry ice to −43° C. Approximately 10 ml. of the sample are placed in the innermost test tube and the apparatus is assembled as in Figure 1. The thermometer is immersed at least 2 cm. above the bulb and the bath level is about 2 cm. above the sample. Throughout the determination the sample is stirred at the rate of about 150 strokes per minute, and the bath is maintained (by addition of small pieces of dry ice) within 0.5° of a temperature selected so that the rate of cooling of the sample, while freezing, is near 0.1° C. per minute. This rate of freezing is produced by temperatures from −42° to −44° C. with the apparatus shown in Figure 1.

The temperature of the sample is read to 0.01° C. at 30-second intervals; at least three readings are taken before freezing begins and from 20 to 25 after freezing begins. The time-temperature readings are plotted on a graph which is scaled so that 1° (on the ordinate) is equal to 10 cm. and 10 minutes are equal to 10 cm.

The uncorrected freezing points are determined by extrapolat-

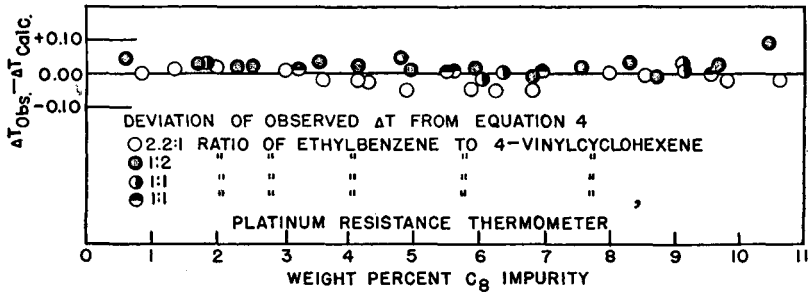

Figure 5. Deviation of Experimental Freezing Points from Empirical Equation 4 for Mixtures with No Butadiene

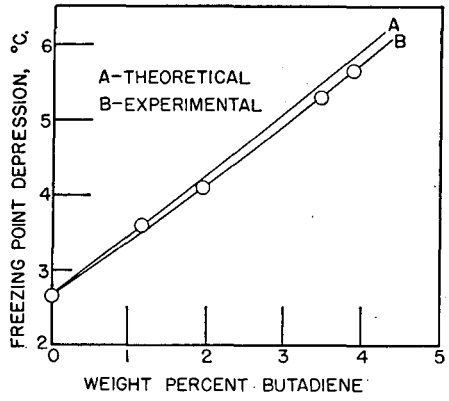

Figure 6. Freezing Point Depression by 1,3-Butadiene of Styrene Containing 6.37 Weight % C_8 Impurity

ing the time-temperature curves by a graphical method described by Taylor and Rossini (*4*). This method is illustrated in Figure 3.

After the liquid cooling curve, *OP*, and a vertical line, *AB*, are drawn at the estimated time, Z_f, that freezing would have begun in the absence of undercooling, three experimental points *G*, *H*, and *I* are chosen about equally spaced and as far apart as possible; *G* is sufficiently far from the beginning of freezing so that essential equilibrium has been established. A horizontal line is drawn through *I*, *DE* is drawn through *H* and *G*, *JI* is drawn through *H* and *I*, and *JK* is drawn parallel to *DE*. The extrapolated freezing point, *F*, is obtained by drawing a line from *K* through *G* to intersection with *AB*.

The corrected freezing points are obtained by adding algebraically to the uncorrected values, the calibration correction, the change in ice-point correction since the original calibration, and the correction for emergent stem, for the thermometer used. The corrected freezing point of the original sample is called t_1, and the corrected freezing point of the sample after removing butadiene is called t_2. The respective freezing-point lowerings, Δt_1 and Δt_2, are obtained by subtraction from −30.63° C., the freezing point of pure styrene (*3*). The per cent styrene is calculated from Equations 4, 5, and 6, or from a nomogram prepared from them.

In order to realize the greatest possible accuracy with liquid-in-glass thermometers it is necessary to check their calibration and the freezing-point procedure being followed. These thermometers are prone to change in bulb volume and rise in ice point, particularly during the first few months after manufacture. It is not unusual for this change to be as great as 0.1° C.

The thermometers are calibrated before use by the National Bureau of Standards.

Calibration corrections are obtained under total immersion conditions at −40°, −35°, −30°, and 0° C., are stated to 0.01° C., and are good to 0.02° C. If the bulb volume changes, the calibration at all points on the scale will be changed by a constant amount; this correction is obtained by taking ice points weekly during the first six months of the life of the thermometer, or until it appears that only small changes are taking place. Ice points are checked every month or two after that. Good ice points are obtained by crushing clean, clear ice, washing it, and making a thick slush with distilled water. The thermometer is immersed to the 0° mark in this slush, in a vessel protected from radiation.

A further control measure is freezing of a standard material of known freezing point. A stable reference substance, having a freezing point close to that of styrene, is bromobenzene. A sample of this substance, with a freezing point certified by the National Bureau of Standards, is frozen in the same way as a sample of styrene, except that freezing is continued until the stirrer begins to labor or until the sample has been freezing about 20 minutes. The points are plotted with a temperature scale about 5 times as sensitive as that for styrene. In following the extrapolation procedure given above, it is important to disregard the initial flat portion of the freezing curve, by choosing points *G*, *H*, and *I* (Figure 3) so that *G* is on the downward-sloping portion of the curve. Errors of several hundredths of a degree were discovered after failure to observe this precaution on two occasions.

By making use of these checks, an accuracy of ±0.02° C. can be obtained.

Separation of Low-Boiling Impurities. Since the low-boiling impurities (principally butadiene) differ so greatly in volatility from the remainder of the impure styrene mixture, they may be effectively removed by the following simple procedure.

About 15 ml. of the original sample are poured into the flask of the evaporating apparatus (Figure 2), and the apparatus is assembled as shown and connected to the pump and a small manometer. Before the clamp above the flask is opened, the system is pumped out to less than 0.5 mm. and the cold trap is cooled with a carbon tetrachloride–chloroform mixture maintained at −65° C. with dry ice. While the flask is shaken gently, the clamp is opened slowly enough so that the pressure does not rise above 10 mm. After the clamp is open, a beaker of warm water placed around the flask aids evaporation. The styrene, ethylbenzene, and 4-vinylcyclohexene are condensed as solid on the walls of the trap, while the light hydrocarbons pass through as vapor. When the sample has completely evaporated, the cold trap is detached, the contents are allowed to melt, and the freezing point of the condensate is determined.

DATA ON SYNTHETIC MIXTURES

In order to investigate the method and develop the required empirical relationships between freezing point and composition, a series of experiments was made on 68 synthetic mixtures ranging from 87 to 98% styrene. The mixtures were made up with known composition and their freezing points determined by the procedure described above. The first thirty of these styrene solutions contained the solutes ethylbenzene and 4-vinylcyclohexene in ratios of 2.2 to 1 and 1 to 2. At a much later time, freezing points of eight solutions containing ethylbenzene and 4-vinylcyclohexene in various ratios were obtained. Some freezing points were also obtained with a platinum resistance thermometer on solutions containing ethylbenzene and 4-vinylcyclohexene in 1 to 1 ratio. The freezing-point lowering is plotted against weight per cent, for all these solutions containing no light hydrocarbons, in Figure 4. Various equations were fitted to these points by the method of least squares. Separate equations through the points representing different ratios of the impurities could be obtained, but at 90% styrene, the difference in per cent styrene found by using 2.2 to 1 ratio of ethylbenzene to 4-vinylcyclohexene instead of 1 to 2 was only 0.16%. Since there does not exist at this time a good independent method for determining.

the ratio of these two impurities, and there is some evidence that the ratio is usually in the neighborhood of 1 to 1 (*2*), a least square fit was obtained using a quadratic function of Δt_2 for all the points together. This equation is

$$p_1 = 2.389\,\Delta t_2 + 0.003\,\Delta t_2^2 \qquad (4)$$

and is shown by curve *B* in Figure 4. Curve *A* is obtained from the laws of ideal solutions. The deviations of observed freezing-point depressions from those calculated by Equation 4 are shown in Figure 5. As expected, most of the points representing 2.2 to 1 ratio of ethylbenzene to 4-vinylcyclohexene give negative deviations, while those of the inverse ratio are positive.

By low-temperature condensation in vacuum, butadiene in various amounts was added to various mixtures of styrene and the 1 to 1 mixture of ethylbenzene and 4-vinylcyclohexene. The nonideality of the butadiene-styrene solutions is shown by the points of one series of solutions in Figure 6. The solutions in this series were made by adding butadiene to styrene containing 6.37 weight % of ethylbenzene and 4-vinylcyclohexene (1 to 1). Other series containing different amounts of heavy impurities gave similar curves. After the freezing-point determinations, the added butadiene was removed (from fresh portions of the solutions) by the vacuum evaporation procedure given above, and freezing points (t_2) were determined. It was found that the weight per cent of butadiene could be represented within the experimental error by the equation

$$p_2 = 1.366(t_2 - t_1) - 0.025(t_2 - t_1)^2 \qquad (5)$$

The deviations of the experimental values of $t_2 - t_1$ from those calculated by Equation 5 are plotted against weight per cent of butadiene in Figure 7. No correlation with content of heavy impurities is observed.

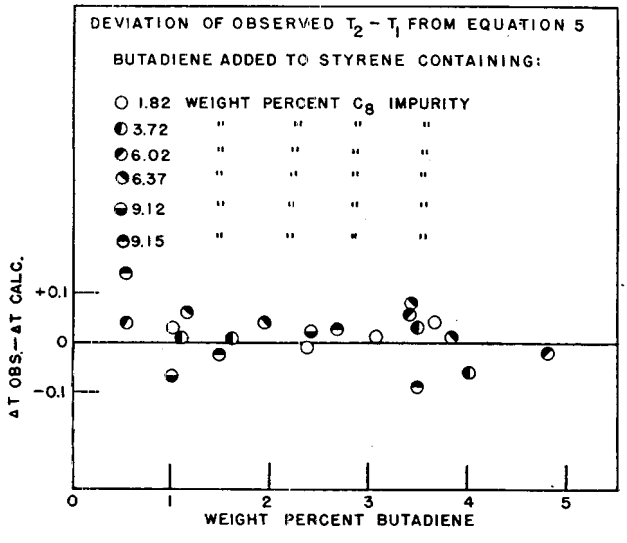

Figure 7. Deviation of Experimental Freezing Point Lowering by Butadiene from Empirical Equation 5

The weight per cent of styrene in the original sample was obtained from the equation

$$p_3 = \frac{(100 - p_1)(100 - p_2)}{100} \qquad (6)$$

The average of the deviation of all the solutions from the calculated value of p_1 obtained by Equation 4 was ±0.06 weight %. The average deviation of p_2, in the synthetic mixtures containing butadiene, from the value calculated by Equation 5, was ±0.05.

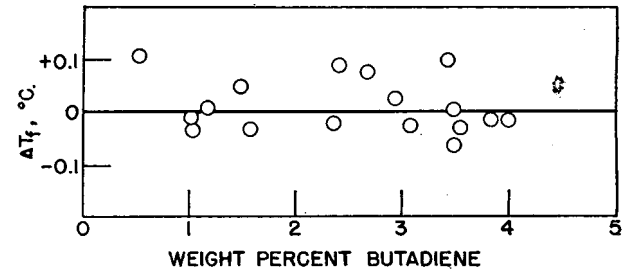

Figure 8. Change in Freezing Point of Impure Styrene Solutions Caused by Adding and Removing 1,3-Butadiene, Plotted against Weight Per Cent of 1,3-Butadiene

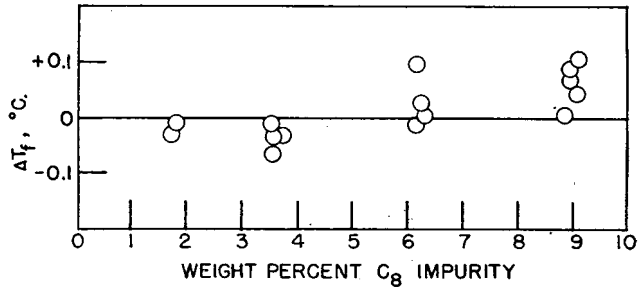

Figure 9. Change in Freezing Point of Impure Styrene Solutions Caused by Adding and Removing 1,3-Butadiene, Plotted against Weight Per Cent of C_8 Impurity

The weight per cent of styrene in all solutions was calculated by use of Equations 4, 5, and 6, and compared with the actual values; the average deviation in p_3 was ±0.06.

The efficiency of the separation of low-boiling impurities is shown by the results obtained on seventeen synthetic mixtures, which are plotted in Figures 8 and 9. The difference in freezing point of the sample before adding butadiene and after removing it by vacuum evaporation is plotted against per cent butadiene in Figure 8. No correlation is observed. In Figure 9 the change in freezing point of the same samples, due to the addition and removal of butadiene, is plotted against per cent heavy impurity in the sample. The positive side of the Δt_f axis is the direction of increase of purity upon separation. From these two graphs, it is evident that the greater the percentage of ethylbenzene and 4-vinylcyclohexene, the more of these substances are removed in the separation, regardless of the amount of butadiene. This observation seems to indicate that the error is caused by distillation of the heavier fraction rather than by the sweeping of solid particles through the trap. The latter effect would be the first error suspected in the arrangement of this apparatus, but such effects are partly dependent upon the amount of gas passing through.

ACCURACY

The error in the freezing-point determinations which is caused by the use of a liquid-in-glass thermometer should not exceed 0.03° C. Reproducibility of freezing points in this laboratory, using different thermometers and different operators, has been of the order of 0.02° C.

There is no evidence that there is any error in the first freezing point (t_1) caused by evaporation of light hydrocarbons from the sample in the freezing tube, at least for samples containing less than 3% butadiene, provided the sample is thoroughly chilled before testing. If the first freezing point is correct, any error in the second freezing point (t_2) such as indicated by Figures 8 and 9, will result in a portion of the heavy impurity's being calculated as butadiene, or the reverse. Thus the error in per cent styrene is

essentially the difference between the error in p_1 and in p_2, or approximately the value of the error in t_2.

The error which can be made by assuming that the ratio of ethylbenzene to 4-vinylcyclohexene is 1 to 1 may be as large as 0.5% if the impurity is entirely one or the other. However, those recycle styrenes which have been investigated have contained these impurities in ratios from 1:2 to 2:1.

An appreciable error in the method is introduced by the assumption that the only impurities are ethylbenzene, 4-vinylcyclohexene, and 1,3-butadiene. The presence of C_4 hydrocarbons other than butadiene is not important, but there is recent evidence that recycle styrene contains C_9 hydrocarbons, particularly isopropylbenzene, in amounts which may be as large as 1.5% (*2*). An error of as much as 0.2 in the weight per cent of styrene may thus be made.

After consideration of all the above sources of error, the probable error in weight per cent of styrene as determined by the double freezing point method is set at ±0.15, for samples of 90% purity.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Daniel R. Stull and the Dow Chemical Company for the specially purified lots of styrene which they furnished for this investigation.

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RECEIVED August 16, 1947. Presented before the Division of Analytical and Micro Chemistry at the 111th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Determination of Silicon in Organosilicon Compounds

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Silicon has been determined in organosilicon compounds by methods ranging from acid digestion with sulfuric acid to newly developed procedures particularly adaptable only to these compounds. The methods are reviewed and evaluated.

THE recent rapid advances in the commercial production and use of organopolysiloxanes have prompted a review and further development of the methods of silicon determination.

Along with the development of the chemistry of organosilicon compounds, investigators have had to establish the technique of analysis of these materials. The approaches used in determining silicon can be generally classed in three distinct groups: wet oxidation, dry combustion, and fusion. Early investigators met with many failures in their tests for silicon because of the high volatility of some of the compounds. Another source of error has been the tendency of the compounds to form some silicon carbide instead of silicon dioxide on combustion.

Knowledge of past applications of the various techniques will place the chemist in a better position to select the method best suited to his particular purpose.

Long ago, Friedel and Crafts (*2*) employed fuming nitric acid in a wet oxidation procedure followed by solution in alkali and reprecipitation. Polis (*11*) achieved some success by decomposing the sample in a mixture of sulfuric acid and potassium permanganate, followed by leaching the manganese oxides with hydrochloric acid and filtering the silica. Kipping and coworkers (*8*, *9*) eliminated the permanganate and substituted nitric acid as an oxidant which, being itself completely volatile, allowed final ignition to silicon dioxide without further transfer of the sample. More recently Hyde and DeLong (*6*) were able to analyze some organosilicon polymers in a similar fashion. Bygden (*1*) accomplished a wet oxidation in a Kjeldahl-type flask, using mercury and sulfuric acid with subsequent filtration of the silicic acid. Perchloric acid has been substituted for sulfuric acid in a wet oxidation procedure proposed by Gilman, Clark, Wiley, and Diehl (*4*).

None of these procedures has been successfully applied to the analysis of compounds of high volatility. Materials boiling below 200° C. are likely to give low results, and care must be taken that silicon carbide does not form in the final ignition step. The present authors have found that ignition in an adequate supply of oxygen at 800° C. almost completely eliminates the formation of silicon carbide.

In a recent monograph on silicone chemistry, Rochow (*12*) suggests a dry combustion method for the determination of carbon, hydrogen, and silicon. The procedure, however, is reported to be slow, conditions depend on the type of compound to be analyzed, and the formation of silicon carbide cannot always be prevented.

Successful fusion of organosilicon derivatives in dry reagents had to await the development of the Parr bomb technique as described by Tsang and Chao (*13*), Whitmore and co-workers (*14*), and Gilliam, Liebhafsky, and Winslow (*3*). By this technique the more volatile samples are best handled. The present paper is an attempt by the authors to sort, out of the present knowledge, the techniques best suited for the determination of silicon in the various types of organosilicon compounds.

DISCUSSION

The most successful analysis of volatile samples has been accomplished by the use of Parr-type bombs. Generally the silicon is determined, after peroxide fusion in a bomb, by the classical dehydration with hydrochloric and/or perchloric acids. However, the unavoidably large volume of salts resulting from the fusion makes evaporation and washing a tedious procedure, and unless the second precipitation and evaporation step is undertaken, results are unreasonably low. On some samples, hydrofluoric acid correction necessitates the use of platinum crucibles. This method of analysis requires on the average 24 hours for completion, which is excessive both from the standpoint of a good control method and from equipment tie-up.

Table I. Summary of Analytical Results in Determination of Silicon

(Per cent silicon)

	Compound	Boiling Point, Atm.	Theory	Parr Bomb				Acid Digestion		Hydrolysis Followed by Acid Digestion, Open Crucible
				Gravimetric (15)	Volumetric (10)	Gravimetric oxine ppt. (15)	Colorimetric (7)	Kjeldahl flask	Open crucible	
1.	$SiCl_4$	57.6	16.52	..	..	...	16.1	...	...	...
2.	CH_3SiCl_3	64.6	18.77	..	..	...	18.7	...	...	...
3.	$C_2H_5SiCl_3$	100	17.16	..	..	...	16.9	...	...	...
4.	$(CH_3)_2Si(OC_2H_5)_2$	111	18.92	18.9	..	...	...	...	...	...
5.	$CH_3(C_6H_5)SiCl_2$	203	14.68	..	..	...	14.72	...	...	14.72
6.	$C_2H_5(C_6H_5)SiCl_2$	230	13.68	..	..	...	...	...	...	13.66
7.	$(CH_3)_2C_6H_5SiCl$	194	16.44	..	..	16.31	...	...	...	...
8.	$[CH_3(C_6H_5)SiO]_n$	X	20.61	..	..	...	...	...	20.62	...
9.	$[C_2H_5(C_6H_5)SiO]_4$	<600	18.70	..	..	...	...	...	18.81	...
10.	$[(C_6H_5)_2SiO]_4$	X	14.16	..	..	...	...	...	14.25	...
11.	$[(CH_3)_2SiO]_3$	133	37.87	36.6	38.1	38.03	36.8	37.83	37.83	...
12.	$[(CH_3)_2SiO]_4$	175	37.87	36.1	38.1	37.54	38.3	38.0	36.8	...
13.	$[(CH_3)_2SiO]_6$	236	37.87	36.5	38.4	38.04	38.2	37.70	37.70	...
14.	$[(CH_3)_2SiO]_n$	X	37.87	38.0	37.8	...	...	...	...	...
15.	$(CH_3)_3SiOSi(CH_3)_3$	99.5	34.57	34.0	34.6	34.58	...	34.33	31.5	...
16.	$(CH_3)_3SiOSi(CH_3)_2OSi(CH_3)_3$	152	35.60	34.8	35.7	...	...	35.43	30.4	...
17.	$(CH_3)_3Si[OSi(CH_3)_2]_2OSi(CH_3)_3$	192	36.14	35.5	36.0	36.12	...	36.06	...	...
18.	$(CH_3)_3Si[OSi(CH_3)_2]_3OSi(CH_3)_3$	229	36.47	36.0	..	...	...	36.37	...	...
19.	$(CH_3)_3Si[OSi(CH_3)_2]_4OSi(CH_3)_3$	247	36.69	36.05	..	...	...	36.68	...	...
20.	$(CH_3)_3Si[OSi(CH_3)_2]_5OSi(CH_3)_3$	270	36.85	36.5	..	...	...	36.60	...	...

Because the peroxide fusion is so useful for decomposing volatile materials, the authors sought to overcome the disadvantages of dehydration of silicic acid.

Wolynez (*15*), after converting soluble silicic acid to the well-known silicomolybdic acid complex, precipitated the complex quantitatively with 8-hydroxyquinoline (oxine). Merz (*10*) later enlarged upon these studies, and prescribed definite conditions for the complex formation and its subsequent precipitation. Prior to forming the complex the silicic acid must be kept in a soluble form. Therefore during solution and neutralization of the peroxide fusion it is essential to keep the reaction mixtures cool and dilute. Kahler (*7*) used the reaction of silicomolybdic acid with sulfite to form molybdenum blue, which can be measured colorimetrically.

These studies establish three useful methods for treatment of a solution of the peroxide fusion: volumetric determination of silicon by oxine, first reported by Wolynez and later thoroughly studied by Merz; gravimetric determination of silicic acid after precipitation as the oxine–silicomolybdic acid complex, also described by Wolynez; and colorimetric determination of silicic acid, recently well summarized by Kahler.

The volumetric method is based on formation of the complex silicomolybdic acid followed by reaction with excess oxine. This gives a precipitate insoluble in dilute hydrochloric acid, which contains 4 moles of oxine, 1 mole of silicon dioxide, and 12 moles of molybdic oxide. Following filtration, the excess oxine is determined by a bromate-bromide titration. If the prescribed conditions are carefully observed, very satisfactory results may be obtained by this method in 3 or 4 hours.

The gravimetric determination given as an alternative method in Wolynez's original report is dismissed by Merz as too tedious. However, the authors have found its use often desirable because of its greater accuracy. The oxine–silicomolybdic acid complex is dried, then ignited to constant weight at 500° C. The residue consists of 1 mole of silicon dioxide to 12 moles of molybdic oxide.

The colorimetric method relies on measurement of the blue color produced when silicomolybdic acid is reduced with sodium sulfite. The developed color is measured by means of a photoelectric colorimeter, using a filter of 715 millimicrons. Samples having a silicon content of 0 to 10% are particularly well adapted to this procedure.

The acid digestion method is subject to certain errors—namely, loss of volatile components and formation of nonoxidizable silicon carbide in the residue. Its simplicity is attractive, however, when one is concerned with the analysis of nonvolatile resins and polymers or of samples which can be readily converted to such polymers by hydrolysis. A study of various factors led the authors to the discovery that by a proper control of conditions during pyrolysis the formation of the objectionable silicon carbide can be prevented. An oxidizing atmosphere must be provided and the temperature of the ignition maintained no higher than 800° C. That higher ignition temperatures favor the formation of silicon carbide may be due to too rapid incineration of the residue before the free carbon, which is nearly always present following the acid digestion, can be oxidized and driven out. A muffle furnace is the most satisfactory means for properly controlling the ignition and even when a furnace is used it may be necessary to pass oxygen through the heated enclosure to ensure the proper atmosphere. The method can be extended to somewhat more volatile types by using a micro-Kjeldahl Vycor flask for the digestion. The narrow neck inhibits volatilization, but such a procedure requires that the free carbon be completely oxidized before the final ignition by the use of perchloric or fuming nitric acids along with the sulfuric acid. Weighings in the micro-Kjeldahl flasks are more cumbersome than weighings made in open-topped crucibles.

For illustrating how representative compounds may best be analyzed, Table I gives actual results and the method or methods used. The scope of the table is somewhat limited, as no samples which boil below or even near room temperature are included. In most instances the results given are only those from methods which gave the best values. Boiling points have been included as an additional guide in the selection of methods for similar materials. All the methods have been compared on some compounds of intermediate volatility as indicated in lines 11, 12, 13, 15, and 16 of the table. Compounds boiling below 200° C. are not properly digested by the acid method and the results are therefore too low. Special techniques must be utilized in handling and weighing low boiling materials. The analyst must not only use the peroxide fusion technique as outlined below, but also observe special precautions in weighing the samples. Gilliam *et al.* (*3*) adequately cover these precautions. The first three detailed methods given below, require preliminary fusion of the sample. This fusion procedure is the same for each method.

PROCEDURE FOR PARR BOMB FUSION

There is considerably less contamination from metals when nickel bomb cases and caps are used. Samples properly bombed in nickel will yield nearly colorless solutions.

In these bombs of 25- to 30-ml. capacity, 2 or 3 grams of sodium peroxide are mixed with 50 to 100 mg. of sugar and 0.050 to 0.250 gram of sample in a size 0 gelatin capsule is added. Following addition of the sample, the bomb case is filled with sodium peroxide and closed with a lead gasket. The bottom of the bomb is heated rapidly to a dull red heat by employing a blast burner that mixes oxygen with natural gas. The hot bomb is immediately cooled in cold water to prevent melting the gasket. The bomb is then opened, the cover is washed with distilled water, and the washings are collected in a 400-ml. Monel or nickel beaker. Any prolonged contact of the hot caustic with the lead gasket will cause troublesome precipitates later.

Distilled water is added to the beaker to a volume of 100 to 125 ml. The bomb case is dropped into the beaker on its side and a nickel cover is placed over the beaker. Following solution, the beaker is cooled, and the bomb is removed and rinsed with water, then with concentrated hydrochloric acid. The volume is made up to 250 to 275 ml. with water, and the alkaline solution is acidified with concentrated hydrochloric acid just to the point of solution of the nickel hydroxide. Litmus paper may also be used. Acidification in too small a volume, at too high a temperature, or to excess may cause precipitation of the silicic acid. The slightly acidified solution may now be safely put in glass and diluted to volume in a 1-liter volumetric flask. From this flask aliquots are taken for volumetric, colorimetric, or gravimetric determination.

VOLUMETRIC DETERMINATION OF SILICON (*10*)

The soluble yellow complex of silicomolybdic acid is produced by the addition of ammonium molybdate to an acidic solution of sodium silicate. A known excess of standard oxine solution is added to this complex to form a dense yellow precipitate. This mixture is made to a known volume and filtered on a dry filter paper. An aliquot is taken from the filtrate and the excess oxine titrated with a standard bromate-bromide solution.

Merz notes that the theoretical factor which is calculated from the formula and equations gives slightly low silicon figures, and advises determination of an empirical factor by each analyst or laboratory. The factor in use in this laboratory is derived from known silicon compounds such as Bureau of Standards samples and pure organosilicon compounds.

Calculation of Theoretical Factor for Volumetric Silicon. The complex precipitate is 1 Si, 4 oxine, 12 MoO_3. 1 mole of Si = 4 moles of oxine. $KBrO_3 + 5KBr + 6HCl = 3Br_2 + 6KCl + 3H_2O$. $C_9H_7ON + 2Br_2 = C_9H_5ONBr_2 + 2HBr$. 4/6 mole of $KBrO_3$.KBr needed to brominate 1 mole of oxine. 1 mole of Si = 16/6 moles of $KBrO_3$. 28.06 grams of Si = 445.36 grams of $KBrO_3$. 1 cc. of 0.1 *N* $KBrO_3$ = 0.002784 gram of $KBrO_3$.

$$1 \text{ cc. of } 0.1\ N\ KBrO_3 = \frac{28.06 \times 0.002784}{445.36} = 0.000175 \text{ gram of Si.}$$

Determination of Empirical Factor for Volumetric Silicon

Sample	% Si	Sample Weight	Cc. of 0.1 *N* Oxine	Factor (Gram of Si per Cc. 0.1 *N* Oxine)
Bureau of Standards feldspar 70	31.13[a]	0.1754 0.2586	310.0 456.8	0.0001763 0.0001763
$Me_3SiO-(Si(Me)-O)_4-SiMe_3$	36.7[b,c]	0.1468 0.1592	304.5 329.2	0.0001770 0.0001770
$Me_3SiO(Si(Me)-O)_3SiMe_3$	36.5[b,c]	0.1498 0.1573	306.0 320.5	0.0001780 0.0001780
$Me_3SiO[SiO(Me)_2]_xSiMe_3$	37.2[c]	0.1529	321.3	0.0001770
$Me_3SiO[SiO(Me)_2]_ySiMe_3$	37.4[c]	0.1649 0.1652	348.0 349.5	0.0001773 0.0001768

[a] B. of S. analysis.
[b] Theoretical.
[c] Acid digestion analysis.

Reagents. 8-Hydroxyquinoline (oxine), 0.4 *N*. Prepare by dissolving 14 grams of oxine in 20 ml. of 1 to 1 hydrochloric acid and diluting to 1 liter. Standardize against 0.2 *N* potassium bromate–potassium bromide solution.

Potassium bromate–potassium bromide solution, 0.2 *N*. Dissolve 5.5 grams of potassium bromate and 20 grams of potassium bromide in distilled water and dilute to 1 liter. Standardize against 0.1 *N* sodium thiosulfate.

Sodium thiosulfate, 0.1 *N*. Prepare and standardize against 0.1 *N* potassium bichromate.

Potassium bromide, 20%. Add 200 grams to 800 grams of distilled water.

Oxalic acid, 8%. Add 80 grams to 920 grams of distilled water.

Potassium iodide, 10%. Add 100 grams to 900 grams of distilled water.

Hydrochloric acid, 1 to 1. Mix 1 part of C.P. concentrated hydrochloric acid with 1 part of distilled water.

Ammonium molybdate solution, 20%. Dissolve 200 grams of reagent ammonium molybdate in 800 grams of distilled water.

Starch solution, 0.5%. Dissolve 5 grams of soluble starch in 1 liter of distilled water. Preserve under a layer of toluene.

Methyl red, 0.1%. Dissolve 0.1 gram of the sodium salt of methyl red in 100 ml. of distilled water.

Sodium peroxide, analytical reagent grade.

Sodium hydroxide, 10%, prepared in a nickel beaker.

Procedure. Transfer a 50-ml. aliquot (5 to 10 mg. of silicon) to a 250-ml. volumetric flask, add 12.5 ml. of 1 to 1 hydrochloric acid to bring the acidity to 2 grams of hydrochloric acid per 100 ml. of solution, and add 50 ml. of distilled water and 10 ml. of 20% ammonium molybdate. Rinse down the molybdate solution with about 1 ml. of water. Stopper and heat 10 minutes at 75° ± 3° C.

Cool, add 21.5 ml. of 1 to 1 hydrochloric acid to bring the acidity to 4 grams of hydrochloric acid per 100 ml. of solution, and slowly add 25 ml. of 0.4 *N* oxine with constant agitation. Heat, stoppered, for 10 minutes at 65° ± 3° C. Cool, dilute to 250 ml., and filter on a dry filter (2 sheets of No. 42 Whatman paper folded together). Keep funnels covered with watch glasses and catch filtrate in a 250-ml. narrow-necked flask to prevent evaporation.

Transfer 100 ml. of the filtrate to a 500-ml. iodine flask, and add 50 ml. of 8% oxalic acid, 100 ml. of 1 to 1 hydrochloric acid, 100 ml. of water, 5 ml. of 20% potassium bromide, and a few drops of 0.1% methyl red. Add a slight excess of 0.2 *N* potassium bromate–potassium bromide solution as shown by the brown bromide color. Allow to stand 1 to 2 minutes, add 5 ml. of 10% potassium iodide solution and 5 ml. of 0.5% starch solution, and titrate the iodine with 0.1 *N* sodium thiosulfate.

Run a blank using sugar, peroxide, and an empty capsule. Treat in same way as a regular sample.

Calculation. 2 (total ml. of 0.2 *N* $KBrO_3$.KBr) − ml. of 0.1 *N* $Na_2S_2O_3$ = net ml. of 0.1 *N* $KBrO_3$.KBr used for 1/25 of original sample.

$$\frac{(25 \times \text{net ml. of } 0.1\ N\ KBrO_3.KBr \text{ for blank}) - (25 \times \text{net ml. of } 0.1\ N\ KrO_3.KBr \text{ for sample}) \times 0.000177 \times 100}{\text{Sample weight in grams}} = \% \text{ silicon}$$

GRAVIMETRIC DETERMINATION OF SILICON (*15*)

Care should be taken to ensure a constant concentration of salts by adding a definite volume of hydrochloric acid to all fusions, then backing up with 10% sodium hydroxide if necessary. Instead of 1-liter flasks, 500-ml. volumetric flasks are used.

Apparatus. Microporous porcelain filtering crucibles (Selas No. 3010).

Reagents. Hydrochloric acid, 1 to 1. Mix 1 part of C.P. concentrated hydrochloric acid with 1 part of distilled water.

Oxine, 0.4 *N*. Dissolve 14 grams of oxine in 20 ml. of 1 to 1 hydrochloric acid, then dilute to 1 liter.

Ammonium molybdate solution. Dissolve at the rate of 20 grams of ammonium molybdate to 30 grams of distilled water. Prepare in nickel beaker as needed. The addition of 2 to 3 ml. of 1 to 1 ammonium hydroxide to each 200 grams of the solution will greatly reduce the precipitation of molybdic oxide during the first heating.

Procedure. Transfer a 50-ml. aliquot to a 250-ml. glass-stoppered flask, add 15 ml. of 1 to 1 hydrochloric acid, and 50 ml. of distilled water, then, after mixing, 15 ml. of 20% ammonium molybdate. Stopper and heat for 10 minutes at 75° ± 3°. Cool to room temperature, and add 20 ml. of 1 to 1 hydrochloric acid, then 25 ml. of 0.4 *N* oxine. Add the oxine from a buret or pipet, shaking the sample during the addition. Restopper and heat

10 minutes at 65° ± 3°. Cool and filter through a tared No. 3010 Selas crucible. Scrub out the flask and wash the precipitate with a dilute solution of oxine in hydrochloric acid (200 ml. of the 0.4 *N* oxine solution plus 50 ml. of concentrated hydrochloric acid diluted to 1 liter). Dry the precipitate 1 hour at 110° to 120°, then ignite for 1 hour at 500° C.

Calculations. The blank varies with each batch of ammonium molybdate solution. It is necessary to determine it for each series of runs.

$$\frac{(\text{Weight of precipitate} - \text{blank})0.1580 \times 100}{\text{Sample weight in grams}} = \% \text{ silicon}$$

COLORIMETRIC DETERMINATION OF SILICON

The molybdenum blue color is measured in a Coleman Universal No. 11 spectrophotometer, using range filters No. PC-4 or 5 and a wave length setting of 715 millimicrons.

A Bureau of Standards sample is used to prepare a series of solutions containing known amounts of silicon and the blue color developed in these solutions measured in the photometer. A calibration curve is then drawn from these measurements.

B. S. feldspar Sample 70 (31.13% silicon) is fusable in sodium hydroxide and is used to make up solutions.

Table II gives some typical data when a cell depth of 50 mm. is used.

Table II. Typical Data

Silicon, Mg.	% Transmittance
0.249	10.0
0.218	12.7
0.187	16.4
0.156	22.0
0.125	30.1
0.093	38.7
0.062	52.3
0.031	69.6
0.000	100.0

Apparatus. Coleman spectrophotometer with filters as previously noted.

Reagents. Hydrochloric acid, concentrated C.P.

Hydrochloric acid, 1 *N*. Dilute 300 ml. of concentrated hydrochloric acid to 4 liters.

Ammonium molybdate, 10%. Dissolve 20 grams of 81% molybdic oxide in 180 ml. of water in a nickel beaker. Prepare as needed.

Sodium sulfite, 17%. Dissolve 170 grams in 830 grams of distilled water.

Sodium peroxide, analytical reagent grade.

Sodium hydroxide, 10%. Prepare in nickel beaker.

Transfer an aliquot containing not more than 0.125 mg. of silicon to a 250-ml. Erlenmeyer flask. If a 50-ml. aliquot is not used, make up to 50 ml. with distilled water. Add 25 ml. of 1 *N* hydrochloric acid, mix, and add 25 ml. of 10% ammonium molybdate. Let stand 2 minutes. Start timer, and start the addition of the 50 ml. of sodium sulfite solution. When it has all been added, mix thoroughly and transfer a portion of the solution to the photometer cell. Take the transmittance reading 7 minutes after the start of the addition of sodium sulfite. Simultaneously, adjust the photometer to zero setting by employing a blank prepared in exactly the same way—i.e., empty capsule, fused, etc. Several samples may be run against the same blank, provided that not more than 1 to 2 hours elapse and that the aliquot size is the same.

The transmittance reading is converted graphically to milligrams of silicon in the aliquot taken.

Calculation.

$$\frac{\dfrac{\text{Mg. of silicon}}{1000} \times \dfrac{1000}{\text{aliquot}} \times 100}{\text{Sample weight in grams}} = \% \text{ Si} =$$

mg. of silicon × 100/ml. of aliquot/sample weight in grams

DIRECT ACID DIGESTION PROCEDURE

High-boiling or resinous samples which will yield 70 to 100 mg. of silicon dioxide are weighed directly in tared platinum or Vycor crucibles, or 20 to 30 drops of the high-boiling chlorides previously noted are dropped from a weighing pipet into a tared crucible containing 3 to 4 ml. of 10% ammonium hydroxide. The water is evaporated, preferably by infrared lamp, and then the digestion is carried out. In either case, 4 or 5 drops of chlorobenzene, which reduces frothing on some samples, are added and the contents are agitated or warmed to solution of the sample. Two milliliters of 15% fuming sulfuric acid are added to the solution at room temperature. Many samples may be speeded up by addition of 0.5 ml. of fuming nitric acid immediately following addition of the sulfuric acid. Digestion is accomplished by holding over a low flame or by several hours' heating on a micro-Kjeldahl rack. When the mass has solidified and danger of frothing is past, the crucible is heated over a Meker burner to complete expulsion of sulfur trioxide fumes. The residue, usually black, is placed in a muffle for 1 hour at 800° C. Residual silicon dioxide is calculated back to silicon in the original sample.

CONCLUSIONS

There is no completely satisfactory routine method for determining silicon in organosilicon compounds, which can be universally applied to all samples. If a small number of widely varying samples is to be handled nonroutinely with a minimum outlay of equipment, the Parr bomb–oxine-volumetric or the Parr bomb–oxine-gravimetric procedures will give very satisfactory results. Fourth figure significance is not expected from either the colorimetric or volumetric determinations.

The best analysis from the standpoint of accuracy and equipment required is the sulfuric acid digestion in Vycor crucibles. Most high-boiling or resinous polymers may be analyzed in this manner. High-boiling chlorides which may be expected to yield high-boiling or resinous hydrolyzates may be successfully attacked by this method following preliminary hydrolysis. The losses by volatilization, which unfortunately limit the applicability of this method, are believed caused by the presence of either or both of two types of structure: the relatively low-boiling, highly stable cyclic materials such as the dimethylsiloxane trimer, tetramer, and pentamer recently reported by Hunter, Hyde, Warrick, and Fletcher (*5*); and hydrolyzates of trisubstituted silicon chlorides or esters. The ether structure which results from the hydrolysis and condensation of trisubstituted silicon chlorides or esters seems analogous to the organic ethers with respect to stability toward sulfuric acid.

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RECEIVED August 8, 1947.

Determination of Phenothiazine in Dust and Mixtures

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Methods for determination of phenothiazine in dusts and mixtures have been re-examined and procedures applicable to commercial dusts and mixtures have been developed.

IN THE administration of the Economic Poisons Article of the Agricultural Code of California, difficulty was encountered in determining phenothiazine in commercial dusts and mixtures. This compound is in common use as an anthelmintic and insecticide and samples are encountered with guarantees ranging from fractions of 1% to 100%. It was thought advisable to re-examine available methods and if possible to develop procedures applicable to the materials encountered.

The method of Smith (*3*) may be used to separate ether-soluble constituents from those insoluble in ether, but cannot be applied with confidence to miscellaneous mixtures possibly containing other organic insecticides such as nicotine, pyridine, or DDT.

The colorimetric procedure of Overholser and Yoe (*5*) was tried, but difficulties were encountered with instability of the colored solutions. Results obtained by the method of Payfer and Marshall (*6*) were somewhat low.

The colorimetric bromine method (*1*) frequently used for this determination was found to be adversely affected by off-colors, as noted by Cupples (*2*). In searching for modifications of this procedure which would eliminate this difficulty it was observed that a modified cuprous chloride reagent produced a color in alcoholic solution of phenothiazine. Work with this reagent also disclosed advantages in sensitivity over the bromine method, as discussed below.

According to Mellor (*3*), white cuprous chloride on exposure to air changes its composition to green basic cupric chloride, $CuCl_2.3CuO.3H_2O$, and it seems possible the production of color by cuprous chloride is effected by this compound.

PREPARATION OF PURIFIED PHENOTHIAZINE

The melting point of phenothiazine is listed in the old handbooks as 180° C., but more recent literature gives 184–185° C. It is soluble in benzene, acetone, alcohol, and ether and slightly soluble in petroleum ether. The commercial product is greenish in color and contains impurities probably due to oxidation. It may be purified (*4*, *7* modified) as follows:

Agitate 10 grams of commercial phenothiazine with 100 ml. of a mixture of benzene and toluene (1 to 9) containing 0.25 gram of Norit activated charcoal. Boil the solution for a few minutes with stirring and filter hot through a heated filter. Cool, filter off the precipitate which separates, wash with ethyl alcohol, dissolve in cold acetone, add 0.25 gram of Norit activated charcoal, and shake for 10 minutes. Filter into twice its volume of water, containing a few milliliters of hydrogen sulfide water. Allow to stand in refrigerator for 24 hours in a closed flask, then filter through a Büchner funnel, wash with water saturated with hydrogen sulfide, then with alcohol, and finally with ether, and dry under vacuum at 80° C. for 8 hours. A melting point of 184.0–184.5° C. was found on material purified in this manner. Exposed to the air, it gradually darkens, but if kept in the dark in a tightly glass-stoppered flask it is stable for several months.

If only an occasional analysis is made, there is no necessity of preparing a large amount of pure phenothiazine. The following method is suitable for preparation of small amounts.

Extract about 0.5 gram of phenothiazine (Eastman thiodiphenylamine No. P-1456) with petroleum ether for about 40 hours in a Soxhlet extraction apparatus. After cooling, a small amount of crystals will be found in the extraction flask. Filter these off on a sintered-glass crucible, wash twice with hot petroleum ether, and once with ethyl ether, and dry at 80° C. under vacuum. The melting point of crystals obtained in this manner was 184.0–184.8° C.

Preserve the insoluble impurities in the sintered-glass crucible for examination as to light absorption as indicated below.

METHOD

Reagents. A. Standard Phenothiazine Solution. Weigh 50 mg. of pure phenothiazine, transfer to a 200-ml. volumetric flask, dissolve, and make to volume in 95% ethyl alcohol (1 ml. = 0.25 mg. of phenothiazine).

B. Modified Cuprous Chloride. Pass sulfur dioxide gas through a solution of 10 grams of copper sulfate pentahydrate and 2.5 grams of sodium chloride in 75 ml. of water. The cuprous chloride will be precipitated according to the equation (*3*):

$$2CuSO_4 + 2NaCl + SO_2 + 2H_2O = 2CuCl + 2NaHSO_4 + H_2SO_4$$

Allow to stand overnight, filter through a Gooch crucible, using a filter paper disk, and wash with 1 to 1 ethyl alcohol until filtrate is free from blue color. Wash with 95% alcohol and finally with ether. Spread the residue on a glass plate, moisten with 1 to 1 ethyl alcohol and about 5 ml. of water, and allow to evaporate slowly. Scrape residue off the glass, grind finely, and wash with alcohol and ether.

C. Copper Reagent. Dissolve 0.2 gram of modified cuprous chloride (B) in 1 ml. of 1 to 1 hydrochloric acid and dilute with 95% ethyl alcohol to 20 ml. Take 5 ml. of this solution in a volumetric flask and make up to 50 ml. with ethyl alcohol. Allow to stand 24 hours before use.

Procedure. Weigh a sample approximately equivalent to 0.05 gram of phenothiazine. If the material contains constituents insoluble in ethyl alcohol, transfer to a Soxhlet apparatus and extract for 24 hours with petroleum ether. Filter the petroleum ether solution if cloudy, and evaporate to dryness on a steam bath. Dissolve the residue in ethyl alcohol, transfer to a 200-ml. volumetric flask, and make up to volume with ethyl alcohol. If the sample is entirely soluble in alcohol, transfer directly to a 200-ml. volumetric flask, dissolve in 95% ethyl alcohol, and make to volume.

Into one 50-ml. volumetric flask deliver 2.00 ml. of the standard solution (A) from a 10-ml. buret. Into another 50-ml. volumetric flask place 2.00 ml. of sample solution. Add 2.00 ml. of reagent C to each flask from a 10-ml. buret, then add to each 5 ml. of ethyl alcohol, washing down the necks of the flasks. Mix well. Stopper the flasks and place in an electric oven for 4 hours at 55° C. Allow to stand for 48 hours in a dark place, dilute to the mark with 95% ethyl alcohol, and mix. Allow to stand 15 minutes and take readings, using a Klett-Summerson photoelectric colorimeter with green filter No. 54.

The standard and sample solutions should be run in duplicate at the same time and the average result taken for calculation of amount of phenothiazine. Duplicate readings on the same solution are usually almost identical. It is important to use fresh standard and unknown solutions for each determination.

Determination of Phenothiazine in Dry Samples in Presence of Pyridine. Pyridine present in some commercial mixtures with phenothiazine interferes with production of the proper color and the procedure should be adapted as follows:

Weigh a sample equivalent to 50 mg. of phenothiazine, transfer to a 150-ml. Erlenmeyer flask, and add about 20 ml. of dilute (1 to 20) sulfuric acid. Mix well and filter through a Gooch crucible, on the bottom of which is a filter paper disk. Wash flask and content in the crucible with small portions of the dilute sulfuric acid using 80 ml. in all. To dissolve phenothiazine in the residue, pour 150 ml. of hot ethyl alcohol (in small portions) through the crucible. Transfer the filtrate to a 200-ml. volumetric flask with ethyl alcohol and make to volume with the same

Table I. Tests of Duplicate Samples of Commercial Phenothiazine

	Duplicates Run Simultaneously		Duplicates Run at Different Times			
			Sample 1		Sample 2	
	Standard	Sample	Standard	Sample	Standard	Sample
Photometric readings	167	163	258	252	167	164
	167	162	251	244	174	170
	...	...	276	268	...	...
Phenothiazine, mg.	0.500	0.488	0.500	0.486	0.500	0.491
	0.500	0.484	0.500	0.486	0.500	0.489
	...	...	0.500	0.485	...	...
Phenothiazine, %	100	97.6	100	97.28	100	98.20
	100	97.0	100	97.21	100	97.70
	...	...	100	97.10	...	...

Klett-Summerson colorimeter used with filter 54, 10-mm. cell.

reagent. Test the solution for phenothiazine as described above, beginning "Into one 50-ml. volumetric flask. . . ."

Analyses of a commercial phenothiazine are tabulated for comparison in Table I. Four other analyses of the same sample gave 97.91, 98.57, 97.89, and 98.70% phenothiazine.

PRELIMINARY EXPERIMENTS

The instrument ordinarily available for colorimetric examination of these solutions was a Klett-Summerson photoelectric colorimeter, although occasional access to a spectrophotometer was possible. It was observed that the color of solutions of pure phenothiazine in alcohol was purple red, while that of impurities was dark blue.

In order to select a filter for the colorimeter which would exclude the effects due to the impurities present, an alcoholic solution of phenothiazine (50 mg. per 100 ml.) and another solution of the impurities were prepared and examined for light absorption in the spectrophotometer. Guided by the results of Table II, a green filter, Klett-Summerson No. 54 (approximate spectral range 500 to 570 millimicrons), was selected for use in the photoelectric colorimeter as suitable for maximum exclusion of effect of impurities present in the phenothiazine.

Table II. Comparison of log I_0/I of Equal Concentrations of Pure Phenothiazine and Impurities

Wave Length, Millimicrons	log I_0/I	
	Phenothiazine	Impurities
525	0.80	0.065
520	0.81	...
515	0.81	...
510	0.81	...
500	0.77	...
490	0.71	0.060
450	0.39	0.075
550	0.68	0.076
600	0.25	0.076

Beckman, Model DU, photoelectric quartz spectrophotometer; 10-mm. cell. Slit width 0.1 mm.

Table III. Sensitivity of Copper and Bromine Methods

Concentration of Phenothiazine, Mg./50 Ml.	Colorimetric Readings			
	Copper Method		Bromine Method	
	Reading	Reading per 0.1 mg. calcd.	Reading	Reading per 0.1 mg. calcd.
0.2	71	35	45	22
0.5	189	38	95	19
1.0	334	33	185	18
1.5	354	23	283	19

Klett-Summerson colorimeter used with filter 54, 10-mm. cell.

To compare relative sensitivity of colors obtained by the bromine method (4), and the copper method, photometric readings of alcoholic solutions of different concentrations were examined in the colorimeter, using the same filter, No. 54, for both methods (Table III).

It is evident from these data that the Klett-Summerson colorimeter gives better response, when phenothiazine solutions are treated with the copper reagent instead of with bromine. There is greater difference in colorimetric readings for the same range of increase in concentration of phenothiazine.

It is stated by Cupples (2) that higher precision in the photometric readings is obtained, when the pointer in the colorimeter rests near the middle of the scale. In the copper method this could be accomplished by using about 0.5 mg. of phenothiazine per 50 ml., as is shown by Table III.

Sensitivities of the copper and of the bromine reagents were also tested by dissolving 25 mg. of phenothiazine in ethyl alcohol, making up to the mark in the 50-ml. volumetric flask, and treating 1 ml. of this solution with the reagents; then 1 ml. of the colored solution (equivalent to 0.01 mg. of phenothiazine) was diluted to 50 ml. The photometric reading was 4 for the copper reagent and 0.5 for the bromine. The blank test was negative.

Table IV. Effect of Time on Color Development of Pure Phenothiazine Solutions

Concentration of Phenothiazine, Mg./50 Ml.	Colorimeter Readings			
	24 hours	48 hours	96 hours	168 hours
0.2	71	75	76	75
0.5	189	196	197	196
1.0	334	355	389	400
1.5	354	407	496	532

Klett-Summerson colorimeter used with filter 54, 10-mm. cell.

If samples contain constituents insoluble in ethyl alcohol, they should be first extracted with petroleum ether. In order to find the period of time for complete extraction, about 5 grams of diatomaceous earth were mixed with 50 mg. of pure phenothiazine and extracted with petroleum ether in the Soxhlet apparatus for 20 hours. The test on the extracted residue showed that the amount of phenothiazine was equal to 50 mg. Therefore, in the regular procedure, a 24-hour period was used for extraction.

It was observed that the colors produced by the copper reagent intensified on standing, particularly in the higher concentrations. To study this factor, four solutions of pure phenothiazine were prepared and treated by the copper method. Solutions with the developed color were allowed to stand in a dark place for different periods of time and colorimetric readings were taken. The results, shown in Table IV, indicate that standing for 48 hours is sufficient time for "full development of color" of 0.5 mg. of phenothiazine solution.

The exact composition of the copper chloride producing these colors has not been determined. When pure cuprous chloride was used no color developed in alcoholic phenothiazine solution. Fresh reagent prepared with Baker's C.P. cuprous chloride produced color slowly with phenothiazine. An old reagent which had been prepared in the same way had a greenish color and gave the characteristic color with phenothiazine more quickly. A commercial fungicide containing 40% copper oxychloride, 53% calcium carbonate, and 7% dispersing agent, produced good color with alcohol solution of phenothiazine.

A mixture was prepared containing 0.8 gram of pure phenothiazine and 9.2 grams of starch. Then 0.3125 gram of this mixture was placed in a 50-ml. beaker and treated with 25 ml. of ethyl alcohol, filtered into a 100-ml. volumetric flask, washed with several portions of ethyl alcohol, and made up to volume. A second solution was prepared by dissolving 0.625 gram in 100 ml. of alcohol and a third solution by dissolving 1.25 grams in 50 ml. of alcohol. The three solutions were tested twice for phenothiazine in the following way: Five milliliters of ethyl alcohol were poured into a 50-ml. volumetric flask, and an aliquot portion of solution equivalent to 0.5 mg. of phenothiazine was added—namely, 2 ml. for the first solution, 1 ml. for the second solution, and 0.25 ml. for the third solution. Then 1 ml. of copper reagent was added and the neck of the flask was washed with an additional 5 ml. of alcohol. A serological 1-ml. pipet divided in 0.01 ml. was used in measuring the phenothiazine solution and the pipet was carefully wiped off before delivering the solution into the flasks.

Table V. Accuracy and Precision of Method

Weight of Mixture, Grams	Volume, Ml.	Photometric Readings		Amount of Phenothiazine	
		Standard	Unknown	Actual, mg.	Found, mg.
0.3125	100	186	186	25	25.0
		184	184	25	25.0
0.625	100	181	179	50	49.4
		171	172	50	50.3
1.250	50	186	186	200	200
		184	184	200	200

Table VI. Comparison of Bromine and Copper Methods, Same Samples of Commercial Phenothiazine

Guarantee, %	Bromine Method, %	Copper Method, %
98.84	98.39	98.13
97.00	96.53	96.00
	98.39	98.84
98.84	98.16	97.62
33.33	33.15	33.43
98.00	98.82	98.20
98.00	98.62	98.00

Table V illustrates that results found are in close agreement with the actual amount of phenothiazine.

A weight of sample should be taken such that the amount of phenothiazine present is approximately equal to that in the standard solution. The recommendations given for use of the Klett-Summerson photoelectric colorimeter were followed in estimating phenothiazine in the regular samples. Concentration of unknown equals concentration of standard divided by reading of standard and multiplied by reading of unknown.

Several samples of commercial phenothiazine were simultaneously tested by the copper and bromine methods. In both methods, the above formula was used instead of the standard curve for calculation of the amounts of phenothiazine. The results of both methods are in fairly good agreement, as indicated in Table VI.

DISCUSSION

Although the copper method is not so rapid as the bromine method, it is more convenient, and the actual time consumed for running it is less. By the copper method a close agreement of results can be obtained on duplicate samples. The sensitivity of the copper method is greater than that of the bromine method.

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Received February 24, 1947.

Determining the Purity of Dicyclopentadiene Concentrates

A Cryoscopic Method

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A cryoscopic procedure is described for determining the dicyclopentadiene content of concentrates having purities of the order of 50 to 80%. Analyses of synthetic samples and of dicyclopentadiene concentrates produced from certain hydrocarbon cracking operations indicate that accuracies of the order of 1 to 2% can be obtained. Within the limits of experimental error, solid solution behavior was not observed in the analyses of these mixtures.

COMMERCIAL quantities of dicyclopentadiene are available as a by-product from certain petroleum refinery operations in which natural gas, petroleum, or its fractions are pyrolyzed. This compound is formed by the dimerization of cyclopentadiene, which is a component of the cracking effluent. In plant-scale operation the dicyclopentadiene is often recovered in concentrates ranging in purity from 50 to 80%. This paper presents a rapid and simple analytical procedure for determining the purity of such concentrates.

