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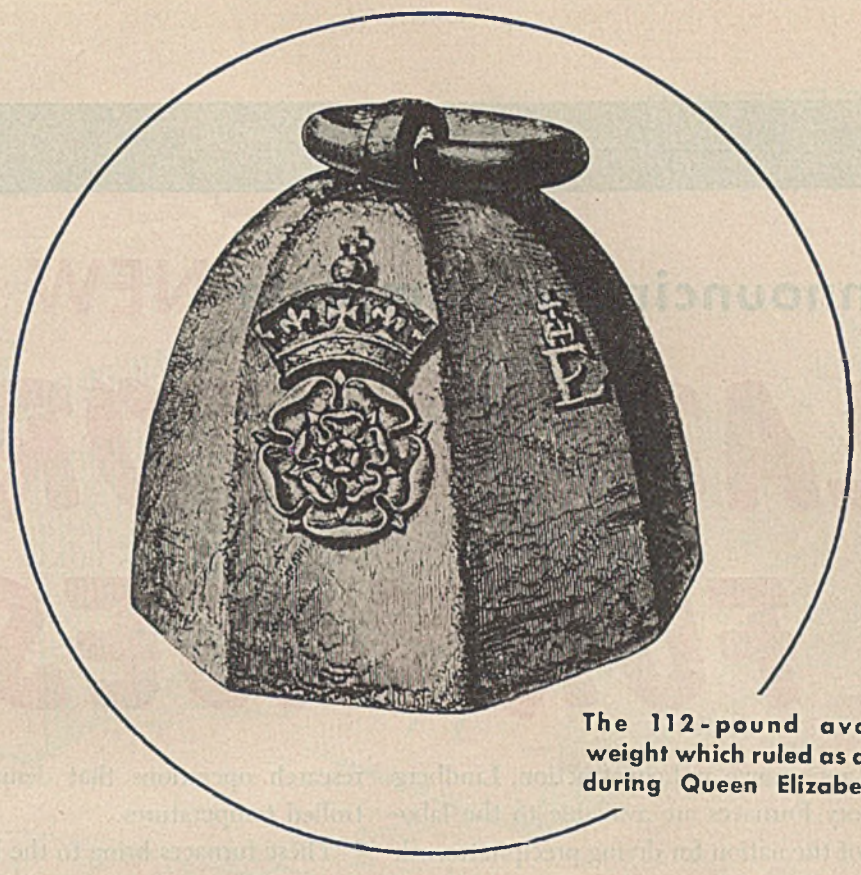

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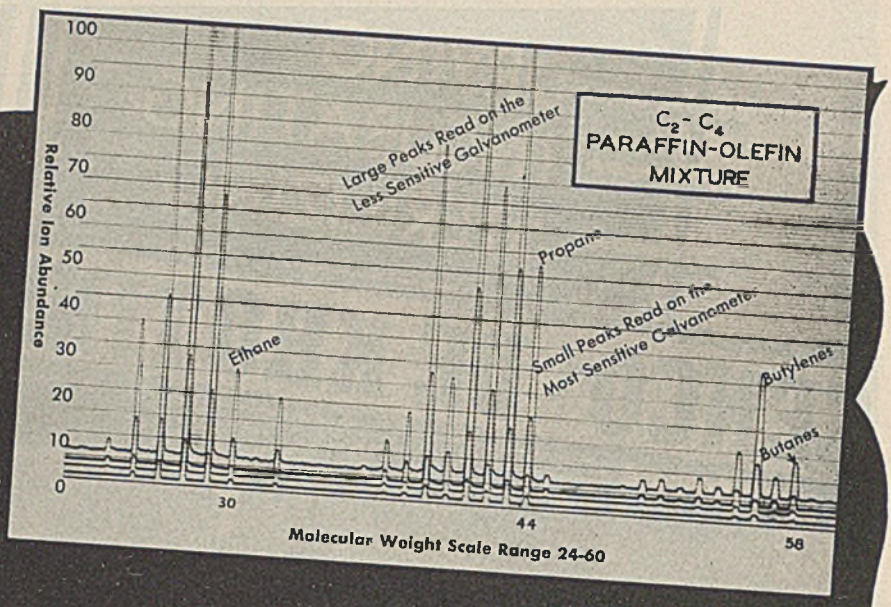

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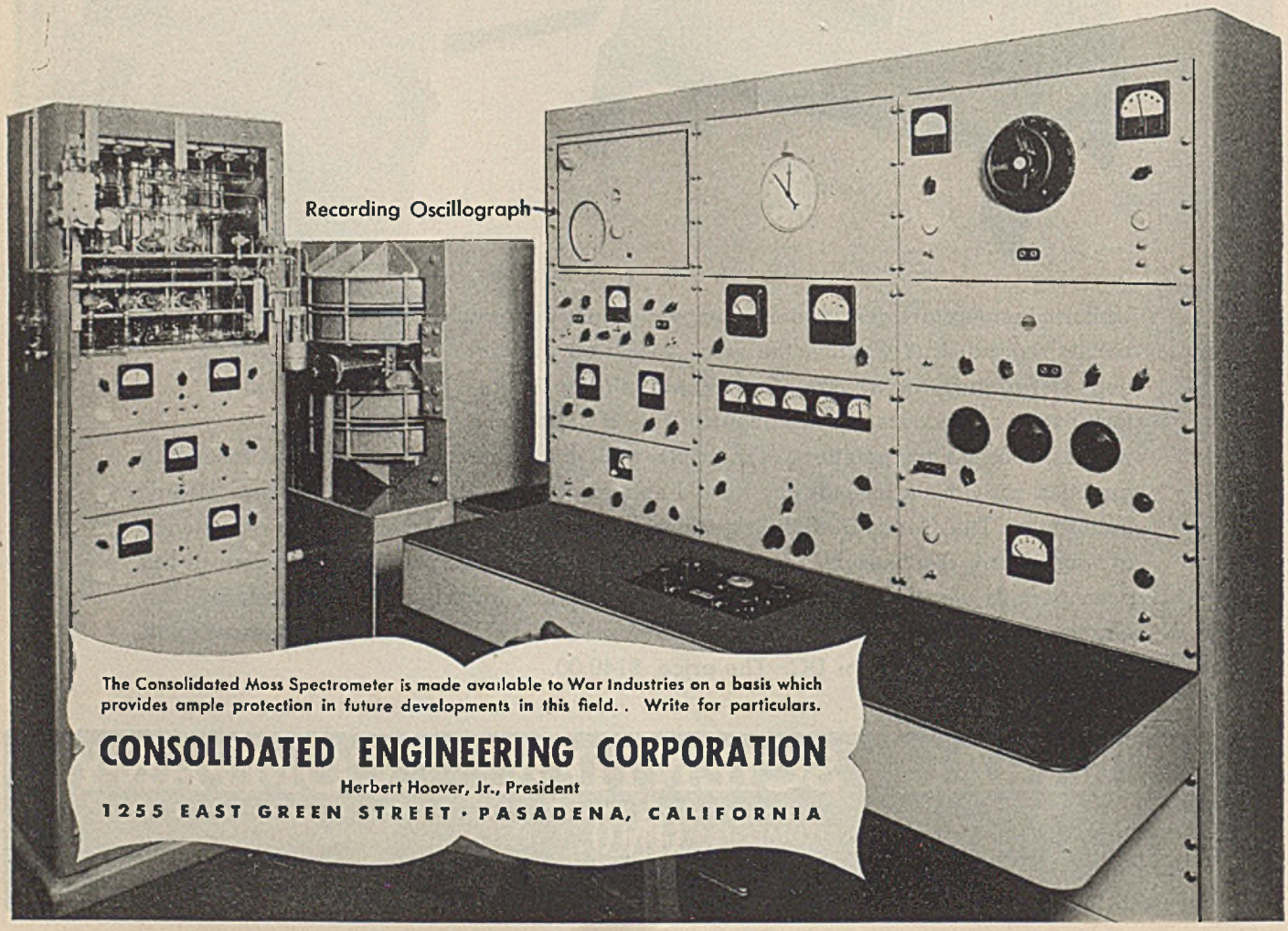

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INDUSTRIAL AND ENGINEERING CHEMISTRY

ANALYTICAL EDITION

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A Vacuum Sublimation and Molecular Distillation Apparatus

BYRON RIEGEL, JOHN BEISWANGER, AND GEORGE LANZL, Northwestern University, Evanston, Ill.

A molecular distillation and vacuum sublimation apparatus, for laboratory use, consists of a manifold maintained at 10^{-5} mm. and an auxiliary degassing line. The manifold is equipped with twelve ground-glass openings of various sizes for use with different types and sizes of stills. A description of some of the stills is included.

INVESTIGATIONS with compounds of high molecular weight, such as steroids and carcenogenic hydrocarbons, led to the construction of a molecular distillation and vacuum sublimation apparatus for purposes of purification and separation. To minimize the time necessary to accomplish these operations, an apparatus was desired which would embody the following features: (1) immediate availability of a high vacuum to a number of operators; (2) distillation and sublimation apparatus useful for macro to micro quantities; (3) arrangement of the apparatus so that the distillations or sublimations could be started or stopped at will; (4) preservation of products in case of mechanical failure of the system; (5) continuous operation of the system with a minimum of attention; and (6) electrically heated stills that would require little or no attention. To fulfill these needs the apparatus shown in Figures 1 and 2 was constructed.

This apparatus consists primarily of two vacuum lines, the manifold which is maintained at a free air pressure of 10^{-5} mm. (McLeod gage), and the exhaust line which is maintained at 10^{-3} mm. The exhaust line serves not only to complete the degassing of the distilland, but also to effect partial evacuation of the still which then may be connected to the manifold without allowing an appreciable rise in pressure. The introduction of a new distillation unit does not, therefore, impair the efficiency of the distillations already in progress. Twelve standard-taper ground-glass outlets are placed along the manifold at intervals of 20 cm. Between the manifold and each outlet are placed wide-bore stopcocks (Eck and Krebs No. 5064), whereas stopcocks of narrower bore (Eck and Krebs No. 5096) connect each outlet with the exhaust line. These are arranged as shown in Figures 3 and 4.

Stopcocks of varying sizes are used. The necessity for this arises from the variation in size of the ground-glass outlets which, in turn, is determined by the design and size of the stills employed. The two largest outlets (size 24/40, stopcock 10 mm.) occupy the center positions on the manifold and thus are nearest the source of vacuum. The two outlets on either side of these are somewhat smaller (size 19/38, stopcock 8 mm.), whereas the smallest outlets (size 14/35, stopcock 6 mm.) occupy the three positions at each end (Figure 2). The stopcocks between the outlet and exhaust are all of the same size (4 mm.). Each outlet is provided with a ground-glass plug which serves to prevent exposure to the atmosphere and dust when not in use.

For construction of the manifold, 40-mm. Pyrex tubing is employed, whereas the exhaust line is constructed of 10-mm. tubing. The assembly of the apparatus is facilitated by constructing it in two halves, which are connected after being mounted on the rack. In this way the possibility of strain is diminished.

The low pressure in the manifold is maintained by an all-steel mercury diffusion pump (Cenco Supervac) and a motor-driven backing pump (Cenco Pressovac). A dry ice trap between the pumps and the manifold prevents condensable gases from entering the pumps and mercury vapors from entering the manifold. A stopcock (Eck and Krebs No. 5044, 15 mm.) between the mercury diffusion pump and the backing pump allows maintenance of the vacuum, even though the pumps are stopped. Para-rubber tape (Cenco) has proved effective for metal-to-glass seals on the pumps and stopcock.

At one end of the manifold is a McLeod gage, protected by a dry ice trap. This gage has been employed for convenience, and does not represent the real pressure of residual vapors in the distillation units. It does, however, record air leakage accurately and give a valuable indication of vacuum-tightness. The true pressure of residual vapors could be measured by the use of a Pirani gage attached to the still with a suitable ground-glass joint.

An ordinary rotary pump (Cenco Hyvac) has proved adequate for the maintenance of the low pressure in the exhaust line. This line is likewise provided with a McLeod gage for which a trap is unnecessary, since the pressure reading is of secondary importance, serving only to indicate when the substance to be distilled has been degassed and the still evacuated.

The rack upon which the apparatus is mounted is constructed 3.75 × 3.75 × 0.3 cm. (1.5 × 1.5 × 0.125 inch) channel iron welded at the corners. The uprights, upon which the manifold, exhaust line, heaters, stills, McLeod gages, and traps are clamped, are 1.25-cm. (0.5-inch) rods mounted 20 cm. apart and bolted to the main frame. In order that the traps may be accessible, no upright is placed in the center (Figures 1 and 2). The rack is mounted 28 cm. from the wall in order to furnish room for a shelf upon which the pumps are placed.

Heaters

To provide a constant and easily adjustable source of heat, without use of an oil or metal bath, electrically heated air baths were constructed. These consist primarily of an asbestos-covered can and lid provided with a Nichrome heating element and a supporting clamp. The dimensions of each heater are determined by the size of the still for which it is to be used. The heating element consists of Nichrome resistance wire (No. 28),

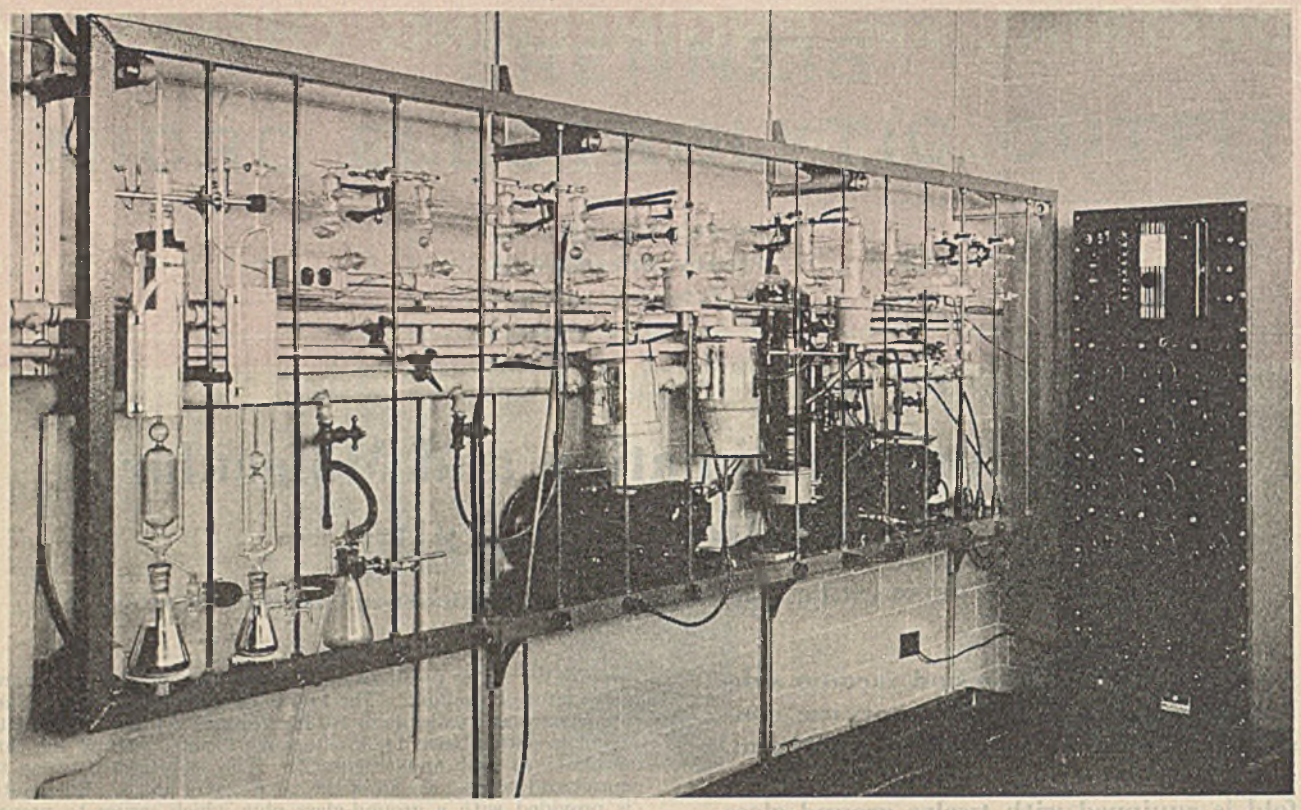

FIGURE 1. MOLECULAR DISTILLATION AND VACUUM SUBLIMATION APPARATUS

one half of which is coiled. A length is chosen (3 meters) which gives about 300 watts at 110 volts. The coiled end of the wire is fastened at *A* (Figure 5) and the coil is distributed over the bottom of the can. The remainder of the wire is threaded through a hole at *B*. Portion *C* is insulated with asbestos, after which the remainder of the wire is coiled around the outside of the can. The outside coils are covered with asbestos, whereas the inner coils are covered with a thin layer of alundum cement. A porcelain washer at *B* serves to insulate the wire. The terminals at *A* and *D* consist of a brass bolt and several brass nuts insulated with mica washers. Porcelain insulators could also have been used. After the asbestos has dried it is coated with water glass to retard abrasion.

The heater support consists of two parts: a brass bracket, *E*, riveted to the can, and a brass rod, *F*, with a vertical hole drilled so that *E* fitted tightly inside it. A brass or iron rod, *G*, is fastened securely to *F* with a set screw. The heaters are supported by clamping *G* to the uprights with standard clamp holders. Figure 4 shows a still and heater in operation.

Temperature Control and Measurement

The temperature adjustment of the distillations is accomplished by the use of autotransformers (General Radio Co., Variac, Type 200-CU). These, provided with switches and pilot lights, are mounted on a panel board placed near the distillation apparatus. A complete wiring diagram is shown in Figure 6. Fuses (5 amperes) protect the Variacs from any overload resulting from failure of the heaters. The leads from the panel board to the heater outlets are placed in the channel of the bottom crosspiece of the main rack. The outlets, which are standard lamp type, are bolted to the frame, as shown in Figure 2. The heaters provided with suitable plug and cord can be used interchangeably at any of the twelve outlets.

A Leeds & Northrup potentiometer type temperature indicator (No. 8674-BC) assures temperature measurements accurate to 2° C. within a range of 0° to 400° C. Each heater cover is provided with an opening into which may be inserted an iron-constantan thermocouple encased in a glass tube.

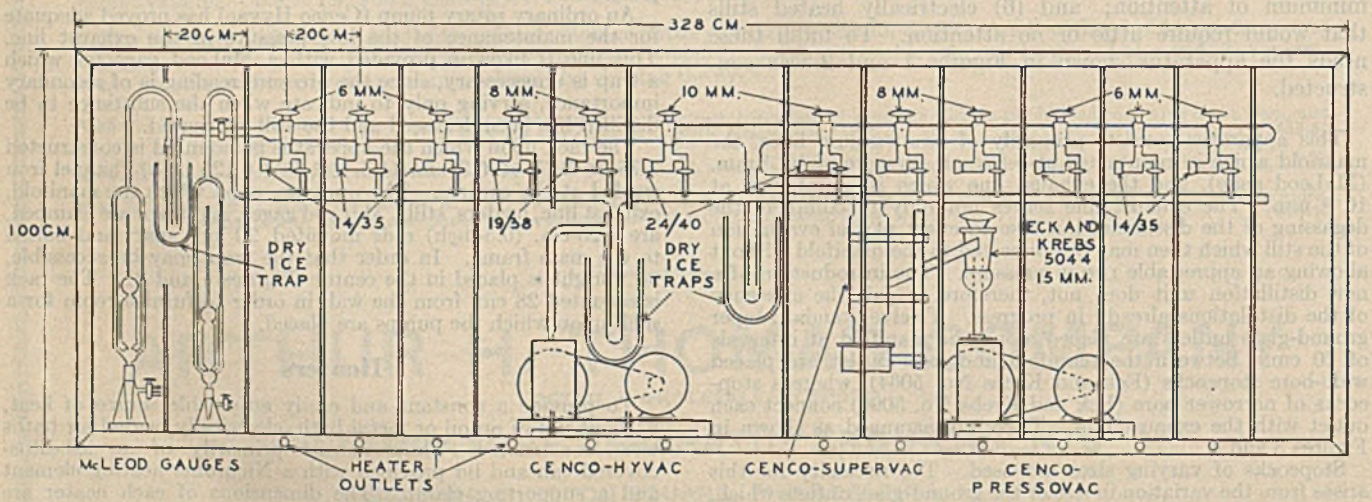

FIGURE 2. DIAGRAM OF APPARATUS

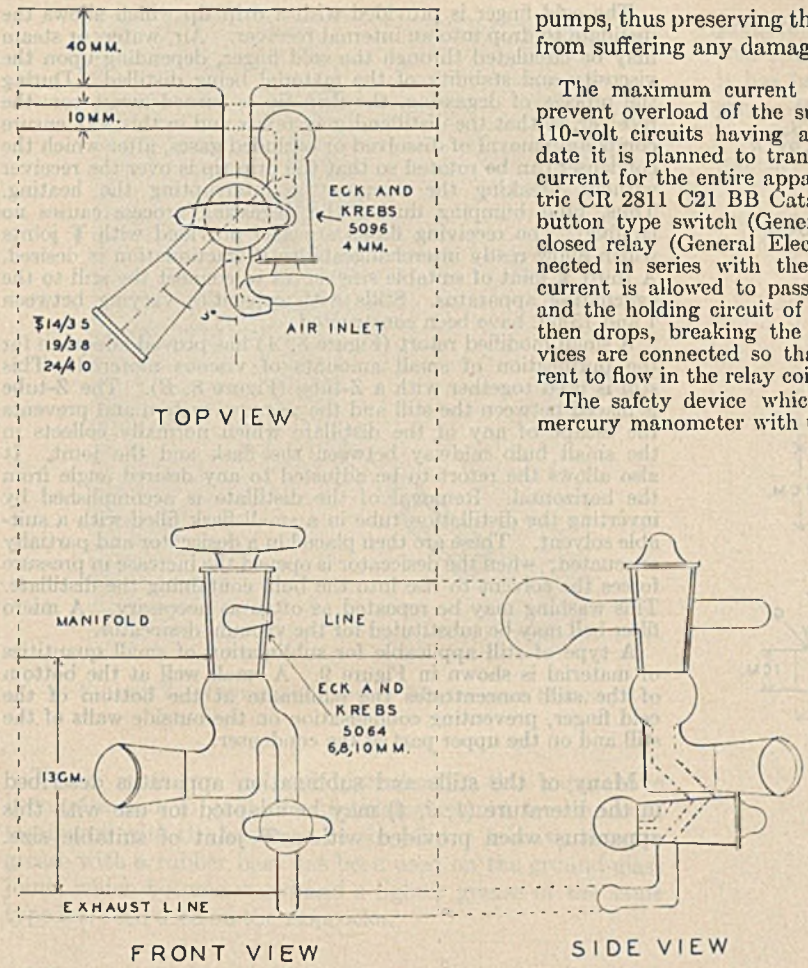

FIGURE 3. ARRANGEMENT OF STOPCOCKS AND OUTLETS

The temperature indicator is placed on the panel with the Variacs. The thermocouple leads, from the panel to the heaters, are fastened along the length of the exhaust line.

Safety Devices

Since many of the distillations and sublimations are allowed to run a day or more without attention, it seemed advisable to provide for the preservation of the products in case of failure of the system. Operation of the system could be interrupted in two ways: (1) distillation of the mercury from the diffusion pump into the backing pump and into the trap, owing to an inadequate water supply in the condenser; (2) breaking of the vacuum by mechanical failure of the backing pump, by development of cracks in the glass system or by sudden leakage of one of the stopcocks.

The safety devices were arranged so that they would interrupt the current to the heaters and to the vacuum pumps, thus preserving the products being distilled and preventing the pumps from suffering any damage.

The maximum current required for the entire apparatus is 45 amperes. To prevent overload of the supply circuit, this total load is divided between three 110-volt circuits having a maximum capacity of 15 amperes each. At a later date it is planned to transfer this load to a 220-volt three-phase circuit. The current for the entire apparatus passes through a magnetic switch (General Electric CR 2811 C21 BB Catalog No. 6,938,875 BB2) which is actuated by a push-button type switch (General Electric CR 2943-A200A start-stop). A normally closed relay (General Electric CR 2811-C9A Catalog No. 4,980,696 G2) is connected in series with the holding current of the magnetic switch. When a current is allowed to pass through the relay coil, the load circuit of the relay and the holding circuit of the magnetic switch are broken; the magnetic switch then drops, breaking the current to the heaters and pumps. The safety devices are connected so that in case of failure of the system they allow a current to flow in the relay coil.

The safety device which operates in case of vacuum failure consists of a mercury manometer with two sealed-in leads. This is placed beside the McLeod gage, so that the dry ice trap will prevent mercury vapor from entering the manifold.

The safety device which operates in case of failure of the water through the condenser of the mercury diffusion pump consists of a mercury-filled flowmeter type of apparatus. The leads are arranged so that they are not connected as long as sufficient water flows. Should this not be the case, however, the two leads are connected by the mercury, thus closing the circuit, which again allows the current to flow through the relay coil. A simple and convenient device of this type was designed by Romeo W. Gouley (*3*).

A switch is provided to break the safety circuit whenever it is desired to start the system. A pilot light indicates whether or not the safety devices are in operation. The magnetic switch, the push-button switch, the relay, the safety switch, and pilot lights are mounted on the panel board (Figure 1).

Types of Stills

The flexibility of the apparatus allows the use of various types and sizes of stills and sublimation apparatus. Figure 7 shows an improved type of still which uses a cold-finger condenser inserted into the distilling flask by means of a $ joint.

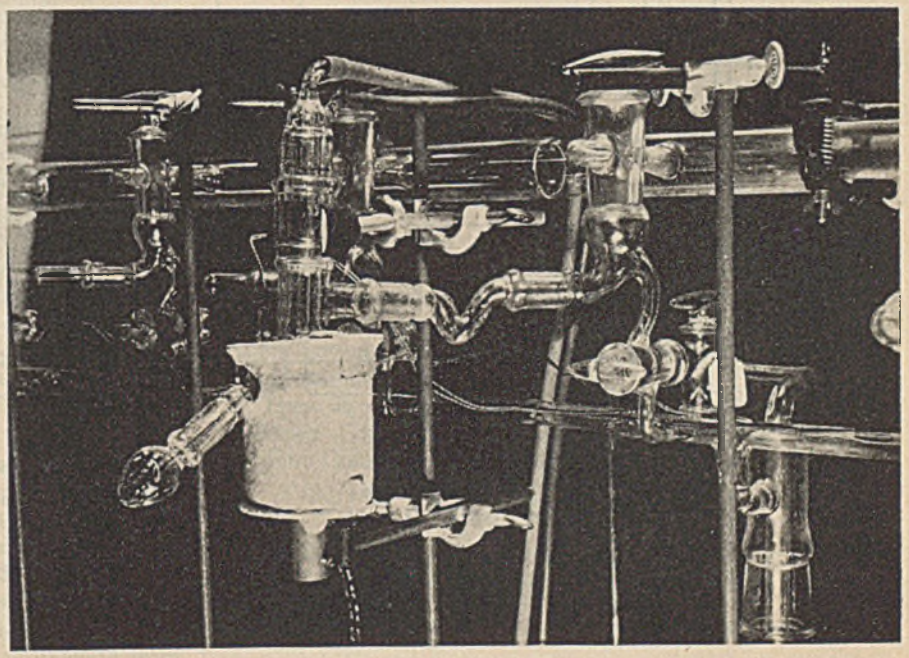

FIGURE 4. STILL AND HEATER ARRANGED FOR OPERATION

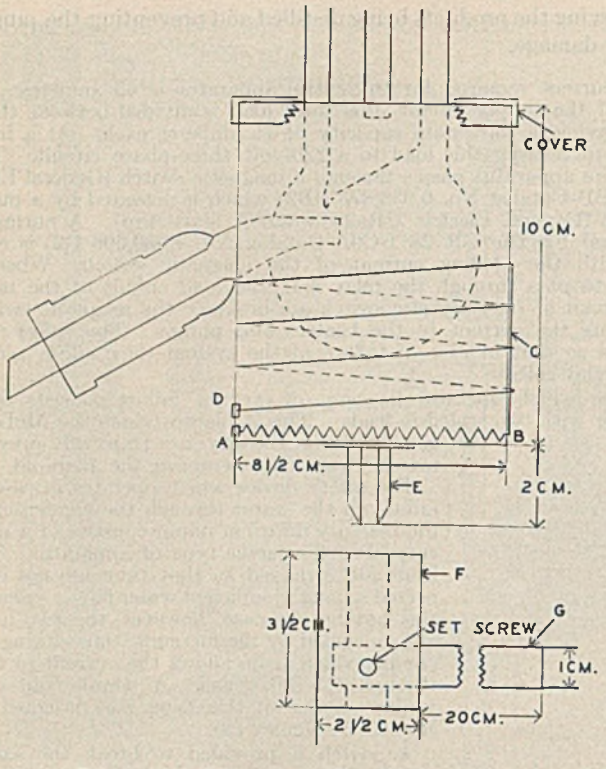

FIGURE 5. DETAILS OF HEATER ASSEMBLY

The cold finger is provided with a drip tip which allows the distillate to drop into an internal receiver. Air, water, or steam may be circulated through the cold finger, depending upon the viscosity and stability of the material being distilled. During the process of degassing, the drip tip is turned away from the receiver, so that the distilland may reflux and in this way ensure complete removal of dissolved or occluded gases, after which the condenser can be rotated so that the drip tip is over the receiver without breaking the vacuum or interrupting the heating. Thus, mild bumping during the degassing process causes no trouble. The receiving flasks are also provided with ⅎ joints which allow ready interchangeability if fractionation is desired. A third ⅎ joint of suitable size serves to connect the still to the evacuating apparatus. Stills with capacities varying between 1 and 15 ml. have been constructed.

A small modified retort (Figure 8, *A*) has proved adequate for the purification of small amounts of viscous material. This still is used together with a Z-tube (Figure 8, *B*). The Z-tube is placed between the still and the vacuum system and prevents the escape of any of the distillate which normally collects in the small bulb midway between the flask and the joint. It also allows the retort to be adjusted to any desired angle from the horizontal. Removal of the distillate is accomplished by inverting the distillation tube in a small flask filled with a suitable solvent. These are then placed in a desiccator and partially evacuated; when the desiccator is opened the increase in pressure forces the solvent to rise into the bulb containing the distillate. This washing may be repeated as often as necessary. A micro filter bell may be substituted for the vacuum desiccator.

A type of still applicable for sublimation of small quantities of material is shown in Figure 9. A small well at the bottom of the still concentrates the sublimate at the bottom of the cold finger, preventing condensation on the outside walls of the still and on the upper part of the condenser.

Many of the stills and sublimation apparatus described in the literature (*1*, *2*, *4*) may be adapted for use with this apparatus when provided with a ⅎ joint of suitable size.

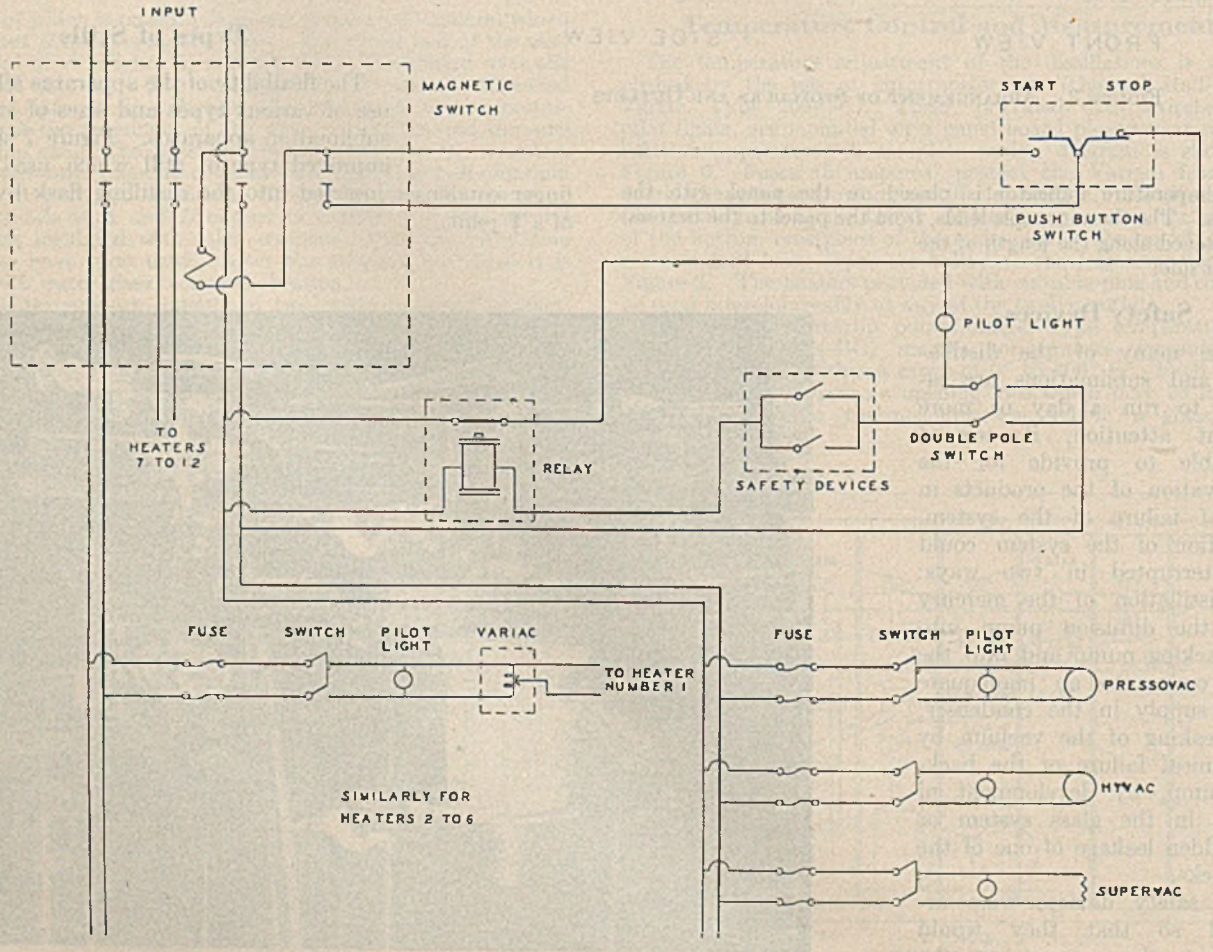

FIGURE 6. WIRING DIAGRAM

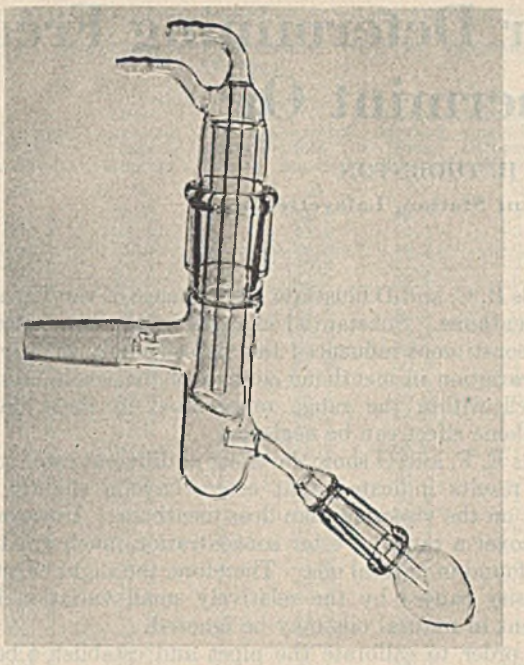

FIGURE 7. IMPROVED TYPE OF MOLECULAR STILL

The ⅎ joints on the stills described in this paper have been attached so that the distillates cannot become contaminated with the lubricant on the joint. A heavy vacuum grease with a rubber base has been used on the ground-glass joints which become warm and a lighter grease of the same type has been used on the stopcocks.

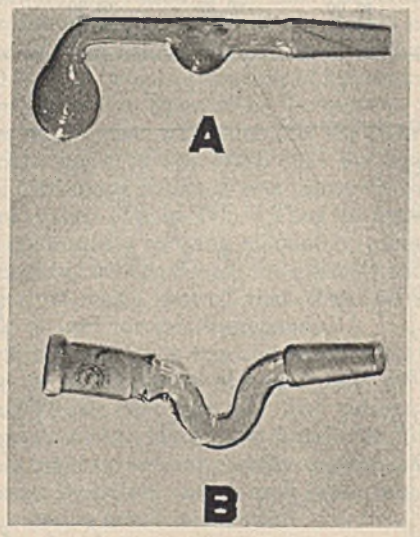

FIGURE 8. STILL FOR SMALL AMOUNTS OF VISCOUS MATERIAL (*A*) AND Z-TUBE (*B*)

The apparatus has been in continuous operation for the past year in this laboratory. During this period a number of compounds of high molecular weight, such as cholestenone, dehydroandrosterone, methyl 6-methoxy-*bisnor-i*-cholenate, 3′-alkyl substituted cyclopentenophenanthrenes, and β-keto adipic ester, have been purified by sublimation and distillation. A number of separations such as vitamin K_1 from alfalfa leaf meal oil and testosterone propionate from sesame oil, have been made. The latter was a commercial preparation from which 92 per cent of the desired compound was obtained in a perfectly pure state. During this period it has been found advantageous to clean the traps and regrease the stopcocks approximately every 2 months. The mercury in the condensation pumps becomes only slightly fouled. The lines, however, have remained completely

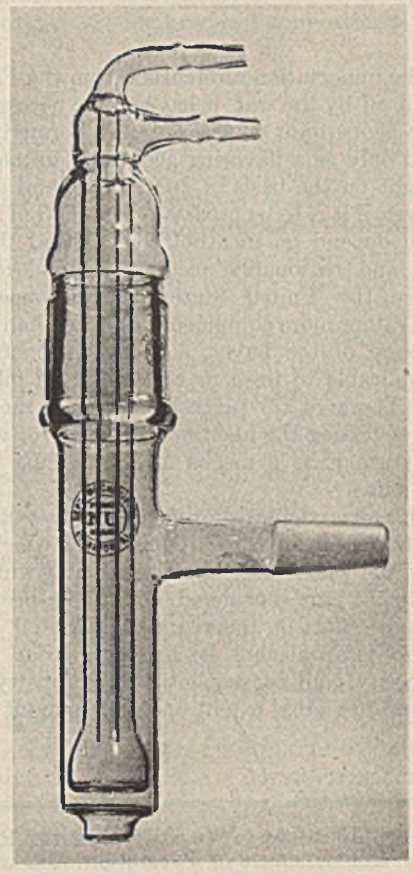

FIGURE 9. SUBLIMATION APPARATUS USEFUL FOR SMALL AMOUNTS

free of contamination. By reducing the vibration of the backing pumps it has been found that the para-rubber connections last almost indefinitely. The frequent and successful use of this apparatus by a number of operators has justified the effort and expense of construction.

Acknowledgment

The authors wish to thank M. N. States of the Central Scientific Company for technical advice and James Morris for the glass work on the apparatus. To one of the referees, K. C. D. Hickman, the authors wish to express their appreciation for suggestions incorporated in the paragraph on the measurement of pressures.

They also wish to thank the Abbott Laboratories, the Glidden Company, the Rockefeller Foundation, and the Upjohn Company for research grants.

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A Viscometric Method for Determining Free Menthol in Peppermint Oil

LYLE JAMES SWIFT AND M. H. THORNTON
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SINCE the importation of menthol from the Far East has been cut off by the war, it has become necessary to turn to American peppermint oil as a source. Formerly American oil was used only as a flavoring agent and in medicine, and so was bought on the basis of odor, taste, and appearance. Now, however, if it is to be processed for menthol its purchase price should depend on menthol content and there should be a rapid and reasonably accurate method for menthol assay. Since the United States Pharmacopoeia method (*5*) requires rather more equipment and time than are usually at the disposal of the buyer, a short viscometric method which is applicable to fresh or well-preserved oils has been developed. Dowzard (*3*) suggested viscosity measurements as a means of testing the identity and purity of volatile oils, but did not use it as a means of determining menthol in peppermint oils.

Peppermint oil is composed principally of menthol, menthyl esters, menthone, and hydrocarbons, all of which have approximately the same molecular weight and which, if only molecular weight were considered, might be expected to have about the same effect on the viscosity of the oil. However, since menthol is an alcohol, its hydroxyl group gives it an exalted viscosity, and consequently this property of the oil should depend to a great extent upon the amount of menthol present.

TABLE I. COMPARISON OF VISCOSITIES OF THE PRINCIPAL CONSTITUENTS OF PEPPERMINT OIL

	Seconds
Menthol	1077.2
Menthone	151.8
Menthyl acetate	210.4

Experimental

When menthol, menthone, and menthyl acetate were allowed to drain from an Ostwald pipet at 50° C., the results shown in Table I were obtained. A sample of 3 ml. was used for each determination.

Thus it appeared that the concentration of free menthol might govern the viscosity of peppermint oils, especially since this compound, together with menthone and menthyl esters, constitutes by far the greater part of the whole oil. This was tested with oils whose compositions were adjusted by additions of the pure substances. The results, all at 30° C., are given in Table II.

It will be noted from Table II that oils A, B, and E had approximately the same composition and did not differ markedly in viscosity as evidenced by time of drainage of the pipet. Since the densities of peppermint oils vary but little, viscosities were considered as proportional to times of drainage. The viscosity of oil H was much lower than that of oils A, B, and E, though this oil differed from them substantially only in its free menthol content. Thus the profound effect of a small amount of free menthol on the total viscosity was demonstrated.

Oils B, C, and D illustrate the influence of varying amounts of menthone. Substantial changes in the concentration of this constituent influenced the viscosity only slightly. Since the variation in menthone content of the whole natural oils is well within the range represented in these data, the menthone effect can be neglected.

Oils E, F, and G show the effect of different ester contents. The results indicated that esters have a slightly greater effect on the viscosity than does menthone. However, these oils cover a range of ester concentration much greater than that found in natural oils. Therefore, the slight effect on the viscosity caused by the relatively small variation in ester content in natural oils may be ignored.

In order to calibrate the pipet and establish a basic reference curve for analytical purposes, a sample of peppermint

TABLE II. EFFECT OF COMPOSITION OF OIL ON VISCOSITY

Oil	Composition of Oil: Free menthol	Esters	Menthone	Time of Drainage at 30° C.
	%	%	%	*Sec.*
A	46.55	4.95	32.00	469.2
B	46.30	4.97	32.12	451.6
C	46.70	4.98	24.60	467.6
D	46.35	4.95	45.50	496.4
E	46.06	5.00	30.30	460.2
F	46.15	9.22	30.30	483.4
G	46.00	16.22	30.30	491.0
H	42.29	4.77	32.92	417.2

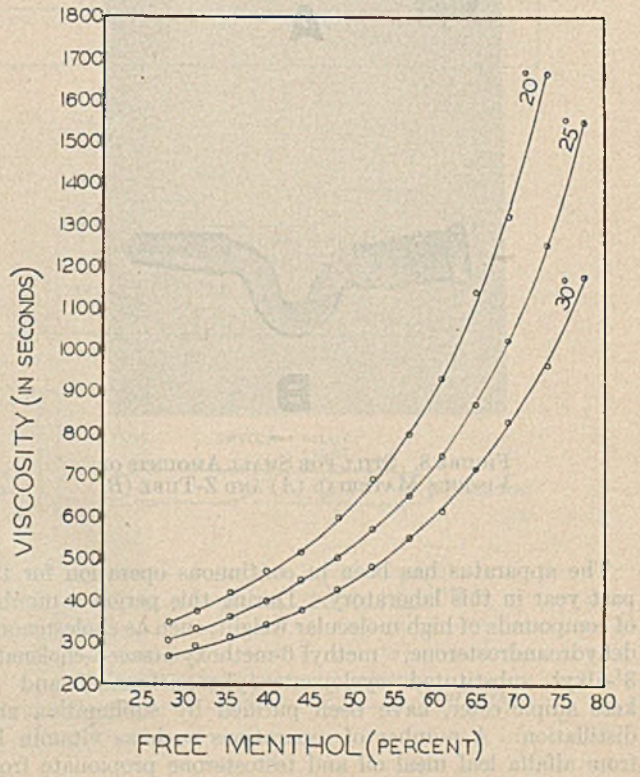

FIGURE 1

Table III. Analysis of Peppermint Oils by Viscometric Method

Oil No.	Viscometric Method: Viscosity Time at 30° C.	Viscometric Method: Uncorrected, free menthol	Viscometric Method: Turbidity index	Viscometric Method: Corrected, free menthol	Chemical Method, Free Menthol	Difference between Viscometric and Chemical Methods
	Sec.	%		%	%	%
1	561.6	57.0	C	51.7	49.7	+2.0
2	394.4	44.5	A	41.2	41.1	+0.1
3	444.2	49.2	A	45.9	46.2	−0.3
4	464.0	50.8	C	45.5	47.9	−2.4
5	578.4	58.0	A	54.7	55.5	−0.8
6	483.6	52.4	C	47.1	47.0	+0.1
7	583.2	58.3	C	53.0	53.1	−0.1
8	391.0	44.2	A	40.9	40.2	+0.7
9	505.4	53.5	A	50.2	50.3	−0.1
10	564.6	57.2	C	51.9	50.3	+1.6
11	468.0	51.1	A	47.8	47.3	+0.5
12	437.6	48.7	A	45.4	45.6	−0.2
13	471.8	51.5	C	46.2	44.3	+1.9
14	476.6	51.8	A	48.5	46.7	+1.8
15	563.0	57.2	A	53.9	55.0	−1.1
16	631.0	60.5	A	57.2	59.0	−1.8
17	701.0	63.5	A	60.2	59.5	+0.7
18	681.0	62.5	A	59.2	60.8	−1.6
19	385.2	43.7	C	38.4	36.9	+1.5
20	458.4	50.4	A	47.1	46.9	+0.2
21	451.0	49.7	A	46.4	46.1	+0.3
22	532.6	55.1	A	51.8	52.6	−0.8
23	425.8	47.7	A	44.4	44.7	−0.3
24	477.4	51.8	A	48.5	50.8	−2.3
25	426.6	47.6	A	44.3	45.6	−1.3
26	385.6	43.7	A	40.4	41.8	−1.4
27	379.4	43.4	A	40.1	41.2	−1.1
28	434.8	48.4	C	43.1	43.0	+0.1
29	489.6	52.8	A	49.5	51.4	−1.9
30	495.0	53.2	A	49.9	49.5	+0.4
31	506.2	57.3	A	54.0	54.0	0.0

oil was distilled with steam. The first quarter of the distillate, which was low in free menthol (27.9 per cent), was adjusted by adding different amounts of pure l-menthol to give a series of mixtures covering a wide range in concentration of this constituent. The viscosity of each oil, expressed in seconds, was measured at 20°, 25°, and 30° C. in the same Ostwald pipet used in the above experiments.

