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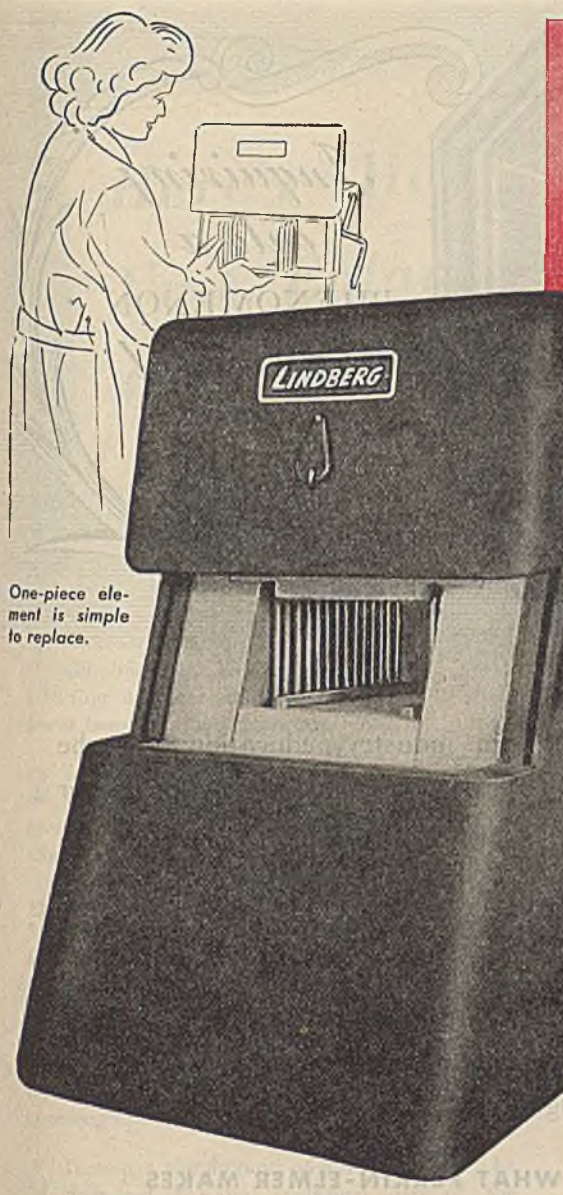

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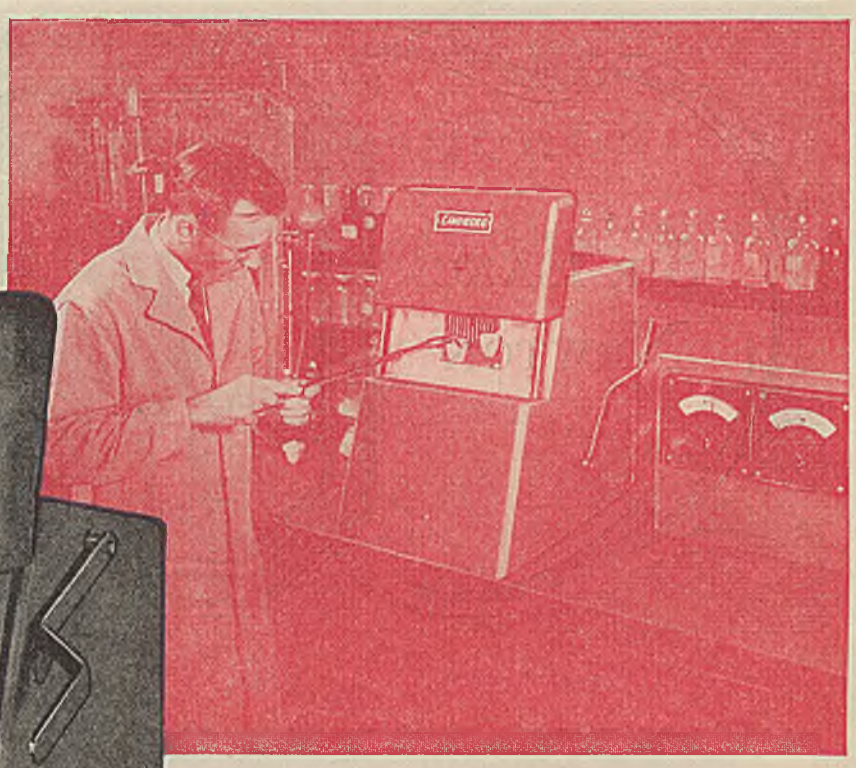

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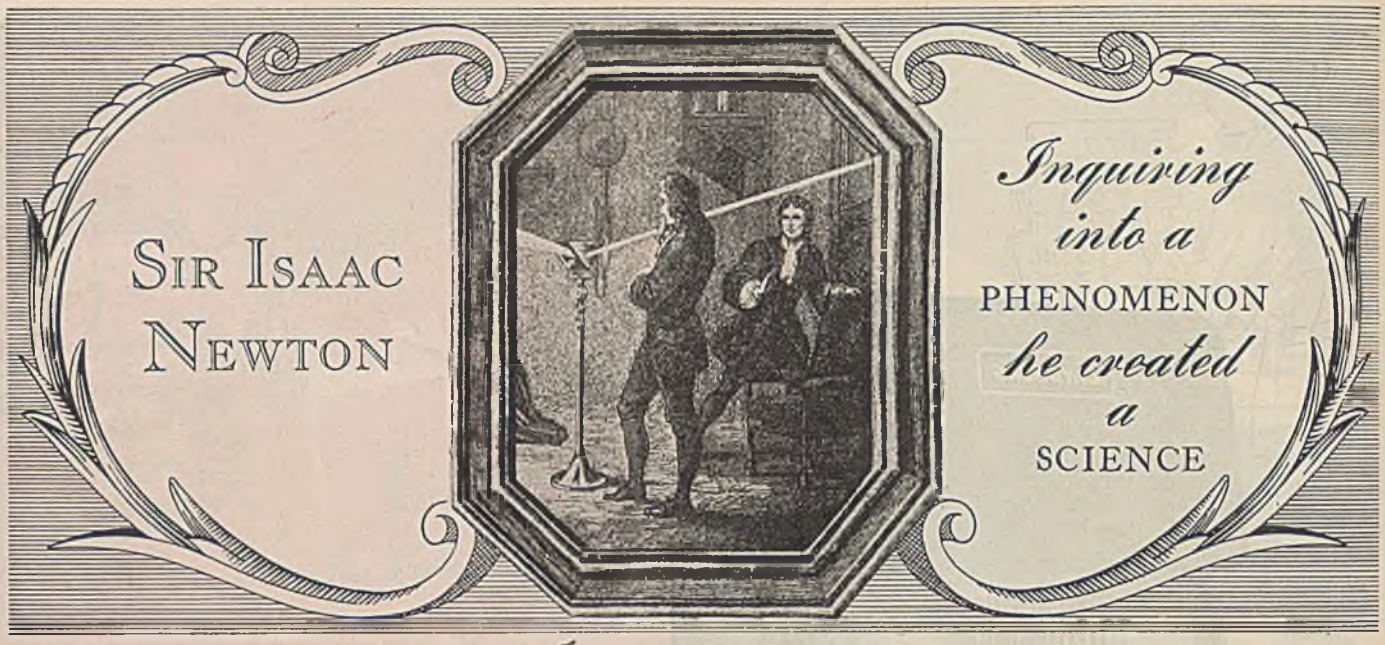

THE flashing colors of a diamond and the clear tints of a rainbow had been seen by countless people before Sir Isaac Newton asked, "How are these colors formed?"

His memoir on "Opticks" records the answer in a quaintly worded Theorem: "The light of the sun consists of rays differently refrangible." This conclusion, based on many experiments with crude glass prisms and a pencil of sunlight slanting into a shuttered room, laid the foundation of all spectroscopy. Further, it led Newton to invent the reflecting telescope, because he thought that his prismatic colors would make an achromatic lens forever impossible.

Today, Sir Isaac Newton's successors are exploring new worlds of astronomy, chemistry, metallurgy, photography and vision with lenses and prisms in instruments of constantly increasing accuracy. Many of these modern explorers—in industry, education and the armed forces—have called on Perkin-Elmer for help in blazing new trails in optical science.

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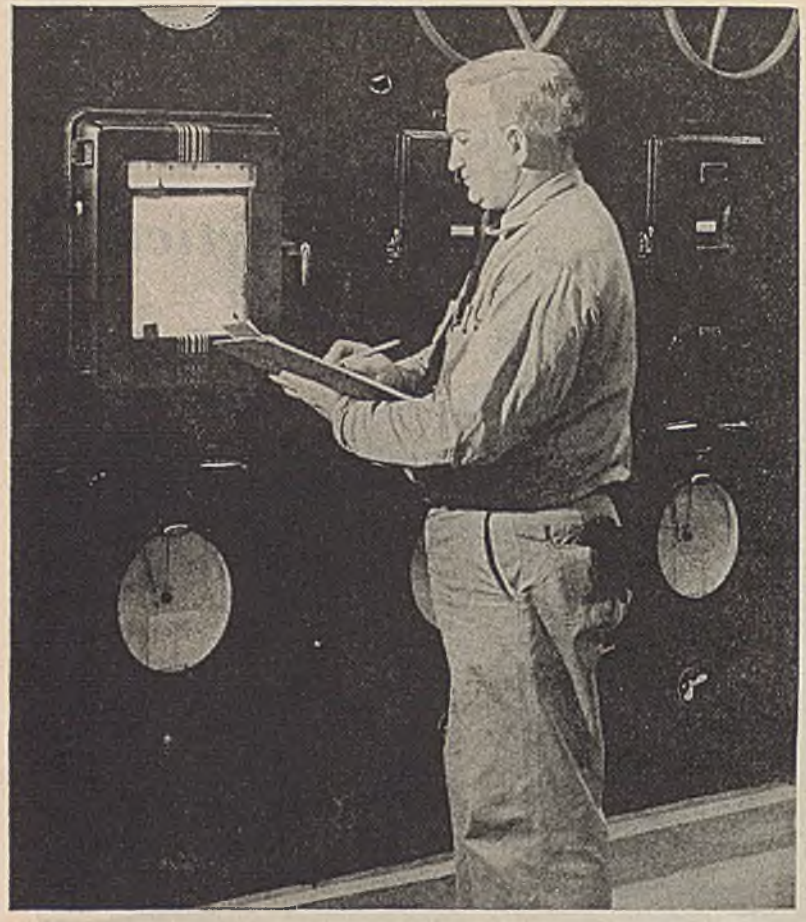

Left: Incoming operators at Butane Products Co., Port Neches, Texas, get a complete temperature history from the wide, easily-read strip-charts of Micromax Pyrometer Recorders. Above: Three of the 60 Micromax Recorders which provide accurate and reliable records of the important fractionating and dehydrogenating temperatures at this butadiene plant.

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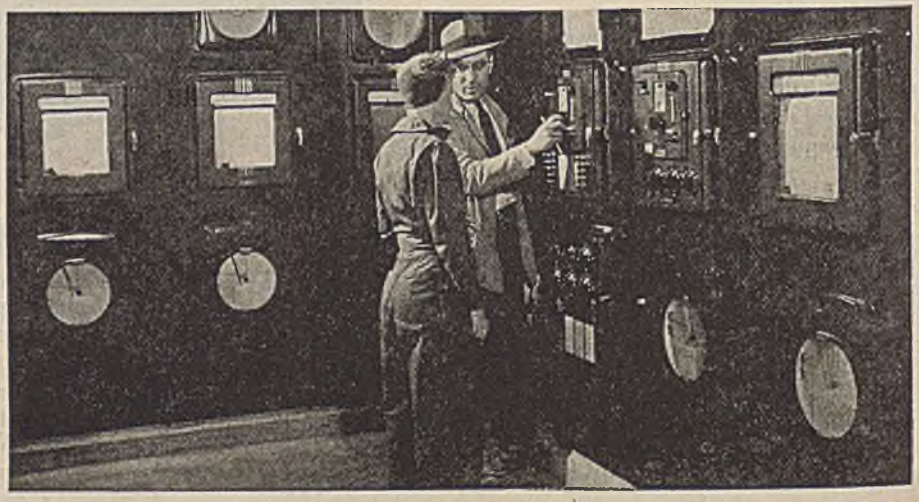

Temperatures of near or distant couples are read with equal reliability on the many L&N Manual Indicators in the Neches plant.

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INDUSTRIAL AND ENGINEERING CHEMISTRY
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WALTER J. MURPHY, EDITOR

Methods of Analysis for Anhydrous Hydrofluoric Acid

Procedures Recommended by Manufacturing Chemists' Association Committee (1944): W. B. Sherry, General Chemical Company, C. F. Swinehart, Harshaw Chemical Company, R. A. Dunphy, Kinetic Chemicals, Inc., and S. C. Ogburn, Chairman, Pennsylvania Salt Manufacturing Company

The Analytical Edition is privileged to present here procedures for anhydrous hydrofluoric acid recommended by the Manufacturing Chemists' Association Committee (1944). While these naturally include contributions from one of its members which have appeared in the ANALYTICAL EDITION [Swinehart and Flisik, 16, 419 (1944)], the methods as published here constitute the association committee's official report and hence are printed in full.

ANHYDROUS hydrofluoric acid is a colorless, fuming, corrosive liquid at relatively cool temperatures. Although it has a pressure of only about 0.5 pound gage at 22° C., it is shipped in pressure containers because its boiling point (19.4° C.) is often exceeded by the temperatures at which it is transported and used. Its freezing point is approximately −83° C., its vapor pressure is approximately 2.5 atmospheres at 50° C., and its density is 1.0 at 0° C. Its heat of vaporization is about 6000 calories per formula weight, and it has a low viscosity and surface tension. For all practical purposes, pipe sizes used in handling the liquid anhydrous hydrofluoric acid may be based on water data. As it is very hygroscopic, due care should be used to prevent moisture pickup in order to avoid errors in analysis, and precautions should be taken to prevent accumulation of water in apparatus, lines, or valves used.

Liquid anhydrous hydrofluoric acid attacks glass and most organic substances. It will react immediately with the skin upon contact, causing serious burns, and its fumes are irritating to the eyes and the mucous membrane. Care should be used to avoid contact with the liquid acid and its fumes, and workers should wear proper rubber-coated coat or apron, neoprene gloves, and face shields. Hoods with adequate ventilation are indicated for working with this acid in the laboratory; otherwise proper respirators should be worn. Water supply should be readily available for thorough and prolonged flushing in case of accidental contact with the skin.

Steel, Monel, copper, silver, and platinum are resistant to the acid, and may be used in the construction of equipment for handling it. Cast iron, or other metals containing silicon, should not be used with anhydrous hydrofluoric acid.

GENERAL PRECAUTIONS

The Division of Industrial Hygiene, National Institute of Health, has issued specific recommendations for the treatment of hydrofluoric acid burns, prepared in consultation with medical and technical personnel of acid manufacturers (*2*, *3*).

SAMPLING PROCEDURES

The taking of the sample in many instances constitutes an important part of the analysis. Obviously the more closely the sample is representative of the material whose composition is sought, the more nearly will the subsequent analysis reflect its true composition. It is fortunate from an analytical standpoint that anhydrous hydrofluoric acid is ordinarily in the form of a homogeneous liquid and the analysis is generally reported on the liquid phase. On the other hand, amounts of impurities present in the anhydrous hydrofluoric acid currently manufactured are of such a low order of magnitude that extreme care is necessary in order that the sample taken for analysis shall not be vitiated by the loss or addition of any of these impurities.

The principal impurities in anhydrous hydrofluoric acid are silicon tetrafluoride, sulfur dioxide, fluosulfonic acid, sulfuric acid, water, and metallic salts. The determination of the total acidity and the percentage of actual hydrofluoric acid in the sample are of fundamental importance.

Anhydrous hydrofluoric acid is now generally stored in steel tanks and shipped in steel tank cars, and it is transferred from the storage tank to the tank car, or vice versa, by two different methods—i.e., by blowing with compressed air (or other suitable gas), or by means of a pump. The use of a pump simplifies the taking of a representative sample from any storage tank, or tank car, if the discharge line from the pump is connected not only to the point where the acid is to be transferred but also back to the tank, or tank car, from which the pump is delivering acid—in other words, if the acid is thus recycled for a sufficient length of time, a sample properly drawn from a suitable connection on the pump line would be truly representative of the material in the tank, or tank car. Where the "blowing" method of transfer is used, a sufficient quantity of acid must first be blown through the lines to eliminate any impurities in the lines in order that the sample, when taken, may be identical with the material in the tank, or tank car.

CYLINDERS FOR TAKING SAMPLES. Cylinders with valves suitable for hydrofluoric acid make convenient containers for drawing samples of anhydrous hydrofluoric acid. These cylinders may then be transported to the laboratory where small amounts are taken out for analysis. Cylinders should not be filled to more than 85% of their water capacity by weight. (They should also be hydrostatically tested before being put in this service; a test pressure of at least 300 pounds per square inch is recommended.)

Cylinders usually used have water capacities of approximately 8.8 pounds or 120 pounds. It is essential that the tare weight and capacity be known for each cylinder. Cylinders should be weighed as they are filled and again when disconnected,

in order to guard against overfilling. In case a cylinder is overweight, some liquid should be run out before it has had an opportunity to warm up. (The usual safety precautions should be taken when running out any liquid hydrofluoric acid.) Finally, the cylinder should be properly labeled with the name of the material it contains, the charge or lot number, and any other pertinent information.

Clean, dry cylinders should be evacuated and the sample run in as liquid. Cylinders already having anhydrous acid in them may be vented at 80° to 100° F., first in an upright position to remove air in the gas phase, then inverted to drain all the liquid out. When all the acid has been run out, the valve should be closed. The cylinder should then be cooled, when it is necessary to draw the sample from a low-pressure line or from another cylinder.

It is preferable to use cylinders prepared by the latter procedure because they introduce less contamination of the sample by iron, etc. (This is because in addition to loose particles of foreign material removed from the cylinder by repeated flushings with anhydrous hydrofluoric acid, the inside of the cylinder becomes passified—that is, a protective film of iron fluoride is formed covering the inside of the cylinder. This passification is destroyed by subsequent washing with water, which should be avoided unless absolutely necessary.)

Samples from Tank Cars. Manual TC-5 gives two methods for unloading anhydrous hydrofluoric acid tank cars (*3*). The piping diagrams in this manual make provision for sample connections, valve *E* in Figure 3 and valve 7 in Figure 4. In the installation of these valves, attention should be given the orientation of the connection, so that only the liquid phase is drawn. Connections from these valves to the sample cylinder should be with small pipe or tubing and a scale provided for weighing the cylinder while being filled. Provision should be made for disposing of the acid used to wash out these lines and that vented from the lines in the unloading schedule.

Drawing the Sample. A representative sample of a tank car, or storage tank, may be obtained by drawing off into the large cylinder approximately 1 pound for each 1000 pounds transferred as the tank car or storage tank is being emptied. The amount of acid transferred can be determined from weights on storage tanks, the time required to transfer the acid, or other means of measuring. This large cylinder is then inverted and the small laboratory sample cylinder filled from it.

Note. In some plants the location of the analytical laboratory may be such that it is not necessary to withdraw samples of anhydrous hydrofluoric acid in cylinders for analysis as described above. This is especially true where the pumping and recycling method is employed. In such cases, the sample weighing tube may be attached directly to a convenient place in the installation, such as at valve 7 in Figure 4 of manual TC-5 (*3*).

Liquid anhydrous hydrofluoric acid from the sample cylinder (or other source) is run into ice in a sample weighing tube of special design, the approximate dimensions of which are 2.25 inches in diameter by 8.5 inches high. A perforated grid divides the bottle into an upper and lower compartment and some ice is placed in each. The acid is introduced through a 0.25-inch tube which extends through the grid. When filled, it weighs less than 500 grams and should be weighed to an accuracy of 15 mg. (A torsion balance of 500-gram capacity is suitable for this purpose.)

Description and Use of Sample Weighing Tube. The plastic weighing tube (Figure 1), designed by The Harshaw Chemical Company, is available as a standard piece of laboratory equipment and may be purchased from Harshaw Scientific Division of The Harshaw Chemical Company, Cleveland, Ohio.

Note. This tube may be constructed, if desired, by machining from 2 inch I.P.S. extra-heavy-wall hard-rubber pipe. If hard rubber is used, it should be lined with paraffin wax to render it impervious to hydrofluoric acid. Quaker State "M" with a melting point of 165° F. is suitable. The inner tube fittings and perforated grid are made of Saran. An adapter should be provided for connecting from the cylinder valve, or pipe connection, to the 0.25-inch SAE flare nut on the Saran tube.

A supporting stand for holding the sample cylinder at the right height over a trip balance is shown in Figure 2. Cylinders, filled to 85% of their water capacity, should be placed horizontally on the stand. The liquid inside should cover the valve outlet. If the cylinder is not sufficiently filled, it may be necessary to tilt it slightly.

Before drawing a sample into the tube for analysis, place the sample cylinder on the support in a position to ensure withdrawal of the liquid phase only. Place a Harvard trip balance

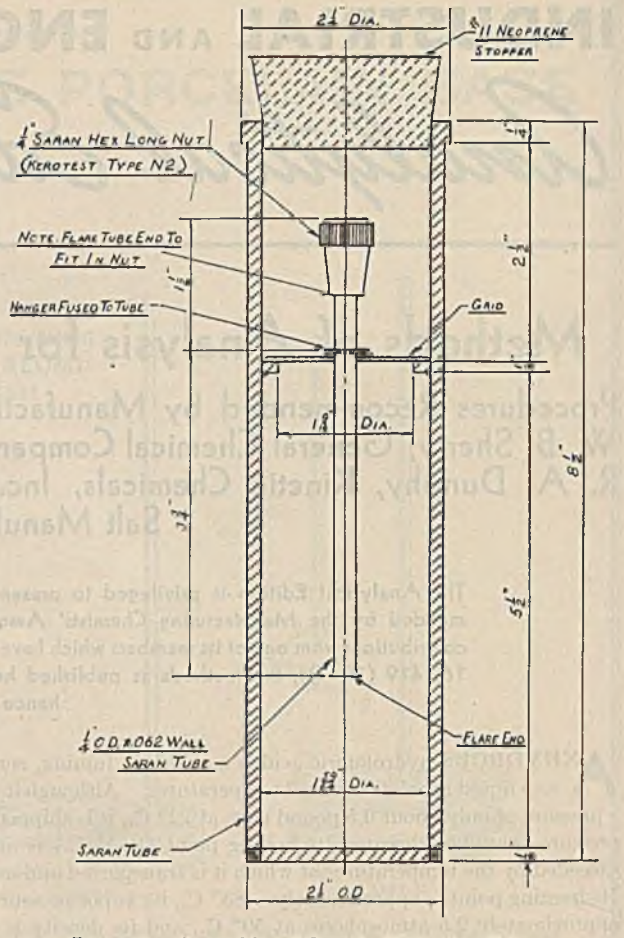

Figure 1. Assembly of Sample Weighing Tube

Maximum weight, complete assembly, 300 grams

(sensitivity need be no greater than ±1.0 gram) so that the left pan is in line with the cylinder valve outlet. Place the sample weighing tube on the left pan and add enough weights to the other pan to overbalance in order to hold the weighing tube at its highest position on the balance. Loosely couple the Saran tube assembly to the cylinder adapter, then raise or lower the balance so that the hook on the Saran tube is in line with the top of the weighing tube. [*Caution.* This adjustment is necessary to prevent suck-back of the liquid into cylinder valve. Fix the cylinder (or pipe connection) securely in order to prevent slipping when the valve is opened. Place the entire setup under a good hood, or in a well-ventilated location.]

Prior to use, dry the sample weighing tube and inner Saran tube at room temperature by forcing dry air into it until globules of water or moist areas are no longer visible. If a hard-rubber tube is used, it should be rewaxed when there are any bare spots in the coating or when any odor of hydrofluoric acid is detected when tube is dry. Plastic tubes do not require a wax coating inside.

Weigh the entire dried sample weighing tube (inner Saran tube and stopper included, but not assembled) on the torsion balance, using rough weights, or make up an approximate tare weight, and balance exactly with the sliding weight. (The accuracy of this torsion balance should be tested for such factors as equality of arms, positioning of weights, etc., and suitable corrections made if the errors are in excess of 20 mg.) Afterwards do not disturb the tare weights. Make all succeeding weighings by adding analytical weights. Carry out subsequent operations quickly in order to avoid changes in weight from evaporation or condensation of moisture.

Place 80 grams of chopped ice in the bottom part of the sample weighing tube. Properly insert the Saran tube so that the perforated grid rests flat on the narrow ledge and the hook rests over the rim. Add 50 grams of chopped ice to the top part of the tube above the grid. Wipe off any droplets of water on the outside of the weighing tube or on the coupling. Carefully weigh the entire assembly, including stopper, adding only analytical weights, and record the total weight of ice added. The ice in the bottom of the tube serves to absorb the heat of dilution of

the hydrogen fluoride, while that in the top serves to trap any vapors formed through local concentration of heat.

The entire weight of ice must not greatly exceed 130 grams, as this amount when melted, plus about 40 grams of sample, will not allow the liquid level to rise above the outlet of the Saran tube. If the setup is exactly as described above, a clearance of about 2 inches is assured. (*Caution.* Never allow the Saran tube outlet to be submerged during the addition of the sample, for then a very rapid suck-back results, which would ruin the sample and may cause an explosion.)

With the sample cylinder in place, open the sample cylinder valve slightly (*Caution.* Rubber gloves should be worn.) and allow a few milliliters of the acid to flow out into a Monel waste beaker to sweep the outlet free from any condensed water that may be present. Place the sample weighing tube (without the rubber stopper) on the Harvard trip balance (as shown in Figure 2) and immediately couple the Saran tube to the cylinder adapter, tightening with a wrench. Balance with the rough weights and make certain that the Saran tube does not hinder the balance swing, then add 40 grams more weights. Carefully open the sample cylinder valve slightly, and adjust the flow of anhydrous hydrofluoric acid to about 10 grams per minute. As the acid strikes the ice, a sizzling sound may be faintly heard and through this guidance the rate of flow may be varied. During the flow, watch the top of the weighing tube for escaping vapors, and, as soon as any are seen, slow down the flow. Test the balance swing to make certain shifting ice in the upper part does not cause sufficient friction against the Saran tube to hinder the swing. Continue the flow of acid sample until the 40 grams is approximately balanced; then close the cylinder valve. After a delay of 15 seconds, disconnect the Saran tube and drop it carefully into the sample weighing tube, which is then stoppered tightly.

Weigh the sample tube accurately on the torsion balance, adding only analytical weights, and record the additional weight over the ice weight as the sample weight. Mix thoroughly by careful inversion until all the ice melts, being certain to keep the tube tightly stoppered, so that none of the solution is lost before the ice melts and the solution becomes homogeneous. Remove the Saran tube and restopper without delay to prevent escape of sulfur dioxide. (The well-mixed diluted acid clinging to the Saran tube will be of no consequence, since aliquot weights will be taken for analysis.) Clean and dry the Saran tube at once. Proceed with determinations covered below without delay, using aliquot portions taken by weight.

ANALYTICAL DETERMINATIONS

Sulfur Dioxide. This constituent must be the first one determined, because the opening of the weighing tube for taking the other aliquot samples may result in loss of sulfur dioxide. Determination should be made as promptly as possible, avoiding standing in the sample bottle prior to analysis.

Provide a well-waxed 250-ml. beaker and a Bakelite, or Saran, stirring rod. To this beaker add 50 ml. of water and exactly 10 ml. of standard 0.1 *N* iodide-iodate solution (*1*). This iodine solution should be standardized at frequent intervals to ensure maximum accuracy. Weigh on a torsion balance. Place a 50-gram weight on the balance pan, then pour an aliquot portion of the sample into the beaker carefully until slightly overbalanced. Weigh accurately to ±0.5 gram. Back-titrate the excess liberated iodine with standard 0.1 *N* thiosulfate, using starch solution as indicator. If no color appears upon adding starch, titrate with iodide-iodate to a blue color. Calculate the percentage of sulfur dioxide in the hydrofluoric acid as follows:

$$\text{Weight of sample} = \text{weight of aliquot} \times \frac{\text{weight of anydrous HF}}{\text{weight of ice} + \text{weight of anhydrous HF}}$$

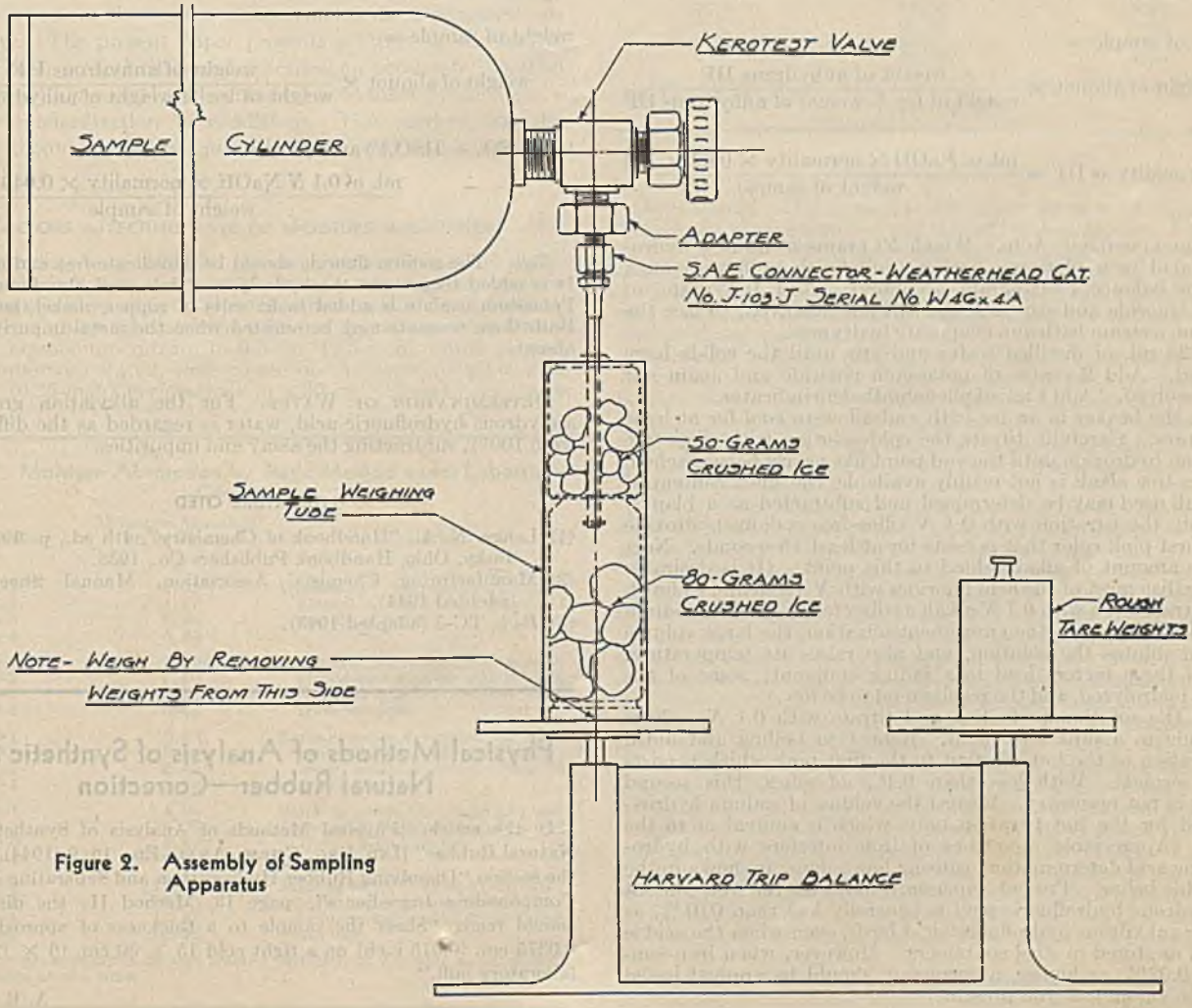

Figure 2. Assembly of Sampling Apparatus

$$\% SO_2 = \frac{\text{ml. of iodate solution} \times \text{normality} \times 3.203}{\text{weight of sample}}$$

Total Acidity. Weigh a dried platinum weighing bottle, with cover, on an analytical balance. (Type bottle as shown in Figure 3 may be used.) Transfer 7 to 8 ml. (7 to 8 ml. of the diluted acid will require between 75 and 95 ml. of *N* sodium hydroxide for titration) of the diluted acid sample with a Saran dropping pipet fitted with a rubber bulb at the top. Cover the weighing bottle and reweigh.

Transfer about 25 ml. of distilled water to a 200-ml. platinum dish. (A silver or Monel dish, or a 250-ml. glass beaker coated with wax, serves as an acceptable substitute.) Add 1 ml. of phenolphthalein indicator (0.1% phenolphthalein solution in denatured alcohol) and just enough *N* sodium hydroxide solution to give a pink color. (Only a fraction of a drop of *N* sodium hydroxide normally will be needed to produce a pink color.) Allow about 75 ml. of *N* sodium hydroxide solution to run from the chamber buret into the platinum dish.

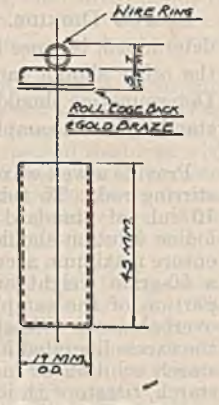

Figure 3. Platinum Weighing Bottle
Approximate weight, 22 grams

Immediately submerge the weighing bottle in the platinum dish and loosen the cover. Stir, and titrate with *N* sodium hydroxide to the first permanent pink color of the indicator. Heat the solution to boiling and if the color fades, add more sodium hydroxide to the first permanent pink color. (If a wax-lined beaker is used, the solution must be transferred to an uncoated beaker before heating.)

Calculations.

Weight of sample =

$$\text{weight of aliquot} \times \frac{\text{weight of anhydrous HF}}{\text{weight of ice} + \text{weight of anhydrous HF}}$$

$$\% \text{ total acidity as HF} = \frac{\text{ml. of NaOH} \times \text{normality} \times 0.02 \times 100}{\text{weight of sample}}$$

Hydrofluosilicic Acid. Weigh 50 grams of diluted hydrofluoric acid in a platinum beaker (dull finish preferred) on a platform balance (±0.5-gram accuracy). Add 0.2 gram of sodium chloride and stir until the salt has dissolved. Place the beaker on a steam bath and evaporate to dryness.

Add 25 ml. of distilled water and stir until the solids have dissolved. Add 2 grams of potassium chloride and again stir until dissolved. Add 1 ml. of phenolphthalein indicator.

Place the beaker in an ice bath and allow to cool for at least 15 minutes. Carefully titrate the cold solution with silica-free *N* sodium hydroxide until the end point has nearly been reached. (If silica-free alkali is not readily available, the silica content of the alkali used may be determined and subtracted as a blank.) Complete the titration with 0.1 *N* silica-free sodium hydroxide to the first pink color that persists for at least 15 seconds. Neglect the amount of alkali added to this point. (It is desirable to neutralize most of the acid fluorides with *N* alkali and to finish the neutralization with 0.1 *N* alkali as directed. If dilute sodium hydroxide is used for the entire neutralization, the large volume required dilutes the solution, and also raises its temperature. Both of these factors lead to a fading endpoint; some of the silica is hydrolyzed, and the results tend to be low.)

Heat the solution to boiling and titrate with 0.1 *N* sodium hydroxide to a pink end point. Reheat to boiling and finish the titration of the hot solution to the first pink which persists for 45 seconds. With less than 0.1% of silica, this second heating is not necessary. Record the volume of sodium hydroxide used for the hot titration only, which is equivalent to the silica. (Appreciable quantities of iron interfere with hydrofluosilicic acid determination, causing low values, as shown in the data table below. Present experience indicates the iron content of anhydrous hydrofluoric acid is generally less than 0.01%, as iron, on anhydrous hydrofluoric acid basis, even when the acid is shipped or stored in steel containers. However, when iron content is 0.02%, or higher, a correction should be applied based upon the amount of iron present.)

Calculations.

Weight of sample =

$$\text{weight of aliquot} \times \frac{\text{weight of anhydrous HF}}{\text{weight of ice} + \text{weight of anhydrous HF}}$$

$$\% \text{ hydrofluosilicic acid} = \frac{\text{ml. of 0.1 } N \text{ NaOH} \times \text{normality} \times 0.036 \times 100}{\text{weight of sample}}$$

Fe Added		H_2SiF_6 Found		Error Caused by Iron,
Grams	%	Grams	%	% H_2SiF_6
		0.198	0.132	0.000
0.003	0.02	0.188	0.125	0.007
0.006	0.04	0.172	0.115	0.017
0.009	0.06	0.151	0.101	0.031
0.012	0.08	0.137	0.091	0.041
0.015	0.10	0.125	0.083	0.049

Sulfuric Acid (Sulfuric Acid and Fluosulfonic Acid, Calculated as Sulfuric Acid). Weigh on a torsion balance a 50.0-gram aliquot of the sample into a 75-ml. platinum dish and evaporate to apparent dryness on a water bath. Add 10 ml. of water and evaporate again to dryness on the water bath. Repeat the evaporations with water until no odor of hydrofluoric acid is detected when hot, then add water and evaporate once more. Usually two evaporations with water are sufficient for sulfuric acid contents below 0.1%.

When all the hydrofluoric acid has been expelled, add 25 ml. of carbon dioxide–free water, 1 gram of sodium fluoride, and 1 gram of potassium oxalate. Titrate with 0.1 *N* alkali using phenolphthalein indicator. The titration is equivalent to sulfuric and fluosulfonic acid. Calculate as sulfuric acid and report to two significant figures.

weight of sample =

$$\text{weight of aliquot} \times \frac{\text{weight of anhydrous HF}}{\text{weight of ice} \times \text{weight of anhydrous HF}}$$

$$\% (H_2SO_4 + HSO_3F) \text{ as } H_2SO_4 = \frac{\text{ml. of 0.1 } N \text{ NaOH} \times \text{normality} \times 0.049 \times 100}{\text{weight of sample}}$$

Note. The sodium fluoride should be fluosilicate-free and neutral. It is added to prevent the hydrolysis of iron and aluminum salts. Potassium oxalate is added to fix salts of copper, nickel, lead, etc. Both these reagents may be omitted when the metal impurities are absent.

Determination of Water. For the alkylation grade of anhydrous hydrofluoric acid, water is regarded as the difference from 100%, subtracting the assay and impurities.

LITERATURE CITED

(1) Lange, N. A., "Handbook of Chemistry", 4th ed., p. 997, Sandusky, Ohio, Handbook Publishers Co., 1938.
(2) Manufacturing Chemists' Association, Manual Sheet H-2 (adopted 1944).
(3) *Ibid.*, TC-5 (adopted 1943).

Physical Methods of Analysis of Synthetic and Natural Rubber—Correction

In the article "Physical Methods of Analysis of Synthetic and Natural Rubber" [Ind. Eng. Chem., Anal. Ed., 16, 9 (1944)] under the section "Dissolving Rubber Hydrocarbon and Separating It from Compounding Ingredients", page 13, Method II, the directions should read: "Sheet the sample to a thickness of approximately 0.0375 cm. (0.015 inch) on a tight cold 15 × 30 cm. (6 × 12) inch laboratory mill."

A. R. Davis

Accelerated Method for Determining Moisture Absorption

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An accelerated method for moisture-absorption measurements is described. Factors such as temperature, the size, depth, and number of dishes, and the arrangement in the humidity chamber influence the final results. Careful standardization of all conditions, therefore, is essential in order to obtain reproducible results. Considerable time can be saved by the accelerated method in making equilibrium moisture-absorption measurements of fertilizers.

THE accurate evaluation of the moisture-absorption tendencies of fertilizer materials and mixed fertilizers is of great importance in predicting the behavior of these materials in storage under humid conditions. Ordinarily, determinations are made by exposing tared samples to humidified air in a desiccator containing either a saturated salt solution or a sulfuric acid solution that gives the desired relative humidity. These tests are generally carried out under conditions existing in the laboratories, with the samples in weighing bottles or watch glasses and without agitation of the air in the desiccator. This method is referred to as the static method in the following discussion.

Results of such determinations are usually not consistent nor readily reproducible. For this reason a common fertilizer material such as sodium nitrate or ammonium sulfate, is often included in each series of determinations to serve as a standard for comparison. Lindsay (*1*) and others have raised objections to making moisture-absorption measurements in a stagnant atmosphere. The present paper presents a study of the factors causing these irregularities and describes an accelerated method in which readily reproducible values are obtained by means of a careful standardization of conditions. This method was developed about three years ago in this laboratory and has been in constant use.

FACTORS AFFECTING RATE OF MOISTURE ABSORPTION

Experiments were made by the ordinary static method to determine the influence of several obvious factors, such as temperature, size of dishes, etc., on moisture absorption. In these tests, carried out under laboratory conditions, 4-gram samples of wax-treated ammonium nitrate in 6.3-cm. (2.5-inch) watch glasses, unless otherwise stated, were placed on the porcelain plate with 0.6-cm. (0.25-inch) perforations in a 30-cm. (12-inch) desiccator containing a saturated solution of sodium nitrate which gives a relative humidity of 74.4% at 25° C. During these tests the temperature in the laboratory fluctuated between 20° and 25° C. The results obtained are tabulated in Table I.

Table I. Moisture Absorption by Static Method under Laboratory Conditions

Series No.	Moisture Absorbed in 24 Hours	Remarks[a]
	%	
A-1	5.35	Desiccator containing samples placed on laboratory bench in middle of room
A-2	4.97	
A-3	5.48	
A-4	5.84	
B-1	7.80	Same as series A, except desiccator placed near window with B-1 and B-2 near and B-3 and B-4 away from window
B-2	7.10	
B-3	5.28	
B-4	5.63	
C-1	5.68	Same as series A, except desiccator protected from drafts to obtain more uniform temperature in desiccator
C-2	5.67	
C-3	5.40	
C-4	5.72	
D-1	4.86	Same as series C, except D-1 and D-2 in 2.5-inch dishes, D-3 and D-4 in 3-inch dishes
D-2	5.26	
D-3	6.59	
D-4	6.82	
E-1	11.51	Same as series C, except perforated plate in desiccator removed and samples suspended from Bakelite top
E-2	12.83	
E-3	12.57	
E-4	10.48	

[a] Four 4-gram samples of wax-treated NH_4NO_3 in 2.5-inch watch glasses in desiccator at one time.

The effect of the number and depth of dishes on moisture absorption is shown in Tables II and III. The data reveal that the temperature, the size, depth, and number of dishes, as well as the arrangement in the humidity chamber, all influence the final results. Careful standardization of all conditions is, therefore, necessary if reproducible results are to be obtained, when determining rates of moisture absorption.

Table II. Effect of Number of Samples in Humidity Chamber

No. of Samples in Humidity Chamber at One Time[a]	Average % Moisture Absorbed 92.9% R.H.	72.4% R.H.
1	15.68	5.22
2	13.25	3.86
3	12.08	3.36
4	10.75	3.23
5	9.62	2.58

[a] 4-gram samples of NH_4NO_3 in 2-inch dishes exposed for 6 hours at 30° C.

Table III. Effect of Depth of Sample Dishes

Depth of Sample Dish[a]	Moisture Absorbed 92.9% R.H.	72.4% R.H.
Inch	%	%
0.25	42.06	13.91
0.5	30.02	9.04
0.75	25.00	7.53
1	19.84	5.96

[a] Four 4-gram samples of NH_4NO_3 in 2-inch dishes of various depths in same humidity chamber for 24 hours at 30° C.

ACCELERATED METHOD

APPARATUS. The humidity chamber (shown in Figure 1) used in the accelerated method consists of a metal can, *A* [an inverted lower portion of a straight-walled, 23-kg. (50-pound) lard can, 20.5 cm. (7 inches) high and 30 cm. (12 inches) in diameter], with an aluminum top, *B*, having six equally spaced holes (6.25 cm., 2.5 inches, in diameter), through which the samples are introduced into the chamber.

The small induction motor, *I* is mounted on a Bakelite plate, *J*, insulating the humidity chamber against the heat developed in the motor. Shaft *K* of the four-blade aluminum fan (7 inches in diameter) is similarly insulated by means of a Bakelite coupling, *M*. Zink (*2*) used small fans on pivots inside a desiccator and induced them to rotate by means of permanent magnets passing near the outside of the desiccator.

The joint between the aluminum top and the can is made airtight by means of two rubber gaskets, *T*, one cemented to the aluminum top and the other to the top edge of the can.

The motor is regulated to run at approximately 350 r.p.m. by means of a voltage regulator. A much higher fan speed than this is not recommended because it will cause the temperature in the humidity chamber to rise unduly, as the result of air resistance against the action of the fan. The use of a metal chamber, raised above the table by legs *N*, permits rapid dissipation of excess heat from this source. In a constant-temperature room where the air is well circulated to maintain uniform temperature, the rise in temperature in the humidity chamber amounts to about 0.2° C. For accurate work, this can be compensated by lowering the room temperature correspondingly, in order to keep the chamber at exactly 30° C. or other desired temperatures.

Dish *G* (20-cm., 8-inch, Pyrex cake plate) contains the saturated salt solution, *H*, with an excess of the solid salt. The aluminum baskets, *D*, holding the sample dishes (with the covers removed) are suspended from glass hook *E*, held by rubber stoppers *F*, arranged as shown in Position I. Position II is drawn to show the details of the aluminum basket and hook arrangement only.

The shallow flat-bottomed sample dishes, having an inside area of 17.16 sq. cm. (2.66 sq. inches), are made by sealing Pyrex rings,

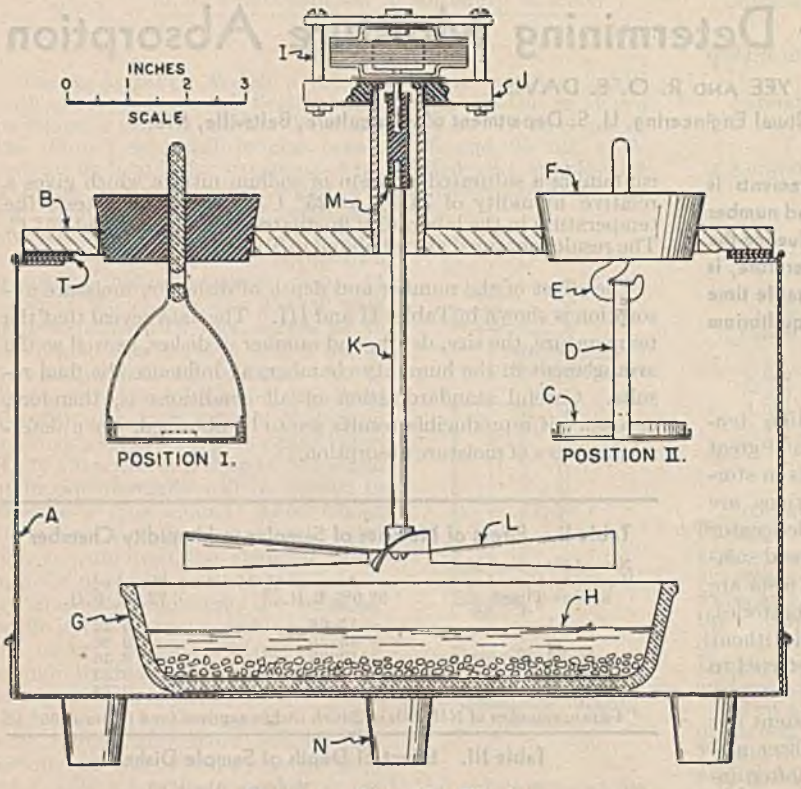

Figure 1. Humidity Chamber

Figure 4 shows the arrangement of the leveling device on top of the sample in a dish. This device consists of a flat aluminum disk, *Q*, slightly smaller in diameter than that of the dish, with a platform, *R*, of similar size to take a kilogram weight, *S*.