DESCRIPTION OF METHOD

The high molal depression constant (K_F = 50.7) and the convenient freezing point (33.6° C.) of dicyclopentadiene (*2*) makes it particularly suited to cryoscopic analysis. However, the usual equations derived from dilute solution laws may not be accurately applied to freezing point data obtained on dicyclopentadiene concentrates having purities as low as 50 to 80%. In the method described in this paper high-purity dicyclopentadiene is added to the concentrate sample in an amount sufficient to raise the purity of the resultant mixture to 93 to 97%. In this concentration range the dilute solution laws hold with reasonable accuracy.

The dicyclopentadiene content of the 50 to 80% concentrate sample can be calculated from a knowledge of the freezing point of the 93 to 97% mixture described above, freezing point of the added high-purity dicyclopentadiene, relative weight of the concentrate sample to the added dicyclopentadiene, and molecular weight of the concentrate sample.

The principles of this method were described by Streiff and Rossini (*3*) and applied to the determination of individual aromatic hydrocarbons in mixtures of aromatics.

APPARATUS

The equipment used in this work is schematically diagrammed in Figure 1. It is essentially the same as that described by Mair, Glasgow, and Rossini (*1*). The main parts are: *A*, freezing cell (clear glass). *B*, constant-speed stirring motor, 108 r.p.m. *C*, drying tower. *D*, thermometer, −10° to +50° C. graduated in 0.1° C. divisions and calibrated for partial immersion. *E*, aluminum stirrer. *F*, clear glass Dewar.

CHEMICALS

High-purity dicyclopentadiene (97 to 100%).

PROCEDURE

The freezing point of the high-purity dicyclopentadiene (97 to 100%) is determined on a separate portion of this material in the

Table I. Analysis of Dicyclopentadiene Concentrates Having Purities of the Order of 60 to 70 Per Cent

Sample No.	Hydrocarbon Added as Impurity	Mol. Wt. of Sample[a]	Synthesis of Freezing Point Mixture: High-Purity Dicyclopentadiene[b]	Purity	Synthetic sample	F.P. of Freezing Point Mixture	Dicyclopentadiene in Freezing Point Mixture	Dicyclopentadiene in Sample: By synthesis	Experimental	Difference
			Grams	*Mole %*	*Grams*	° *C.*	*Mole %*			
1	*p*-Cymene[c]	132.9	32.6220	99.02	4.9281	11.9	94.50	65.97	64.42	−1.55
2	*m*-Xylene[c]	123.7	34.5432	98.45	4.2167	13.0	94.78	66.42	66.65	+0.23
3	Cumene[c]	127.4	37.1717	98.60	3.6779	13.7	94.95	59.66	59.39	−0.27
4	Dodecane[d]	142.9	30.8838	98.50	5.0869	13.3	94.85	70.93	70.90	−0.03
									Av.	−0.4

[a] Computed from sample synthesis. [b] Supplied by Phillips Petroleum Co. [c] Eastman pure grade. [d] Connecticut Hard Rubber Co., best grade.

Table II. Cryoscopic Analyses of Dicyclopentadiene Concentrates Obtained from Hydrocarbon Cracking Operations

Sample No.	Mol. Wt. of Sample	Sample Used	High-Purity Dicyclopentadiene Added	Purity of High-Quality Dicyclopentadiene Added	F.P. of Freezing Point Mixture	Dicyclopentadiene in Freezing Point Mixture	Dicyclopentadiene in Sample: Determined Independently		Difference
		Grams	*Grams*	*Mole %*	° *C.*	*Mole %*			
1	140[a]	3.6786	35.7790	98.53	11.3	94.36	51.41	52.0[b]	−0.6
2[d]	135.5[c]	10.5293	27.3916	98.48	5.5	92.94	78.17	78.27[c]	−0.1
3	134[a]	5.0426	26.6825	98.99	11.2	94.33	69.34	69.0[b]	+0.3
4	142[a]	3.7677	36.0064	98.99	13.2	94.83	52.13	52.8[b]	−0.7
5	145.3[a]	3.8657	35.1514	98.58	21.0	96.77	78.68	....	...
6[e]	141.4[c]	12.7744	26.3079	98.53	10.8	94.24	84.79	84.58[c]	+0.2
								Av.	−0.2

[a] Determined by Beckmann method in benzene.
[b] Determined by method based on thermal depolymerization of dicyclopentadiene.
[c] Computed from previous analysis and sample synthesis.
[d] Known increment of high-quality dicyclopentadiene added to sample 1 to prepare sample 2.
[e] Known increment of relatively pure dicyclopentadiene added to sample 5 to prepare sample 6.

manner described below. If solid at room temperature, it is melted prior to introduction into the freezing cell.

A sample of about 4 to 10 grams of 50 to 80% dicyclopentadiene concentrate is weighed on the analytical balance. To this material is added a weighed quantity of the high-purity dicyclopentadiene in such an amount that the concentration of the resultant mixture is about 93 to 97 mole %. The freezing point of this mixture is measured to the nearest 0.1°, and the highest temperature recorded after the first appearance of crystals is taken to be the freezing point. Supercooling of the order of 0.05° to 0.1° C. may be observed. Cold water serves as a satisfactory cooling bath and should be adjusted to a temperature such that a cooling rate of about 0.1° to 0.2° C. per minute is obtained in the freezing cell.

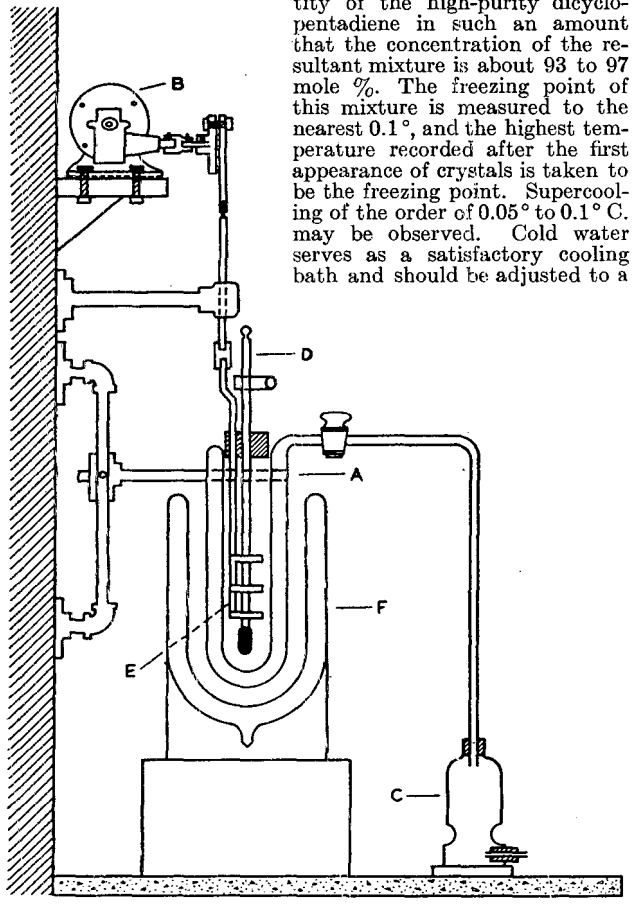

Figure 1. Diagram of Equipment

An independent molecular weight determination is made on a separate portion of the 50 to 80% dicyclopentadiene concentrate using the Beckmann method.

CALCULATION OF RESULTS

The purities of the 93 to 97% mixture (subsequently referred to as the freezing point mixture) and of the high-purity dicyclopentadiene used in its preparation are computed from the following relation:

$$\log_{10} P = 2 - \frac{A}{2.303}(T_{F_0} - T_F)$$

where P = purity, mole per cent dicyclopentadiene
T_{F_0} = freezing point of pure dicyclopentadiene, 33.6° C.
T_F = experimentally determined freezing point, ° C.

$$A = \frac{\Delta H}{RT_{F_0}^2} = 0.002605$$

[This value of A was calculated using a value for ΔH of 487 calories per mole, computed from the molal depression constant (K_F = 50.7) reported by Smoker and Burchfield (*2*).]

The purity of the 50 to 80% concentrate sample is calculated from the following expression derived from a weight balance on dicyclopentadiene:

$$Z = P - \frac{WC}{BM}(E - P)$$

where Z = mole per cent dicyclopentadiene in 50 to 80% concentrate sample
B = grams of concentrate sample taken for analysis
C = molecular weight of concentrate sample taken for analysis
W = grams of high-purity dicyclopentadiene used in preparing freezing point mixture
E = mole per cent purity of dicyclopentadiene W
M = mole weight of dicyclopentadiene W (assumed to be 132.2 when E = 97 − 100)
P = mole per cent dicyclopentadiene in freezing point mixture

EXPERIMENTAL DATA

Several synthetic mixtures of essentially pure dicyclopentadiene with various hydrocarbons were analyzed by the procedure de-

scribed. Data from these experiments are summarized in Table I.

A number of commercial products available from hydrocarbon cracking operations were also analyzed. Results from these experiments are summarized in Table II.

DISCUSSION

Ideal behavior is assumed for the mixtures on which the freezing point measurements are made. Data obtained on samples 2 and 6 in Table II show normal freezing point depressions for the added dicyclopentadiene. This indicates that within the limits of experimental error the formation of solid solutions did not occur.

In the analysis of dicyclopentadiene concentrates of the order of 50 to 80% purity, accuracy of the order of 1 or 2% may be obtained with the equipment and procedure described.

With the use of more elaborate cryoscopic equipment and techniques, such as the utilization of a platinum resistance thermometer for temperature measurements and time-temperature melting or freezing curves for the determination of the true freezing point (*4*), better accuracies could perhaps be realized.

ACKNOWLEDGMENT

The author is indebted to H. J. Hepp and to M. R. Cines for helpful suggestions while conducting this work and to the Phillips Petroleum Company for permission to publish this paper.

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RECEIVED July 28, 1947. Presented before the Division of Petroleum Chemistry at the Midwest Regional Meeting, Kansas City, Mo., June 23 to 25, 1947.

Determination of Total Aromatic Plus Olefin

By Sulfonation in the Presence of Phosphorus Pentoxide

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A procedure is presented for the determination of the total olefin and aromatic content of hydrocarbon mixtures in the gasoline and kerosene boiling range by absorption in sulfuric acid containing 30% by weight of phosphorus pentoxide. The method consists of treating the sample with the sulfonation mixture at ice temperature, which converts the unsaturated hydrocarbons (olefins and aromatics) to acid-soluble compounds and leaves the paraffins and naphthenes in a separate phase that can be measured quantitatively.

THERE is considerable information in the literature on the use of sulfuric acid as a means of determining olefin and aromatic content. The previous methods, although satisfactory for some mixtures, are not equally accurate for all types of hydrocarbon mixtures (*7, 8, 10*). Fisher and Eisner (*8*) have shown that adjustment of acid concentration and experimental conditions to overcome this failing has not been entirely successful. If fuming sulfuric acid is used, complete sulfonation of unsaturated compounds is usually obtained, but some of the saturated components may also be sulfonated (*19, 21*). If less concentrated acid is used, the reaction with saturated hydrocarbons is decreased but aromatics may be incompletely sulfonated (*9, 10*) and olefins tend to produce polymers or alkylates which are not completely absorbed by the acid layer (*6, 11, 13, 18*).

The use of sulfuric acid modified with various added "catalysts" has also been proposed. Silver sulfate has been used for this purpose (*17*). Kattwinkel (*12*) proposed a mixture of concentrated sulfuric acid containing 14% by weight of phosphorus pentoxide. Although these reagents were an improvement over sulfuric acid alone, they were not entirely successful. Berg and Parker (*4*) recently suggested the use of fuming sulfuric acid and glacial acetic acid.

The purpose of the present paper is to show that if the sulfonation mixture contains 30% by weight of phosphorus pentoxide and 70% by weight of 95 to 96% sulfuric acid, and the reaction is carried out under carefully controlled conditions on hydrocarbon mixtures boiling under 600° F., side reactions are virtually eliminated and an accurate measure of the total olefin and aromatic content is obtained.

This method of determining total olefin and aromatic content has been used in this laboratory for some time and during the war was submitted to the American Society for Testing Materials and incorporated in emergency method ES-45A (*2*), and more recently in tentative method D875-46T (*3*). This procedure was also used for analysis of gasoline by Kurtz, Mills, Martin, Harvey, and Lipkin (*14*). However, a detailed evaluation of the method is not given in any of these references.

In brief, the method involves treating the sample with the sulfonating mixture at ice temperature to convert the unsaturated hydrocarbons to acid-soluble compounds. The saturated hydrocarbons are not sulfonated and can be centrifuged from the reaction mixture and measured quantitatively as "raffinate." Since the sulfonating mixture described herein is a strong corrosive chemical, the operator should take suitable safety precautions. A safety mask should be worn during the test period, and the mechanical shaker should be equipped with a safety hood.

APPARATUS

Sulfonation Flask. The flask used in this work (*1*) is shown in Figure 1. The calibrated neck of the 10-ml. flask is graduated in 0.1-ml. increments and readings can be estimated to 0.02 ml., a precision of 0.2%. A modified Babcock bottle (*3*) can also be used if precision of 1% is sufficient.

Centrifuge. A centrifuge should be suitable for handling the type of sulfonation flask described above.

Mechanical Shaker. A mechanical shaking device is required that has a horizontal stroke 7.5 ± 1.25 cm. (3 ± 0.5 inches) in length and a speed of 250 ± 25 cycles per minute, each cycle consisting of one forward and one return stroke. [Shaking by hand is practical only for samples of low olefin content (*2, 3*).] The bottles or flasks are supported in a vertical position during

the shaking period, immersed in ice water to a depth of 10 to 12.5 cm. (4 to 5 inches). A mechanical shaker suitable for this work (Figure 2) is a modified Arthur H. Thomas shaker 8916. The device used to hold the sulfonation flasks during the shaking period is shown in Figure 3. A desirable addition to the shaker is a cover that protects the operator in case a flask breaks during sulfonation. If a cover is not used, a face mask should be worn during the shaking period.

REAGENTS

Sulfuric Acid. Reagent or C.P. grade, containing 95 to 96 weight % sulfuric acid.

Sulfonation Mixture. Prepare a mixture consisting of 70% by weight of sulfuric acid and 30% by weight of C.P. or reagent grade phosphorus pentoxide, by adding the phosphorus pentoxide at the rate of 30 to 55 grams every 3 minutes to the sulfuric acid. Stir the mixture during the addition of phosphorus pentoxide. The resulting solution should contain only a small amount of insoluble residue.

Because this reagent is highly hygroscopic, precautions should be taken to prevent absorption of moisture.

TEST PROCEDURE

Add 25 ± 1 ml. of sulfonation mixture to the sulfonation flask, stopper the flask, and place it in the shaker filled with ice water which is maintained at a temperature of 0° to 5° C. After the flask has been in the bath for at least 5 minutes, record the temperature of the sample and pipet 5 or 10 ml. of the sample into the sulfonation flask, taking special care to allow the sample to run down the side wall of the flask, so that the two layers do not mix. Pipet highly volatile samples at ice temperature, others at room temperature. The choice of 5- or 10-ml. sample depends on the olefin content, which may be determined by bromine number (*3*) if not known. If the sample contains less than 40% olefin, 10 ml. should be treated; otherwise 5 ml. should be used.

Though the procedure is applicable to most olefins without any difficulty, certain ones like cyclohexene and isoprene in high concentration react violently, causing venting or even breakage of the flask. If such difficulties are encountered, dilution of the sample with a saturated hydrocarbon such as iso-octane is advisable.

Cool the flask containing the acid-hydrocarbon mixture in the ice water bath for an additional 5 minutes. Clamp the flask(s) in place by securing the brass cups over the stoppers. The depth of ice water should be such that the flask is immersed 4 to 5 inches.

During the preliminary 5-minute shaking period, shake the flask for one full cycle every 10 seconds. One cycle at full speed is required. Usually the shaker will complete 1 to 2 cycles at reduced speed after the motor is turned off. At the end of the 5-minute period, shake continuously for 20 minutes.

Vent the stopper momentarily, remove the flask from the ice bath, and place it in the centrifuge, and rotate at approximately 1000 r.p.m. for 10 minutes. Remove the flask from the centrifuge and add sufficient sulfuric acid to bring the hydrocarbon within the graduated portion of the neck.

If the volume of the unreacted material exceeds the volume of the lower graduated portion of the neck, add sufficient acid to bring the upper meniscus within the graduated portion above the bulb. Centrifuge for an additional 10 minutes.

Adjust the temperature of the acid-hydrocarbon mixture to within 1° C. of the original temperature of the test sample when pipetted into the flask. Place the flask in front of a plain, nonglary, white or light colored background illuminated by diffused light, and read the scale to the nearest 0.02 ml. at the upper and lower levels of the hydrocarbon layer. The appearance of the two menisci in the neck of the flask is shown in Figure 4. Read the lower meniscus at the lowest point of the dark zone between the hydrocarbon and acid layers: 4.34 ml., as shown in Figure 4, *A*. Read the upper meniscus at the lowest point of the dark zone above the hydrocarbon layer: 9.80 ml., as shown in Figure 4, *B*. Calculate the milliliters of unreacted sample by subtracting the lower meniscus reading from the upper meniscus reading. Centrifuge for an additional 10-minute period, adjust to temperature, and repeat the readings. If the volume of unreacted material does not agree with the previous reading to within 0.05 ml., repeat centrifuging and reading until agreement is obtained. For nonviscous samples such as gasolines one 10-minute centrifuging period after addition of acid is usually sufficient.

Calculation Calculate the volume per cent absorption as follows:

$$S = \frac{(V - \nu)100}{V}$$

where S = volume % absorption
V = ml. of sample charged
ν = ml. unreacted sample

Calculate the volume of olefin plus aromatic as follows:

For 10-ml. samples: Olefin + aromatic = $S - C$

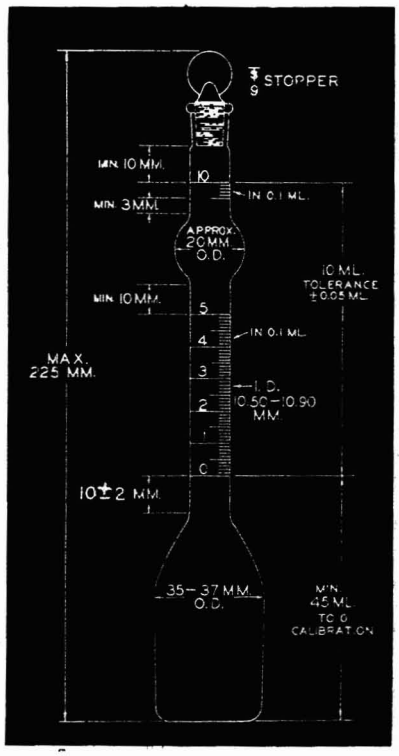


Figure 1. Sulfonation Flask

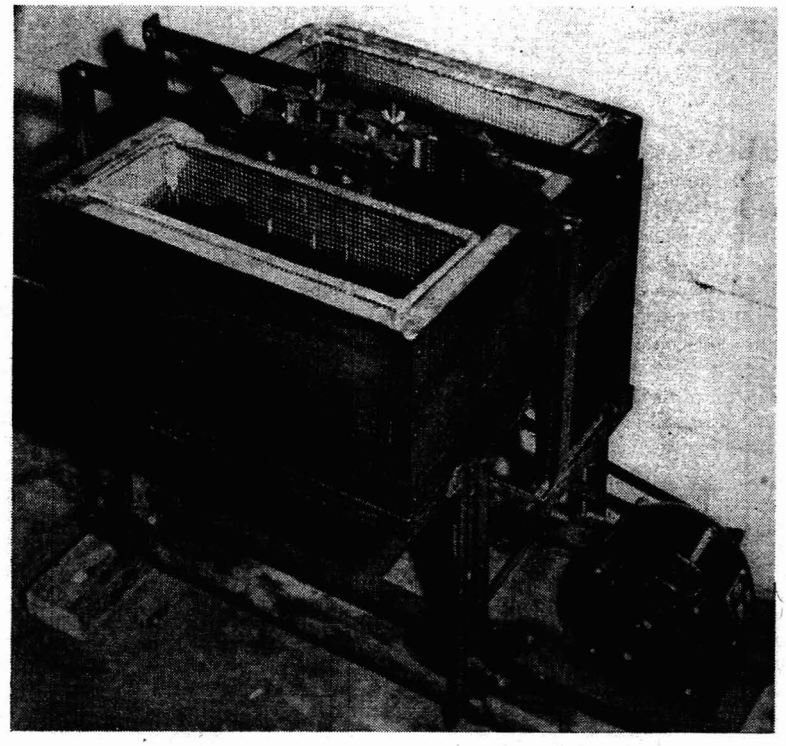


Figure 2. Mechanical Shaker

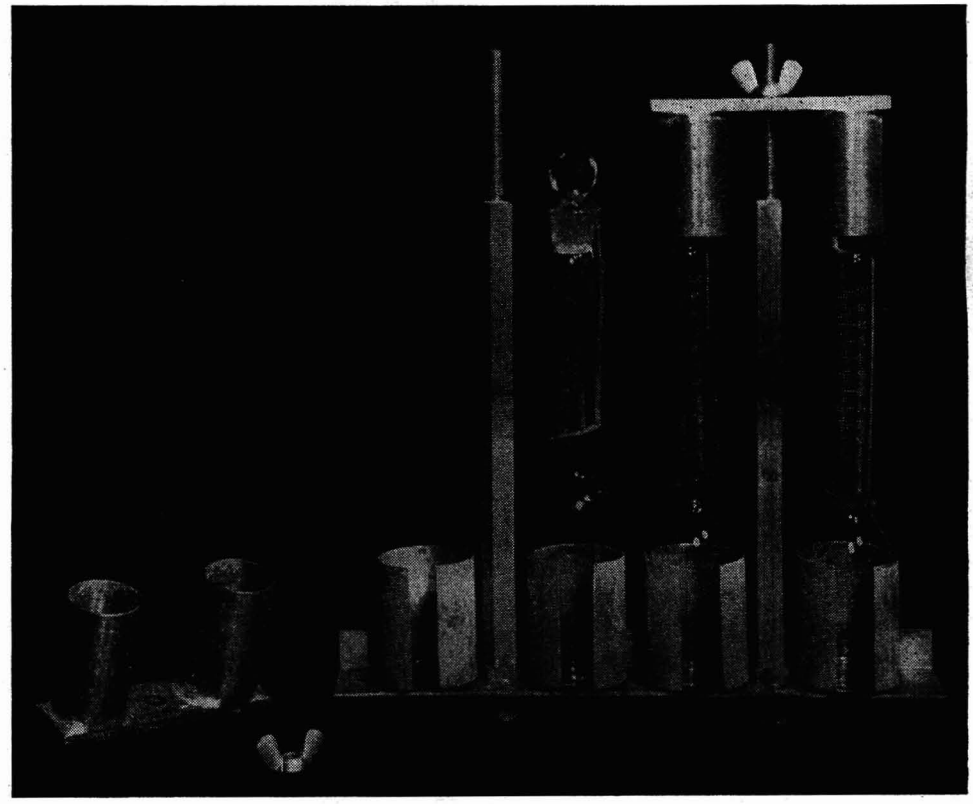


Figure 3. Flask Holder

For 5-ml. samples: Olefin + aromatic = $S - 2C$

where C is correction obtained from Figure 5.

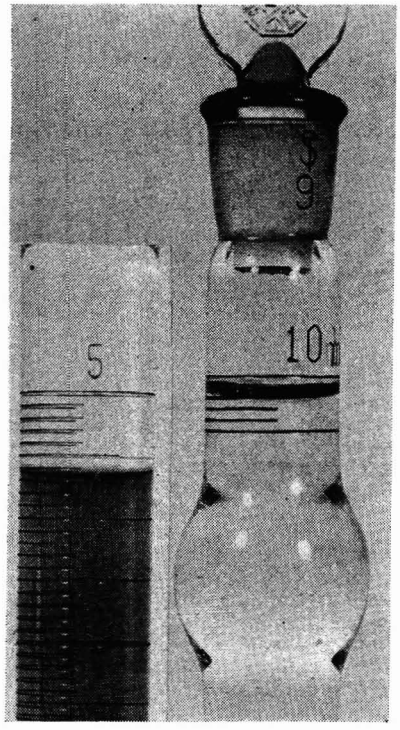
Figure 5 was developed by testing a series of known blends and plotting the deviation obtained against per cent absorption. It compensates for several effects, such as solubility of saturated hydrocarbons in the acid layer and reaction of the sulfonating mixture with some of the saturated components.

DISCUSSION

In order to show that this procedure does not sulfonate saturated hydrocarbons to any appreciable extent, a series of tests was run on a group of pure paraffins and naphthenes. Data given in Table I show the acid absorption and refractive index change for treating periods of 20 minutes (recommended) and 30 minutes at 0° C. and also for 60 minutes at 25° C. The compounds used in this work were for the most part of technical purity but were freed of unsaturated contaminants by percolation through silica gel. The absorption in 20 minutes' acid treating at 0° C. was in all cases 1% or less. In 30 minutes' treating at 0° C. the acid absorption was 1.5% or less. The maximum index change in any case for the 20- and 30-minute treats at 0° C. was 0.0003.

These data indicate that in using the recommended procedure there is virtually no sulfonation of saturated hydrocarbons, even when the treating time is extended to 30 minutes. If, however, the temperature is raised to 25° C., there is evidence of considerable reaction with saturated hydrocarbons. The data in Table I indicate that for treating times of 60 minutes at 25° C. the maximum absorption for the paraffins investigated was 10.5%. The naphthenes tested, excluding methylcyclohexane, gave a maximum absorption of 11%. Methylcyclohexane showed an absorption of 21%. This is the highest absorption yet encountered for a saturated hydrocarbon. Further investigation confirmed the fact that the sulfonation of methylcyclohexane with 30% phosphorus pentoxide in sulfuric acid is very sensitive to temperature. Some difficulty may therefore be encountered in the analysis of mixtures of high concentrations of both olefins and methylcyclohexane. If methylcyclohexane is shaken with the sulfonation mixture without cooling, the reaction may be very violent.

The investigation of the behavior of saturated hydrocarbons in the test procedure was continued to include a series of cuts of a gasoline which had been pre-



A *B*

Figure 4. Appearance of Menisci in Flask

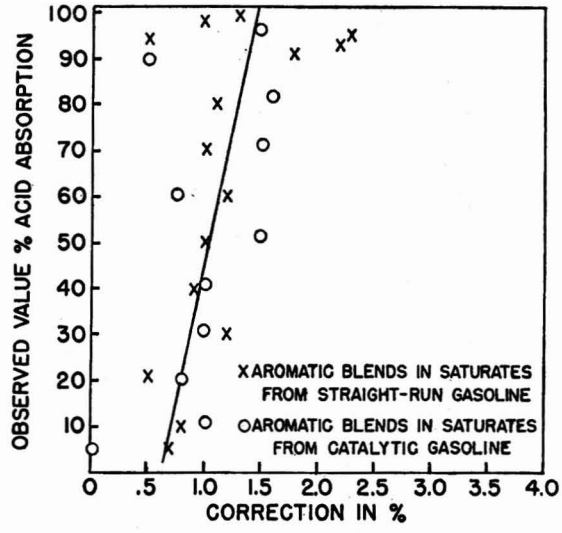


Figure 5. Solubility Correction

Table I. Effect of Sulfonation Mixture on Paraffins and Naphthenes

Hydrocarbon	n_D^{20}[a]	Volume % Absorbed[b] 20 min. at 0° C.	30 min. at 0° C.	60 min. at 25° C.	Change in n_D^{20} 20 min. at 0° C.	30 min. at 0°. C.	60 min. at 25° C.
n-Hexane[c]	1.3823	0.5	1.5	6	0	−0.0001	−0.0023
Mixed Hexanes[c]	1.3795	1	1	9	0	−0.0003	−0.0017
Neohexane[d]	1.3692	1	1.5	3	−0.0002	−0.0001	−0.0005
n-Heptane[e]	1.3880	0	0.5	0.5	−0.0001	−0.0001	0
Heptane[f]	1.3957	1	1	8	0	−0.0002	−0.0017
Mixed Heptanes[c]	1.3980	0	0.5	10.5	0	−0.0002	−0.0018
Trimethylbutane[g]	1.3895[h]	1	...	..	0	.....	.....
2,2,4-Trimethylpentane[i]	1.3935[h]	0	...	..	−0.0001	.....	.....
2,2,4-Trimethylpentane[g]	1.3917[h]	0.5	...	..	0	.....	.....
2,2,4-Trimethylpentane[j]	1.3917	1	1	1	0	0	+0.0002
Cyclohexane[g]	1.4264	0	0	0.5	0	0	−0.0004
Methylcyclohexane[f]	1.4230[h]	0	0	21	0	0	−0.0020
Ethylcyclohexane[g]	1.4333	0	0	5	−0.0001	−0.0001	−0.0008
Decahydronaphthalene[f]	1.4755	0	0	11	0	−0.0001	−0.0013
Dicyclohexyl[f]	1.4796	0	0	0	−0.0001	−0.0001	−0.0002

[a] All samples except [h] were freed of unsaturated hydrocarbons by percolation through silica gel.
[b] No correction factors applied.
[c] Phillips Petroleum Co., commercial grade.
[d] Phillips Petroleum Co., pure grade.
[e] Westvaco Chlorine Products Corp., octane standard.
[f] Eastman Kodak Co., practical grade.
[g] Eastman Kodak Co., C.P. grade.
[h] Treated with 95% sulfuric acid to remove unsaturated hydrocarbons.
[i] Eastman Kodak Co., technical grade.
[j] Rohm and Haas Co., octane standard.

Table II. Effect of Sulfonating Mixture on Olefin- and Aromatic-Free Gasoline Fractions

Boiling Range, °C.	n_D^{20}	Volume % Absorbed[a] 20 min. at 0° C.	30 min. at 0° C.	60 min. at 25° C.	Change in n_D^{20} 20 min. at 0° C.	30 min. at 0° C.	60 min. at 25° C.
40–70[b]	1.3807	2	2	16.5	−0.0005	−0.0003	−0.0035
70–100[b]	1.4035	1	2	12.5	−0.0002	−0.0002	−0.0023
100–125[c]	1.4107	1	1	5.5	−0.0003	−0.0001	−0.0019
125–150[b]	1.4178	1.5	1.5	7	−0.0003	−0.0001	−0.0017
150–175[c]	1.4247	1	1	2.5	−0.0002	−0.0001	−0.0010
175–200[c]	1.4314	1	0	3	−0.0002	−0.0001	−0.0012
200[c]	1.4425	1	1.5	4	0	−0.0003	−0.0011
70–100[d]	1.3994	0	...	..	−0.0001	.....	.....
100–125[d]	1.4103	0	...	..	−0.0001	.....	.....
125–150[d]	1.4160	0	...	..	−0.0001	.....	.....
150–175[d]	1.4238	0	...	..	−0.0001	.....	.....
175–200[d]	1.4297	0	...	..	−0.0002	.....	.....

[a] No correction factor used.
[b] Freed of unsaturated hydrocarbons by treatment with 66 Bé. sulfuric acid.
[c] Same treatment as [b] followed by 5-minute treatment with P_2O_5-H_2SO_4.
[d] Freed of unsaturated hydrocarbons by percolation on silica gel.

viously freed of olefins and aromatics. The data given in Table II indicate essentially the same conclusions as in the investigation of the individual paraffins and naphthenes.

A series of blends, each containing an aromatic and saturated hydrocarbon, was tested to determine whether or not different aromatics would be completely removed by the proposed procedure. The data are shown in Table III for eight different aromatics representing 26 blends. The maximum deviation is 1.5% and the average deviation 0.5%.

As more trouble is usually encountered with the sulfonation of olefins, the method was tested on a series of blends containing up to 90% olefin. The olefins were a mixture of equal parts of diisobutylene, octene-2, and cyclohexene and were blended with the paraffin-naphthene portion of a straight-run gasoline. The aromatic component was an equal volume mixture of benzene, toluene, and xylenes. The experimental values for olefin-aromatic contents and refractive indices after acid treating were obtained on both 5- and 10-ml. samples and are shown in Table IV. Maximum deviation for olefin plus aromatic content is 1.5% for 10 ml. of sample and 3.0% for 5 ml. of sample. The errors in volume per cent olefin plus aromatic for 5-ml. samples are about twice as large as for 10-ml. samples because of larger errors of reading the meniscus levels. Similar data presented by Kurtz *et al.* (*14*) obtained under routine conditions show that different operators can obtain comparable results.

Considering these data from the standpoint of the refractive index of the "raffinate," the 10-ml. treating gives good agreement on samples containing 40% or less of olefin. If the olefin content is higher, considerable deviation may be encountered. If 5 ml. of sample are used on these highly olefinic samples, the deviation is considerably less. Even where the physical properties do not agree, the volumetric results are essentially correct. In such cases better physical properties of the saturated component can usually be obtained for materials in the gasoline boiling range, by using the nitrogen tetroxide procedure (*5, 14, 20*). This procedure involves removing the olefins by reaction with nitrogen tetroxide and treating the olefin-free residue with the sulfonating mixture.

Up to this point, the evaluation of the method has been limited to fractions in the gasoline boiling range. Data given in Table V show that the method can be applied to samples boiling up to 315° C. (600° F.). The aromatic and saturated fractions of a cut from East Texas crude were separated by using silica gel (*15, 16*) and distilled to obtain the cuts indicated in Table V. Known amounts of each aromatic fraction were blended with the corresponding saturate fraction. The aromatic concentrations are approximately 25, 50, and 75%. The acid absorption is accurate to within 1.0% in every case. The refractive index deviation for the highest boiling cut is somewhat high. However, if a 5-ml. sample is used, the index of the raffinate is satisfactory. The index deviation in this range is probably due to decreased contact of acid and sample caused by increased viscosity of the sample, although other factors may contribute. In general, the method is not recommended for cuts having 90% boiling points above 315° C. (600° F.).

The preceding data show that the method can be used for samples of relatively high boiling range. It can also be used for volatile samples. Blends have been prepared containing as much as 80% added isopentane in a low boiling thermal gasoline. That the maximum error in acid absorption was found to be 2% indicates that low-boiling samples can be tested in this procedure without introducing abnormal errors due to volatility losses.

Table III. Determination of Various Types of Aromatics

Aromatic	Known Vol. % Aromatic[a]	Determined Vol. % Aromatic	Vol. % Deviation
Benzene	70	69.5	−0.5
	80	79.5	−0.5
	90	89.5	−0.5
	100	100.0[b]	0.0
Toluene	70	69.0	−1.0
	80	79.0	−1.0
	90	89.0	−1.0
	100	100.0[b]	0.0
Ethylbenzene	70	71.5	+1.5
	80	80.5	+0.5
	90	91.0	+1.0
	100	100.0[b]	0.0
Xylene (mixed)	20	20.5	+0.5
	40	40.5	+0.5
	60	60.5	+0.5
	80	80.0	0.0
	100	100.0[b]	0.0
Cumene	70	71.0	+1.0
	80	81.5	+1.5
	90	91.0	+1.0
o-Ethyltoluene	50	50	0.0
	75	75.5	+0.5
m-Ethyltoluene	50	50.5	+0.5
	75	75.0	0.0
p-Ethyltoluene	50	50.5	+0.5
	75	75.0	0.0

[a] Blended in light alkylate.
[b] No correction factor applied to samples containing 100% aromatics.

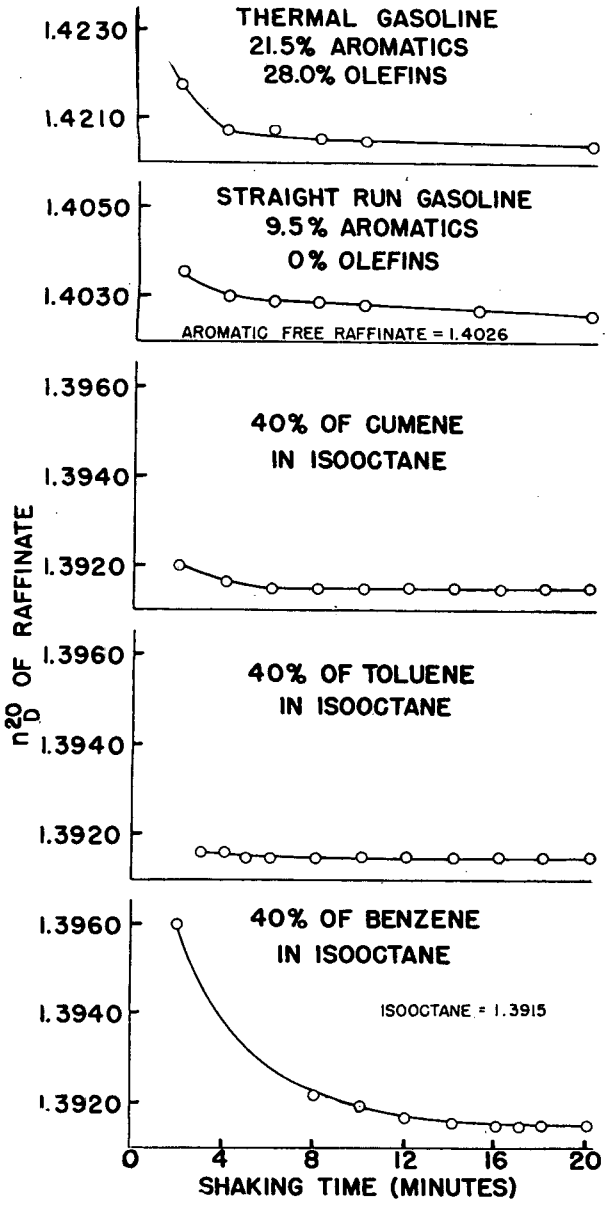


Figure 6. Rate of Removal of Unsaturates

Table IV. Accuracy for Blends of Olefins, Aromatics, and Saturates

Approximate Blend Composition, Volume Per Cent			Vol. % Aromatic + Olefin			n_D^{20} Raffinate		
			Exptl. − Known			(Exptl. − Known) × 10^4		
Olefin[a]	Aromatic[b]	Saturate[c]	Known	10 ml. treated	5 ml. treated	Known	10 ml. treated	5 ml. treated
0	20	80	20.0	0.5	0.5	1.4125	+1	0
20	0	80	20.0	1.5	2.5	1.4126	−2	−7
0	40	60	39.5	1.5	1.0	1.4126	+1	−1
20	20	60	39.5	1.5	3.0	1.4126	0	−4
40	0	60	39.5	1.0	2.5	1.4127	−3	−8
0	60	40	59.0	0.5	0.5	1.4126	+1	−1
20	40	40	59.0	1.0	2.5	1.4127	0	−1
40	20	40	59.0	1.0	2.5	1.4129	−1	+7
60	0	40	59.0	1.0	2.0	1.4130	−9	−9
0	80	20	79.0	0.5	0	1.4128	0	0
20	60	20	79.0	0.5	0.5	1.4130	−1	−3
40	40	20	79.0	0.5	2.0	1.4133	+3	0
60	20	20	78.5	1.0	2.0	1.4136	+29	0
80	0	20	78.5	1.0	2.5	1.4139	+15	−4
20	70	10	89.0	0.5	0	1.4136	+3	−2
40	50	10	88.5	1.0	0.5	1.4141	+9	−4
60	30	10	88.5	0.5	1.0	1.4147	+29	+7
80	10	10	88.5	0.5	1.5	1.4152	+37	+4
90	0	10	88.5	0	1.0	1.4154	+60	−13
80	20	0	98.5	0	0	No raffinates		

[a] Mixture of equal volumes of cyclohexene, diisobutylene, caprylene.
[b] Mixture of equal volumes of benzene, toluene, xylene.
[c] Paraffin-naphthene portion from straight-run gasoline.

Table V. Accuracy through Boiling Range for Synthetic Blends[a]

Boiling Range	Vol. % Olefin + Aromatics		n_D^{20}	
	Known	Exptl. − known	Saturate fraction	Raffinate − saturate
100–150° C.	24.5	+0.7	1.4136	−0.0002
(212–302° F.)	49.0	+0.9	1.4136	−0.0001
	73.6	+0.2	1.4136	0
150–205° C.	24.8	+1.0	1.4275	−0.0004
(302–400° F.)	49.7	+0.2	1.4275	−0.0004
	74.5	0	1.4275	0.0004
205–260° C.	24.9	+0.1	1.4430	0
(400–500° F.)	49.7	+0.2	1.4430	0
	74.6	0	1.4430	−0.0001
260–315° C.	24.8	+0.8 (−0.4)[b]	1.4479	+0.0011 (0)[b]
(500–600° F.)	49.5	−0.2 (−0.1)[b]	1.4479	+0.0019 (+0.0002)[b]
	74.3	+1.0 (+1.5)[b]	1.4479	+0.0019 (+0.0003)[b]

[a] Aromatic and saturates separated from straight-run East Texas crude by silica gel.
[b] 5 ml. of sample treated.

The data in Tables I and II show that an increase in shaking time from 20 to 30 minutes did not appreciably increase the absorption of saturated hydrocarbons. Similar data for samples containing aromatics and olefins, showing the refractive index change during the test period, are given in Figure 6. The shape of the index *vs.* time curve indicates that most of the unsaturated hydrocarbons are removed within the first few minutes. With the flask in the vertical position, as in this work, benzene requires 16 minutes for complete sulfonation.

Figure 7 shows the temperature versus time curves obtained during the sulfonation of a 5-ml. sample of a 50% mixture of cyclohexene in isooctane. The data compare maximum temperatures obtained by: (1) no preliminary shaking, (2) the recommended procedure using a 5-minute preliminary shaking period of 1 to 2 shakes every 10 seconds, and (3) the A.S.T.M. procedure (*3*) using a 5-minute preliminary shaking period of 1 to 2 shakes every minute. Using the recommended pro-

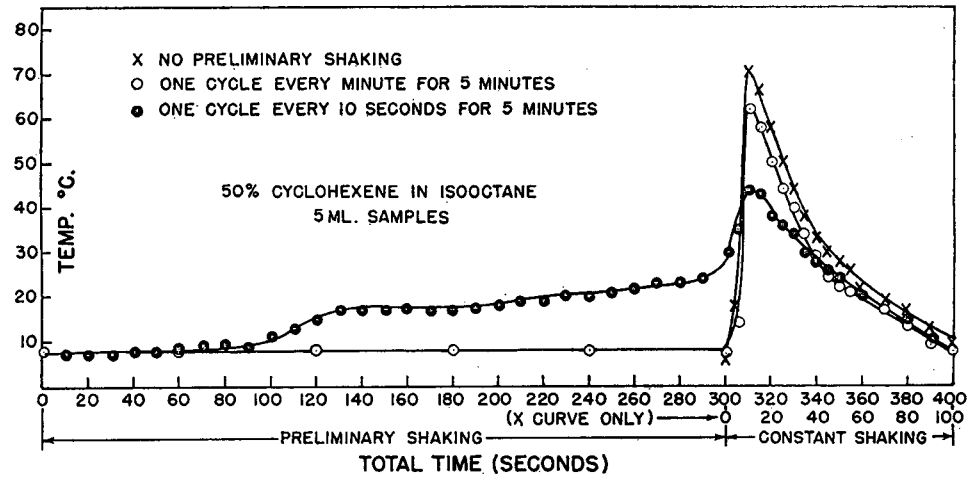


Figure 7. Reaction Temperatures with Different Shaking Techniques

cedure the olefin mixture reached a maximum temperature of 45° C. compared with 60° and 70° C. for the A.S.T.M. procedure and the procedure using no preliminary shaking, respectively. Using 50% toluene in iso-octane, maximum temperatures of 16°, 23°, and 23° C. were obtained by the three shaking procedures. The temperatures of the reaction mixture were measured using an iron-constantan thermocouple in direct contact with the reaction mixture.

The test method has been applied to a wide range of samples with satisfactory results.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of many members of the laboratory, particularly the following: S. C. T. McDowell for much of the recent laboratory work, especially that relating to temperature rise; R. E. Ledley and S. J. Hetzel for work on the solubility correction and proper method of reading meniscus; Miss D. J. Bellar for much accurate work; and C. C. Martin and W. T. Harvey for helpful discussion.

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RECEIVED July 25, 1947.

Electrical Heating and Bottom Receivers for Distillation Equipment

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The construction and application of electrical heating and bottom receivers for distillation equipment are discussed.

THE procedures described here for heating still flasks have been infrequently employed because of misconceptions as to their operation and safety characteristics and also because some of the modifications in design which make their operation practical have not been generally known among laboratory men.

Correct boiling is best obtained by an arrangement that induces rapid circulation of the boiling liquid. A soft, even heat over a large surface may give rise to bumping and foaming unless some type of ebullator is used. A sharp source of heat which causes very rapid circulation gives very good boiling characteristics in most cases.

Ease of control is mainly dependent upon the heat capacity of the heating device and the temperature at which it runs.

Safety depends upon a heating element which runs at a relatively low temperature and is free from the possibility of breakage. When large quantities of inflammable liquids are being distilled, the vaporizer should contain only a small amount of liquid and should be so arranged that, in case of breakage, the main body of liquid will be automatically isolated.

The boilup rate should be relatively constant. Half-shell heaters usually have a decreased boilup rate as liquid is distilled from the flask, because a part of the heat is radiated to the surroundings. Whole-shell heaters will give a relatively constant boilup but will run very hot as the still liquid becomes low. This is undesirable from the standpoint of safety.

Most of the heating flasks described below do not allow the charge to be distilled to dryness; this is not a serious disadvantage. If the contents are to be completely distilled over, some high-boiling scavenger liquid must be added to push the heavy ends through the column. For total reflux operation the heaters can be run with approximately 25 ml. or less, which will usually allow cuts to be taken of the heavy ends.

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IMMERSION HEATER OF BARE WIRE

An immersion heater of bare wire is illustrated in Figure 1, *B*. The heating element consists of a 60-cm. length of No. 30 Chromel wire wound in a tight spiral spring of 6-mm. diameter. The spring is stretched around three sides of a rectangular form of 4-mm. glass rod. The ends of the element are connected by twisting and braiding two lengths of copper wire, which are in turn fastened by small couplings to tungsten leads sealed through a $ joint.

This heater is closest to the ideal type when vaporizing noncorrosive liquids. Its negligible heat capacity allows instantaneous control response. There is no fire hazard with inflammable liquids, as the fine wire burns through when out of contact with liquid. There is apparently little superheating even when the element runs red hot, because it is surrounded by a rapidly moving blanket of vapors. The heater has been used in vacuum distillations without an ebullator although, in this case, it is advisable to furnish the major part of the heat by means of an external shell heater. There is little tendency for liquids to froth, probably because of slight superheating of vapors surrounding the wire. The main disadvantage, besides unsuitability for corrosive liquids, is carbonization of organic solvents if the element is exposed.

Table I. Heat Loss and Operating Temperatures of External Heating Elements

	Watts	
Total power input	350	490
Heat out in steam	225	345
Heat loss from entire flask (including boiling tube)	105	120
Heat loss from external heating wire	20	25

Total Input, Watts	Thermocouple, ° C.
0 (immersion heater)	97
100	128
200	156
300	183
350	197
395	212
445	232
495	244

EXTERNAL BOILING TUBE

One of the most useful heaters, because of its ease of construction, consists of a 35- to 45-mm. tube sealed at an angle to one side of the bottom center of a round-bottomed flask (Figure 1, *C*). The heating element is preferably wrapped directly on the boiling tube with little outside insulation. Insulation is ineffective, as there is negligible heat loss from the element (Table I) and it may cause overheating and breakage if the flask runs dry. The heater gives good boiling, though not so smooth as bare wire, and requires an ebullator for high-vacuum operation.

In four years of use the writer has had no case of breakage during operation when using this type of heater. The location of the tube off-center causes the liquid to slide over the side of the tube and froth up, thus protecting the tube from thermal shock if the still flask runs dry.

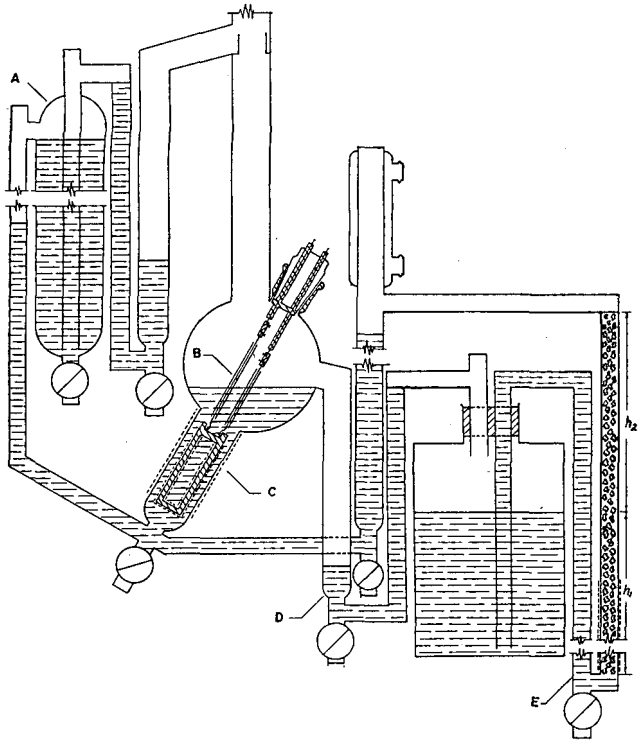

Figure 1.

A. **Bottom receiver for total reflux, vented to outside pressure by barometric legs**
B. **Bare wire immersion heater**
C. **External boiling tube**
D. **Bottom receiver for finite reflux, recycle overflow line**
E. **Bottom receiver for finite reflux, heat pump**

The tests (Table I) were conducted with 1 liter of water in a 1-liter still flask supplied with both an external boiling tube (Figure 1, *C*) and an immersion heater of bare wire (Figure 1, *D*). The heat-out in the steam was determined by condensing and removing the water. The heat loss from the still flask was determined by the difference between the power input and the heat-out when the immersion heater was used. The difference in heat-out for a given power input when the immersion heater and external boiling tube were used was taken to be the heat loss from the latter heating element.

The external boiling tube was 12 cm. long and 40 mm. in diameter. The heating element was 90 cm. of Chromel ribbon, 1.6 × 0.10 mm., covered with two thin strips of Alundum cement, 12 mm. wide.

The temperatures were determined by placing a thermocouple over several turns of the winding and coating it with a little Alundum cement. The readings, as measured and recorded, are higher than the bare wire. Using the figures for heat loss above and assuming 100% insulation by the Alundum cement over the thermocouple, when the thermocouple reads 200° C. the bare wire would read 197° C.

HEATER WITH EXTERNAL CLOSED CIRCUIT (FIGURE 2, *B*) (*3*)

The best results are obtained with a 15-mm. loop which gives rapid circulation and is resistant to thermal breakage if the heating is accidentally supplied above the liquid level. A heater of Chromel ribbon covering 20 cm. of 15-mm. tubing can supply more than 600 watts and multiple loops can be used to obtain greater capacity. The boiling characteristics are intermediate to the two types described above and it may be used for vacuum distillations, though it has an appreciable pressure drop. There is little tendency to foam or bump. The heater may be used to evaporate thermally unstable liquids in place of a steam jacket (*2*, *5*). Two-phase systems whose components differ appreciably in boiling point give rise to violent explosions in this heater.

PROTECTED IMMERSION HEATER (*7*)

The simplest construction consists of the element wound on a glass tube with flanged ends to center it inside the protection tube. Copper leads connected to the ends of the element are brought out of the protection tube through a rubber stopper. This seals the end of the protection tube, so that there is no fire hazard in case of breakage.

An alternative construction method with more control lag is use of a loose winding packed in dry Alundum powder. The leads are taken out through ceramic sleaves. No breakage has occurred with these heaters as long as the heat is supplied below the liquid level. They are best used inside of an external boiling tube or a loop heater. Their boiling characteristics are almost as good as the bare wire heater. However, they are more difficult to construct than either external heater.

Construction of Heating Elements. The heating element is wrapped directly on the glass and the ends are fastened with bands of copper wire wrapped over thin strips of asbestos paper. The ends of the element are braided into the twisted ends of copper wire. The coils of the element are held in place by thin strips of refractory cement; the writer prefers a temporary cement consisting of a thin slurry of Alundum cement and water. This can be painted on with a brush, sets almost immediately, and can be washed off. If a permanent cement is desired the Alundum slurry is made with diluted water glass. The glass is coated with paraffin wax or grease before the element is wound and cement applied. The heater can be made as a detachable shell.

Where autotransformers are not available, the element can be made a variable resistance by providing several taps of twisted copper wire, into which the element is braided. If the heating is to be distributed over the entire heating tube, the coils can be wound in multiple-pitch spirals. In even-pitch spirals the power leads are together at one end of the element. They are more troublesome to wind and care has to be exercised that adjacent coils do not come loose and cause a short circuit.

Leads. The power leads are connected to the element by battery clips covered with rubber tubing. The rubber tubing prevents the clips from shorting out when disconnected and makes the connection to the element incapable of accidental disconnection. The length of the twisted copper ends prevents the rubber tubing from overheating.

SHELL HEATERS FOR ROUND-BOTTOMED FLASKS

These heaters (*1, 3, 6*) are preferably made as detachable shells, formed by winding asbestos rope around a flask and coating the outside with Alundum cement and water glass. A buried element can be wound on the outside of this shell, which is then covered with another similar shell. An exposed element, if desired, can be wound on the inside of the first shell and tacked in place with Alundum cement and water glass. These shells are light and strong and can stand a high operating temperature. They fit closely enough to be used interchangeably with different flasks of the same size.

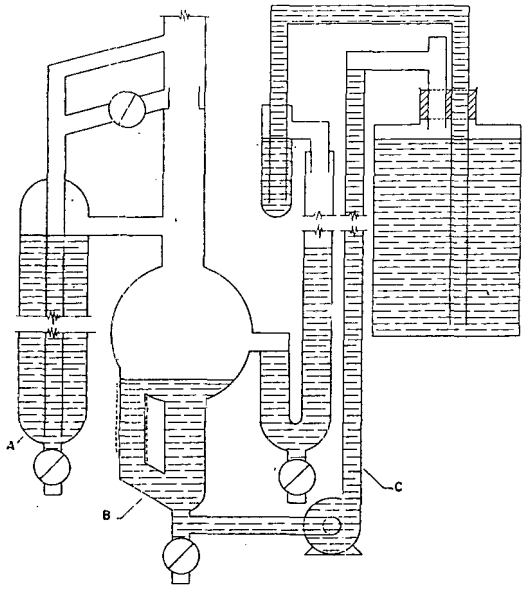

Figure 2.

A. **Bottom receiver for total reflux, running under an atmosphere of still vapors**
B. **External closed circuit heater**
C. **Bottom receiver for total reflux, mixing with still flask contents effected by means of a pump**

Shell heaters have a great tendency towards bumping and are best used to insulate the still flask. They are very useful for supplying the major portion of heat in the case of thermally unstable liquids or viscous liquids, whose circulation is poor. They have considerable control lag and, unless the flask is totally enclosed by the shell, the boiling rate decreases with the liquid level.

TOTAL VAPORIZER

A liquid may be vaporized in backward-feed distillations (*4*) in a packed or indented tube surrounded by a heated bath of liquid. This will vaporize any liquid that does not contain an appreciable amount of nonvolatile material to plug the vaporizer. It has better heating characteristics than any of the other methods discussed. Since it is a film type of vaporizer, there is less superheating in the liquid phase and no bumping or frothing. Rate of vaporization is controlled by the flow of liquid from the receiver and is independent of the power to the vaporizer. This control is more troublesome than that by means of heat input. However, by using a calibrated orifice in the flow line a more constant distillation rate can be obtained, independent of fluctuations in the power line and heat losses around the column base. While the liquid flow to the vaporizer may be varied instantaneously, the distillation rate lags because of condensation in the vapor lines to the column. The vaporizer has excellent safety characteristics, since there is very little liquid in it and the flow to it from the receiver may be stopped instantly. The possibility that heating liquid in the bath around the vaporizer may decompose if the distillation stops while the column is unattended, may be avoided by using a stable, high-boiling liquid for the heating bath. This liquid is contained in a jacket sealed to the vaporizer and vented at the top through an external condenser. A simpler but less effective method when a paraffin oil bath is used is to put a copper cooling coil just above the surface of the oil. This will remove most of the heat when the oil expands and covers the coil.

