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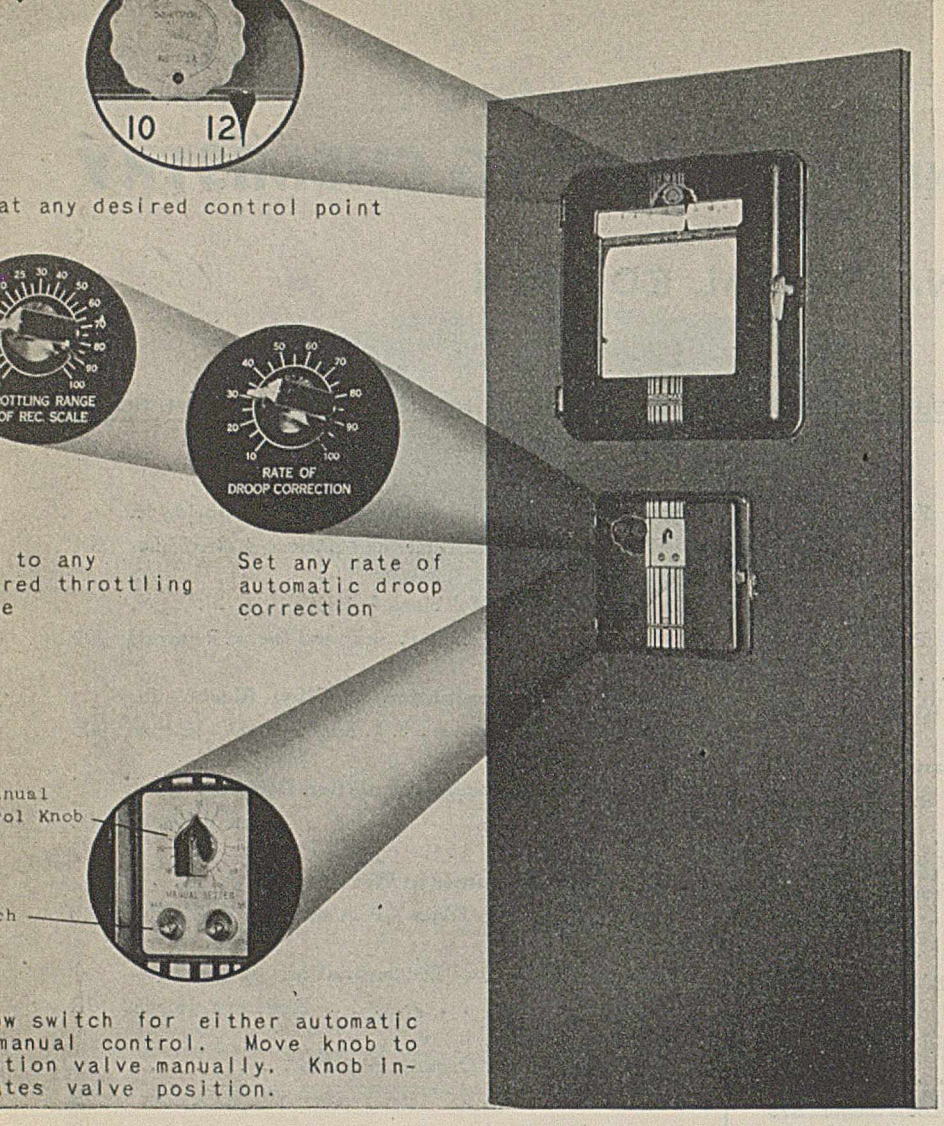

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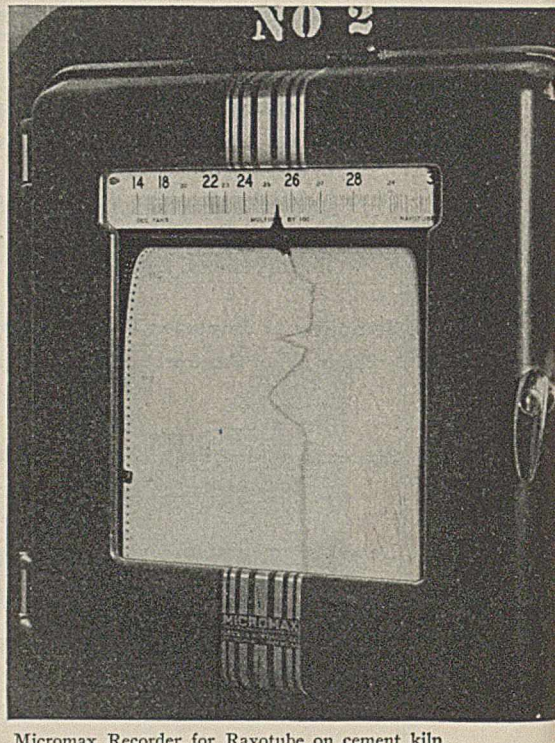

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INDUSTRIAL AND ENGINEERING CHEMISTRY

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PUBLISHED BY THE AMERICAN CHEMICAL SOCIETY • HARRISON E. HOWE, EDITOR

Determination of Moisture in Sugar Products[1]

Filter Paper Method for Corn Sirup

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PROBABLY no class of products has created so much controversy over moisture content as the one containing sugars. Not only is the stability of the sugars themselves involved (with respect to temperature) but sirups and impure sugars contain residual acids, proteins, gums, and mineral matter which complicate the usual problem of drying, so that peculiar results are often attributed to decomposition when the variable involved is in an improper method. The problem is an old one and the numerous papers which have been published give ample evidence of the fact that it is an analytical problem in all laboratories dealing with sugar products.

The usual variables in past papers which have been published on this subject are: (1) temperature, and (2) extenuated drying surface.

Temperature is not only an important variable with respect to the stability of the sugars themselves, but becomes more significant when impurities are present. The usual safeguard is to use temperatures under 100° C. However, this prolongs the period of drying, which is critical in that many products which are comparatively stable over a given period of heat treatment develop instability over prolonged heating. In order to offset the lower temperature, vacuum ovens are employed to speed the removal of water.

Necessity for dispersion of the sample in order to provide an extenuated surface for drying, particularly in the case of sugar products, has long been recognized, the primary purpose being to speed and to ensure the completeness of moisture removal.

Carr and Sanborn (*3*) in 1895 employed pumice and their method is essentially the A. O. A. C. method of today. Rolfe and Faxon (*9*) over 40 years ago suggested paper rolls, but the method never gained acceptance, probably because of insufficient interest at that time. More recently, Rice and Boleracki (*8*) proposed the use of two silver plates to increase the surface area of the sample. Although extending the surface area, this method failed when applied to corn sirup, because on drying, the dextrin in the corn sirup formed flakes that tended to curl and "dust off" from the silver surface.

In the A. O. A. C. method, employing sand, wherein a 1- or 2-gram sample is used, weight constancy is never obtained. In order to have some criterion by which moisture determinations can be discontinued, drying is allowed to proceed "until the change in weight does not exceed 2 mg. per interval", with a 2-hour drying period in general acceptance. Such a criterion gives a precision no greater than 0.2 per cent, which becomes of considerable magnitude in the corn products industry, which sells millions of pounds of corn sirup and crude sugar annually. This criterion is also particularly unfortunate, in that the looseness of the definition of constancy has given use to many statements of decomposition and instability of the product when the essential point has been the inadequacy of the method for that particular product. As a natural result of this, research has been stopped on more adequate methods for determination of true moisture.

Rice (*7*) departed from the oven methods for sugar products by applying to refiner's sirup Bidwell and Sterling's (*2*) modification of the distillation method of Dean and Stark (*4*). He changed their technique by introducing Filter-Cel in the flask as a bed for the sample of refiner's sirup. When the distillation was started, the refiner's sirup spread through the Filter-Cel, thus combining the sample dispersion found necessary in the oven methods to a method yielding measured water. The present authors (*6*) applied Rice's distillation technique to corn sirup and found that the corn sirup did not spread but formed a hard ball, necessitating the incorporation of the sample manually in the Filter-Cel before distillation was started. This work with tables for Baumé-moisture values has been published (*5*); it gave moisture values 0.7 to 1.8 per cent higher than values by the usual A. O. A. C. sand method.

From the standpoint of a further reduction in losses, this discrepancy became of enormous importance to the corn products industry wherein factory yield is determined from incoming and outgoing dry substance. As a result, for the past 8 years an extended investigation of true moisture has been carried out on the products of the industry—corn sirup, crude sugar, steep water, feeds, hydrol—with the refinement of old moisture methods and the development of new drying technique. These have such general interests that they have been extended to other sugar products. This work on analytical methods for true moisture will be recorded in a series of papers, of which this is the first.

Corn Sirup and Corn Sugar

Corn sirup is the thick viscous sirup obtained from the partial hydrolysis of starch. It is a mixture of carbohydrates and, in addition, contains small amounts of residual acidity, crude protein, and ash. Corn sirup is of several types, and the degree of hydrolysis is defined by the amount of reducing sugars expressed as dextrose on a dry-substance basis, to which the expression "dextrose equivalent", or simply D. E., has been assigned by the industry. The authors' average analysis of 42 dextrose equivalent corn sirup, the commonest

[1] This is the first article in a series on this subject. The second follows on page 858.

type, known generally as confectioners' sirup, is as follows on a dry-substance basis:

Reducing sugars as dextrose	42
Dextrin	37
Maltotetrose	5
Maltotriose	15
Maltose	21
Dextrose	22
Residual acidity as hydrochloric acid	0.02
pH, 50% by weight	5.0
Ash	0.28
Crude protein	0.05

The dextrin content of corn sirup makes a moisture test by the A. O. A. C. method difficult, as the sirup does not spread easily, tends to form a skin by surface drying, and sets to a concretelike mass from which the final traces of moisture are removed with difficulty or not at all. The presence of ash, residual acidity, and protein, particularly since dextrin is present, has always cast serious doubts as to whether the slowly removed last traces of moisture were moisture of the sirup or water of decomposition.

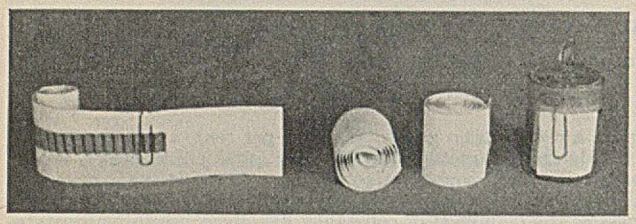

FIGURE 1

Crude corn sugar, more familiarly known in the trade as "70" and "80" sugars, may be said to represent the complete acid hydrolysis of starch in commercial practice. The product cannot be so closely defined as corn sirup because of trade uses, but the composition may be expressed as follows:

Reducing sugars as dextrose	82 to 92
Acidity	0.30 to 0.40
pH, 50% solids	4.2 to 5.2
Ash	0.6 to 1.25
Crude protein	0.08 to 0.20

There is little if any dextrin in crude sugar, so the drying problem differs from that of corn sirup, but it is further complicated by the larger percentage of reactive dextrose and by the increasing quantities of residual acid, ash, and protein.

The method of the present authors, employing toluene distillation, has a decided advantage over the A. O. A. C. sand method, as the size of the sample used is tenfold, increasing the accuracy of the test. The higher values obtained for corn sirup by this method as compared to the A. O. A. C. method evoked criticism of decomposition because of the higher temperature involved (110° to 112° C.), despite the fact that the values for corn sugar were in relatively close agreement with those of the A. O. A. C. method. It would normally be thought that corn sugar would be more susceptible to decomposition because of the larger percentage of dextrose and residual traces of acidity, ash, and protein. As a result, the initial stages of this investigation of true moisture center on comparison of the data by toluene distillation with the A. O. A. C. method at 70° and 100° C., together with new technique at these temperatures.

Method by Drying on Sand

The investigation of moisture determination in corn sirup by drying on sand was carried out in a vacuum oven at 74-mm. (mercury) pressure and in an air oven. The temperatures in the vacuum oven were 70° C., conforming to the A. O. A. C. (*1*), and 100° C., a practice that has become part of the routine analytical procedure in the corn products industries. The air oven temperature was 100° C.

Acid-washed quartz sand was used. It was screened for size, that passing a 20-mesh and retained on a 40-mesh being used. Approximately 35 to 40 grams were run into an aluminum dish, 7.5 cm. (3 inches) in diameter. Original weights included a small pestle and a cover. Immediately on removing the test from the oven for cooling in a desiccator, the cover was placed in position, as it was found that the material was hygroscopic. Dishes, sand, and pestle were dried in an air oven at 100° C. overnight and removed to a desiccator the following morning previous to use.

The corn sirup was added to the sand in two ways:

1. According to the A. O. A. C. method (*1*). A sample of sirup was diluted to a known volume, and from it was pipetted a known quantity containing approximately 1 gram of dry substance which was added to the sand in the dish. The dish was then placed on a steam bath in order to remove the excess water, and the contents were stirred with the pestle until the sand became stiff. It was then removed to a vacuum oven and dried to constant weight.
2. Direct addition to the sand. The approximate amount of sirup was run on the sand in a dish previously weighed, and then reweighed. The dish was placed in the oven for approximately 15 minutes, after which it was removed and the sirup was thoroughly incorporated with the sand. The dish was then returned. At the end of 4 hours, the first weight was made for moisture loss and thereafter periodically every 2 hours until the loss was nearly constant, after which a longer interval was taken.

A large quantity of corn sirup was obtained and run into 118-ml. (4-ounce) wide-mouthed screw-capped bottles, each bottle being used for a set of tests and discarded, thus eliminating the possibility of moisture loss by evaporation of the stock material. The dextrose equivalent of the sirup was 43.1 with acidity, ash, and protein as above.

The data in Table I show that the moisture by air oven at 100° C. is less than that obtained in a vacuum oven at the same temperature, but equal to or greater than that obtained by the A. O. A. C. procedure for incorporation with the sand employing vacuum at 70° C. The vacuum oven at 100° C., with direct incorporation of the sirup with the sand, gave results at the end of 20 hours equal to those obtained by toluene distillation. The higher results obtained by this method are attributed to the greater surface area produced by stirring the hot sirup in the sand. The resulting material was furrowed and comparatively loose, this structure being maintained during the drying process. Twenty hours, the time necessary to secure a constant weight, is much too long for laboratory procedure, and it was thought that if a greater effective surface area could be produced, a moisture determination could be

TABLE I. MOISTURE OF CORN SIRUP BY DRYING ON SAND

(Moisture by toluene distillation, 19.30%)

Type of Oven	Temperature of Oven	Approximate Weight of Sample	Method of Adding Sirup to Sand	Moisture					
				4 hours	6 hours	8 hours	10 hours	12 hours	20 hours
	° C.	Grams		%	%	%	%	%	%
Vacuum	70	1	A. O. A. C.	17.22	17.57	17.85	17.96	18.07	18.41
	70	1	A. O. A. C.	17.64	17.80	18.01	18.03	18.04	18.39
	100	2	Direct	19.06	19.10	19.22	19.25	19.27	19.27
	100	2	Direct	19.13	19.21	19.27	19.28	19.29	19.31
Air	100	1	Direct	17.62	18.07	18.43	18.63	18.73	18.81
	100	1	Direct	18.56	18.65	18.79	18.84	18.91	18.97
	100	2	Direct	17.77	18.00	18.10	18.17	18.24	18.36
	100	2	Direct	17.80	18.04	18.12	18.25	18.28	18.36

TABLE II. MOISTURE OF CORN SIRUP BY DRYING ON FILTER PAPER

(Moisture by toluene distillation, 19.30%. Temperature of oven, 100° C.)

Type of Oven	Approximate Weight of Sample	Weight of Water Added	Area of Filter Paper	Moisture 4 hours	6 hours	8 hours	10 hours	20 hours
	Grams	*Grams*	*Sq. in.*	%	%	%	%	%
Vacuum	1	1	20	18.75	18.97	19.09	19.13	19.26
	1	1	40	19.07	19.16	19.21	19.22	19.25
	1	1	60	19.14	19.20	19.24	19.26	19.29
	1	1	70	19.15	19.26	19.28	...	19.31
	1	2	70	19.20	19.30	19.30	19.30	19.31
	2	2	40	18.93	19.06	19.14	19.15	19.18
	2	2	60	19.03	19.16	19.17	...	19.28
	2	2	70	19.05	19.19	19.24	19.26	19.29
Air	1	1	40	18.67	18.92	19.01	19.03	19.23
	1	1	60	18.82	19.05	19.08	19.08	19.29
	1	1	70	19.00	19.10	19.18	19.22	19.28
	1	2	70	19.10	19.19	19.24	19.25	19.29
	2	2	70	18.30	18.42	18.50	18.54	19.07

made on corn sirup within 6 or 8 hours, which also would remove the criticism that decomposition occurs through prolonged heating.

Moisture by Drying on Filter Paper

During the investigation for such a condition, the authors considered various proposals but finally settled on one which has given highly satisfactory results.

The sirup was diluted and dried on filter-paper coils (Figure 1), held together with a paper clip. The coils were made by rolling a strip of filter paper onto a corrugated phosphor-bronze separator, the paper being longer and wider than the separator, in order that the ends and the outer layers of the coils might be made of filter paper. The filter paper must show a pH of less than 7 when an indicator is applied to it. This precaution is sufficient when corn sirup is dried, since this material contains a small amount of residual acid and is fairly well buffered, but when pure sugar solutions are dried, it often becomes necessary to acidify the solution slightly with an acid such as lactic in order to prevent decomposition. Several types of filter paper were examined and Whatman No. 1 proved to be the most satisfactory.

APPARATUS. Filter paper. A strip of crepe filter paper 4.375 × 50 cm. (1.75 × 20 inches) with a pH of less than 7.

Separator. Corrugated strip of phosphor-bronze 1.25 × 40 cm. (0.5 × 16 inches) No. 36 B. & S., made by running the original strip through small-size meshed gears.

Weighing bottle. Medium form, 40 × 65 mm. with a 40/20 standard-taper ground-glass stopper.

Paper clip. Common type, to hold the filter paper in position with reference to the metal separator.

PROCEDURE. A paper coil and weighing bottle were dried in an air oven at 100° C. for 6 hours, cooled in a desiccator, and weighed. The weighing bottle must always be closed with its stopper before it is removed from the oven to the desiccator, in order to prevent the adsorption of moisture not only by the filter paper itself but also when it is covered by corn sirup, which is quite hygroscopic. The coil was removed, approximately 1 gram of sirup was run into the weighing bottle, and the exact weight was obtained. To this sirup was added 1 or 2 ml. of distilled water; the bottle was warmed, and the water and sirup were mixed to form a dilute solution. The paper coil was then introduced and allowed to absorb the sirup solution. The last trace was taken up by shaking the coil across the bottom of the weighing bottle. The weighing bottle should not be placed in the oven until the filter paper has thoroughly absorbed the sirup, as evidenced by the disappearance of excess sirup where the edge of the filter paper touches the bottom of the weighing bottle.

The steps in the determination of the proper amount of sirup for filter paper are shown in Table II. In computing the area of the filter paper, both sides are counted as effective.

Table II shows clearly that as the area of the filter paper per gram of sirup increases, the time for effecting a moisture determination correspondingly decreases. With 451 sq. cm. (70 sq. inches) and 1 gram of sirup, the time for a test should not exceed 6 hours in a vacuum oven at 100° C. Again, the moisture content is found to be equal to that obtained by toluene distillation.

The above procedures were applied to "70" sugar. The dextrose equivalent of the sample used was 80.8; otherwise the sugar conformed to the general specifications above. The data obtained are similar to those for corn sirup and are found in Table III.

Conclusions

The A. O. A. C. method does not seem particularly adapted to corn sirup and sugar for accurate work.

The usual moisture determinations of corn sirup and sugar on sand in an air oven are invariably low.

Moisture determinations of corn sirup and corn sugar on sand in a vacuum oven can be made satisfactorily, provided sufficient time is taken and the ratio of sample to sand is correct. The latter involves dish size.

Moisture determinations of corn sirup and corn sugar by means of the proposed filter paper method are more accurate and much more rapid.

Moisture determinations on corn sirup by the toluene distillation method, although higher than the A. O. A. C. method, have the same precision as vacuum oven methods at 100° C., provided the latter are carried out under conditions adaptable to the viscous characteristics of corn sirup. However, both these methods are subject to the general criticism that both temperatures might produce decomposition, which could be avoided at lower temperatures.

TABLE III. MOISTURE OF CORN SUGAR

(Moisture by toluene distillation, 17.45%. Vacuum oven used)

Temperature of Oven	Approximate Weight of Sample	Method of Adding Sirup to Sand	Weight of Water Added	Area of Filter Paper	Moisture 4 hours	6 hours	8 hours	10 hours	20 hours
° *C.*	*Grams*		*Grams*	*Sq. in.*	%	%	%	%	%
		By Drying on Sand							
70	1	A. O. A. C.			17.00	17.11	17.14	17.15	17.30
	1	A. O. A. C.			16.90	17.02	17.08	17.11	17.22
100	2	Direct			17.25	17.28	...	17.30	17.42
	2	Direct			17.30	17.31	...	17.31	17.38
		By Drying on Filter Paper							
100	1		2	70	17.36	17.44	17.44	17.45	17.45
	1		2	70	17.37	17.40	17.42	17.44	17.44

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Determination of Moisture in Sugar Products

Use of Filter-Cel for Corn Sirup

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IN THE belief that the controversy over the moisture content of corn sirup would never be settled until lower temperatures were used, which in turn would require a more complete sample dispersion or a still thinner surface film for drying than was obtained in the filter paper method, it was decided to attempt to apply Filter-Cel, used in the toluene distillation as a dispersing medium, to a straight vacuum-oven method, thereby not only obtaining a very large surface area but also employing a larger sample weight. The technique proved unusually successful, and since the procedure has been adopted as official for the corn products industry by the Technical Advisory Committee of the Corn Industries Research Foundation, it is described here in detail.

TABLE I. DATA ON CORN SIRUP

Time	Moisture Found at 100° C. 10.6382-gram sample	10.0545-gram sample
Hours	%	%
8	14.89	14.87
18	14.98	15.03
39	14.99	15.02
	At 60° C.	
	10.5626-gram sample	10.6021-gram sample
15	14.55	14.54
38	14.88	14.89
60	14.96	14.92
100	14.98	14.96

Materials and Apparatus

FILTER-CEL. A large quantity of Filter-Cel is washed by percolation with distilled water that has been slightly acidulated with hydrochloric acid. This treatment is continued until the effluent is acid to litmus. Washing with distilled water follows until the effluent is essentially neutral, and the Filter-Cel is then air-dried. A quantity, usually a quart, is transferred to an air oven at 105° C. and kept for use.

SAMPLE CONTAINERS. For the determination, two types of apparatus are used—one for referee samples, or those requiring the highest degree of precision, and one for factory or routine procedure.

REFEREE SAMPLES. A 250-ml. Pyrex Erlenmeyer or wide-mouthed Erlenmeyer flask, neck ground to 40/50 standard taper is used. This is the same flask that is used for distillation tests. The closure is a 40/12 standard-taper weighing bottle stopper.

ROUTINE SAMPLES. A 7.5-cm. (3-inch) aluminum dish with deep slip cover, which is sold as a small crucible desiccator, or 1-pound friction-top tins in use in corn products plants for packaging of mixed table sirups are used.

STIRRER. The stirrers or pestles are 100 × 15 mm. Pyrex test tubes. The stirrer extension is a glass rod, fitted at one end with two rubber rings, cut from tubing and so spaced that when inserted into the test tube a rather snug fit is obtained at top and bottom of tube. The rod, with its glass test tube end, makes an easily manipulated stirrer. The rod is removed after the stirring operation, leaving the test tube with the Filter-Cel sample mass.

Procedure

Filter-Cel (25 grams) is run in duplicate flasks, the test tube is added, and the stopper is set at a 90° angle in the mouth of the flask, which is brought to constant weight in the vacuum oven at the temperature at which the drying test is to be carried out. A third flask, with stopper only, is used as a tare in weighing and put through the same procedure as flasks used for samples.

Because of surface moisture adsorption, it was found desirable to use individual desiccators: 5- or 10-pound friction-top cans, commonly used for packaging mixed table sirup. The desiccant is phosphorus pentoxide.

The sample weight always is taken so that 5 to 8 grams of solids result. Samples below 35° Bé. are handled by means of a pipet (from which the tip has been cut off) by running approximately 20 to 40 ml. of solution on the Filter-Cel, stoppering, and reweighing for sample weight. For samples over 35° Bé., 8 to 10 grams are weighed in a nickel scoop, to which 10 ml. of distilled water are added. The scoop is warmed on a steam bath to facilitate the formation of a homogeneous sirup, which is run onto the Filter-Cel. The scoop is washed successively with three 5-ml. portions of distilled water. The Filter-Cel–sirup mixture is then gently worked to a damp mass. This is an important step, as the ratio of Filter-Cel to sample plus washings must always yield a damp mass, so that the Filter-Cel retains its powdered form. A wet mass will produce a concretelike structure after drying.

In order to remove the excess water, the flasks are then placed in a vacuum oven maintained at a pressure of 50 to 75 mm. by a water pump. This usually requires 2 to 4 hours. The flasks are removed, the Filter-Cel mass is quickly reworked to a powder, and the flasks are replaced in the vacuum oven, this time actuated by a Hyvac or Megavac pump. It is important to rework at an early stage, as the mass is then very friable and easily handled, whereas it may become difficult if left until the final stage of dryness. Constant weight—i. e., within 1 mg. or 0.01 per cent—is usually obtained in 15 hours or more, depending upon the original condition of the Filter-Cel mass.

The precision by this method is surprisingly good, checks often agreeing in the second decimal place. The dispersion of the sample is so complete that lower temperatures in the vacuum oven can be used for drying, a particularly valuable technique with heat-sensitive materials. Some idea of results obtainable can be gained from experimental data (Table I) on a sample of 45° Bé. corn sirup, which is nearly a plastic at room temperature, and from which a sample can be obtained with difficulty.

The method has been in successful use in the corn products industry for 3 years.

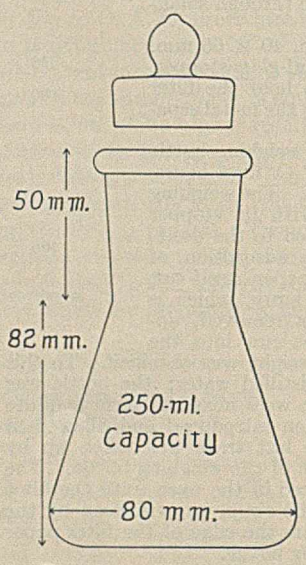

FIGURE 1. APPARATUS

Weighing bottle ground-glass stopper $\frac{\text{S}}{}$ 40/12. Standard flask used in toluene distillation method for corn sirup $\frac{\text{S}}{}$ 40/50. 15 × 100 mm. test tube

Objections to Filter-Cel

It is difficult to bring to constant weight. The material used here did not present this problem, as it had been calcined by the manufacturer and washed as described above.

It is prone to dust under vacuum or during manipulation. This was not found to be the case when used with reasonable care.

It is a good heat insulator and hence permits only a slow heat transfer to the sample. The mass is wet in the initial stages of drying and the heat transfer is good, while in the latter stages, as the Filter-

TABLE II. HYGROSCOPICITY OF FILTER-CEL

Elapsed Time, Hours	Gain in Weight at Relative Humidities of: 35%	45%	52%	66%	78%
5	0.020	0.031	0.036	0.041	0.075
15	0.028	0.041	0.041	0.058	0.085
72	0.039	0.048	0.048	0.063	0.096
119	0.060	0.071	0.079	0.096	0.12
143	0.060	0.071	0.079	0.096	0.13
167	0.060	0.071	0.079	0.096	0.13

Cel mass becomes drier, the heat transfer is slower. A maximum recording thermometer placed in the mass showed that approximately 1 hour was required for the center to reach the oven temperature, either 70° or 100° C. Pieces of fine copper wire (1.25 to 2.5 cm., 0.5 to 1 inch) incorporated with the Filter-Cel did not sufficiently improve the heat transfer to warrant their use. The heat transfer can be improved in a vacuum oven by soldering the tray to the wall support.

It is hygroscopic. The Filter-Cel used had a notable lack of hygroscopicity, despite its large surface area. This was shown by placing 10-gram samples of Filter-Cel in aluminum moisture dishes (25 × 75 mm.) and bringing them to constant weight in a vacuum oven at 100° C. The dishes were then transferred to humidity chambers with relative humidities of 35, 45, 52, 66, and 78 per cent (average temperature 30° C.). The dishes were weighed periodically, the mass being stirred after each weighing. Equilibrium was reached in 119 hours, as shown in Table II.

Adaptation of Filter-Cel Method

The Filter-Cel method can be adopted to usual routine conditions.