When percentage of free menthol was plotted against viscosity in seconds, the regular curves shown in Figure 1 were obtained. However, when natural oils of known free menthol content were allowed to drain from the pipet at one of the temperatures used in the calibration and the time applied to the appropriate curve, it was found that the corresponding free menthol percentage obtained was somewhat higher than the chemical value (Table III). Thus it appeared that natural oils contained a varying amount of some substance other than menthol which affected the viscosity substantially. Determination of resins showed that these substances accounted only in part for the discrepancy.

The interfering substance was correlated with the insolubility of the oil in a mixture of equal parts by volume of methanol and 70 per cent ethanol. The various degrees of turbidity produced when the oil was mixed with four volumes of the solvent mixture are very easily distinguished. They were designated by letters and to each letter was assigned a numerical correction factor as follows:

Turbidity Designation		Correction Factor
A	Clear to opalescent	3.3
B	Cloudy	4.5
C	Very cloudy to partly insoluble	5.3

The following formula indicates the use of these factors:

Apparent % free menthol from curve − turbidity correction factor = true % free menthol

The menthol contents of a number of natural peppermint oils were determined by both the viscometric and chemical methods (Table III). These oils were from the 1941 crop but were stored in 4-ounce tins sealed at the still. The absence of color in these oils indicated that they were in excellent condition. The samples were measured directly from the tins into the Ostwald pipet without preliminary treatment of any kind.

The results of the analysis of 31 different oils show reasonably good agreement between the chemical and viscometric methods. The greatest difference is 2.4 per cent free menthol in the case of oil 4. However, most of the values are in much closer agreement and the accuracy of the viscometric method is sufficient to give it wide practical application.

Discussion

The viscometric method for the determination of free menthol in peppermint oils was developed primarily for use in the field. The equipment required is simple and inexpensive and can be used under conditions in which the chemical method is impracticable. The use of this method will effect a great saving in time, since a determination of free menthol can be made in less than 20 minutes. The technique is easily acquired and a knowledge of chemistry on the part of the operator is not necessary. The accuracy of the method is sufficient for field purposes and for many applications in the laboratory where extreme accuracy is not essential. A further advantage of the viscometric method is that a free menthol determination can be made on very small samples (2 to 3 ml.) of oil. The sample can be recovered for other determinations.

This method should facilitate work in the breeding of mints for high menthol content, since the breeder will be able to make his own analyses. Since only small samples are required, the use of the viscometric method will materially lessen the number of plants to be grown before selections are made. The rapidity with which results can be obtained by the viscometric method should facilitate the marketing of peppermint oils on the basis of their free menthol content.

The menthol content of the oil furnishes valuable information concerning the proper time to harvest mint for maximum menthol yield (*4*). In the past it has not been possible to analyze sufficient samples so that all growers could have the benefit of this information. The viscometric method, which is both rapid and simple, should be of great help in solving this problem.

The application of the viscometric method is limited to fresh or well-preserved natural oils of normal composition. It is not applicable to oils of high resin content because the resins greatly increase the viscosity of the oil. However, the chemical method also gives unreliable results when preformed resin content is high (*4*) as well as when heating during saponification causes resinification to take place (*1*, *2*). The viscometric method can be used only to determine free menthol. In order to obtain total menthol, it is necessary to use the chemical method for the determination of the ester value, but, in many cases, a determination of free menthol gives all the information desired.

Acknowledgment

The authors are indebted to N. K. Ellis, of the Horticulture Department, Purdue University Agricultural Experiment Station, for the peppermint oil samples used in this work. It is largely due to his stimulus that this work was undertaken.

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Thixotropic Behavior of Oils

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Various types of oils in the viscosity range of 1 to 800 poises were measured on a rotational viscometer capable of imparting shearing stresses over a wide range. From these oils, flow curves were obtained extending from low to high rates of shear. All these oils showed a definite thixotropic behavior and exhibited all the characteristics of thixotropic plastics above a certain rate of shear designated as "the limiting rate of shear". Below this critical point the oils behaved like true Newtonian liquids, showing no signs of thixotropic structure. The limiting rates of shear were found to be related to the measured true Newtonian viscosities of the oils. The product of limiting rate of shear and viscosity was a constant for all the oils tested.

OILS have been generally assumed to be true Newtonian liquids, even to the extent that industrial laboratories are satisfied to determine the viscosity of an oil by a one-point measurement. It has been suggested by some workers in the field that certain oils exhibit a pseudoplastic characteristic.

Extensive studies in this laboratory have shown that the many different oils investigated are not pseudoplastics but are true Newtonian liquids for a limited range of rate of shear and behave, beyond the limited range, at least for the heavier oils, like thixotropic plastics.

In the literature (*2*, *9*) thixotropy is defined as an isothermal gel-sol-gel transformation. Thixotropic behavior does not require a complete transformation; it is considered a sufficient condition for the presence of thixotropy that a material changes its plastic viscosity from a higher to a lower value as a result of mechanical agitation and regains its original high viscosity upon rest. However, the thixotropic characteristic of a material is also a function of time, which means that the viscosity of the thixotropic material depends not only on previous mechanical agitation but also on the time period during which the material has been subjected to such mechanical agitation.

Thixotropy is found in paints (*8*, *10*, *11*), where it often proves to be useful, in bentonite suspensions (*7*), in gelatin sols (*8*), in iron oxide sols (*13*), and in printing inks (*3*). Little or no reference has been made to the thixotropy of oils. This thixotropic characteristic of oils probably has not been recognized because most instruments used for viscosity measurements have not permitted the application of sufficiently high rates of shear, or only one-point viscosity determinations have usually been performed, and these are insufficient to show thixotropy. The rotational viscometer developed in this laboratory, described by Green (*3*), was well suited for studying the thixotropic behavior of oils.

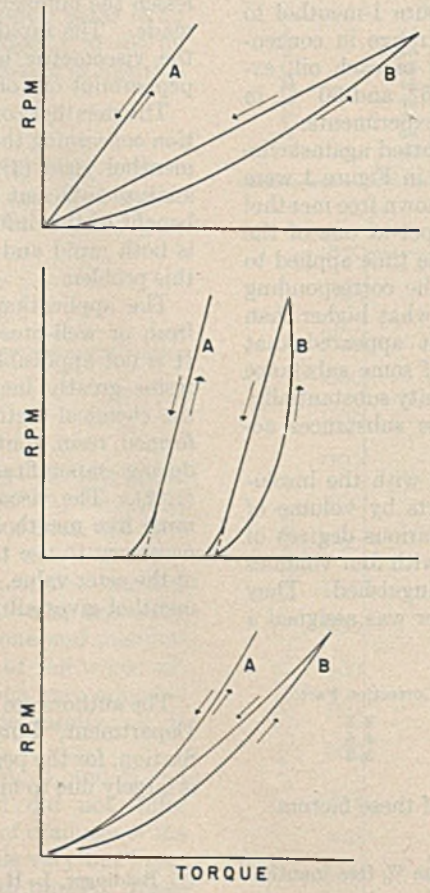

FIGURE 1. FLOW CURVES

Upper. *A.* Nonthixotropic true Newtonian liquid
B. Thixotropic true Newtonian
Center. *A.* Nonthixotropic plastic
B. Thixotropic plastic
Lower. *A.* Nonthixotropic pseudoplastic
B. Thixotropic pseudoplastic

Instrument

The viscometer has played an important part in obtaining the results reported below. It is built on the rotational principle, where the cup is rotated at various speeds and the bob is stationary, being suspended from a helical spring. The torsional modulus of this spring is calibrated by weights. Various springs are used to cover a wider range of viscosity measurements. The dimensions of the cup and the bob have been chosen to minimize the effect of plug flow and turbulence. A lid on the cup prevents the oils from climbing up the shaft of the bob and from being thrown out of the cup at higher rates of shear. No "channeling" could be evidenced, provided the bob was perfectly in center with the cup. The end effect introduced by the bottom of the bob is about 2 per cent of the whole shearing effect and therefore can be disregarded in calculating viscosities and yield values. A constant temperature bath keeps the temperature of the material to be investigated within ±0.2° C.

Flow Curves

The viscosities and the yield values of the different materials are obtained from flow curves, found by plotting the number of revolutions per minute as a function of the resulting torque. In the process of getting such flow curves, the speed of the cup is changed and the corresponding deflection of the bob, a measure of the torque, is marked down. It is understood that the amount of deflection depends upon the angular velocity of the cup. Since the angular velocities or

the revolutions per minute are proportional to the rate of shear or to the velocity gradient, in the flow curve diagram, the revolutions per minute of the ordinate may be replaced by a rate of shear ordinate. The relationship between the revolutions per minute (r. p. m.) and velocity gradient or rate of shear (dv/dr) in sec.$^{-1}$ for a rotational instrument is

$$1 \text{ r. p. m.} = 60\, r^2 h S dv/dr \qquad (1)$$

where r is any radius between the cup and the bob, h is the immersed height of the bob, and S is an instrumental constant equal to

$$(1/R_c^2 - 1/R_b^2)/4\pi h$$

where R_c is the radius of the cup and R_b is the radius of the bob.

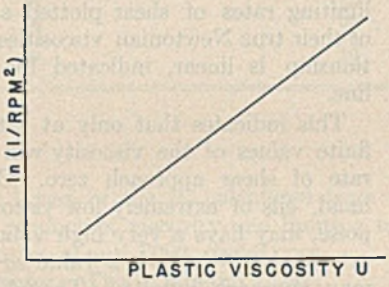

FIGURE 2. SCHEMATIC CURVE SHOWING CHANGE IN PLASTIC VISCOSITY WITH TOP R. P. M. FOR THIXOTROPIC PLASTIC

The mean rate of shear can be calculated for a given r. p. m. by substituting a mean radius in Equation 1. Then the equation for the mean rate of shear expressed in reciprocal seconds is

$$dv/dr = 4 \text{ r. p. m.}/60\, hS\,(R_c + R_b)^2 \qquad (2)$$

Though the measurements were made with cups and bobs of various sizes, the reported revolutions per minute were recalculated for a cup of 1.5-cm. radius and a bob of 1.3-cm. radius and 5.1-cm. immersed height. Then, the relationship between the revolutions per minute and the mean velocity gradient or the mean rate of shear (dv/dr) in sec.$^{-1}$ is

$$1 \text{ r. p. m.} = 1.36\,(dv/dr) \qquad (3)$$

The torque, also called the shearing stress, may be expressed in dynes-centimeter, a value which can be obtained by multiplying the deflection by the torsional constant of the helical spring.

Flow curves are obtained by increasing the rates of shear to any desired maximum value and then decreasing them until the starting value is reached. Following this procedure hysteresis loops (*3*, *6*, *8*, *10*) are obtained for thixotropic materials, while in nonthixotropic materials the up- and downcurves coincide. In Figure 1 six typical flow curves are shown. *A* (upper) is representative of a nonthixotropic true Newtonian liquid; the up- and downcurves coincide and form a straight line passing through the point of origin. *B* (upper) is representative of a thixotropic liquid; its upcurve has a continuous curvature, while its downcurve is again a straight line passing through the point of origin. *A* and *B* (center) are comparable to *A* and *B* (upper), but are obtained from true plastic materials and are representative of such substances. The downcurve of a true plastic material has a large linear portion, but at lower rates of shear shows a development of some curvature, explained (*1*, *4*, *12*) as caused by plug flow. Experimental data have been given by Green (*3*). However, the curvature in most cases where pigment suspensions and oils were investigated shows a greater extension than would be expected from plug flow. This extended curvature at lower rates of shear is believed to be caused mostly by thixotropy. The plastic viscosities and the yield values relating to the straight portion of the downcurve may be obtained from Reiner's equation as follows:

$$U = \frac{T - T_2}{\omega} S \qquad (4)$$

$$f = T_2 C \qquad (5)$$

where U is the plastic viscosity in poises and T is the torsion in dynes-centimeter. T_2 is the torsion corresponding to the intercept which is obtained by extending the straight portion of the flow curve to the torsion axis, ω is the angular velocity, f is the yield value in dynes per square centimeter, and C is an instrumental constant equal to $S/\ln(R_c/R_b)$.

A (Figure 1, center) represents a flow curve obtained from a nonthixotropic true plastic, and it is characterized by the fact that the up- and downcurves coincide. The criterion of any plastic is the presence of an intercept of the downcurve with the torsion axis, indicating the existence of yield value. *B* (center) is a flow curve obtained from a thixotropic true plastic, where the upcurve has a curvature throughout all rates of shear and does not coincide with the downcurve.

Finally (Figure 1, lower) two flow curves are shown, obtained from pseudoplastic materials. *A* represents a nonthixotropic and *B* a thixotropic material. Such pseudoplastic flow curves are characterized by the fact that even their downcurves have a curvature throughout all speeds.

Thixotropy of Oils

Any thixotropic plastic will show a viscosity which depends upon the highest rate of shear to which the material has been subjected before starting on the downcurve. These downcurves are always found to be straight lines, thus indicating a stable condition. This stable condition has been termed thixotropic level by Green (*3*) and can be identified by its top rate of shear or top r. p. m.

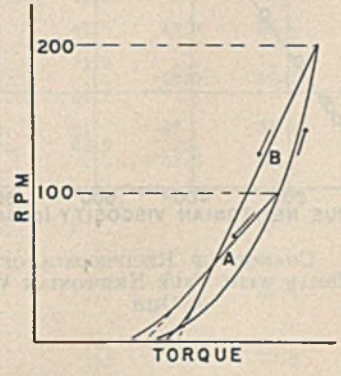

FIGURE 3. FLOW CURVES
Hysteresis loops obtained for thixotropic plastic measured to top r. p. m. of (*A*) 100, (*B*) 200

The correlation found between the plastic viscosity and the respective top rate of shear or top r. p. m. is shown in Figure 2, drawn schematically according to an equation given in another paper (*5*):

$$U = \ln (K/\text{r. p. m.}^2)/m \qquad (6)$$

where $2/m$, also designated as M, is the coefficient of thixotropic breakdown and is defined as the loss in shearing force

TABLE I. LIMITING RATES OF SHEAR FOR OILS OF VARYING VISCOSITY (FIGURE 4)

(Temperature constant, 30° C.)

Oils	Viscosity (True Newtonian)	Limiting Revolutions per Minute (*RPM*)	Limiting Rates of Shear	1/Limiting Rates of Shear	Viscosity × Limiting Rates of Shear
	Poises		*Sec.*$^{-1}$	*Sec.*	*Dynes/sq. cm.*
Mineral oil[a]	780	12	9.0	0.113	6900
Isobutylene oil	770	12	9.0	0.113	6800
Linseed oil (low acid)	380	25	18.4	0.054	7000
Linseed oil	250	38	28.0	0.036	7000
Isobutylene oil	180	52	38.3	0.026	6800
Linseed oil	125	75	55.5	0.018	6800
Linseed oil	115	81	59.8	0.017	6800
Esso lubricant 3000	103	95	70.0	0.014	7200
Mineral oil and gum varnish	72	135	100	0.010	7100
Linseed oil	48	205	151	0.007	7200
Mineral oil	31	310	228	0.004	7100
Mineral oil	30	315	231	0.004	6900
Isobutylene oil	23	405	298	0.003	6800
Mineral oil	21	455	335	0.003	7000
Mineral oil[a]	20	490	360	0.003	7200
Mineral oil	19	500	370	0.003	7000
Linseed oil	16	580	430	0.002	6800
Linseed oil[b]	10	725	532	...	..
Castor oil[b]	4	1450	1070	...	..
Mineral oil[b]	3	1450	1070	...	..
Isobutylene oil[b]	1	1450	1070	...	..
Mineral oil (medical)[b]	1	1450	1070	...	..

[a] Standard viscosity oils supplied by National Bureau of Standards as refined mineral oil.
[b] Since, owing to instrument limitations, limiting rates of shear could not be reached, maximum *RPM* and rates of shear to which oils could be measured are tabulated. At these rates of shear, below limiting rates of shear, oils still behave like true Newtonian liquids.

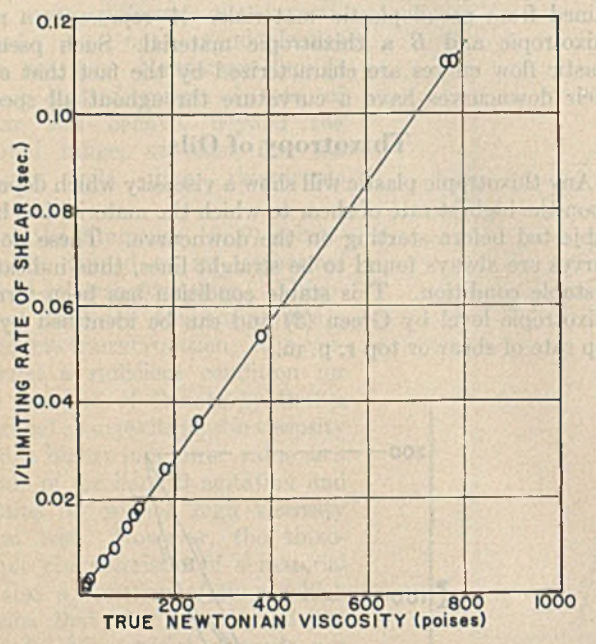

FIGURE 4. CHANGE OF RECIPROCALS OF LIMITING RATE OF SHEAR WITH TRUE NEWTONIAN VISCOSITIES OF OILS

per unit area per unit increase in velocity gradient, and K is an integration constant and is constant for each material.

When flow properties of thixotropic materials are investigated, a relationship is found between the intercept on the torque axis and the respective top rate of shear. In most cases the intercept increases with an increase in top rate of shear or top r. p. m., which is shown in Figure 3 schematically. This subject will be treated in more detail by H. Green and the author in a subsequent paper.

The flow curves obtained from the oils seem to be a combination between A (Figure 1, upper) and B (Figure 1, center). The outstanding fact concerning the oils which have been investigated in this laboratory is their apparent normal behavior (true Newtonian) up to a certain rate of shear. Only above this rate of shear, which will be called the "limiting rate of shear", does the thixotropic behavior of oils become apparent. This limiting rate of shear is not a fixed value but depends essentially upon the viscosity of the oil measured in its true Newtonian region. These limiting rates of shear, even for highly viscous oils, are so high that most standard methods of measuring viscosities of oils did not permit using sufficiently high rates of shear to detect thixotropy. These limiting rates of shear are not very sharply defined, but approximate values are given in Table I for various oils. Figure 4 shows the reciprocal of the limiting rates of shear plotted as a function of their true Newtonian viscosities. This relationship is linear, indicated by the straight line.

This indicates that only at extremely large finite values of the viscosity will the limiting rate of shear approach zero. On the other hand, oils of extremely low viscosity, below 1 poise, may have a very high value of limiting rate of shear—indeed, a value so high that it may approach infinity. Though it has not been possible to determine the limiting rate of shear value for oils of low Newtonian viscosities because of limitations imposed by the viscometer, it may very well be that all oils are thixotropic.

The force (torque) acting between two adjacent layers of the oil is equal to the product of viscosity and rate of shear. This product for the limiting rate of shear was found to be approximately constant for all oils (Table I) if the measurements were performed by increasing the rates of shear (or r. p. m.) very fast and by immediately decreasing them without waiting at the top r. p. m. The shearing force acting between

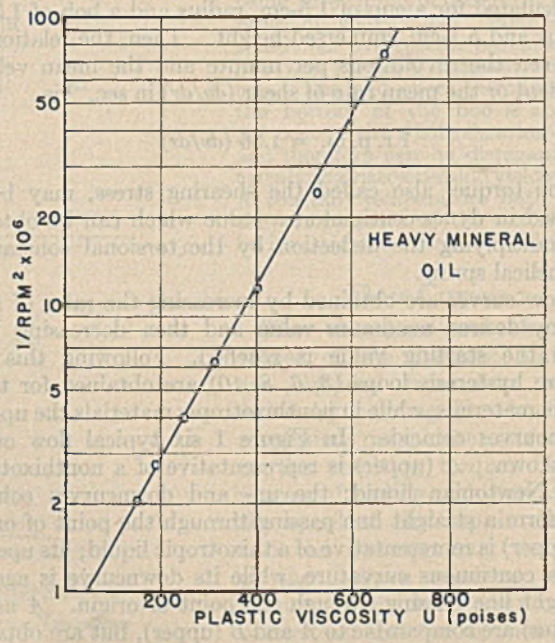

FIGURE 5. EXPERIMENTAL CURVE SHOWING CHANGE OF PLASTIC VISCOSITY WITH CHANGE IN TOP R. P. M.

TABLE II. VISCOSITIES OF HEAVY MINERAL OIL

Top Rates of Shear	Top R. P. M.	Plastic Viscosity	Torque Intercept
Sec.$^{-1}$		*Poises*	*Dynes cm.*

A. Plastic viscosities and intercepts at various thixotropic levels. Temperature constant, 30° C.

83	112	665	1.0×10^3
150	205	538	6.7
222	302	402	14.5
290	393	316	20.5
365	495	250	24.0
440	595	197	28.0
515	700	150	31.0

B. True Newtonian viscosities at various temperatures. Top r. p. m. below "limiting *RPM*"

Temperature ° C.	True Newtonian Viscosity *Poises*
25	1130
30	780
40	290
50	130
60	60
80	15
90	10

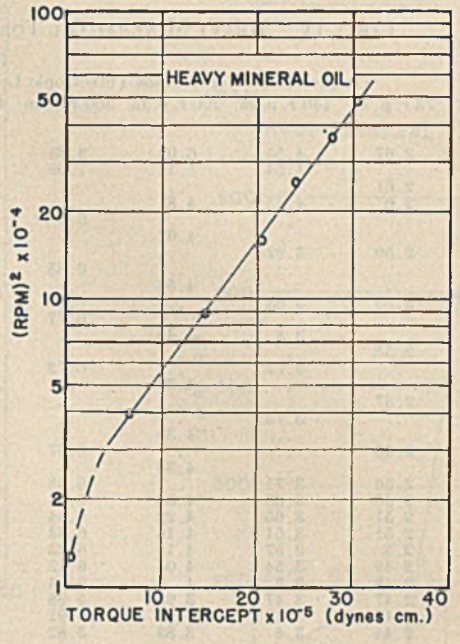

FIGURE 6. EXPERIMENTAL CURVE SHOWING CHANGE OF INTERCEPT WITH CHANGE IN TOP R. P. M.

two adjacent layers of the oil calculated from the average value of the product of viscosity and limiting rate of shear was found to be about 7000 dynes per square centimeter. This minimum shearing force of 7000 dynes per square centimeter may be required to overcome an energy barrier before the alignment of the micelles in the direction of rate of shear can start.

Little has yet been said about the flow curves of oils above the limiting rates of shear. In this region these flow curves are very much like flow curves obtained from true plastic materials; in fact, an intercept can be found for the downcurves of the hysteresis loops of oils. These downcurves contain fairly large straight-line portions very similar to the straight-line portions of downcurves of true plastics.

Measurements furthermore indicate that thixotropic levels exist which control the plastic viscosity of the oil as a function of the top rate of shear or top r. p. m. Figure 5 and Table II, A, show the relationship of the plastic viscosity of a typical oil to the respective top r. p. m. Any doubt regarding the plasticity of oils above the limiting rates of shear is removed by the similarity of Figures 2 and 5.

But there is further indication that oils can be identified as true plastics above the limiting rates of shear, since in accordance with expectation the intercept increases with an increase in top rate of shear, or top r. p. m., as shown in Figure 6 and Table II, A. Measurements also show that a complete recovery of structure takes place if the oils are left at rest for a period of time following shear agitation. Since this is one of the most important characteristics of thixotropy, it bears out the statement that oils show thixotropic behavior above their limiting rates of shear.

TABLE III. HEAVY MINERAL OIL FLOW CURVES MEASURED TO VARIOUS TOP R. P. M. (FIGURE 7)

(Temperature constant, 30° C.)

		Downcurve Torque $\times 10^{-5}$ for Various Thixotropic Levels						
	Upcurve Torque $\times 10^{-5}$	112 r. p. m.	205 r. p. m.	302 r. p. m.	393 r. p. m.	495 r. p. m.	595 r. p. m.	700 r. p. m.
R. p. m.	*Dynes cm.*				*Dynes cm.*			
14.5	..	4.6	4.25	3.55	3.36	3.0	2.48	2.3
22	..	6.5	6.0	5.7	4.95	4.6	3.9	3.5
36	10.6	11	10.6	9.7	9.0	8.1	7.2	6.5
63.5	18.4	18.8	18.0	16.6	...	...	...	...
76	..	..	..	...	18.6	16.6	...	13.4
85	25.8	25.8	24.8	23.3	...	..	17.4	..
112	34	..	31.9	29.9	27.6	24.9	...	...
130	39.9	..	36.6	36.4	...	...	27.2	24.2
145	...	..	...	...	36.1	32.9	...	..
158	46.0	..	44.2	41.5	...	..	...	..
180	52.3	..	50	47	43.9	39.7	35.9	..
192	...	..	...	...	...	..	...	33.9
205	56.0	..	...	51.9	...	...	...	..
218	...	..	...	...	52.0	46.0	...	..
234	60.9	..	...	56.7	...	..	43.5	..
252	64	..	...	61	57	53.0	...	42.4
278	67.5	..	...	64.9	...	...	52	..
289	...	..	...	...	62.5	57.0	...	..
302	70	..	...	...	...	..	...	...
312	..	..	...	...	66	..	...	51.5
325	73	..	...	...	...	61	57	..
349	74.8	..	...	...	...	...	...	..
360	..	..	...	...	...	65	...	..
370	76	..	...	...	74.0	..	61	56.5
393	77.8	..	...	...	...	69.1	...	..
414	..	..	...	...	...	..	65.2	..
420	80.2	..	...	...	...	...	...	..
423	..	..	...	...	...	72.8	...	59.8
445	80.2	..	...	...	...	...	...	..
454	..	..	...	...	...	75.5	69	..
467	80.7	..	...	...	...	..	...	..
474	..	..	...	...	...	..	...	64.3
495	81	..	...	...	...	..	73	..
520	82.3	..	...	...	...	..	...	67.5
523	..	..	...	...	...	..	75.9	..
544	81.9	..	...	...	...	..	...	..
568	81.7	..	...	...	...	..	...	..
569	..	..	...	...	...	..	79	71
595	81.5	..	...	...	...	..	...	...
621	81.5	..	...	...	...	..	...	73.8
648	81	..	...	...	...	..	...	..
668	..	..	...	...	...	..	...	76.3
676	80.5	..	...	...	...	..	...	..
700	79.3	..	...	...	...	..	...	..

TABLE IV. HEAVY MINERAL OIL TORQUE-TIME CURVES AT VARIOUS CONSTANT R. P. M. (FIGURE 8)

(Temperature constant, 30° C.)

Time	Torque × 10^{-6} for Various Thixotropic Levels					Time	Torque × 10^{-6} for Various Thixotropic Levels				
	73 r. p. m.	140 r. p. m.	200 r. p. m.	300 r. p. m.	400 r. p. m.		73 r. p. m.	140 r. p. m.	200 r. p. m.	300 r. p. m.	400 r. p. m.
Sec.						*Sec.*					
0	2.67	4.55	6.09	8.23	10.6	130	2.43	3.37	3.84	5.78	6.43
3	..	4.54	5.12	7.09	..	140	2.41	3.35	3.8	5.72	6.33
4	2.61	..	..	..	8.5	150	2.4	3.34	3.77	5.7	6.28
7	2.6	4.35	4.81	..	..	160	2.39	3.3	3.75	5.61	6.19
8	..	..	..	6.9	8.14	170	2.38	..	3.71	5.6	6.12
10	..	..	4.67	..	..	180	2.37	3.26	3.67	5.56	6.08
11	2.59	3.92	..	..	..	190	2.36	..	3.65	5.5	6.01
13	..	..	..	6.83	7.9	200	2.35	..	3.63	5.48	5.97
14	..	..	4.56	..	..	210	2.35	3.18	3.61	5.41	5.91
15	2.58	3.83	..	..	..	220	2.34	..	3.57	5.4	5.88
18	..	..	..	6.77	7.8	230	2.33	..	3.54	5.38	5.83
19	..	3.8	4.49	..	..	240	2.32	3.13	3.52	5.33	5.8
20	2.58	..	..	..	..	270	2.3	3.08	3.45	5.27	5.7
23	..	3.77	..	6.72	..	300	2.28	3.05	3.4	5.2	5.61
24	..	..	4.39	..	..	330	2.26	3.01	3.35	5.13	5.54
25	2.57	..	..	..	7.68	360	2.28	2.98	3.34	5.11	5.49
28	..	3.75	..	..	..	390	2.24	2.94	3.3	5.08	5.46
29	..	..	4.39	..	..	420	2.21	2.91	3.28	5.05	5.4
30	2.56	..	..	6.67	7.63	450	2.2	2.9	3.26	5.02	5.38
34	..	..	4.35	..	..	480	2.2	2.87	3.23	5.0	5.33
35	2.56	3.71	..	6.58	7.58	510	2.2	2.85	3.2	4.98	5.32
40	2.55	3.68	4.32	6.53	7.51	540	..	2.84	3.19	4.98	5.3
50	2.51	3.65	4.22	6.44	7.38	570	..	2.84	3.15	4.95	5.3
60	2.51	3.61	4.16	6.33	7.21	600	..	2.8	3.14	4.95	5.28
70	2.5	3.57	4.1	6.23	7.1	630	..	2.8	3.11	4.94	5.28
80	2.49	3.54	4.04	6.12	6.95	660	..	2.78	3.1	4.92	5.28
90	2.48	3.5	4	6.04	6.81	690	..	2.76	3.08	4.92	5.27
100	2.47	3.47	3.95	5.98	6.71	720	..	2.73	3.06	4.92	5.27
110	2.45	3.43	3.91	5.91	6.61	750	..	2.73	3.05	4.92	5.27
120	2.44	3.4	3.89	5.82	6.51	780	..	2.71	3.05	4.92	5.27

Experimental Curves

A large number of oils have been measured at various top r. p. m. Since all are of a similar nature, only one representative flow curve is shown in Figure 7 and Table III.

Figure 8 and Table IV show the decrease in torque as a function of time for constant rates of shear or r. p. m. This decrease is typical for thixotropic plastics.

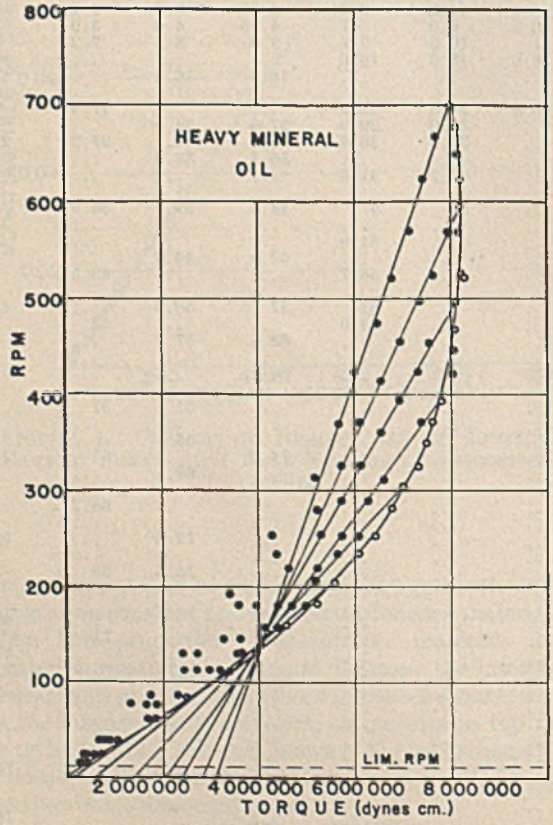

FIGURE 7. EXPERIMENTAL FLOW CURVES OBTAINED FROM VARIOUS TOP R. P. M.

Temperature, Turbulence, and Slippage

It is evident that the type of flow curves obtained from oils is not caused by factors like change of temperature, turbulence, or slippage.

Slippage can be immediately discarded as a result of experiments made with a grooved bob and cup (*3*).

Turbulence can be eliminated as cause for the particular structure of the oil flow curves, since turbulence would have tended to increase rather than decrease the forces (torques) at higher rates of shear.

Finally there remains the question of temperature. Increase of temperature undoubtedly decreases viscosity. The point then is, how much rise in temperature is required to decrease the oil viscosity to the same plastic viscosity obtained by increasing the rate of shear. To determine this, a heavy mineral oil was chosen and its viscosity below the limiting rate of shear was measured at various temperatures. Though the decrease in viscosity is substantial, it is not large enough to account for the rapid decrease in viscosity with an increase in top rate of shear. In Table II the true Newtonian viscosities are given for various temperatures and the plastic viscosities are given for various thixotropic levels and are designated by their respective top rates of shear and top r. p. m.

Table II makes it obvious that the decrease in plastic viscosity resulting from an increase in top rate of shear is too great to be entirely caused by an increase in temperature.

This point is more clearly shown by plotting the plastic viscosities at various top r. p. m. against the true Newtonian viscosities obtained at various temperatures (Figure 9 and Table V). For example, an increase from 100 to 700 top r. p. m. decreases the plastic viscosity from 710 to 150 poises, which in turn requires a temperature increase from 31° to 48° C., if the temperature is responsible for the entire increase in viscosity. However, no appreciable temperature increase could be observed if the temperature was taken before and immediately after the measurement. Therefore temperature can also be ruled out as a determining factor for the particular shape of the oil flow curves.

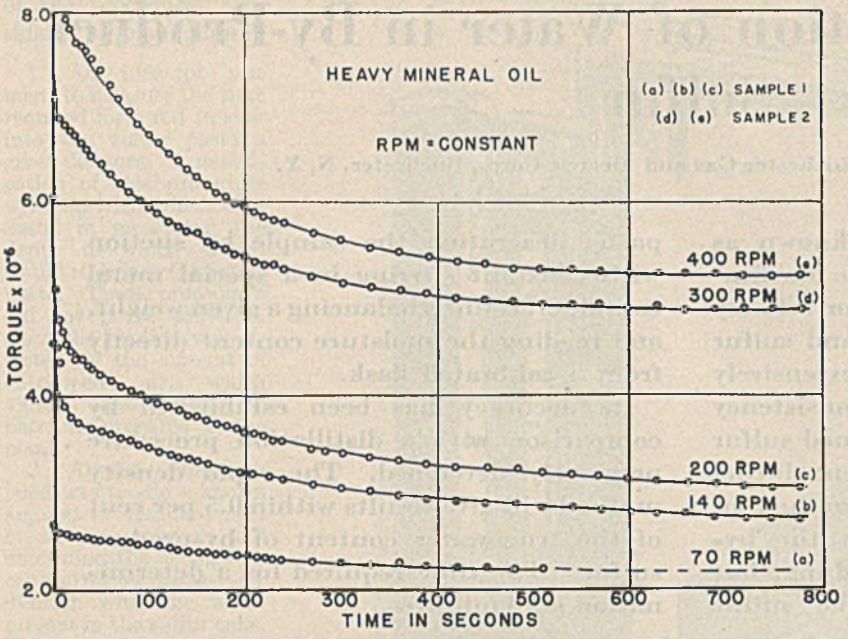

FIGURE 8. TORQUE-TIME CURVES AT VARIOUS CONSTANT R. P. M.

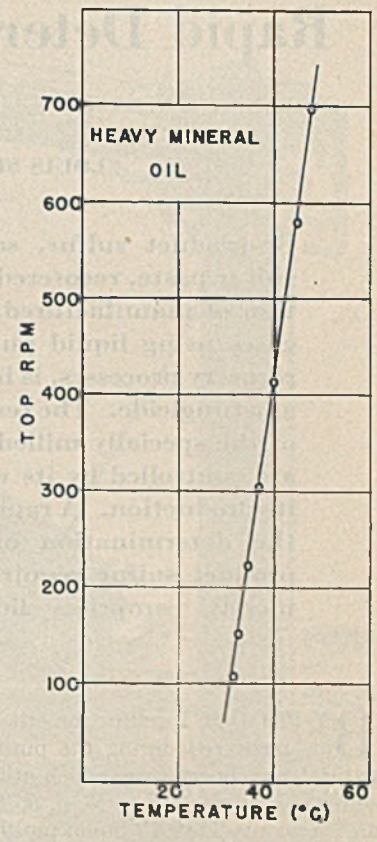

FIGURE 9. CURVE CONSTRUCTED TO SHOW INCREASE IN TEMPERATURE REQUIRED TO OBTAIN SAME VISCOSITY AS OBTAINED BY INCREASE IN TOP R. P. M.

TABLE V. INTERPOLATED DATA FROM FIGURE 9

Viscosity *Poises*	Top R. P. M.	Temperature ° *C.*
660	110	31
600	155	32
500	225	34
400	305	36.5
300	415	39.5
200	580	44.5
150	700	48

Conclusions

A full theoretical treatment of the phenomenon of the thixotropic behavior of oils has not yet been developed, but a few suggestions may elucidate the results so far presented.

At rest all molecules are distributed at random, taking on a statistic average position. Applying a rate of shear beyond the limiting rate of shear, which is equivalent to developing a minimum directional force, the molecules may start an alignment in the direction of shear; hence the original random structure of the oil breaks down, as indicated by a decrease in plastic viscosity. Upon rest the molecules will slowly return to their original random position.

Discussion

According to the literature, oils are often recommended for use in calibrating viscometers, but this may lead to a serious error (*3*). The present paper shows that unreliable results may be obtained if oils are used above their limiting rates of shear, since most oils behave like true Newtonians only below this critical point. The danger of an unreliable calibration with oils is particularly great if highly viscous oils are used, since their limiting rates of shear are very low.

However, any such oil can be used for calibration if the applied rates of shear are kept below the limiting rate of shear of the oil, in the range where it behaves like a true Newtonian liquid.

Although the oils tested showed no evidence of impurities, some oils contain waxes and other contaminants; therefore, the question of a possible separation at high rates of shear remains to be discussed. Separation would probably have a decreasing effect on the torque and therefore would show phenomena like those due to a thixotropic breakdown of the oil structure. However, the effects described above are not due to any detectable separation, since repeated measurements after short periods of time yield identical results, and the elapsed time intervals, of a few minutes, are long enough to allow a thixotropic recovery, but not a redistribution of any separated materials. It is highly improbable that a redistribution or redispersion could take place while the material is at complete rest, even if a longer time for recovery were allowed.

Acknowledgment

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Rapid Determination of Water in By-Product Sulfur

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By-product sulfur, sometimes known as sulfur paste, recovered during the purification of manufactured, natural, or refinery gases using liquid purification and sulfur recovery processes, is being used extensively as a fungicide. The texture and consistency of the specially milled and creamed sulfur are controlled by its water content during its production. A rapid density method for the determination of water in the by-product sulfur requires no weighing, but merely comprises liquefying the sulfur paste, deaerating the sample by suction with constant stirring in a special metal container, counterbalancing a given weight, and reading the moisture content directly from a calibrated flask.

Its accuracy has been established by comparison with a distillation procedure previously developed. The rapid density method will give results within 0.5 per cent of the true water content of by-product sulfur. The time required for a determination is 10 minutes.

BY-PRODUCT sulfur, sometimes known as sulfur paste, is recovered during the purification of manufactured, natural, or refinery gases which utilize liquid purification and sulfur recovery processes (*5*, *6*, *9*, *10*). At Rochester, N. Y., an "ammonia-Thylox" liquid purification system is in operation. The details of the process have been described by Bowman (*1*). The chemistry of the process as studied by Gollmar (*5*) and in the author's laboratory can be briefly summarized as follows:

Certain thioarsenates are capable of absorbing oxygen from the air, replacing part of the sulfur in the thioarsenate molecule, and precipitating it as elemental sulfur. This lower sulfur thioarsenate molecule can absorb hydrogen sulfide, forming the original thioarsenate, and the cycle can be repeated continuously. In practice theoretical quantities of arsenic trioxide and soda ash are dissolved in boiling water and added to the system as required to maintain an arsenic trioxide concentration of 6.0 grams per liter. The gas containing some 25.9 grams (400 grains) of hydrogen sulfide per 2.83 cubic meters (100 cubic feet) is scrubbed by the Thylox (arsenic) liquor containing the thioarsenates as indicated in the reactions that follow:

A. Preliminary reactions:

$$As_2O_3 + 2Na_2CO_3 + H_2O \longrightarrow 2Na_2HAsO_3 + 2CO_2 \quad (1)$$

$$2Na_2HAsO_3 + 5H_2S \longrightarrow Na_4As_2S_6 + 6H_2O \quad (2)$$

$$Na_4As_2S_5 + O_2 \longrightarrow Na_4As_2S_5O_2 \quad (3)$$

B. In the hydrogen sulfide absorber the following reactions take place in the ammonia-Thylox process:

$$(NH_4)_4As_2S_5O_2 + H_2S \longrightarrow (NH_4)_4As_2S_6O + H_2O \quad (4)$$

$$(NH_4)_4As_2S_6O + H_2S \longrightarrow (NH_4)_4As_2S_7 + H_2O \quad (5)$$

Reaction 4 is faster than 5 and represents the main reaction in the absorber.

C. In the thionizer the Thylox liquor is treated with air under pressure, where oxygen is absorbed and sulfur liberated as follows:

$$(NH_4)_4As_2S_7 + {}^1/_2O_2 \longrightarrow (NH_4)_4As_2S_6O + S \quad (6)$$

$$(NH_4)_4As_2S_6O + {}^1/_2O_2 \longrightarrow (NH_4)_4As_2S_5O_2 + S \quad (7)$$

Reaction 7 is faster than 6 and is believed to be the main reaction taking place in the pressure thionizer.

When the pH of a solution of $(NH_4)_4As_2S_6O$ drops below 7.3, arsenic trisulfide and free sulfur precipitate out, indicating some reduction of pentavalent As^{+++++} to trivalent As^{+++}.

Figure 1 presents a view of the hydrogen sulfide removal system with the two absorbers at the left, the pressure thionizer on the right, and the operating building in the center. The plant is capable of handling some 566,300 cubic meters (20,000,000 cubic feet) of gas per day. The sulfur slurry released at the top of the thionizer flows to a supply tank, thence to a continuous vacuum filter where the by-product sulfur is separated and washed.

The nature and general properties of by-product sulfur have been described previously (*2*, *3*, *4*, *7*, *8*). By-product sulfur consists essentially of water, free sulfur, less than 1 per cent of iron oxide, and less than 1 per cent of water-soluble salts. The by-product sulfur as it comes from the filters consists of a yellow-gray sticky paste containing about 45 per cent of water. To prepare this material for market, the sulfur is milled and creamed in special equipment and the moisture content is raised to 52 to 60 per cent, depending upon the type of product desired. In this condition, it is being used extensively today as a fungicide not only for apple orchards but for other fruits as well.

In preparing the final milled sulfur at the plant, it is necessary to keep the water content within definite limits. This requires a rapid and reliable method for determination of the water in the sulfur paste. The author (*8*) previously described a distillation method for determination of water in by-product sulfur, which was based upon the separation of water from the sulfur by the use of a mixture of light oil and gas oil which aided in distilling over of the water into a calibrated trap using a reflux condenser. This method proved to be accurate and convenient. However, the 2 to 3 hours required for the determination by the distillation procedure were too long when the by-product sulfur was being processed at the plant, and the water content was required within a short time in order not to hold up production.

Experimental Work

At the outset the operating engineers requested that a method be developed which would be simple, accurate, and reliable, and have the result available within 10 minutes or less. If such a method could be developed, plant operation and production could proceed normally and without delay.

To meet these requirements a number of procedures were studied and some preliminary work was performed. Some

of the procedures considered were as follows:

1. An attempt was made to measure the time required for a rod to sink into the sulfur paste a given distance. A modification of this procedure was also tried which consisted in measuring the depth to which a rod would sink into the sulfur paste. These procedures did not prove practical, since the reading was affected by the amount of entrapped air, which varied from batch to batch as prepared at the plant.
2. Another procedure tried was to add a known amount of standard acid or alkaline solution and determine the change in concentration caused by dilution with the water present in the sulfur cake. This procedure gave uncertain results and appeared to have too many difficulties to develop further.
3. The next procedure attempted was similar to No. 2, except that the change in concentration was determined by measuring the refractive index. This likewise proved impractical.
4. Attempts were made to separate the water present in the sulfur paste by centrifuging, with and without addition of other substances to aid in such separation. Further work showed that the addition of a small quantity of carbon disulfude in sulfur paste gave a fair separation, but the results obtained were erratic. Further work showed that this procedure was impractical and was abandoned.
5. The determination of the density of the sulfur paste was tried. The density method was given further consideration because it showed from the start that results could be obtained that were within 1 to 2 per cent of the true moisture content when using sulfur from the same batch. However, when sulfur paste from other batches was employed, results were erratic. It was found that the density of sulfur in the by-product material was close to that of sulfur reported in the literature.