PROCEDURE

Determination of Rate of Moisture Absorption. The tared dish (with cover removed) containing the sample is placed under the spreading device, as shown in Figure 3. Disk *O* is then rotated 7 or 8 revolutions at a moderate speed, while the sample dish is being turned in the opposite direction. This procedure is repeated with a change in the direction of rotation. If the spreading disk is properly adjusted, the pins should just touch the surface of the leveled sample in the dish. If furrows are formed in the sample, the spreading disk is too low and should be raised.

Next the leveling device with a kilogram weight is placed on top of the spread sample, as shown in Figure 4, and allowed to stand for 2 minutes (arbitrarily chosen), so as to produce a uniformly smooth surface and packing of the sample in the dish. This leveling step is necessary for very accurate determinations because the rate of moisture absorption depends on the surface area exposed to the humid atmosphere.

If the sample is in coarsely granular form, it is impossible to obtain a uniformly smooth surface as with finer materials. Such coarse granules can be spread simply with a spatula.

With the fan going at the proper speed, the samples are placed in the humidity chamber, as shown in Position I in Figure 1. At the end of a desired time interval (1 or 2 hours) the samples are taken out and weighed with the covers on to determine the increases in weight and then replaced in the chamber for another interval before reweighing. The increases in weight for the various time intervals are calculated as the moisture-absorption rate in per cent of the sample.

Equilibrium Moisture-Absorption Measurements. The same procedure is followed, except that it is sufficient to spread the samples uniformly with the aid of a spatula, and to keep them in the humidity chamber for 2 days. This is sufficient time for equilibrium to be established in practically all cases. Fertilizers containing an unusually large amount of hygroscopic components, however, may require an extra day. Such fertilizers can be easily recognized by their high absorption values.

Figure 2. Sample Dish with Cover

Table IV. Moisture Absorption Rate by Accelerated Method at 30° C.

No.	Sample[a]	Relative Humidity in Chamber	Moisture Gained in Various Time Intervals: 1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
		%	%	%	%	%	%	%
1	$NaNO_3$	75.2	0.86	1.73	2.71	3.60	4.39	5.49
			0.87	1.75	2.71	3.77	4.46	5.57
2	$NaNO_3$	75.2	0.82	1.75	2.72	3.63	4.41	5.42
			0.82	1.77	2.70	3.61	4.45	5.36
3	$CO(NH_2)_2$	75.2	1.72	3.14	4.36	5.61	6.69	7.66
			1.78	3.16	4.39	5.70	6.71	7.80
4	$(NH_4)_2SO_4$	84	1.30	2.71	3.99	5.41	6.82	8.35
			1.26	2.60	3.94	5.31	6.87	8.38
5	NH_4NO_3, sample A	65.2	..	6.82	..	13.23	..	19.21
			..	6.77	..	13.40	..	19.52
6	NH_4NO_3, sample B, wax-coated	72.4	..	0.36	..	0.63	..	0.87
			..	0.39	..	0.67	..	0.92
7	NH_4NO_3, sample B, wax-coated	81.4	..	0.64	..	1.18	..	1.87
			..	0.71	..	1.20	..	1.62
8	NH_4NO_3, sample B, wax-coated	96.3	..	1.41	..	2.30	..	3.09
			..	1.34	..	2.15	..	2.90
9	Fertilizer X	72.4	0.43	0.39	0.88	1.22	3.56[b]	3.72[c]
			0.61	0.34	0.87	1.22	3.58	3.73
10	Fertilizer Y	65.2	1.98	2.91	3.56	3.93	..	...
			1.70	2.48	2.98	3.52	..	...

[a] 4-gram samples in 2-inch dishes used in all tests, except No. 2, 2.5-gram samples.
[b] 21-hour result.
[c] 23-hour result.

Table V. Comparison of Results Obtained by Static and Accelerated Methods

No.	Sample[a]	% Moisture Absorbed: Static Method[b] 24 hours	Accelerated Method 2 hours	4 hours	6 hours
1	NH_4NO_3, tech.	20.32	10.91	20.20	29.22
2	NH_4NO_3 wax-coated and dusted, sample A	4.96	2.97	4.35	5.46
3	NH_4NO_3 coated and dusted, sample B	10.61	5.74	10.74	16.08
4	NH_4NO_3 wax-coated	0.76	0.48	0.71	0.92
5	NH_4NO_3 dusted only	19.16	10.29	19.82	29.15

[a] 4-gram samples in 2-inch dishes exposed to atmosphere of 72.4% relative humidity at 30° C.
[b] Fan in humidity chamber stopped.

Table VI. Effect of Temperature on Moisture Absorption

Sample[a]	Temperature °C.	Moisture Absorbed %
Urea[b]	29.5	2.86
	30.0	3.16
	30.5	3.43
$NaNO_3$[c]	29.5	1.76
	30.0	1.90
	30.5	2.07

[a] Samples in 2-inch dishes exposed to atmosphere of 75% R.H. for hours.
[b] 2.5-gram samples.
[c] 3.7-gram samples.

0.6 cm. (0.25 inch) high, onto flat Pyrex plates, 5 cm. (2 inches) in diameter. The covers for these dishes are inverted watch glasses of the same size, with glass knobs cemented to them to facilitate handling. Since such Pyrex plates are not easily obtained now, aluminum dishes (as shown in Figure 2) of similar dimensions are also used. For protection against the corrosive action of certain types of fertilizers, the inside surfaces of these aluminum dishes are well lacquered.

Figure 3 shows the details of the aluminum spreading device used to distribute the samples uniformly on the dishes. It consists, essentially, of a disk, *O*, with two rows of pins, 0.3 cm. (0.125 inch) in length at staggered distances from the center of the disk and intersecting at right angles to each other as shown in section *A'–A'*, Figure 3.

Table VII. Relation between Moisture Absorption and Fineness of Sample

Fineness of Sample[a]	Moisture Absorbed
Mesh	%
20– 48	1.78
48–100	1.78
100–150	1.77
150–200	1.79
Through 200	1.77

[a] 2.5-gram samples of $NaNO_3$ in 2-inch dishes exposed to atmosphere of 75% R.H. at 30° C. for 2 hours.

Table VIII. Relation between Moisture Absorption and Amount of Sample Used

No.	Sample Used, A[a]	Moisture Absorbed, B	Moisture Absorbed, B/A
	Grams	*Gram*	%
1	5.0	0.0462	0.93
2	4.5	0.0456	1.02
3	4.0	0.0460	1.15
4	3.5	0.0453	1.30
5	3.0	0.0458	1.53
6	2.5	0.0463	1.85
7	2.0	0.0453	2.27
8	1.5[b]	0.0387	2.58

[a] $NaNO_3$ (48- to 100-mesh) in 2-inch dishes exposed to atmosphere of 75% R.H. for 2 hours at 30° C.
[b] Just enough sample to cover dish at beginning of test.

Because of variations in the density of the various materials and the need for an approximately constant volume of sample, the amount of fertilizer taken for such a test has to be adjusted in some extreme cases, but in general 4-gram samples work out well for the size of dish chosen. The relative humidity used likewise has to be chosen according to requirements in individual cases. For testing ammonium nitrate that has undergone various conditioning treatments, 72% relative humidity has been adopted. This represents about the average condition encountered locally.

EXPERIMENTAL RESULTS

Rate of Moisture Absorption. In order to test the accuracy and reproducibility of the results obtained by the accelerated method, the moisture-absorption rates of a number of fertilizer materials and mixed fertilizers were determined in duplicates at different relative humidities at 30° C. The results, tabulated in Table IV, show good agreements between duplicate samples. Experiments 1 and 2, made at different times and in different humidity chambers, show the reproducibility of such results.

Table V shows that under identical conditions as much moisture is absorbed in 4 hours by the accelerated method as in 24 hours by the static method.

Variations of only 0.50° C. in temperature produce distinct changes in the results, as shown by Table VI. These are, undoubtedly, brought about by change in the relative humidity over the salt solution as well as in the absorption rate of the samples.

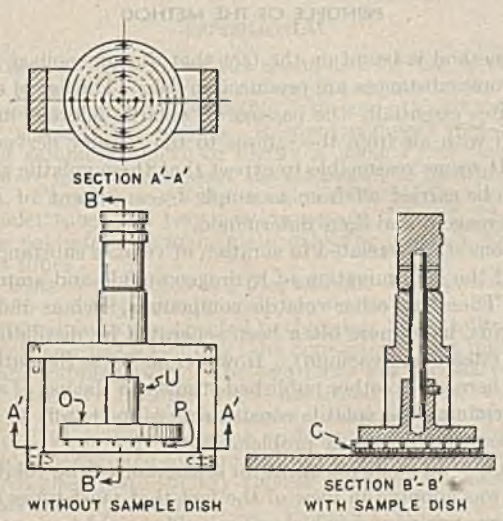

Figure 3. Spreading Device

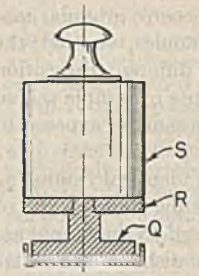

Figure 4. Sample Dish with Leveling Device

Sodium nitrate samples of different particle sizes, ranging from 20- to 48-mesh to through 200-mesh, give identical absorption values when tested under the same conditions (Table VII). The reason for this is that the absorption values obtained are actually not values for the solid phase, but for the saturated solution surrounding the solid particles. Therefore, once a thin layer of saturated solution is formed over the leveled surface layer of solid particles, regardless of the size of the particles, the rate of absorption of the saturated solution surrounding the solid is the same, as long as there is enough solid phase present to keep this solution saturated; otherwise the absorption rate will gradually decrease as the solution becomes more dilute.

For the same reason the amounts of moisture absorbed by varying sized samples (1.5 to 5 grams) of 48- to 100-mesh sodium nitrate are practically identical under the same conditions, although the percentage of moisture absorbed (column 4 Table VIII) varied from 0.93 to 2.58%. It appears that the amount absorbed is governed by the surface exposed rather than by the amount of sample. The low value of experiment 8 was due to insufficient amounts of solid salt present to keep the solution satu-

Table IX. Constancy of Moisture Absorption per Unit Weight of Sample per Unit Area of Surface Exposed

Sample Dish	Area in Dish	Sample in Dish[a]	Gain per 2-Hour Intervals[b] 1st	2nd	3rd
	Sq. in.	*Grams*	%	%	%
A	1.5920	3.5942	0.95	0.82	0.83
B	2.6577	6.0	0.84	0.82	0.81

[a] Same weight of sample per sq. inch in each dish.
[b] At 75% relative humidity and 30° C.

Table X. Total Moisture in Fertilizers at Equilibrium

Fertilizer Sample[a]	% Moisture in Original Samples	% Total Moisture at Equilibrium[b] 59.4% R.H.	65.2% R.H.	72.5% R.H.
A	3.86	2.94	5.76	19.45
		3.13	5.70	19.26
B	4.61	3.42	7.47	19.86
		3.61	7.50	19.79
C	4.28	3.80	9.15	20.67
		3.65	9.25	20.74
D	5.76	3.35	9.72	17.93
		3.55	9.82	17.97
E	5.76	4.60	12.84	20.55
		4.59	12.79	20.97
F	4.08	3.08	25.37	40.87
		3.05	24.85	42.78

[a] 4-gram samples in 2-inch dishes in humidity chamber for 48 hours at 30° C. at three relative humidities.
[b] % total moisture at equilibrium = % moisture absorbed + % moisture in original sample.

Table XI. Comparison of Time Required to Come to Equilibrium at 30° C.

Fertilizer Sample[a]	Relative Humidity in Chamber	Method Used	% Moisture Absorbed 2 days	4 days	6 days	8 days	11 days
A	59.4	Accelerated	4.68	...	...	...	...
A	59.4	Static	3.63	4.24	4.51	4.66	4.62
A	65.2	Accelerated	17.00	...	...	...	...
A	65.2	Static	12.97	15.50	16.65	17.05	17.13
A	72.4	Accelerated	29.42	...	...	...	...
A	72.4	Static	19.55	24.57	27.20	28.67	29.49
B	59.4	Accelerated	2.65	...	...	...	...
B	59.4	Static	1.61	2.00	2.14	2.33	2.48

[a] 4-gram samples in 2-inch dishes used.

rated during the test. In similar tests with coarse granular materials having large interstices between granules, so that the moist air can penetrate deeply into the mass, different absorption results may be obtained with different amounts of sample taken, because in such cases the effective area of the samples exposed to the moist atmosphere also varies.

Various sizes of dishes can be used to give identical results, as demonstrated by Table IX, provided the same unit weight of sample is taken per unit area of surface exposed to the humid atmosphere. This applies, however, only to dishes having flat bottoms and straight side walls. It does not hold in the case of watch glasses, because in this case the solution around the edges may become locally undersaturated while there is still solid salt in the center of the dish.

EQUILIBRIUM MOISTURE-ABSORPTION MEASUREMENTS. Table X shows that the method gave good precision on the total moisture content of several fertilizers in equilibrium with three relative humidities at 30° C. The particular relative humidities were chosen because most of the common fertilizers begin to absorb moisture around 65% relative humidity.

Results, shown in Table XI, reveal that considerable time can be saved by the accelerated method in making equilibrium moisture-absorption measurements of fertilizers.

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Determination of Spoilage in Protein Foodstuffs, with Particular Reference to Fish

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Based on the determination by an aeration technique of volatile reducing substances, a method has been developed for estimation of spoilage in proteinaceous foodstuffs, with particular reference to fish. The oxidizing solution is a 0.1 *N* potassium permanganate solution in *N* sodium hydroxide. Values for California sardines, mackerel, and tuna at various stages of spoilage are given. Further applications of the procedure for the estimation of volatile principles in other foods and food products are indicated.

WIDELY varying methods have been proposed and employed for the evaluation of "borderline" freshness or incipient spoilage in fish and fish products where subjective organoleptic opinions are likely to differ and definite decisions are made with difficulty.

The fact that new methods and modifications of old ones are reported from time to time implies the lack of a sufficiently sensitive and reliable procedure for the determination of the state of incipient spoilage.

The majority of chemical methods used for this purpose are dependent upon the presence of specific substances, or groups of related substances, such as indole, hydrogen sulfide, volatile nitrogenous bases, and volatile acids. The production of these compounds, predominantly by bacterial action, is dependent upon the particular flora present. Furthermore, considering the wide range of bacterial species with diverse biochemical activities which are encountered in and around fish during spoilage, the mere absence of specific products will not necessarily indicate the actual state of freshness.

The use of a method designed for the detection of a specific type of spoilage product assumes that it has been produced in greater amounts than all others. This may or may not be the case, since fish may be spoiled and still not yield a positive test for the substance upon the presence of which a particular method depends, because the organisms responsible for the decomposed condition have not produced the compound.

Fresh fish have relatively little odor, and as fish undergo spoilage unpleasant odoriferous substances are formed, the amount and variety being dependent upon the extent and kind of decomposition.

Investigators responsible for evaluation of the condition of fish and other food products, recognizing these facts, have resorted to the more simple, rapid, and relatively sensitive organoleptic method. However, certain factors adversely affect its use, such as the influence of temperature on odor, the masking of odors, personal opinion or preference, the ability of one person to detect an odor more readily than another, and olfactory fatigue.

A chemical method to serve as a sensitive and reliable measure of the freshness of fish has been developed, in which the volatile substances largely responsible for odor are estimated and by which the state of preservation of a fish sample can be determined. A close correlation has been observed between the chemical values and the organoleptic judgment—namely, low values for fresh material and progressively higher values for material after progressive stages of spoilage. However, changes in fish have been determined chemically before they could be detected organoleptically.

This report describes the principle and operation of the method, with data illustrative of its application to various proteinaceous foodstuffs. A more detailed account of its application to determination of spoilage in various Pacific Coast and other fishes, as well as an extensive critical review of the literature on the spoilage of fish and fish products, will be presented in subsequent papers.

PRINCIPLE OF THE METHOD

The method is based on the fact that during spoilage volatile odoriferous substances are produced in fish. The act of smelling constitutes essentially the passage of volatile odorous materials admixed with air from the sample to the sensory nerves of the nose. It seems reasonable to expect that these volatile constituents can be carried off from as ample by a current of air into suitable reagent and there determined.

Previous studies related to aeration of volatile substances have included the determination of hydrogen sulfide and ammoniacal bases. These and other volatile compounds, such as indole and fatty acids, have more often been separated by distillation procedures (steam and vacuum). However, as far as the authors are aware, there are no other published studies on the use of aeration for determining the volatile constituents of foodstuffs as a whole in relation to the spoilage problem.

The use of an oxidizing agent for estimation of the volatile substances was adopted in view of the fact that most types of volatile organic compounds which conceivably might be formed during spoilage are oxidizable. The reducing action of organic com-

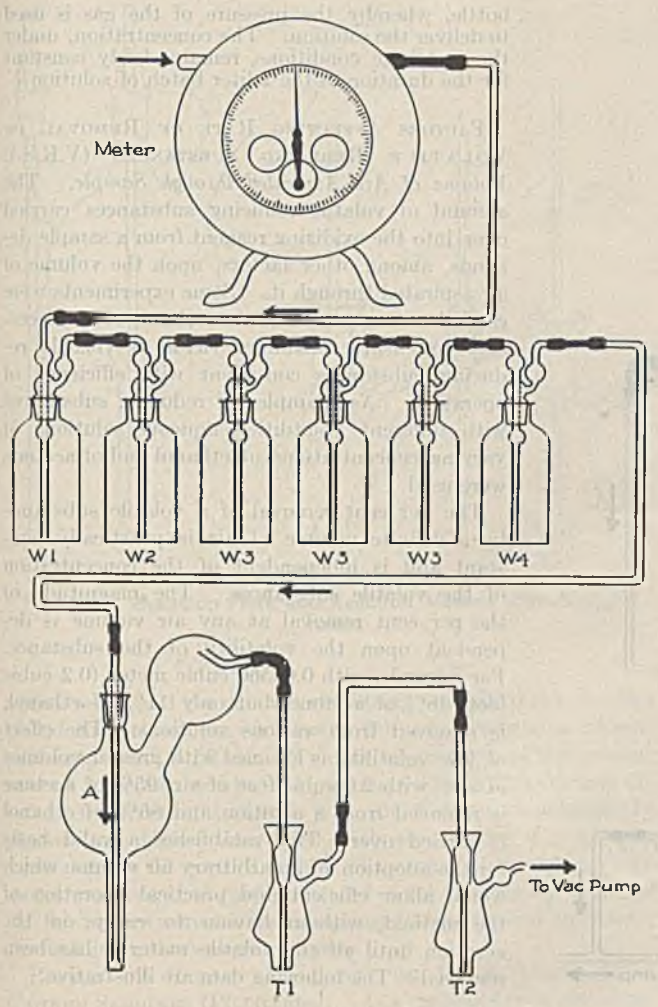

Figure 1. Diagram of Original Apparatus

A. Aeration flask
*T*1, *T*2. Reaction vessels
*W*1. Concentrated H_2SO_4
*W*2, *W*4. Redistilled water
*W*3. 10% alkaline $KMnO_4$ or activated carbon
Meter. Wet-test air meter

pounds has been utilized frequently in their determination. The work of Quitmann (*1*) on the determination of the reducing value of air as a measure of its cleanliness, and of Stamm (*2*) on the estimation of organic compounds, may be cited in this connection.

EXPERIMENTAL

Apparatus. The apparatus assembled for use is shown in Figures 1 and 2. Figure 1 illustrates the apparatus as originally set up, using a sensitive gas meter to measure the air volume aspirated through the sample. A more compact and portable two-unit sample is shown in Figure 2, using Pyrex flowmeters, which were calibrated against the air meter, to measure the air volume. Figure 3 shows the aeration flask, *A*, for the sample, its air-inlet tube, and two reaction vessels, *T*1 and *T*2, for the alkaline potassium permanganate oxidizing solution with their air-inlet tubes.

A consists of two bulbs of 1-liter and 500-ml. capacities and a 20 × 125 mm. Pyrex tube sealed onto the 1-liter bulb. The upper 500-ml. bulb is connected to the lower 1-liter bulb by a 60-mm. neck and has an outlet tube connected to an inner spray and foam trapping tube. The two bulbs are necessary to take care of excessive frothing which sometimes occurs, as, for example, with very fresh fish samples. The air-inlet tube of the aeration flask is kept in place with an interchangeable 24/40 standard-taper ground-glass male joint and its end is closed with a sintered-glass plate slightly smaller than the diameter of the tube portion.

The reaction vessel, *T*, is a Pyrex 125-ml. Erlenmeyer with a deep gutter around the neck (an iodine number determination flask), to the base of which has been sealed a 20 × 35 mm. Pyrex well. The reaction flask is fitted with an air-inlet tube which is an interchangeable ground-glass 24/40 standard-taper male joint with a reduced tube of 12-mm. diameter at both ends, one end of which is closed with a sintered-glass plate.

All rubber connections used in the apparatus were originally boiled in 5% sodium hydroxide solution and thoroughly washed with several changes of boiling redistilled water. They should be periodically rewashed.

Sampling Procedures. In sampling raw fish a sufficient portion of the material under examination, such as 12 California sardines (pilchards) or 12 mackerel or a 5- to 10-cm. (2- to 4-inch) transverse cross-sectional slice from a tuna, is ground and well mixed. Portions of this ground mixture are wrapped in a double layer of cheesecloth or gauze, placed in the cylinder (*C*, Figure 4) of the squeezing apparatus on top of a perforated steel disk, a Chromel wire screen, a 0.3-cm. (0.125-inch) white felt pad, and a second Chromel wire screen, in the order named, and a pressure of 3 to 5 tons is applied to piston (*P*, Figure 4). The expressed juice is collected through the spout, *S*, at the base of the cylinder. The dimensions of the various parts of the squeezing apparatus are: cylinder *C*, 10 × 11.5 cm. (4 × 4.5 inches) outer dimensions, with a 1.25-cm. (0.5-inch) wall, piston *P* 7.5 × 10.2 cm. (3 × 4 inches) perforated disk 7.3 × 0.6 cm. ($2^{15}/_{16}$ × $^1/_4$ inches). All are made of steel.

For canned fish, the entire contents of a can is squeezed by hand or in a small press of the fruit and vegetable juice extractor type, through two layers of cheesecloth or gauze, and the press liquor collected. All press juices are allowed to stand or are centrifuged to separate the aqueous solution. A uniform representative sample of the water-soluble constituents of the fish is thus obtained.

Aeration of Sample. Five milliliters of the fish press juice sample are pipetted into the test tube portion of flask *A* and aerated 40 minutes with 0.0566 cubic meter (2 cubic feet) of air, measured by the air meter, *M*, or by the flowmeter, *F*, into two reaction vessels, *T*1 and *T*2. Each reaction vessel contains 10 ml. of 0.1 *N* potassium permanganate solution in *N* sodium hydroxide solution. The air is cleaned by passage through six Drechsel gas-washing bottles containing, in order, concentrated sulfuric acid, *W*1, redistilled water, *W*2, 10% potassium permanganate in 10% sodium hydroxide solution *W*3 (3 bottles), and redistilled water, *W*4. Recently activated granular carbon has been substituted successfully for the 10% alkaline potassium permanganate solution. The air is aspirated by means of a laboratory vacuum pump through a stabilizing storage tank to eliminate stroke fluctuations. A water suction pump may be used as long as the water pressure is constant and not subject to fluctuations.

The concentrated sulfuric acid in the Drechsel bottle, during prolonged aeration, absorbs moisture and some organic matter from the air, becoming diluted and brown. When in constant use, it should be renewed every week, either by using fresh acid or by boiling down the diluted acid with a trace of an oxidizing agent, such as sodium nitrate. The 10% alkaline potassium permanganate solution eventually develops a brown precipitate and likewise should be replaced every week or two. The use of activated carbon obviates this cleaning procedure.

Oxidizing Reagent. The potassium dichromate–sulfuric acid reagent of Quitmann (*1*) was originally used as the oxidizing solution in the reaction vessels, *T*. However, it was found that a 0.1 *N* potassium permanganate in *N* sodium hydroxide was a much more sensitive reagent. An acid potassium permanganate solution was less effective than the acid dichromate mixture, while ceric sulfate in sulfuric acid was completely ineffective as an oxidizing solution at room temperature for the volatile reducing substances from fish press juice. The greater oxidizing power for organic compounds of alkaline over acid potassium permanganate solution has been reported (*3*). The normality of the alkali in the potassium permanganate solution affects the amount of reduction obtained; somewhat lower values were found with 0.1 *N* than *N* sodium hydroxide.

The alkaline potassium permanganate solution is prepared with water redistilled from an alkaline potassium permanganate solution in an all-glass distilling apparatus. The solution is boiled for 5 to 10 minutes, allowed to stand covered overnight at room temperature, and filtered through asbestos on a sintered-glass funnel. The alkaline potassium permanganate solution is usually made up in 2-liter quantities and stored in an amber glass bottle with a screw cap. Under these conditions, the solution is stable as long as it lasts.

Determination of Amount of Reduction. The amount of reduction of the permanganate is determined as follows:

To 10 ml. of oxidizing reagent in vessel *T*, after aeration, are added 5 ml. of 6 *N* sulfuric acid followed by 10 ml. of 0.11 *N* fer-

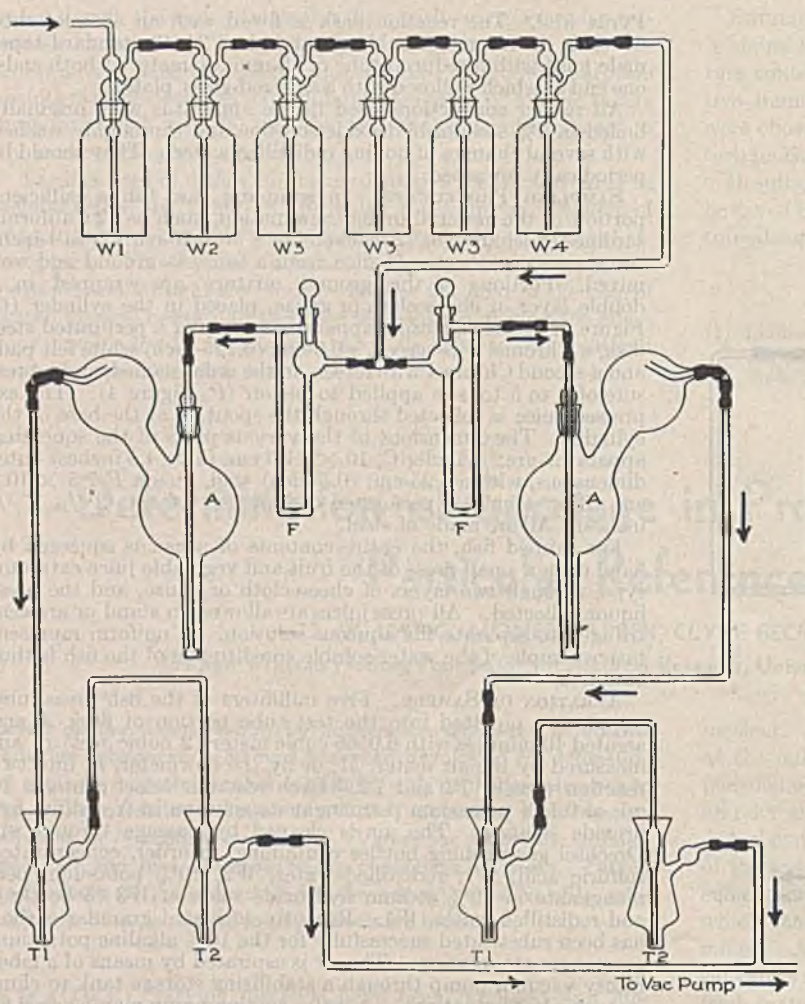

Figure 2. Diagram of Portable Two-Sample Unit

A. Aeration flask
F. Flowmeter
T1, *T2*. Reaction vessels
W1. Concentrated H_2SO_4
W2, *W4*. Redistilled water
W3. 10% alkaline $KMnO_4$ or activated carbon

rous ammonium sulfate in 0.01 *N* sulfuric acid. The excess of Mohr's salt is titrated with 0.01 *N* ceric sulfate in *N* sulfuric acid solution, using *o*-phenanthroline ferrous complex as indicator. Ten milliliters of the original oxidizing solution in a trap with a sintered-glass tube, after standing at room temperature for the duration of an aeration (40 minutes), are acidified with 5 ml. of 6 *N* sulfuric acid and mixed with 10 ml. of the ferrous ammonium sulfate solution, and the excess Mohr's salt is titrated with the ceric sulfate solution. This titer serves as the control for the unreacted oxidizing solution.

The difference between the test and the control titers is directly proportional to the amount of reduction of the potassium permanganate, which is expressed as microequivalents of reduction. A titer difference of 1 ml. of the 0.01 *N* ceric sulfate solution corresponds to a reduction of 10 microequivalents. The concentration of the volatile substances in a sample is directly proportional to the amount of reduction of the oxidizing agent. Studies are in progress on the determination of the reduction spectrophotometrically and preliminary experiments indicate its feasibility.

Stability of the Mohr's Salt Solution. The 0.11 *N* ferrous ammonium sulfate in 0.1 *N* sulfuric acid decreases in the concentration of ferrous ion on standing exposed to the air in the storage bottle. It was found that by replacing the air in and above the solution with illuminating gas, the rate of deterioration of the solution was reduced. (The authors wish to thank Paul L. Kirk, Division of Biochemistry, University of California, for this suggestion.) The stability of the solution was further markedly increased by the addition of 0.001% of sodium hydrosulfite or sodium sulfite. To facilitate having an atmosphere of illuminating gas in contact with the solution of Mohr's salt at all times, a dispensing arrangement similar to that of a water wash-bottle was inserted into the neck of the amber glass storage bottle, whereby the pressure of the gas is used to deliver the solution. The concentration, under these storage conditions, remains fairly constant for the duration of the 2-liter batch of solution.

Factors Affecting Rate of Removal of Volatile Reducing Substances (V.R.S.). *Volume of Air Aspirated through Sample.* The amount of volatile reducing substances carried over into the oxidizing reagent from a sample depends, among other factors, upon the volume of air aspirated through it. Some experiments were carried out to ascertain the volume of air necessary to ensure maximum yields of volatile reducing substances consistent with efficiency of operation. As examples of reducing substances with different volatilities, aqueous solutions of varying concentrations of ethanol and of acetone were used.

The per cent removal of a volatile substance by a definite volume of air is practically constant and is independent of the concentration of the volatile substances. The magnitude of the per cent removal at any air volume is dependent upon the volatility of the substance. For example, with 0.00566 cubic meter (0.2 cubic foot) 48% of acetone, but only 11% of ethanol, is removed from various solutions. The effect of low volatility is lessened with greater volumes of air: with 2.0 cubic feet of air, 95% of acetone is removed from a solution and 86% of ethanol is carried over. This establishes a valid basis for the adoption of an arbitrary air volume which would allow efficient and practical operation of the method, without having to carry on the aeration until all the volatile material has been removed. The following data are illustrative:

With 2.0 cubic feet of air, 86 and 87% of the total volatile reducing substances were recovered, respectively, from two samples of raw sardine (pilchard) and raw tuna press juices. The actual amounts of reduction for these two samples were 129.5 and 20.6 microequivalents per 5 ml. The per cent recoveries can be regarded as uniform, considering the diversity in the concentration of volatile reducing substances in these two preparations.

Temperature. A factor of possible influence is temperature, both of the sample and of the oxidizing reagent. No significant or practical advantage was found in warming the sample to 60° C. At higher temperatures, the raw press juice coagulates and tends to block the sintered-glass plate. The alkaline potassium permanganate oxidizing solution apparently undergoes some decomposition at higher temperatures, since controls with the oxidizing solution maintained (for 40 minutes) at 60° to 100° C. gave much higher values than controls kept at room temperature. The effect of temperature was not studied further.

Other Gases as Aspirating Media. Since gases might behave differently as sweeping agents for volatile reducing substances from aqueous solution, various gases were compared with air. No significant difference in the recovery of volatile reducing substances was found with nitrous oxide, oxygen, and nitrogen, all of which apparently act as mechanical sweeping agents for the volatile reducing substances.

Pressure of Air during Aspiration. In the arrangement of the apparatus described above, the air meter is at the start of the air-washing train—that is, the air is metered at atmospheric pressure and passes through the meter before entering the washing system. When the meter was placed between the second re-

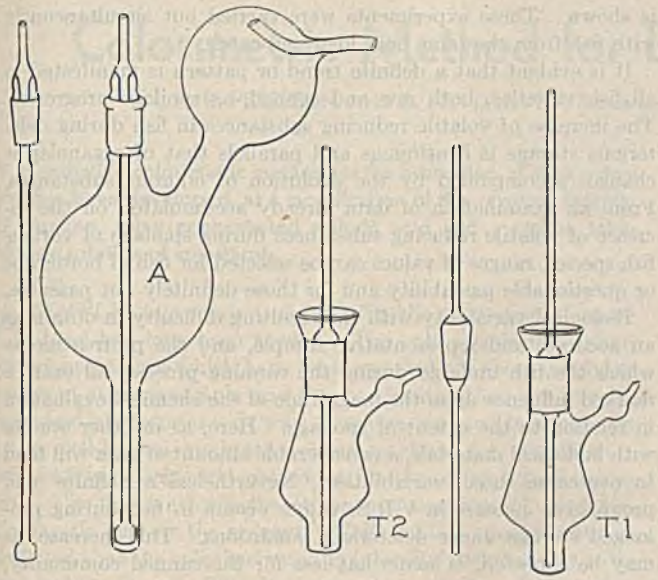

Figure 3. Aeration Flask and Reaction Vessels with Air-Inlet Tubes

action vessel and the vacuum stabilizing tank the meter was in a closed circuit and the air passing through it was at a reduced pressure, equal to 50 mm. (2 inches) of mercury at the aeration rate of 2.0 cubic feet in 40 minutes. The V.R.S. values obtained by aerating a sample with air metered at atmospheric pressure are higher than those with air metered at a vacuum equivalent to 50 mm. (2 inches) of mercury. Accordingly, the meter was kept at the air-intake end of the air-washing train and the flowmeters were calibrated against the meter with air passing through it at atmospheric pressure.

Effect of Retort Processes on V.R.S. Content of Canned Sardines (Pilchards). As a preliminary to the application of the method for the determination of spoilage in canned fish, it was deemed necessary to ascertain if any volatile reducing substances were formed during sterilization.

California sardines packed in No. 1 tall cans, natural style, were given the standard retort process of 90 minutes at 115.56° C. (240° F.). A number of cans were then subjected to additional processes up to a total of six. The last batch of cans, therefore, received a heating of 540 minutes or 9 hours at 240° F. All cans were cooled with water between individual cooks.

The extensive heat treatment had no significant effect on the content of volatile reducing substances in the canned sardines. Similarly, higher process temperatures do not influence the V.R.S. values.

Table I. Effect of Storage Temperature on Volatile Reducing Substances in California Sardines

Hours Stored	Microequivalents Reduction per 5 Ml. of Press Juice of Fish: Sea Water at 80.6° F. (27° C.) Raw	Canned	Organoleptic condition	Sea Water at 37° F. (2.8° C.) Raw	Canned	Organoleptic condition
5	31.8	6.8	Passable	..	....	...
9	45.1	19.3	Raw not passable, canned, borderline	..	....	...
13	56.2	39.2	Not passable	..	....	...
17.75	123.3	73.0	Not passable	..	....	...
28	..	...	...	35.7	6.9	Passable
73	..	...	...	35.1	9.8	Passable
98	..	...	...	33.0	7.7	Passable

Fish at 80.6° F. were no longer fit for canning after 9 hours' storage, whereas fish at 37° F. remained fit for canning until the end of the experiment.

Application of Method to Fish. A large number of experiments have been performed on the progressive spoilage of various commercially canned Pacific Coast fish, such as sardines (pilchards), tuna, mackerel, and shad. The fish were examined in the raw state and after various canning operations, both organoleptically and for volatile reducing substances. Some representative data are presented in Tables I to V to illustrate the scope of the method and its usefulness as a means of evaluating progressive spoilage.

Table II. Volatile Reducing Substances Increase in Pacific Mackerel during Storage at 63.5° F. (17.5° C.)

Hours Stored	Microequivalents Reduction per 5 Ml. Raw	Canned	Condition of Raw Sample (Organoleptic Evaluation)
1.5	6.2	13.7	Passable
6.0	19.1	15.7	Slight sour odor, near borderline passability
8.8	19.6	19.2	Slight sour odor, near borderline passability
11.3	22.5	21.6	Sour odor, of doubtful passability
14.8	67.0	30.5	Not passable
31.3	163.5	164.0	Not passable

Table III. Volatile Reducing Substances Increase in Bluefin Tuna Canned after Various Storage Periods

Hours Stored	Microequivalents Reduction per 5 Ml. of Canned Press Juice	Condition of Sample (Organoleptic Evaluation)
0	8.4	Passable
5.1	15.7	Passable
9.4	18.0	Passable
13.5	23.1	Doubtful passability
17.2	24.2	Not passable
21.5	34.8	Not passable

Table IV. Volatile Reducing Substances in Canned Atlantic Mackerel

Microequivalents Reduction per 5 Ml.	Condition of Sample (Organoleptic Evaluation)
14.4	Passable
24.5	Incipient spoilage, not passable
44.1	Putrid odor

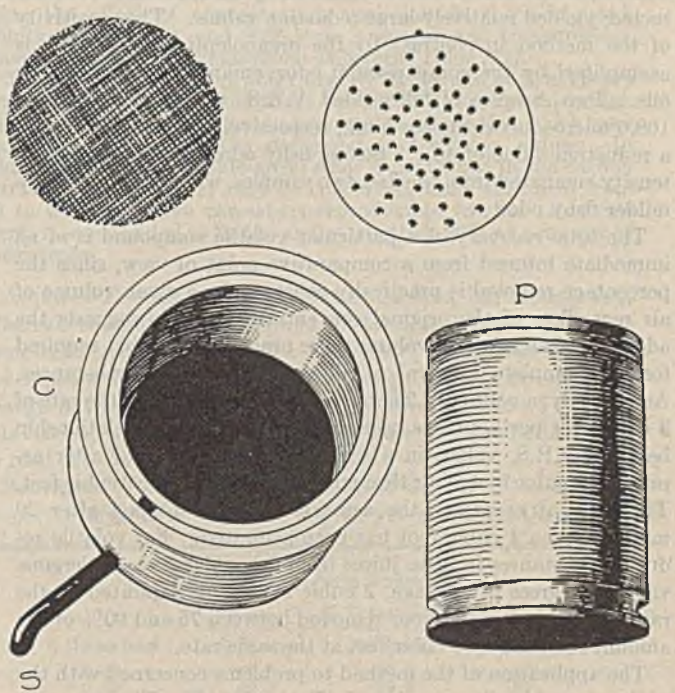

Figure 4. Apparatus for Obtaining Fish Press Juice

Cylinder *C*, piston *P*, perforated disk, and Chromel screen

Application of Method to Other Foodstuffs. Some experiments were conducted to ascertain the relationship between the content of volatile reducing substances and the state of preservation of foodstuffs other than fish.

Ground round steak was incubated in the original wrapping paper at 37.5° C. and periodically sampled as follows: A 5-gram sample was well triturated in a mortar with 10 ml. of redistilled water. The mixture was squeezed through cheesecloth and a 5-ml. aliquot of the press liquor was aerated with 2 cubic feet of air through two reaction vessels containing 10 ml. of oxidizing agent. The results obtained are shown in Table V.

Table V. Increase in Volatile Reducing Substances in Ground Round Steak during Storage at 37.5° C.

Hours Stored	Microequivalents Reduction	pH of Press Liquor	Organoleptic Condition
0	9.1	5.57	Normal meat odor
19	10.4	5.01	Normal meat odor
26	38.8	5.48	Strong, sour odor; meat has brown color; no longer appears edible
43	49.0	6.92	Definite tainted odor
50	57.4	7.33	Foul, putrid odor

Some preliminary experiments were also carried out on the increase in volatile reducing substances in eggs during storage and the relationship to the organoleptic condition. The results indicated the possible usefulness of the method for this commodity.

DISCUSSION

This method, in contrast to others proposed as measures of spoilage in such proteinaceous foodstuffs as fish and meat, is designed for determining only the volatile constituents which may be assumed to be responsible for the odors encountered. Thus is obtained a chemical and objective evaluation of the amount of odor which, when estimated organoleptically, is qualitative or at best only roughly quantitative and subjective.

The determination may be considered sensitive, since very dilute solutions of acetone and ethyl alcohol, from which no trace of the characteristic odors of the pure substances was detected, yielded relatively large reduction values. The sensitivity of the method in contrast to the organoleptic examination is exemplified by the comparison of odors emanating from fish liver oils. Two samples of oil yielded V.R.S. values of 102.5 and 108.6 microequivalents per 5 ml., respectively, while a third gave a reduction value of 46.1. Strong fishy odors of comparable intensity emanated from the first two samples, while the third had a milder fishy odor.

The total recovery of a particular volatile compound is of no immediate interest from a comparative point of view, since the percentage removal is practically constant for a given volume of air regardless of the original concentration. This suggests the adoption of an aeration volume more practical than that required for the complete removal of all volatile reducing substances. Accordingly, a volume of 2 cubic feet of air aspirated at the rate of 3 cubic feet per hour has been adopted. The same relationship between V.R.S. values in a series has been observed after aspirating 1 cubic foot of air through the sample as after 2 cubic feet. Therefore, if necessary the aeration may be stopped after 20 minutes when 1 cubic foot has been aspirated. For volatile reducing substances in press juices from fish which have undergone varying degrees of spoilage, 2 cubic feet of air aspirated at the rate of 3 cubic feet per hour removed between 75 and 90% of the amount removed by 6 cubic feet at the same rate.

The application of the method to problems concerned with the evaluation of spoilage is given in Tables I to V. The data presented in Table I may be of particular interest since the prolongation of keeping qualities through storage at lower temperatures is shown. These experiments were carried out simultaneously with fish from the same boat load and catch.

It is evident that a definite trend or pattern is manifested in all fish varieties, both raw and canned, as spoilage progresses. The increase of volatile reducing substances in fish during deleterious storage is continuous and parallels that of organoleptic changes accompanied by the evolution of odorous substances. From an examination of data already accumulated on the increase of volatile reducing substances during spoilage of various fish species, ranges of values can be selected for fish of borderline or questionable passability and for those definitely not passable.

Biological variability with the resulting difficulty in obtaining an accurate and representative sample, and the pretreatments which the fish undergo during the canning process, all exert a marked influence upon the magnitude of the chemical evaluation in relation to the extent of spoilage. Here, as in other studies with biological materials, a considerable amount of data will tend to overcome these variabilities. Nevertheless a definite and progressive increase in V.R.S. values occurs in fish during prolonged storage under deleterious conditions. This increase, as may be expected, is somewhat less for the canned commodity, largely because of the volatilization of some of the gases through the exhaust or other pretreatment which the commodity undergoes during canning.

The accuracy of the method is not influenced by the type of spoilage. For instance, under one set of experimental conditions fish spoiled with the formation of sour, fatty acid odors, while under another set of conditions foul and putrid odors were produced. The specific products formed were different in these two experiments, yet both gave high values for volatile reducing substances and indicated advance degrees of spoilage.

The application of this aeration technique is not limited to spoilage determination. It has proved of value in estimating the concentration of volatile constituents in a variety of foods, where the amount of volatile material is used as an index of quality or desirability of a product.

SUMMARY

Based on the fact that proteinaceous foodstuffs emit odorous substances as they undergo spoilage during deleterious handling and storage, a method has been developed which determines these volatile materials and can be used to measure the state of freshness or the degree of spoilage in such foodstuffs.

A measured volume of washed air is aspirated through a suitably prepared sample of a material and through an alkaline potassium permanganate solution, all solutions being at room temperature. The amount of reduction of this oxidizing reagent is used as the measure of the concentration of the volatile substances.

The procedure has been applied particularly to determine state of freshness of raw and canned fish.

This method may be applied to the evaluation of odorous principles in other food commodities, where palatability and desirability are dependent upon the concentration of volatile substances.

ACKNOWLEDGMENT

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Colorimetric Method for Determination of Ergosterol

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A quantitative colorimetric method for the estimation of pure ergosterol has been developed, as a modification of the reversed Salkowski reaction, using concentrated sulfuric acid and a carbon tetrachloride solution of ergosterol.

A SIMPLE, inexpensive method for the estimation of ergosterol has long been needed. The only satisfactory quantitative procedures which have been reported are those involving the use of a spectrograph, and this has limited their use to relatively few laboratories. It was, therefore, decided to adapt one of the well-known qualitative color reactions for sterols to a quantitative colorimetric procedure for the estimation of ergosterol. The first tried was the Rosenheim reaction (*1*) using trichloroacetic acid as the reagent. This was abandoned when it was found that the color increased in intensity progressively with time, making it very difficult to reproduce results. It was also noted that in the chloroform used as a solvent for this method there was a rather rapid autoxidation of the ergosterol, with the formation of a yellow color further interfering with the color developed.

It was then decided to try a modification of the "reversed Salkowski" reaction (*2*) using concentrated sulfuric acid. This proved to give a satisfactory, stable color, orange by transmitted light and having a strong green fluorescence. A carbon tetrachloride solution of ergosterol was used to avoid the autoxidative effects which were observed when chloroform was used as the solvent.

Table I. Per Cent Transmission

Mμ	20 min.	30 min.	40 min.	50 min.	60 min.
400	2	2	2	2	2
450	2	2	2	2	2
500	2	2	2	2	2
550	15.5	15	14.5	14.5	14.5
600	54	53.5	53	52.5	52
650	77.5	76.5	77	77.5	77

EXPERIMENTAL

The Coleman Universal spectrophotometer Model 11 and its 13-mm. square tube cuvette were used in these studies.

Nissen and Petersen (*3*) have given a general discussion of the methods and problems of colorimetric studies.

PURIFICATION OF ERGOSTEROL. The ergosterol used was the "Puriss. in Nitrogen" grade prepared by the Glaxo Laboratories, Ltd., Greenford, Middlesex, England. This had turned yellow on standing, and it was necessary to recrystallize it by dissolving 50 grams in 400 ml. of hot, pure ethyl acetate and allowing it to crystallize overnight at 30° C. The product retained a slight yellow tinge, and the crystallization was repeated, resulting in a pure white product consisting of short needles. This was sucked free of solvent and dried at 30° C. under reduced pressure in a stream of nitrogen. $(\alpha)_D^{25} = -126°$, found $(\alpha)_D^{25} = -128.7°$ (purified through benzoate) reported (*1*). Purity, approximately 98% (spectrograph).

The above observed rotation was the same as was obtained for the Glaxo ergosterol before it had yellowed and the specific rotation of the yellowed material was $(\alpha)_D^{25} = -115.5°$ before recrystallizations.