Ten grams of prepared Filter-Cel were run into aluminum moisture dishes (25 mm. in height, 75 mm. in diameter) and brought to constant weight. Five grams of corn sirup, weighed in a nickel scoop, were diluted with 5 ml. of distilled water and run onto the Filter-Cel. The scoop was then washed with three successive 2-ml. portions of distilled water which were added to the Filter-Cel. These proportions yielded a damp workable mass which, after thorough incorporation of sirup and Filter-Cel, was dried in the vacuum oven at 100° C.

The results in five dishes run simultaneously were as follows:

Per Cent Moisture	
42.0 dextrose equivalent	55.0 dextrose equivalent
19.46	20.48
19.46	20.49
19.44	20.50
19.49	20.47
19.44	20.49

These results show satisfactory precision. However, considerable patience and care are necessary to obtain checks within 0.1 per cent.

The success of the method at 60° C. suggested that the drying might be carried out at still lower temperatures if suitable apparatus could be devised to handle the Filter-Cel method under higher vacuums than obtainable in a vacuum oven. This evidence would provide the final proof of the stability of corn sirup at temperatures of usual moisture determinations.

The experimental work went through several stages of development. The design which has proved most successful from the standpoint of manipulation is shown in Figure 1.

A review of the literature shows that a similar problem confronted Lobry de Bruyn and Van Laent (*3*). Their apparatus was essentially two small flasks, connected by a curved glass tube employing rubber tubing joints. One flask contained the sample and the other phosphorus pentoxide. Vacuum was applied to the apparatus through a stopcock sealed in a curved tube. Brown, Morris, and Millar (*1*), Rolfe and Faxon (*4*), and later Walker (*6*) applied the device. None of these investigators had employed dispersion of sample in the use of the apparatus.

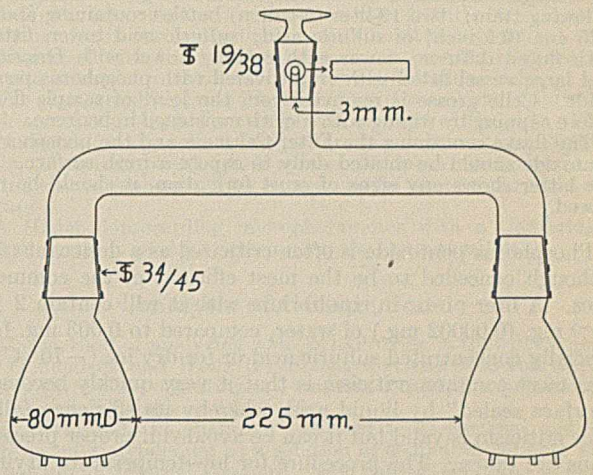

FIGURE 2. IMPROVED APPARATUS

The basic idea of the original authors was reincorporated in a new design, which made possible the application of the Filter-Cel method to the sample in one of the flasks. Several designs were tried, and from the experience gained a type was developed which has proved exceptionally easy to manipulate and, through standard tapered joints, holds high vacuums for extended periods without recourse to the vacuum pump (Figures 2 and 3).

Procedure with New Apparatus

The Filter-Cel and flasks are prepared as for the procedure at 100° C. Success in drying is obtained by having sufficient water present in the sample, so that a damp powder can be worked easily with the pestle.

The flasks containing the damp Filter-Cel mass are placed in a Weber vacuum oven at 38° C. (100° F.) which is connected, through a receiver containing a large quantity of calcium chloride, to a Hyvac pump and allowed to remain there overnight. The next morning the Filter-Cel mass in the flask is reworked to a fine powder, phosphorus pentoxide is run into the other flask, the flask joints are lubricated with a small amount of Cello grease, and the apparatus is connected to a high-vacuum pump, until the system is thoroughly pumped down. The apparatus is dis-

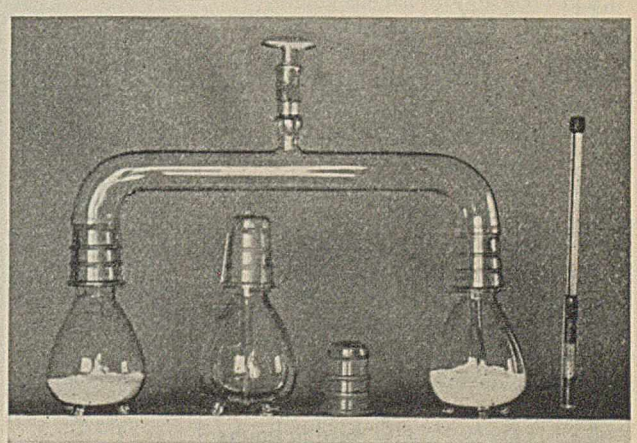

FIGURE 3. PHOTOGRAPH OF APPARATUS

connected from the pump, the flask containing the Filter-Cel mass is placed in an air bath at 37–38° C. (100° F.), and the other flask in a bath of running water for cooling, in order to maintain a temperature drop through the apparatus.

The vacuum on the apparatus is checked daily by means of a McLeod gage, and repumped when necessary. For weighing, the vacuum is released by carefully introducing air through the following train: two 19-liter (5-gallon) bottles containing about 1.25 cm. (0.5 inch) of sulfuric acid; sulfuric acid tower, fitted with fluted diffuser; tower with cotton; tower with Drierite; and large vessel fitted with trap covered with phosphorus pentoxide. Cello grease is removed from the joint of sample flask before capping by wiping with a cloth moistened in benzene.

The flasks containing the Filter-Cel mass and the phosphorus pentoxide should be rotated daily to expose a fresh surface. If the latter shows any signs of crust formation, it should be renewed.

Phosphorus pentoxide is often criticized as a desiccant (*5*), although conceded to be the most efficient of the common ones. A liter of air in equilibrium with it will contain 2×10^{-5} mg. (0.00002 mg.) of water, compared to 0.003 mg. for specially concentrated sulfuric acid or for dry ice (−70° C.). The most common criticism is that it very quickly becomes "surface sealed" by liquid acid, whereby its efficiency falls. This criticism is valid but it can be avoided if proper precautions are taken. The procedure for low-temperature drying (38° C.) recognized the desirability of maintaining a fresh surface of phosphorus pentoxide. In the first place, it was not used until evacuation had reduced the moisture as far as possible. The phosphorus pentoxide was changed frequently and between drying periods the flask was tapped or rotated so as to expose a fresh surface.

The method proved to be most successful and the technique is easily acquired. Some idea of the data obtained can be observed from Table III.

Other typical data on corn sirup, employing 100° C. in the vacuum oven by the Filter-Cel method are given in Table IV.

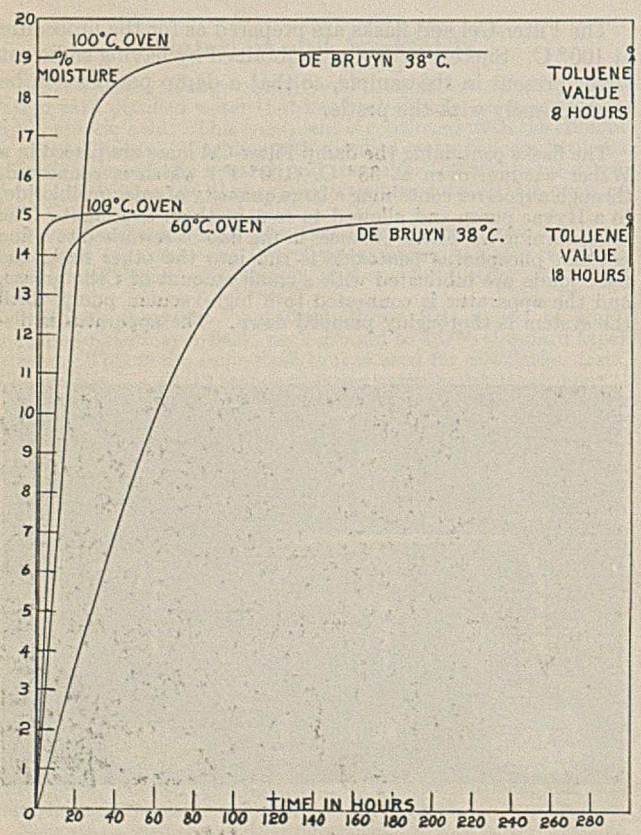

FIGURE 4. DATA ON CORN SIRUP

TABLE III. DETERMINATION OF MOISTURE

(Material, Corn Sirup, Commercial Baumé 43.22°, D. E. 42.6)

Elapsed Time	Conditions		Moisture 10.3065-gram sample	Moisture 10.1260-gram sample
Hours			%	%
	Filter-Cel with 100° C. Vacuum Oven			
6	27-inch water vacuum and sample reworked			
9	Hyvac pump		19.14	19.17
34	Hyvac pump		19.20	19.17
66	Hyvac pump		19.22	19.19
	Lobry de Bruyn–Filter-Cel Method, 38° C. (100° F.)			
				9.4712-gram sample
27	Vacuum oven			17.13
48	Lobry de Bruyn pressure	40 mm.		18.72
92	Lobry de Bruyn pressure	2 to 4 mm.		19.00
113	Lobry de Bruyn pressure	<0.1 mm.		19.14
157	Lobry de Bruyn pressure	<0.1 mm.		19.14
177	Lobry de Bruyn pressure	<0.1 mm.		19.20
222	Lobry de Bruyn pressure	<0.1 mm.		19.20
	Flask transferred to vacuum oven at 100° C.			
227	Hyvac pump			19.20
	Lobry de Bruyn–Filter-Cel Method, 38° C. (100° F.)			
				8.8289-gram sample
20	Vacuum oven			18.38
41	Lobry de Bruyn pressure	<0.2 mm.		19.02
69	Lobry de Bruyn pressure	<0.2 mm.		19.10
150	Lobry de Bruyn pressure	<0.2 mm.		19.16
216	Lobry de Bruyn pressure	<0.2 mm.		19.19
373	Lobry de Bruyn pressure	<0.1 mm.		19.22
	Flask transferred to vacuum oven at 100° C.			
378	Hyvac pump			19.22

Two further examples are shown in Figure 4, one a 43° Bé. and the other a 45° Bé. grade corn sirup.

Conclusions

New methods of dispersion of viscous sirups for standard vacuum oven procedure and of drying at low temperatures have been developed.

The stability of corn sirup at 100° C. in vacuum and at 112° C. with boiling toluene has been demonstrated by essentially identical results obtained through drying at 38° C. under low pressure.

The moisture-Baumé–dextrose equivalent relationship as shown in the original Fetzer-Evans (*2*) tables is correct.

Filter-Cel (diatomaceous earth) is an excellent medium for dispersal of viscous materials.

TABLE IV. DETERMINATION OF MOISTURE

Elapsed Time	Pressure	Sample Weight		Moisture	
Hours	*Mm.*	*Grams*	*Grams*	%	%
Commercial Baumé 39.68° (Bé. = Bé. 140°/60° F. + 1.00° Bé.), D. E. 55.0. Stream of air passing through oven, 2 hours. Small stream of dry air passing through oven, 2 hours.					
4	...	11.2014	12.2193	24.85	23.13
15	<1			25.41	25.44
22	<1			25.43	25.44
37	<1			25.45	25.45
44	<1			25.45	25.45
	Commercial Baumé 41.03°, D. E. 42.0				
4	Through calcium chloride trap, Megavac pump	12.2920	11.7820	23.48	23.40
19	<1			23.60	23.60
24	<1			23.61	23.62
39	<1			23.62	23.63

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(1) Brown, H. T., Morris, G. H., and Millar, J. H., *J. Chem. Soc. Trans.*, 71, 76 (1897).
(2) Fetzer, W. R., and Evans, J. W., IND. ENG. CHEM., ANAL. ED., 7, 41 (1935).
(3) Lobry de Bruyn, C. A., and Van Laent, F. H., *Rec. trav. chim. Pays-Bas*, 13, 218 (1894).
(4) Rolfe, G. W., and Faxon W. A., *J. Am. Chem. Soc.*, 19, 698 (1897).
(5) Snyder and Sullivan, IND. ENG. CHEM., 16, 1164 (1924).
(6) Walker, P. H., *J. Am. Chem. Soc.*, 29, 541 (1907).

Spectrochemical Analysis of Alkali Products

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THIS laboratory was installed for making daily control analyses of sodium hydroxide, soda ash, sodium bicarbonate, and sodium chloride brine. Additional quantitative analyses are made on precipitated calcium carbonate, calcium hydroxide, and calcium chloride.

The analyses use the internal control method (*3*, *4*), in which an element not already present in the solution to be analyzed is added in a fixed and constant amount and the spectral lines of this element are used for control. The relative intensities of the line of the element to be tested and a line of the control element are measured and the log ratio between them is determined. Because the control element is present in a constant amount, the ratio between the lines is a function of the test element only.

Working curves are made by plotting the log of the ratio of the line intensities against the log of the concentration of the impurity, giving a linear function.

Equipment

A Bausch & Lomb Littrow spectrograph equipped with quartz optics is used. A quartz condensing lens is placed between the light source and the slit of the spectrograph to shorten the exposure time. An image of the arc is focused on the collimator lens.

Two fixed slits (30 and 40 microns wide) mounted on a single slide are used in place of the adjustable slit on the spectrograph; because of the constant changing of the slit width, the adjustable slit on the instrument became inaccurate and required frequent checking. The slide, containing the two fixed slits, is so mounted that either slit opening can be placed directly in front of the adjustable slit, open at 1.5 mm. This wide opening of the adjustable slit is used when placing the calibrating pattern on the plate. The 40-micron slit is used for spectrograms in the ultraviolet, while the 30-micron slit is for the visible region of the spectrum.

The arc source is the high-voltage arc developed by Duffendack and Thomson (*1*), and is capable of operating up to 5 amperes at 2300 volts or 2.2 amperes at 4600 volts. A small inductance is placed in the primary side of the circuit.

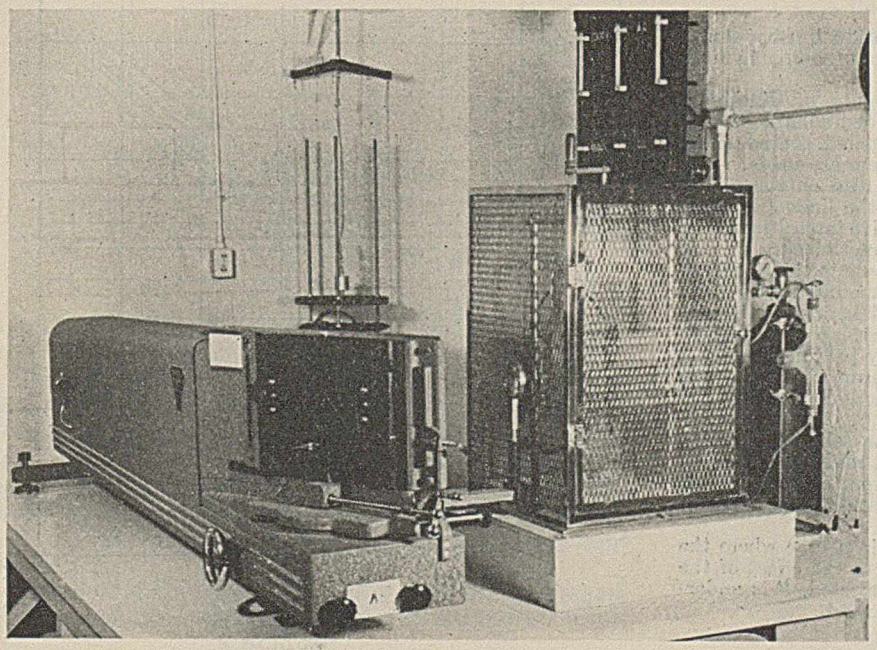

FIGURE 1

The arc stand is the same design as that used by Duffendack and Wolfe (*2*). Figure 1 shows the stand, with other parts of the source in the background. A 110-volt direct current arc is connected with the same stand and can be used in place of the alternating current source by throwing a single switch.

The photographic plates (Eastman Polychrome) are calibrated with a rotating stepped sector placed in front of the slit. The light source for the calibrating pattern is a high-voltage (20,000 volts, 25 milliamperes) spark across aluminum electrodes in a hydrogen atmosphere. This source can be seen to the right in Figure 1.

A Hilger nonrecording microphotometer with a Cambridge galvanometer is used for determining the line intensities.

Analysis of Caustic Soda

The method of analyzing sodium hydroxide is that developed by Duffendack and Wolfe (*2*), except that the 2300-volt alternating current arc operating at 5 amperes is used after preburning the coated electrodes at 2.5 amperes.

The sample to be analyzed is diluted to 25 per cent and 25 p. p. m. of molybdenum as a solution of sodium molybdate are added for an internal standard. For caustic solutions weaker than 25 per cent, the sample is diluted to 10 per cent and 10 p. p. m. of molybdenum are added. Separate sets of calibration curves are necessary for the 25 and 10 per cent solutions.

The coating and burning procedures have been described (*2*).

Calibration curves for silicon, aluminum, calcium, magnesium, iron, manganese, copper, nickel, strontium, and lead have been completed.

Because of the small amount of iron and manganese present, the faint lines are influenced by the background of the spectrum. This effect was overcome by using a faint molybdenum line (2649.5) for standardizing. In this manner both the impurity line and the molybdenum line are equally affected by the background.

In the determination of nickel and copper in sodium hydroxide, the heavy background around the principal impurity lines was again a serious problem. There is no faint molybdenum line near to calibrate against, which will correct for a change in background intensity. The changing background will shift the bottom of the calibration curve in the manner shown in Figure 2. The resulting error may be as great as 50 per cent in the range from 0.8 to 2.5 p. p. m. of nickel.

This error was overcome by determining the log ratio of the line with the background next to the line instead of using a molybdenum line. Such a calibration is used only in the lower range of analysis where a correction of background is necessary.

Preparation of Working Curves

Working curves for analysis are determined by running the prepared solutions, in which the amounts of the test elements are varied, over the range of analysis desired. The lines of the test element and the internal standard are read and the log ratios of the various concentrations are determined.

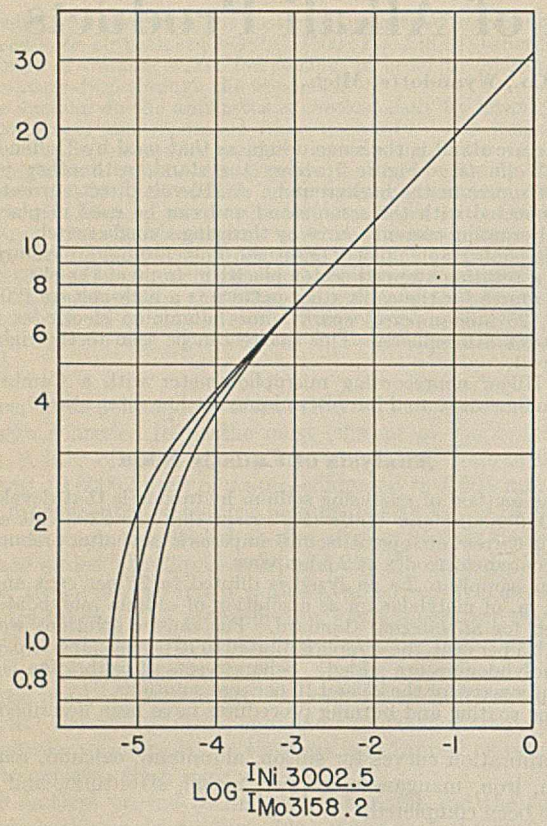

FIGURE 2

The test element is added to a solution of the purest product obtainable to keep the residual amount of the element to a minimum. In many cases this minimum amount is in the range of analysis desired. Therefore, it is desirable to determine this residual amount as accurately as possible. This is not always possible by chemical methods because of the small amount of the element present.

The procedure used for determining the residual, using aluminum as the impurity in a solution of 25 per cent of sodium hydroxide as an example, is as follows:

The solutions, containing varying amounts of aluminum (beginning with a solution containing no added aluminum) and the constant amount of molybdenum, are prepared, coated on the electrodes, and burned, and the spectrograms are made. The lines and the plate-calibrating pattern are read on the microphotometer and the relative intensities of the lines are determined. Then the log differences of the relative intensities of the aluminum line and the molybdenum line are determined.

A value is assumed for the residual aluminum in the first solution and this is added with the additional amounts in the other solutions. These concentrations are plotted against the respective log intensity ratios, using semilog paper. If the assumed quantity of aluminum is higher than actually present, the curve will bend upward (Figure 3, A). If the assumed value is low, the curve will bend down, *B*. When the actual amount is assumed, the curve will become a straight line, *C*.

By overcorrecting and undercorrecting the calibrating curve, and approaching the corrected value with a series of curves on both sides (Figure 3, *E*), the amount of the impurity in the sample used for calibration can be determined to within 20 per cent of the actual amount present.

This method of correction is shown also in a case where the lower end of the curve bends down, owing to the effect of the background (Figure 4). Curve *A* is overcorrected, *B* is undercorrected, while in *C* the correct value is assumed.

This method of preparing working curves is based on the linear relation obtained when plotting the log ratio of the in-

TABLE I. PRECISION AND RANGE OF ANALYSES

	Spectral Lines Impurity	Mo	Range of Analysis	Deviation from Mean
	Å.	*Å.*	%	%
		25% Sodium Hydroxide		
Si	2881.6	3158.2	0.0005 –0.0050	3.2
Si	2987.7	3158.2	0.0190 –0.0700	4.2
Al	3092.7	3158.2	0.00015–0.0010	2.0
Al	3082.2	3158.2	0.0002 –0.0015	2.6
Al	2660.4	3158.2	0.0010 –0.0040	4.0
Ca	3968.5	3903.0	0.0001 –0.0015	4.5
Mg	2802.7	2816.2	0.0004 –0.0010	4.3
Fe	2483.3	2649.5	0.00007–0.0005	5.0
Mn	2794.8	2649.5	0.00002–0.00005	3.0
Mn	2794.8	3158.2	0.00005–0.0001	1.8
Cu	3247.6	[a]	0.00004–0.0002	2.5
Ni	3002.5	[a]	0.00008–0.0006	2.6
Ni	3050.8	[a]	0.00008–0.0006	3.2
Sr	4077.7	3903.0	0.0001 –0.0020	7.8
Pb	2833.1	3158.2	0.00002–0.0003	8.0
		10% Sodium Hydroxide		
Si	2881.6	3158.2	0.0002 –0.0030	2.2
Al	3092.7	3158.2	0.00005–0.0006	4.6
Al	3082.2	3158.2	0.0001 –0.0015	3.9
Ca	3006.9	3158.2	0.0010 –0.0110	6.8
Mg	2779.9	3158.2	0.0001 –0.0036	7.4
Mg	2783.0	3158.2	0.0009 –0.0050	6.9
Fe	2483.3	3158.2	0.00005–0.0002	7.3
Mn	2794.8	3158.2	0.00002–0.0002	3.0
		10% Sodium Carbonate		
Si	2881.6	3208.9	0.00006–0.0005	5.9
Ca	3006.9	3208.9	0.0004 –0.0050	7.6
Al	3092.7	3208.9	0.00001–0.0003	5.2
Mg	2779.9	3208.9	0.0002 –0.0020	5.6
Mg	2783.0	3208.9	0.0002 –0.0020	5.5
Fe	3020.7	3208.9	0.00004–0.0004	6.6
		20% Sodium Chloride		
Mg	2802.7	2816.2	0.00006–0.0006	3.1
Ca	3968.5	3903.0	0.0001 –0.0007	4.8

[a] Background next to line.

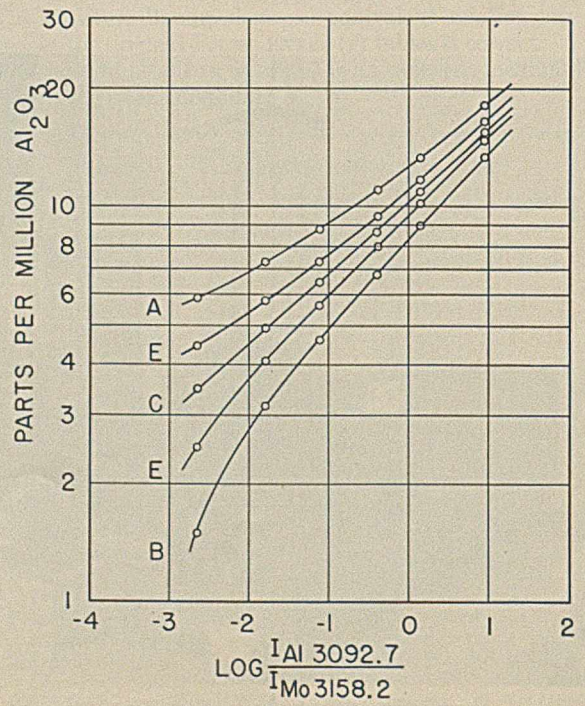

FIGURE 3

TABLE II. ANALYSIS OF SODIUM HYDROXIDE

Analysis No.	Iron	Deviation[a]	Manganese	Deviation	Magnesium	Deviation	Silicon	Deviation	Aluminum	Deviation
	P. p. m.	%	P. p. m.	%	P. p. m.	%	P. p. m.	%	P. p. m.	%
1	2.0	4.2	0.36	2.2	3.7	8.0	19	5.6	4.9	3.2
2	1.9	1.0	0.39	6.0	4.2	12.6	18	0.0	5.2	2.8
3	1.85	3.6	0.36	2.2	3.4	8.8	18	0.0	5.1	2.0
4	1.9	1.0	0.36	2.2	3.3	11.5	17	5.6	4.9	3.2
5	2.0	4.2	0.36	2.2	3.5	6.2	18	0.0	5.2	2.8
6	1.95	1.6	0.37	0.5	4.0	7.2	18	0.0	5.0	1.2
7	1.9	1.0	0.37	0.5	3.3	11.5	18	0.0	5.1	0.8
8	2.15	11.4	0.38	3.2	3.9	4.6	19	5.6	5.2	2.8
9	1.95	1.6	0.36	2.2	3.6	3.5	18	0.0	5.1	0.8
10	1.7	11.4	0.36	2.2	3.5	6.2	17	5.6	5.1	0.8
11	1.85	3.6	0.37	0.5	3.9	4.6	18	0.0	5.0	1.2
12	1.9	1.0	0.37	0.5	3.8	1.9	18	0.0	5.0	1.2
13	2.0	4.2	0.36	2.2	4.0	7.0	18	0.0	4.9	3.2
14	1.95	1.6	0.37	0.5	3.9	4.6	18	0.0	5.2	2.8
15	1.7	11.4	0.37	0.5	3.9	4.6	18	0.0	5.0	1.2
16	1.95	1.6	0.37	0.5	3.8	1.9	18	0.0	5.0	1.2
	Av. 1.92	4.0	0.368	1.8	3.73	6.6	18	1.4	5.06	2.0

[a] % deviation from average.

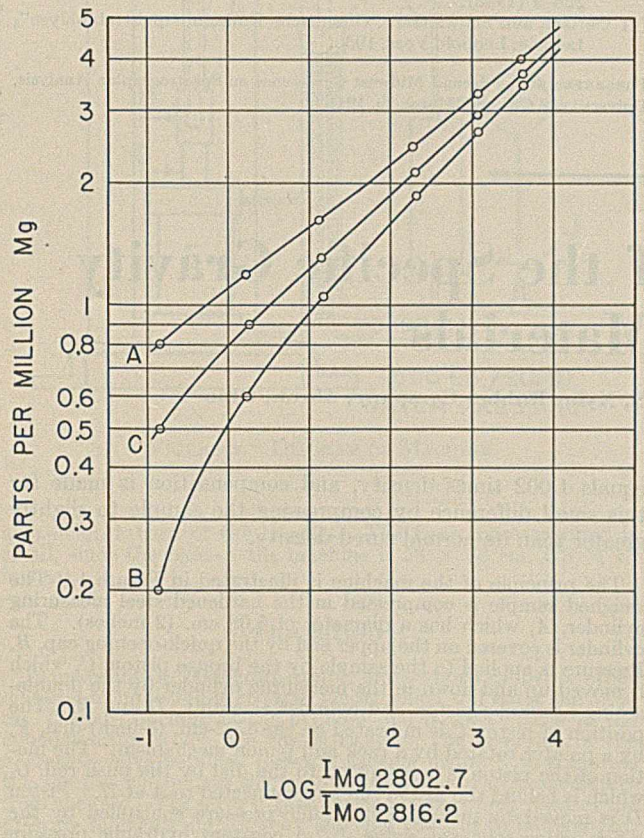

FIGURE 4

tensities of the two lines (impurity and control) against the log of the concentration.