BOTTOM RECEIVERS FOR TOTAL REFLUX OPERATIONS

The simplest receiver (Figure 2, *A*) consists of a holdup device inserted between the fractionating section and the still flask. Because the receiver illustrated runs under an atmosphere of the column vapors, the connecting lines must usually be sealed to the column. The fractionating effect of the still flask (one plate) is lost but it does not contain any heavy residues which accumulate in the still flask proper. In case of a leak elsewhere in the system, only the liquid contained in the still flask is lost.

The same arrangement, except that the receiver is allowed to run under atmospheric pressure (Figure 1, *A*), allows more flexibility. The difference between the pressure at the column base and the atmosphere is compensated for by small barometric legs.

A third method (Figure 2, *C*) consists of maintaining the major portion of the still liquid in an outside receiver whose contents are circulated through the still flask by means of a pump. The arrangement illustrated allows, in the case of leakage, only the contents of the still and the small amount of overflow liquid to be lost.

The above methods emphasize safety by isolating the major portion of the charge and limiting liquid loss to a small fraction. They permit any amount of liquid to be distilled without special oversized flasks. The heat loss is less from these receivers than for the usual operation with all the charge kept at the boiling point in the still flask, and cooling of the liquid recycle decreases the decomposition of thermally unstable liquids.

BOTTOM RECEIVERS FOR FINITE REFLUX

Batch operations may be effected as illustrated in Figure 1, *E*, by circulating through the still flask the contents of an outside receiver not supplied with a limiting overflow. Figure 1 shows the circulation being effected by means of a heat pump which permits the liquid recycle to enter at its boiling point. The pump consists of a U-leg from the receiver, the outer side wrapped with a heating element. The static liquid height, h_1, is usually made at least 150% of the height, h_2, to which the liquid is lifted. However, in small-bore tubing the former height may be severalfold smaller. An uninsulated 8-mm. tube with h_1 and h_2 both equal to 60 cm. (2 feet) will pump approximately 8 liters per hour of benzene with a power input of 600 watts.

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RECEIVED April 9, 1947.

Determination of Aspartic Acid

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A method is described for the quantitative determination of aspartic acid in complex biochemical mixtures such as protein hydrolyzates. The hydrolyzate is concentrated to a small volume, treated with alkaline dimethyl sulfate for 1.5 hours, and acidified with concentrated sulfuric acid. Aspartic acid is converted to a mixture containing approximately 20% maleic acid and 80% fumaric acid. The sum of the mixture of unsaturated dicarboxylic acids is quantitatively determined by the polarograph.

IN a search for a satisfactory method for the determination of aspartic acid in amino acid mixtures, it became evident that no simple, reliable chemical procedure existed. Particularly striking was the fact that values differing in some instances by as much as several hundred per cent were obtained when a protein hydrolyzate was analyzed for aspartic acid by several methods available for this purpose (*3*).

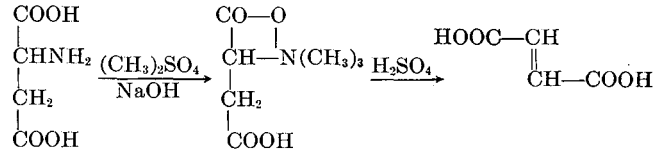
Until recently, the only available methods for the quantitative isolation of aspartic acid from amino acid mixtures were modifications of the Ritthausen-Foreman method (*10*)—precipitation of the calcium salt with alcohol. The work of Bailey *et al.* (*2*), however, proved that this technique is unsatisfactory for quantitative measurements. The highly insoluble copper salt also has been frequently used in the last stages of the isolation of aspartic acid. The application of this procedure to quantitative work is limited by the fact that in the presence of other amino acids there is the probability of mixed complex formation, mixed crystallization, and mutual solubility. This latter effect has been clearly illustrated by Bailey, who noted an appreciable difference in the solubility of copper aspartate in the presence of copper serine. A colorimetric method for estimating aspartic acid which has been reported by Arhimo (*1, 19*) consists of precipitating the acid from a protein hydrolyzate by the Ritthausen-Foreman method, oxidation and bromination with potassium permanganate and bromine to dibromoxaldehyde, and subsequent colorimetric determination of the dibromoxaldehyde with dinitrophenylhydrazine.

As a result of the application of recently developed techniques to the problem, reliable, though tedious, determinations can now be made.

Stokes and Gunness (*18*) have described a microbiological method for the determination of aspartic acid using *Lactobacillus delbruckii* as a test organism and titrating the resulting lactic acid with standard alkali after incubating for 72 hours. The isotope dilution method was used by Shemin (*17*) for ascertaining the aspartic acid content of bovine and human serum albumin. Gordon, Martin, and Synge (*11*) used the partition chromatographic procedure for isolating aspartic acid from tyrocidin. Several workers have utilized chromatographic methods for the separation of aspartic acid from other amino acids, including glutamic acid. The amino acid mixture was adsorbed on a column of treated aluminum oxide (*8, 20, 23*) or barium sulfate (*21*) and the aspartic acid was selectively eluted by the proper solvent. The resulting aspartic acid was then determined by the Kjeldahl method or colorimetrically by the use of the ninhydrin reagent. Adsorption on anion-exchange synthetic resins of the polyamine-formaldehyde type (amberlites) has been used by Cannan (*5*) for the isolation and separation of the monoamino dicarboxylic acids. Kibrick (*14*) separated the dicarboxylic acids by Cannan's method and then estimated the aspartic acid in the mixture by the ninhydrin–carbon dioxide determination.

It is obvious from the foregoing survey that a simple and accurate method for determining aspartic acid in complex mixtures, without a preliminary separation from other constituents, would be extremely valuable.

In this regard, a method based on the procedure of Engeland (*9*) and Dakin (*7*) was developed. The reactions involve the conversion of aspartic acid to fumaric and maleic acids by treatment with dimethyl sulfate and subsequent determination of the resulting unsaturated dicarboxylic acids by means of the polarograph. The following reactions have been proposed by Dakin, who estimated the resulting fumaric acid by extracting it from the reaction solution with ether, distilling off the ether, and weighing the residue:



Because ether extraction of complex mixtures is far from specific, a significant error can result if other ether-soluble materials are present in the solution, and are calculated as fumaric acid. In addition, insufficient evidence was advanced regarding the quantitative conversion of aspartic acid to fumaric acid exclusively; no quantitative estimation of the probable presence of small amounts of maleic acid was reported. Braunshtein *et al.* (*4*) used the dimethyl sulfate reaction to convert aspartic acid to the unsaturated dicarboxylic acids, which were then reduced quantitatively to succinic acid. The resulting succinic acid was determined gasometrically by the specific enzyme succinic acid dehydrogenase.

The present work proposes to standardize the conditions involving the reaction with dimethyl sulfate, to investigate the possible production of a mixture of the cis-trans isomeric dicarboxylic acids, and to determine polarographically the products formed by the oxidative-deaminization reaction with alkaline dimethyl sulfate. In this way, only those substances present, which are polarographically reducible under the conditions used, would interfere with the determination.

The polarographic determination of fumaric and maleic acids in aqueous solutions has been reported by several authors (*16, 22*) and, when conducted with the usual precautions of polarographic techniques, offers no particular difficulties.

APPARATUS AND MATERIALS

Polarograph. A Heyrovský polarograph, model XII, manufactured by E. A. Sargent and Company, was used for the polarographic measurements. The drop time for the capillary in the base solution used was 3.2 seconds at zero applied voltage. The temperature of the cell was maintained constant by means of a large water bath. Any other variation in the environmental conditions was corrected by the use of a standard sample of aspartic acid which was treated in the same manner as the unknown.

Reagents. Standard solutions of Eastman Kodak's *dl*-aspartic acid, City Chemical Corporation's fumaric acid, and a hydrolyzed sample of Merck's maleic anhydride was used. The concentration of these stock standard solutions was 1.50×10^{-2} *M*, 1.72×10^{-2} *M*, and 1.72×10^{-2} *M*, respectively. A base solution of pH 8.2 was prepared by adjusting the pH of a 1.0 *M* ammonium chloride solution to the desired value by the addition of concentrated ammonium hydroxide. The following additional chemical reagents were also used: dimethyl sulfate, Merck's practical grade; sodium hydroxide, 40% aqueous solution; concentrated sulfuric acid, specific gravity 1.84; 6 *N* hydrochlo-

Table I. Determination of Aspartic Acid in Aqueous Solutions

Aspartic Acid Taken	Diffusion Current	Found: Maleic + fumaric acid	Found: Aspartic acid	Recovery
Mg.	*Microamperes*	*Mg.*	*Mg.*	%
2.00	1.13	1.76	2.01	100.5
4.00	2.24	3.50	4.00	100.0
6.00	3.34	5.17	5.94	99.0
8.00	4.21	6.70	7.70	96.4
10.0	5.28	8.36	9.60	96.0
		Av.	98.4	
		Av. deviation	1.8	
		Av. deviation of mean	0.8	

ric acid solution, and phenolphthalein solution, 0.1% in 95% ethanol.

ANALYTICAL PROCEDURE

Dimethyl Sulfate Reaction. A sample containing 2 to 10 mg. of aspartic acid dissolved in a minimum volume of water (2 to 5 ml.) in a 50-ml. Erlenmeyer flask is treated with approximately 2 ml. of the dimethyl sulfate reagent. Several drops of the phenolphthalein indicator are added and the solution is rendered alkaline by the dropwise addition of the sodium hydroxide solution; the mixture is kept alkaline in this manner by the repeated addition of drops of sodium hydroxide for about 1.5 hours. The solution should be shaken frequently during this time and the temperature kept below 25° C. Temperatures which are significantly higher than this value cause a rapid rate of hydrolysis of the dimethyl sulfate to sulfuric acid and methyl alcohol. This tends to neutralize the slight excess of alkali, and necessitates the addition of more sodium hydroxide. Then there is a further increase in the temperature, which in turn accelerates the hydrolysis of dimethyl sulfate. The simple precaution of keeping the flasks immersed in a water bath at the prescribed temperature reduces this undesirable effect to a minimum. During the course of the reaction there is a gradual decrease in the intensity of the indicator color due to a simultaneous destruction of the phenolphthalein. It is therefore necessary to add small increments of the indicator periodically in order to observe properly the alkalinity of the solution. After the reaction has been permitted to proceed for the specified length of time, 1 ml. of concentrated sulfuric acid is added to the mixture and shaken, and the contents are allowed to stand for 0.5 hour.

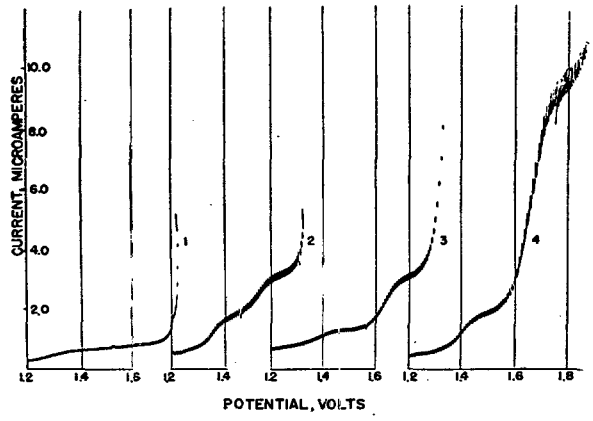


Figure 1. Polarograms Showing Formation of Maleic and Fumaric Acid from Aspartic Acid

Temperature, 25° C. pH, 8.2. Sensitivity, 1/20
Supporting electrolyte, 0.7 *N* NH_4Cl in 0.04 *N* NH_4OH
Curves (1) base solution, (2) 2 mg. of maleic and 2 mg. of fumaric acids, (3) solution resulting from dimethyl sulfate treatment of 6 mg. of aspartic acid, (4) 18 mg. of treated aspartic acid

Polarographic Analysis. The material resulting from the dimethyl sulfate treatment is neutralized to phenolphthalein with a concentrated solution of sodium hydroxide and transferred to a 100-ml. volumetric flask with distilled water. Sufficient hydrochloric acid is added to bring the pH of the solution to approximately 1.5; this requires about 2 ml. of 6 *N* hydrochloric acid. The solution is diluted to the 100-ml. volume and an oxygen-free aliquot is polarographed in the applied voltage range of −0.5 to −1.1 volts (dropping mercury electrode *vs.* mercury pool).

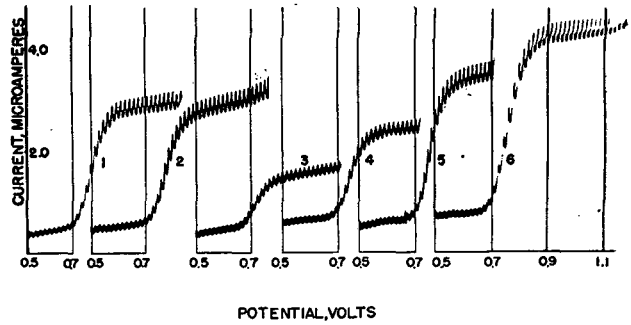


Figure 2. Current-Potential Curves for Treated Aspartic Acid

Temperature, 25° C. Sensitivity, 1/20. pH, 1.5
Supporting electrolyte, 0.05 *N* HCl
Curves (1) 4 mg. of fumaric acid, (2) 2 mg. of fumaric and 2 mg. of maleic acid, (3) 2 mg. of aspartic acid, (4) 4 mg. of aspartic acid, (5) 6 mg. of aspartic acid, (6) 8 mg. of aspartic acid

For simple solutions of aspartic acid it was found satisfactory to calculate the unsaturated dicarboxylic acid wave height by subtracting the galvanometer reading at −0.55 volt from the reading at −1.0 volt. However, for unknown solutions of complex mixtures it is advisable to plot the entire polarogram, as the plateaus at the potentials specified for the spot readings may be slightly displaced from the values given. The true wave heights of the dicarboxylic acids are obtained by subtracting the galvanometer deflection equivalent to the residual current of the base solution from the observed diffusion current of the test solution. This is compared to the true wave height of either a standard solution of fumaric or maleic acid, or preferably, to pure aspartic acid which has been treated in the same manner as the unknown sample.

CALCULATIONS

Using Aspartic Acid as the Standard. Per cent by weight of aspartic acid $= \dfrac{W_s \times H_u \times 100}{H_s \times S}$

where W_s = weight of aspartic acid in the standard, mg.
H_s = true wave height of the total unsaturated dicarboxylic acids produced from the standard
H_u = true wave height of the total unsaturated dicarboxylic acids produced from the sample
S = weight of sample polarographed, mg.

If either maleic or fumaric acid is used as the standard, the value obtained by the above calculation is multiplied by the factor 1.147 in order to express the standard readings in terms of aspartic acid.

POLAROGRAPHIC EXAMINATION FOR MALEIC AND FUMARIC ACIDS

For the purpose of identifying maleic and fumaric acids in the reaction mixture, a slight modification of the method recently described by Warshowsky and Elving (*22*) was followed.

The solution resulting from the dimethyl sulfate treatment is carefully neutralized with strong sodium hydroxide to phenolphthalein and transferred to a 100-ml. volumetric flask with 30 ml. of distilled water. Fifty milliliters of the ammonium chloride–ammonium hydroxide base solution are added, and the contents are diluted to volume with water. A portion of this mixture is transferred to the polarographic cell, and the dissolved oxygen is removed by rapidly passing a stream of nitrogen through the solution for several minutes. The sample is then examined polarographically for maleic and fumaric acids in the applied voltage range of −1.1 to −1.8 volts.

As shown by the polarograms in Figure 1, a mixture containing approximately 20% maleic and 80% fumaric acid is definitely produced by the treatment outlined in the preceding paragraphs. Therefore, in attempting to devise an analytical method

for the determination of aspartic acid by these reactions, it is important that this fact be considered. Inasmuch as it has been shown that maleic and fumaric acids display a similar polarographic behavior in acid medium (*13*), it is not necessary to determine separately the amount of each of these products in the final reaction mixture. Instead, if the unsaturated acids are polarographed in a strongly acid medium, both compounds are reduced at the same potential and yield the same diffusion current. This appears as a single wave and the diffusion current measured for the sum of the two substances will be equal to the current produced for an equivalent amount of either maleic or fumaric acid (Figure 2). Therefore, for the purpose of determing aspartic acid, the polarographic procedure is performed in an acid solution of an approximate pH of 1.5. The concentration of the sum of the unsaturated acids can be calculated by using either a maleic or fumaric acid standard; in the present work, fumaric acid was used as the standard.

The determination of the aspartic acid in simple aqueous solutions of this amino acid by the procedure outlined above showed that the conversions to the unsaturated dicarboxylic acids are quantitatively complete. The experimental evidence of this observation was established by comparing the wave height of the solutions of treated aspartic acid to the wave height obtained with a solution of an equivalent concentration of fumaric acid which had been subjected to the same conditions. The direct linear relationship between the diffusion current measured and the resulting dicarboxylic acid concentration of the final solution, in the range of 1.7×10^{-4} to 8.6×10^{-4} M, was also experimentally established and is shown in Figure 2 and Table I.

The same numerical values were obtained when the aspartic acid was determined in the presence of a synthetic mixture of pure amino acids. For this purpose, a mixture containing approximately 10 to 20 mg. of each of the following amino acids was used in addition to the aspartic acid: Eastman Kodak's *dl*-alanine, glycine, and *l*-tryosine; Merck's *dl*-leucine, *dl*-isoleucine, *l*-lysine hydrochloride, *dl*-phenylalanine, *dl*-glutamic acid monohydrate, *dl*-histidine, *l*-tryptophan, and *dl*-valine. In the absence of aspartic acid, the same amino acid mixture gave a curve identical to that of the base solution, confirming the fact that these amino acids did not interfere with the accuracy of the determination. The results obtained in these studies with synthetic mixtures of pure amino acids (Table II) show that an accuracy and precision of 2 to 3% relative can be achieved.

Table II. Determination of Aspartic Acid in Amino Acid Mixtures

Amount Taken	Found	Average Recovery
Mg.	*Mg.*	%
2.00	1.86, 1.93	95.0
4.00	4.17, 4.00	102.0
6.00	5.82, 5.82	96.8
8.00	7.60, 7.66	95.3
10.0	9.89, 9.41	96.5
	Av.	97.1
	Av. deviation	2.0
	Av. deviation of mean	0.9

EFFECT OF EXPERIMENTAL CONDITIONS

Preliminary investigations on the effect of time of standing in the presence of alkaline dimethyl sulfate and concentration of sulfuric acid were also made. These studies indicate that at least 1.5 hours are required to complete the reaction with dimethyl sulfate. When the length of time was only 15 minutes, nonreproducible and very low values—i.e., 30 to 40% conversion to fumaric-maleic acid—was found. If a reaction time of 1 hour was selected, repeated values of only 85 to 92% of the theoretical conversion were obtained. The acid concentration was also revealed to be an important factor. If, as suggested by Dakin, no acid was added to augment the acidity resulting from the hydrolysis of the dimethyl sulfate, a maximum conversion of only 40% was detected. The conversion, however, was consistently complete when 1 ml. of concentrated sulfuric acid in a total volume of approximately 10 ml. of the reaction mixture was used.

Results obtained with larger volumes of solution were not only unsatisfactory from the point of view of manipulation, but they gave significantly lower recoveries. For example, when the reaction was carried out in a volume of 30 ml., only approximately 90% of the aspartic acid was found. If, however, the solution was first evaporated to about 2 to 4 ml., the theoretical quantity was detected. In view of this observation it is recommended that for the determination of aspartic acid in proteins and other mixtures, the volume of the aliquot sample first be reduced by evaporation of the water.

Aspartic acid was determined in several proteins after hydrolyzing the sample for 24 hours with 6 N hydrochloric acid and concentrating the hydrolyzate to a final volume of about 4 ml. before reaction with dimethyl sulfate. No apparent difficulties were encountered with the determination of the aspartic acid content of crystalline egg albumin, bovine serum albumin, Type B Botulinum toxin (all electrophoretically pure), and Difco's Bacto-isoelectric casein. The values obtained in this study, shown in Table III, are compared with the results reported in

Table III. Determination of Aspartic Acid in Acid-Hydrolyzed Proteins

Protein	Weight of Sample Hydrolyzed	Aspartic Acid Found		Literature Values		
					Method	Reference
	Mg.	*Mg.*	%	%		
Crystalline egg albumin	48.8 48.4	4.16 4.00	8.5 8.3	8.2 9.3	Isolation Microbiological	(*6*) (*12*)
Bovine serum albumin	26.7 32.0	2.74 2.94	10.3 9.3	10.2	Isotope dilution	(*17*)
Difco Bacto-isoelectric casein	107 214	6.55 13.1	6.3 6.3	6.3, 7.2 6.1	Microbiological Isolation	(*12, 18*) (*2*)
Type B, botulinum toxin, sample 9 B 10%	17.9 17.9	2.33 2.35	13.0 13.1	13.4	Microbiological	(*15*)
Type B, botulinum toxin, sample 10 B 4	13.3 13.3	2.04 2.06	15.3 15.5	15.3	Microbiological	(*15*)

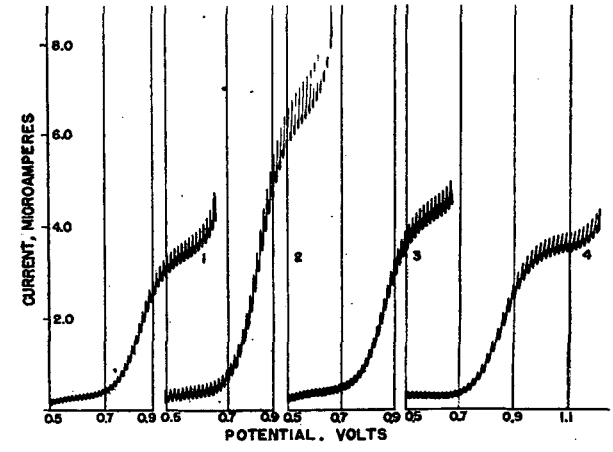


Figure 3. Determination of Aspartic Acid in Acid-Hydrolyzed Difco Bacto-isoelectric Casein

Temperature, 25° C. Sensitivity, 1/20. pH, 1.5
Curves (1) 107 mg. of casein, (2) 214 mg. of casein, (3) 110 mg. of casein plus 2 mg. of aspartic acid, (4) 62 mg. of casein plus 4 mg. of aspartic acid

Table IV. Recovery of Aspartic Acid Added to Hydrolyzed Proteins

Protein	Weight of Protein Sample	Aspartic Acid Present			Total Aspartic Acid Found	Recovery
		In sample	Added	Total		
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	%
Crystalline egg albumin	48.2	4.04	2.00	6.04	6.34	104.9
	46.1	3.87	4.00	7.87	7.82	99.4
Bovine serum albumin	19.9	1.93	2.00	3.93	3.80	96.4
	27.9	2.71	4.00	6.71	6.53	97.3
Bacto - isoelectric casein	110	6.93	2.00	8.93	8.80	98.8
	62	3.90	4.00	7.90	8.00	101.2
					Av.	99.5

the literature for the same proteins using other previously described methods.

Recovery experiments, in which known amounts of aspartic acid were added to a sample of three of the proteins mentioned in the preceding paragraph and hydrolyzed for 24 hours with 6 *N* hydrochloric acid, were also performed. In these experiments, an average recovery of 99.5% of the total aspartic acid content was obtained (Table IV). Typical polarograms obtained in the studies with casein and casein plus added aspartic acid are shown in Figure 3.

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RECEIVED September 10, 1947.

Determination of Water in Alkylation Sulfuric Acid

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The Karl Fischer method for determination of water has been adapted to determination of water in alkylation sulfuric acid. Pretreatment of sample with anhydrous ammonium chloride prevented decomposition of Fischer reagent and an electrometric technique permitted end-point detection. Accuracy on chemically pure acid solutions was sufficiently good to show average deviation from known water content of less than 0.1% absolute. Reproducibility on alkylation acid was good, as was the recovery of known increments of water.

ALTHOUGH many tests have been proposed for determining water by physical properties, only three chemical methods of test of relative importance have been proposed in recent years: the acetyl chloride–pyridine (*5*), the acetic anhydride–pyridine (*6*), and the Fischer reagent (*2*) methods. The first two determine water by acidimetric titrations and require corrections for acidic or basic constituents in the sample. The Fischer reagent method is highly specific for water and operates independently of the acidity of the sample.

The method of detecting the end point is described by Almy, Griffin, and Wilcox (*1*).

APPARATUS

A length of Bakelite rod turned to the size of a No. 6 rubber stopper is bored with four holes to fit two electrodes, a stirrer, and a buret tip (Figure 1).

An end-point detection apparatus consists of a laboratory model Beckman pH meter or other suitable vacuum tube millivoltmeter, two electrodes, and shielded copper leads from the electrodes to the input terminals of the pH meter. The two electrodes are clean pieces of platinum and tungsten wire, No. 10 B. & S. gage, each about 19 cm. in length. Daily polishing with steel wool cleans the electrodes sufficiently to keep them in working order.

A variable-speed stirring motor with glass stirrer.

Two 50-ml. burets, one automatic, the other offset to permit the tip to pass through the Bakelite stopper.

A number of 150-ml. extraction flasks are used for titrating flasks.

A 1-liter bottle reservoir for the Fischer reagent is fitted with a standard-taper pump from a gas-washing bottle. The aspirator and reservoir are separated by a tube of Drierite. The top openings of the buret are protected by similar tubes.

A weighing pipet of the Smith or Lunge type. Other end-point

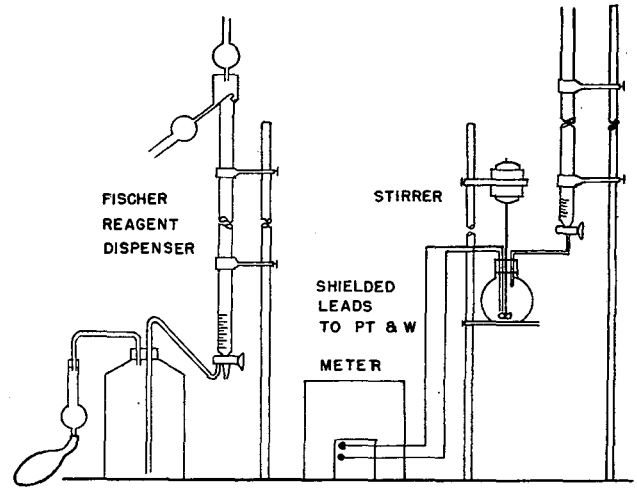

Figure 1. Titration Equipment

detection devices are described by Wernimont and Hopkinson (7) and McKinney and Hall (3). Complete titration assemblies for Fischer reagent are commercially available.

REAGENTS

The chemicals used to prepare the Fischer reagent must be of suitable quality in regard to their water content. Any water contained in the ingredients will consume the reagent itself, with a correspondingly low water equivalence for the final solution.

The Fischer reagent used contained a ratio of 1 mole of iodine to 2 moles of sulfur dioxide to 8 moles of pyridine and was dissolved in about an equal weight of methanol. This formula gives an equivalence of about 5 mg. of water per ml. of Fischer reagent and is prepared as follows:

A stock solution of sulfur dioxide–pyridine is prepared by weighing about a gallon of pyridine into a flask, adding 203 grams of liquid sulfur dioxide per kilogram of pyridine, and storing in a glass-stoppered bottle.

To 1359 grams of the stock sulfur dioxide–pyridine solution are added 1786 grams of methanol and 453.6 grams (1 pound) of iodine slowly and with frequent coolings, and the solution is swirled to prevent caking in the bottom. If allowed to heat up, the reaction may become explosively violent. It is allowed to stand for at least 24 hours before standardization.

The standard water in methanol solution is prepared by adding 5 ml. of water to 1 liter of anhydrous alcohol. This gives about 5 mg. per ml. and approximately matches the Fischer reagent.

The water equivalents of the solutions are determined as described by Almy, Griffin, and Wilcox (1).

In preparation, storage, and subsequent use, the two solutions must always be protected from atmospheric moisture, especially in locations with highly humid atmospheres.

PROCEDURE

The acid to be analyzed is weighed quickly to the nearest milligram into a clean, dry titrating flask and stoppered. Enough acid is used to give 0.04 to 0.10 gram of water; over 2 grams of acid are not used because there would not be an excess of ammonium chloride, which is added as the next step. Five grams of ammonium chloride, which has been dried overnight at 105° C., are added to the acid in the flask. This should all be done as quickly as possible and with the care necessary to prevent picking up moisture from the air. The ammonium chloride and acid are mixed with a dry flattened stirring rod, and any soft lumps which form are crushed. Then 20 ml. of anhydrous methanol are pipetted into the flask and used to rinse the stirring rod. The ammonium chloride–sulfate mix will not go into solution but will remain as a finely divided solid which does not interfere with the titration, if it is not allowed to cake at the bottom of the flask. A 2- or 3-ml. excess of Fischer reagent is added, the flask is placed upon the titration assembly, and the excess Fischer reagent titrated with the standard water in methanol solution. A blank determination is made, using the 5 grams of ammonium chloride and 20 ml. of methyl alcohol. The water content is calculated as follows:

$$\% \text{ water} = \frac{[A - (B + C)] \times F \times 100}{W}$$

where A = ml. of Fischer reagent added
B = ml. of water in methanol solution used in back-titration $\times$ R
R = ratio, ml. of Fischer reagent per ml. of water in methanol solution
C = ml. of Fischer reagent for blank
F = grams of water equivalent to 1 ml. of Fischer reagent
W = weight of sample in grams

Typical data collected on the duplicate determination of water in two samples are given in Table I.

Table I. Typical Data

Weight of Sample, Grams	Fischer Reagent, Ml.	Water in Methanol Solution, Ml.	Fischer Reagent for Blank, Ml.	Water Equivalent of Fischer Reagent, G. H_2O/Ml.	Ratio of Fischer Reagent to Methanol Solution	H_2O, %
2.055	18.70	3.60	5.11	0.00428	1.212	1.92
2.029	19.00	3.96	5.11	0.00428	1.212	1.92
2.057	21.62	3.35	5.20	0.00415	1.250	2.47
2.026	21.86	3.73	5.20	0.00415	1.250	2.46

Table II. Analysis of White Acids by Fischer Reagent Method

H_2SO_4 %	Check H_2SO_4 %	Average H_2SO_4 %	H_2O %	Check H_2O %	Average H_2O %	Total of Averages %	Deviation from 100.00 %
98.16	98.14	98.15	1.87	1.79	1.83	99.98	−0.02
96.24	96.26	96.25	3.75	3.79	3.77	100.02	+0.02
93.71	93.72	93.72	6.34	6.42	6.38	100.09	+0.09
93.32	93.31	93.32	6.81	6.73	6.77	100.09	+0.09
93.22	93.26	93.24	6.83	6.85	6.84	100.08	+0.08
79.47	79.47	79.47	20.54	20.47	20.51	99.98	−0.02
36.43	36.40	36.42	63.47	63.51	63.49	99.91	−0.09
					Average deviation		±0.07

EXPLORATORY WORK ON WHITE ACIDS

White acids (C.P. sulfuric acid and water) were used in the initial work, because the water content could be determined by subtracting the acidity as sulfuric acid from 100. The results of the acidity as sulfuric acid plus the water by this method gave 100.0 ± 0.1%. Total impurities on the white acids were less than 0.01%. The reproducibility of the test is shown in Table II.

ANALYSES OF ALKYLATION ACID SAMPLES

Analyses of some alkylation acids are shown in Table III. The percentage of water was determined by this method. The titratable acidity as sulfuric acid was determined by immediate titration at room temperature of a weighed diluted sample with 0.25 N carbonate-free sodium hydroxide.

The butyl hydrogen sulfate was determined by the difference between the titratable acidity and the total acidity after a diluted portion of the sample had been refluxed 24 hours. The increase in acidity was calculated as one half of the butyl hydrogen sulfate present. This, of course, assumed no dibutyl sulfate to be present as was indicated by Roby (4).

The actual percentage of sulfuric acid was calculated by correcting the titratable acidity for the butyl hydrogen sulfate present.

Table III. Analyses of Alkylation Acid Samples

Sample No.	H_2O	Titratable Acid as H_2SO_4	Actual H_2SO_4	$BuHSO_4$	Oxidizables as Carbon	Hydrolysis No. *Mg. NaOH/g.*	H_2O Added *Gram*	H_2O Recovered *Gram*	Total Percentage
	%	%	%	%	%				
1	2.73	88.39			6.37				
1	2.78	88.39	87.00	4.38	6.33	11.4	0.0652	0.0641	100.49
2	3.03	88.90							
2	3.00	88.91	87.91	3.14	5.06	9.1	0.0616	0.0612	99.13
3	3.11	88.90							
3	3.17	88.94	88.31	1.93	5.87	5.0	0.0624	0.0632	99.22
4	3.01	89.54			5.47				
4	3.06	89.55	88.44	3.46	5.48	8.7	0.0641	0.0640	100.42
5	3.15	88.17			6.65				
5	3.15	88.23	87.47	2.44	6.69	5.8	0.0619	0.0625	99.69
6	2.75				7.15				
6	2.81	88.20	87.47	2.32	7.27	6.0	0.0711	0.0706	99.78
7	2.95	87.61			7.21				
7	2.93	87.58	86.73	2.69	7.21	7.6	0.0616	0.0625	99.57
8	1.92	97.08			0.53				
8	1.92	97.03	96.78	0.99	0.54	2.8	0.0636	0.0643	100.16[a]
9	0.79	97.94			0.68				
9	0.82	98.01	97.84	0.43	0.68	1.1	0.0640	0.0628	99.74[a]
10	2.46	91.46			4.61				
10	2.47	91.45	91.00	1.45	4.59	4.1	0.0637	0.0627	99.52
11	1.85	93.65			3.16				
11	1.89	93.67	93.20	1.41	3.08	3.7	0.0622	0.0624	99.60

[a] Fresh acids.

The oxidizables as carbon were determined by total wet reduction of potassium dichromate by the sample.

The hydrolysis numbers are the milligrams of sodium hydroxide needed to neutralize the acid formed by hydrolysis of 1 gram of acid sample. These hydrolysis numbers could be calculated to percentage of alkyl sulfate.

Known increments of water were analyzed as follows:

The acid was analyzed for the original water content. Another sample of the acid was taken and a weighed amount of water was added after the ammonium chloride was mixed with the acid sample. The total water was determined and this value corrected for the amount of water originally present in the sample. The difference was taken as the water recovered.

The total percentages contain water, actual sulfuric acid, butyl hydrogen sulfate, and oxidizables as carbon. The total percentages do not include the ash (normally 0.3 to 0.5%) or take into account the fact that some carbon is totaled twice, in both the butyl hydrogen sulfate and the oxidizables as carbon.

DISCUSSION

No method of known accuracy and acceptable convenience was available for comparison with this method. The accuracy of the white acid analyses was indicated as within 0.1% absolute. The reproducibility was good on alkylation acids. The water recovered as compared to a known amount added was in close agreement.

The range covered was from 98.5% to about 30% in a white acid; the black acid from 98% to about 85% titratable acidity as sulfuric acid.

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RECEIVED August 1, 1947. Presented before Petroleum Group Sessions, Fifteenth Midwest Regional Meeting, AMERICAN CHEMICAL SOCIETY, Kansas City, Mo., June 1947.

Determination of the Gamma-Isomer Content of Benzene Hexachloride

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The gamma isomer of benzene hexachloride has recently become the object of considerable interest because of its outstanding effectiveness as an insecticide. Technical benzene hexachloride contains the gamma isomer, usually to the extent of about 10 to 12%, along with varying proportions of at least four other less active isomers and small amounts of other compounds. A cryoscopic method has now been developed for the determination of the gamma isomer content of mixtures of the isomers or of technical benzene hexachloride. This can be carried out with equipment available to any laboratory and requires a relatively short time. It involves measurement of the depression which a sample of the material to be analyzed produces in the freezing point of pure gamma benzene hexachloride.

BENZENE hexachloride has recently been the subject of extensive investigations as an insecticide and is now being produced in considerable quantities for that purpose. The production of a new material for large-scale use soon necessitates the development of an analytical procedure for its determination. Technical benzene hexachloride, or 1,2,3,4,5,6-hexachlorocyclohexane, is composed of at least five isomers, which differ in their toxicity to insects. The gamma isomer has a much greater insecticidal value than any of the others. Consequently, the determination of this isomer is of primary importance in analysis of the technical product. Several methods for determining the percentage of the isomers in benzene hexachloride have been described, including weighing them after separation by fractional extraction and selective precipitation (*7*), weighing after separation by partition chromatography (*6*), bioassay (*4*), and infrared spectroscopy (*1*).

This paper describes a cryoscopic method of determining the gamma isomer in technical benzene hexachloride. If a mixture of isomers is dissolved in a much larger quantity of one of the isomers, a depression in freezing point of the solvent isomer will be caused by the other isomers. This method has been applied by Haller *et al.* (*3*) to the determination of the *o,p'*- and *p,p'*-DDT content of a DDT oil. It is also applicable to the determination of the isomers of benzene hexachloride, but is most useful when the isomers are present in substantial amounts. For the DDT analyses Haller *et al.* used a solvent unrelated to DDT. For the analysis for γ-benzene hexachloride this method has been modified in order to eliminate the unrelated solvent.

If a known weight of a mixture of the benzene hexachloride isomers is dissolved in a known weight of pure gamma isomer, a freezing-point depression results, which is dependent upon the content of the other isomers. If a depression for the alpha isomer in the pure gamma isomer is obtained, the amount of gamma isomer present in the mixture may be calculated from the difference between the apparent numbers of moles in 1 gram of the solute in the two cases.

Suppose that the adding of 1 gram of the pure alpha compound to 10 grams of the pure gamma compound depresses the freezing point of the gamma compound by 6° C. The other isomers would give the same depression when present in the same amounts, with the exception of the gamma isomer, which would give no depression. If 1 gram of an unknown mixture, known to be made up of the isomers, is added to 10 grams of the gamma isomer, any effect on the freezing point of the solvent will be due only to the alpha, beta, delta, and epsilon isomers in the mixture. Suppose the depression to be 4°; approximately one third of the mixture would then consist of the gamma compound.

PROCEDURE FOR A CRYOSCOPIC METHOD

Apparatus. An oil bath that can be maintained at 120° to 130° C., consisting of a 1000-ml. beaker in which the oil should

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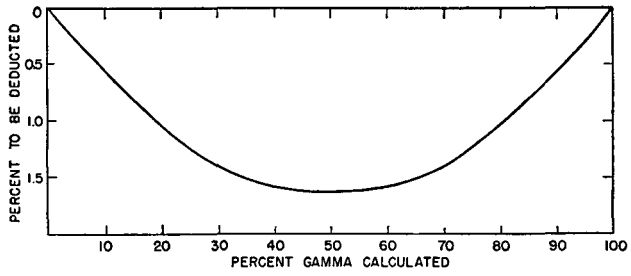

Figure 1. Correction Curve for Cryoscopic Analysis of Benzene Hexachloride

Table I. Determination of Gamma Isomer in Known Mixtures of Benzene Hexachloride Isomers

Sample No.[a]	Gamma Present, %	Gamma Found, %
1	6.3	6.2
2[b]	10.0	10.3
3	10.1	10.1
4	15.1	15.6
5	19.2	19.3
6	20.1	19.4
7	25.0	25.9
8	30.0	29.7
9	50.0	49.5
10	70.1	70.8
11[c]	70.0	70.3
12[d]	80.0	80.3
13	85.0	85.8
14	90.0	89.8

[a] Unless otherwise indicated, all samples contain alpha isomer.
[b] Contains 70% alpha, 10% beta, 10% delta.
[c] Contains 20% alpha, 10% delta.
[d] Contains 20% delta.

have a depth of about 110 mm. A freezing tube, of heat-resistant glass, with external dimensions 20 × 150 mm. A freezing-tube jacket, of heat-resistant glass, with external dimensions 38 × 150 mm. A thermometer, mercury in glass, graduated to 0.1°, with a range of +70° to +120° C. The thermometer is inserted through a rubber stopper to hold it in position in the freezing tube. A spiral stirrer made from a 3-mm. glass rod or a 2-mm. metal rod such as Monel metal or Nichrome. The stopper for holding the thermometer has a longitudinal groove on one side to allow for free operation of the stirrer.

Reagents. Gamma isomer of benzene hexachloride. The isomer should be recrystallized until the melting point obtained by the conventional capillary-tube method is 112.4–113° C. and the freezing point obtained by a cooling curve is 112.5° C. or higher.

Alpha isomer of benzene hexachloride known to contain no gamma isomer.

Determination. A 0.5000-gram sample is introduced into the bottom of the freezing tube, and 10.000 grams of pure γ-benzene hexachloride are then placed on top of the sample. The freezing tube is placed in the oil bath at 120° to 130° C. When the γ-benzene hexachloride and sample are melted, the stirrer and the thermometer are put in position, so that the thermometer reaches to about 0.5 cm. from the bottom of the tube, and the freezing tube is removed from the oil bath and placed in the freezing-tube jacket, which is also being kept warm in the oil bath. The rubber stopper around the freezing tube should be tight enough to create a warm dead-air space. The stirrer is worked slowly so as to intimately mix the sample throughout the γ-benzene hexachloride. When the temperature of the melt reaches 113° C., the freezing-point apparatus is raised until it is about 1 cm. above the surface of the oil bath. The stirrer is manually operated at the rate of about 100 complete strokes per minute. The temperature continues to rise for a few minutes before cooling begins. When the temperature reads 114° C., the temperature is recorded, with the aid of a good hand lens to permit interpolating to 0.01° C. (if possible), at 15-second intervals until the stirrer freezes. The freezing tube is removed from the jacket and the solution remelted in the oil bath. The melting-tube jacket is warmed and the operation repeated as described above, until consistent results are obtained.

Freezing-point data for the γ-benzene hexachloride alone are obtained in the same manner.

Freezing-point data for 0.5000 gram of the α-benzene hexachloride (known to contain no gamma isomer) in 10.000 grams of the gamma isomer are obtained in the same manner.

Calculation. Freezing-point curves are plotted for the γ-benzene hexachloride, the sample dissolved in the γ-benzene hexachloride, and the alpha isomer in the γ-benzene hexachloride. From the plateaus of the curves the freezing-point depressions caused by the alpha isomer and the sample are obtained.

The total number of moles per gram of alpha isomer, designated as a, is obtained from the equation

$$m = \frac{W\ \Delta t}{1000\ wK_f}$$

where m = moles of material other than solvent in 1 gram of solute
W = weight of solvent in grams
w = weight of sample in grams
Δt = freezing-point depression in ° C.
K_f = cryoscopic constant for solvent (gamma isomer) = 16.8

From the same equation the apparent number of moles per gram of unknown mixture are calculated and designated as b.

$(a - b) \times 290.8 \times 100$ = apparent % gamma isomer in sample

The gamma isomer present in the sample acts as an addition to the gamma isomer used as the solvent. Consequently, the results obtained on calculation are high, and a correction may be made by either of two methods. The apparent calculated amount of gamma isomer may be added to the weight of the solvent actually used and the results recalculated. A much simpler method, however, is to use a table or a curve. Figure 1 shows such a curve prepared by calculating the differences in the apparent and the corrected percentages, in which the ordinate gives the percentage to be deducted from the apparent calculated percentage of gamma isomer in the sample, which is the abscissa.

Theoretically a is the number of moles per gram of alpha isomer and may be obtained by simply dividing one by the molecular weight of benzene hexachloride—namely, 290.8. This theoretical value cannot, however, be used for a in the calculations, because a small amount of other isomers may be present in the gamma isomer that is used as the solvent in the determination. The cryoscopic constant may vary with different samples of the gamma isomer, and must also be determined. The simplified calculation may then be performed. The freezing-point and melting-point specifications for the gamma isomer are the minimum values for materials sufficiently pure to be used in the determination.

ANALYSIS OF KNOWN MIXTURES

In order to prove the method, known mixtures of the benzene hexachloride isomers were analyzed by the foregoing procedure. The isomers used in these determinations had the following melting points, which were used as criteria of their purity:

	° C.
Alpha	157.5–158
Beta	289–304
Gamma	112.4–113
Delta	138–139.5

The composition of the mixtures and results of the determinations are shown in Table I.

The agreement of determinations on two samples of technical benzene hexachloride analyzed by the cryoscopic method and the infrared spectrometer is indicated below:

Method	Sample 1, %	Sample 2, %
Infrared spectrometer	14.8	12.8
Cryoscopic	15.0	12.3

DISCUSSION OF METHOD

The cryoscopic constant for the gamma isomer, obtained as an average of 20 determinations with the alpha isomer, was 16.8. Consequently, this value is given for use in the above calculation. Each experimenter should, however, calculate this constant from the data at hand when the depression caused by the alpha isomer has been obtained. The constant calculated from the depressions caused by the delta and epsilon isomers is in agreement with that obtained by use of the alpha isomer. The alpha isomer is used in this determination because it is the main component of

technical benzene hexachloride and is easily obtained in a pure form. Cooling curves of paired mixtures of the various isomers indicate that no association or compound formation occurs on heating them together.

There are three points in the manipulation that require close attention. (1) Parallax must be avoided in reading the thermometer; an error of 0.05° C. may easily occur when a hand lens is used. (2) The sample must be dry, free of organic solvents, and completely dissolved and evenly distributed throughout the solvent (gamma isomer). The spiral stirrer (*5*) has been found more effective in accomplishing this than an ordinary ring stirrer. The freezing points will be successively higher until a homogeneous solution is obtained. (3) The supercooling must be reduced to a minimum if the correct freezing point is to be obtained.

The method presented here has the advantage over the usual cryoscopic method, in which an unrelated solvent is used and the total number of moles of the solute (sample) in grams is calculated, in that when the value for a has been obtained it may be used repeatedly without redetermination for each sample. This is also true for the determination of the freezing point of the gamma isomer that is used as the solvent. As long as gamma isomer of the same lot and the same thermometer are available, only one freezing-point determination is necessary for the analysis of each sample.

After several readings of the points on the cooling curve have been made and the freezing-point curves plotted, it becomes evident that when supercooling occurs, the high point, or plateau, may be observed directly without making a time-temperature curve.

For the rapid determination of an approximate gamma-isomer content, a temperature depression–gamma content curve may be prepared by the use of known mixtures of the benzene hexachloride isomers or by calculation. Percentages read from such a curve do not require any correction for the gamma content, as the values on the curve have been obtained from actual readings instead of being calculated.

The accuracy of the cryoscopic method depends upon the care with which the temperature readings are made and the precision of the instrument used to make the readings. A Beckmann thermometer permits readings with interpolation to 0.001° C. A 1 to 20 ratio of sample to solvent is equivalent to 0.03% in the calculations. The use of a Beckmann thermometer has one important disadvantage—a much larger quantity of solvent is necessary than when a thermometer with a smaller mercury content is used. Each 0.01° C., as in the case of the method presented, is equivalent to 0.3%. Other methods for determining the freezing points, such as the use of a platinum resistance thermometer (*8*), offer means for increased precision in reading the temperatures.

The gamma isomer is at present available only in limited quantities, but the production of this material in a reagent grade for this determination is practical. The development of a micro- or semimicromethod will obviate to some extent this disadvantage. The cryoscopic constant of 16.8 for the gamma isomer would indicate that such modifications are practical.

Other sources of error ordinarily present in freezing-point determinations (*2*, *5*) may be disregarded in this method for determining the gamma isomer of benzene hexachloride, since they fall outside of the apparent precision.

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RECEIVED November 22, 1947. Presented before the Division of Agricultural and Food Chemistry, Symposium on Insecticides in Food Production, at the 112th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y. Other papers in this symposium were published in the April issue of *Industrial and Engineering Chemistry*, pages 673 to 717.

Evaluating Fungal Amylolytic Materials for Saccharifying Fermentation Mashes

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BIOLOGICAL saccharifying agents such as barley malt and mold bran vary more or less in enzymic potency from one batch to another, depending upon raw materials and processing variables. Saccharifying agents are much more expensive than the starchy materials processed; hence, for their most efficient and economical use a means must be afforded for testing the enzymic activity, thus making it possible to adjust the proportions to be employed for maximum yield and minimum expense.

Physical and chemical tests have been devised for determining the amylase content of amylolytic agents—for example, polarimetric measurements (*7*), viscometric measurements (*2*), iodometric methods such as those of Wohlgemuth (*19*), Windisch and Kohlbach (*10*, *17*, *18*), and Sandstedt, Kneen, and Blish (*12*, *13*), and reducing sugar methods such as the Lintner determination (*1*, *4*, *11*). Methods for estimating α-amylase and β-amylase individually have also been devised by Sandstedt, Kneen, and Blish (*12*, *13*), and Kneen and Sandstedt (*9*), and have been tested and modified by others (*5*, *6*, *8*).

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Although these procedures afford relatively rapid measure of the amylase potency of saccharifying materials, no exact correlation can be found between any of these physical and chemical tests and actual alcohol yields obtained from the saccharified fermentation mashes. For instance, chemical tests give neither accurate information as to the suitability of a malt for fermentation mashes nor any indication of the required levels for most efficient use. Thorne, Emerson, Olson, and Peterson (*14*) made extensive tests with a large number of malts and came to the conclusion that "probably only fermentation tests can give an accurate evaluation." They found that malts which would normally be rejected as distillers' malts on the basis of Lintner values frequently gave better alcohol yields from wheat mashes than malts with very high Lintner values. Evidently other factors besides those measured by chemical tests for amylase enter into the effectiveness of malt for saccharifying fermentation mashes.

The situation as regards correlation of chemical tests for amylase activity with value for saccharifying fermentation mashes is even less favorable with mold bran than with malt. With the

A rapid fermentation method has been developed for evaluating samples of mold bran as to their relative saccharifying abilities and their optimum requirements for maximum alcohol yields from grain mashes. The test fermentations employ standardized procedures with a simple medium containing cornstarch and yeast extract and an incubation period of 24 hours. Relative activities are given by the numerical values of the Y-axis intercepts of the parallel straight lines obtained when the weights of mold bran used divided by the weights of ethanol produced are plotted against the weights of mold bran used. The minimum optimal requirements for maximum alcohol production for different mold brans are directly proportional to these intercepts.

successful introduction of mold bran for saccharifying grain mashes in the alcohol fermentation industry (*3*, *15*, *16*), methods for evaluating different fungal amylase preparations for this purpose have become important. The best chemical test method for use with mold bran is the α-amylase determination of Sandstedt, Kneen, and Blish (*13*). Mold brans with high α-amylase values are certain to be satisfactory for saccharifying fermentation mashes, but the α-amylase value for a particular mold bran does not permit a prediction of the optimum level of mold bran to be used. There has been continued effort in many laboratories to find a rapid laboratory method that would serve to evaluate mold bran, as well as other saccharifying agents, for fermentation purposes—that is, a method to indicate the amount of amylolytic agent required and the alcohol yield that could be expected. However, no method except actual fermentation tests on a series of mashes with several levels of the saccharification agent has given satisfactory results. Such fermentation tests are tedious, necessitate much labor, and require three or four days before the evaluation is completed.

DEVELOPMENT OF STANDARD EVALUATION TEST PROCEDURE

Materials employed in the fermentation industries vary somewhat in composition as received, especially as regards moisture content. The same is true of the materials used in the course of the work reported in this paper, such as corn meal, cornstarch, yeast extract, and the mold bran samples. It is possible to compensate for these variations by employing all materials on a dry weight basis. However, this complicates procedures by making necessary a moisture determination on each material before it is used. Moreover, the industrialist is interested in the results obtainable with the materials as received by him. Hence, during the course of this investigation all materials used were stored in air-tight containers and weighed out as needed in the form and condition received without making any corrections for their composition or altering them in any way. This particular approach was made in an attempt to devise a method which would be most useful for practical application and would eliminate or compensate for as many variables as possible while still retaining accuracy and speed.

A graphical analysis of ordinary grain fermentation test data with mold bran showed the ethanol yield, at a given time, to be a rectangular hyperbolic function of the weight of mold bran employed. This relation means that the weight of mold bran required to produce a unit yield of ethanol is a linear function of the weight of mold bran used. In the form of the usual straight-line equation, $y = mx + b$, the linear relation is:

$$\frac{\text{Weight of mold bran}}{\text{ethanol yield}} = m\,(\text{weight of mold bran}) + b$$

The value of the slope, m, is positive—that is, the weight of mold bran required to produce a unit weight of alcohol increases in a linear fashion with the weight of mold bran used. With corn mash fermentations different mold brans gave straight lines which had practically the same slopes but different intercepts, b—that is, the lines were nearly parallel. The more efficient the mold bran, the lower was the intercept value obtained. The discovery of this function gave promise of furnishing a method for an accurate evaluation of a given sample of mold bran as a saccharifying agent in mashes for alcoholic fermentation. Based upon this mathematical principle, it was found possible to develop a procedure which has all the advantages of the tedious fermentation method but requires the use of a minimum number of levels of the saccharifying agent, and gives an exact fermentation evaluation in a much shorter period of time.

Corn, as a test substrate, was abandoned because its lack of uniformity would render standardization very difficult. To secure a more uniform test substrate it was decided to try pure food-grade cornstarch with Difco yeast extract to supply nutrients; both materials are readily available and highly uniform in quality. It was first necessary to determine the best amounts of starch and yeast extract and the proper acid concentration to be used in cooking which would result in a mash not too thick to handle and ferment at the lower levels of mold bran, but still containing inappreciable amounts of fermentable sugars.

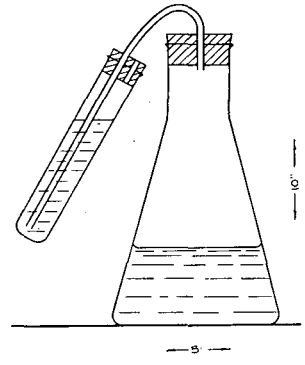

Figure 1. Fermentation Flask Assembly

Standard Evaluation Test. In each 1-liter wide-mouthed Erlenmeyer flask are placed 100 grams of food quality cornstarch, 5.0 grams of Difco yeast extract, and 250 ml. of 0.05 *N* hydrochloric acid at about 70° C. The contents are well mixed with a glass stirring rod and all flasks of the series are placed in a water bath which is heated by means of Fisher burners until the temperature of the mashes has risen from about 60° to 85° C., with occasional stirring. The flasks are then quickly transferred to the hot autoclave; to prevent irreversible retrogradation of the starch it is important not to let the mashes cool below 80° C. The mashes are cooked for 60 minutes at 20 pounds' steam pressure. The autoclave is then blown down to atmospheric pressure, after which the flasks are steamed continuously in the autoclave at atmospheric pressure, and removed one at a time for saccharification.

To the hot mash in the flask are quickly added the requisite amount of concentrated sodium carbonate solution to adjust the pH within the range of 5.0 to 5.3, and then a slurry of the desired quantity of mold bran in 250 ml. of cold water; the original temperature of the mold bran slurry is adjusted so that the temperature of the mixed mash is about 55° C. The contents of the flask are immediately mixed with a high-speed blade mixer for about 1 minute. The flask is then placed in a cold water bath to reduce the mash temperature to 30° C.