SELECTION OF WAVE LENGTH FOR READING COLOR AND TIME OF COLOR DEVELOPMENT. A 0.1% solution of ergosterol in pure, dry carbon tetrachloride was prepared and the color developed as follows: five milliliters of the solution were pipetted into each of five 25-ml. amber mixing cylinders and 10 ml. of concentrated sulfuric acid were added. These were mixed by inverting several times. The color formed in the lower acid layer, which was then read by pipetting this layer into the cuvette. A transmission curve for each sample was determined after they had stood for 20, 30, 40, 50, and 60 minutes, respectively (Table I).

Constancy of transmission is reached in about 40 minutes at 550 and 650 mμ.

A further check of the best wave length to be used was made by determining transmission curves as before where the concentration of ergosterol was varied in the range 0 to 0.1% with the color development time fixed at approximately 50 minutes (Table II).

The greatest reading differential in this concentration range is found at 550 mμ. It may therefore be expected that the greatest sensitivity will be obtained at this wave length.

A plot of the log transmission values obtained at 550 mμ against the concentration results in a smooth curve which is substantially a straight line in its middle portion. This portion was used in the remainder of the work, using a color development time of from 50 to 60 minutes.

Table II. Per Cent Transmission

	Ergosterol				
Mμ	0.02%	0.04%	0.06%	0.08%	0.10%
400	4	2	2	2	2
450	5	2	2	2	2
500	13.2	4.5	3	2.2	2
550	59	40	27.2	19.2	14
600	83.5	77	67.5	59	52.2
650	89.5	89	85	81	78

FACTORS AFFECTING INTENSITY OF COLOR DEVELOPED

ACID. There are two ways in which the acid used might affect the color developed with ergosterol—the color of the acid and its concentration.

Color. Sulfuric acid is frequently discolored because of contact with the gasket material in the plastic closures. It is therefore important to refer all readings made to the particular acid used in developing the color.

Concentration. Several experiments were made to determine the effect of concentration variation in the acid used. Dilutions of 94, 90, and 86% were prepared and transmission-concentration curves determined as before (Table III).

The colors developed in this range by the 94 and 90% acids are practically identical, while those by the 86% are much darker. They also have a redder tinge, and tend to be cloudy, which may account, in part, for the decreased transmission values. In addition, a secondary blue-green color is developed in the carbon tetrachloride layer. It may be concluded, then, that acid from 90 to 94% will give consistent reproducible results. For this work, it was decided to fix the acid strength at 90% as a matter of consistency.

AGING OF ERGOSTEROL SOLUTIONS IN CARBON TETRACHLORIDE. When these solutions are allowed to stand, a change takes place even in the dark, resulting in a progressive increase in the color developed with sulfuric acid, even though there is no apparent change in the color of the solution as shown by Table IV. For this reason, solutions of ergosterol in carbon tetrachloride must be read as soon as they are prepared.

SOLVENTS. The question of the best solvent to be used in preparing ergosterol solutions has as yet not been thoroughly

Table III. Effect of Acid Concentration

	% Transmission		
% Ergosterol	94% acid	90% acid	86% acid
0.02	59.8	60.5	41.5
0.04	39.2	39.8	31
0.06	26.5	27	19.5
0.08	18.8	18.5	10
0.10	13.9	14.1	7

Table IV. Effect of Aging

	% Transmission				
% Ergosterol	0 days	1 day	2 days	7 days	28 days
0.10	14.1	9.8	...	1.0	Too dark
0.10	14.5	...	3.5	...	....

Table V. Per Cent Transmission

% Ergosterol	I	II	III	Average
0.01	..	78	77.2	77.6
0.02	59	60.5	61.5	60.3
0.03	..	49.5	48.7	49.1
0.04	40	39.8	39.5	39.8
0.05	..	31.5	32.0	31.7
0.06	27.2	27	26.8	27
0.07	..	21.5	21.6	21.5
0.08	19.2	18.5	18.8	18.8
0.09	..	15.5	15.6	15.5
0.10	14	14.1	13.9	14

studied. Carbon tetrachloride was used in this work, because ergosterol apparently does not autoxidize as readily in it as in chloroform. It is not at all impossible that other solvents such as cyclohexane or cyclohexanone, etc., would be useful.

TRANSMISSION-CONCENTRATION CURVE FOR ERGOSTEROL

Three separate 0.10% solutions of ergosterol in carbon tetrachloride were prepared and read immediately, using 90% sulfuric acid. In preparing the various concentrations of ergosterol, the volume of carbon tetrachloride solution was maintained constant at 5 ml. Thus, in preparing the 0.02% solution, 1 ml. of the 0.10% solution was diluted in the mixing cylinder with 4 ml. of pure carbon tetrachloride, etc., then 10 ml. of 90% sulfuric acid were added to each by pipet and mixed by inverting five times. This mixing was repeated three more times at 10-minute intervals to maintain an even distribution of color. The total time from the addition of the sulfuric acid averaged about 50 minutes (Table V).

The per cent transmission (log) when plotted against the concentration results in a curve indicating obedience to Beer's law in the range 0.02 to 0.08% ergosterol. The reproducibility of the values is well shown by the close agreement obtained in the separate determinations. It is thus possible to determine an unknown quantity of pure ergosterol in a carbon tetrachloride solution by developing a color as above, reading the per cent transmission at 550 mμ, and applying this value to the standard curve, from which the concentration of ergosterol equivalent to the value may be read. By simple proportion, the fact that the standard ergosterol is but 98% pure may be compensated for, and the result reported in terms of 100% ergosterol.

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Determination of Formaldehyde

In the Presence of Acrolein and Other Aldehydes by the Polarographic Method

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Formaldehyde can be rapidly determined in the presence of acrolein and acetaldehyde by the polarographic method with an accuracy of ±2%. The propionaldehyde wave does not overlap that of formaldehyde but its presence tends to cause low results unless determinations are run immediately. The most satisfactory results are obtained in 0.1 *N* lithium hydroxide containing 0.01 *N* lithium chloride, at constant temperature and constant pH, without removal of dissolved oxygen.

ALTHOUGH many methods are available for the determination of formaldehyde, little attention has been given to its determination in the presence of other aldehydes.

The Romijn potassium cyanide method (*2*, *4*, *10*, *11*, *12*) has been reported (*1*) to be suitable when acetaldehyde is present, if, in the procedure, the silver salt of the excess cyanide is immediately filtered off. According to Jahoda (*6*), however, this method is unsatisfactory for formaldehyde in the presence of acetaldehyde, and this investigator reports an accurate polarographic method. The polarographic method for this purpose has been confirmed by Grimaldi and Wells (*5*). Jonescu and Slusanschi (*7*) have worked out a time-precipitation relationship for the determination of certain aldehydes in aldehydic mixtures using dimethyldihydroresorcinol (dimedon) as a reagent. This method, however, is too time-consuming for industrial use.

This paper describes the determination of formaldehyde in the presence of acrolein by the polarographic method. The effect of the additional presence of acetaldehyde and propionaldehyde is also brought out.

It has been previously pointed out that in the polarographic determination of acrolein (*9*) in the presence of formaldehyde and acetaldehyde, the formaldehyde wave occurs between two acrolein waves when working in an alkaline medium. Although there is an encroachment of the formaldehyde wave on the second acrolein wave, for the determination of acrolein such an encroachment offers no difficulty because the first acrolein wave gives reliable results. In the case of formaldehyde, however, this encroachment is important because this aldehyde gives only the one wave and its overlapping by the acrolein wave renders formaldehyde determinations inaccurate. Polarography, however, offers a reliable method since there is no overlapping when the concentration of acrolein does not appreciably exceed that of formaldehyde. When overlapping does occur, this may be corrected by repeating the determination with a much smaller sample.

A 0.1 *N* lithium hydroxide solution, 0.01 molar in lithium chloride, is most satisfactory for the determination of formaldehyde in the presence of acrolein within the limits stated above. No perceptible change in the pH occurs with dilution by the addition of the sample nor by traces of acid in the sample. The advantageous use of this solution confirms the previous report of Jahoda (*6*) that the use of a high pH made for greater sensitivity in the determination of formaldehyde in the presence of acetaldehyde.

SOLUTIONS

All indifferent salt solutions contained maxima inhibitor, consisting of 1 ml. of 0.2% methyl red alcoholic solution and 1.5 ml. of 0.02% bromocresol green alcoholic solution added per liter. They also contained 0.01 molar lithium chloride.

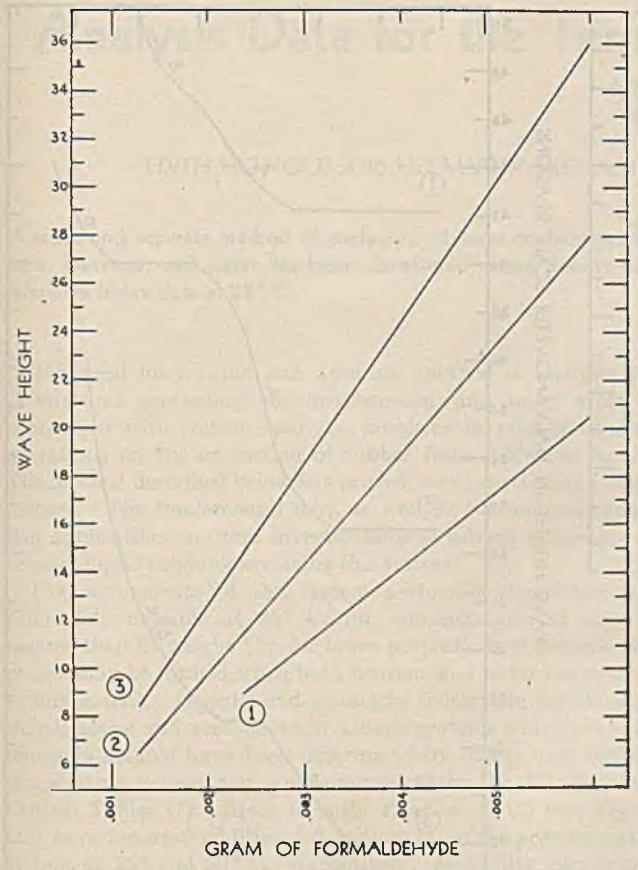

Figure 1. Effect of Temperature on Wave Height

Sensitivity 1/5. Volume 105 ml. pH 12.9
Temperature (1) 20°, (2) 25°, (3) 30° C.

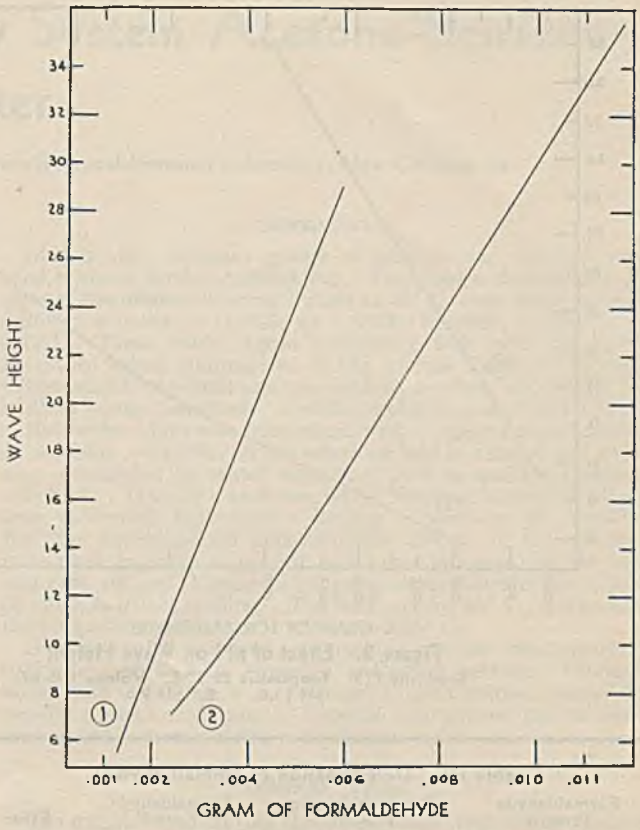

Figure 2. Effect of pH on Wave Height

Sensitivity 1/5. Temperature 25.5° C. Volume 105 ml.
1. pH 12.9. 2. pH 12.2

Solution at pH 9.6 was prepared by adding carbon dioxide to a saturated solution of lithium carbonate.

Solution at pH 11.6 was a saturated lithium carbonate solution.

Solution at pH 12.2 was a 0.005 molar lithium hydroxide solution.

Solution at pH 12.9 was a 0.1 molar lithium hydroxide solution.

ALDEHYDE SOLUTIONS. The acrolein solution was prepared from commercial acrolein by collecting a 52° distillation fraction in water containing 0.01% hydroquinone as polymerization inhibitor. This solution was standardized by the Ripper bisulfite method as modified by Kolthoff and Furman (*8*). The formaldehyde solution was prepared by diluting Heyden's commercial formalin and analyzed by the hydrogen peroxide method (*8*).

The propionaldehyde was that of Eastman Kodak Company. All weighings were carried out in ampoules and the aldehyde analyzed by the Ripper bisulfite method.

Table I. Determination of Formaldehyde

Formaldehyde Present	Acetaldehyde Present	Formaldehyde Found	Error
Mg.	*Mg.*	*Mg.*	%
1.19	2.74	1.19	0.0
1.19	1.64	1.28	+7.5
1.19	1.09	1.19	0.0
1.19	0.55	1.19	0.0
2.38	0.55	2.38	0.0
2.38	1.09	2.40	+0.8
2.38[a]	1.64	2.38	0.0
2.38	2.74	2.40	+0.8
3.56	0.55	3.45	−3.1
3.56	1.09	3.45	−3.1
3.56	1.64	3.45	−3.1
3.56	2.74	3.55	−0.3
5.94	5.48	5.63	−5.2
5.94	1.09	5.94	0.0
5.94	1.64	5.94	0.0
5.94	2.74	5.94	0.0

[a] See curve 2, Figure 4.

ANALYSES

The polarograph used in this work was the Elecdropode (manufactured by the Fischer Scientific Company, Pittsburgh, Pa.). All work was carried out at one fifth of the galvanometer sensitivity, using 125-ml. lipless beakers without removal of dissolved oxygen from the solutions. The removal of oxygen was not

Table II. Determination of Formaldehyde

Formaldehyde Present	Acrolein Present	Formaldehyde Found	Error
Mg.	*Mg.*	*Mg.*	%
1.19	0.742	1.23	+3.4
1.19	1.48	1.21	+1.7
1.19	2.22	1.19	0.0
1.19	3.71[a]	...	...
2.38	0.742	2.43	+2.1
2.38	1.48	2.34	−1.7
2.38	2.22	2.34	−1.7
2.38	3.71	2.38	0.0
3.56	0.742	3.51	−1.4
3.56	1.48	3.40	−4.5
3.56	2.22[b]	...	...
3.56	3.71[b]	...	...
5.94	0.742	5.94	0.0
5.94	1.48	5.94	0.0
5.94	2.22[b]	...	...
5.94	3.71[b]	...	...

[a] See curve 1, Figure 4.
[b] Acrolein wave overlaps that of formaldehyde.

Table III. Determination of Formaldehyde

Formaldehyde Present	Acrolein Present	Acetaldehyde Present	Formaldehyde Found	Error
Mg.	*Mg.*	*Mg.*	*Mg.*	%
1.19	1.48	2.74	1.19[a]	0.0
2.38	1.48	2.74	2.38	0.0
3.56	1.48	2.74	3.54	−0.6
5.94	1.48	2.74	5.72	−3.7
1.19	2.22	2.74	1.19	0.0
2.38	2.22	2.74	2.76[b]	+16.0
3.56	2.22	2.74	3.34[b]	−6.2
5.94	2.22	2.74	5.72[b]	−3.9

[a] See curve 3, Figure 4.
[b] Waves overlap and determinations were calculated using apparent minimum between two waves.

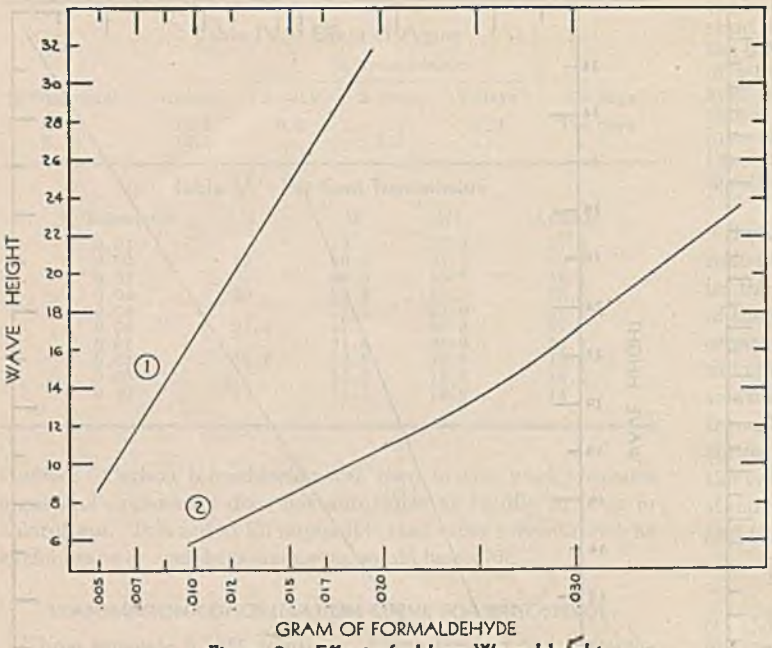

Figure 3. Effect of pH on Wave Height

Sensitivity 1/5. Temperature 25.5° C. Volume 105 ml.
1. pH 11.6. 2. pH 9.6

Table IV. Determination of Formaldehyde

Formaldehyde Present	Propionaldehyde Present	Formaldehyde Found	Error
Mg.	*Mg.*	*Mg.*	%
5.94	None	5.90	−0.6
5.94	2.48	5.51[c]	−7.2
5.94	1.24	5.51[a]	−7.2
5.94	6.20	5.42[a]	−8.7
2.38	6.20	2.47[b]	+3.7
2.38	6.20	2.06[c]	−13.4
2.38	6.20	1.76[d]	−26.0
2.38	2.48	2.36[b]	−0.8

[a] Samples analyzed routinely, within 5 to 10 minutes.
[b] Samples run immediately on addition of propionaldehyde.
[c] 10-minute standing before analyzing.
[d] 15-minute standing before analyzing.

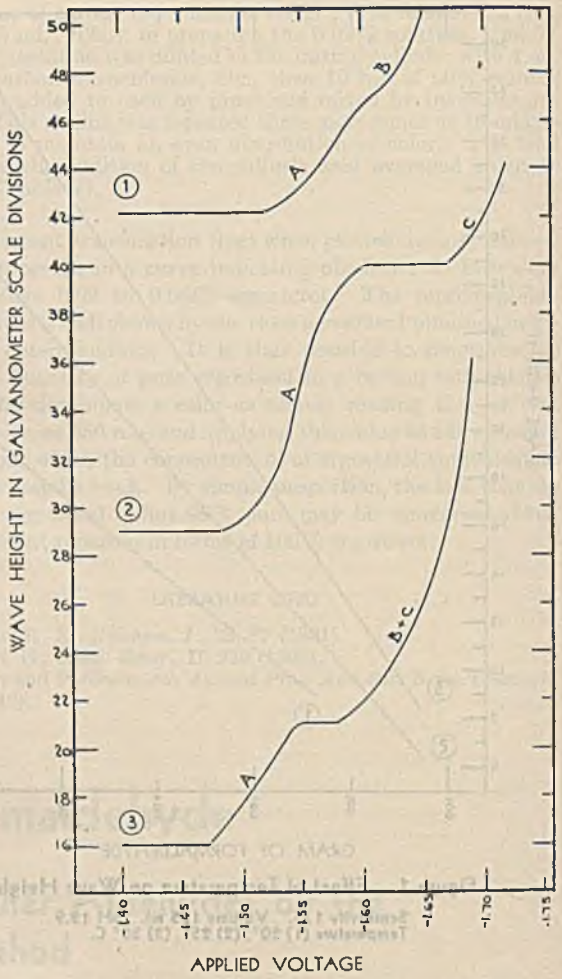

Figure 4. Current Voltage Curves for Formaldehyde

Sensitivity 1/5. Volume 105 ml. pH 12.9. Temperature 25.5° C.
A. Formaldehyde wave. *B*. Acrolein wave. *C*. Acetaldehyde wave
1. 1.19 mg. formaldehyde, 3.71 mg. acrolein
2. 2.38 mg. formaldehyde, 1.64 mg. acetaldehyde
3. 1.19 mg. formaldehyde, 1.48 mg. acrolein, 2.74 mg. acetaldehyde

required, as no change in residual current was observed in the voltage increment used with the pH 12.9 lithium hydroxide base solution.

After addition of the sample to the base solution the cell can stand for at least 0.5 hour without loss of formaldehyde. This is an added advantage, in that one can prepare several cells without danger of loss in accuracy. Acetaldehyde and acrolein, however, must be determined immediately after adding the sample to the base solution.

For the most accurate quantitative work the temperature of the solution must be constant as observable by an ordinary laboratory thermometer.

The temperature effect on the polarographic wave is shown in Figure 1. The effect of pH on the polarographic wave is shown in Figures 2 and 3, which indicate the necessity of constant pH. Figure 4 shows typical polarographic curves taken on solutions containing both formaldehyde and acrolein.

RESULTS

A 0.1 *N* lithium hydroxide solution containing lithium chloride and maximum inhibitor giving a pH near 13 was selected as best for reproducibility, high sensitivity, and noninterference of acrolein and acetaldehyde in the determination of formaldehyde. The formaldehyde wave appears in the voltage range −1.50 to −1.65, having a corrected half-wave potential of −1.56 volts, a drop weight of 0.0045 gram, and at a drop rate of 6.3 seconds.

The determination is limited by the ratio of the quantities present in the aliquot taken for analysis. With 6 mg. of formaldehyde, no more than 0.75 mg. of acrolein may be present. However, with 1.2 mg. of formaldehyde, 2.2 mg. of acrolein do not interfere. Acetaldehyde may be present up to 2.74 mg. The presence of propionaldehyde renders the formaldehyde determination low. If the sample is analyzed immediately after addition to the base solution the error is slight. The propionaldehyde wave occurs in the same voltage range as that of acetaldehyde and showed no overlapping with the formaldehyde wave.

The representative data shown in Tables I, II, III, and IV were obtained at pH 12.9, 25.5° C., and a galvanometer sensitivity of one fifth.

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Analysis Data for the Ternary System Acetone-Benzene-Water

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A rapid and accurate method of analyzing mixtures containing acetone, benzene, and water has been developed, using density and refractive index data at 25° C.

THE need for a rapid and accurate method of analysis for mixtures containing acetone, benzene, and water arose in connection with solvent-recovery problems in pilot-plant-scale operations on the extraction of rubber from goldenrod leaves. The method described below has proved very practical and satisfactory. The fundamental data as well as the method should find applications in other investigations of solvent extraction or ternary liquid behavior involving this system.

The components of the system acetone-benzene-water are completely miscible at 25° C. for concentrations of acetone greater than 65 weight %; for lower proportions of acetone two phases may be formed when both benzene and water are present to any extent. Density and refractive index data for the acetone-benzene and acetone-water binary systems which are each mutually soluble have been determined by others over limited temperature ranges and are summarized in the International Critical Tables (*7*). More recently Ernst *et al.* (*6*) and Young (*11*) have reported additional density data on the acetone-water system at 25° and 20° C., respectively. Solubility curves and tie line data for the ternary system at 15°, 30°, and 45° C. were obtained by Briggs and Comings (*4*) by means of refractive index measurements. In the present investigation density and refractive index data at 25° C. for this system in the region of complete miscibility were obtained to permit analysis of mixtures in the homogeneous portion of the ternary diagram.

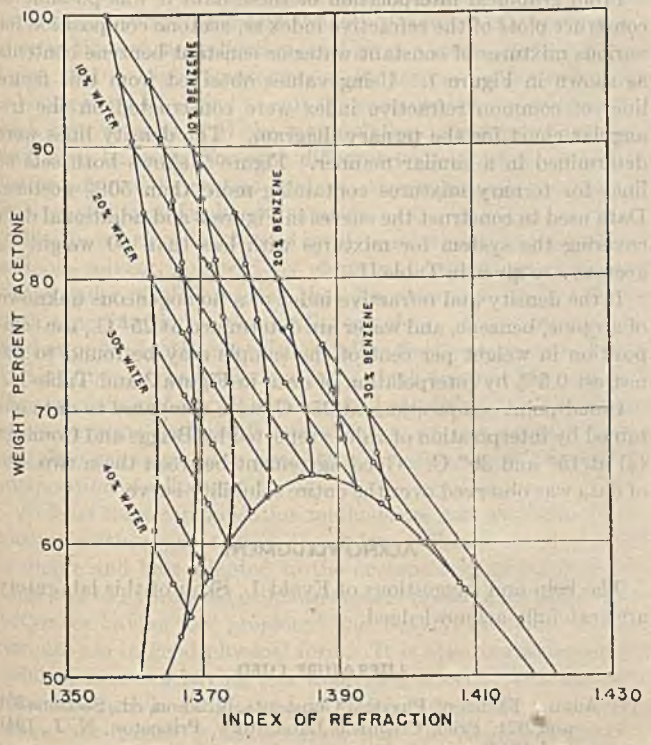

Figure 1. Refractive Indices for Mixtures of Acetone, Benzene, and Water

EXPERIMENTAL

MATERIALS. Reagent grades of acetone and benzene were used without further purification. Densities and refractive indices of the original starting liquids at 25° C. were found to be as follows: acetone, d. 0.7857, n_D 1.3562; benzene, d. 0.8731, n_D 1.4972. These values agree sufficiently well with the better literature values summarized in the Annual Tables of Physical Constants (*1*) to permit analyses with an accuracy of 0.5% by the method herein described. Distilled water was used throughout.

Refractive index was determined with a dipping refractometer of precision ±0.00005 on the solutions held in a metal cup which was surrounded by water circulated from a constant-temperature bath. Density was measured by National Bureau of Standards calibrated hydrometers having a precision of ±0.0002. For this determination approximately 250 cc. of the solutions were used in large stoppered test tubes immersed in the bath and were allowed to come to temperature equilibrium for at least 30 minutes before reading. The bath, set for 25° C., maintained the temperature constant to within ±0.05° C.

Solutions were prepared by weighing out the components to 0.01 gram on a 2-kg. capacity analytical balance. Mixtures amounting to 300 cc. were prepared to give ample material for density measurement and to decrease inaccuracies due to evaporation of the more volatile components.

TERNARY SYSTEM

The method of analyzing three component systems by measuring two independent properties has been described by Berl and Ranis (*3*) and others (*2, 5, 8, 9, 10*). In the present method re-

Table I. Experimental Data

Weight % Acetone	Weight % Benzene	Weight % Water	n_D^{25}	Density at 25° C.
100	.....	.....	1.3562	0.7857
94.96	5.04	...	1.3625	0.7897
86.28	4.58	9.14	1.3654	0.8165
77.44	4.11	18.45	1.3671	0.8423
69.72	3.70	26.58	1.3675	0.8632
62.03	3.29	34.68	1.3666	0.8831
54.04	2.86	43.10	1.3650	0.9023
49.07	2.60	48.33	1.3634	0.9138
44.50	2.36	53.14	1.3617	0.9243
90.03	9.97	....	1.3688	0.7938
82.07	9.09	8.84	1.3710	0.8194
73.75	8.17	18.08	1.3718	0.8441
66.21	7.33	26.46	1.3714	0.8650
80.13	19.87	....	1.3812	0.8021
71.42	17.71	10.87	1.3823	0.8314
64.98	16.11	18.91	1.3814	0.8510
69.57	30.43	....	1.3950	0.8107
66.89	29.26	3.85	1.3952	0.8208
64.84	28.36	6.80	1.3952	0.8286
54.16	45.84	....	1.4166	0.8242
49.41	50.59	....	1.4229	0.8282
40.61	59.39	....	1.4350	0.8358
19.76	80.24	....	1.4666	0.8543
.....	100	....	1.4972	0.8731
95.44	....	4.56	1.3581	0.7995
79.82	16.36	3.82	1.3784	0.8103
74.91	21.50	3.59	1.3850	0.8139
69.25	27.43	3.32	1.3927	0.8178
61.56	35.49	2.95	1.4029	0.8236
53.81	43.61	2.58	1.4145	0.8294
47.16	50.58	2.26	1.4233	0.8346
43.95	53.94	2.11	1.4288	0.8372
90.47	....	9.53	1.3600	0.8143
81.53	9.88	8.59	1.3719	0.8192
73.60	18.65	7.75	1.3828	0.8236
80.14	....	19.86	1.3626	0.8435
76.50	4.54	18.96	1.3678	0.8441
68.39	14.66	16.95	1.3795	0.8455
69.75	....	30.25	1.3634	0.8704
67.92	2.63	29.45	1.3664	0.8702
65.75	5.74	28.51	1.3699	0.8696
60.98	....	39.02	1.3629	0.8918
57.80	5.21	36.99	1.3686	0.8892
49.10	....	50.90	1.3606	0.9181
48.10	....	51.90	1.3600	0.9201
39.85	....	60.15	1.3572	0.9366
39.77	....	60.23	1.3573	0.9367
29.85	....	70.15	1.3524	0.9541
21.47	....	78.53	1.3477	0.9668
20.02	....	79.98	1.3467	0.9702
.....	....	100	1.3325	0.9970

Table II. Derived Data for Ternary Diagram at 25° C.

Refractive Index	Weight % Acetone	Weight % Benzene	Weight % Water	Refractive Index	Weight % Acetone	Weight % Benzene	Weight % Water
1.335	3.2	96.8	0.0	1.390	73.3	26.7	0.0
1.340	9.9	91.1	0.0		70.1	24.9	5.0
1.345	17.5	82.5	0.0		69.9	25.0	5.1
1.350	25.6	74.4	0.0		66.3	23.7	10.0
1.355	35.0	65.0	0.0		64.8[a]	19.2	16.0
1.360	97.0	3.0	0.0	1.395	69.5	30.5	0.0
	93.7	1.3	5.0		68.6	30.0	1.4
	90.4	0.0	9.6		66.2	28.8	5.0
	47.9	0.0	52.1		63.5[a]	27.5	9.0
	43.4[a]	2.1	54.5				
				1.400	65.8	34.2	0.0
1.365	93.0	7.0	0.0		62.6	32.4	5.0
	89.6	5.4	5.0		61.4[a]	31.5	7.1
	88.7	5.0	6.3				
	86.0	4.0	10.0	1.405	62.1	37.9	0.0
	82.2	2.8	15.0		59.0	36.0	5.0
	77.9	2.1	20.0		58.6[a]	35.8	5.6
	73.4	1.6	25.0				
	68.5	1.5	30.0	1.410	58.5	41.5	0.0
	50.8[a]	3.8	45.4		55.6[a]	40.0	4.4
1.370	89.0	11.0	0.0	1.415	55.0	45.0	0.0
	87.0	10.0	3.0		52.5[a]	43.8	3.7
	85.5	9.5	5.0				
	81.9	8.1	10.0	1.420	51.4	48.6	0.0
	78.0	7.0	15.0		49.3[a]	47.7	3.0
	73.6	6.4	20.0				
	68.9	6.1	25.0	1.425	47.7	52.3	0.0
	63.8	6.2	30.0		46.2[a]	51.2	2.6
	56.7[a]	6.8	36.5				
				1.430	44.2	55.8	0.0
1.375	85.1	14.9	0.0		43.1[a]	54.9	2.0
	81.6	13.4	5.0				
	77.8	12.2	10.0	1.435	40.7	59.3	0.0
	73.8	11.2	15.0	1.440	37.3	62.7	0.0
	69.3	10.7	20.0	1.445	33.9	66.1	0.0
	64.5	10.5	25.0	1.450	30.6	69.4	0.0
	60.9[a]	11.0	28.1	1.455	27.3	72.7	0.0
				1.460	24.1	75.9	0.0
1.380	81.1	18.9	0.0	1.465	20.7	79.3	0.0
	77.6	17.4	5.0	1.470	17.4	82.6	0.0
	73.9	16.1	10.0	1.475	14.0	86.0	0.0
	69.8	15.2	15.0	1.480	10.7	89.3	0.0
	65.1	14.9	20.0	1.485	7.4	92.6	0.0
	63.7[a]	15.2	21.1	1.490	4.1	95.9	0.0
				1.495	0.8	99.2	0.0
1.385	77.1	22.9	0.0				
	73.8	21.2	5.0				
	71.1	20.0	8.9				
	70.1	19.9	10.0				
	65.8	19.2	15.0				
	65.0[a]	19.2	15.8				
Density				Density			
0.790	98.4	0.0	1.6	0.850	77.7	22.3	0.0
	94.6	5.4	0.0		74.0	5.0	21.0
					70.5	9.5	20.0
0.800	95.2	0.0	4.8		70.2	10.0	19.8
	91.6	5.0	3.4		66.3	15.0	18.7
	88.0	10.0	2.0		64.7[a]	17.7	17.6
	84.3	15.0	0.7		24.5	75.5	0.0
	82.6	17.4	0.0				
				0.860	73.9	0.0	26.1
0.810	91.8	0.0	8.2		70.0	5.0	25.0
	88.3	5.0	6.7		66.1	10.0	23.9
	84.6	10.0	5.4		62.9[a]	14.4	22.7
	83.4	11.6	5.0		13.5	86.5	0.0
	80.9	15.0	4.1				
	77.3	20.0	2.7	0.870	69.8	0.0	30.2
	73.7	25.0	1.3		66.1	5.0	28.9
	70.7	29.3	0.0		62.0	10.0	28.0
					61.0[a]	11.5	27.5
0.820	88.4	0.0	11.6		2.9	97.1	0.0
	84.4	5.6	10.0				
	84.8	5.0	10.2	0.880	65.6	0.0	34.4
	81.2	10.0	8.8		62.0	5.0	33.0
	77.5	15.0	7.5		58.7[a]	8.9	32.4
	73.8	20.0	6.2				
	71.0	24.0	5.0	0.890	61.7	0.0	38.3
	70.2	25.0	4.8		56.1[a]	6.9	37.0
	66.7	30.0	3.3				
	63.1	35.0	1.9	0.900	57.4	0.0	42.6
	59.6	40.0	0.4		53.4[a]	5.3	41.3
	58.6	41.4	0.0				
				0.910	52.8	0.0	47.2
0.830	84.9	0.0	15.1		50.3[a]	3.8	45.9
	81.2	5.0	13.8				
	77.6	10.0	12.4	0.920	48.2	0.0	51.8
	73.9	15.0	11.1		46.6[a]	2.8	50.6
	71.1	18.9	10.0				
	70.3	20.0	9.7	0.930	43.3	0.0	56.7
	66.8	25.0	8.2		42.2[a]	1.9	55.9
	63.3	30.0	6.7				
	59.5	35.0	5.5	0.940	38.0	0.0	62.0
	58.5	36.5	5.0		37.3[a]	1.1	61.6
	56.1	40.0	3.9				
	52.6	45.0	2.4	0.950	32.3	0.0	67.7
	47.3	52.7	0.0	0.960	26.1	0.0	73.9
				0.970	19.5	0.0	80.5
0.840	81.3	0.0	18.7	0.980	12.3	0.0	87.7
	77.6	5.0	17.4	0.990	5.0	0.0	95.0
	74.0	10.0	16.0				
	70.8	14.2	15.0				
	66.5	20.0	13.5				
	65.1[a]	22.3	12.6				
	35.7	64.3	0.0				

[a] Cloud point data.

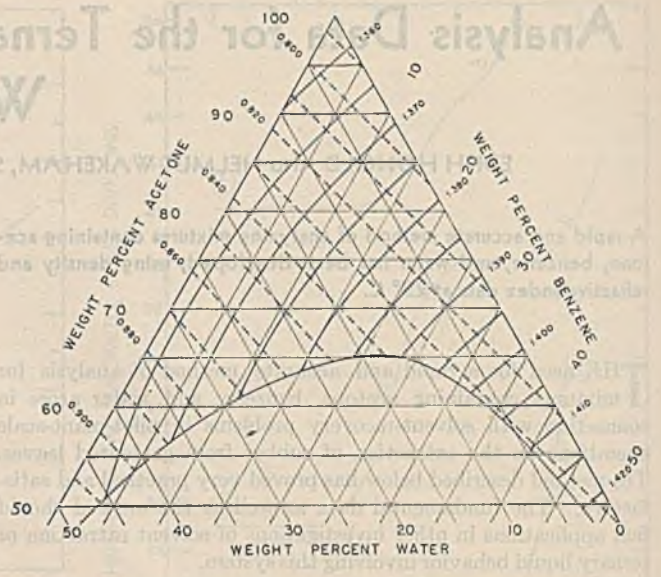

Figure 2. Analysis Diagram for Acetone-Benzene-Water Mixtures Containing More Than 50% Acetone

fractive indices and densities were determined for a number of solutions of known composition, so that lines of equal density and of equal refractive index could be constructed. These lines were plotted on a triangular chart in such a way that the composition of an unknown could be found by interpolation of its measured density and refractive index values.

Two series of mixtures were prepared, one starting with acetone and water in different proportions, the other with acetone and benzene; the third component was added stepwise until the cloud point was reached, measurements of refractive index and density being made after each addition. The data of the one series thus overlapped and served to check the data of the other over most of the region of homogeneous mixture. Table I includes experimental data for the ternary system at 25° C.

From graphical interpolation of these data it was possible to construct plots of the refractive index *vs.* acetone composition for various mixtures of constant water or constant benzene contents as shown in Figure 1. Using values obtained from this figure lines of common refractive index were constructed on the triangular chart for the ternary diagram. The density lines were determined in a similar manner. Figure 2 shows both sets of lines for ternary mixtures containing more than 50% acetone. Data used to construct the curves in Figure 2 and additional data covering the system for mixtures with less than 50 weight % acetone are given in Table II.

If the density and refractive index of a homogeneous unknown of acetone, benzene, and water are determined at 25° C., the composition in weight per cent of the sample may be found to the nearest 0.5% by interpolation of data in Figure 2 and Table II.

Cloud-point compositions at 25° C. were compared to data obtained by interpolation of values reported by Briggs and Comings (*4*) at 15° and 30° C. Good agreement between these two sets of data was observed over the entire solubility curve.

ACKNOWLEDGMENT

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Analysis of Cellulose Derivatives

Total Acyl in Cellulose Organic Esters by Saponification in Solution

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Most saponification methods for the determination of total acyl in cellulose organic acid esters involve heterogeneous conditions. A method has been developed in which the sample is saponified in solution. As a result, homogeneous saponification conditions exist which eliminate errors due to the condition of the sample, improve the accuracy, shorten the time of saponification, and give a better end point. The effects of time, temperature, and alkalinity were studied and the optimum conditions for each were established. The range of applicability of this method is discussed and compared with the Eberstadt and alcoholic alkali methods. The basic principle followed involves solution of the sample in a suitable solvent, followed by stepwise additions of alkali and water under conditions such that the ester remains in solution until saponification is practically complete. The regenerated or only slightly esterified cellulose finally precipitates in a soft finely divided form which does not interfere with completion of the reaction or the back-titration. The precision obtainable by this method has been evaluated and limits of uncertainty (maximum range within which nearly all carefully run values should fall) are ±0.1 to 0.2% acetyl, depending on the type of ester being analyzed. A measure of the accuracy of the method was obtained by analyzing samples for free hydroxyl and for acetyl or apparent acetyl (saponification value calculated as acetyl) and complete acyl in the case of cellulose mixed esters. The observed acetyl or apparent acetyl values were compared with those calculated by difference from observed free hydroxyl contents and molar ratios of the acyl groups in the case of cellulose mixed esters, assuming exactly 3 hydroxyls per glucose unit of cellulose. The agreement was well within experimental error.

THE determination of total acyl in cellulose organic esters by saponification in solution overcomes some of the difficulties encountered in the heterogeneous saponification methods such as the Eberstadt (or Knoevenagel) and the alcoholic alkali methods described in the first paper of this series (*5*). A solution method has been developed which eliminates the effect of the physical form of the sample, permits the use of a lower alkalinity and a shorter time of saponification, and gives a more satisfactory end point and a slight improvement in accuracy. However, the solvents must be varied to suit the composition and solubility of each type of ester, and the manipulation is somewhat involved and must be varied for each different type of ester. It is thus better adapted to routine analyses of familiar samples than to isolated analyses of unknown samples.

The basic principle followed involves solution of the sample in a suitable solvent, followed by stepwise additions of alkali and water under conditions such that the ester remains in solution until saponification is practically complete. When the regenerated or only slightly esterified cellulose finally comes out of solution it is in a soft finely divided form which does not interfere with the completion of the reaction or the back-titration. Conditions of temperature and alkali concentration are chosen to ensure a complete reaction and to avoid the formation of acids by the decomposition of cellulose.

At least three saponification methods are now available for the analysis of the organic esters of cellulose. The Eberstadt method is simple and best adapted to the acetone-soluble cellulose acetates and to hydrolyzed cellulose acetate propionates and acetate butyrates having low propionyl and butyryl contents, if these samples are in good physical form. It is also recommended for cellulose acetates having less than 15% acetyl. The alcoholic alkali method is simple and is widely applicable to cellulose esters, but usually is less accurate than the Eberstadt method. Both methods are inaccurate when the liquid medium used swells and softens the ester excessively but does not dissolve it. The saponification in solution method described in this paper may be used on solvent-soluble cellulose acetates, propionates, butyrates, acetate propionates, and acetate butyrates. It is particularly useful for esters not readily analyzable by the Eberstadt method and where good accuracy is desired.

[1] Now in service of U. S. Coast Guard.

There are several references in the literature to methods in which the cellulose ester is dissolved in a solvent, such as acetone or pyridine, and the alkali added dropwise. Partial saponification is thus carried out in solution, but no precautions are given for maintaining a solvent system and for holding the saponifying ester in solution as long as possible.

Barnett (*1*) described a procedure, applied only to acetone-soluble cellulose acetates, by which a 0.3-gram sample was dissolved in 30 ml. of acetone and was saponified with 50 ml. of 0.1 *N* sodium hydroxide for 24 hours at room temperature. After diluting with water, the excess alkali was back-titrated with 0.1 *N* acid using phenolphthalein indicator. It was necessary to run a blank on the reagents and on cellulose and a considerable correction was applied. Battegay and Penche (*2*) analyzed cellulose acetate by dissolving a 0.3 to 0.5-gram sample in 30 ml. of pyridine at 40° C. The solution was cooled to 25° C., and 50 ml. of 0.5 *N* alkali were added. After shaking for 0.5 hour the excess alkali was back-titrated. Murray, Staud, and Gray (*9*) developed a rapid acetyl method in which a 0.5-gram sample was dissolved in 20 ml. of pyridine and was saponified for 0.5 hour at about 50° C. with 20 ml. of 0.5 *N* alkali. This method is rapid and applicable to a certain range of cellulose acetates, but the temperature used is too high. Charriou and Valette (*3*) determined the acetyl in cellulose acetates by dissolving a 1.5-gram sample in 100 ml. of acetone and adding 50 ml. of 0.5 *N* sodium hydroxide dropwise with agitation. The flask was stoppered and agitated vigorously for 0.5 hour and the excess sodium hydroxide titrated with 0.5 *N* sulfuric acid using phenolphthalein indicator. A Du Pont specification (*4*) gives a method in which a 1.5-gram sample is dissolved in 100 ml. of acetone, 50 ml. of 0.5 *N* sodium hydroxide are added dropwise, and after 3 hours of agitation the excess is titrated with 0.5 *N* hydrochloric acid using phenolphthalein indicator.

In earlier experiments in this laboratory (*10*) various solvents were tried including acetone, acetone-water, and acetone-alcohol mixtures with aqueous alkali at various times and temperatures. Each of these methods is applicable to a narrow range of cellulose esters. In some cases a temperature of 40° to 55° C. was used, but this does not give satisfactory results. Side reactions occur which require exact control of the time to obtain accurate results. Because of these limitations and their specific character these methods have not been widely accepted.

PROCEDURE

The more common cellulose lower fatty acid esters have been divided into the following groups, based on their solubility differences, and variations made in the procedure to meet the requirements of each:

Group I. Solvent-soluble cellulose acetates having about 15 to 30% acetyl, which require water in the solvent mixture to effect solution of the sample. These solutions tolerate a considerable amount of water during saponification.

Group II. Cellulose acetates having 30 to 44.8% acetyl, most cellulose acetate propionates and acetate butyrates having less than 30% propionyl or butyryl, and similar esters whose solutions will tolerate some water.

Group III. Cellulose propionates, butyrates, acetate propionates, acetate butyrates, etc., having 30 to 45% propionyl or butyryl, which are very water-resistant, and the solutions of which tolerate little water.

Group IV. Cellulose propionates, butyrates, and mixed esters having more than 45% propionyl or butyryl whose solutions will tolerate practically no water.

The samples are dried about 2 hours at 100° to 110° C., cooled in a desiccator, and accurately weighed into 250-ml. glass-stoppered Erlenmeyer flasks. Each sample is dissolved in a solvent mixture which meets the following requirements: It must not react with the alkali, must tolerate the addition of considerable amounts of water, preferably should be a solvent for a wide range of cellulose esters, and the resulting system must be a solvent for the alkali. Blanks are run on each solvent combination used, and are carried through all steps of the manipulations

In the procedure variations for the four groups, solvent mixtures are specified which have been used successfully. Definite additions of water and alkali are also recommended. Other solvents meeting the requirements listed above and other amounts and orders of addition of water and alkali should yield satisfactory results if the additions are made at a rate consistent with the rate of saponification and change in solubility, so the steadily changing ester will remain in solution as long as possible. Enough alkali must be added at the start to produce appreciable diminution in acyl content, and before precipitation occurs enough water must be added to maintain a solution. More or perhaps all of the alkali can then be added, together with enough water to produce the desired dilution. If the manipulations are carried out properly, the partly esterified or regenerated cellulose separates slowly from solution and settles in a soft bulky form. If the precipitate is coarse or gummy the analysis is probably spoiled and should be repeated. In general, a total of 120 ml. of solvent and 30 ml. of alkali is added, which produces a dilution of the 0.5 *N* alkali to 0.1 *N*.

Group I. A 1-gram sample is dissolved in 50 ml. of the solvent mixture. In the case of cellulose acetates in the lower acetyl range of this group a 2:1:1 by volume mixture of water, pyridine, and acetone is recommended, while in the upper acetyl range of this group the same solvents in a ratio of 1:1:1 are suggested. During saponification 70 ml. of water (making a total of 120 ml. of added solvent) and 30 ml. of 0.5 *N* alkali are added. The distilled water is added to the solution with vigorous shaking until a temporary precipitate is observed at the point where the water first comes in contact with the solution or until the entire 70 ml. have been added. A 15-ml. portion of alkali is added, or alkali is added until a temporary precipitate forms. Then the remainder, if any, of the 70 ml. of water is added and the flask shaken until the solution becomes turbid. Finally alkali is added to give a total of 30 ml. The flasks are stoppered and allowed to stand at room temperature for 6 hours or more.

Group II. A 0.5-gram sample is dissolved in 50 ml. of a 1:1 by volume mixture of pyridine and acetone. A 20-ml. portion of distilled water and 10 ml. of 0.5 *N* alkali are added with swirling, which is continued until a slight turbidity appears. Then the remaining 50 ml. of distilled water and 20 ml. of alkali are added. Six hours or more at room temperature are allowed for saponification.

Group III. A 0.5-gram sample is dissolved in 100 ml. of a 1:1 by volume mixture of acetone and methyl alcohol. A 10-ml. portion of 0.5 *N* alkali is added slowly with swirling, until a definite turbidity develops. The remaining 20 ml. of alkali and 20 ml. of water are then added slowly. Six hours or preferably overnight at room temperature must be allowed for saponification.