This relationship may be affected by extraneous factors, causing it no longer to hold true. Therefore, it has been customary with this laboratory, when making a new calibration, to calibrate two lines of the impurity so that one line may be checked against the other. However, the only interfering factor encountered in the low concentration range described here has been the effect of the background on the very faint impurity line.

The effect of the background can be overcome to an appreciable extent, by working for the following conditions:

Choose impurity and control lines near each other, so that there is little difference in the background around the lines.

Adjust the concentration of the internal standard, so that the intensity of the control line is near the intensity of the impurity line.

Analysis of Sodium Carbonate and Bicarbonate

The sodium carbonate is dissolved in distilled water to give a 10 per cent solution and 25 p. p. m. of molybdenum standard are added. The electrodes are coated, dried, and burned as in a caustic analysis.

Because of the wide range of analysis covered by various grades of ash, it is necessary to use several lines for some of the elements. The lines used and the range of analysis are shown in Table I.

Sodium bicarbonate is first calcined to sodium carbonate, and then the sodium carbonate procedure and curves are used. If the sodium bicarbonate is burned at 200° C. for 1 hour, there is no loss nor gain of impurities.

Analysis of Sodium Chloride

The sodium chloride comes into the laboratory as a saturated solution (25 to 26 per cent sodium chloride), which is diluted to 20 per cent sodium chloride with 25 p. p. m. of molybdenum as internal standard.

If this 20 per cent salt solution is coated on an electrode and burned, the arc will burn through one spot on the electrode, and after the salt in this spot is gone, the arc burns on bare carbon only. This tends to make very erratic analytical results. It can be avoided by adding sufficient redistilled nitric acid to make the final solution approximately 7 per cent nitric acid. The arc will then wander over the entire face of the electrode. Using this method, very reproducible results are obtained.

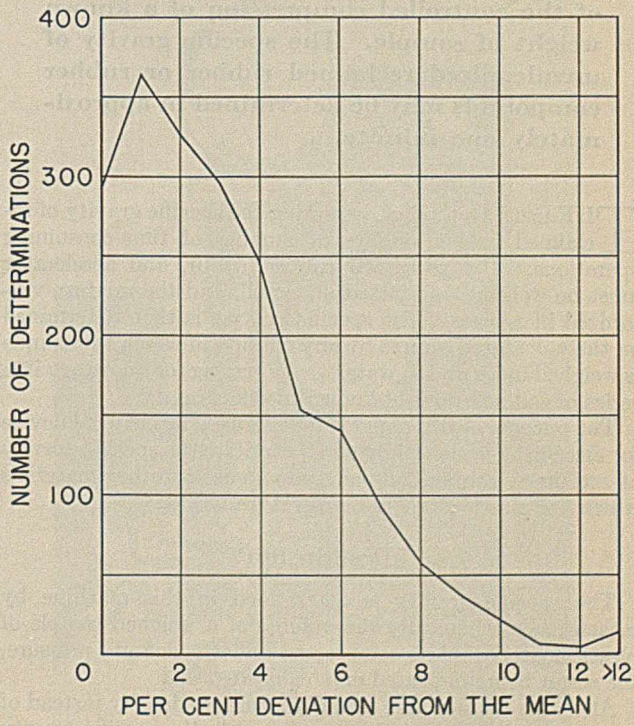

FIGURE 5

Accuracy and Precision

It is possible to determine the amount of impurity in the sample used for calibration to within 20 per cent of the amount present. Therefore, if the sample to be analyzed is as pure as the sample from which the calibration was made, the analysis may be ±20 per cent from the actual amount. This error decreases on analyses made farther up on the calibration curve. If there is again as much impurity in the sample analyzed as in the sample from which the calibration was made, the possible error will be half, or 10 per cent.

The precision of analysis, the range of analysis, and the spectral lines used are shown in Table I.

The precision (per cent deviation from the mean) was calculated from control analyses made in duplicate over a period of 3 months. During this time, 3600 determinations were made.

The distribution of the deviations from the mean for 25 per cent sodium hydroxide is given in Figure 5. Any duplicate determinations having deviations greater than 10 per cent are not accepted and the analysis is repeated. Less than 2 per cent of the analyses lie in this range.

The precision of analysis was also calculated for a sample of 25 per cent sodium hydroxide analyzed sixteen times over a period of 24 days (Table II). No two of these analyses were made on the same photographic plate. Calcium and copper were not determined because a separate exposure is necessary. Nickel, lead, and strontium were below the limit of detection.

Acknowledgment

The author wishes to thank O. S. Duffendack and R. A. Wolfe for suggestions and assistance rendered in the installation and operation of the laboratory and the Michigan Alkali Company for permission to publish the analytical data included in this paper.

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PRESENTED at the Second Midwest Conference on Spectrographic Analysis, University of Chicago, March 29, 1941.

Rapid Determination of the Specific Gravity of Plastic Materials

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The specific gravity of a plastic material is indicated directly by a machine as a result of the controlled compression of a known weight of sample. The specific gravity of unvulcanized reclaimed rubber or rubber compounds may be determined in approximately one minute.

THE usual method of measuring the specific gravity of reclaimed rubber requires a number of time-consuming operations. The reclaimed rubber, sulfur, and accelerator must be weighed and mixed on a mill, and the mixture vulcanized in a press. The specific gravity is then determined on the vulcanized sample by any method in which the sample is weighed in air and in water, or by testing for buoyancy in a series of salt solutions of known specific gravity.

The purpose of this paper is to describe a mechanical device for measuring very rapidly and accurately the specific gravity of reclaimed rubber, rubber compounds, or other materials which are plastic at room temperature.

Description

The specific gravity is determined in this machine by measuring mechanically the volume of a weighed sample of plastic material when compressed under a definite pressure, the result being indicated on a calibrated dial.

Although the machine actually measures density instead of specific gravity, the results correspond to the specific gravity as measured by other methods. Specific gravity at 20° C. equals 1.002 times density, and compensation is made for this small difference by compressing the sample to slightly greater than its normal cured density.

The principle of the machine is illustrated in Figure 1. The weighed sample is compressed in the hardened steel measuring cylinder, *A*, which has a diameter of 5.08 cm. (2 inches). The cylinder is covered on the upper end by the quick-opening cap, *B*. Pressure is applied to the sample by the bronze piston, *C*, which is moved up and down in the measuring cylinder by the double-acting hydraulic piston and cylinder assembly, *D* and *E*. The position of piston *C* is indicated on the 15.2-cm. (6-inch) dial, *F*, by a pointer rotated by a rack and pinion mechanism. The motion of the piston is transmitted to the dial by the push rod, *G*, which is behind the piston rod and connected to it at *H*. Piston *D* is moved by means of hydraulic pressure controlled by the quick-acting four-way valve, *I*. A constant hydraulic pressure of 28.1 kg. per sq. cm. (400 pounds per sq. inch), indicated on gage *N*, is maintained by diaphragm valve *J*, controller *K*, and air cushion *L* to give a pressure on the sample of 112.5 kg. per sq. cm. (1600 pounds per sq. inch). The compressive force on the sample is 2280 kg. (5020 pounds) with no correction for friction loss.

Since density is defined as mass per unit volume and since the weight of the sample and the diameter of the measuring cylinder are constant, the length of the space occupied by the compressed sample varies inversely with its density. This length is indicated on dial *F*, which is calibrated to read specific gravity directly in the range of 0.960 to 1.850 in intervals of 0.010. The divisions range in size from 8.90 to 2.54 mm. (0.35 to 0.10 inch), and the third decimal place is easily estimated over the entire scale.

Convenience and speed of operation were dominant factors in the design of the machine. The indicating dial is at eye level for easy and accurate reading. The compression chamber and operating valve are placed at a convenient working height. The cap for the cylinder is suspended by a spring on a swinging arm fitted with positioning stops so that the cap may be swung into place and out of the way rapidly, as shown in Figures 2 and 3. An interrupted thread allows locking or releasing of the cap with

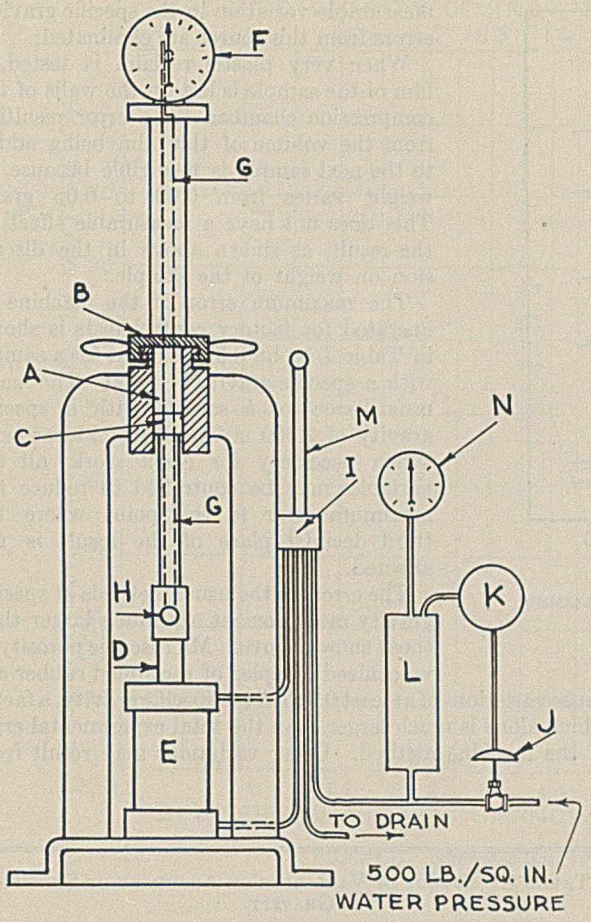

FIGURE 1. DIAGRAM OF MACHINE

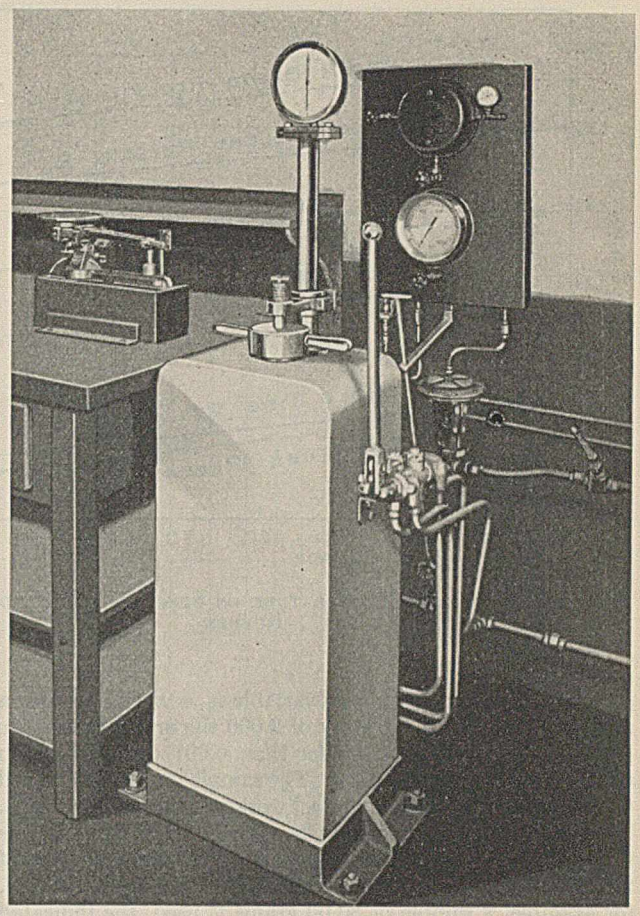

FIGURE 2

a one-eighth turn of the handles. The floor space required is small, since the base of the machine is 58 × 33 cm. (23 × 13 inches).

Operation

In operation, a 100.0-gram sample of reclaimed rubber or other material to be tested is cut into pieces which will enter the 5.08-cm. (2-inch) opening of the cylinder and is then placed in the cylinder, after which the cap is closed. The lever *M*, of the operating valve is pulled forward, causing piston *C* to compress the sample, whereupon the specific gravity is read immediately from the dial. The lever is then pushed back, which releases the pressure momentarily, thereby allowing the cap to be removed. By bringing the lever forward again, the sample is ejected by the piston. The piston is lowered and the machine is ready for the next determination.

The complete operation of weighing a sample and determining the specific gravity is accomplished in approximately one minute. In the case of reclaimed rubber, the sample is frequently hot and must be cooled to room temperature before testing, and this increases the elapsed time of a determination to 10 minutes. With the older methods of measuring the specific gravity of reclaimed rubber, 5 to 10 minutes of working time were required per determination, and the elapsed time was about 30 minutes.

Factors Influencing Accuracy

Several factors influence the accuracy of the machine. The sample must be weighed to 100.00 ± 0.05 grams to eliminate variations from this source. For a specific gravity

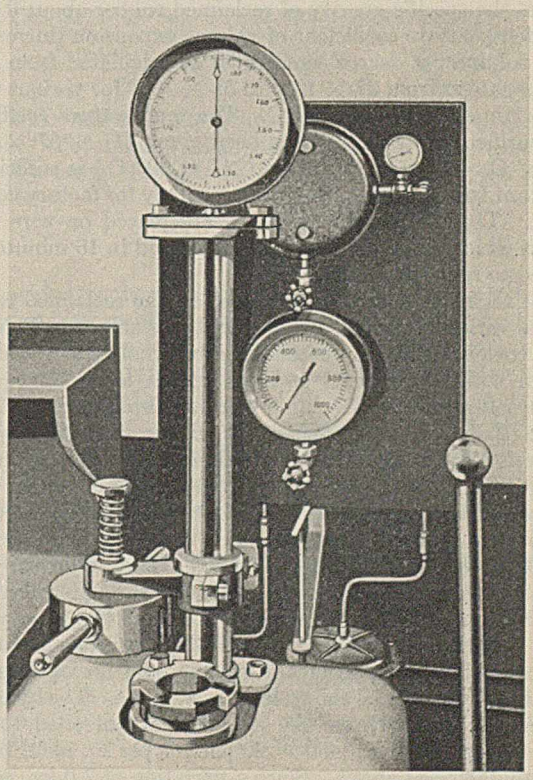

FIGURE 3

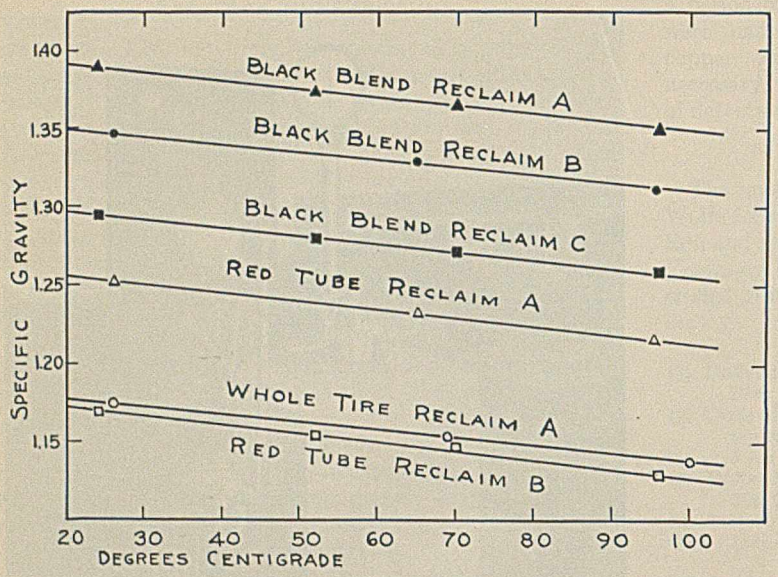

FIGURE 4. EFFECT OF TEMPERATURE ON SPECIFIC GRAVITY OF RECLAIMED RUBBER

of 1.000 the error from this variable is ±0.0005 and for a sample with a specific gravity of 2.000 the error amounts to ±0.001. Since changes smaller than 0.001 cannot be seen on the dial, this error is small. The sample is usually weighed to ±0.1 gram for factory control work in order to save time. The weight of the sample may be changed from 100.00 grams to test materials with a specific gravity outside the range of the dial (0.960 to 1.850) if proper corrections are made. If, for example, a sample of 80.00 grams is used, the indicated reading is multiplied by 0.8.

Temperature has an appreciable effect on the specific gravity of reclaimed rubber, as shown in Figure 4. A rise of 1° C. lowers the specific gravity of reclaimed rubber about 0.0005. The temperature coefficient of volume expansion (increase in unit volume per degree centigrade) as calculated from these curves ranges from 37×10^{-5} to 51×10^{-5} for various types of reclaimed rubber at 25° C. The temperature coefficient for purified crude rubber has been given (*1*) as 67×10^{-5} at 25° C. Room temperature (24° to 30° C.) is sufficiently constant to maintain satisfactory accuracy for factory control work. The samples to be tested are placed on wire trays across which air is circulated by a fan, and in 10 minutes are cooled to room temperature.

Variation in the pressure exerted on the reclaimed rubber causes variation in the specific gravity, as shown in Figure 5. The coefficient of compressibility of whole-tire reclaim is shown within the limits of these curves to be 52×10^{-6} (contraction in unit volume per atmosphere) at 25° C. Scott (*2*) found the coefficient for rubber vulcanized with 3 per cent sulfur to be 48×10^{-6} in the same pressure range. A pressure of 112.5 kg. per sq. cm. (1600 pounds per sq. inch) was selected by experiment as that which compressed the sample to a density corresponding to the specific gravity results obtained with other methods. The air in the compression cylinder is pressed out at the cap through a clearance of 0.025 mm. (0.001 inch), which is too small to pass any rubber. A small amount of air is probably entrapped, but it is compressed to an insignificant volume, and no variations in specific gravity can be shown from this source. The hydraulic pressure which operates the machine is controlled to 28.1 ± 1.4 kg. per sq. cm. (400 ± 20 pounds per sq. inch) by the system described above. Since a variation of 7.0 kg. per sq. cm. (100 pounds per sq. inch) is necessary to cause a measurable variation in the specific gravity, errors from this source are eliminated.

When very plastic reclaim is tested, a film of the sample is left on the walls of the compression chamber. The error resulting from the volume of this film being added to the next sample is negligible because its weight varies from 0.00 to 0.05 gram. This does not have a measurable effect on the result, as shown above in the discussion on weight of the sample.

The maximum error of the machine as operated for factory control tests is shown in Table I to be 0.32 per cent for a sample with a specific gravity of 1.000. The maximum error for a sample with a specific gravity of 2.000 is 0.0042 or 0.21 per cent. When necessary for exact work, all the variables may be controlled to reduce the maximum error to the point where the third decimal place of the result is not affected.

The errors in the usual methods of specific gravity measurement are much larger than those shown above. Microscopic porosity of vulcanized samples of reclaimed rubber can cause variations of at least 0.010 in the specific gravity, a factor which alone is much larger than the total experimental error of the machine method. Other variations may result from

TABLE I. EFFECT OF VARIABLES ON ACCURACY OF SPECIFIC GRAVITY

	Factory Control Tests		Exact Tests	
Variable	Maximum error	Effect on specific gravity	Maximum error	Effect on specific gravity
Weight of sample	±0.1 gram	±0.0010[a]	±0.001 gram	±0.0000
Temperature of sample	±3° C.	±0.0015	±0.5° C.	±0.0003
Hydraulic pressure	±1.4 kg. per sq. cm.	±0.0002	±0.7 kg. per sq. cm.	±0.0001
Residue in cylinder	+0.05 gram	−0.0005	±0.000 gram	±0.0000
Total maximum error	.....	+0.0027 −0.0032	.....	±0.0004

[a] For a sample with a specific gravity of 1.000.

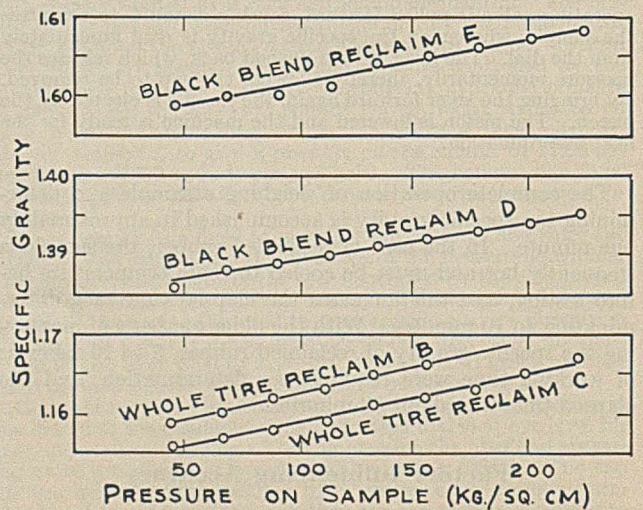

FIGURE 5. EFFECT OF PRESSURE ON SPECIFIC GRAVITY OF RECLAIMED RUBBER

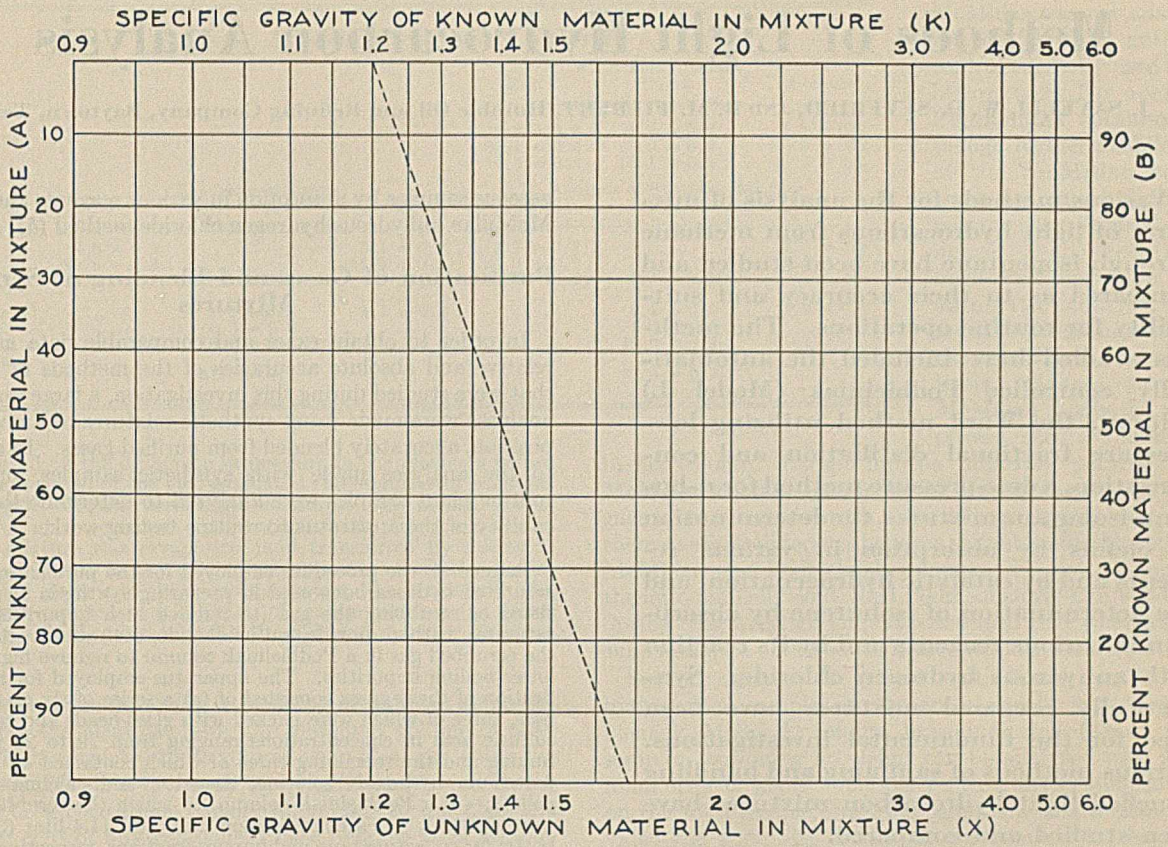

FIGURE 6. SPECIFIC GRAVITY OF A TWO-COMPONENT MIXTURE

$$X = \frac{A}{\frac{100}{M} - \frac{B}{K}}$$

M = specific gravity of mixture (on horizontal lines)

Directions. Mark specific gravity of known component on top scale and specific gravity of mixture on horizontal line which corresponds to percentage composition of mixture. Connect these points with a straight line and read specific gravity of unknown on bottom scale.

Example (dotted line). Specific gravity of known = 1.20. Specific gravity of 50 per cent mixture with unknown = 1.40. Specific gravity of unknown = 1.68.

errors when weighing and mixing the sulfur and accelerator with the sample, from varying vulcanization periods and temperatures, and from incomplete wetting of the vulcanized sample when weighing in water. The method which makes use of a series of salt solutions of known specific gravity is the most dependable and rapid of the older methods for checking the specific gravity of vulcanized rubber samples, but the accuracy is limited to the interval between consecutive solutions, which for practical purposes is usually 0.010. Hence, in these methods a total error of 0.020 is not uncommon.

Applications

This machine can be used to measure the specific gravity of unvulcanized natural or synthetic rubber compounds or devulcanized scrap rubber in process of manufacture without additional treatment. Its value is most apparent when testing materials which ordinarily must be mixed with vulcanizing ingredients to obtain a cured sample for the usual specific gravity methods.

The specific gravity of nonplastic materials such as rubber compounding materials or ground scrap rubber may be determined if mixed with a plastic material such as crude or reclaimed rubber. The plastic material must be present to flow under pressure and fill the voids between the nonplastic particles. It is usually convenient to mix equal parts of the nonplastic material and reclaimed rubber of a known specific gravity on a mill and to test the mixture in the specific gravity machine. The unknown component in such a mixture may be found by using the formula or alignment chart shown in Figure 6.

Summary

A machine for the determination of the specific gravity of reclaimed rubber or other plastic materials has been described. The determination is made on an unvulcanized sample which is compressed to 112.5 kg. per sq. cm. (1600 pounds per sq. inch) in the case of reclaimed rubber and rubber compounds, and the specific gravity is indicated on a dial. The time required for a determination is approximately one minute. The weight and temperature of the sample and the pressure applied to it affect the result, but with proper control of these variables the specific gravity is reproducible to the third decimal place. This is much more accurate than other specific gravity methods for rubber compounds.

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PRESENTED before the Division of Rubber Chemistry at the 101st Meeting of the American Chemical Society, St. Louis, Mo.

Methods of Light Hydrocarbon Analysis

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Various methods for the analysis of mixtures of light hydrocarbons from methane through isopentane have been studied and compared as to their accuracy and suitability for routine operations. The methods studied have included the automatically controlled Podbielniak (Model L) column, the Ward method utilizing low-pressure fractional distillation and condensation, a dew-pressure method for *n*-butane–isobutane mixtures, the determination of olefins by absorption in various reagents and by catalytic hydrogenation, and the determination of isobutene by absorption in various reagents and by its reaction with anhydrous hydrogen chloride. Synthetically prepared mixtures have been used for the fundamental investigations. Various methods of sampling and handling liquefied light hydrocarbon mixtures have been studied and compared.

IN RECENT years, the various light hydrocarbons have assumed an increasingly important role as raw materials and there is every indication that this trend will continue. The application of conversion and polymerization processes for the production of gasoline stocks has been a prime factor in the demand for certain light hydrocarbon stocks of definite composition. Concurrently with these developments, there has been an increasing interest in the reproducibility and accuracy of methods of analysis for these light paraffins and olefins, especially the group of four-carbon-atom hydrocarbons.

The present work has included studies of a number of the more widely accepted methods of analysis. Where results indicated it to be desirable, the methods were modified. In many cases, the methods were subjected to a theoretical analysis of the maximum accuracy to be expected. Consideration was given to accuracy, reproducibility, simplicity, speed, and economy of materials. Synthetically prepared mixtures were used for the fundamental investigations, and studies were made of methods of sampling and handling the mixtures prior to analysis.

The types of apparatus studied for the fractional analysis of light hydrocarbon mixtures were the automatically controlled Podbielniak column, the modified California Natural Gasoline Association's (McMillan) column (*6*), and a modification of the Ward apparatus (*15*) for the fractionation of light hydrocarbons at reduced temperatures and pressures. In conjunction with the study of these apparatus, a dew-pressure apparatus for the determination of isobutane and *n*-butane in their two-component mixtures was developed. Included in the study of methods for unsaturate analysis were the determination of total unsaturates in gaseous samples by absorption in various reagents and by the McMillan catalytic hydrogenation method (*7*), and the determination of isobutene in gaseous samples by absorption in various reagents and by the McMillan anhydrous hydrogen chloride method (*5*).

Purification of Gases and Blending of Synthetic Mixtures

In order to obtain exact and comparable data as to the relative and absolute accuracies of the methods of analysis that were studied during this investigation, a large number of analyses were made using synthetic mixtures of known composition, accurately blended from purified gases. In addition to the analyses made with synthetic samples, numerous routine plant samples were analyzed to determine the applicability of the apparatus to routine testing work.