When all the mashes for a series have been saccharified and cooled, each is inoculated with 20 ml. of an active 24-hour culture of yeast in 20% malt extract medium. The yeast employed in the authors' work was No. 567 of the Northern Regional Research Laboratory collection. Each flask is fitted with a rubber stopper bearing a water trap containing about 30 ml. of water (see Figure 1) and the fermentations are incubated at 30° C. for 24 hours. At the end of this period the water from the trap is added to the flask and the final volume of each beer is measured. An aliquot of 250 ml. of each beer is distilled from a Kjeldahl flask after addition of about 0.5 gram of calcium carbonate and 200 ml. of wash water; in each case about 99 ml. of distillate are made up to exactly 100 ml. at 25° C., and the specific gravity is determined at 25°/25° with a Chain-o-matic Westphal balance.

Table I. Amylolytic Preparations Tested

Source	Designation	Lab. No.
L. A. Underkofler	Lab. preparation	FCPC-1
Farm Crops Processing Corp.	Semicommercial	FCPC-2
L. M. Christensen	Pilot plant	UN
Iowa State College	Lab. preparation	ISC-1
Iowa State College	Lab. preparation	ISC-2
Jacques Wolf Corp.	Commercial Protozyme	JWC
Schwarz Laboratories, Inc.	Commercial Polidase	SLI
Mold Bran Co., Inc.	Commercial Eaglezyme	MB-1
Mold Bran Co., Inc.	Mycelium concentrate	MB-2
Mold Bran Co., Inc.	Plant experimental	MB-3
Mold Bran Co., Inc.	Plant experimental	MB-4
Mold Bran Co., Inc.	Plant experimental	MB-5
Mold Bran Co., Inc.	Plant experimental	MB-6
Mold Bran Co., Inc.	Commercial Eaglezyme, feed quality	MB-7
Mold Bran Co., Inc.	Plant experimental, contaminated	MB-8
Mold Bran Co., Inc.	Commercial Eaglezyme, Blue Label	MB-9
Mold Bran Co., Inc.	Commercial Eaglezyme, Blue Label	MB-10
Mold Bran Co., Inc.	Commercial Eaglezyme, Red Label	MB-11
Mold Bran Co., Inc.	Commercial Eaglezyme, Green Label	MB-12

Ethanol contents of the distillates are then read from an appropriate table and the total weight of ethanol from each test fermentation is calculated.

A large number of runs were required in developing the cornstarch test medium and the standardized procedure given above. After various proportions of the materials employed had been tested, 100 grams of starch and 5.0 grams of Difco yeast extract per flask were found most satisfactory, these quantities ensuring an adequate excess of starch and of nutrients for the test period employed. In preparing the mashes it was found that the best procedure was to use 250 ml. of 0.05 *N* hydrochloric acid per flask and to gelatinize the starch before cooking for 60 minutes at 20 pounds' steam pressure. Mashes so prepared worked well in the subsequent mechanical operations and contained less than 3.0%, based on original weight of starch, of reducing substances calculated as dextrose. The fermentation period of 24 hours was chosen after a series of runs, which were analyzed following incubation periods of 12, 18, 24, 36, and 48 hours, had shown this period to be the minimum for obtaining good agreement in the results for duplicate fermentations, and a wide spread of ethanol yields with different levels of mold bran.

EVALUATION OF ENZYMIC ACTIVITY OF MOLD BRAN SAMPLES

After development of a satisfactory test method and procedure, it was next necessary to determine whether the method could be used to evaluate amylolytic preparations having different enzymic activities for the saccharification of fermentation mashes. The 19 different fungal amylase preparations tested are listed in Table I along with the type or name designation and the laboratory number.

As laboratory facilities did not permit parallel fermentations employing all of the 19 amylolytic agents in a single series, it was necessary to choose a reference agent for comparison. Preparation FCPC-2 was chosen because it gave not only excellent ethanol yields but also very consistent results the several times it was used as a comparison standard. Maximum deviations were always less than 2%.

Fermentation series, using the standard evaluation test procedures, were run with each of the 19 amylolytic agents, employing triplicate fermentations with the four levels of 1.0, 2.0, 3.0, and 4.0 grams of amylolytic agent in each series. The data obtained, given as the averages for the triplicate fermentations, are presented in Table II.

The data for all of the 19 amylolytic agents tested were plotted as $\frac{\text{weight of mold bran used}}{\text{weight of ethanol produced}}$ against weight of mold bran.

Table II. Standard Evaluation Test Data

Mold Bran, Lab. No.	Mold Bran, G./100 G. Starch	Ethanol, G./Flask	Weight of Mold Bran / Weight of Ethanol
FCPC-2	1.0	24.98	0.0402
	2.0	28.65	0.0698
	3.0	31.07	0.0965
	4.0	32.50	0.1230
FCPC-1	1.0	26.30	0.0380
	2.0	30.57	0.0653
	3.0	32.36	0.0927
	4.0	33.33	0.1200
UN	1.0	23.00	0.0435
	2.0	28.47	0.0703
	3.0	30.41	0.0988
	4.0	32.18	0.1244
ISC-1	1.0	18.15	0.0550
	2.0	24.55	0.0815
	3.0	27.28	0.1095
	4.0	29.89	0.1338
ISC-2	1.0	19.44	0.0513
	2.0	25.58	0.0780
	3.0	27.79	0.1096
	4.0	30.30	0.1320
JWC	1.0	25.48	0.0392
	2.0	28.45	0.0704
	3.0	30.83	0.0972
	4.0	31.72	0.1260
SLI	1.0	25.87	0.0387
	2.0	29.08	0.0688
	3.0	30.69	0.0977
	4.0	32.50	0.1230
MB-1	1.0	20.95	0.0479
	2.0	26.27	0.0761
	3.0	29.70	0.1010
	4.0	30.34	0.1317
MB-2	0.5	17.45	0.0286
	1.0	23.08	0.0433
	1.5	25.65	0.0583
	2.0	27.51	0.0726
MB-3	1.0	19.90	0.0502
	2.0	23.92	0.0836
	3.0	26.31	0.1139
	4.0	28.26	0.1412
MB-4	1.0	22.90	0.0436
	2.0	27.00	0.0740
	3.0	28.89	0.1039
	4.0	31.38	0.1278
MB-5	1.0	21.03	0.0476
	2.0	25.30	0.0789
	3.0	27.85	0.1076
	4.0	30.20	0.1325
MB-6	1.0	21.17	0.0472
	2.0	24.21	0.0827
	3.0	27.83	0.1079
	4.0	29.82	0.1340
MB-7	1.0	7.53	0.1328
	2.0	15.83	0.1264
	3.0	22.27	0.1350
	4.0	24.90	0.1603
MB-8	1.0	6.21	0.1610
	2.0	13.85	0.1445
	3.0	19.79	0.1519
	4.0	23.21	0.1722
MB-9	1.0	24.51	0.0408
	2.0	29.26	0.0673
	3.0	31.73	0.0944
	4.0	33.03	0.1210
MB-10	1.0	21.48	0.0466
	2.0	26.49	0.0757
	3.0	29.46	0.1019
	4.0	30.61	0.1309
MB-11	1.0	20.92	0.0477
	2.0	25.92	0.0772
	3.0	28.60	0.1048
	4.0	30.60	0.1308
MB-12	1.0	15.81	0.0632
	2.0	21.03	0.0950
	3.0	24.47	0.1226
	4.0	27.03	0.1478

Representative curves are shown in Figure 2. All the curves were found to be straight lines except for samples MB-7 and MB-8; if higher levels of these samples had been used, curves such as the dotted line of Figure 2 might have been obtained by extrapolation. However, mold brans of such low activity would be useless in saccharifying fermentation mashes in commercial practice. The data for the lowest levels of some of the other mold bran samples also do not fit the curves very well. This is

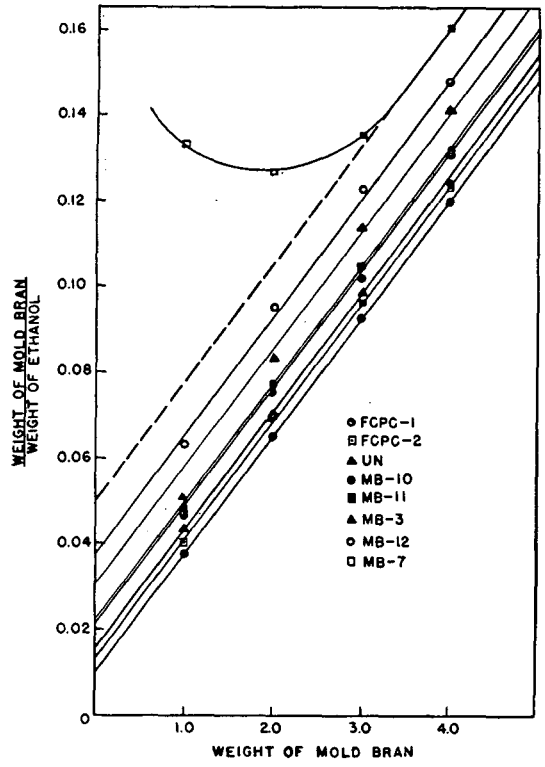

Figure 2. Curves for Standard Evaluation Tests

typical for samples with weak amylolytic potency, since saccharification is erratic at the low levels with resultant unreliable fermentations.

It is apparent from the figure that the lines tend to have approximately the same slopes—that is, they are essentially parallel—and the better the mold bran the lower is the intercept value obtained by extrapolating the curves to the *Y*-axis. The slopes for the curves are practically identical, having a value of about 0.0274, but the intercepts are inversely proportional to the amylolytic activities of the different samples. This is true because, in accordance with the mathematical relationship, the weight of mold bran required to produce a unit of alcohol increases in a linear fashion with the weight of mold bran used. Hence, mathematically, the potency of a mold bran is expressed by the value of the zero-axis intercept, the point of theoretical maximum efficiency—that is, the intercepts serve to evaluate the enzymic activity of different mold bran samples. The intercept values, taken from the plotted data, in the order of their increasing numerical values, representing decreasing amylolytic activities, are included in Table V.

Since the slopes of all the standard evaluation test curves are practically the same, about 0.0274, a simplification in the procedure suggested itself. The standard evaluation test could be modified by using a single level of mold bran, say 3.0 grams per flask, and then computing the intercept from the straight-line equation. Three mold bran samples, MB-10, MB-11, and MB-12, were selected for testing this procedure. Data representing the averages of quadruplicate fermentations using the single level of 3.0 grams of each of these samples and the standard evaluation test procedure are shown in Table III. Using the slope value 0.0274, obtained from the curve in Figure 2 for reference sample FCPC-2, the intercept values were calculated from the data of Table III. These are compared in Table IV with the graphical intercepts for the same samples taken from Figure 2. Greatest variation between graphical and calculated intercept values was for MB-11. This mathematical method, although apparently not so accurate as the graphical method employing four levels of

Table III. Single Level Standard Evaluation Test Data

Mold Bran, Lab. No.	Mold Bran, G./100 G. Starch	Ethanol, G./Flask	Weight of Mold Bran / Weight of Ethanol
MB-10	3.0	29.15	0.1028
MB-11	3.0	28.71	0.1045
MB-12	3.0	25.09	0.1199

Table IV. Intercepts Determined Graphically from Four Mold Bran Levels and Calculated from a Single Level

Mold Bran, Lab. No.	Graphical Intercept Value	Calculated Intercept Value
MB-10	0.0210	0.0206
MB-11	0.0213	0.0223
MB-12	0.0378	0.0377

mold bran, seems promising for use in industrial control laboratory procedures for the rapid evaluation of the enzymic activities of fungal amylase products employed in the saccharification of fermentation mashes.

In order to eliminate the troublesome variables of differences in composition commonly encountered in working with agricultural products all materials were used on the "as received" basis. In this way the enzymic activities of the mold bran samples are evaluated on the as received basis just as they will be employed in industrial operations. In other words, moisture content and other composition variables are compensated for in the intercept values obtained by the standard evaluation test procedure. For example, if two agents have the same amylolytic potency on a dry weight basis but one has a higher moisture content, the numerical intercept value will be larger for the one with higher moisture. If desired, moisture determinations could be made and all results expressed on the dry basis. However, in any event, to obtain reliable comparative data the same substrate should be used for all the samples of amylolytic agents evaluated—that is, reference curves with the reference standard mold bran should be obtained with each new batch of starch employed. Obviously materials should be stored in air-tight containers in order to prevent changes in moisture content.

OPTIMUM REQUIREMENT OF FUNGAL AMYLOLYTIC AGENTS FOR MAXIMUM ETHANOL PRODUCTION

Preliminary information indicated that, because the amylolytic activities of mold bran samples were inversely proportional to the intercept values obtained by plotting data from the standard evaluation tests, the optimal requirements for two different mold brans for maximum ethanol production would be proportional to the two intercept values. Hence, if any one good mold bran is selected as a reference standard and conventional grain fermentations are run, employing various levels of this mold bran to determine the minimum optimal requirements for maximum ethanol production, the minimum optimal levels for other fungal amylolytic agents might be calculated by direct proportion from the intercept values obtained by the standard evaluation test method. The proportion used in such calculations would be:

$$\frac{\text{Optimal level (unknown agent)}}{\text{optimal level (standard agent)}} = \frac{\text{intercept (unknown agent)}}{\text{intercept (standard agent)}}$$

Ethanol yield data from ordinary 72-hour grain fermentations were obtained with each of the mold brans previously evaluated by the standard evaluation test procedure for which sufficient sample was available. These grain fermentations were conducted in a manner similar to the procedures of the standard evaluation test, except that 100 grams of ground whole yellow corn were substituted for the starch and yeast extract, 0.02 *N* instead of 0.05 *N* hydrochloric acid was used since this gave a final pH of 5.0 to 5.3 and eliminated the necessity of further pH adjustment, and the fermentation period was increased to 72 hours.

A series of preliminary 72-hour corn fermentations using several levels of reference sample FCPC-2 indicated that a maximum

yield of ethanol was obtained when about 2.5 to 3.0 grams per 100 grams of corn were used. The average ethanol yields from series using 0.1-gram increments between these limits were plotted and the minimum optimal level for maximum ethanol production for reference sample FCPC-2 was judged to be 2.66 grams per 100 grams of corn.

The minimum optimal levels for the samples to be tested were calculated from the proportion given above, using the graphical intercepts taken from Table V and the minimum optimal level of 2.66 grams per 100 grams of corn for reference sample FCPC-2. The 72-hour corn fermentations were then conducted with each mold bran sample; duplicate fermentations were employed at each of six mold bran levels, using 0.5-gram increments with the computed minimum optimal level about midway in the series. It is unnecessary to give detailed data for all these series, but typical results are shown in the curves of Figure 3.

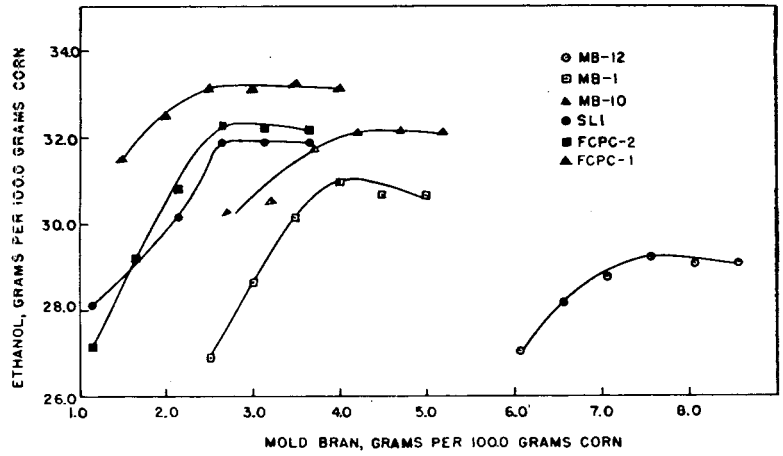

Figure 3. Ethanol Yields from Corn Saccharified with Mold Brans

In Table V are summarized the calculated and experimental minimum optimal levels for maximum ethanol yields from 100 grams of corn for the mold brans tested.

It is apparent from the data that the calculated optima agree closely in all cases with the optima determined experimentally. The only deviations were found with samples FCPC-1, JWC, and UN. It is also apparent from Table V that there is no correlation between the α-amylase values, determined by the method of Sandstedt, Kneen, and Blish (*18*) and the minimum optimal levels of mold brans required for saccharifying fermentation mashes.

Table V. Evaluation Data for Mold Bran Samples Tested

Mold Bran, Lab. No.	Graphical Intercept Value	Optimal Mold Bran Level, G. per 100 G. Corn		α-Amylase Value, Units
		Calculated	Experimental	
FCPC-1	0.0100	2.00	2.5	384
MB-9	0.0131	2.62	2.6	417
SLI	0.0132	2.64	2.6	800
FCPC-2	0.0133	(Reference)	2.66	457
JWC	0.0144	2.88	3.0	480
UN	0.0156	3.12	3.5	196
MB-2	0.0163	3.26	...	223
MB-4	0.0177	3.54	...	267
MB-1	0.0200	4.00	4.0	148
MB-10	0.0210	4.20	4.2	300
MB-11	0.0213	4.26	4.3	260
MB-5	0.0236	4.72	...	240
ISC-2	0.0240	4.80	4.8	113
MB-6	0.0250	5.00	...	253
ISC-1	0.0264	5.28	5.3	141
MB-3	0.0303	6.06	...	218
MB-12	0.0378	7.56	7.6	160

The data clearly indicate that not only can the relative total enzymic activities of fungal amylolytic agents for the saccharification of fermentation mashes be evaluated from the intercepts obtained by plotting the data from the standard evaluation tests, but also the minimum optimal levels to obtain maximum ethanol production from corn mashes can be calculated from these intercepts. Any good mold bran sample is selected as a reference standard. Conventional 72-hour grain fermentation tests, employing grain of average moisture content, are run to determine the minimum optimal requirement of this reference mold bran for maximum ethanol yields.

If desired, by means of moisture analyses, the results may be put on a dry weight basis. A series of 24-hour standard evaluation test fermentations on the cornstarch–yeast extract medium is then run with the reference mold bran, employing duplicate or triplicate fermentations at mold bran levels of 1.0, 2.0, 3.0, and 4.0%. The data are plotted on graph paper, and a straight line is drawn through the points, extending to its intercept with the Y-axis. This is the reference curve, and from this line the slope and the value of the intercept for the reference mold bran are obtained. To evaluate any other mold bran, its intercept value is then obtained from standard evaluation test fermentations. For best results three or four levels of the mold bran should be employed, and the intercept obtained graphically in the same way as for the reference mold bran. For rapid routine work standard evaluation test fermentations may be run at a single mold bran level, and the intercept calculated from the straight line equation, using the slope derived for the reference mold bran. The minimum optimal requirement for maximum ethanol yields for the mold bran under test is then calculated by proportion. In this way an accurate evaluation is obtained on the day following the start of the tests. Inasmuch as the calculated values are minimum optimum levels, for plant use it would probably be desirable, as a safety factor, to employ slightly more than the calculated proportion of mold bran. This would also provide for fluctuations in the moisture content and quality of the grain used in the plant—for example, if the calculated minimum optimum amount for a particular batch of mold bran were 3.0 grams per 100 grams of grain, 3.3 to 3.5 grams per 100 grams of grain might be recommended. A safety factor of 10 to 15% more than the calculated amount should prove adequate to compensate for normal plant processing variations.

ACKNOWLEDGMENT

The work reported in this paper was supported by an industrial fellowship established by the Farm Crops Processing Corporation, Omaha, Neb. The authors are also indebted to the Mold Bran Company, Eagle Grove, Iowa, for furnishing many samples of varying amylolytic activity, and to E. H. LeMense of that company who provided the α-amylase determinations on the samples used.

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RECEIVED June 30, 1947. Presented before the Fermentation Section, Division of Agricultural and Food Chemistry, at the 111th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Determination of Amino Nitrogen in Corn Sirup

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In the manufacture of corn sirup very small amounts of amino nitrogen contribute considerably to formation of undesirable yellow color. The present method shows that a modified Van Slyke method for amino nitrogen can be applied to corn sirup with good results. A 100-cc. reaction vessel is combined with a 3-cc. measuring buret, permitting the use of 25-ml. samples. Sugars in the solution have an inhibiting effect on the reaction, but this can be overcome by proper selection of the quantities of reagents. Determination of amino nitrogen with satisfactory precision is possible in the range from 10 to 40 p.p.m.

ONE of the greatest problems in the manufacture of corn sirup is the development of color in process solutions and in the finished sirup. A portion of this color is formed by the condensation of amino acids and sugars (*2*, *6*, *8*).

Determination of amino nitrogen in the corn sirup is of value in controlling color formation, but, the small quantities involved (as low as 10 p.p.m.) are difficult to measure by ordinary means. Colorimetric methods such as Folin's (*3*) are strongly affected by the presence of sugars. An additional difficulty is the selection of a standard for comparison; each amino acid gives a different degree of color with the reagent.

A modification of the Van Slyke amino nitrogen method gives good results on corn sirup.

APPARATUS

Harral (*4*) combined a macro reaction vessel of a new type with a micro measuring buret, permitting the estimation of very small concentrations of amino acids by the use of a large sample. With a few changes, the Harral apparatus has been used here.

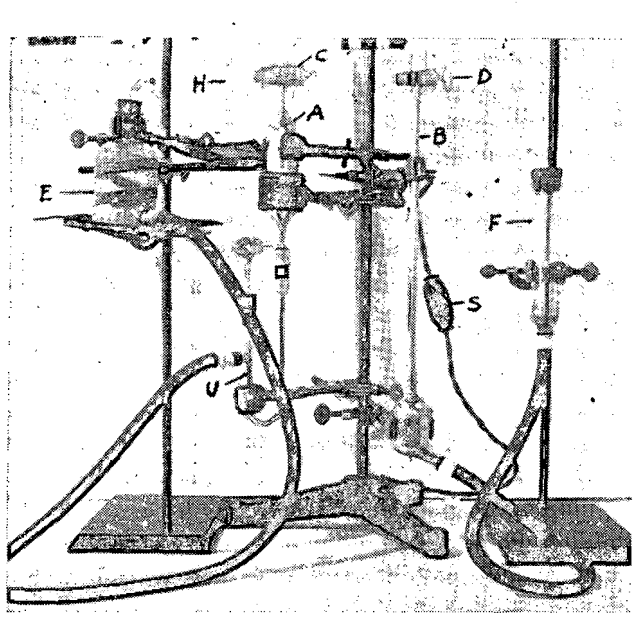

Figure 1. Modified Amino Nitrogen Apparatus

In Figure 1, *A* is the reaction vessel, of 100-cc. capacity. *B* is a 3-cc. measuring buret graduated in 0.01 cc. and having a reservoir bulb of about 50-cc. capacity. *F* is the leveling bulb for the buret; *E* is the leveling bottle for the reaction chamber. *C* is a three-way stopcock leading through stopcock *D* to *B* or directly to *H*, the inlet tube. *U* is the air trap protecting *A*. The lower end of the reaction vessel is surrounded by a heating element consisting of a closely fitting copper cylinder wrapped with resistance wire surrounded by asbestos. The current is regulated by a rheostat (not shown) and switch *S*.

REAGENTS

Saturated sodium nitrite was made by dissolving 80 grams of sodium nitrite sticks in 100 ml. of hot water, cooling, and decanting.

The alkaline permanganate was made by dissolving 50 grams of potassium permanganate and 25 grams of sodium hydroxide, and making them up to 1 liter.

The amino acids used as standards were obtained from the Eastman Research Laboratories. *dl*-Leucine was used without further purification. *d*-Glutamic acid was recrystallized from water. Its melting point was then found to be 199–200° C. The reported value is 199° C. (*1*). These acids were dried for 1 hour at 100° C. and 29-inch vacuum, cooled, weighed, dissolved, and made up to volume. Aliquots were used for the determinations.

PROCEDURE

After all air in the apparatus is replaced by mercury, the sample and acetic acid are drawn into the reaction chamber, where they are shaken and heated until substantially air-free. The air is removed; sodium nitrite is drawn in and mixed with the solution by shaking. The reaction is allowed to proceed for 30 minutes, after which the mixture of nitrogen and nitric oxide is removed to the measuring buret. The reaction chamber is then cleaned, and alkaline permanganate is introduced and made air-free. The gases are forced back into the reaction chamber, where the nitric oxide is rapidly absorbed, leaving only nitrogen, which is then forced back into the gas buret and measured.

The stopcocks are carefully greased and the system is rinsed thoroughly with water by letting it in at *H* and driving it back and forth between *A* and *B* by means of leveling bulb *E*. The water is forced out at *H* and mercury is made to fill the reaction vessel, the gas buret, the connecting capillary, and the entrance capillary, *H*. The sample (25 ml.) is drawn in at *H* from a small beaker, which is washed twice with 5-ml. portions of water; these are drawn in also, followed by 4 ml. of glacial acetic acid and finally by 1 ml. of water from small graduated tubes. The total volume is always kept at 40 ml.

With *C* closed bulb *E* is lowered until the solution is surrounded by the heating element, leaving a vacuum space above the solution. The current is turned on and the solution is warmed and shaken. After a few minutes *E* is raised and the removed air shows up as a bubble above the solution. This is forced out the

Table I. Effect of Reaction Time on Recovery of Amino Nitrogen from Glutamic Acid

Reaction Time	Nitrogen Taken	Nitrogen Found	Recovery
Min.	*Mg.*	*Mg.*	%
10	0.880	0.657	74.3
30	1.760	1.680	95.5
30	0.880	0.845	96.0
30	1.596	1.520	95.3
45	1.596	1.517	95.0

stopcock and the process is repeated until only a very small bubble of air exists above the solution. This takes 20 to 30 minutes. The solution is allowed to cool to about room temperature and the sodium nitrite solution (4 ml.) is drawn in from a graduated tube, care being taken to admit no air. The nitrite in the capillary is not washed in. The solutions are shaken until thoroughly mixed, then bulb *E* is left at the upper level and the reaction is allowed to go on for 30 minutes. The gas is then forced over into *B* by adjusting *C* and *D* and raising *E*. *C* is closed and *E* is lowered; more gas is shaken out and passed over into *B;* this is repeated until very little gas comes out of the solution. *C* is then turned so that the solution is removed through *H*.

A is cleaned by rinsing with three portions of water, and 8 ml. of permanganate solution are drawn in and freed from air. When all the air is out, *A* and *B* are connected and *E* is lowered. The gas is drawn over into *A* and absorbed by shaking. When no more gas will dissolve, *A* and *B* are connected again and the permanganate is forced up to stopcock *C*, which is then closed while the permanganate is boiled out. *C* is again opened and *E* raised, forcing permanganate through the capillary to *D*. By careful manipulation the hole in *D* can be filled with permanganate without allowing any to come through into *B*. *D* is then closed and the mercury in level bulb *F* is brought to the same level as that in *B*. The volume of gas, the temperature, and the barometer reading are taken.

A blank must always be run on the reagents.

CALCULATIONS

Standard tables (*7*) give the weight of amino nitrogen corresponding to 1 cc. of nitrogen gas at 11° to 30° C. and 728 to 772 mm. pressure. It is only necessary to multiply the figure obtained from the table by the volume of nitrogen found to get the weight of amino nitrogen in the sample.

EXPERIMENTAL

Optimum reaction time was determined for a solution of glutamic acid, using 2 ml. of saturated sodium nitrite and 4 ml. of glacial acetic acid. The results (Table I) showed that a 10-minute reaction period was too short and a 45-minute period unnecessarily long. A standard reaction time of 30 minutes was decided upon, and further trials were made to improve the determination.

A solution of *dl*-leucine, run under conditions similar to those for glutamic acid, yielded only the same amount of nitrogen (Table II). These results indicated that the incompleteness of reaction was not due to the amino acid, but to the conditions of analysis. It was recognized that the presence of corn sirup in the reaction mixture would probably have an inhibiting effect on the reaction. In order to evaluate this effect, determinations were made on corn sirup and a mixture of corn sirup with standard glutamic acid solution under the same conditions. Corn sirup in the mixture reduced the recovery of amino nitrogen from 95 to 69% (Table II).

Accordingly, the acid addition was increased to 10 and then 15 ml., with corresponding increases in recovery to 97 and 92%. At the highest acid concentration a heavy white material, probably a mercury salt, appeared and seemed to interfere with the reaction. The acid concentration was reduced to its original value and the nitrite addition was increased to 4 ml. Under these conditions the reaction went to completion even in the presence of corn sirup. The slightly high results were probably due to a very slight amount of air not removed from the sample solution at the beginning of the analysis.

Table II. Effects of Quantities of Reagents and Presence of Corn Sirup on Recovery of Added Amino Nitrogen

Sample	Glacial Acetic Acid	Sodium Nitrite	Nitrogen Taken	Nitrogen Found	Recovery
	Ml.	*Ml.*	*Mg.*	*Mg.*	%
dl-Leucine	4	2	1.544	1.439	93.2
	4	2	1.544	1.462	94.7
Solution 1[a]	4	2	?	0.408	...
Solution 1 plus glutamic acid	4	2	0.408 + 0.760	0.932	69
Glutamic acid	10	2	1.059	1.069	100.9
Solution 2	10	2	?	0.293	...
Solution 2 plus glutamic acid	10	2	0.293 + 0.760	1.029	97.0
Solution 3	15	2	?	0.30	...
Solution 3 plus glutamic acid	15	2	0.30 + 0.53	0.80	95
Solution 4	15	2	?	0.21	...
Solution 4 plus glutamic acid	15	2	0.21 + 0.53	0.68	89
Glutamic acid	4	4	1.06	1.07	101
Solution 4	4	4	?	0.25	...
Solution 4 plus glutamic acid	4	4	0.25 + 0.53	0.79	102
Solution 5	4	4	?	0.52	...
Solution 5 plus glutamic acid	4	4	0.52 + 0.54	1.06	100

[a] These solutions were corn sirup process liquors at about 22° Bé.

DISCUSSION

The experiments show that an adaptation of the Van Slyke amino nitrogen method can be used for the determination of very small amounts of amino nitrogen in corn sirup liquors.

Although several amino acids yield high results by this method (*5*), these are either absent from or present in amounts less than 2% in corn proteins. Glutamic acid and leucine account for the major portion of corn proteins, and these react normally.

Ammonia nitrogen interferes, but it can be removed with Permutit before analysis. The Van Slyke manometric apparatus can also be used in the analysis, but the effect of sugars has not yet been worked out.

The error in the determination is about ±0.02 cc. of nitrogen gas, which amounts to ±1% when 2 cc. (40 p.p.m.) are obtained and about ±4% when 0.5 cc. (10 p.p.m.) is obtained. This is about the same precision as that of the Kjeldahl method for total nitrogen at similar concentrations.

The tendency towards slightly high results was also found by Van Slyke in some cases; his results on glutamic acid were high by about 1% in the macromethod (*9*) and high on leucine by about 0.2% in the micromethod (*10*).

CONCLUSIONS

Amino nitrogen can be determined in corn sirup liquors by a modified Van Slyke method with a precision of ±1% when 40 p.p.m. are present and of ±4% when 10 p.p.m. are present.

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RECEIVED September 4, 1947.

Quantitative Estimation of GR-S in Rubber Reclaim

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A practical method for estimating the amounts of GR-S or natural rubber reclaims in mixtures containing these two polymers has been worked out on the basis of determination of the rubber hydrocarbon content by difference and by direct analysis (chromic acid oxidation). A correction factor must be applied before estimation can be attempted for mixtures rich in natural rubber but not for reclaim mixtures rich in GR-S. This correction factor accounts for the deviation normally encountered in natural rubber reclaim between the determinations of the hydrocarbon content by direct analysis and by difference. The factor was determined by the origin of the scrap from which the natural rubber reclaim had been prepared. A tentative chemical explanation of this deviation is given. Limits of error in the estimation are listed and two simple working examples illustrate the procedure.

THE identification of GR-S and its estimation in scrap rubber and reclaimed rubber have been the object of considerable research during recent years. Although it has become economically and practically impossible to segregate synthetic and natural rubber scrap, it still is of interest to determine the amount of GR-S in the finished reclaim for the purpose of evaluating its properties.

Accurate methods have been worked out for the quantitative determination of GR-S in mixtures with natural rubber, but they require either a considerable amount of time or the use of expensive apparatus not accessible to the average reclaim manufacturer. Several of these methods can be carried out only after the hydrocarbon polymers have been brought into solution, and this, as far as reclaim is concerned, introduces difficulties. The average reclaim manufacturer or compounder is in general not concerned in obtaining a highly accurate estimation of GR-S in reclaim mixtures, but rather prefers the application of a method which, while fairly easy to carry out, will permit estimation of GR-S within 5 parts of the total hydrocarbon present.

RUBBER HYDROCARBON CONTENT

For some time the evaluation of reclaim had been based on either the direct or indirect determination of its rubber hydrocarbon content. The direct determination was carried out by chromic acid oxidation (*1*), which gives the quantity of natural rubber hydrocarbon; the indirect, or difference method, by complete analysis of the sample and deducting the sum total of the acetone extract, ash, carbon black content, etc., from 100. The difference was considered to be the total rubber hydrocarbon content, which includes that of both the natural rubber and the GR-S.

Inasmuch as the presence of the methyl group in the position as encountered in the isoprene unit is mandatory as far as the chromic acid oxidation method is concerned, it was not surprising that butadiene-styrene copolymers would not yield acetic acid by this method. Repeated analysis of GR-S yielded only at the most 3 to 4% of rubber hydrocarbon, and values of 1 to 2% were found more generally. Reclaiming of vulcanized GR-S could not be expected to change this condition. Experiments have confirmed this point of view.

Therefore, it was felt that if the percentage of rubber hydrocarbon as obtained for any given sample by the above analysis were a direct function of either of the two kinds of polymers present, the proportions of each could be estimated by means of a simple plot based on the two rubber hydrocarbon contents, one obtained by difference and one by direct analysis. Inasmuch as GR-S practically does not enter into the formation of acetic acid by chromic acid oxidation, the value obtained for the rubber hydrocarbon content by difference can be expected to represent the sum total of the two polymers present, whereas the rubber hydrocarbon obtained by direct analysis would be a function of the content of natural rubber. Thus the values obtained by direct analysis of various mixtures containing GR-S would fall on a straight line proportional to the per cent of GR-S and natural rubber present in these mixtures.

For this purpose GR-S and natural rubber reclaims were prepared separately, and carefully and completely analyzed. Seven predetermined mixtures of these reclaims were then prepared with analytical accuracy covering the whole range from 100% GR-S to 100% natural rubber. The per cent of rubber hydrocarbon by direct analysis of these mixtures was determined. Figure 1 (dashed line) shows the actual data obtained for such a series plotted against the known proportions of GR-S and natural rubber for each mixture. Except for mixtures very high in GR-S a straight line was obtained. The aberration at high GR-S contents is a consequence of the slight amount of rubber hydrocarbon obtained for GR-S. This effect is bound to cause an error in the estimation of reclaims containing 90% or more GR-S. Practically, however, the straight line can be extrapolated to the zero point without changing the results to any greater extent in mixtures less rich in GR-S.

To apply this method for industrial use the esti-

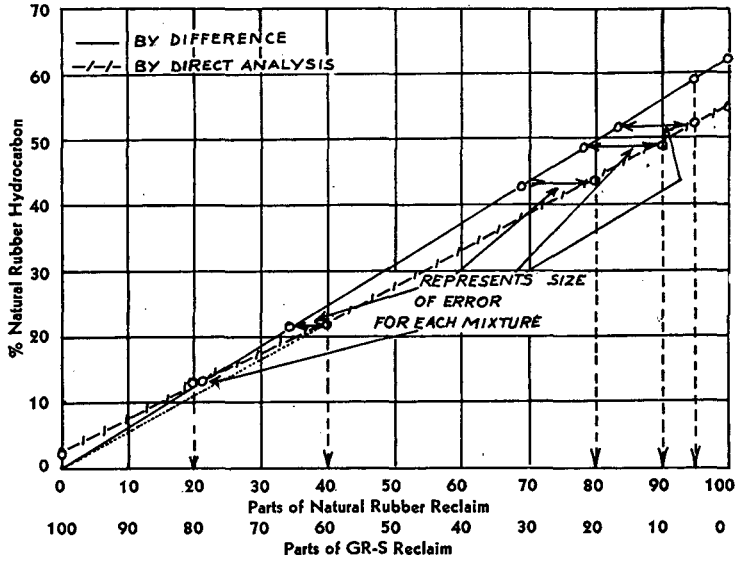

Figure 1. Rubber Hydrocarbon in Mixtures

mation of a mixture of natural and synthetic rubber of unknown proportions will have to be based also on the percentage of total rubber hydrocarbon as determined by the difference method, because this latter value will represent the sum of both natural and synthetic rubber hydrocarbon. In the case represented by Figure 1, this value was found to be 62.9%. As such it can be considered and recorded on the graph as 100 parts of polymers. This value must then be the end point of the straight line originating at the zero point. The data obtained from the various mixtures analyzed were replotted on this basis and are also shown in Figure 1 (solid line). If the data found by direct rubber hydrocarbon analysis are placed on that line, the parts of GR-S and natural rubber reclaim which can now be derived from the abscissa of this plot do not correspond to the actual analytical mixtures prepared. A mixture actually containing 100% of natural rubber reclaim seems to contain 88.5%; 95% natural rubber reclaim, only 83.5%; 80% natural rubber reclaim, 69%; 40% natural rubber reclaim, 34.5%; 20% natural rubber reclaim, 21.5%; and no natural rubber, 3%. The greatest error in estimation occurs for mixtures high in natural rubber. The less the amount of natural rubber, the smaller the error appears to be. However, the error introduced in this way was so great that unless the reason for it and some correction factor could be found, the method could not be considered useful.

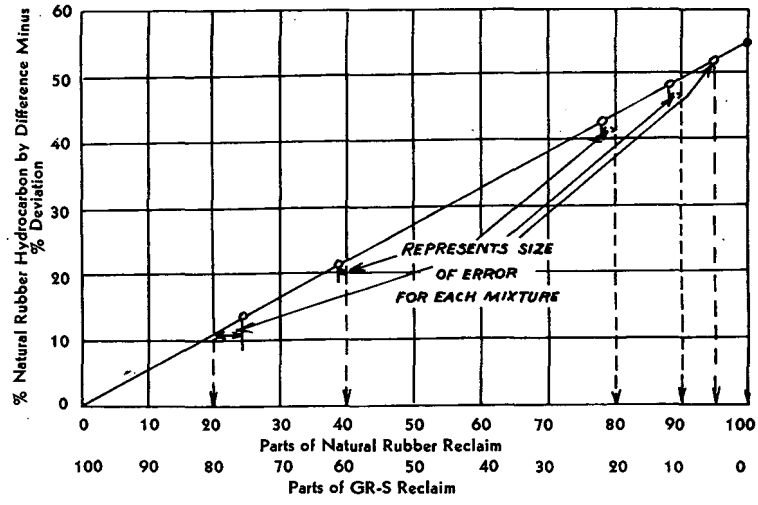

Figure 2. Rubber Hydrocarbon in Mixtures

The rubber reclaiming industry has known for some time that the values obtained for the rubber hydrocarbon content of natural rubber reclaim by direct analysis are in almost all cases lower than those obtained for the same reclaims by the difference method. This difference is referred to below as deviation.

DEVIATIONS

It has often been assumed that the deviation between the two values of the natural rubber hydrocarbon determinations was due to analytical errors and as such had to be considered entirely accidental. However, if this were true, the rubber hydrocarbon content obtained by direct analysis should not be practically always lower in value than that obtained by difference. The sum total of errors introduced in the latter case would be cumulative, owing to errors obtained in each single analysis of the various ingredients in the reclaim. Analytical errors, however, like any other errors, can be positive as well as negative. Thus, if analytical errors were the sole cause for the deviation between the two rubber hydrocarbon determination methods, they should not cause the rubber hydrocarbon determination by difference to be constantly of higher value than that obtained by direct analysis.

The yield of acetic acid obtained by chromic acid oxidation is expressed in fractions of gram moles of acetic acid per gram mole of methyl groups present. For natural rubber the yield amounts to 0.75, which indicates that 4 isoprene units form 3 moles of acetic acid. Combined sulfur can be expected to interfere with this reaction, necessitating the introduction of a correction factor. Results obtained by the oxidation of hard rubber (*3*) have indicated that for about 3 atoms of combined sulfur 2 isoprene units fail to yield acetic acid, or in other words, that for every 3 atoms of combined sulfur 1.5 moles of acetic acid are lost.

Inasmuch as the combined sulfur is not removed during the reclaiming procedure, part of the rubber hydrocarbon in reclaim is not available for further vulcanization. It is only logical to assume that this is the same part of the rubber hydrocarbon that does not enter into the chromic acid oxidation.

Thus the deviation between the two determinations of rubber hydrocarbon might be explained by the fact that a small amount of unsaturation is lost during vulcanization of the original crude rubber and that some unsaturation might also have been lost through oxidation, both of which could be expected to disturb the quantitative chromic acid oxidation to acetic acid. The factor of oxidation cannot be discounted. Burger (*1*) found the deviation much greater for reclaims than for vulcanizates not containing reclaim. Experiments on a natural rubber gumstock, which did not contain antioxidant, showed a considerable amount of deviation when this stock was subjected to exaggerated reclaiming conditions permitting considerable oxidation. The original vulcanized compound yielded 86% of natural rubber hydrocarbon as determined by direct analysis, but when subjected to the highly oxidizing reclaiming conditions yielded only 76% of natural rubber hydrocarbon. Naturally, such exaggerated conditions do not exist under actual reclaiming procedures. The scrap subjected to reclaiming contains antioxidant and in most cases carbon black, both of which permit a certain amount of protection against oxidation. The conditions under which reclaiming is carried out usually do not permit any sizable amount of oxidation; therefore such large deviations as mentioned above cannot be expected to occur.

Inasmuch, however, as a deviation occurs at all times in natural rubber reclaim, it will also play a part in any estimation of the GR-S or natural rubber present in mixtures such as analyzed above. If this is true, the error in the estimation of the components of the mixtures will be the larger, the greater the deviation between the rubber hydrocarbon content by difference and by direct analysis of the natural rubber reclaim present. This deviation was considerable for the natural rubber reclaim used in Figure 1. Furthermore, the error in the estimate of the components of the mixtures will be much greater if these mixtures contain considerable amounts of natural rubber. Figure 1 shows that the error in estimation decreases gradually as the amount of GR-S present in the mixture increases.

Therefore, it was decided to examine this deviation for natural rubber reclaims, such as peels, carcass, tire, and tube reclaims, in greater detail. Ample data for such critical scrutinization are available in any control laboratory of the rubber reclaiming industry. These laboratories have carried out complete analyses of natural rubber reclaims for product control and sales purposes over a number of years.

For this purpose the various natural rubber reclaims were grouped together according to the origin of the scrap from which they had been prepared. The same kind of graphic representation was used; the value of the rubber hydrocarbon content was assigned by difference on the ordinate of the system to a value equivalent to 100 parts of natural rubber reclaim on the abscissa. Inasmuch as the value of the rubber hydrocarbon by difference represented at all times the total amount of polymers present, it could be considered as 100 parts of natural rubber. A straight

line was then drawn through the origin, assigning a zero rubber hydrocarbon content to a zero content of natural rubber reclaim.

Each natural rubber reclaim was in this way represented by a line, the slope of which was determined by its natural rubber hydrocarbon content by difference. On each line the value for the rubber hydrocarbon obtained by direct analysis for the particular reclaim was recorded. The fact that in each case the parts of rubber which could be read in this way on the abscissa were less than 100, indicated that the rubber hydrocarbon content obtained by direct analysis was less than that obtained by difference analysis. A great number of natural rubber reclaims were plotted in this way.

If this graphic representation is carried out separately for the reclaims originating from different kinds of scrap rubber, each kind of reclaim seems to possess a particular and characteristic range of deviation. For example, three natural rubber peel reclaims showed a range of deviation between 1.2 and 3% of natural rubber; three black carcass reclaims, one between 6.0 and 8.0%; three light-colored carcass reclaims, one between 10 and 12.5%; and out of the twelve first, second, and third line natural rubber tire reclaims, nine showed a range of deviation between 3.4 and 7%. The range of deviation is characteristic for each kind of reclaim and seems to be a function of the origin of the reclaim more than of the reclaiming formulas used during the reclaiming process. This behavior of the reclaims is generally true, but it will fail in the case of certain specific compounding ingredients which interfere with the direct rubber hydrocarbon analysis. The difference in the deviation between carcass and peel reclaims cannot be ascribed to errors in analysis due to the possible presence of cellulose or its decomposition products in the carcass reclaims. Reclaims prepared from cellulose-free scraps, such as tube reclaims and peel reclaims, also differ widely in their deviations; the tube reclaims exhibit much larger deviations than those encountered for peel reclaims.

Table I. Deviations of Reclaims

Reclaim	Av. Deviation Found by Analysis over 2–4 Years	Mean Deviation
Peel reclaim		
I	1.2	
II	1.6	1.5
III	1.6	
IV	2.9	
Carcass reclaim		
I	6.1	
II	5.9	
III	6.9	6.6
IV	7.2	
V	6.8	
First line whole tire reclaim		
I	4.2	
II	3.7	
III	4.0	
IV	2.3	
V	3.8	
VI	3.6	
VII	2.7	
VIII	6.8	3.8
IX	5.4	
X	3.3	
XI	3.7	
XII	3.0	
XIII	4.7	
XIV	5.0	
XV	2.1	

Table I shows the average deviations of the three main groups of reclaims as found by actual analysis over a period of 2 to 4 years.

The mean deviation of tire reclaims falls between those of the peel and the carcass reclaims. This can hardly be called coincidence, but rather was to be expected. Tire reclaims must, after all, be basically considered as mixtures of peel and carcass reclaims. Reclaims prepared from highly reinforced compounds such as peel show the smallest deviation between the two hydrocarbon values, and reclaims which contain no carbon black as reinforcing agent, such as light-colored carcass reclaims, show the greatest. A tentative explanation of this phenomenon can be attempted in regard to the loss of acetic acid due to vulcanization (loss of unsaturation) during the chromic acid oxidation. Furthermore, Thornhill and Smith (*2*) found that the smallest loss in unsaturation occurred during the vulcanization of highly reinforced carbon black compounds, which age better than nonreinforced compounds. A drop in unsaturation ascribed to oxidation has been noted by several investigators during accelerated aging experiments.

The above seems to indicate that the deviation between the values of the rubber hydrocarbon by difference and by direct analysis cannot be regarded as entirely accidental or due to cumulative errors occurring during analysis. On the contrary, a certain amount of statistical order is evident, indicating that these deviations are largely dependent on the compounds from which the reclaims have been prepared.

If the original data obtained by analysis are replotted, taking the deviation for the kind of reclaim used into consideration, it can be seen immediately that the magnitude of error, so predominant before this correction was applied, has become considerably smaller (Figure 2). Now a mixture known to contain 90% of natural rubber seems to contain 88%; 80% of natural rubber, 78%; 40% of natural rubber, 39%; and 20% of natural rubber, 24.5%. The higher the content of natural rubber, the smaller the error seems to be. The biggest error is encountered in mixtures rich in GR-S. However, the magnitude of the error has decreased to such an extent that the method becomes applicable for quantitative estimation of the components, at least for practical purposes. Correction for the deviation in proportion to the amount of natural rubber present in each mixture is automatically obtained by the slope of the line.

Algebraic calculation of the content of natural rubber or GR-S reclaim in their mixtures is equally possible. If no correction factor had to be taken into consideration, $S = T - R$, where S represented the per cent of GR-S, T the total rubber hydrocarbon content by difference, and R the per cent natural rubber. However, because of the slight amount of acetic acid obtained by chromic acid oxidation from GR-S, the experimental data obtained for R would be too high. Therefore, the actual value for the natural rubber present in the mixture can be expressed by $R_1 = R - aS$, where a is the amount of acetic acid obtained from GR-S. Introducing this correction into the first equation, we obtain $S = \frac{T - R}{1 - a}$.

At the same time, however, the yield of acetic acid from vulcanized natural rubber and reclaim prepared therefrom has been found to depend on the amount of combined sulfur and to a certain extent on the oxidation of the rubber hydrocarbon. Therefore, another correction factor has to be introduced. $S = \frac{T - bR}{1 - a}$, would then constitute the correct scientific formulation for calculation of the content of synthetic rubber reclaim in mixture with natural rubber. The size of factor b would depend on the kind of scrap used (whole tires, tube, etc.).

Practically, however, factor a can in most cases be neglected. The limits of accuracy of the carbon black and cellulose analysis, both of which would enter into this formula by means of value T, are, in the case of reclaim, far from ideal. In determination of the direct rubber hydrocarbon content of reclaim, check analyses varying about 0.5% are considered very good indeed. Thus, as the reclaim mixtures become richer in natural rubber the increment of acetic acid due to the presence of GR-S will rapidly fall within the experimental limits of error, disqualifying the application of factor a. On the other hand, a mixture rich in GR-S will not justify the application of factor b. Practical experiments on such mixtures have shown that the smallest amount of error was actually obtained if factor b was not used. Actually, the estimation of GR-S reclaim could be much more accurate than ±2.5%

if the limits of accuracy of the various analyses entering into the calculations were more favorable. Thus, it has been proved of practical expediency to use the correction for the deviation only in cases where direct analysis shows a comparatively large amount of rubber hydrocarbon, indicating the presence of mixtures rich in natural rubber. Mixtures containing 15% or less direct rubber hydrocarbon, or large amounts of GR-S, should be plotted without accounting for the deviation. Because only a small amount of natural rubber reclaim is present, the error due to the deviation between the rubber hydrocarbon content by difference and that by direct analysis is proportionately small and even compensates for the error due to the small amount of direct rubber hydrocarbon obtained from GR-S. Such mixtures are more accurately determined if no correction for the deviation is applied. Figure 3 shows the simplicity of the method.

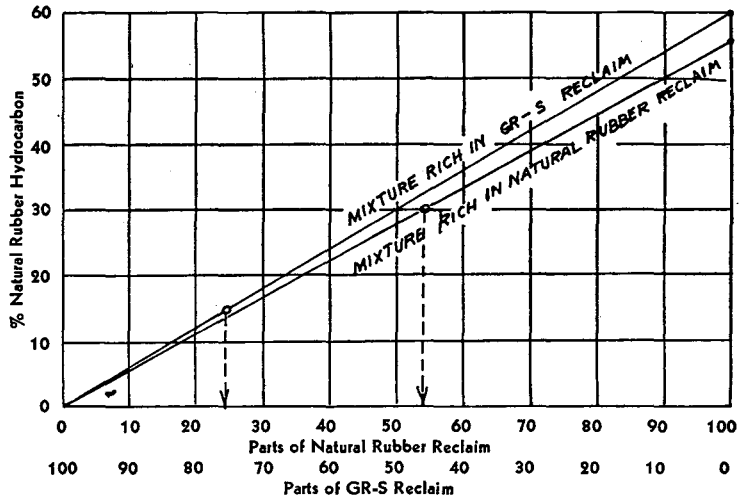

Figure 3. Rubber Hydrocarbon in Mixtures

PROCEDURE FOR ESTIMATING GR-S

Assuming that two unknown mixtures of tire reclaims have to be analyzed for their content of natural and synthetic polymer, the practical procedure for the estimation of GR-S would amount to the following steps:

1. Determination of the rubber hydrocarbon by difference.
2. Determination of the direct rubber hydrocarbon by chromic acid oxidation.
3. Ascertaining the kind of reclaim under consideration (tire, peel, carcass, tube, etc.).
4. Ascertaining the average deviation for this kind of reclaim.
5. Drawing the graphic plot. Plotting the percentage rubber hydrocarbon on the ordinate, the parts of natural rubber reclaim on the abscissa.

Assuming for the sake of illustration that one of the unknown mixtures gave:

1. A total rubber hydrocarbon content by difference of 60%.
2. A direct natural rubber hydrocarbon content of 30%.
3. First line tire reclaim.
4. Normal deviation for a natural rubber first line tire reclaim, 3.8.
5. The direct natural rubber hydrocarbon content of 30% indicates a reclaim mixture fairly rich in natural rubber. Therefore the correction for the deviation should be applied (60 − 3.8 = 56.2):56.2% of rubber hydrocarbon constitutes the anchor point of the line going through the zero point of the graph. That line is followed down to an ordinate reading of 30% natural rubber hydrocarbon, and the parts of GR-S reclaim are read at that point on the abscissa (see Figure 3).

Assuming for the sake of illustration that the second of the unknown mixtures gave:

1. A rubber total hydrocarbon content by difference of 60%.
2. A direct natural rubber hydrocarbon content of 15%.
3. Not necessary.
4. Not necessary.
5. The direct natural rubber hydrocarbon content of 15% indicates reclaim mixtures rich in GR-S. Therefore, it is not necessary to apply any correction for the deviation. As 60% rubber hydrocarbon constitutes the anchor point of the line going through the zero point on the graph, that line is followed down to an ordinate reading of 15% hydrocarbon. The parts of GR-S reclaim are read at that point on the abscissa (see Figure 3). Figure 3, as well as these examples, must be considered a working example only.

First line tire reclaims show a smaller mean deviation than second or third line tire reclaims. Thus the estimation can be much closer if it is known whether the reclaim in question is classified as first, second, or third line reclaim. If the method is used by a reclaim manufacturer to determine the GR-S content on one of the reclaims of his line, still greater accuracy can be obtained because the history of the production of this reclaim is probably known over a number of years. In general, specific gravity and total rubber hydrocarbon content by difference will give the experienced worker some indications of the kind of reclaim with which he is working, and the correction for the deviation can thus be adapted properly. The method should not be expected to be more accurate than ±2.5 parts of polymer, unless the origin of the reclaim and its behavior had been established. This is possible only if a reclaimer is trying to evaluate reclaim mixtures of his own production.

The originators of the rubber hydrocarbon determination by direct analysis have pointed out that certain substances, such as mineral rubber and greater amounts of cellulose, will obscure this analysis and render it inaccurate. Consequently, the presence of such materials will naturally also affect the estimation of GR-S or natural rubber unless they are removed prior to determination of the rubber hydrocarbon content.

CONCLUSION

The method is none too accurate above 90% of either natural or synthetic rubber present, unless the reclaim analyzed is well known. If the degree of deviation for a given reclaim is well established, it is possible to determine the mixtures up to 95% of either component. However, if the degree of deviation is an average figure only, derived by such critical scrutinization of a number of reclaims as shown in Table I greater accuracy cannot be expected in the estimation of the components of mixtures containing more than 90% of either natural or synthetic rubber.

The GR-S or natural rubber content of reclaims containing a great variety of scraps, such as some of the so-called victory reclaims, can be estimated only with a lesser degree of accuracy than ±2.5 parts. Difficulties in the estimation of the polymer components may also be encountered in light-colored reclaims. However, such estimations are seldom necessary because of the peculiar compounding technique required by GR-S for proper physical performance.

ACKNOWLEDGMENT

The author wishes to express appreciation to N. Novakovich, who prepared the reclaim mixtures and carried out the analysis.

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RECEIVED May 13, 1947. Presented before the Division of Rubber Chemistry, AMERICAN CHEMICAL SOCIETY, Cleveland, Ohio, May 1947.

Determination of Water in Bleaching Powder

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A distillation method is described for the determination of water in bleaching powder and high-test calcium hypochlorites, involving heating the sample with *o*-dichlorobenzene to drive all the water into the graduated separator tube of a specially constructed one-piece apparatus.

THE water content of bleaching powder, which is used for bleaching, disinfection, and decontamination, is an important factor in its stability (*7, 8*). Of lesser importance is its contribution to corrosion in containers and to caking in cold weather. This applies to bleaching powder made by the chamber or rotary processes and to the high-test calcium hypochlorites. For these reasons, knowledge of the water content is particularly desirable to users of bleaching powder for procurement and specification purposes.