Group IV. A 0.5-gram sample is dissolved in 100 ml. of a 1:1 by volume mixture of pyridine and methyl alcohol. A 30-ml. portion of 0.5 *N* methyl-alcoholic alkali is added slowly with swirling. A 20-ml. portion of water is added slowly and the flask swirled until the solution becomes turbid, so that the regenerated cellulose will settle in a finely divided form. The flasks are allowed to stand overnight at room temperature for completion of saponification.

In all cases, the excess of alkali is back-titrated using standard 0.5 *N* hydrochloric acid and phenolphthalein indicator.

The result may be calculated as follows:

$$\frac{\left[\left(\begin{matrix}\text{Ml. of HCl for}\\ \text{blank}\end{matrix}\right) - \left(\begin{matrix}\text{ml. of HCl for}\\ \text{sample}\end{matrix}\right)\right] \times \text{HCl normality} \times 4.3}{\text{sample weight}} = \% \text{ apparent acetyl}$$

This result is given in terms of per cent acetyl in the case of cellulose acetate and as apparent acetyl (saponification value calculated as acetyl) in the case of other esters.

STUDY OF THE METHOD

The effects of some of the variables of the method were studied using four samples, one representative of each of the four groups described. The analyses of these samples are given in Tables I,

Table I. Effect of Time and Temperature of Saponification

Sample	Time	Temperature of Saponification: 0° C.	20–35°C.	40° C.	50–55° C.	
		Acetyl or apparent acetyl				
	Hours	%	%	%	%	%
Low-acetyl cellulose acetate 16.8% acetyl	1	16.0	16.4	...	16.3	...
		16.0	16.4	...	16.4	...
	3	16.1	16.5	...	16.8	...
		16.2	16.5	...	16.9	...
	6	16.2	16.8	...	16.9	...
		16.2	16.8	...	17.0	...
	16	16.2	16.8	16.8	17.6	18.5[a]
		16.2	16.8	16.8	17.8	18.7[a]
	24	16.3	16.8	...	16.3	17.8[a]
		16.4	16.8	...	16.5	17.9[a]
	48	16.5	16.8	17.0	17.3	19.7[a]
		16.5	16.8	17.1	17.4	19.8[a]
Acetone-soluble cellulose acetate 40.4% acetyl	1	39.7	40.0	...	39.8	...
		39.8	40.0	...	39.9	...
	3	40.0	40.1	...	40.3	...
		40.0	40.0	...	40.3	...
	6	39.9	40.4	...	40.7	...
		40.0	40.4	...	40.7	...
	16	40.0	40.4	40.4	39.2	43.3[a]
		40.0	40.5	40.5	39.3	43.4[a]
	24	40.1	40.4	...	36.0	43.3[a]
		40.1	40.5	...	36.1	43.5[a]
	48	40.2	40.4	40.7	37.2	45.8[a]
		40.3	40.4	40.7	37.3	45.9[a]
Cellulose acetate butyrate I 35.0% apparent acetyl 12.6% acetyl 37.0% butyryl	1	22.0	32.0	...	34.5	...
		22.2	32.1	...	34.5	...
	3	22.2	34.7	...	35.3	...
		22.3	34.7	...	35.4	...
	6	28.1	35.0	...	35.8	...
		28.2	35.0	...	35.9	...
	16	33.0	34.9	35.4	37.6	39.2[a]
		33.1	35.0	35.5	37.6	39.2[a]
	24	34.5	35.0	...	34.0	39.7[a]
		34.5	35.0	...	34.1	39.7[a]
	48	35.0	35.0	36.1	36.0	39.3[a]
		35.0	35.0	36.3	36.2	39.5[a]
Cellulose acetate butyrate II 34.4% apparent acetyl 0.7% acetyl 55.6% butyryl	1	4.4	18.5	...	31.9	...
		4.5	18.7	...	32.0	...
	3	4.6	25.7	...	35.1	
		4.6	25.8	...	35.1	...
	6	10.5	33.5	...	35.4	...
		10.7	33.8	...	35.5	...
	16	17.8	34.3	34.6	38.5	...
		17.9	34.4	34.6	38.7	...
	24	21.3	34.4	...	39.3	...
		21.3	34.4	...	39.4	...
	48	30.6	34.4	36.1	42.7	...
		30.8	34.4	36.0	42.9	...

[a] Calculations made using titration figure for unheated blank.

II, and IV; two are cellulose acetates and two cellulose acetate butyrates, the last of which is essentially a butyrate with only a very little acetyl.

In these experiments good commercial grades of methyl alcohol and acetone were used. A good commercial grade of pyridine was treated with flake sodium hydroxide and fractionated. The water azeotrope was removed and the fraction taken which boiled at 115.5° to 116.0° C.

Effect of Time and Temperature of Saponification. The effects of the time and temperature of saponification were studied as shown in Table I. The above procedure was followed with these two variations.

These data show that better results are obtained at the room temperature range (20° to 35° C.) than at 0°, 40°, or 50° to 55° C. In the case of cellulose acetates and cellulose acetate propionates or butyrates of low propionyl or butyryl content, acceptable results may be obtained after 6 hours at room temperature which do not change even after 48 hours. About 16 hours at this temperature must be allowed for cellulose acetate propionates or acetate butyrates having a high propionyl or butyryl content.

At 0° C. the reaction is very slow and in most cases is not complete even after 48 hours. Erratic results are obtained at 50° to 55° C. Changes in the titer of the blank, as a result of heating, indicate a side reaction. There is a considerable reaction after 16 hours, and as much as 2 ml. of alkali was consumed in some of the blanks. Results are too high when calculated using the titration figure of an unheated blank. There is also some side reaction in 48 hours at 40° C. After 16 hours there has been a reaction in some cases, and results were acceptable in other cases. Because of this erratic behavior, saponification temperatures should be kept below 40° C.

Effect of Time of Saponification and Alkali Concentration. The effects of the time of saponification and the alkali concentration were studied as shown in Table II. The nominal concentration is that produced by dilution of the added alkali by the solvents added. The actual concentration is probably below this value at all times.

Table II. Effect of Time and Alkali Concentration

Sample	Time	Alkali Concentration: 0.05 N	0.1 N	0.2 N
	Hours	Acetyl or Apparent Acetyl %	%	%
Low-acetyl cellulose acetate, 16.8% acetyl	1	15.9	16.4	16.8
		15.9	16.4	16.8
	3	16.1	16.4	16.8
		16.2	16.5	16.8
	6	16.4	16.8	16.8
		16.4	16.8	16.8
	16	16.3	16.8	16.8
		16.4	16.8	16.8
	24	16.4	16.8	16.8
		16.4	16.8	16.8
	48	16.5	16.8	16.8
		16.5	16.8	16.8
Acetone-soluble cellulose acetate, 40.4% acetyl	1	39.6	40.0	40.4
		39.7	40.0	40.4
	3	39.9	40.0	40.4
		40.0	40.1	40.4
	6	40.2	40.4	40.4
		40.2	40.4	40.4
	16	40.2	40.4	40.4
		40.2	40.5	40.4
	24	40.2	40.4	40.4
		40.3	40.5	40.4
	48	40.3	40.4	40.4
		40.3	40.4	40.4
Cellulose acetate butyrate I 35.0% apparent acetyl 12.6% acetyl 37.0% butyryl	1	27.0	32.0	34.7
		27.2	32.1	34.8
	3	30.4	34.7	35.0
		30.6	34.7	35.0
	6	34.1	35.0	35.0
		34.1	35.0	34.9
	16	34.7	34.9	35.3
		34.8	35.0	35.3
	24	35.0	35.0	35.3
		35.0	35.0	35.4
	48	35.0	35.0	35.4
		35.1	35.0	35.5
Cellulose acetate butyrate II 34.4% apparent acetyl 0.7% acetyl 55.6% butyryl	1	8.2	18.5	26.6
		8.4	18.7	26.8
	3	13.1	25.7	31.9
		13.3	25.8	32.0
	6	22.1	33.5	34.3
		22.3	33.8	34.4
	16	24.8	34.3	31.0 34.1
		24.9	34.4	31.2 34.2
	24	31.7	34.4	30.7 34.0
		31.9	34.4	30.5 34.2
	48	34.1	34.4	32.6 32.8
		34.2	34.4	32.4 32.5

Table III. Precision Studies

Test No.	Low-acetyl cellulose acetate	Acetone-soluble cellulose acetate	Cellulose acetate butyrate I	Cellulose acetate butyrate II
	Per Cent Acetyl or Apparent Acetyl			
1	16.80	40.40	34.96	34.42
2	16.80	40.40	34.99	34.36
3	16.79	40.42	34.90	34.50
4	16.79	40.42	34.96	34.42
5	16.76	40.44	34.98	34.48
6	16.77	40.41	34.94	34.37
7	16.87	40.40	35.05	34.35
8	16.86	40.49	34.98	34.40
9	16.86	40.40	35.01	34.51
10	16.87	40.38	35.07	34.30
Numerical average = $\bar{X}_1$				
$\bar{X}_1$ =	16.81	40.41	34.98	34.41
Sum of squares of deviations from average = Σd^2				
Σd^2 =	0.0173	0.0088	0.023	0.042
Standard deviation = $\sigma_{10} = \sqrt{\frac{\Sigma d^2}{10}}$				
σ_{10} =	0.042	0.030	0.048	0.065
Limit of uncertainty of average = $LU_{av.} = \bar{X}_1 \pm \frac{3\sigma_{10}}{\sqrt{10}}$				
$LU_{av.}$ =	16.77 to 16.85	40.38 to 40.44	34.93 to 35.03	34.35 to 34.47
Limit of uncertainty of individual result = $LU_1 = \pm \frac{3\sigma_{10}}{0.923}$				
LU_1 =	±0.13	±0.10	±0.16	±0.21

When 0.5 N alkali, diluted by the solvents added to produce a concentration of 0.1 N, is used. In this case the reaction is complete in from 6 to 16 hours. The results obtained during this time are not changed even after 48 hours.

When 0.25 N alkali, diluted by the solvents to 0.05 N, is used the reaction is very slow, and in most cases is not complete after 48 hours. In the case of 1.0 N alkali diluted to 0.2 N, acceptable results are obtained in one hour on cellulose acetates, and this value remains constant for 48 hours. The reaction is complete in the case of cellulose acetate butyrate I after 3 hours, but the results are too high after about 16 hours. Results on cellulose acetate butyrate II are very erratic. When 2.5 N alkali is used to give a nominal concentration of 0.5 N after dilution, suitable additions of alkali cannot be made. The alkalinity is so high that the sample precipitates immediately, giving unsatisfactory results.

PRECISION STUDIES

The precision attainable by this procedure was evaluated by the method described by Moran (*8*). The same four samples used in the above studies were analyzed ten times by the same operator under the most favorable conditions, and the data obtained are shown in Table III. The average of the ten values ($\bar{X}_1$) was calculated for each sample, individual variations from the average were squared, and the sums of these squares (Σd^2) then used in the following calculations:

Standard deviation (σ) is the most accurate measure of dispersion about an arithmetical mean, and mathematically is the square root of the average of the squares of the individual deviations:

$$\sigma_{10} = \sqrt{\frac{\Sigma d^2}{10}}$$

The limit of uncertainty of the average ($LU_{av.}$) is the narrowest range within which any one result can be guaranteed:

$$LU_{av.} = \bar{X}_1 \pm \frac{3\sigma_{10}}{\sqrt{10}}$$

The limit of uncertainty of the method under the best possible conditions (LU_1) is the precision range within which a high proportion of results (997 out of 1000) should fall when good samples are analyzed by a skilled technician working under closely controlled conditions:

Table IV. Test of Accuracy

Sample	Low-Acetyl Cellulose Acetate	Acetone-Soluble Cellulose Acetate	Cellulose Triacetate	Cellulose Acetate Butyrate I		Cellulose Acetate Butyrate II
Acyl content						
% acetyl	16.8	40.4	44.4		12.6	0.7
% butyryl	...	...	...		37.0	55.6
Per cent free hydroxyl observed	18.16[a]	3.03	0.15		2.20	0.15
Hydroxyl groups per glucose unit						
Esterified	0.757	2.513	2.955	Acetyl	0.927	0.059
				Butyryl	1.650	2.853
Free, observed	2.070	0.472	0.025		0.410	0.032
Observed, total	2.827	2.985	2.980		2.987	2.944
Apparent acetyl						
Observed	16.8	40.4	44.4		35.0	34.4
Calculated from hydroxyl and molar ratio	18.9[b]	40.5	44.6		35.1	34.6

[a] Value low. Good accuracy not attained on samples of such high hydroxyl content by method employed.
[b] Calculated acetyl value high because based on inaccurate hydroxyl value.

$$LU_1 = \pm \frac{3\sigma_{10}}{0.923}$$

When this test was applied to a low-acetyl cellulose acetate, a limit of uncertainty of ±0.13% acetyl was found. In the case of acetone-soluble cellulose acetate this range is ±0.10% acetyl, as compared to ±0.16% acetyl when this test was applied to the same sample analyzed by the Eberstadt method. The solution method is thus a little better than the Eberstadt method. The precision attainable on the cellulose acetate butyrate I is within ±0.16% apparent acetyl. The corresponding range for cellulose acetate butyrate II is ±0.21% apparent acetyl. Both the precision and accuracy have been found to be a little better than obtained by the alcoholic alkali method.

The limit of uncertainty under routine conditions (LU_2) is evaluated from similar data collected over a period of a year, and will probably be found to be somewhat larger—i.e., poorer precision.

TEST OF ACCURACY

It is difficult to establish the accuracy of an analytical method for cellulose derivatives because it is practically impossible to prepare a sample of known composition. A practical measure of accuracy was obtained by analyzing the four samples used in the above tests and a sample of cellulose triacetate for their free hydroxyl contents by the acetic anhydride and pyridine method (*6*), and the cellulose acetate butyrates for their molar ratios of acetyl and butyryl and their acetyl and butyryl contents in weight per cent (*7*). Acetyl values for the cellulose acetates were calculated from the observed free hydroxyl contents assuming 3 replaceable hydroxyls per glucose unit of cellulose. Apparent acetyl values for the acetate butyrates were calculated from the observed free hydroxyl contents and molar ratios of acetyl and butyryl, making the same assumption. When acetyl or apparent acetyl values, calculated in this way, agree with the observed values, a measure is obtained of the combined accuracies of all the methods involved.

Table IV shows the analytical data obtained on five samples, the number of esterified and free hydroxyls per glucose unit, and their totals. The accuracy attained in these analyses is shown by the agreement between these totals and 3 hydroxyls per glucose unit and by the agreement between the observed and calculated acetyl values.

In the case of the low-acetyl cellulose acetate the acetyl calculated does not agree well with the observed acetyl. The free hydroxyl value, however, is probably low because high accuracy of the free hydroxyl determination is not to be expected for samples having more than about 1.5 free hydroxyls. The procedure (*6*) does not provide drastic enough conditions to produce complete acetylation of cellulose esters having such high free hydroxyl contents. The accuracy of the analysis of this sample is therefore not established. In the other cases the observed acetyl or apparent acetyl and the calculated values agree practically within the precision limits attainable by these methods, and the accuracy probably lies within these same limits.

SUMMARY

A method for the analysis of total acyl in cellulose esters of organic acids is presented in which the sample is saponified in solution. This method overcomes some of the difficulties encountered in the commonly used heterogeneous saponification methods in that it is independent of the condition of the samples, can be run in a shorter elapsed time, and is a little more accurate. It does, however, involve somewhat more complicated manipulation.

This method has been applied to solvent-soluble cellulose acetates (containing from 15 to 44.4% acetyl), cellulose acetate propionates and acetate butyrates, and cellulose propionates and butyrates up to and including tributyrates. These cellulose esters have been divided into four groups based on composition and solubility. Variations are recommended for each group such that the sample is dissolved in a suitable solvent, and alkali and water are added alternately to maintain a solvent system until saponification is nearly complete. The regenerated cellulose is then in a finely divided form and does not interfere with the completion of the reaction or the back-titration.

The effects of time and temperature of saponification and time and alkali concentration were studied and the optimum conditions established. Usually 0.5-gram samples are saponified with 30 ml. of 0.5 *N* alkali with a total of 120 ml. of added solvent, allowing 6 to 16 hours for saponification at room temperature (20° to 35° C.). The reaction is complete in 6 hours or less in enough cases, particularly the cellulose acetates, so that a fairly accurate value could be obtained in one working day, if required.

Precision studies were made on samples typical of each of these groups, and it was found that precision limits of from ±0.10% acetyl for cellulose acetates to ±0.20% apparent acetyl for cellulose acetate butyrates may be attained when working under carefully controlled conditions. These ranges are the limits of uncertainty within which practically all carefully made determinations should fall; however, duplicate values usually agree closer than this, as shown by data reported.

A measure of the accuracy of the methods has been obtained by analyzing samples completely for acetyl, acyl groups in the case of cellulose mixed esters, and free hydroxyl. The observed acetyl or apparent acetyl results were compared with values calculated by difference from observed free hydroxyl contents and molar ratios of the acyl groups present in mixed esters, assuming exactly 3 hydroxyls per glucose unit of cellulose.

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Determination of Vanillin and Coumarin in Flavoring Extracts

Ultraviolet Absorption Method

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A method for the analysis of genuine and imitation vanilla extracts involves a dilution, precipitation with lead acetate, extraction with ethyl ether, and dilution of the ether extract to a large volume with water. The quantity of total vanillin and coumarin in the solution is estimated from the absorption at 2875 Å. and the quantity of each from a simple equation involving values at 2313 Å. The method is rapid and convenient and the results are of good reproducibility. Tests with mixtures of known composition indicate good accuracy. A slightly modified and simpler procedure not employing ether extraction is employed for pure vanilla extracts.

ONE of the early methods for determination of vanillin and coumarin in flavoring extracts was that proposed by Hess and Prescott (*7*), a modification of which is in general use today (*11*). This method is based on the difference in the chemical structure of vanillin and coumarin. Vanillin is separated from coumarin through the addition of aqueous ammonia to an ether extract of the vanilla extract. The vanillin forms a water-soluble aldehyde-ammonia while the coumarin remains unaffected in the ether layer. Upon acidification and extraction of the aqueous layer with ether, the vanillin is recovered in the ether layer. By evaporating the ether solutions, the amounts of vanillin and coumarin can be determined. However, the residues are usually contaminated and need further purification, thus reducing the convenience and accuracy of the method.

Several colorimetric methods for the determination of vanillin (*2, 3, 6, 10*) and coumarin (*4, 8, 9*) involve chemical reactions in which substances showing absorption in the visible spectral range are produced. However, both vanillin and coumarin show pronounced absorption in the ultraviolet region of the spectrum. Vanillin exhibits a maximum at a wave length where coumarin shows only slight absorption. It was the purpose of this work to discover if the general principle in spectrophotometry used by Englis and Skoog (*5*) for determination of sulfanilamide and sulfathiazole in mixtures could be similarly applied to determination of vanillin and coumarin in commercial flavoring extracts.

EQUIPMENT

Some absorption data were obtained by means of a Bausch & Lomb medium quartz spectrograph supplemented with a Leeds & Northrup recording microphotometer. Other measurements were obtained with a Beckman photoelectric spectrophotometer.

The spectrograph was operated with a slit width of 0.07 mm. A Wood's type of hydrogen discharge tube served as a source of illumination. The hydrogen tube was placed with the exit window at a distance of 8 cm. from the slit. A cell of 1-cm. length with quartz windows was used to hold the liquids during their examination. Separate exposures of the solvents and the samples were taken for a period of 1.5 minutes each. The spectra were recorded on Eastman Polychrome plates, each of which was calibrated by making a series of separate exposures in which the time interval was varied in a regular manner: 2, 4, 8, 16, 32, and 64 seconds. The plates were developed for 5.5 minutes in Eastman x-ray developer at 18° C., then fixed, washed, and dried. After drying, the densities of the spectrograms at selected wavelength intervals were determined with a Leeds & Northrup recording microphotometer. A family of plate calibration curves for the selected wave lengths was then constructed, by reference to the appropriate curve the relative intensity values for the pure solvent and sample were found, and from these the extinction value for the solution was calculated.

The Beckman instrument was operated with a slit width of 1.0 to 1.1 mm. in the range 2250 to 2500 Å. and of 0.5 to 0.6 mm. in the range 2500 to 3000 Å. The extinction values were read directly.

ABSORPTION CHARACTERISTICS

In establishing the absorption curves of vanillin and coumarin, solutions were prepared in concentrations of 10 mg. of each per liter in water containing about 10% alcohol. The E values are expressed for a cell length of 1 cm. The curves are shown in Figure 1; the greatest difference in absorption occurs at 2313 Å. At wave length 2875 Å. the extinction value for an equal weight of either constituent is the same. Thus the total concentrations for a mixture of the constituents can be found by determining the extinction at 2875 Å. and the amount of each individual constituent can be obtained by use of a simple equation rom the extinction value at 2313 Å. The equation to be us d is:

$$xE_c + (t - x)E_v = E_m$$

In the equation

x = concentration of coumarin in mg. per liter
$t - x$ = concentration of vanillin in mg. per liter
t = total concentration of both constituents as found by preliminary observation of E value at 2875 Å.
E_c = extinction value for 1 mg. of coumarin per liter at 2313 Å.
E_v = extinction value for 1 mg. of vanillin per liter at 2313 Å.
E_m = extinction value observed for mixture at 2313 Å.

Subsequent testing indicated that the E values at the two wave lengths selected showed no significant change when water containing an amount of ether equivalent to that present under the conditions of analysis of later experiments was employed as a solvent.

As indicated in Figure 2, the solutions and the mixture follow the Beer-Lambert law.

ANALYSIS OF PURE VANILLA EXTRACTS

The first portion of the experimental work was concerned with examination of samples containing only one constituent: pure vanilla extracts containing 37% alcohol. The first of these was analyzed by the official Hess-Prescott method. Percentages

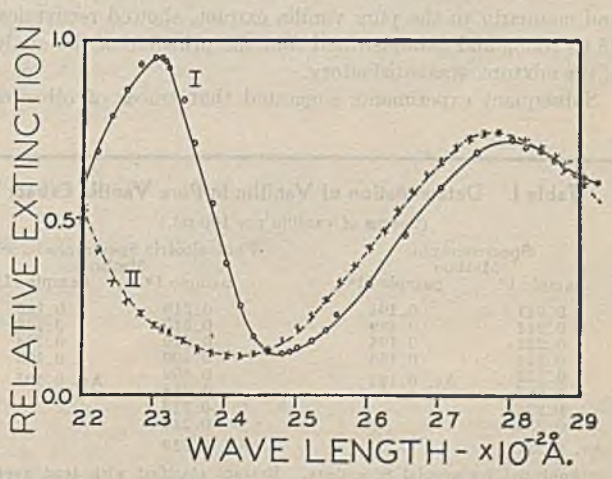

Figure 1. Absorption Curves
I. 10 mg. of vanillin per liter
II. 10 mg. of coumarin per liter

of unpurified vanillin indicated for duplicate samples were 0.21 and 0.20%.

The object of the first experiment was to learn if vanillin could be determined on a very small sample without removing the alcohol, by precipitating the resins with lead, diluting to a larger volume, filtering, and determining the absorption of the filtrate.

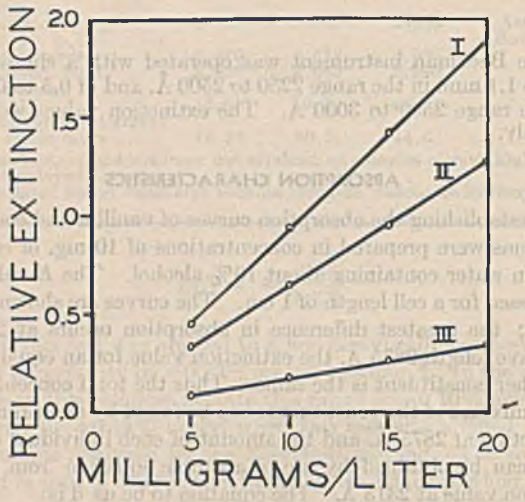

Figure 2. Absorption Values

I. Vanillin at 2313 Å.
II. Vanillin and coumarin at 2875 Å.
III. Coumarin at 2313 Å.

SPECIAL PROCEDURE FOR GENUINE EXTRACTS. NO ETHER EXTRACTION. Five milliliters of the pure vanilla extract were measured into a 1-liter volumetric flask, 10 ml. of lead acetate (80 grams per liter) were added, and the mixture was allowed to stand for 30 minutes. At the end of this period the mixture was diluted to volume with distilled water and filtered, and the filtrate examined with the spectrophotometer. In subsequent experiments the excess lead acetate showed some absorption in the selected range. To remove the lead 20 ml. of the filtrate were treated with approximately 0.5 gram of solid sodium dihydrogen phosphate monohydrate and filtered before the absorption was determined. From the observed E values at wave length 2313 Å. the quantity of vanillin in 100 ml. of extract was calculated by reference to the E values for solutions of known concentration at this wave length. The results of the tests are given in column one of Table I.

An experiment was also carried out to determine if the method could give complete recovery of vanillin and satisfactory results if coumarin were present also.

The analyses, after addition of known amounts of pure vanillin and coumarin to the pure vanilla extract, showed recoveries of 98 to 100% and demonstrated that the principle of the analysis of the mixtures was satisfactory.

Subsequent experiments suggested that traces of other constituents may have increased the absorption at the selected wave lengths and the analysis of sample I was repeated later by an alternate procedure involving an extraction with ether.

GENERAL PROCEDURE FOR VANILLA EXTRACTS. A 20-ml. portion of the genuine extract was measured into a 50-ml. volumetric flask and diluted to volume with distilled water. The mixture was then transferred to a 50-ml. glass-stoppered Erlenmeyer flask, 1 gram of crystalline lead acetate was added, and the mixture was shaken thoroughly and allowed to stand for 1 hour. After filtration, a 10-ml. portion of the filtrate was placed in a small separatory funnel, 10 ml. of ethyl ether (previously washed with water) were added, and the mixture was shaken. The aqueous layer was carefully withdrawn into another separatory funnel and again treated with ether. The procedure was carried out four times using successively 10-, 8-, 5-, and 5-ml. portions of the ether. After each separation the ether portion was washed into a 1000-ml. volumetric flask by means of a stream of distilled water and the funnel completely washed out. When all the ether extractions had been transferred to the volumetric flask, the mixture was diluted to volume and the ultraviolet absorption of the solution was determined. At this dilution ether is completely soluble and the solvent mixture has essentially the same absorption as water. However, a similar solution of pure water and ether is recommended for establishing the I_0 value of the solvent. The ultraviolet absorption was determined at 2313 Å. as before (Table I).

The values obtained for sample I by the procedure employing the Beckman instrument are slightly lower and of somewhat better reproducibility than those obtained by the first procedure with the spectrograph. Repeating the second procedure with a new sample of similar nature and determining absorption with both types of instruments proved satisfactory, although perfect agreement was not obtained.

Table I. Determination of Vanillin in Pure Vanilla Extract

(Grams of vanillin per 100 ml.)

Spectrographic Method		Photoelectric Spectrophotometric Method	
Sample I[a]	Sample II[b]	Sample I[b]	Sample II[b]
0.211	0.191	0.210	0.195
0.211	0.189	0.210	0.198
0.223	0.195	0.210	0.194
0.223	0.188	0.209	0.193
0.223	Av. 0.191	0.209	Av. 0.195
0.223		0.218	
0.224		0.218	
0.236		0.214	
Av. 0.227		Av. 0.212	

[a] Analyzed by special procedure. Extract clarified with lead acetate. No ether extraction used.
[b] Analyzed by general procedure. Extract clarified with lead acetate. Filtrate extracted with ethyl ether.

Table II. Analysis of Imitation Vanilla Extracts[a]

(Caramel, 0.200 gram of vanillin, and 0.200 gram of coumarin per 100 ml. Ether extraction used to remove vanillin and coumarin)

Vanillin	Coumarin	Total
	Gram per 100 ml.	
Spectrographic Method		
0.210	0.188	0.398
0.192	0.204	0.396
0.198	0.198	0.396
0.198	0.198	0.396
0.198	0.198	0.396
0.198	0.198	0.396
0.188	0.228	0.416
0.198	0.206	0.404
0.198	0.206	0.396
0.198	0.198	0.396
Photoelectric Spectrophotometric Method		
0.200	0.200	0.400
0.200	0.200	0.400

[a] Total vanillin and coumarin found by ultraviolet absorption at 2875 Å. and proportion of each by absorption at 2313 Å.

The results, after the extraction procedure, agree with the quantity indicated by the Hess-Prescott method without any purification of the residue. Perhaps there is a slight loss of vanillin in the Hess-Prescott method during removal of the alcohol and in the first drying of the sample, after evaporation of the ether. This loss may be compensated by the small amount of impurity, so that the values indicated without purification may be nearer correct.

ANALYSES OF IMITATION VANILLA EXTRACTS

An attempt was first made to find a clarifying agent which would remove the color but neither of the primary constituents sought. To simulate the color material in imitation vanilla extracts, samples of caramel were prepared by heating glucose (*1*). These were added in appropriate quantities to solutions of vanillin and coumarin, and decolorization was attempted. None of the agents tried was found satisfactory; impurities absorbing in the ultraviolet still remained or some of the constituents sought were removed. It was imperative to use an ether extraction for the separation of the vanillin and coumarin from other compo-

Table III. Analysis of Commercial Imitation Vanilla Extracts

(Ether extraction used to remove vanillin and coumarin)

Sample I			Sample II		
Vanillin	Coumarin	Total	Vanillin	Coumarin	Total
Gram per 100 ml.			*Gram per 100 ml.*		
Spectrographic Method			Photoelectric Spectrophotometric Method		
0.098	0.138	0.236	0.117	0.110	0.227
0.094	0.146	0.240	0.117	0.110	0.227
0.092	0.148	0.240	0.117	0.111	0.228
0.094	0.142	0.236	0.117	0.112	0.229
0.092	0.144	0.236	0.117	0.110	0.227
0.090	0.146	0.236	...	...	...
0.094	0.136	0.230	...	...	...
0.094	0.144	0.238	...	...	...
Hess-Prescott Gravimetric Method			Spectrographic Method		
0.098	0.14	0.231	0.113	0.117	0.230
0.094	0.114	0.208	0.117	0.116	0.233

Table IV. Analysis of Mixture of Genuine and Imitation Vanilla Extract by Ultraviolet Absorption Method

Sample I			Sample II		
Vanillin	Coumarin	Total	Vanillin	Coumarin	Total
Gram per 100 ml.			*Gram per 100 ml.*		
0.168	0.053	0.221	0.153	0.055	0.208
0.168	0.053	0.221	0.155	0.053	0.208
0.163	0.055	0.218	0.153	0.055	0.208
0.163	0.056	0.218	0.158	0.053	0.209
0.163	0.055	0.218	Av. 0.155	0.054	0.208
0.163	0.055	0.218			
Av. 0.165	0.054	0.219			
Calculated Quantities Present on Basis of Previous Analysis of Component Extracts					
0.165	0.055	0.220	0.156	0.055	0.211

nents of the imitation extracts before the spectrophotometric analysis could be carried out.

The laboratory samples of imitation vanilla extract were prepared by dissolving 0.2000 gram each of vanillin and coumarin in 10 ml. of alcohol in a 100-ml. flask, adding enough caramel to give a proper color value, and diluting to volume.

Since no resins were present, no lead clarification was carried out. A 5-ml. portion of the solution was extracted with ethyl ether, following the general procedure previously described, and the spectrophotometric evaluation made at 2313 and 2875 Å. The quantities of vanillin and coumarin were calculated (Table II).

The results, given in Table II, show very good agreement for total vanillin and coumarin with the 0.400-gram total known to be present. Reproducibility of results is slightly less satisfactory for the individual constituents, but still very good.

The next experiment was examination of a commercial imitation extract represented as containing vanillin, coumarin, sugar, artificial color, and 2% alcohol, by the method applied to the laboratory sample (Table III). Reproducibility of results is very good for the total of the flavoring constituents and only slightly less satisfactory for the individual materials.

Duplicate analyses by the Hess-Prescott method were in good agreement with the ultraviolet absorption method.

BLENDS OF GENUINE AND IMITATION VANILLA EXTRACTS

It is very desirable that any method proposed for vanilla extracts be applicable to a genuine or imitation product or a blend of the two. Accordingly, the general procedure for analysis of pure vanilla extract was applied to a blend of genuine samples and commercial imitation samples already analyzed. The two were mixed in equal proportion by volume and a 20-ml. portion of the mixture was analyzed (Table IV).

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Colorimetric Determination of Chromium in Steel

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Chromium is determined in steel by a method based on the fact that ferric perchlorate, which is itself colorless, intensifies the color of the dichromate ion. The method is not subject to interference by iron or usual alloying constituents.

IN PRACTICALLY all the colorimetric methods that have been employed, chromium in steel is determined after it has been separated from iron. The determination is then made by measuring the intensity of the chromate or dichromate color or the color produced with dichromate and a suitable organic reagent.

When large amounts of steel are taken for analysis, iron is first separated from chromium by extraction with ether. Small amounts of iron are precipitated and chromium is simultaneously oxidized and dissolved by the use of an alkaline peroxide solution. Yoe (*2*) describes the use of disodium-1,8-dihydroxynaphthalene-3,6-disulfonate (Koenig's reagent), diphenylcarbazide, and diphenylsemicarbazide for the colorimetric analysis of chromium. These reagents are very sensitive to small amounts of chromium but are subject to interference by iron and other alloying constituents that may be present in certain steels, thus making chemical separations necessary. However, Mal'tsev and Temirenko (*1*), using diphenylcarbazide, take a small sample weight and determine up to 0.1% chromium in steel without previous separation. Organic reagents have been employed in cases where the chromium content of steel is very low, as the color produced by small amounts of chromate or dichromate ion alone is too weak to allow accurate results.

This paper describes a method for the colorimetric determination of chromium in steel, which is not subject to interference by iron or alloying constituents usually present. The method is suitable for steel containing between a few thousandths and 1% chromium and is rapid, as chemical separations are not used. The basis of the method lies in the fact that ferric perchlorate, which is itself colorless, intensifies the color of the dichromate ion.

EXPERIMENTAL

Solutions were prepared containing known amounts of chromium and iron, using a standard dichromate solution and National Bureau of Standards sample 22b. These samples were dissolved and boiled with perchloric acid to oxidize chromium. After being cooled and diluted to a definite volume they were transferred to an absorption tube and a colorimeter reading was taken. The solutions were then reduced with a small crystal

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Table I. Effect of Iron on Intensity of Dichromate Color

	1.000 Gram of Iron			0.500 Gram of Iron			No Iron		
Chromium, Mg.	Oxidized solution	Reduced solution	Difference	Oxidized solution	Reduced solution	Difference	Oxidized solution	Reduced solution	Difference
0.070	13	0	13	...	..	...	...	..	...
0.240	42	2	40	...	..	...	...	..	...
0.570	91	3	88	...	..	...	...	..	...
0.870	132	4	128	...	..	...	...	..	...
0.950	150	5	145	78	1	77	37	0	37
1.15	170	6	164	...	..	...	...	..	...
1.85	272	7	265	152	4	148	72	0	72
2.30	332	10	322	188	5	183	90	1	89
2.75	382	11	371	222	7	215	109	2	107
3.65	464	13	451	289	8	281	145	3	142
4.55	...	..	...	354	8	346	182	5	177
5.55	...	..	...	427	11	416	222	7	215

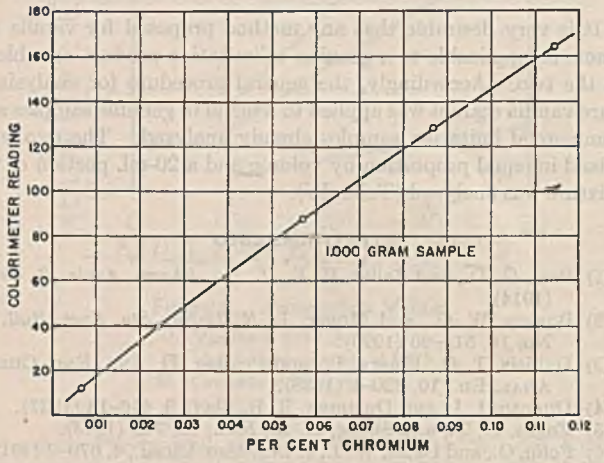

Figure 1

of ferrous ammonium sulfate and a second reading was taken. The difference between these two readings represents the colorimeter reading due to the dichromate.

Results in Table I show that ferric perchlorate itself is colorless but increases the color intensity of the dichromate.

It was found from subsequent experiments that a 1-gram sample was most suitable when the chromium content of the steel was between a few thousandths and 0.1%. When the percentage of chromium was between 0.1 and 1%, a 0.5-gram sample was taken for analysis.

Accordingly, solutions were prepared using a steel of known chromium content and a standard solution of potassium dichromate. Readings were taken on these solutions after carrying them through the procedure described below. Graphs (Figures 1 and 2) were drawn from the results obtained. In each case the line is a slight curve.

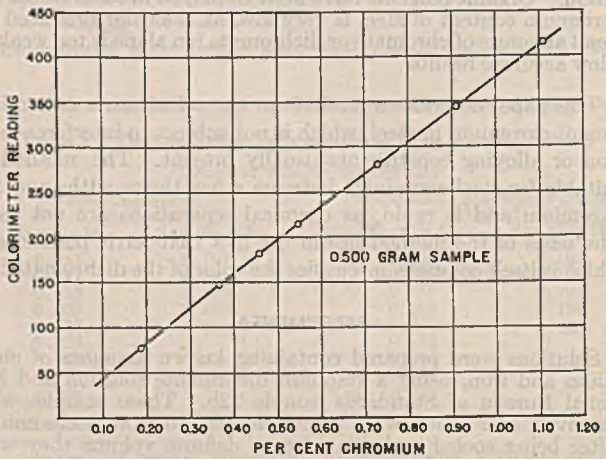

Figure 2

In order to determine the accuracy of the method, various samples of Bureau of Standard steels were analyzed. Artificial standards were also prepared by adding nickel, copper, cobalt, molybdenum, aluminum, titanium, and vanadium to weighed mixtures of Bureau of Standard steels and analyzing the resulting mixtures (Table II).

PROCEDURE

Preparation of Solution. Transfer 1.000 gram of sample, for steel containing up to 0.1% chromium, to a 125-ml. Phillips beaker and dissolve in 10 ml. of dilute nitric acid (1 to 1) and 20 ml. of perchloric acid (70 to 72%). For steel containing between 0.1 and 1% of chromium take a 0.500-gram sample and dissolve in 10 ml. of nitric acid (1 to 1) and 15 ml. of perchloric acid (70 to 72%). Evaporate to dense fumes of perchloric acid and boil gently for 5 minutes to oxidize chromium (boiling for 8 minutes does no harm). Cool the beaker and contents rapidly in tap water. Dissolve soluble salts with 20 ml. of water and transfer the solution to a 50-ml. glass-stoppered volumetric flask. Cool the solution to room temperature and dilute to 50 ml.

Taking the Colorimeter Reading. Transfer a portion of the solution to the absorption tube and reduce with a small crystal (about 10 to 20 mg.) of ferrous ammonium sulfate. Adjust the colorimeter so that the reading on this solution is zero. Discard the solution in the absorption tube, refill with the oxidized solution, and take a second reading. This reading is a measure of the color due to the dichromate. In the absence of elements which form highly colored ions, such as copper, nickel, cobalt, etc., it is not necessary to reset the colorimeter at zero.

Table II. Determination of Chromium in Bureau of Standards Samples

Composition	Alloying Element Added	Chromium Present	Chromium Colorimetrically
Gram	%	%	%
1.000 8d	None	0.007	0.007
			0.008
1.000 11d	None	0.008	0.009
			0.009
1.000 13d	None	0.023	0.023
			0.023
1.000 15c	None	0.055	0.055
			0.056
0.500 30c	None	0.977	0.98
			0.98
0.500 72	None	0.911	0.91
			0.91
0.500 72a	None	0.655	0.66
			0.66
0.500 100	None	0.180	0.19
0.300 20d			
0.700 22b	None	0.087	0.087
0.400 20d			
0.600 22b	None	0.115	0.115
0.970 13d	3 Cu	0.022	0.022
0.500 100			
0.470 11d	3 Cu	0.095	0.099
0.485 100	3 Cu	0.175	0.187
0.485 72	3 Cu	0.883	0.90
0.970 13d	3 Ni	0.022	0.020
0.500 100			
0.470 11d	3 Ni	0.095	0.096
0.485 100	3 Ni	0.175	0.18
0.485 72	3 Ni	0.883	0.87
0.500 11d			
0.500 68[a]	1.38 Mo	0.108	0.092
0.400 72			
0.100 68[a]	0.67 Mo	0.764	0.75
0.100 106			
0.900 11d	0.1 Al	0.137	0.137
0.250 106			
0.250 11d	0.5 Al	0.65	0.64
0.990 13d	1 Co	0.023	0.021
0.500 100			
0.490 22b	1 Co	0.092	0.095
0.495 100	1 Co	0.178	0.186
0.495 72	1 Co	0.902	0.90
0.495 72	1 V	0.902	0.88
1.000 HJN[b]	1.5 Ti	0.115	0.102
0.250 HJN[b]			
0.25 72	0.75 Ti	0.51	0.49
0.167 HJN[b]			
0.333 72	0.50 Ti	0.646	0.63

[a] Authors' alloy, contains 2.76% molybdenum and 0.208% chromium.
[b] Authors' alloy, contains 1.50% titanium and 0.115% chromium.

All subsequent colorimeter measurements are made by taking a reading on an oxidized solution, reducing the solution in the absorption tube with a small crystal of ferrous ammonium sulfate, and taking a second reading. The difference between the first and second readings then represents the color due to the dichromate present. If appreciable amounts of highly colored ions are present, the colorimeter is adjusted to read zero on the reduced solution before a reading is taken on the oxidized sample.

DESCRIPTION OF COLORIMETER

A Klett-Summerson photoelectric colorimeter was used. This colorimeter has a logarithmic scale, and when Beer's law applies, the scale readings are proportional to the concentration of colored ion. Measurements were made in an absorption tube of 12.5-mm. inside diameter, with a color filter transmitting between 410 and 480 millimicrons.

DISCUSSION OF RESULTS

Reference to Table II shows that duplicate determinations agree well and the results obtained compare favorably with the Bureau of Standard certificate values. Moderate amounts of the ordinary alloying constituents do not affect the accuracy of the method. Since the color intensity of dichromate is dependent upon the concentration of ferric perchlorate, it is evident that large amounts of alloying constituents would produce an appreciable error in this method. In the case of silicon, for example, experience has shown that amounts in excess of 1% cause a perceptible error if the normal graph of per cent *vs.* colorimeter reading is used. However, supplementary graphs for the determination of chromium in high-silicon iron or steel may be prepared, by using samples which contain known chromium and known, similar, high-silicon percentages. An additional precaution deserves mention: it is necessary to allow the silica present in the colorimeter tube to settle for 1 or 2 minutes before taking a reading.

This method is applicable to the great majority of steels without any modification whatever. Inasmuch as iron is not separated from chromium, the analysis is more rapid than most methods for the colorimetric determination of chromium in steel.

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Quantitative Separation of Alcohol and Ester Forms of Vitamin A

By Solvent Extraction and Chromatographic Methods

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If vitamin A alcohol and vitamin A palmitate are distributed between equal volumes of 95% aqueous methanol and petroleum ether, 27% of the alcohol form and 98% of the ester form remain in the petroleum ether phase. A simple formula allows calculation of the percentage of either form of vitamin A if an unknown mixture is distributed between the solvents. Vitamin A alcohol can be separated quantitatively from its esters by filtration of a solution in ethylene dichloride through chromatographic columns of activated alumina. A procedure for carrying out this analytical separation is described.

IT HAS been claimed (*4*) that esterified vitamin A in its natural state in fish liver oils has a higher biological potency than equivalent amounts of vitamin A alcohol in the unsaponifiable fractions of these oils. Investigation of these claims necessitated the development of a fast and simple method for the analytical separation of vitamin A alcohol from its esters. Molecular distillation of oils has been used (*6*), but the equipment is not so simple in operation as is desirable for an analytical method. (A method for the assay of both forms by fluorophotometry was published after the completion of this manuscript, *8*.)

The analytical separation by solvent fractionation was studied by Gillam (*5*) who reported the distribution of vitamin A (alcohol) between light petroleum ether and aqueous methanol. Claussen (*3*) separated the alcohol and ester forms by distribution between hexane and 81.9% aqueous ethanol. A search of the literature did not reveal data concerning the advantages of different alcohols for the separation or details of the analytical procedure.

The distribution of crystalline vitamin A alcohol and crystalline vitamin A palmitate was determined by shaking a solution of about 0.40 mg. of vitamin A alcohol or 0.70 mg. of vitamin A ester in 50 ml. of petroleum ether (Skellysolve B) with an equal volume of an aqueous aliphatic alcohol. The change in volume was noted and the percentage distribution was calculated from determinations of vitamin A in the petroleum ether phase by measuring the ultraviolet absorption at 328 mμ. The results are summarized in Figure 1.

The percentage distributions agree well with those reported by Gillam (*5*, 70, 80, and 95% aqueous methanol), and Claussen (*3*, 82% aqueous ethanol), and Baxter (*1*, 83% aqueous ethanol). The distribution refers to the amount of vitamin A in each phase rather than to its concentration, as the volume of the phases changes owing to mutual solubility of the solvents. The volume of 50 ml. of 95% methanol increases on shaking with 50 ml. of petroleum ether to 57 ml. at 5.5°, 59 ml. at 28.5°, and 60 ml. at 36°.

The best separation for analytical purposes can be obtained with 95% (by volume) aqueous methanol, as only 27% of the al-

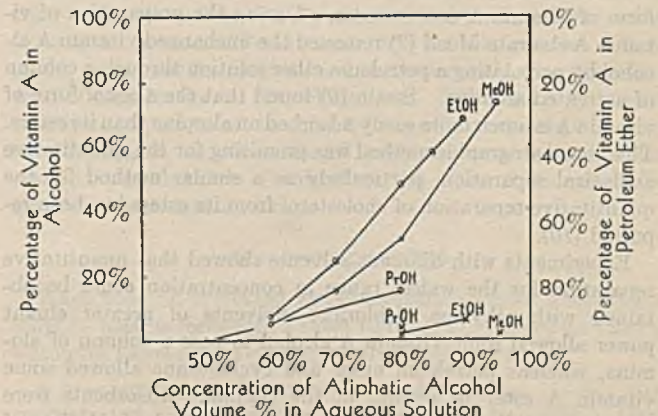

Figure 1. Distribution of Vitamin A Alcohol and Ester Forms between Aliphatic Alcohols and Petroleum Ether

O. Vitamin A alcohol
X. Vitamin A ester

Table I. Adsorption of Vitamin A

(40 I.U. per ml.)