In general, the procedure employed for the purification of the saturated hydrocarbons used in preparing synthetic blends consisted of scrubbing the gas (to remove such impurities as unsaturates, carbon dioxide, sulfur dioxide, etc.) and fractionating the scrubbed gas in a Podbielniak column to remove higher- and lower-boiling impurities. The apparatus employed for the purification of these gases consisted of (*a*) a series of six glass scrubbers, three of which were packed with glass beads and contained sulfuric acid in concentrations ranging from 79 to 20 per cent fuming and the remaining three of which contained 43 per cent potassium hydroxide solution, Ascarite, and calcium chloride pellets; (*b*) a Podbielniak column, to which the scrubbed gases were charged; (*c*) a condensation bulb and 11.4-liter (3-gallon) bottle for collecting pure and impure fractions, respectively, from the Podbielniak distillation; and (*d*) suitable connections to the storage and blending apparatus and to the vacuum line.

The ethane, propane, isobutane, and *n*-butane used in this work were purified from commercial gases according to the procedures described by McMillan (*6*). Isopentane was purified by a series of prolonged distillations in which the front and tail ends from each distillation were discarded. Isobutene was prepared by contacting *tert*-butyl alcohol and oxalic acid in equal parts by weight in a suitable apparatus equipped with a stirring device and reflux condenser, the evolved gases being repeatedly scrubbed with caustic and condensed before being tested for purity by absorption in 94 per cent sulfuric acid. The 2-butene was prepared by contacting *sec*-butyl alcohol and phosphoric acid, the apparatus and procedure for treating the evolved gases being the same as for isobutene. Constancy of boiling point (within 0.1° C.) after repeated fractionations in which the front and tail ends from each distillation were discarded was the criterion employed for determining the purity of saturated hydrocarbons; in the case of isobutane and *n*-butane, this test was usually supplemented by dew-pressure determinations.

After purification, the gases were transferred to the storage and blending system. This system consisted essentially of four banks of five 11.4-liter (3-gallon) bottles each, all the bottles in each bank being connected to a common manifold, which was in turn connected to the purification system and to the vacuum line. Each bank of bottles was connected to a condensation bulb, a Y-type manometer, and a calibrated receiver (immersed in a water bath) of approximately 2000-ml. capacity. Each receiver was connected through a second manifold to the vacuum line and to the analytical equipment. A Toepler pump and condensation bulb, together with manometers and calibrated receivers, were also connected to this manifold. All apparatus used in the blending and transferring of synthetic mixtures was constructed of fused glass.

When preparing a synthetic mixture, the desired portion of gas in each bank of bottles was transferred to its respective receiver by condensation and revaporization in the condensation bulb. To ensure the removal of any air that may have leaked through stopcocks into the storage bottles during prolonged storage periods, the condensation bulbs were always opened to vacuum for approximately 15 minutes after the gas had been condensed with liquid nitrogen. The molar volume of each gas was determined by manometer readings in the respective receivers, all of which were immersed in a common water bath to eliminate errors caused by possible temperature differences. Inasmuch as all receivers were calibrated with respect to volume, a simple calcu-

TABLE I. AUTOMATIC PODBIELNIAK ANALYSES

	Refinery Sample		
	Run 1	Run 2	Average
	Mole per cent		
Propane	2.3 ± 0.2	1.9 ± 0.2	2.1 ± 0.2
Isobutane	83.1 ± 0.2	83.0 ± 0.2	83.0 ± 0.2
n-Butane	14.6 ± 0.2	15.1 ± 0.2	14.9 ± 0.2

	Synthetic Mixture			Analysis of Synthetic Mixture		
	1	2	3	1	2	3
	Mole per cent			*Mole per cent*		
Propane	2.6 ± 0.2	....	3.1 ± 0.2	2.7 ± 0.2	....	3.2 ± 0.2
Isobutane	80.8 ± 0.2	....	95.8 ± 0.2	80.6 ± 0.2	....	95.6 ± 0.2
n-Butane	16.6 ± 0.2	4.8 ± 0.2	....	16.7 ± 0.2	4.7 ± 0.2	....
Butenes	....	....	....	....	....	....
Isopentane	....	8.4 ± 0.2	1.1 ± 0.2	....	8.4 ± 0.2	1.2 ± 0.2
n-Pentane	....	6.5 ± 0.2	....	....	6.4 ± 0.2	....
Hexanes	....	19.2 ± 0.2	....	....	19.0 ± 0.2	....
Heptanes and heavier	....	61.1 ± 0.2	....	....	61.5 ± 0.2	....

lation, based on the difference in pressure in the receivers before and after removal of the gas, evaluated the quantity of each gas in the blend. All gases used in the blend were, after measurement, condensed in a common condensation bulb, from which the blended mixture was revaporized and transferred by means of the Toepler pump to any part of the apparatus desired. Before introducing gases into the blending system, the pressure in the system was reduced to 0.001 mm. of mercury or less (as determined by a McLeod gage) with the aid of a mercury diffusion pump.

To compensate for errors in blending introduced by deviations from the ideal gas laws, the measured volume of each gas has been corrected to its volume as an ideal gas, using the compressibility data of Jessen and Lightfoot (*3*) and Roper (*10*). These corrections usually amounted to less than 0.4 mole per cent of any component based on the total sample. Assuming manometric readings to be accurate to within ±0.5 mm. of mercury, the over-all probable uncertainty in blending has been computed to be between ±0.1 and ±0.2 mole per cent on each component based on the total sample. The probable uncertainties in composition incurred when measuring gas volumes or pressures in blending and in analyzing by the various methods have been included in the data tables.

Automatically Controlled Microfractionation Columns

Criticism has been directed in recent years toward standard microfractionation columns because of their failure to give consistent, reproducible analytical results. In this laboratory, serious discrepancies were sometimes encountered when duplicate samples were analyzed on different columns, or by different operators on the same column. Although it was suspected that these discrepancies were attributable more to variations in the operating technique employed by different operators and minor differences in the construction of the columns themselves than to any fundamental fault of the columns, the lack of definite data as to the absolute accuracy of the columns when operated under different conditions precluded, until recently, the establishment of a standardized operating procedure.

Inasmuch as Podbielniak (*8*, *9*) and McMillan (*6*) have reported thorough studies of the various factors affecting the analytical accuracy of the manually controlled Podbielniak and modified California Natural Gasoline Association's columns, this report will be limited to a discussion of results from the automatically controlled columns only.

Although analyses of synthetic mixtures demonstrated that more accurate results could be obtained from the manually controlled modified C. N. G. A. column than from the manually controlled Model L Podbielniak column, it was found that the modified C. N. G. A. column as developed by McMillan was not adaptable to the type of automatic control currently used in this laboratory for operating Podbielniak columns. Although further modifications of the McMillan column (such as removing the aluminum slugs from the condenser head) resulted in improved operation under automatic control, these modifications necessitated the removal of many of the basic features to which McMillan attributed the improved accuracy obtainable in his column. Except for the improved packing in the McMillan column (consisting of a six-turn-per-inch No. 13 gage brass spiral wire with a No. 23 gage straight wire insert), there was little difference between the automatically controlled McMillan and Podbielniak columns. In view of this, only the automatically controlled Podbielniak column was studied during this investigation; it is assumed that comparable results would be obtained from the modified McMillan column.

Several analyses of typical routine and synthetic light hydrocarbon samples in the automatically controlled Podbielniak Model L column have indicated (Table I) that samples containing no olefins can be analyzed with a maximum error of about ±0.3 per cent on any component based on the total sample, including isobutane and *n*-butane, provided the operating conditions are adapted to the type of sample being analyzed and are carefully controlled throughout the distillation. These results compare with an average maximum error of about ±0.7 per cent reported by McMillan for the manually operated Podbielniak "micro" precision column.

This investigation has indicated also that light hydrocarbon samples containing unsaturates may be separated satisfactorily into groups of the same number of carbon atoms in this column, but that fractionation between unsaturates and saturates containing the same number of carbon atoms is extremely difficult except possibly in the case of ethene-ethane. (No attempt has been made to evaluate, in this investigation, the accuracy of the ethene-ethane separation.) It is feasible, of course, to analyze a sample containing unsaturates by operating a fractionating column in conjunction with auxiliary equipment, analyzing segregated fractions by absorption, hydrogenation, or the method described by Lang (*4*).

In order to achieve consistently accurate results from the Podbielniak column, the operator must adhere to the following standardized procedure, with modifications as noted, according to the nature of the sample.

After charging the sample, the temperatures in the still and head of the column are so balanced that the column is operated under total reflux at slightly reduced pressure (about 100 mm. less than atmospheric) for a period of time depending upon the nature of the sample. From 30 to 60 minutes of refluxing is usually sufficient to bring the column to equilibrium. (If the sample contains large quantities of methane, the distillation may be started without bringing the column under total reflux.) The pressure in the column is then slowly increased to atmospheric, and the distillation is begun with the temperature in the head of the column at the boiling point of the lightest component in the sample.

During the distillation, the temperature in the condenser section of the column, as recorded by the potentiometer, is taken as an indication of the purity of the overhead product and, on plateaus, is maintained as near as possible to the true boiling point of the component being withdrawn. If, on a plateau, the temperature should rise above the boiling point of the component

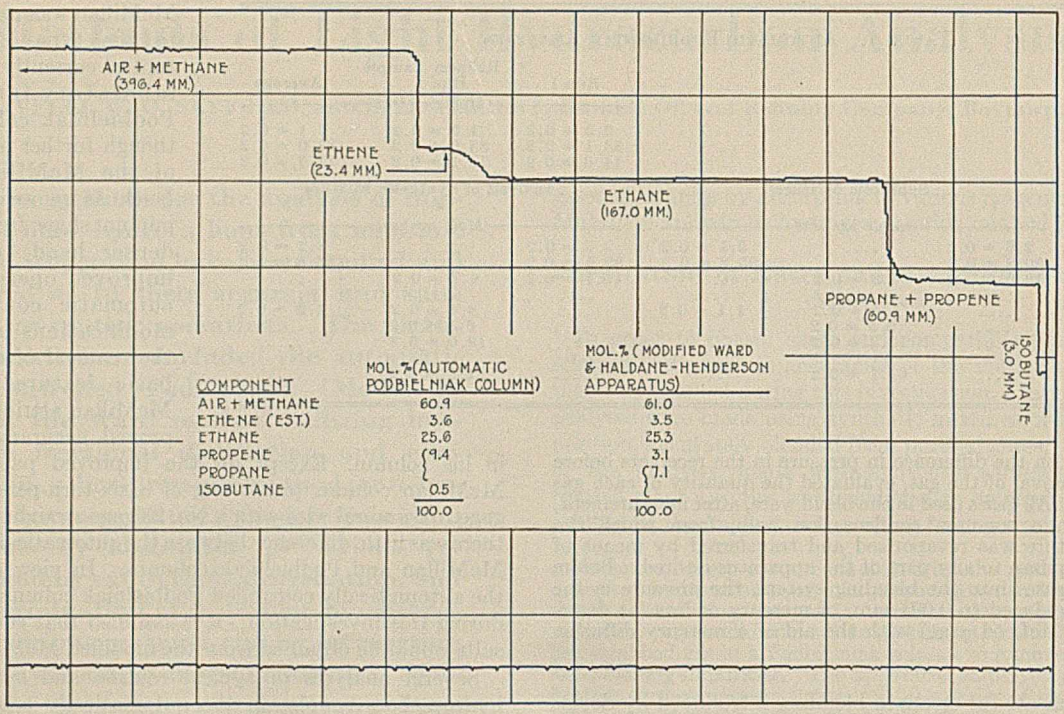

FIGURE 1. AUTOMATIC PODBIELNIAK COLUMN (MODEL L) DISTILLATION CHART

being withdrawn, the take-off should be discontinued immediately and the column operated under total reflux until the temperature returns to its former value. The take-off should then be recommenced at a slower rate and the distillation allowed to proceed. If the temperature should again rise, this procedure should be repeated until a take-off rate which will maintain the plateau temperature at a constant value has been established or until a break is certain.

When a break between components is imminent (as indicated by a decrease of pressure in the column, a "thinning out" of the reflux on the column packing, or both) the column should be operated under total reflux for several minutes while the boiling rate in the still is reduced. By reducing the boiling rate the liquid reflux on the column packing is further thinned out, thus materially reducing the column holdup (while maintaining a high reflux ratio) and increasing the sharpness of the break. After these conditions have been established, the take-off is again begun at a very slow rate and continued until the break is completed. This procedure may be considerably modified for different types of samples. For samples containing large amounts of methane, ethane, or propane, the distillation conditions may be so controlled on plateaus (by controlling the heat input to the still) that the boiling rate in the still is automatically reduced when a break is approached. Under these conditions, the reflux will thin out and the take-off rate will be lowered automatically, thus obviating the necessity for operating the column under total reflux.

In the analysis of gaseous samples, the pressure in the column is reduced to 500 to 600 mm. of mercury and the still is filled with mercury toward the end of the distillation in order to minimize dead space errors within the column. In the analysis of liquid samples, the pressure in the column is reduced to 500 mm. of mercury after the *n*-butane plateau has been reached, and to 250 mm. of mercury after the isopentane plateau has been reached. After each reduction in pressure the column is operated under total reflux for at least 15 minutes to bring it to equilibrium.

When a sample is distilled under the above conditions, the appearance of the finished distillation chart is taken as the best indication of the fractionation, and hence, analytical accuracy, obtained during the analysis. In appearance the distillation curve should have the following characteristics: (*a*) the plateaus should be horizontal, with few fluctuations (such as are caused by excessive pressure variations, flooding in the column, and other undesirable operating conditions), and should show no rise in temperature even when approaching a break; (*b*) breaks between components should be sharp, exhibiting little sloughing off at the plateaus, except in the case of separations between isomers or between saturates and unsaturates containing the same number of carbon atoms. A typical distillation curve, obtained in this laboratory when employing this standardized operating procedure for the analysis of a refinery sample containing unsaturates, is shown in Figure 1. (The percentage of ethene in this distillation was estimated by the equal-area method; however, the accuracy of the ethene-ethane separation has not been evaluated in this laboratory and ethene is usually determined by hydrogenation of the C_2 fraction.)

It is obvious that the accuracy of automatic Podbielniak analyses depends not only upon the skill and experience of the operator but also upon the cleanliness and mechanical conditions of the column and automatic equipment.

Although smooth operation of the automatic Model L type column used in this investigation was considerably enhanced by several minor changes in the automatic control equipment and in methods employed for inducing reflux and controlling the heat input to the still, the necessity for the majority of these modifications has been obviated by the introduction of the new Podbielniak "supercool" column. This column includes certain construction features (such as the extension of the vacuum jacket to cover the still as well as the column, and

TABLE II. CONDITIONS FOR SEPARATION OF COMPONENTS

Separation	Final Pressure *Mm. Hg*	No. 5	Condenser Temperatures No. 4	No. 3	No. 2	No. 1
			° *C.*	° *C.*	° *C.*	° *C.*
C_1-C_2	0.03	Liquid nitrogen	−175	−160	−142	−128
C_2-C_3	0.03	Liquid nitrogen	−160	−143	−128	−113
C_3-C_4	0.03	Liquid nitrogen	−136	−122	−107	Room temp.
C_4-C_5	0.03	Liquid nitrogen	−112	−96	Room temp.	Room temp.

TABLE III. MODIFIED WARD APPARATUS ANALYSES

	Synthetic Mixture				Analysis			
	Run 1	Run 2	Run 3	Run 4	Run 1	Run 2	Run 3	Run 4
	Mole per cent				*Mole per cent*			
Ethane	15.7 ± 0.2	7.6 ± 0.2	70.7 ± 0.2	2.3 ± 0.2	15.4 ± 0.2	7.7 ± 0.2	71.1 ± 0.2	2.4 ± 0.2
Propane	16.2 ± 0.2	41.6 ± 0.2	2.8 ± 0.2	3.4 ± 0.2	16.2 ± 0.2	41.7 ± 0.2	3.1 ± 0.2	3.3 ± 0.2
Butanes	68.1 ± 0.2	42.1 ± 0.2	4.5 ± 0.2	89.9 ± 0.2	68.4 ± 0.2	42.0 ± 0.2	4.3 ± 0.2	90.2 ± 0.2
Isopentane	.....	8.7 ± 0.2	22.0 ± 0.2	4.4 ± 0.2	.....	8.6 ± 0.2	21.5 ± 0.2	4.1 ± 0.2

an improved method for applying liquid nitrogen) which practically eliminate superheating within the still and column.

High-Vacuum Gas Analysis

In general, methods for the fractional analysis of light hydrocarbon mixtures may be divided into two classifications, both of which are dependent upon the relative volatilities (alpha) of the various hydrocarbons in the mixture and to some extent upon rectification. In the type of apparatus developed by Ward (*15*) as a modification of the apparatus originally developed by Shepherd and Porter (*14*) and improved upon by Shepherd (*13*), separation of the components in a mixture is achieved through a series of simultaneous partial distillations and condensations in a series of highly evacuated condenser tubes across which is maintained a temperature gradient (each condenser being maintained at a constant temperature) throughout a given separation. The final separation of each component from the remainder of the mixture is achieved in these apparatus at pressures below 0.1 mm. of mercury absolute, at which pressure the relative volatilities of the various hydrocarbons are several times greater than they are at atmospheric pressure. Thus the fractionating efficiency of this type of apparatus is obtained as a result of these large values of alpha; in a microfractionation column, where the values of alpha are relatively small, effective fractionation is achieved primarily through rectification at low temperatures and at pressures ranging from about 100 mm. of mercury to atmospheric. Consequently, many of the inherent difficulties of a column analysis, such as holdup, maintenance of reflux ratios, etc., are not encountered in the type of apparatus developed by Shepherd and Porter and by Ward. Because of these and other intrinsic advantages, an apparatus similar to that used by Ward was investigated.

As used in this investigation, the modified Ward apparatus (Figure 2), constructed of Pyrex glass and mounted on Transite board, consisted essentially of a condenser train consisting of four condenser tubes for fractionation, plus a fifth condenser for collecting gases distilled from the train; a constant-volume buret with a calibrated capacity of 256.4 cc. for measuring gas quantities; an internal pumping system consisting of a mercury diffusion pump and a Toepler pump (controlled with compressed air);

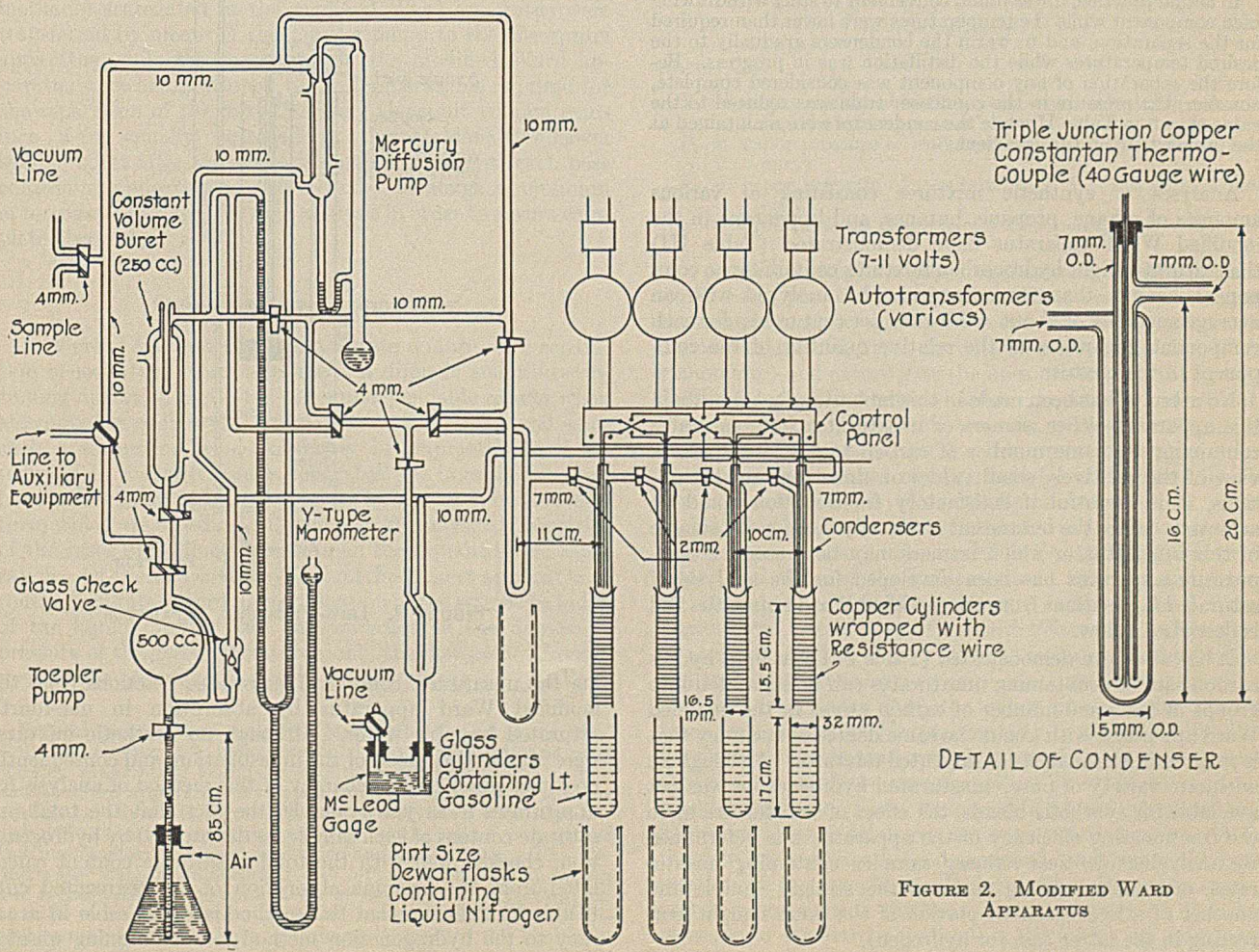

FIGURE 2. MODIFIED WARD APPARATUS

TABLE IV. MODIFIED WARD APPARATUS ANALYSES

	Straight Sample		Hydrogenated Sample[a]		Average	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
	Mole per cent		*Mole per cent*		*Mole per cent*	
Ethane and ethene	3.6 ± 0.2	.....	3.7 ± 0.3	.....	3.6 ± 0.2	.....
Propane and propene	82.0 ± 0.2	2.1 ± 0.2	82.4 ± 0.3	2.7 ± 0.3	82.2 ± 0.2	2.4 ± 0.2
Butanes and butenes	14.4 ± 0.2	97.5 ± 0.2	13.9 ± 0.3	96.9 ± 0.3	14.2 ± 0.2	97.2 ± 0.2
Isopentane and heavier	.....	0.4 ± 0.2	.....	0.4 ± 0.3	.....	0.4 ± 0.2
Total unsaturate content	20.1 ± 0.2	31.2 ± 0.2	.....	.....	.....	.....

[a] Results calculated on hydrogen-free basis.

and a McLeod gage for measuring the pressure in the condenser trains (controlled by vacuum).

The following system of temperature control was used on each of the four condenser tubes: A copper cylinder wrapped with resistance wire and containing light gasoline was slipped over each condenser and the wire was attached to contact points on the control panel. The contacts for each cylinder were in turn connected to an autotransformer (Variac) and transformer in series, the latter giving a range of 7 to 11 volts and the former giving 0 to 100 per cent control of the transformer voltage. A glass cylinder containing light gasoline and a pint-size Dewar flask containing liquid nitrogen were then slipped concentrically over the copper cylinder. The temperature in the condenser was adjusted and maintained at any desired value by adjusting the Variac. Temperatures were read by means of triple-juncture copper-constantan thermocouples constructed of 40-gage wire, having an ice-and-water cold junction, and calibrated against the boiling point of nitrogen, the sublimation point of carbon dioxide, and the freezing point of water. The procedure employed during the analysis of samples in this apparatus was essentially the same as that described by Ward, except that the temperatures and pressures shown in Table II were used for the separation of the various components in a mixture rather than the values given by Ward.

In actual practice, it was found convenient to start withdrawing each component while the temperatures were lower than required for the separation, and to warm the condensers gradually to the desired temperatures while the distillation was in progress. Before the separation of any component was considered complete, however, the pressure in the condenser train was reduced to the value shown in Table II while the condensers were maintained at the proper temperature gradient.

Analyses of synthetic mixtures consisting of various amounts of ethane, propane, butanes, and isopentane in the modified Ward apparatus have demonstrated (Table III) that saturated light hydrocarbon mixtures containing no component heavier than isopentane can be analyzed with an average accuracy of about ±0.3 mole per cent or less for each component, regardless of the relative quantity of the component in the mixture.

No attempt has been made in this laboratory to separate in this apparatus either isomers or unsaturates from saturates containing the same number of carbon atoms; however, in view of the relatively small values of alpha for such separations, it is doubtful if satisfactory fractionation could be achieved. Since the butane cut is the only fraction obtainable in this apparatus in which isomers may be present, a dew-pressure apparatus has been developed for the analysis of saturated C_4 fractions from the modified Ward apparatus and is described below.

It has also been demonstrated (Table IV) that light hydrocarbon samples containing unsaturates can be separated into groups of the same number of carbon atoms in the modified Ward apparatus with about the same degree of accuracy that is realized in the analysis of saturated mixtures. Although an adequate variety of pure, unsaturated hydrocarbons was not available for synthetic blends, the effect of unsaturates upon the fractionating efficiency of the apparatus was determined by analyzing identical refinery samples containing unsaturates, one analysis being made of the straight sample and another of a hydrogenated portion of the same sample (correcting in the latter case for hydrogen).

In view of the fact that hydrocarbon samples containing unsaturates can be accurately separated into groups of the same number of carbon atoms in the modified Ward apparatus, a further investigation has been made of the possibility of determining the unsaturate content of segregated fractions from the apparatus by absorption in auxiliary equipment. Because of the small sample volumes (about 200 gaseous ml.) employed for an analysis, it was necessary to choose an apparatus for the unsaturate determinations in which gas samples of 10 ml. or less could be analyzed accurately. A Haldane-Henderson gas analysis apparatus was selected for this investigation; a 40 per cent solution of potassium hydroxide was used as the confining liquid in the buret, and one-fourth saturated bromine solution (containing 50 grams per liter of potassium bromide) was used as the absorption reagent in the pipet.

Analysis of several refinery light hydrocarbon samples containing unsaturates have indicated (Tables V and VI) that such samples can be analyzed satisfactorily by determining the unsaturate content of segregated fractions from the modified Ward apparatus by absorption in one-fourth saturated bromine water. Although no synthetic mixtures were used in this phase of the investigation, and consequently no direct proof of the accuracy of this method of analysis for unsaturates is as yet available, the fact that the total unsaturate content of each sample, as determined by hydrogenation, checked well with the total unsaturate content calculated from the bromine absorption of the segregated cuts (Table V) indicates that this method is comparable in accuracy to the hydrogenation method for determining unsatu-

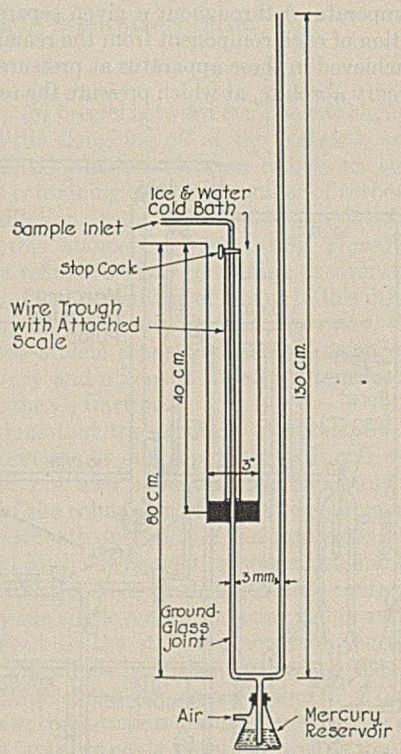

FIGURE 3. DEW-PRESSURE APPARATUS

TABLE V. COMBINED MODIFIED WARD AND HALDANE-HENDERSON APPARATUS ANALYSES

	Run 1	Run 2
Methane	....	61.0 ± 0.2
Ethene	0.4 ± 0.2	3.5 ± 0.2
Ethane	9.4 ± 0.2	25.3 ± 0.2
Propene	21.1 ± 0.2	3.1 ± 0.2
Propane	68.3 ± 0.2	7.1 ± 0.2
Butanes	0.8 ± 0.2	
Total unsaturates:		
By hydrogenation	21.7 ± 0.2	6.7 ± 0.2
By bromination	21.7 ± 0.1	....
By bromination of segregated fractions	21.6 ± 0.1	6.6 ± 0.1

TABLE VI. AUTOMATIC PODBIELNIAK AND COMBINED MODIFIED WARD (AND DEW-PRESSURE OR HALDANE-HENDERSON APPARATUS) ANALYSES

	Automatic Podbielniak Column		Modified Ward Apparatus and Auxiliary Equipment	
	Run 1	Run 2	Run 1	Run 2
Methane	60.9 ± 0.2	....	61.0 ± 0.2	....
Ethene (estimated)	3.6 ± 0.2	....	3.5 ± 0.2	....
Ethane	25.6 ± 0.2	....	25.3 ± 0.2	....
Propene	9.4 ± 0.2	....	3.1 ± 0.2	....
Propane	....	2.1 ± 0.2		2.6 ± 0.2
Isobutane	0.5 ± 0.2	83.0 ± 0.2	7.1 ± 0.2	83.0 ± 0.2
n-Butane	....	14.9 ± 0.2	....	14.4 ± 0.2

rates in the C_2 and C_3 fractions. The relative merits of various absorption reagents for olefin determination are discussed below.