A variety of methods have been tried for the determination of water in bleaching powder, such as by heating the powder quickly in a tube at 200° to 250° and absorbing the liberated chlorine in potassium iodide solution (*9*), by difference between 100% and the total percentages of calcium hypochlorite, calcium chloride, sodium chloride (if present), calcium hydroxide, calcium carbonate, calcium sulfate, and insolubles in acid, and by passing dry air over the bleaching powder, then over phosphorus pentoxide, and obtaining water by increase in weight of the phosphorus pentoxide. In general, such methods are considered unsatisfactory because they are either lengthy or inaccurate. Oven-drying methods cannot be used because the calcium hydroxide in the bleaching powder combines with carbon dioxide in the atmosphere and the calcium hypochlorite of the powder decomposes on heating.

The method of direct determination of water content by distillation (*3, 4, 11*) has been most widely used. Somiya (*11*) modified the apparatus devised by Dean and Stark (*2*) for water determination by distillation and used solvents heavier than water, such as carbon tetrachloride and tetrachloroethylene. The federal specification method (*3*) for water in bleaching material involves distillation of the water from the bleaching powder with *o*-dichlorobenzene and collection of the distillate in a 100-ml. centrifuge tube graduated at the bottom for at least 1 ml. in 0.2-ml. divisions and containing petroleum ether. The Pennsylvania Salt Manufacturing Company uses a modification of the federal specification method, in which some minor changes are made in the distillation apparatus and a 100-ml. Goetz centrifuge tube having a stem with the first milliliter graduated in 0.05-ml. divisions is used as a receiver. The excellent method of Suter (*12*) for determination of water in caustic soda and other alkaline materials, involving distillation followed by a Fischer moisture titration of the distillates, cannot be applied as bleaching powder releases volatile oxidants on heating.

In the distillation methods of Lundin (*6*) and Thielepape (*13*), the vapor enters the top of the condenser and the separated water is continuously washed down into the trap by the condensing solvent. Lundin used a mixture of solvents containing tetrachloroethane, which was much higher in density and boiling point than water. A similar method developed by Greene (*5*) for the determination of moisture in tetrachloroethane, hexachloroethane, and chlorinated paraffin has been adapted to the determination of water in bleaching powder for Chemical Corps experimental work and procurement specifications. This method and apparatus as modified for bleaching powder have some advantages over the methods previously mentioned in that the condenser and receiver of the apparatus are in one unit, only one solvent is used, and inflammable solvents, such as petroleum ether, are not required, and after the distillation has been started no further attention of the operator is needed until the final reading is made. No reference to this method as applied to bleaching powder is given in the literature, and it might be useful to analysts concerned with bleaching powder.

At first the standard Chemical Corps method of determining water content by distillation (*5*) was used with *s*-tetrachloroethane as the distillation liquid. Some modifications were made of the apparatus, such as use of a 500- or 1000-ml. boiling flask in place of the 2-liter flask used for the larger samples of other materials, addition of a spherical joint for ease of changing the flasks, and replacement of the plain separator tube with a graduated tube. Results which agreed with those obtained by the federal specification method (*3*) were obtained providing the distillation was not continued for longer than 30 to 40 minutes. For longer periods of time, high results were obtained which were thought to be due to reaction of the calcium hydroxide of the bleaching powder with the *s*-tetrachloroethane or hydrochloric acid liberated by the *s*-tetrachloroethane. At the suggestion of W. L. Drahenstatt of the Diamond Magnesium Company, *o*-dichlorobenzene was used as the distillation liquid in place of *s*-tetrachloroethane and results were obtained which reached a maximum value in about 30 minutes and did not increase over a period of several hours.

APPARATUS

The apparatus is constructed as shown in Figure 1. The dimensions given should be carefully duplicated to obtain satisfactory results.

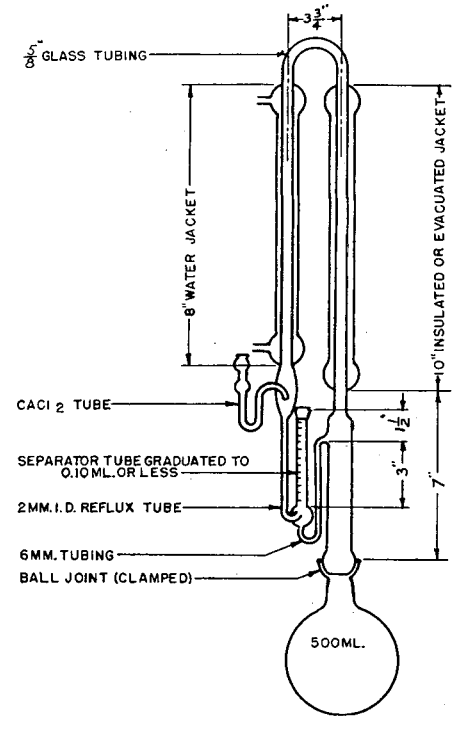

Figure 1. Apparatus

The flask is a round-bottomed, short-necked 500-ml. flask with spherical joint (such as Ace Glass Corp. No. 6902 round-bottomed boiling flask with joint No. 35/20), and is fastened to the apparatus with a quick-fastening pinch clamp (such as A. H. Thomas Company No. 3241 pinch clamp, size No. 35). The condensing

part of the apparatus is a one-piece all-glass unit with a portion of a 5-ml. Mohr type Pyrex pipet graduated in intervals of 0.1 ml. (Corning Glass Works No. 57060 pipet) as the separator tube. A calcium chloride tube is attached with rubber tubing as a guard tube to prevent atmospheric moisture from entering the apparatus. The portion shown as "insulated or evacuated jacket" may be either insulated by wrapping with asbestos paper or constructed with an evacuated jacket as shown to prevent refluxing. The flask may be heated with a gas burner, but an electric heater with rheostat (such as Fisher Scientific Co. No. 11-425) is preferable.

METHOD

Dry the apparatus carefully before use. Place 20.00 to 50.00 grams of sample, depending on the water content of the sample (preferably weighed by difference in a weighing bottle) in the dry flask by means of a powder funnel. Add 100 to 250 ml. of *o*-dichlorobenzene, depending on the sample weight taken, and connect the condensing apparatus. Apply heat slowly at first while air is being displaced from the apparatus, and then heat to boiling at a rate sufficiently rapid to carry any drops of water separated on the condenser wall downward into the separator tube. Close the separator tube with a stopper when the water rises to within about 1.25 cm. (0.5 inch) of the top. Continue the distillation until readings taken at 10-minute intervals show no further increase in the volume of water separated. Read the volume of water separated and calculate the per cent water in the sample.

NOTES. To check the performance of the apparatus, add 100 ml. of *o*-dichlorobenzene to the boiling flask, connect the condensing apparatus, and distill as described above. Remove any water which may accumulate in the separator tube and allow the contents of the flask to cool. Add 1.00 ml. of water to the *o*-dichlorobenzene in the boiling flask, connect the condensing apparatus, and distill as before, applying heat slowly at first until most of the water is distilled over. Not less than 95% of the added water should be recovered in the separator tube.

Water in the *o*-dichlorobenzene may be removed by distilling as described above, or the water content of the *o*-dichlorobenzene may be determined and used as a blank correction.

The apparatus may be dried before use by distilling *o*-dichlorobenzene in it until all the water has accumulated in the separator tube. In a series of determinations to prepare for each test, it is only necessary to remove the water in the separator tube from the previous determination by absorbing it with a strip of filter paper, and to attach another boiling flask containing the next weighed sample and a fresh portion of the solvent.

If difficulty is experienced in flushing all the droplets of water condensed on the walls of the condenser down into the separator tube, the condenser water may be shut off near the completion of the determination to allow the condenser to heat up just enough to volatilize this water and drive it into the separator tube. Before making further determinations, the condenser walls should be cleaned thoroughly with cleaning solution.

EXPERIMENTAL

When the water content of specially prepared calcium chloride hexahydrate was determined by this method, all the water of hydration was recovered, and no water was obtained in a similar trial with calcium hydroxide. None of the other possible hydrates in bleaching powder, such as the compound $CaCl_2.Ca(OH)_2.H_2O$ (*1*, *10*), was tested. However, the results (Table I) of comparative determinations, by this method and by difference, of the water content of a number of bleaching powder samples from several manufacturers indicate that all the water of hydration in bleaching powder is determined by this method. These samples were mixed thoroughly to make them more homogeneous.

The distillation method described in this paper was also compared with the federal specification distillation method (*3*) with the results shown in Table II.

DISCUSSION

The method described in this paper has been checked against water contents determined by difference and by the standard federal specification method. The agreement between the values obtained is considered satisfactory and the discrepancies are thought to be due mainly to the nonhomogeneous nature of the bleaching powder and error in reading the separated water volumes. The method permits determination of 0.1 to 2.0 grams of water, which requires bleaching powder samples of about 20 to 50 grams in weight.

Table I. Water Content of Bleaching Powder

Sample No.	1	2	3	4	5
	%	%	%	%	%
Calcium hypochlorite	72.8	11.8	31.3	32.1	36.6
Calcium chloride	4.6	39.6	33.1	32.2	31.3
Calcium chlorate	1.1	8.3	1.6	0.9	0.6
Sodium chloride	11.3	...	...	...	...
Calcium hydroxide	3.9	16.0	24.0	29.5	23.2
Calcium carbonate	2.9	6.2	3.0	2.4	2.8
Calcium sulfate	0.2	0.0	0.5	0.4	0.2
Insolubles in acid	0.5	0.4	0.2	0.2	1.0
Total	97.3	82.3	93.7	97.7	95.7
Water, by difference	2.7	17.7	6.3	2.3	4.3
Water by distillation method (av. of duplicate determinations)	2.5	17.9	6.4	2.5	4.6

Table II. Comparison of Two Distillation Methods for Determination of Water in Bleaching Powder

Sample No.	Authors' Method: Individual determinations	Authors' Method: Average	Federal Specification Method
	%	%	%
1	2.4, 2.3	2.4	2.5
2	1.1, 1.1	1.1	0.9
3	0.7, 0.7	0.7	0.6
4	2.3, 2.2	2.3	2.4
5	5.6, 5.7	5.7	5.5
6	2.6, 2.3	2.5	2.7
7	3.1, 3.1	3.1	3.1
8	9.8, 9.4	9.6	9.2
9	4.2, 4.0	4.1	4.3
10	2.3, 2.3	2.3	2.3
11	1.6, 1.8	1.7	1.8

Suter (*12*) states that in distillation apparatus in which the vapors are introduced into the top of bulb or spiral type condensers, as much as 0.3 ml. of water may be held up in the condenser without being flushed down by the continuous washing of the solvent liquid. In the apparatus described here, which has a straight tube condenser, it has been the experience that, if the condenser walls are clean, only a negligible amount of water remains on the condenser walls. However, if difficulty is experienced in flushing down all the droplets of water held on the condenser walls, the condenser water may be shut off near the completion of the determination to allow the condenser wall to heat up just enough to volatilize the droplets and drive the water completely down into the separator tube.

The fire hazard is reduced, and the solvent employed, *o*-dichlorobenzene, shows no tendency to react with the bleaching powder as has been reputed to happen with such solvents as toluene.

The method has been used satisfactorily for several years and it is believed that, with minor modifications, it could be used for determining water in materials similar to bleaching powder.

ACKNOWLEDGMENT

H. C. Biggs constructed the apparatus, and some of the determinations were made by John Coffman and Mary Torrans.

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RECEIVED October 3, 1947.

Distillation Equipment Suitable for Centigram and Decigram Quantities

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The simple and effective transfer of microliter volumes of distillate from condenser to receiver through the use of capillary siphons is described. Stills embodying this principle have been used successfully for the fractional and molecular distillation of centigram and decigram quantities.

ONE of the problems encountered in the fractional distillation of centigram or decigram quantities is the efficient transfer of distillate from condenser to receiver. Dropwise transfer which is suitable for stills of larger capacity (*1, 2, 3, 5, 7, 9, 10, 12*) is not feasible, as one drop of distillate may represent about 10% of a 200-microliter charge. In addition it is desirable that a microstill possess not only low column holdup but also low holdup between still head and receiver, a feature which would appear to preclude the use of stopcocks or other ground surfaces for the control of the reflux ratio and the transfer of distillate.

The technique used by Morton and Mahoney (*6*) and by Craig (*4*) for the collection of fractions in the distillation of decigram or smaller quantities is based upon the periodic removal of distillate from a single receiver with the aid of a capillary pipet.

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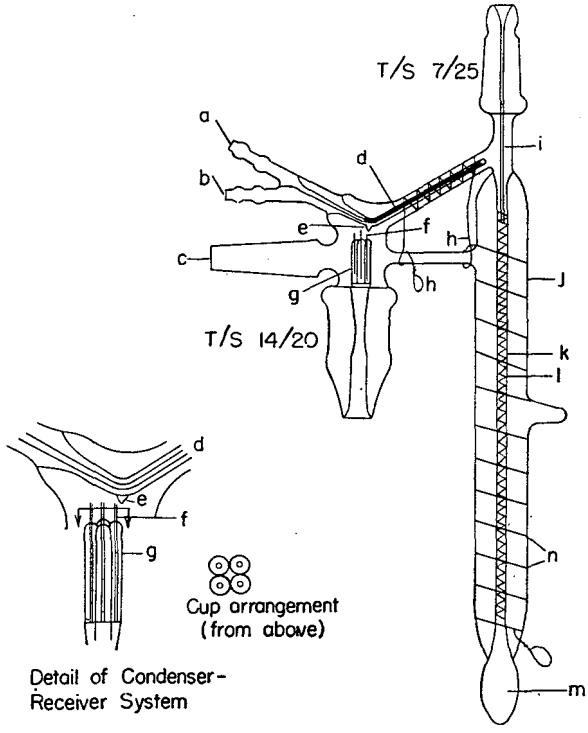

Figure 1. Microstill

a. Condenser inlet. *b.* Condenser outlet. *c.* To vacuum system. *d.* Cold finger condenser. *e.* Collecting tip for condensate. *f.* Capillary tubes, 0.2-mm. bore. *g.* Receivers, capacity 45 μl. *h.* Ends of condenser-housing heater coils, 6 to 7 turns spaced 4 to 5 mm. apart of No. 28 Chromel-A wire. *i.* Thermocouple well. *j.* Column jacket, evacuated to 10^{-6} mm. and silvered to leave clear strips 2 mm. wide in front and back. *k.* Column tube, 2.5 mm. i.d. × 150 mm. *l.* Packing, 3 to 4 turns per cm. of No. 30 Chromel wire. *m.* Boiler, capacity sufficient for 100- to 300-μl. charge. *n.* Column heater coil, 8 to 9 turns spaced 12 to 13 mm. apart of No. 28 Chromel-A wire

This procedure, while useful at times, sacrifices one or more of the desirable features associated with fractional distillations conducted on a larger scale: convenient operation at either atmospheric or reduced pressures, collection of a number of fractions without interruption of the distillation, and continuous indication of the progress of the distillation.

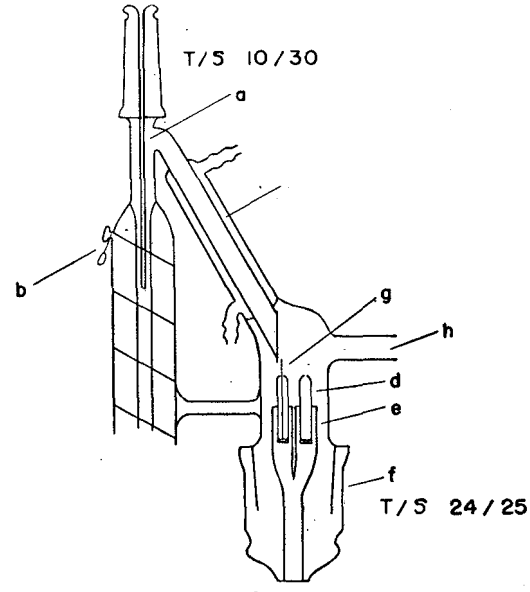

Figure 2. Semimicrostill

a. Thermocouple well. *b.* End of jacket heating coil. *c.* Condenser. *d.* Replaceable receiver cups, 3 × 20 mm. o.d. *e.* Receiver holder, 6 mm. o.d., to hold four receiver cups. *f.* Inner joint cut to give adequate clearance for receiver holder. *g.* Capillary tube, 0.2-mm. bore. *h.* To vacuum system

In this paper several stills are described which operate effectively at both atmospheric and reduced pressures and which permit the efficient collection of microliter quantities of distillate up to a maximum of four fractions without interruption of the distillation. Provision is also made for the measurement of the temperature at the still head with reflux rates as low as 5 μl. per minute. Although these stills possess low holdup, control of the reflux ratio is limited to that which is possible by careful control of the temperature of the bath surrounding the boiler, and of the condenser housing.

MICROSTILL

The microstill is illustrated in Figure 1. The condenser-receiver system is so constructed that the condensate accumulates at the collecting tip, fills the 0.2-mm. bore capillary tube which is in incipient contact with this tip, and thus passes into the receiver. At the recommended distillation rate of 5 to 20 μl. per minute the surface of the condenser will appear dry, yet distillate will flow steadily into the receiver. At higher distillation rates a drop of distillate may form on or near the collecting tip, flow down the

outside of the capillary tube, and thus escape collection in the receiver. The dimension of the capillary tube must be such as to avoid rupture of the liquid column during the course of a distillation. For liquids of high density and low surface tension—e.g., carbon tetrachloride—the maximum length of a capillary tube 0.2 mm. in inside diameter is about 18 mm.

The receivers, each 2.5 × 15 mm., are mounted upon a supporting rod sealed to a female 14/20 standard taper, so that as the joint is rotated, the center of the top of each receiver will pass under one side of the collecting tip. The tops of the receivers are constricted so as to leave a hole about 1 mm. in diameter; this constriction serves to center the capillary tube in the receiver and to minimize loss of distillate by diffusion. The amount of distillate in a receiver may be estimated visually to within 10 to 20% or determined more accurately with the aid of a telescope equipped with a filar eyepiece. Fractions are removed from the receivers with weighed capillary pipets.

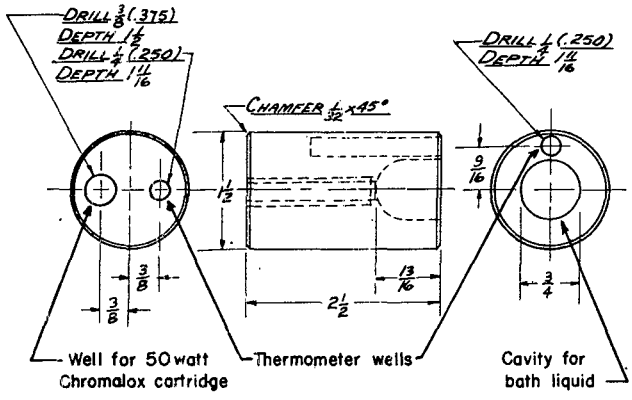

Figure 3. Heating Block

The fabrication of the condenser-receiver system illustrated in Figure 1 is difficult, though at least one manufacturer (Ace Glass Co., Vineland, N. J.) has made a number of satisfactory stills of this construction (*11*). The straight condenser of the semimicrostill (Figure 2) is considerably easier to construct and in principle would appear to be reasonably satisfactory for use in the microstill.

The column, 2.5 mm. in inside diameter × 150 mm. with a wall thickness of 0.2 mm., contains a tightly fitting helix of 3 to 4 turns per cm. of No. 30 Chromel wire. The column is surrounded by a jacket 15 mm. in diameter, evacuated to 10^{-5} mm., and silvered so as to leave two transparent strips, 2 mm. wide, so aligned as to permit observation of the column during a distillation. The heating coils on the column jacket are not ordinarily used during a distillation but serve to remove less volatile substances from the column after a distillation has been completed. At ordinary distillation rates and when the condenser-housing coils are not in operation the 5- to 10-mm. section between the jacket ring seal and the side arm functions as an air condenser, thereby permitting conditions approximating total reflux. When the condenser-housing coils are placed in operation, the temperature of the condenser housing and adjacent regions may be raised to the point where a fraction of the vapors will pass into the side arm and be collected on the condenser.

The temperature at the still head may be measured with the aid of a single-junction iron-constantan (4 mil) thermocouple inserted into the thermocouple well, 0.5-mm. bore and 0.1-mm. wall thickness, which extends 5 to 10 mm. below the top of the silvering of the column jacket. At reflux rates of more than 5 μl. per minute accurate temperature measurements may be made. Distillation rates of 5 to 20 μl. per minute can be maintained with a quiet distilland surface and for this reason no boiling aid is required.

Accessories needed for the operation of the microstill include a vacuum line, a potentiometer, and a heating block. The block (Figure 3) is used as an oil bath for the microstill or horizontally as an air bath for the molecular still (Figure 4). The temperature of the block is controlled with the aid of a variable autotransformer.

The microstill was found to have an efficiency equivalent to ca. 9 theoretical plates with a column holdup of 20 to 30 μl.

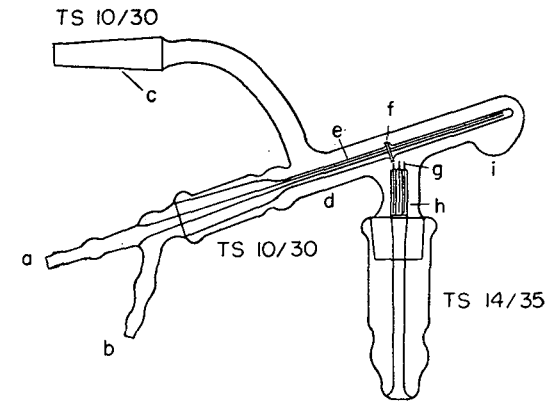

Figure 4. Molecular Still

a. Condenser inlet. *b.* Condenser outlet. *c.* To vacuum system. *d.* Still housing. *e.* Cold finger condenser. *f.* Flange for collecting condensate. *g.* Capillary tubes, 0.2-mm. bore. *h.* Receivers, capacity 45 μl. *i.* Boiler, capacity 200 μl.

when tested with carbon tetrachloride–benzene mixtures (*8*). The performance of the still in several typical distillations may be judged by the data given in Figure 5.

SEMIMICROSTILL

The semimicrostill (Figure 2) is simply a larger version of the microstill, except for the modifications in the receiver housing.

The column is 300 mm. in length and 5.5 mm. in inside diameter, and is made of Pyrex thin-walled tubing. Without any packing the column showed about the same efficiency as the microstill. In the semimicrostill, because of the need for greater flexibility, the boiler flask is attached to the column with a standard taper. A second and desirable modification in this still is the use of replaceable receiver cups (capacity about 150 μl.) which obviated

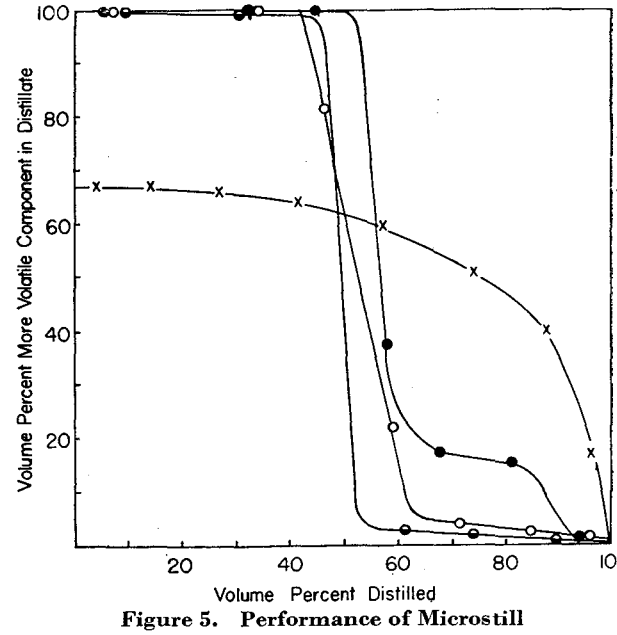

Figure 5. Performance of Microstill

●. 1:1 mixture of 2-chlorovinyldichloroarsine and benzyl chloride; vol. of distillate, 160 μl.; pressure, 10 to 25 mm.; bath temp., 96.5–122° C; time, 160 min.

○. 1:2 mixture of 2-chlorovinyldichloroarsine and bis(2-chloroethyl) sulfide; vol. of distillate, 283 μl.; pressure, 1.5 to 10 mm.; bath temp., 92–127° C.; time, 210 min.

◒. 2:1 mixture of 2-chlorovinyldichloroarsine and crude bis(2-chloroethyl) sulfide; vol. of distillate, 293 μl.; pressure, 2 to 10 mm.; bath temp., 115–125° C.; time, 240 min.

×. 1.8:1 mixture of carbon tetrachloride and benzene; vol. of distillate, 213 μl.; pressure, 745 mm.; bath temp., 92–98° C.; time, 200 min.

Composition of fractions determined by specific gravity measurements.

the use of a number of receiver assemblies in case more than four fractions were desired.

MOLECULAR STILL

The receiver of the molecular still (Figure 4) is identical with that of the microstill.

The distillate is collected upon the flanged section of the straight, inclined cold-finger condenser and transferred to the receivers with the aid of capillary tubes 0.2 mm. in inside diameter. In operation, the boiler, containing a 50 to 150-μl. charge, is inserted into the cavity of the heating block clamped with its long axis horizontal. At the pressure obtainable with a diffusion pump, or with a good mechanical pump, high boiling oils—e.g., dibutyl phthalate—could be distilled at a rate of 20 μl. per minute at temperatures below 50° C. when ice water was passed through the condenser.

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RECEIVED October 27, 1947. Contribution 1155 from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, Calif. Based upon work done for Office of Scientific Research and Development under Contract OEMsr-325 with the California Institute of Technology.

Microdetermination of Bismuth in Biological Material

An Improved Photometric Dithizone Method

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An improved photometric dithizone method has been developed for the determination of bismuth in biological material, applicable to 100 ml. or less of urine and 20 grams or less of blood. Bismuth is originally isolated from the highly diluted digested material, using a solution of dithizone in chloroform (60 mg. per liter), lead is separated with a buffer solution of pH 3.4, and bismuth is finally determined spectrophotometrically. The accuracy is within ±0.1 microgram of bismuth for the range 0 to 10 micrograms and ±0.5 microgram for the range 0 to 50 micrograms. Only one strength of dithizone solution is used throughout the complete procedure. Although used specifically for urine and blood, the method can be applied to other biological material. Bone samples may require special treatment.

AN IMPROVED photometric dithizone method for the determination of bismuth in biological material has been developed recently and applied in this laboratory. Following preparation of samples by the regular wet-ashing procedure (*5*), bismuth need not be isolated from mixtures of extraneous salts as the sulfide (*4*), but can be extracted directly and quantitatively as bismuth dithizonate. Separation from the lead dithizonate extracted simultaneously is effected at pH 3.4, as indicated by Bambach and Burkey (*1*), the final estimation of bismuth being made at high pH, as described by Snyder (*6*). The sensitivity of the procedure is equivalent to that of the sulfide separation (*4*).

REAGENTS AND APPARATUS

Ordinary high-grade chemicals may be used throughout the method. Eastman dithizone may be used without further purification. The chloroform used may be a freshly obtained product after redistillation from Pyrex or used chloroform reclaimed by a procedure previously described (*1*).

All Squibb-type (Pyrex) separatory funnels used must be cleaned meticulously with hot 50% by volume nitric acid and distilled water to ensure removal of any bismuth or lead present as surface contamination from previous use (*4*). The same applies to the matched pairs of cells used for density measurements.

Any standard spectrophotometer may be used for measurements of density. Instruments must be capable of isolating a narrow band centered at 505 mμ.

PROCEDURE

Preparation of Samples. Samples of biological material, 100 ml. or less of urine, or 20 grams or less of blood or tissue, are prepared for analysis by a wet-ashing method (*5*). The digestion is made in a distilling flask, 1 liter in volume, provided with three necks possessed of interchangeable ground-glass connections, into which are fitted the distilling head and two separatory funnels. This method of digestion involves the addition of 20 ml. of concentrated sulfuric acid (specific gravity 1.84), 5 ml. of perchloric acid (70 to 72%), and 20 ml. or more of nitric acid (specific gravity 1.42). The digested sample is allowed to cool, 50 ml. of distilled water are added, and the solution is transferred quantitatively to a 400-ml. Pyrex beaker. The beaker and contents are placed in an ice water bath, 15 ml. of 40% w/v ammonium citrate solution and 50 ml. of 20% w/v sodium sulfite solution are added; then, with stirring, 100 ml. of ammonium hydroxide (specific gravity 0.9) are also added.

Extraction 1. The prepared sample is transferred quantitatively to a 500-ml. Squibb-type separatory funnel. After the addition of 5 ml. of 10% w/v potassium cyanide solution, the mixture is diluted to 400 ml. with distilled water and mixed thoroughly. Bismuth and lead are extracted as the dithizonates by successive additions of 10-ml. portions of dithizone in chloroform (60 mg. per liter) until the last portion shows no color change. For amounts of bismuth not exceeding 10 micrograms, three portions of dithizone solution suffice. If the amount of bismuth exceeds 10 micrograms but is not over 50 micrograms, four portions of dithizone solution are necessary. Each additional 10-ml. portion is roughly equivalent to 50 micrograms of bismuth. The combined extract is collected in a 125-ml., graduated, Squibb-type separatory funnel.

Depending upon the amount of bismuth present, either the whole or an aliquot of the total previous extract, containing not more than 50 micrograms of bismuth, is taken for Extraction 2, and washed with 50 ml. of distilled water. After separation of the two phases, the chloroform phase is transferred to a clean separatory funnel and the aqueous phase is shaken with 5 ml. of dithizone in chloroform (60 mg. per liter); the latter is added to the chloroform phase but the wash water is discarded.

Extraction 2 (Removal of Lead.) The chloroform phase containing bismuth and lead dithizonates plus excess dithizone is shaken for 30 seconds with 50 ml. of buffer solution, pH 3.4 (*1*), and the chloroform phase is transferred to a clean separatory

Table I. Analysis of Urine

Range Used, Micrograms	Bismuth Added, Micrograms	Bismuth Found, Micrograms
0–10	0	0.1
0–10	0	Nil
0–10	1	0.9
0–10	1	1.0
0–10	5	5.0
0–10	5	5.1
0–10	10	10.1
0–10	10	9.9
0–50	10	10.5
0–50	10	10.0
0–50	30	30.0
0–50	30	29.5
0–50	50	50
0–50	50	50
0–50	500	505
0–50	500	495

funnel. (Separatory funnels used must be scrupulously clean.) The buffer solution is shaken with an additional 5 ml. of dithizone in chloroform (60 mg. per liter) and this is added to the main chloroform fraction. The aqueous buffer solution containing lead is discarded. (In the presence of large amounts of lead, it will be necessary to repeat the above treatment in order to make certain that all of the lead has been removed.) The combined lead-free chloroform fraction is next shaken for 30 seconds with 25 ml. of 1% v/v nitric acid and the chloroform fraction is removed to another clean separatory funnel. The treatment with 25 ml. of 1% v/v nitric acid is repeated, the acid fractions being combined while the chloroform fraction is discarded. The combined acid phase is washed with 5 ml. of clear chloroform to remove traces of dithizone, and all the excess chloroform is drawn off. (Care must be taken not to allow the dilute acid phase to enter the bore of the stopcock.) The bismuth is now present in 50 ml. of 1% v/v nitric acid.

Extraction 3 (Estimation of Bismuth). Twenty milliliters of ammonium hydroxide (specific gravity 0.9) containing 0.2 gram of potassium cyanide (10 grams of potassium cyanide per liter of ammonium hydroxide w/v) and 15 ml. of dithizone in chloroform (60 mg. per liter) are added and the mixture is shaken for one minute. A small pledget of cotton is placed in the tip of the separatory funnel stem, and 5 ml. of the chloroform phase are filtered through and discarded. The proper sized cell is then filled with the filtered chloroform layer and its optical density (or transmittancy) is obtained at 505 mμ. A blank consisting of 50 ml. of 1% v/v nitric acid is also treated as in Extraction 3 in order to determine the zero bismuth reading. (One blank is sufficient for a series of samples dealt with on any one day.) The readings are then evaluated from charts prepared beforehand from known amounts of bismuth treated as in Extraction 3. Two ranges are used, 0 to 10 micrograms and 0 to 50 micrograms, depending on the cell lengths. One calibration chart is derived for each range. The zero in each case is used to determine the chart zero and readings are evaluated from a fresh curve drawn parallel to the original calibrated curve, passing through the zero of the blank. The cells used are cylindrical, having optically plane ends and an internal diameter of 14 mm. These cells must be meticulously cleaned and dried before use.

ANALYTICAL RESULTS

In Table I are listed results obtained by the analysis of 100-ml. urine samples (in duplicate) containing known added quantities of bismuth. In Table II are listed results obtained by the analysis of 20-gram samples of rabbit blood (in duplicate) containing known added quantities of bismuth.

DISCUSSION

Stannous tin and monovalent thallium do not interfere and lead is removed during Extraction 2 with the buffer solution, pH 3.4. Stannous tin as such is oxidized during the sample preparation and therefore it does not interfere (*3*). The same holds for thallium (I). Thallium (III) is not extracted during the initial extraction step which involves a pH of approximately 10. Should thallium (I) by chance avoid oxidation and thus be extracted during the initial extraction step, it would be subsequently removed at pH 3.4 during the removal of lead.

Bismuth is quantitatively extracted (Extraction 1) even in the presence of ammonium citrate, calcium, ferric, and phosphate ions, and chloride and other extraneous ions present in the original digest, because of a high dilution factor, a high pH of approximately 10, and a relatively strong solution of dithizone in chloroform.

In dealing with minute quantities of bismuth in the presence of unusually large quantities of lead (Extraction 2), one may find that the bismuth cannot be shaken completely into 1% v/v nitric acid because the great excess of free dithizone liberated from the lead dithizonate during the pH 3.4 wash tends to hold bismuth in the chloroform phase. However, if a stronger acid solution, such as 5% v/v nitric acid, is used, all the bismuth will strip into the stronger acid. When such samples are encountered, it will be necessary to re-extract the bismuth, including the pH 3.4 wash, before proceeding to the final colorimetric extraction. For samples examined by this laboratory, such a step has not been required, but it is given as a precautionary procedure for determining small amounts of bismuth in the presence of large amounts of lead.

Table II. Analysis of Blood

Range Used, Micrograms	Bismuth Added, Micrograms	Bismuth Found, Micrograms
0–10	0	Nil
0–10	0	Nil
0–10	1	1.0
0–10	1	0.9
0–10	5	5.0
0–10	5	4.9
0–10	10	9.8
0–10	10	10.0
0–50	10	10.5
0–50	10	10.0
0–50	30	30.5
0–50	30	30.5
0–50	50	50
0–50	50	50
0–50	500	505
0–50	500	500

For Extraction 3, one strength of dithizone in chloroform (60 mg. per liter) is used for both ranges—namely, 0 to 10 micrograms and 0 to 50 micrograms of bismuth. The high pH of approximately 11.5 obtained in Extraction 3 permits the removal of the major part of the excess dithizone to the aqueous phase, and, in effect, ammonium dithizonate is actually used for the quantitative extraction of bismuth. It has been found convenient to store the solution of potassium cyanide in ammonium hydroxide (10 grams of potassium thiocyanate per liter of ammonium hydroxide w/v) in a refrigerator. The sensitivity of extraction 3 is ±0.1 microgram of bismuth for the range 0 to 10 micrograms and ±0.5 microgram for the range 0 to 50 micrograms.

Although used specifically for urine and blood, the method can be readily applied to other biological material. Some difficulty may be encountered in analyzing bone, but small samples may be analyzed, provided the phosphate content is not greater than that normally found in 100 ml. of urine. Larger bone samples are best handled by introducing an initial step of bismuth separation as the sulfide, solution of the sulfide in nitric acid (*2*, *4*), and subsequent treatment as outlined above, starting with "Preparation of Samples."

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RECEIVED October 11, 1947. Presented before the Division of Analytical and Micro Chemistry at the 112th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y.

Trace Metal Determination in Fats

With Special Reference to Copper in Milk Fat

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A simple method of sample preparation for the determination of copper in fats is described. A new approach is used in which 50-gram samples of the fat can be prepared for dry ignition in a muffle furnace in a reasonably short time. As many as twelve samples can be prepared for ignition by a single operator at one time. Copper is finally determined polarographically or colorimetrically. Precision of the method utilizing a polarographic procedure is estimated by treatment of collaborative data. Presumably the method is applicable to the determination of other traces of metals in fats and oils.

TO STUDY the deleterious effect of a minute amount of copper on the stability of milk fat it became necessary to assay a great many samples with the greatest possible precision. A monocolor dithizone procedure (*1*) has been more or less adapted as standard for the determination of copper in foods in this laboratory. Although highly satisfactory for the usual types of foods, this method lacks precision when the copper content is extremely low, as demonstrated specifically for dry whole milk containing copper in concentrations below 2.0 p.p.m. (*2*).

The limiting factor in the determination of copper in foods is the size of sample that can be handled satisfactorily. In many cases precision can be improved by increasing the size of the sample taken for analysis. However, in nearly all procedures a necessary first step is the destruction of the organic matter by either acid digestion or ignition, and because of labor and loss of time only a small sample size is practical. Increasing the sample size to obtain a satisfactorily measurable amount of copper will also extend the time and labor involved in destruction of organic matter.

Milk fat, or for that matter any fat or oil, presents a particularly troublesome problem in that it contains copper in the extremely low concentrations of 0.1 to 0.25 p.p.m. The destruction of organic matter in fats by acid digestion is exceedingly long and tedious. Increasing the sample size beyond 10 or 15 grams extends the time and labor involved in the digestion beyond practicable limits. Ignition techniques are also rather long and tedious, and usually result in a substantial loss of the sample during the charring procedure. This loss is undoubtedly attributable to a mechanical carry-off of sample by volatilization or creepage. The proposed method involves a new type of charring technique prior to ignition in a muffle furnace and makes possible the preliminary ashing in approximately 2 hours. Furthermore, as many as twelve samples can be prepared for ignition by a single operator at one time. The proposed procedure is primarily a different approach to sample preparation, and the discussion of the mechanics of the method is largely limited to this phase.

It is highly desirable that any new method be compared with one or more accepted methods. For the purpose of this investigation, acid digestion of the sample followed by spectrophotometric estimation of the copper as a dithizone complex (*1*) was chosen as the standard by which reliable comparative data could be obtained. Although the comparative method selected is one of the most sensitive, it was found necessary to digest 50 grams of milk fat in order to obtain a satisfactorily measurable amount of copper with any degree of precision. This was accomplished by digesting five 10-gram samples and combining the digests. This operation required constant attention for 12 to 16 hours. Thus only a single determination could be made at one time, with accompanying exceedingly high blanks. Various attempts to digest 40- to 50-gram samples singly—i.e., not in increments—gave no savings in time nor labor, and, in the majority of the cases, the digestions had to be abandoned before they were completed. Though the data presented in Table I for the comparative method are few in number, they are considered the best obtainable by other than the proposed method.

The assumption was made that the copper in milk fat would be in a state similar to that in other dairy products—namely, bound in the protein. Thus, the first attempt to arrive at a rapid means of sample preparation was direct extraction with perchloric acids at pH 1.0 (*2*). This technique accounted for only 25 to 50% of the copper found by either the proposed or comparative method. Various combinations of mineral acids and solvents yielded low results, varying from 10 to 40% of the indicated true value. Attempts at extraction utilizing complexing agents such as cyanide and a mixture of pyridine and thiocyanate were equally unsuccessful. In all, some eighteen different attempts at extracting the copper from the milk fat were tried; none was found satisfactory.

Charring of a large sample of oil is difficult (*3*), tedious, and time consuming but it appeared that it would have to be relied upon, followed by ignition in a muffle furnace. Low and varying copper data indicated that charring techniques resulted in mechanical loss of ash through creepage and volatilization. Dropping the oil into a hot Vycor crucible gave low and varying results, but was rapid in that a large portion of the oil was eliminated by volatilization, leaving only a small amount of char. It was reasoned that the low data obtained were due to a mechanical carry-off of sample during the volatilization. This was prevented

Table I. Comparative Analytical Data

	No. of Detns.	Copper Content			Standard Deviation
		Mean	Max.	Min.	
		P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*
Milk fat A					
Proposed method	8	0.11	0.15	0.05	0.029
Comparative method	4	0.10	0.17	0.05	0.071
Milk fat B					
Proposed method	6	0.11	0.14	0.08	0.024
Comparative method	1	0.11	...	..	...
Milk fat C					
Proposed method	22	0.09	0.16	0.05	0.029
Proposed method, modified[a]	21	0.09	0.14	0.05	0.028
Milk fat D					
Proposed method, modified[a]	14	0.17	0.31	0.10	0.063
Comparative method	2	0.21	0.21	0.20	...
Hydrogenated cottonseed oil (200-hour A.O.M.[b] stability)					
Proposed method	9	0.19	0.25	0.16	0.024
Comparative method	5	0.14	0.25	0.04	0.084

[a] Modification consisted of estimating copper content by monocolor dithizone procedure following ashing technique as outlined in proposed procedure.
[b] Active oxygen method.

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by inserting a mat of glass wool (Figure 1) about midway between the top and bottom of the crucible. The oil was dropped below the mat by employing a small funnel. As the oil dropped on the bottom of the hot crucible, it underwent a combination of charring and volatilization, but the vapors were filtered through the glass wool, which in effect acted as a filter for the retention of nonvolatile matter. This technique, followed by polarographic estimation, gave comparable results as shown in Table I.

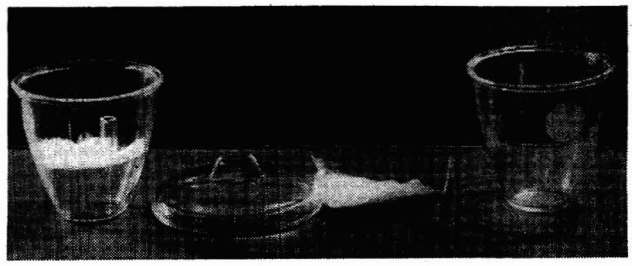

Figure 1. Ignition Crucible, Assembled and Unassembled

The method proposed below is based on the charring techniques described. Final ignition is accomplished in a muffle furnace at 450° C. Although a polarographic procedure has been adapted for the final estimation, other procedures may be employed. This is demonstrated in Table I for milk fat C; the proposed method was compared with a modification in which the ashing technique outlined in the proposed procedure was followed by application of a monocolor dithizone method. The data compare well.

As many as twelve determinations can be carried out at one time by one analyst. A convenient arrangement for conducting multiple charring is shown in Figure 2.

SAMPLE PREPARATION

Apparatus. Pyrex Ware. All Pyrex ware, Vycor crucibles, and crucible covers must be thoroughly cleaned by washing with concentrated nitric acid, and rinsed thoroughly with tap water and finally several times with redistilled water.

Glass Wool. Glass wool is cleaned in the same manner as Pyrex ware and stored in a covered container to prohibit metal contamination.

Vycor Crucibles, 50-ml. capacity with covers. It is not advisable to use crucibles that have been noticeably etched.

Pyrex Microfunnels, prepared by drawing out a piece of 5-mm. Pyrex tubing so that the drawn end has an internal diameter of 1 to 2 mm. The glass tubing is cut so that the over-all length is approximately 20 to 30 mm. (see Figure 1).

Pyrex Dropping Funnels, 125-ml. capacity.

Assembling Apparatus for Preliminary Ignition. Prepare Vycor crucibles by inserting a mat of glass wool about 5 mm. thick approximately equidistant from top and bottom of crucible. Insert a microfunnel so that the small end completely passes through the glass wool mat, near the wall of the crucible (Figure 1). Set up the crucibles and dropping funnels so that the melted milk fat may be dropped through the microfunnel at a rate of about 1 drop every 4 or 5 seconds while the crucible is heated over a Bunsen burner (Figure 2).

PROCEDURE

Melt the fat and stir thoroughly to ensure a homogeneous sample. Transfer a small portion of the liquefied fat to the dropping funnel, revolve the funnel so that the inner surface is thoroughly wetted, and allow to drain completely, discarding the drained fat. This step is necessary to account for that portion of the weighed sample which because of incomplete drainage would otherwise remain in the dropping funnel.

Transfer 50 grams of the melted fat, weighed by difference, to the dropping funnel. Adjust the burner so that with continuous heating the bottom of the crucible will remain just below redness. Allow the oil to drop through the microfunnel on the bottom of the hot crucible, but not directly on the glass wool. Charring and volatilization will occur. The glass wool mat filters the vapors and prevents mechanical carry-off during volatilization. Check frequently to ensure a drop rate of 1 drop every 4 to 5 seconds. Do not allow an excessive accumulation of oil in the bottom of the crucible, since creepage over the sides may occur with subsequent loss of copper. Continue charring and volatilization until all the oil has drained into the crucible. After complete drainage continue heating for 5 minutes.

Place cover on crucible in such a manner that the contents will have free access to air. A small, bent Pyrex rod adequately serves this purpose. Transfer to an electric furnace and ignite at 450° C. until all carbon particles are destroyed.

When ignition is complete, remove from furnace, cool, and cautiously add 5 ml. of concentrated hydrochloric acid. Using a Pyrex stirring rod, press the glass wool firmly into the bottom of the crucible and add just sufficient metal-free redistilled water (about 10 ml.) to cover the glass wool. Transfer the acid solution to a 50-ml. Pyrex beaker by filtering through a small pledget of glass wool in a small funnel, leaving the glass wool in the crucible. Complete the transfer by washing with small portions of redistilled water until the final volume of solution in the beaker is approximately 40 ml. Cautiously evaporate the solution in the beaker to dryness. At the same time evaporate 5 ml. of concentrated hydrochloric acid which is to be carried through all subsequent steps as a blank. Infrared heating lamps are ideally suited to this purpose, as they will allow rapid evaporation without loss due to spattering.

DETERMINATION OF COPPER

Any of the more sensitive methods may be used for determining the copper, once the sample has been prepared. The authors have used both colorimetric and polarographic estimation, but prefer the latter.

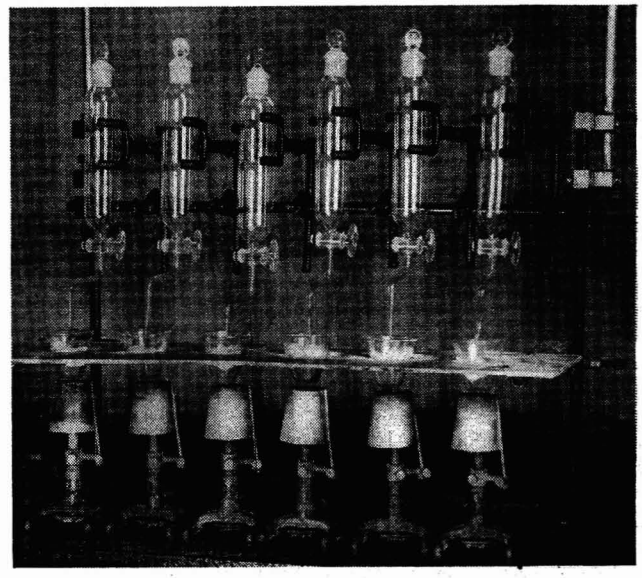

Figure 2. Convenient Arrangement for Preparing Six Samples at One Time

The polarographic technique employed in obtaining the data presented in this paper is essentially the same as used by Cranston and Thompson (*2*). However, very small step heights were obtained, due to the extremely low concentrations of copper in fat samples. The original procedure, using an internal standard technique, required the addition of a standard to the electrolyte solution of the sample, resulting in dilution and lowered precision. For this reason an internal standard technique was used in which the copper standard was combined with the electrolyte. Thus, additional dilution was avoided and better precision in instrumentation was made possible by measuring greater concentrations of copper, the concentration of the copper due to the sample being represented by the difference in the step heights of sample plus standard and standard alone.

The addition of the combined standard-electrolyte to the ashed samples of some types of fats results in the precipitation of phosphates, and when samples much in excess of 50 grams are employed, some of the copper tends to be occluded by the phos-

Table II. Recovery of Copper Added to a Single Sample of Milk Fat

Copper Added[a] P.p.m.	Total Copper Calculated[b] P.p.m.	Total Copper Found P.p.m.
0.26	0.36	0.33
0.26	0.36	0.30
0.26	0.36	0.37
0.26	0.36	0.30
0.26	0.36	0.33
0.26	0.36	0.32

[a] Copper oleate dissolved in oleic acid, aliquots added to 50-gram sample of milk fat.
[b] Mean of 6 determinations of sample used as base copper content.

Table III. Collaborative Data on Copper

Lot	Sample No. 1	2	3	4	Sample No. 1	2	3	4
	Laboratory A, P.p.m.				Laboratory B, P.p.m.			
A	0.05	0.06	0.09	0.10	0.05	0.05	0.14	..
B	0.08	0.03	0.06	0.09	0.09	0.05	0.11	0.11
C	0.09	0.08	0.07	0.08	..	0.05	0.08	0.13
D	0.10	..	0.12	0.08	0.12	0.14	0.12	0.19
E	0.15	0.05	0.16	0.08	0.11	0.14	0.05	0.17
F	0.09	0.08	..	0.07	0.05	0.06	0.12	0.11

phate, which may result in low copper recovery. No difficulty has been encountered with the small amounts of precipitate formed when the sample size is confined to 50 grams.

The proposed procedure was applied to a sample of hydrogenated cottonseed oil (Table I) and found as satisfactory as for the milk fat. This type of sample preparation should have wide application in studies on fats and oils, and should be applicable to the determination of traces of metals other than copper.

ESTIMATION OF PRECISION

Where there exist no adequate methods of assaying for comparative purposes, it is difficult to prove the adequacy of a new method, particularly when the concentration of the substance sought is so low that it is nearly quantitatively undetectable. The concentration of copper in milk fat is around 0.1 p.p.m. and seldom exceeds 0.2 p.p.m. It is usually conceded that replicate data checking within ±0.05 p.p.m. are satisfactory, but when the concentration is 0.10 p.p.m., a deviation of ±0.05 p.p.m. becomes enormous on a percentage basis—for example, with milk fat containing 0.1 p.p.m. of copper the data might be expected to vary from 0.05 to 0.15 p.p.m., or from 50 to 150% of the actual copper content. The problems of proving the precision of such methods are obvious. The difficulty becomes more apparent when no satisfactory methods exist by which comparative data can be obtained. In such instances the data must be obtained under the best possible conditions and treated in such a manner that the precision of the method can be indicated.

The difficulties of obtaining comparative dithizone data utilizing acid digestion for sample preparation have been described. Complete recovery of copper added to the milk fat would be considered valuable evidence, if the added copper is approximately similar to the naturally occurring copper. Data on the recovery of copper added to milk fat in the form of copper oleate (Table II) indicated that the method was adequate.

The burden of proving the precision of the method was placed upon data obtained on identical samples run by two laboratories (Table III). Six lots of milk fat were prepared by a collaborating laboratory. Eight samples were obtained from each lot under conditions which would ensure the best possible uniformity. Four samples were run by each laboratory and the results were critically examined for both intra- and interlaboratory replication. Each laboratory ran one determination on each sample, and only those data which were obviously in error (spilled, instrument failure, etc.) were discarded.

The inter- and intralaboratory precisions of the method are indicated in Table IV, which shows means and standard deviations; the two laboratories were able to obtain consistently comparable results. The interlaboratory checks are nearly identical with the intralaboratory checks, and it appears that the precision of the method is such, that by careful application of the techniques it is possible to check interlaboratory data as readily as intralaboratory data.

In order to check the precision further, data were obtained on the same samples, utilizing the identical techniques, except that the ash was taken up in hydrochloric acid and determined colorimetrically by the dithizone procedure. Unfortunately the collaborating laboratory was not equipped to run colorimetric determinations, so interlaboratory comparison by this procedure was not possible. As shown in Table I, milk fat sample D, the agreement was satisfactory.

If the means and standard deviations are obtained by utilizing all the data irrespective of lots, it is possible to estimate the precision that may be expected over a large number of samples within a laboratory and between laboratories. This can be considered valid only on the basis that the lots were approximately the same and that the mean of any one lot was well within one standard deviation of the mean of any other lot. Table V presents the "probable precision" of the method, utilizing all the data.

It can be reasonably assumed that the precision estimations would improve with increasing copper contents. If, however, the deviations remained in the same order regardless of copper content, the analytical data obtained on samples of higher copper content would be expected to be even more precise. For example, if a sample of 0.10 p.p.m. is analyzed it can be assumed that 68% of the results would fall within 0.10 ± 0.04 p.p.m. (60 to 140% of the mean); but if a sample of 0.50 p.p.m. is analyzed the results will be within 0.50 ± 0.04 p.p.m. (92 to 108% of the mean). The deviation is far more significant in the former than in the latter. Thus if the precision is estimated at the low level of copper content and found satisfactory, concern regarding the precision of the method at higher levels of copper concentration appears needless.

Table IV. Indicated Precision of Method

	Intralaboratory					
	Laboratory A		Laboratory B		Interlaboratory	
Lot	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
A	0.08	0.020	0.09	0.043	0.08	0.032
B	0.07	0.023	0.09	0.024	0.08	0.028
C	0.08	0.002	0.09	0.033	0.08	0.023
D	0.10	0.016	0.14	0.033	0.12	0.032
E	0.11	0.046	0.12	0.045	0.11	0.049
F	0.08	0.008	0.09	0.031	0.08	0.024

Table V. Probable Precision of Method

	Lab. A	Lab. B	Labs. A and B
Mean	0.09	0.10	0.09
Standard deviation	0.029	0.041	0.037

Particularly significant when working with samples of low copper content is the number of determinations required to give a reliable mean. This number can be predicted by calculating the standard error of the mean. The standard error, assuming normal distribution, is a measure of the variation to be expected in future determinations of the mean—the standard error is to the mean and its variation as the standard deviation is to the individual determination. The mean of any number of determinations will not deviate from the true mean by more than two standard errors 95% of the time. Presented in Table VI are the standard errors and the expected variations for a varying number of determinations. Calculations were made using the standard

Table VI. Predicted Maximum Variation from True Mean with Varying Number of Assays

No. of Determinations per Sample	Standard Error of Mean	Predicted Maximum Variation from True Mean
2	0.040	±0.08
3	0.028	±0.06
4	0.024	±0.05
5	0.020	±0.04
6	0.018	±0.04

deviation 0.04 (mean 0.10) which appears to be the greatest deviation for either laboratory or for the total data of both laboratories. Table VI ignores the 5% of the cases which will exceed two standard errors.

Thus the advisability of running more than two determinations is apparent. At least three and preferably four determinations are necessary, whereas little can be gained in precision by running five or more.

ACKNOWLEDGMENT

Sincere acknowledgment is made to E. K. Nielsen, Kraft Foods, who prepared the milk fat samples and participated in the collaborative assays by which the precision of the procedure was established.

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RECEIVED July 24, 1947.

Microdetermination of Alkoxyl Groups

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A modification of the alkoxyl determination has given excellent results with esters and volatile substances. Apparatus and technique have been modified to include the features of the setups described by two previous authors. A new simple method of obtaining a satisfactory quality of hydriodic acid is described. The silver iodide precipitate is purified in a new manner that makes the results more accurate.

THE determination of alkoxyl groups using the original apparatus of Pregl (*5, 9–12*) gives low results with many compounds, such as volatile substances and esters which split off the alcohol on immediate contact with hydriodic acid. Low values have also been reported for substances containing more than one methoxyl group.

Elek (*2*) reviewed various modifications of the determination proposed to overcome this difficulty and described apparatus and a modified technique which gave good results. The essential parts of his apparatus are the introduction of a water-cooled reflux condenser and a more efficient absorption tube for the alkyl iodide. He allowed more time for the reaction to take place and used propionic in place of acetic anhydride.

Furter (*4*) described a setup for the determination of either alkoxyl or alkimide groups which gave excellent results with both esters and very volatile substances, including ethyl ether. The essential feature of his apparatus is an extra reaction vessel through which the gases pass, ensuring complete conversion to the alkyl iodide.