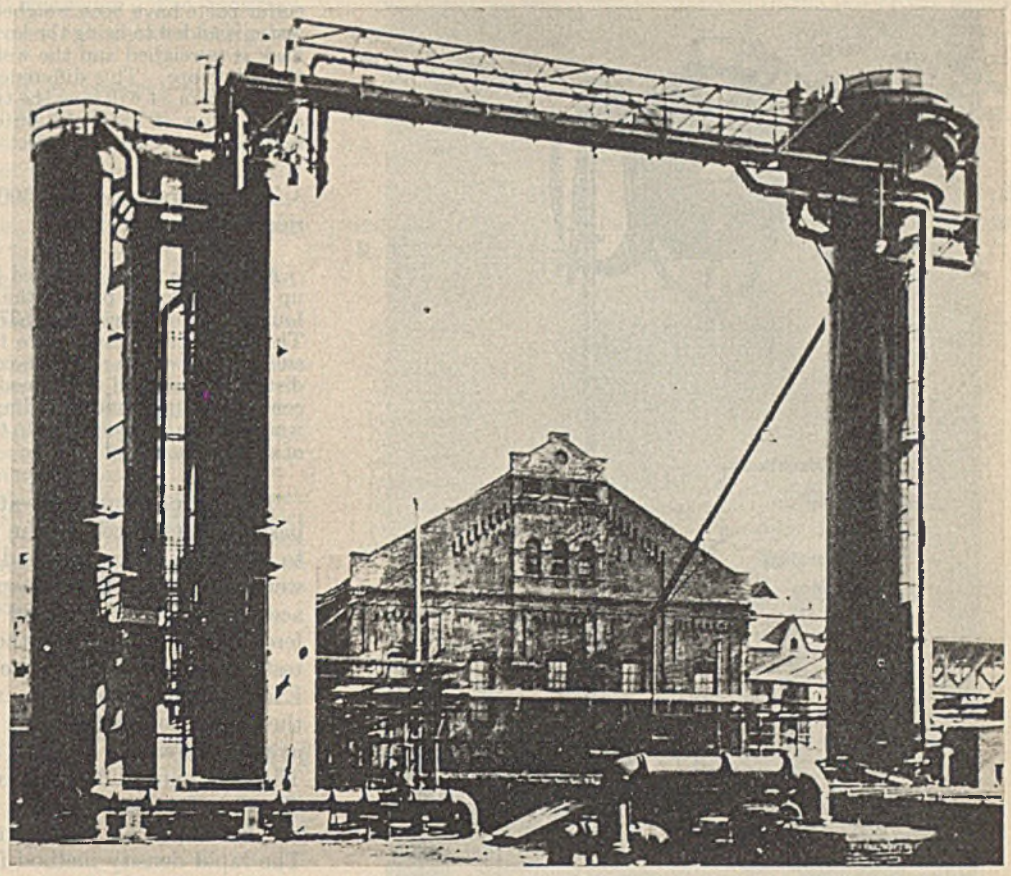

FIGURE 1. HYDROGEN SULFIDE REMOVAL SYSTEM

From previous work carried out in this laboratory on the properties and behavior of by-product sulfur, it was found that various dispersing agents possessed the property of liquefying the sulfur paste, thereby converting it into a creamy solution that could be readily handled. One of the dispersing agents found best for this purpose was Bindarene flour, made by the International Paper Company. The analysis of this Bindarene flour dispersing agent was as follows:

Total solids	96.90
Ash	8.52
Silica and insoluble	0.04
Oxides of iron and alumina	0.11
Calcium oxide	4.60
Magnesium oxide	1.70
Sulfur trioxide	0.36
Sulfur dioxide	4.10
Total sulfur	3.06
pH (of 10% solution)	5.7
Organic matter (100% water ash)	88.39

One procedure employed early in this study was to liquefy the sulfur paste with the Bindarene flour dispersing agent and measure the volume of a known weight of sample. It was observed that the liquefied sulfur paste contained large quantities of small air bubbles which were held in suspension. When these air bubbles were removed, consistent results were obtained.

The procedure finally adopted consisted in general in liquefying the sample of sulfur paste with the dispersing agent, deaerating the sample by suction with constant stirring in a special metal container, and then weighing the sample to determine its moisture content. The detailed procedure is described below.

Apparatus

Figure 2 shows the apparatus partly disassembled. This apparatus consists of:

1. A metal container or deaerating cylinder 31 cm. (12.189 inches) high, 10 cm. (4 inches) in outside diameter, and 0.5 cm. (0.189-inch) in wall thickness.
2. A No. 15 rubber stopper through which the stirrer and paddles pass is used to close the deaerating cylinder, and to support the stirrer and packing gland, and an outlet to the vacuum pump.
3. A proper type of stirrer is required as indicated in Figure 2, where the arrangement and distribution of the paddles are shown, a total of six being used. More paddles are present in the top of the cylinder in order to provide better agitation for deaerating the froth or foam that may be present at the top of the sulfur paste.
4. A heavy-duty, completely enclosed, $^1/_6$ horsepower motor, 1750 r. p. m., is connected to the stirrer by a flexible coupling as shown in the photograph. The motor, stirrer, and cylinder are mounted on a suitable support as indicated. The flexible coupling affords an easy means for removing and inserting the stirrer in the cylinder.
5. A calibrated 500-cc. Florence Pyrex flask.
6. A trap (shown in the right-hand corner) consists of a bottle with rubber stopper in which are two connections, one to the deaerating cylinder, the other to the water suction pump not shown, and a two-way stopcock used to break the suction at the end of the deaeration period.

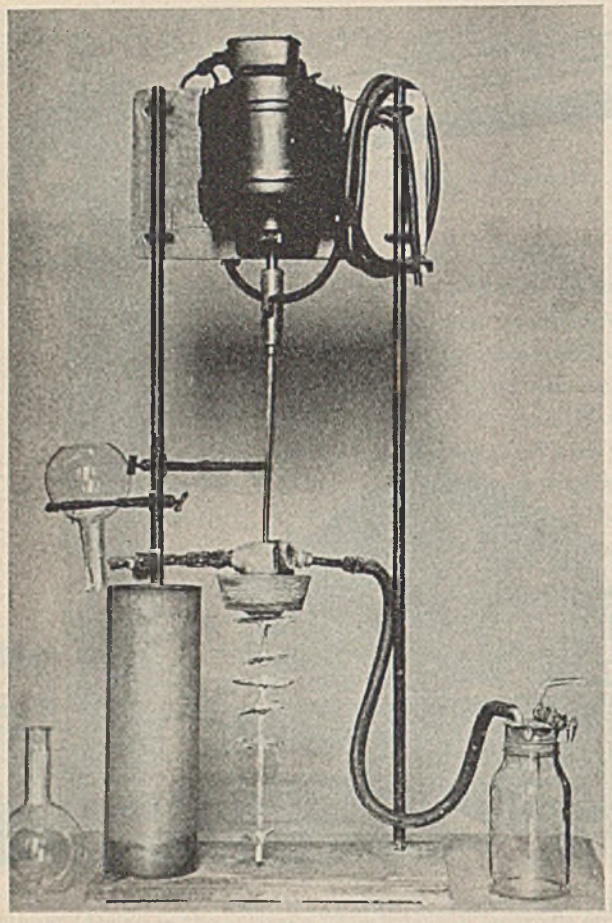

FIGURE 2. DISASSEMBLED APPARATUS

7. Heavy-walled rubber pressure tubing for connections.
8. A suitable torsion or dispersing balance and weights not shown in the photograph are required, and an 800-ml. Pyrex beaker.
9. A 45-cm. (18-inch) wooden spatula has been found most satisfactory for transferring the sulfur paste from the sample can to the metal cylinder. One-gallon enamel milk pails are satisfactory for holding the sample taken from each batch.

Procedure

The procedure as finally adopted consists in placing approximately 800 grams of sulfur paste in the metal cylinder (about half full). This need not be weighed, as a little experience will soon tell how much is right. Next, 4 or 5 grams (a heaping teaspoonful) of Bindarene flour dispersing agent are added. The Bindarene flour is distributed through the paste by mixing with the wooden spatula for about 10 seconds. The metal cylinder with the sulfur paste is then connected to the apparatus, the water suction is started, and the stopcock in the trap is closed. Finally, the motor is started to operate the stirrer. The sulfur paste sample is deaerated under suction with constant stirring for 5 minutes. The motor is then cut off, stopping the stirrer, the stopcock in the trap is opened, and the water suction is turned off. The metal cylinder is disconnected from the apparatus, and the liquefied sulfur paste is poured into a 800-ml. beaker. The calibrated Florence flask is placed on the balance, tared, and 703 grams of the liquefied and deaerated sulfur paste are weighed into the flask. The author has found it simpler to make a special weight weighing exactly 703 grams. The neck of the flask is then washed down with 1 ml. of methyl alcohol to which a small quantity of methyl orange indicator has been added. The water content of the sulfur paste in per cent is then read directly from the calibration on the flask at the level where the top of the meniscus reaches in the neck.

An occasional sample is received with a moisture content below 54 per cent, which is the lowest amount that can be read directly from the flask calibrations. In such a case the procedure is the same, except that after the 703 grams of liquefied and deaerated sulfur paste have been weighed into the calibrated flask, distilled water is added to bring the level up to the 54 per cent mark. The flask is reweighed and the weight of added water is obtained by the difference. This difference divided by 4 and the result subtracted from 54 will give the true moisture content, since, as indicated below, 4 grams of water are equivalent to 1 per cent of moisture in the sulfur paste.

The calibration of the 500-ml. Pyrex Florence flask is carried out as follows:

A Florence flask is selected so that the 54 per cent mark comes up into the lower part of the neck. The 54 per cent mark was found to be the height of 537 grams of distilled water at 25° C. The calibration marks were then determined empirically, using sulfur paste whose moisture content had been determined by the distillation method mentioned above (*8*). Each additional 2 per cent mark, up to and including the 60 per cent mark on the flask, was found to be equivalent to the height of an additional 8.1 grams of distilled water at 25° C.

Table I presents the water content of by-product sulfur paste as determined by the rapid density method, compared to the distillation method (*8*). Some forty-one samples were analyzed. These represent results that have been accumulated over a period of 4 years by at least eight different analysts. The author has on his records over 2200 tests made on sulfur paste by this rapid density method. Examination of these data indicates close agreement between the distillation method and the rapid density method. The average difference between the two methods is 0.1 per cent. The maximum difference, one sample only, is 1.0 per cent. The data further show that results within 0.5 per cent of the true moisture can be obtained by the rapid density method. The rapid density method for moisture in by-product sulfur paste has a number of advantages: No weighing of the original sample is required. It is easily and readily liquefied and deaerated. There is no weighing or measuring to obtain

TABLE I. WATER CONTENT OF BY-PRODUCT SULFUR PASTE

Test No.	Distillation Method	Rapid Density Method	Difference between Methods
	%	%	%
1	57.0	56.5	−0.5
2	57.5	57.0	−0.5
3	57.5	58.0	+0.5
4	58.0	57.5	−0.5
5	56.5	56.5	0.0
6	57.0	56.5	−0.5
7	58.5	58.0	−0.5
8	56.5	56.0	−0.5
9	58.7	58.5	−0.2
10	56.7	56.5	−0.2
11	55.5	55.0	−0.5
12	57.5	58.0	−0.5
13	59.0	59.5	+0.5
14	59.0	58.5	−0.5
15	58.0	58.5	+0.5
16	59.5	59.5	0.0
17	60.5	60.5	0.0
18	57.0	57.5	+0.5
19	57.5	57.5	0.0
20	59.0	59.0	0.0
21	58.5	58.5	0.0
22	59.0	59.0	0.0
23	60.0	59.5	−0.5
24	59.5	59.5	0.0
25	59.0	59.0	0.0
26	57.5	58.5	+1.0
27	57.0	57.0	0.0
28	59.0	59.5	+0.5
29	57.0	57.0	0.0
30	58.0	57.5	−0.5
31	59.0	59.5	+0.5
32	56.0	56.0	0.0
33	56.5	56.5	0.0
34	53.0	53.5	+0.5
35	56.0	55.5	−0.5
36	57.5	57.0	−0.5
37	57.0	56.5	−0.5
38	59.0	59.5	+0.5
39	51.0	50.5	−0.5
40	55.0	54.5	−0.5
41	61.0	61.0	0.0
	Av. 57.6	57.5	−0.1

the final results, but merely a balancing. The moisture content is read directly, no further calculations being required.

After the analyst has acquired experience, a moisture determination can be made within 10 minutes. To speed up the results further, the author has employed two metal containers, so that while one sample is being deaerated, the sample in the other container is being cleaned and made ready for the new determination. Thus by rotating, the time for the determination can be further reduced.

Certain precautions must be taken in the use of this method:

1. Sufficient dispersing agent must be added to liquefy the sample.
2. The entrapped air must be completely removed; otherwise the results will be high. A good test for the completeness of air removal is to tap on the bottom of the beaker or flask containing the liquefied sulfur paste with the finger. If the sound has a ring, the air has not been completely removed. A little experience soon accustoms the analyst to the difference in sound.
3. The distillation method should be used as a periodic check until the analyst has assured himself that the results are correct and that the samples are uniform. An occasional sample has been received from which it was impossible to remove the entrapped air. The samples are generally from sulfur recently made from the sulfur by-product recovery system.

No difficulty has been encountered in the use of the rapid density moisture method, even though used by various members (at least eight) of the laboratory staff. This method is in constant use at this laboratory, especially during the spring when the sulfur paste is being prepared for market.

Acknowledgment

The author wishes to acknowledge the cooperation of members of the laboratory staff, and in particular Theodore R. McCann in carrying out this work.

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Qualitative Test for Methoxy and Other Alkoxy Groups

Compounds Encountered in Pharmacology and Toxicology

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ALTHOUGH numerous modifications of the Zeisel method (*5*) for the quantitative determination of methoxy and other alkoxy groups have been described, and much elaborate apparatus has been designed for the determination, a simple and reliable qualitative test for such groups has hitherto been lacking. Beckmann (*2*) and Feist (*3*) detected methoxy groups in a few compounds by splitting off the methyl radical in such a way as to methylate known compounds, then determining the melting point of the resulting methylated derivative. Such a procedure is slow and inconvenient. Neave and Heilbron (*4*) merely described a very crude qualitative Zeisel procedure, heating compounds to 140° C. with hydriodic acid and passing the evolved methyl or ethyl iodide into silver nitrate, without provision for removing interfering substances which might distill over.

The present paper gives a satisfactory qualitative method for the detection of lower alkoxy groups in soluble compounds of relatively low molecular weight. It is based on the Zeisel method, but requires only a test tube for apparatus, together with a porous plug, impregnated to remove interfering substances. The alkyl iodides produced in positive tests are detected by means of the vermilion color (mercuric iodide) which they give with mercuric nitrate.

Method

PREPARATION OF POROUS PLUGS. Cut double-thickness cheesecloth (gauze) from a roll 18 inches (45 cm.) wide into strips 2 inches (5 cm.) wide and 18 inches long. Fold each strip once, to get a strip 2 by 9 inches. Place the strips on a sheet of glass. Thoroughly moisten each strip by pipetting 5 ml. of the impregnating solution onto it, taking care that the strips do not curl. Dry the strips on the glass, either by standing overnight or by leaving it in a warm place for 2 or 3 hours.

IMPREGNATING SOLUTION. Dissolve 1 gram of lead acetate in about 10 ml. of water and pour it into 60 ml. of 1 *N* sodium hydroxide in a 100-ml. cylinder. The heavy precipitate which appears soon redissolves. Add a solution of 5.0 grams of sodium thiosulfate in about 10 ml. of water, and 1 ml. of glycerol. Dilute the mixture to 100 ml. with water. The glycerol prevents any of the dried salt mixture from flying off as dust when the strips are rolled before insertion in the test tubes.

CONDUCT OF THE TEST. Place about 0.1 gram of the substance to be tested (preferably well powdered if a solid), in a 16 by 150 mm. test tube. Carefully pipet 1 ml. of glacial acetic acid and 1 ml. of hydriodic acid (57 per cent, sp. gr. 1.7) onto it. It is desirable, but not essential, to add a small (1 to 2 mm.) granule of unglazed porcelain to promote ebullition. Roll one of the impregnated gauze strips rather loosely into a cylindrical shape and insert it in the mouth of the test tube with a rotary motion. When most of the plug has been inserted into the tube, twist it in the opposite direction to expand it in the bore to a fairly tight fit. There must be no obvious channels by which vapors can bypass the plug. Push the plug down until its top is about 1.5 inches (4 cm.) below the mouth of the test tube. Put a small piece of nonabsorbent cotton (cotton batting) over the plug and press it down into a disk about 2 to 3 mm. thick. Give a strip of filter paper about 2 cm. wide and 10 cm. long a slight longitudinal fold, moisten about a third of its length with a saturated solution of mercuric nitrate in 2 per cent by volume nitric acid, and rest the paper upon the cotton wad.

Place the test tube in a glycerol bath about 4 to 5 cm. deep, maintained at 120° to 130° C. The bath may be heated initially above 130° C., if several tubes are to be immersed in it. Condensed vapors will gradually work up the tube walls into the impregnated plug, which will show a gray discoloration.

With a positive test, a yellow color will spread upward from the bottom edge of the test paper, while changing to a bright

TABLE I. DETECTION OF ALKOXY GROUPS

Compound	Alkoxy Groups	Color on Test Paper	Time in Minutes	Result	Explanation
Anisole	1 MeO—	V	7	++++	
Phenetole	1 EtO—	V	5	++++	
Guaiacol	1 MeO—	V	8	++++	
Potassium guaiacol sulfonate	1 MeO—	V	8	++++	S does not interfere in this case
Vanillin	1 MeO—	V	6	++++	
Vanillin acetate	1 MeO—	V	7	++++	
Eugenol	1 MeO—	V	4	++++	
Acetophenetidine	1 EtO—	V	8	++++	
Methyl salicylate	1 MeO—	V + O	4	++++	MeOH hydrolyzed off forms MeI
n-Butyl ether	2 BuO—	Y + O	10	+	B. p. of BuI is 131° C. Ether resists HI
n-Hexyl ether	2 HexO—	Y + light green	10	(—)	B. p. of hexyl iodide is 180° C.
α-Methyl glucoside	1 MeO—	V	2	++++	
Antipyrine	None	Faint buff	10	—	
Piperine	None	Y (very strong)	10	—	Yellow unaccounted for
Dimethoxytetraethylene glycol	2 MeO—	O (strong)	3	+++	
Plasmochin	1 MeO—	Y + O + V	10	+++	
Atebrin dihydrochloride	1 MeO—	O + V	10	+++	
Nupercaine hydrochloride	1 BuO—	O	5	++	B. p. of BuI is 131° C.
Dibutylaminopropyl-*p*-aminobenzoate sulfate (Butyn)	None	Faint gray	10	—	Discolorations probably due to reduction by decomposition products
Azochloramide (Dichloroazodicarbonamidine)	None	Gray	10	—	Discolorations probably due to reduction by decomposition products
Picrolonic acid	None	Y + pale gray	10	—	Discolorations probably due to reduction by decomposition products
Codeine (alkaloid)	1 MeO—	O (strong)	3	+++	
Codeine sulfate	1 MeO—	Y + gray	10	(—)	Discoloration due to S
Brucine (alkaloid)	2 MeO—	V	7	++++	
Quinidine (alkaloid)	1 MeO—	Y + V	10	+++	
Quinidine sulfate	1 MeO—	Y + V	10	+++	No interference by S
Quinine (alkaloid)	1 MeO—	Y + V + O	10	++	Only small spots of V and O
Quinine hydrochloride	1 MeO—	Y + V + gray	10	++	Slight interference by HCl
Quinine sulfate	1 MeO—	Y + gray	10	(—)	S interferes strongly
Narcotine	3 MeO—	V + O	5	++++	
Cotarnine hydrochloride	1 MeO—	Y + O + gray	4	+++	Slight interference by HCl
Thalline sulfate	1 MeO—	Black + gray	10	(—)	S interferes very strongly
Ethylmorphine hydrochloride (Dionine)	1 EtO—	V + O	5	++++	
Cocaine hydrochloride	1 MeO—	O	5	+++	MeOH hydrolyzed off forms MeI
Hydrastine (alkaloid)	2 MeO—	V	3	++++	
Hydrastine hydrochloride	2 MeO—	O	3	+++	No interference by HCl
Morphine sulfate	None	Y (faint)	10	—	No interference by S
Strychnine sulfate	None	Y (strong)	6	—	No interference by S
Atropine sulfate	None	Gray	10	—	S obscures negative reaction

V = vermilion, O = orange, Y = yellow
++++ = strong positive, +++ = moderate positive, ++ = weak positive, + = doubtful positive
(—) = false negative, — = negative

orange or vermilion. Less frequently, the orange or vermilion color will appear rather suddenly over all the moist portion of the test paper. The vermilion may appear as a solid band of color, or as spots against a background of yellow or yellow-orange. A permanent yellow color indicates a negative or a doubtful positive test. The faint yellowish color obtained when the mercuric nitrate solution dries on the test paper is without significance.

The test should be continued until a distinct positive reaction is evident, or until 10 minutes have elapsed. In certain cases, particularly in tests which have been heated for 10 minutes with negative results, a slight gray discoloration may appear on the test paper, probably owing to the action of volatile substances which slightly reduce the mercuric nitrate with the production of finely divided mercury. This discoloration usually fades in an hour or so. Permanent dark gray discolorations due to sulfur compounds are discussed under "Results".

Limits of Sensitivity

The test was applied to 5-, 10-, and 25-mg. portions of vanillin and codeine (alkaloid). The colors were fairly strong with the 25-mg. portions. With vanillin the test was still positive, although weak, with 5 mg. With codeine, the test was still positive (moderate orange color) with 10 mg. but 5 mg. gave only a lemon-yellow color. Accordingly, the limits of sensitivity are about 5 mg. for vanillin and 10 mg. for codeine.

For the detection of methoxy and ethoxy groups in smaller amounts of material (1 to 10 mg.), the A. O. A. C. method (*1*) is undoubtedly superior; sulfur is automatically taken care of, but special equipment and a longer time are required to run the test.

Results

Table I shows that methoxy and ethoxy groups are rather readily detected. With solids, 100 mg. were used; with liquids, 0.10 ml. The "time in minutes" shows when each test was discontinued. In most cases a distinct positive test was obtained in a shorter time. Sulfur often interferes rather seriously with the test, apparently by reduction to volatile mercaptans which are not held back by the impregnated plug. Since the test is invariably satisfactory with free alkaloids, it is desirable to convert most alkaloid sulfates to the free alkaloid before testing. The slight gray discoloration produced by hydrochlorides does not seriously interfere.

Although the butoxy group in nupercaine is readily detected, the test is weak with *n*-butyl ether. Hexyl ether does not react at all. This is undoubtedly due to the high boiling point of the higher iodides, although lower solubility in water and resistance of the simple ethers to the action of hydriodic acid may be a factor.

The "false negative" reactions given in Table I refer to cases in which an alkoxy group is present in the compound, but in which a negative test is obtained, because of either the low volatility of the alkyl iodide or the action of sulfur in obscuring the vermilion color of a positive test.

Acknowledgments

The author wishes to thank Eileen C. Asmuth for technical assistance. Thanks are also extended to Joseph Levine of the U. S. Bureau of Narcotics for samples of certain alkaloids, and to the Union Carbide and Carbon Chemicals Corporation for furnishing other compounds.

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Delivery of Liquids at Low and Constant Rates

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The design of an apparatus for the delivery of small batches of fluid at low and constant rates is presented. Minimum delivery rates are easily and accurately regulated at flows of fractions of cubic centimeters per minute.

Full range control of flow rates is possible. There are no moving parts in the entire unit. Individual parts are obtainable without difficulty or conflict with strategic materials. Only the simplest glass-blowing techniques are involved. Construction and calibration are rapid.

Statics of the apparatus are analyzed to produce an equation useful in design of a unit of proper size for any particular installation desired.

THE apparatus herein presented was suggested by an experiment in which absolutely constant rates of addition of a reagent were required. Moreover, these rates were to be relatively low, in the range of a few cubic centimeters per minute (4 to 5 maximum). Devices which have previously been used with success in other experiments in this laboratory include constant-head tank-meter devices and small pumps of centrifugal or piston design. The main difficulty with the former lies in their lack of adaptability to operation with small batches. Pumps of either design require materials high on priority lists. Centrifugals require auxiliary metering devices, while all piston types, either reciprocating or continuously advancing types, require complicated gearing. In the latter case, full range control is impossible. The present design involves no moving parts, no head tanks, and no meters, and provides full range control.

The design selected relies upon controlled rate of vaporization as the method of regulation. However, the apparatus described is not limited to a pure liquid, since the fluid being vaporized may be completely dissimilar to that whose flow is being regulated. Thus, water may be the material vaporized while a solution may be fed to the final receiver.

[1] Present affiliation, U. S. Naval Reserve.

The maximum capacity of the unit illustrated, with 60 cm. (2 feet) of 32-gage Nichrome wire in the heater, is 4 to 5 cc. per minute, the requirement noted above. Increase of this maximum may be obtained by increase of electrical input (limited in the illustrated unit only by heater design).

Description of Apparatus

As shown in Figure 1, two main units are involved: a still and condenser combination, and a U-tube feeding device. The material to be fed is discharged from the left-hand arm of this latter tube, 11, into any desired receiver. Into the right-hand arm, 9, is fed water, or other suitable liquid, condensed from vapor as delivered by the still. These two liquids are separated by a denser fluid in the bottom of the U-tube, carbon tetrachloride in the illustrated unit. Liquid delivered to the top of the right-hand arm will raise the level of the carbon tetrachloride in the left-hand arm, thereby causing the discharge from the top of a volume of liquid equivalent to the rise in level multiplied by the cross section of the tube.

DETAILS. The still, 1, is an ordinary Dewar flask, so that radiation is minimized and rates of vaporization depend solely upon electrical input. Flask capacity should be that of the amount of water which must be vaporized, plus an amount to keep the heater covered, plus a suitable disengaging area above

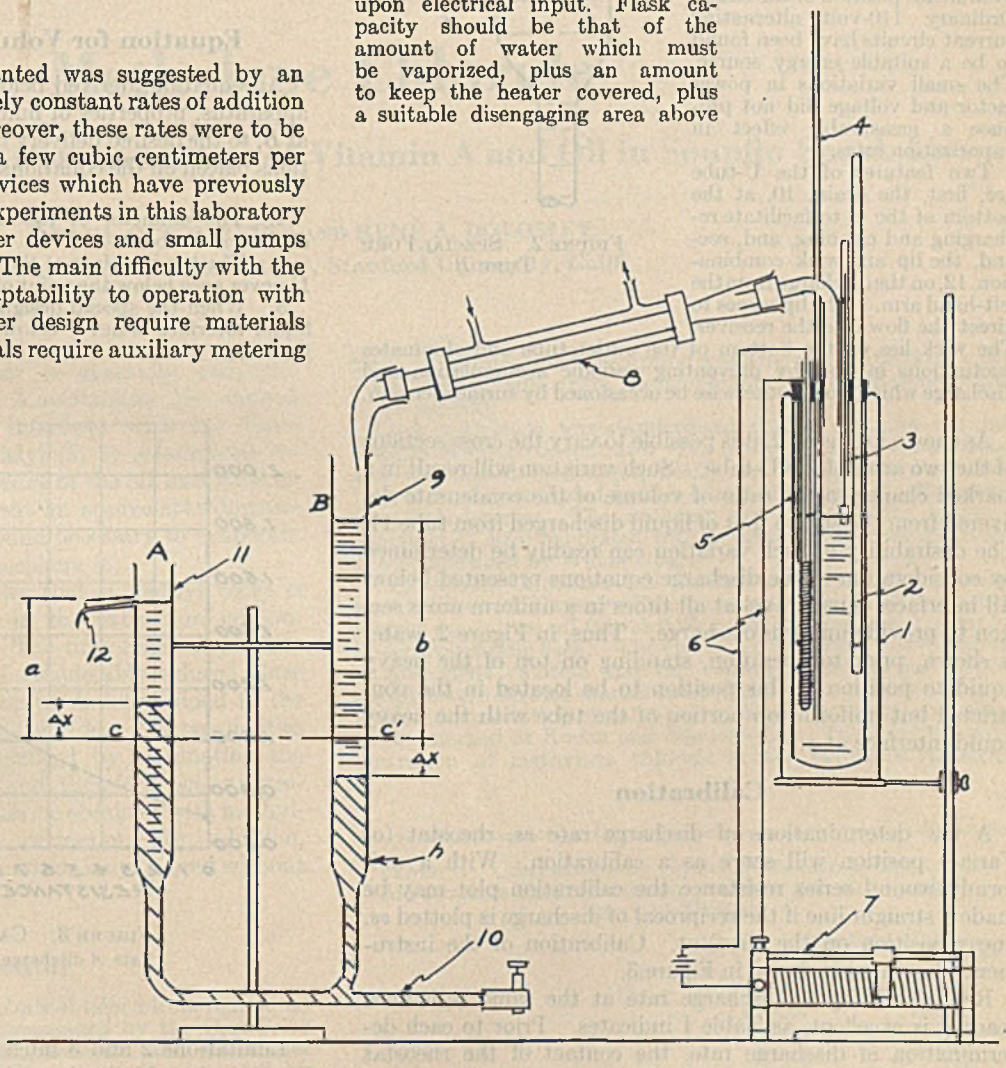

FIGURE 1. DIAGRAM OF ASSEMBLED APPARATUS

1. Insulated flask
2. Thermometer
3. Water level gage
4. Safety tube
5. Heating unit
6. Electric leads
7. Slide-wire resistance
8. Condenser
9. Receiver tube
10. Drain
11. Delivery tube
12. Glass lip

the liquid surface to prevent entrainment. The amount of liquid to be vaporized, which may be calculated from the equations presented at the end of the paper, will vary with density of the three fluids involved, and with the cross sections of the two arms of the U-tube. The still is equipped with any type of electrical submergence heater. Because of the present shortage of equipment, the heater used consisted of a length of fine resistance wire wound on a glass tube, 5, one lead emerging from the inside of the tube. An ordinary knife heater would be suitable, although occupying more space. The level indicator, 3, consists of a piece of cork into which a straight length of wire of moderately heavy gage is fastened. The top of the wire projects above the top of the flask and is enclosed in a glass tube with a sealed top. A long open tube, 4, acts as a pressure safety valve. Figure 1 also indicates a thermometer, 2, extending through a cork into the liquid in the still. Its purpose is only convenience in the prediction of the imminence of boiling.

Control of the heat supplied to the still may be regulated by any combination of resistances or, preferably, by some instrument similar to a Variac transformer which contains a graduated scale. If resistances are used, a scale should be mounted to indicate position of the slides. Ordinary 110-volt alternating current circuits have been found to be a suitable energy source. The small variations in power factor and voltage did not produce a measurable effect in vaporization rates.

Two features of the U-tube are, first, the drain, 10, at the bottom of the U to facilitate recharging and cleaning, and, second, the lip and wick combination, 12, on the discharge from the left-hand arm. The lip serves to direct the flow into the receiver. The wick lies on the bottom of the outlet tube and eliminates fluctuations in flow by preventing periodic accumulation and discharge which would otherwise be occasioned by surface tension.

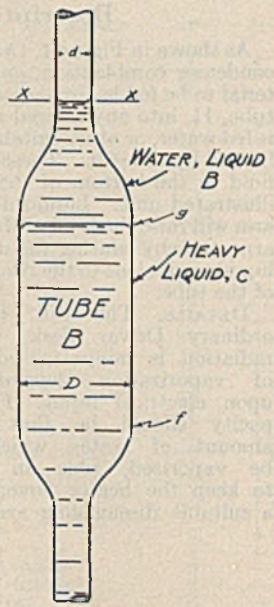

FIGURE 2. SPECIAL FORM TUBE *B*

As shown in Figure 2, it is possible to vary the cross sections of the two arms of the U-tube. Such variation will result in a marked change in the ratio of volume of the condensate delivered from the still to that of liquid discharged from tube 11. The desirability of such variation can readily be determined by consideration of the discharge equations presented below. All interfaces must travel at all times in a uniform cross section to provide uniform discharge. Thus, in Figure 2, water is shown, prior to operation, standing on top of the heavy liquid to position x, this position to be located in the constricted but uniform top portion of the tube with the heavy liquid interface at g.

Calibration

A few determinations of discharge rate *vs.* rheostat (or Variac) position will serve as a calibration. With a uniformly wound series resistance the calibration plot may be made a straight line if the reciprocal of discharge is plotted *vs.* linear position on the rheostat. Calibration of the instrument illustrated is shown in Figure 3.

Reproducibility of discharge rate at the same resistance reading is excellent, as Table I indicates. Prior to each determination of discharge rate, the contact of the rheostat was moved from and returned to the given setting.

Operation

The U-tube is filled with a heavy liquid and the left-hand side charged with the material to be fed. Since the first few seconds of operation usually give irregular results, this last fluid should be charged in sufficient quantity to bring the level nearly, but not exactly, to the point of discharge. The short time thereby allowed after vaporization commences in the still before fluid flows from the left arm of the U-tube will be sufficient to prevent these initial irregularities from influencing the otherwise constant delivery rate.

The still is also charged with the requisite amount of fluid, presumably water, and current is passed to the heater. As the temperature of the fluid in the still nears the boiling point, current is regulated to the point which, by previous calibration, is shown to correspond to the desired delivery rate.

TABLE I. REPRODUCIBILITY OF DISCHARGE RATE

Slider Setting	Volume Collected	Time Required	Volume Rate	Maximum Variation
	Cc.	*Sec.*	*Cc./min.*	%
(1) 0	5.0	420.3	0.714	
0	5.0	417.0	0.720	0.8
0	5.0	417.9	0.718	
(2) 5	5.0	335.8	0.895	1.1
5	5.0	339.7	0.885	
(3) 10	5.0	250.0	1.200	
10	5.0	250.0	1.200	0.8
10	5.0	248.0	1.210	
(4) 14	10.0	308.5	1.945	
14	10.0	308.5	1.945	0.4
14	10.0	307.5	1.953	

Equation for Volume Rate of Discharge

An equation, derived below, relates the dimensions of the apparatus, properties of fluids involved, and the rate of feed at B, to the desired delivery rate at A (see Figure 1). Limitations placed on the equations are:

1. The diameter of the main body of tube B equals the diameter of tube A.
2. The top interface of the heavy liquid on side B (see Figure 1) never goes below the point of constriction h.
3. When the special design of tube B (Figure 2) is used, the liquid interface is never below point of constriction f nor above that at g.

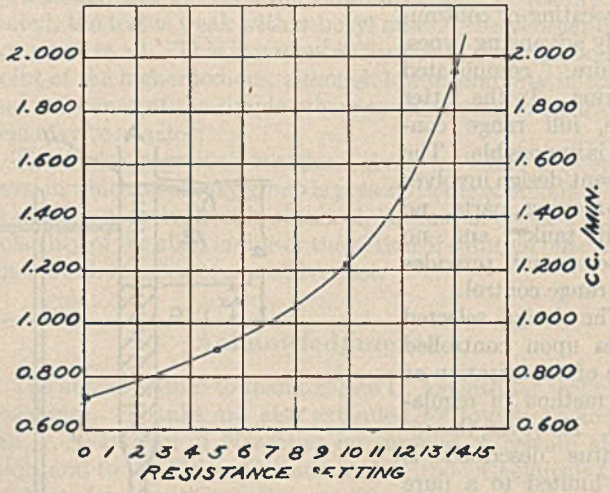

FIGURE 3. CALIBRATION CURVE
Rate of discharge *vs.* resistance position

Limitations 2 and 3 must be maintained for constant delivery rates. Limitation 1 is not required but is convenient in obtaining a simple equation.

If the surface of the heavy liquid moves down a distance ΔX in side B, the general equation for static displacement, as calculated by hydrostatics, is

$$(b + \Delta X)\rho_B = (a - \Delta X)\rho_A + 2\rho_C \Delta X$$

or

$$b = \frac{a\rho_A + \Delta X(2\rho_C - \rho_A - \rho_B)}{\rho_B} \quad (1)$$

b, a, and ΔX are dimensions shown in Figure 1, ρ_A and ρ_B are the densities of the liquids in sides A and B, and ρ_C is the density of heavy liquid.

Let L_1 be the volume rate at which light liquid is introduced at B and let tube B be designed as shown in Figure 2. Then, since ΔX is the displacement of the heavy liquid at C, the volume of light liquid entering tube B is $A\,\Delta X$, where A is the cross section of tube B where its diameter is D.

Of the liquid charged, $L_1 - A\,\Delta X$ remains to increase head b and this increase, Δb, may be written

$$\Delta b = \frac{L_1 - A\,\Delta X}{a}$$

where a is the cross section of tube B where diameter is d.

From Equation 1

$$\Delta b = \left(\frac{2\rho_C - \rho_A - \rho_B}{\rho_B}\right)\Delta X$$

$$\therefore\ L_1 = \left(\frac{2\rho_C - \rho_A - \rho_B}{\rho_B}\right) a\,\Delta X + A\,\Delta X$$

and

$$A\,\Delta X = L_2, \text{ since } D_A = D_B$$

Dividing L_1 by L_2

$$L_1 = L_2 \frac{(2\rho_C - \rho_A - \rho_B)a + A\rho_B}{\rho_B A}$$

or

$$L_1 = L_2 \frac{(2\rho_C - \rho_A - \rho_B)(d/D)^2 + \rho_B}{\rho_B} \quad (2)$$

If the design is that shown in Figure 1, with tubes A and B of equal and uniform diameter, Equation 2 simplifies to

$$L_1 = L_2\left(\frac{2\rho_C - \rho_A}{\rho_B} - 1\right) + L_2$$

In this case, the capacity of the still must be increased for a given delivery rate, L_2.

Methylene Chloride

In the Extraction and Determination of Vitamin A and Oil in Soupfin Shark Livers

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PEROXIDE-free diethyl ether is generally employed in the extraction of vitamin A–containing oils derived from fish livers. This solvent interferes with the Carr-Price (*1*) and the Rosenthal-Erdelyi (*6*, *7*) colorimetric reactions used to determine the potency of the oil, and must be removed. Furthermore, to prevent an appreciable destruction of the vitamin it has been found necessary to evaporate the solvent in an oxygen-free atmosphere (*3*).

The present investigation shows that if diethyl ether is replaced by methylene chloride in the extraction process, several advantages are realized. The time required for routine colorimetric determinations is considerably reduced, since the solvent containing the oil may be directly added to the chloroform solution of antimony trichloride. The possibilities of vitamin A destruction are reduced by eliminating the necessity of removing the solvent and, therefore, better agreement between duplicate results can be obtained with methylene chloride than with ether extraction. In addition, "refrigeration grade" methylene chloride may be used without further purification.

Experimental

The potency of pooled oils and of oils obtained from individual soupfin shark liver samples was determined by the Carr-Price reaction, its Rosenthal-Erdelyi modification, and ultraviolet absorption. In each case the dilution principle advocated by Norris and Church (*5*) was employed. The color intensity of the Carr-Price reaction was measured at 620 mμ with a Coleman 10-S spectrophotometer (5 mμ slit width), while that of the Rosenthal-Erdelyi reaction was evaluated with a Klett-Summerson photoelectric colorimeter equipped with a green (No. 54) filter. The ultraviolet absorption was determined at 328 mμ with a Beckman quartz prism spectrophotometer, using isopropanol as the solvent.

Each instrument was standardized against the same natural vitamin A ester concentrate. The potency of the concentrate was established spectrographically by Distillation Products, Inc., and was confirmed in this laboratory using the Beckman spectrophotometer. The Beckman and the Coleman spectrophotometers were calibrated with potassium chromate solutions as recommended by Wilkie (*10*).

METHYLENE CHLORIDE AND CHLOROFORM IN THE CARR-PRICE AND ROSENTHAL-ERDELYI REACTIONS. A sample of extracted soupfin shark liver oil (about 0.25 gram) was weighed in a tared 25-ml. volumetric flask. The experimental sample was dissolved in methylene chloride and the control in chloroform. Both samples were further diluted as required and were treated with a chloroform solution of antimony trichloride prepared according to the method of Koehn and Sherman (*4*). The resulting concentration of methylene chloride in the experimental series

TABLE I. COMPARISON OF CHLOROFORM AND CHLOROFORM–METHYLENE CHLORIDE AS SOLVENTS IN THE COLORIMETRIC ASSAY OF VITAMIN A

Reaction	Oil Pool	Colorimetric Determinations: Chloroform	Chloroform–methylene chloride
		I. U./g.	*I. U./g.*
Rosenthal-Erdelyi	3	42,900	42,500
	4	386,000	388,000
	x	5,350	5,400
Carr-Price	3	41,800	39,300
	4	370,000	369,000
	5	166,000	166,100
	x	6,100	6,200

amounted to 10 per cent by volume. The same conversion factor was used to calculate the potency of both series shown in Table I.

METHYLENE CHLORIDE EXTRACTION OF OIL AND VITAMIN A. Methylene chloride was compared with ether in the extraction of oil and vitamin A from shark livers. Liver samples previously frozen in dry ice were homogenized in a Waring Blendor and 2- to 3-gram aliquots were extracted by the short method of Stansby and Lemon (*8*). A 5- or 10-ml. aliquot of the supernatant solution was evaporated to dryness in a tared 50-ml. beaker and the percentage of oil in the liver was calculated from Formula 3.

When methylene chloride was used as the extracting agent, a 1-ml. aliquot of the extract was diluted with chloroform for the colorimetric procedures, or with isopropanol for direct absorption. Ether, when used as the extractant, was removed from the aliquot by immersing the flask in a 40° to 50° C. water bath and blowing a stream of an inert gas (methane) over the solution. The ether-free oil was then diluted to 25 ml. with the appropriate solvent and the weight of oil in each aliquot was calculated from Formula 2. Typical results are shown in Table II.

CALCULATION OF RESULTS. [The procedure of relative aliquots for the simultaneous evaluation of oil and vitamin A in a determination is that used in the Technical Fisheries Laboratory, U. S. Department of Interior, Seattle, Wash. (*2*).]

If the aliquot method outlined above is used, one must bear in mind that the volumes of solvent and oil are nearly additive. The volume of solvent in any given aliquot is then calculated from the approximate formula:

$$V_s = V_a - \frac{W_0}{D} \qquad (1)$$

where V_s is the volume of solvent, V_a is the aliquot volume, W_0 is the weight of oil, and D is the average density of the oil.

Using the value for V_s as determined in Formula 1, the oil extracted by 100 ml. of solvent may be found from:

$$\frac{W_0 \times 100}{V_s} \qquad (2)$$

and the percentage of oil in the original sample becomes:

$$\frac{W_0 \times 100}{V_s} \times \frac{100}{W_L} \qquad (3)$$

where W_L is the weight of the liver sample.

The percentage of oil determined and calculated in this manner was in agreement with values obtained by Soxhlet extraction, and vitamin A potencies were in agreement with determinations made on weighed samples of the extracted oil.

Discussion

Wokes and Willimott (*11*) studied the antimony trichloride–vitamin A reaction in solvents other than chloroform. They dissolved not only the oil, but also the antimony trichloride in these solvents. In all cases the sensitivity of the reaction was diminished, making the substitution inadvisable for many investigations. It was found in this laboratory that antimony trichloride was more readily soluble in methylene chloride than in chloroform. This advantage was offset by the fact that the resulting color intensity was 30 per cent less; however, partial substitution of methylene chloride for chloroform had no effect on the sensitivity of the reaction, as shown in Table I.

According to the results shown in Table II, methylene chloride is as efficient as ether in the extraction of oil and vitamin A. Although no benefit is gained in the subsequent ultraviolet method of assay, a distinct advantage is realized in colorimetric procedures. Moreover, better agreement can be obtained between duplicate determinations when methylene chloride is used in place of ether. The essential validity of this substitution is indicated in the agreement between the results obtained by the physical and chemical methods of analysis.

Attempts to use methylene chloride to extract the unsaponifiable fraction proved unsatisfactory, since persistent emulsions were encountered. Oils of potency less than 10,000 I. U. per gram are seldom received in this laboratory and, therefore, saponification is required in but very few cases.

Even though methylene chloride is less volatile than diethyl ether, great care in handling this solvent is required to prevent loss by evaporation. Moreover, because of the toxicity of this solvent all operations should be conducted in a well-ventilated place. Attempts are being made by others in this laboratory to find solvents which will reduce these disadvantages.

Confirmation of the value of methylene chloride in extracting oils containing vitamins is afforded in the proposed method of the U. S. Pharmacopoeia (*9*) for the assay of capsules of halibut liver oil.

TABLE II. COMPARISON OF ETHER AND METHYLENE CHLORIDE IN THE EXTRACTION OF OIL AND VITAMIN A

Sample	Oil in Liver: Ether	Oil in Liver: Methylene chloride	Assay Method	Vitamin A[a]: Ether	Vitamin A[a]: Methylene chloride
		%		*I. U./g.*	*I. U./g.*
2	56.0	55.2			
			C-P[b]	...	...
			R-E[c]	127,400	134,500
			U-V[d]	132,000	134,800
4	61.1	61.5			
			C-P	112,600	106,000
			R-E	115,200	113,000
			U-V	109,800	108,200
5	60.3	59.7			
			C-P	104,000	104,000
			R-E	104,000	101,000
			U-V	105,500	104,700
118	79.6	79.3			
			C-P	234,000	236,000
			R-E	232,000	233,000
			U-V	...	...
120	68.2	68.5			
			C-P	27,000	27,200
			R-E	27,000	26,900
			U-V	...	...
122	70.4	70.6			
			C-P	32,950	33,100
			R-E	33,400	33,000
			U-V	...	...
126	53.0	53.3			
			C-P	184,700	185,000
			R-E	183,500	183,000
			U-V	...	...