No operating difficulties were experienced when analyzing samples in the combined modified Ward and Haldane-Henderson apparatus. Approximately 10 ml. of each fraction (except methane) segregated in the modified Ward apparatus were transferred by means of the Toepler pump to the absorption apparatus while the condensers in the modified Ward apparatus were being warmed to the temperatures required for the separation of the next fraction. Constant volume readings were usually obtained in the Haldane-Henderson sample buret after six passes through the reagent (each pass consuming approximately 20 seconds), although a minimum of ten passes was used for each analysis in order to ensure complete absorption.

Dew-Pressure Apparatus

In view of the fact that the accurate quantitative separation of isobutane from *n*-butane is a difficult and time-consuming operation and often gives undependable results when attempted in ordinary microfractionation columns, and since such a separation would probably be impracticable if attempted in a high-vacuum apparatus, an investigation has been made of methods of analysis for isobutane–*n*-butane mixtures which are based on differences in the physical properties of the gases but do not depend upon their quantitative separation. Of the two such physical methods most applicable to routine laboratory work (determination of the refractive index of the liquid mixture and determination of the dew-point pressure of the gaseous mixture), only the dew-point pressure method has been investigated in this laboratory.

Several investigators, notably Hachmuth (*2*) and Woog, Sigwalt, and Gomer (*16*), have developed apparatus for determining the dew pressures of isobutane–*n*-butane mixtures at 0° C. The dew-pressure apparatus developed during the course of this investigation (shown in Figure 3) consists simply of a Y-type manometer connected to a mercury reservoir, with half of one arm of the manometer immersed in an ice-and-water bath. The ice-and-water bath is constructed of 7.5-cm. (3-inch) glass tubing and is connected to the manometer by means of a rubber stopper. In order to read the mercury level in that portion of the manometer surrounded by the bath, a scale is attached to the back of a metal trough which is placed around the manometer arm and extends to the wall of the bath. This scale is calibrated with respect to the scale on the other arm of the manometer by raising the mercury in both arms under atmospheric pressure and taking blank readings on both scales.

The following procedure is followed during the analysis of an isobutane–*n*-butane mixture in this apparatus:

After evacuating the apparatus and connecting lines to an absolute pressure of 0.001 mm. or less and filling the bath with chipped ice and water (the temperature in the bath must be maintained at 0° C. throughout an analysis by agitating the ice-water mixture and adding ice if necessary), enough sample is admitted through the stopcock into the left arm of the manometer so that the mercury level in the sample arm of the manometer is about even with the bottom of the ice-and-water bath under atmospheric pressure. The mercury in the manometer is then raised in increments of 5 to 10 mm. by increasing the air pressure in the mercury reservoir (by means of a pressure-regulating valve), allowing sufficient time after each increase for the temperature of the sample to readjust itself to 0° C.

The dew pressure is determined by plotting the pressure on the sample in millimeters of mercury, against $\Delta V/\Delta P$, $\Delta V/\Delta P$ being the ratio of the incremental decrease in sample volume (plotted as the incremental increase in the height of mercury in the sample side of the manometer) to the incremental increase in pressure on the sample for each rise of mercury in the manometer. This ratio, which remains nearly constant as long as the sample remains in the gaseous phase, increases rapidly as the dew pressure is reached and passed. The dew pressure is taken as the point where the change in slope of the pressure *vs.* $\Delta V/\Delta P$ curve begins, and the composition of the sample is computed from the formula:

$$Y_1 = \frac{P_1}{\pi}\left(\frac{\pi - P_2}{P_1 - P_2}\right) \qquad (1)$$

where

Y_1 = mole per cent of isobutane in vapor in equilibrium with liquid at dew pressure
P_1 = vapor pressure of isobutane at 0° C. (1190 mm. of mercury)
P_2 = vapor pressure of *n*-butane at 0° C. (770 mm. of mercury)
π = total pressure on system at dew point, mm. of mercury

A somewhat simpler method of obtaining the dew pressure would be to plot the pressure, in millimeters of mercury, on the sample against the decrease in volume (plotted as the increase in the height of mercury in the sample side of the manometer) and extrapolate the lines plotted from the points obtained before and after the dew pressure is passed until they intersect. This would, however, necessitate the use of an empirical table in which the dew pressures corresponding to various percentages of isobutane (as determined by the dew-pressure analyses of synthetic mixtures) were given; such a table has not yet been developed in this laboratory.

The vapor pressure values used in Equation 1 differ somewhat from some of the values quoted by Sage and Lacey (*11*, *12*, obtained by extrapolation), Dana (*1*), and Woog, Sigwalt, and Gomer (*16*). Since these literature values differ by as much as 10 to 15 mm. for both isobutane and *n*-butane, and there seemed to be no way of selecting the correct vapor pressures from these data, the vapor pressures for the butanes were determined experimentally in this laboratory by the method described above. In view of the accurate results obtained by the dew-pressure method on synthetic mixtures when using these values, it appears that the experimentally determined vapor pressures are very nearly correct. After the dew pressure has been determined, and before the composition of the sample is calculated from Equation 1, it is necessary to correct the manometer readings at the dew pressure to standard conditions. By taking an imaginary

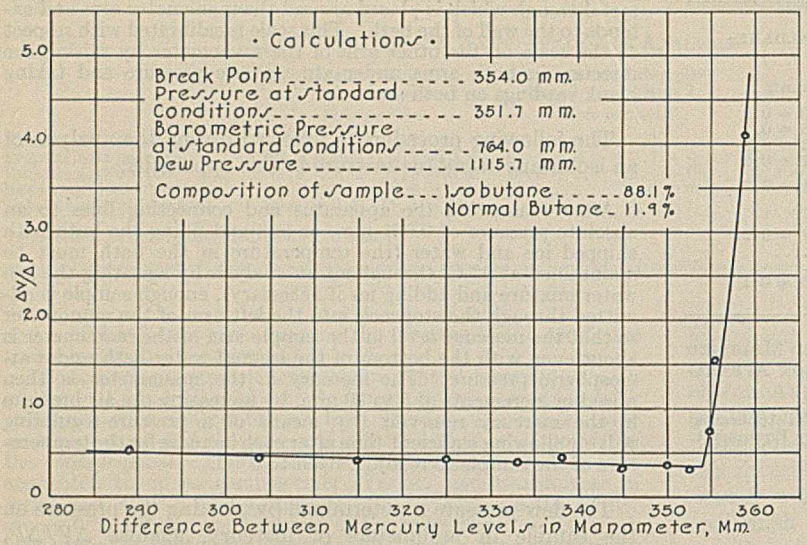

FIGURE 4. DEW-PRESSURE CURVE AND CALCULATIONS

reference plane through the bottom of the ice-and-water bath on the dew-pressure apparatus (Figure 3), the following temperature correction formula may be derived:

$$P_0 = H_R K - H_L$$

where

P_0 = corrected dew pressure, mm. of mercury
H_L = height above reference plane of mercury in left arm (sample side) of manometer at dew pressure, mm.
H_R = height above reference plane of mercury in right arm of manometer at dew pressure (equal to H_L plus difference in mercury heights in manometer at dew pressure), mm.
K = coefficient of reduction for correcting mercury heights at room temperature to 0° C.

A typical dew-pressure curve and calculations are shown in Figure 4.

Analyses of synthetic isobutane–*n*-butane mixtures by the dew-pressure method have resulted (Table VII) in an average analytical accuracy of about ±0.5 per cent (about the limit of experimental accuracy for this method of analysis). From an inspection of Equation 1, however, it is evident that the inclusion of even small amounts of higher- or lower-boiling impurities with the butanes would have an appreciable effect upon the accuracy of the analysis. In order to ascertain quantitatively the effects of small quantities of impurities upon the accuracy of the dew-pressure method of analysis, several synthetic samples containing small amounts of either propane or isopentane in addition to the butanes were analyzed by dew-pressure determinations, the composition of the samples being calculated on the assumption that no gases other than the saturated butanes were present. It was found (Table VIII) that the presence of small quantities of either gas caused errors in the analysis about three times greater than the magnitude of their concentrations. (In the case of very small concentrations of impurities, the error could not be determined accurately because the probable experimental error in blending the synthetic mixtures was about ±0.2 mole per cent for each component; however, the errors introduced by less than 0.2 per cent of an impurity are probably within the limit of experimental accuracy of the dew-pressure determinations.) It is also evident that the extent to which these impurities affect the dew-pressure determination is dependent not only upon the concentration of the impurity but also, to a lesser extent, upon the proportion of isobutane to *n*-butane in the sample.

In view of the marked effect of higher- and lower-boiling impurities upon the accuracy of the dew-pressure determination for isobutane–*n*-butane mixtures, it is evident that, in order to obtain reliable results from the analysis of hydrocarbon samples containing components other than the butanes, the butane fraction must be segregated from the other constituents of the mixture in such a way that it contains substantially no impurities. In this laboratory, no attempt has yet been made to segregate impurity-free C_4 fractions from a Podbielniak distillation; however, it is doubtful if a pure fraction could be obtained from a column distillation unless the cut were collected well after the propane-isobutane break and before the *n*-butane–isopentane break. Although, in such a procedure, the accuracy of the dew-pressure determination would still depend to a large extent upon the fractionating efficiency of the column, it is probable that a considerable saving in time and liquid nitrogen would be realized (over attempting the isobutane–*n*-butane split on the Podbielniak-type apparatus).

The feasibility of segregating saturated butane mixtures from samples containing propane and air in addition to the butanes in the modified Ward apparatus has been demonstrated in this laboratory with synthetic mixtures; analyses of such mixtures in the combined modified Ward and dew-pressure apparatus have resulted in average deviations from the true composition of about ±0.6 mole per cent or less for each component in the mixture (Table IX). Inasmuch as propane-butanes and butanes-isopentane separations are achieved in the modified Ward apparatus with about the same degree of accuracy, and small amounts of isopentane have about the same effect (although in the opposite direction) as equal amounts of propane upon the accuracy of dew-pressure determinations, it appears reasonably certain that saturated butane cuts, when properly segregated in the modified Ward apparatus, do not contain sufficient quantities of these impurities to affect materially the accuracy of the dew-pressure determination.

Although no attempt has been made to determine experimentally the effects of C_4 unsaturates upon the accuracy of dew-pressure determinations, it is obvious that appreciable quantities of these unsaturates would introduce a considerable error into the analysis. When analyzing samples containing unsaturates, therefore, it is necessary to remove the unsaturates by absorption or to saturate them by hydrogenation before analyzing them by the dew-pressure method. The practicability of analyzing hydrogenated samples in the combined modified Ward and dew-pressure apparatus has been demonstrated by numerous analyses of routine samples.

TABLE VII. DEW-PRESSURE ANALYSES OF ISOBUTANE–*n*-BUTANE MIXTURES

	Run 1	Run 2	Run 3	Run 4
	Mole per cent			
	Synthetic Mixture			
Isobutane	84.3 ± 0.2	56.3 ± 0.2	49.4 ± 0.2	19.5 ± 0.2
n-Butane	15.7 ± 0.2	43.7 ± 0.2	50.6 ± 0.2	80.5 ± 0.2
	Analysis			
Isobutane	84.4 ± 0.4	56.8 ± 0.4	49.8 ± 0.4	19.3 ± 0.4
n-Butane	15.6 ± 0.4	43.2 ± 0.4	50.2 ± 0.4	80.7 ± 0.4

TABLE VIII. EFFECT OF IMPURITIES UPON DEW-PRESSURE ANALYSES

	Synthetic Mixture					Analysis				
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 1	Run 2	Run 3	Run 4	Run 5
	Mole per cent					*Mole per cent*				
Propane	.....	.....	.....	0.2 ± 0.2	0.5 ± 0.2	.....	.....	.....	.....	.....
Isobutane	49.8 ± 0.2	48.6 ± 0.2	49.6 ± 0.2	89.9 ± 0.2	81.3 ± 0.2	49.4 ± 0.4	46.5 ± 0.4	43.3 ± 0.4	90.5 ± 0.4	82.8 ± 0.4
n-Butane	49.9 ± 0.2	50.0 ± 0.2	47.7 ± 0.2	9.9 ± 0.2	18.2 ± 0.2	50.6 ± 0.4	53.5 ± 0.4	56.7 ± 0.4	9.5 ± 0.4	17.2 ± 0.4
Isopentane	0.3 ± 0.2	1.4 ± 0.2	2.7 ± 0.2	.....	.....	.....	.....	.....	.....	.....

TABLE IX. COMBINED MODIFIED WARD AND DEW-PRESSURE ANALYSES

	Synthetic Mixture			Analysis		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
	Mole per cent			*Mole per cent*		
Propane	3.9 ± 0.2	4.1 ± 0.2	17.1 ± 0.2	4.1 ± 0.2	10.0 ± 0.1	17.1 ± 0.2
Isobutane	80.3 ± 0.2	80.8 ± 0.2	59.7 ± 0.2	80.9 ± 0.4	81.1 ± 0.4	59.0 ± 0.4
n-Butane	15.8 ± 0.2	9.2 ± 0.2	23.2 ± 0.2	15.0 ± 0.4	8.9 ± 0.4	23.9 ± 0.4

Comparison of Standard Microfractionation Columns with High-Vacuum Analysis Apparatus and Auxiliary Equipment

In view of the wide differences between the types of apparatus discussed above, it might be well to compare these apparatus with respect to accuracy, speed and economy of operation, versatility, and adaptability to routine laboratory operation. Although final conclusions regarding the relative merits of the automatically controlled Podbielniak column, modified Ward apparatus, and dew-pressure apparatus cannot be made until each apparatus has been further tested with synthetic mixtures under routine laboratory operating conditions, the following comparisons, on the basis of data obtained thus far, appear to be justified.

ACCURACY. When properly operated, the automatic Podbielniak Model L type column and combined modified Ward and dew-pressure apparatus are comparable in accuracy (about ±0.5 per cent or less maximum deviation from the true composition on any component through isopentane) in the analysis of saturated light hydrocarbon mixtures. Although samples containing unsaturates can be satisfactorily separated into groups of the same number of carbon atoms in either apparatus, individual olefins must be determined by analyzing the segregated fractions in auxiliary equipment. Unsaturates must be removed from C_4 fractions before satisfactory analyses for isobutane or *n*-butane can be made in either apparatus.

OPERABILITY. Consistently accurate analytical results from either apparatus are dependent to a large extent upon the proper condition and operation of the apparatus. Operating conditions for the modified Ward and dew-pressure apparatus are standardized and, after a little practice, easily followed; the establishment of optimum operating conditions in the automatic column depends to a large extent upon the skill of the operator and the mechanical condition of the equipment.

SPEED AND ECONOMY OF OPERATION. The time and liquid nitrogen required for an accurate analysis in the combined modified Ward and dew-pressure apparatus are, for nearly every type of sample, considerably less than for a comparable analysis in the automatic Podbielniak (Model L) column; this is especially true of the analysis of butane samples.

VERSATILITY. Both the microfractionation column and the modified Ward and dew-pressure apparatus have certain limitations in the analysis of light hydrocarbon mixtures. Since, in the modified Ward apparatus, the original sample and all the individual components thereof are measured as gases, no sample containing appreciable amounts of any hydrocarbon heavier than isopentane—i. e., liquid under ordinary atmospheric conditions—can be analyzed without the use of auxiliary equipment. It would, of course, be possible to operate the apparatus in conjunction with a calibrated topping still, analyzing the overhead by fractional condensation and, if desirable, analyzing the residue by a column distillation.

On the other hand, it is possible to analyze small gaseous sample volumes (200 ml. or less) in the modified Ward and dew-pressure apparatus; samples of at least 1500 ml. (gaseous) and preferably several times larger are required for a column analysis. Small amounts of individual components, especially if they are the lightest or heaviest components of a mixture, are detected and measured much more easily in the modified Ward apparatus than in a column distillation.

In this laboratory, it has often been found convenient to operate the combined modified Ward and dew-pressure apparatus in conjunction with a microfractionation column for some types of samples.

For example, a cracked gas sample containing a small proportion of butanes-butenes is fractionated in a column; the butanes-butenes cut, along with some propane and isopentane, is segregated from the other components of the sample. This cut is then hydrogenated, the C_4 hydrocarbons are separated from the hydrogen, propane, and isopentane in the modified Ward apparatus, and the iso–*n*-butane ratio is determined in the dew-pressure apparatus. From the dew-pressure analysis, the hydrogenation of the total C_4 cut, and an analysis for isobutene (by the anhydrous hydrochloric acid method or by absorption) the amount of isobutane, isobutene, *n*-butenes, and *n*-butane in the sample may be determined with a far greater degree of accuracy than would have been possible by the column analysis alone.

Determination of Total Unsaturates in Gaseous Samples

Among the available methods for the determination of total unsaturates in gaseous samples are absorption in saturated bromine solution, absorption in solutions containing bromine in the form of a complex salt, absorption in various concentrations of sulfuric acid, and catalytic hydrogenation at room temperatures and atmospheric pressures according to the procedure developed by McMillan *et al.* (*7*).

Because bromine reacts rapidly and completely with gaseous unsaturated hydrocarbons, this reagent is often used for the determination of total unsaturates. However, it is apparent that saturated bromine solution as it is usually prepared (containing excess free bromine) attacks isobutane and, to a lesser extent, *n*-butane and therefore does not give accurate determinations of unsaturate content. It was found (Table X) that on refinery butane-butenes samples (which had been analyzed previously by hydrogenation), three passes into the reagent gave approximately correct results, whereas determinations in error by 20 per cent or more were experienced if nine or more passes were used. Reproducible results were, however, obtained by using one-fourth saturated bromine solutions (with and without excess potassium bromide, or containing mercuric nitrate) as the absorption reagent. These solutions do not appreciably attack saturated butanes, as indicated by the fact that approximately the same un-

TABLE X. COMPARATIVE ANALYSES FOR TOTAL UNSATURATE CONTENT OF PLANT SAMPLES USING BROMINE SOLUTIONS

Absorption Reagent	Number of Passes through Reagent	Total Unsaturates Found by Analysis
		Mole per cent
Saturated bromine solution	3	10.4 ± 0.1, 10.7 ± 0.1
	6	11.3 ± 0.1, 11.8 ± 0.1
	9	12.4 ± 0.1, 12.6 ± 0.1
	3	10.8 ± 0.1
	9	14.8 ± 0.1
	15	17.7 ± 0.1
One-fourth saturated bromine solution	12	34.1 ± 0.1
	15	34.5 ± 0.1
	40	34.3 ± 0.1
One-fourth saturated bromine solution containing excess KBr	12	34.1 ± 0.1
	12	34.7 ± 0.1
	12	34.8 ± 0.1
	12	34.8 ± 0.1
	16	35.4 ± 0.1
	17	35.7 ± 0.1
	22	35.7 ± 0.1
	22	35.8 ± 0.1
	40	35.2 ± 0.1

saturate content was found after twelve to forty passes through the reagent (Table X). In addition, these reagents failed to react with pure gaseous isobutane. It is believed that failure to obtain exactly reproducible results with dilute bromine solutions is attributable to the absorption of small quantities of saturated components in the liquid dibromides formed from the reaction of bromine with unsaturates.

In general, sulfuric acid solutions are not desirable reagents for the determination of total gaseous unsaturates. These reagents (especially concentrated solutions) apparently dissolve different amounts of saturated hydrocarbons, depending upon the types and concentrations of unsaturates absorbed, and either high or low analytical results are possible (Table XI). In the absence of ethene, fairly accurate results are obtainable when using 86 per cent sulfuric acid as the absorption reagent (Table XI), but the procedure is time-consuming (sixty to eighty passes through the reagent are necessary in order to obtain constant readings).

The McMillan catalytic hydrogenation method for the determination of total gaseous unsaturates was found to be both rapid and accurate in the analysis of synthetic butanes-butenes mixtures (Table XI), provided suitable correction factors were applied to compensate for the deviations of the various hydrocarbons from the perfect gas laws. Five passes over the nickel catalyst were usually sufficient to react all the unsaturates in the majority of the samples, and complete reaction was obtained in all cases after ten passes. This method possesses the disadvantage, however, that the nickel catalyst employed is easily poisoned and must be activated frequently. In order to determine the state of activation of the catalyst, frequent analyses of samples of known unsaturate content should be made.

Determination of Isobutene in Gaseous Samples

Absorption methods for the determination of isobutene in gaseous samples usually employ 60 to 70 per cent sulfuric acid, or sulfuric acid solutions, as the absorbing medium. In all such procedures, the reagent usually absorbs small quantities of *n*-butenes and butadienes in addition to isobutene, thus necessitating the employment of correction factors to compensate for errors introduced by the absorption of the *n*-olefins and diolefins. Such corrections are usually applied by passing the sample through the reagent until a constant rate of absorption per pass is obtained, and extrapolating the curve of "per cent absorbed *vs.* number of passes" back to 0 pass (Figures 5 and 6).

In this laboratory, the following reagents were investigated: 60 per cent sulfuric acid (2-minute passes), 60 per cent sulfuric acid saturated with copper sulfate (2-minute passes), and 65 per cent sulfuric acid containing 0.5 per cent by volume of *tert*-butyl alcohol (20- and 45-second passes). Analyses of synthetic mixtures by each of these procedures have shown (Table XII and Figures 5 and 6) that comparable analytical results, accurate to within a few tenths of 1 per cent, are obtainable by each of these methods, provided the procedure is carefully carried out and a sufficient number of passes are employed. Since the rate at which isobutene is absorbed by these reagents decreases as the isobutene content of the sample decreases, it is important that a sufficient number of passes be made to ensure that the absorption values used in the extrapolation do not represent the absorption of small amounts of isobutene as well as *n*-butenes; otherwise, the curve of per cent absorbed *vs.* number of passes for samples containing appreciable amounts of *n*-butenes would become difficult to interpret. For this reason, at least twenty passes should be made before extrapolating the curve obtained in this type of analysis.

TABLE XI. COMPARATIVE ANALYSES FOR TOTAL UNSATURATE CONTENT OF SYNTHETIC LIGHT HYDROCARBON MIXTURES

Analytical Method	Isobutene in Sample	2-Butene in Sample	Total Unsaturates in Sample	Unsaturates Found by Analysis
		Mole per cent		
Absorption in 94% H_2SO_4	10.4 ± 0.2	0.0 ± 0.2	10.4 ± 0.2	10.3 ± 0.1
	0.0 ± 0.2	19.4 ± 0.2	19.4 ± 0.2	21.3 ± 0.1
	0.0 ± 0.2	20.9 ± 0.2	20.9 ± 0.2	20.1 ± 0.1
	0.0 ± 0.2	21.2 ± 0.2	21.2 ± 0.2	22.4 ± 0.1
Absorption in 86.3% H_2SO_4	0.0 ± 0.2	28.0 ± 0.2	28.0 ± 0.2	28.5 ± 0.1
	0.0 ± 0.2	28.1 ± 0.2	28.1 ± 0.2	28.9 ± 0.1
McMillan catalytic hydrogenation method	0.0 ± 0.2	30.9 ± 0.2	30.9 ± 0.2	31.0 ± 0.2
	0.0 ± 0.2	40.5 ± 0.2	40.5 ± 0.2	40.2 ± 0.2
	50.1 ± 0.2	0.0 ± 0.2	50.1 ± 0.2	50.2 ± 0.2
	41.1 ± 0.2	26.7 ± 0.2	67.8 ± 0.2	67.9 ± 0.2
	0.0 ± 0.2	93.6 ± 0.2	93.6 ± 0.2	93.1 ± 0.2

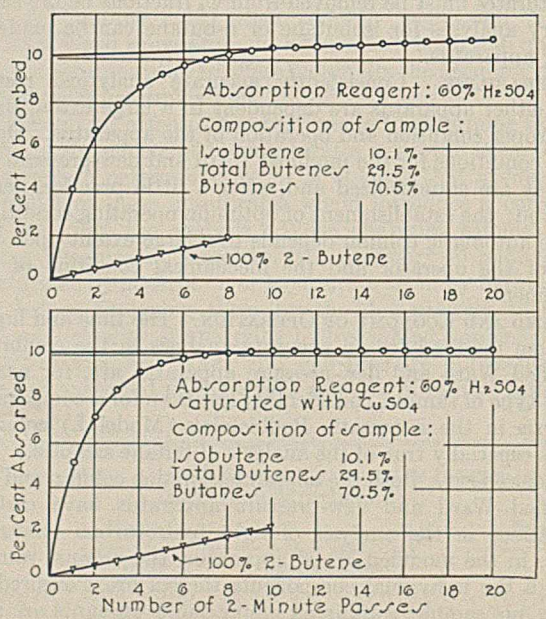

FIGURE 5. ABSORPTION OF ISOBUTENE IN 60 PER CENT SULFURIC ACID

Lower curve, acid saturated with copper sulfate

TABLE XII. COMPARATIVE ANALYSES FOR ISOBUTENE CONTENT OF SYNTHETIC LIGHT HYDROCARBON MIXTURES

Analytical Method	Composition of Synthetic Sample: Isobutene	2-Butene	Isobutene Found by Analysis
	Mole per cent		
Absorption in 60% H_2SO_4 + $CuSO_4$	10.2 ± 0.2	20.1 ± 0.2	10.4 ± 0.1
	11.6 ± 0.2	64.4 ± 0.2	11.8 ± 0.1
	19.1 ± 0.2	53.0 ± 0.2	19.2 ± 0.1
Absorption in 60% H_2SO_4 + 0.5 volume % of *tert*-butyl alcohol	6.0 ± 0.2	22.2 ± 0.2	6.1 ± 0.1
	7.3 ± 0.2	20.0 ± 0.2	7.4 ± 0.1
	7.9 ± 0.2	81.7 ± 0.2	7.8 ± 0.1
	10.1 ± 0.2	11.8 ± 0.2	10.0 ± 0.1
	21.0 ± 0.2	0.0 ± 0.2	20.9 ± 0.1
McMillan anhydrous HCl method	0.0 ± 0.2	100.0 ± 0.2	0.0 ± 0.2
	20.1 ± 0.2	79.1 ± 0.2	20.2 ± 0.2
	22.3 ± 0.2	77.7 ± 0.2	22.2 ± 0.2
	30.8 ± 0.2	62.6 ± 0.2	30.1 ± 0.2
	100.0 ± 0.2	0.0 ± 0.2	100.6 ± 0.2

Of the above reagents, the 65 per cent sulfuric acid containing 0.5 per cent *tert*-butyl alcohol was found preferable for use in routine analyses because of its greater rapidity and the smaller absorption of *n*-butenes per pass (thus permitting more accurate extrapolation).

The McMillan method (*5*) for the determination of isobutene by its reaction, in the liquid phase, with anhydrous hydrogen chloride, was found to be accurate within about ±0.2 per cent for concentrations of isobutene below about 25 mole per cent (Table XII). The reagent is, apparently, specific for isobutene, as evidenced by its failure to react with 2-butene. However, the apparatus in its original form is not well adapted to routine laboratory work because of the indefiniteness of the end point of the reaction; also, appreciable quantities of air or moisture in the sample introduce errors in the determinations. When properly operated by experienced operators, however, this method is probably superior to any of the absorption procedures now available for the determination of isobutene.