Adsorbent	Ester Adsorbed	Alcohol Adsorbed
	%	%
From 100 ml. of ethylene dichloride on 5 grams of adsorbent		
Powdered dextrose	0	0
ZnO (activated)	0	0
Magnesol	0	17
$Ca_2P_2O_7 . 4H_2O$	0	13
$Ca(OH)_2$	0	33
Al_2O_3 (activated)	0	70
On chromatographic columns, 1.1-cm. diameter, 10.5-cm. length		
Powdered dextrose	0	0
ZnO (activated)	0	26
$Ca_3(PO_4)_2$	0	58
Al_2O_3	0	100

Table II. Ester Content of Oils and Concentrates

Sample	Ester	Ester
	I.U./g. (approx.)	%
Vitamin A acetate	3,500,000	100
Vitamin A palmitate	3,300,000	98
Fish liver oil (shark)	115,000	98
Vitamin A distillate	200,000	98
Halibut liver oil	130,000	94
Cod liver oil	2,500	93
Vitamin A alcohol	4,000,000	1
Fish liver oil concentrate	1,000,000	0

cohol form and as much as 98% of the ester form remain in the petroleum ether phase.

Analytical Solvent Separation. Dissolve a sample of fish liver oil or concentrate containing about 0.5 to 5 mg. of vitamin A (in the following, the weight of vitamin A esters is expressed as the weight of its contents of vitamin A alcohol) in 50 ml. of petroleum ether. Shake vigorously with 50 ml. of 95% aqueous methanol in a 100-ml. glass-stoppered measuring cylinder for 1 minute, allow the phases to separate, and note the volumes. Determine the concentration of vitamin A in each phase with the antimony trichloride reaction or spectrographically after proper dilution. Calculate the milligrams of vitamin A in each phase and find the milligrams of vitamin A alcohol and ester from the following formulas:

$$a = 1.380 \times A_m - 0.028 \times A_e$$

$$e = 1.028 \times A_e - 0.380 \times A_m$$

where a represents mg. of vitamin A alcohol, e mg. of vitamin A ester, A_m mg. of vitamin A in the methanol phase, and A_e mg. of vitamin A in the petroleum ether phase.

The solvent separation cannot be applied if vitamin A is present as the acetate ester, as only 78% of the acetate remains in the petroleum ether phase. The presence of 1% of cholesterol affects the distribution considerably (*5*). The presence of more than 1% of the fish liver oil or concentrate tends to shift the distribution of vitamin A alcohol to the petroleum ether phase.

Alumina has been widely used for the adsorption of the alcohol form of vitamin A concentrates. During the preparation of vitamin A stearate Mead (*7*) removed the unchanged vitamin A alcohol by percolating a petroleum ether solution through a column of activated alumina. Swain (*9*) found that the alcohol form of vitamin A is much more easily adsorbed on alumina than its esters. This chromatographic method was promising for the quantitative analytical separation, particularly as a similar method for the quantitative separation of cholesterol from its esters has been reported (*10*).

Experiments with different solvents showed that quantitative separation for the widest range in concentration could be obtained with ethylene dichloride. Solvents of greater eluant power allowed some vitamin A alcohol to pass a column of alumina, whereas petroleum ether and cyclohexane allowed some vitamin A ester to remain in the column. Adsorbents were tested by shaking a sample of 5 grams with 100 ml. of solutions of vitamin A alcohol or palmitate (about 1 mg. in 100 ml. of ethylene dichloride). (The author is obliged to J. G. Baxter for a sample of pure vitamin A palmitate.) The results are shown in Table I, which also shows the adsorption on chromatographic columns, using the first 5 ml. of filtrate for analysis.

The degree of activation of the alumina is of the greatest importance. Alumina which has been too highly activated will destroy part of the vitamin A ester during passage. The destruction can easily be recognized by the shape of the ultraviolet absorption curve. It is not always evident in the antimony trichloride reaction, as some decomposition products will still develop a blue color with the reagent. Columns of alumina which were well suited to the quantitative separation did not adsorb azobenzene from a 1 to 4 mixture of benzene and petroleum ether. They permitted the separation of 4-methoxyazobenzene and benzene-azo-β-naphthol from the same solvent into two distinct bands on the column. Benzene-azo-β-naphthol and aminoazobenzene-azo-β-naphthol were so strongly adsorbed that they could not be separated on the column. This indicates a degree of activation of group 2 in Brockmann's standardization of alumina (*2*).

Analytical Separation by Chromatography. Introduce activated alumina, Alorco Grade A, <80-mesh, into a 50-ml. stopcock buret and secure a column of 15 ml. (about 15 grams) in the lower part with plugs of Pyrex glass wool. Activate the column at from 110° to 120° C. for 1.5 hours in a stream of nitrogen. Draw about 30 ml. of ethylene dichloride with gentle suction through the column in order to avoid any deleterious effect by the heat of wetting, allowing the level of the liquid to stay just above the column. Add exactly 2 ml. of a solution of a sample of fish liver oil or concentrate (containing about 5 mg. of vitamin A in 10 ml. of ethylene dichloride) and pass through the column. Follow immediately with 40 to 50 ml. of ethylene dichloride, never allowing the column to become dry. Transfer the filtrate to a 100-ml. measuring flask and fill up to volume. Assay this solution and the original solution (after diluting 1 to 50) with the antimony trichloride reaction. The concentration of vitamin A found in the filtrate is due to the ester form alone. The concentration of the alcohol form is found by subtraction from the total concentration.

With the above concentrations of vitamin A, the alcohol form will not form a colored band on the column, so that its progress cannot be observed directly. If the column is allowed to stand for one day, a yellow-orange band, due to vitamin A decomposition products, will become visible about 2 to 3 cm. below the top of the column. The ultraviolet absorption at 328 mμ can be used for the estimation of vitamin A in the filtrate, but allowance must be made for the fact that the absorption of vitamin A in ethylene dichloride is about 13% less than in isopropanol.

Crystalline vitamin A alcohol does not pass the column under the above experimental conditions and no increase of the concentration in the filtrate is observed after the addition of the alcohol to fish liver oils. Crystalline vitamin A palmitate and acetate pass the column without changing concentration and can be recovered in the filtrate after addition to fish liver oils. Results obtained by this method are reproducible within 2% and agree within these limits with results obtained by the solvent extraction procedure. Table II shows the ester content of some representative samples of oils and concentrates.

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Estimation of Tung Oil as an Adulterant

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TUNG oil is sometimes adulterated with soybean, sesame, peanut, rapeseed, stillingia, and other oils, which are much cheaper than tung oil during normal times. Since the present Sino-Japanese War, the situation has been reversed. As the facilities for exporting tung oil from China abroad have been greatly reduced and almost cut off, its price in China has become far lower than the other kinds of ordinarily available vegetable oils. In spite of the fact that large quantities of tung oil have been consumed by the new-born tung oil cracking industries in the manufacture of gasoline and fuel oil substitutes, its price, relative to other oils, still remains low. As a consequence it is not an uncommon practice of some dishonest dealers to use tung oil as an adulterant in other higher-priced edible oils—e.g., rapeseed, peanut, and sesame oils.

On account of its strong purgative and emetic action tung oil is not edible. The presence of a small amount of tung oil in other edible oils, even as little as 0.5%, may cause severe disturbances, if the oils are taken as food. On the other hand, when rapeseed and other oils are used industrially, especially to make lubricating oil components or substitutes, the presence of tung oil is highly objectionable because of its tendency to gelatinize. It is obvious that the detection and determination of adulterating tung oil in these oils are of considerable importance.

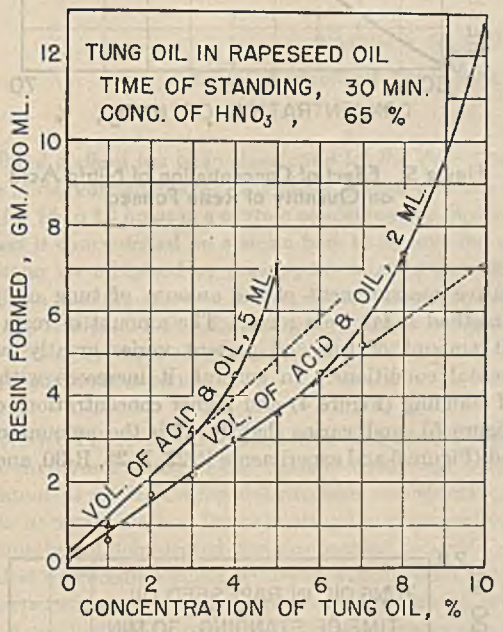

Figure 1. Resins Formed from Samples of Rapeseed Oil Adulterated with Tung Oil

Several analytical methods for testing the purity of tung oil and determining the amount of its adulterants present are available (*4*, *5*), largely based on the gelatinization of tung oil upon heating. When the percentage of tung oil in the mixtures is small, these methods cannot be applied. Tung oil is characterized by its high refractive index and high iodine number, and an examination of these values of the oils under consideration may throw some light on the presence or absence of tung oil. However, as these values of the unadulterated oils cover a comparatively wide range, they do not give conclusive evidence. A test based on some reaction specific only to tung oil will be much more desirable.

COLORIMETRIC QUALITATIVE TEST

A qualitative test for tung oil has been worked out by Wan (*3*, *6*), its procedure being essentially the following:

The testing reagent is prepared by dissolving 10 grams of antimony trichloride in 100 ml. of chloroform, 5 to 10 ml. of this reagent are poured into a test tube, and 1 drop of the oil to be examined is placed on the surface. On standing for 10 minutes to 1 hour, the formation of a dark red ring indicates the presence of tung oil.

This test is very sensitive. Although castor oil and sesame oil in this reagent after long standing may also produce a pinkish tint, the intensity of color due to the presence of tung oil is far more pronounced.

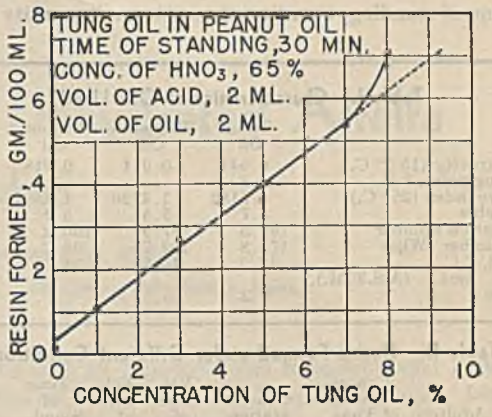

Figure 2. Resins Formed from Samples of Peanut Oil Adulterated with Tung Oil

The present authors attempted to develop this test into a quantitative colorimetric method. Samples of 0.1 to 0.2 gram of rapeseed oil containing a few per cent of tung oil were weighed out in test tubes, and 10-ml. portions of the antimony trichloride reagent were added to each. They were thoroughly shaken and let stand for 2 or 3 hours. The solutions were then filtered and immediately compared in a colorimeter. It was found that the color intensity was not directly proportional to the concentration of tung oil present. Furthermore, the reaction seemed still to go on, and precipitates continued to form even during the short time of examination in the colorimeter. This greatly interfered with the comparison of the color. Only after very long standing, say, overnight, did the filtered solution remain clear. This reaction was therefore discarded as the basis of a method for quantitative determination.

GRAVIMETRIC METHOD

When tung oil is treated with concentrated nitric acid a solid jellylike mass is formed in a short time (*1*, *2*). Based on this reaction the following gravimetric method was worked out.

A few milliliters of the oil sample under test are treated with concentrated nitric acid in a glass-stoppered test tube. After being thoroughly shaken, it is immersed in ice water for some time. Then the reaction product is filtered through an asbestos-matted Gooch crucible, washed thrice with petroleum naphtha (50° to 150° C.), dried at 100° C., and weighed.

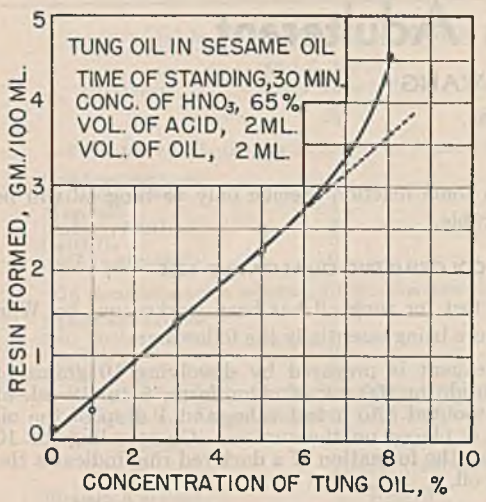

Figure 3. Resins Formed from Samples of Sesame Oil Adulterated with Tung Oil

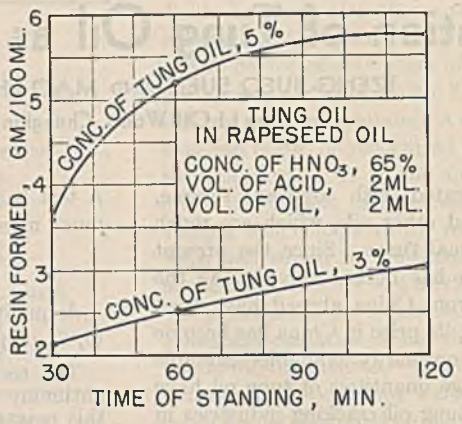

Figure 4. Effect of Time of Standing on Quantity of Resin Formed

It was found that the result was affected by the following variables: nature of the adulterated oil, concentration of nitric acid, time of standing, quantity of sample, and quantity of nitric acid. They were studied in detail and the best conditions sought.

The experimental results under various conditions are given in Table II and Figures 1 to 6. The nearly straight-line relationship between the weight of the resinous matter formed and the percentage of tung oil in the adulterated oils (Figures 1 to 3), when its concentrations are low, indicates that this method can be used as a quantitative measurement of the amount of tung oil present.

This method is far from ideal. The amount of resin formed per unit amount of tung oil present varies greatly with the experimental conditions. In general, it increases with longer times of standing (Figure 4) and higher concentrations of nitric acid (Figure 5), and varies slightly with the amount of nitric acid used (Figure 6 and experiments R-22, R-28, R-30, and R-31).

Table I. Characteristics of Oils Used

	Tung Oil	Rapeseed Oil	Peanut Oil	Sesame Oil
Specific gravity (15.5° C.)	0.941	0.914	0.918	0.922
Solidifying point, ° C.	2.8	....	....	....
Refractive index (25° C.)	1.5183	1.4720	1.4691	1.4719
Acid number	5.7	5.5	5.2	9.3
Saponification number	192.5	187.9	196.2	186.6
Iodine number (Wijs)	171.8	102.7	98.2	112.4
Titer test, ° C.	32.2	15.8	34.6	21.2
Heating test (A.S.T.M.), minutes	9.3	....	....	....

Table II. Resins Formed under Different Conditions

Expt. No.	Adulterated Oil	Concentration of Tung Oil %	Concentration of HNO_3 %	Volume of Oil Ml.	Volume of Acid Ml.	Time of Standing Min.	Resin Formed G./100 ml.
R-0	Rapeseed	Blank	65.0	2	2	30	0.15
R-1	Rapeseed	1	65.0	2	2	30	0.65
R-2	Rapeseed	2	65.0	2	2	30	1.70
R-3a	Rapeseed	3	65.0	2	2	30	2.05
R-3b	Rapeseed	3	65.0	2	2	30	2.25
R-3c	Rapeseed	3	65.0	2	2	30	2.10
R-4	Rapeseed	4	65.0	2	2	30	3.00
R-5	Rapeseed	5	65.0	2	2	30	3.60
R-6	Rapeseed	6	65.0	2	2	30	4.55
R-7	Rapeseed	7	65.0	2	2	30	5.25
R-8	Rapeseed	8	65.0	2	2	30	7.36
R-10	Rapeseed	10	65.0	2	2	30	12.75
R-11	Rapeseed	Blank	65.0	5	5	30	0.44
R-12	Rapeseed	1	65.0	5	5	30	0.99
R-13	Rapeseed	3	65.0	5	5	30	3.20
R-14	Rapeseed	4	65.0	5	5	30	4.50
R-15	Rapeseed	5	65.0	5	5	30	7.22
R-21	Rapeseed	3	65.0	2	2	60	2.53
R-22	Rapeseed	3	65.0	2	2	120	3.05
R-23	Rapeseed	5	65.0	2	2	60	5.20
R-24	Rapeseed	5	65.0	2	2	120	5.75
R-25	Rapeseed	3	65.0	2	5	30	2.30
R-26	Rapeseed	3	65.0	2	10	30	2.35
R-27	Rapeseed	3	65.0	2	10	60	2.55
R-28	Rapeseed	3	65.0	2	10	120	2.75
R-29	Rapeseed	8	65.0	1	1	30	7.70
R-30	Rapeseed	10	65.0	1	1	30	12.40
R-31	Rapeseed	10	65.0	1	2	30	14.00
R-32	Rapeseed	5	70.0	2	2	30	7.95
R-33	Rapeseed	5	60.0	2	2	30	0.20
P-0	Peanut	Blank	65.0	2	2	30	0.16
P-1	Peanut	1	65.0	2	2	30	1.10
P-3	Peanut	3	65.0	2	2	30	2.70
P-5	Peanut	5	65.0	2	2	30	4.01
P-7	Peanut	7	65.0	2	2	30	5.35
P-8	Peanut	8	65.0	2	2	30	7.10
S-0	Sesame	Blank	65.0	2	2	30	0.15
S-1	Sesame	1	65.0	2	2	30	0.35
S-3	Sesame	3	65.0	2	2	30	1.40
S-5	Sesame	5	65.0	2	2	30	2.25
S-7	Sesame	7	65.0	2	2	30	3.40
S-8	Sesame	8	65.0	2	2	30	4.55

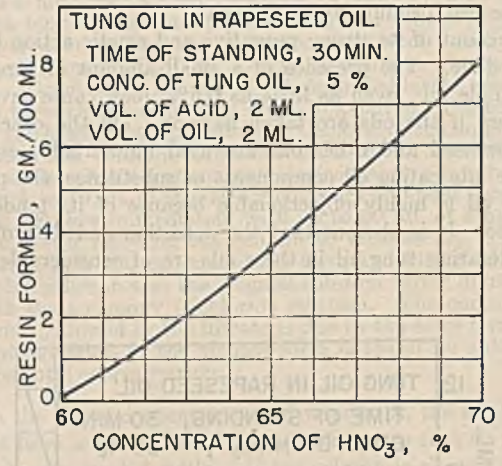

Figure 5. Effect of Concentration of Nitric Acid on Quantity of Resin Formed

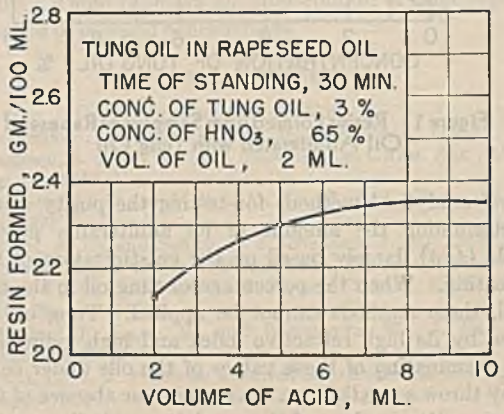

Figure 6. Effect of Volume of Nitric Acid on Quantity of Resin Formed

Among these factors the concentration of nitric acid has a tremendous effect. If the concentration is below 60%, there is hardly any resin formed. If it is above 70%, the jellylike mass is so voluminous and thick that is difficult to handle. It was found that 65% strength is about right. In the actual determinations of tung oil content, the concentration of nitric acid should obviously be accurately adjusted.

It seems that the resin is not formed solely from the tung oil. The adulterated oil is also somehow affected, probably coprecipitated, but to different degrees for different oils. The quantity of resin formed per unit quantity of tung oil present differs appreciably in rapeseed, peanut, and sesame oils. This shows that the adulterated oil also takes part in the process of resinification. With the same kind of adulterated oil, the quantity of resin formed becomes higher when the tung oil concentration or the total amount of resin goes beyond a certain limit. The results with 1-ml. and 2-ml. portions of oils and acid are fairly close to each other (experiments R-8 and R-10, R-29 and R-30). When the volume of the samples and acid used is increased to 5 ml., however, the quantity of resin formed becomes noticeably higher.

The mechanism of the reaction between tung oil and nitric acid has not been clear. Considering all the variations mentioned above, it appears to be complicated. For our present purpose, however, it can be utilized as a basis for quantitative estimation of tung oil as an adulterant. By weighing the resin obtained under controlled conditions and comparing it with a previously prepared curve with known samples under identical conditions, the percentage of tung oil present can be readily ascertained. The reproducibility of the experimental results is reasonably high, as evidenced by experiments R-3a, R-3b, and R-3c. If the percentage of tung oil in the adulterated oil is too high, it may be first diluted with clean oil. However, such cases would be rare. The recommended experimental conditions are 65.0% nitric acid, 2.0 ml. each of oil sample and acid, and 30-minute standing in ice water.

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Determination of Carotene in Dehydrated Alfalfa

A Simplified Method

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A simplified method has been developed for the determination of carotene in dehydrated alfalfa. The finely ground alfalfa is allowed to stand for 16 to 18 hours in a mixture of acetone and Skellysolve B, the extract is concentrated on a steam bath to remove the acetone, the pigments are adsorbed by drawing the mixture through a magnesia column, and the carotene is then eluted with a 4% solution of acetone in Skellysolve B. Refluxing and phasic separations are avoided and many more samples can be handled per day than by other methods in common use.

IN CONNECTION with a research project at this laboratory which involves a study of the changes occurring in the carotene content of alfalfa during dehydration and storage, a rapid as well as accurate method for estimating the carotene content of a large number of dehydrated samples became desirable. It was hoped that a procedure could be found which would not require phasic separations, since both time and equipment would be saved. A method which required little heating was also desired, in order to hold the isomerization of carotene to a minimum during the analytical procedure.

A survey of the more common methods of analysis showed no one procedure that completely met the needs. It was not possible to extract all the carotene by the method suggested by Kernohan (*6*) which involves allowing the sample to stand in petroleum ether. It was found, however, that the carotene could be extracted by allowing a sample of dehydrated alfalfa to stand in a mixture of Skellysolve B and acetone. Wall and Kelley (*13*) used these solvents in the extraction of dehydrated materials in a Soxhlet. It has been found convenient to weigh out the samples in the afternoon and allow them to stand overnight. This procedure makes it possible to determine the carotene content of a large number of samples per day. Results are reproducible with an accuracy equal to or somewhat better than those obtained in this laboratory by other methods. Isomerization of the carotene is minimized and a saving in equipment is effected, since phasic separations may be eliminated and complicated extraction apparatus is not required.

PROCEDURE

EXTRACTION OF CAROTENE. One or 2 grams of finely ground alfalfa (preferably through a 40-mesh screen) are weighed into an Erlenmeyer flask or sample bottle and covered with 60 ml. of a mixture of 1 part acetone to 2 parts Skellysolve B. The mixture is shaken and the tightly stoppered container then set aside in the dark for 16 to 18 hours, usually overnight. The extract is filtered through a Büchner funnel and the residue thoroughly washed, by decantation, with several small portions of Skellysolve, followed by heating on a steam cone to drive off most of the remaining acetone and to concentrate the solution to a volume of approximately 40 ml. (15 minutes' heating is usually sufficient).

SEPARATION OF CAROTENE FROM OTHER PIGMENTS. A chromatographic separation of carotene from other pigments is made on a column of 2 parts Hyflo Super-Cel and 1 part magnesia (Micron brand No. 2641). The columns are made as described by Wall and Kelley (*13*) but are shorter in length (8 to 10 cm.). After adsorption of the pigments, the carotene is eluted with a 4% acetone–Skellysolve B mixture.

ESTIMATION OF CAROTENE. The solution of carotene is made up to volume and analyzed with a Beckman (*2*) spectrophotometer, for β-carotene and neo-β-carotene B, using the wave lengths and absorption coefficients suggested by Beadle and Zscheile (*1*).

DISCUSSION

The procedure has been checked against two of the more common methods of analysis. The method of Peterson, Hughes, and Freeman (*10*) gave slightly higher carotene values in most cases, but the thoroughness of the methanol wash has considerable

Table I. Comparison of Three Methods for Determination of Carotene in Dehydrated Alfalfa

Sample No.	P.H.F. Method Total carotene	P.H.F. Method β-Carotene	Moore and Ely Extraction MgO Column Total carotene	Moore and Ely Extraction MgO Column β-Carotene	Proposed Method Total carotene	Proposed Method β-Carotene
	Mg./100 g.	%	*Mg./100 g.*	%	*Mg./100 g.*	%
316[a]	38.2	70	38.5	89	37.6	86
	38.0	69	38.7	85	38.2	86
321	17.4	67	16.8	84	16.6	83
	16.1	73	16.1	83	16.3	82
325[a]	37.8	75	37.8	86	36.5	88
	38.0	74	38.0	86	36.5	88
331	13.0	67	13.0	76	13.0	80
	12.0	67	12.5	75	12.8	78
341	20.4	67	20.3	83	20.2	84
	19.6	66	19.8	85	19.4	89
510	11.4	58	11.1	81	10.9	81
	11.2	56	11.0	84	10.6	86

[a] Steam-blanched before drying.

Table II. Effect of Removing Noncarotenoid Pigments from Carotene Extracts Obtained by Phasic Separation

Sample No.	P.H.F. Method Total carotene	P.H.F. Method β-Carotene	P.H.F. Method Followed by Adsorption Total carotene	P.H.F. Method Followed by Adsorption β-Carotene	Adsorption Procedure Total carotene	Adsorption Procedure β-Carotene
	Mg./100 g.	%	*Mg./100 g.*	%	*Mg./100 g.*	%
831	13.0	67	11.9	72	12.8	78
382	16.1	57	12.6	72	11.8	76
383	16.8	53	12.4	67	12.6	70
384	23.0	67	18.9	73	16.3	72
385	9.1	57	7.6	70	8.2	83

influence on the results obtained by this method. The data in Table I were obtained with a more rigorous washing procedure and agree closely with chromatographic techniques. The apparently low percentage of β-carotene calculated when the Peterson, Hughes, and Freeman (*10*) technique of extraction was used is shown in Tables I and II. These low values are due, largely, to the presence of small amounts of noncarotenoid pigments, which cause the absorption measurements at 478 mμ to be low. It has been pointed out by several workers (*3*, *7*, *14*) that some noncarotenoid pigments remain after phasic separations. Table II shows the increase in per cent of β-carotene and the lower carotene values following the removal of the noncarotenoid pigments on a magnesia column.

The other method used for comparison was an extraction procedure, using a Waring Blendor and a foaming solvent mixture of alcohol and Skellysolve B similar to that of Moore and Ely (*8*). This was followed by adsorption and elution from a magnesia column according to the method of Wall and Kelley (*13*).

There is good agreement between the data obtained by the proposed method and the other two methods, as shown in Table I. Samples 316 and 325 have a much higher total carotene content than any of the other samples listed in Table I. These samples were collected at the same time and differ from No. 321 only in that they were blanched with steam for 7 and 10 minutes, respectively, before drying. It has been pointed out by the authors (*12*) that steam blanching stabilizes the carotene in fresh alfalfa, so that the usual loss which accompanies drying is avoided. The percentage of β-carotene is somewhat greater in the blanched samples than in the corresponding unblanched sample. Complete extraction of carotene could be effected from coarsely ground unblanched alfalfa, but a finer grind was necessary for extraction of the blanched material. Excellent results were obtained with all types of samples which had passed through a 40-mesh screen.

The new procedure causes some isomerization, but less than methods which require refluxing. A sample of β-carotene (S. M. A. Corporation) was allowed to isomerize slightly and was then carried through the entire analytical procedure. The additional isomerization for three such determinations averaged 6%. Recovery tests, carried out at the same time, gave excellent results and agree well with those of Wall and Kelley (*13*). The magnesia columns cause very little loss of carotene.

The samples must be placed in the dark while standing. As pointed out by Pepkowitz (*9*), carotene, in petroleum ether, is subject to photochemical destruction in the presence of chlorophyll and acetone. No losses seem to occur if the solutions are protected from light.

Possible procedures for the removal of the acetone after filtration have also been compared. Most of this acetone must be removed at this point in the procedure in order to obtain a good separation of pigments on the column. The acetone may be removed by washing with water or by evaporation. Since heating on a steam cone caused only a slight increase in per cent of isomerization as compared with evaporation under reduced pressure or washing with water, this procedure has been followed because of its convenience. Data obtained by these three methods for the removal of acetone are recorded in Table III for comparisons. These data differ from the data recorded in Table I because the analyses were made after prolonged storage.

The acetone present in the final solution need not be removed for spectrophotometric estimation of the β-carotene–neo-β-carotene B system. The concentration of acetone in this solution varies from 2 to 4%. This amount of acetone has no effect on the absorption spectra of β-carotene in the range used for carotene estimation. The two curves are identical in the range from 380 to 510 mμ. The specific absorption coefficients at the critical wave lengths, as obtained by Beadle and Zscheile (*1*) in hexane, and the authors' measurements in hexane, redistilled Skellysolve B, and a mixture of redistilled Skellysolve B and 5% acetone, are shown in Table IV. Acetone does exhibit considerable absorption at lower wave lengths and would have to be removed if measurements in this range were desired.

While isomers of β-carotene, other than the neo-β-carotene of Beadle and Zscheile, have been found in alfalfa, the percentage of β-carotene as calculated by their method has been regarded as a measure of the extent of change in the nature of the carotenoids present. This is considered significant because of the probable reduced nutritional value of the isomeric pigments. Kemmerer and Fraps (*4*) have recently reported that neo-β-carotene B has about one half the vitamin A activity of β-carotene. They have also reported (*5*) another constituent of the carotenoid fraction of plant materials which is not separated from β-carotene on a magnesia column, but which can be separated on a column of calcium

Table III. Effect of Different Procedures for Removal of Acetone from Carotene Extracts

Sample No.	Steam Cone Total carotene	Steam Cone β-Carotene	Method of Removal of Acetone—Vacuum Evaporation Total carotene	Vacuum Evaporation β-Carotene	Water Wash Total carotene	Water Wash β-Carotene
	Mg./100 g.	%	*Mg./100 g.*	%	*Mg./100 g.*	%
325	36.7	89	36.4	89	36.4	89
	36.6	87	36.1	90	37.0	89
	36.6	89	36.1	90	35.6	89
331	10.4	81	10.8	82	10.3	80
	10.4	79	10.7	83	10.2	82
	10.7	80	10.8	83	10.3	77
341	19.7	85	19.6	85	19.5	85
	19.5	84	19.3	88	19.4	83
	19.5	85	19.8	87	19.9	81

Table IV. Specific Absorption Coefficients of β-Carotene

Solvent	Wave Length 436.0 mμ	450.0 mμ	466.0 mμ	478.0 mμ
Hexane[a]	196	258	206	228
Hexane	198	259	210	231
Redistilled Skellysolve B	193	253	203	224
5% acetone in Skellysolve B	193	253	205	224

[a] Coefficients as given by Beadle and Zscheile (*1*).

hydroxide. They have identified this pigment as the neo-β-carotene U of Polgár and Zechmeister (*11*). They report that this carotenoid has no vitamin A activity. It may, therefore, become necessary to calculate the carotene composition on the basis of more than two components. This may be possible by the use of spectrophotometric data obtained at other critical points on the absorption curve of the extract. It is hoped that a continuation of spectral studies will furnish data that will be useful in this connection.

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Determination of Citric Acid in Fermentation Media and Biological Materials

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Several modifications have been made in the colorimetric pentabromoacetone method for the determination of citric acid as proposed by Pucher, Vickery, and associates. The time required per sample is reduced, while accuracy has not been diminished. This method has been applied to the determination of citric acid in fermented media and tissue extracts with satisfactory results.

CERTAIN strains of the mold, *Aspergillus niger*, convert a large proportion of carbohydrate substrates to citric acid. With most strains of this organism some oxalic acid is also produced. In studying the factors affecting the production of citric acid by this mold (*3*), it was necessary to have rapid and reasonably accurate methods for the determination of these two acids in the presence of each other.

Since both acids have rather insoluble calcium salts, these salts have been used for their determinations in fermentation media by Doelger and Prescott (*2*), Wells, Moyer, and May (*8*), and others. Calcium oxalate is much less soluble than calcium citrate in cold, slightly acid solution, and the two salts may be separated in this manner. Calcium citrate is insoluble in hot neutral solutions. These two acids are usually the main acidic products of this fermentation, and for routine work the difference in acidity between the total acid and that due to oxalic acid may be considered to be due to citric acid. This is a somewhat uncertain procedure, as the mold produces many other acids, and an independent method for the determination of citric acid is desirable.

The volumetric method of Wilkinson, Siphard, Fulmer, and Christensen (*9*) involves preliminary precipitation of the lead salts of the oxalic and citric acids. The acids are then regenerated and titrated with alkali and ceric sulfate. This method is not very specific for citric acid, as many fermentation acids form rather insoluble lead salts.

A more specific method for the determination of citric acid depends upon its conversion to pentabromoacetone, which may be estimated gravimetrically or colorimetrically. This conversion is based on the fact that when citric acid is oxidized with potassium permanganate in the presence of bromine, under controlled conditions, the acid is converted quantitatively to pentabromoacetone. Deysher and Holm (*1*) have discussed the difficulties in the gravimetric determination of this derivative of citric acid.

Pucher, Vickery, and associates (*4*, *5*, *6*) have estimated the pentabromoacetone colorimetrically by a method based on the yellow color formed by the addition of pentabromoacetone to a sodium sulfide solution. This colorimetric method is somewhat involved and requires quantitative extraction of the pentabromoacetone from the reaction solution. Purinton and Schuck (*7*) have modified the original method slightly but still require quantitative extraction of the pentabromoacetone and several other rather involved procedures. In the modification described, no attempt has been made to extract the pentabromoacetone quantitatively from the reaction solution. Instead, advantage is taken of the distribution coefficient and a single extraction is made. A single extraction has been found to remove a constant amount of the total pentabromoacetone in any series of samples of uniform volume. This modification has made possible the development of a more convenient procedure.

EXPERIMENTAL

Reagents. Sulfuric acid, equal volumes of 95% sulfuric acid and water. 1 *M* potassium bromide, bromine water (saturated), 3% hydrogen peroxide, petroleum ether (acid-washed Skellysolve B).

Dioxane-water mixture, equal volume of dioxane and water.

Sodium sulfide solution, 4 grams of sodium sulfide nonahydrate per 100 cc. of solution.

1.5 *N* and 0.1 *N* potassium permanganate.

Procedure. If the samples are known to contain reducing material, aliquots preferably containing less than 25 mg. of citric acid are placed in 2.5 × 20 cm. (1 × 8 inch) Pyrex test tubes and 2 cc. of the sulfuric acid solution are added. The total volume is adjusted to about 20 cc. and the samples are boiled for a few minutes. The solutions are then cooled and 3 to 5 cc. of bromine water are added. After 10 minutes, any precipitate

Table I. Recovery of Added Citric Acid from Solutions

Citric Acid Added per Sample	Solution A[a]		Solution B[b]		Solution C[c]	
	Citric acid found	Recovery of added acid	Citric acid found	Recovery of added acid	Citric acid found	Recovery of added acid
Mg.	*Mg.*	%	*Mg.*	%	*Mg.*	%
0	0	.....	0.35	.....	0.24	.....
			0.37		0.25	
			Av. 0.36		Av. 0.24	
0.20	0.19	95.0	0.57	105.0	0.44	100.0
	...	100.0	0.56	100.0	0.43	95.0
0.40	0.39	97.5	0.75	97.5	0.63	97.5
	0.41	102.5	0.74	95.0	0.65	102.5
0.80	0.78	97.5	1.18	102.5	1.04	100.0
	0.80	100.0	1.16	100.0	1.07	103.8
1.00	1.01	101.0	1.36	100.0	1.25	101.0
	1.02	102.0	1.37	101.0	1.24	100.0
1.20	1.23	102.5	1.56	100.0	1.44	100.0
	1.19	99.2	1.58	101.7	1.47	102.5
1.60	1.58	98.8	1.95	100.0	1.85	100.6
	1.59	99.4	1.99	102.5	1.88	102.5
1.80	1.79	99.4	2.10	96.3	2.00	97.8
	1.77	98.3	2.17	100.6	2.06	101.1

[a] Citric acid added to distilled water.
[b] Citric acid added to fermented synthetic medium.
[c] Citric acid added to fermented molasses medium.

that has formed is removed by centrifugation. The supernatant liquids are decanted off, and adjusted to known volumes. If the samples do not contain appreciable amounts of reducing material they may be adjusted to known volumes without this preliminary bromine treatment.

Aliquots of these solutions, preferably containing 0.2 to 1.8 mg. of citric acid, are placed in test tubes (the 18 × 150 mm. size is convenient) and 0.3 cc. of the sulfuric acid, 0.2 cc. of the potassium bromide, and 1 cc. of the strong potassium permanganate solutions are added. The total volumes are adjusted to about 5 cc. and the tubes are allowed to stand for 5 minutes at room temperature. At the end of this period they are chilled in an ice bath, and the excess permanganate is decolorized with the hydrogen peroxide solution. Care must be taken to keep the reaction mixtures below 5° C. during this step. Any excess peroxide is removed with the weak permanganate. The total volumes are then adjusted to 10 cc. (the test tubes should have a 10-cc. calibration mark for this purpose) and 13 cc. of the petroleum ether are added. The tubes are stoppered, shaken vigorously, and centrifuged (to break any emulsion that might be formed).

Colorimeter test tubes are prepared containing 5 cc. of the water-dioxane mixture and 5 cc. of the sodium sulfide solution, and 10-cc. portions of the petroleum ether extract containing the pentabromoacetone are added. The colorimeter tubes are then stoppered, shaken vigorously, and centrifuged. The color produced should be a light yellow and will be fully developed in 5 minutes. It is stable for several hours. The absorption is determined in a photoelectric colorimeter at 450 mμ. Light absorption by the solution has been found to be reasonably constant from 400 to 450 mμ. A tube containing no citric acid, but which has gone through the same procedure, is used as a 100% transmission standard. At least two known samples of citric acid should be run with each set of analyses. The color follows Beer's law, as is shown in Table I, column 3, and the standards are used to calculate k in the equation $\log T = kc$.

If too large a sample of citric acid has been used, a second smaller aliquot of the petroleum ether extract may be taken, thus avoiding another complete analysis.

As is shown in Table I, the method gives satisfactory results when between 0.2 and 1.8 mg. of citric acid is present in the sample. Larger samples of citric acid may be used. In such cases larger volumes of petroleum ether for extraction of the pentabromoacetone, or smaller aliquots of the petroleum ether extract containing the pentabromoacetone, may be used. However, several known solutions must always be determined in exactly the same manner as the unknown samples.

Recoveries of added citric acid from two types of fermentation media are also shown in Table I. Solution B contained citric acid, oxalic acid, and unfermented sucrose as the major organic constituents. The solution had been prepared for analysis by removing the mycelium, and adjusting the fermented liquor to a known volume. Various amounts of citric acid were added, as shown in Table I, to an aliquot of this solution corresponding to 0.02 cc. of the original fermented liquor. The same procedure was repeated with solution C, a partially fermented beet molasses medium.

This method has also been applied to the determination of citric acid in tissue extracts. Quantitative recoveries were obtained when citric acid was added to muscle extracts and human seminal fluid. Isocitric acid, *cis*-aconitic acid, *trans*-aconitic acid, and oxalacetic acid do not interfere with this method (Table II) when present in biological samples. Gluconic acid, which is often found in media fermented by *A. niger*, does not interfere. Pucher *et al.* (*6*) list several other acids often found in fermentation media which are not converted to pentabromoacetone under the conditions of this method and thus do not interfere with the determination of the citric acid.

The following critical points have been noticed:

An excess of hydrogen peroxide in the solution before the petroleum ether extraction leads to low recoveries. The presence of excess potassium permanganate leads to high recoveries.

The solution must be thoroughly chilled before excess permanganate is removed; otherwise, recoveries are erratic.

Some stabilizing agent must be present to stabilize the colored reaction product of the pentabromoacteone and the sodium sulfide. Both 50% dioxane-water and 50% pyridine-water solutions have proved satisfactory.

Interfering materials may be removed from the petroleum ether by acid washing.

The pentabromoacetone should not be allowed to remain in the petroleum ether for more than 15 minutes.

Table II. Specificity of Method

Substance Tested	Maximum Sample Used	Equivalent of Citric Acid Found
	Mg.	*Mg.*
Isocitric acid	18.8	<0.01
cis-Aconitic acid	1.74	<0.01
trans-Aconitic acid	1.74	<0.01
Oxalacetic acid	6.0	<0.01
Gluconic acid	5.0	<0.01

DISCUSSION

The advantage of these modifications over the method as originally proposed by Pucher *et al.* (*4*) are: (1) All manipulations are done in two or at most three test tubes. (2) The extraction procedure which required the use of separatory funnels and the repeated extraction has been simplified. (3) The technique does not have to be rigidly standardized, as several known samples are included with every set of analyses.

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Analysis of Acetylsulfanilyl Chloride by the Karl Fischer Reagent

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A method is presented for the analysis of acetylsulfanilyl chloride (an intermediate in the production of sulfonamide drugs) for both acetylsulfanilyl chloride content and moisture. The former is obtained by reacting the sample with water in pyridine, measuring the unreacted water by means of the Karl Fischer reagent, and calculating, by difference, the water which has reacted. This is corrected for the water present in the sample, which also reacts. The water in the sample is determined by a titration in methanol with the Fischer reagent in the absence of pyridine. The water determination has an accuracy represented by a standard deviation of ±0.012% of water. The precision of the acetylsulfanilyl chloride determination is represented by a standard deviation of ±0.18% of acetylsulfanilyl chloride, with probably no systematic error. The effect upon the values of such impurities as acetone in the methanol reagent, sulfanilic acid in the sample, and other substances is discussed. The method may be applicable to other acid chlorides.

ACETYLSULFANILYL chloride has assumed importance as an intermediate in the production of sulfonamide drugs, where it is used to form acetylated sulfonamides by condensation with various amines:

$$CH_3CO.NH.C_6H_4.SO_2Cl + NH_2R \longrightarrow CH_3CO.NH.C_6H_4.SO_2NHR + HCl$$

In this connection, it became desirable to develop a method for determining both the acetylsulfanilyl chloride and the moisture content of the intermediate.

The development of the Karl Fischer reagent as an almost specific reagent for the determination of water (*2, 3, 4, 6, 11*) has been a noteworthy achievement. This reagent has not merely been used to determine the water content of substances, but has been applied to the determination of many classes of compounds which will react either with water or with appropriate reagents to liberate water. The amount of substance can thus be determined by measuring with the Fischer reagent the loss or gain of water in the reaction mixture. In this way, substances such as alcohols (*1*), organic acids (*7*), acid anhydrides (*12, 13*), and carbonyl compounds (*8*) have been analyzed. Since sulfonyl chlorides, such as acetylsulfanilyl chloride, can react with water

$$CH_3CO.NH.C_6H_4.SO_2Cl + H_2O \longrightarrow CH_3CO.NH.C_6H_4.SO_3H + HCl$$

it seemed reasonable to suppose that the Fischer reagent could be used for the analysis. It was found that the sample could be treated with a solution of water in pyridine, the water content of which had been found by the Fischer reagent, and the consumption of water determined by a titration with the Fischer reagent. Acetylsulfanilyl chloride may, however, contain varying amounts of free water, and so it is necessary to correct for its presence. When the sulfonyl chloride was dissolved in methanol, the water reacted with the chloride only to a slight extent (for which a correction could be applied), thus permitting determination of the water. This agrees with Schroeter's (*9*) observation that the chloride can be precipitated essentially unchanged from ethanol by addition of water. The authors have confirmed this observation, using methanol instead of ethanol. The obvious explanation of the difference in reactivity of the sulfonyl chloride with water in the presence or absence of pyridine is that the pyridine acts as a necessary intermediary by removing the hydrogen chloride formed in the reaction.

This work was restricted to analysis of the acetylsulfanilyl chloride, because of specific interest in that compound, but it would seem a reasonable expectation that other acid chlorides, including sulfonyl chlorides, could be analyzed in a similar manner. (After this paper was written, the authors learned that a method had been reported, *10*, for determining acetyl chloride by hydrolysis, employing the Fischer reagent.)

METHOD OF ANALYSIS

REAGENTS. The Karl Fischer reagent is prepared according to the directions of Smith, Bryant, and Mitchell (*11*) and is dispensed from an automatic buret provided with tubes of Indicating Grade activated alumina to protect it from access of moist air. It is standardized by weighing 2 or 3 drops of water from a Lunge pipet into 10 ml. of anhydrous methanol and titrating with the Fischer reagent. (In this and in the following titrations the end points were observed visually.) From the difference between the volumes of reagent consumed in this titration and by the anhydrous methanol, the weight of water (grams) equivalent to 1 ml. of the reagent, T, may be calculated.

This laboratory has found this method preferable to standardization through a solution of water in methanol. There is less chance of error because of the possible danger of changes in the water content of a standard water solution. The two methods of standardization are equally precise, being equal to a standard deviation of about ±0.0035 mg. of water per ml. of reagent, or about ±0.1% of the standardization value.

The methanol used must be of the highest quality in respect to freedom from acetone, since the presence of acetone causes errors, discussed below.

A standard solution of water in pyridine is prepared, to contain close to 0.015 gram of water per ml. of solution. This solution is dispensed from an automatic buret protected from access of moisture from the air, and 10-ml. portions with 25 ml. of methanol added are titrated with the Fischer reagent in order to get the exact water content. Let E = ml. of Fischer reagent for 10 ml. of the pyridine solution plus 25 ml. of methanol. The water content of the reagent will not change if the buret is well protected, except in so far as the water content of the methanol changes and the volume of the pyridine solution alters because of temperature changes. Under normal conditions and except for work of the most extreme accuracy, the change due to this cause may be neglected. E will change with any deterioration in the Fischer reagent itself, and must, therefore, be checked frequently.

ANALYSIS OF SAMPLE FOR ACETYLSULFANILYL CHLORIDE. From a glass-stoppered weighing bottle, weigh by difference 1 to 2 grams of the sample into a dry 125-ml. Erlenmeyer flask, which is stoppered at once with a two-hole rubber stopper. One hole is fitted with a drying tube, while the other fits over the tip of the buret. Cool the flask containing the sample in an ice bath in order to prevent excessive heating upon addition of the pyridine-water solution. (Such overheating causes condensation of moisture on the upper part of the flask and spoils the analysis.) With the flask in the ice bath, introduce 10 ml. of the pyridine-water solution, stopper the flask immediately, and swirl gently to dissolve the sample completely. Keep the flask in the ice bath only long enough to prevent the temperature from rising. Set it aside after complete solution of the sample for 10 minutes from the time the pyridine-water solution is added. The temperature of the solution at the end of this period should be only slightly below room temperature to avoid condensation of moisture in it when the flask is opened to introduce, from a protected automatic buret, 25 ml. of the methanol which has been used in determining E. (The methanol is added to dilute the yellow color of the solution in order to avoid difficulty with the end point.) Allow the methanol to wash down the side of the flask and immediately titrate with the Fischer

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reagent to obtain F, the number of milliliters of Fischer reagent required for the water left unconsumed by the sample. In order to ensure the presence of sufficient water to hydrolyze the sample completely, F should be greater than the blank for 25 ml. of methanol. Otherwise repeat the determination with a smaller sample.

ANALYSIS OF SAMPLE FOR WATER. To a well-dried 125-ml. Erlenmeyer flask rapidly add from a weighing bottle 2 to 2.5 grams of the sample. Minimize exposure of the sample to the air. Keep the flask well stoppered except when adding reagents or titrating, and then substitute for the solid stopper a stopper containing two openings—one provided with a drying tube, the other for introduction of a buret tip. From an automatic buret protected from moisture, add 25 ml. of absolute methanol. Swirl the mixture to get complete solution of the sample and immediately titrate rapidly with the Fischer reagent to the first end point which persists for at least 1 minute. Call this volume K. Obtain a titration value for the 25 ml. of methanol. Call this volume L.