[a] Assays for a given sample were run on aliquots of same extract.
[b] Carr-Price.
[c] Rosenthal-Erdelyi.
[d] Ultraviolet absorption.

Summary

Methylene chloride is comparable to ether for the extraction of oil in soupfin shark livers, but not for the separation of the unsaponifiable fraction. Vitamin A may be determined directly on an aliquot of the methylene chloride extract. Through the use of methylene chloride it is possible further to simplify an already rapid routine method for the simultaneous determination of oil and vitamin A in these livers.

Acknowledgment

The authors wish to express their gratitude to J. Murray Luck for the keen interest he has shown and the helpful suggestions he has offered while supervising this project. They are indebted to Distillation Products, Inc., for the vitamin A ester concentrate, to the Dow Chemical Company for the methylene chloride (refrigeration grade) used in this work,

and to C. R. Noller of the Department of Chemistry for indicating the possible use of methylene chloride for the work presented in this paper.

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This investigation is incidental to a survey on the vitamin A potency of soupfin shark livers. The survey is part of a program investigating the entire California soupfin shark fishery and is conducted by the California Division of Fish and Game.

Rapid Determination of Sodium Chloride in the Presence of Protein

Application to Salt-Cured Food Products

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Sodium chloride in protein-containing material and solutions is determined by direct titration with 0.0856 *N* silver nitrate, using dichlorofluorescein as an adsorption indicator (40 to 100 mg. of sodium chloride in a volume of 400 ml.). A 0.2 *N* sodium acetate–acetic acid buffer of pH 4.5 is added to the solution or minced sample and allowed to stand or boiled and cooled. The salt is completely extracted from the sample and it is shown that silver is not adsorbed by the protein present at this hydrogen-ion concentration. Results obtained agree closely with the common Volhard method of digestion with nitric acid in the presence of silver nitrate.

PROTEIN interferes in all the usual methods for the determination of chloride, and must be removed by some wet- or dry-ashing procedure. This requires considerable time and inconvenience when large numbers of routine determinations are to be carried out. Attempts to remove protein by various precipitants have not been satisfactory (*2*).

Recently, adsorption indicators have come into common use for the direct titration of halide ions, and in relatively simple solutions the method is very much more rapid and equally as accurate as the common Volhard procedure in which the chloride is precipitated by addition of silver nitrate, and the excess silver ion titrated with thiocyanate (*1*, *3*, *12*).

Dichlorofluorescein seems to be the most suitable adsorption indicator for the chloride ion in dilute solution (*6*, *7*, *9*, *10*). Here also, low results are obtained in the presence of protein, apparently because of an adsorption of silver on the protein (*6*, *11*).

In fish muscle the principal proteins have isoelectric points at about pH 5 and 6 (*8*), and it was reasoned that this adsorption should be at a minimum, and negligible in amount, at a hydrogen-ion concentration near the isoelectric point of the protein micelles. Muscle suspensions and extracts were titrated in sodium acetate and phosphate buffer solutions over the range pH 3 to 9. Theoretical results were obtained between about pH 4 and 4.8 in the presence of protein, while in pure salt solution the indicator was satisfactory between pH 4 and 7. Results have been checked on salt fish, canned fish, fish meals, bacon, etc., and on pickles and brines. The optimum pH range was from 4.3 to 4.8 in each case, and the method as finally developed was found to be entirely satisfactory from an analytical standpoint. In the author's experience, the use of organic solvents as sometimes recommended did not result in any improvement over the above method.

Reagents

Dichlorofluorescein indicator, 0.1 per cent in 50 per cent ethanol.

Acetate buffer, equal volumes of 0.2 *N* sodium acetate and 0.2 *N* acetic acid, adjusted to pH 4.5 (±0.2) if necessary.

Silver nitrate, 14.52 grams in 1000 ml. of solution standardized against pure dry sodium chloride, 1 ml. = 5 mg. of NaCl.

Procedure

Pickles and Solutions. To an aliquot of solution containing about 40 to 100 mg. of sodium chloride, add approximately 20 ml. of acetate buffer solution, and dilute to about 400 ml. with distilled water. Add 10 drops of indicator and titrate with the silver nitrate solution, while slowly swirling the flask, until a pinkish red coloration appears throughout the solution. Titration must be fairly rapid, since the adsorption of the dye considerably increases the light-sensitivity of the colloidal silver chloride, thereby rapidly increasing the rate of aging and decreasing the sharpness of the end point. If the pickle contains much protein, a much sharper end point will be obtained if the solution is boiled for a minute or more after addition of the buffer and cooled to 20° C. before dilution and titration.

Salt Fish and Other Cured Food Products. A well-comminuted sample is mixed with 25 ml. of buffer, and diluted to about 75 ml. with water. The mixture is boiled for a few minutes, and cooled. The whole may then be diluted to 400 ml. and titrated as before, or the solution may be made to 100 ml. and an aliquot of the supernatant liquid used for the titra-

tion. The weight of sample used will depend on the salt content and degree of subdivision of the sample. If it is desirable to use a large sample for the sake of uniformity in sampling, the titration of an aliquot containing 40 to 100 mg. is the preferable procedure. Either fresh or dried samples may be used, so that moisture and salt analyses are often conveniently made on the same sample. If a 0.5-gram sample is used, as is convenient with salt fish, the percentage salt content is read directly on the buret, 1 ml. being equivalent to 1 per cent of sodium chloride in the original material.

Table I. Recovery of Added Sodium Chloride

Sample	NaCl Added	NaCl Found	Recovery of Added NaCl
	Mg.	*Mg.*	%
Fresh cod, 0.5 gram	0	0.5[a]	...
	25	25.3	101
	50	51.0	101
Light salt cod, 0.5 gram	0	60.0	...
	25	85.2	101
	50	109.5	99
	75	135.0	100
Hard salt cod, 0.5 gram	0	97.5	...
	25	122.0	98
	75	170.3	97

[a] Volhard method.

Results and Discussion

A series of titrations was conducted on pure sodium chloride solutions and on extracts of salt fish and of fish meal with sodium chloride added, over a range of pH 3 to 8 in sodium acetate and phosphate buffers. The results are shown in Figure 1.

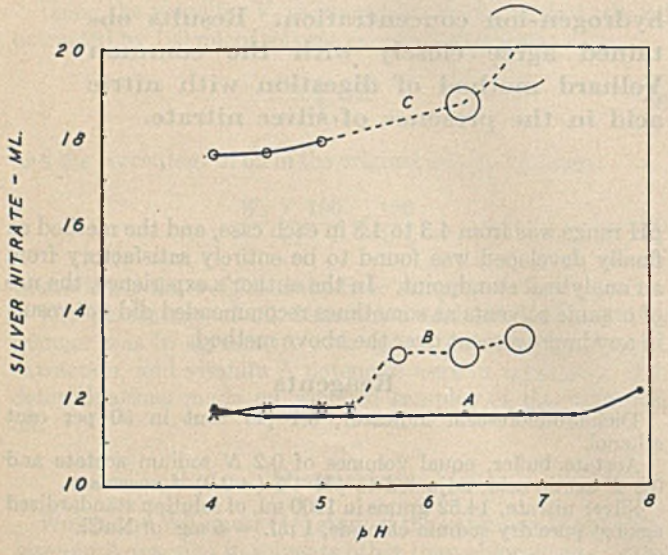

Figure 1. Influence of pH on Titration End Point

1 ml. of $AgNO_3$ soln. = 5 mg. of NaCl

A. Standard solution of sodium chloride. Titer = 11.60 ml.
B. Standard solution of sodium chloride + 1 gram of fresh cod muscle. Titer = 11.80 ml.
C. Standard solution of sodium chloride + extract of 1 gram of fish meal. Titer = 17.60 ml.
Dotted lines show indefinite end points—approximate limits shown.

At acidities below pH 4 the indicator showed no color change, and above pH 7.5 the change became indefinite, an orange-yellow or yellow color being obtained. In the presence of protein the end point becomes indefinite above approximately pH 6, at an acidity just below the point where an increased adsorption of silver occurs. The amount of silver and dye ions adsorbed by the protein increases as the solution is made more alkaline, and thus no definite end point is obtained in this range.

It is indeed shown that between pH 4 and 5 a range probably slightly more acid than the isoelectric point of the known proteins present, little or no adsorption of silver or of chloride occurs. In solutions containing considerable soluble protein, such as those from fish meal where the salt content is low and consequently a high dilution cannot be used, a pH of 4.2 to 4.8 gives results agreeing with the Volhard method. A buffer of pH 4.5 was accordingly selected for routine use.

This indicates that cod and haddock muscle has an apparent isoelectric point near pH 4.5. Other work on the salting of cod muscle (*4*) has also shown an apparent isoelectric point between pH 4.0 and 4.5. Further study of the proteins present is needed, although the isoelectric point does not necessarily coincide with the point of minimal acid and base-binding capacity (*5*).

Recoveries of added sodium chloride are shown in Table I and agreement of results with those of the standard Volhard method in Table II, on samples of salt cod, fish meal, and bacon. The fish meal samples were analyzed by boiling a suspension of 5 grams of meal in 100 ml. of buffer and water, then adding 50 mg. of sodium chloride (standard solution), to a 20-ml. aliquot of the suspension. Otherwise the salt content was too low, and insufficient colloidal silver chloride was formed to allow easy observation of the end point.

Table II. Comparison of Adsorption Indicator and Volhard Methods

Sample	Sodium Chloride Found	
	Adsorption indicator	Volhard
	%	%
Hard salt cod, 0.5 gram	19.8 (±0.2)[a]	19.9 (±0.3)[a]
Light salt cod, 0.5 gram	11.3 (±0.2)[a]	11.2 (±0.2)[a]
Bacon, 5 grams	1.30 (±0.03)[a]	1.31 (±0.03)[a]
Fish meal, cod	3.00, 2.95	2.93, 2.91
Fish meal, herring	0.75, 0.70	0.72, 0.73

[a] Average of 4 determinations.

Recovery of added salt is very satisfactory, and the results agree with the standard method.

Complete extraction of salt from a minced sample by the buffer solution was obtained. Boiling for 2 to 5 minutes, followed by cooling to room temperature, gave the same results as extracting for approximately 3 hours at room temperature, and the end point was considerably more satisfactory. Decreasing the extraction time to less than 3 hours at room temperature resulted in incomplete extraction, as found by comparison with the wet-digestion methods.

The presence of fat or oil in fish meal or meats had no influence on the results. The method has been in continuous use for more than a year in the research and routine analyses of salt fish and other fishery products.

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Spectrophotometric and Biological Assay of Vitamin A in Oils

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IN AN earlier publication (*2*) comparisons were made between the biological and spectrophotometric methods of assay of vitamin A in a series of fish liver oils. Since that publication a new U. S. P. reference cod liver oil for biological assay of vitamin A has been introduced. Spectrophotometric tests on the stability of this reference have been performed and new comparisons between the two methods of assay have been made.

Experimental Procedure

PHYSICAL. The instrument used was a Judd Lewis ultraviolet photometer with the different photometer units, including the source, rigidly mounted to the frame of a medium Bausch & Lomb ultraviolet spectrograph. The light source was a tungsten-steel spark. The density scale of the lower sector of the photometer was calibrated over a range of density values of 0.60 to 1.00 against three different concentrations of potassium chromate solution for each sample tested. Isopropanol was used as a solvent for all the oils. Two weighings and two dilutions of each weighing were made for each sample. Match points were read from the image (magnification factor 6) of the plate projected on a white screen by a modified Bausch & Lomb projector.

The assays of the oils of lower potency were made on the unsaponifiable fractions and in some cases also on the whole oils. Two methods of saponification (*2*) were used for each oil.

BIOLOGICAL. The U. S. P. XI procedure was followed in all cases and the oils were handled in the manner previously reported (*2*).

TABLE I. U. S. P. REFERENCE COD LIVER OIL 2

No.	$E^{1\%}_{1\ cm.}$ (Unsaponifiable Fraction)	No.	$E^{1\%}_{1\ cm.}$ (Whole)
1	0.78	1	0.86
2	0.83	2	0.86
3	0.75	3	0.86
4	0.75	4	0.86
5	0.81	5	0.88
6	0.69	6	0.85
7	0.70	7	0.85
8	0.70	8	0.82
9	0.71	9	0.79
10	0.69	10	0.86
11	0.73	11	0.85
12	0.74		
13	0.77	$E^{1\%}_{cm.}$ = 0.85 (coefficient of variation 2.86)	
14	0.76		

$E^{1\%}_{1\ cm.}$ = 0.74 (coefficient of variation 5.95)
Conversion factor (unsaponifiable fraction), 2300
Conversion factor (whole), 2000
Based on value of 1700 U. S. P. per gram.

TABLE II. STABILITY OF U. S. P. REFERENCE COD LIVER OIL 2

Bottle No.	Date	$E^{1\%}_{1\ cm.}$	No. of Measurements
1	11/1/40	0.79 (unsaponifiable fraction)	1
		0.86 (whole)	1
	12/1/40	0.79 (unsaponifiable fraction)	1
		0.88 (whole)	1
2	4/4/41	0.77 (unsaponifiable fraction)	3
		0.87 (whole)	3
	4/6/42	0.68 (unsaponifiable fraction)	3
		0.82 (whole)	3
3	4/7/41	0.72 (unsaponifiable fraction)	1
		0.84 (whole)	1
	11/7/41	0.64 (unsaponifiable fraction)	1
		0.86 (whole)	1
	4/15/42	0.60 (unsaponifiable fraction)	3
		0.77 (whole)	3

Results

In Table I are listed values obtained by spectrophotometric assay of U. S. P. reference oil 2. These measurements were made over a period of 2 years on freshly opened samples of the reference oil and each sample was tested within the date labeled as safe. No significant variation of measurement was obtained among the samples tested.

Table II includes data on the stability of standard reference 2 as indicated by assays carried out on three bottles. The assays were made when the bottles were first opened and also after intervals varying from one month to a year. After each measurement the bottles were flushed with carbon dioxide, stoppered, and stored in the refrigerator. A loss in vitamin A potency after several months was indicated.

TABLE III. FISH LIVER OILS

Source of Vitamin	No. of Samples	Potency Range, Biological Units	Average Conversion Factors	Coefficient of Variation[a]
Cod liver oil (unsaponifiable fraction)	32	1,000–4,850	2170	6.54
Cod liver oil (whole)	12	1,000–4,850	2070	9.32
		Higher Potency Oils		
Capsules	4	1,330–9,700	1900	3.23
Tablets	8	2,070–11,800	2030	7.58
Shark liver oil	12	4,500–98,000	1910	6.54
Tuna liver oil	9	12,500–85,000	1910	7.27
Halibut liver oil	10	38,000–201,000	1960	8.06
Vitamin A ester concentrates	7	202,000–520,000	1970	4.82
Vitamin A concentrates	7	205,000–1,200,000	1850	6.00
Miscellaneous	4	7,800–9,200	2050	3.93

Weighted average conversion factor for higher potency oils, 1940.

[a] Coefficient of variation = $\frac{\text{standard deviation} \times 100}{\text{average conversion factor}}$

Table III lists values obtained with 93 samples of fish liver oils varying from cods of biological potency of 1000 units to concentrates of over 1,000,000 units per gram. These are segregated into groups. The number of samples tested, potency range in biological units, average conversion factors, and coefficients of variation are listed. The conversion factors for the oils of higher potency are in good agreement, but the average factor of 1940 for this group is smaller than that of 2170 for the unsaponifiable fraction of the cods. The 93 samples represent all the samples tested over a definite period, with the exception of two multiple vitamin capsules. Absorption curves for these two atypical samples, along with that of a sample giving a typical vitamin A curve, are shown in Figure 1.

Table IV compares the conversion factors of the two reference oils based on the claimed unit contents and also the conversion factors of the test oils which are computed from the biological assay results obtained with the two reference samples.

Discussion of Results

The data in Table IV show that the average conversion factors for the test oils have undergone a significant decrease

TABLE IV. CONVERSION FACTORS

	No. of Measurements	Conversion Factor	Coefficient of Variation
U. S. P. reference 1 (unsaponifiable fraction)	14[a]	2180[b]	2.40
U. S. P. reference 2 (unsaponifiable fraction)	14	2300[c]	5.95
Cod liver oil (unsaponifiable fraction), Series I[d]	22	2700[b]	10.30
Cod liver oil (unsaponifiable fraction), Series II	32	2170[c]	6.54
Higher potency oils, Series I[d]	31	2260[b]	9.60
Higher potency oils, Series II	61	1940[c]	7.01

[a] Measurements made on four freshly opened bottles of U. S. P. reference 1, averages given in (2). 1-gram samples used.
[b] Based on biological value of 3000 U. S. P.
[c] Based on biological value of 1700 U. S. P.
[d] See (2).

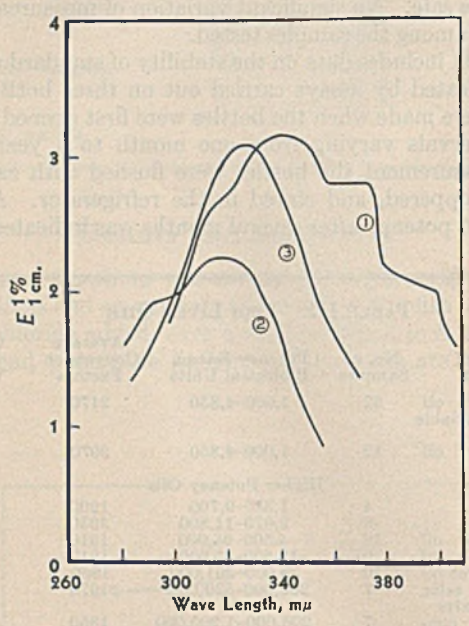

FIGURE 1. ABSORPTION CURVES IN ISOPROPANOL SOLUTION

1, 2. Experimental multiple vitamin capsules giving atypical vitamin A absorption curves
3. Experimental multiple vitamin capsule giving a normal vitamin A curve

with the advent of U. S. P. reference cod liver oil 2. Since there has been no change in the spectrophotometric technique, it is probable that this change is associated with the biological test. Such a lowering of the conversion factor could result from differences in the size of the unit of vitamin A in the two reference samples, but such an explanation is not consistent with all the facts. The conversion factors of reference samples 1 and 2 computed on the basis of the claimed unit content are in fair agreement; in fact, that of the former is slightly lower than the latter. It would appear more probable that such a lowering of the conversion factors is due to differences in the stabilities of the two reference oils. A relatively short time elapses between the opening of a fresh bottle of reference and the completion of a spectrophotometric test, whereas there is considerable opportunity for loss of activity to occur before and after the feeding of the diluted reference to the test rats. Thus, if reference 2 were more stable than reference 1, it would produce the same effect in the biological test as would a reference containing larger units.

Spectrophotometric data listed in Table II on reference oil 2 and data previously published (2) on reference oil 1 indicate that neither of these oils as received from the U. S. P. committee has shown any loss in potency; however, spectrophotometric measurements on reference 1 suggest a rapid loss of vitamin A potency in samples which had been opened and allowed to stand in the refrigerator, as evidenced by a drop in the $E^{1\%}_{1\,cm.}$ value of about 14 per cent. Similar measurements on the new reference also suggest a loss of vitamin A potency with time as indicated in Table II, but the deterioration appears to be less rapid than that for reference 1, a drop of 14 per cent occurring after several months rather than weeks.

It would thus seem that improvements could be made on the stability of the reference oil, possibly by the use of antioxidants. It was pointed out by Dyer *et al.* in 1934 (3) that the biological activity of carotene or cod liver oils was affected by the oil which was used as a diluent for feeding. Recently Hickman (5) and Quackenbush (6) have shown that vitamin E possesses antioxidant properties for vitamin A or carotene in vivo as well as in vitro. These studies suggest that some of the variations in biological assays may be due to differences in the content of vitamin E or other antioxidants in different samples. Studies of the problem are now in progress in this laboratory.

A survey of the data listed in Table III indicates a high degree of correlation between the spectrophotometric and biological assays of vitamin A. The average conversion factor of 1940 for the higher potency fish liver oils is lower than the value of 2000 recently proposed as a working value for comparisons between measurements made in different laboratories. This factor is lower than that of 2170 found for the unsaponifiable extract of cods. All these factors are lower than that of 2460 reported by Baxter and Robeson (1) for crystalline vitamin A.

There were only two samples in this survey which did not give comparable results by the spectrophotometric and biological methods. Absorption curves of these samples are given in Figure 1. Curve 1 is the absorption curve of an experimental multiple vitamin capsule, in which the biological assay gave a lower value than did the spectrophotometric assay. The absorption curve is resolved into three distinct "flats" in the regions of 340 mμ, 365 mμ, and 390 mμ and resembles the absorption curve of a mixture of vitamin A and cyclized vitamin A as reported by Embree (4). The interpretation was that the vitamin in the capsule had been partially cyclized and hence a measure of the absorption at 328 mμ did not give a true measure of the vitamin A potency.

The second case was that of another experimental multiple vitamin capsule, the absorption curve of which is plotted as curve 2 in Figure 1. In this case the absorption peak was shifted to 318 mμ; in addition there was a rather high flat at 300 mμ and the ratio of biological assay to $E^{1\%}_{1\,cm.}$ was low. This seems to compare with a case of destruction due to oxidation as reported by Robinson (7). In neither case could the spectrophotometric method give an accurate value of the vitamin A potency of the sample, and the inaccuracy of the assays was indicated by the absorption curves.

Summary

Spectrophotometric measurements on fresh samples of U. S. P. reference sample 2 over a period of 2 years are recorded. The average conversion factor, computed on the basis of claimed content of 1700 U. S. P. per gram, is 2280 for the unsaponifiable fraction and 2000 for the whole oil.

The conversion factors computed from biological and spectrophotometric measurements on 32 cods and 61 oils of higher vitamin A potency show a decrease of from 20 to 14 per cent from those computed when the older standard reference oil 1 was used in biological assays. The new conversion factors are 2170 for the unsaponifiable fraction of the cods and 1940 for the higher potency oils. The suggestion is made that such a decrease in the conversion factors is due to a difference in the stability of the two references.

There was good agreement between the results of the biological and spectrophotometric tests of all but two samples. The absorption curves of these were not typical for vitamin A and thus served as an indication that the spectrophotometric method could not give an accurate assay in such cases.

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Laboratory Cooking, Mashing, and Fermentation Procedures

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A procedure is presented for conducting test laboratory fermentations for the production of alcohol from corn, wheat, or rye. The analytical procedures and methods of calculating results are described. The procedure is sufficiently flexible to be used for testing a variety of fermentation conditions or fermentation raw materials.

THERE has been no generally accepted method for laboratory studies of the grain alcohol fermentation process. During the course of numerous studies involving the fermentation of corn, wheat, and rye mashes, it was necessary to develop an accurate procedure yielding reproducible results. The objective was a method which would yield experimental results closely comparable with production data. Thus a standard control by this technique should yield as much alcohol as could be expected in an efficiently operated distillery when the same grains are processed. Furthermore, since it was necessary to evaluate the results obtained when varying one processing condition such as a conversion, cooking conditions, or type of grain, the method had to encompass a high degree of accuracy. As a result of the continued improvement and extensive use of this procedure, the authors are now convinced of the accuracy of the method and feel that the experimental error is less than 1 per cent. Only ordinary laboratory equipment is required.

This paper presents detailed procedures for the preparation of the mash and the analytical methods employed to obtain essential data. Since distillery nomenclature is often confusing, the terms are defined and all calculations are explained and shown by example. Typical laboratory fermentation data are presented. Below is the procedure for preparing 1.5 liters of mash at a concentration of 38 gallons of mash per bushel of grain (gallons of mash per bushel, the total volume in gallons which contains 1 bushel of grain).

Cooking and Conversion Procedures

Pressure Cooking. *Grain Bill*, 92 per cent corn or wheat, 8 per cent distillers' barley malt (Lintner value—minimum 175° dry basis).

Meal Analysis. These grinds (Table I) can be obtained by grinding through the medium screen of the Wiley mill. Other mills may be substituted, provided comparable grinds are obtained.

Moisture. At the time of mashing weigh a 10-gram sample of each of the ground grains employed into a tared dish, dry this sample at 110° C. for 3 hours, and reweigh.

Starch Content. Starch analysis is made by the A. O. A. C diastase–hydrochloric acid method (*1*).

Cooking. Place 883 cc. of tap water in a 2-liter beaker. Clamp the beaker in a water bath (any suitable vessel), so that the beaker is at least half submerged. The water in the beaker should be mechanically agitated with a propeller driven by a variable-speed laboratory motor.

Heat to 100° F. (temperature of water in beaker) and add 2.33 grams of distillers' barley malt. (Fahrenheit temperatures are used in conformity with industrial practice.)

Heat to 130° F. and add 241.2 grams of corn or wheat.

Remove source of heat.

Check pH, taking care to keep the entire procedure as quantitative as possible.

Adjust pH to 5.6 with *N* sulfuric acid.

Heat to 170° F. (at such a rate that this temperature is attained in about 20 minutes), measure, and record the volume by dry inches (the number of inches from the surface of the mash to the top of the container).

Increase the temperature to approximately 200° F. as rapidly as possible and hold at this temperature for 1 hour. However, start timing when the temperature reaches 185° F.

At the end of 1 hour make up the volume to equal that at 170° F.

Autoclave at 22 pounds for 1 hour. (The mash should be mechanically agitated at all times except when checking dry inches and when the cook is autoclaved.)

Table I

Grind	Mesh	Corn, %	Wheat, %	Barley Malt, %
On	12	0.0	0.0	0.0
On	16	0.5	0.0	0.5
On	20	2.0	6.0	1.5
On	30	25.5	55.5	11.0
On	40	32.0	22.0	36.5
On	60	37.5	8.0	26.5
Through	60	12.0	14.5	23.5

Conversion. After removing the cook from the autoclave place it in a water bath and agitate mechanically. It is important to have the temperature of the water bath between 160° and 170° F. at the time the cook is added. Cool the water bath to 145° F. and add the conversion slurry, consisting of 140 cc. of water and 20.95 grams of distillers' barley malt, when the temperature of the cook reaches 152° F. Make the malt slurry 30 minutes before using and heat to 130° F. immediately before adding it to the cook; warm the slurry in a water bath with occasional stirring.

Flash Conversion Method. As soon as the malt slurry is added to the cook, stir until there are no lumps (manual as well as mechanical stirring is frequently required) and then cool as rapidly as possible to 72° F. The conversion pH should be 5.4 to 5.5 (this need not be checked, since the adjustment of the pH for cooking will place the conversion pH in this range).

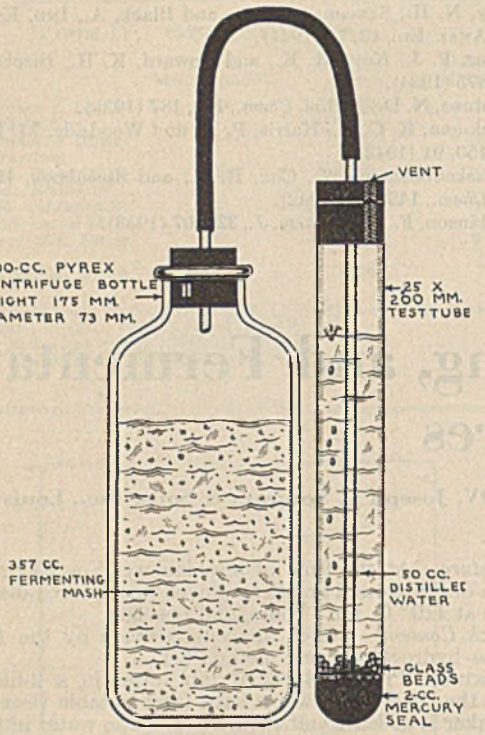

FIGURE 1. LABORATORY FERMENTATION FLASK EQUIPPED WITH CARBON DIOXIDE SCRUBBER

Sixty-Minute Conversion Method. Flash conversion is a superior production method which is gaining favor in the industry and normally results in increased alcohol yields. However, this method occasions more difficulty in the laboratory if efficiency data, based on sugar analysis, are desired (see method B, section on calculation). As an alternate, the 60-minute conversion method (comparable with the old standard plant procedures) may be employed. In this case, the mash is mechanically agitated after addition of the malt slurry for 60 minutes while held at 145° F., then cooled rapidly to 72° F.

INFUSION PROCESS (ATMOSPHERIC MASHING). The procedures described above are used frequently in research and control work in connection with modern grain alcohol plants. However, it is occasionally desirable to employ the infusion process when testing the fermentation of wheat, rye, or malt mashes. These grains do not require pressure-cooking for efficient gelatinization of the starch.

Wheat. Heat 883 cc. of tap water to 100° F. as above, add 241.2 grams of wheat slowly, raise the temperature to 155° F. in 45 minutes, and hold for 60 minutes; then cool to 152° F. and add the malt slurry containing 23.3 grams of malt prepared as described above. Hold the mash another 30 minutes at 145° F. and then cool to 72° F. The mash must be mechanically agitated at all times.

Rye. Heat 883 cc. of tap water to 100° F., add 23.3 grams of malt slowly, agitate the slurry 5 minutes, then add 241.2 grams of rye slowly. Raise the temperature to 130° F. in 30 minutes, hold 15 minutes, raise to 145° F. in 15 minutes, and hold at 145° F. for 60 minutes. At the end of this period cool the mash rapidly to 72° F. The mash is to be mechanically agitated at all times.

Fermentation Conditions

PREPARATION OF YEAST FOR INOCULUM. Transfer one loop of yeast from slant to test tube containing 10 cc. of 16° Balling malt extract. Transfer every 24 hours for 2 days through the same medium, transfer contents of the last tube to 200 cc. of the same medium, incubate for 20 hours at 86° F., and then use for inoculum.

PROCEDURE FOR SETTING FERMENTERS. This is a critical step in the procedure. Failure to observe reasonably aseptic conditions will result in contaminated fermentations and nullify the final data. Lack of observance of quantitative technique has the same result.

Pour cooled converted cook into clean, sterile 2-liter graduated cylinder.

Add 300 cc. of sterile thin stillage (whole residue from distillation of alcohol from fermented mashes. Thin stillage is screened.) Water may be used when stillage is unavailable.

Mix thoroughly with propeller.

Adjust pH to 4.8 with *N* sulfuric acid if necessary.

Make volume up to 1500 cc. with tap water.

Mix well by pouring back and forth from graduate to beaker once or twice.

Check pH; if above 4.85 set back to 4.8 with a few drops of 4 *N* sulfuric acid.

Add 7 cc. of yeast (2 per cent of total mash volume) to each sterile, tared fermenter.

Measure mash into fermenters as follows: Rinse dry sterile 500-cc. graduate with 50 cc. of mash. Measure out 350 cc. of mash and pour into fermenter (Figure 1), allowing to drain until there is only 1 drop every 2 seconds.

Fill three fermenters from each cook as described above. Use a different graduate for each cook. Mix mash in beaker well each time before pouring into cylinder.

Attach trap to fermenter as follows (see Figure 1): Place 2 cc. of mercury, glass beads to a depth of about 1.25 cm. (0.5 inch) and 50 cc. of distilled water in a 25 × 200 mm. test tube. A glass tube extends to the bottom of this trap and is connected with a one-hole rubber stopper in the fermenter by a short piece of rubber tubing. The connection between trap and fermenter should be sterile.

INCUBATION. It has been the practice to incubate mash fermentations in a constant-temperature water bath (Figure 2). Air incubation may be used, but it is believed that baths are more satisfactory.

Incubate at 72° F. for the first 20 hours.

Incubate at 86° F. for the next 30 hours.

Incubate at 90° F. for the final 16 hours.

At least twice during fermentation, at 20 and 50 hours, shake the fermenter thoroughly by swirling until all mash adhering to the bottom of the flask is in suspension.

Fermentation Analysis

The majority of the work in these laboratories has necessitated only initial final analyses. Periodic analyses may be made, but when maximum accuracy is desired only final analyses should be determined. The individual will vary these procedures to fit his own requirements. These again are critical, since work of this type frequently involves slight differences in yield, which are significant and must be so established. The analyses to be made will depend on the data desired, which can be selected after reading the section on calculations.

INITIAL DATA. *Sample.* The initial data are obtained by the analysis of the mash remaining after the fermenters are set. All analyses are run on the supernatant obtained by centrifuging the mash, with the exception of initial total sugar analyses of flash converted mashes, which are treated as described below. The mash is centrifuged at 2000 r. p. m. for 10 minutes. Filtration through coarse paper may be substituted.

a. pH. Take pH using potentiometer and glass electrode.

b. Titratable acidity. Titrate 10 cc. of centrifuged sample to the phenolphthalein end point with 0.1 *N* sodium hydroxide. Record as cc. of 0.1 *N* sodium hydroxide per 10 cc. of sample.

c. Sugar concentration.

1. Maltose. Determine reducing sugar in centrifuged sample of mash by the method of Stiles, Peterson, and Fred (*2*), (20 minutes' oxidation and employ correction factor determined for reagent on maltose).

2. Total sugar, flash converted mashes. As soon as fermenters are set, weigh 100 cc. of excess mash into small Erlenmeyer flask. Adjust pH to 5.4 to 5.5 with a drop of concentrated sodium hydroxide and complete conversion by holding in the water bath to 145° to 150° F. for 1 hour. Shake every 5 minutes, being careful not to splash mash high on the side of the flask. Adjust to initial weight with water and centrifuge sample. Hydrolyze exactly 5-cc. sample with 5 cc. of 1.38 *N* hydrochloric

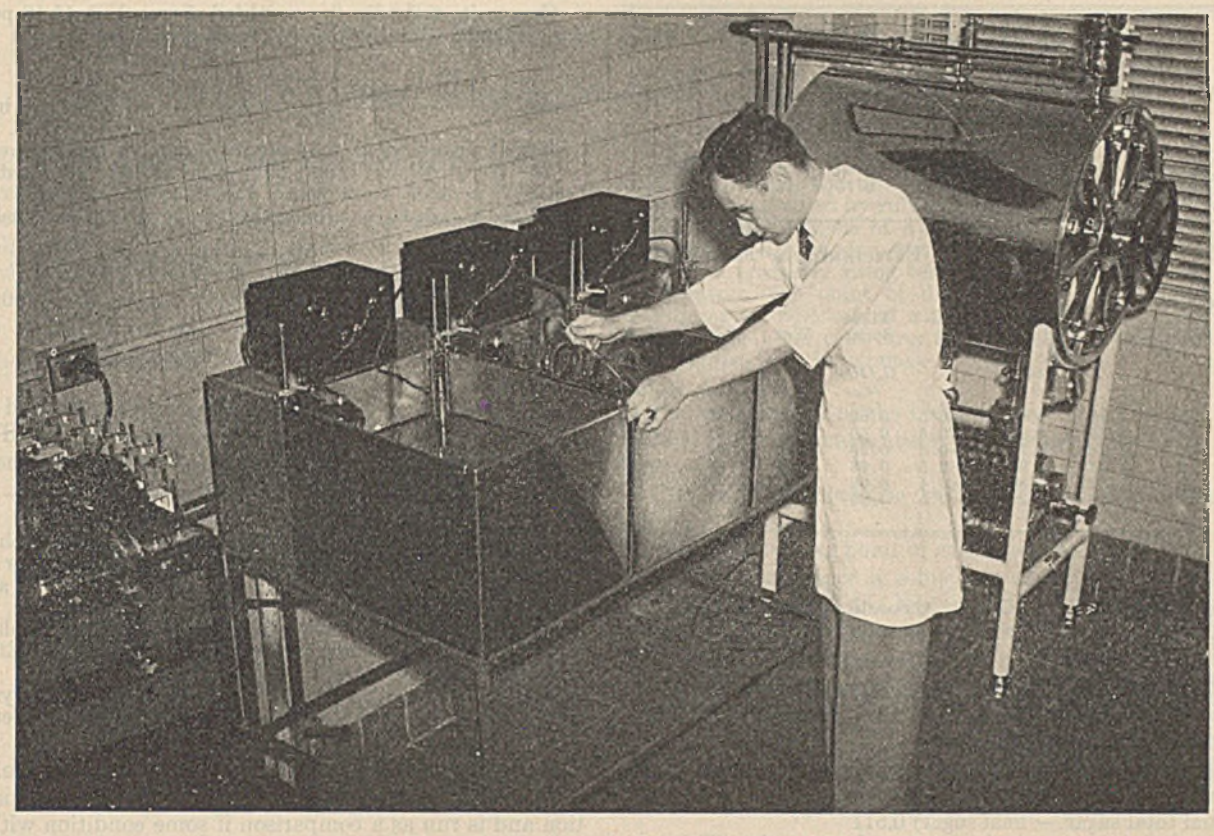

FIGURE 2. LABORATORY WATER BATHS

Equipped with agitators, thermoregulators, heating units, and cooling coils for accurate temperature control

acid by boiling for 2.5 hours, then run sugar by method of Stiles, Peterson, and Fred.

3. Total sugar, 60-minute converted mashes. Centrifuge or filter sample of excess fermenter mash. Hydrolyze with hydrochloric acid as above and run sugar.

d. Balling. Determine on the undiluted supernatant with an 8° to 16° Balling hydrometer.

FINAL DATA. *Preparation of Sample.* Mix the fermented mash thoroughly, weigh the fermenter, and calculate the weight of mash. Weigh exactly one half of the mash into a 1-liter distillation flask and add 50 per cent of the water in the trap to this sample, which is used for whole mash alcohol analyses for proof gallon per bushel yield calculations as follows (proof gallon per bushel is a term used to denote gallons of 100° proof alcohol yield from 1 bushel of grain. In distilling practice corn, rye, wheat, and malt are measured in 56-pound bushels. The bushel is measured on an air-dry basis unless otherwise stated.)

a. Distill into a 100-cc. volumetric flask with 5 cc. of water in the bottom. The condenser should be provided with a tip so that all vapors pass through the water in the receiver. (Receiver should be immersed in an ice bath.) Distill up to neck of volumetric receiving flask.

b. Warm distillate to room temperature, make up to volume, and determine alcohol with the immersion refractometer (usually used at 17.5° C.).

Centrifuge the remainder of the mash at 2000 r. p. m. for 10 minutes and analyze the supernatant as follows:

a. pH, as initial.

b. Titratable acidity, as initial.

c. Balling. Final Ballings should be determined with a Balling hydrometer, range −2.0° to +2.0°, of such size that it may be used in a 50-cc. graduate for convenience.

d. Final sugar. Place a 5-cc. sample in a 25 × 200 mm. Pyrex test tube, add 5 cc. of 1.38 *N* hydrochloric acid, and hydrolyze the sample in a boiling water bath for 2.5 hours. Determine the sugar content, calculated as glucose, by the method of Stiles, Peterson, and Fred (*2*).

e. Alcohol for efficiency calculation by Method B. Pipet 50 cc. (volumetric pipet) into distillation flask, add enough 0.5 *N* sodium hydroxide calculated from titratable acidity to neutralize beer, and add 1 cc. of mineral oil to prevent foaming. Distill over 50 per cent of total volume into 50-cc. volumetric flask, make to volume, and determine alcohol as in proof gallon per bushel analysis.

Calculations

1. CALCULATION OF PROOF GALLON PER BUSHEL. Proof gallons per bushel = (per cent by volume of alcohol as read in 100 cc. of distillate) − (initial sugar concentration of yeast mash as grams of maltose per 100 cc. × 0.0208) ×

$$\frac{100 \text{ (cc. of distillate)}}{175 \text{ (cc. of mash distilled)}} \times \text{concentration of mash (gallons of mash per bushel)} \times \frac{2}{100}$$

For calculation of proof gallons per bushel from fermentations made by the process described above, in which the mash concentration is 38 gallons per bushel, the factor is as follows: Proof gallons per bushel = (per cent by volume of alcohol as read) − (initial sugar concentration of yeast mash as grams of maltose per 100 cc. × 0.0208) × 0.4343.

The proof gallons per bushel yield should be expressed on a dry basis for comparison. This is determined by dividing the above figure by the per cent of dry grain mashed.

2. DERIVATION OF CORRECTED FACTOR FOR YEAST MASH IN PROOF GALLONS PER BUSHEL ALCOHOL ANALYSIS.

y = sugar concentration of yeast mash (grams per 100 cc.).

$0.511y$ = grams of absolute alcohol from 100 cc. of yeast mash.

$0.511y \times 0.035$ = grams of absolute alcohol from 3.5 cc. of yeast mash.

$(0.511y \times 0.035)\ 0.92$ = grams of absolute alcohol from 3.5 cc. of yeast mash, assuming 92 per cent efficiency.

$\frac{0.511y \times 0.035 \times 0.92}{0.791}$ = per cent of alcohol in 100 cc. of distillate due to yeast mash.

∴ Correction factor = $0.0208y$.

3. CALCULATIONS OF EFFICIENCY. *Method A* (based on starch analysis). The composite starch content of the grain bill is calculated in pounds per dry bushel of grain by weighted averages.

TABLE II. INITIAL DATA

Cook No.	pH	Balling	Titration of Acid	Initial Sugar	Maltose	Conversion	Moisture of Corn
			Cc. 1 N NaOH / 10 cc.	*G./100 cc.*	*G./100 cc.*	*%*	*%*
1	4.83	11.6	1.82	12.08	8.44	70.6	13.41
2	4.86	11.9	1.88	12.03	8.75	73.5	13.41

TABLE III. EFFICIENCY OF FERMENTER

Cook No.	Fermentation No.	pH	Titration of Acid	Balling	Final Sugar	Wet	Dry	Alcohol	Plant Efficiency	Fermentation Efficiency
			Cc. 1 N NaOH/ 10 cc.		*G./100 cc.*	*Proof gal./bu.*		*% by vol.*	*%*	*%*
1	4	4.18	4.80	−0.5	0.60	5.48	6.32	7.19	92.2	97.0
	12	4.16	4.81	−0.5	0.60	5.47	6.31	7.16	91.7	96.5
	131	4.16	4.70	−0.6	0.59	5.46	6.30	7.17	91.8	96.7
2	10	4.34	3.95	−0.5	0.68	5.54	6.38	7.23	92.9	98.0
	27	4.32	3.82	−0.4	0.68	5.54	6.38	7.24	93.1	98.2
	111	4.36	4.22	−0.5	0.68	5.51	6.35	7.22	92.8	97.9

Pounds of starch × 0.1725 = theoretical proof gallons per bushel (0.1725 is the factor for conversion of pounds of starch to proof gallons of alcohol).

$$\frac{\text{Actual yield (dry basis)}}{\text{theoretical (dry basis)}} \times 100 = \text{per cent efficiency}$$

Method B (based on sugar analysis).

a. Fermentation efficiency (based on sugar fermented)

$$\frac{\text{Actual grams of absolute alcohol obtained}}{(\text{initial total sugar} - \text{final sugar})\ 0.511} \times 100 =$$

per cent fermentation efficiency

b. Plant efficiency (based on total sugar present)

$$\frac{\text{Actual grams of absolute alcohol obtained}}{\text{initial total sugar} \times 0.511} \times 100 =$$

per cent plant efficiency

c. Grams of absolute alcohol per 100 cc. =

per cent of alcohol × 0.791

SAMPLE CALCULATIONS. The calculation of the results of experiments presented in Tables II and III is shown in detail in the following examples:

Example I. Method B was used for calculating efficiencies.

1. Calculation of proof gallons per bushel yield from fermenter No. 4 (Table II).

Initial sugar on yeast mash = 15.60 grams per 100 cc.

Alcohol as read in 100 cc. of distillate distilled from 0.5 volume of mash in fermenter = 12.93 per cent.

$12.93 - (15.60 \times 0.0208) \times 0.4343 =$

5.48 proof gallons per bushel (wet basis)

$$\therefore \frac{5.48}{86.59} \times 100 = 6.32 \text{ proof gallons per bushel (dry basis)}$$

2. Method B. Calculation of efficiency of fermenter No. 4 (Table III).

a. Fermentation efficiency. Per cent of alcohol by volume = 7.19.

$7.19 \times 0.791 = 5.69$ grams of absolute alcohol per 100 cc.

$$\frac{5.69}{(12.08 \times 0.60)\ 0.511} \times 100 = 97.0 \text{ per cent}$$

fermentation efficiency

b. Plant efficiency.

$$\frac{5.69}{12.08 \times 0.511} \times 100 = 92.2 \text{ per cent plant efficiency}$$

Example II. Calculation of Efficiency by Method A. In this case only starch, moisture, and proof gallons per bushel alcohol (whole mash alcohol) data are obtained.

Grain bill, 92.0 per cent corn, 8.0 per cent barley malt

Starch content of corn = 61 per cent (wet basis). Moisture of corn = 11.5 per cent.

Starch content of malt = 51.5 per cent (wet basis). Moisture of malt = 7.0 per cent.

Composite moisture (by weighted average) = 11.18 per cent.

Composite starch content of grain bill in pounds per dry bushel $= \frac{\left(92 \times \frac{61}{0.885}\right) + \left(8 \times \frac{51.5}{0.93}\right)}{100} = 67.91$ (dry basis)

Actual alcohol yield, 5.33 proof gallons per bushel (wet basis) and 6.00 proof gallons per bushel (dry basis). $67.91 \times 56 \times 0.1725 = 6.56$ theoretical proof gallons per bushel.

$$\text{Per cent efficiency} = \frac{6.00}{6.56} \times 100 = 91.4$$

Discussion

Either the complete method or one of the three alternates listed below may be used, dependent on the factor under investigation.

Starch analysis of grain with determination of proof gallons per bushel alcohols, no micro sugars or alcohols. Efficiency is calculated by Method A.