TABLE XIII. CALCULATED CHANGE IN COMPOSITION OF LIQUID SATURATED HYDROCARBON SAMPLE DURING SAMPLING FROM CONTAINER

Component	Original Sample Volume Remaining in Bomb: 100%	90%	70%	50%	30%
	Mole per cent				
Sampling under Vapor Pressure of Sample					
Ethane	10.00	9.89	9.58	9.06	...
Propane	80.00	80.08	80.30	80.65	...
Isobutane	10.00	10.03	10.12	10.29	...
Sampling by Displacement with Tap Water					
Ethane	10.00	9.99	9.98	9.94	9.89
Propane	80.00	80.01	80.02	80.05	80.09
Isobutane	10.00	10.00	10.00	10.01	10.02

Methods of Sampling and Handling Light Hydrocarbon Stocks

It has been the usual practice in many laboratories to transfer liquefied hydrocarbon samples from containers to analytical equipment by (1) withdrawing the liquid sample under its own vapor pressure, or (2) eliminating the vapor space within the bomb by water repressuring and withdrawing the liquid sample by water displacement. (It is also customary to collect liquid samples by water displacement.) Under certain conditions, described below, the employment of either of these methods may effect a change in the composition of the sample sufficient to cause measurable errors in the subsequent analysis.

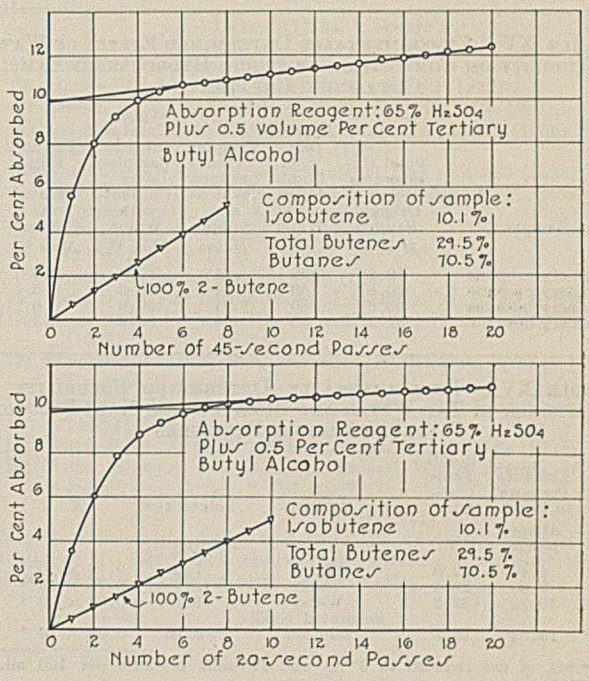

FIGURE 6. ABSORPTION OF ISOBUTENE IN 65 PER CENT SULFURIC ACID PLUS 0.5 VOLUME PER CENT *tert*-BUTYL ALCOHOL

TABLE XIV. CALCULATED CHANGE IN COMPOSITION OF LIQUID HYDROCARBON SAMPLE CONTAINING UNSATURATES DURING SAMPLING FROM CONTAINER

Component	Original Sample Volume Remaining in Bomb: 100%	90%	70%	50%	30%
	Mole per cent				
Sampling under Vapor Pressure of Sample					
Propene	50.00	49.99	...	49.90	49.75
Propane	50.00	50.01	...	50.10	50.25
Sampling by Displacement with Tap Water					
Propene	50.00	49.98	49.92	49.82	49.58
Propane	50.00	50.02	50.08	50.18	50.42
Sampling by Displacement with Saturated NaCl Solution					
Propene	50.00	50.00−	49.98	49.96	49.92
Propane	50.00	50.00+	50.02	50.04	50.08

When method 1 is employed, the magnitude of the error introduced depends upon several factors, including the composition of the sample, the amount of liquid withdrawn, and the size of the container. From the calculated data in Table XIII it is apparent that, when a sample consists of a mixture of hydrocarbons having widely separated boiling points, a detectable change in composition occurs when more than 10 per cent of the contents of the container is withdrawn. On the other hand (Table XIV), a negligible change in composition is experienced when more than 50 per cent of the contents is withdrawn from a bomb containing a sample whose constituents boil closely together.

When method 2 is employed, the magnitude of the error introduced depends upon the relative water solubilities of the components in the sample, the amount of water added to the bomb, and the size of the bomb. From data reported in the literature and obtained by experiment (Table XVII), it is evident that there is a wide variation in the water solubilities of the different hydrocarbons, especially between paraffins and olefins. According to calculated data based on experimentally determined solubilities (Table XIII and XIV), water repressuring causes no appreciable change in the com-

TABLE XV. EXPERIMENTALLY DETERMINED EFFECT OF WATER SOLUBILITY ON COMPOSITION OF LIQUID HYDROCARBON SAMPLES CONTAINING UNSATURATES

Sample	Total Unsaturates in Original Sample	Approximate % of Bomb Filled with Water	Contact Time between Water and Sample	Total Unsaturates in Sample after Contact with Water	Reduction in Unsaturate Content
	Mole %		*Hours*	*Mole %*	*Mole %*
Refinery propane	19.1	90	24	17.8	1.3
Refinery propane	20.8	50	24	20.3	0.5
Refinery butanes	30.6	80	24	30.0	0.6
Refinery butanes	31.2	50	24	30.9	0.3

TABLE XVI. EXPERIMENTALLY DETERMINED SOLUBILITY OF NITROGEN IN TAP WATER AND IN SATURATED NaCl SOLUTION UNDER VARIOUS PRESSURES

Absolute Pressure in Bomb	Bomb Temperature	Solvent	Solubility[a]	K
Atmospheres	*° C.*		*Ml./100 cc.*	
4.40	22.8	Water	7.5	0.76×10^{-4}
7.80	23.1	Water	13.6	0.78×10^{-4}
10.32	22.2	Water	18.7	0.81×10^{-4}
10.32	18.7	Saturated NaCl solution	2.15	0.09×10^{-4}

[a] Ml. of gas reduced to 0° C. and 760-mm. pressure per 100 ml. of solvent.

position of a sample consisting of saturated hydrocarbons, although the composition of samples containing unsaturates may be materially changed. Direct experiments (Table XV) on the effects of water repressuring on the composition of refinery samples containing unsaturates (made by determining, by hydrogenation, the unsaturate content of the samples before and after repressuring) have qualitatively confirmed the calculated data in Table XIV.

For many types of liquid samples, either of the above methods of transferring may cause a measurable change in the composition of the sample, especially if the sample container is small enough to necessitate the removal of a considerable percentage of its contents for analysis. Alternative methods of transferring samples are repressuring with mercury, or repressuring with a saturated solution of salt in water. Although mercury repressuring is probably the best method of transferring liquid samples contained in small bombs, no experiments have yet been made in this laboratory to determine the effect of this method upon the composition of various types of samples. Laboratory experiments have demonstrated, however (Tables XVI and XVII), that the solubility of various gases is about 80 per cent less in saturated sodium chloride solution than in water and calculations based on this fact have shown that repressuring samples with this solution results in comparatively small changes in the composition of liquid hydrocarbon samples, even when such samples contain a large proportion of unsaturates (Table XIV).

In evaluating the changes in composition of liquid hydrocarbon samples effected by the various methods of transferring such samples, an attempt has been made, whenever possible, to base all calculations upon experimental data and to check the calculated results experimentally. Calculations on the effects of water repressuring of samples were based on water-solubility values for hydrocarbons quoted in the literature and obtained by direct experiment in this laboratory.

It was assumed that Dalton's law and Henry's law (expressed in this case as $K = C/P$, where K is the solubility coefficient, C the concentration of dissolved gas in moles per 100 ml. of solvent, and P the partial pressure of the gas in atmospheres) applied to the hydrocarbon-water systems studied.

Values of K determined by experiment for the system nitrogen-water were nearly constant for pressures ranging from 4.4 to 10.32 atmospheres; values of K for various hydrocarbon-water systems were found to be slightly higher at pressures under which the hydrocarbon existed as a liquid than at atmospheric pressure (Table XVII).

The extent to which hydrocarbon-water systems followed Dalton's law was ascertained roughly by determining the water-solubility of a refinery propane-propene sample which had been analyzed in the combined modified Ward and Haldane-Henderson apparatus, and comparing the observed solubility (52.6 ml. of gas per 100 ml. of water) with the solubility calculated from the K values for the individual components in the sample (54.5 ml. of gas per 100 ml. of water).

Finally, the calculated changes in composition were checked qualitatively by observing the reduction in unsaturate content (as determined by several check hydrogenations) of several refinery samples that were repressured with various proportions of water (Table XV). It will be noted from Table XV that a detectable analytical error is introduced when 50 per cent or more of the bomb is filled with water, and that the error increases considerably as the water content of the bomb is increased.

The following procedure was employed for determining the solubilities of hydrocarbons in water and in saturated sodium chloride solution:

A 3.785-liter (1-gallon) bomb was filled with water (or solution) and approximately half the liquid was then displaced by the liquid hydrocarbon sample. The bomb, with vigorous intermittent shaking to ensure thorough contact between liquid and sample, was allowed to stand for 24 hours or more under the vapor pressure of the sample (it was found that the systems reached equilibrium within 24 hours), and then connected to an evacuated apparatus consisting of a 1-liter flask connected through a reflux condenser (through which ice water was circulated) and a calcium chloride tube to a calibrated (300-ml.) glass bulb, to which were attached a Y-type manometer and Toepler pump. A portion of the water in the bomb was then admitted to the flask and heated under partial vacuum, the evolved gases being transferred to the calibrated bulb by means of the Toepler pump and measured by noting the pressure change recorded on the manometer. Vapors were withdrawn until a constant pressure was obtained in the flask. By computing the volume of gas obtained and by weighing the flask and calcium chloride tube before and after admitting water to the system, the volume of dissolved gas per 100 ml. of water (or saturated sodium chloride solution) could be computed easily. In order to apply corrections for dissolved gases originally

TABLE XVII. EXPERIMENTALLY DETERMINED WATER SOLUBILITIES

(Liquefied hydrocarbons and mixtures of hydrocarbons under their own vapor pressure)

Sample	Absolute Pressure in Bomb	Bomb Temperature	Contact Time between Water and Hydrocarbons	Solubility[a]	K
	Atmospheres	*° C.*	*Hours*	*Ml./100 ml.*	
n-Butane	2.09	20.2	24	10.9	2.33×10^{-4}
Propane	9.43	20.9	24	27.9	1.32×10^{-4}
Refinery butane[b]	7.32	21.7	24	25.3	.....
Refinery propane[c]	13.32	22.9	24	57.1	.....
Refinery propane[c]	12.22	20.0	48	57.1	.....
Refinery propane[d]	9.16	19.8	24	52.6	.....
Isobutene	3.04	18.6	24	59.1	8.67×10^{-4}
Solubility of Isobutene in Saturated NaCl Solution					
Isobutene	2.77	17.4	72	12.1	1.95×10^{-4}

[a] Ml. of gas reduced to 0° C. and 760-mm. pressure per 100 ml. of solvent.
[b] Total unsaturates (by hydrogenation) 30.6%.
[c] Total unsaturates (by hydrogenation) 20.8%.
[d] Total unsaturates (by hydrogenation) 19.1%.

present in the tap water, blank determinations of this water were also made in the apparatus.

In computing the change in composition of various liquid light hydrocarbon samples caused by the progressive removal, under their own vapor pressure, of the samples from their containers, it was assumed that this removal consisted of a succession of small single flashes, in each of which a volume of vapor equal to the volume of liquid withdrawn was formed. By assuming Raoult's law to apply and setting up material balances between vapor and liquid after each withdrawal of sample, a series of equations was obtained which could be solved either directly or by trial and error.

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Chemical Determination of Nicotinic Acid Content of Flour and Bread

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THAT the new synthetic vitamins would ultimately be used on a major scale for the nutritional enhancement of some of our more refined foods was almost a foregone conclusion. Much emphasis has been directed toward the nutritional improvement of white flour by the restoration of the members of the vitamin B complex to the level of whole wheat. Nicotinic acid is one of the principal vitamins used in the enrichment of flour and bread, but because of limitations in the biological method for testing foods of low nicotinic acid content, no reliable data have been available up to the present time on the concentration of this vitamin in flour and related products. Previously reported chemical methods lack specificity when applied to materials of plant origin (*14*).

In the present study the Melnick-Field chemical method (*8*) for the determination of nicotinic acid has been adapted for use in analyzing flours and breads. The nicotinic acid content of a variety of such products is presented. Particular attention has been directed toward study of the reliability of the extraction procedure and of the subsequent chemical analysis, the specificity of the method for nicotinic acid in these materials, and the fate of the vitamin during the fermentation of the dough and baking of the bread.

Principle of Method

A concentrated aqueous extract is digested with hydrochloric acid to liberate any nicotinic acid from its compounds. The hydrolyzate is made alcoholic and is subjected to preferential charcoal adsorption for the removal of most of the interfering pigments without loss of nicotinic acid. The neutralized filtrate is treated with cyanogen bromide and aniline reagents, yielding a yellow color which is measured. Calculation is based on suitable correction for residual interfering color and on the increment in photometric density obtained with a known addition of nicotinic acid to an aliquot of the test solution.

Reagents

CYANOGEN BROMIDE. Water saturated with bromine at 5° to 10° C. is just decolorized in the cold by the addition of a 10 per cent potassium cyanide solution. From 70 to 75 cc. of the potassium cyanide solution are used in the titration of 500 cc. of bromine water. This reagent when stored in the refrigerator at about 5° C. will keep almost indefinitely (more than 5 months).

ANILINE SOLUTION. Redistilled aniline is dissolved in absolute ethyl alcohol to make a 4.0 per cent solution. When stored in a brown glass bottle at room temperature, this reagent will keep for months.

STANDARD NICOTINIC ACID SOLUTION. This contains 100 micrograms per cc. of absolute ethyl alcohol and will keep permanently.

BUFFER SOLUTION. Composed of 1960 cc. of water, 10 cc. of phosphoric acid (85 per cent), 30 cc. of 15 per cent sodium hydroxide, and 333 cc. of absolute ethyl alcohol.

HYDROCHLORIC ACID SOLUTIONS. Concentrated (specific gravity about 1.18), and approximately 1 *N*.

SODIUM HYDROXIDE SOLUTIONS. Concentrated (approximately 18 *N*), and approximately 1 *N*.

PHENOLPHTHALEIN SOLUTION. 1 per cent in alcohol.

CHARCOAL. Darco-G-60, a vegetable charcoal (obtainable from the Darco Corp., 60 East 42nd St., New York, N. Y.).

Extraction of Nicotinic Acid

A 2-gram sample of flour or air-dried bread is weighed into a 250-cc. Erlenmeyer flask, 100 cc. of distilled water are added, and the suspension is autoclaved for 30 minutes at 7 kg. (15 pounds) pressure. The material is centrifuged and the precipitate washed twice with 50 cc. of boiling water. The pooled extract and washings may be concentrated *in vacuo* (if speed is a factor) or in an oven at 115° C. overnight to a volume of 5 cc. or less. [The distillation apparatus described by Melnick and Field (*7*) with slight modifications lends itself readily to concentration of extract. Caprylic alcohol (10 drops) is added to the pooled extracts to prevent foaming. An air-inlet tube extending to the bottom of the distillation flask controls bumping. While concentration to dryness involves no loss of nicotinic acid, subsequent

solution is facilitated by evaporating short of dryness.] Five cubic centimeters of concentrated hydrochloric acid are added and the material is substantially dissolved by immersing the flask or beaker in a boiling water bath. The solution, or suspension, is transferred to a calibrated test tube and diluted to the 15-cc. mark with water previously used to rinse the flask (or beaker). The acidity is approximately 4 *N*.

Hydrolysis, Decolorization, and Chemical Reaction

The test tube is immersed in a boiling water bath and the hydrolysis allowed to proceed for 30 to 40 minutes with occasional stirring. If foaming occurs, a few drops of caprylic alcohol may be added. The sample is cooled to room temperature and the volume restored to the original 15 cc. Ten cubic centimeters of absolute ethyl alcohol are added, and the solution is transferred to a 125-cc. Erlenmeyer flask followed by 300 mg. of charcoal. The mixture is shaken and filtered at room temperature. (Whitall Tatum filter paper, 3-inch, manufactured by the Armstrong Cork Co., Lancaster, Penna., is suitable.) A one-half aliquot of the filtrate, 12.5 cc., is pipetted into the graduated test tube, one drop of phenolphthalein is added, and the solution is neutralized in the cold to pH 7, and diluted to 15 cc. (Concentrated sodium hydroxide solution is used at first; the final pH adjustment is made with the 1 *N* reagents, using litmus paper as an outside indicator.)

For the colorimetric tests 3-cc. portions of this test solution (equivalent to one-tenth aliquots of the original sample) are used. (*a*) To the first sample 7 cc. of the alcoholic buffer solution are added. (*b*) To the second aliquot, 6 cc. of the cyanogen bromide reagent are added from a buret, followed immediately with the addition of 1 cc. of the aniline solution. The solutions are stirred after the addition of each reagent. (*c*) To the third aliquot of the test solution, 0.1 cc. of the standard nicotinic acid solution (10 micrograms) is added, followed by the reagents as in (*b*).

Table I. Nicotinic Acid Values of Aqueous Extract and Entire Test Material

Sample	Flour	Ash Content	Nicotinic Acid Values: Direct hydrolysis of sample	Nicotinic Acid Values: Hydrolysis of concentrated aqueous extract
		%	*Mg. %*	*Mg. %*
A	Whole wheat, hard, spring	1.82	9.25	6.49
B		1.84	9.60	6.20
C	Whole wheat, soft, winter	1.92	9.80	5.43
D		1.77	9.50	5.24
E	Entire wheat	0.82	4.02	2.09
F	Patent, hard (bread flour)	0.45	2.27	1.90
G		0.44	2.56	1.78
H	Patent, soft (cake flour)	0.40	2.19	1.73
I	Patent, soft (pastry flour)	0.43	2.30	1.32
J	Rye, medium	1.22	3.63	2.46
K	Rye, light	0.85	3.35	1.78
L	Rye, white	0.83	2.30	1.40
M	Clear, hard, spring	0.71	3.32	2.51
N		0.73	3.08	2.17

Readings and Calculations

The maximal yellow color, developed within 3 to 5 minutes and generally stable for at least the next 5 minutes, is read in a photoelectric colorimeter with a 420 millimicron filter. By subtracting from the photometric density of solution (*b*) that of solution (*a*) due to the color remaining after decolorization, the photometric density of the reacted nicotinic acid is obtained. This is converted into absolute units of nicotinic acid by correlating the increment in photometric density, solution (*c*) minus solution (*b*), with the amount of nicotinic acid added.

In the colorimetric measurements it is necessary to have two center settings, one for evaluating the residual color in the test solution, the other for the color developed by the chemical reaction. The photoelectric colorimeter is set to give a galvanometer reading of 100.0 (zero photometric density) with a solution containing 2 cc. of water, 1 cc. of alcohol, and 7 cc. of alcoholic buffer solution. Using the resulting center setting (No. 1, galvanometer reading with the test tube or cuvette containing the pure solution now removed), the blank solutions are read in turn to determine residual color of each of the test solutions (blank). The colorimeter is then set to give a galvanometer reading of 100.0 with a solution containing 2 cc. of water, 1 cc. of alcohol, 6 cc. of cyanogen bromide reagent, and 1 cc. of aniline solution. All the subsequent solutions containing reacted nicotinic acid are read using the new center setting (No. 2). Galvanometer readings are converted into photometric density by the formula

$$L = 2 - \log G$$

where L = photometric density and G = galvanometer reading. (Most instruments have a dual scale which allows direct reading in terms of photometric density; others are supplied with a conversion table.)

If the colorimeter cell requires more than 10 cc. (and less than 20 cc.) of solution, the alcoholic, decolorized, neutralized test solutions are diluted to double volume with a 33 per cent ethyl alcohol solution and 6-cc. aliquots are used for testing. Double quantities of reagents are also used. If in place of the test-tube type of cell, a flat cuvette is used, the center settings will be greater than 100.0 and therefore will be off the scale. In such cases the instrument is set to give a galvanometer reading of 50.0 instead of 100.0 and all readings are multiplied by 2 to give the correct value for conversion to photometric density.

Typical Calculation. Test substance = 2.000 grams of whole-wheat flour, cell = flat cuvette, and volume of solutions to be read = 20 cc.

Center setting (No. 1) = 53.5 galvanometer reading (observed) = 107.0 galvanometer reading (corrected).

Center setting (No. 2) = 57.5 galvanometer reading (observed) = 115.0 galvanometer reading (corrected).

Solution (*a*) using center setting (No. 1) = 46.5 galvanometer reading (observed) = 93.0 galvanometer reading (corrected) = 0.032 photometric density.

Solution (*b*) using center setting (No. 2) = 20.25 galvanometer reading (observed) = 40.5 galvanometer reading (corrected) = 0.392 photometric density.

Solution (*c*) using center setting (No. 2) = 9.75 galvanometer reading (observed) = 19.5 galvanometer reading (corrected) = 0.710 photometric density.

0.392 − 0.032 = 0.360 photometric density due to reacted nicotinic acid.

0.710 − 0.392 = 0.318 photometric density due to 10 micrograms of reacted nicotinic acid.

$\frac{0.360}{0.318} \times 10 = 11.3$ micrograms of nicotinic acid in test solution.

$11.31 \times 10 = 113$ micrograms in sample of test material.

$113 \times \frac{1}{2.000} = 56.5$ micrograms per gram of whole-wheat flour.

Validity of Method for Analysis of Flour and Bread

Nicotinic Acid Content. Fourteen different samples of flour were analyzed. In one series the 2-gram samples were subjected to direct acid hydrolysis according to the procedure originally reported (*8*); in the second series the samples were subjected to aqueous extraction as described above. The results are presented in Table I.

The figures obtained by direct acid hydrolysis of the test materials are from 50 to 100 per cent higher than those obtained on the aqueous extract. It was conceivable that incomplete extraction rather than increased specificity of the method for nicotinic acid was responsible for the smaller values noted in the latter series. The original procedure of direct acid treatment eliminates the possibility of low values due to faulty extraction, inasmuch as the test material is almost completely dissolved during the hydrolysis. Accordingly, both series of analyses were repeated after the addition of nicotinic acid to flours E to N, in amounts sufficient to bring the vitamin content to 5.0 milligrams per cent. In both series good recovery values were obtained, varying from 90 to 110 per cent of the added nicotinic acid.

However, a satisfactory recovery experiment does not necessarily prove that the nicotinic acid naturally present within the particles themselves has been extracted. The existence of this possibility was emphasized by experience with the chemical analysis for thiamine in foods. In order to test further the efficiency of the procedure for effecting complete extraction of nicotinic acid from within the flour granules themselves, analyses were conducted on breads baked with ordinary and nicotinic acid–enriched flours. The results are

summarized in Table II. As expected, the basal breads yielded higher values when subjected to the procedure of direct acid hydrolysis. Approximately 85 per cent of the added nicotinic acid was recovered by this method, which involves solution rather than extraction of the sample. When the tests were conducted on the aqueous extracts, smaller values were obtained, but the recovery of the added nicotinic acid was the same as that noted in the initial tests. This agreement is good evidence of the complete removal of nicotinic acid by the preliminary aqueous extraction procedure. The low recoveries of the added nicotinic acid (85 per cent) noted in testing the enriched breads are not due to errors in either method, since direct additions of nicotinic acid to air-dried bread samples are followed by complete recoveries. The smaller values obtained by the modified method appear to be due to improvement in the specificity of the procedure for nicotinic acid in plant products.

Reproducibility tests using the modified method were conducted with the fourteen flours listed in Table I and with ten ordinary and enriched breads. These have indicated that, on the average, duplicate values agree to within ±3 per cent of the mean, the maximal difference being ±7 per cent of the mean.

Table II. Completeness of Aqueous Extraction of Nicotinic Acid from Enriched Bread

Sample	Direct Hydrolysis of Sample: Found	Increment	Recovered	Hydrolysis of Concentrated Aqueous Extract: Found	Increment	Recovered[a]
	Mg./lb.	*Mg./lb.*	%	*Mg./lb.*	*Mg./lb.*	%
Basal bread A	7.9	..	..	5.7	..	..
Enriched bread[b]	16.3	8.4	83	14.5	8.8	87
Basal bread B	7.8	..	..	5.8	..	..
Enriched bread[c]	16.5	8.6	84	14.9	9.1	88

[a] Recoveries expressed as per cent of added nicotinic acid.
[b] Increment expected, 10.12 mg. of nicotinic acid per pound (assuming no loss in baking).
[c] Increment expected, 10.35 mg.

Specificity for Nicotinic Acid

This chemical reaction with cyanogen bromide and aniline is not specific for nicotinic acid alone but for the pyridine ring with the alpha position unsubstituted (*14*). The method as originally published by Melnick and Field (*8*) has received much study in the University of Wisconsin laboratory, where it gave good agreement with the black-tongue assay on dogs in the case of animal and yeast products. For this reason the simpler and less time-consuming procedure of direct acid hydrolysis is used in analyses of such products. The earlier papers (*6*, *8*) indicate what quantity of the test materials may be taken for testing and how much charcoal should be used for the decolorization. Other points are discussed, such as the stability of the reagents, the necessity for using acid in preference to alkaline hydrolysis, the validity of the method of calculation, and the necessity for carrying out the two independent blank corrections. The much higher values obtained when testing products of plant origin (*14*) can be attributed to the widespread occurrence of pyridine compounds in plant materials. The animal organism does not synthesize pyridine compounds but has a marked ability to destroy and excrete such compounds, regardless of how they are administered (*10*).

The values obtained in the present study agree very well with those found by Snell and Wright (*12*) using their microbiological assay for nicotinic acid (Table III). The fact that two totally unrelated methods, one involving a chemical reaction, the other the growth and metabolism of bacteria, yield essentially the same values, is highly presumptive that

Table III. Agreement between Chemical and Microbiological Methods

Material	Nicotinic Acid Content: Chemical assay	Microbiological assay[a]
	Mg. %	*Mg.* %
Whole-wheat flour	5.2–6.5	4.5–7.0
Patent flour	1.3–1.9	1.0–2.0

[a] Method of Snell and Wright (persona communication).

both procedures measure the same substance, and that both methods are specific for nicotinic acid in these products. More recently at the University of Wisconsin biological assay of whole-wheat flour with dogs has indicated a value of 5.4 mg. per cent (*13*), in good agreement with the values listed in Table III.

Some of the published chemical methods may yield lower values than those recorded in the present paper, notwithstanding their good reproducibility and good recoveries of added nicotinic acid. However, it can be readily shown that in such cases the smaller values are not due to greater specificity for nicotinic acid but to errors inherent in the methods.

Data obtained for nicotinic acid in flour and bread by a chemical procedure which does not involve preliminary hydrolysis are unreliable. Nicotinic acid exists in nature almost entirely as free and combined nicotinamide (*4*) and in such forms reacts in variable degrees with the reagents to give colors of much smaller intensity than would be expected for an equivalent amount of free nicotinic acid (*4*, *8*). Accordingly, total nicotinic acid in enriched bread and flour cannot be evaluated under such circumstances. Only through hydrolysis to the free nicotinic acid stage can all these related compounds be converted to a common denominator and thus be capable of chemical estimation.

Improper evaluation of the blank value (residual color of the test solution) can also yield erroneous—i. e., smaller—values. The blank described by Bandier (*2*) for making this correction involves the addition of all reagents except the aromatic base to an aliquot of the test solution. However, since cyanogen bromide reacts to give the same color to an appreciable extent with nicotinic acid even in the absence of the base, false low results are obtained. It was found in tests with pure solutions containing 3 to 50 micrograms of nicotinic acid, that the yellow color developed without aniline is equivalent to 33 to 20 per cent, respectively, of that obtained when the base is included.

In other methods (*1*, *3*, *5*, *6*) the cyanogen bromide reagent is omitted but the aromatic amine included in the blank test. The base was observed to react directly with substances in the hydrolyzates to yield colors indistinguishable from that of the reacted nicotinic acid. Though this observation was confirmed, it has been shown that in the presence of cyanogen bromide these interfering side reactions do not occur (*9*). Thus, inclusion of the base in the blank test will give too high a value for the blank correction with an associated decrease in the calculated nicotinic acid content. In the earlier study (*9*) which established this point, a wide variety of test materials was investigated, but flours and breads were not included. It was of interest, therefore, to conduct comparable studies on these products, especially since the present procedure includes the use of the concentrated aqueous extract rather than the whole test material for the hydrolysis.