CALCULATIONS. There is a slight consumption of water by the sample during this procedure which is proportional to the total amount of water present (including that in the methanol) and must be corrected for. The total number of grams of water in the titrated solution equals KT. The correction to be added to the water content of the sample, expressed as grams of water, equals $47.3 \times 10^{-3}KT - 0.298 \times 10^{-3}$. (The derivation of this correction is described below.) If the total water found is greater than 25 mg., the correction factor is $50.6 \times 10^{-3}KT - 0.337 \times 10^{-3}$.

Let the % water in the sample equal M.

$$\text{Then } M = \frac{KT + 47.3 \times 10^{-3}KT - 0.298 \times 10^{-3} - LT}{\text{weight of sample in grams}} \times 100$$

$$= \frac{T(1.0473K - L) - 0.298 \times 10^{-3}}{\text{weight of sample in grams}} \times 100$$

The consumption of water from the pyridine-water reagent by the sulfonyl chloride (equivalent to $E - F = G$) is less than the total amount of water consumed by the amount of water present in the sample itself, which is also consumed in the reaction. Let the percentage of acetylsulfanilyl chloride, uncorrected for the water in the sample, equal H. Then

$$H = \frac{GT \times 233.67 \times 100}{\text{weight of sample in grams} \times 18.016}$$

where 233.67 = the molecular weight of acetylsulfanilyl chloride and 18.016 = the molecular weight of water. Or

$$H = \frac{12.97GT}{\text{weight of sample (grams)}}$$

H must be corrected for the water in the sample by means of the expression:

$$\% \text{ acetylsulfanilyl chloride} = H + 12.97M$$

CORRECTION FOR WATER CONTENT

It was suspected that a slight reaction took place between the acetylsulfanilyl chloride and the water contained in it when the sample was dissolved in alcohol to determine the water content. The extent of this error was determined by adding water and determining how much could be found by analysis.

Acetylsulfanilyl chloride was recrystallized twice from chloroform, washed with petroleum ether, and dried under vacuum over sodium hydroxide and paraffin. The water content (uncorrected) of this sample was 0.019% at the start of the series of experiments and 0.036% at its conclusion. Methanol was dried according to the method of Lund and Bjerrum (*5*) by refluxing with magnesium and iodine and then distilling until successive cuts gave a constant titration with the Fischer reagent. The methanol which was used had a blank value that varied between 1.1 and 1.4 mg. of water per 25 ml.

In order to conform as closely as possible to the actual conditions of the water determination with respect to the time allowed for reaction of the sample with the water in the solution, the tests were carried out as follows: The weighed sample in a stoppered flask was placed near a weighed Lunge pipet containing water and also near the automatic buret containing the methanol. Twenty-five milliliters of methanol were added to the sample, the mixture was swirled for a few seconds to effect solution, and some water was immediately added from the Lunge pipet (which was equipped with a rubber stopper after weighing in order to minimize absorption of water from the air by the methanol during the addition of water from the pipet). The contents of the flask were immediately titrated with the Fischer reagent and finally the Lunge pipet was reweighed to determine the exact weight of water added.

Table I gives the data from these determinations. Column 2 is the sum of the water present in the methanol and in the sample (as determined by the Fischer titration) and of the added water.

The values in columns 2, 3, and 4 would seem to indicate some correlation between the water lost and the total water found or present. On the assumption that this correlation could be expressed as a linear function and that column 3 contains the independent variable, the best linear equations were calculated (by the method of least squares) using all 21 values in one case, and only the first 17 values in the other case. The latter equation was calculated because usually the determinations have involved a total water content of less than 25 mg.

Correction in grams of water =

$$50.6 \times 10^{-3} \times \text{grams of water found} - 0.337 \times 10^{-3} \quad (1)$$

Correction in grams of water =

$$47.3 \times 10^{-3} \times \text{grams of water found} - 0.298 \times 10^{-3} \quad (2)$$

Table I. Determination of Error in Analysis for Water

Acetylsulfanilyl Chloride Added	Total Water Present	Total Water Found	Water Lost (Present Minus Found)
Grams	*Mg.*	*Mg.*	*Mg.*
2.594	3.0	3.0	0.0
2.589	3.3	3.0	0.3
2.096	3.3	3.8	−0.5
2.496	6.1	6.3	−0.2
2.717	7.5	7.7	−0.2
2.277	8.3	8.2	0.1
2.721	12.5	12.2	0.3
2.021	12.7	12.4	0.3
2.432	13.3	13.1	0.2
1.961	15.2	14.6	0.6
1.770	15.6	15.2	0.4
2.163	15.9	15.3	0.6
1.927	16.0	15.4	0.6
2.075	18.2	18.0	0.2
2.358	20.8	19.9	0.9
2.464	22.8	22.1	0.7
1.974	25.5	24.7	0.8
1.952	33.3	32.4	0.9
2.263	44.4	42.3	2.1
2.173	60.1	57.0	3.1
2.098	71.1	68.4	2.7

PRECISION AND ACCURACY

The deviations of the values in column 4 of Table I from the corresponding values calculated from Equations 1 and 2, obtained by substituting in the equations the appropriate values in column 3, measure the precision of the water determination. This also measures the accuracy, provided one neglects the slight error due to the fact that a part of each value in column 2 is obtained by an uncorrected titration of the water present in the sample; but since the latter is low, this error is negligible.

Using Equation 1, the average deviation (for all the 21 values in Table I) is ±0.20 mg. of water (the maximum deviations being +0.57 and −0.40 mg.) and the standard deviation (root-mean square) is ±0.27 mg. of water. This would be equivalent to ±3.5 mg. of acetylsulfanilyl chloride and, for an average sample weight for the water determination of 2.1 grams, it would amount to ±0.17% of acetylsulfanilyl chloride.

Using Equation 2, for 17 values (with maximum deviations of +0.46 and −0.38 mg.), the average deviation is ±0.17 mg. of water and the standard deviation is ±0.22 mg. of water. The equivalent values for acetylsulfanilyl chloride would be ±2.9 mg. or ±0.14% of acetylsulfanilyl chloride.

From duplicate analyses of 17 actual samples, a standard deviation from the respective means of ±0.23 mg. of water was calculated; or, for a 2-gram sample, ±0.012% of water. From duplicate analyses for acetylsulfanilyl chloride on the same 17 samples, a standard deviation of ±2.3 mg. of acetylsulfanilyl

chloride was calculated; or, for an average sample weight of 1.3 grams, ±0.18% of acetylsulfanilyl chloride. There is no reason to believe that the analysis has any systematic error, but because of the difficulty of preparing a sample of known purity this cannot be stated with certainty. Some samples have, however, been analyzed which gave values not far from 100%.

EFFECT OF VARIATIONS IN TIME OF HYDROLYSIS

The method calls for a period of 10 minutes after the addition of the pyridine-water solution for the completion of the hydrolysis. Actually, the reaction is much more rapid. A series of determinations was made on a single sample in order to test this point. In order to separate the time variable from that which would be introduced by the presence toward the end of the reaction of variable concentrations of Fischer reagent, the volume of pyridine-water solution was varied according to the size of the sample (since it was not convenient with a substance like acetylsulfanilyl chloride to keep the sample weight constant), so that the back-titration with the Fischer reagent would be fairly constant. The values obtained, all of which agreed within the experimental error of the method, showed that it is immaterial whether the hydrolysis is allowed to proceed for 3 or 25 minutes.

EFFECTS OF IMPURITIES OTHER THAN WATER

The Fischer reagent is specific for water, with the exception that certain inorganic oxides will also be titrated (*6*) and occasionally a substance may be met with, such as hydroxylamine (*8*), which will react and so will require special treatment to avoid interference. In acetylsulfanilyl chloride, some possible impurities may be acetylsulfanilic acid, hydrochloric acid, acetic acid, sulfanilic acid, and water. Obviously, neither acetylsulfanilic acid nor hydrochloric acid interferes in the determination of acetylsulfanilyl chloride because they are products of the reaction, and the reaction has been shown to proceed as expected with recrystallized material. No additions of these two substances were made to test their effect upon the determination of water, but no difficulty was obtained in analyzing for water in rather impure samples which would contain these substances if they were normal impurities. A method has been reported for determining acetic acid by its esterification with methanol (*7*) to liberate water, but since this requires a boron trifluoride catalyst, it does not interfere here.

Sulfanilic acid does not introduce any error into the water determination. It does introduce an error into the acetylsulfanilyl chloride determination. Presumably, it reacts with the compound as follows:

$$HO_3S.C_6H_4.NH_2 + ClO_2S.C_6H_4.NH.COCH_3 \longrightarrow HO_3S.C_6H_4.NH.O_2S.C_6H_4.NH.COCH_3 + HCl$$

thus preventing an equivalent quantity of the chloride from reacting with the water.

Table II gives data showing the quantities of the sulfonyl chloride which have been consumed by varying amounts of added sulfanilic acid. The ratio of chloride consumed to sulfanilic acid present averages 0.99, with considerable variation of the individual ratios from this average. The variations from the 1 to 1 ratio are probably not indicative of a real departure from the stoichiometric proportions, but point to certain experimental difficulties. For example, the mixtures with small amounts of sulfanilic acid might show variations because of the low equivalent net volume of Fischer reagent for the sulfanilic acid. In other cases, the difficulty may be that the sulfanilic acid, which is added as a solid, dissolves more slowly than the sulfonyl chloride in the water-pyridine solution, so that the chloride may react preferably with the water. There is also additional exposure to atmospheric moisture.

The sulfanilic acid present may be determined by acidification and titration with sodium nitrite solution at room temperature.

Table II. Effect of Sulfanilic Acid

(1) Sulfanilic Acid Added	(2) Acetylsulfanilyl Chloride Taken	(3) Acetylsulfanilyl Chloride Found	(4) Acetylsulfanilyl Chloride Consumed (2) − (3)	(5) Ratio (4) to (1)
Millimole	*Millimoles*	*Millimoles*	*Millimole*	
0.0583	7.006	6.942	0.064	1.10
0.1243	5.853	5.742	0.111	0.89
0.1942	4.243	4.099	0.144	0.74
0.4131	5.079	4.651	0.428	1.04
0.6390	5.923	5.189	0.734	1.15
0.6674	4.380	3.690	0.690	1.03
				Av. 0.99

A correction for the sulfonyl chloride consumed may then be applied. All the samples examined by the authors had so little sulfanilic acid that the possible variations in the stoichiometric ratio just mentioned were of no practical significance.

Another impurity which may cause difficulty is acetone in the methanol reagent.

A sample of chloride was analyzed for water, using in one set of determinations methanol containing 0.003% of acetone, and in the other set methanol containing 0.34%. The first set gave a value of 0.06% and the second set a value of 0.50% of water in the chloride. Another sample was analyzed in the same manner, in one case using methanol with only a trace of acetone in it and in the other the same methanol to which 0.35% of acetone had been added. The latter methanol solution gave a value of 0.79% water in the chloride in comparison with a value of 0.13% water obtained using the pure methanol.

Acetone may interfere with the Fischer titration for water in alcohol because of ketal formation, which causes the liberation of water. However, addition of acetone to methanol in the concentrations mentioned did not cause an increase in the apparent water value of the methanol. There would seem to be some specific effect of the chloride involved, possibly in catalyzing ketal formation. Since a blank value would thus not correct for this effect, the presence of any but the merest trace of acetone in the methanol will lead to high water values for the chloride, with a consequent high value for chloride.

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Osmometry of High-Polymer Solutions

APPARATUS

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A glass osmometer of simple design is described which is especially useful for osmotic pressure measurements of high-polymer solutions that tend to form a stable foam. The assembly and operation of the osmometer are described and data are presented to illustrate the order of reproducibility obtained.

THE determination of the osmotic pressure of high-polymer solutions is, at present, the most feasible method of measuring the colligative properties of these systems, since ultracentrifuge equipment is very expensive and its use is accordingly restricted.

The osmotic pressure of a solution may be determined by either the static-elevation method, which involves the measurement of the liquid head developed by the influx of solvent into the solution through a suitable semipermeable membrane by the operation of osmotic forces arising from the difference in the activities of the solvent molecules in the two phases, or the dynamic-equilibrium method, in which the magnitude of an externally applied pressure necessary to counterbalance the osmotic pressure is determined.

While it is possible to use either method or a combination of them with any osmometer, the designs of the various instruments described in the literature are sufficiently different, depending upon which method is to be used, to permit classifying them as static-elevation osmometers (*1, 4, 7, 8, 12, 14, 22, 23, 24*) and dynamic-equilibrium osmometers (*2, 3, 5, 6, 11, 13, 15, 21, 25*). The principal advantage of the dynamic method is the rapidity with which a measurement can be made. In order to obtain this advantage effectively, it is necessary to employ a large membrane, with adequate support to minimize "ballooning" effects. This requires a relatively complex instrument, involving specially milled channels, metal-to-glass seals, needle valves, stopcocks, etc. These latter factors increase the possibility of leaks and the entrapment of air, especially if the solution being measured has a tendency to form a stable foam.

The static-elevation instrument avoids most of these difficulties because of its inherent simplicity of design and of operation. The disadvantage of the longer period of time required to complete a single determination can be mitigated by proper selection of instrument dimensions and, since this type of instrument is compact and inexpensive, by operating a number of them simultaneously, using solutions of different concentration or of different polymers.

In Figure 1 is shown a static-elevation osmometer which has been used in this laboratory for the osmometry of a number of high polymers in a variety of solvents. This instrument is a modification of the osmometer described by Schulz (*24*). The transparency obtained by the use of a heavy-walled glass cell and the use of a glass-stoppered cylinder of sufficient height to enclose the inner assembly completely and assure a vertical position are the principal improvements. An etched scale on the capillary is optional and is suggested as a possible means of avoiding the use of a cathetometer.

OSMOMETER AND MODE OF ASSEMBLY

The inner assembly (Figure 1) consists of a 4-mm. wall glass cell, *A*, to which the capillary, *B*, of about 0.7-mm. bore is fastened through a 7/25 ground-glass joint. The metal portions of the inner assembly consist of a clamp base, *C*, a clamp yoke, *D*, and their fasteners. Although brass is satisfactory for many systems, it is advisable to use either 18–8 stainless steel or nickel, the latter being preferred. (Specifically, nickel-plated brass and 18–8 stainless steel have been found unsatisfactory when used with aqueous phosphate or phthalate buffers.) The base should be perforated as shown, using a No. 1 drill. The flat membrane of the assembled cell is shown at *E*, resting on a support of ash-free filter paper, *F*.

Before a new cell is put into service, the footing of cell *A* should be inspected for flatness against a piece of plate glass, and, if necessary, ground flat using 1600-mesh emery powder. The glass joint should also be tested for possible leaks. This test is conducted as follows: The joint is wet with acetone and seated immediately. The end of a low-pressure 0.3 kg. per sq. cm. (5 pounds per sq. inch) air hose is held at the place inside *A* where the inner joint of the capillary tube comes through, forcing the hose against the shoulder of the cell. This will tend to displace the liquid seal and will quickly do so if the joint is even slightly leaky. If the joint is satisfactory, the seal will withstand this pressure for 30 to 60 seconds without drying out. If the joint fails to pass this test, it should be ground with 1600-mesh emery powder until it does. It is important that the inner portion of the joint extend beyond the grinding of the outer portion of the joint, if grinding is to produce any improvement. The cell and its capillary should then be marked, so that this particular pair will always be used together.

The process of assembling the osmometer is very simple. A disk of filter paper is cut out or punched out with a hardened steel punch of sufficient size to cover the floor of the clamp base, *C*. The base and the paper are wetted with solvent and a similar disk of previously prepared membrane material is laid on the paper, care being taken not to allow the membrane to become dry. A clean cell is placed on the membrane and the yoke, *D*, is put into place and tightened. It will not be necessary to tighten the yoke screws excessively—a little more than finger-tight will suffice.

The interior parts of the assembly are rinsed several times with the solution and then completely filled with it. Solution is then sucked into the capillary tube until it is about half full and the tube inserted into the glass joint of the cell, the excess solution being allowed to flow around the unseated joint and not through the upper end of the capillary tube. The final position of the capillary meniscus can be adjusted to any desired level by allowing the solution to flow out around the joint in the manner just

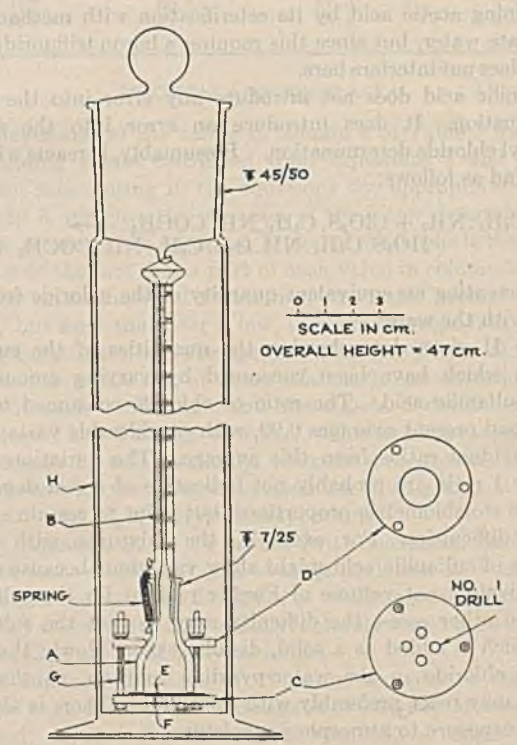

Figure 1. Osmometer

described. (If necessary, the liquid can be forced out by means of a little pressure.) After the desired level is obtained, the joint is firmly seated and secured with a suitable fastener, such as a light spring or rubber band, depending on the solvent. It is important that no air be trapped inside the instrument, and this can easily be avoided after a little experience. No lubricant is used in the joint, for obvious reasons. Pliers whose jaws are covered with sections of medium-walled rubber tubing will be found helpful in seating and unseating the joint (the upper hook, Figure 1, must be sufficiently robust).

The outer surfaces of the assembly are thoroughly washed with solvent to remove all traces of solute and then lowered into the cylinder, *H*, containing about 100 cc. of solvent. This operation will trap some air in the holes of the base, which can easily be removed by raising and lowering the (inner) assembly more or less rapidly while holding the cylinder at an angle of 45 degrees. The level of the external liquid is adjusted to a suitable height and the cylinder closed. In order to obtain a vertical position of the capillary, it is recommended that the inner assembly be suspended from the top of the cylinder. The osmometer should then be thermostated to within ±0.1° C. of the desired temperature, until no further change in the head of liquid is observed.

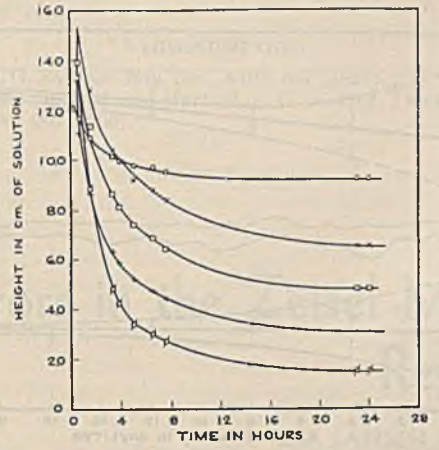

Figure 2. Equilibration Curves at Various Polymer Concentrations

System, polyvinyl alcohol in dilute salt solutions

	Grams/100 ml.
o—o—o.	2.00
x—x—x.	1.60
□—□—□.	1.20
+—+—+.	0.80
ϕ—ϕ—ϕ.	0.40

It will be found advantageous to adjust the initial positions of the inner and outer levels to as near the anticipated final head of liquid as prior information regarding the systems permits. This procedure reduces the time required for equilibration which, in extreme cases, may amount to from 8 to 24 hours, depending on the temperature, the solvent, the initial head, and the permeability of the membrane. Equilibrium may be approached from either above or below if the solution readily wets glass; solutions composed of nonaqueous solvents, such as acetone, benzene, alcohols, chloroform, etc., are examples. With aqueous solutions, however, it is essential to approach the equilibrium position from above—i.e., the determination should be begun with the inner meniscus near the top of the capillary. This will assure a receding angle of contact at the solution-glass-air interface throughout the determination, which is essential in order to secure the proper correction for capillarity (see below).

It is very important that the glass parts of the osmometer be scrupulously clean in making determinations involving aqueous solutions. The following treatment has been found adequate: All glass parts are immersed in a hot concentrated nitric-sulfuric acid bath (1 to 1 by volume) for several hours or overnight, rinsed with distilled water, and immersed in a warm 20% caustic solution for about 5 minutes. They are then rinsed thoroughly with distilled water after a dilute acid rinse; all contact with organic solvents is avoided for drying. The capillary may be dried by sucking clean, dry air through it.

CAPILLARY CORRECTION CONSTANT

The observed height at equilibrium must be corrected for the capillary rise of the solution. This capillary correction constant is easily determined by a direct measurement, using the capillary (without the cell) and the solution used in the osmotic-pressure determination. The capillary correction constant obtained by use of the pure solvent may be employed if the surface tension of the solution does not differ from it by more than 5% (calculated for a capillary of 0.7-mm. bore). Since this is usually, if not always, the case, it is not necessary to measure this correction for each individual solution. The precautions regarding cleanliness of glass and the direction of approach to equilibrium discussed above apply to the measurement of the capillary correction constant. A receding water-air-glass angle of contact is, in general, more stable and reproducible than the advancing angle, and it is essential, therefore, to base the capillary correction on it.

The relatively unstable water-air-glass interface can be eliminated altogether by a somewhat more complicated procedure using, in the capillary, an immiscible liquid, such as toluene or xylene. A liquid-glass interface is formed in the inner part of the glass joint by first sucking a small quantity of organic liquid into the joint, followed by some of the solution being measured. A little experience will be necessary to get the correct quantity of organic liquid, so that the liquid-liquid interface is in the wide part of the joint and the liquid-air interface is near the top of the capillary. The capillary tube and the cell are then assembled as previously described. The final head of liquid, in pressure units of centimeters of liquid of unit density, will be the algebraic sum of the height-density products of the two liquid columns.

MEMBRANES

Regenerated cellulose, which has not been dried out, is probably the most satisfactory material.

Commercial cellophane in this form can be obtained from several manufacturers (Sylvania Industrial Corp., Fredericksburg, Va., and E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). The material should not be waterproofed and should have a wet thickness (in water) of about 0.1 mm. (0.004 inch). It is important to specify that the material be free of wrinkles. Undried commercial cellophane has been used in the osmometry of such diverse systems as polyisobutylene in cyclohexane (*11*), polyvinyl acetate in acetone and in benzene, polyvinyl alcohol in water and in dilute salt solutions, and various cellulose esters in acetone (*26*).

Denitrated collodion may be used, although it is, generally speaking, not so uniform as cellophane. Flory (*11*) states that denitrated collodion membranes are more permeable than swollen cellophane, although this depends largely on the manner of preparation (*9*, *10*).

Membranes are conditioned for use by washing thoroughly with distilled water to remove all formaldehyde (the stock should be stored in a dilute formaldehyde solution to prevent bacterial action), and the water is then displaced with acetone if the membrane is to be used with organic liquids. The water should be displaced gradually by washing the material in successive aqueous acetone solutions of increasing acetone concentration—e.g., in four solutions of 25, 50, 75, and 100% acetone. This method does not materially alter the permeability of the membrane (*20*). Pure acetone can be displaced directly with any desired liquid.

Occasionally, a membrane behaves erratically, which is manifested by inconsistently low final pressure results or by an irregular equilibration curve. This is presumably due to some structural anomaly in the material itself. The possibility of solute permeation should not be overlooked, especially if the average molecular weight of the polymer is very low. (Flory, *11*, measured polymers with molecular weights as low as 6000, using swollen cellophane membranes. Measurements of polymers of 10,000 to 15,000 have been made in this laboratory.) This can be determined by a suitable test of the external liquid (dialyzate) at the conclusion of an osmotic-pressure determination.

TREATMENT OF DATA

After the capillary correction constant, h_σ, has been subtracted from the observed equilibrium height, $h_{obs.}$, the resultant height is the osmotic pressure of the polymer in centimeters of solution. It is desirable to express this pressure in units which are independent of the density, so that values obtained for various

Table I. Reproducibility of Osmotic-Pressure Determinations

Solute	Solvent	Concentration G./100 cc.	Temperature ° C.	Osmotic Pressure $\pi \times 10^3$ atm.
Cellulose acetate (40.4% acetyl)	Acetone	1.00	25	4.5, 4.7
Cellulose acetate-butyrate (13.0% acetyl, 37.0% butyryl)	Acetone	1.35	25	6.9, 7.0
Cellulose nitrate (11.95% N)	Acetone	1.19	25	10.1, 10.3
Cellulose butyrate (55.6% butyryl)	Acetone	1.00	25	5.0, 5.0, 4.9
Cellulose acetate-phthalate (19.6% acetyl, 30.3% phthalyl)	Acetone-methyl cellosolve (80–20 by volume)	1.00	25	5.0, 5.2, 5.2
Polyvinyl acetate, RH 361	Acetone	1.00	25	1.9, 1.8
	Benzene	1.00	25	1.9, 1.9
Polyvinyl acetate, Gelva V-60	Acetone	2.00	25	9.0, 9.3
	Acetone	2.50	25	14.7, 14.8
Polyvinyl alcohol, RH 393	Water	1.20	25	7.8, 8.2, 7.7, 8.2
Gelatin, lime-processed deashed calf, (B461-48D)	Water	2.00	40	6.5, 5.9, 6.3
	Water	2.50	40	8.9, 8.7, 8.7

systems can be compared directly. The pressure in atmospheres, π, can be calculated by using the following equation:

$$\pi = \frac{(h_{obs.} - h_\sigma)d_s}{1033} \qquad (1)$$

where d_s is the density of the solution in grams per cubic centimeter, which should be known to about ±0.005. It will be sufficiently accurate to use density values obtained by interpolation from a straight line drawn between the known or measured density of the solvent and the measured density of a solution of approximately the maximum concentration to be studied.

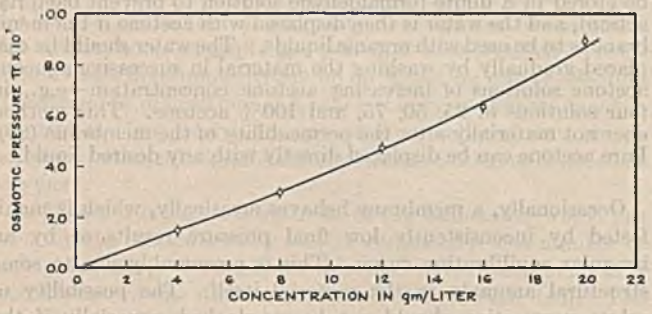

Figure 3. Dependence of Osmotic Pressure on Polymer Concentration
System, polyvinyl alcohol in dilute salt solution

It is recommended that the osmotic pressure be plotted as a function of the polymer concentration, and the best average curve through these points and the origin then drawn. The pressure values at any desired concentration may then be read off for the calculation of any specific function of pressure and concentration. This process yields a "graphical average", and is more satisfactory in many cases than calculating the function directly from the data.

Probably the most accurate manner of plotting osmotic data for general high-polymer studies is according to the following equation, suggested by Huggins (*17*, *19*):

$$\frac{\pi}{C_2} - \frac{RTd_1}{3M_1d_2^3} \times C_2^2 = \frac{RT}{M_2} + \frac{RTd_1}{M_1d_2^2}\left(\frac{1}{2} - \mu_1\right)C_2 \qquad (2)$$

where π is the pressure in atmospheres, C_2 is the polymer concentration in grams per cubic centimeter, R is the gas constant in cubic centimeters atmospheres per degree per mole, T is the absolute temperature, d_1 and d_2 are the densities of solvent and solute, respectively, M_1 and M_2 are the molecular weights of solvent and solute, respectively, and μ_1 is a constant depending on the nature of the solvent and solute. The second term on the left in Equation 2 is negligible for many systems, but may be significant in others (*17*, *18*, cf. Figure 4). By plotting the term, or terms, on the left against C_2, a straight line should be obtained (at least at the lower concentrations), whose intercept is inversely proportional to the number-average molecular weight of the polymer (cf. Equation 3):

$$\lim_{c \to 0}\left(\frac{\pi}{C_2}\right) \cong \lim_{c \to 0}\left(\frac{\pi}{C_2} - \frac{RTd_1}{3M_1d_2^2} \times C_2^2\right) = \frac{RT}{M_2} \qquad (3)$$

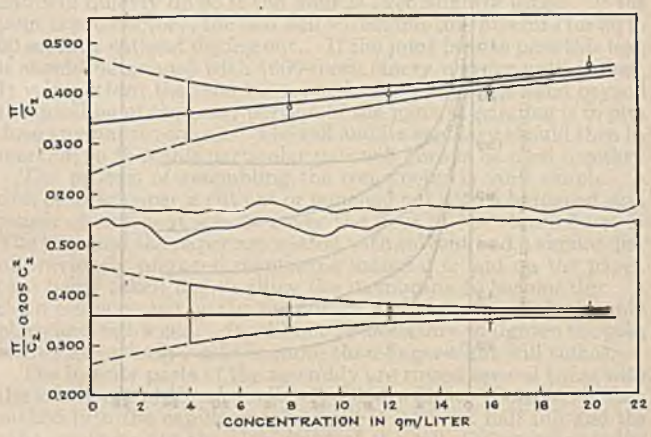

Figure 4. Dependence of Osmotic Pressure–Concentration Ratio (with and without Correction Term) on Polymer Concentration
System, polyvinyl alcohol in dilute salt solution

For a discussion of the significance of the μ_1 value, which is related to the slope, reference is made to the original papers of Huggins (*16*, *18*, *19*).

REPRODUCIBILITY OF EXPERIMENTAL RESULTS

The precision obtained with aqueous solutions is lower than that obtained with organic liquid systems. This is shown in Table I. Aqueous systems usually show an uncertainty of about 2 or 3 × 10^{-4} atmosphere (2 or 3 mm. of water), which amounts to 10 or 20% of the total pressure in the very low pressure region. The effect of this uncertainty is shown graphically by the vertical lines in Figures 3 and 4. The results obtained with a commercial "high-viscosity" polyvinyl alcohol in dilute salt solution (Du Pont's No. RH 630 in a phosphate buffer solution containing 1.4 × 10^{-3} mole of sodium monohydrogen phosphate, 7.8 × 10^{-3} mole of sodium dihydrogen phosphate, and 1.3 × 10^{-2} mole of sodium chloride per liter. The ionic strength of the "solvent" = 0.025 mole per liter) are collected in Table II and shown in Figure 2 (equilibration curves), Figure 3 (pressure-concentration dependence), and Figure 4 (reduced pressure-concentration dependence) (cf. Equation 2).

Extrapolation of either plot—i.e., with and without the 0.205 C_2^2 term—yields an intercept from which may be calculated a number-average molecular weight of 70,000, with an uncertainty of approximately 10%. For similar systems of higher molecular weight, this uncertainty increases rapidly and soon becomes prohibitively large—for example, at M_2 = 225,000, the uncertainty will amount to more than 50%.

Table II. Osmotic Pressure of Polyvinyl Alcohol, RH 630, in Dilute Salt Solution

C_2, Gm./Cc.	$h_{cm.}$, Water	$\pi \times 10^4$ Atm.	π/C_2	$0.205 \times C_2^2$	$\frac{\pi}{C_2} - 0.205 \times C_2^2$
0.0040	1.5_0	1.4_5	0.36_3	0.00_3	0.36_0
0.0080	3.0_6	2.9_6	0.37_0	0.01_3	0.35_7
0.0120	4.8_8	4.7_2	0.39_4	0.02_9	0.36_5
0.0160	6.5_4	6.3_2	0.39_5	0.05_2	0.34_3
0.0200	9.2_7	8.9_7	0.44_9	0.08_2	0.36_7

R = 82.1 cc. atm. per degree per mole. T = 298° A. d_1 = 1.00 gram per cc. M_1 = 18. d_2 = 1.62 grams per cc.

The reproducibility of measurements of nonaqueous polymer solutions is about two to three times better, and therefore a considerably greater range of molecular weight determinations can be obtained with an accuracy of 10% or less.

ACKNOWLEDGMENT

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Errors in the Zeisel Methoxyl Values for Pectin Due to Retained Alcohol

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Retained ethanol causes the methoxyl content of pectins, as measured by the Zeisel method, to be as much as 20% higher than saponification values. This ethanol cannot be removed by the usual drying techniques but can be removed by humidification followed by drying. Acetone rather than ethanol precipitation results in good agreement between the two methods of analysis. Retained isopropyl alcohol can be removed by drying at 100° C.

DISCREPANCIES between results obtained by the Zeisel and saponification methods of determining methoxyl in pectin have been observed in this laboratory. The former method has yielded results as much as 20% higher—e.g., a sample analyzed by the two methods showed methoxyl contents of 12.5 and 10.5% by the Zeisel and saponification methods, respectively. An investigation of these differences showed that the disagreement was due to alcohol that could not be removed by ordinary drying procedures but could be removed by humidification prior to drying. When the retained alcohol was removed, the saponification procedure gave results equal to or higher than those by the Zeisel method, the extent of alkali hydrolysis being a function of alkali concentration, time, and temperature.

METHODS

SAPONIFICATION PROCEDURE. The method was a modification of that of Olsen *et al.* (7); stoppered flasks instead of beakers were used in order to decrease the error caused by absorption of carbon dioxide, and the Hinton (4) indicator, which is a mixture of 3 parts of phenol red to 1 part each of bromothymol blue and cresol red, was used instead of phenolphthalein. A 1-gram sample of pectin was placed in a 500-ml. Erlenmeyer flask, a few milliliters of alcohol were added to wet it, followed by 300 ml. of water, and the mixture was allowed to stand until the pectin dissolved. After the solution had been titrated to the indicator end point with 0.1 N sodium hydroxide, 20 ml. of 0.5 N sodium hydroxide were added, the flask was stoppered, and the reaction mixture allowed to stand for 2 to 3 hours at room temperature, whereupon 20 ml. of 0.5 N hydrochloric acid were added and the solution was back-titrated with 0.1 N sodium hydroxide. This last titer corresponds to the saponification value.

ZEISEL METHOD. Clark's (1) modification of the Viebock and Schwappach method for the determination of methoxyl and ethoxyl groups was used. This consists in a volumetric determination of the alkyl iodide formed by the action of hydriodic acid on methoxyl and ethoxyl groups.

HUMIDIFICATION PROCEDURES. The pectin samples in shallow dishes were placed in a humidor comprising a desiccator in which water replaced the drying agent. Toluene was kept on the water to prevent mold growth. Humidification of the pectin could be accomplished by allowing the samples to stand in the humidor or by bubbling a slow stream of air through the water.

RESULTS

Since it seemed likely that the high Zeisel results were due to ethanol as an impurity in the pectin, an attempt was made to remove the ethanol by drying in an Abderhalden dryer. As can be seen from Table I, this decreased the Zeisel methoxyl content determination to only a small extent.

Mease (5) showed that cotton, wool, silk, and rayon absorb alcohol and hold an appreciable amount of it, even when dried to constant weight at temperatures considerably above the boil-

ing point of alcohol. The alcohol could be removed from the fibers by rinsing in water and again drying. Hilbert and Jansen (2) later found that glucosidocytosine attaches alcohol with such great tenacity that the alcohol could not be quantitatively removed by drying in an Abderhalden dryer at 135° C. at a pressure of 1 mm. or even 10^{-5} mm. of mercury. However, when the material was placed in an atmosphere of high humidity, the alcohol was gradually displaced by water, which was then easily removed by the Abderhalden procedure.

This humidification technique was adopted for several pectin samples selected at random. As can be seen from Table II, the technique brought the results by the Zeisel method into agreement with those by the modified Olsen saponification method. The observation that the disagreement in methoxyl values was less than those reported in Tables I and III was to be expected, since some atmospheric humidification had undoubtedly taken place during storage.

Table I. Effect of Abderhalden Drying on Methoxyl Content of Pectins Having Higher Zeisel than Saponification Methoxyl Values

Sample[a]	OCH_3 Before Drying: Saponification	OCH_3 Before Drying: Zeisel	Loss in Weight on Drying	OCH_3 After Drying[b]: Saponification	OCH_3 After Drying[b]: Zeisel
	%	%	%	%	%
1. Citrus, 200 grade, purified	10.5	12.5	5.95	10.5	12.1
	10.6	12.4	....	10.4	12.1
2. Citrus, 185 grade	9.1	9.8	7.27	9.1	9.9
	9.1	9.8	....	9.0	9.6
3. Citrus 178 grade	10.1	11.6	2.35	10.2	11.2
	10.2	11.7	....	10.3	11.2

[a] Sample 1 purified according to method of Olsen *et al.* (7) including drying in vacuum oven. Sample 3 dissolved, reprecipitated with ethanol, and dryed in vacuum oven at 70° C. overnight previous to analysis and Abderhalden drying. Samples 1 and 2 dried in P_2O_5-containing Abderhalden dryer at 78° C. at pressure less than 1 mm. of Hg for 8 hours. Sample 3 was dried in dryer at 100° C. to constant weight; total heating time, 8 hours.
[b] Calculated on original moisture basis.

The error due to retained solvent can be avoided not only by use of the humidification treatment but also by the use of solvents for precipitation that are practically inert in the Zeisel methoxyl determination—e.g., acetone—or solvents, such as isopropanol, that are less strongly retained than is ethanol and therefore can be removed in an Abderhalden dryer (Table III). It is evident also from Table III that 2 days' humidification is sufficient to remove retained ethanol. Determinations made after 4 and 6 days' humidification agreed with those made after 2 days. It is to be expected that pectin samples which have been allowed to stand open in a room of high humidity for some time will no longer contain combined alcohol.

Table II. Effect of Humidification on Methoxyl Content of Pectins

Sample	Without Humidification Treatment: Zeisel	Without Humidification Treatment: Saponification	With Humidification Treatment[a]: Zeisel	With Humidification Treatment[a]: Saponification
	%	%	%	%
Apple, 300 grade	6.4	6.0	6.0	6.2
	6.5	6.0	6.1	6.1
Apple, 285 grade	7.8	7.6	7.5	7.4
	7.9	7.5	7.6	7.4
Citrus, 178 grade	11.6	10.1	10.0	10.3
	11.7	10.2	10.0	10.4
Citrus Pectinum NF VII	9.8	9.7	9.3	9.5
	9.8	9.7	9.3	9.5
Citrus, 200 grade, purified	10.3	10.1	9.5	9.5
	10.4	10.1	9.5	9.6

[a] Humidification treatment: Pectin samples in shallow dishes placed in desiccator over water and slow stream of air bubbled through water for one month. Toluene kept on water to prevent mold growth. Samples then dried in a vacuum oven at 70° C. for 24 hours.

Table III. Methoxyl Values for Pectin[a] Precipitated by Acetone, Ethanol, and Isopropanol as Related to Similar Values after Humidification

Solvent Used to Precipitate Pectin from Solution[b]	Humidification Treatment	Methoxyl Content: Zeisel	Methoxyl Content: Saponification
		%	%
Acetone	None	10.1	10.2
	None	10.2	10.2
Isopropanol	None	11.1	10.2
	None	11.0	10.3
Isopropanol	2 days	10.0	10.5
	2 days	10.0	10.5
Isopropanol	None	10.1[c]	....
Dried at 100° C. in Abderhalden	None	10.1[c]	....
Ethanol	None	11.6	10.1
	None	11.7	10.2
Ethanol	2 days	10.2	10.4
	2 days	10.1	10.5
Ethanol	None	11.2[c]	10.2[c]
Dried at 100° C. in Abderhalden	None	11.2[c]	10.3[c]

[a] All samples, freshly precipitated and humidified, dried at 70° C. in vacuum oven overnight previous to analysis. Humidification carried out by placing 2-gram samples of pectin, contained in crystallizing dishes having inside diameter of 45 mm., in large Pyrex desiccator containing 1 liter of water with a little toluene added.
[b] Sample of citrus pectin, 178 grade, dissolved in water to give concentration of 1%. Aliquots precipitated with indicated solvents.
[c] Calculated to moisture content after drying in vacuum oven at 70° C.

DISCUSSION

Errors due to retained alcohol should be guarded against, particularly in studies of pectin structure, since most frequently pectin and its derivatives are precipitated from solution with ethanol, and ordinary drying technique does not remove all the alcohol. Pectinic acids prepared with pectinesterase showed the same tendency to retain ethanol. In this connection Morell, Baur, and Link (6) observed that citrus polygalacturonide, purified by extraction with 70% alcohol, still contained 0.4 to 0.6% methoxyl by the Zeisel method after treatment with alkali. Olsen *et al.* (7) obtained results in agreement with those of Link.

An attempt was made to use the de-esterification of pectin by alfalfa pectinesterase (pectase) as an analytical method for methoxyl determination in pectin. However, the enzyme method gave lower values than the Zeisel method on humidified pectins. In every case the enzyme hydrolyzed the pectins to a residual methoxyl content of 0.5%. Subsequent saponification accounted for most of the difference. Orange and tomato pectinesterases gave similar results, although the latter contained some pectinase. This extent of enzyme hydrolysis is not in accord with the findings of Hills, White, and Baker (3), who reported that tomato pectinesterase will hydrolyze pectin only to 1.8% of residual methoxyl, without describing their method for the determination of total methoxyl content of pectin.

The significance of the residual methoxyl not hydrolyzed by pectinesterase and a more detailed study of saponification will be reported at a later date.

ACKNOWLEDGMENT

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Automatic Distillation Apparatus for Gasoline Analysis

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An apparatus is described which makes possible the automatic operation of an efficient laboratory distillation unit. While the device was designed for gasoline analysis, it can be adapted to the control of other distillations. It automatically collects the fractions of distillate and plots the distillation curve. It has been operated 24 hours per day for well over a year with an operator present only 8 hours per day.

THE high level of the quality requirements specified for aviation gasoline makes it necessary to determine the composition of gasoline stocks in terms of individual hydrocarbons, in order to be able to produce the maximum amount of the desired components, and to eliminate or minimize production of materials of low quality. The first step in the analytical procedure is separation of the sample by distillation into fractions consisting of substantially pure hydrocarbons where possible, or, as a compromise, a mixture of a very few components.

Analytical distillation of gasoline requires a fractionating column with a large number of theoretical plates, and at the same time, small holdup per theoretical plate. In addition, as Rose (*2, 3, 4*) and his associates have shown, analytical distillations frequently require operation at reflux ratios of over 100 to 1, and use of a charge that is large compared to the holdup of the column in order to be able to isolate components present in small amounts. Under these conditions the distillation of a single sample may require several weeks of continuous operation. For this reason an automatic distillate-collecting device was constructed which makes possible the operation of a laboratory distillation unit with only occasional attention from the operator. This device uses some of the principles of the unit described by Bruun and Falconer (*1*) but is more nearly fully automatic. It was developed to be used with Podbielniak Super-Cal Model B distillation unit with a column containing 90 cm. (3 feet) of 22-mm. Heligrid packing, but it could, of course, be used with any type of laboratory distillation units.

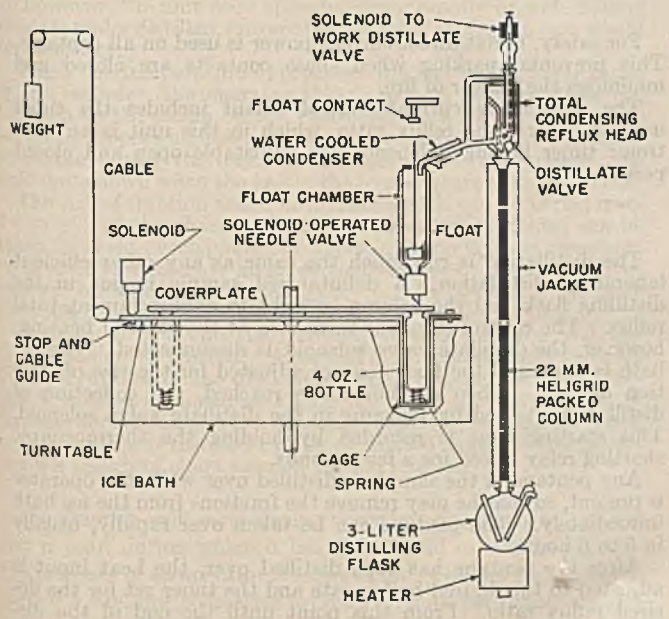

Figure 1. Sketch of Distillate-Collecting Device

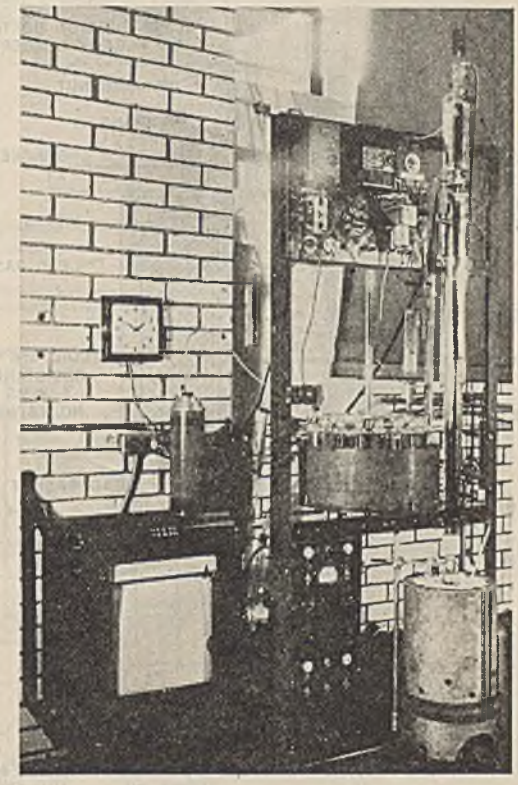

Figure 2. Automatic Distillation Apparatus

DESCRIPTION

A sketch of the distillate-collecting device is given in Figure 1, and a photograph of the entire unit in Figure 2. The photograph was taken during the development of the apparatus and does not show several minor improvements.

As shown in Figure 1, the distillate from the total condensing reflux head passes through a cooler into a float chamber. When the desired amount of fraction has been collected in this chamber, the float strikes a contact, the solenoid-operated valve on the bottom of the float chamber opens, and the fraction drains into one of 18 bottles held in a turntable. The valve then closes and the turntable moves, bringing an empty bottle under the float chamber.

The float chamber is made of Pyrex and has a jacket through which cold water or other cooling medium can be circulated. The float is also glass and is sealed to a brass guide rod.

The solenoid-operated needle valve on the float chamber was made from an automotive carburetor needle valve (Figure 3), the seat of which was reground and the needle spring loaded to ensure a tight seal.

The fractions are collected in 120-ml. (4-ounce) oil sample bottles held in cages fastened to a turntable. Springs in the bottom of the cages press the bottles firmly against a cover plate. An effective seal between the bottle tops and the cover plate is obtained by using petrolatum as a lubricant on the cover plate.

The cover plate is stationary and has a hole in it under the float chamber through which the bottles are filled. When the three bolts holding the plate are removed, it can be slid back and the bottles removed from the turntable.

Next to each of the 18 bottles and close to the edge of the turntable is a pin 1.25 cm. (0.5 inch) high and 0.6 cm. (0.25 inch) in diameter. One pin is always resting against the stop attached to the turntable solenoid (Figure 4). When this solenoid is energized the stop is raised and one pin allowed to pass under it.