No starch analysis, proof gallons per bushel alcohols determined as well as efficiency alcohols and micro sugars. Efficiency is calculated by Method B.

No proof gallons per bushel alcohols, no starch analysis, and results evaluated only by efficiency as calculated by Method B.

This represents the standard or control procedure. It is used when evaluating grains, yeast strains, or yeast propagation and is run as a comparison if some condition within the procedure itself is under investigation.

Interpretation of Results

As with all new laboratory procedure, the analyst should run a sufficient number of control tests to determine his ability to secure good fermentations and reproducible results before attempting to introduce any variables. The criteria of a good fermentation are as follows:

CORN MASH. *Initial Sugar*, about 12 grams per 100 cc. Low sugar indicates poor cooking or poor grain.

Per Cent Conversion, about 70. Low per cent conversion may indicate poor cooking or poor grade of malt.

Final pH, above 4.0. A low final pH is the result of bacterial contamination.

Titratable Acidity, 3.5 to 4.5. High acidity, like low pH, indicates bacterial contamination.

Final Balling, 0.3 to 0.7. A high final balling indicates incomplete fermentation.

Proof Gallons per Bushel Yield, 5.2 or above.

Plant Efficiency, 92 per cent to 94 per cent.

WHEAT MASH. The above limits also hold good for wheat mashes, except that final Ballings are often somewhat higher and the final yield is usually lower (5.0 to 5.1 proof gallons per bushel).

RYE MASH. Because of the viscosity of rye mash, the final Balling is unreliable as an index to the completeness of fermentation. The yield from a rye fermentation should be 4.8 proof gallons per bushel or above.

Acknowledgments

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Analysis of Butane-Isobutane Mixture

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MIXTURES of *n*-butane and isobutane, such as might result from isomerization processes or from a preliminary fractional distillation of natural or casinghead gas, are commonly analyzed by low-temperature fractional distillation, as with a Podbielniak still. For accurate results this requires a trained technique, at least 3 hours' time, and at least 10 grams of sample. For control purposes the time factor especially is a detriment.

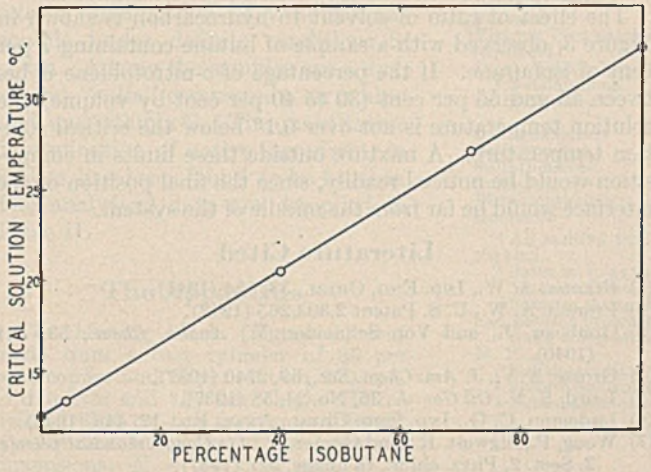

FIGURE 1

Alternative methods proposed are the percentage of a gaseous mixture condensed at dry ice temperatures (*5*), the shape of the pressure-volume isothermal curve (*7*), the index of refraction at −25° C. (*4*), and Raman spectra (*3*). The first two seem crude and inaccurate, the third requires special technique, and the last requires expensive apparatus. Another method tried is the temperature of disappearance of the interface between the liquefied butanes and water (19° C. for *n*-butane, 3° C. for isobutane, *1*), but it was found difficult to observe this temperature with sufficient precision.

The method here proposed (*2*) is the observation of the critical solution temperature of the butane mixture with pure *o*-nitrotoluene.

TABLE I. COMPOSITION OF BUTANES

Critical Solution Temperature	Isobutane Present	Isobutane Found
° C.	%	%
12.5	0	0
13.3	4	4.0
20.5	39.8	39.4
27.0	71.4	71.4
32.8	100	100

Apparatus and Procedure

The apparatus consists of glass tubes or ampoules, 10 to 20 cm. long and 5 to 8 mm. in diameter, sealed at one end and drawn down to a narrow neck at the other, about 1 to 2 mm. in diameter. These are filled about 30 per cent full with *o*-nitrotoluene through a capillary funnel, and immersed in a cold bath of dry ice and acetone (which freezes the *o*-nitrotoluene). A sample of butanes to be analyzed is condensed into a tube through a capillary funnel, filling the tube to about 1.7 times the depth of the solid *o*-nitrotoluene—i. e., the tube is about 75 per cent filled.

The tube is then sealed at the neck with the fine flame of a blast lamp while the tube is still immersed in the cold bath. The tube is attached by rubber bands to a thermometer reading preferably in fractions of a degree, and immersed in a water bath such as a large glass test tube. By frequent tipping of the tube with gradually rising temperature or twirling with falling temperature, the point of disappearance or reappearance of cloudiness can be observed within 0.1° or 0.2° C., corresponding to less than 1 per cent error in percentage of isobutane. The test requires 10 to 15 minutes.

The composition of the butanes is a linear function of the critical solution temperature, as illustrated in Table I and Figure 1.

Other solvents could be used in place of *o*-nitrotoluene, as shown in Table II.

The high freezing point of acetophenone, 19.7° C. makes it less convenient, since it sometimes crystallizes out during a determination; and the higher critical solution temperature with isobutane in the case of the other solvents means a higher pressure and greater risk of breaking the tubes, as well as less precise temperature reading. Moreover, cresol and eugenol are less readily available in pure form.

TABLE II. CRITICAL SOLUTION TEMPERATURE

Solvent	*n*-Butane ° C.	Isobutane ° C.
Acetophenone	10.6	24.5
o-Chloroaniline	29.8	50.5
Cresol (technical)	14.2	45.5
Eugenol	23.0	43.0
Nitrobenzene	40.0	61.0
Aniline (*6*)	84.1	109.0

TABLE III. CRITICAL SOLUTION TEMPERATURES WITH AROMATIC SOLVENTS

Paraffin	*o*-Nitrotoluene ° C.	Nitrobenzene ° C.	Aniline ° C.
Propane	65	None	None
Isobutane	32.8	61	109
n-Butane	12.5	40	84.1
Neopentane	30[a]	54[a]	102[a]
Isopentane	9	32	78.4
n-Pentane	2	24	71.5
Neohexane	11[a]	33[a]	81
n-Hexane	−1	21	69.1

[a] Estimated.

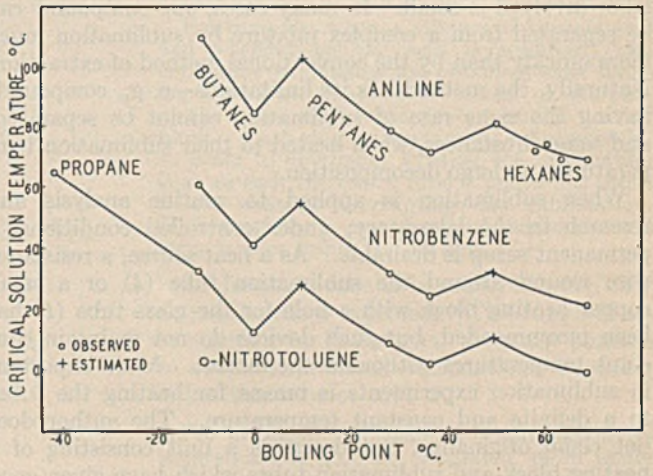

FIGURE 2

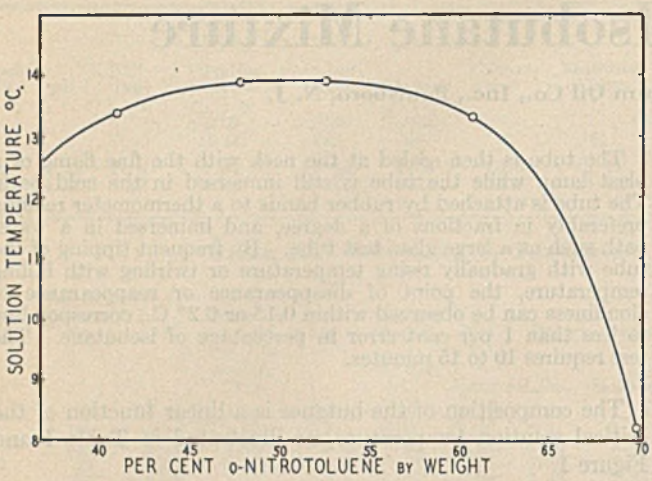

FIGURE 3

Effects of Contaminants

The method is intended for analysis of a wholly butane mixture. Olefins must be absent, since a small percentage would lower the critical solution temperature considerably. This condition is met readily as isomerization catalysts destroy any olefin gas present. Presence of water in the sample is objectionable—it freezes and plugs up the capillary funnel, but it does not affect the critical solution temperature, probably because it is so slightly miscible with either the nitrotoluene or the butane. It can be removed with a calcium chloride drying tube in the gas line before condensation.

The presence of other paraffins affects the determination to some extent, as illustrated in Table III and Figure 2.

Thus the presence of 1 per cent propane in the "butane mixture" would cause an error of about 2.6 per cent isobutane too high. One per cent of isopentane would make the percentage of isobutane only 0.2 per cent too low, and a similar amount of *n*-pentane about 0.5 per cent too low. If the amounts of these paraffin impurities are sufficient to interfere with the determination, it is probably necessary to run a distillation analysis. Table III and Figure 2 include the other two solvents to show the remarkable parallelism for different aromatic solvents. Propane is omitted in those cases because its critical temperature is reached without mixing with aniline (*1*) or nitrobenzene. The aniline critical solution temperature of neopentane was estimated in a previous paper (*1*), and the other estimates were made by paralleling those with aniline.

The effect of ratio of solvent to hydrocarbon is shown in Figure 3, observed with a sample of butane containing 7 per cent of isobutane. If the percentage of *o*-nitrotoluene is between 45 and 55 per cent (30 to 40 per cent by volume) the solution temperature is not over 0.1° below the critical solution temperature. A mixture outside these limits in composition would be noticed readily, since the final position of the interface would be far from the middle of the system.

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Vacuum Sublimation

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APPARATUS for sublimation on a macro scale is seldom encountered in chemical laboratories. Almost all sublimation apparatus described in the literature is designed for micro work (*2*, *3*). Sublimation in vacuo is an excellent method for purifying compounds and is worthy of greater consideration. The advantage of this method over crystallization is that the loss of material is negligible, the sublimed compound is dry and free from solvents, and the actual labor involved is small. In many cases, one compound can be separated from a complex mixture by sublimation much more quickly than by the conventional method of extraction. Naturally, the method has its limitations—e. g., compounds having the same rate of sublimation cannot be separated, and some substances, when heated to their sublimation temperatures, undergo decomposition.

When sublimation is applied to routine analysis and research in the laboratory, under controlled conditions, a permanent setup is desirable. As a heat source, a resistance wire wound around the sublimation tube (*4*) or a small copper heating block with a hole for the glass tube (*1*) has been recommended, but such devices do not maintain constant temperatures without a thermostat. Most important in sublimation experiments is means for heating the tubes to a definite and constant temperature. The author does not claim originality, but describes a unit consisting of a heating block and sublimation tubes which have given good service in this laboratory.

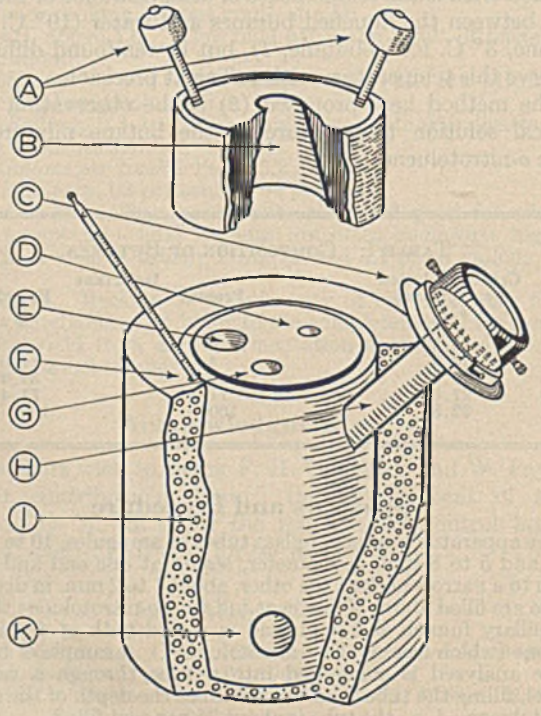

FIGURE 1. HEATING BLOCK

Originally, a heating block to accommodate but one size of tube was used, but later an improved block was constructed for a range from room temperature to 275° C. To enhance the utility of this block, three holes were drilled for three different sizes of tubes, and an auxiliary top was provided, so that 25-ml. and 50-ml. Erlenmeyer flasks could be heated to specified temperatures. It can also be used as a constant heat source for laboratory apparatus.

Two tables show the application of vacuum sublimation. In Table I, the rate of sublimation of various compounds under definite conditions is given. Among the samples are a dye, several "sulfa" compounds, an amino acid, and vitamin C. The quantitative determination of active ingredients in tablets has been found to be feasible. The analytical data are compiled in Table II.

TABLE I. RATE OF SUBLIMATION

Compound (0.200 Gram)	Temperature ° C.	Pressure Microns	Time Hours	Weight Gram	Sublimate Melting point[a] ° C.
Salol	35	9	6	0.192	41.7– 42.2
Azobenzene	40	9	2	0.155	67.5– 68.5
8-Hydroxyquinoline	40	8	1	0.154	73.5– 74.2
2-Methyl-1,4-naphthoquinone	60	7	2	0.184	105.4–106.3
	80	8	0.5	0.189	
2-Hydroxy-3-methyl-1,4-naphthoquinone	80	11	3	0.191	172.6–172.9
Nicotinamide	80	6	5	0.183	128.2–129.9
Pyramidon	90	15	1.5	0.200	106.0–106.5
Acetylsalicylic acid	100	10	1	0.198	130.4–131.5[b]
Phenacetin	100	13	1.5	0.167	134.1–134.8
Caffeine	120	8	1	0.167	236.2–236.8
1-Hydroxyanthraquinone	130	9	0.33	0.165	194.4–195.2
2-Hydroxyanthraquinone	130	7	24	0.063	310.5–311.1
1,8-Dihydroxyanthraquinone or Istin	130	10	1.5	0.171	193.6–194.1
Saccharin	130	10	2	0.195	225.2–227.8
Phenobarbital or 5-ethyl-5-phenyl-barbituric acid	140	9	1.5	0.199	175.7–176.3
Pentacrythrite	150	10	3	0.193	256.4–260.6
Fluoran	150	12	1.5	0.181	183.8–184.3
Vitamin C or ascorbic acid[c]	150	8	24	0.052	186 –187[d]
l-Leucine	150	8	10	0.139	287.0–288.2
D. C. Yellow No. 11 or 2-(2-quinolyl)-1,3-indandione	150	5	20	0.189	242.3–242.8
Sulfanilamide[e]	120	4	24	0.170	164.6–165.2
	150	5	2	0.199	
Sulfaguanidine[f]	150	8	24	0.001	183.6–185.0
Sulfapyridine	150	6	10	0.108	190.3–191.1
Sulfathiazole[g]	150	7	24	0.015	192.1–198.7
Sulfadiazine[h]	150	6	24	0.038	252.2–252.8
Phenolphthalein	180	7	24	0.072	260.6–261.5
Quercetin	200	7	16	0.042	317.4–317.9

[a] All melting points are corrected and were determined in a Hershberg precision melting point apparatus.
[b] Same m. p. as that obtained in same bath on a sample of pure acetylsalicylic acid.
[c] Vitamin C courtesy of Chas. Pfizer & Co., Inc.
[d] Same m. p. as that obtained in same bath on a sample of pure ascorbic acid. Nonsublimed part had become brownish and melted 1° lower.
[e] Sulfa compounds and tablets courtesy of Maurice E. Avery, Lederle Laboratories, Pearl River, N. Y.
[f] Sulfaguanidine charged melted at 187.6–188.7°, nonsublimed part at 189.6–190.5°.
[g] Sulfathiazole charged melted at 200.0–200.7°, nonsublimed part at 199.9–201.0°.
[h] Sulfadiazine charged melted at 254.5–254.8°, nonsublimed part at 253.9–254.5°.

The Apparatus

The heating block (Figure 1) was made from a cast cylinder of 85 per cent copper and 15 per cent tin, 100 mm. in diameter and 150 mm. high. Three holes drilled on top for three different sizes of test tubes are of the following dimensions: $E = 23 \times 90$ mm., $G = 14 \times 80$ mm., and $D = 11 \times 62$ mm. Thermometer well F, 8×82 mm., is inclined towards the center. A hole with an extension tube, H, 19 mm. in inside diameter, is likewise inclined towards the back, leaving the front free for working. H is threaded on the main block and takes the thermostat, C. Hole K, 20×70 mm., is for the heating unit.

To enable one to heat 25-ml. and 50-ml. Erlenmeyers, an auxiliary top was added, 100 mm. in diameter and 55 mm. high. This top is recessed to fit over the main block and by means of two wooden knobs, A, can be removed easily. Conical hole B has a top diameter of 25 mm. and a bottom diameter of 52 mm.

The block is lagged on the bottom and on the sides by a 30-mm. layer of magnesia, I. The side of the auxiliary block is protected by a layer of asbestos paper.

The electrical parts consist of a G-E cartridge-type heater, 200-watt, 115-volt, No. 151-H, 1.88 cm. (0.75 inch) in diameter and 5.9 cm. (2.375 inches) long, fitting into K; a Quick-Set bimetal thermoregulator, C, range 25° to 275° C. (No. 4-239, American Instrument Co., Silver Springs, Md.) fits into tube H, which is 205 mm. deep; a relay, 115-volt (No. ABYT 8, Struthers, Dunn, Inc., Philadelphia, Penna.), is placed in a wooden box and provided with the necessary electrical connections to the heating unit and the thermoregulator.

Table III shows the performance of the heating block.

The sublimation tubes are shown in Figure 2. Tube B is air-cooled; A and C have an inner water-cooled condenser on which the sublimate collects. The tubes fit into hole E (Figure 1).

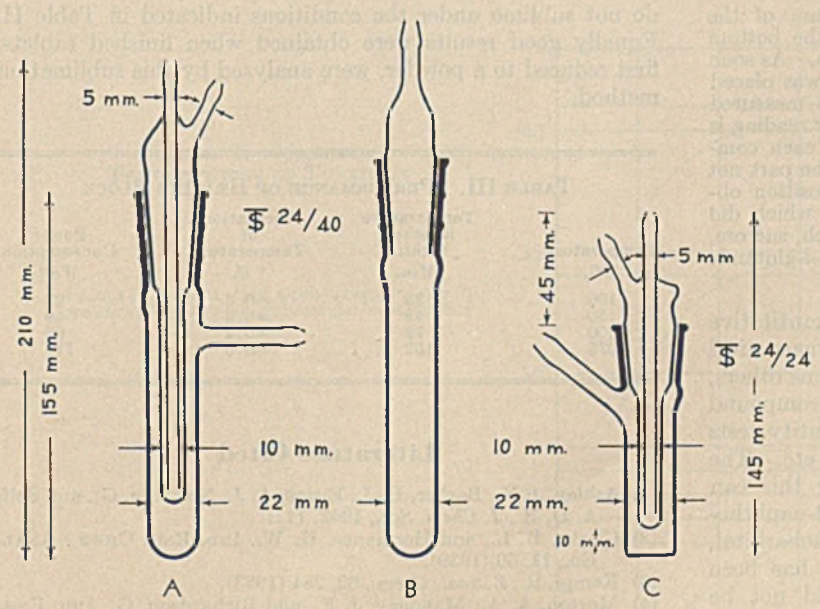

FIGURE 2. SUBLIMATION TUBES

Method

The air-cooled sublimation tube, B, is used mainly in cases where two sublimates are expected.

For example, when a mixture of benzoic acid and *o*-benzoyl-benzoic acid is sublimed, two crystalline sublimates are obtained, each forming a separate band on the cooler or the part of the tube which extends out of the heating block. Each ring is then scraped out separately. In order to determine whether the sublimation is at an end, the entire tube is pulled out about 1 cm. without interrupting the sublimation. If incomplete, a new band of sublimate will form farther inside the old ring.

Sublimation tube A is preferred when only one sublimate is expected. This tube will accommodate up to 2 grams. It is also possible to conduct fractional sublimations by using either this tube or tube C.

For example, a mixture of 1- and 2-hydroxyanthraquinone can be separated more easily and with practically no loss of material by first keeping the tube at 100° C. and 10 microns.

TABLE II. ANALYTICAL DATA

Composition of Sample[a]	Weight of Sample	Sublimation			Sublimate			
		Temperature	Time	Pressure	Weight average of (15 determinations)	Melting Point[a]	Percentage	
							Min.	Max.	Average and mean deviation
	Gram	° *C.*	*Hours*	*Microns*	*Grams*	° *C.*			
50.00% salol, m. p. 41.7–42.2°, and 50.00% phenacetin, m. p. 134.0–134.5°	0.0500	35	2	11	0.0248	41.1– 41.8	48.0	50.8	49.60 ± 0.56
		120	0.25	13	0.0252	132.2–134.2	50.0	52.0	50.41 ± 0.28
2.22% 2-methyl-1,4-naphthoquinone, m. p. 104.6–106.1° (also lactose, calcium gluconate, starch, sucrose, and small quantities of talcum and magnesium stearate)	0.5000	80	0.5	14	0.0107	104.8–105.8	2.04	2.26	2.14 ± 0.05
84.29% acetylsalicylic acid and starch	0.0500	110	0.33	13	0.0422	132 –133[b]	83.8	85.2	84.40 ± 0.34
33.31% nicotinamide, m. p. 128.8–129.4° (also lactose, starch, sucrose, and small quantities of talcum and magnesium stearate)	0.0500	110	0.33	11	0.0169	128.5–129.0	33.0	34.2	33.77 ± 0.30
75.00% phenacetin, m. p. 134.2–134.8° (also sucrose, starch, and small quantities of talcum and magnesium stearate)	0.0500	120	0.5	10	0.0376	133.9–134.6	74.6	76.0	75.25 ± 0.40
46.29% phenobarbituric acid, m. p. 175.4–176.4°, and 1.46% stearic acid and starch	0.1000	150	0.67	11	0.0482	173.6–175.1	46.8	49.5	48.17 ± 0.57
81.20% sulfanilamide, m. p. 164.4–164.8° (also starch, sucrose, and small quantities of talcum and magnesium stearate)	0.1000	155	3	10	0.0815	164.3–164.8	80.7	82.2	81.56 ± 0.31

[a] All melting points are corrected.
[b] Same m. p. as that obtained in same bath on a sample of pure acetylsalicylic acid.

The sublimate will be the more volatile 1-hydroxyanthraquinone. The experiment is now interrupted and the first sublimate is collected. The temperature of the heating block is then raised to 180°, or a second heating block previously heated to this temperature is used, and the sublimation is continued. The sublimate which now forms is the 2-isomer. The separation of the two isomers by this method is much sharper than by fractional crystallization.

Another example of fractional sublimation, the mixture of salol and phenacetin, will be found in Table II. Salol was first sublimed from the mixture at 35° C. and then the phenacetin was sublimed at 120°.

The second water-cooled tube, *C*, is used for smaller samples and mainly for quantitative determinations.

It has a flat bottom, so that the substance is exposed in a thin layer. The distance between the bottom and the condenser tip is but 10 mm.; therefore, the vapors have but a small distance to travel. The inside condenser weighs 23 grams and is small enough to be hung up on a balance arm by means of a wire attached to the constriction. The sublimate will collect on the tip of the condenser and will adhere firmly, as was observed by Kempf (*3*). There need be no fear that the sublimate will fall off when handling the condenser. When the clean ground joints are lubricated with Cenco Vacu-Seal, they will never stick.

To illustrate the variation in the rates of sublimation, data on twenty-seven organic compounds are shown in Table I.

The figures were obtained as follows: 0.200 gram of the compound (purest grade available) was placed on the bottom of tube *A* and then connected with the vacuum pump. As soon as the necessary low pressure was reached, the tube was placed in hole *E* of the heating block. The pressure was measured every hour or more on a McLeod gage. The average reading is shown in Tables I and II. The melting points of each compound before sublimation, of the sublimate, and of the part not sublimed, were determined. There was no decomposition observed except in the cases mentioned. Substances which did not sublime at 150° C. and 10 microns were: starch, sucrose, lactose, talcum, magnesium stearate, l-cystine, and *d*-glutamic acid.

Sublimation is used in this laboratory for the quantitative determination of active ingredients in pharmaceutical tablets. The method may not be so accurate as some others, but it is time-saving in many instances, and the compound is obtained in dry and pure form suitable for identity tests such as mixed melting point determinations, etc. The results are slightly high, but no explanation for this can be advanced now. The estimation of 2-methyl-1,4-naphthoquinone, acetylsalicylic acid, phenacetin, phenobarbital, nicotinamide, salol, and sulfanilamide in tablets has been found to be practical. The same method could not be applied to sulfapyridine, sulfaguanidine, sulfathiazole, and sulfadiazine tablets, because these four sulfa compounds do not sublime to any extent below 160° C. at 10 microns pressure; and above that temperature, some of the other tablet constituents decompose slowly. The data in Table II were obtained as follows:

A mixture containing the same percentage of ingredients as in commercial tablets was prepared on an analytical balance. A weighed sample of from 0.05 to 0.50 gram was placed on the flat bottom of tube *C* and was spread evenly by tapping. The temperature of the cooling water varied from 9° to 17° C. The time required for complete sublimation of the compound in question from the mixture had previously been determined, and in the actual quantitative determination, the time was lengthened by one third to assure complete sublimation. At the end of the procedure, the condenser was removed, most of the water was blown out of the condenser, the two inlet tubes were stoppered, and the sealing wax was wiped from the ground surface. The condenser with the adhering sublimate was weighed, the sublimate then scraped off, the tube tip wiped clean with a solvent, and the condenser minus the sublimate weighed again.

The phenobarbital tablets contain 1.46 per cent of stearic acid; therefore the sublimate will be a mixture of 97 per cent phenobarbital and 3 per cent of stearic acid. In this mixture the phenobarbital may be estimated by one of the standard methods. Other common tablet ingredients like sucrose, lactose, starch, talcum, and magnesium stearate do not sublime under the conditions indicated in Table II. Equally good results were obtained when finished tablets, first reduced to a powder, were analyzed by this sublimation method.

TABLE III. PERFORMANCE OF HEATING BLOCK

Temperature	Temperature Reached within:	Fluctuations of Temperature	Power Consumption
° *C.*	*Min.*	° *C.*	*Watts*
100	28	±0.3	23
150	49	±0.3	46
200	72	±0.4	69
275	132	±0.5	114

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Estimation of Water in Alcohol with Aid of Dicyclohexyl

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Water content in otherwise pure ethyl alcohol of nearly absolute grade may be determined by measurement of critical solution temperature in the system alcohol-water-dicyclohexyl. The "dicyclohexyl point", easily obtained in a few minutes without necessity of standard solutions or special apparatus, is referred to a graph for percentage value.

THE critical solution temperature of a kerosene-alcohol mixture serves as a remarkably precise index of water content, as reported by Crismer (*3*), Andrews (*2*), and the Bureau of Standards (*5*). Each lot of kerosene, however, must be calibrated by a laborious process. The method is, therefore, of little use to the majority of laboratory workers requiring only occasional determinations of water in alcohol. A single substance, similar in physical properties to kerosene but which could be calibrated once for all, would be a desirable substitute. For this purpose a paraffin hydrocarbon with molecular weight of about 170 would be ideal—for example, a dodecane. Unfortunately, pure open-chain paraffins of such high molecular weight are available only as costly academic curiosities.

A new solution of this problem has become possible through the commercial appearance of dicyclohexyl (bicyclohexyl, dodecahydrodiphenyl). The system ethanol-dicyclohexyl has the convenient critical solution temperature of 23.4° C., with an elevation of 18° for the first 1 per cent of water added to the alcohol so tested. Provided any given alcohol preparation and the dicyclohexyl are mixed in or near the ratio of critical composition during calibration, no great precision in measurement of volumes is required in subsequent determinations, as shown in Figure 1. Since the main aim of this work is to test alcohol that is nearly absolute, the simple volume ratio of 1 to 2 was chosen for the final graph of Figure 2.

Sources of Error

The shift of peak of the miscibility curve toward the dicyclohexyl axis with increase of water content calls for increasing care in measurement of volume, and the probable error grows. Since the precise determination of water in alcohol over the range 96 to 99 per cent (by weight) seems to be an unimportant problem, no attempt is made here to complicate matters by reporting data at other volume ratios than the uniform 1 to 2 value adopted. Determinations below 99 per cent are thus only approximate.

Unfortunately, mixtures of dicyclohexyl and alcohol, either of the critical composition or nearly that ratio, markedly display the phenomenon of critical opalescence (*8*) at temperatures just above the maximum temperature of genuine turbidity. This cuts down slightly the extreme precision characteristic of the Crismer technique using kerosene. With dicyclohexyl and a good thermometer it is nevertheless easy to distinguish alcohol preparations as close to each other, for example, as 99.90 and 99.91 per cent; by the Crismer method, 99.900 and 99.903 per cent.

More important is the problem of purity of the dicyclohexyl. Fortunately, the present industrial product is a synthetic individual of high grade, derived by hydrogenation of diphenyl. Several lots of the hydrocarbon, both "technical" and purified, were tested against a standardized alcohol of 99.9+ per cent grade. No significant difference in critical solution temperature was found in this series; certainly

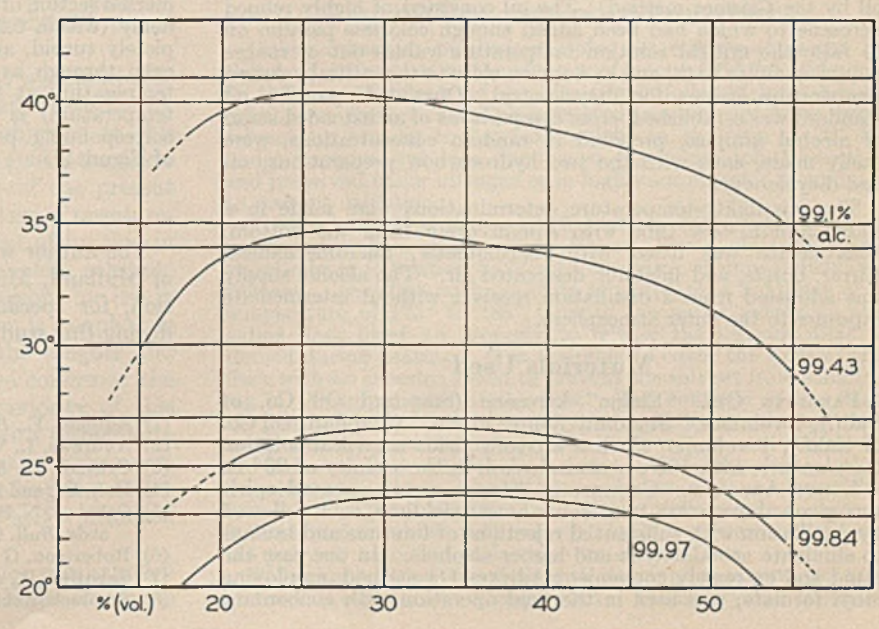

FIGURE 1. MAXIMUM TEMPERATURES OF TURBIDITY

Solutions of aqueous ethyl alcohol preparations in dicyclohexyl. Abscissa, volume per cent alcohol

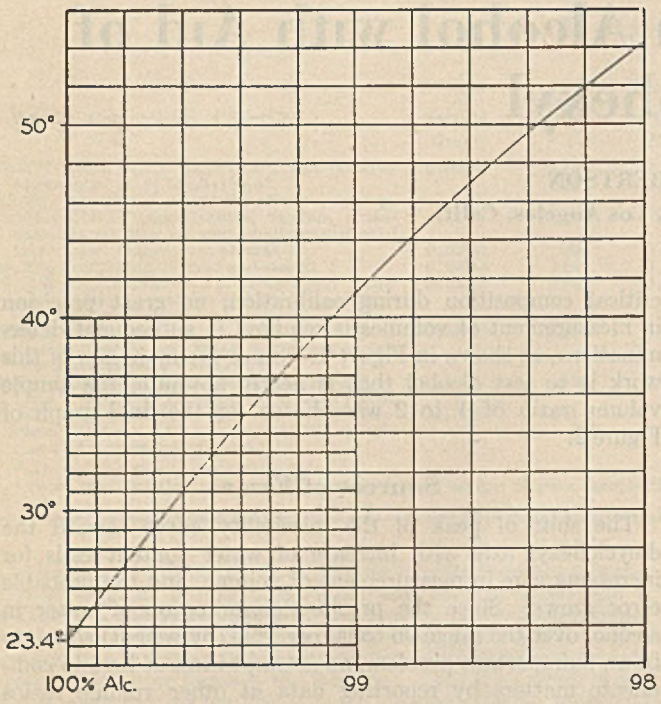

FIGURE 2. PERCENTAGES OF ETHYL ALCOHOL AND CORRESPONDING DICYCLOHEXYL POINTS
1 to 2 alcohol-dicyclohexyl ratio

none equivalent to the difference between 99.90 and 99.91 per cent alcohol.

Water content in the dicyclohexyl is not a serious problem. Simple filtration of the clear liquid product through dry filter paper removes all stray suspended globules of water, and perhaps even some of the extremely small content of dissolved water. The data used in preparing Figure 2 refer to clear samples of the hydrocarbon which have been exposed freely to ordinary atmospheric conditions. When such material was thoroughly dried, no significant change in melting point could be detected with a Beckman thermometer.

A series of purified alcohol preparations (98 to 99.99 per cent), the densities of which at 25.00° were determined with a graduated pycnometer (*6*), was first used to standardize a special paraffin oil by the Crismer method. The oil consisted of highly refined kerosene to which had been added enough colorless paraffin oil to raise the critical solution temperature values out of ranges requiring an ice bath, and to a region close to the critical solution temperature values for dicyclohexyl. Once this paraffin oil standard was established, cross comparisons of an extended series of alcohol samples, prepared in random concentrations, were easily made, each with the two hydrocarbon preparations, oil and dicyclohexyl.

The miscibility-temperature determinations were made in a short 20-mm. test tube with siphon drain from the bottom. This device was fitted with thermometer, micromechanical stirrer, burets, and inlet for desiccated air. The alcohol supply was admitted from a distillation receiver without intermediate exposure to the outer atmosphere.

Materials Used

PARAFFIN OIL. "Elaine" kerosene (Standard Oil Co. of Calif.), 7 volumes. Standard White Oil No. 7 (Standard Oil Co. of Calif.), 1 volume. Such a mixture yields a critical solution temperature, with 100 per cent alcohol, in the vicinity of 23° C.

ETHYL ALCOHOL. Commercial 95 per cent fermented spirit was given three reflux treatments over quicklime, each followed by distillation with substantial rejections of foreruns and tailings to eliminate acetaldehyde and higher alcohols. In one case the rapid and extremely convenient Adickes (*1*) method, employing ethyl formate, was used in the final operation with concordant results. Particular attention was paid to the most highly dehydrated alcohol which was attainable. Six lots, prepared on different days by the two methods, had critical solution temperature values (with the paraffin oil) varying from 23.27° to 23.37°, and densities at 25.00° C. from 0.78506 to 0.78508, there being no consistency nor correlation within those ranges; average 23.31° and 0.78507+. By short extrapolation, 23.2° was taken as the critical solution temperature for 100 per cent alcohol, for which the extremely reliable density value of 0.78506 is known (*5*).

DICYCLOHEXYL. Three lots of industrial origin were investigated:

	Melting Point ° C.
1. Eastman Kodak Co., P4641, merchandise of 1941	3.5
2. Dow Chemical Co., technical product of 1943	3.4
3. Special preparation made by fractionation of 2 through a 30-plate column, followed by recrystallization	3.63

The melting-point value for No. 3 refers to the constant equilibrium temperature of a mush of the hydrocarbon alternately freezing and melting slowly in a bath varied from 3° to 4°, there being from $^1/_3$ to $^2/_3$ of solid present. A National Bureau of Standards certified Beckman thermometer was used, with corrections for setting, certificate, and stem emergence, and the figure 3.63° signifies merely the elevation above the ice point determined just before and after the main experiment.

Product 1 was recrystallized without use of solvent, yielding product 4, a part of which was washed with concentrated sulfuric acid, yielding 5. Both 4 and 5 agreed exactly with 3 in melting point. In view of the constancy of the temperature, it was judged that the theoretical melting point (disappearance of last crystal) is not over 3.65°, and that this value ±0.03° is the melting point of pure dicyclohexyl. Apparently the figures of 4° (*4*) and "above 4°" (*7*) reported for this compound in the literature were not determined with special precision.

Presumably the impurities in any of the products so far encountered are not only of small amount, but of physical nature not entirely foreign to dicyclohexyl itself. Accordingly, the critical solution temperature values obtained with the five preparations are even more closely concordant than the melting points. Thus Nos. 1 and 2 are satisfactory for ordinary accuracy in estimation of water in alcohol.

Dicyclohexyl Point

PROCEDURE. To 2.0 cc. of the alcohol being tested, in a dry 15-mm. test tube, add 4.0 cc. of dicyclohexyl and stir with a dry thermometer. Heat until the mixture becomes a clear solution, and then allow to cool slowly, with continued stirring. As the critical solution temperature is approached, the liquid becomes opalescent, suggestive of very dilute soap solution. It is still clear enough so that the mercury thread in the immersed section of the thermometer is readily distinguished. Suddenly (within 0.2° temperature range) the liquid becomes completely turbid, and the mercury thread is no longer discernible even through as little as 5-mm. thickness of the liquid. The temperature at this stage (approximately the critical solution temperature) is recorded as the dicyclohexyl point, and the corresponding percentage of alcohol is read from the graph of Figure 2.

Acknowledgment

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Unsaturation of Butadiene and Related Polymers

As Determined by Iodine Chloride Addition

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This paper describes procedures which have been developed to determine the unsaturation of various butadiene and related polymers and copolymers, as well as mixed vulcanizates of Buna S and rubber. These methods are based on the use of *p*-dichlorobenzene as a solvent and iodine chloride as the addition agent, following the general technique employed in the standard Kemp-Wijs method for the determination of the unsaturation of natural rubber.

The ratio of butadiene to styrene in copolymers has been calculated from the iodine value and from the carbon-hydrogen ratio; however, the accuracy of these procedures is subject to several variables which are discussed.

Unsaturation data are presented on highly purified emulsion-type polymers of butadiene-isoprene and butadiene-styrene which agree closely with the presence of one double bond for each diolefin molecule present. The reaction rate of Buna S with halogens is shown to agree closely to that of natural rubber hydrocarbon.

SINCE iodine chloride adds quantitatively to the double bonds in natural rubber hydrocarbon (*5*, *6*), it was considered important to study the reaction of various unsaturated synthetic elastomers with this reagent. Recently Cheyney and Kelley (*3*) found that the Wijs reagent reacted so slowly with polybutadiene and its copolymers swollen in chloroform or carbon disulfide, that the addition reaction required 48 hours or longer to approach completion. Butadiene and butadiene-styrene polymers are frequently only partially soluble in chloroform or carbon disulfide and, if soluble, they are partially precipitated by the addition of the glacial acetic acid in the Wijs reagent. This requires that the reaction proceed between the swollen polymer and the iodine chloride solution, which accounts in part for the long period needed to complete the reaction. Other complications, such as emulsion formation and occlusion of iodine in the precipitate during titration of the unreacted iodine chloride, are likely to be involved in the standard Kemp-Wijs procedure (*4*, *5*, *6*) which was developed for natural rubber.

The present investigation was undertaken to overcome these difficulties and to develop a rapid and accurate method which could be applied to various types of synthetic rubber-like polymers. It was also hoped that data obtained on the unsaturation of these polymers would throw some light on their chemical structure as related to polymerization procedure.

A study of numerous solvents showed that *p*-dichlorobenzene heated from 165° to 172° C. was the most satisfactory for general use. Cheyney and Kelley (*3*) objected to this solvent on the ground that cyclization of the synthetics takes place as the result of heating. The results of the present investigation, however, show that very little loss of unsaturation occurs in the polymers during the period of heating in *p*-dichlorobenzene necessary to attain a complete solution. The use of *p*-dichlorobenzene is also advantageous for use in the case of crude natural rubber, since solution is complete in less than one hour, which is important in the tougher and less soluble types. The present authors have confirmed the finding of Blake and Bruce (*1*) on the superiority of this solvent for vulcanized rubber as compared with tetrachloroethane (*5*). Soft vulcanized butadiene-styrene copolymers are also soluble in hot *p*-dichlorobenzene, making it possible to determine the total rubber content of a soft vulcanized mixture of natural and GR S rubber.

In order to avoid or substantially to reduce precipitation upon addition of Wijs solution, the iodine chloride was made up using carbon tetrachloride as a solvent in place of glacial acetic acid. In the case of the butadiene-nitrile copolymers the standard Wijs solution was employed, since the iodine chloride reaction product was more soluble in the presence of the glacial acetic acid.

Emulsions which formed during titration were broken by the addition of 25 cc. of alcohol. In the case of vulcanized GR S tire tread stocks containing channel black, it was found that by omitting the water and adding 75 cc. of alcohol, excellent results were obtained. In this case the presence of the larger quantity of alcohol caused the carbon black to settle rapidly, so the end point could be quickly obtained.

In the case of polybutadiene 0.10-gram samples gave low results, since the excess of iodine chloride is not sufficient to complete the reaction. Sample weights of 0.06 gram were found to give consistent results. In the case of the high-nitrile type of polymer, solution in *p*-dichlorobenzene is difficult; 20 to 60 passes through a clean tight mill before extraction greatly aided solution. This milling has been found to have no significant effect on the iodine value of any of the synthetics or of natural rubber and can be practiced to advantage whenever the polymer requires too long a period to dissolve.

General Recommended Procedure

Unless otherwise purified, samples are acetone-extracted in the standard manner in the absence of strong light for 16 hours. In the case of vulcanized samples this is followed by a 4-hour chloroform extraction. The extracted samples are freed from solvent by heating to constant weight in an oven at 70° C. under vacuum, cut into fine pieces (approximately 15-mesh), and preserved under nitrogen or in high vacuum prior to analysis to prevent oxidation.

The finely divided sample (0.10 gram, or 0.06 gram in case of polybutadiene) is placed in a 500-cc. Pyrex glass-stoppered vapor-release iodination flask with 50 grams of pure *p*-dichlorobenzene. The flask with contents is placed on a hot plate at a temperature of 175° to 185° C. with the vapor release on the iodine flask lined up properly to release the pressure that is formed during heating. It is desirable to cover the sides of the flask with an asbestos shield to prevent the solvent from solidifying in the upper part of the flask. The contents of the flask are gently whirled from time to time to facilitate solution, care being taken to avoid causing particles to adhere and scorch on the sides of flask above the solvent. The time for solution depends upon the nature of the polymer and usually varies from about 20 to 180 minutes. If the time required exceeds 3 hours and a rubber mill is available, it is recommended that the polymer be milled to increase its solution rate.

Following solution, the flask with its contents is removed and allowed to cool to room temperature. Before crystallization of

the *p*-dichlorobenzene is complete the partially solidified solution is liquefied by adding 50 cc. of chloroform. Twenty-five cubic centimeters of iodine chloride in carbon tetrachloride are added from a pipet, using a vacuum to suck the solution up uniformly and very slowly to avoid loss of iodine chloride by evaporation. A thin film of 15 per cent potassium iodide is placed on the stopper of the iodine flask just before closing and the solution allowed to stand for one hour at room temperature in the dark to complete the reaction. Twenty-five cubic centimeters of 15 per cent freshly prepared potassium iodide solution are added, followed by the addition of 50 cc. of distilled water. The excess iodine is immediately titrated with standard 0.1 *N* sodium thiosulfate solution, using freshly prepared starch indicator which is added towards the end of the titration. Twenty-five cubic centimeters of ethyl alcohol are added towards the end of the titration to break the emulsion.

TABLE I. EFFECT OF VARYING EXPERIMENTAL CONDITIONS OF IODINE VALUE OF POLYBUTADIENE

Solvents Employed	Polybutadiene	Heating Period in $C_6H_4Cl_2$	Iodine Chloride Solution Used	Time of Reaction	Iodine Value
	Gram	*Hours*		*Hours*	
75 cc. of CS_2	0.1040	...	Wijs	3	399.0[a]
	0.1034	...	Wijs	24	407.9[a]
75 cc. of $CHCl_3$	0.1031	...	Wijs	3	388.1[a]
	0.1026	...	Wijs	24	406.3[a]
50 grams of $C_6H_4Cl_2$ + 50 cc. of CS_2	0.1201	1.0	CCl_4	1	385.3
	0.1017	1.0	CCl_4	1	413.7
	0.1014	1.0	CCl_4	1	412.6
	0.0824	1.0	CCl_4	1	435.7
	0.0756	1.0	CCl_4	1	436.5
	0.0759	1.0	CCl_4	1	436.1
50 grams of $C_6H_4Cl_2$ + 50 cc. of $CHCl_3$	0.0753	1.0	CCl_4	1	435.0
50 grams of $C_6H_4Cl_2$ + 50 cc. of CS_2	0.0668	1.0	CCl_4	1	438.6
	0.0615	1.0	Wijs	1	409.1
	0.0613	6.0	CCl_4	1	435.9
	0.0604	1.0	CCl_4	1	438.5
	0.0606	1.0	CCl_4	1	440.9
	0.0511	1.0	CCl_4	1	440.1
	0.0510	1.0	CCl_4	1	440.5
	0.0313	1.0	CCl_4	1	441.7
	0.0221	1.0	CCl_4	1	440.4
	0.0218	1.0	CCl_4	1	441.8

[a] Heavy precipitation occurs upon adding Wijs reagent.