In Table IV representative data are presented. For convenience the procedure already reported (*8*) for making the blank correction is referred to as the "dilution blank" while the other, which includes the addition of the aromatic amine, is called the "aniline blank". (In the latter case, 6 cc. of

TABLE IV. EFFECT OF ANILINE IN BLANK IN NICOTINIC ACID DETERMINATION

(Indication of Specificity)

Test	Sample (2.000 Grams)	Time of Reading[a]	Photometric Density (2 − log G) Dilution blank	Aniline blank	Test with cyanogen bromide and aniline	Nicotinic Acid Using dilution blank	Nicotinic Acid Using aniline blank
						Mg. %	*Mg. %*
A	Wheat germ[b]	5 min.	0.058	0.119	0.211	6.3	3.8
		18 hours	0.051	0.248	0.105		
		48 hours	0.056	0.310	0.066		
B	Whole-wheat flour C	5 min.	0.048	0.168	0.475	5.9	4.2
		18 hours	0.051	0.426	0.168		
		48 hours	0.056	0.538	0.061		
C	Patent flour I	5 min.	0.071	0.181	0.174	1.4	0.0
		18 hours	0.071	0.420	0.100		
		48 hours	0.071	0.545	0.076		
D	Bread from ordinary patent flour	5 min.	0.046	0.155	0.194	2.1	0.6
		18 hours	0.060	0.409	0.094		
		48 hours	0.051	0.553	0.056		
						Micrograms	
E	10 micrograms of nicotinic acid	5 min.	0.000	0.000	0.323	10	10
		18 hours	0.000	0.000	0.066		
		48 hours	0.000	0.000	0.000		

[a] Reaction between nicotinic acid and reagents reaches maximum in 5 minutes.
[b] Solution from this sample was diluted 1 to 3 (1 + 2) for colorimetric tests. All readings were made on 1/10 aliquots of final test solutions.

0.03 *M* orthophosphoric acid, in addition to the aniline, were added in place of the buffer to 3 cc. of the test solution in order to simulate test conditions with respect to pH and titratable acidity.)

The data in Table IV indicate that the initial blank value (read after 5 minutes) for the solution containing aniline is in every case greater than that obtained following simple dilution to volume with the buffer. For this reason the nicotinic acid values show a marked discrepancy between the two series, depending upon which blank value is used in the calculations. In every case smaller values are obtained when the aniline blank is used.

When solutions tested for nicotinic acid are allowed to stand, there is a fading of the yellow pigment (test E, Table IV). The dilution blanks remain constant. However, the aniline blanks progressively increase in color intensity, so that at the end of 48 hours they are exceedingly large and bear no possible relationship to the solutions tested with the reagents. (Use of *p*-aminoacetophenone in an equivalent amount gave the same results.) What is especially pertinent is that in these latter tests the same solutions were used with the same amount of aniline. Apparently, the cyanogen bromide added in order to test for the nicotinic acid inhibited the aniline side reactions. Only by carrying out an independent blank determination of the residual color subsequent to decolorization, in addition to that on the reagents, can the color due to reacted nicotinic acid be evaluated.

Study of the rate of fading of the reacted nicotinic acid yields further evidence of the specificity of the present method for nicotinic acid in flour and bread. In tests with pure solutions of nicotinic acid (E, Table IV) the color produced reaches a maximum in 5 minutes, is stable for the next 5 to 10 minutes, and then slowly fades. Within 18 hours after the initiation of the reaction 80 per cent of the original color is lost and within 48 hours it completely disappears. The same rate of development and fading of color was observed in testing each of the materials described in this paper. At the end of 48 hours, the residual color in each test solution was practically the same as that for the dilution blank, indicative of complete fading of the color produced by the chemical reaction. This parallelism in the course of the reaction and in reaction velocities further supports the view that the material measured by the chemical method is actually nicotinic acid.

Fate of Nicotinic Acid during Fermentation of Dough and Baking of Bread

Using the chemical method described in this paper, analyses were conducted on aliquots of mixed bread ingredients prior to the addition of water and on the final baked loaves. Two methods of enrichment were used in these tests: the use of flour containing added nicotinic acid; and the use of ordinary flour, the extra nicotinic acid being supplied by a high-vitamin yeast. The values obtained are presented in Table V. From 83 to 99 per cent of the total nicotinic acid present in the bread ingredients was recovered on analysis of the air-dried bread samples. This small loss in nicotinic acid during the fermentation of the doughs and the baking of the breads is in keeping with the known stability of this vitamin to oxidation (*11*) and heating, even in the presence of strong acid or alkali (*10*).

Summary

The Melnick-Field method for the chemical determination of nicotinic acid has been adapted for use in testing flour and bread. The completion of the preliminary aqueous extraction, reproducibility of results, recovery of added nicotinic acid, and specificity of the method for nicotinic acid in such products have been found to be highly satisfactory.

Whole-wheat flour contains from 5.2 to 6.5 mg. of nicotinic acid per cent; patent flour from 1.3 to 1.9 mg. per cent. The more flour is refined, the lower is its nicotinic acid content. During the fermentation of dough and baking of bread only small losses of nicotinic acid occur.

Acknowledgment

The authors are indebted to Harry A. Waisman of the University of Wisconsin for suggesting the use of the aqueous extraction in analyzing plant products for nicotinic acid, and to W. A. Lohman, Jr., General Mills, Inc., New York, N. Y.,

TABLE V. FATE OF NICOTINIC ACID DURING FERMENTATION OF DOUGH AND BAKING OF BREAD

Sample	Means of Enrichment	Nicotinic Acid Increment	Total Nicotinic Acid Values Expected	Found	Recovery
		Mg./lb. bread	*Mg./lb. bread*		*%*
A-1, white bread	None	0.0	6.1[a]	5.7	93
A-2, white bread	Nicotinic acid added to flour	10.12	16.22	14.5	89
B-1, white bread	None	0.0	6.4[a]	5.8	91
B-2, white bread	Nicotinic acid added to flour	10.35	16.75	14.9	89
C-1, white bread	None	0.0	7.0[a]	6.4	92
C-2, white bread	High-vitamin yeast	9.7	16.7	14.7	88
C-3, white bread	High-vitamin yeast	9.7	16.7	13.9	83
C-4, white bread	High-vitamin yeast	6.0	13.0	12.2	94
C-5, white bread	High-vitamin yeast	4.1	11.1	10.8	97
D-1, whole-wheat bread	None	0.0	19.7[a]	19.4	99

[a] Based on analyses of bread ingredients prior to addition of water.

and R. S. Swanson and H. S. Cullens, Thomas Baking Co., Long Island City, N. Y., for kindly supplying the flours used in this study.

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Sampling of Imported Wool for the Determination of Clean Wool Content

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RAW wool is a composite of wool fiber, grease, suint, excreta, dirt, sand, clay, vegetable matter, moisture, and occasionally paint. Its content of clean wool varies from 20 to 80 per cent, averaging around 50 per cent. In this condition it is marketed in bales or bags ranging in weight from 90 to 540 kg. (200 to 1200 pounds).

Commercially, the determination of "shrinkage", or its opposite, the percentage of clean wool content, of a lot of raw wool is based upon visual examination by specialists, an admittedly unsatisfactory procedure. When differences between the opinions of specialists exist, settlement tests are often made by scouring from 5 to 25 per cent of the bales in controversy. Sometimes one or more "representative" fleeces are drawn for test.

The accurate determination of the wool content of a lot of raw wool is of great importance to the textile industry, wool growers, the Government, and others. No completely satisfactory laboratory method has hitherto been developed, primarily because of the lack of a practical procedure for obtaining a representative sample. Several factors have contributed to this, most important being the nonuniform character of the material. The impurities are not uniformly distributed over a fleece, very wide variations from portion to portion being common. Furthermore, the percentage of "clean wool" content of the individual fleeces in a package varies more or less widely, and this is also true of the different packages in a lot. The second major difficulty is the practical one of removing portions of wool from heavy, bulky, tightly compressed bales or bags under congested conditions where the time element is of the utmost importance.

Some experimental work on the sampling of raw wool has been done by various investigators. Jones and Lush (*3*) studied the representativeness of a sample composed of 10 per cent of the fleeces, drawn at fixed numerical intervals at the time of shearing. Wilson (*7*) composited small handfuls taken from random locations in each fleece. Spencer, Hardy, and Brandon (*6*) compared the shrinkage of specific areas of fleeces with that of the entire fleeces. The Bureau of Agricultural Economics of the U. S. Department of Agriculture is conducting investigations along similar lines.

The present investigation was undertaken to determine the possibility of obtaining small samples of known and controlled levels of accuracy by the application of well-known and accepted principles of statistics.

Theoretical

RANDOM SAMPLES FROM HOMOGENEOUS LOTS. The mean of the observed values of a given quality of a number of units or small portions taken at random from a large homogeneous lot of merchandise is, in general, closer to the true average value of the quality for the entire lot than is any single observed value. The greater the number of such observations and the lesser the degree of nonuniformity among the observed values, the closer will the mean of the observations be to the true value for the lot. This

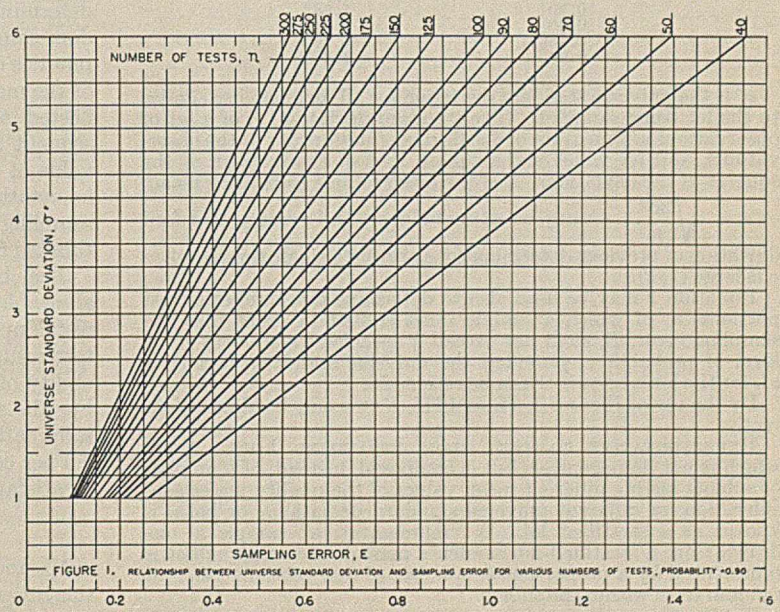

FIGURE 1. RELATIONSHIP BETWEEN UNIVERSE STANDARD DEVIATION AND SAMPLING ERROR FOR VARIOUS NUMBERS OF TESTS, PROBABILITY =0.90

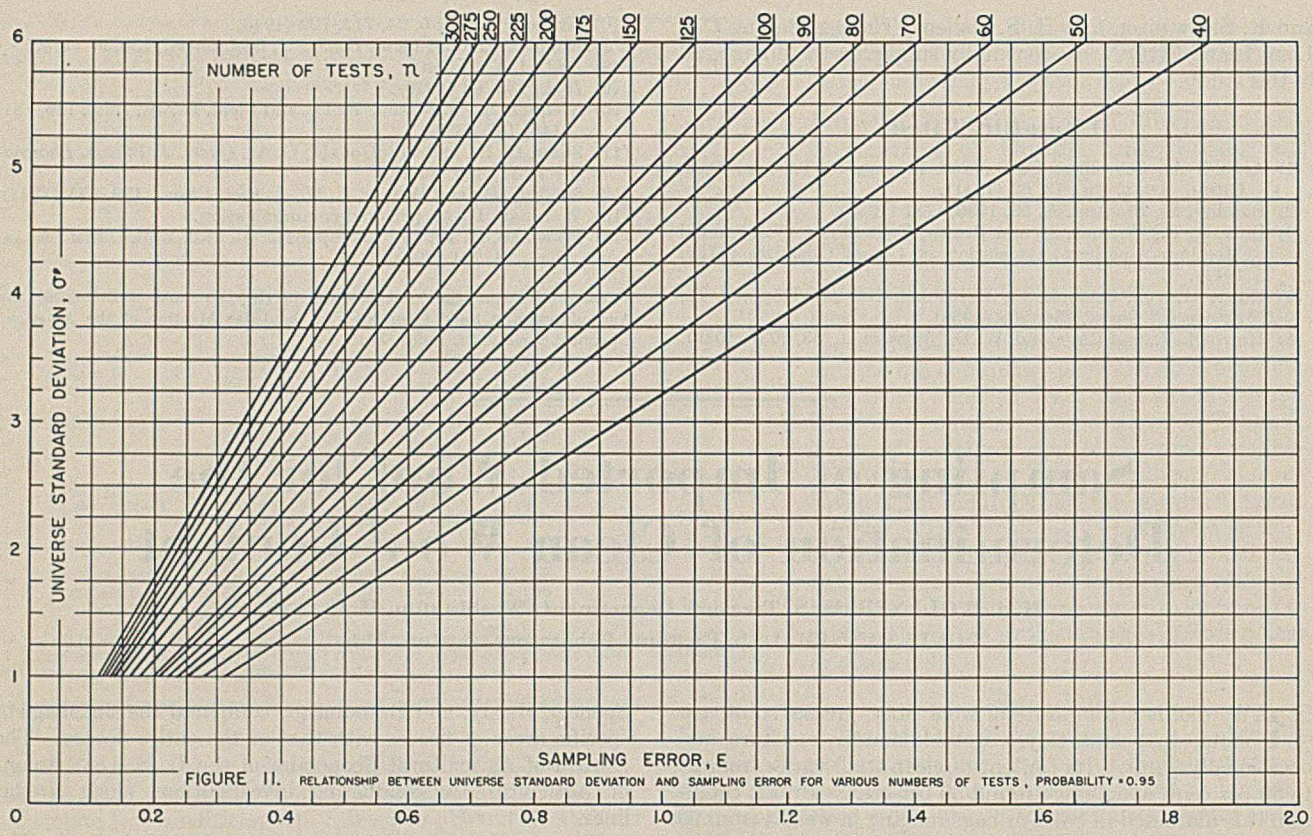

FIGURE II. RELATIONSHIP BETWEEN UNIVERSE STANDARD DEVIATION AND SAMPLING ERROR FOR VARIOUS NUMBERS OF TESTS, PROBABILITY = 0.95

can be expressed by the following closely approximate relationship, which is based upon the theory of probability:

$$E = \frac{t\,\sigma'}{\sqrt{n}} \qquad (1)$$

where E is the sampling error, n is the number of random portions tested, and t is the probability factor. The value chosen for this factor determines the theoretical probability that the mean of the test values will not differ from the true value for the lot by more than the sampling error, E. Several such factors for various probabilities follow:

Probability	t
0.50	0.675
0.60	0.842
0.70	1.036
0.80	1.282
0.90	1.645
0.95	1.960
0.99	2.576

σ' is the universe standard deviation for all merchandise similar to the lot being sampled. The standard deviation, σ, of a set of observations is a measure of the degree of uniformity of the observations, and its value is the square root of the average of the squares of the deviations, d, of the observations from their mean, or $\sigma = \sqrt{\frac{\Sigma d^2}{n}}$. σ' may be estimated from the average standard deviation of previous determinations or from a single sample when n is large (*1*, *2*).

Equation 1 may be used also to determine the number of test portions, n, of which a sample must consist in order that the sampling error, E, shall not exceed a specified value for a given level of probability, provided the universe standard deviation, σ', for the lot is known or can be estimated.

By the use of Figures I, II, and III, E or n may be obtained directly.

Representative Samples from Stratified Lots. A lot which is not homogeneous but is composed, instead, of a number of sublots having different mean values of the quality under consideration, or different universe standard deviations, or both, is known as a stratified lot. A "representative" sample is one drawn from a stratified lot in such a manner that each sublot is represented by a proportionate number of test portions taken at random from within the sublot.

When the universe standard deviations within the sublots are equal—i. e., $\sigma'_{w1} = \sigma'_{w2} = \ldots = \sigma'_{w}$—it can be shown (*4*) that for representative samples of n portions the sampling error, E, depends only upon the variability within a sublot, σ'_w, and not upon differences between the mean values of the sublots or upon the proportions of the sublots in the entire lot. Equation 1 applies, using for σ' the universe standard deviation within a sublot.

From Equation 1 it is obvious that, given the limit of permissible sampling error, E, for a specified probability, the number of random test portions, n, to be taken from a lot of material to constitute a sample thereof can be calculated, provided the universe standard deviation, σ', is known. This is practically never the case in actual practice, but a reasonably close value can be selected when sufficient previous determinations of standard deviation have been made on random samples from similar material. Any serious change of practice on the part of the producer, or variation in the nature of the material, or difference in the sampling procedure, is reflected in the changed level of the standard deviations of subsequent samples, so that necessary revision of the selected value of σ' can be made. Whatever the selected value may be, statistical procedure provides a method whereby the sampling error at any level of probability may be reliably estimated from the actual data obtained by testing the samples.

The choice of particular values for the probability and the allowable sampling error is a matter for judgment and depends upon many factors, such as the use to be made of the test results, the cost of sampling and testing, etc. A probability of 95 per cent, or chances of 95 in 100, is often used. For the sampling of lots of imported greasy wool for the determination of wool content a maximum sampling error of 0.5 per cent of wool content, with a probability of 95 in 100, is satisfactory for most purposes.

Experimental

Nature of Wool Investigated. The initial phase of this investigation was limited to Australian and South Ameri-

can wools, each of which represents a major type of imported raw apparel wool, in so far as methods of preparation and packaging are concerned. The former type includes wool not only from Australia, but also from New Zealand and South Africa; the latter type includes wool from both Argentina and Uruguay. Bales of Australian type wool contain about forty graded fleeces from which the inferior portions and the grosser impurities have been removed. The average weight is about 135 kg. (300 pounds), and average dimensions about 90 × 75 × 75 cm. (36 × 30 × 30 inches). South American bales are larger; they weigh about 450 kg. (1000 pounds) and measure approximately 150 × 90 × 75 cm. (60 × 36 × 30 inches). They are more tightly compressed than are bales of Australian wool and the fleeces are not, as a rule, so carefully prepared and graded. The outstanding characteristic of both these types of imported wool is the relative uniformity, as compared with domestic wool, of the contents of each package, reflective of the greater care with which the foreign fleeces are graded and prepared for packing. This higher degree of uniformity aids greatly the development of a practicable method for sampling imported wool.

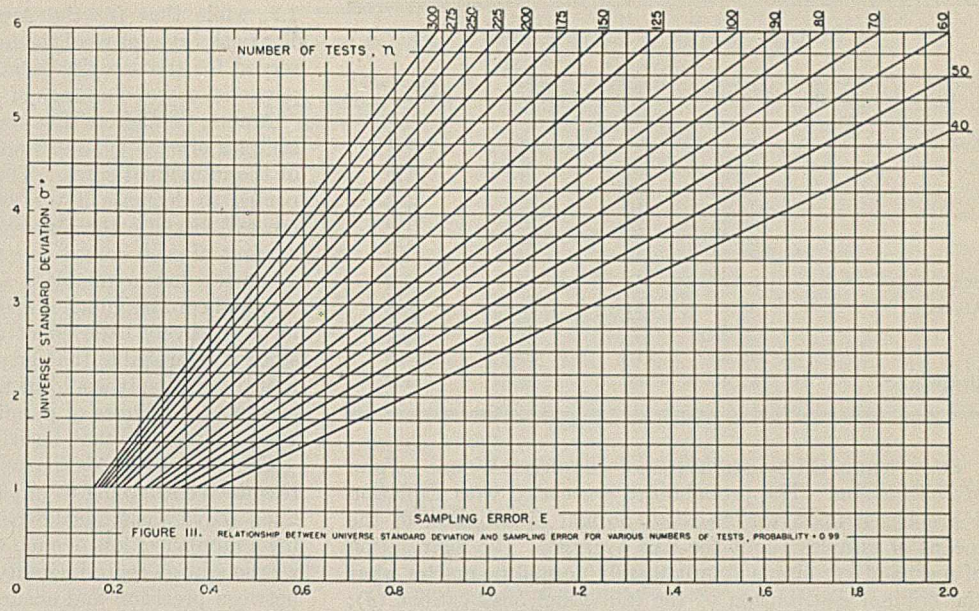

FIGURE III. RELATIONSHIP BETWEEN UNIVERSE STANDARD DEVIATION AND SAMPLING ERROR FOR VARIOUS NUMBERS OF TESTS, PROBABILITY = 0.99

Lots of Australian and South American greasy apparel wool, as imported, are usually composed of a number of sublots of one or more bales. It is generally accepted that all the bales in a sublot contain wool similar in character, quality, and grade, have the same level of percentage clean content, and have been produced, treated, and packed in substantially the same manner. Such sublots, therefore, are the "universes" to which the universe standard deviation, σ', of Equation 1 applies.

Method of Sampling Used. The experimental work was directed toward the determination of the possibilities of representative sampling of imported greasy apparel wool.

An equal number of cores (one or more) were taken from each bale by means of specially designed equipment, consisting essentially of a hand-operated, motor-driven, revolving tube provided with easily replaceable cutting edges. The locations of the borings in any bale were chosen at random, limited by the presence of baling bands or metal closure devices. Practical considerations led to the adoption of 5 cm. (2 inches) as the most suitable diameter for the tube. The maximum feasible depth of penetration was 40 cm. (16 inches); usually, however, a depth of 20 to 25 cm. (8 to 10 inches), yielding a core weighing 100 to 150 grams, was found satisfactory. Contamination of the sample with burlap was avoided by cutting a V-shaped slit in the bale covering and raising the flap before boring. Each core was immediately put into a labeled waxed paper bag which was kept in a moisture-proof drum container until weighed.

Laboratory Procedure in Determination of Clean Content. The weighed cores, in labeled cotton net bags of fine mesh, were degreased with a volatile solvent, then fluffed by hand. In this condition the wool, still in the cotton bags, was thoroughly washed by suspension in warm dilute soap solutions and passage between the rolls of a wringer. During this process the bulk of the vegetable impurities was carefully removed by hand. The wool was then rinsed, hydroextracted, and dried to constant weight at 105–110° C. in electric conditioning ovens. Total extractives and ash in the dried wool were determined on test portions. Remaining vegetable matter was determined by picking it out by hand or by comparing with prepared standards. All percentages of clean wool content were computed to the same content of moisture, extractives, and ash in the final product.

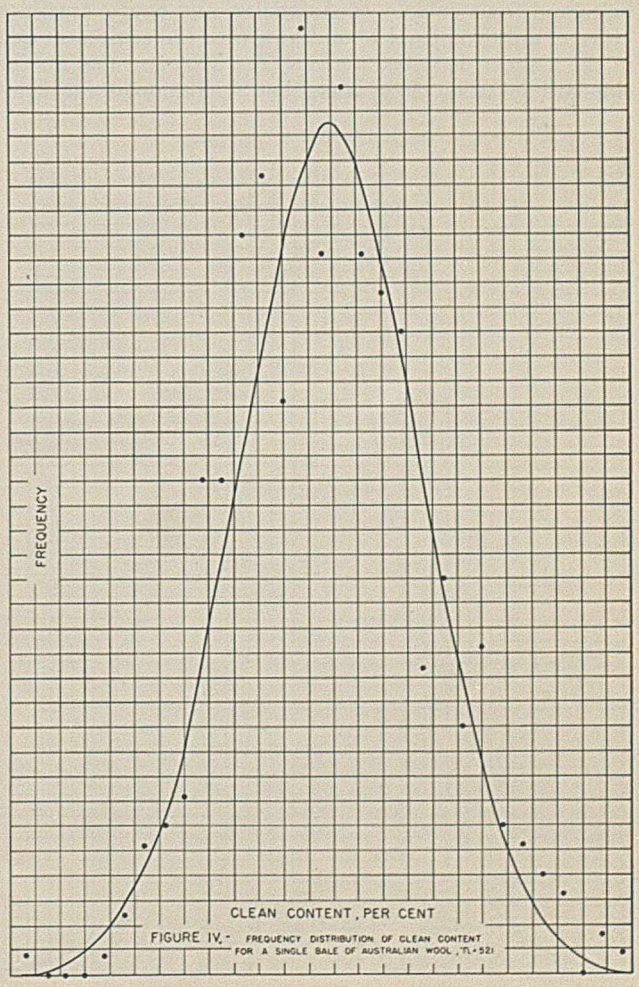

FIGURE IV. FREQUENCY DISTRIBUTION OF CLEAN CONTENT FOR A SINGLE BALE OF AUSTRALIAN WOOL, n = 521

TABLE I. REPRESENTATIVE VALUES

Origin	No. of Bales	Clean Content %	Average Weight per Core *Grams*	No. of Test Portions, n	Standard Deviation, σ
Australia	4	48.6	96	40	2.1
	3	56.0	112	54	1.9
	1	60.2	124	18	1.6
	1	61.0	130	16	1.1
	1	62.4	110	16	1.9
	1	67.0	93	18	1.9
	1	68.6	128	18	1.2
	1	71.3	117	18	1.8
South Africa	5	54.2	145	30	2.2
Argentina	1	48.1	211	20	2.3
	1	50.4	200	20	2.8
	1	46.8	210	20	2.4
	1	48.5	218	20	1.8
Uruguay	1	57.4	162	15	1.7

STATISTICAL CHARACTERISTICS OF IMPORTED WOOL. *Form of Distribution.* The probabilities associated with Equation 1 are based upon the so-called normal distribution of the means of samples about the true average. The distribution of means of samples is approximately normal no matter what the original distribution of the individual observations (δ); however, the more nearly "normally" these observations are distributed, the more exact is the relationship.

Figure IV is typical of the distribution of the observations of percentage clean content in individual bales of Australian wool. Figure V shows the distribution for a large lot of such wool. The theoretical "normal" curves corresponding to the experimental data are shown for comparison.

Standard Deviation. Table I shows some representative values of standard deviation found for samples from single bales and from sublots of Australian and South American types of greasy apparel wool drawn with the equipment and in the manner described above. The average value for the standard deviation of the Australian wool samples was about 1.7, while that for the Argentine samples was about 2.3. For both types of wool the individual standard deviations observed for the samples (a majority comprising 15 to 20 test portions) covered a range of 1.0.

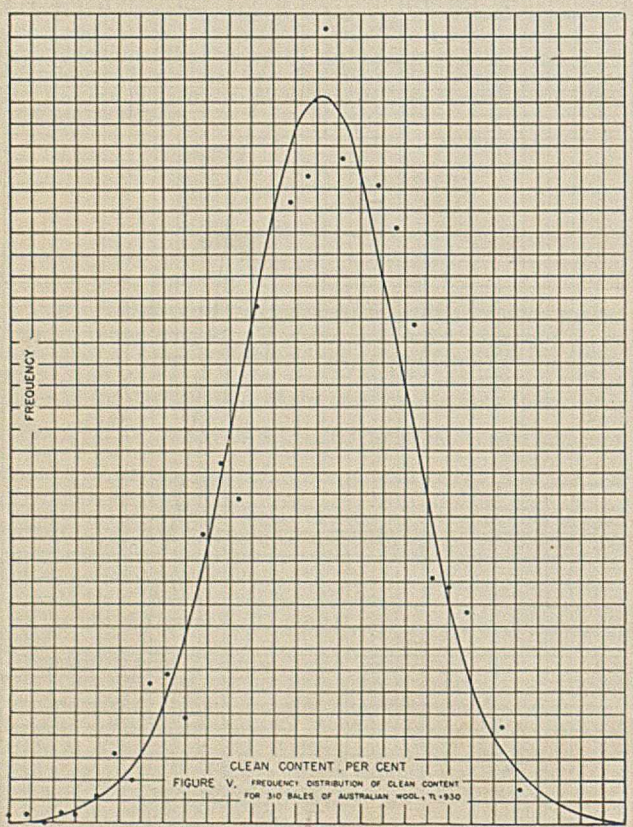

FIGURE V. FREQUENCY DISTRIBUTION OF CLEAN CONTENT FOR 310 BALES OF AUSTRALIAN WOOL, '11-'930

Among the factors that contribute to the variations in σ for samples of each type of wool is nonuniformity in the weight of the individual cores. The standard deviation may be expected to decrease if the weight of the test portions is increased, because the observed value of the percentage clean content of each core is in itself of the nature of an average. From the data available it appears that variations in the individual core weights not exceeding 25 per cent of the average have relatively little effect upon the value of σ. However, since it is probable that greater variations may produce significant effects, it would be necessary to specify the weight of cores and the limits of permissible variation in any standard wool sampling method based upon the statistical approach.

EXPERIMENTAL VERIFICATION. The applicability of representative sampling to imported wool by the procedure outlined above was studied experimentally by sampling and testing a number of lots of such wool. In these tests information was sought on reproducibility and accuracy. The distinction between precision and accuracy is well known. The former is a measure of the reproducibility of the results; it may or may not be reflective of the accuracy, depending upon the adequacy of the test methods and the homogeneity of the material with respect to the particular method of sampling used. The ultimate proof of the accuracy of a sample is a comparison of the results found by testing the sample and then by testing the entire quantity of material.

Table II shows the results of tests of triplicate representative samples from each of three lots of imported greasy wool. Table III compares the percentage clean content of representative samples from each of ten lots of wool with the actual clean content found by carefully controlled scouring and carbonizing tests in a commercial scouring plant. Strict control of the commercial tests was essential if the comparison was to be significant, since it was found that precisions of the order desired were not normally obtained in ordinary practice. As a result of the special precautions taken, the commercially determined percentage clean contents have a precision of the order of ± 0.5 per cent of clean content.