The motive power for the turntable is supplied by a weight connected to the turntable by a cable through a system of pulleys.

The pins extend through the turntable and serve as cable guides on the underside.

An ice bath surrounds the bottles in the turntable. Ice is placed in a screened-off portion in the center of the bath, so that an ice jam cannot prevent the table from turning. The cover plate and turntable have holes through which the ice bath can be filled.

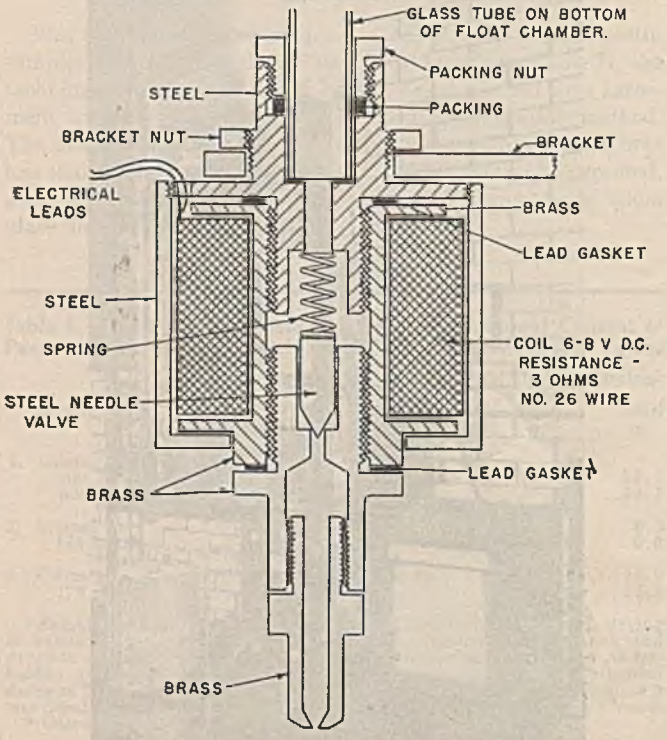

Figure 3. Cross Section of Solenoid-Operated Needle Valve

The only temperature recorder available was a potentiometer-type instrument covering the range of 0° to 400° F. with an iron constantan thermocouple. In order to improve the accuracy of the temperature measurement, a two-junction thermopile was used, thus doubling the sensitivity of the instrument for a given temperature change. The internal cold-junction compensator was balanced out by the use of a manganin resistor. For the low range of the instrument the external cold junction was placed in an ice bath, and for the high range in a steam bath. In this manner temperature measurements precise to ±0.6° F. were obtained. The measurements must, of course, be corrected to a standard barometric pressure.

ELECTRICAL CIRCUIT

The electrical circuit as shown in Figure 5 has three different functions: (1) to control the collection of fractions, (2) to supply power to the recorder and cause the time at which a fraction is taken to be recorded, and (3) to stop the distillation when necessary.

When the float rises far enough to strike the adjustable float contact, a sensitive 6-volt direct current relay is energized. The closing of this relay causes a latch-in relay to close, and starts a vacuum tube timer. While the latch-in relay is closed, power is supplied to the float chamber needle valve, holding it open. After about 1 minute the vacuum tube timer releases the latch-in relay. This allows the needle valve to close and energizes the turntable solenoid just long enough to allow an empty bottle to move into position. A push-button is provided, so that this cycle can be started manually.

During the period that the latch-in relay is closed, a small 110-volt alternating current relay is also closed. The thermocouple leads are connected across this relay, so that when it is closed the thermocouple is shortened. This causes a break in the temperature record that shows when a fraction was bottled.

A circuit is provided to shut down the unit (1) when all 18 receiving bottles have been filled, (2) when the vapor temperature reaches some predetermined point, or (3) when the temperature goes off either end of the recorder scale.

As an alternative the unit can be made to go on total reflux.

There are two alternating current circuits, one supplying the heater load and the other supplying the control load. The heart of the shutdown circuit is a sensitive direct current relay having one side of the alternating current control line across its contacts. This relay is energized from the battery booster when the reset switch is closed. There are four contacts, any of which when closed will short out this relay and thus break the alternating current control circuit. One contact is attached to the turntable, so that contact is made after the last bottle is filled. A contact is attached to each end of the recorder scale to prevent the temperature from going off the scale. The fourth contact is also in the recorder and can be set to make contact at any desired temperature, thus stopping the operation.

Since the contacts on the sensitive direct current relay are not large enough to carry the entire alternating current load, another relay is used to break the alternating current heater circuit. This relay is energized by the alternating current power from the control circuit. Thus, both safety relays are opened when any of the four safety contacts are closed. A switch is provided, so that the heater circuit may be kept on even if the alternating current control circuit is off. By the use of this switch the unit can be made either to go on total reflux or to shut down entirely when one of the four safety contacts is closed.

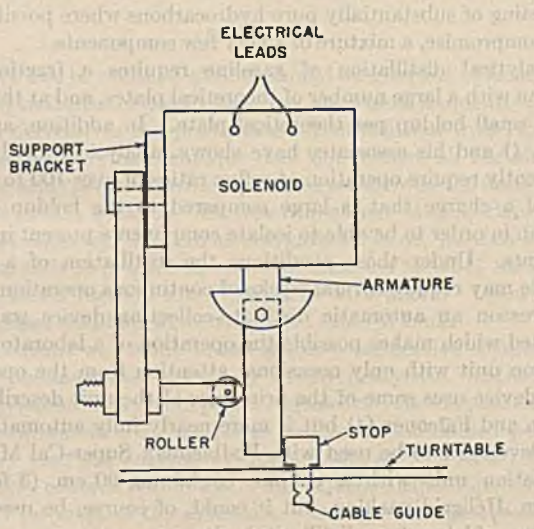

Figure 4. Turntable Solenoid Arrangement

For safety, 6-volt direct current power is used on all contacts. This prevents sparking when these contacts are closed and minimizes the danger of fire.

The alternating current control circuit includes the timer used to control the reflux ratio, which in this unit is an electronic timer having independently adjustable open and closed periods.

OPERATION

The distillation is run much the same as any other efficient laboratory distillation. A debutanized sample is put in the distilling flask and the column brought to equilibrium at total reflux. The control circuit is turned on at the start of heating; however, the distillate valve solenoid is disconnected. The ice bath is filled and the float contact adjusted for the size of fraction desired. When equilibrium is reached, the collection of distillate is started by plugging in the distillate valve solenoid. This starting time is recorded by holding the thermocouple shorting relay closed for a few seconds.

Any pentane in the sample is distilled over while the operator is present, so that he may remove the fractions from the ice bath immediately. The pentane can be taken over rapidly, usually in 5 to 6 hours.

After the pentane has been distilled over, the heat input is adjusted to the desired boilup rate and the timer set for the desired reflux ratio. From this point until the end of the distillation the operator gives little attention to the unit. Every morning and evening he removes fractions from the turntable,

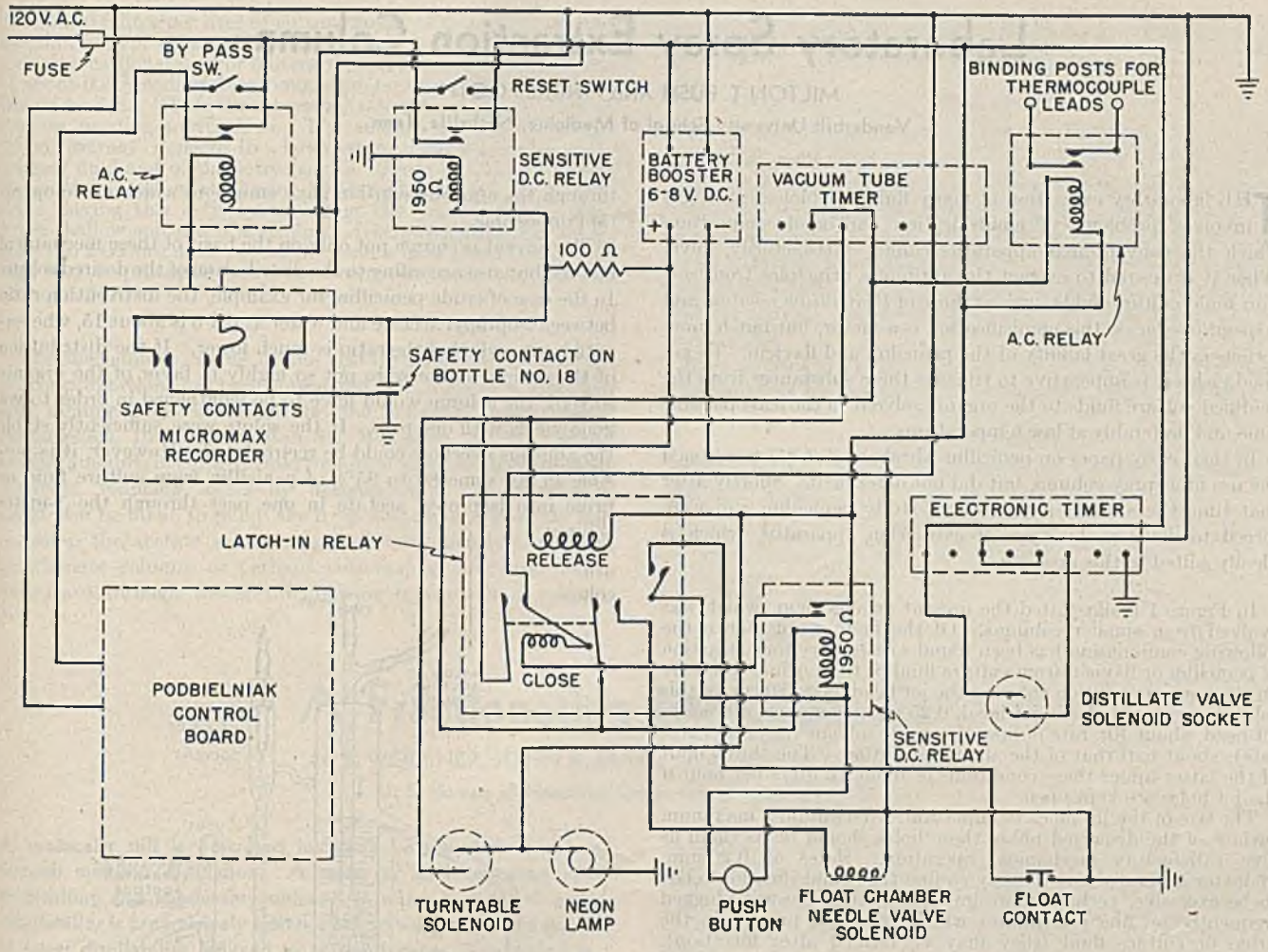

Figure 5. Wiring Diagram of Distillate-Collecting Device

winds up the weight, and checks the heat input to the column and the flask. Since less than 10% of the charge is normally taken overhead per day the heat inputs need only occasional adjustment. A variation of 5% in a reflux ratio of 100 to 1 has no appreciable effect on the efficiency of separation obtained. If, however, the unit were operated more rapidly or wide boiling range samples distilled, automatic control of the boilup rate would be necessary.

When the temperature approaches the end of the lower range of the recorder, the operator shifts the cold junction from the ice bath to the steam bath. Since there is a 25° F. overlap between the upper and lower scales, this change can usually be made sometime during the day. If the operator is not present, the unit shuts down when the end of the temperature scale is reached.

The size of fraction taken in this apparatus can be varied from 0.6 to 3% of the charge. Thus, small enough fractions can be taken to avoid overlapping of components and it is not necessary to be able to take cuts at given temperatures. If a number of cuts are obtained on a plateau, they can be blended to simplify the subsequent inspection work.

At the end of a run the recorder chart is removed and the distillation curve plotted. A section of a recorder chart showing the interruption in the distillation curve obtained when a fraction is bottled is shown in Figure 6. Since the chart speed of the recorder is fixed at 5 cm. (2 inches) per hour, the rate at which each fraction was distilled can be calculated from the size of cut and the length of chart covered by one fraction.

The fraction-collecting device described has been in use for over a year, during which it has been found extremely reliable. The presence of an operator is required for an 8-hour period at the start of a run, but thereafter for only an hour each morning and evening. Duplicate distillations check within the accuracy of the temperature recorder.

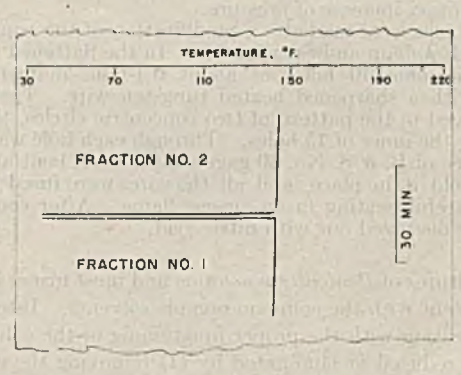

Figure 6. Section of Recorder Chart

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of A E. Traver, who designed the electrical circuit, and to express their appreciation to J. B. Rather for permission to publish this paper.

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Presented before the Division of Petroleum Chemistry at the 106th Meeting of the American Chemical Society, Pittsburgh, Pa.

Laboratory Spray Extraction Column

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THE laboratory extraction of many fluids of biological origin involves problems of emulsification and/or decomposition which the conventional apparatus cannot satisfactorily solve. When it is desired to extract the antibiotic principles from certain mold culture fluids, such as those of *Penicillium notatum* and *Aspergillus flavus*, this emulsification is a factor, but much more serious is the great lability of the penicillin and flavicin. To get good yields it is imperative to transfer these substances from the acidified culture fluids to the organic solvent in the least possible time and preferably at low temperatures.

In their early paper on penicillin Abraham *et al.* (*1*) mentioned the use of a spray column, but did not describe it. Shortly after that time the authors undertook to study penicillin, and were forced to develop this type of extraction apparatus, which is ideally suited to this problem.

In Figure 1 is illustrated the present arrangement, which was evolved from smaller columns. Of the factors not shown the following combination has been found satisfactory for extraction of penicillin or flavicin from culture fluid or from urine: pressure on the aqueous solution entering the jet head, 60 to 80 cm. of this solution; size of holes in jet head, 0.25 mm.; number of holes in jet head, about 40; rate of flow of organic solvent (isopropyl acetate), about half that of the aqueous solution. The throughput of the latter under these conditions is about 10 liters per hour if the jet holes are kept open.

The size of the jet holes is important. To obtain a maximum surface of the dispersed phase these holes should be as small as give satisfactory mechanical operation. Holes of 0.20-mm. diameter generally give a spray so fine that emulsification tends to be excessive, perhaps more important, they become plugged frequently by fine precipitates which are often present in the urine or culture fluid (they may be formed after filtration). With 0.25-mm. holes, on the other hand, emulsification is generally unimportant (even with culture fluid containing corn steep water) and the sediment usually passes through the holes readily. When the holes do become plugged they can usually be opened by a momentary increase of pressure.

The jet holes were made by a modification of the copper wire method of Branham and Sperling (*2*). In the flattened end of a Pyrex tube some 40 holes of about 0.4-mm. diameter were punched with a sharpened heated tungsten wire. These holes were arranged in the pattern of two concentric circles, the outer of 25 holes, the inner of 15 holes. Through each hole was placed a short piece of B. & S. No. 30 gage copper wire, bent at a right angle to hold it in place, and all the wires were fused into the glass by careful heating in an air-gas flame. After cooling the copper was dissolved out with nitric acid.

Most cultures of *Penicillium notatum* and most urines emulsify to some extent with the common organic solvents. Interference of emulsification with the proper functioning of the column can usually be reduced or eliminated by (1) removing the emulsion which accumulates below the solvent-inflow tube (the emulsion is withdrawn through the drain without interrupting the operation of the column by clamping the aqueous-overflow tube until the interface regains its proper position) or (2) choosing a satisfactory organic solvent. In the authors' experience isopropyl acetate is the solvent of choice. If the commercial product (95% pure) is washed with water, treated with anhydrous calcium chloride, and redistilled, the solvent gives a minimum of emulsification. Similar treatment of commercial *n*-butyl acetate (90%) gives a slightly less satisfactory solvent. Similar purification of various grades of amyl acetate gave a solvent which was distinctly less satisfactory than commercial *n*-butyl acetate.

Modifications of the spraying technique in which acetate was sprayed upward or ethylene dichloride was sprayed downward through the aqueous solution gave emulsions which made operation impossible.

The solvent is chosen not only on the basis of these mechanical factors, but also according to the distribution of the desired solute. In the case of crude penicillin, for example, the distribution ratio between isopropyl acetate and water at pH 3 is about 15, whereas with isopropyl ether the ratio is much lower. If the distribution of the desired solute were not so highly in favor of the organic solvent, the column would have to be lengthened in order to get good yields with one pass. If the solute were sufficiently stable the aqueous overflow could be recirculated. However, it is possible to get some 80 to 85% of penicillin from culture fluid or urine into isopropyl acetate in one pass through the 1-meter column.

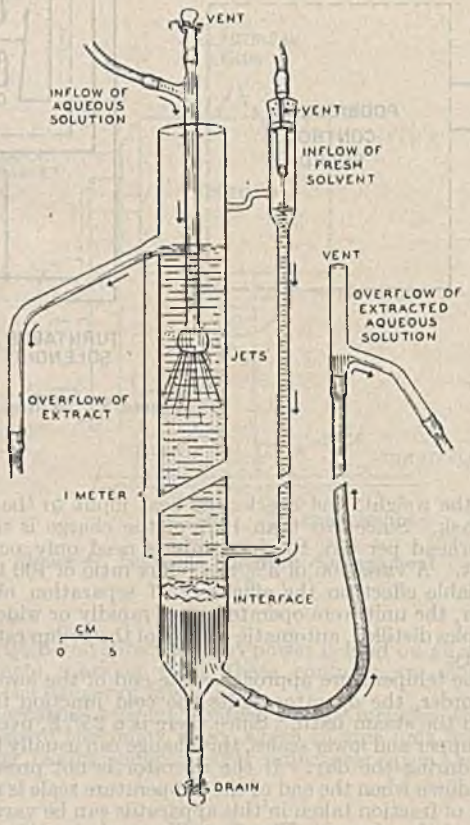

Figure 1. Spray Extraction Column

A batch of culture fluid or of urine containing penicillin or flavicin can be extracted as follows:

The fluid is poured through a fine "glass cotton" filter to remove coarse particles, then cooled to about 5° C. The column is filled with water and cold purified isopropyl acetate and the aqueous overflow tube adjusted so that the interface is just below the solvent-inflow tube. The culture is acidified in 400-cc. batches with phosphoric or hydrochloric acid to pH 3.0 ± 0.2, poured into a funnel some 60 to 80 cm. above the jet head, and allowed to spray into the acetate. Approximately 2 minutes are required for most of the 400 cc. to flow through. By acidifying and pouring in successive batches at intervals just often enough to maintain operation, decomposition of the penicillin is minimized. Fresh acetate is run countercurrentwise through the column at a rate

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of about 0.5 liter per liter of culture fluid. The acetate solution overflow is kept cold and is shaken out in batches with aqueous sodium bicarbonate. For 5 liters of acetate, three 50-cc. portions of about 0.3 *N* sodium bicarbonate are needed. The pH of each should be 7–8. These three bicarbonates are pooled and kept on dry ice pending purification. The amount of activity in this "crude extract" depends to a large extent upon the nature of the original fluid and of the active solute. From shallow cultures of *Penicillium notatum* containing 30 Oxford units per cc. or from urine having this activity or higher the yield is 80 to 85%. From shallow cultures of a strain of *Aspergillus flavus* containing only 1 to 2 Oxford units per cc. the yield is only about 50%. This high loss is principally due to decomposition in the acidified aqueous solution.

The mechanical mixing of the culture fluid and acid in the proper proportions has been considered, and it appears that suitable equipment is available for the purpose (from Wilson Pulsafeeders, Inc., 205 Clinton St., Buffalo 5, N. Y.). Much smaller amounts of culture could thus be acidified at more frequent intervals, reducing losses by decomposition. A Pulsafeeder could also be made to pump the fresh acetate, and possibly also to pump the acetate solution upward continuously through a bicarbonate column, or perhaps better spray the bicarbonate downward through the acetate flowing upwards in a column. Possibly on a larger scale these continuous operations could be carried out more efficiently than is feasible on a small laboratory scale.

SUMMARY

A laboratory spray extraction column is particularly useful for extracting aqueous fluids of biological origin with solvents lighter than water. Advantages over the more common laboratory extraction apparatus are that emulsification interference can usually be entirely circumvented, and transfer of solute to the organic solvent takes place in a minimum of time. It offers these possible disadvantages: (1) relatively large volumes of solvent are needed; (2) the distribution of the desired solute must be favorable.

In recovering penicillin or flavicin from culture or urine these disadvantages are unimportant compared with the advantages.

ACKNOWLEDGMENT

Funds for this work were kindly given by the Mallinckrodt Chemical Works.

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A Fractionating Molecular Still

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A molecular still is described in which fractionation is effected through multiple distillation. A series of communicating stages of distilling and condensing surfaces, in which material of greater distillability is progressively advanced to forward stages and material of lesser distillability refluxed to rearward stages, is provided in a single unit.

IN THE course of a program instituted in this laboratory to isolate and characterize the active principle (or principles) of marihuana, *Cannabis sativa L.* (*11*), it was proposed to separate the components of the resin, in which the physiologically active material is contained, by fractional molecular distillation. This operation was performed initially in a pot-type molecular still, similar to that described by Mair, Schicktanz, and Rose (*9*). In this type of still, material which is evaporated from the surface of the distilland condenses on the roof, flows into an annular gutter which prevents its return to the distilland, and is conducted directly to receivers.

Two major factors limit the extent to which fractionation can result from molecular distillation in a still of this type. One results from the lack of provision for constant renewal of the distilling surface, such as is provided by ebullition in ordinary distillation. Diffusion of molecules to the surface layer is retarded by the usually viscous nature of the distilland. Molecules of greater volatility, which normally would preferentially distill from the surface, are thus entrapped in the body of the distilland, and the distilling surface becomes correspondingly enriched in molecules of lesser volatility. Since only molecules evaporated from the surface of the distilland reach the condenser, the extent to which a representative portion of the whole distilland is subjected to distillation is seriously affected. Redesign of the molecular still has been necessary to circumvent this limitation. The so-called "falling-film" type of still, in which the distilland flows by gravity over a vertical heating surface, was first described by Hickman (*8*); a number of other stills employing this principle have also been designed (*7*, *10*, etc.). In these stills, the combined effect of thin layer of distilland and constant movement of the distilland results in a material improvement in the extent to which the distilland surface will be representative of the entire charge.

The second major factor limiting the extent to which fractionation can take place in the pot-type still is the absence of means for multiple distillation. In molecular distillation, as in distillations conducted at higher pressures, the degree of separation is a function of the respective rates of evaporation of the various molecular species. The ratio of the components of a mixture in the distillate from any single distillation is proportional to the partial pressures of the components in the distilland; in molecular distillation it is also inversely proportional to the square roots of their molecular weights.

In order to improve the degree of separation in ordinary (equilibrium) distillations, fractionating columns are used which provide, in effect, successive stages of distillation, the number of effective redistillations being expressed in terms of theoretical plates. In molecular distillation, however, the type of fractionating column dependent upon the establishment of equilibrium between liquid and vapor obviously cannot be employed. In the falling-film type of molecular still, fractionation is achieved through passage of the distilland over the heating surface maintained at a definite temperature; the maximum theoretical fractionation which can result in this way is equivalent to one perfect plate. To attain further fractionation, complex arrangements of combinations of still units designed to simulate the performance of fractionating columns have been employed (*4*, *5*, *6*).

The present communication describes a simple apparatus which provides for multiple distillation, while retaining the conditions necessary for molecular distillation. This is accomplished by providing within a single unit a series of communicating stages of distilling and condensing surfaces. As in the simple still, distillate from an initial stage is condensed on the roof of the still and engaged by a trough during its downward flow. In-

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stead of being removed from the still, however, the distillate is directed by the troughs to a succeeding heating surface, located at a higher level than the preceding one. Here the more readily distilled portions are again preferentially evaporated, and again carried to the next stage. While the more volatile material progressively advances, the less volatile material continuously regresses on the floor of the still toward rearward heating surfaces. By applying heat in graduated amounts to the several sections of the still, with the greater amounts applied at the initial stages, the desired rate of distillation and ratio of reflux may be maintained.

The heating areas are defined by means of transverse ridges on the floor of the still, which act as dams checking the rearward flow of liquid and causing it to form shallow pools. The troughs are so located in relation to the pools as to engage distillate from each pool and conduct it to the succeeding pool. Liquid in each area is constantly replenished both by distillate from preceding areas, conveyed by the troughs, and by reflux from the superiorly located areas. The constant rearward flow of liquid along the heating surface and the merging of the counterflowing distillate and distilland at each stage serve to agitate and continuously renew the surface of the distilland.

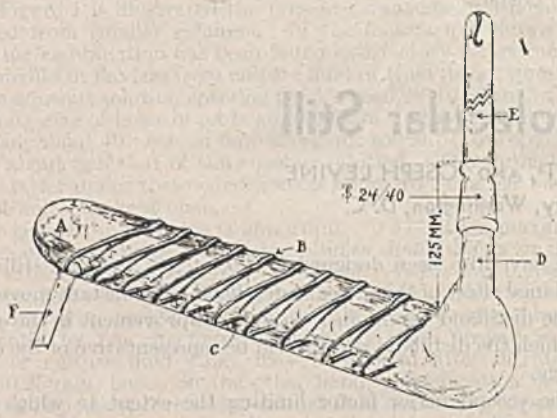

Figure 1

The several heating areas are not distinct; a continuous film of liquid covers the entire floor of the still, thereby providing the large distillation surface which is important in molecular distillation. The condensing areas on the roof of the still are, however, essentially distinct, each stage being the area between successive troughs. Material evaporated from any point on the heating surface is condensed on the surface immediately above it, and is necessarily carried to a forward point for redistillation.

The uppermost trough leads directly to a take-off tube, through which the distillate is conveyed to the receiver system. The still is connected to the usual high-vacuum system, in which operating pressures of 10^{-4} mm. of mercury or less are maintained.

DESCRIPTION OF APPARATUS

The authors' still (Figures 1 and 2) is constructed of a cylindrical Pyrex tube, *A*, 38 cm. long and 50 mm. in diameter. The interior surface of the tube is provided with a series of 10 channels, *B*, approximately 3 mm. deep, placed at an angle of 45° with the axis of the tube and 25 mm. apart. These extend around the tube except for a space of 20 mm. at the bottom, where lateral ridges, *C*, 2 mm. high are situated 5 mm. behind the termini of the channels. A vertical tube, *D*, 22 mm. in diameter and 125 mm. in length, and provided with a male $ 24/40 joint, is sealed onto the top of the tube, at the lower end. The cap, *E*, of this opening is 30 cm. in length. A thermometer is suspended on a glass hook sealed onto the top of the cap, reaching to within several millimeters of the floor of the still. An outlet tube, *F*, 12 mm. in diameter is sealed onto the bottom of the tube, at the upper end. This leads directly to the vacuum and receiver system. The termini of the uppermost internal channel lead directly into this tube.

Heat is provided by a Nichrome wire heater, built in four sections. Each section consists of four parallel coils, separated by mica strips, connected in series. The individual coils are approximately 75 mm. in length and 4 mm. in diameter, and are made by wrapping 60 cm. (2 feet) of No. 22 B. & S. Nichrome wire around a rod. Each heater unit is connected through a common ammeter to an individual switch and 3.5-ampere rheostat; the current passing through each unit can be read separately on the ammeter. The heater is fastened to an asbestos-board floor of a metal box, 36 × 7.5 × 5 cm., one end of which is partially cut out to adapt it to the still. The still is seated on asbestos paper covering the heating coils, and is cemented in the box with magnesia-asbestos plaster. The box

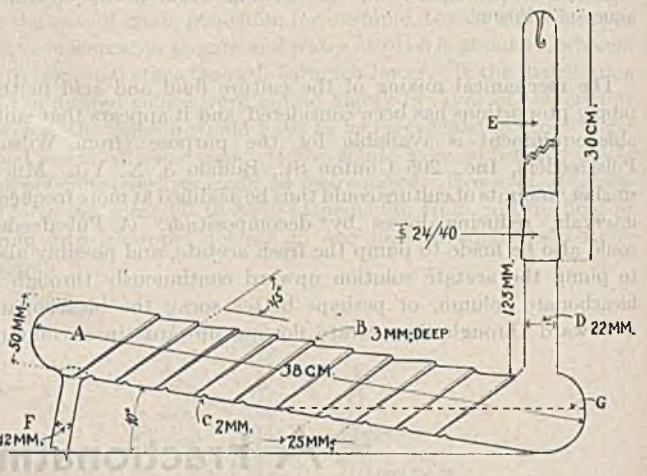

Figure 2

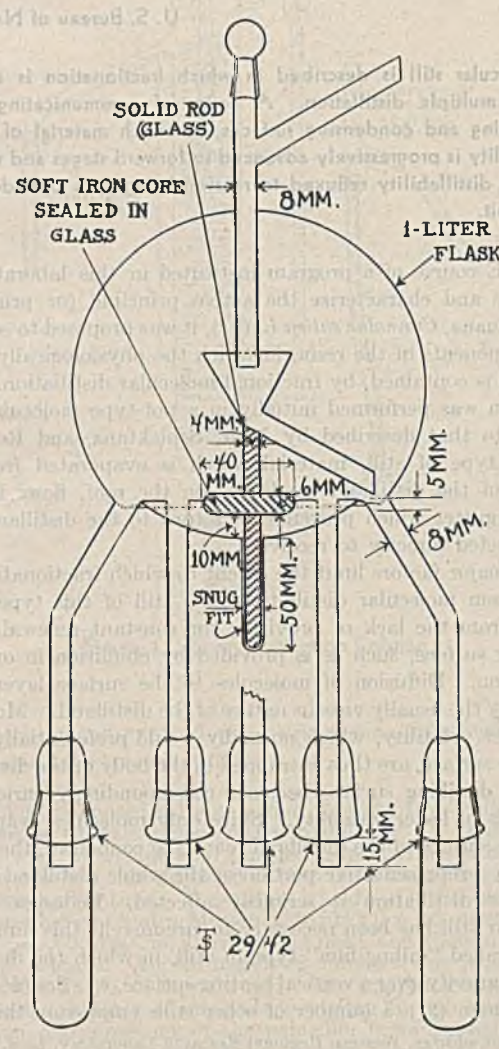

Figure 3

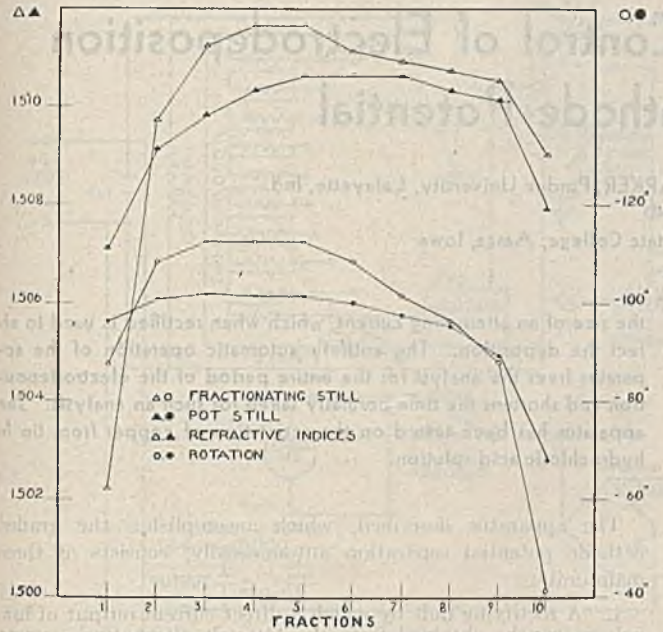

Figure 4

is clamped on a rigid frame, in such a position as to fix the still at an angle of 10° with the horizontal.

The outlet tube of the still is connected directly to a vacuum line and to the receiver system, in which fractions may be collected in individual receivers without interruption of vacuum. This apparatus (Figure 3) is a modification of the Brühl apparatus (*12*). (The apparatus used in this laboratory was designed by S. T. Schicktanz.) It consists of a central chamber to which are attached ten radial arms, each bearing a ⅌ 29/42 joint. Distillate is directed to the arms through a funnel, the stem of which is bent at an angle as shown in the drawing. The funnel is mounted on a vertical glass rod, which serves as a pivot and bearing. A soft iron rod is sealed horizontally into the mounting of the funnel; an external electromagnet is used to turn the funnel, thus directing the stem toward the desired receiver arm. Distillate is collected in test tubes resting in the caps which are used as closures of the arms.

The vacuum system, consisting of a 1-liter ballast flask, dry ice trap, McLeod gage, and mercury vapor diffusion pump, backed by a fore-pump, is assembled in the usual manner.

OPERATION

In operation, the still is charged through opening *D* to the level indicated by the broken line, *G*, and the system is closed. The first heater unit is turned on low, and the charge is degassed by gradual reduction of pressure. During the degassing operation a small amount of material may be spattered on the roof of the still, especially if the pressure is reduced too rapidly; this will be washed down by the first portions of distillate during the actual distillation.

After the charge has been thoroughly degassed, the diffusion pump is started and a greater amount of heat applied to the first heater unit until distillation proceeds. Material evaporated from the surface of the main charge will condense on the roof of the still and flow laterally around the inner surface of the still until engaged by a channel, *B*, along which it will flow downward and forward to a succeeding heating area. As distillation progresses, the condensate forms pools in these areas and overflows, forming a continuous flowing film on the floor of the still. The succeeding heating units are cut in as the areas they govern become supplied with distilland. Distillation from each area proceeds in the manner described, and the more volatile components are progressively carried forward while the less volatile components flow in a rearward direction. The rate of distillation and reflux is governed by the heat input at the several heater sections; by the application of a proper differential in the input of the succeeding sections, as indicated on the ammeter, a proper rate of reflux may be maintained.

Distillate at the uppermost stage is conducted by the final trough in the series to the outlet tube and thence to the receiver system, where it is directed to the desired individual receiver by manipulation of the funnel. Fractions may be cut on a basis of equal volume or in accord with an obvious change in some physical property, such as color or viscosity.

The extent of the improvement in fractionation attained with the fractionating still as compared with the pot-type still is illustrated in Figure 4.

Portions of an acetylated marihuana "red oil", twice previously distilled to remove unlike components, were distilled in each type of still at a pressure (McLeod gage) of 10^{-4} mm. Ten fractions of approximately 8 grams each were collected. The specific rotations and refractive indices shown indicate a significant differential between the two series of fractions. An evaluation of the significance of this differential may be made by comparison with the results obtained in the distillation of marihuana red oil by other workers. Bergel (*1*), for example, construed failure to obtain differences in the physical constants of the several fractions resulting from ordinary high-vacuum distillation as evidence that the red oil was an individual compound.

DISCUSSION

The apparatus as described may readily be adapted to considerable variation, while retaining the features by virtue of which fractionation results. By appropriate modification, for example, distilland could be introduced in a continuous fashion at some intermediate point in the still, rather than being placed in the still initially as a single charge. The charge could be provided from a reservoir of predegassed material; or distillate from a "falling-film" type of apparatus could be conducted directly to a point on the heating surface perhaps one third of the distance from the initial stage. Apparatus constructed in this manner would be especially desirable for the distillation of thermally labile material.

During the distillation of marihuana oils, the McLeod gage regularly indicated a residual gas pressure of less than 10^{-4} mm. of mercury. It is recognized that this does not indicate the actual distillation pressure; it does, however, indicate air-tightness of the system and absence of decomposition of the charge to gaseous products. Considerations of the magnitude of the actual distillation pressure must not, in any event, be made in accord with the ordinary concepts of pressure. The flow of evaporated molecules during molecular distillation is essentially unidirectional, while the usual concepts of pressure relate to random movement of molecules. During unidirectional distillation, the mean free path of the molecules is, as pointed out by Brønsted and Hevesy (*2*), much greater than the mean free path under ordinary conditions. Indeed, the interrelation between direction of movement and magnitude of mean free path is incorporated in the definition of mean free path which was proposed by Langmuir (*3*). There may be, then, considerable latitude in the dimensions of the still and in the rate of distillation in so far as they relate to adherence to the principles of molecular distillation.

ACKNOWLEDGMENT

The authors wish to thank S. T. Schicktanz for furnishing the design of the receiver, and Edith J. Poindexter for preparation of the drawings.

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Application has been made for patent on this still.

Apparatus for Automatic Control of Electrodeposition with Graded Cathode Potential

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AND
HARVEY DIEHL, Iowa State College, Ames, Iowa

An apparatus is described for carrying out graded cathode potential electrodepositions automatically, whereby a metal may be separated by cathodic deposition from a metal lying closely above it in the electromotive series. The device consists of a vacuum tube amplifier which magnifies the cathode-calomel voltage sufficiently to actuate a relay and motor which drives a Variac, the Variac governs the size of an alternating current, which when rectified is used to effect the deposition. The entirely automatic operation of the apparatus frees the analyst for the entire period of the electrodeposition and shortens the time normally taken for such an analysis. The apparatus has been tested on the separation of copper from tin in hydrochloric acid solution.

IN THE normal practice of analysis by the electrodeposition of a metal at the cathode, the voltage necessary to yield a current of convenient size is applied initially to the cathode and anode and no further attention paid to it other than to change its value occasionally to maintain the current as the composition of the electrolyte changes. By such a constant current electrodeposition the possible separations are limited to those metals below hydrogen in the electromotive series from those above hydrogen, hydrogen being evolved after the deposition of the lower metal in preference to deposition of the higher metal. The change in the cathode-anode voltage during the electrolysis is no clue to the extent of the deposition of a metallic ion, but is the algebraic difference of the voltages between the solution and the cathode and anode and the IR drop through the solution, all of which may undergo change during the electrolysis.

By inserting a reference half-cell into the solution and measuring the voltage between the cathode and the reference cell it becomes possible to isolate the effect at the cathode. The voltage between the solution and the cathode consists of the equilibrium voltage of the electrode metal toward the solution containing its ions and concentration polarization caused by the flow of current. Neglecting the latter for the time being, there is thus provided a means of following the change in the concentration of the metal ion during the deposition, the reversible voltage being given by the Nernst equation:

$$E = E_0 + \frac{RT}{nF} \ln [M^{n+}]$$

Thus, for example, to separate copper from tin, the apparatus shown in Figure 1 is employed, a calomel half-cell being used as the reference electrode. The cathode-calomel voltage, measured by the potentiometer, increases as the copper is deposited. Using a 0.1 N calomel reference half-cell, this voltage is not allowed to exceed 0.45 volt, the electrolyzing current being decreased progressively by increasing the resistance, R, to accomplish this. The last copper will be gradually deposited by the successively smaller current without the deposition of any tin.

Since their first use by Sand (*3*) such graded cathode potential separations have not become popular, even though a number of very useful applications of the method have been devised, notably by the English workers Sand, Lindsey, Collin, and Torrance. The continuous attention of the analyst is required throughout the electrolysis and the deposition can seldom be completed in less than 60 minutes, since it is necessary to use a relatively low initial current, less than 2 amperes. At greater currents the changes of the cathode voltage occur so rapidly that the manual operations of balancing the potentiometer and adjusting the current cannot be carried out sufficiently rapidly. Clearly then, this is a case for automatic control.

The apparatus described, which accomplishes the graded cathode potential separation automatically, consists of three main units:

1. A rectifying unit by which a direct current output of low voltage can be obtained from the 110-volt alternating current line to perform the electrolysis.
2. A control circuit consisting of a vacuum tube amplifier, a relay, and a motor-driven Variac by which the cathode voltage is made to govern the electrolyzing current.
3. A vacuum tube voltmeter for convenience in measuring the cathode-calomel voltage.

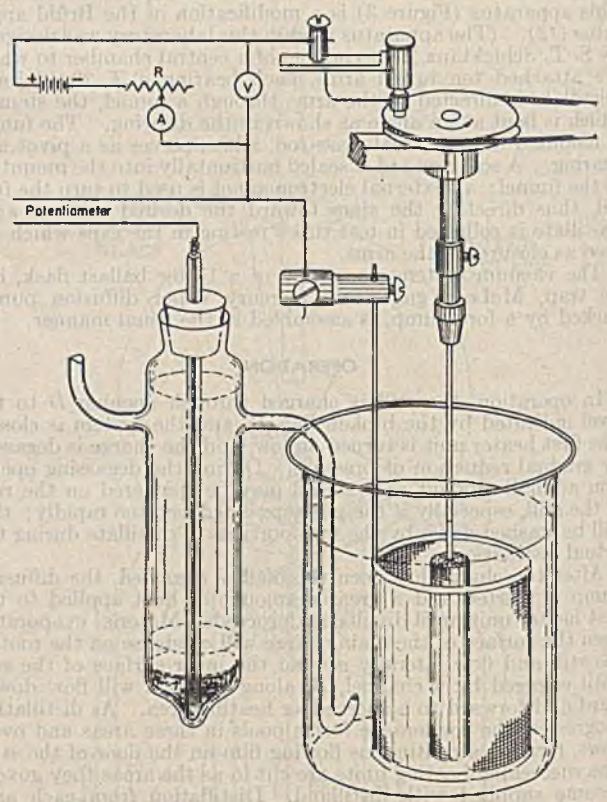

Figure 1. Circuit and Apparatus for Graded Cathode Potential Electrodeposition

The circuit and appearance of the apparatus are shown in Figures 2 and 3.

RECTIFYING CIRCUIT

The direct current needed for the electolysis is obtained from the 110-volt alternating current line, the circuit elements being, successively, a switch in the 110-volt alternating current input, a Variac, a step-down transformer, a selenium rectifier, a milliammeter-shunt combination, and a filter.

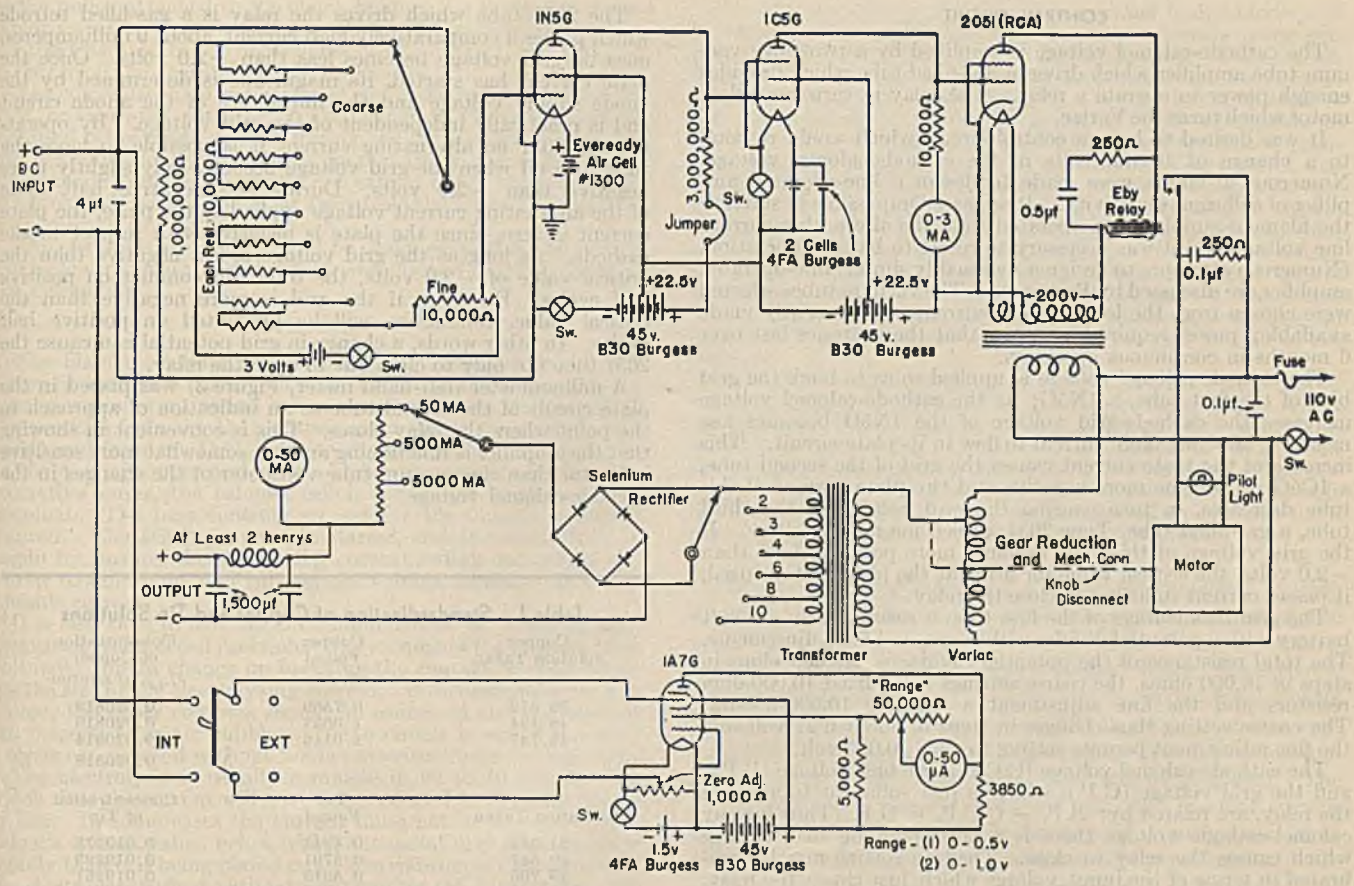

Figure 2. Circuit of Apparatus for Automatic Graded Cathode Potential Electrodeposition

Relay H. H. Eby Co., Philadelphia, Type ER12, 10,000 ohms. Variac, General Radio Co., Cambridge, Mass., Type 200B. Motor, 1/50 h.p. nduction motor with 1200 to 1800 r.p.m., gear reduction to turn Variac about 0.5 r.p.m. Rectifier, I.T. and T. selenium rectifier, Type 4B1C4

The Variac is driven by a motor activated in turn by the amplifier-relay (control) circuit and is the mechanism whereby the electrolyzing current is decreased as a result of increases in the cathode-calomel voltage. The Variac may be set by hand after turning a knob which disengages it from the motor.

The secondary of the step-down transformer has taps to provide voltages of 2, 3, 4, 6, 8, and 10 volts when 110 volts are supplied the primary of the transformer by the Variac. Thus, no more than a safe load can be supplied the selenium rectifier which is capable of handling up to 10 volts.

The rectifier is followed by a filter consisting of an inductance of 2 henrys in series and two capacitances of 1500 µf in parallel, one before and one after the choke. The purpose of the filter is to smooth out the 120-cycle ripple which otherwise affects the control circuit adversely.

The direct current drawn is measured by a milliammeter suitably arranged with three shunts, so that it can be made to read three current ranges, 0 to 0.05 ampere, 0 to 0.5 ampere, and 0 to 5.0 amperes, by changing the position of the range selector knob on the lower right front panel of the instrument.