The end point is reached when the color change passes from a light brown to a light purple and finally to a colorless solution. When the end point is near, moderate shaking of the solution is necessary after each drop or partial drop of sodium thiosulfate is added. Violent shaking should be avoided to prevent breaking the flask. A blank is carried through all the operations of heating, etc. The difference in cubic centimeters of 0.1 *N* thiosulfate between the blank and the sample titration is used to calculate the iodine value:

$$\text{Iodine value} = \frac{\text{cc. of 0.1 } N \text{ } Na_2S_2O_3 \times 1.2692}{\text{wt. of sample in grams}}$$

Iodine Value of Polybutadiene

The effect of using different solvents on the iodine value of polybutadiene (German sodium Buna "85") under various conditions is shown by Table I. In the experiments where 0.1-gram samples are employed the iodine values are too low because the addition reaction is not complete. Some precipitation occurred in all cases where solution was effected by hot *p*-dichlorobenzene supplemented by carbon disulfide or chloroform and where iodine chloride in carbon tetrachloride was used. Where heavier precipitation occurred using Wijs solution, the increased time of reaction did not overcome the low results. Heating for 6 hours in *p*-dichlorobenzene resulted in about 1 per cent reduction in the iodine value. This shows that the effect on the unsaturation as the result of heating for 1 hour in *p*-dichlorobenzene can be neglected.

Since the excess iodine chloride appeared important, a series of determinations was carried out varying the sample weights. The samples were dissolved in 50 grams of boiling *p*-dichlorobenzene for 1 hour, 50 cc. of carbon disulfide and 25 cc. of 0.2 *N* iodine chloride in carbon tetrachloride were added, and the reaction was carried out for 1 hour at room temperature. The results are plotted in Figure 1 and show that a weight of sample of 0.05 to 0.06 gram is satisfactory. These weights correspond to an excess of iodine chloride of 50 to 60 per cent. About the same excess was found to be necessary to complete the iodine chloride reaction in 1 hour in the case of natural rubber (*6*).

To determine the tendency of polybutadiene to substitute, a series of determinations was carried out at room temperature and at 3° C. with variations in reaction periods from 1 to 24 hours. The sample weight was 0.06 gram and the procedure the same as the other series shown in Figure 1. Figure 1 indicates that substitution takes place slowly even at 3° C. A reaction period of 1 hour therefore appears to be justified, since the addition reaction apparently is completed in this period, provided the proper excess of reagent is employed. The high iodine values obtained by Cheyney and Kelley after a reaction period of 336 hours are undoubtedly due in part to substitution.

The theoretical iodine value for polybutadiene is 469.6. The acetone-extracted sodium butadiene polymer was not a pure hydrocarbon as judged from its combustion analysis. The nonhydrocarbon portion appears to be made up largely of ash and combined oxygen.

Two butadiene and one isoprene emulsion polymers were prepared under the supervision of B. S. Biggs of these laboratories. The latex was coagulated by pouring it into an excess of alcohol and the coagulum was washed in warm 50 per cent alcohol and finally in pure alcohol. It was finally dried to

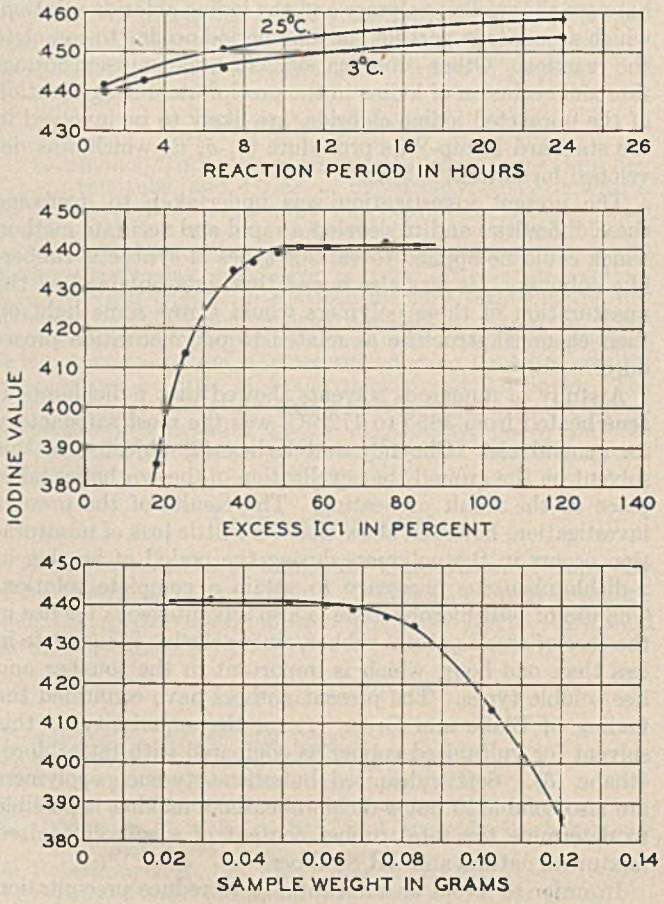

FIGURE 1. IODINE VALUE OF POLYBUTADIENE

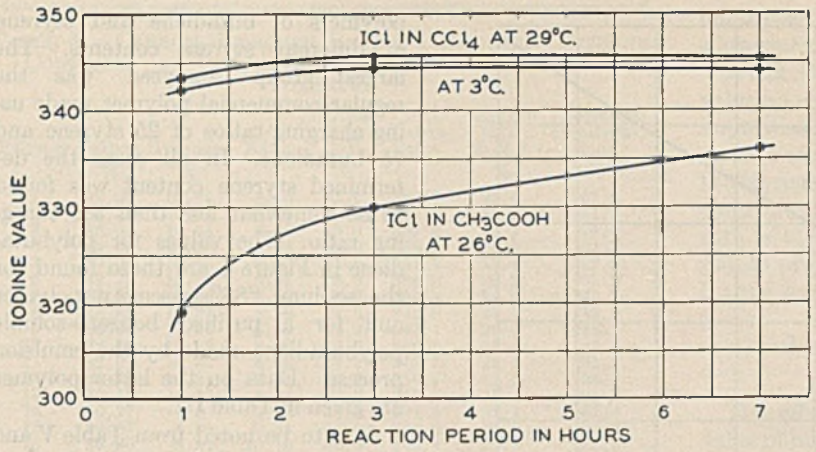

FIGURE 2. IODINE VALUE OF BUTADIENE-STYRENE COPOLYMERS

TABLE II. COMBUSTION ANALYSES AND IODINE VALUES OF BUTADIENE AND ISOPRENE POLYMERS

Polymer	Carbon	Hydrogen	C/H Ratio	Iodine Value	Theoretical Iodine Value
	%	%			%
Polybutadiene (German Na 85)[a]	88.08	10.87	8.10	440.5	93.7
Polybutadiene (emulsion method)	88.86	11.21	7.93	455.1	96.6
Polyisoprene (emulsion method)	...	...	..	369.1	99.0

[a] Acetone extracted before analysis.

constant weight under high vacuum at 60° C. A highly purified lot of isoprene was also polymerized by the soap emulsion method.

The iodine values and analysis of these polymers are given in Table II in comparison with the acetone-extracted German Buna "85" viscosity polymer made by mass polymerization, using sodium as a catalyst. Whereas the German sodium polymer of butadiene has an iodine value somewhat below the theoretical value, the unsaturation of the emulsion polymers agreed fairly closely with theory.

The iodine value of 440 for the extracted sodium Buna "85" is lower than theory, which is 469.6, even when the non-hydrocarbon portion is considered. The unsaturation of sodium Buna "85" is calculated to be 93.69 per cent of theory. This low unsaturation value, together with its low solubility and plasticity, may be taken as evidence of the presence of some type of interlinkage between the polymer chains; however, knowledge of these structural details is lacking. Chain branching, oxygen bridging, or cyclization during polymerization has been suggested to account for the differences in the properties of butadiene and isoprene polymers as compared with natural rubber.

Iodine Value of Butadiene-Styrene Copolymers

The effect of using different solvents and experimental conditions on the iodine value of an acetone-extracted 75/25 butadiene-styrene copolymer is shown in Table III. These results indicate that the use of either chloroform or carbon disulfide to supplement the *p*-dichlorobenzene is satisfactory, provided iodine chloride in carbon tetrachloride is employed in place of Wijs solution. Chloroform, however, is preferred over carbon disulfide because of the objectionable odor and fire hazard of the latter solvent.

Data from Table II, plotted in Figure 2, show evidence of very little substitution in the case of the butadiene-styrene copolymer. Reaction conditions of 1 hour at room temperature appear to be a satisfactory selection when iodine chloride in carbon tetrachloride is employed. The use of Wijs solution gives low results even after a 48-hour reaction period. Under these conditions the iodine values obtained are in agreement with those of Cheyney and Kelley (*3*). The data in Figure 2 show that Wijs solution is unsatisfactory for use with butadiene-styrene copolymers, whereas iodine chloride in carbon tetrachloride appears to meet the necessary requirements. Iodine bromide (Hanus solution) was also tried but was found to be slower than Wijs solution under identical conditions. After 7 hours' reaction period, the Hanus value was 318, in contrast with 336 for the Wijs value for the same period.

Reactivity of Double Bonds in Buna S

Since some of the double bonds in Buna S appear to be located in side-chain vinyl groups as a result of 1,2 addition polymerization of the butadiene, it was thought that this might affect their reactivity as compared with the double bonds in the main chain resulting from 1,4 addition. In order to detect such a difference, the reactivity of Buna S with iodine, iodine chloride, and iodine bromide was compared with rubber under identical conditions. These experiments are outlined in Table IV and the data show that very little difference exists between the reactivity of Buna S and rubber hydrocarbons towards iodine, iodine chloride, or iodine bromide.

Determination of Styrene in Butadiene-Styrene Copolymers

Since no method was available for the determination of the styrene content of GR S rubber or other butadiene-styrene

TABLE III. IODINE NUMBER OF A BUTADIENE-STYRENE COPOLYMER UNDER VARIOUS EXPERIMENTAL CONDITIONS

Solvents Employed	Butadiene-Styrene Copolymer	Heating Period in $C_6H_4Cl_2$	Iodine Chloride Solution Used	Temp. of Reaction	Time of Reaction	Iodine Value
	Gram	*Hours*		° *C.*	*Hours*	
75 cc. of $CHCl_3$	0.10	...	Wijs	20-30	48	334.1[a]
75 cc. of CS_2	0.10	...	Wijs	20-30	48	330.0[a]
50 grams of $C_6H_4Cl_2$ + 50 cc. of CS_2	0.10	1.0	Wijs	26	1	319.3
	0.10	1.0	Wijs	26	3	330.2
	0.10	1.0	Wijs	26	7	336.0
50 grams of $C_6H_4Cl_2$ + 50 cc. of $CHCl_3$	0.10	1.0	Wijs	26	7	335.8
50 grams of $C_6H_4Cl_2$ + 50 cc. of CS_2	0.05	1.0	CCl_4	29	1	343.5
	0.10	1.0	CCl_4	29	1	343.5
50 grams of $C_6H_4Cl_2$ + 50 cc. of $CHCl_3$	0.10	1.0	CCl_4	29	1	344.0
50 grams of $C_6H_4Cl_2$ + 50 cc. CS_2	0.10	6.0	CCl_4	29	1	343.2
	0.10	1.0	CCl_4	29	3	345.4
	0.10	1.0	CCl_4	29	7	345.5
	0.10	1.0	CCl_4	3	1	342.2
	0.10	1.0	CCl_4	3	3	344.0
	0.10	1.0	CCl_4	3	7	343.8

[a] Solid phase present throughout reaction.

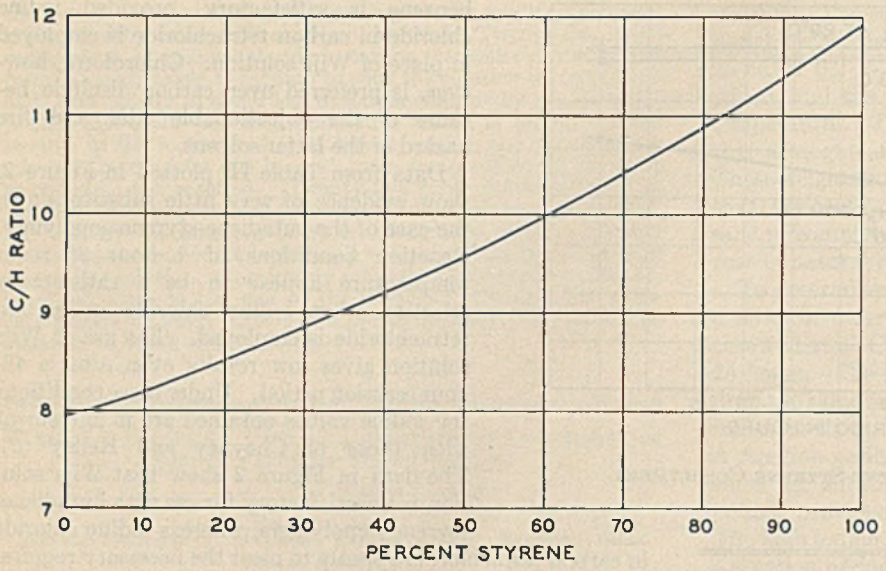

FIGURE 3

TABLE IV. RELATIVE REACTIVITY TOWARDS HALOGENS OF BUNA S AND RUBBER[a]

Polymer	0.2 *N* Addition Agent Added	Reaction Period	Reagent Consumed	Addition Agent Consumed
		Min.	*Cc.*	%
Rubber	25 cc. of I_2 in CCl_4	240	0.70	2.8
Buna S	25 cc. of I_2 in CCl_4	240	0.56	2.3
Rubber	11 cc. of ICl in CCl_4	10	10.56	96.0
Buna S	11 cc. of ICl in CCl_4	10	10.00	91.0
Rubber	5 cc. of ICl in CCl_4	10	4.90	98.0
Buna S	5 cc. of ICl in CCl_4	10	4.88	97.5
Rubber	3 cc. of ICl in CCl_4	60	2.94	98.0
Buna S	3 cc. of ICl in CCl_4	60	2.97	99.0
Rubber	3 cc. of IBr in CCl_4	10	1.90	63.2
Buna S	3 cc. of IBr in CCl_4	10	1.90	63.2

[a] 0.1000 gram of acetone-extracted crepe or Buna S dissolved in 75 cc. of chloroform and reaction carried out in dark at 25° C.

copolymers, the present authors employed combustion analyses, using the carbon-hydrogen ratio to calculate the styrene content. As the styrene content increases from 0 to 100 per cent the carbon-hydrogen ratio will vary from 7.943 to 11.915, as shown in Figure 3. A variation of ±0.2 per cent in carbon will change the carbon-hydrogen ratio in a 21 per cent styrene-79 per cent butadiene copolymer by ±0.017, which is equivalent to ±0.5 per cent styrene. A variation of only 0.023 per cent in the hydrogen content will be equivalent to the same change in carbon-hydrogen ratio and styrene content; therefore, special precautions must be taken in the combustion analyses to avoid errors. The present authors depended upon a carefully conducted micromethod combustion. These analyses were carried out by F. C. Koch of these laboratories. In the case of soap-free polymers, the accuracy is believed to be sufficient to establish the styrene content to within 1 or 2 per cent.

Data giving the variation of iodine value and per cent of theoretical unsaturation are shown in Table V and Figure 4 for copolymers of butadiene and styrene of different styrene contents. The largest group analyzed was the regular commercial polymer made using charging ratios of 25 styrene and 75 butadiene. In all cases the determined styrene content was found to be somewhat less than the charging ratio. The values for polybutadiene in Figure 4 are those found for the sodium "85" viscosity polymer and for a purified benzene-soluble polybutadiene made by the emulsion process. Data on the latter polymer are given in Table III.

It is to be noted from Table V and Figure 4 that the iodine values of butadiene-styrene copolymers are approximately proportional to their styrene content. The iodine value can therefore be employed to calculate the styrene content. However, variations in the amount of unsaturation lost during polymerization and the presence of soap and other impurities will influence the accuracy of such a procedure. The commercial emulsion polymers contain variable amounts of inorganic matter, their ash contents usually varying from 0.3 to 1.2 per cent. Combustion analyses indicate that they contain some combined oxygen, in some cases possibly as much as 0.2 to 0.5 per cent.

The data in Table V on purified polymers are evidence that there is very little loss in unsaturation in the butadiene beyond the one double bond per butadiene molecule which would result from linear chain polymerization. The data on the per cent of theoretical unsaturation for the butadiene present in the acetone-extracted commercial polymers are undoubtedly influenced somewhat by errors in the results for styrene content based on microcombustion analyses. These data indicate that 2 to 10 per cent of the theoretical unsaturation of the butadiene after one of its double bonds enters the chain is used up during the polymerization process. However, it was found, as shown in Table V, that by careful fractionation of a benzene solution of a soap-free benzene-soluble type polymer the unsaturations of the higher molecular fractions are close to the theoretical value. The whole polymer, No. 12 in Table V, was unsaturated to 98.3 per cent of theory. In this case the styrene content was determined by an interferometer method recently developed by W. O. Baker and

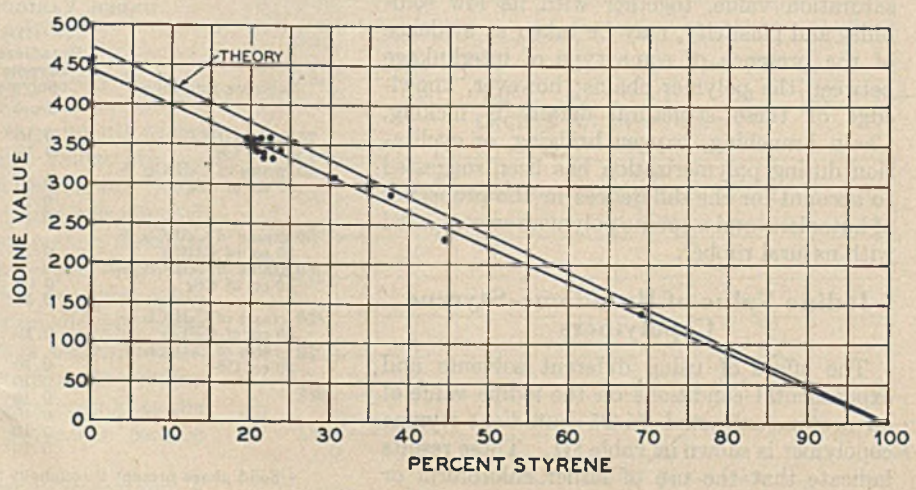

FIGURE 4

TABLE V. CARBON-HYDROGEN RATIOS, CALCULATED STYRENE CONTENTS, AND IODINE VALUES OF BUTADIENE-STYRENE POLYMERS

Polymer	Carbon	Hydrogen	C/H Ratio	Styrene from C/H	Iodine Value	Styrene from I. V.[a]	Theoretical Unsaturation[b]
	%	%		%		%	%
1	89.00	10.39	8.56	21.0	360.0	23.4	97.0
2	88.28	10.34	8.54	20.8	342.0	27.2	91.9
3	88.65	10.44	8.50	19.0	357.8	23.9	94.0
4	89.00	10.36	8.59	22.0	338.2	28.0	92.3
5	89.04	10.40	8.56	21.0	341.2	27.4	92.0
6	89.70	10.42	8.61	22.5	334.0	28.9	91.8
7	88.08	10.36	8.50	19.0	352.8	24.9	92.7
8	88.85	10.37	8.58	21.6	342.0	27.2	92.9
9	88.35	10.31	8.56	21.0	343.1	27.0	92.2
10	88.38	10.28	8.60	22.2	340.0	27.6	93.0
11	89.09	10.32	8.63	23.2	343.5	26.9	95.2
12	88.52	10.29	8.61	22.7	356.7	24.1	98.3
13	89.52	10.13	8.84	29.6	313.4	33.4	94.8
14	89.27	9.83	9.08	36.4	291.1	38.1	97.4
15	89.47	9.59	9.33	44.0	234.0	50.0	89.0
16	89.80	8.81	10.19	66.0	142.4	69.8	89.2
				Purified Polymers			
P-1[c]	89.24	10.28	8.68	25.0	353.5	24.8	100.4
P-2[c]	89.48	10.23	8.73	26.3	346.5	26.3	100.1
P-3[c]	89.34	10.25	8.72	25.8	353.3	24.8	101.3
P-4[d]	89.80	10.42	8.62	23.0	356.1	24.2	98.5
P-5[d]	89.87	10.32	8.61	22.7	359.3	23.6	98.7
P-6[d]	89.33	10.35	8.63	23.2	359.1	23.6	99.5
P-7[d]	89.26	10.25	8.70	25.3	359.8	23.5	100.3
P-8[d]	89.27	10.29	8.67	24.5	359.6	23.5	100.1
P-9[d]	88.76	10.35	8.58	21.9	355.5	24.4	97.0
P-10[d]	88.06	10.30	8.55	20.7	352.1	25.0	94.8

[a] $\%\ \text{styrene} = 100 - \left(\frac{\text{iodine value}}{469.6} \times 100\right)$.

[b] Calculated by employing 469.6 as the theoretical iodine value for polybutadiene—i. e., $\frac{\text{iodine value of polymer} \times 100}{\text{wt. proportion of butadiene in polymer} \times 469.6} = \%$ of theoretical unsaturation.

[c] Prepared by coagulation of polymer emulsion with alcohol and washing with alcohol. Addition of antioxidant was omitted.

[d] Prepared from benzene-soluble soap-free commercial polymer No. 12 by fractional coagulation from dilute benzene solution upon addition of methyl alcohol. These fractions represent about 90 per cent of the polymer and are given in order of decreasing molecular weights.

J. H. Heiss of these laboratories, giving a styrene content of 23.4 per cent and a theoretical unsaturation of 99.1 per cent.

In the case of benzene-soluble emulsion-type polymers purified by coagulation and washing with ethyl alcohol, the data in Table V show that very little loss in the unsaturation of butadiene beyond one of its double bonds occurs during polymerization. In this case the styrene contents given were also checked by the above-mentioned interferometer method. The iodine values of the alcohol precipitated and purified emulsion polymers from pure butadiene and specially purified isoprene also showed close agreement with theory.

Since soap is not removed by acetone extraction, its presence in the polymer will reduce the iodine value. The soap content is generally less than 1 per cent, but in some cases more may be present. In a special case where a polymer was found to contain 5.25 per cent soluble soap calculated as sodium stearate the iodine value of the acetone-extracted residue was found to be 345.1. When the acetone-extracted polymer was refluxed with a mixture of 2 volumes of benzene and 1 of alcohol for 1 hour to remove this soap, the iodine value of the polymer increased to 350.6. This is a lower value than expected, which can only be accounted for on the basis that the soap acids were unsaturated.

The presence of soap will lower the carbon-hydrogen ratio, resulting in calculated styrene contents which are too low. Consequently the calculated per cent of theoretical unsaturation will be too low. The presence of soap and other impurities will reduce the iodine value, which also results in a lower calculated theoretical unsaturation.

When the styrene content is calculated from the iodine value as shown in Table III, it is seen that the presence of impurities giving a low iodine value will result in a calculated styrene content which is too high. This method for obtaining the styrene content will also give too high results if the butadiene present does not have theoretical unsaturation. If care is taken to remove the soap, the iodine value method should prove to be a fairly accurate procedure for determining the styrene content of commercial GR S.

Iodine Value of Butadiene–Acrylic Nitrile Polymers

The effect of different reaction conditions on the iodine value of butadiene–acrylic nitrile copolymer is shown in Table VI. These data show that either of two procedures is satisfactory. In one procedure the polymer can be passed 60 times through a tight mill roll and rendered soluble in chloroform, thereby avoiding the use of *p*-dichlorobenzene. However, to save time a lesser amount of milling together with the use of *p*-dichlorobenzene will be found advantageous. In either case the use of Wijs solution is preferred, along with addition of 50 cc. of chloroform to the *p*-dichlorobenzene solution. The polar nature of the glacial acetic acid increases the solubility of the polymer and its iodine chloride addition product.

The results of chemical analysis and iodine value of these polymers are given in Table VII, together with butadiene contents calculated from both carbon-hydrogen ratios and nitrogen contents. The loss in unsaturation upon polymerization is higher than in the case of the butadiene-styrene polymers. This is also reflected in the low plasticity and difficulty in processing the nitrile copolymers, since cross-linking or cyclization reactions during polymerization would be expected to result in loss of unsaturation and decreased processibility.

Iodine Value of Polychloroprene

Tests made on GN neoprene using the present method showed that in 2 hours at room temperature the reaction had proceeded to 56.9 per cent completion (iodine value 169.1)

TABLE VI. IODINE VALUE OF BUTADIENE-NITRILE COPOLYMERS UNDER VARIOUS EXPERIMENTAL CONDITIONS

Solvents Employed	Butadiene-Nitrile Co-polymers	No. of Passes through Mill	Heating Period in $C_6H_4Cl_2$	Iodine Chloride Solution Used	Time of Reaction	Iodine Value[a]
	Gram		*Hours*		*Hours*	
	(Copolymer containing 6.54% nitrogen)					
50 grams of $C_6H_4Cl_2$ + 50 cc. of CS_2	0.1	0	2.5	Wijs	1	315.3[b]
50 grams of $C_6H_4Cl_2$ + 50 cc. of $CHCl_3$	0.1	0	2.5	CCl_4	1	306.0[b]
	0.1	20	1.25	Wijs	1	302.1 (303.0)
	0.1	0	2.5	Wijs	1	300.0 (302.5)
50 grams of $C_6H_4Cl_2$ + 75 cc. of $CHCl_3$	0.1	0	2.5	Wijs	1	302.2
50 grams of $C_6H_4Cl_2$ + 50 cc. of $CHCl_3$	0.1	0	2.5	Wijs	3	300.5
	0.1	0	2.5	Wijs	7	306.0 (307.2)
	0.05	0	2.5	Wijs	7	306.2
	0.1	0	4.5	Wijs	1	300.1
50 grams of $C_6H_4Cl_2$ + 75 cc. of $CHCl_3$	0.1	0	4.5	Wijs	1	300.4
50 grams $C_6H_4Cl_2$ + 50 cc. of $CHCl_3$	0.1	0	9	Wijs	3	239.0 (245.3)[c]
	0.1	20	3	Wijs	3	264.8 (263.8)
	0.1	60	2	Wijs	3	262.0
	0.1	60	1	Wijs	3	265.8
	0.05	60	1	Wijs	3	265.5
75 cc. of $CHCl_3$	0.05	60	..	Wijs	3	264.2
	0.05	60	..	Wijs	30	268.2

[a] Reaction carried out at room temperature.

[b] Heavy ppt. formed.

[c] Polymer undissolved.

TABLE VII. ANALYSES OF BUTADIENE–ACRYLIC NITRILE POLYMERS

	Polymers	
	Regular nitrile content	High nitrile content
Carbon, per cent	81.53	79.80
Hydrogen, per cent	9.53	8.90
C/H ratio	8.55	8.97
Butadiene content from C/H (A), per cent	79.2	66.5
Nitrogen, per cent	6.54	10.19
Acrylic nitrile, per cent	24.8	38.5
Butadiene from N (B), per cent	75.2	61.5
Iodine value	302.2	266
Theoretical iodine value from A, per cent	81.0	85.0
Theoretical iodine value from B, per cent	86.0	92.3
Average % theoretical iodine value from A and B	83.5	88.6

and to 64.6 per cent (iodine value 191.9) after a 24-hour reaction period. The theoretical iodine value for polychloroprene is 297.1. As no difficulties were experienced in the procedure, it is concluded that the chlorine atom attached to the carbon atom in the second position in the butadiene base unit offers hindrance to the iodine chloride addition reaction. This was previously noted (*6*), when it was found that addition of iodine chloride by the Kemp-Wijs method took place to the extent of only 30 per cent. A comparison of these results also shows the greater activity of the iodine chloride in carbon tetrachloride. Another experiment employing the present procedure using 0.05 gram of neoprene and a 24-hour reaction period at room temperature resulted in forcing the addition to 67.3 per cent, corresponding to an iodine value of 199.9.

Iodine Value of Butyl Rubber

The present method, or the Kemp-Wijs procedure, was found not to be applicable to butyl rubber, since substitution occurs so readily in both procedures as to introduce large uncertainties in the low unsaturation values. Various procedures have been studied (Table VIII).

Samples of 0.5 gram were used in all cases. In the case of chloroform only as the solvent, it required overnight to dissolve the butyl rubber. When hot *p*-dichlorobenzene was employed, as in the case of the butadiene polymers, the solution time ranged from 20 to 30 minutes. The concentration of sodium thiosulfate solution was reduced to 0.05 *N* to increase the accuracy of the titration. In other respects the procedure was the same as previously outlined.

The best choice of analytical procedure appears to be to employ 50 grams of *p*-dichlorobenzene and 50 cc. of chloroform as a solvent to obtain maximum speed. The use of 5 cc. of Wijs solution and carrying out the reaction for 2 hours in the dark at ice-water temperature appear to keep substitution at a minimum, and at the same time complete the addition reaction with a series of butyl rubbers of varying unsaturation.

It is seen that a sample of polyisobutylene having an average molecular weight of about 30,000 has an iodine value of 1.36, corresponding to an unsaturation of 0.36 per cent. If this unsaturation arises from free end valences, the unsaturation of the butyl rubber arising from the addition of the diolefin should be corrected by subtracting this value. On the other hand, polyisobutylene may contain some diolefin.

Hanus solution and iodine chloride in carbon tetrachloride were tried but were found to be more reactive than Wijs solution, causing considerable substitution. In the case of a 1.45 butyl rubber, the iodine values at room temperature and 1-hour reaction period in the dark were: Hanus, 6.86; iodine chloride in carbon tetrachloride, 6.57; and Wijs, 4.25.

Estimation of Natural and Synthetic Rubber in Mixed Vulcanizates

The chromic acid oxidation method of Kheraskova and Korsunskaya (*7*) which was improved by Burger, Donaldson, and Baty (*2*) serves to determine the natural rubber content of a mixture of GR S and natural rubber. This method, however, will not give the combined amount of natural and GR S rubber in an unknown vulcanized mixture. For this purpose the authors have applied the present method to finely divided samples which were first acetone- and chloroform-extracted in the regular manner.

The composition tested was a tread formula given in Table IX. The results of analysis presented in this table appear to be satisfactory.

Duplicability of Method

The duplicability of the present iodine chloride method is indicated by Table X, which shows that the present procedure as applied either to crepe or butadiene-styrene copolymers is probably duplicable within ±0.25 per cent. Care must be taken, however, to guard against nonhomogeneity in the

TABLE VIII. IODINE NUMBER AND ISOPRENE CONTENT OF BUTYL RUBBER AND POLYISOBUTYLENE UNDER VARIOUS EXPERIMENTAL CONDITIONS

Polymer	Solvents Used	Iodine Chloride Solution Used, Wijs	Reaction Period	Reaction Temp.	Iodine Value	Unsaturation[a]
		Cc.	*Hours*	*° C.*		%
Butyl B-1.45	100 cc. of $CHCl_3$	20	0.5	24	5.37	1.44
		20	1	24	6.00	1.61
		20	4	24	7.10	1.90
		10	0.5	24	4.90	1.31
		10	1	24	5.49	1.47
		10	4	24	6.65	1.78
		5	0.5	24	4.13	1.11
		5	1	24	4.50	1.21
		5	4	24	5.62	1.51
		2	0.5	24	3.22	0.86
		2	1	24	3.22	0.86
		2	4	24	3.48	0.93
		10	0.5	3	3.87	1.04
		10	1	3	4.24	1.14
		10	4	3	4.88	1.31
		5	0.5	3	3.36	0.90
		5	1	3	3.44	0.92
		5	4	3	3.86	1.04
		3	0.5	3	3.29	0.88
		3	1	3	3.32	0.89
		3	4	3	3.49	0.94
		2	0.5	3	3.17	0.85
		2	1	3	3.22	0.86
		2	4	3	3.17	0.85
Polyisobutylene, 30,000 molecular weight		20	1	24	1.37	0.37
		10	1	3	1.37	0.37
		2	0.5	3	1.39	0.37
		2	1	3	1.37	0.37
		2	4	3	1.39	0.37
	50 grams of $C_6H_4Cl_2$ + 50 cc. of $CHCl_3$	5	0.25	3	1.09	0.30
		5	1	3	1.15	0.31
		5	2	3	1.36	0.36
		5	3	3	1.36	0.36
Butyl X		5	0.25	3	3.00	0.81
		5	1	3	3.20	0.86
		5	2	3	3.32	0.89
		5	3	3	3.32	0.89
Butyl X-1		5	0.25	3	4.58	1.23
		5	1	3	5.16	1.38
		5	2	3	5.36	1.44
		5	3	3	5.46	1.46
Butyl X-2		5	0.25	3	6.50	1.74
		5	1	3	7.35	1.97
		5	2	3	7.80	2.09
		5	3	3	7.91	2.12

[a] Unsaturation = $\frac{\text{iodine number of polymer} \times 100}{372.8}$

Table IX. Analysis of Natural and GR S Vulcanizates

	I	II	III
Rubber Tread Compositions[a]			
GR S (21% styrene)	100.00	50.00	25.00
Smoked sheets	....	50.00	75.00
Stearic acid	3.00	3.00	3.00
Kosmobile 77	45.00	45.00	45.00
Zinc oxide	5.00	5.00	5.00
Sulfur	3.00	3.00	3.00
Santocure	1.25	1.25	1.25
El. Sixty	0.50	0.50	0.50
Total	157.75	157.75	157.75
Calculated Rubber Contents			
Natural rubber, per cent	0.00	31.8	47.6
Natural rubber hydrocarbon (% natural rubber × 0.95), per cent	0.00	30.2	45.2
GR S rubber, per cent	63.4	31.7	15.8
GR S rubber hydrocarbon (% GR S × 0.925), per cent	58.7	29.3	14.7
Chemical Analysis			
Acetone extract, per cent	7.8	5.6	4.0
Chloroform extract, per cent	2.0	1.0	0.75
Sulfur, combined, per cent	1.27	1.86	2.13
Iodine value	225.1	232.6	235.2
Natural R. H. (chromic acid method), per cent	1.45	31.3	45.3
Natural rubber, per cent	1.53	33.0	47.6
GR S hydrocarbon, per cent	58.8	29.0	15.9
GR S rubber (GR S hydrocarbon ÷ 0.93), per cent	63.1	31.2	17.1

$$\% \text{ GRS hydrocarbon} = \frac{A(100-B) - 372.8\,C}{D} \times 100$$

A = iodine value of extracted vulcanizate
B = combined per cent acetone and chloroform extracts
C = per cent natural rubber hydrocarbon. Determined by chromic acid method
D = iodine value of extracted GR S. Average for 13 lots GR S rubber = 347.0

[a] Vulcanized 20 minutes at 142° C.

samples. Errors in sample weight should be kept within ±0.0001 gram and the titration within ±0.03 cc. Since the end point in the titration is sensitive to 0.01 cc. it is not difficult to duplicate results within the above limits.

Substitution takes place more readily with the new method than with the Kemp-Wijs procedure, owing to the greater chemical activity of the iodine chloride in carbon tetrachloride as compared with the Wijs reagent. By limiting the time of reaction to 1 hour, the error due to substitution is very small and as judged from the results on crepe rubber the addition reaction appears to be completed in this time.

Table X. Iodine Value of Crepe and Butadiene-Styrene Copolymer Rubber under Various Conditions

Polymer	Method	Reaction Time *Hours*	Reaction Temp. *° C.*	Iodine Value	Difference %
A	Present	1	24	356.1	0.34
		1	24	357.3	
B	Present	1	24	312.7	0.49
				314.2	
C	Present	1	24	296.6	0.27
				297.4	
Crepe rubber	Present	1	24	352.3	0.09
Crepe rubber[a]	Present	1	24	352.0	
Crepe rubber	Present	16	24	373.0	
Crepe rubber[a]	Kemp-Wijs	2	3	352.5	
Crepe rubber[a]	Kemp-Wijs	1	24	353.9	

[a] 12 passes through tight mill rolls.

Conclusion

The procedures outlined in this paper make it possible to determine accurately the unsaturation of a wide range of synthetic rubberlike substances prepared by the polymerization of mono- and diolefins.

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Preparation of Phosphomolybdic Acid from Phosphoric Acid and Pure Molybdic Acid

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METHODS of preparation of phosphomolybdic acid appearing in the literature are intricate and indirect, usually involving several intermediate steps (*2*). This reagent can, however, be directly prepared from pure molybdic oxide and phosphoric acid (*1*). The molybdic oxide used by the author contained not more than 0.05 per cent impurities.

The method given below saves both time and materials in this preparation. It is applicable also to the preparation of phosphotungstic acid by substitution of tungstic for molybdic oxide. By changing the molecular proportions, any one of the heteropoly acids of phosphorus and molybdenum can be produced. The same holds true for tungsten.

Pure molybdic oxide (144 grams, 1 mole) is weighed out and to it are added 9.61 grams (1/12 mole) of 85 per cent phosphoric acid (U. S. P.) and enough water to make a total volume of approximately 1.5 liters. On stirring, the suspension is white, having the appearance of milk. It is placed on a hot plate and heated to boiling. As the temperature increases the color changes from white to a canary yellow. After boiling for approximately 3 hours, some molybdic oxide still remains in suspension. It is allowed to settle 2 minutes and filtered on an 11-cm. No. 52 Whatman filter paper. The residue is washed with 50 cc. of distilled water. The dried residue weighs 27.6 grams, so that only 116.4 grams of molybdic oxide have gone into solution. The filtrate is clear and deep yellow in color. It is evaporated in a 1500-cc. beaker on the hot plate (requiring about 3 hours) to a volume of approximately 100 cc. The solution at this point is deep orange in color and boils at 106° C. It is then removed from the hot plate and allowed to cool to room temperature overnight. The crystals which form occupy almost the entire volume, leaving only a small amount of supernatant liquid. The crystals are filtered without washing and dried somewhat on a 9-cm. No. 41-H Whatman filter paper.

The filtrate has a volume of 18 cc. and contains some 10 to 15 grams of phosphomolybdic acid. The damp crystals weigh 130 grams when dried at room temperature in a vacuum, and the final weight is 111.7 grams. The product is deep yellow to orange in color and has lost some of its crystalline appearance.

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Improved Electrometric Apparatus

For Use with the Karl Fischer Method for Determination of Water

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WATER is one of the materials most frequently determined in analytical laboratories; the control of many plant operations and the evaluation of many research data are dependent on a knowledge of the water content of the products and intermediates concerned.

One of the best of the numerous methods so far developed, first proposed by Fischer in 1935 (*2*), involves the direct determination of water by titration with a solution of iodine, sulfur dioxide, and pyridine in methanol. Since that time, Smith, Bryant, and Mitchell (*5*) have studied the nature of the reaction and established the proper stoichiometric relationship of the various constituents of the reagent and their relation to the water removed. In their work the end point was determined visually—i. e., taken at the point where the color of the solution being titrated changed from pale yellow to a dark brown, owing to a small excess of the reagent.

For dark-colored samples, Almy, Griffin, and Wilcox (*1*) developed a potentiometric method for determining the end point by measuring the potential difference of a platinum-tungsten electrode system. Although this method gave good results, the laboratory model Beckman pH meter used for measuring the electromotive force is expensive and the change of e. m. f. at the end point is comparatively small, only about 20 millivolts.

Wernimont and Hopkinson (*6*) recently utilized the "dead-stop" end-point method of Foulk and Bawden (*3*) and pointed out that this method gives sharper and more reproducible end points than either the visual or the potentiometric method. The dead-stop method is based on the fact that when an electromotive force of about 10 millivolts is impressed upon two platinum electrodes immersed in Karl Fischer reagent, current flows so that the galvanometer is deflected off the scale. During the titration the galvanometer remains deflected until at the end point it suddenly comes to zero reading. The work of these authors was duplicated in this laboratory with excellent success.

Apparatus and Reagents

This paper describes a simpler, less expensive electrometric apparatus using the dead-stop technique for determining the end point. The parts can be procured for about ten dollars. The assembly is rugged and is suitable for use in either research or plant control laboratories.

In this apparatus the rather delicate and expensive galvanometer used by Wernimont and Hopkinson (*6*) is replaced by a cathode ray "magic eye" tube of the type used in a great number of laboratory instruments. The circuit used is essentially the titrimeter circuit of Serfass (*4*), modified and adapted to the determination of the Karl Fischer end point. Two platinum electrodes with a small polarizing voltage are placed in the solution being titrated. The electrical changes on these electrodes are amplified by a 6F5 vacuum tube. The amplifier is coupled to the 6E5 cathode ray tube which serves as the indicator.

The power for this apparatus is furnished by the 110-volt alternating current lines through a 6H6 tube acting as a rectifier-doubler. The original Serfass circuit did not use the rectifier as a doubler, thus allowing his titrimeter to be used on both alternating and direct current supply lines. The authors' rectifier-doubler circuit gives approximately a 250-volt direct current supply for the operation of the amplifier and cathode ray tubes. Although it cannot be used on direct current supply lines, this circuit has the advantage of giving a much brighter fluorescence on the target of the 6E5 cathode ray tube. The proper setting of the sensitivity control, a control for varying the bias of the grid of the 6F5 tube, was found in all cases to be the position of minimum resistance; therefore, this control was omitted from the finished instrument. Since it is necessary to use a polarizing voltage at all times, no provision is made for disconnecting this polarizing voltage.

The polarizing voltage is controlled by the current flowing through the 6F5 tube and varies with this current. At the beginning of the titration, this voltage is less than 1 millivolt and increases to about 15 millivolts at the end point. The current flowing between the electrodes during the titration is about 5 microamperes and becomes practically zero at the end point.

It was found that fluctuations of 5 volts or less in the line voltage do not have an appreciable effect on the end point. Such fluctuations do cause small changes in the eye position, but the end point is still readily discernible. If the supply voltage is unstable, a constant-voltage transformer should be used or a voltage regulator tube should be incorporated into the circuit.

The electrometric assembly is wired in accordance with Figure 1 and is mounted in a small metal cabinet. The complete setup is shown in Figures 2 and 3.

Titrations are made by adding an excess of Karl Fischer reagent and back-titrating the excess with a standard solution of water in methanol. The direct titration of the sample with Karl Fischer reagent was tried but was found to give premature and fading end points. It was decided, therefore, to adopt the back-titration with standard methanol. The titration flask containing the sample with excess Karl Fischer reagent is placed in position beneath the standard methanol buret. The eye control is turned carefully to the point where the "magic eye" is completely open. The eye control must not be turned

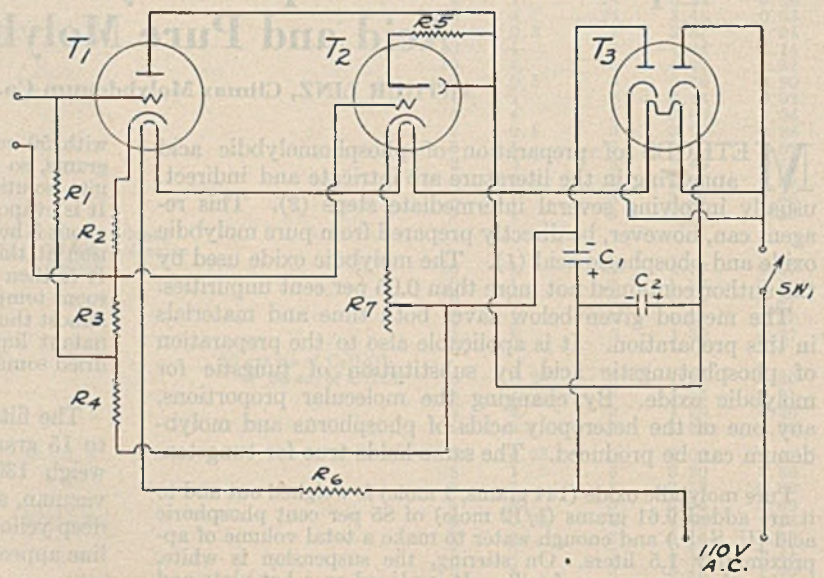

FIGURE 1. WIRING DIAGRAM FOR ELECTROMETRIC APPARATUS

R_1. 100,000-ohm fixed resistor, 0.5 watt
R_2, R_3. 1000-ohm fixed resistors, 1 watt
R_4. 25,000-ohm fixed resistor, 1 watt
R_5. 500,000-ohm fixed resistor, 1 watt
R_6. 350-ohm resistor (in line cord)
R_7. 25,000-ohm volume control
T_1. 6F5
T_2. 6E5
T_3. 6H6
SW_1. Single-pole single-throw toggle switch
C_1, C_2. 4-μf. electrical condenser, 450 volts

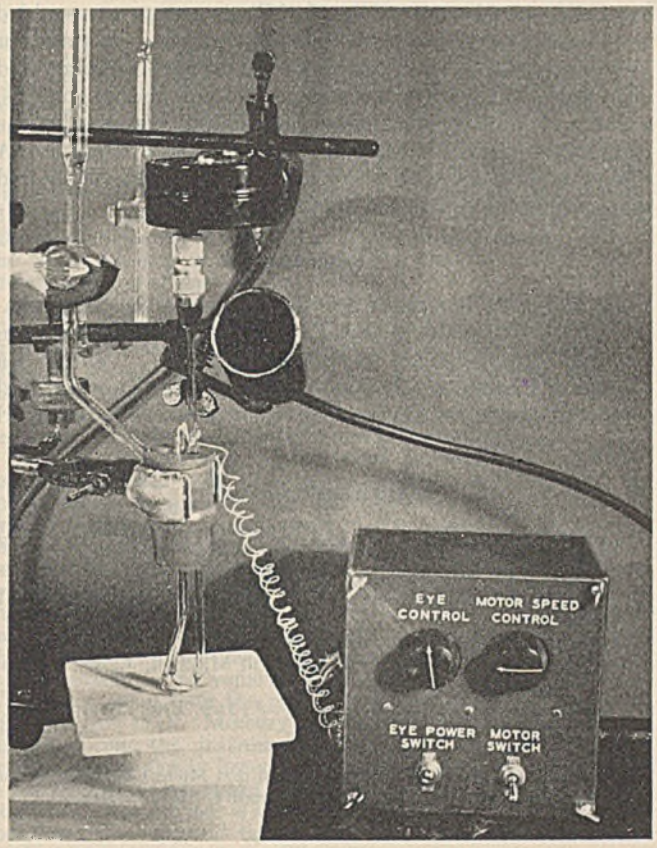

FIGURE 2. ELECTROMETRIC PORTION OF APPARATUS

beyond this point. The standard methanol is added at a rate of 1 to 2 drops per second until the eye starts to close, when the addition of methanol is stopped. The eye will completely close within 2 to 5 seconds. This slight lag is a characteristic of the reaction and has been observed by other workers using both the potentiometric and dead-stop methods.