The apparatus described here is a modification of the one used by Furter, to which the Elek reflux condenser has been added. The reaction mixture is allowed to stand at room temperature for some time before heating begins and, in general, the time of the determination has been considerably increased, as in the Elek procedure.

The hydriodic acid generally purchased was found unsuitable for use without purification, whether reagent grades or those designated as special for the microdetermination of methoxyl were used. They often contain hydrogen sulfide, phosphine, and possibly alkyl iodides formed from the reaction of the acid vapors on the material used to cover the bottle stoppers. These give large blanks, sometimes the amount of precipitate of silver iodide is equal to or greater than that obtained in actual determinations. Clarke (*1*) describes a procedure for preparing a colorless acid suitable for the determination, which uses hypophosphorous acid followed by distillation. However, for the past seven years the author has been treating ordinary reagent grades of hydriodic acid by a simple procedure suggested by Knoll (*6*). The acid, specific gravity 1.7, is refluxed (air condenser) for about 2 hours while a stream of carbon dioxide or nitrogen is bubbled through. The color of the acid is of no importance; even the very dark grades give no blank. When stored in brown glass-stoppered bottles at laboratory temperatures, the acid continues to give good results for a number of

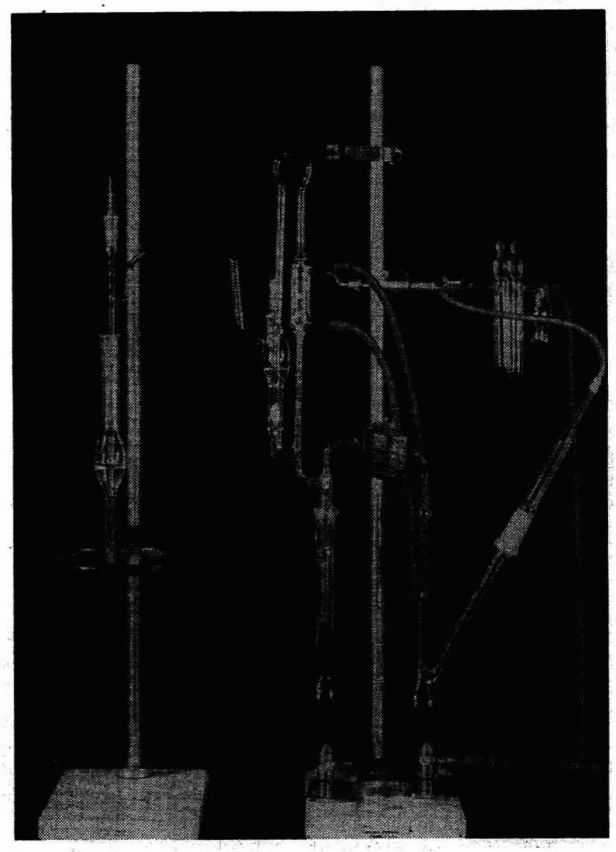

Figure 1. Apparatus

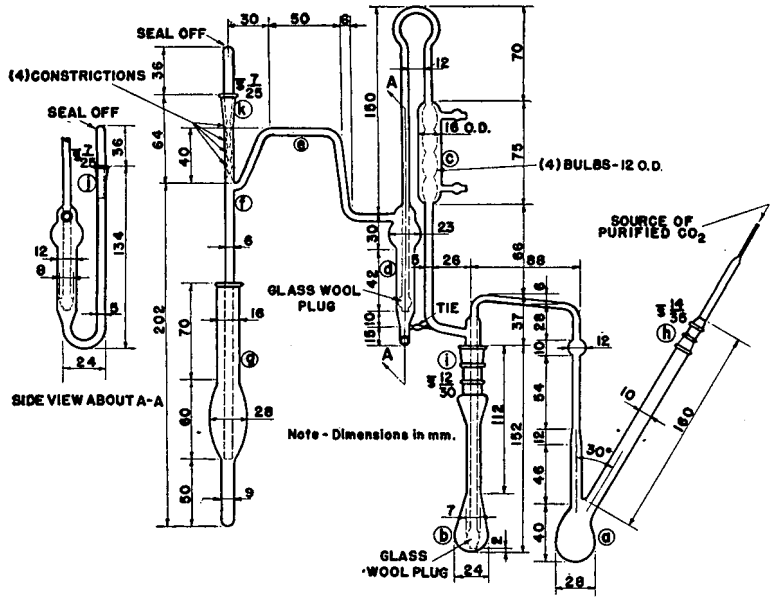

Figure 2. Diagram of Apparatus

weeks (test samples should be run at frequent intervals), although it is repeatedly stated in the literature that the acid should be colorless and fresh (*1*, *5*, *8–12*). Any dissolved iodine present should be advantageous, since Elek (*2*) showed that addition of iodine to the reaction mixture converts organic bound sulfur to the elementary form, preventing its interference.

In this laboratory, the gravimetric procedure is preferred and for about seven years the silver iodide has been washed with cold concentrated nitric acid, specific gravity 1.42. This treatment removes any silver sulfide which might be obtained during the determination, as well as any reduced silver caused by allowing the acid alcoholic silver nitrate to stand too long before filtration. This procedure always yields a pure yellow precipitate without danger of loss by solution in the nitric acid. Samples of pure silver iodide precipitate were washed many times with large amounts of acid in a filter tube with no loss in weight. However, silver iodide is soluble in cold fuming nitric acid.

Figure 1 is a photograph of the apparatus, while Figure 2 gives details of construction.

The apparatus consists of a reaction flask, *a*, which has an inlet tube for the passage of carbon dioxide and a distilling head connected to a tube passing to the bottom of a second reaction flask, *b*. Glass wool or sintered glass is used at the point shown to ensure small bubbles. *b* is connected to the reflux condenser, *c*, which in turn is connected to the scrubbing tower, *d*. Glass wool or sintered glass is again used as shown for breaking up bubbles. *d* is connected by means of a side arm, *e*, to the tube, *f*, which passes into the test tube, *g*, used for collecting the precipitate. Ground joints are present at *h*, *i*, *j*, and *k*. The Pregl-type bumping stick may be used if desired, but very satisfactory results are obtained without it when the sample is weighed in a platinum boat and inserted into the apparatus.

This method has given excellent results for the past four years, during which a very large number of determinations have been made by nine different microanalysts. Some of these results are shown in Table I.

No determinations of alkimide were made with this apparatus. However, since Furter determined both alkoxyl and alkimide with his apparatus, it is reasonable to assume that the setup described in this paper could also be used for this purpose.

REAGENTS

The reagents used are identical to those described by Roth (*11*, *12*), Niederl (*9*), and Grant (*5*) with the exception of the hydriodic acid, the treatment of which is described below.

Treatment of Hydriodic Acid. One pound of reagent grade hydriodic acid, specific gravity 1.7, is placed in a round-bottomed flask which, in turn, is connected by means of a ground joint to an air condenser. The acid is heated to gentle boiling for about 2 hours, during which a slow stream of carbon dioxide or nitrogen is bubbled through by means of a glass tube extending to the bottom. At no time should the acid vapors be allowed to come in contact with organic material, which would cause recontamination. When heating is stopped the flow of gas likewise should be discontinued, as fuming acid is formed by passing the gas through at room temperature (*7*). A blank methoxyl determination should then be carried out to determine the quality of the acid. If even a trace of silver iodide precipitate is obtained, the refluxing with carbon dioxide or nitrogen should be repeated, as it is possible to obtain a reagent which gives a perfect blank. The acid is stored in a brown glass-stoppered bottle at laboratory temperature and gives good results for a number of weeks (the color of the product is of no importance). Test samples should be run at frequent intervals to make certain that the reagent is still efficient.

PROCEDURE

The sample, if solid or a high-boiling liquid, is weighed in a platinum boat and placed in reaction flask *a*. Volatile samples are weighed in the customary capillaries and inserted with a platinum boat or tetrahedra for the prevention of bumping.

Table I. Determination of Alkoxyl Groups by New Apparatus and Techniques

Compound[a]	Empirical Formula	No. of Alkoxyl Groups	Weight of Sample, Mg.	Weight of AgI Corrected, Mg.	Found, % Alkoxyl	Calculated, % Alkoxyl
			Methoxyl			
Vanillin	$C_8H_8O_3$	1 (ether)	5.393	8.634	20.80	20.38
			5.212	8.049	20.40	20.38
			6.742	10.299	20.18	20.38
			5.657	8.586	20.05	20.38
			6.877	10.466	20.10	20.38
			5.792	8.911	20.32	20.38
			5.163	8.140	20.83	20.38
			6.415	9.871	20.32	20.38
Research	$C_{10}H_{11}O_5N$	1 (ester)	8.253	8.370	13.40	13.78
	$C_{28}H_{43}O_6N$	1 (ether)	8.122	3.742	6.09	6.34
	$C_{19}H_{16}O_2$	1 (ether)	7.932	11.210	18.67	18.44
	$C_{19}H_{14}O$	1 (ether)	6.240	9.680	20.49	20.66
			Ethoxyl			
Research	$C_{23}H_{26}O_4N_2$	1 (ester)	5.971	3.528	11.32	10.98
		1 (ester)	2.822	1.731	11.76	10.98
	$C_{18}H_{26}O_3N_2$	1 (ester)	5.701	3.390	11.41	11.31
	$C_{18}H_{24}O_3N_2$	1 (ester)	6.681	3.971	11.40	11.37
	$C_{11}H_{16}O_5N_2$	2 (ester)	3.829	7.069	35.41	35.17
	$C_6H_8O_4N_2$	1 (ester)	5.872	8.050	26.29	26.18
	$C_7H_{10}O_4N_2$	1 (ester)	5.208	6.528	24.04	24.07
	$C_7H_9O_3N_2Br$	1 (ester)	4.365	4.107	18.05	18.09
	$C_7H_{10}N_2O_4$	1 (ester)	4.221	5.448	24.75	24.20
	$C_{16}H_{24}O_6N_2$	2 (ester) 2 (ether)	4.218	11.670	53.06	52.95
	$C_9H_{14}N_2O_6$	1 (ester)	6.782	6.471	18.30	18.30
	$C_9H_{14}O_4N_2$	2 (ester)	6.346	13.973	42.23	42.07
			5.731	12.609	42.20	42.07
	$C_{32}H_{37}O_{16}N_{11}$	2 (ether)	7.536	4.311	10.97	10.84
	$C_{17}H_{20}N_4O_4$	1 (ether)	7.717	5.147	12.79	13.09
	$C_{24}H_{28}N_9O_{16}$	1 (ether)	5.876	1.972	6.45	6.48
			5.326	1.736	6.25	6.48

[a] All compounds listed gave acceptable values for elementary analyses.

Several crystals of phenol and 5 to 6 drops of acetic or propionic (*2*) anhydride are added and the mixture is warmed to obtain solution. Hydriodic acid is added to the second reaction flask, *b*, so that the bulb portion is about one-half full, and the flask is put into place. Equal parts of 5% solutions of cadmium sulfate and sodium thiosulfate are added to the scrubbing compartment, *d*, to fill it half way. Water is run into tube *f* through ground joint *k*, and the top is closed quickly so as to have the constrictions filled with water. The source of carbon dioxide is attached at ground joint *h*, and the gas is bubbled through for a couple of minutes until the excess water in tube *f* has been forced out. Two cubic centimeters of 3.85% alcoholic silver nitrate are placed in test tube *g* and the tube is put into place so that delivery tube *f* goes to the very bottom. The ground joint at *h* is opened and tinfoil about the size of a dime is placed in reaction flask *a*, and then enough hydriodic acid to fill the bulb part of the flask approximately half full. A bumping tube of the Pregl type may be inserted into *a* if desired but is not necessary, as the platinum boat reduces bumping. The source of carbon dioxide is immediately attached.

The flow of gas is regulated so that the bubbles pass through the silver nitrate in test tube *g* at the rate of about one bubble per second. Water is run through condenser *c* and a microburner is used to heat the acid in *b* to gentle boiling. (*Caution.* Fuming acid is formed upon passing gas through at room temperature, *7*.) In this condition, the apparatus is allowed to stand for 30 minutes. The mixture in *a* is then brought to boiling by means of a microflame and boiling of the contents of *a* and *b* is continued for one-half hour.

After this period, the water is drained from the condenser and boiling of the contents of both flasks is continued for at least an additional half hour. The stopper at ground joint *k* is then opened, test tube *g* lowered, and the precipitate washed into the flask alternately by means of 1 to 200 nitric acid and alcohol. Then 1 to 200 nitric acid is added to the contents of the test tube *g* until it is about four-fifths full, followed by 5 drops of concentrated nitric acid, specific gravity 1.42. The contents of the tube are then brought just to the boiling point on a steam bath, immediately cooled, and filtered, using a Pregl filter tube and suction flask. The silver iodide precipitate is washed with 1 to 200 nitric acid and then with cold concentrated nitric acid, specific gravity 1.42, by filling up the filter tube with the concentrated acid, allowing it to soak through for several minutes, and then sucking off. This should be repeated several times. The precipitate is then washed with 1 to 200 nitric acid followed by alcohol and dried in an oven at 120° C., after which the precipitate is weighed. Silver iodide (0.120 mg.) is added to the weight of the precipitate to give the corrected value before calculation (*3, 5, 9, 11, 12*).

ACKNOWLEDGMENT

The author is indebted to the following persons for their work in connection with this paper. The photograph of the setup (Figure 1) was made by Lester Hodax, while the sketch of the apparatus (Figure 2) was made by W. D. O'Connor, Jr. The apparatus used was fabricated by John Deakin and Emil Wiegand. The analyses shown in Table I were done by Esther Bass, Samuel Blackman, Claire Farkas, Janet Farnow, Marian Faulkner, Gerhard Kisch, Bella Littman, Margaret Sullivan, and Marie Walker. All the above-mentioned are in the employ of this company or were at the time of their work.

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RECEIVED July 12, 1947. Presented before the Division of Analytical and Micro Chemistry at the 112th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y.

Colorimetric Microdetermination of Zirconium

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This paper comprises the development of an accurate colorimetric method for determination of zirconium in clays or silicate rocks, using the pink lake formed by the zirconium alizarin sulfonate complex. The method applies to a range of zirconium oxide content up to 0.275 mg. with an accuracy to 0.003 mg. of zirconium oxide.

AN URGENCY existed for developing an accurate determination of small amounts of zirconium in naturally occurring clays and their activated products. The gravimetric method by precipitation of zirconium as a phosphate (*3*) usually employed in silicate rock analysis, or as a selenite (*5*) in the steel industry, fails to detect zirconium oxide in amounts less than 0.01%, using at least a 1-gram sample. The steel industry developed a rough colorimetric determination of zirconium using *p*-dimethylaminoazophenylarsonic acid (*2*) which permits slightly greater accuracy (to 0.005% zirconium). However, as stated by the authors, very apparent difficulties arise in the standardization of this method. Recent spectrographic techniques (*4*) also give comparable accuracies to 0.005% zirconium.

Consequently, existing qualitative tests for zirconium as compiled by Feigl (*1*) and as given by Yoe and Overholser (*10*) were examined for possible quantitative development. The zirconium alizarin sulfonate lake was selected as a color complex least subject to interference, and specific in its reaction for clay analysis. It proved to be entirely satisfactory and very accurate, and fills a need in analytical chemistry for a determination of zirconium oxide to an accuracy of 0.0006%.

EXPERIMENTAL

Development. The zirconium alizarin colors in this investigation were read with a Coleman Universal spectrophotometer, Model 11, and the pH values with a Beckman pH meter. The optical densities recorded by this spectrophotometer are the logarithms to base 10 of the ratio of the intensity of the incident light to that of the transmitted light. The test solutions were made up to 100 ml. in volumetric flasks. A stock solution of 0.05% sodium alizarin sulfonate and a standard solution of zirconium oxychloride (1 ml. equivalent to 0.05 mg. of zirconium oxide), adjusted to a pH of 2.2 with hydrochloric acid were utilized.

Table I. Solution Densities with Interfering Substances

(To 100 ml., 1.00 mg. of sodium alizarin sulfonate, color developed in 1 hour, 5200 Å.)

Interfering Substances Added to Test Solution, Mg.						
$AlCl_3 \cdot 6H_2O$			474.0			
$FeCl_3$			50.7			
$TiCl_4$ as TiO_2			1.5			
$Th(NO_3)_4 \cdot 4H_2O$ as ThO_2			0.2			
Test Condition, 5 Ml. of 1 *N* HCl Added to Give pH 1.5						
	Blank	Al^{+++}	Fe^{+++}		Ti^{++++}	Th^{++++}
	0.004	0.023	0.118		0.015	0.004
0.11 mg. of ZrO_2 added	0.065	0.080	0.112		0.068	0.066
Test Condition, 10 Ml. of 1 *N* HCl Added to Give pH 1.1						
	0.004	0.004	0.079		0.004	0.004
0.11 mg. of ZrO_2 added	0.065	0.066	0.088		0.066	0.065
Test Condition, pH 1.1, Fe^{+++} Reduced in Silver Reductor						
				Fe^{++}		
	0.004	0.004	0.089	0.004	0.004	0.004
0.11 mg. of ZrO_2 added	0.066	0.065	0.108	0.065	0.065	0.066

Since the pH of the hydrolysis of a zirconium salt to the hydroxide is 2.8 (*9*), an initial survey was made in the acidic range from 1.5 to 4.0 pH to determine the effect of pH on the color of a 0.001% sodium alizarin sulfonate solution. It was found that change in solution pH over this pH range had only a negligible effect on the density of the yellow alizarin color at the point of minimum transmittance of 4180 Å. The pH of the solutions was adjusted with hydrochloric acid.

With the pH constant at 1.5 and the zirconium oxide content at 0.275 mg., transmittance curves were run with varying amounts of sodium alizarin sulfonate. Since the alizarin at the two lowest percentages was entirely consumed in the formation of the zirconium complex, these two transmittance curves gave the point of minimum transmittance for the zirconium pink lake at 5200 Å.

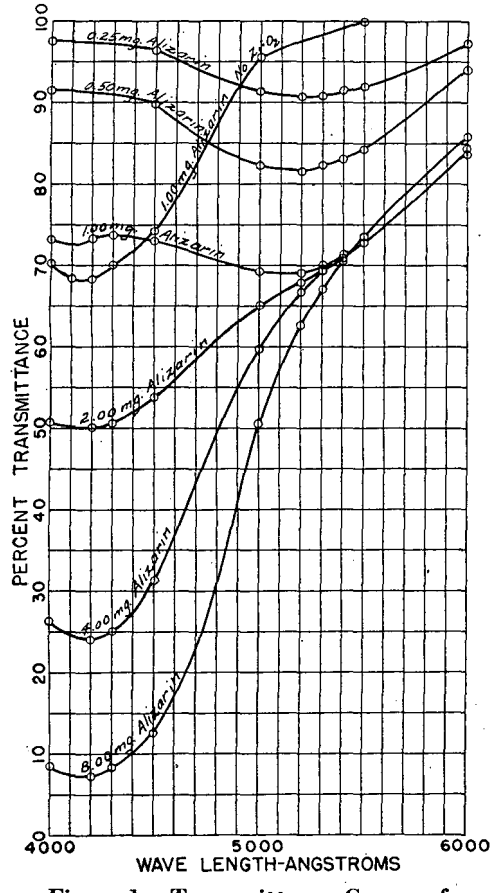

Figure 1. Transmittance Curves of Zirconium-Alizarin Complex

Solutions containing 0.275 mg. of zirconium oxide with varying amounts of sodium alizarin sulfonate

For convenience, the transmittance curve for 1.00 mg. of sodium alizarin sulfonate and no zirconium oxide at a pH of 1.5 is plotted in Figure 1 along with the curves containing variable amounts of alizarin and 0.275 mg. of zirconium oxide. In subsequent determinations, a 1.00-mg. addition of alizarin was selected not only to eliminate interference in reading densities of the developed lake from the yellow of the alizarin but also to permit full development of the lake by retaining the indicated slight excess of alizarin reagent. This excess of alizarin is shown by the slight dip in the transmittance curve at 4180 Å. Zirconium lake densities are then read at 5200 Å.

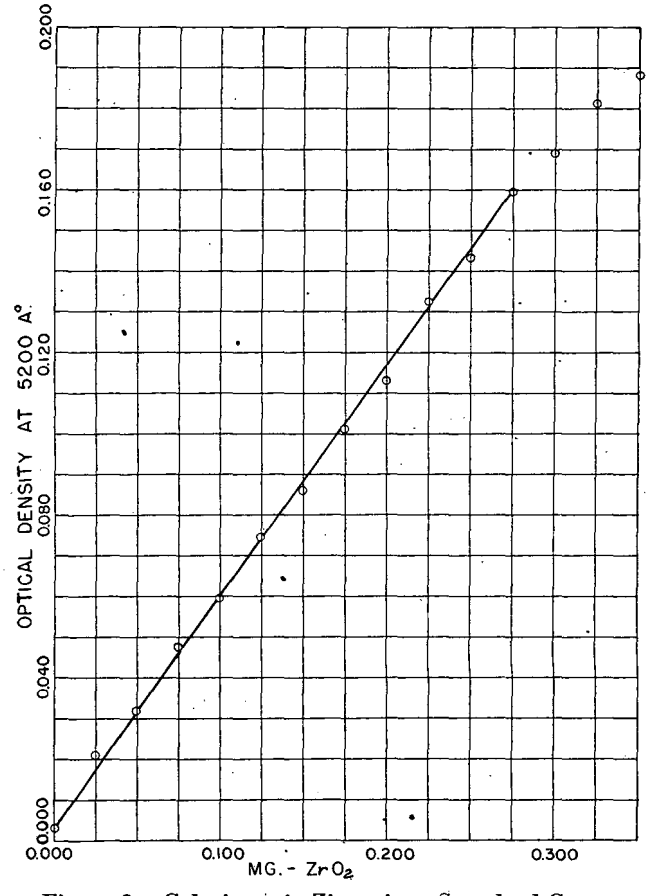

Figure 2. Colorimetric Zirconium Standard Curve

Density *vs.* mg. of zirconium oxide

Standard Curve. Test solutions (100 ml.) were made up with additions of zirconium from the standard zirconium oxychloride solution. The zirconium lake color densities were read at intervals of time at 5200 Å. Color development is complete in 1 hour and is stable for at least 4 hours. However, upon standing overnight, the lake precipitated and settled out. The zirconium alizarin sulfonate lake follows Beer's law. Zirconium oxide contents over 0.275 mg. show insufficient alizarin to permit the full development of the color complex. The standard curve of color density versus milligrams of zirconium oxide is plotted in Figure 2.

Interfering Substances. The following substances (*6*) in solution are considered to interfere with the formation of the zirconium lake and the measurement of its density: fluorine sulfates, phosphates, silicon, molybdenum, antimony, tungsten, and organic hydroxy acids. These substances for meither com-

plex radicals or precipitates with zirconium (*1*). However, they were not investigated because they were not present in the clay material, were eliminated by the outlined analytical method, or occurred in such small quantities that the procedure as outlined eliminated their interference.

Interference from aluminum, ferric iron, titanium, and thorium, which cause coloration with sodium alizarin sulfonate, was investigated. Acid conditions in the test solutions eliminated interference from thorium, and the lowering of the pH to 1.1 by the addition of 10 ml. of 1 *N* hydrochloric acid from titanium and aluminum. Iron interference was overcome by reduction from the ferric to the ferrous state by passing the solution through a silver reductor (*8*) of 12-ml. capacity. In these tests, quantities of aluminum, iron, and titanium were taken to approximate the analysis of the clays using a 0.5-gram sample—namely, 20, 5, and 0.3%, respectively, calculated to the oxide. The results are recorded in Table I.

Procedure. A 0.5-gram sample of clay is weighed into a 20-gram nickel crucible, approximately 4 grams of sodium hydroxide pellets are added, and the fusion is made. The completed fusion is then digested on a hot plate with distilled water. The insoluble portion of the digestion is filtered out with a 9-cm. No. 40 Whatman filter paper and thoroughly washed with distilled water. Since the insoluble will contain the sodium zirconate, the filtrate is rejected. The insoluble is digested with about 12 ml. of hot concentrated hydrochloric acid and washed with hot distilled water into a 100-ml. beaker. The total amount of solution should not exceed 75 ml. The filter paper with residue is retained for further analysis.

The solution is cooled to room temperature, 2 drops of phenolphthalein indicator are added, and the solution is neutralized cautiously, not permitting the temperature to rise, with a 50% sodium hydroxide solution. Five milliliters of 1 *N* hydrochloric acid are added, and the mixture is passed through a silver reductor of 12-ml. capacity. The reductor is rinsed out with 5 ml. of 1 *N* hydrochloric acid and 5 ml. of distilled water. The reduced solution is received in a 100-ml. volumetric flask, 2 ml. of 0.05% sodium sulfonate are added, and the solution is brought up to 100-ml. volume with distilled water. The final solution is mixed well by shaking. The pH should be 1.1. It is allowed to stand 20 hours or overnight for color development. The density of the solution is read at 5200 Å.

The filter paper from the hot hydrochloric acid digestion of the sodium zirconate is burned at 925° C., and the residue is fused with a few pellets of sodium hydroxide. This second fusion is digested and filtered in the same manner as the initial fusion. The insoluble residue is similarly leached with hydrochloric acid and washed. The resulting leachate is also adjusted to a pH of 1.1. The small amount of iron present permits the omission of the reduction step. The number of milligrams of zirconium oxide contained in the two solutions are taken from the previously plotted standardization curve of density versus milligrams of zirconium oxide and added for the final zirconium oxide content of the sample. The method is accurate to 0.0006% zirconium oxide with the 0.5-gram sample used.

DISCUSSION

Dissolution of Sample. In clay material, zirconium usually occurs as a silicate, the mineral zircon, which is broken down and put in solution with difficulty. Sodium carbonate fusions of a clay which contained about 0.035% zirconium oxide gave only about 60% of the zirconium oxide found by using a sodium hydroxide fusion. This substantiates the facts as given by Venable (*7*).

Triple sodium hydroxide fusions were made on National Bureau of Standards plastic clay 98 and a raw Cheto, Ariz., clay. The third fusion of the residue from the hydrochloric acid digestion of the sodium zirconate shows (Table II) no additional zirconium oxide. Therefore, two fusions must be made on every sample to ensure complete breakdown of the zirconium compounds present. Plastic clay 98 was found to contain 0.0250% zirconium oxide. The Bureau of Standards certificate of analysis shows an average of 0.041% zirconium oxide or variation in separate analyses between 0.032 and 0.05%.

Table II. Triple Fusions

	Undried, 0.5-Gram Sample, Cheto, Ariz., Clay		Dried at 140° C., 2 Hours, 0.5-Gram Sample, Bureau of Standards Clay 98	
	Density	ZrO_2, mg.	Density	ZrO_2, mg.
1st fusion	0.097	0.164	0.068	0.114
2nd fusion	0.008	0.010	0.009	0.011
3rd fusion	0.003	0.000	0.003	0.000
Triple fusion total		0.174		0.125
			% ZrO_2 found	0.0250
			% ZrO_2 certified av.	0.041

Table III. Density Readings

	Undried, 0.5-Gram Sample, Cheto, Ariz., Clay		Dried at 140° C., 2 Hours, 0.5-Gram Sample, Bureau of Standards Clay 98	
	Density	ZrO_2, mg.	Density	ZrO_2, mg.
Single fusion	0.096	0.163	0.072	0.121
0.055 mg. of ZrO_2 added	0.128	0.219	0.103	0.176

It is most important in the procedure to neutralize the hydrochloric acid, used in the digestion of the sodium zirconate, at room temperature, to form zirconium hydroxide, $Zr(OH)_4$; otherwise, at higher temperatures the acid-insoluble zirconyl hydroxide, $ZrO(OH)_2$ (*7*), will be formed.

Color Development. Zirconium lake color development is rapid but limited to 4 hours for the standard and test interfering substance solutions. However, the solutions made up with the clay samples show a very slow development in color and remain stable for at least 30 hours before precipitation takes place. The high concentration of sodium chloride in these solutions possibly influences this behavior.

When a standard zirconium solution is added to a clay solution sample in which the color has already been developed, the development of additional color is also slow. Density readings taken after addition of this standard zirconium solution were exactly equivalent to the amount of zirconium oxide added, as shown in Table III.

CONCLUSION

This newly developed colorimetric method for the quantitative determination of zirconium, by using the pink lake formed by the zirconium alizarin sulfonate complex, permits the detection of as little as 0.003 mg. of zirconium oxide in clays.

It is believed that this investigation has laid the foundation for the accurate determination of small amounts of zirconium in rock analysis. With possible modifications, it undoubtedly can be extended to advantage in other fields of chemistry.

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RECEIVED April 26, 1947.

Inorganic Spot Test for Copper

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The appearance of a violet color when concentrated hydrobromic acid reacts with salts of divalent copper is used as the basis of a spot test for copper. It requires only common inorganic reagents and has a sensitivity comparable to that of copper tests with organic reagents.

ONE of the numerous specific reactions for copper which have been proposed is the appearance of a violet color when concentrated hydrobromic acid reacts with salts of divalent copper.

As early as 1877 Cresti (*3*) applied this color reaction for the identification of copper metal, which he exposed to the vapors of hydrobromic acid and bromine. Sabatier (*6*) used concentrated hydrobromic acid or a mixture of potassium and phosphoric acid in order to detect the Cu (II)-ion in aqueous solution. He attributed the violet color which developed to the formation of a complex compound of cupric bromide and hydrogen bromide, as he succeeded in preparing an unstable dark-colored crystalline product of the approximate formula $3CuBr_2.2HBr.6H_2O$, probably identical with the metastable compound observed by Carter and Megson (*2*) in the system cupric bromide–hydrobromic acid–water at 25° C. The color of the solution depends on the concentration of the hydrobromic acid; at concentrations of 26% or more, the color is a pure violet, below 26% brown to brown-violet.

Scheringa (*7*) applied the reaction to the detection of copper in organic substances, and reported a sensitivity of 0.5 microgram. Augusti (*1*) used it as a microtest, and was able to detect 0.15 microgram of copper in one macrodrop. He stated that the presence of lead, cadmium, trivalent iron, silver, monovalent mercury, and monovalent copper interferes with the test, but that the influence of the two latter cations can be eliminated by oxidation. Kirchhof (*5*) used the reaction for the detection of copper in rubber.

The authors have developed the reaction into a spot test for copper, and especially studied the interferences of other ions and their possible elimination. The method requires only common inorganic reagents and yet is of a sensitivity comparable to that of the known copper tests with organic reagents (*4*). The reagent used is an aqueous solution of ammonium bromide and phosphoric acid. This acid has proved superior to sulfuric acid, as it does not lead to the undesirable formation of free bromine, does not attack the filter paper, and largely suppresses the interference by Fe (III) and Bi (III) ions.

REAGENTS

1. Ammonium bromide–phosphoric acid. Ammonium bromide (5 grams) is dissolved in a few cubic centimeters of water; sirupy phosphoric acid (d = 1.75) (4 cc.) is added, and the liquid is diluted with water to a total volume of 100 cc.
2. Saturated bromine water.
3. Silver
4. Sodium acetate, 1% solution.
5. Sodium fluoride, 4% solution.

PROCEDURE

One drop of the solution to be tested is placed on filter paper (the authors used Green No. 803; Whatman filter paper gave a slight copper reaction with the reagent). After the spot is dried in a current of warm air, one drop of reagent 1 is brought into the spot and dried in the same way. In the presence of copper a violet color appears on the paper; on addition of a drop of water the copper compound is concentrated at the outside border of the spot in form of a violet ring. The color fades rather quickly under the influence of atmospheric moisture and at very low copper concentration may even disappear. It returns, however, when the spot is dried again with warm air. (Cold air is not very effective.) In this way it is possible to detect 0.1 microgram of copper in one macrodrop (0.05 cc.). The limit concentration is therefore 1 to 500,000. With one microdrop of 0.05 cc. even 0.025 microgram of copper can be detected.

The reaction can be used in presence of ions of gold, bismuth, Cr (III), iron, nickel, and cobalt, when the quantity of these metals does not exceed the maximum limits indicated in Table I.

ELIMINATION OF DISTURBING INFLUENCES

The color reaction may not appear or the test becomes uncertain for the following reasons: (1) the reducing properties of Hg (I), Sn (II), or Fe (II) compounds; (2) the color of Au (III), Bi (III), or Fe (III) bromides which are present or are formed in the solution to be tested in concentrations higher than those indicated in Table I; (3) the photochemical color change of silver compounds; and (4) the characteristic color of Cr (III), Ni (II) and Co (II) compounds present in concentrations higher than those indicated in Table I.

Table I. Permissible Quanitities of Metals

Foreign Cation Present in Copper Solution to Be Tested	Maximum Quantity of Foreign Cation per Macrodrop	Minimum Quantity of Copper Ion Detectable per Macrodrop	Ratio of Copper Ion to Foreign Cation
	Grams	*Gram*	
Au (III)	2.5	0.2	1:12.5
Bi (III)	50	1.0	1:50
Cr (III)	5	0.2	1:25
Fe (III)	5	0.2	1:25
Ni (II)	25	0.2	1:125
Co (II)	1	0.2	1:5

1. To eliminate the interference of the reducing substances, the solution to be tested is oxidized with saturated bromine water (reagent 2), and the test is carried out as follows: One drop of the solution to be tested is dried on the filter paper, one drop of saturated bromine water is dropped onto the spot and dried as before, one drop of reagent 1 is added and dried, and finally one drop of water is added and dried with a current of warm air.
2. In case the quantity of Au (III) compounds in the solution is higher than shown in Table I, a yellow-brown ring appears on the paper, and the reaction is not clear. This effect can be avoided if one boils, in a small test tube, one drop of the solution to be tested with one drop of reagent 1 and a few grains of reagent 3. When the brown color has disappeared, the ordinary test can be made with one drop of this diluted solution. It is now possible to detect 0.2 microgram of copper in the presence of 500 microgram of gold.

To eliminate the disturbing influence of the yellow color of Bi (III) bromide, one drop of a 1% solution of sodium acetate (reagent 4) is added to one drop of the test solution and one drop of reagent 1. White bismuth phosphate precipitates, and the test is now made as usual with one drop of this suspension. The main portion of bismuth phosphate collects in the center of the spot. In this manner, 0.2 microgram of copper can be detected in a solution containing 5.0 micrograms of bismuth.

The phosphoric acid present in reagent 1 converts the red-brown Fe (III) bromide into a complex ferriphosphoric acid, so that the spot test is possible, if the maximum quantity of iron indicated in Table I is not exceeded. In the presence of higher concentrations of iron, the test has to be modified as follows: One drop of the solution is boiled with one drop of saturated bromine water (reagent 2), so as to convert any Fe (II) salt present into Fe (III) compounds. To this solution, one drop of reagent 1 and one drop of a 4% sodium fluoride solution (reagent 5 are added); the latter is used to diminish the Fe (III) concentration. Upon heating the yellow-white ferric phosphate precipitates. The liquid is cooled and one drop of it is used for the test. Like bismuth (III) phosphate, ferric (III) phosphate collects in the center of the spot, and continued heating produces a

Table II. Maximum Quantities of Cations

Foreign Cation Present in Copper Solution to Be Tested	Highest Quantity of Foreign Cation Used per Macrodrop	Minimum Quantity of Copper Ion Detectable per Macrodrop	Ratio of Copper Ion to Foreign Cation
Au (III)	500	0.2	1:2500
Bi(III)	500	0.2	1:2500
Fe (III)	200[a]	1	1:200
Ag (I)	2500	0.2	1:12,500

[a] Maximum quantity permissible.

Table III. Sensitivity of Test

Anion Present in Copper Solution	Quantity[a] of Anion per Macrodrop	Minimum Quantity of Copper Ion Detectable per Macrodrop
	γ	γ
Nitrate	1550	0.1
Chlorate	2090	0.5
Bromate	3200	0.5
Iodate	4380	0.5
Periodate	4800	0.5

[a] These quantities correspond to solutions to 0.5 mole.

brownish coloration of the center, surrounded by the violet ring. Thus, 1.0 microgram of copper can be detected in the presence of 200 micrograms of iron.

3. In the presence of silver compounds one drop of the solution to be tested is boiled for a short while with one drop of reagent 1. Silver bromide precipitates, and one drop of the liquid is taken for the ordinary test. This reaction allows detection of 0.2 microgram of copper in presence of 2500 micrograms of silver. Upon exposure to light, the silver bromide which collects in the center of the spot immediately turns gray.

4. If such colored substances as Cr (III), Ni (II), and Co (II) compounds are present, the test is applicable only if their concentration is not higher than that indicated in Table I. If large quantities are present, they must be removed from the solution by one of the ordinary analytical methods.

Table II indicates the maximum quantities of foreign cations per macrodrop which will not interfere with the modified specific procedures.

West (*8*) observed that nitrates, chlorates, bromates, iodates, and periodates give an intense yellow stain under the conditions of this reaction. The color is obviously due to free bromine liberated by these highly oxidizing substances. In the case of nitrates present in the solution, the test is not affected adversely, as the violet ring is formed before the yellow color appears.

In the presence of chlorates and bromates the violet ring appears first, but is immediately obscured by a deep yellow stain. Fortunately, the color fades quickly, and after renewed drying, only the violet ring, characteristic of copper, reappears.

The situation is somewhat more complicated in the case of iodates and periodates, which give a very persistent yellow stain. In this case, the reaction is performed in the following manner, utilizing the difference in capillarity of copper ions and these anions:

One drop of the solution to be tested is dried on the paper, and one drop of reagent 1 is added and dried. Another two drops of reagent 1 are then brought onto the yellow spot. Upon drying, a yellow ring appears and, after some time, an outer violet ring.

The authors have determined the sensitivity of the method in the presence of the oxidizing anions; their results are summarized in Table III.

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RECEIVED September 30, 1946.

NOTES ON ANALYTICAL PROCEDURES

Melting-Point Bath Liquids

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THE paucity of published material relating to substitutes for concentrated sulfuric acid as the bath liquid in the common melting-point apparatus is difficult to account for in view of the disadvantages of this almost universally used liquid, chief of which are its hygroscopicity and its hazards. In particular, it is annoying and time-consuming when a melting-point determination must be interrupted because absorption of water vapor has lowered the boiling point of the bath below its expected value, and it is dangerous when one is working with unstable compounds or with compounds that melt near the boiling point of the acid. Yet the advantages of the type of apparatus employing a liquid heat-transfer medium, especially the Hershberg apparatus (*1*) for the accurate determination of corrected melting points by total thermometer immersion, seemed so great that a search was made for a substance that would be generally superior to sulfuric acid.

The classic treatises on organic chemical methods suggest only phosphoric acid, paraffin oil, paraffin, glycerol, castor oil, rapeseed oil, mixtures of sulfuric acid with inorganic sulfates, and mixtures of inorganic salts. Some of these have the same disadvantages as sulfuric acid; others are not liquid at room temperature. More recently there have been recommended HB-40 (*2*), a product consisting mainly of a mixture of terphenyls, and silicone fluid type 550 (*4*), an aromatic silicone.

After over 60 different organic substances distributed among several types which seemed to offer promise had been investigated, two were ultimately selected as superior to the others and to those described in the literature—Aroclor 1248 and silicone oil 9981-LTNV-40. Both products are liquid at room temperature, boil above 350° C. (corrected), are colorless, noncorrosive, highly stable to heat, nonhygroscopic, without objectionable odor at elevated temperature, have a high flash point, and are commercially available in large quantities. The former gives toxic effects only upon prolonged inhalation of the vapor, while the latter is nontoxic.

EXPERIMENTAL

Preliminary Screening. A large number of substances were heated to boiling in an open beaker with a thermometer immersed in the liquid. Of these, 62 registered a temperature of 298° C. (corrected) or above. Some were solids, as it was thought pos-

sible that a mixture of a solid compound with just enough liquid compound to render the mixture liquid at room temperature might yield the best result.

Of the 62, 24 were colored, usually yellow, and 38 were either colorless or only yellow tinted. The substances which possessed color (Table I) were set aside temporarily and, when it was found that the colorless substances were yielding the desired results, nothing more was done with them.

Further Screening. The 36 compounds that were colorless or only yellow tinted before heating, with Nos. 6 and 9 (Table III) which were light yellow in color but were studied when it was found that silicones were so stable to heat, were then subjected to a prolonged heat-stability test designed to accelerate the production of color or other effects produced on continuous use in the melting-point apparatus (*1*). Samples were placed in test tubes which dipped into an electrically heated Wood's metal bath. In one tube was placed a standardized Anschütz thermometer, the mercury column was totally immersed, and all tubes were lightly stoppered. The liquids were first brought to 200° (corrected). Within 24 hours, 27 substances had become yellow to dark brown and were rejected (Table II).

The eleven remaining, which did not darken in color at 200° C. (corrected), were left in the Wood's metal bath and brought to 250° (corrected). After 48 hours the temperature was increased to 300° (corrected) and maintained there for 48 hours. The results with these compounds, with concentrated sulfuric acid for comparison, are shown in Table III.

Table II. Substances Rejected on Further Screening

Substance	Supplier	Description
Hydrocarbons		
n-Octadecane	Connecticut Hard Rubber Co.	
Embedding paraffin	R. F. Revson Co.	
Technical ceresin	Eimer and Amend	
Santowax MH	Monsanto Chemical Co.	Hydrogenated *m*-terphenyl
HB-40	Monsanto Chemical Co.	Mainly a mixture of terphenyls
Alcohols		
Cetyl alcohol	Eastman Kodak Co.	
Heptadecanol	Carbide and Carbon Chemicals Corp.	
Ethers		
Di-*n*-decyl ether	Connecticut Hard Rubber Co.	
Di-*n*-dodecyl ether	Connecticut Hard Rubber Co.	
Esters		
Cetyl acetate	Eastman Kodak Co.	
n-Octadecyl acetate	Eastman Kodak Co.	
Butyl stearate	Arnold, Hoffman and Co.	
n-Butyl sebacate	Eastman Kodak Co.	
Amoil-S	Distillation Products, Inc.	*n*-Amyl sebacate
Octoil-S	Distillation Products, Inc.	2-Ethylhexyl sebacate
n-Butyl phthalate	Eastman Kodak Co.	
Octoil	Distillation Products, Inc.	2-Ethylhexyl phthalate
Flexol plasticizer DOP	Carbide and Carbon Chemicals Corp.	2-Ethylhexyl phthalate
Santicizer B-16	Monsanto Chemical Co.	Butyl phthalyl butyl glycollate
Santicizer R-15	Monsanto Chemical Co.	Ethyl phthalyl ethyl glycollate
Santicizer M-17	Monsanto Chemical Co.	Methyl phthalyl ethyl glycollate
Ether esters		
Butyl Cellosolve stearate	Arnold, Hoffman and Co.	
p-tert-Amylphenoxyethyl laurate	Sharples Chemicals, Inc.	
Metal organics		
Tri-*o*-cresyl phosphate	Eastman Kodak Co.	
Tri-*m*-cresyl phosphate	Eastman Kodak Co.	
Tri-*p*-cresyl phosphate	Eastman Kodak Co.	
Kronitex A	Kavalco Products	Tricresyl phosphate

Table I. Rejected Group of Colored Substances

Substance	Supplier	Description
Hydrocarbons		
Santowax DO	Monsanto Chemical Co.	Mixture of diphenyl and *o*-terphenyl with probably some *m*-terphenyl
Santowax O	Monsanto Chemical Co.	*o*-Terphenyl
Amyldiphenyl	Eastman Kodak Co.	
Dinonylnaphthalene	Sharples Chemicals, Inc.	
Halogenated hydrocarbons		
Aroclor 1262	Monsanto Chemical Co.	Chlorinated diphenyl containing 62% chlorine
Alcohols		
Ceryl alcohol	Eastman Kodak Co.	
Ethers		
Di-*n*-tetradecyl ether	Connecticut Hard Rubber Co.	
Esters		
Isoamyl stearate	Eastman Kodak Co.	
sec-Octyl stearate	Eastman Kodak Co.	
Tripalmitin	Eastman Kodak Co.	
Tristearin	Eastman Kodak Co.	
Diphenyl phthalate	Monsanto Chemical Co.	
Peanut oil	Eimer and Amend	
Rapeseed oil	Eimer and Amend	
Nitriles		
Hexadecyl nitrile	Columbia Organic Chemicals Co.	
Arneel 18 D	Armour and Co.	Octadecyl nitrile
Ether alcohols		
Polyethylene glycol 300	Carbide and Carbon Chemicals Corp.	$HOCH_2(CH_2OCH_2)_xCH_2OH$, av. mol. wt. 300
Di-*tert*-amylphenoxyethanol	Sharples Chemicals, Inc.	
Ether esters		
Methyl Cellosolve oleate	Kessler Chemical Co., Inc.	
Flexol plasticizer 4GO	Carbide and Carbon Chemicals Corp.	Polyethyleneglycol di-2-ethylhexoate
Di-*tert*-amylphenoxyethyl acetate	Sharples Chemicals, Inc.	
Metal organics		
Tri-*p-tert*-amylphenyl phosphate	Sharples Chemicals, Inc.	
Tri-1,2,4-xylenyl phosphate	Sharples Chemicals, Inc.	
Tri-*o*-phenylphenyl phosphate	Eastman Kodak Co.	

DISCUSSION

From Table III it is clear that, after sulfuric acid has been rejected because of its unsuitability for use at 250° C., the compounds that stand out for their heat stability are the Aroclors (Nos. 1, 2, and 3) and the silicones (Nos. 6 to 11). As a class, the former show the effect of prolonged heating by darkening in color, while the latter do not darken in the slightest but become viscous and even glassy. A choice from each class may be made on the basis of other properties. Table IV gives a survey of some other pertinent properties of the most interesting Aroclors and silicones compared with sulfuric acid.

The data on the Aroclors and silicones were taken mainly from the technical bulletins issued by the manufacturers; the data on sulfuric acid were obtained from secondary reference works. While the Aroclors show no appreciable differences among themselves in heat stability, No. 3 possesses the highest boiling point and the highest flash point; these favorable properties would appear to outweigh its relatively high viscosity and mark it as the best of the Aroclors. Of the silicones, only Nos. 7 and 8 are colorless and remain liquid after prolonged heating at 250° C. There is little to distinguish between these in boiling point or flash point, but better stability at 300° C. and lower viscosity indicate No. 7 as superior. Thus Nos. 3 and 7 stand out as the two most satisfactory liquids of those studied. Their heat stability is remarkable when it is realized that paraffin, among the colorless substances, turns yellow within 24 hours when held at 200° C. Heating for 48 hours at 200° C., followed by 48 hours at 250° C., the minimum conditions under which these substances exhibit very apparent chemical change, corresponds to a period of normal use in a melting point apparatus of from several months to a few years. Both liquids may be heated several times to 300° C. without showing chemi-

Table III. Color of Liquids on Prolonged Heating

No.	Substance	Supplier	Description	Color at 200° C.[a] 24 hours	Color at 200° C.[a] 48 hours	Color at 250° C. 24 hours	Color at 250° C. 48 hours	Color at 300° C. 24 hours	Color at 300° C. 48 hours
	Sulfuric acid	J. T. Baker Chemical Co.	c.p. coned.	Colorless	Colorless	Colorless	Colorless		.....
	Halogenated hydrocarbons								
1	Aroclor 1232	Monsanto Chemical Co.	32% chlorine	Colorless	Colorless	Yellow tint	Light yellow	Dark yellow	Yellow brown
2	Aroclor 1242	Monsanto Chemical Co.	42% chlorine	Colorless	Colorless	Yellow tint	Light yellow	Dark yellow	Yellow brown
3	Aroclor 1248	Monsanto Chemical Co.	48% chlorine	Colorless	Colorless	Yellow tint	Light yellow	Yellow	Yellow brown
4	Halowax 1012	Bakelite Corp.	Chlorinated naphthalene, about 50% chlorine	Brown tint	Light brown	.....	.....	.....	.....
	Metal organics								
5	Diphenyl *o*-chlorophenyl phosphate	Eastman Kodak Co.		Yellow tint	Light brown	.....	.....	.....	.....
6	DC 550 fluid	Dow Corning Corp.	A silicone	Light yellow[b]	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow[c]
7	Silicone oil 9981-LTNV-40	General Electric Co.	A silicone	Colorless	Colorless	Colorless	Colorless[c]	Colorless[c]	Colorless[c]
8	Silicone oil 9981-LTNV-70	General Electric Co.	A silicone	Colorless	Colorless	Colorless	Colorless[c]	Colorless[d]	.....
9	Silicone oil 9981-LTNV-70 OX	General Electric Co.	A silicone, contains oxidation inhibitor	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow[e]
10	Silicone oil 9981-LTNV-100	General Electric Co.	A silicone	Colorless	Colorless	Colorless[c]	Colorless[d]	.....	.....
11	Silicone oil 9981-LTNV-200	General Electric Co.	A silicone	Colorless	Colorless	Colorless[c]	Colorless[d]	.....	.....

[a] Temperatures in ° C. (corr.) [b] Clear, not turbid. [c] Becomes more viscous. [d] Sets to a gel. [e] Incipient boiling with copious fumes.

Table IV. Physical Properties of Liquids

No.	Boiling Temp.[a]	Specific Gravity	Viscosity[b]	Flash Point[c]	Fire Point[c]	Coefficient of Expansion[d]	Specific Heat
H_2SO_4	...	1.83[e]	74[f]	None	None	0.000576	0.34[g]
1	301	1.26–1.27[h]	48–50	152–154	238	0.000725	.....
2	327	1.38–1.39[h]	80–93	176–180	334	0.000678	ca. 0.29[i]
3	365	1.45–1.46[h]	185–240	193–196	None	0.000702	ca. 0.27[i]
7	428	0.97[j]	185	ca. 316	ca. 316	0.001	0.47
8	439	0.97[j]	325	ca. 316	ca. 316	0.001	0.47

[a] Temperature of liquid, ° C. (corr.), when vigorously boiling.
[b] Saybolt Universal seconds at 100° F.
[c] ° C.
[d] Cc./cc./° C.
[e] 25° C./4° C.
[f] Calculated from data in other units
[g] Cal./g. at 25–45° C.
[h] 25° C./25° C.
[i] Cal./g. at 20° C.
[j] At 20° C.

cal change. No. 3 has been used at 343° C. (corrected) in the Hershberg apparatus while No. 7 may be heated to 400° C. (corrected) or a little higher.

The choice between Nos. 3 and 7 will depend on the particular type of melting point apparatus used. Prolonged heating causes the former to turn light yellow and the latter to become more viscous; thus, for apparatus without provision for stirring, No. 3 would be preferable. The toxic effects for animals found (*3*) on prolonged exposure to Aroclor vapors evolved at high temperatures would probably rule out the use of No. 3 in apparatus using an open beaker, but should not be a consideration in apparatus, including the Hershberg apparatus, where there is little or no escape of vapors into the air. The lower flash point of No. 3 would also make it less desirable for use in open beakers.

Application to Hershberg Apparatus. While both No. 3 and No. 7 are superior to sulfuric acid in this apparatus, the former has been preferred by the author because of the smaller coefficient of expansion and the smaller energy input requirement. If the product of the specific gravity and specific heat (Table IV) is taken as a measure of the energy required to heat the melting-point bath to a given point, it will be seen that No. 3 requires the least energy and sulfuric acid the most. The figures for the products are: No. 3, 0.39; No. 7, 0.46; and sulfuric acid, 0.62. Otherwise expressed, a given heat input will raise the bath to a given temperature most rapidly with No. 3 and least rapidly with sulfuric acid. Although a liquid of low heat capacity may be disadvantageous, especially in an apparatus heated by a gas flame, because the temperature of the bath is less easily controlled, no difficulty was experienced with the electrically heated Hershberg apparatus.

A slight modification in the length and diameter of the Nichrome wire used in the apparatus has been found desirable in order to attain rapidly the higher temperatures made possible by these liquids. Eight feet of 26-gage (B. and S., 2.550 ohms per foot) wire, wound in two layers of about equal length, are recommended. With this heating element and with No. 3 as the liquid, starting from room temperature a bath temperature of 200° C. can be reached in 4.5 minutes and 300° C. in 10 minutes. Both No. 3 and No. 7 were found unsuitable for use with an internal heating element of bare Nichrome wire; the former turned yellow, while the latter became very viscous.

SUMMARY

Two commercially available liquids, Aroclor 1248 and silicone oil 9981-LTNV-40, have been found superior to other compounds tested for use as heat-transfer liquids in apparatus for the determination of melting points. Both substances are colorless, relatively heat-stable, noncorrosive, and nonhygroscopic, and their boiling points are above 350° C. Aroclor 1248 can be used at temperatures as high as 340° C. and silicone oil 9981-LTNV-40 as high as 400° C.

ACKNOWLEDGMENT

The author wishes to express his thanks to the industrial firms listed in the tables, many of which provided samples of their products for this study, and to Ralph F. Wiseman for assistance in the experimental work.

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RECEIVED March 22, 1947.

A Large Soxhlet-Type Extractor

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DURING a series of investigations involving the removal of wax from cotton fabric and yarn in various stages of manufacture, a need developed for a large Soxhlet-type extractor. Large extractors have been described (*1*, *2*), but they are only about 40 times the capacity of the standard Soxhlet (about 200 ml.) while a capacity of approximately 200 times was desired. The extractor described here was designed to handle 100 to 200 skeins of yarns or 3 to 5 pounds of raw cotton, sliver, or fabric. The extractor was operated successfully with nine different solvents, from diethylether (boiling point 34.4° C., 93.9° F.) to a light naphtha (boiling point 150° C., 302° F.). The operation was similar to a standard Soxhlet, the solvent being "dumped" from the sample and renewed every 4 minutes for 8 hours. Ethyl and methyl alcohols had minimum cycle times of 8 minutes because of their relatively high heats of vaporization. Carbon tetrachloride gave some trouble due to the slow formation of hypochlorous acid which attacked the galvanized sheet iron, the copper tubes, and condensers. Chloroform gave no difficulty.

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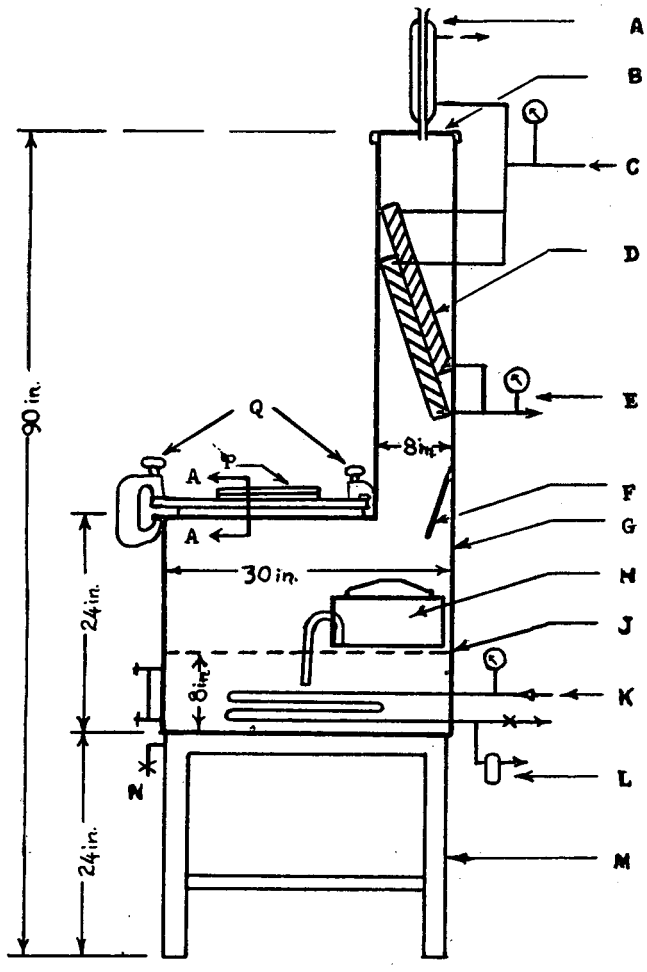

Figure 1. Cut-Away View of Soxhlet-Type Extractor

A. Glass condenser
B. Removable lid
C. Cooling water inlet with pressure gage
D. Automobile radiator condensers
E. Water outlet with temperature gage
F. Deflector skirt
G. Body of 24-gage galvanized sheet iron, 18 inches wide to fit snug on both sides of radiators
H. Sample tray and rack
J. 1-inch angle iron supports for tray
K. Steam coils with pressure gage
L. Steam trap
M. Angle iron legs
N. Drain
P. Observation port
Q. C clamps on lid

DESIGN

The principal features and general dimensions of the extractor are illustrated in Figures 1 to 3.