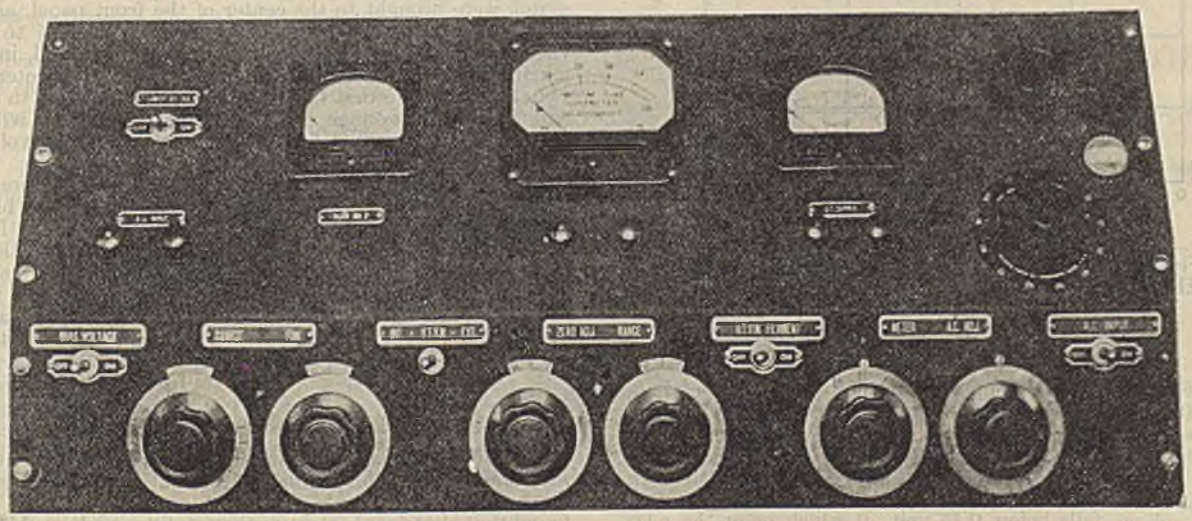

Figure 3. Apparatus for Automatic Graded Cathode Potential Electrodeposition

CONTROL CIRCUIT

The cathode-calomel voltage is amplified by a two-stage vacuum tube amplifier which drives a gas-filled tube which provides enough power to operate a relay. The relay in turn controls a motor which turns the Variac.

It was desired to have a control circuit which would respond to a change of 10 millivolts in the cathode-calomel voltage. Numerous attempts were made to design a line-operated amplifier of sufficient sensitivity. It appeared impossible to stabilize the filament supply when operated from the alternating current line voltage and it was necessary to resort to battery operation. (Numerous attempts to design a reasonably simple, line-operated amplifier are discussed by Parker, *2*.) The vacuum tubes selected were chosen from the low filament current tubes recently made available; power required is so low that the batteries last over 6 months in continuous operation.

The cathode-calomel voltage is applied so as to buck the grid bias of the first tube, a 1N5G; as the cathode-calomel voltage increases the cathode-grid voltage of the 1N5G becomes less negative, allowing more current to flow in its plate circuit. This increase of the plate current causes the grid of the second tube, a 1C5G, to become more negative and the plate current of this tube decreases, in turn causing the grid voltage of the third tube, a gas-filled tube, Type 2051, to become more positive. If the grid voltage of this tube becomes more positive (less) than −2.0 volts, the critical value for firing at the plate voltage used, it passes current sufficient to close the relay.

The grid bias voltage of the first tube is secured from a 3-volt battery with a potential divider with coarse and fine adjustments. The total resistance of the potential divider is 110,000 ohms in steps of 10,000 ohms, the coarse settings being fixed 10,000-ohm resistors and the fine adjustment a variable 10,000-resistor. The coarse setting thus changes in steps of about 0.27 volt and the fine adjustment permits setting to about 0.005 volt.

The cathode-calomel voltage (C.C.P.), the bias voltage (B.P.), and the grid voltage (G.P.), which is just sufficient to activate the relay, are related by: B.P. − C.C.P. = G.P. Thus for any calomel-cathode voltage there is a corresponding bias voltage which causes the relay to close. The bias setting may be calibrated in terms of the input voltage which just closes the relay, by impressing a known voltage on the input terminals and adjusting the bias until the relay just closes. The calibration is essentially linear and usually holds for about 2 weeks, after which the unit must be recalibrated. The use of the vacuum tube voltmeter makes such readjustments a simple matter.

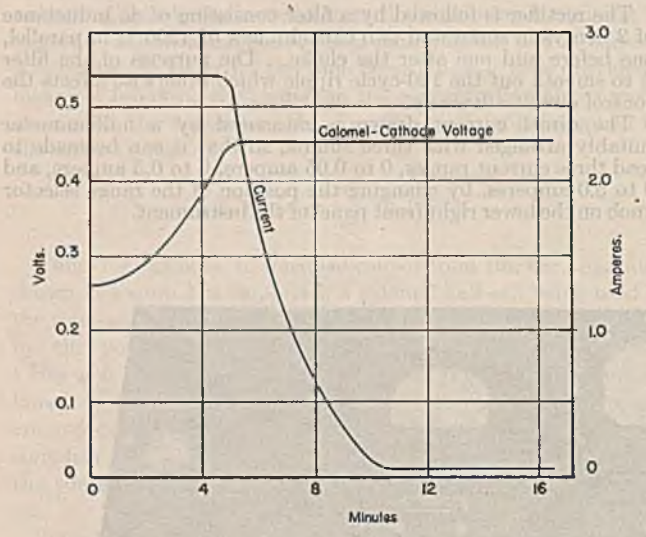

Figure 4. Course of Cathode-Calomel Voltage and Electrolyzing Current during Deposition of Copper in Presence of Tin

In the separation of copper from tin, the calomel-cathode voltage (0.1 *N* calomel) must be limited to 0.45 volt. This corresponds to a certain bias setting—for example, coarse reading 8, fine 27.0. With the bias so adjusted any voltage greater than 0.45 volt applied to the D.C. input causes the relay to close. The electrolyzing current is then reduced until the calomel-cathode voltage falls below 0.45 volt, at which point the relay opens.

The 2051 tube which drives the relay is a gas-filled tetrode which passes a comparatively high current, about 9 milliamperes, once its grid voltage becomes less than −2.0 volts. Once the plate current has started, its magnitude is determined by the anode supply voltage and the impedance of the anode circuit and is practically independent of the grid voltage. By operating the 2051 on alternating current it is possible to have the tube turn off when the grid voltage becomes only slightly more negative than −2.0 volts. During the negative half cycle of the alternating current voltage applied to the plate, the plate current is zero, since the plate is negative with respect to the cathode. As long as the grid voltage is less negative than the critical value of −2.0 volts, the tube will conduct on positive half cycles. However, if the grid is more negative than the critical value, conduction will be prevented on positive half cycles. In other words, a change in grid potential can cause the 2051 tube not only to close but to open the relay.

A milliammeter (left-hand meter, Figure 3) was placed in the plate circuit of the second tube as an indication of approach to the point where the relay closes. This is convenient in showing that the amplifier is functioning and is a somewhat more sensitive indicator than the vacuum tube voltmeter of the changes in the cathode-calomel voltage.

Table I. Standardization of Copper and Tin Solutions

Copper Solution Taken	Copper Found		Concentration of Copper
Grams	*Grams*		*G./g. of solution*
36.313	0.7560		0.020819
48.194	1.0033		0.020819
48.747	1.0146		0.020814
		Av	0.020818
Tin Solution Taken	**Tin Found**		**Concentration of Tin**
21.982	0.4243		0.019278
29.547	0.5701		0.019295
30.706	0.5913		0.019261
30.405	0.5852		0.019246
38.065	0.7335		0.019270
		Av.	0.019270

VACUUM TUBE VOLTMETER

For convenience in measuring the cathode-calomel voltage, a vacuum tube voltmeter was incorporated in the apparatus. The recent design of Garman and Droz (*1*) was used, employing a single, battery-operated tube and making use of a bridge-type circuit. Because of the low power consumption (0.3 watt) the inconvenience and cost of frequent battery replacement are reduced to a minimum and the added advantage is secured of greater stability and greater simplicity than with line operation. The circuit is shown in Figure 2.

The vacuum tube voltmeter was placed in the center of the apparatus and shielded by enclosure in a separate metal box. The zero and range adjustment controls, meter, and filament switch were brought to the center of the front panel, as will be seen from a close inspection of Figure 3. In order to use the vacuum tube voltmeter for other purposes if desired, input terminals were placed on the panel (just below the meter) and a switch provided so that the voltmeter could be made to read the cathode-calomel voltage (int.) by direct connection within the apparatus with the D.C. input, or to read some other voltage applied to the voltmeter input terminals (ext.).

The vacuum tube voltmeter is calibrated by first shorting the input terminals and varying the zero adjustment to bring the meter to read zero. The desired voltage to cause full-scale reading is then applied and the range adjusted until the meter reads full scale. The zero is then checked and adjustment made if necessary. The full-scale setting is then checked and adjustment made if needed. The voltmeter may be calibrated over any range up to 1 volt—for example, 0 to 0.5 volt or 0 to 1.0 volt.

AUXILIARY EQUIPMENT

In graded cathode potential electrodepositions, vigorous stirring is essential. This is most conveniently accomplished with a rotating platinum anode, driven at about 800 r.p.m. It was found that electrical contact to the rotating anode could be made conveniently by a carbon brush bearing on the flat upper surface

of the pulley attached to the chuck holding the electrode; some minor variation of a few milliamperes in the electrolyzing current occurred because of the unevenness of this contact, but this had no effect on the operation of the apparatus.

The conventional form of calomel electrode may be used, 0.1 *N*, 1 *N*, or saturated, the appropriate change in the limited potential used being made on substituting one for another. The calomel cell must be placed with its contact tip on the outer side of the cathode and the tip should not extend below the lower edge of the cathode, so as to be as far away from the lines of flow of electrical current as possible.

The electrode assembly should be designed so that the beaker can be lowered away and the electrodes washed quickly following the electrolysis.

OPERATION

The filament voltage, bias voltage, and vacuum tube voltmeter filament are turned on and the apparatus is given a 20-minute warming up period before starting the electrodeposition. Connections from the D.C. output are made to the cathode and anode and from the D.C. input to the cathode and calomel cell. In the case of copper and metals higher than copper in the electromotive series, the calomel cell is connected to the positive terminal. The bias controls are set for the limited potential wanted. The stirring motor is started and the electrolysis is begun by turning the alternating current switch on, setting the Variac to full value, and turning the voltage regulator to give a suitable current.

It is best then to test the D.C. input circuit by breaking contact at the calomel cell junction. The reading of the vacuum tube voltmeter should change on breaking the contact or upon altering the size of the electrolyzing current. If a variation is not observed, the other contacts should be examined and the calomel cell inspected for air bubbles. If the circuit is closed the electrolysis can proceed without further attention from the operator.

The electrolysis is usually complete in 20 to 40 minutes, by which time the current will have been reduced to 20 milliamperes or less. In some cases the current must not be allowed to fall below a certain value, below which the metal may dissolve more rapidly than it is being plated out. The solution is then removed, the electrodes washed without interrupting the current, and the determination concluded in the usual manner.

Table II. Separation and Determination of Copper and Tin

Copper Taken				Tin Taken			
Solution	Copper content	Copper Found	Copper Error	Solution	Tin content	Tin Found	Tin Error
Grams	*Gram*	*Gram*	*Mg.*	*Grams*	*Gram*	*Gram*	*Mg.*
13.819	0.2877	0.2876	−0.1	25.5	0.48	Not determined	
22.700	0.4726	0.4721	−0.5	25.5	0.48	Not determined	
22.902	0.4768	0.4763	−0.5	25.5	0.48	Not determined	
6.332	0.1318	0.1318	0.0	42.420	0.8175	0.8169	−0.6
12.701	0.2644	0.2649	−0.5	25.872	0.4986	0.4992	+0.6
16.620	0.3460	0.3467	+0.7	26.527	0.5112	0.5121	+0.9
16.997	0.3539	0.3539	0.0	27.749	0.5347	0.5354	+0.7
16.619	0.3460	0.3458	−0.2	26.401	0.5088	0.5078	−1.0
28.104	0.5851	0.5856	+0.5	15.230	0.2934	0.2942	+0.8
30.820	0.6417	0.6416	−0.1	16.415	0.3163	0.3173	+1.0

APPLICATION TO SEPARATION OF COPPER FROM TIN

The apparatus was tested on the separation of copper from tin. The electrode potentials of these metals are +0.345 and −0.136, respectively, and a separation of copper from appreciable amounts of tin by the ordinary electrodeposition process is impossible. The deposition was carried out from a hydrochloric acid solution containing hydroxylammonium chloride to prevent the liberation of chlorine at the anode, a procedure first described by Schoch and Brown (4).

Standard solutions of copper and tin were prepared as follows:

Electrolytic copper prepared by electrolysis of C.P. copper sulfate was dissolved in dilute nitric acid and converted to copper sulfate by evaporation with a slight excess of sulfuric acid. The gray, anhydrous copper sulfate remaining after fuming was dissolved carefully and diluted. The copper in this solution was determined electrolytically. A weight buret was used to measure out the solution. To each determination were added 3 grams of ammonium nitrate and 3 ml. of nitric acid. The electrolysis was carried out in the usual way and continued sufficiently long to ensure the deposition of all copper. The results are given in Table I.

Pure tin was dissolved in hot, concentrated hydrochloric acid in contact with metallic platinum, a reflux condenser being employed to prevent the loss of tin by volatilization. The solution was diluted carefully and the tin determined electrolytically. The solution was measured out with a weight buret, diluted to 250 ml., and treated with 10 grams of hydroxylammonium chloride. The tin was then deposited on a copper-plated platinum electrode, using a current of 1.5 amperes. Just before discontinuing the electrolysis the solution was neutralized with ammonia to avoid any solvent action of hydrochloric acid on the tin during the washing process. Following the deposition the solution was checked for the complete removal of tin. The results are given in Table I. This solution contained about 5 ml. of free, concentrated hydrochloric acid per 25 ml. It was found that if the solution was not diluted to about 250 ml. the tin deposited in part as large crystals at the top and bottom of the electrode.

Quantities of these solutions, delivered from a weight buret, were mixed and to the resulting solution were added 10 ml. of concentrated hydrochloric acid and 2 grams of hydroxylammonium chloride. The solution was then diluted to 150 ml. and the electrolysis begun at a current of 2 to 4 amperes. The apparatus was set to limit the cathode-calomel voltage to 0.45 volt (0.1 *N* calomel). The electrolysis was allowed to continue until the current had been decreased to about 0.020 ampere.

The course of the current and the cathode-calomel voltage during a typical deposition of copper are shown in Figure 4, the quantities of copper and tin present in the particular determination from which the data was obtained being, respectively, 0.3222 and 0.5190 gram. The action of the apparatus is clearly apparent. Once the critical potential, as set by the bias controls, is reached the calomel-cathode voltage is held constant and the current decreased to a residual value approaching zero. The electrolysis was generally continued until the residual current was about 20 milliamperes, the time required being from 20 to 40 minutes, depending on the amount of copper present.

Following the deposition of copper, about 8 grams of hydroxylammonium chloride were added, the solution was diluted to about 250 ml., and the tin deposited on a copper-clad platinum cathode.

The results of a series of separations of copper from tin are given in Table II. The separation is very successful as long as a large amount of tin is present, as in the analyses reported in Table II. When the amount of tin is less than that equivalent to the copper present the separation becomes erratic, low results being frequently obtained for copper. This peculiar behavior resides in the electrochemistry involved, however, and not in the action of the apparatus described, since similar erratic results were obtained on low-tin mixtures by the conventional, manual method of carrying out the graded cathode potential electrodeposition. Additional tin can be added when necessary, as, for example, in the direct determination of copper in bronze.

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St. Louis Plant of Fisher

Fisher Scientific Co. has established a plant at 2109 Locust St., St. Louis, Mo., to serve laboratories in the central states area with supplies. The 4-story building was engineered from its receiving platform to shipping room to expedite movement of orders. All floors and departments are furnished with modern equipment for efficient handling of merchandise and, as at Pittsburgh and New York, there is available a staff trained to render sales and technical service.

Microdetermination of Sulfate

A Colorimetric Estimation of the Benzidine Sulfate Precipitate

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IN CONNECTION with another investigation, the need arose for a simple, rapid, and accurate method for the determination of small amounts of sulfate, using only the limited equipment and reagents then available. The following method was developed and proved extremely satisfactory: The sulfate in the sample was precipitated as the benzidine sulfate, purified, and dissolved in 0.2 *N* hydrochloric acid, diazotized, and coupled, after destroying the excess nitrous acid, with Bratton and Marshall's reagent (*1*), *N*-(1-naphthyl)ethylenediamine dihydrochloride. The resultant intense purple color was read in a Klett-Summerson photoelectric colorimeter.

Colorimetric methods for the determination of sulfate are not new. Yoshimatsu (*8*) first treated the dissolved benzidine sulfate precipitate with a mixture of iodine, potassium iodide, and ammonia, and compared the resultant brown color with a benzidine sulfate standard similarly treated. Hubbard (*2*) and later Wakefield (*7*) used the yellow color produced by the action of hydrogen peroxide and ferric chloride on the dissolved benzidine sulfate precipitate. Kahn and Lieboff (*3*), thus far, were the only ones to couple diazotized benzidine sulfate with an alkaline solution of phenol. Lentonoff and Reinhold (*4*) used the color produced by the action of sodium β-naphthoquinone-4-sulfonate on an alkaline solution of benzidine sulfate as a basis for comparison.

Since its initial introduction by Bratton and Marshall (*1*) as a coupling agent for the determination of sulfonamides, *N*-(1-naphthyl)ethylenediamine dihydrochloride has found increasing popularity. Shinn (*6*) found that it gave superior results for the determination of nitrite in water, sewage, and foods. Recently, Looney and Dyer have used this reagent with success in the determination of potassium in serum (*5*).

REAGENTS

Sodium nitrite, 0.1%, prepared fresh daily.

Ammonium sulfamate, 0.5%.

N-(1-naphthyl)ethylenediamine dihydrochloride, Eastman Kodak Company No. 4835, 0.1%. The solution is made up in distilled water and kept in a dark bottle. The reagent keeps best if refrigerated.

Benzidine hydrochloride reagent, 4.0 grams of pure benzidine hydrochloride dissolved in a small amount of distilled water and made up to 250 ml. with 0.2 *N* hydrochloric acid.

Standard Sulfate Solutions. Stock, 0.5437 gram of reagent potassium sulfate dissolved in distilled water and diluted to 1 liter; 1.0 ml. is equivalent to 0.3 mg. of sulfate. Dilute standard, 10.0 ml. of the stock standard diluted to 100 ml. with distilled water; 1.0 ml. is equivalent to 0.03 mg. of sulfate.

Standard Benzidine Hydrochloride. Stock, 0.4014 gram of pure benzidine hydrochloride dissolved in a small amount of 0.2 *N* hydrochloric acid and diluted to 100 ml. with the same solvent; 1.0 ml. is equivalent to 1.5 mg. of sulfate. Dilute standard, 1.0 ml. of stock solution diluted to 100 ml. with 0.2 *N* hydrochloric acid; 1.0 ml. is equivalent to 0.015 mg. of sulfate.

Acetone-ethanol, 1 to 1, using 95% ethanol and reagent acetone.

PROCEDURE

To a 15-ml. centrifuge tube with a narrowed conical bottom, containing 1.0 ml. of glacial acetic acid and 1.0 ml. of benzidine hydrochloride reagent, an amount of sample containing between 0.015 and 0.15 mg. of sulfate is added, the solution is mixed, 2.0 ml. of acetone-alcohol are added, the tube is capped, and the contents are thoroughly mixed by rolling between the hands. The tube is allowed to stand in an ice-water bath for a half hour to complete precipitation.

At the end of this period, the tube is centrifuged at 2500 r.p.m. for 10 minutes and the supernatant poured off with one rapid motion. While still inverted, the mouth of the tube is carefully touched with a pad of clean filter paper to blot off excess liquid. The tube is permitted to drain on the filter paper pad for 5 minutes.

The walls of the tube are washed with 2.0 ml. of acetone-alcohol in a manner to avoid disturbing the tightly packed precipitate. The tube is recentrifuged for 5 minutes, and the supernatant poured off and permitted to drain for 5 minutes on the filter paper pad. The washing and draining procedure is repeated once more.

Two milliliters of 0.2 *N* hydrochloric acid are blown into the centrifuge tube. The precipitate dissolves almost instantly. The tube is placed in an ice-water bath, 1.0 ml. of sodium nitrite solution is added, and the tube is shaken to mix the solutions and allowed to stand for 3 minutes. One milliliter of ammonium sulfamate solution is added, and the tube is shaken and allowed to stand for an additional 2 minutes. Finally, 1.0 ml. of *N*-(1-naphthyl)ethylenediamine dihydrochloride solution is added to develop the color. After mixing, the violet solution is permitted to stand for 20 minutes, transferred quantitatively to a 50-ml. volumetric flask, and diluted to mark with distilled water. The solution is read in a photoelectric colorimeter using a green filter. The Klett No. 54 is satisfactory for the Klett-Summerson instrument.

It is recommended that a standard solution of benzidine hydrochloride or standard sulfate solution containing 0.03 mg. as sulfate be carried through, at the same time. The former solution need only be diazotized and coupled, while the latter must be carried through the entire procedure as described above. A blank, using 2.0 ml. of 0.2 *N* hydrochloric acid, diazotized and coupled with the color reagent, serves as zero reading on the photometer.

Table I. Determination of Sulfate

SO_4 *Mg.*	SO_4 Recovered *Mg.*	Error *Mg.*	Error %
0.0150	0.0152	0.0002	1.3
0.030	0.0300	...	0
0.030	0.0303	0.0003	1.0
0.060	0.060	...	0
0.060	0.0597	0.0003	0.5
0.090	0.0921	0.0021	2.3
0.150	0.1470	0.0030	2.0

CALCULATION

Reading of unknown ÷ reading of standard × concentration of standard = mg. of sulfate in sample

DISCUSSION

This method offers many advantages. The ease and simplicity with which the determination is carried out, together with the availability of the reagents used, are foremost. No extreme precautions are necessary beyond attention to the use of narrowed conical tips on the centrifuge tubes. These are readily fashioned by drawing the tips of ordinary glass centrifuge tubes in a flame until the diameter of the tip is about 2 mm. After centrifugation, the precipitated benzidine sulfate packs tightly and can be easily washed without disturbance or loss. Tubes which have been soaked in dichromate–sulfuric acid mixture must be carefully washed to remove all traces of sulfate.

The color obtained on final coupling with Bratton and Marshall's reagent has remarkable stability. In initial runs, stability studies revealed no decrease in intensity even after 12 hours. The color follows Beer's law closely. This is true of both solutions of pure benzidine hydrochloride and known sulfate solutions used in recovery studies. The precision of triplicate determinations was excellent, not varying by more than 0.1%.

The limitations of benzidine sulfate precipitation in the presence of large amounts of chloride and phosphate should be pointed out. At the time this study was carried out the sulfate concentration in a dialyzate which had no, or at most, traces of chloride

and phosphate present was being checked. This provided no interference. In the case of biological materials, phosphate should be completely removed. Chloride should not be present in amounts such that the weight ratio of chloride to sulfur exceeds 30; else, precipitation of benzidine sulfate is not complete.

The method is accurate in a range extending from 0.05 to 0.150 mg. of sulfate with a maximum error of 2%. The results obtained on pure sulfate solutions are summarized in Table I. Each value is the mean of triplicate determinations.

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NOTES ON ANALYTICAL PROCEDURES

An Observation of Possible Value for Sugar Determinations

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IN AN attempt to modify the Sichert and Bleyer reagent (*1*) for micro use, potassium iodide was included in the reagent. When this reagent was heated with glucose, a precipitate formed which was identified as cuprous iodide. The presence of the iodide ion speeds up the reduction reaction, presumably because of the removal of the cuprous ion from solution as cuprous iodide. The observation may have value for the determination of sugars, for the cuprous iodide can be determined iodometrically in the solution or separated and weighed directly. Its weight is of the order of ten times that of the glucose taken.

The reagent may be made up as follows: To 250 cc. of water are added 500 grams of hydrated sodium acetate, 75 cc. of 5% acetic acid, and 5 grams of potassium iodide. The solids are dissolved by warming the solution, and 25 grams of crystalline cupric sulfate are added as a 10% solution. About 40 grams of glucose are now added, the volume is made up to 1 liter, and the mixture is heated in boiling water for 45 minutes, let cool slowly, and filtered to remove the crystalline precipitate of cuprous iodide which had separated during the heating. For the determination, the solution is heated for 30 minutes at 100° with an equal volume of glucose solution.

Further work on this reagent is not contemplated.

Lowering of the pH by the addition of more acetic acid results in the formation of less precipitate for a given amount of glucose; if the amount of acetic acid is decreased, a white precipitate (apparently cupric hydroxide or a basic cupric salt, as it is nonreducing) is formed on heating, even in the absence of glucose. Omission of the preliminary heating with glucose causes a slight black precipitate to form during the determination if the quantity of glucose being determined is very small. The use of more than 5 grams of potassium iodide per liter increases the speed of the reaction, but makes iodometric estimation of cuprous iodide in the solution difficult. Less than 0.2 gram of glucose will give no reduction. The reagent is affected by maltose, whose reduction, under the conditions mentioned, is about 1/6 that of an equal weight of glucose. The maltose reduction is not complete even after 50 minutes of heating, whereas the glucose reduction shows no increase after 30 minutes.

LITERATURE CITED

(1) Sichert and Bleyer, *Z. anal. Chem.*, 107, 328 (1936).

THIS work was aided by a grant from Mead Johnson and Co.

Improved Distillation Receiver

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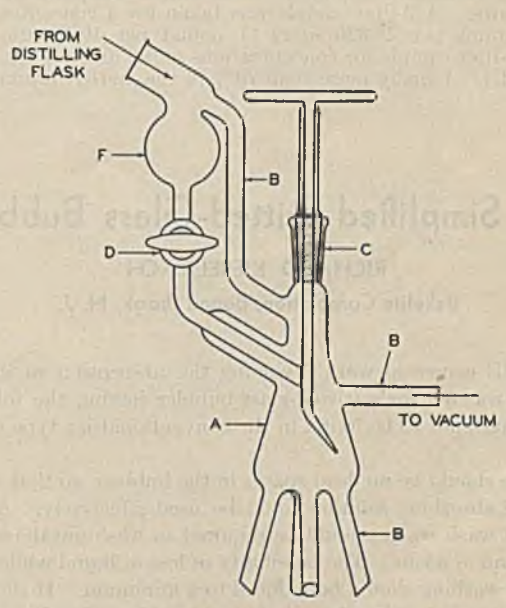
Georgia School of Technology, Atlanta, Ga.

THE usual variety of cow's-udder fraction cutter (*1*) has the inherent disadvantage that each drop of distillate must be collected in one of the receivers attached to the cutter. The modification described makes it possible to interrupt the flow of a distillate while the operator follows the course of a temperature change in the fractionating column. Since the fraction cutter can be clamped in a stationary position, it is possible, in the case of low-boiling fractions, to surround the entire receiver with a freezing mixture.



In practice bulb *F* is approximately 10 ml. in diameter. Ground joint *C* has a standard taper 14/35, and the body, *A*, is made from a 50-ml. Erlenmeyer flask. All glass tubing, *B*, is 8 mm. The inside rod that conducts the distillate to the receivers must rotate freely and must not leave the surface of the tube coming from stopcock *D*, 2-mm. bore.

LITERATURE CITED

(1) Reilly and Rae, "Physico-chemical Methods", 3rd ed., Vol. II p. 94, New York, D. Van Nostrand Co., 1939.

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A Modification of the Ethanolamine Hydrolysis Method for Determination of Methyl Bromide

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THE study of the insecticidal value of methyl bromide as a fumigant requires a method for determination of this compound mixed with air. A modification of the method described by Stenger *et al.* (*1*) has been developed for its determination in the atmosphere of chambers during fumigations.

The sampling methods described by Stenger *et al.* (*1*) for various reasons could not be applied directly under some of the conditions which the authors encountered in their work, which required taking successive samples from several locations simultaneously. The modification developed involves withdrawal by aspiration of a portion of the atmosphere of the chamber, passing it through a series of absorption tubes packed with sand wet with ethanolamine, and subsequent determination of the bromide ion by the Volhard method. In this way gas losses during sampling are avoided.

Various types of absorption tubes may be used. The one which the authors found most satisfactory was made from 1-cm. (inside diameter) glass tubing bent in the form of a V, with the bend flattened so that the packing could be retained in one arm. The sand used for packing was of such a size that it would pass through a 10-mesh screen but be retained on a 16-mesh screen. It was prepared for use by digesting with concentrated nitric acid, washing free of acid, and igniting. The absorption tubes, each containing a column of sand 25 cm. high, moistened with 2 ml. of ethanolamine, were connected by means of inverted U-tubes, and all joints were rubber-covered glass to glass. The number of absorption tubes in series is dependent upon the concentration of methyl bromide and the rate of aspiration. The authors used four absorption tubes and a sampling period of 20 minutes. A 2-liter sample was taken for a concentration of 453.6 grams per 28,320 liters (1 pound per 1000 cubic feet) and a 1-liter sample for concentrations of 0.9 and 1.8 kg. (2 and 4 pounds). Usually more than 70% of the methyl bromide was recovered from the first absorber, about 20% from the second, 6% from the third, and 2 % from the fourth.

Following sampling, the contents of the absorption tubes were washed into Erlenmeyer flasks, and the bromide ion was determined by the Volhard method. Since ethanolamine retards the end point, a blank on the same amount of ethanolamine should be carried through the procedure and allowed for in calculating the results.

This method has given results reproducible with a standard deviation of ±0.01 pound with methyl bromide concentrations of approximately 1 pound per 1000 cubic feet.

The amounts of methyl bromide recovered from a fumigation chamber of 1000 cubic feet capacity ranged from the equivalent of 96 to 100% of a 1-pound charge (mean recovery 97%), 95 to 100% of a 2-pound charge (mean recovery 97%), and 91 to 98% of a 4-pound charge (mean recovery 95%). The samples were taken 15 minutes after introduction of the fumigant. However, this chamber was exposed to the effect of wind. A statistical analysis was made, using the results obtained from 88 individual samples, to calculate the normal rate of leakage and the increased rate due to wind of different velocities. Using the calculated concentrations in the chamber at the time of sampling and comparing with the analytical results, the recoveries were between 1 and 2% greater than those presented.

ACKNOWLEDGMENT

The authors are indebted to W. E. Fleming for the statistical analysis of certain of the data.

LITERATURE CITED

(1) Stenger, V. A., Shrader, S. A., and Beshgetoor, A. W., IND. ENG. CHEM., ANAL. ED., 11, 121 (1939).

A Simplified Fritted-Glass Bubbler

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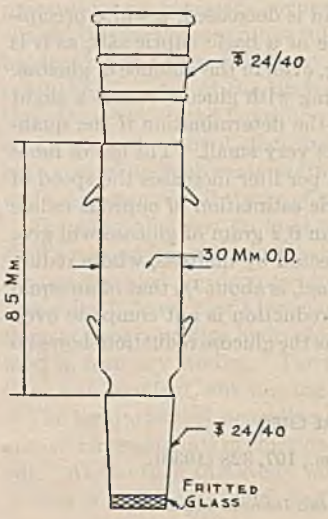
IN THE course of work involving the absorption of gases, a need was felt for a fritted-glass bubbler having the following advantages not to be found in the conventional jar type of bubbler.

There should be no dead spaces in the bubbler, so that a minimum of absorbing solution could be used effectively. A minimum of wash water should be required to wash out the solution at the end of a run. The possibility of loss of liquid while draining and washing should be reduced to a minimum. It should be possible to connect a series of bubblers quickly and easily, without the use of rubber tubing, and without difficulties of alignment. The bubbler should preferably be compact, rugged, and inexpensive.

The bubbler shown in the illustration proved to be the answer to the problem, and is of the simplest possible design. When a gas flow rate of 300 ml. per minute is used, the bubbler operates efficiently with as little as 15 ml. of absorbing solution. For special applications, the dimensions could, of course, be altered.

In operation, a series of bubblers is set up in the following manner: The No. 1 bubbler is inserted in the standard taper neck of a flask bearing a tubulature for the entrance of the gas. A slight pressure (lung power is enough, where permissible) is applied at the tubulature, and the absorbing solution poured into the bubbler. The tubulature is closed by means of a stopcock or pinchclamp to maintain the pressure, and a second bubbler inserted in the neck of the first. The process is repeated for as many bubblers as are required, and the apparatus is ready for use.

At the completion of a run, a suction is applied at the tubulature of the flask, pulling the solution into the flask. A very small amount of water from a wash bottle is required to wash down the bubblers, this being sucked down in the same way. Obviously, loss of liquid is next to impossible at this stage. Ideally, the receiving flask should be so designed that it can be used as a vessel for subsequent reactions.



This type of bubbler has one disadvantage—the necessity of maintaining a gas pressure while filling, and until putting into operation—which may preclude its use in certain applications. For many purposes, however, this inconvenience is more than compensated for by the advantages listed above.

Apparatus for Washing Selas Crucibles

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Lucky Heart Laboratories, Inc., Memphis, Tenn.

A RECENT article (*1*) outlines an arrangement for washing Selas filtering crucibles by reverse flow. This laboratory has performed this task in a slightly different manner, which the author believes to be more convenient and perhaps more economical of material.

The crucible to be cleaned is placed in an inverted position with the top fitting into a circular groove cut into the top of a Walter-type crucible holder held in a standard filter flask. The bottom of the crucible is covered with another holder of the same type from which the regular stem has been removed. A short-stemmed funnel is placed upright in the opening of the holder. The groove in the lower holder is filled with a suitable liquid, the crucible is pressed down as suction is applied to the filter flask, and the desired washing medium is poured into the funnel. Strong acids or solvents which would damage the rubber connection should be added at such a speed that the volume does not build up in the reservoir, and the liquid runs through the filter as soon as it is added. Unnecessary damage to the rubber connections between filter flask and trap is also avoided by the stem in the lower holder which conveys the wash liquid down into the flask and prevents splashing into the side arm.

This arrangement utilizes equipment readily available and requiring no modification other than cutting the small groove in one of the holders, which in no way interferes with its subsequent use in the usual manner.

LITERATURE CITED

(1) Benne, E. J., IND. ENG. CHEM., ANAL. ED., 16, 277 (1944).

LABORATORIES and NEW EQUIPMENT

Hotel Association Testing Laboratory

The American Hotel Association testing laboratory, which is part of the Department of Hotel Administration at Michigan State College, East Lansing, Mich., is located on the college campus. Bernard R. Proulx is head of the department and Bruce Hartsuch is in charge of the laboratory and all tests.

Established about a year ago, the laboratory is as yet meagerly equipped and uses considerable equipment from another department of the college, but when war conditions permit it is planned to equip it fully and make it one of the outstanding laboratories of the country. Tests of textiles and cleaning compounds, as well as other physical tests, are now being made.

Specimen Holder for Abraser

A specimen table for the Taber abraser, that permits testing paper products for wear resistance in moist or wet condition, has been announced by Taber Instrument Corp., North Tonawanda, N. Y. Results of tests are reported either numerically as the number of wear cycles to produce a given amount of wear, or as loss in weight when weighed on a precision laboratory balance.

Autoclave

A 1-gallon autoclave, made by the Industrial Machinery Co., Bloomfield, N. J., is now available for prompt shipment. It combines in its design, construction, and equipment the functions and usual features of large high-pressure autoclaves, sulfonators, nitrators, chlorinators, kettles, and similar equipment.

Tin-Plated Panels

Bright tin-plated sheet steel panels, approximately 31 gage and measuring 3 × 5 inches, are available in limited amounts from the Stewart Research Laboratory, P. O. Box 173, Washington, D. C. They are used in flowout and flexibility tests of paints and varnishes, in kauri-reduction tests, and for filing samples for reference.

BOOK REVIEWS

Colorimetric Determination of Traces of Metals. *E. B. Sandell.* xvi + 487 pages. 15 × 23 cm. Interscience Publishers, Inc., 215 Fourth Ave., New York, N. Y., 1944. Price, $7.00.

This book is Volume III of "Chemical Analysis", the new series of monographs on analytical chemistry and its applications being published by Interscience Publishers. It is a worthy addition to their list, published or in preparation.

The author's aim is presentation of a "limited number of methods which . . . appear to be best suited for dealing with traces of metals. No one reagent is necessarily the best for the determination of an element in all kinds of samples or under all conditions, and consequently two or three methods are described. . . for a number of the metals. The treatment is to a considerable extent based on the experience of the writer . . ."

Part I is a general introduction. The four chapters deal with trace analysis (17 pp.), methods of separation (15 pp.), methods of measurement (40 pp.), and 19 general colorimetric reagents (36 pp.). Anyone not widely experienced in colorimetric methods of measurement will find much valuable information in this section. The chapter on measurement seems the weakest, but obviously the present range and variety of instruments could not be covered in the space available. A comprehensive critical discussion of this subject is definitely needed. In connection with the stated limit of range of colorimetric methods, Mehlig showed the practicability of working with far higher concentrations of iron and of copper in ores.

Part II, covering analytical procedures, includes methods for 45 separate metals and the rare earth elements. Since various metals (Ac, Cs, Hf, Ma, Pa, Ra, Rb, Sr, Th, Y) are omitted and only selected procedures are described, perhaps a more accurate title might have been "Selected Methods for the Colorimetric Determination of Certain Metals". It seems likely that many analysts will wish that here had been included methods for nonmetallic elements, such as the halogens, silicon, nitrogen, and phosphorus (those omitted are B, Br, C, Cl, F, H, I, N, O, P, Po, S, Se, Si, Te). A second volume might be devoted to these elements.

In general, the information for each element is classified under separations, methods of determination, and industrial applications, such as rocks, ores, minerals, metals, soils, water, and biological materials. Adequate operating directions are given for applying the

selected methods, and many references and notes document and amplify the material. As stated in the preface, balanced treatment is still impossible because of the lack of information on, and critical study of, many methods. Most colorimetric methods need such investigation.

In view of Professor Sandell's contribution to colorimetry in this meritorious book, the reviewer hesitates to offer an adverse comment. The following statements and questions are included, therefore, with the sole aim of helping to clarify and improve analytical literature. Grid lines are generally desirable in using graphs, and observed points should be shown if possible. One likes to know the spectral band width used in determining curves such as that for the permanganate solution (see Figure 4, which does not show the characteristic small bands. Why?). Consistency is desirable in formulating the heteropoly compounds, preferably in accordance with Keggin's work (including naming in terms of the central atom). There is possible uncertainty regarding the plotting of the spectral curves because of the use as ordinate legends of extinction, $\log I_0/I$, $\log \epsilon$, transmission, transmittancy, and absorption. Is it transmission or transmittancy (the terms are not synonymous)? Is it ϵ or $\log \epsilon$ (Figure 27)? Is it absorption—that is, $100 - T$ (Figure 49)? Is transmittancy a logarithmic value (Figure 64)? Is it not simpler to plot transmittancy directly on semilogarithmic paper, rather than $\log 1/T$ (or $\log I_0/I$) on linear paper, for curves to test conformity to Beer's law or to use in determinations?

The author's justified caution about using others' extinction coefficients (p. 57) implies the necessity of determining one's own calibration data for any specific instrument.

Very few typographical errors were noted. Optical density is meant on page 34. The symbol Ti appears twice in the table on page 75. Although hardly an error, the use of the abbreviation "etc." leaves something to be desired from the standpoint of concise scientific writing.

Conversations with industrial analysts have confirmed the reviewer's initial opinion that this book is now the first compilation to which to turn in the hope of finding a tested colorimetric method, clearly presented for use. Analytical chemists are indeed indebted to Professor Sandell for such a monograph.

M. G. MELLON

Experimental Spectroscopy. *Ralph A. Sawyer.* 323 pages. Prentice-Hall, Inc., New York, N. Y. Price, $5.00.

This book fills a long-felt need for a general text and reference book on applied spectroscopy. The author deals with spectroscopy as a tool for the chemist or physicist, and not as a science in itself. The primary emphasis is placed on those principles and techniques that are fundamental to the applications of spectroscopic equipment as a general research tool. Highly theoretical topics are avoided and no attempt is made to cover phases of experimental spectroscopy requiring highly specialized knowledge of mathematics or atomic physics. The origin of spectra, the Raman effect, absorption spectrophotometry, and similar topics of rather limited interest or requiring extensive mathematical treatment are largely omitted.

A brief historical sketch of the development of spectroscopy and a short nontechnical discussion of light sources make up the first two chapters. Chapters 3 to 7 provide the most complete, readable presentation of the principles of design, adjustment, and use of prism and grating spectrographs which has appeared in a single book or paper. If the book contained nothing else of merit, this section alone would make it a valuable contribution to spectroscopic literature. The reviewer could not escape the impression that the author underestimates the proportion of spectrographs employing original gratings now in use. Dr. Sawyer's industrial experience being largely limited to prism spectrographs, he is apparently not fully aware of the fact that spectrographs employing original gratings are now produced in greater quantity than prism spectrographs in this country.

Chapter 8 covers the photographic process as used in spectrography. The author makes no attempt to deal with the theoretical aspects of photographic chemistry and presents little new material on the application of photography to spectroscopy. However, the familiar properties and behavior of the emulsion are adequately covered and correlated with spectroscopic applications. Interesting and useful data, assembled from previous publications of Kodak Research Laboratories, are presented.

The determination of wave length is treated in some detail in Chapter 9, with particular attention to practical methods based on comparison with primary, secondary, and tertiary standards. Conventional methods of extrapolation are discussed and the practical methods of identifying lines in analytical work are described. References to all the most important sources of wave-length data are given. Fundamental methods of wave-length determination, such as interferometry, are mentioned only casually.

Chapter 10, dealing with the measurement of light intensity, provides an excellent general treatment of the problem of photometry in both emission and absorption spectroscopy and covers both visual and objective instrumental methods of photometry. Valuable notes on technique and sources of error are included. A number of commercially available microphotometers are described and, while these descriptions are for the most part reasonably accurate, the author was apparently unfamiliar with one of the makes discussed, the Applied Research Laboratories comparator-densitometer. The author classified this instrument as a projection-type instrument and describes a projection-type microphotometer as one in which "a considerable length of the spectrum is projected on a screen carrying an adjustable slit, behind which the light-sensitive element is placed". The Applied Research Laboratories instrument is not a projection instrument in this sense and employs projection only as a means of providing the comparator feature and locating the line to be measured. Moreover, the film or plate is not moved during the scanning of the line, as implied by the author, and the remarks concerning the relative advantages and disadvantages of the projection-type instrument do not strictly apply to this microphotometer. Aside from this rather minor confusion, the treatment of photometers is unbiased, accurate, and effective.

The last three chapters, 11, 12, and 13, deal with infrared spectroscopy, vacuum ultraviolet spectroscopy, and "spectrochemical" analysis, respectively. These chapters give a brief but reasonably complete treatment of the practical aspects of the selection and design of the instruments and the fundamental techniques employed.

The reviewer noted several deviations from currently accepted spectroscopic nomenclature. Among the words or phrases used, to which the scientific lexicographer might take exception, are "linear dispersion" and "spectrochemical analysis". The author's use of such words and phrases detracts infinitesimally from the value of the book and is objectionable only because the book will undoubtedly become one of the standard references on the subject and is likely to be widely quoted.

Not only is the book valuable for the specific spectroscopic information it contains, but also it provides an excellent bibliography. The references given are well selected and few important references are omitted. The bias and prejudices common to most discussions of spectroscopic apparatus and techniques are notably absent in this work, and the author's impartial factual treatment of the controversial phases is highly praiseworthy. Remarkably complete, considering the scope of the subjects embraced, the book should be of great help in broadening the knowledge and interests of workers already in the field. While highly theoretical phases have been avoided, the author presents sufficient fundamental theory and correlates it so well with practical considerations that the book should contribute towards rationalizing the now overly empirical art of spectroscopy.

J. RAYNOR CHURCHILL

Experiments in General Inorganic Chemistry. *J. L. Maynard* and *T. I. Taylor.* 550 pages. D. Van Nostrand Co., 250 Fourth Ave., New York, N. Y., 1944. Price, $2.90.

A laboratory manual designed to accompany "General Inorganic Chemistry" by Sneed and Maynard, which may be adapted for use with any standard textbook by changing the order of experiments. Laboratory techniques and descriptive aspects of chemistry are emphasized.

Specifications for Analytical Reagents

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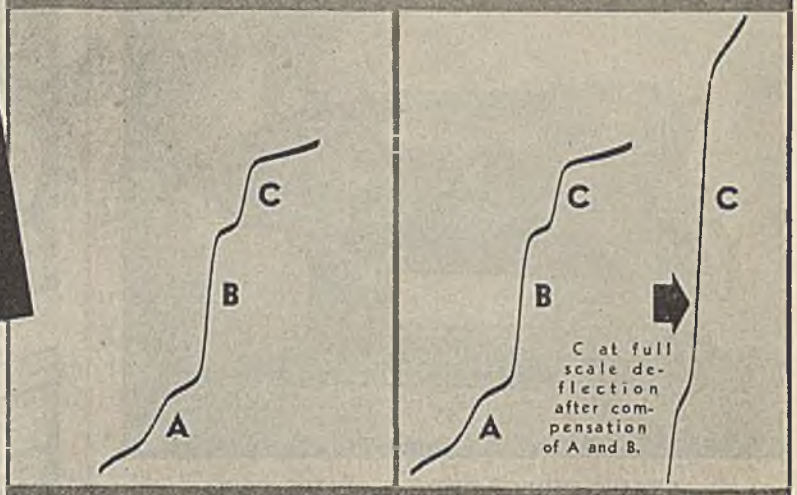

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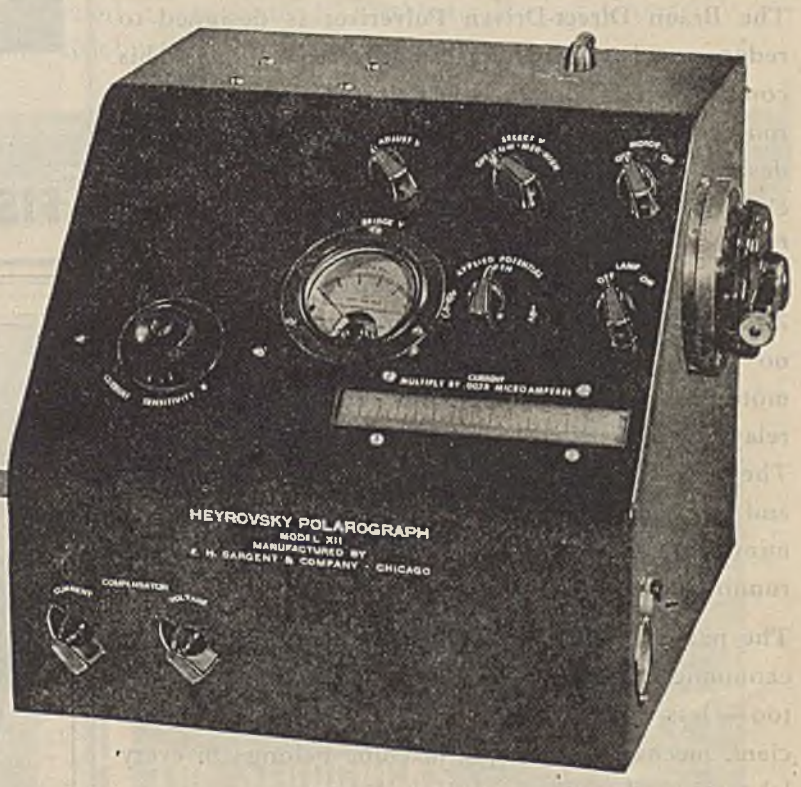

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