Since it is essential that atmospheric moisture be carefully excluded from the reagents, an all-glass Schilling buret (Ace Glass Co., Catalog No. 3325) is used to dispense the Karl Fischer reagent. The methanol solution of water used for the back-titration is measured in a buret with a two-way stopcock, using a 2-liter bottle for a reservoir. The tip of this buret was modified by sealing on a tip of the shape necessary to fit the Bakelite stopper used to support the electrodes and prevent the entrance of atmospheric moisture. Both reagent reservoirs and burets are protected from atmospheric moisture with drying tubes filled with Drierite.

The reagents and the Bakelite stopper are the same as those used by Almy, Griffin, and Wilcox (*1*). The Karl Fischer reagent is prepared so that the molar ratio of iodine–sulfur dioxide–pyridine is 1:3:8.

Procedure

Weigh a sample containing 50 to 150 mg. of water into a titration flask. A 150-ml. extraction flask is convenient for this titration. If the sample is a solid or a viscous liquid, add 50 ml. of anhydrous methanol to serve as a solvent. The water content of this methanol must be determined by titrating a 50-ml. portion and correcting the results accordingly.

If the solid is insoluble in methanol, allow the sample to stand in contact with the solvent for 30 minutes. This procedure is adequate for finely divided solids such as wood pulp, nitrocellulose, and cotton linters. For dense granular samples such as proteins and ammonium nitrate, the methanol and sample must be heated to boiling, stoppered, and cooled to room temperature before titrating with the Karl Fischer reagent.

Add the standardized Karl Fischer reagent to the sample until this reagent is present in a 1- to 2-ml. excess, indicated by the change of color of the solution being titrated from pale yellow to dark brown. Place the titrating flask beneath the standard methanol buret, adjust the "magic eye" as described above, and titrate until the eye closes.

In making the calculations, the equivalence factors of the solutions are most conveniently expressed as milligrams of water per milliliters of solution.

TABLE I. ANALYSIS OF KNOWN SOLUTIONS OF WATER IN METHANOL

Sample No.	Water Present	Water Added	Total Water	Water Found	Difference
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
1	11.7	56.5	68.2	67.3	−0.9
2	11.7	47.7	59.4	59.5	+0.1
3	11.7	55.0	66.7	66.8	+0.1
4	11.7	139.1	150.8	151.3	+0.5
5	11.7	121.4	133.1	132.3	−0.8
6	11.7	123.2	134.9	134.3	−0.6
7	11.7	168.2	179.9	179.5	−0.4
8	11.7	184.6	196.3	196.4	+0.1

Application of Method

The Karl Fischer method, using this electrometric apparatus for determining the end point, has been applied in this laboratory to the determination of water in a variety of samples, including such liquids as methanol, ethanol,

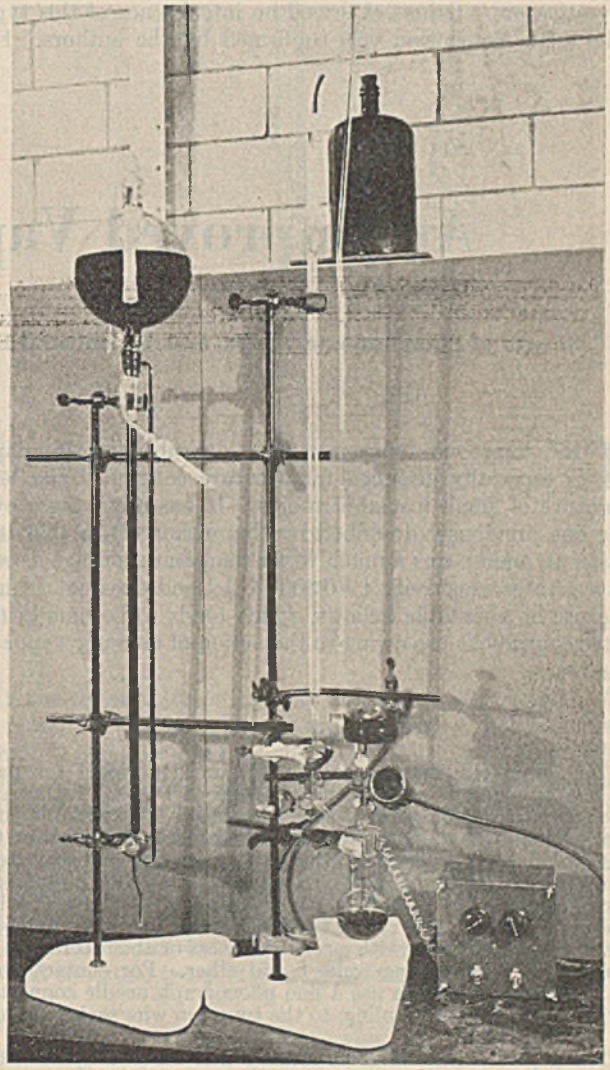

FIGURE 3. COMPLETELY ASSEMBLED ELECTROMETRIC APPARATUS

TABLE II. COMPARISON OF ELECTROMETRIC KARL FISCHER METHOD WITH OTHER METHODS

Sample	Water Found: Karl Fischer	Water Found: CCl_4 Distillation	Water Found: Oven
	%	%	%
Protein	11.1, 11.1	11.1, 10.8	10.9, 10.9
Vinsol resin	1.2, 1.2	....	1.4, 1.4
Pine oil	0.66, 0.64	0.5	.....
Nitroglycerin	0.28, 0.29	0.29[a]	.....
Wood pulp	6.5, 6.6	....	6.3, 6.3
Nitrocellulose	2.8, 2.8	2.9	.....
Glycerol	2.6, 2.6	2.6[b]	.....
Rosin size	30.4, 30.5	30.9	29.3, 29.3
Ammonium nitrate	0.26, 0.26	....	0.33, 0.36

[a] By desiccation method.
[b] By specific gravity.

glycerol, pyridine, nitroglycerin, solutions of resins, pine oil, and pinene, and in solids such as protein material, wood pulp, cotton linters, nitrocellulose, and ammonium nitrate. The results of the determination of water in known mixtures of methanol and water are given in Table I. A comparison of the results obtained on several miscellaneous materials by the Karl Fischer method and by other standard methods is given in Table II.

The possibility that highly unsaturated compounds will interfere with the titration because of the addition of iodine to the ethylenic double bonds has been suggested by earlier investigators. Others observed no interference of this type. The latter conclusion was confirmed by the authors. For example, a 10-ml. excess of the Karl Fischer reagent was added to the sample of pine oil and the mixture allowed to stand for 14 minutes before back-titrating. Under these exaggerated conditions, which are never encountered in a normal analysis, a value of 0.67 per cent water was found, whereas by the regular procedure a value of 0.65 per cent was obtained. A similar experiment with a sample of pinene likewise showed that a negligible absorption of iodine took place.

Summary

An improved apparatus for the electrometric determination of the end point in the Karl Fischer method has been constructed and applied to the determination of water in a variety of materials. The moisture content of unsaturated compounds can be successfully determined by the Karl Fischer method.

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An Improved Vapor Thermoregulator

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THE improved thermoregulator described in this paper is especially designed for accurate control of the temperature of small insulated rooms. It has advantages over the one previously described by the author (*2*) in that it is easier to make; has a much wider temperature range as well as a greater sensitivity (±0.005° C.); and does not require the use of a metallic bellows, which tends to become brittle after continuous use owing to the action of mercury vapor on the brass.

Design of Regulator

The improved thermoregulator (Figure 1), except for parts of the adjustment mechanism, is made of Pyrex. Essentially, the regulator is a modified U-tube manometer filled with mercury. The levels of the mercury in arms *A* and *B* are controlled by the change of vapor pressure of ethyl ether with the change in temperature. At the upper part of *B* is a small evacuated bulb (about 2 cm. in diameter) containing an electric contact point, *C*, while the upper part of *A* is connected to a thin-walled bulb, *F* (about 4 cm. in diameter and with a wall thickness of about 0.15 mm. at the bottom), containing some liquid ether. For contact point *C*, it was found best to use a fine phonograph needle connected, by means of a steel coupling, to the tungsten wire sealed through at *U*.

The adjustment mechanism connected to the lower part of this modified manometer is similar to that used in the previous instruments described by the writer (*2*, *3*). It consists of a closely fitting Fernico alloy plunger, *L* (about 0.015 mm. smaller than the bore), inside a very uniform-bore (±0.001-mm.) glass tube, *J* (8.04-mm. bore, 50 mm. long). The movement of the plunger is regulated by a finely threaded brass rod through a brass plug fastened to the top of the glass tube by means of wires around the projections on the side of the tube.

K is a mercury reservoir (25 × 35 mm.) with an opening, *Q*, for removing or introducing more mercury if necessary. Below *J* is another reservoir, *M* (10 × 2 cm.), holding enough mercury to take care of any change in levels in *A* and *B* occasioned by a sudden drop in temperature, so no air can be sucked into the upper parts of these tubes.

In order to make it possible for the adjustment mechanism to operate at atmospheric pressure while the upper part of *B* is evacuated and that of *A* is under the vapor pressure of ether, it is necessary to make the length of the mercury column between *C* and the middle of *J* about 800 mm., which is greater than the highest atmospheric pressure ordinarily encountered.

Filling and Adjustment

After assembling the various parts, which should be thoroughly cleaned and dried, into a unit, the thermoregulator is filled with mercury in the following manner: With stopcock *O* turned off and *G* opened to bulb *H*, and the Fernico plunger raised above *J*, the instrument is connected to a good mercury pump at *D* and evacuated thoroughly. Mercury is then distilled into the instrument from flask *E* until reservoir *K* is about three-fourths full. After the instrument has been sealed off at *R*, air is introduced into the space above the mercury in the adjustment mechanism side by carefully opening stopcock *O* until the mercury levels in *A* and *B* rise nearly halfway up in their respective tubes. At the same

time mercury in K is allowed to run into J to keep the mercury level from going down much below J; otherwise air might accidentally get into tubes A and B, which would render the instrument unworkable. To prevent accidentally letting too much air into the apparatus, a cork, P, with a small slot at the side is placed in the inlet tube above O.

Next, about 1 ml. of dried ethyl ether, which has been stored over mercury to remove any peroxide, is transferred through I by the method previously described (*3*) into bulb F, the bottom of which is kept in a cold-water bath. With stopcock G turned off and the water bath replaced by some powdered dry ice to lower the vapor pressure of the ether in the system to about 2 cm. of mercury, the instrument is sealed off at S.

After the dry ice has been removed and the ether in F has come to room temperature, more air is admitted through O until the pressure inside the adjustment mechanism is equal to atmospheric pressure, so cap N can be removed. The manometer reading, shown by the difference between the mercury levels in A and B, is then equal to the vapor pressure of ethyl ether at room temperature. The thermoregulator is now ready for use.

Suppose, for example, it is desired to operate a constant-temperature room at 30° C. As the room is being warmed up to the desired temperature, the vapor pressure of the ether in the system increases and forces some of the mercury back into K, as the Fernico plunger is still above J. When the room temperature reaches a little above 29° C., the Fernico plunger is lowered into J so as to force the mercury level up in B, almost to make contact with C. The amount of mercury in the system can be adjusted so that the Fernico plunger is operating in about the middle of J, when the desired temperature is reached. In case it is necessary to put some mercury back in K, it is best to put N in place again after the plunger has been raised above J and apply suction to O. Mercury is withdrawn from the reservoir by simply tilting the instrument. The final adjustment is accomplished by slowly lowering the plunger until the mercury in A barely makes contact at C when the room temperature is exactly at 30° C. The plunger is then locked in position by means of the lock nut, T. Sufficient time should be allowed for the mercury in the system to come to equilibrium.

Sensitivity and Temperature Range

Since glass is not a good thermal conductor, the sensitivity of the instrument depends largely on the thickness of the wall of bulb F. It should be as thin as possible, but strong enough to stand the pressure encountered at the temperature range at which the instrument is intended to be used. For this reason, this bulb should be tested before being sealed to the instrument.

The lower limit of temperature range of this type of thermoregulator is the freezing point of mercury (−38.87° C.), but at this temperature the sensitivity of the instrument is greatly reduced, since the vapor pressure of ethyl ether at −40° C. changes more slowly with the temperature than at 30° C. For such low temperature, therefore, a liquid such as ethyl chloride is more suitable than ethyl ether. The upper limit of the temperature range is determined by the lengths of A and B. For this particular instrument, the upper limit is a little above 40° C., because the distance between the contact point, C, and the bottom of A is about 940 mm., while the vapor pressure of ethyl ether at this temperature is about 921 mm. If the instrument is intended to be used for higher temperatures, arms A and B should be made longer and the wall of F should be made thicker to stand the correspondingly higher pressure. In place of the glass bulb, F, however, a thin-walled bulb or tube of some metal that does not react with mercury may be connected to the instrument by means of a Fernico coupling. This will permit higher operating temperatures and at the same time give improved sensitivity due to the better thermal conduction of the metal.

This improved thermoregulator together with a sensitive relay (*1*) was able to maintain a constant-temperature room at 30° ±0.005° C. at the regulator as shown by a differential thermometer. In order to do this, the heat input should be properly balanced with the heat loss of the room, so that there is practically no lag in the heating system.

In case it is desired to lower the temperature in the room, the Fernico plunger is raised above tube J and more mercury from the reservoir is added to the system until the plunger is again operating at about the middle of J when the mercury makes contact at C at the desired temperature.

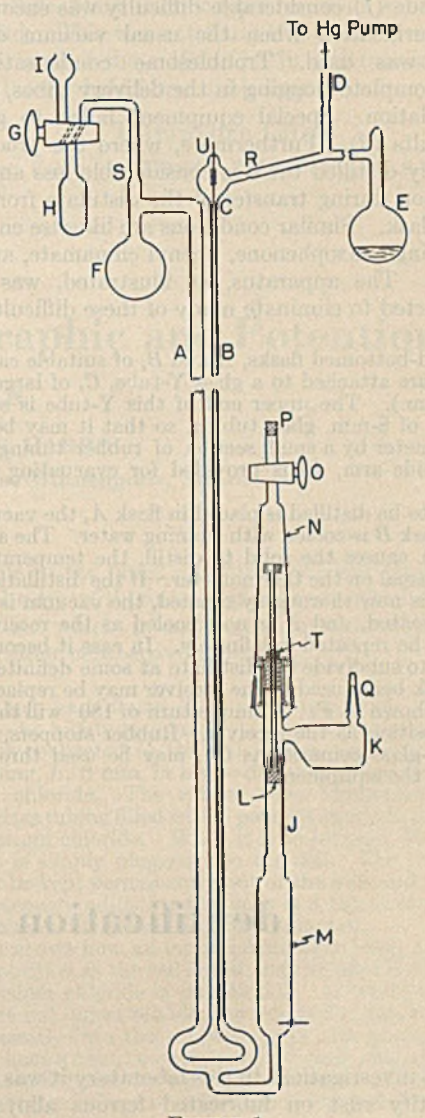

FIGURE 1

In the summer, when the outside temperature was 35° C. or more, it was possible to maintain in a well-insulated (5-cm., 2-inch, cork insulation) room (7 × 13 × 10 feet) to ±0.03° C. at the regulator, with the aid of a small refrigerator unit and two thermoregulators of this type, one controlling the heater and the other the cooling unit. In order to accomplish this, the heat input was carefully balanced against the cooling unit and fans were placed at different levels of the room to ensure proper circulation. Temperature in reaction chambers located on opposite sides of the room showed the same temperature variations.

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Vacuum Distillation Equipment for Volatile Solids

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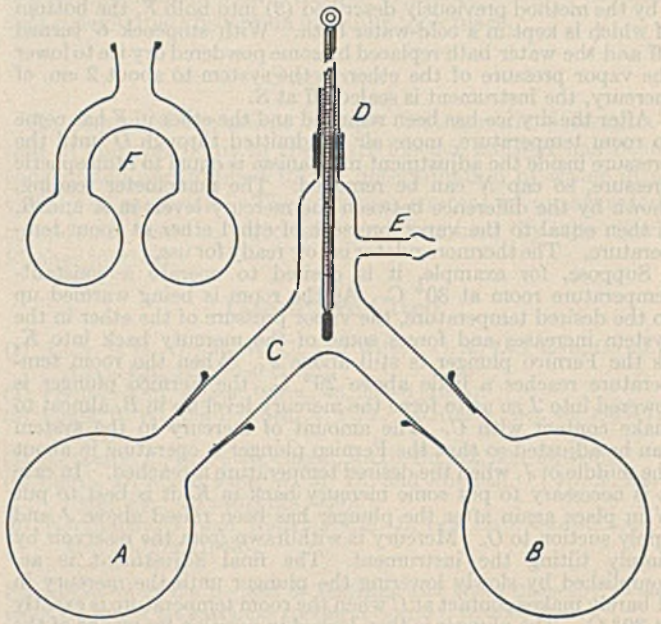
DURING the preparation of a quantity of aluminum ethoxide (*1*) considerable difficulty was encountered in the final purification when the usual vacuum distillation equipment was used. Troublesome condensation, even leading to complete clogging in the delivery tubes, often prevents distillation. Special equipment failed to give satisfactory results (*3*). Furthermore, where the product must be repeatedly distilled there is considerable loss and possible contamination during transfer of the distillate from receiver to original flask. Similar conditions are likewise encountered when distilling benzophenone, phenyl cinnamate, and similar compounds. The apparatus, as illustrated, was designed and constructed to eliminate many of these difficulties.



Two round-bottomed flasks, *A* and *B*, of suitable capacity (50 to 500 ml.) are attached to a glass Y-tube, *C*, of large diameter (15 to 25 mm.). The upper end of this Y-tube is sealed to a short length of 8-mm. glass tubing, so that it may be attached to a thermometer by a small section of rubber tubing at *D*. A convenient side arm, *E*, is provided for evacuating the entire equipment.

The solid to be distilled is placed in flask *A*, the vacuum is applied, and flask *B* is cooled with running water. The application of heat to *A* causes the solid to distill, the temperature being recorded as usual on the thermometer. If the distillation is to be repeated, *A* is now thoroughly cleaned, the vacuum is again applied, *B* is heated, and *A* is now cooled as the receiver. This process may be repeated indefinitely. In case it becomes necessary further to subdivide the distillate at some definite temperature, the flask being used as the receiver may be replaced by one of the type shown at *F*. A simple turn of 180° will throw either flask into position as the receiver. Rubber stoppers, or preferably ground-glass connections (*2*), may be used throughout in constructing the equipment.

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Identification of Rust on Iron and Steel

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IN SOME investigations in this laboratory it was necessary to identify rust on lubricated ferrous alloys. Visual inspection could not be relied upon because of the similarity in appearance between rust and petroleum gum. Ordinary chemical tests were not applicable, since most of them brought about the removal of some of the base metal along with the rust. Removal of the rust by pressing solvent-impregnated filter or gelatin paper onto the specimen proved unsatisfactory; with all the solvents tried, the base metal was preferentially dissolved. While these tests were unsatisfactory on the whole, those obtained with gelatin paper were much better than those in which ordinary filter paper was used. This suggested the use of gelatin paper moistened with water rather than a rust solvent. Subsequently it was found that gelatin paper moistened only with water was capable of removing an amount of iron rust sufficient for testing, without affecting the metal itself.

Procedure

The gelatin paper was prepared by fixing unexposed glossy photographic paper in sodium thiosulfate solution (200 grams in 1 liter of solution), treating in a hypo-eliminating solution (*1*) and drying. The hypo-eliminator (Kodak Formula HE-1) is prepared as follows:

Water	500 ml.
Hydrogen peroxide, 3%	125 ml.
Ammonium hydroxide, 3%	100 ml.
Water to make	1 liter

After fixing, the paper is washed 30 minutes in running water, immersed for 6 minutes in the above solution, washed for 10 minutes in water, and dried. About 6450 sq. cm. (1000 sq. inches) of paper may be processed in 1 liter of solution. Paper processed in this fashion may be stored in stoppered bottles indefinitely.

To carry out a test the gelatin surface of the dry paper is moistened slightly with distilled water and pressed firmly against the specimen. Continuous pressure on the paper is unnecessary, owing to the inherent adhesive property of the moist gelatin. After 15 to 30 seconds the paper is removed, care being taken to avoid stripping the gelatin coating from the paper base. Should it be impossible to remove the paper without this happening, the back should be moistened with distilled water and allowed to

stand for a minute or so, before another attempt at removal is made.

After removal from the specimen, the test paper is immersed in 10 per cent hydrochloric acid containing 0.05 per cent potassium ferrocyanide. Upon development a Prussian blue pattern of the rusted surface is obtained. The development time should be relatively short (10 to 15 seconds) in the case of freshly rusted surfaces if an accurate rust pattern is desired. With aged or worn rust it may be necessary to extend the time of development to one minute or more. The pattern is usually more distinct if the paper is dried with heat.

The Prussian blue developed in the gelatin has little tendency to diffuse and, contrary to filter paper prints, clear, sharp patterns result. Potassium ferrocyanide is preferred to potassium thiocyanate as a developing agent since the latter tends to give a blurred and indistinct image.

Experimental

Tests performed on solvent-cleaned, specially prepared, rusted iron and steel strips using the procedure described gave satisfactory analytical patterns of the rusted surface. Tests made on clean, freshly polished iron strips failed to give a Prussian blue color even upon prolonged development.

The test apparently is specific for iron oxide; attempts to remove sulfide films from copper or lead specimens were unsuccessful. This was attributed to the fine structure and close adherence of the sulfide film in contrast to the rather flaky and loosely held ferric oxide layer.

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An Improved Salt Bridge for Polarographic and Potentiometric Measurements

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AN EXTERNAL reference electrode is frequently used in potentiometric and polarographic measurements. The connection to the solution is by some type of a salt bridge. The ideal salt bridge, in addition to eliminating the junction potential, should have a low resistance (especially in polarography), should not contaminate the solution being used, and should be easy to handle. A number of bridges have been described in the literature, none of which incorporates all the desired features. The inverted-U bridge of Irving and Smith (*2*), with ground-glass plugs at the ends, has been widely used in potentiometric work but has an undesirably high resistance for polarography if the reference electrode is to be used as the anode. A bridge of this type, 40 cm. long, when filled with saturated potassium chloride, was found to have a resistance of about 7000 ohms, some 6500 ohms being attributable to the plugs. Laitinen (*3*) has described an inverted-U bridge with sintered-glass ends which has a lower resistance but which, in common with other all-glass designs (*1*, *4*), is rather cumbersome to use.

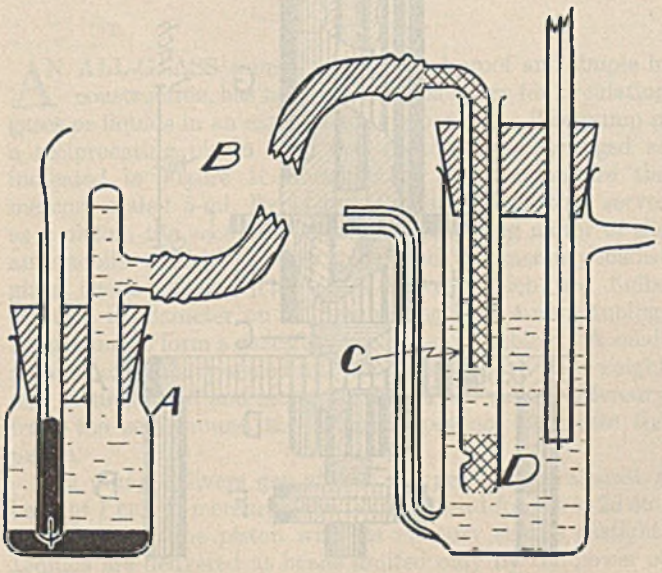

FIGURE 1

The authors have found the very simple bridge illustrated in Figure 1 to be highly satisfactory. The saturated calomel electrode, *A*, is connected to the cell with a piece of ordinary soft rubber tubing, *B*, 6 mm. in inside diameter, filled with saturated potassium chloride. The rubber tube terminates in a short length of glass tubing filled with 3 per cent agar jell, also saturated with potassium chloride. When it is to be used, the free end of the bridge is simply plugged into the cell. The reference electrode may be kept permanently out of the way and the necessity of simultaneously adjusting two cells in a thermostat to connect with an unbendable glass bridge is eliminated.

Figure 1 shows how an intermediate agar plug, *D*, having the same composition as the cell liquid, may be used if contamination with potassium chloride is undesirable. If traces of potassium chloride are not objectionable, the free end of the bridge may be inserted directly into the solution. For this purpose it is convenient to have a thin, coarsely-sintered glass plug at the tip of *C*. The agar is still desirable to prevent mixing by convection. The end of the bridge is kept in potassium chloride solution when not in use.

The resistance of such a bridge 56 cm. long was found to be only 600 ohms. For a polarogram showing a diffusion current of 10 microamperes, the error in half-wave potential due to *IR* drop in the bridge would be about 3 millivolts. This error is negligible for most purposes and may, of course, be decreased by the use of a shorter bridge or larger tubing. If exact values of half-wave potentials are desired, the resistance of the cell and bridge may be measured and the correction applied whenever necessary. For potentiometric titrations, in which a low bridge resistance is not essential, plug *D* is conveniently replaced by one of ground glass, as in the bridge of Irving and Smith (*2*).

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A Briquetting Press and Electrode Loader for Spectrochemical Analysis

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An inexpensive and easily constructed apparatus is described, for briquetting a powdered sample for spectrographic analysis and loading the briquet into an electrode crater without subsequent manual handling. The apparatus minimizes contamination and loss of sample, as well as danger of electrode breakage upon loading.

SAMPLES submitted for spectrochemical analysis are often in the form of powder or material which must be brought to a powder in preparation for the analysis. This is particularly true in the case of rocks, ores, slags, refractories, ashes, residues, ceramic products, and pigments. The dried residue of a dissolved metal or alloy is also sometimes used for analysis.

When a powdered sample is placed in the crater of a carbon or graphite electrode, some difficulties may be encountered. An unpacked sample requires a deeper electrode crater than a tightly packed sample, and an excessive amount of electrode wall must be consumed if the sample is burned to completion. A powdered sample placed loosely in the crater does not burn so uniformly as a sample packed in the electrode under pressure. A light and fluffy powder, or one containing certain compounds, tends to be blown from the crater during arcing. Considerable time is often spent in transferring a weighed sample to the crater without any loss of sample. Preburned electrodes tend to be fragile and extra care must be taken in filling them.

Packing the powdered sample into the electrode crater or briquetting the sample into a firm pellet and placing the pellet in the crater has become common practice. The packing operation is performed by means of a steel rod (*5*); and the briquetting is done with the aid of a punch and die (*2*, *4*), a micro pellet press, or a briquetting press recently described (*1*). In all these briquetting methods the pellet must be handled in placing it in the electrode crater.

The apparatus described here briquets the sample, discharges the pellet directly into the electrode crater, and presses the pellet firmly into the crater without subjecting the pellet to handling by the operator. Repeated checks have shown a negligible loss of sample during these operations. The apparatus is inexpensive, requires very little metal for its construction, and can be easily built in any well-equipped machine shop. The sample to be briquetted may be a powdered sample or a mixture of sample and other material added to serve as arc stabilizer, binder, or carrier of the internal standard. Electrodes prepared by means of either of the two most commonly used electrode cutting tools (*3*, *5*) may be used.

Apparatus

DESIGN DETAILS. Figure 1 shows the construction and Figure 2 shows the apparatus assembled and unassembled.

The base, *A*, is a circular piece of 0.25-inch steel plate, 2.75 inches in diameter, supported by three 0.25-inch steel legs, *B*, 1 inch in length from the bottom of the base. The electrode holder, *C*, is a hollow bolt with a 0.75-inch hexagonal head, *D*, 1.375 inches long and having a 0.5-inch threaded outside diameter. It has a 0.25-inch inside diameter which is threaded through the head end to a depth of 0.5 inch. The upper end is beveled flat to fit tightly against the briquetting mold. The bolt is screwed up tight through the center of the base. The electrode height adjuster, *E*, is a threaded steel rod 0.75 inch in length and 0.25 inch in diameter, at one end of which is a knurled nut, *F*, 1 inch in diameter and 0.25 inch thick. The adjuster screws into the electrode holder from below.

The briquetting mold, *G*, is a cylindrical piece of 0.875-inch steel rod 2.375 inches in length. A threaded hole 0.5 inch in diameter and 0.688 inch in depth is bored in the center of the lower end of the mold. Two rectangular slits, *H*, 0.25 inch wide and 0.688 inch in length, are cut on opposite sides of the threaded end of the mold. A 0.188-inch hole is bored in the center of the upper end of the mold and extends through the mold to the threaded end. The top of the mold is reamed out to a funnel shape 0.25 inch deep and 0.75 inch in diameter. A 1.5 × 0.25 × 0.125 inch steel plate, *I*, with rounded ends fits into the slit at the bottom of the mold and separates the top of the electrode holder from the bottom of the 0.188-inch hole in the mold when the mold is screwed down firmly over the electrode holder. A tool steel rod, *J*, 0.188 inch in diameter and 2 inches in length, slightly concave at the lower end, and having a head, *K*, at the upper end made of a circular piece of 0.25-inch steel 1.44 inches in diameter, serves as a plunger. A small wire spring, *L*, 1.75 inches in length, compressible to 0.688 inch, is placed over the plunger and is held in place by the plunger head.

OPERATION. The briquetting mold is removed from the electrode holder and the plunger is removed from the mold. A 0.25-inch spectroscopic electrode, less than 1.25 inches in length, having square-cut ends and a crater of desired depth, is placed in the electrode holder with the crater end up, but not extending above the top of the electrode holder. The mold is screwed down over the electrode holder, the steel plate is placed in its slot, and the mold is tightened. The electrode is

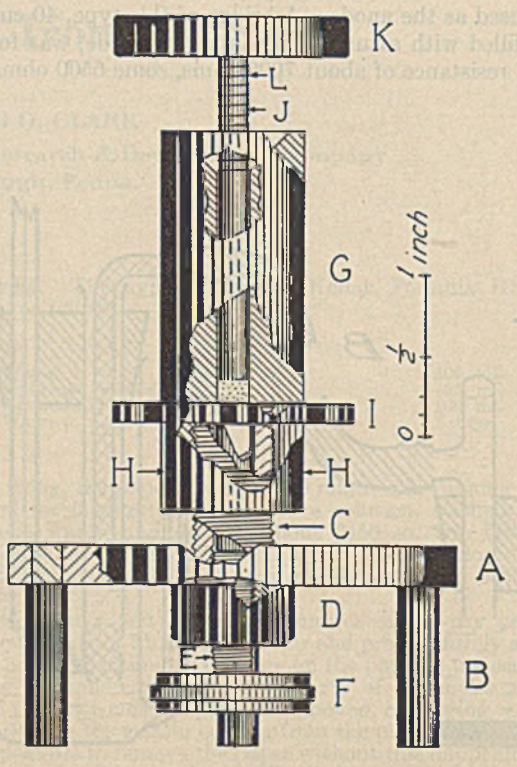

FIGURE 1. CONSTRUCTION OF APPARATUS

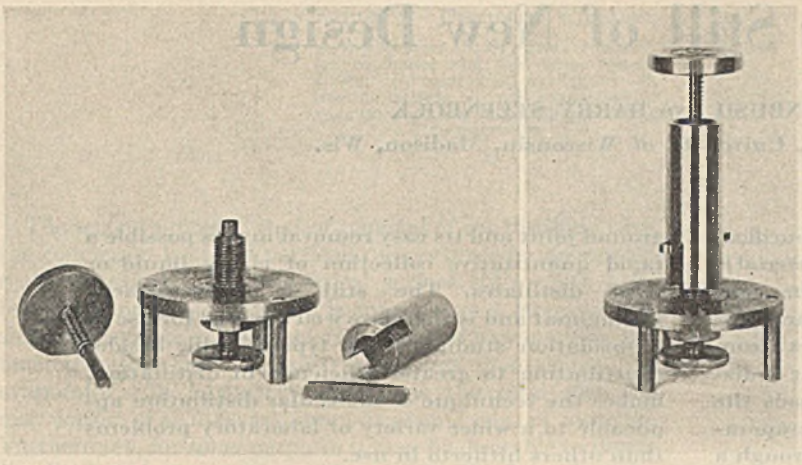

FIGURE 2. BRIQUETTING PRESS, UNASSEMBLED AND ASSEMBLED

raised by turning the height adjuster until the top of the electrode presses firmly against the bottom of the steel plate. The powdered sample or sample mixture is placed in the 0.188-inch hole in the top of the mold, using the top of the mold as a funnel, and is brushed into the mold with a camel's-hair brush. If it is desirable that the sample not touch the upper part of the sidewalls of the mold, a small glass funnel may be used to introduce the sample into the bottom of the mold. The plunger is placed in the mold and pressure is applied to the plunger head, molding the sample between the bottom of the plunger and the top of the steel plate, *I*.

The spring raises the plunger from the sample when the pressure is released. The mold is loosened one turn, the steel plate is removed, and the mold is screwed down until the inside of the mold is in firm contact with the electrode holder and the top of the electrode. A slight pressure on the plunger head discharges the pellet into the electrode crater and additional pressure forces the pellet firmly into the crater. The mold is removed from the electrode holder and the height adjuster is turned up until the filled electrode projects sufficiently from the top of the electrode holder to be easily removed.

The apparatus as described is fitted to electrodes of 0.25-inch diameter with drilled craters larger than 0.18 inch, but dimensions can be adjusted for construction of a similar apparatus fitted to electrodes of other size. The apparatus is easy to take apart and can be cleaned with the aid of a small brush and pipe cleaner. Fragile, preburned electrodes may easily be filled without breaking. The samples may be briquetted by hand pressure or by means of a press operated under known pressures (*4*).

Acknowledgment

The authors wish to thank Wallace Lowry of the Oregon State Department of Geology and Mineral Industries for technical assistance.

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A Glass Laboratory Pump for Gases or Liquids

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AN ALL-GLASS pump, which is leakproof and simple in construction, has been found satisfactory for circulating gases or liquids in an experimental apparatus. The pump is a reciprocating piston with two check valves arranged as indicated in Figure 1. Features of construction are the mercury-sealed 5-ml. Pyrex hypodermic syringe that serves as a piston, the vacuum-operated reciprocating motor of the automobile windshield-wiper type, and the mercury-loaded glass check valves. The check valves, which are bulbs 10 mm. in diameter on a 15-mm. length of 4-mm. tubing, are ground to form a gastight seat on 8-mm. tubing. A small amount of mercury sealed into each check valve adds weight and ensures rapid and secure seating of the valve. Mercury from the seal around the plunger does not penetrate the piston.

The pump delivers gas at 230 ml. per minute against a head of 1 cm. of mercury; the limiting head for gas is 25 cm. of mercury. The piston with its mercury seal is gastight. Liquids are delivered at heads limited only by the power of the motor.

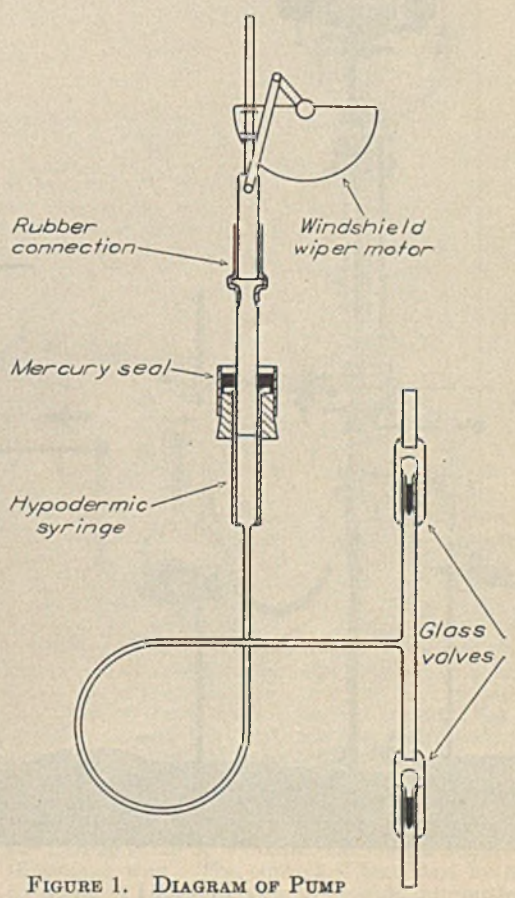

FIGURE 1. DIAGRAM OF PUMP

A Molecular Still of New Design

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College of Agriculture, University of Wisconsin, Madison, Wis.

A cyclic still of simple design for use in the purification of either large or small quantities of material is described. The evaporator surrounds the condenser, thus providing a large surface area for evaporation and a relatively small area from which the condensate must drain. During a distillation a magnetically driven rotor spreads the incoming distilland in a thin film over the evaporator surface. The condenser is attached through a ground joint and its easy removal makes possible a rapid quantitative collection of either liquid or solid distillates. The still is glass-surfaced throughout and is therefore well adapted for use in autoxidation studies. This type of still, besides contributing to greater efficiency in distillation, makes the technique of molecular distillation applicable to a wider variety of laboratory problems than others hitherto in use.

IN THE application of molecular distillation to the separation or purification of compounds, two recognized technical problems involve (1) the continuous renewal of the surface of the distilland, and (2) the removal of the distillate from the condenser. The first is of importance because the process of molecular distillation is essentially evaporation from the surface of the distilland and it is evident that only those molecules which reach the surface have the opportunity to pass to the condenser during the heating interval. It is therefore essential that the surface be continuously renewed to prevent the less volatile components of the distilland from imprisoning molecules which would normally distill if allowed to reach the surface. Such imprisonment is exemplified in the pot type of still, in which a thick layer of distilland is commonly employed without special provision for effecting surface renewal. In such a still relatively high temperatures are required to effect distillation and since the heating is necessarily continuous, heat-labile compounds are subjected to a great risk of decomposition. Nevertheless, the degree of fractionation is low.

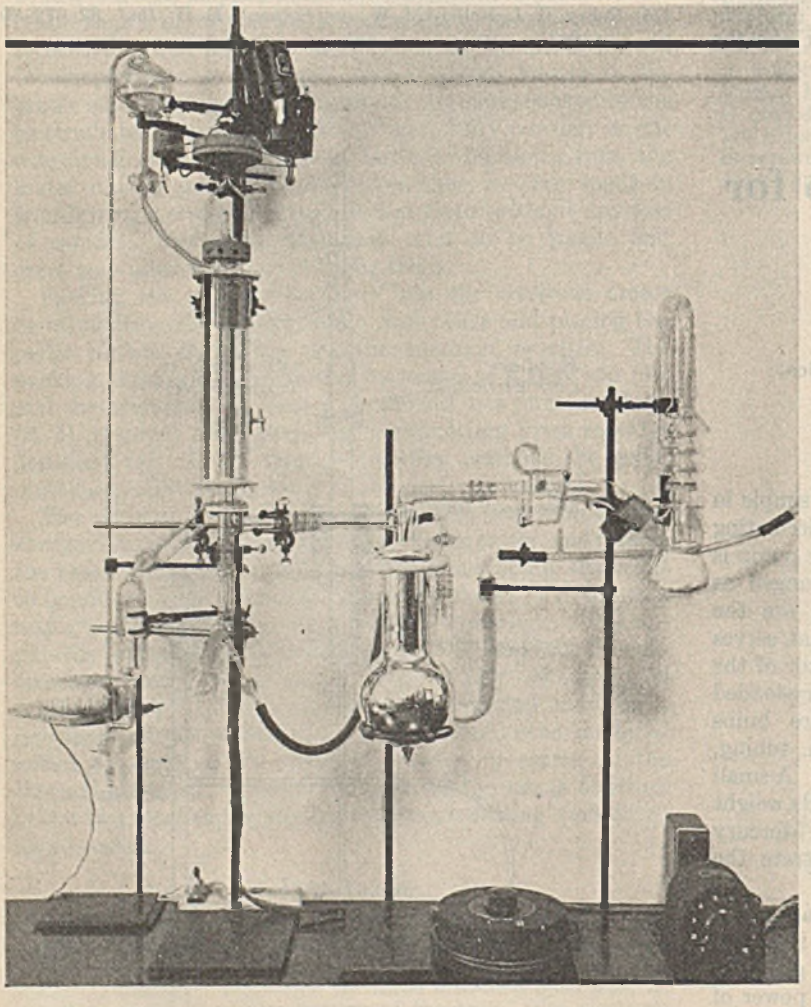

FIGURE 1

A considerable advance in distillation technique has resulted with the shifting of the evaporator from the horizontal to a vertical position, in which form the distilland is allowed to flow over a cylindrical or dome-shaped evaporator (*3–6*). Such vertical-surface stills depend upon gravitational flow to control the thickness of the layer of the distilland and to effect renewal of the distilling surface.

One of the chief problems is to induce the distilland to spread over the evaporator. In attempting to solve this problem, experimenters have resorted to the use of concentric wire gauze distributors and spiral embossing of the evaporator surface (*3*), spirally wound wire (*5*), or fragment glass which is fused to a glass evaporator to roughen its surface (*1*). While these devices are reported to effect spreading, it is to be expected that they should obstruct the free flow of the distilland and thus create zones of variable thickness and regions of sluggish flow with consequent subjection of localized areas to a relatively long period of heating. A further difficulty which may arise in simple gravitational flow is that of stratification of the distilland (*2*), the inner strata nearest the evaporator moving very slowly while the colder outer strata, from which evaporation is possible, move with relative rapidity. This difficulty is likely to become of importance with a distilland of high viscosity and especially at the lower temperatures.

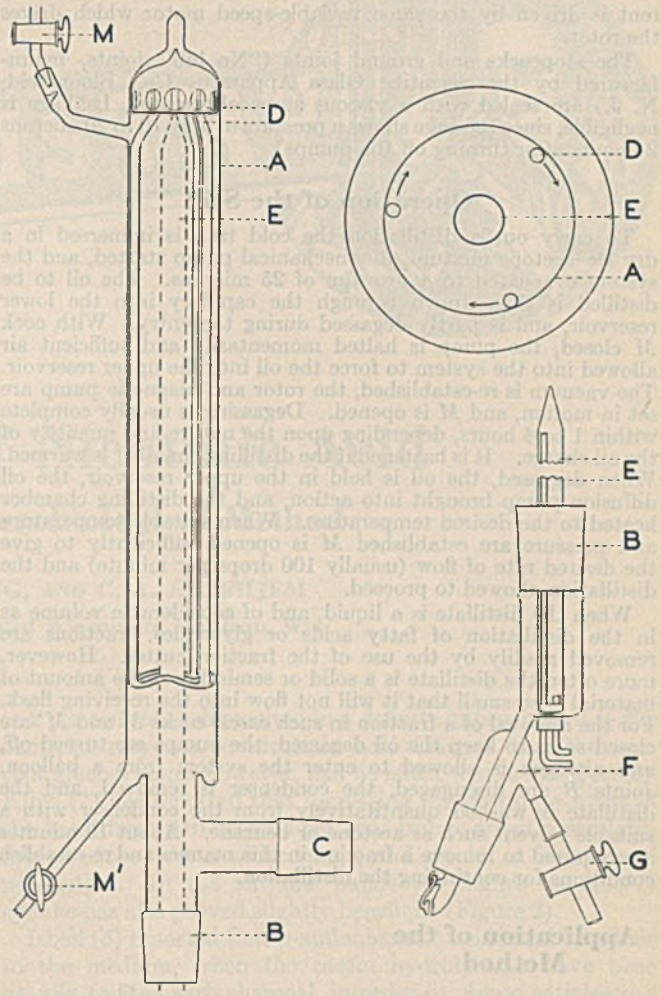

FIGURE 2. DISTILLING CHAMBER

A, evaporator; *B*, *C*, joints for attaching condenser and cold trap, respectively; *D*, rotor; *E*, condenser; *F*, fraction cutter; *G*, cock connecting to vacuum line; *M*, *M'*, cocks connecting to reservoirs.

The second problem, that of removal of the distillate from the condenser, is one of particular importance in laboratory-scale operations. In the vertical surface stills which have been employed, the distillate is spread over a large surface, since the condenser commonly surrounds the evaporator and must therefore have a larger surface area than that of the evaporator. In laboratory operations the total distillate may consist of as little as 1 gram or even 1 mg. of material. Furthermore, for some purposes it may be desirable to divide the distillate into a number of fractions. If it happens to be a liquid, some of it may find its way through the delivery tube and fraction cutting device into the receiver. However, if it is a solid or semisolid it is likely to remain quantitatively on the walls of the condenser. To circumvent this difficulty Hickman (*3*) introduced the use of a constant yield oil, the function of which was to entrain the distillate and carry it out of the still. In effect, this technique involves the transfer of the distillate from one oil to another, from which it is usually not more readily removable than from the first.