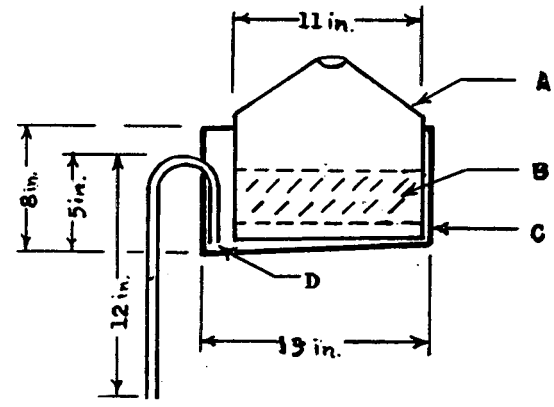

Figure 2. Detail of Sample Tray

A. Sample rack
B. Sample space
C. Sample tray
D. Siphon

Two automobile radiator cores provided adequate condenser capacity. It was not necessary to seal these radiators to the extractor, since a snug fit on all sides retained all the vapors. The glass condenser on top of the extractor served to indicate by cloudy vapors when the boiling rate was in excess of the condenser capacity. The condensed solvent poured off the deflector into the sample tray across its entire width, thus reducing the possibility of any channeling through a large sample. The liquid was boiled in the bottom by the steam coil (15 feet of $^5/_8$ inch copper tubing) and the vapors rose from a surface area of 540 square inches (0.35 square meter), reducing entrainment and ensuring a pure solvent feed to the sample.

The sample tray was simply a box with a siphon tube in one side. Tests of several different sizes and numbers of siphon tubes resulted in the selection of the $^5/_8$ inch copper tube as shown. This tube proved the most satisfactory, emptying 3 gallons of solvent from the tray in little more than one minute. As the liquid level reached the bottom of the tray, the siphon was broken because the open end of the tube cleared the bottom of the tray by 0.125 inch. With some solvents, as in the conventional Soxhlet extractor, it was possible to condense liquid into the tray so rapidly that the liquid could not be removed fast enough to break the siphon. However, this presented no serious difficulty, since this condition occurred only for cycle times less than the standard time of 4 minutes. The cycle time was regulated by adjusting the steam supply.

The sample was held on a rack which had a screen bottom and no sides and was provided with a handle at the top to facilitate convenient carrying. The bottom of the rack cleared the tray bottom by 1 inch, leaving a drain space which aided in removing dirty solvent from the sample each time the tray emptied. The total contact volume available for sample and filled with solvent was 11 × 17 × 4 inches or 748 cubic inches (12 liters). It would be a simple matter to provide screen sides and top for the rack to hold chips, leaves, or pieces of any other material one desired to extract.

The principal difficulty in design was the lid seal. Several modifications were tried until the one shown proved satisfactory. The lid seat, *J* in Figure 3, was made from four pieces of angle iron laid carefully in a square on a flat surface and gas-welded at

the corners with the upright web on the inside. The upper edge was then squared and finished flat with a hand file. The lid frame *H* in Figure 3, was made from four pieces of 1 × 2 inch steel, slotted and clamped in place on top of the angle-iron frame, in which position the four frame pieces were electric-welded to one another. These two surfaces (inside the slot) were then sufficiently true; a double gasket of felt 1/16 inch thick and 3/16 inch wide provided a seal that prevented the escape of any solvent vapors from the edge of the lid during operation. An 18-inch square sheet of copper was laid on top of the lid frame and bolted to it every 3 inches, and the seam was soldered over to prevent leaks.

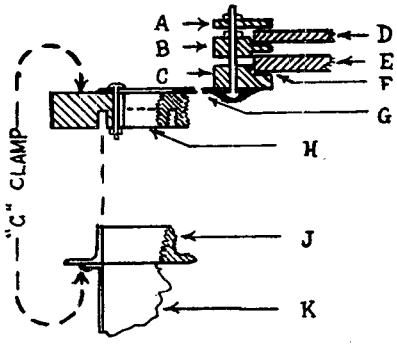

Figure 3. Detail of Lid Section *A-A* of Figure 1

A. Steel ring of 1/4 inch plate iron
B. Steel ring of 1/2 inch plate iron
C. Steel ring of 5/8 inch plate iron
D. 10-inch diameter glass disk 1/4 inch thick
E. 10-inch diameter glass disk 3/8 inch thick
F. Felt or neoprene gaskets
G. Cover sheet of lid
H. Lid frame
J. Lid seat of 1-inch angle iron
K. Extractor body

The porthole in the lid enabled the entire interior, boiling solvent, and solvent level in sample tray, to be observed during operation. Thus the cycle time was determined by watching the rise and fall of solvent in the sample tray and taking the time between two rises.

The 18-inch square opening provided by the lid facilitated easy charging and removal of the trays used.

EXTRACTION OF SAMPLE

As with the laboratory Soxhlet, operation consisted of adjusting the heat input for proper solvent rate. The factor limiting solvent rate was the condensing capacity of the radiators, which in turn depended upon the rate of flow and temperature of the inlet cooling water. The maximum operating pressure of the inlet water to the radiators was about 20 pounds per square inch (58 kg. per sq. cm.) gage. With most solvents the pressure was set at 15 pounds per square inch (44 kg. per sq. cm.) gage, but with the alcohols it was necessary to operate at maximum. With most solvents 0.5 to 2 pounds per square inch (0.15 to 6 kg. per sq. cm.) gage steam pressure was sufficient to maintain optimum solvent rate, but with the high-boiling naphtha it was necessary to use 125 pounds per square inch (366 kg. per sq. cm.) gage steam. The solvent rate was never allowed to get any higher than that which gave an outlet cooling water temperature 20° F. (11° C.) below the boiling point of the solvent.

PURIFICATION OF SOLVENT

Prior to the extraction of a sample, the solvent was purified by use of a special tray (not shown in figures) in place of the sample tray. This was 18 inches square and 10 inches deep and had a 1-inch bronze check valve soldered in the middle of the bottom. A piece of string was clipped to a short wire loop soldered to the seat of the check valve and run out through the condensers at the top. Pulling the string raised the valve seat and drained the tray. Starting with about 10 gallons of impure solvent in the bottom of the extractor, pure vapors were boiled off and condensed into the tray, leaving about a quart of concentrated solution containing the impurities. This residue was then drained off and the bottom washed with about a pint of pure solvent from the tray by raising the check valve slightly. The drain valve was closed and all the clean solvent drained back into the bottom. Cooling water was then circulated through the steam coils for several minutes in order to cool the solvent enough to allow removal of the lid without excessive vapor loss. The special tray could then be removed and the sample tray inserted ready for operation. At the end of the extraction the solvent was cooled as above prior to removal of the lid and sample. The steam was always turned off just as the tray began to empty, in order to allow for complete drainage of solvent from the sample.

CONCENTRATION OF EXTRACT

After the 8-hour extraction period, the steam was turned off and the solvent cooled as described above. The sample tray with sample was then removed and the special square tray inserted. All but about 2 or 3 pints (1 to 1.5 liters) of the solvent were then boiled off and collected in the tray. These 2 or 3 pints still in the bottom and containing the extracted wax were then drained into an appropriate container, and an extra pint of clean solvent from the tray above was allowed to wash over the bottom and also drain into the container. Since this left practically no wax in the extractor, a fairly accurate estimate could be made of the amount of extract.

Each time the extractor was started, the body was full of air which had to be expelled from the top by the rising vapors. Some vapors were entrained by this air and the solvent odor was strong for a few minutes. As soon as the air was out, however, no more odors could be detected. After the rate was adjusted by the steam valve, the extractor required no more attention for the 8-hour extraction period.

COST

The Chevrolet radiators used cost about $20 each and the galvanized sheet iron, steel, glass, and fittings cost about $30. A mechanic assembled the unit in two weeks. The total cost, then, was approximately $150. Stainless steel would be more satisfactory but was unavailable at the time. Had it been used the cost would have been roughly doubled.

Cooling water was available from a circula ing system utilizing a cooling tower supplying water to this and other units. Should it be necessary, however, to use tap water and discard the effluent, the maximum consumption would be approximately 250 gallons per hour. At 30 cents per 1000 gallons, the cost would amount to about 70 cents for one 8-hour extraction period, including solvent purification and concentration of extracts.

SUMMARY

A large-size Soxhlet-type extractor provides very thorough solvent extraction of samples up to 748 cubic inches (12 liters) in volume at atmospheric pressure. Three gallons of hot solvent are added to and removed from the sample every 4 minutes (for most solvents, 8 minutes for alcohols) from a total solvent volume of about 38 liters (10 gallons). The large condensing capacity is obtained by means of two automobile radiators. A special tray in place of the sample tray facilitates purification of solvent prior to extraction and concentration of extract afterwards. A large lid with vapor-tight seal provides easy charging of sample.

ACKNOWLEDGMENT

Acknowledgment is hereby made to J. W. Moore and C. M. Furgason for many helpful ideas and suggestions.

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RECEIVED January 11, 1946. Developed for a project sponsored by the Texas Cotton Research Committee at the Bureau of Industrial Chemistry, University of Texas, Austin, Tex.

Rapid Determination of Specific Gravity of Liquids under Pressure

J. I. LACEY

Hooker Electrochemical Company, Niagara Falls, N. Y.

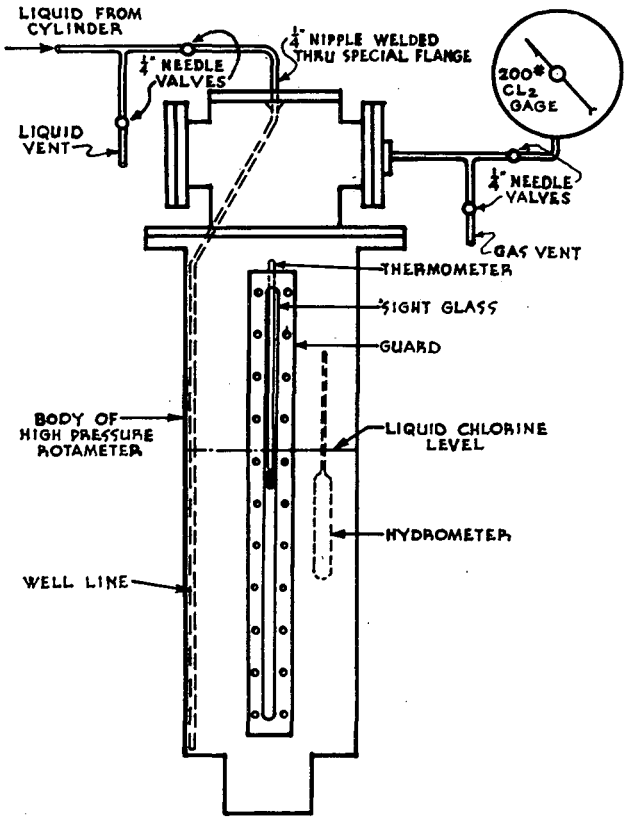


Figure 1. Apparatus for Determining Density of Liquid Chlorine

A METHOD for the rapid determination of the specific gravity of a liquid under pressure at various temperatures involves placing the sample in a high-pressure vessel that is fitted with a sight glass and reading the specific gravity by means of a hydrometer floating in the liquid. This method has been used to check the densities of commercial liquid chlorine.

Apparatus. The sample was held in the case of a Fischer and Porter armored, horizontal line flow rotameter, modified as described (see Figure 1). The metering tube, gaskets, and float were removed, the bottom outlets were blanked off, and a well line and a gas outlet were provided through the top flange. The connections were made so that either liquid or gas could be withdrawn or charged. A −10° to +110° C. thermometer subdivided to 1° C. was suspended in the rotameter case, so that the bulb and part of the stem, but not the calibration, were immersed in liquid chlorine when the vessel was half full. A 0 to 200 pounds per square inch silver diaphragm pressure gage was provided on the gas phase. A hydrometer was placed in the case before filling it with chlorine, and it floated in the liquid after filling. To cover the range indicated, it was necessary to use two hydrometers, 1.300 to 1.400 and 1.400 to 1.500 specific gravity at 60° F.; the scale on each hydrometer was divided into 100 parts. When the gravity changed from 1.399 to 1.401 it was necessary to expel the sample, change hydrometer, and then put a fresh sample into the vessel. The temperature was controlled by means of a water bath, the level of which was kept just below the level of the chlorine in the vessel.

Procedure. A cylinder (100 or 150 pounds) of commercial liquid chlorine was connected to the well line and inverted. Liquid chlorine was then run into the vessel until it was half full. During the filling, sufficient gas was vented to allow the liquid to enter, but no special effort was made to eliminate all air from the system. The temperature of the water bath was raised to 50° C. and allowed to cool slowly. The testing equipment was located out of doors, and radiation to the atmosphere was the source of cooling except in the extreme lower range, where ice water was occasionally used.

The temperature and specific gravity were read at frequent intervals. The vessel was moved gently between readings to ensure a uniform liquid temperature. At the end of the determination, the instruments were checked against Bureau of Standards instruments and found to be accurate within the limits to which they could be read.

This method may be adapted to other liquids under pressure, providing the pressure does not collapse the instruments. The liquid being tested must not appreciably attack the confining vessel or instruments.

In this instance the confining vessel was a high-pressure rotameter case, but any vessel of appropriate dimensions having a suitable sight glass may be used.

RECEIVED March 28, 1947.

Nomograph for Particle Size Determination with the Sharples Supercentrifuge

ELERINGTON SAUNDERS, *Monsanto Chemical Company, Merrimac Division, Everett, Mass.*

THE Sharples supercentrifuge has been used for the determination of particle size and particle distribution in colloidal systems (*2-6*). Mathematical relations for calculating the size of particles sedimented out under definite operating conditions have been presented (*3, 4*). Since these calculations are somewhat laborious, graphical methods have been found convenient. Fancher, Oliphant, and Houssiere (*2*) have presented an alignment chart, but this construction does not consider several variables and requires a new chart for each system under consideration. An alignment chart has also been presented for the Svedberg ultracentrifuge (*7*). It is believed, therefore, that the following nomograph, which is applicable to any system and can be applied to centrifuges of varying dimensions with a simple correction, will simplify these calculations still further.

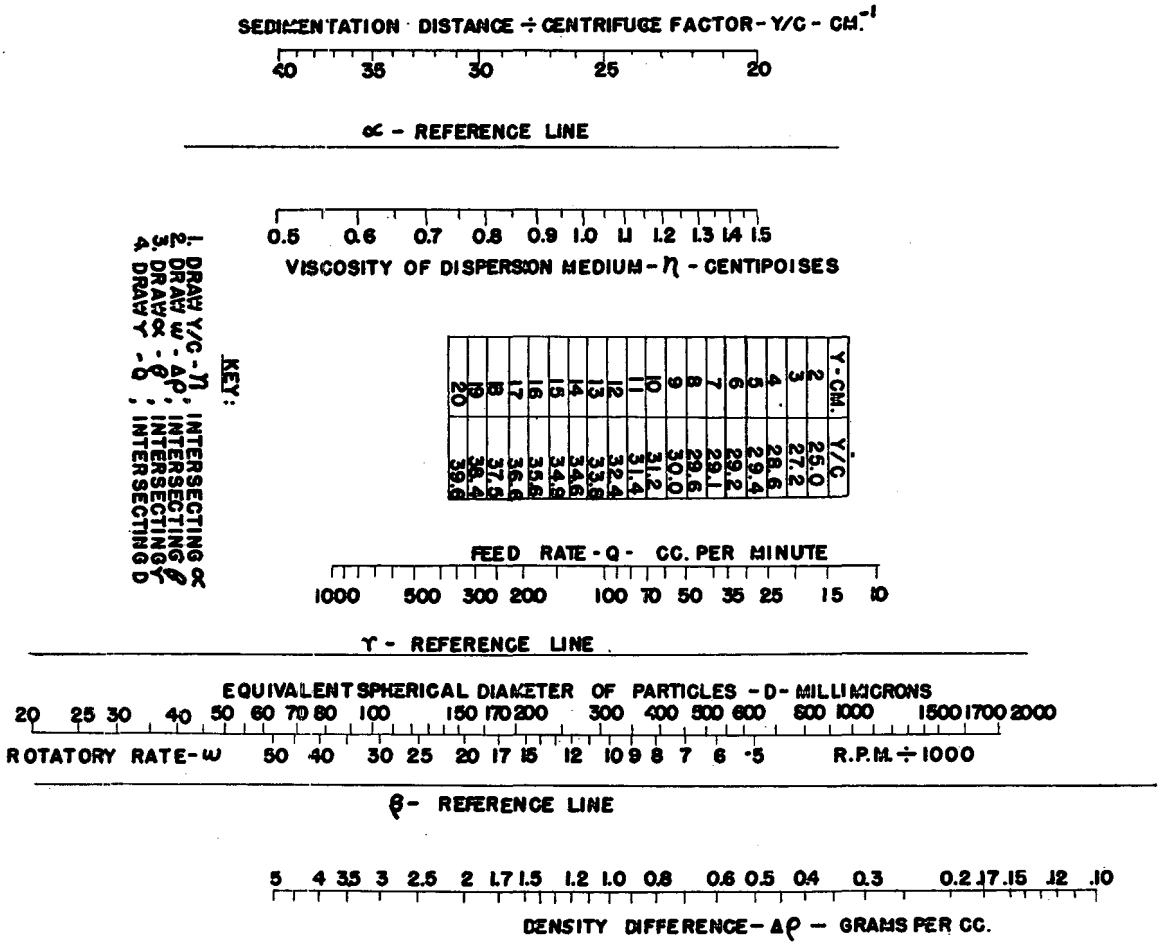
Hauser and Lynn (*3*) have developed methods for calculating the size of particles deposited by the supercentrifuge under definite operating conditions by assuming that the particles obey Stokes' law, and that the flow parallel to the rotatory axis is streamlined. Their equation is expressed as:

$$Y = \frac{18\,QK_1\,\eta}{\pi\,(R_2^2 - R_1^2)\,D^2\omega^2\,\Delta\rho}\left[\frac{R_2^2}{2}\ln\frac{R_2}{X_0} - \frac{R_1^2}{2}\left(\ln\frac{R_2}{X_0}\right)^2 + \frac{X_0^2 - R_2^2}{4}\right] \quad (1)$$

where Y = vertical distance of deposition of particle of given size in cm., measured from bottom of centrifuge bowl
Q = rate of feed of suspension, cc. per second
K_1 = function of construction of bowl and equal to:

$$K_1 = \frac{R_2^2 - R_1^2}{3/4\,R_1^4 + \frac{R_2^4}{4} - R_2^2R_1^2 - R_1^4\ln\frac{R_1}{R_2}}, \frac{1}{\text{cm.}^2} \quad (2)$$

R_2 = distance from axis of rotation to bowl wall, cm.



R_1 = distance from axis of rotation to overflow weir, cm.
η = viscosity of dispersion medium, poises
D = equivalent spherical diameter of particle sedimented out at Y, cm.
ω = angular velocity of rotation, radians per second
$\Delta\rho$ = difference in density between dispersed particles and dispersion medium, $\rho_1 - \rho_2$
ρ_1 = density of dispersed particles, grams per cc.
ρ_2 = density of dispersion medium, grams per cc.
X_0 = distance from axis of rotation at which a given particle will begin to sediment out toward bowl wall, cm.

For a given suspension, and fixed operating conditions, Equation 1 becomes:

$$Y = F(X_0, D) \tag{3}$$

but is implicit in X_0 and unsolvable except by a family of curves, determinants, or an alignment chart. However, under conditions of narrow particle size range and appropriate feed rate, D no longer tends to be an independent variable and Equation 3 approaches:

$$Y = f(X_0) \tag{4}$$

Under these conditions, since Y depends only on X_0, the terms in the brackets in Equation 1, called C, can be given values for each value of Y and a curve of C *vs.* Y drawn (*3*). The tabulation of Y *vs.* Y/C shown on the nomograph has been taken from this curve.

If the factors $\frac{18 K_1}{\pi (R_2^2 - R_1^2)}$ in Equation 1 are called A, a constant, Equation 1 becomes:

$$Y/C = \frac{A \eta}{D^2 \omega^2} \frac{Q}{\Delta\rho} \tag{5}$$

Since a value of Y/C for every value of Y may be given, Equation 5 is one of six variables and may be nomographed (*1*). In the accompanying nomograph, the calculations have been simplified further by conversion to more convenient units of the variables in Equation 5. This conversion was accomplished as follows:

$$\frac{Y}{C} = \frac{A\ \eta\ \text{(centipoises)}\ Q\ \text{(cc. per minute)}}{D^2\ \text{(millimicrons}^2)\ \omega^2\ \text{(revolutions}^2\ \text{per minute}^2)\ \Delta\rho\ \text{(grams per cc.)}} \times$$

$$\frac{1\ \text{poise}}{100\ \text{centipoises}} \times \frac{1\ \text{cc. per second}}{60\ \text{cc. per minute}} \times \frac{1\ \text{millimicron}^2}{(10^{-7})^2\ \text{cm.}^2} \times \frac{1\ \text{revolution}^2\ \text{per minute}^2}{\left(\frac{2\pi}{60}\right)^2\ \text{radians}^2\ \text{per second}^2}$$

or

$$\frac{Y}{C} = \frac{A\ \eta\ Q}{D^2\ \omega^2\ \Delta\rho} \times \frac{(60)^2 \times 10^{14}}{(2\pi)^2 \times 6000}$$

$$\frac{Y}{C} = \frac{1.52 \times 10^{12}\ A \eta Q}{D^2\ \omega^2\ \Delta\rho} \tag{5A}$$

where η is expressed in centipoises
Q is expressed in cc. per minute
D is expressed in millimicrons
ω is expressed in r.p.m.
$\Delta\rho$ is expressed in grams per cc.
Y is expressed in cm.
C is expressed in cm.^{2}

The method of nomographing referred to (*1*) includes only equations containing up to five variables, but may be extended to plot six variables as follows:

Equation 5 may be reduced to four equations of three variables each:

$$\log \alpha = \log \eta - \log Y/C \quad (6)$$

$$\log \beta = -\log \Delta\rho - 2 \log \omega \quad (7)$$

$$\log \gamma = \log \alpha + \log \beta \quad (8)$$

$$2 \log D = \log \gamma + \log Q \quad (9)$$

where α, β, and γ are introduced parameters.

If each of these three variable equations is nomographed on the same sheet, according to the method of Davis (*1*), and the parameters represented by reference lines, Equation 5 may be solved by drawing four lines, each representing the solution of one of these equations. The constant factor, A, is included in the nomograph by locating one scale to correspond to an arbitrary numerical solution of Equation 5. This scale location is then checked by comparing graphic and numerical solutions of Equation 5 over different areas of the nomograph. Changes in the units in which the variables are expressed will only result in shifting the position of one scale up or down, without affecting the relations between scales.

The value of A in Equation 5 depends only upon the dimensions of the centrifuge bowl. The bowl dimensions of each centrifuge of the same type are, of course, the same. The value of A should, therefore, be the same for all centrifuges of the same type; in the author's laboratory this is the Sharples Type T-66-24 1 HY, for which $R_1 = 2.175$ cm. and $R_2 = 0.7348$ cm. A for this instrument was calculated to be $1.61 \frac{1}{\text{cm.}^4}$; using the more convenient units of Equation 5A, the entire constant becomes 2.44×10^{12}. For other centrifuges of different dimensions, Equation 10 applies:

$$D_2 = \frac{D_1}{10^6} \sqrt{\frac{A_2}{2.44}} \quad (10)$$

where D_2 = size of particles obtained in different centrifuge, mμ
D_1 = size of particles obtained from nomograph, mμ
A_2 = constant for different centrifuge, $\frac{1}{\text{cm.}^4}$

Sample Calculation. A colloidal dispersion is to be centrifuged. The following data are obtained:

Viscosity of dispersion medium = 0.8 centipoise.

Difference in density between particle and medium = 4.07 grams per cc. If the centrifuge is operated at 40,000 r.p.m. with a feed rate of 100 cc. per minute, what is the size of particle which will sediment out 9 cm. above the bottom of the bowl? (Y/C = 30 from tabulation on nomograph.)

By calculation: $D = \sqrt{\frac{2.44 \times 10^{12} (0.8) (1000)}{30 (40{,}000)^2 (4.07)}} = 100$ mμ

From nomograph: $D = 99$ mμ

The variation in these values is within the limits of operating precision.

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RECEIVED March 27, 1947.

Kjeldahl Determination of Nitrogen without Distillation

Application to Samples Containing Phosphorus

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A TIME-saving modification of the Kjeldahl method (*3*) has been published recently, in which the digested material is diluted and adjusted to the methyl red end point with sodium hydroxide. Thus, the free sulfuric acid is neutralized. Then formaldehyde is added, and the ammonium ion is titrated to the phenolphthalein end point with standard 0.1 N sodium hydroxide.

The chief disadvantage of this method lies in the interference of phosphorus. When the ammonium ion is titrated from the methyl red end point to the phenolphthalein end point, the phosphate is converted from the primary to the secondary salt, thus introducing a positive error. A minor disadvantage is that the precipitation of sulfates of calcium and barium and of hydroxides of iron and aluminum (when these elements are present in the sample) makes the end points less distinct. This paper describes a procedure that eliminates both these difficulties.

After the usual digestion, dilution, and addition of sodium bromide, zirconyl chloride is added, and the solution is brought to the methyl red end point. Zirconyl hydroxide is precipitated, carrying with it all the phosphorus as zirconyl phosphate. The solution is diluted in a volumetric flask and filtered. An aliquot portion of the filtrate is then titrated as usual with sodium hydroxide to the phenolphthalein end point in the presence of formaldehyde. This procedure will also remove calcium, barium, aluminum, and iron.

The carbonate, introduced as a contaminant of the sodium hydroxide, is quantitatively precipitated with the zirconyl hydroxide (*1*, *4*). Therefore, the boiling just prior to the adjustment to the methyl red end point may be omitted.

This procedure takes about 10 minutes more than is required in the absence of phosphate (*3*), but, it is still shorter than the classical Kjeldahl method with the distillation.

Reagents. In addition to the reagents previously listed (*3*), zirconyl chloride solution, about 1.0 M is required. Dissolve 322 grams of zirconyl chloride octahydrate in 600 ml. of 1.0 N hydrochloric acid and dilute to 1 liter with the same acid.

Procedure. Weigh a sample containing about 10 milliequivalents of nitrogen. Perform the digestion and dilution as previously described (*3*), then transfer the solution to a 250-ml. volumetric flask, rinsing the digestion flask with five 10-ml. portions of water. Add 15 ml. of sodium bromide, 5 ml. of zirconyl chloride, and 3 drops of methyl red. Add 10 N sodium hydroxide dropwise until the solution turns yellow, then add N sulfuric acid dropwise

Table I. Nitrogen in Pure Organic Compounds
(2% of phosphorus added as Na_3PO_4)

Compound	% N Theory	% N Mean	Mean Deviation
Acetanilide	10.36	10.36	0.06
Anthranilic acid	10.22	10.20	0.02
Diphenyl amine	8.28	8.22	0.04
s-Diphenylurea	13.21	13.18	0.03
Sulfanilic acid	8.08	8.07	0.04

Table II. Nitrogen in Dried Blood

Sample No.	% N by Standard Kjeldahl Method[a]	% N by Proposed Method	Mean Deviation
2	7.15	7.12	0.02
14	3.58	3.62	0.05
20	4.61	4.62	0.05

[a] Analyzed by Thorn Smith, 5719 Woodward Ave., Detroit, Mich.

until it is just pink. Cool the solution to room temperature, and dilute to the mark. After thorough mixing, filter a portion of the solution through a rapid, fluted, 15-cm. paper. Discard the first 5 ml. of the filtrate, then pipet 100 ml. into a 250-ml. Erlenmeyer flask. Add a drop of methyl red to replace that adsorbed on the precipitate. Adjust the solution to the methyl red end point with 0.1 *N* sodium hydroxide. Read the buret, and continue as directed (*3*).

Run a blank determination, which usually amounts to 0.10 to 0.20 ml.

RESULTS AND DISCUSSION

Table I shows the results obtained with pure organic compounds. Each entry in the third column is the mean of two determinations. Table II shows the results obtained with samples of dried blood. Each entry in the third column is the mean of two determinations.

These tables show that the procedure is accurate and precise.

The specified amount of zirconyl chloride is sufficient for samples containing 150 mg. of phosphorus. Should samples of greater phosphate content be encountered, the quantity of zirconyl chloride should be increased proportionally.

Micro and semimicromodifications of the Kjeldahl procedure without distillation have been developed successfully for samples free from phosphorus (*2*). However, the distillation in the classical Kjeldahl micromethod is so rapid that no advantage is gained by eliminating it, and for this reason the microtechnique was not extended to samples containing phosphorus.

ACKNOWLEDGMENT

The authors are grateful to the Research Council of Rutgers University for financial aid in this investigation.

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RECEIVED August 16, 1947. Presented before the Division of Analytical and Micro Chemistry at the 112th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y.

Preparation of Phosphomolybdic Acid from Phosphoric Acid and Molybdic Trioxide

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IN THE course of investigating methods for manufacturing phosphotungstic and phosphomolybdic acids for analytical reagent use, irrespective of the relative amounts of phosphoric acid and molybdic trioxide initially employed the product obtained by the method suggested by Linz (*5*) was found to be the 1 to 12 acid. It has not been possible to prepare other ratios by this method, nor can the 1 to 12 acid be prepared in "reagent grade" quality by this process with a single recrystallization. Despite the chemical similarity between tungsten and molybdenum, it has not been found possible in this laboratory to prepare the corresponding phosphotungstic acid by the mere interaction of phosphoric and tungstic acids in aqueous solution as suggested by Linz.

The compound containing phosphorus and molybdenum was initially prepared by Debray (*1*) by the digestion with aqua regia of the yellow precipitate formed by the interaction of phosphoric acid and ammonium molybdate. Finkener, Pemberton, von der Pfordten, Gibbs, and Hundeshagen (*2, 3, 4, 6, 7*) showed that this compound had phosphorus and molybdenum present in a ratio of 1 to 12. All their methods of preparation were at best very tedious and Wu (*8*) worked out a synthesis in which sodium molybdate and phosphoric acid reacted in the presence of excess hydrochloric acid and the heteropoly acid was isolated from the reaction mixture by an ether extraction.

In 1943, Linz (*5*) prepared the acid by the reaction of molybdic and phosphoric acids in aqueous solution. In view of the fact that Linz's method uses water as the only solvent, it was thought to be a good one for the preparation of commercial quantities of the acid. The method is somewhat unsatisfactory because the product is contaminated with phosphoric acid after its crystallization from aqueous solution, but ether extraction yields the desired 1 to 12 acid. Even crystals of the acid so impure as to have an approximate 1 to 6 ratio (by analysis) yielded the 1 to 12 acid of high purity upon a single recrystallization from ether.

EXPERIMENTAL

A. One mole (144 grams) of C.P. molybdic trioxide, 1/12 mole (9.6 grams) of C.P. 85% phosphoric acid, and 1500 ml. of water were heated to boiling in a Coors casserole. After 6 hours' boiling the color changed from white to yellow and some molybdic trioxide remained undissolved. The residue was filtered onto a No. 52 Whatman paper and washed several times with water. The dried residue weighed 26 grams; 118 grams of molybdic trioxide had gone into solution. The yellow filtrate was evaporated in a Coors evaporating dish until crystals appeared and then cooled to 25° C., whereupon the acid crystallized out in bulk. The phosphorus was determined as magnesium pyrophosphate and the molybdenum as lead molybdate. Duplicate analyses gave: 1 phosphorus to 10.10 molybdenum and 1 phosphorus to 10.12 molybdenum.

The product (crystals) obtained was dissolved in 200 ml. of water and extracted with 300 ml. of U.S.P. ether in a 1000-ml. separatory funnel. The remaining aqueous layer was extracted with ether in the presence of 5 ml. of concentrated hydrochloric acid to salt out the ether complex, and the remaining layer of water was extracted again with ether. One hundred milliliters of water were then added to the united ethereal extracts and crystallization of the aqueous solution in the presence of a little nitric acid yielded 129.2 grams of acid after removal of the ether. The analysis gave a ratio of 1 phosphorus to 11.90 molybdenum and 1 phosphorus to 11.85 molybdenum. By calculation 118 grams of reacted molybdic trioxide would give 131.7 grams of $H_7P(Mo_2O_7)_6 \cdot 10H_2O$; this formula was calculated from the analytical data. Therefore a 98% yield was obtained from the combined ethereal fractions.

B. One mole (144 grams) of C.P. molybdic trioxide, 0.1 mole (11.5 grams) of C.P. 85% phosphoric acid, and 1500 ml. of water were maintained at a boiling temperature for approximately 10 hours. A residue of 16 grams of molybdic trioxide remained when the reaction mixture was filtered. Crystallization of the filtrate gave an acid having a ratio of 1 phosphorus to 8.68 molybdenum and 1 phosphorus to 8.80 molybdenum by analysis. By calculation 128 grams of reacted molybdic trioxide would give a ratio of 1 phosphorus to 8.88 molybdenum. The impure phosphomolybdic acid obtained was purified by an ether extraction. Analysis of the acid gave a ratio of 1 phosphorus to 11.91 molybdenum and 1 phosphorus to 12.00 molybdenum.

C. One mole (144 grams) of C.P. molybdic trioxide and 0.166 mole (19.2 grams) of C.P. 85% phosphoric acid were permitted to react in the presence of 1500 ml. of water at a boiling temperature for 6 hours. At the end of this time all the molybdic trioxide had dissolved. On evaporation a sirupy liquid was obtained which was difficult to crystallize. Analysis of the solid product obtained gave 1 phosphorus to 5.62 molybdenum and 1 phosphorus to 6.68 molybdenum. Ether extraction then yielded an acid with the following ratios: 1 phosphorus to 11.85 molybdenum and 1 phosphorus to 11.95 molybdenum.

D. One hundred and fifty grams of C.P. tungstic trioxide, 6.23 grams of C.P. 85% phosphoric acid, and 1500 ml. of water were maintained at a boiling temperature for 12 hours. No reaction

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occurred. These are theoretical amounts of reactants to produce a phosphotungstic acid having a ratio of 1 phosphorus to 12 tungsten. Increasing the phosphoric acid concentration ten times did not make the reaction proceed. The authors have not had the opportunity of investigating the results obtained when freshly precipitated tungstic acid is used instead of the readily available trioxide.

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RECEIVED October 1, 1946.

Volumetric Determination of Free Sulfuric Acid in Crude Sulfonic Acids

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OIL-soluble sulfonic acids produced when petroleum oils are treated with strong sulfuric acid contain varying amounts of the free mineral acid which, upon neutralization with alkali, results in the contamination of the finished soap with inorganic salts (sodium sulfate). Hence a rapid method for the determination of the free mineral acid (sulfuric acid) content of sulfonated oil or crude sulfonic acids is needed for plant control as well as in laboratory investigations.

A procedure for the determination of sulfuric acid in the presence of sulfonic acids as described by Burton and Robertshaw (*1*) involves repeated extractions of the sample with sodium chloride solution and gravimetric determination of the extracted sulfuric acid by precipitation as barium sulfate. Although fairly accurate, this method is subject to the objection inherent in most gravimetric procedures—it is time-consuming.

Marron and Schifferli (*2*) have presented a method for the quantitative determination of the organic sulfonate content of synthetic detergents which may be used when the sulfuric acid content is not of prime importance.

Laboratory studies have shown that the sulfuric acid contained in a mixture of sulfonic acids, oil, and water can be determined rapidly and with a fair degree of accuracy by water extraction (and titration) of the mineral acid from a *n*-amyl alcohol–benzene solution of the crude sulfonic acid sample. The sulfonic acid–oil mixtures encountered are soluble in the *n*-amyl alcohol–benzene solution, and repeated extraction with water will remove all the mineral acid and only traces of the organic acids. Sulfonic acid–oil mixtures which contain impurities insoluble in *n*-amyl alcohol–benzene solution obviously cannot be analyzed conveniently by this method.

PROCEDURE

Measure by means of a pipet exactly 10 ml. (or weigh 10 grams) of the sulfonic acid into a 125-ml. separatory funnel.

Add 30 ml. of *n*-amyl alcohol and 60 ml. of benzene, and mix by shaking.

Extract the solution with two or more successive 10-ml. portions of distilled water, allowing sufficient time (2 to 15 minutes) after each extraction for the water layer to become clear before separating and proceeding with the next extraction.

Titrate each water extract separately with standard 0.1 *N* or 0.05 *N* alkali. Continue the extractions until an approximately constant titration value is obtained (three to four extractions).

The major portion of the sulfuric acid is always present in the first water extract, and a good, quick estimate of the mineral acid content can be obtained by titration of this first extract. The titer of the successive water extracts diminishes rapidly and becomes essentially constant after the second or third extraction. This constant residual value is due to the extraction of traces of sulfonic acid after removal of the sulfuric acid has been completed.

RESULTS

Table I presents the data obtained when three different sulfonic acid samples are analyzed by the volumetric method described above.

Data presented in Table I are calculated as follows:

Sample No.	1		2			3		
Analysis No.	1	2	1	2	3	1	2	3
Ts (as grams per liter of sample)	12.59	11.61	32.84	32.73	32.54	10.02	10.45	10.71
Tk (as grams per liter of sample)	0.30	0.18	0.26	0.19	0.15	0.10	0.11	0.17
No. of titers averaged	4	4	3	3	2	2	2	2
N	6	6	6	6	6	5	5	5
$T = [Ts - Tk \times (N - 1)]$	11.09	10.71	31.54	31.78	31.79	9.62	10.01	10.03

Samples 1 and 2 were prepared by adding known amounts of sulfuric acid to sulfuric acid–free sulfonic acids. Sample 3 (plant produced) contained an unknown amount of sulfuric acid and was evaluated by the gravimetric method mentioned above (*1*). These data indicate that in evaluating the sulfuric acid content of sulfonic acid–oil mixtures, deviations from true values of no more than ±2% can be expected.

Table I. Evaluation of Sulfuric Acid in Sulfonic Acid Samples

Method employed	Volumetric								Gravimetric	
Sample No.	1		2			3			3	
Volume of sample analyzed, ml.	10		10			10			10	
H_2SO_4 in sample, gram per liter	10.90		31.80			Unknown			Unknown	
Analysis No.	1	2	1	2	3	1	2	3	1	2
Water used, ml. per extraction	20	10	20	10	5	20	5	5	..	..
H_2SO_4 extracted, gram per liter										
First extraction	10.75	10.05	30.60	30.55	29.70	9.00	8.76	9.32	..	..
Second extraction	0.65	0.83	1.08	1.35	1.70	0.65	1.13	0.61	..	..
Third extraction	0.34	0.22	0.37	0.27	0.44	0.17	0.34	0.44	..	..
Fourth extraction	0.30	0.17	0.27	0.22	0.39	0.13	0.12	0.17	..	..
Fifth extraction	0.30	0.17	0.27	0.17	0.19	0.07	0.10	0.17	..	..
Sixth extraction	0.25	0.17	0.25	0.17	0.12	..	..	..	..	..
H_2SO_4 found, gram per liter	11.09	10.71	31.54	31.78	31.79	9.62	10.01	10.03	9.50	9.55
Deviation from known amount present										
Gram per liter	+0.19	−0.19	−0.26	+0.02	−0.01	..	..	..	..	..
%	+1.75	−1.75	−0.82	−0.06	−0.03	..	..	..	..	..

CALCULATION OF RESULTS

The corrected titer to be used in calculating the quantity of mineral acid in the sample is computed by means of the following expression:

$$T = Ts - Tk(N - 1)$$

where N = total number of extractions
Ts = sum of titers of all extracts
Tk = average of the small, essentially constant titers obtained after the first two or three extractions
T = corrected titer

Tk is an average of titers of extracts in which traces of sulfonic acid are present. It is assumed that this trace of organic acid is present in all extracts except the first, where tests have shown the mineral acid content is strong enough to "salt out" the organic acid. Therefore Tk is multiplied by $(N - 1)$ rather than by N to correct for the organic acid extracted.

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RECEIVED February 21, 1947.

BOOK REVIEWS

Organic Analytical Reagents. *Frank J. Welcher.* Vol. III. xi + 593 pp. D. Van Nostrand Co., Inc., 250 Fourth Ave., New York, N. Y., 1947. Price, $8 single volume; $7 series.

The third volume of this valuable series covers material representing more important procedures of analytical chemistry than the previous two volumes [reviewed in ANAL. CHEM., **20**, 88 (1948)]. The volume is divided into three parts: Heterocyclic Nitrogen Compounds, 153 pages; The Oximes, 258 pages; Acidic Imino Compounds, 164 pages, with name and synonym index as well as an index to reagent uses.

The binding is substantial and attractive, the paper stock sturdy of dull finish, and the format attractive with legible type. All that has been said in the review of the first two volumes may be said of the third volume. In Volume III there seem to be included more extensive and complete directions for the preparation of the materials and much more complete directions for their use, probably because much of the material included in this volume is the product of domestic and English origin.

In, Part I, 48 pages are devoted to pyridine. The analytical applications of pyridine have been, with 2 exceptions out of 170, references of foreign origin. Sixteen pages are devoted to quinoline and to derivatives, 40 pages to bipyridine and related compounds, and 98 pages to pyrazolone and miscellaneous heterocyclic ring nitrogen compounds.

Part II on the oximes gives 330 literature references to dimethyl glyoxime in 67 pages and 30 pages to other oximes, and 48 pages are devoted to cupferron and neo-cupferron with 161 literature citations. This section is completed with references to the use of nitroso amines.

Part III is of principal interest because of the procedures outlined in the use of dithizone; 212 literature references are included on this subject alone.

Volume III of this series represents the most valuable individual volume for those who wish to select one of the first three volumes. To have this work as a part of the library of every research and routine laboratory is a wise investment.

There are a few unfortunate errors. The functional group to which the bipyridines, phenanthrolines, and terpyridines owe their remarkable properties is the (=N—C—C—N=) group and not as given (page 101) as (—N=C—C=N—).

G. FREDERICK SMITH

The Analysis of Fermentation Acids. *James B. McNair.* xi + 290 pages. Westernlone Press, Los Angeles, Calif., 1947. Price, $7.50.

Hitherto information concerning the qualitative and quantitative determinations of the fatty acids commonly produced by fermentation, such as formic, acetic, propionic, butyric, and lactic, has been found widely scattered through the literature and in books on physiological or medical chemistry. This includes such biological material as foods, vegetable products, silage, honey, wine, alcohols, vinegar, esters, sour milk, cheese, blood, urine, and feces. In this book the author has collected analytical information from many sources and has codified, edited, and condensed it in the light of his long analytical experience. The general plan followed is the description of an analytical method for an acid selected from the literature for its apparent utility. Criticisms and modifications as suggested by various workers are reviewed and the accuracy of the method is discussed, usually from the author's own findings.

The literature of the field has been so thoroughly searched that it seems doubtful to the reviewer whether any worth-while analytical method concerning fermentation acids has escaped notice. Both chemical and physical methods are given. Analyses of single acids and mixtures of two or more are discussed. In the analyses of complex mixtures the algebraic method of Gillespie and Walters for the calculation of results is emphasized. In the preliminary separation of acids from media the author considers that ether is superior to steam distillation. That the treatment of a topic is adequate is shown by the requirement of 40 pages to explain the Duclaux method, apparatus, and technique; lactic acid occupies 102 pages. The book should be in every laboratory where analyses of fermentation acids are required.

L. E. WARREN

Metodi di Analisi Chimica Siderurgica. *Gaetano Gavioli.* xix + 379 pages. Ulrico Hoepli, Corso Matteotti 12, Milano, Italy, 1947. Price, 1200 lire.

This is a clear and well organized account of modern analytical procedures for iron, steel, and related materials. It deals essentially with classical chemical methods, but brought up to date. Spectrographic methods, for example, are only briefly mentioned in the introductory chapter on elementary techniques, qualitative analysis, spot tests, and various empirical methods.

Each procedure carries a brief explanation of the chemistry, followed by adequate details, discussion of interferences, and estimates of accuracy and of time required. In the chapter (132 pp.) on cast iron and steels, quantitative determinations are described for 28 elements, with some comparison in most cases of the relative merits of different methods. Another principal chapter (57 pp.) covers ferrous alloys and pure metals, followed by seven special chapters (111 pp.) on materials used for electrical resistance, hardness alloys, ores, slags, refractory materials, fluxes, and solid fuels. Some of the details (standard specifications, sampling conventions, etc.) represent official methods of Italian national industrial organizations, but most of the material comes from American and German official publications and from the general literature, which seems to be covered up to 1945, about 60 of the 175 references being dated 1935–45.

The methods are well organized, critically considered, and clearly described. The printing is good, the diagrams are clear, errors negligible. The binding, however, especially for what is essentially a manual, is extremely poor. It could otherwise prove a useful book despite the foreign language, particularly because of its coverage of recent literature.

JOHN E. RICCI

Proceedings for the Society for Experimental Stress Analysis. *C. Lipson* and *W. M. Murray*, editors. xix + 136 pages. Vol. V., No. 1. Addison-Wesley Press, Inc., Kendall Square Building, Cambridge 42, Mass., 1947. Price, $6.

CRYSTALLOGRAPHIC DATA

Contributed by Armour Research Foundation of Illinois Institute of Technology

THIS is the second in a series of monthly summaries of crystallographic data. The first paper [ANAL. CHEM. 20, 274 (1948)] covered the organization of this project, the conditions for cooperation by outside organizations, the conventions to be used for crystallographic descriptions, and crystallographic data for the two polymorphic forms of *p,p'*-DDT.

2. Adipic Acid

Well formed crystals of adipic acid (hexanedioic acid) can be obtained from ethyl acetate. Crystals from water either macroscopically or on a microscope slide are not well formed, although they are recognizable as basal pinacoid tablets elongated parallel to *b*. The crystals from sublimation on a microscope slide are well formed and usually give rhombs lying on the basal pinacoid (Figure 1). There is no evidence from either the thermal work or recrystallization that adipic acid exists in more than one polymorphic form.

CRYSTAL MORPHOLOGY (determined by A. Smedal and W. C. McCrone).

Crystal System. Monoclinic.

Form and Habit. Tablets from ethyl acetate elongated parallel to *b* with well developed basal pinacoid {001}; other forms present are: prism {320}, orthopinacoid {100}, and occasionally the clinopinacoid {010}.

Axial Ratio. $a:b:c = 2.014:1:1.965$.

Interfacial Angles (Polar). $100\Lambda001 = 42°55'$; $110\Lambda\bar{1}10 = 52°48'$.

Beta Angle. $137°5'$; $(137°5')$ (*2*).

X-RAY DIFFRACTION DATA (determined and checked by I. Corvin and J. Whitney).

Space Group. $C_{2h}^5(P2_1/a)$ (*3*).

Cell Dimensions. $a = 10.27$; $b = 5.10$; $c = 10.02$. ($a = 10.07$; $b = 5.16$; $c = 10.00$) (*3*). ($a = 10.27$; $b = 5.16$; $c = 10.02$) (*1*).

Formula Weights per Cell. 2; (2) (*1, 3*).

Formula Weight. 146.14.

Density. 1.344 (measured by flotation and pycnometer); 1.37 (calculated).

Principal Lines

Index	*d*	I/I_1
001	6.82	0.41
$\bar{2}02$, $20\bar{2}$	4.67	0.02
...	4.49	0.02
011, $0\bar{1}1$	4.13	1.00
101	3.79	Very weak
200	3.52	Very weak
002	3.45	0.31
...	3.31	0.05
210	2.86	0.14
020	2.53	Very weak
201	2.51	0.07
120	2.41	0.07
300	2.32	0.10
003	2.26	0.08
...	2.19	0.02
...	2.14	0.08
220	2.10	Very weak
022	2.04	Very weak
$\bar{5}05$	1.92	Very weak
202	1.87	Very weak
$\bar{2}22$	1.80	Very weak
400	1.76	Very weak
004, 030	1.71	Very weak
222	1.50	Very weak
420	1.45	Very weak
024	1.42	Very weak
500	1.40	Very weak
040	1.28	Very weak
402, 303	1.24	Very weak
240	1.22	Very weak

OPTICAL PROPERTIES (determined and checked by A. Smedal and W. C. McCrone).

Refractive Indices (5893 Å.; 25° C.). $\alpha = 1.466 \pm 0.002$. $\beta = 1.506 \pm 0.002$. $\gamma = 1.590 \pm 0.002$.

Optic Axial Angle. $2V = 76°$.

Dispersion. Very slightly inclined dispersion, $v > r$.

Optic Axial Plane. 010.

Acute Bisectrix. γ.

Extinction. $\gamma\Lambda c = 3°$ in acute β.

Sign of Double Refraction. Positive.

Molecular Refraction (*R*). *R* (calcd.) = 32.97. *R* (obsd.) = 33.00.

THERMAL DATA (determined by W. C. McCrone).

1. Adipic acid sublimes readily just below the melting point to give well formed rhombs lying on the basal pinacoid and showing an almost centered optic axis interference figure and a profile angle of 54°.

2. Melting occurs at 151–153° C. with no decomposition; the melt crystallizes on cooling with only slight supercooling. Sharp pointed rods grow rapidly to form feathery crystals; the growth rate increases as the temperature falls to room temperature.

3. All three principal views can be obtained; shrinkage cracks and interference figures are characteristic of each view (Figure 2). An almost centered optic axis figure shows $2V = 76°$, (+), with very slight dispersion, $v > r$.

4. Adipic acid is almost insoluble in thymol, although crystals in the zone of mixing during a mixed fusion show a profile angle of 44°, parallel extinction and one index less than the thymol melt.

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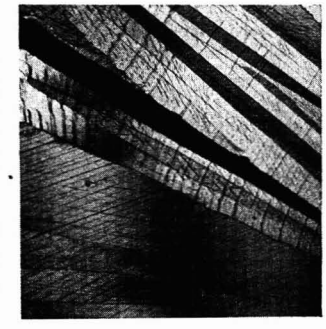
3. *trans*-Azobenzene

Excellent crystals of azobenzene can be obtained from alcohol and ethylacetate, by sublimation or on a microscope slide by recrystallization from thymol. For most purposes the sublimed

Figure 1. Crystals of Adipic Acid Formed by Sublimation on a Microscope Slide

Figure 2. Fusion Preparation of Adipic Acid
Crossed Nicols



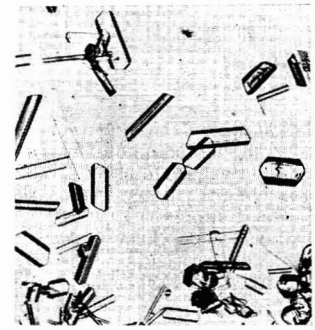


Figure 3. Crystals of Azobenzene from Thymol on a Microscope Slide

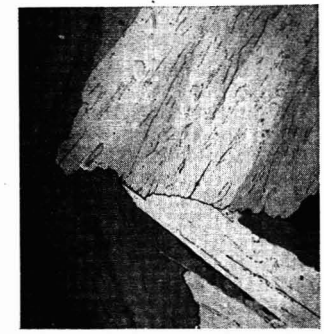


Figure 4. Fusion Preparation of Azobenzene
Crossed Nicols

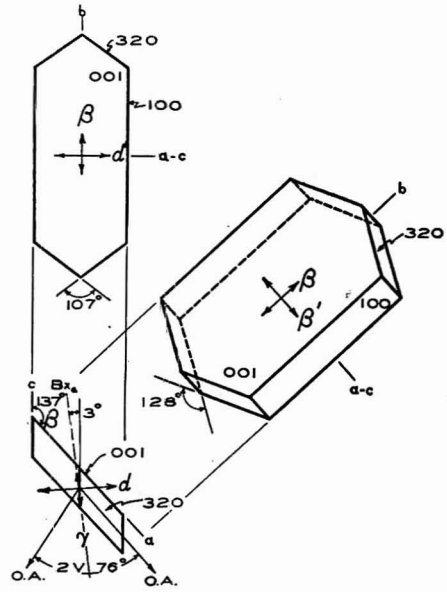


Figure 5. Orthographic Projection Showing Principal Views of Adipic Acid

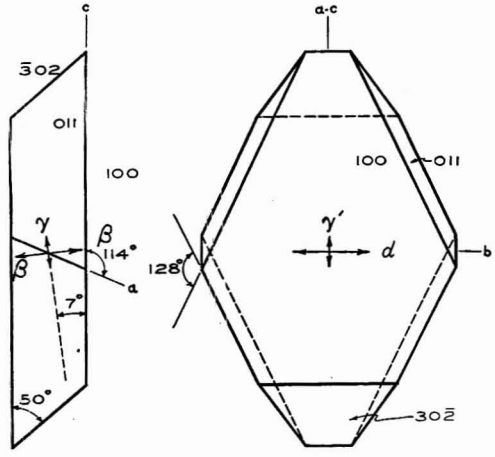


Figure 6. Orthographic Projection Showing Principal Views of Azobenzene

crystals are most satisfactory; no evidence of the existence of polymorphic forms of azobenzene was obtained.

Azobenzene has been set up differently from the orientation reported in the literature (*1*, *2*): The reorientation involves reversing the *a* and *c* axes to follow the convention $c < a$. Although this change is made with reluctance we feel that any convention based on crystal habit is impractical, as habit depends on so many factors other than cell dimensions—e.g., solvent, temperature, impurities, etc. If the convention $a < c$, rather than $c < a$, had been chosen the orientation of adipic acid would have had to be changed.

Crystal Morphology (determined and checked by W. C. McCrone and J. Cook).

Crystal System. Monoclinic.

Form and Habit. Usually tablets lying on the orthopinacoid {100}, slightly elongated parallel to *b*. Other common forms are clinodome {011}, orthodomes {$\bar{3}02$}, and the basal pinacoid {001}.

Axial Ratios. $a:b:c$ = 2.676:1:2.114 (calculated from x-ray data). 2.662:1:2.108 (calculated from goniometry) (*2*).

Interfacial Angles (Polar). $011\Lambda01\bar{1}$ = 55°; $30\bar{2}\Lambda100$ = 50°.

Beta Angle. 114°; (114° 26′) (*2*).

X-Ray Diffraction Data (determined and checked by J. Whitney and I. Corvin).

Space Group. C_{2h}^5 ($P2_1/a$) (*1*).

Cell Dimensions. a = 15.40; b = 5.77; c = 12.20. (a = 15.40 ± 0.04; b = 5.77 ± 0.02; c = 12.20 ± 0.04) (*1*).

Formula Weights per Cell. 4; (4) (*1*).

Formula Weight. 182.22.

Density. 1.220 ± 0.001 (1.22) (*1*).

Principal Lines

Index	d	I/I_1
002	7.03	0.25
200	5.59	0.12
011	5.33	0.07
110	5.12	1.00
...	4.88	0.05
003	4.62	0.44
111	4.54	0.92
012	4.47	0.41
...	4.30	0.04
...	4.18	0.17
210	4.02	0.10
...	3.82	0.58
300	3.75	0.50
202	3.67	0.49
004	3.51	0.08
...	3.39	0.04
...	3.29	0.10
...	3.19	0.23
...	3.09	0.25
...	3.00	0.12
020	2.88	0.07
005, 400	2.80	0.04
022	2.68	0.17
220	2.57	Very weak
204	2.51	Very weak
303	2.45	Very weak
006	2.34	Very weak
222	2.27	Very weak

Optical Properties (determined and checked by W. C. McCrone and J. Cook).

Refractive Indices (5893 Å.; 25° C.). α = 1.706 ± 0.005. β = 1.720 ± 0.008. γ = 1.85 ± 0.02.

Optic Axial Angles

	$2E$	$2V$	$2H$
Red	48°	27°	31°
Blue	88°	49°	54°
5893 Å.	64° (59.5) (*1*)	36°	41° (39° 21′) (*1*)

Dispersion. Very strong horizontal dispersion of the optic axial angle, $v > r$; very slight dispersion of the optic axial plane.

Optic Axial Plane. ⊥010 with $\gamma\Lambda c$ = 7° in obtuse beta.

Acute Bisectrix. γ.

Sign of Double Refraction. Positive.

Molecular Refraction (R). R (calcd.) = 59.71. R (obsd.) = 62.26.

Pleochroism. Very light yellow (X); orange (Y); and deep orange (Z).

Thermal Data (determined by W. C. McCrone).

1. Azobenzene melts at 68° C. with no decomposition and only a slight tendency toward sublimation. The melt always crystallizes spontaneously and very rapidly; the rate increases as the temperature falls.

2. The crystal front is angular, usually with a profile angle of 50°. This view shows γ and β with oblique extinction of 7° and an obtuse bisectrix interference figure. Figure 4 shows a completely crystallized fusion preparation of azobenzene.

3. Azobenzene is very soluble in thymol and can be crystallized from this solvent (Figure 3). A thymol mixed fusion gives good profile angles; 129°, 115°, and 155° are prominent with a flash interference figure.

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Optical Society of America

L. T. HALLETT, Associate Editor

SEVERAL papers were presented at the meeting of the Optical Society of America in New York City, March 4, 5, and 6, which are of interest to analysts. They are abstracted below:

Some Techniques and Accessories to Facilitate the Application of a G.E. Recording Spectrophotometer. E. E. RICHARDSON, Kodak Research Laboratories, Rochester, N. Y.

Fiducial marks for quickly placing the graph paper, a method for checking wave length, a system for cooling the light source, a convenient provision for replacing lamps, and an air-drying unit attached to the amplifier are described. A modified integrating sphere and its complete assembly having two hemicylindrical tubes with a portion extending from the split lenses to the sphere wall offer a means of making transmittance (total and specular) and reflectance (total and diffuse) measurements, and reduce stray-light errors in testing the transmittance of interference filters and fluorescent filters and solutions. Aluminum plates for supporting magnesium oxide surfaces, plates for the specular ports, and specular light traps are described. A prism system has been constructed for testing small areas in the center of transparent objects 8 inches square and 5 inches in length and the axial area of lenses, especially photographic objectives. With this system reflectance can be measured at 45°, with light polarized in the plane of incidence. Accessories are used for measuring the transmittance at oblique angles for polarization both in and perpendicular to the plane of incidence. A method for testing small areas without and with auxiliary lenses, a means of increasing the wave-length range from 425 to almost 1000 mμ, and its wave-length calibration are explained.

Standards of Reflectance. HARRY J. KEEGAN, National Bureau of Standards, Washington, D. C.

In 1931, the International Commission on Illumination adopted a resolution (3a) which may be translated as follows: "For the colorimetric measurement of opaque materials the luminance of the specimen studied ought to be expressed as a function of the luminance of a surface of the oxide of magnesium considered under the same conditions of illumination and observation." Accordingly, the National Bureau of Standards further developed its technique for the preparation of magnesium oxide from freely burning magnesium turnings and published a letter circular (present number LC-547) describing the advantages and disadvantages of such a standard. Reference is made to this procedure in three engineering test method specifications: the spectral characteristics and color of objects and materials, a colorimetric test and pulp, and certain spectrophotometric and colorimetric test methods for paint, varnish, lacquer, and related materials. Although magnesium oxide standards of reflectance are widely accepted in principle, it is understood that relatively few laboratories follow a technique of preparation that will yield the most accurately reproducible standards.

The details of the technique developed at the National Bureau of Standards are shown, together with calibrations of a working standard of reflectance made of machine-ground and polished white glass. These repeated calibrations made over a period of about 10 years show, from the minor random fluctuations, the degree to which the successively prepared magnesium oxide standards are reproducible. The maximum spread in spectral reflectance is about 0.5%. The calibrations also show from the lack of a significant regular trend in these fluctuations that the master working standards are permanent to a degree even exceeding the best reproducibility that we have been able to obtain with magnesium oxide; and it is far more permanent than magnesium oxide, which may yellow by a detectable amount within one day after preparation. The bureau is now prepared to issue reflectance standards of fire-polished white glass calibrated by comparison with the master standards. These standards of spectral reflectance are available both to public and private agencies for spectrophotometry and colorimetry.

An Application of Spectrophotometry in the Textile Industry: Testing Dyestuff Shipments. E. P. MERSEREAU AND J. L. BARACH, Alexander Smith and Sons Carpet Co.

Traditionally, dyestuff shipments have been tested by visual comparison of yarns or fabrics dyed by standard procedures. The comparison is based on a standard dyestuff mutually accepted by the supplier and user. Because this method is both time-consuming and insensitive, many consumers and suppliers are turning to spectrophotometric measurements of the absorption of dye solutions for standardization and testing. The application makes use of the well-known Beer-Bouguer-Lambert law. Allowing for the infrequent occurrence of errors caused by a lack of ability of the method to show differences in substantivity of the dyestuff for the fiber, absorption spectrophotometry has been found of great value.

In the solution preparation, standard chemical analytical techniques are used with the addition of certain precautions such as control of pH, solution preparation temperature, time of standing, dye interaction effects, and concentration. All data and pertinent information are placed on a card for each dyestuff. The strength values expressed as per cent of standard concentration are entered on a control chart, the limits of which are chosen to indicate the range of acceptable variation in relation to the observed precision of both the dyeing process and the variation of one shipment to another. The standard deviation for five determinations of one sampling is 0.33, expressed in per cent of standard, while that for one shipment to another is approximately 2.0. Correlation is obtained between this method and actual dyeing experience. It is of clear-cut value to an operating textile company consuming dyestuffs to use spectrophotometric solution measurements to give not only an increased accuracy and precision over the test dyeings, but also a saving of time of as much as 0.5 hour per test on the average.

Applications of Spectrophotometry in a Textile Plant. FREDERICK T. SIMON, Shelton Looms, Sidney Blumenthal and Co., Inc.

Certain special methods of spectrophotometric technique have been developed or applied in connection with the problems encountered in a textile mill. These mainly concern the measurement of dyed fabrics and the solutions of the dyes that are used to color them. Inasmuch as the methods of handling water-soluble dyes in aqueous solution are well known, only the procedures for preparing vat and naphthol dyes are discussed. Multicomponent dye mixtures are the usual case encountered in dyeing and printing of textiles. In order to obtain quantitative data on these mixtures, a polyethylene oxide condensate must be added to the solution to produce additivity. The concentration of each component of the mixture then can be accurately computed by means of a reciprocal matrix when a given combination is analyzed repeatedly. Some of the phenomena of the dyeing process are studied, using the spectrophotometer as a tool for the measurement of dye baths while the dyeing is proceeding. A miniature dye box was developed for simulation of production dyeing and is connected with a continuous flow cell placed in the spectrophotometer. These are discussed and demonstrated.

Spectrophotometric Method for Determination of Lead. GEORGE L. REYMANN, KENNETH H. SMITH, AND N. F. BARR, Stoner-Mudge, Inc.

The application of a recording spectrophotometer as a useful tool in the development of techniques and methods of quantitative analysis has been demonstrated. In the standardization of a procedure for rapid and precise determination of microquantities of lead in lacquers by the dithizone method, it was observed in measuring the absorption at 520 millimicrons that at low pH ranges high results and poor reproducibility were obtained. By a study of the transmission curves drawn by a G.E.-Hardy recording spectrophotometer throughout the entire visual range, a second absorption band at 620 millimicrons was observed. As the pH was raised the absorption at 620 millimicrons decreased, reproducibility was improved, and values approaching the theoretical were obtained. An optimum pH range of 10.5 to 11 was thus quickly and easily established.

Evaluation of the Elementary Causes of Deviations in Spectrochemical Analysis. JOSEPH GEFFNER, Weirton Steel Co.

The difference between an individual spectrochemical determination and the "true" composition of a sample may be divided into two parts: (1) a constant amount called the bias, equal to the difference between the "true" composition and the average of all such spectrochemical determinations made under essentially identical conditions, and (2) a variable amount called the deviation, equal to the difference between the individual determination and the average of all such determinations. The frequency distribution of the deviations of all determinations generally approximates the normal, and its sigma (standard deviation) is a measure of the uncertainty of the analytical method. The deviation of an individual determination may be subdivided further into components due to elementary causes such as sample inhomogeneity, photographic response variations, excitation instability, and others. Using the statistical technique of analysis of variance, the contribution of each of several such elementary causes to the total uncertainty may be estimated, together with fiducial limits within which the true value of each estimated contribution may reasonably be expected to lie. The results of a comprehensive experiment for evaluating the total uncertainty, the uncertainty components, and their fiducial limits for the spectrochemical analysis of copper in steel are reported.

Quantitative Spectrographic Analysis of the Rare Earth Elements. VELMER A. FASSEL AND H. A. WILHELM, Iowa State College, Ames, Iowa.

The spectrophotometric methods generally used for the quantitative analysis of the rare earths, do not permit the determination of low concentrations—i.e., less than 2% of rare earth impurities in another rare earth, and the determination of major amounts of those members of the group which possess no strong absorption bands in the wave-length region 2200 to 10,000 Å. Emission spectra methods which are designed to supplement the spectrophotometric methods have been developed. The specific examples described are the determination of samarium in neodymium (0.1 to 2%), europium in samarium (0.01 to 4%), and yttrium and gadolinium in complex mixtures (10 to 1000%). The methods, however, should be readily adaptable to similar determinations involving the other members of the series.

For the determination of minor constituents, the procedures involve the high amperage direct current arc excitation of rare earth oxide–powdered graphite mixtures. Selected lines of the major rare earth constituent serve as almost ideal internal standards. For the determination of major constituents in complex mixtures, similar excitation conditions are used. The selection of cerium as the internal standard for these determinations was based on the relative ease with which any cerium present in a mixture could be preferentially separated from the mixture and then reintroduced in a standard amount. Studies on the variation of the intensity ratios during excitations and the effect of large changes in arc current, per cent graphite, and weight of sample charge on the integrated intensity ratios are included. Precision and accuracy data indicate that the experimental error for duplicate determinations is about ±4% of the amount present.

Wide Range Analysis for Zinc Using Spectrographic Line Widths. E. JOHN EASTMOND AND BERNICE E. WILLIAMS, U. S. Department of Agriculture, Albany, Calif.

A spectrographic method has been developed for determination of zinc in biological ashes by use of line width as a concentration index. Synthetic standards and analytical samples, in the form of sulfates, are mixed with a potassium sulfate–graphite buffer containing barium and cadmium as internal controls. Samples are excited in a direct current arc and spectra recorded with a Littrow quartz spectrograph. The contour of Zn 2138 Å. is recorded with a recording microphotometer and the width determined at the maximum density of the control lines Ba 2255 Å. or Cd 2288 Å. A plot of the logarithm of this width *vs.* logarithm of zinc concentration prepared from standard samples containing 0.004 to 10% zinc serves as an analytical curve. Results determined by ordinary density measurements have been compared with those from width measurements. Although the two methods show comparable reproducibility of repeated exposures at the lower limit of the analysis range, the width method is more precise at higher ranges and has proved applicable even at concentrations where the line density method is limited by self-reversal and low photographic plate contrast. Concentrations as high as 10% have been determined, indicating possible application of such a method to the estimation of major constituents in such biological ashes or inorganic powders.

Practical Applications of a Replica Grating Spectrograph Installation. J. T. ROZSA, National Spectrographic Laboratories, Inc.

Techniques for analyses by a Wadsworth mounted replica grating spectrograph with mated accessories have been worked out on a variety of applications, including oil, low alloy steel, lead, aluminum, and zinc alloys, siliceous ceramic materials, blood, and general qualitative analysis. Working curves, analytical line pairs, exposure times, and general performance data are given. For applications not involving high spectral line count materials or extreme sensitivity quantitative work a replica grating will be found as satisfactory as an original grating.

Image Contrast Control with Phase Microscopy. OSCAR W. RICHARDS, American Optical Co.

Four types of diffraction plates are possible, depending on whether the absorbing material is on (*A*) the conjugate, or (*B*) the complementary area, and the retarding medium is on the conjugate (+), or on the complementary area (−). Bright contrast occurs with *A*+ and *B*+ diffraction plates, because regions of longer optical path in the specimen appear brighter than those of lesser path; conversely, dark contrast occurs in the image when *A*− and *B*− plates are used. Dark contrast is useful when the image should resemble that from a stained preparation and for measurement. However, the dense contrast from *A*− plates may obscure some of the underlying detail in the specimen, which would be visible with the more transparent dark contrast *B*− or bright contrast *A*+ diffraction plates. Bright contrast is useful for counting, motion study, and for stereophotomicrographic analysis. Smaller objects are seen more easily in the image with bright than with dark contrast.

Application of the Weber-Fechner law and the periodic principle reduces the many possible combinations of diffraction plates to those needed in practical microscopy. The image of small biological particles, as virus and certain cancer cells, has best contrast with 0.1λ retardation. Many specimens show best with λ/4, but other highly refractile materials like glass require greater retardations. At low magnifications (±100×) a retardation of λ/3 is often better than λ/4. While different retardations could be obtained with one plate and light of different wave length, the differential sensitivity of the eye to different colors precludes generally satisfactory application. Absorbing material on the diffraction plate increases the contrast in the image of both unstained specimens containing optical path differences and of weakly absorbing, poorly pigmented specimens. Increased visibility is often possible by the use of a diffraction plate which increases the contrast in the image from the principal object and decreases the image contrast of interfering material. Further increased contrast is attained by combining color contrast and phase contrast in the image of specimens with selective wavelength absorption. Photomicrographs illustrate these principles.

The Multipupil in Phase Microscopy. HAROLD OSTERBERG, American Optical Co.

In the conventional phase microscope the diffraction plate is located at or inside the microscope objective and at the plane conjugate to the condenser diaphragm. Condenser diaphragm and diffraction plate act as entrance and exit pupils, respectively. Additional real images of the condenser diaphragm may be formed by means of auxiliary lens systems. These images are called multipupils. Diffraction plates may be placed at one or all of these multipupils. The increase in the length of a bipupil system can be moderate and can afford ample space at the second pupil for the more complex elements of variable phase systems. As expected, multipupil systems function as effective phase contrast systems. It is shown on general theoretical grounds that the multipupil system is equivalent to a single system of phase microscopy whose over-all magnification is equal to the product of the magnifications of the multipupil system and whose pupil function is the product of the complex pupil functions associated with each of the multipupils. The physical interpretation of the composite pupil function as a product is that the optical paths through corresponding areas of the multipupils are additive, while the corresponding amplitude transmissions are multiplied. This is another expected result. The theory will illustrate the usefulness of Fourier transformations in considering problems of phase microscopy.

Modern Instrumental Methods of Analysis

L. T. HALLETT, Associate Editor

THE three-day (March 22, 23, and 24) symposium on Modern Instrumental Methods of Analysis, sponsored by the Minnesota Section of the A.C.S. and the Institute of Technology of the University of Minnesota, drew a registration of 300. The papers presented by experts in the field were well received and promoted formal and much informal discussion. The arrangements and housing facilities provided by the committee headed by W. N. Lipscomb at the University of Minnesota Center for Continuation Study made the meeting pleasant as well as instructive.

Instrumental Methods of Analysis. R. H. MÜLLER, New York University, New York, N. Y.

In the past several years many and varied new techniques of instrumental methods of analysis have been developed.

One of the more interesting techniques of recent years is a method for analyzing homologous series of fatty acids by a continuously flowing chromatograph, developed in Sweden during the war by Claesson. This is accomplished with a filtered and collimated beam of light, passing through a small analyzing cell which contains two compartmented sections divided by a plate of glass at an angle of 45°. In one section is a reference solution; in the second flows the solution from the adsorption column, which is to be analyzed. As the various components of the homologous series are forced through the cell, changes in refractive index take place which are proportional to concentration. These changes are recorded on permanent records.

Also discussed were various mechanoelectronic schemes for automatic pipetting and for automatic titration end-point determinations. The methods discussed were of two general varieties, those of a purely electronic nature and those employing mechanical differentials in conjunction with electronic circuits.

This discussion points the way toward the practically unexplored field not only of analytical instrumentation but of instruments for many purposes and uses in and out of the laboratory.

General Optical Methods of Analysis. E. J. MEEHAN, School of Chemistry, University of Minnesota, Minneapolis, Minn.

Methods of chemical analysis based upon optical measurements were discussed. They included spectrochemical analysis by means of emission spectra, absorption spectrophotometry in the visible and ultraviolet regions of the spectrum, use of infrared and Raman spectra, analysis based upon the measurement of the intensity of scattered light, analysis by means of fluorescence, photoelectric colorimetry, and refractometry. The principles upon which each of the methods is based and a brief description of the practical utilization of each method were given.

The primary purpose of the paper was to compare and evaluate the general optical methods, considering especially the following aspects: the type of analytical problem to which each method is applicable, the kind and accuracy of the analytical result obtained, and the comparison with nonoptical methods of analysis.

It was pointed out that absorption spectra give relatively less specific information to analysts because of the broad bands obtained. An exception to this is the bands obtained from the rare earths. The millionfold increase in the intensity of spark spectra in going from 1000° to 4000° C. was cited as an important factor which permitted the determination of trace elements by emission spectroscopy.

Because of the possibility of the overlapping of spectral lines, the importance of selecting several lines for positive identification of elements was stressed.

In fluorescence analysis questionable results can be obtained when self-quenching or quenching by electrolytes or ions of molecules takes place. While the speaker did not specifically mention the large amount of work published in this field, this basic work should permit the analyst to appraise the influence of foreign ions on fluorescence quenching. Little of this information has found its way into the analytical literature on fluorescence.

Molecular Weight Determination by Light Scattering. P. DEBYE, Cornell University, Ithaca, N. Y.

Pure liquids scatter light because of their molecular structure, which causes thermal fluctuation of density and refractivity (Einstein). A solution scatters more because on top of the density fluctuations we are now also having fluctuations in concentration with corresponding additional fluctuations of the refractive index. For small concentrations this excess scattering is first proportional to the concentration, c. It also depends on how much change in refractive index results from a deliberate change in concentration. If measurements are made which determine the quotient of change in refractive index over concentration, this quotient defines a refractive constant, H, which is a second factor in the relation between excess turbidity and molecular weight. The third and last factor in this relation is the molecular weight, M, itself, such that the product HcM is exactly equal to the excess turbidity. By measuring this excess turbidity and combining it with a measurement of the change in refractive index with concentration, we, therefore, have an absolute method for determining molecular weights. As the excess turbidity is proportional to M, the method is especially useful for substances of high molecular weight. Osmotic pressure is proportional to $1/M$, which makes the classical osmotic method not so desirable for this case.

For substances of high molecular weight and especially for long-chain molecules, deviations of van't Hoff's classical osmotic relation begin to be important at very low concentrations. The same is true for the turbidity relation. It can be shown that both these deviations are intimately related to each other. They can be handled satisfactorily and used as a source of information about molecular interaction (good and bad solvents). The molecular weight of a polymer dissolved in a mixture of two solvents may seem to depend on the mixture. This may be a real association effect; however, it has been shown in some important cases that the variability of the molecular weight is only apparent and is a result of preferential absorption of one of the components of the solvent on the polymer.

With substances of high molecular weight another deviation of the limiting turbidity relation, which has no counterpart in the osmotic pressure relation, becomes apparent. It concerns the refraction constant, H. In the usual formula which tells how to calculate H from the observed change of refractive index with concentration, it is supposed that the dimensions of the solute molecule are small compared with the wave length of the light. If this no longer holds, an angular dissymmetry of the scattered light can be observed (more forward than backward scattering). The interference theory of this effect shows how the relative magnitude of the scattered intensity in two directions, say, 45° and 135°, can be used to measure the size of the particle. For this purpose it is not necessary to know the absolute intensity of the scattered light. At the same time H becomes smaller than in the limiting case of very small particles and so the molecular weight derived from the simple formula has to be corrected by a correction factor larger than unity.

For high enough molecular weights the turbidity may become large enough to be measured as an apparent absorption with the usual instruments. In this case a spectrophotometer can be substituted for the special instrument in which the scattered light is measured directly. The total scattering is measured as an apparent absorption for different wave lengths. If we call α the absorption coefficient, it is found that Hc/α in the limit for small concentrations depends on the wave length and, therefore, cannot represent $1/M$. However, plotting Hc/α against the square of the reciprocal wave length a curve appears which is practically a straight line, which extrapolated determines an intercept equal to $1/M$. The slope of this straight line is a measure of the size of the particle.

Infrared Absorption Spectrometry. R. BOWLING BARNES, Physics Division, American Cyanamid Co., Stamford, Conn.

The infrared spectrum of a given organic compound may be used to characterize the particular compound in question. It is one of the most truly unique of the physical constants and as such it serves well as a "fingerprint." In contradistinction to many other physical properties, the infrared spectrum of a mixture of two compounds, A and B, does not lie somewhere between A and B, but consists of a direct superposition of the spectrum of A plus the spectrum of B. This is true for a mixture of any number of components, provided the components exist individually and do not exhibit any physical or chemical interactions among themselves. Thus the infrared spectrum of a mixture may be used for purposes both of qualitative and of quantitative analyses. As generally presented, such a spectrum is a plot of per cent transmittance as ordinates versus the wave length (in microns) or the

frequency (in cm.$^{-1}$, $V = 1.104$), as abscissas. The latter furnish the data for qualitative analyses, whereas the former supply the information needed for making a quantitative analysis.

Early during the past war, obtaining very rapid analyses of gas streams for their content of iso- and *n*-butane became extremely urgent. The fact that this and many other critical analyses could be performed by infrared methods in a matter of minutes caused instrument manufacturers to consider the production of relatively small and inexpensive infrared spectrometers. Several excellent spectrometers are now on the market and still others are soon to be offered. Today there are probably one hundred infrared spectrometers in use for every one in use ten years ago. Analytical papers on infrared are appearing at a rapid rate, and the general usefulness of this instrumental method is becoming more widely recognized.

The infrared method is applicable to materials in the solid, liquid, or gaseous state; requires a very small sample which is neither destroyed nor damaged during the analysis; is generally far less time-consuming than conventional methods; and may frequently be rendered automatic, or used for purposes of control. Depending as it does upon the changes of electric moment which accompany certain of the vibrations of the atoms within the molecule, an infrared absorption spectrum may be used either to characterize structurally or to fingerprint a molecule, to detect the presence or absence of certain specific reactive groups within the molecule, or to serve as a basis for qualitative or quantitative analyses.

A new technique was described which permits the use of water as a solvent. It involves the use of heavy hydrogen, which shifts the water vapor band away from the portion of spectrum under investigation. The details of this method will be published in the near future.

Raman Spectra. E. J. ROSENBAUM, Sun Oil Co., Norwood, Pa.

The Raman spectrum of any substance under analysis is obtained by a light-scattering process and is in no way connected or concerned with absorption or reradiation. The Raman spectrum of every compound or material is unique and distinct; hence Raman spectra effectively "fingerprint" each chemical compound.

Vibrational energy levels are the truly important characteristic quantity of a given molecule. Some small portion of light is always scattered in passing through a sample. The greater part of this light does not have a change in frequency; a small fraction of this light does have a frequency change.

When light attempts to pass through a substance under consideration, collisions occur between photons of light and molecules of the compound. The greater part of these collisions are elastic and hence no transfer of energy occurs. A few of the collisions, however, are not elastic, and there occurs a transfer of energy between the particular photon and molecule involved. This energy is vibrational in nature, and the transfer of energy may occur in either direction—i.e., the photon may transfer energy to the molecule or vice versa, depending on which has the higher energy level. Where energy is given up by the photon, the scattered light is of a lower frequency. Conversely, the light is of a higher frequency when the energy is transferred to the photon from the molecule. This scattered light of greater or lesser frequency, when detected and recorded, forms the Raman spectrum of the substance under question. Lines on the long wave side are called Stokes waves; those on the short wave side are called anti-Stokes waves.

The invariant in Raman spectra is the frequency difference between the Raman lines and the exciting frequency; this is called the Raman shift, and is unique for each kind of molecule and invariant for a particular molecule, regardless of the exciting frequency.

This method of analysis, like all methods, has its limitations. Most serious is the fact that the sample under consideration must be colorless when visible light (such as the mercury arc) is used as the exciting frequency. Furthermore, the sample may not be turbid, even to the smallest degree, and it can contain no fluorescent impurity. While Raman spectra of gases can be obtained, the scattering effect of gases is of such a low order of magnitude as to render Raman analysis of gas samples untrustworthy and impractical.

The best type of sample to consider for a projected Raman analysis is a good cut from an analytical column. For best results and for the convenience of the analyst, the sample should not contain more than five components, although more complex samples can be accurately analyzed.

Samples can be analyzed qualitatively by the distinctive fingerprint of the spectrum itself, and furthermore, the intensity of the Raman line is proportional to the concentration of a given substance. Because the absolute intensity of the Raman spectra is so very weak, the only truly satisfactory method of recording these phenomena is on photographic plates; even here relatively long exposure times are required, and because photographic emulsions are nonlinear, the problem is further complicated. However, quantitative results with Raman spectra photographically recorded, are in general, accurate to the nearest per cent.

Analytical Applications of Electron Microscopy. JAMES HILLIER, Radio Corporation of America, Princeton, N. J.

Because electric or magnetic fields possessing rotational symmetry behave as true lenses for electrons traveling approximately parallel to the axis of symmetry and the wave length associated with the periodic behavior of electrons is very small, microscope systems using electrons and possessing high resolving power can be constructed. Images demonstrating resolution of the order of 10 Å. have now been obtained under optimum instrumental and specimen conditions. In the discussion it was shown that the resolving power of the lenses used and the resolution demonstrated at specific points of an image may differ by orders of magnitude. Information made available through the use of the electron microscope is completely dependent on the resolution demonstrated in the image and on a number of other factors relating to the suitability of the preparation of the specimen.

The electron microscope as an analytical tool was contrasted against the other types of instruments described in the symposium in that it provides specific information regarding the heterogeneities of a system rather than the average values of some quantities which are common to a very large number of nearly identical entities. It was shown that the electron microscope provides accurate measurements of lengths and of angles—i.e., of size and shape. Thus, measurements on nearly similar particles can be compiled statistically and it is possible to plot size and shape distributions accurately. This method is particularly useful when complicated shapes are encountered and when the particle size distribution characterizes the behavior of a material.

The electron microscope was shown to be of greatest value as a means of conveying to the brain through the sense of vision, qualitative information regarding the organization of structures in the range of dimensions 10 to 10,000 Å. Its major limitation at the present time was shown to be the lack of suitable criteria by which the accuracy of representation presented by the electron microscope may be judged.

Use of Ultraviolet and Visible Spectrometry for Identification and Determination of Structure of Organic Compounds. R. NORMAN JONES, National Research Council, Ottawa, Ontario, Canada.

The talk dealt principally with the relation between the electronic absorption spectra and the structure of organic molecules, and examples were given to illustrate the application of this knowledge to the elucidation of the structure of organic compounds.

The absorption of radiation in the visible and ultraviolet regions of the spectrum excites the covalent bonding electrons of the absorbing molecule. For instrumental reasons such measurements are normally limited to the range between 2000 and 8000 Å., and absorption in this region of the spectrum is associated with excitation of double bonds. Consequently, ultraviolet spectrometry provides information about the unsaturated centers in the molecule.

Examples were given of the spectra associated with carbonyl groups and with various types of conjugated dienes and polyenes. The effect of *cis-trans* isomerism on the spectra of polyenes was illustrated and the influence of ionic groups on the ends of the conjugated system was discussed. These observations provide the basis of the modern theory of color and chemical constitution of dyestuffs.

The spectra of aromatic hydrocarbons of the anthracene and phenanthrene type are much more complex than those of linear unsaturated compounds. Examples were given to show how such spectra have been used to establish the structure of new aromatic compounds and to follow the reaction of aromatic compounds with proteins.

Polarography and Amperometric Titrations. I. M. KOLTHOFF, School of Chemistry, University of Minnesota, Minneapolis, Minn.

Voltammetry involves the determination of interpretation of current-voltage curves. When the dropping mercury electrode is used as the indicator electrode the subject is called polarography, the term introduced by J. Heyrovský of Charles University, Prague. The principles of the use of a stationary and a rotating platinum electrode in current-voltage measurements were

discussed. Although of limited use, it appears that the rotating platinum electrode had great advantages in the rapid determination of traces of reducible substances—for example, traces of dissolved oxygen, bromine and iodine, etc.

The theoretical fundamentals of polarography were discussed and a brief discussion was given of the use of a polarograph in inorganic and organic analysis. A review was given of amperometric titrations using the dropping mercury electrode or the rotating platinum electrode as indicator electrode.

Mass Spectroscopic Methods. ALFRED O. C. NIER, University of Minnesota, Minneapolis, Minn.

The mass spectrometer and mass spectrograph are finding more and more application as analytical tools. Isotopic analyses of all the elements can be made without too much trouble. Modern mass spectrophotometers can differentiate abundance ratios of isotopes to better than 1%, and they can detect extremely minute amounts of isotopes; for instance, the ratio of He^3 to He^4 in well gas is 1.5×10^{-7} to 1, yet the He^3 can be readily detected. The work by Dempster at Chicago on the analysis of solids was mentioned. This technique was described in the column "Instrumentation" for February. The author pointed out that the fundamentals of this technique are well established, and it remained an engineering problem to design instruments for wider, more general use. Quantitative analyses of gaseous mixtures, even those containing isomeric organic compounds, have been reduced to a laboratory routine. Automatic recording mass spectrometers have been employed in making continuous analyses of the process gas in industrial processes. Some of the processes at Oak Ridge were controlled by such a method.

Partial pressures down to 10^{-17} mm. of mercury can be measured, so long as some other substance of the same mass number is not present in the mixture. For example, if water vapor is present in the mixture, it would be hopeless to try and detect some other substance of mass number 18.

A brief discussion of the principle of operation of modern mass spectrometers and mass spectrographs was given. Methods and problems encountered in applying these instruments to various problems were discussed.

Analysis by Emission Spectroscopy. J. R. CHURCHILL, Aluminum Co. of America, New Kensington, Pa.

Spectrochemical analytical methods received a tremendous impetus from the war because they are time- and labor-saving, both important considerations under wartime pressure.

First to be considered in any discussion of emission spectroscopy are the emission sources. There are two general classifications of sources—the conventional arcs and various types of sparks, including the "simple" spark, Feussner spark, Hasler spark, and Wolfe spark. The Hasler spark, which is a recent development, is supplied by a rectified 240-cycle alternating current. Rectification is accomplished by a four-diode circuit which assists in maintaining a constant light source. The Wolfe spark, an air interrupter type, affords excellent control of the breakdown voltage. Consequently, it gives more reproducible results.

Originally, as in Raman spectra, satisfactory recording was accomplished only by photographic means. However, since the advent of photomultiplier tubes, the photographic method of spectroscopic recording is gradually being replaced by the more satisfactory photoelectric means.

The use of emission spectroscopy was discussed from the viewpoint of modern metallurgical analytical procedure, in which field emission spectroscopy has proved an invaluable time- and labor-saving boon.

X-Ray Methods of Analysis. LUDO K. FREVEL, The Dow Chemical Co., Midland, Mich.

Two types of x-ray methods of analysis were discussed: diffraction techniques for single crystals and polycrystalline materials, and x-ray spectroscopic methods applied to liquids or solids.

The single crystal methods (Laue, oscillation, Weissenberg) are most effective in the field of structure determination. Applications of these techniques to the complex cyclodiphenylsiloxanes showed that classical molecular weight determinations and melting point criteria were inadequate to characterize these substances. The variation in melting point behavior and erratic molecular weight values were found to be due to polymorphism, solvent of crystallization, and eutectic formation. The powder diffraction method gives considerably less structural information than the single crystal methods; but its simplicity and general applicability make it attractive for the chemical identification of solids, for crystallite-size determination of submicroscopically fine powders, and for phase diagram studies. The utility of the powder method resides in its ability to detect the state of chemical combination of each crystalline component in a mixture. Typical problems solved by this diffraction method are: identification of corrosion products, constitution of fluxes, detection of pigments or fillers in plastics or elastomers, analysis of minerals, recognition of polymorphic modifications; determination of quartz in siliceous dusts, and study of chemical reactions in the solid state. In applying the fingerprint method to a large number of actual analyses, one encounters cases where the patterns sought are not found in a collection of 2000 to 3000 cataloged patterns. In those instances the matching method can be augmented by systematically comparing the diffraction pattern of an unidentified phase with representative patterns of the various known crystal structures in an attempt to establish isomorphism between the unknown phase and one of the standard structures.

X-ray spectrochemical analysis can be carried out by either emission spectroscopy or absorption spectroscopy. A new development in the field of spectrochemical analysis is the Dow x-ray absorption spectrometer. The optical system of the instrument consists of a point source of x-rays, an absorption cell, and a cylindrical multiple-crystal "lens" situated midway between the source and the receiver slit of a Geiger-Müller tube. For automatic recording of the absorption spectrum of a substance the receiver assembly containing the Geiger-Müller tube travels along the optical axis of the instrument at exactly twice the velocity of the lens. Accurate quantitative analyses of the chlorine content in hydrocarbon mixtures can be carried out with this spectrometer.

Applications of Radioactive Isotopes to the Field of Analytical Chemistry. PAUL R. O'CONNOR, School of Chemistry, University of Minnesota, Minneapolis, Minn.

A radioactive atom will show exactly the same chemical properties as a stable atom of the same element up until the time that radioactive decay occurs, as the chemistry of an element is dependent only on its atomic number. Consequently, the radioactivity can serve as a means of tracing a given chemical type through various procedures and offers unique advantages in many cases of ease and rapidity of analytical determinations, the abiiity to determine materials at very low concentrations, and finally the means of identifying the properties of a given atom when mixed with other atoms of the same element.

In the relationship $dN/dt = -\lambda N$, $-\lambda N$ is defined as the "activity" of the isotope in question, where λ is the decay constant of the isotope, N is the number of atoms involved, and t is time. Furthermore, $N = N_0 e^{-\lambda t}$, where N is the number of atoms at any time, and N_0 is the original number; $N_0/2 = N_0 e^{-\lambda t_{1/2}}$, where $t_{1/2}$ is the half life of the isotope being considered. The decay constant λ is defined:

$$\lambda = \frac{0.693}{t_{1/2}}$$

The concentrations of isotopes that can be handled analytically by radioactivity measurement methods are 10^{-10} M and below.

When the activity of a radioactive isotope is to be measured with a Geiger counter, it is expressed in counts per minute, 1000 counts per minute being considered the standard. The concentration of an isotope required to give the standard count of 1000 per minute is related to the half life of the isotope.

$$-\lambda N = 1000 \text{ counts per minute}$$

$$\frac{0.693}{t_{1/2}} N = 1000, t \text{ in minutes}$$

If either concentration or half life is known, the other may be calculated. For example, an isotope with a half life of one day will give the standard 1000 counts per minute when it is present in a concentration of only 3×10^{-17} M ($N = 2 \times 10^7$ molecules). Conversely, a concentration of 2×10^{-11} M is required for C^{14} to give a standard count, indicating a half life of 4500 years.

Next, some of the instruments used in radioactive measurements were discussed. First to be considered was the quartz fiber electroscope used for following gamma-rays in the analysis of radioactive material. It is very simple to construct and uses a voltage in the neighborhood of 2000 volts.

Next to be discussed were the various types of Geiger-Müller tubes and their particular functions. It was pointed out that in the newer tubes the quenching gas (usually ethyl alcohol vapor) used to suppress secondary side wall emission is omitted, as in present-day instruments the desired quenching is done electronically with the so-called quenching circuits.

Finally, the construction and functions of the vibrating-reed electrometer were explained. This device is used in working with such isotopes as C^{14}, S^{35}, and H^3 whose low energy beta-particles are ordinarily absorbed in the window of conventional Geiger-Müller counters. The vibrating reed electrometer is capable of measuring extremely minute amounts of current.

Many radioactive isotopes can be made by neutron irradiation and the availability of such isotopes as C^{14}, P^{32}, S^{35}, Ca^{45}, and H^3 offers great advantages in many lines of research.

Methods of Laboratory Molecular Distillation. EDMOND S. PERRY, Distillation Products, Inc., Rochester, N. Y.

Molecular distillation has become a useful analytical tool for separating and measuring substances of high molecular weight. Standard types of apparatus range from micro pot stills to highly specialized cyclic batch centrifugal stills which spread the distilland in a very thin film. A single molecular distillation has a separatory power of 0.5 to 1.0 operating molecular plate but because these "plates" are much larger than when measured at the higher pressures of ordinary distillation, the separations in the molecular still are considerably better than generally supposed.

In considering the relative value of various types of molecular stills, an important factor to be reckoned with is the so-called decomposition hazard. This is expressed in the relationship $D = t \times P$, where D = the decomposition constant for the still, t = time in seconds that a given molecule is being heated in the still, and P = the pressure in microns.

The laboratory molecular still allows some mixtures to be separated into pure components by various procedures. Monoglycerides, for example, can be obtained pure from an equilibrium mixture of the mono-, di-, and triglycerides. Mixtures of naturally occurring unsaturated acids of high molecular weights have been resolved into the pure components. Distillation of the multicomponent natural oils and fats usually effects only an increase in the concentration of the constituents. Wool grease yields fractions having different melting points, whereas with petroleum oils only a gradual change in viscosity is noticed.

A general use of the micro pot still is in the concentration of the substances that occur naturally in dilutions too high to permit reliable analysis. The vitamin E content of edible fats and vegetables can be determined successfully because in one molecular evaporation the vitamin concentration can be increased about 250 times in 100% yield.

A.S.T.M. Symposium on Organic Reagents for Metal Analysis

GRANT WERNIMONT, *Eastman Kodak Co., Rochester, N. Y.*

A.S.T.M. Committee E-3 on Chemical Analysis of Metals is concerned with the formulation of standard methods of chemical analysis of metals, including methods of sampling and tolerances in values obtained in chemical analysis of metals. Division D of Committee E-3 has been set up to help the other divisions to study general analytical methods of all types. One of the important activities of Division D has been arranging symposia on various topics which are of general interest to all members of Committee E-3. On March 4, a Symposium on Organic Reagents for Metal Analysis was held in connection with the spring meeting of Committee E-3 in Washington, D. C.

S. E. Q. Ashley, General Electric Co., Pittsfield, Mass., chairman of Division D, presided at the morning session and J. W Stillman, E. I. du Pont de Nemours and Co., Wilmington, Del., secretary of Committee E-3, presided at the afternoon session.

Abstracts of the five papers presented at the symposium follow:

Organic Reagents for Gravimetric Analysis. JOHN F. FLAGG, General Electric Co., Schenectady, N. Y.

Two types of organic compounds are useful as reagents for inorganic ions: compounds that form chelate complexes and compounds that form salts. Examples of chelate complexes are dimethylglyoxime with nickel and 8-hydroxyquinoline with aluminum. The insoluble precipitate formed when phenylarsonic acid is added to a solution of zirconium illustrates the saltlike compounds. The discussion was limited to two reagents which fall in the first classification.

Thionalide (*p*-aminonaphthalide of thioglycolic acid) is a reducing agent and it forms stable complex compounds with metals that form slightly soluble sulfides. This crystalline solid compound was studied rather thoroughly during the last decade by R. Berg and his co-workers but not much has been published about it in English. It is not available commercially.

The following advantages of thionalide over hydrogen sulfide as a metal precipitant were cited:

It can be used in solutions of nitric acid up to 0.5 *N* concentrations.

The metal precipitates do not drag down soluble metal ions by coprecipitation as is the case with sulfides.

Lead and cadmium do not form precipitates in acid solution, which makes possible certain separations that are impossible when hydrogen sulfide is used.

The filtrate from a thionalide separation can be conveniently used for analysis of nonprecipitated metals because the excess reagent can be converted to an insoluble flocculent compound by means of iodine.

The main disadvantage to the use of thionalide is its reducing property, which makes the removal of strong oxidizing agents absolutely necessary. The precipitate with copper also forms rather slowly. Copper, silver, bismuth, thallium, ruthenium, and palladium have all been determined gravimetrically by means of thionalide. By precipitating in the presence of different combinations of hydroxide, tartrate, and cyanide many convenient separations of metals among the sulfide groups are possible.

Picrolonic acid (1-*p*-nitrophenyl-3-methyl-4-nitro-5-pyrazolone) is another new organic reagent. It forms a crystalline precipitate with calcium which is slightly more soluble than calcium oxalate; calcium can be separated from other alkaline earth ions (10 times magnesium does not interfere) using this reagent. The precipitate is conveniently dried by drawing filtered air through the precipitate on a fritted-glass filter; it cannot be oven-dried.

Picrolonic acid forms insoluble compounds with copper, lead, potassium, and thorium. It is advantageous in the case of lead because the resulting conversion factor is favorable. To separate lead from interfering ions, it is precipitated with thiourea at 0° C., redissolved, and then precipitated with picrolonic acid.

Use of Organic Solvents in Metal Analysis. C. J. RODDEN, National Bureau of Standards, Washington, D. C.

Solvent extraction has long been used for detecting iodine by means of chloroform. From the quantitative point of view, ethyl ether serves to separate thallium, iron, arsenic, germanium, and gold as the chlorides. The concentration of chloride is very important, as is shown by the fact that iron is not extracted when the concentration of hydrochloric acid is low. Isopropyl ether has been suggested as a substitute for ethyl ether. Best results on these extractions are obtained when some kind of continuous extractor is used. Other inorganic compounds which can be extracted with organic solvents are the thiocyanates of molybdenum, iron and cobalt, and chromic acid as a complex peroxy compound.

Many metals can be separated by extraction after they have been converted to complex organic compounds. Thus the cupferron complex of uranium can be separated from many interfering substances by means of chloroform. Chelate compounds of metals with 8-hydroxyquinoline are soluble in chloroform and some of the resulting compounds (aluminum and gallium) fluoresce in ultraviolet light.

The dithizone complex compounds are well known and much used for making quantitative separations by extraction with chloroform. The solubility of dithizonates can be regulated by controlling the pH of the water layer, and adding suitable complex-forming reagents such as chloride, iodide, and cyanide.

Other metallic organic precipitates that can be extracted are the compounds of α-nitroso-β-naphthol, of diphenyl carbazide, and of salicylic acid.

Solvent extraction methods have found wide use in connection with the analysis of uranium and they can be used a great deal more widely in connection with the analysis of other metals than has been done in the past.

Organic Reagents in Colorimetric Analysis. JOHN H. YOE, University of Virginia, Charlottesville, Va.

More than 5000 organic compounds have been investigated in a comprehensive study of possible colorimetric reagents now being made by a group of educational institutions in the South. It has been the aim to chart the behavior of various kinds of organic compounds with all metals in the periodic table under both acid and alkaline conditions. A frequency table of the useful reactions of organic reagents has been drawn up for more than 600 of the 5000 compounds and it turns out that the transition elements are high in the list of those which form useful colored compounds.

A systematic method of studying the usefulness of an organic reagent is followed. For the reagent, suitable solvents must be found in which the reagent is soluble and stable. The color reaction is then studied as follows:

1. Effect of pH on stability and color of the compounds
2. Stability of the compounds to light
3. Nature of the color reaction
4. Role of color formation
5. Behavior as to conformity of the Lambert-Beer law
6. Sensitivity of the color reaction
7. Optimum concentration range
8. Effect of the presence of foreign ions
9. Effect of temperature between 15° and 35° C. on the color

Several examples of studies were discussed. *p*-Nitrosoaniline was specific in its color reaction with palladium. By systematic substitution into the organic molecule, it was found that other compounds gave good color reactions as long as the nitro group was in the para position. Some of these compounds were superior to others with regard to the systematic studies listed above and it is possible to pick the best reagent for use under different working conditions.

It was pointed out that almost too many reagents have been found for iron; nearly 25% of the 5000 compounds studied react (without oxidation or reduction) with this ion. An interesting reaction is that of iron with disodium-1,2-dihydroxybenzene-2,5-disulfonate. Three complex compounds are formed between ferric ion and this compound: (1) a blue complex formed in the pH range 4.0 to 4.9, (2) a violet complex formed in the pH range 5.6 to 6.9, and (3) a red complex formed in the pH range 7.2 to 9.4. The red and blue complex compounds both obey the Lambert-Beer law but the red complex is more sensitive for the determination of iron. The red complex compound can be used as an acid-base indicator in the pH range 7 to 9. Very few inorganic ions interfere with the determination of iron by this method. Titanium forms a stable yellow colored compound which does not interfere with the determination of iron and it is possible to determine titanium in the presence of iron by reducing the iron with hyposulfurous acid.

Organic Reagents for Volumetric Analysis. JOHN F. FLAGG, General Electric Co., Schenectady, N. Y.

Organic reagents are used in two ways as volumetric reagents for determining metals:

1. Standardized solutions of the reagents are used to react directly with the metals with some means of detecting the equivalence point in the titration.
2. The metals are precipitated with excess of organic reagent, the precipitate is filtered and washed, and the organic part of the complex metal compound is determined.

An example of the first procedure is that of the amperometric titration of cobalt with α-nitroso-β-naphthol. The cobalt solution is buffered to a pH of 4.5 to 5.0 and titrated directly with a standardized solution of the reagent. At a potential of −1.5 volts (relative to a saturated calomel electrode) the current decreases in a linear fashion as the reagent is added, until the cobalt is entirely precipitated. Further additions of the reagent produce increasing currents and the intersection of the two lines (milliliters of reagent plotted against current) gives the equivalence point. In cases where easily reducible ions are present in the solution it is advantageous to use a potential of about −0.06 volt. In this case the current remains constant until the end point is reached, after which it increases with the further addition of the reagent. The method is precise and accurate to within 0.5% of the amount of cobalt present and can be used in the range of 1.5 to 12 mg. of cobalt.

Other examples of direct volumetric methods using amperometric titrations are titration of nickel with dimethylglyoxime and titration of copper with α-benzoinoxime.

The equivalence point in these titrations can be determined conductometrically. For example, if a standardized solution of cupferron is added to a copper solution, the conductivity of the solution increases as the reagent is added because ammonium ions are liberated. After the copper has all precipitated, the conductivity decreases with the addition of reagent because hydrogen ions are then being removed from the solution. The intersection of the two straight lines (milliliters of reagent plotted against conductivity) corresponds to the equivalence point. This method is rapid; the acid concentration is not critical but iron interferes because it forms a precipitate with cupferron. It has been reported that nickel can be titrated conductometrically with dimethylglyoxime.

Most of the indirect titration methods depend upon the bromination of the organic part of the metal-complex compound Copper can be precipitated with anthranilic acid, filtered, washed, and dissolved in dilute hydrochloric acid. A standardized bromine solution (from potassium bromate) is added and the excess of reagent back titrated iodometrically. One of the advantages of these bromination methods is the favorable stoichiometric factors that result. When copper is precipitated with salicylaldoxime, the equivalent weight of copper is $^1/_{14}$ its atomic weight; when nickel is precipitated with dimethylglyoxime, and the precipitate titrated with bromine, the equivalent weight of nickel is $^1/_{24}$ its atomic weight.

The organic part of a metal complex can be titrated with standardized cerate solutions, although sometimes the course of the reactions is not known. In the case of the oxine complex with magnesium, the reaction is reproducible and 1 mole of oxine is equivalent to 30 equivalents of cerate ion, so that the equivalent weight of magnesium is $^1/_{60}$ its atomic weight. A disadvantage of this method for magnesium is the fact that blanks will often run high and they must always be determined.

It seems likely that volumetric determinations of metals with organic reagents will be used more extensively in the future because in many cases they offer distinct advantages over other methods used at the present time.

Oxidation-Reduction Indicators. G. FREDERICK SMITH, University of Illinois, Champaign, Ill.

The ferroin type of heterocyclic organic compounds (1,10-phenanthroline, 2,2′-bipyridine, 2,2′,2″-terpyridine) were synthesized 45 years before chemists became aware of their usefulness in chemical analysis. The following divalent metal ions form complex compounds:

Iron[a]	Cobalt[a]	Beryllium
Ruthenium[a]	Nickel	Copper
Osmium	Zinc	Cadmium
	Manganese	Silver
		Chrhmium[a]

[a] Complex compound is colored.

The organic compounds themselves have been used as oxidation-reduction indicators, color "detectives," masking reagents, and anion precipitants.

The most important use of the ferroin compounds is that of oxidation-reduction indicators. Usually the ferrous complexes are used for this purpose. By introducing suitable molecular groups into the more simple ferroin compounds, it is possible to obtain oxidation-reduction indicators which are color-sensitive to narrow voltage changes between the limits of 0.85 and 1.25 volts (referred to the hydrogen electrode). The color intensity of the oxidized form of these indicators is not great, so that approximately 90% of the indicator must be oxidized to give a visual color change.

As an example of how important it is to pick the right indicator, it was pointed out that chromate ions can be titrated with ferrous solutions using 1,10-phenanthroline as the indicator. For the reverse titrations (ferrous ions with dichromate solutions), 4,4′-dimethylbipyridine is superior to any indicator now in use. The reason for this lies in the fact that each indicator changes color at the potential corresponding to the equivalence point for that titration.

The oxidation-reduction of cerate solutions depends a great deal upon the kind of acid present in the solution and upon its concentration. Perchloric acid gives the largest oxidizing potential, and it is possible to titrate 0.001 *N* ferrous solutions with dilute standard cerate solutions using 5-nitro-1,10-phenanthroline as the indicator.

The ferroin-type compounds can be used as masking agents. For example, small amounts of aluminum in iron can be determined by forming the ferrous-bipyridine complex and precipitating the aluminum as hydroxide with ammonium carbonate.

The systematic study of the ferroin-type compounds should continue to yield improvements to existing analytical procedures as well as useful new ones.

AIDS FOR THE ANALYST . . . . .

Optical System for Melting-Point Apparatus. Nelson Whitman, Jackson Laboratory, E. I. du Pont de Nemours and Co., Wilmington, Del.

An optical system has been devised for use with melting-point apparatus which overcomes the major faults of systems previously used. The magnification has been inadequate and the short focal-length lenses used have been subject to breakage through rapid changes in temperature and to deterioration of cemented surfaces through exposure to high temperatures. Morton and Mahoney [IND. ENG. CHEM., ANAL. ED., **13**, 498 (1941)] described an apparatus which eliminated the closely placed lens system by projecting the silhouette of the melting-point capillary upon a screen. This required a brilliant light source, a projection lens, and a darkened room. Moreover, such physical changes as change in color of the sample could not be readily observed.

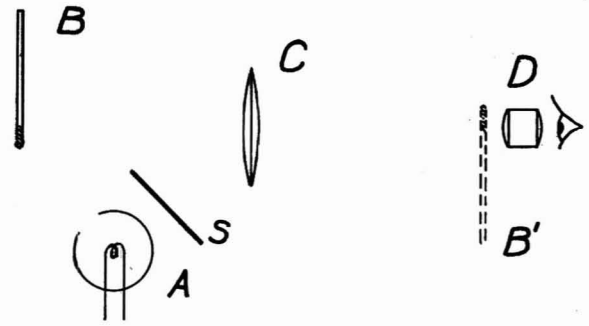


Figure 1. Schematic Diagram

The new system eliminates these disadvantages by the use of a relatively long focal-length lens to form a real image of the melting-point capillary, which image is then magnified to the desired degree. This image is located in a plane at least four times the focal length of the lens away from the melting-point capillary. At this distance from the heated melting-point apparatus any type of magnifier or microscope can be used without danger from heat effects. A schematic diagram of the system is shown in Figure 1.

The melting-point capillary, *B*, is illuminated by light source *A*, which is shielded by *S* from the observer. The real image, *B'*, is formed by the positive lens, *C*, and is magnified by the microscope, *D*. In its simplest form *C* can be a double convex lens (f = 60 mm.) mounted 120 mm. from the object. The image will then be located approximately 240 mm. from the object and be of the size, though inverted. This image can be magnified by a magnifying glass, *D*, mounted slightly beyond the location of the real image.

The basic instrument can be refined by the use of corrected lenses and erecting lenses or prisms, which can be obtained at present through salvage houses. One instrument has been constructed from two objectives, one set of erecting prisms, one ocular, and one half of the body of a binocular. The second objective was mounted to face the first objective, located in its original position in the half binocular. The capillary melting-point tube was located in the focal plane of the outside lens. With this mounting, full advantage was taken of the color corrections of the lenses and the full-sized image was formed erect and in a proper plane for magnification by the ocular. This system could be improved by the use of a single objective of one half the focal length of the binocular objectives and corrected for use at an object distance of twice the focal length.

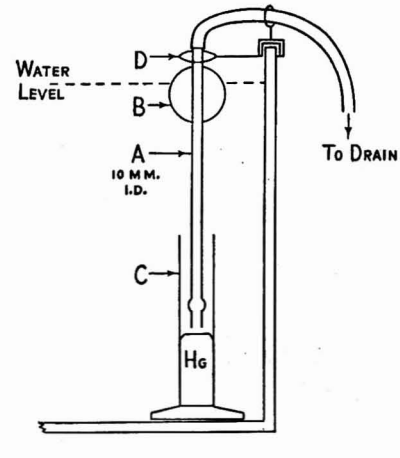
The instrument was made for use by students in courses in qualitative organic analysis and organic synthesis. It has been in use for over six years. Aside from the utility of such instruments to laboratories where large numbers of melting-point determinations are made, students took more interest in determining melting points when this instrument was available to them than when each was required to set up his own equipment.

Constant-Level Siphoning Device. R. D. Maguire, Canadian General Electric Co., 940 Lansdowne Ave., Toronto 4, Ontario, Canada.

A CONSTANT-temperature water bath operating around room temperature is normally cooled by means of a copper coil through which tap water circulates. It may be desirable in some cases, for the sake of efficiency or economy, to dispense with this coil and add the cooling water directly, removing it at the same rate. However, if the bath is a Pyrex cylinder, or has glass sides, the outgoing water cannot be removed by an overflow orifice at the desired water level, and the water must be removed by siphoning.

The following device was originated in this laboratory to give a simple but entirely reliable method of maintaining a constant water level, by siphon action, regardless of the rate of water-inlet flow.

The siphoning tube, *A*, is fitted with a float, *B*, a blown glass sphere or large waterproofed cork. A gum rubber discharge tube is fitted to the top and leads to drain, while near the bottom of the siphoning tube an enlargement prevents entrainment of mercury. The siphoning tube floats so that the lower end is inside a cylinder, *C*, such as the bottom half of a 100-ml. graduate, partially filled with mercury. A loop of stiff wire, *D*, fastened to the side of the bath retains the upper part of the siphoning tube.



When filled with water and floating, the siphoning tube takes out water at a rate dependent upon its diameter and also upon the height of the water level above the end of the discharge tube. This causes the water level to go down until the end of the siphoning tube is immersed in mercury, when the flow stops. There is absolutely no discharge of water in this condition. When there is enough water in the bath to float the siphoning tube again, the cycle is repeated.

The height of mercury in the cylinder and the position of the float will determine the water level height and the amount of its variation. In order to maintain a small water level fluctuation, the end of the discharge tube should not be more than a few inches below the level of the water in the bath. If it is more, a column of mercury is drawn part way up the tube by the increased head, necessitating a greater rise in the float to overcome it.