A third problem in still construction, the elimination of exposed metallic surfaces, arose in this laboratory in the application of molecular distillation to the study of autoxidation of fats. Since it is well known that mere traces of certain metals markedly accelerate autoxidation, it seemed inadvisable to attempt such studies with a still in which the fats were continuously in contact with metallic surfaces.

Effective advances have been made toward the solution of these three problems in a still now in use in this laboratory. A description of the construction and operation of the still (Figure 1) are given here.

Construction of the Still

The functional part of the still, the distilling chamber, consists of (1) a cylindrical evaporator which is electrically heated from the outside, (2) a magnetically driven rotor which spreads the distilland over the inner surface of the evaporator, (3) a removable condenser which serves as a bearing for the rotor, and (4) a fraction cutter for the removal of liquid distillates. The chamber is connected on one side through a dry ice trap to an oil diffusion pump outfit (Cenco No. 93,270A) and a modified McLeod gage, and on the other side to two reservoirs and a magnetic pump.

The details of the distilling chamber are shown diagrammatically in Figure 2. The evaporator, *A*, is a Pyrex tube, 50 × 450 mm., which is closed at the upper end and connects through an annulus to the two male joints, *B* and *C* (⅋ 29/42), at the lower end. A side arm from the annulus connects with the lower reservoir, and another side arm near the top of the evaporator connects through a drop-regulator to the upper reservoir. The evaporator is heated by a jacket which contains a series of ten vertical coils comprising a total of 60 feet of No. 22 Nichrome wire. A thermometer is mounted in contact with the evaporator wall and the temperature is controlled by a rheostat in a 110-volt circuit.

The rotor, *D*, consists of three 5-mm. rods fused to a magnet chamber fitted closely to the evaporator surface, with just enough clearance to allow rotation (see cross-sectional view at upper right of Figure 2). The magnet chamber contains a ring of ten permanent magnets (Alnico, General Electric Co., 0.938 cm., 0.375 inch, in diameter and 0.469 cm., 0.188 inch thick) which are held in position by decagonal sheets of asbestos and sealed in the chamber under high vacuum. A conical indentation in the center of the lower surface, ground to fit the tip of the condenser, serves as the bearing for the rotor. The rotor is turned by a rotating magnetic collar which is driven by a variable-speed motor. The collar fits snugly around the outer surface of the evaporator and contains 10 Alnico magnets identical with and placed in juxtaposition to those of the magnet chamber.

The condenser, *E*, is a 14 × 600 mm. tube, the lower end of which is fused to the female part of joint *B*. The condenser and rotor are in position for operation when male and female joints *B* are brought together. The condenser is equipped with inlet and outlet tubes for either air or liquid cooling. The fraction cutter, *F*, has a delivery tube with a check valve extending through the ground joint (⅋ 24/40) into the receiving flask (not shown). The check valve is a glass bead ground to fit the opening of the delivery tube. A glass rod attached to the bead forms a hook and eye hinge with a rod fused to the delivery tube directly above, so that the bead rests lightly against the opening. The receiver is evacuated through a stopcock, *G*. With the distilling chamber evacuated, readmittance of air through *G* causes the bead to press firmly into position. An adhering drop of distillate serves as lubricant and seal for the check valve. When the distillate is to be small in quantity or solid in nature, the bead is removed from the hook, and cock *G* is replaced by a glass stopper.

The lower reservoir has a capacity of 160 cc.; the upper, 500 cc. Between the reservoirs is the magnetic pump which is shown diagrammatically in Figure 3. The pump has a glass plunger, *H*, which is ground to fit its cylinder, and two check valves, *I* and *I'*, which are glass balls at the ends of small rods. Within the plunger, sealed under vacuum, is a steel cylinder, *L*, which weighs 14 grams, to make possible its magnetic operation. A stopcock, *J*, serves for the introduction of the distilland into and its removal from the still. For the introduction of the distilland a capillary extension is attached through the ground joint (⅋ 10/30). During the distillation this is replaced by a cap. In removing the distilland, the pump is drained by inserting the glass rod, *K*, through the cock, *J*, thus dislodging valve *I* and pushing the plunger upward finally to dislodge valve *I'*.

The pump is operated by an electromagnet which consists of a core of soft iron 20 × 20 × 400 mm. and several hundred turns of No. 16 magnet wire. The current is furnished by a 6-volt storage battery. A circuit breaker to provide intermittent cur-

rent is driven by the same variable-speed motor which drives the rotor.

The stopcocks and ground joints ("No lub" joints, manufactured by the Scientific Glass Apparatus Co., Bloomfield, N. J.) are sealed with a viscous stopcock grease. Leakage is negligible, since the gage shows a pressure of only 10 to 20 microns 24 hours after turning off the pumps.

Operation of the Still

To carry out a distillation the cold trap is immersed in a dry ice–acetone mixture, the mechanical pump started, and the system evacuated to a pressure of 25 microns. The oil to be distilled is then drawn through the capillary into the lower reservoir, and is partly degassed during the entry. With cock *M* closed, the pump is halted momentarily and sufficient air allowed into the system to force the oil into the upper reservoir. The vacuum is re-established, the rotor and magnetic pump are set in motion, and *M* is opened. Degassing is usually complete within 1 to 4 hours, depending upon the nature and quantity of the oil charge. It is hastened if the distilling chamber is warmed. When degassed, the oil is held in the upper reservoir, the oil diffusion pump brought into action, and the distilling chamber heated to the desired temperature. When suitable temperature and pressure are established *M* is opened sufficiently to give the desired rate of flow (usually 100 drops per minute) and the distillation allowed to proceed.

When the distillate is a liquid, and of considerable volume as in the distillation of fatty acids or glycerides, fractions are removed readily by the use of the fraction cutter. However, more often the distillate is a solid or semisolid or the amount of material is so small that it will not flow into the receiving flask. For the removal of a fraction in such cases, cocks *M* and *M'* are closed so as to keep the oil degassed, the pumps are turned off, and nitrogen is allowed to enter the system from a balloon. Joints *B* are disengaged, the condenser is removed, and the distillate is washed quantitatively from the condenser with a suitable solvent such as acetone or benzene. About 10 minutes are required to remove a fraction in this manner and re-establish conditions for continuing the distillation.

Application of the Method

The effectiveness of the still in concentrating a substance from its dilute solution was shown in the analytical distillation of two standard dyes (purchased from Distillation Products, Inc., Rochester, N. Y.), celanthrene red and dibutyl aminoanthraquinone, after the method of Hickman (*3*). The diluent was a residue oil, corn oil from which the volatile constituents had been removed by distillation at 210° C.

In each case 1 mg. of dye was dissolved in 50 grams of the residue oil and distillation was allowed to proceed for 20 minutes, during which time the oil made one complete cycle through the still. The distillate was removed from the condenser with acetone and the quantity of dye determined colorimetrically. Subsequent fractions were removed at progressively increasing temperatures until the dye had distilled completely from the oil.

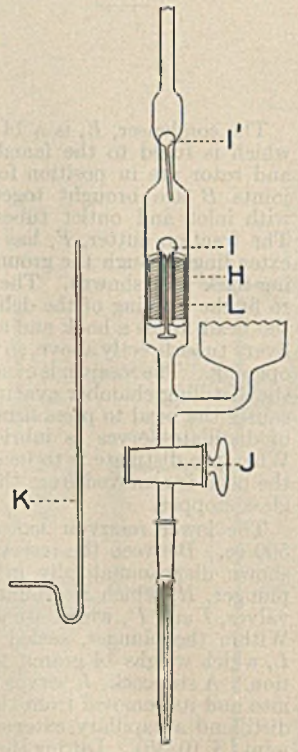

FIGURE 3. ALL-GLASS SURFACED MAGNETIC PUMP

H, plunger; *I*, *I'*, check valves; *J*, cock for introduction of distilland; *K*, tool for releasing valves to drain pump; *L*, iron cylinder

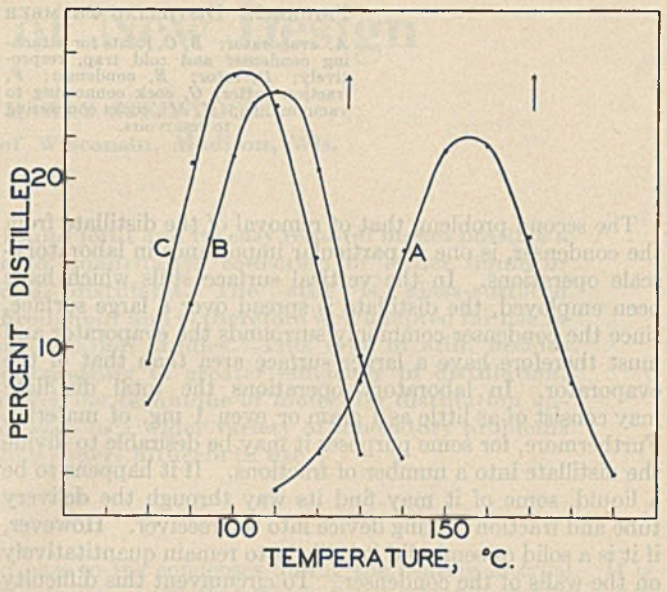

FIGURE 4. DISTILLATION CURVES OBTAINED WITH STANDARD DYES

A, dibutyl aminoanthraquinone, 20-minute cycle; *B*, celanthrene red, 20-minute cycle; *C*, celanthrene red, 30-minute cycle. Arrows indicate temperature of maxima reported by Hickman for the same dyes with a 10-minute cycle.

The curves thus obtained (Figure 4) showed maxima at 110° and 155° C., respectively, for the two dyes, or 17° and 16° lower, respectively, than those reported by Hickman (*3*), who used a 10-minute cycle. The actual differences in distilland temperatures were probably even greater, since the authors' values represent temperatures within the heating jacket. Under these conditions variable results were obtained with a 10-minute cycle, probably because of the low heat conductivity of the glass evaporator. It is evident, however, that the authors' lower distillation maxima are attributable in part to the longer cycle and in part to the improved spreading of the distilland over the evaporator surface.

In the application of the still to practical problems of the laboratory its adaptability has been further demonstrated. It has been used successfully in the concentration of antioxidants (*7*), tocopherols (*8*), and other vitamins and sterols from natural oils, in analytical distillations, and in the distillation of higher fatty acids, natural fats and oils, and synthetic glycerides.

Acknowledgment

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Determination of Nicotinic Acid

Modifications in the Microbiological Method

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THE Snell-Wright microbiological assay for nicotinic acid (*13*) has provided a valuable method for the determination of the vitamin, which has been widely employed in nutritional research. The method has been in use in this laboratory for over 2 years, and has been applied to many hundreds of samples. While the results on the whole have been fairly satisfactory, a number of difficulties have been encountered. This paper sets forth certain modifications in the procedure which have led to more consistent and uniform results, and describes the method in detail as it is now being carried out in this laboratory.

The difficulties that have arisen in the use of the original Snell-Wright method have been associated in the main with the standard curve. When the assay is employed routinely over an extended period of time, it has been the authors' experience that the different standard curves vary considerably, the growth of the bacteria sometimes being so poor that it is scarcely possible to arrive at any results at all. Even the better curves, such as curve 1, Figure 1, are decidedly rounded, and frequently are not linear to levels of nicotinic acid higher than 0.2 microgram. While results read from the higher parts of such curves may be reliable, the nonlinearity of the response raises the suspicion that substances other than nicotinic acid are limiting the growth at the higher levels, and hence implies the possibility of a lack of specificity of the assay.

A minor trouble, which has caused much unnecessary labor in many laboratories, has been the difficulty in securing satisfactorily low blank values. High blanks have been caused chiefly by the presence of nicotinic acid in the casein and biotin concentrates formerly used, and may now be easily avoided.

The discovery that fatty acids interfere with the microbiological determinations of riboflavin (*15*) and pantothenic acid (*10*) naturally raised a question regarding the possibility of similar interference in the determination of nicotinic acid. Fortunately *Lactobacillus arabinosus*, when grown on the nicotinic acid basal medium, seems to be insensitive to fatty acids.

Experimental

COMPOSITION OF THE BASAL MEDIUM. Increasing the glucose and sodium acetate in the Snell-Wright basal medium to 2 per cent of each resulted in a definite improvement in the bacterial response to nicotinic acid (Figure 1). Two per cent glucose alone, however, had no effect (curve 1, Figure 2). Kline (*7*) has reported that increasing the cystine content of the basal from 0.01 to 0.02 per cent brings about a straight-line response over a wider range, and results in higher acid production. In the authors' hands the higher amount of cystine has also proved slightly beneficial (Figure 2).

Isbell (*5*) reported that *p*-aminobenzoic acid must be added to the medium, when the casein hydrolyzates have been heavily treated with charcoal, in order to obtain satisfactory growth. The authors have not been able to observe such an effect, no doubt because of a contamination of some of their reagents with this factor, but have considered it advisable to include *p*-aminobenzoic acid in the modified basal medium.

The composition of the medium finally adopted is shown in Table I. Aside from the exceptions noted above and the use of 0.2 instead of 0.4 part per billion of biotin, the composition is the same as that recommended by Snell and Wright. This modified medium is referred to in the remainder of this paper as the basal medium.

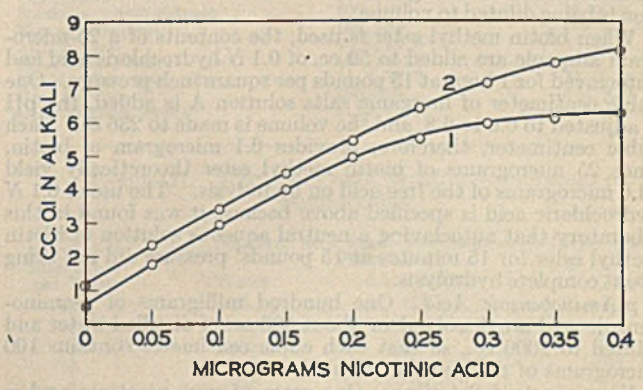

FIGURE 1. RESPONSE TO PURE NICOTINIC ACID (72 HOURS' INCUBATION)

1. Snell-Wright medium
2. Snell-Wright medium modified to contain 2 per cent glucose and 2 per cent sodium acetate

TABLE I. BASAL MEDIUM FOR NICOTINIC ACID ASSAY

Acid-hydrolyzed casein	0.5%
l(-)Tryptophan	0.01%
l(-)Cystine	0.02%
Glucose, anhydrous	2.0%
Sodium acetate, anhydrous	2.0%
Calcium pantothenate	0.1 p. p. m.
Thiamine chloride	0.1 p. p. m.
Pyridoxine	0.1 p. p. m.
Riboflavin	0.2 p. p. m.
Biotin	0.2 p. p. m.
p-Aminobenzoic acid	0.1 p. p. m.
Adenine	10 p. p. m.
Guanine	10 p. p. m.
Uracil	10 p. p. m.
Inorganic salts solution A	See text
Inorganic salts solution B	See text

PREPARATION OF STOCK SOLUTIONS. The stock solutions described below are stored in the dark in the presence of chloroform and toluene, and preferably in the refrigerator. The toluene is added until a thin layer is visible over the surface of the solution, and the quantity of chloroform used is such that a few drops are visible at the bottom of the flask.

Acid-Hydrolyzed Casein. One hundred grams of "vitamin-free" casein—e. g., Labco—are twice extracted by stirring 15 minutes at room temperature with 2 to 3 volumes of 95 per cent ethanol and filtering. The casein is then gently refluxed over a low flame with 300 cc. of concentrated hydrochloric acid for 16 to 20 hours. The casein hydrolyzate is concentrated to a paste in vacuo from a 70° to 80° C. water bath, 200 cc. of water are added, and the concentration is repeated. The residue is dissolved in about 700 cc. of water, adjusted to pH 4.0 (yellow-green to bromocresol green, outside indicator) with saturated sodium hydroxide solution, 20 grams of an activated charcoal (Norite A or Darco G-60) are added, and the mixture is stirred for 1 hour at room temperature. The charcoal is removed by gravity filtration, the pH adjusted to 6.6 to 6.8 (bromothymol blue, outside indicator), and the filtrate diluted with distilled water to 1000 cc. Each cubic centimeter therefore is equivalent to 100 mg. of the original casein. The final solution should be no darker than a pale straw color. Any sediment occurring in the casein hydrolyzate on standing may in general be eliminated by autoclaving for 1 hour at 10,500 kg. per square meter (15 pounds per square inch) pressure.

l-Cystine Solution. Four grams of *l*-cystine are suspended in 500 cc. of hot water and the smallest amount of concentrated hydrochloric acid needed to effect solution is added dropwise with stirring. The solution is then diluted to 1000 cc., so that each cubic centimeter contains 4 mg. of *l*-cystine.

l(-)Tryptophan. Two grams of *l*(-)tryptophan are dissolved in about 700 cc. of warm water, with the aid of a few drops of concentrated hydrochloric acid. The solution is diluted to 1000 cc. so that each cubic centimeter contains 2 mg. of *l*(-)tryptophan.

Anhydrous C. P. glucose and sodium acetate are weighed out as needed.

Biotin. The contents of an ampoule containing 25 micrograms of biotin as the free acid are diluted to 250 cc., so that each cubic centimeter contains 0.1 microgram of biotin. One cubic centimeter of inorganic salt solution A is added as the solution is being diluted to volume.

When biotin methyl ester is used, the contents of a 25-microgram ampoule are added to 50 cc. of 0.1 *N* hydrochloric acid and autoclaved for 1 hour at 15 pounds per square inch pressure. One cubic centimeter of inorganic salts solution A is added, the pH is adjusted to 6.6 to 6.8, and the volume is made to 236 cc. Each cubic centimeter, therefore, provides 0.1 microgram of biotin, since 25 micrograms of biotin methyl ester theoretically yield 23.6 micrograms of the free acid on hydrolysis. The use of 0.1 *N* hydrochloric acid is specified above because it was found in this laboratory that autoclaving a neutral aqueous solution of biotin methyl ester for 15 minutes at 15 pounds' pressure did not bring about complete hydrolysis.

p-Aminobenzoic Acid. One hundred milligrams of *p*-aminobenzoic acid are dissolved in about 500 cc. of distilled water and diluted to 1000 cc., so that each cubic centimeter contains 100 micrograms of *p*-aminobenzoic acid.

Nicotinic Acid Solutions. One gram of pure nicotinic acid is dissolved in about 700 cc. of warm distilled water and diluted to exactly 1000 cc. This stock solution should be made up fresh at least every 2 months as a precaution against possible change in potency. Exactly 10 cc. of the above solution are diluted to 1000 cc. to give a 10 microgram per cc. solution of nicotinic acid. This solution is made up fresh each time that the standard (see below) is prepared.

Ten cubic centimeters of the 10 microgram per cc. solution are diluted to 1000 cc. to make the standard solution containing 0.1 microgram of nicotinic acid per cc. This standard is freshly prepared each week.

Commercial C. P. nicotinic acid has been used as received for the primary standard throughout this investigation. A sample taken from a half-used, 115-gram (quarter-pound) bottle was examined for moisture by heating over phosphorus pentoxide at 100° C. and 0.08-mm. pressure for 5 hours. The loss in weight amounted to 0.91 per cent.

Thiamine Chloride, Calcium Pantothenate, and Pyridoxine Solution. A stock solution is prepared in distilled water, and is so diluted as to contain 100 micrograms of each of these vitamins per cc.

The following solutions are made up according to the directions given by Snell and Wright (*13*): riboflavin solution; adenine, guanine, uracil solution; inorganic salts solution A; and inorganic salts solution B.

STOCK CULTURES AND INOCULUM. Stock cultures are carried as stabs in an agar medium made up as outlined by Snell and Wright, except that the medium is modified to contain 0.5 per cent dextrose and 0.5 per cent sodium acetate. These cultures are transferred at weekly intervals and at each weekly transfer 3 to 4 extra stab cultures are prepared. Each culture is incubated 24 to 32 hours at 37° C. and then held in the refrigerator until needed.

To grow inoculum for the assay tubes a transfer is made from one of the above stab cultures into a tube containing 10 cc. of the basal medium plus 1 microgram of nicotinic acid. This liquid culture is incubated for 24 hours at 37° C. and the cell suspension so obtained is used for inoculation, either as it is or after diluting with an equal volume of 0.9 per cent saline. This procedure eliminates centrifugation and resuspension of the cells.

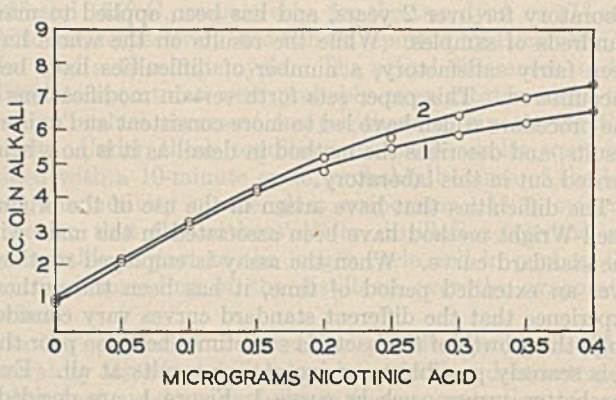

FIGURE 2. RESPONSE TO PURE NICOTINIC ACID (72 HOURS' INCUBATION)

1. Snell-Wright medium modified to contain 2 per cent glucose
2. Snell-Wright medium modified to contain 0.02 per cent cystine

Inoculum so prepared is not used for growing further inocula by serial transfers in the liquid medium. On the contrary, one should return to a stab culture each time inoculum is to be prepared. One such stab may be used several times if proper precautions are taken to exclude contamination with other organisms.

PREPARATION OF SAMPLES. Dry materials are finely ground, and fresh materials are mechanically homogenized in water—e. g., with a Waring Blendor or Potter-Elvehjem homogenizer (*11*). One gram of material is suspended in 50 cc. of *N* hydrochloric or sulfuric acid, and the suspension is autoclaved 20 minutes at 15 pounds per square inch pressure. The cooled mixture is adjusted to pH 6.6 to 6.8 with *N* sodium hydroxide, and diluted to such a volume that each cubic centimeter contains approximately 0.05 microgram of nicotinic acid. Filtration is unnecessary, but does no harm. Water-soluble materials are dissolved in the acid, autoclaved, neutralized, and diluted as above.

PROCEDURE. To prepare medium sufficient for 100 tubes, the following quantities of the above stock solutions and reagents are required:

Acid-hydrolyzed casein solution	50 cc.
l-Cystine solution	50 cc.
l(-)Tryptophan solution	50 cc.
Anhydrous glucose	20 grams
Anhydrous sodium acetate	20 grams
Biotin solution	2 cc.
p-Aminobenzoic acid solution	1 cc.
Thiamine, calcium pantothenate, pyridoxine solution	1 cc.
Riboflavin solution	2 cc.
Adenine, guanine, uracil solution	10 cc.
Inorganic salts, solution A	5 cc.
Inorganic salts, solution B	5 cc.

These materials are mixed, adjusted to pH 6.6 to 6.8, and diluted with distilled water to 500 cc. Five cubic centimeters of this solution, which is exactly double the concentration of the basal medium, are measured into each tube. Twenty tubes are reserved for blanks and for the standard curve. To these are added 0.0, 0.0, 0.5, 0.5, 1.0, 1.0, 1.5, 1.5, 2.0, 2.0, 2.5, 2.5, 3.0, 3.0, 3.5, 3.5, 4.0, 4.0, 5.0, and 5.0 cc., respectively, of the standard nicotinic acid solution (0.1 microgram per cc.).

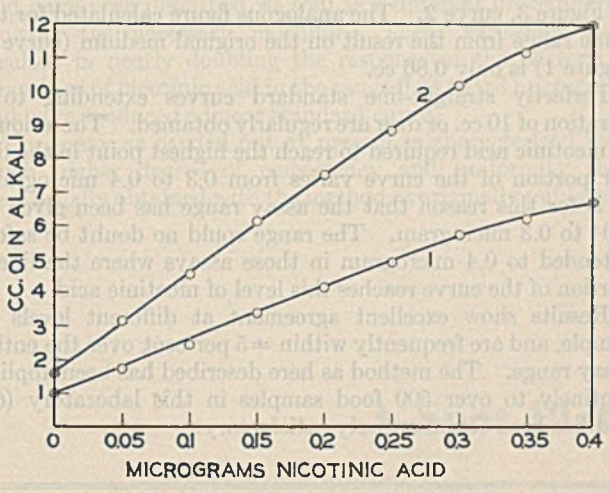

FIGURE 3. RESPONSE TO PURE NICOTINIC ACID ON PRESENT BASAL MEDIUM

1. 24-hour incubation
2. 72-hour incubation

The remaining tubes are used for the assay of the test samples. For each sample 8 tubes are used, and to them are added, respectively, 1.0, 1.0, 2.0, 2.0, 3.0, 3.0, 4.0, and 4.0 cc. of the solution prepared as described above. The volume of liquid in each tube is then brought to 10 cc. by the addition of distilled water, the tubes are plugged, autoclaved at 15 pounds per square inch pressure for 15 minutes, cooled to room temperature, and inoculated with 1 drop of inoculum. After 72 hours' incubation at 37° (±1°) C. the contents of each tube are titrated according to the directions given by Snell and Wright (*13*).

CALCULATION OF RESULTS. In evaluating the results of an assay, the agreement between the titration values of duplicate tubes is first noted. These ordinarily do not differ by more than 0.2 to 0.3 cc. of 0.1 *N* alkali, and any tubes which do differ by 0.5 cc. or more are discarded. Such variations probably are attributable either to bacterial contamination or to errors in measuring out the liquids added to the tubes. If the duplicate tubes agree satisfactorily, the titration values are averaged and used as one value in subsequent calculations.

The standard curve is plotted in the usual manner—e. g., curve 2, Figure 3—and from it the nicotinic acid content of each assay tube is determined. Tubes which contain less than 0.04 or more than 0.30 microgram of nicotinic acid are rejected as being outside the useful assay range. The nicotinic acid contents of tubes falling in this range are then converted to micrograms of nicotinic acid per cubic centimeter of test solution, and the agreement at different levels is noted. The final nicotinic acid content of the sample is calculated from the average of at least three such values, none of which deviates more than ±10 per cent from the average.

An alternative procedure for setting up samples and calculating results has been used in this laboratory, in case a great many routine assays are being done by an experienced analyst. In this procedure five tubes are used for each sample, and to them are added 1.0, 2.0, 3.0, 4.0, and 5.0 cc., respectively, of the test solution. The results from at least three of these tubes must fall in the assay range, and must agree to within ±10 per cent to permit calculation of a reliable value for the nicotinic acid content of the sample. The results from tubes which do not satisfy the above requirements are rejected.

TIME OF INCUBATION. In practically all this work the tubes have been incubated 72 hours and this period is recommended for general use. However, it is possible to secure usable results in shorter periods, even after as short a period as 24 hours' incubation. A standard curve obtained in this way is shown in Figure 3. It will be noted that this curve is very similar to that obtained in 72 hours by the original Snell-Wright method (Figure 1), but is more nearly linear. Comparative results obtained after 24 and 72 hours' incubation are shown in Table II.

EFFECT OF CORNSTARCH AND FATTY ACIDS ON *L. arabinosus*. Five grams of commercial cornstarch and 50 cc. of 0.1 *N* hydrochloric acid were mixed and autoclaved for 20 minutes at 15 pounds per square inch pressure. The mixture was adjusted to pH 6.6 to 6.8 and diluted to 100 cc. Two other similar preparations were also made in which water and 0.1 *N* sodium hydroxide, respectively, were substituted for the hydrochloric acid. Each solution was assayed for nicotinic acid by the original Snell-Wright method, both directly and after the addition of nicotinic acid equivalent to 5 micrograms per gram of the original starch. The results are shown in Table III.

Samples of oleic, linoleic, stearic, and palmitic acids were also tested for their effect on the nicotinic acid assay. These samples were the same as those used by Strong and Carpenter (*15*) and were tested at the levels found by them to have the

TABLE II. COMPARISON OF 72-HOUR AND 24-HOUR ASSAY RESULTS

Samples	24-Hour Assay	72-Hour Assay
	Micrograms per gram	
Defatted corn germ	46.4, 45.7	44.0, 45.5
Raw corn germ	34.8, 35.1	31.8, 30.0
Rice Krispies	108, 107	106, 104
Corn flakes	19.2, 19.5	18.4, 17.0
Solubilized liver extract	102, 101	98.0, 94.0
Wheat middlings	108, 103	104, 99.3
Alfalfa meal	40.8, 39.0	43.2, 38.0
Skim milk powder	10.2, 9.70	8.20, 8.20
Dried yellow split peas	31.8, 32.7	31.8, 33.0
Rice polishings	880, 900	864, 850
Canned green beans	4.70, 4.70	4.50, 4.60

TABLE III. RECOVERY OF NICOTINIC ACID ADDED TO CORNSTARCH[a]

Volume of Autoclaved Suspension per Tube	Nicotinic Acid Recovered[b] after Autoclaving Starch Suspended in:		
	Water	0.1 *N* HCl	0.1 *N* NaOH
Cc.	%	%	%
1	92	107	102
2	102	102	109
3	109	105	105
4	102	102	107
5	98	102	104

[a] Starch contained 0.4 μg. of nicotinic acid per gram and 5 μg. per gram was added after autoclaving.
[b] Corrected for nicotinic acid content of original starch.

TABLE IV. EFFECT OF FATTY ACIDS ON RECOVERY OF KNOWN AMOUNTS OF NICOTINIC ACID

Substances Added per Tube			Ratio of Fatty Acid to Nicotinic Acid	Nicotinic Acid Recovered
Fatty Acid Kind	Fatty Acid Amount	Nicotinic Acid		
	Micrograms	*Micrograms*		%
Oleic	48	0.05	960	100
	96	0.10	960	100
	144	0.15	960	100
	192	0.20	960	96
	24	0.05	480	110
	48	0.10	480	100
	72	0.15	480	95
	96	0.20	480	92
Linoleic	40	0.05	800	125
	80	0.10	800	117
	120	0.15	800	115
	160	0.20	800	105
	20	0.05	400	125
	40	0.10	400	122
	60	0.15	400	107
	80	0.20	400	80
Stearic	20	0.05	400	100
	40	0.10	400	100
	60	0.15	400	114
	80	0.20	400	108
	100	0.25	400	106
Palmitic	13	0.05	260	120
	26	0.10	260	110
	39	0.15	260	108
	52	0.20	260	111
	65	0.25	260	106

greatest influence on the riboflavin assay. The method of testing also was the same as that previously used. The results are presented in Table IV.

Discussion

The decision to employ a strong acid to effect extraction of the sample has been based mainly on two considerations. First, the demonstrated existence of combined forms of nicotinic acid which are not measured by *L. arabinosus* (*1*, *4*, *8*) makes some kind of a hydrolytic procedure imperative if total nicotinic acid is to be determined. Secondly, sodium hydroxide has proved undesirable for general use on account of soap formation with high-fat samples. The data in Tables III and IV indicate that little interference is to be expected from fatty acids in the sample, with the possible exception of linoleic acid. Materials high in this acid should probably be extracted with ether before assay.

If crystalline biotin preparations are used, the casein hydrolyzate will be the only important factor contributing to high blanks. Removal of nicotinic acid from the casein must be made as complete as possible before hydrolysis. Fifty per cent alcohol and 0.1 *N* hydrochloric acid have been suggested for extraction of the casein, but appear to have little if any advantage over 95 per cent alcohol. It is very important that the hydrolyzate be treated with an active charcoal. In the authors' hands Darco G-60 has given the best results.

It is their experience that inocula grown by repeated transfer in the Snell-Wright basal medium are less satisfactory than cells taken directly from the stab culture. This undoubtedly is attributable to deficiencies in that medium. Whether or not the present basal would be more successful in this regard has not been investigated, since the recommended procedure uniformly produces a very active inoculum.

The basal medium recommended in this paper is the result of extensive trials made with many different combinations of ingredients. Additional substances which were tested but appeared not to be beneficial include a folic acid preparation, asparagine, glutamine, ammonium sulfate, ammonium phosphate, glycine, serine, threonine, isoleucine, and various combinations of these materials. A few runs were made in which pyridoxine, thiamine, and riboflavin were omitted from the medium. In agreement with previous observations (*2*, *12*) satisfactory results were obtained, and it may well be that these ingredients are superfluous. However, the authors do not feel justified at the present time in recommending that they be left out. The beneficial effect of the increased concentration of sodium acetate is in line with similar observations by Stokes and Martin on *Lactobacillus casei* (*14*).

The main advantages of the modified procedure are the enhanced response of the bacteria to pure nicotinic acid, the linearity of the response, and the greater reliability and reproducibility of the assay results. The bacterial response may be expressed by the average additional acid production elicited by each increment of nicotinic acid. This amounts to 1.40 cc. per 0.05 microgram of nicotinic acid over the range 0.04 to 0.30 microgram for the standard curve given in Figure 3, curve 2. The analogous figure calculated for the same range from the result on the original medium (curve 1, Figure 1) is only 0.86 cc.

Perfectly straight-line standard curves extending to a titration of 10 cc. or over are regularly obtained. The amount of nicotinic acid required to reach the highest point in the linear portion of the curve varies from 0.3 to 0.4 microgram. It is for this reason that the assay range has been given as 0.04 to 0.3 microgram. The range could no doubt be safely extended to 0.4 microgram in those assays where the linear portion of the curve reaches this level of nicotinic acid.

Results show excellent agreement at different levels of sample, and are frequently within ±5 per cent over the entire assay range. The method as here described has been applied routinely to over 500 food samples in this laboratory (*6*), and has been found entirely satisfactory.

TABLE V. EFFECT OF OXIDIZING AGENTS ON MICROBIOLOGICAL ASSAY OF NICOTINIC ACID IN WHEAT

Material Assayed	Preliminary Treatment of Extract[a]	Nicotinic Acid Found: Brown *et al.* (*3*)	Nicotinic Acid Found: Present authors
		μg./g.	*μg./g.*
Whole wheat flour (collaborative sample)[b]	None	43.8	40.0
	H_2O_2, HCl	24.75	42.4
	H_2O_2, Lloyd's reagent	23.75	...
	H_2O_2, NaOH	48	47.5
	$KMnO_4$[c]	....	36.8
Wheat bran, 1	None	140	...
	H_2O_2, HCl	138	...
Wheat bran, 2	None	....	188
	H_2O_2, HCl	....	172
	NaOH,[d]	....	288
	NaOH, then H_2O_2, HCl	....	292

[a] Extracts were prepared and treated with Superoxol in acid or alkaline solution exactly according to directions of Brown *et al.* (*3*). Extract of wheat bran 2 was made by boiling for 1 hour with 100 parts of 0.1 *N* HCl.

[b] Obtained from John Andrews—same sample as that used by Brown *et al.* (*3*).

[c] Heated 10 minutes at 100° with excess *N* $KMnO_4$, then decolorized with hydrogen peroxide.

[d] To 20 cc. of 0.1 *N* HCl extract were added 3 cc. of 15 per cent NaOH, mixture was allowed to stand 10 minutes at room temperature and neutralized. This treatment hydrolyzes alkali-sensitive precursor of nicotinic acid which is present in wheat (*1*), and is extracted but not hydrolyzed by 0.1 *N* HCl at 100° (*3*).

A serious criticism has recently been leveled at the microbiological method for the determination of nicotinic acid by Brown, Thomas, and Bina (*3*), who have reported that much lower values were found in various wheat fractions after oxidation with hydrogen peroxide. The authors have repeated their work on whole wheat flour, and are unable to corroborate their findings. A summary of the results is given in Table V. It is apparent that in the authors' hands no variations greater than the experimental error of the method were found after oxidation.

The figure of about 40 micrograms per gram for the whole wheat sample seems to be the correct nicotinic acid content as determined on the extract prepared according to Brown *et al.* There is little doubt that this is much lower than the true value, which is about 64 micrograms per gram (*9*), and that the discrepancy is attributable to incomplete extraction. A similar difference is evident in the case of wheat bran, and is again probably due to the method of extraction used.

The value of 34 micrograms per gram for the nicotinic acid content of wheat germ (*3, 16*) is probably too low. Assay of a more extended series of wheat germ samples in this laboratory has shown that the germ contains approximately the same amount of nicotinic acid as does whole wheat.

Summary

Modifications in the basal medium proposed by Snell and Wright (*13*) for the microbiological determination of nicotinic acid have been described, as well as a different procedure for growing the necessary inoculum. These variations have resulted in nearly doubling the response of the bacteria to quantities of nicotinic acid in the range 0.04 to 0.3 microgram, and have produced a linear standard curve.

The assay as carried out at present is characterized by a much higher degree of consistency and uniformity than were usually obtained with the method as originally published.

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A Spot Plate for Drop Tests

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AT PRESENT two common types of spot plates are in use. When a soluble colored reaction product is anticipated, a white spot plate is used. If the reaction product anticipated is a precipitate (other than black, blue, or dark red), a black spot plate is ordinarily selected. During investigations of various drop reactions it became apparent that a combination black and white spot plate would be of advantage, and a plate of the design shown in Figure 1 was ordered. Such a plate is obtained by placing a black glaze on half of an ordinary three-depression white spot plate. The line of demarkation between the black and the white should run exactly through the centers of the three depressions.

The plate as shown is adaptable for either colored solutions or precipitates. Its great advantage lies in the hands of the experienced analyst to whom each intermediate precipitate and color is significant, regardless of the form of the final test product. By observing intermediate colors or precipitates possible interferences due to complex ion formation, competitive reactions, etc., can be anticipated (*1*). A further use is suggested by the work of West and Houtman (*2*), who propose a test for orthophosphate which depends on the formation of a yellow precipitate, best seen over a black surface, which is differentiated from interfering precipitates by converting it to a blue compound which should be observed over a white surface. The combination spot plate has proved invaluable, both in research and in the routine application of drop reactions.

Spot plates of this design can be obtained after the war from the Fisher Scientific Company, Pittsburgh, Penna.

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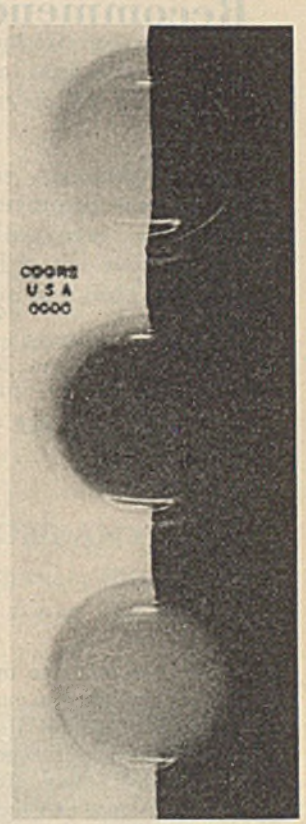

The top depression contains a clear colorless solution, the center a blue colored compound, and the lower a yellow precipitate. The advantage of the combination plate is best observed by alternately covering the white and black halves.

FIGURE 1. COMBINATION BLACK AND WHITE SPOT PLATE

Anhydrous Copper Sulfate in the Kjeldahl Nitrogen Determination

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THE Association of Official Agricultural Chemists (*1*) recommends the use of crystallized copper sulfate (pentahydrate) in the official Kjeldahl-Gunning-Arnold method for the determination of organic nitrogen. Bradstreet (*2*) in a survey of the Kjeldahl method of analysis mentions the use of copper sulfate as a catalyst for the Kjeldahl digestion, but in every case this is the copper sulfate pentahydrate. Niederl and Niederl (*3*) recommend, for the micro-Kjeldahl technique, the use of 1 part of potassium sulfate, 3 parts of copper sulfate pentahydrate, and a small amount of selenium.

This latter procedure, somewhat modified for semimicro-quantities, was used for routine determinations of nitrogen in organic compounds. It was found, however, that if the copper sulfate pentahydrate were replaced by anhydrous copper sulfate several advantages were apparent. The anhydrous copper sulfate did not dissolve completely in the sulfuric acid (unlike the pentahydrate) and the fine crystals served as "boiling stones", allowing a much more rapid and smooth digestion, free from all bumping. The size of the flame used was of less importance, since there was no tendency to bump.

Copper sulfate as a catalyst is likewise useful in the distillation of the ammonia as an internal indicator, since the blue color disappears when the solution is made basic, while black cupric oxide is formed.

The semimicromethod of analysis used calls for a sample size of 20 to 35 mg., to which are added 8 ml. of concentrated sulfuric acid, 2 grams of copper sulfate–potassium sulfate mixture and about 20 mg. of powdered selenium. The mixture is digested for 15 to 25 minutes. A finely ground mixture of 1 part of anhydrous copper sulfate and 2 parts of potassium sulfate, with a small amount of selenium is used as catalyst in the semimicro-Kjeldahl analyses run in this laboratory.

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Recommended Specifications for Microchemical Apparatus

Correction in the Design of the Dumas Nitrogen Stopcock

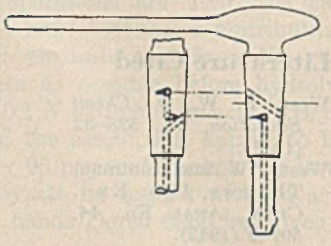

FIGURE 1. INCORRECT

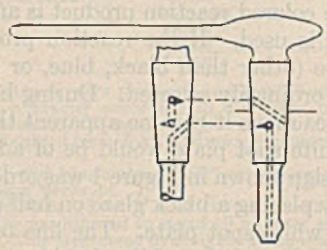

FIGURE 2. CORRECT

IT HAS been called to our attention that the design of the stopcock published in our previous report [IND. ENG. CHEM., ANAL. ED., 13, 580 (1941)] is incorrect, in that it reduces the safe zone of possible leakage from one fourth of the circumference to one eighth or less. To overcome this, we suggest that the stopcock be designed as shown in Figure 2 instead of the previous incorrect procedure shown in Figure 1.

In the incorrect view, the groove on the lower end opening of the straight bore is directed toward the opening of the bent outlet bore. In the correct form, Figure 2, the direction of the groove on the lower end of the straight bore must be away from the opening of the bent bore, so that a safe zone of at least one fourth of the circumference of the stopcock plug remains. The groove of the bent bore may remain as specified.

G. L. ROYER, *Chairman*
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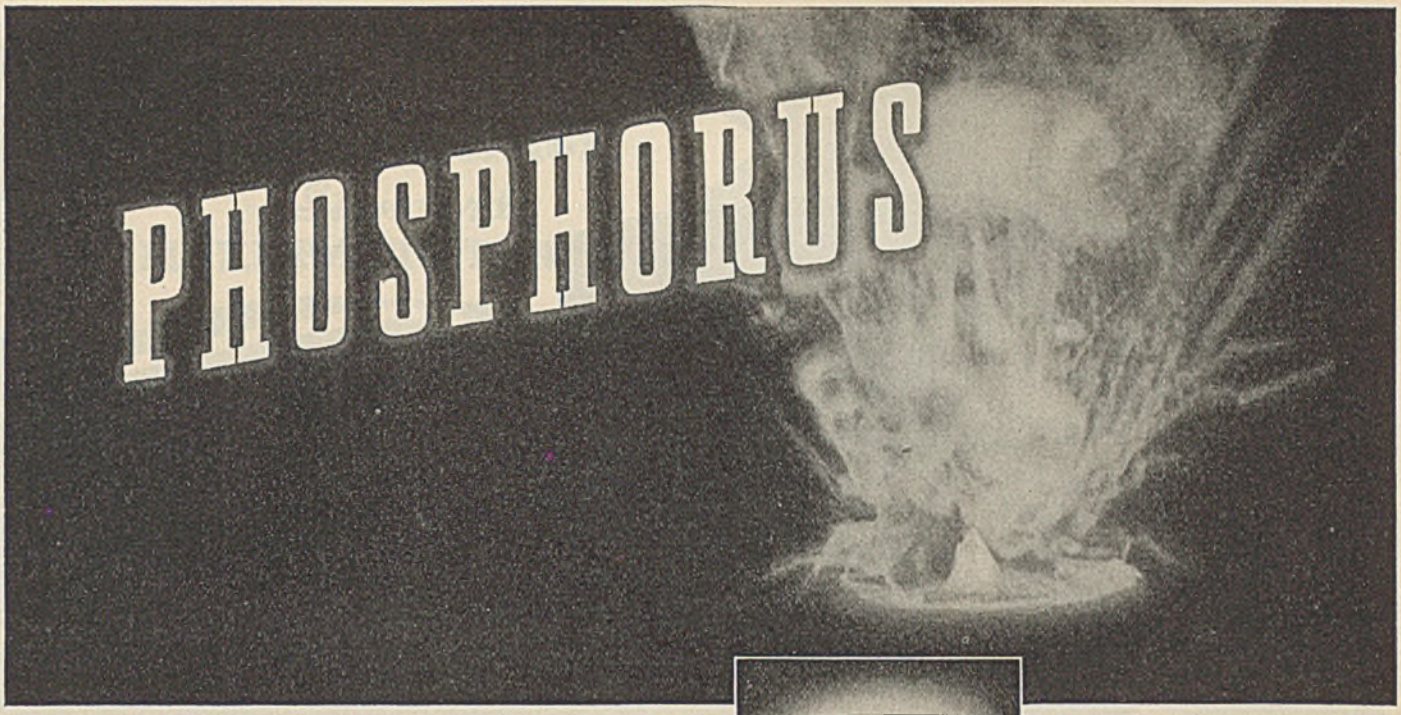

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