For a probability of 95 per cent, assuming a universe standard deviation, σ', of 2.0 the anticipated maximum sampling errors, E, for samples of size n = 103, 89, and 129 are ± 0.39, ± 0.42, and ± 0.35 per cent, respectively, or ranges of 0.8, 0.8, and 0.7 per cent. Reference to Table II shows that the test results are well within the computed ranges.

The agreement found between the percentage clean contents of the samples and the entire lots (Table III) is considered very good, particularly in view of the fact that the commercially determined values are not absolute, their precision

TABLE II. TESTS ON TRIPLICATE SAMPLES OF IMPORTED WOOL

Sample	Average Weight per Core *Grams*	No. of Cores	Clean Content %
	From 103-Bale Lot of Australian Wool		
1	99	103	55.4
2	103	103	55.2
3	104	103	55.6
	From 89-Bale Lot of Australian Wool		
1	75	89	56.4
2	74	89	55.9
3	76	89	56.4
	From 129-Bale Lot of Cape Merino Wool		
1	105	129	50.9
2	106	129	50.7
3	101	129	50.9

TABLE III. COMPARISON OF PERCENTAGE CLEAN CONTENT OF SAMPLES WITH THAT OF ENTIRE LOTS

Lot No.	Origin and Type	No. of Bales	Weight of Lot	No. of Cores in Sample	Average Weight per Core	Clean Content		
						Sample	Entire lot	Difference
			Pounds		*Grams*	%	%	%
1	B. A. 5's	5	4789	50	244	67.1	67.6	−0.5
2	B. A. Cotts	5	4824	75	194	71.3	71.7	−0.4
3	Aus. merino	17	4917	102	97	54.7	55.5	−0.8
4	Cape, pulled	16	4866	111	102	61.0	60.0	1.0
5	B. A. 58–60's	5	4856	50	215	51.6	51.5	0.1
6	Greasy Chubut	7	5569	70	217	41.5	41.1	0.4
7	B. A. lambs	5	4769	100	237	69.7	70.5	−0.8
8	B. A. 2nd clip	5	4805	50	234	63.1	64.3	−1.2
9	M. V. 58–60's	5	4752	50	196	57.9	57.7	0.2
10	M. V. 60–64's	5	4980	50	202	58.1	58.0	0.1
					Mean	59.60	59.79	0.55

TABLE IV. UNIFORMITY OF WOOL IN BALE

	Weight	No. of Fleeces	Average Clean Content	Standard Deviation
	Lbs.		%	%
Middle half	117	15	60.1	3.5
Outer half	114	15	59.7	4.1

being no better than ±0.5 per cent of clean content. Particularly significant is the fact that the means of the sample results and of the mill results are in practical agreement (difference less than 0.2 per cent). It indicates that in these tests the sampling method was free of significant constant errors and therefore suitable for obtaining representative samples for the determination of the clean content of the lots of wool.

Discussion

The success of the sampling method outlined is dependent upon truly random sampling within the bale. By taking cores, complete randomness is somewhat limited, because the boring always takes place from the surface of the bale to the interior, generally about one quarter of the depth. Obviously, if we define the exterior of the bale as that depth to which the tube penetrates, then if the interior of the bale were to have a constant different level of clean content than the exterior, the accuracy of the result would be affected. Such a condition did not exist in any of the bales tested in the course of this investigation. The results of the comparative tests on the ten lots of wool (Table III) give strong evidence that no significant constant difference existed between the exterior and interior of those bales.

Additional evidence of the comparative uniformity of the wool throughout a bale is afforded by the data obtained in another line of investigation of sampling methods. All the fleeces in a bale of Australian wool were removed individually and their respective clean contents determined. Those in the middle half of the bale were compared with the remainder. Table IV summarizes the results.

While uniformity in the statistical sense has been found to obtain in the past within bales of Australian and South American apparel wools, there is no assurance that temporary local conditions affecting this uniformity may not occur, or that commercial practice may not change in the future. Adequate precautions must be taken to ascertain any changes in the statistical character of the material and, if present, suitable modifications in sampling procedure must be introduced, as indicated in the suggested practice discussed below.

When the universe standard deviation, σ', for any type of wool has been established after sufficiently broad experience, and when statistical uniformity is known or judged to obtain, the individual cores need not be tested separately. Under such conditions all the cores may be composited into a single sample for the determination of the average percentage clean content. The work of laboratory testing may thus be greatly facilitated and reduced.

SUGGESTED PRACTICE FOR SAMPLING IMPORTED GREASY APPAREL WOOL. 1. Establish the universe standard deviation, σ', for single bales, or for sublots of similar bales having the same average clean content, for each type of wool, by determining the standard deviations of a sufficient number of samples of 25 or more cores under the conditions of sampling that obtain.

2. From these data select representative values of σ' for each type of wool. Conservative judgment makes it advisable to select approximately the highest values found.

3. Decide upon the limit of permissible sampling error, E, and the probability factor, t, which meet the requirements of the use to which the test results are to be put. It should be borne in mind that sample size and, hence, sampling costs, rise rapidly with lower limits of permissible sampling error.

4. From Equation 1 or Figure I, II, or III, ascertain the number of cores, n, to be taken for a sample. Consider this number as a minimum.

5. If the lot does not consist of bales of wool approximately equal in weight, containing the same kind of wool or hair (sheep, goat, camel, alpaca, etc.) from the same country of origin, having the same general character (in fleeces, matchings, etc.) and in the same general condition (greasy, scoured, skirted, pulled, etc.), subdivide the shipment into "sampling units" in each of which these conditions are met. Consider each such unit as a separate lot for sampling and testing purposes.

6. From random locations in each bale in a sampling unit take an equal number of cores 5 cm. (2 inches) in diameter and 22.5 cm. (9 inches) long; the total number of cores should not be less than the calculated number, n. For routine tests the cores may be composited and an average percentage of clean content determined thereon.

7. Periodically redetermine the universe standard deviation, σ', to establish its constancy and continued applicability. At these times the relative uniformity of the wool within the bales should be verified. This may best be accomplished by taking a second core, by means of a longer sampling tube, from the same borings made in sampling for verification of σ'. The mean percentages of clean content and the standard deviations of such duplicate samples may then be compared for evidence of "control" (*1*, *5*)—that is, agreement within the limits of normal variation.

The procedure outlined above should be followed if many routine sampling tests are to be made. As a guide for the occasional sampling of shipments of Australian or South American apparel greasy wool the data obtained in the experimental work described in this paper may be used, provided the sampling tool and procedure described above are employed. A value of 2.5 for the universe standard deviation, σ', which is about the highest value observed in these tests, is suggested. Under these conditions the sampling error of the average of 100 cores should not exceed 0.5 per cent of clean content, approximately 95 times in 100.

Acknowledgment

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Spectrographic Studies of Coprecipitation

Fourth-Period Elements with Barium Sulfate, and Copper and Zinc with Lead Sulfate

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The coprecipitation of iron, cobalt, nickel, chromium, and manganese with barium sulfate and copper and zinc with lead sulfate was studied spectrographically. All precipitates of barium sulfate were prepared by the method of Popoff and Neuman, which consists of addition of the sulfate ions to the solution of the barium ions. It was found that cobalt and nickel do not coprecipitate with barium sulfate, whereas iron, manganese, and chromium do. Copper and zinc coprecipitate with lead sulfate.

IN QUANTITATIVE analysis it is of extreme importance that the precipitate used in the determination of any element or group be as pure as possible. In practically all cases, other ions are present in addition to the one being determined. It is, therefore, of prime importance to know whether the elements or groups present will coprecipitate with the substance being precipitated and to what extent the weight of the resulting precipitate will be affected.

Since the elements in the fourth period are frequently encountered in the determination of sulfate as barium sulfate and since lead is commonly determined as the sulfate in brasses with both copper and zinc in solution, the problem deserves careful study.

Spectrographic methods as carried out by Popoff, Waldbauer, and McCann (*8*) and Waldbauer and Gantz (*11*) were used in this study.

Materials Used

Water, sulfuric acid, and nitric acid were purified by distillation. All water used in making solutions and in recrystallizations was redistilled from alkaline permanganate solution in an all-Pyrex still having a block tin condenser. Nitric acid and sulfuric acid were distilled in a Pyrex still. Upon examination in the spectrograph, the water and acids showed no trace of the elements to be studied.

All salts were recrystallized from redistilled water to remove any impurities that were initially present.

Fifty per cent alcohol was prepared from commercial 95 per cent ethyl alcohol decanted from silver nitrate, and distilled from calcium hydroxide.

A sulfuric acid solution, made up from the distilled acid so that 40 grams of solution were equivalent to approximately 0.8 gram of barium sulfate, was used in all barium sulfate precipitations. This solution was standardized against Bureau of Standards potassium acid phthalate (*7*). The barium chloride solution was made to contain 21 grams per liter.

When the presence of any of the cations was desired as an impurity in the solution of barium chloride, solid chlorides of the metals were added instead of stock solutions, to eliminate any change, such as hydrolysis, which would make it difficult to determine exactly the quantity of metal being added. In the case of iron, ferric ammonium chloride was used because it is a crystalline substance of definite composition, whereas ferric chloride tends to hydrolyze.

The lead, copper, and zinc standard solutions were prepared by dissolving spectrographically pure metals in distilled nitric acid. Ten milliliters of each solution contained a weight of other metal equivalent to 0.5 gram of lead sulfate.

All barium sulfate precipitates for spectrographic analysis were obtained by the method of Waldbauer and Gantz (*11*). Purified salts were used in the preparation of spectrographic standards, the range of which was 0.01 to 1 per cent of each cation. The spectrographic standards had the same barium sulfate and lead sulfate content as the precipitate under examination.

Apparatus

The apparatus for the preparation and study of precipitates is essentially that used by Popoff and Neuman (*7*) and Popoff, Waldbauer, and McCann (*8*). Platinum Gooch crucibles were used in the barium sulfate precipitations, while Jena glass crucibles were used in the study of lead sulfate precipitations in order that the precipitates studied gravimetrically could be used in the spectrographic study without the danger of contamination with asbestos. It was necessary to use asbestos in the case of barium sulfate, since no Jena crucibles suitable for use with barium sulfate were available.

The graphite electrodes were found to show a slight lead contamination. Line 2614.2 Å. appeared when a photograph of the spectrum, emitted by the pure electrodes alone, was taken. This is one of de Gramont's "*raies ultimes*" for lead (*1*). No traces of the other elements involved were found.

General Methods of Analysis

BARIUM SULFATE PRECIPITATIONS. Two hundred fifty milliliters of water were added to an acidified (3 to 4 ml. of 6 *N* hydrochloric acid) solution containing a 10-ml. excess of barium chloride and that amount of cation impurity equivalent to all the barium to be precipitated as the sulfate. The barium chloride solution was kept just below the boiling point by means of a hot plate while 40 grams of the standard sulfuric acid were added dropwise with constant stirring. The precipitates were digested for one hour at 65° C. on a constant-temperature air bath. The mother liquor was decanted, and the precipitate was washed with four 15-ml. portions of water, and then washed into the crucible. The precipitates were heated for one hour at 800° C.

For spectrographic determination, the electrodes and solutions were prepared as previously described (*8*). An equal number of drops of standard, or of solution to be analyzed, were placed in each electrode, and the electrodes dried in an oven for 8 hours at a temperature of 250° to 300° C. The high temperature is necessary to dry the electrodes completely, for they do not burn well when there is even a small quantity of sulfuric acid in the graphite.

LEAD SULFATE PRECIPITATIONS. Stock solutions of the nitrates of lead, copper, and zinc were prepared. Equivalent portions of lead solution were then placed in each of four beakers. The first and third portions were analyzed for lead sulfate content merely with the addition of 10 ml. of nitric acid followed by 5 ml. of concentrated sulfuric acid. The second and fourth portions contained 10 ml. of the foreign metallic solution (either copper or zinc) added previous to the addition of the sulfuric acid.

The method of precipitation of lead sulfate was essentially the same as described for barium sulfate. The solutions containing the precipitates were evaporated on hot plates until all the nitric acid was removed. The beakers and contents were then cooled to room temperature, each watch glass was rinsed off into its respective beaker with 50 ml. of redistilled water, and the solution and precipitate were warmed gently for 5 minutes and then cooled for one hour in an ice bath. The precipitates were then filtered through the Jena crucibles, and washed with 100 ml. of 2 per cent sulfuric acid solution and then with 30 ml. of the 50 per cent alcohol solution. Finally they were dried in the oven between 130° and 140° C. for 4 hours, weighed, replaced in the oven for one hour, and reweighed. In no analysis was it necessary to dry the precipitates more than three times for them to reach constant weight.

The procedure for spectrographic determination was essentially that of Nitchie (*5*) and Popoff, Waldbauer, and McCann (*8*). The visual analysis of the plates was made with a bifocal microscope (magnifying power of ×7) using transmitted light reflected from a sheet of white paper.

Lines Used	
Copper	2961.18 and 2961.89 Å.
Zinc	3282.3, 3302.6, and 3345 Å.
Cobalt	2694 Å.
Nickel	2416 Å.
Iron	2598 Å.
Manganese	2576, 2593.7, and 2605.7 Å.
Chromium	2843.25, 2835.64, and 2830.47 Å.

Experimental Results

Of the elements in the fourth period, titanium and vanadium were not studied because of the inherent difficulties in their use, no scandium salts were available, and potassium (*7*) and calcium (*11*) had previously been studied.

The first procedure was to determine qualitatively whether or not the element in question did coprecipitate. The precipitates of barium sulfate were prepared from barium chloride solutions containing amounts of barium chloride and impurity, each equivalent to 0.8 gram of barium sulfate. Lead sulfate precipitates were prepared from solutions containing lead nitrate and impurity, each equivalent to 1.0 gram of lead sulfate. Spectrograms were taken, and the presence of elements being studied was determined by the presence or absence of the most persistent lines. The absence of the lines of the element in question was considered as conclusive evidence that the element was not present only after careful check by recognized analytical procedures.

Cobalt and nickel were the first elements studied. Mellor (*4*) states that cobalt coprecipitates with barium sulfate, and Johnston and Adams (*2*) and Lange and Berger (*3*) report that nickel coprecipitates with barium sulfate. However, neither cobalt nor nickel could be found in the precipitates by spectrographic methods. When regular qualitative methods using organic reagents were used, no trace of either element could be found. The organic reagents used were: for cobalt, nitroso-beta-naphthol and for nickel, dimethylglyoxime (*6*).

Table I. Contamination of Iron, Manganese, and Chromium in Barium Sulfate

Element	Barium Sulfate Calculated	Barium Sulfate Found	Difference from Theoretical	Element in $BaSO_4$ by Spectrograph
	Gram	*Gram*	*Mg.*	%
None	0.8236	0.8234	− 0.2	..
	0.8315	0.8317	+ 0.2	..
	0.8015	0.8015	0.0	..
	0.7860	0.7861	+ 0.1	..
			Av. + 0.025	
Iron	0.8604	0.8512	− 9.2	0.75
	0.8195	0.8096	− 9.9	0.75
	0.8624	0.8521	−10.3	0.75
	0.8128	0.8024	−10.4	0.75
			Av. −10.0	
Manganese	0.8115	0.8099	− 1.6	0.01
	0.8165	0.8146	− 1.9	0.01
	0.8244	0.8220	− 2.4	0.01
	0.8567	0.8542	− 2.5	0.01
			Av. − 2.1	
Chromium	0.8859	0.8795	− 6.4	..
	0.8075	0.8008	− 6.7	..
	0.8050	0.7975	− 7.5	..
	0.7957	0.7868	− 8.9	..
			Av. − 7.4	

In like manner iron, manganese, and chromium with barium sulfate and copper and zinc with lead sulfate were studied. Iron and manganese were found to be coprecipitated with barium sulfate. Because of the juxtaposition of other lines with the "*raies ultimes*" of chromium, the spectrographic study of the coprecipitation of this element was considered of little value. Indications, however, pointed strongly toward coprecipitation of chromium.

It was also found that copper and zinc were coprecipitated with lead sulfate. The gravimetric study, therefore, was devoted to iron, manganese, chromium, copper, and zinc; the quantitative spectrographic study was limited to iron, manganese, copper, and zinc for the reasons mentioned above.

Standards were prepared for the elements in question, and by comparison with these standards the approximate per cent of the element coprecipitated was determined. The results are recorded in Tables I and II.

Discussion

In the cases of iron, manganese, and chromium the weights of precipitates obtained were less than the theoretical, indicating that some phenomenon other than adsorption took place. This phenomenon may possibly be due to the formation of complex ions containing iron, manganese, and chromium similar to those discussed by Recoura (*9*, *10*) in which iron and chromium are in the form of "ferrisulfuric acid" and "chromosulfuric acid".

Table II. Contamination of Copper and Zinc in Lead Sulfate

	$PbSO_4$ Calcd.[a]	$PbSO_4$ Found	Difference from Calcd.	Difference from Actual	Element in $PbSO_4$
	Gram	*Gram*	*Mg.*	*Mg.*	%
Pure Pb	0.4106	0.4107	0.1	...	...
	0.4106	0.4107	0.1	...	...
Cu present	0.4106	0.4124	1.8	1.7	0.4
	0.4106	0.4120	1.4	1.3	0.3
	0.4106	0.4121	1.5	1.4	0.3
Pure Pb	0.2875	0.2876	0.1	...	...
	0.2875	0.2878	0.3	...	...
Zn present	0.2875	0.2888	1.3	1.1	0.4
	0.2875	0.2890	1.5	1.3	0.4

[a] Calculated $PbSO_4$ value was determined electrolytically by depositing lead in a known weight of standard lead nitrate solution as PbO_2 on anode.

The data seem to indicate that zinc contamination of lead sulfate could be due to mixed crystal formation, since zinc sulfate and lead sulfate crystallize in the orthorhombic system. It does not seem probable, however, that copper contamination was due to mixed crystal formation, since the pentahydrate of cupric sulfate crystallizes in the triclinic system. It is more likely that adsorption, occlusion, or possibly postprecipitation occurred in these cases.

Conclusions

Iron, manganese, and chromium are coprecipitated with barium sulfate. Cobalt and nickel are not coprecipitated with barium sulfate. The data for iron, manganese, and chromium indicate the formation of complex ions containing these elements.

Copper and zinc are coprecipitated with lead sulfate. The data for zinc indicate the possibility of mixed crystal formation, whereas the data for copper indicate the possibility of adsorption, occlusion, or postprecipitation.

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Extracts from theses submitted by Frederick Rolf and Harold Frediani to the State University of Iowa for the degree of M.S.

Determination of Mercury in Organic Compounds

An Iodometric Procedure Based upon the Method of Rupp

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IN THE iodometric method of Rupp (*20*) mercury is precipitated as metal by reduction of the double iodide with formaldehyde in alkaline solution, the liquid is acidified, and the mercury is dissolved in standard iodine solution, excess of which is finally titrated with thiosulfate. The procedure is less convenient than appears, because long shaking may be required to dissolve the globulated mercury in iodine solution, and because the presence of minute particles of undissolved mercury is hard to detect in the liquid strongly colored with iodine. Results are in general inaccurate: negative errors were reported by Kolthoff and Keijzer (*14*) and by Bruchhausen and Hanzlik (*3*), and persistently high results by Fenimore (*7*) and by Deal (*4*).

Low results due to incomplete reduction of mercury to the metallic state or to incomplete solution of the metal in iodine should be preventable (*21*). In order to facilitate the dissolution of mercury, by precipitating and keeping the metal in finely divided condition, various additions have been suggested—viz., ether and alcohol (*1*), calcium chloride (*2*), and gelatin (*11*), and Kolthoff (*13*) recommended precipitation by hydrogen peroxide added to the solution alkaline with barium hydroxide (*13*). These devices were found to be either only partially effective or not conveniently applicable.

The high results generally obtained by Rupp's method, hitherto unexplained, are probably due to reduction of iodine by formic acid (*5*) produced from formaldehyde by action of alkali and during the reduction of mercuric salt, and present in formalin. Kolthoff and Keijzer (*14*) reported that after heating formalin with alkali the acidified solution absorbed iodine, and attributed this result to formation of "a polymer or a condensation product of formalin". A better interpretation is that formic acid is produced by the Cannizzaro reaction and is the compound which combines iodine. The ability of formic acid to interfere was shown by the series of tests recorded in Table I.

Twenty-five milliliters of 0.1 *N* iodine were pipetted into an iodine flask and 5 ml. of 50 per cent acetic acid added. Formic acid (99 per cent) was introduced in the amount indicated in column 1, and the stoppered flask was allowed to stand in the dark for the period indicated, after which the solution was diluted and the iodine titrated with 0.1 *N* sodium thiosulfate.

Table I shows that formic acid is not immediately oxidized by iodine under the conditions of analysis, but that continued contact leads to appreciable reaction. In Rupp's method this interference would be operative during the more or less prolonged shaking required to dissolve the mercury, and would be the cause of positive error.

TABLE I. REDUCTION OF IODINE BY FORMIC ACID

Formic Acid *Ml.*	Time *Min.*	0.1 *N* $Na_2S_2O_3$ *Ml.*
0 (blank)	45	25.60
1	45	25.20
3	0	25.60
3	45	24.95
3	60	24.80

Unsuccessful attempts were made to replace formaldehyde by a reducing agent such that neither the excess of reagent nor its oxidation product would introduce later complication. The substances tried were acetaldehyde, chloral hydrate, glucose, glycerol (*22*), and ethanolamine (*19*, *23*). As no satisfactory agent was found, it became obvious that the metal must be isolated by filtration. When the liquid is acidified with acetic acid before filtration the mercury globulates to an extent which makes its rate of dissolution in iodine solution prohibitively slow. It had been expected that this difficulty could be overcome by use of bromine (from Koppeschaar's bromide-bromate solution, *15*) instead of iodine, but this was shown not to be the case under the conditions of analysis. It was found, however, that bromine is superior to iodine for the purpose, as its lighter color makes possible the easy detection of traces of undissolved mercury, and because under similar conditions it dissolves mercury more rapidly than does iodine (*24*). Filtration of the precipitated mercury from the still alkaline solution is not a uniformly satisfactory operation, as the metal is in part so finely divided that it creeps badly and may not be wholly retained on the filter. This remaining difficulty is avoided by precipitating with the finely divided mercury some basic magnesium carbonate, presence of which facilitates the operations of decantation and filtration and in no way interferes with completion of the analysis. In this manner the mercury is separated from all substances which might interfere. unless the sample contains some other metal readily reducible to the metallic state, a condition seldom encountered. Removal of mercury by filtration is advantageous also because dissolution of the metal by bromine can be effected in a restricted volume and hence without undue dilution of the bromine.

Comparative trials of several reducing agents, especially formaldehyde and hydrazine (*6*, *12*), led to selection of the latter as the reagent which most uniformly caused rapid and complete separation of mercury in satisfactory physical condition from alkaline solutions of the double iodide.

It was found that an immoderate excess of potassium iodide may partially or wholly prevent reduction of potassium mercuric iodide to mercury in presence of the usual amount of alkali, but that this effect can be counteracted by addition of more alkali.

A solution of 0.1 gram of mercuric chloride in about 50 ml. of water, to which were added 3 ml. of concentrated sulfuric acid and several drops of Koppeschaar's solution, to simulate the initial conditions of analysis, was treated with 15 ml. of 20 per cent potassium iodide solution (instead of the usual 5 ml.). The solution was neutralized with 20 per cent sodium hydroxide and the usual excess of 5 ml. was added. Introduction of 15 ml. of 2 per cent hydrazine sulfate solution caused no precipitation of mercury. Addition of a second 5 ml. of the alkali produced a slight turbidity, and a third 5 ml. of alkali caused a heavy precipitation of mercury. After removal of the metal by filtration no mercury could be detected in the filtrate (sodium stannite test).

In the application of the entire procedure to the analysis of organic compounds, the samples are decomposed by heating with potassium persulfate and concentrated sulfuric acid

(*9*) in an all-glass apparatus with air-cooled condenser. The sulfur dioxide finally present in the mixture may be sufficient to cause separation of mercurous sulfate or metallic mercury upon dilution, to prevent which enough Koppeschaar's solution is added to generate bromine in excess, assuring oxidation of sulfur dioxide and presence of mercury as soluble mercuric salts. The effectiveness of a simple air-reflux (Figure 1) in preventing volatilization losses during decomposition of halogen-containing samples was tested by attaching a water trap to the upper end of the condenser. After normal decompositions of chloromercuriphenol and of diacetoxymercuriphenol mixed with sodium chloride no mercury could be detected in the trap, and the results for mercury were satisfactory.

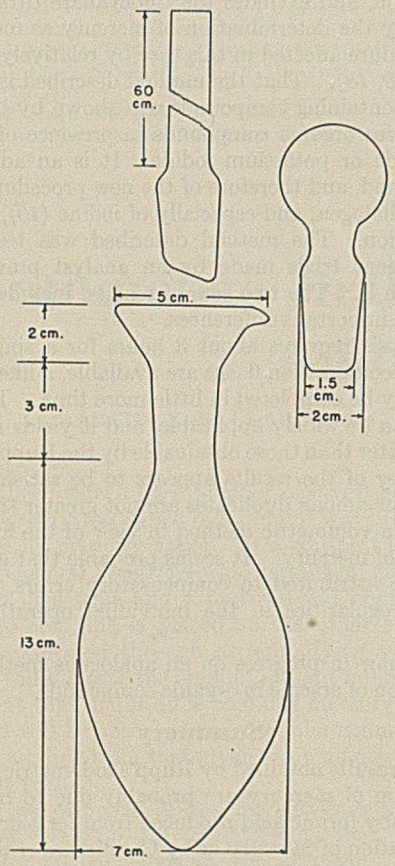

FIGURE 1

Mercury can be precipitated from the alkaline solution of the double bromide (K_2HgBr_4) by formaldehyde, an operation which would permit a small economy with respect to potassium iodide. Tests presented in Table II showed this procedure to be accurate enough for the purpose. It could not be employed in the method developed, because the concentration of salts in the solution (after decomposition by persulfate and sulfuric acid) was sufficient to decrease the stability of the double bromide to such an extent that precipitation of mercuric oxide by alkali could not be prevented by any reasonable excess of potassium bromide.

A 25-ml. aliquot of a solution of mercuric sulfate (from the pure metal), or a weighed sample of purified mercuric chloride in 25 ml. of water, was treated with 10 ml. of 50 per cent potassium bromide solution in an iodine flask. The solution was neutralized with sodium hydroxide, and an excess of 5 ml. of 20 per cent sodium hydroxide solution was added. Mercury was precipitated by addition of 5 ml. of 18 per cent formalin followed by warming. The metal so precipitated was bulky and somewhat granular in appearance. It was removed by filtration and determined by the procedure described below.

TABLE II. PRECIPITATION OF MERCURY FROM ALKALINE SOLUTION OF POTASSIUM MERCURIBROMIDE BY FORMALDEHYDE

Mercury Taken	Mercury Found	Error
Mg.	*Mg.*	*Mg.*
19.7	19.3	−0.4
39.4	39.2	−0.2
39.4	39.2	−0.2
50.5	50.8	+0.3
50.9	50.5	−0.4
89.2	89.3	+0.1
98.8	98.1	−0.7
98.8	98.0	−0.8
98.8	98.4	−0.4
98.8	98.5	−0.3

Apparatus

The 250-ml. flask (Figure 1) is in effect a lemon-shaped iodine flask, with a ground-glass seat for the air-condenser interchangeable with a ground-glass stopper. The flange above the mouth of the flask is provided with a lip for convenience in pouring. It is an advantage to have several flasks for each condenser. In the procedure described the flask serves for decomposition of the sample and for precipitation of the mercury, solution of the metal by bromine, and the final titration.

Procedure

Weigh the sample (about 50 to 100 mg. of mercury) on a piece of cigaret paper or in other convenient manner and transfer to the flask. Introduce 0.5 gram of potassium persulfate, and from a Mohr pipet add 3 ml. of concentrated sulfuric acid in such a way as to wash into the flask any solid particles adherent to the neck. Attach the air-condenser and apply to the tip of the flask the flame of a "micro" burner. Heat gently at first until action subsides, and then heat more vigorously (in presence of chlorine the upper half of the condenser should not become hot; see note *b*, Table III) until organic matter is completely destroyed and the mixture is colorless. This ordinarily requires 20 to 30 minutes. If after heating for some time it becomes obvious that decomposition is slow, allow the mixture to cool somewhat, add through the condenser a suspension of 0.5 gram of potassium persulfate in 1 ml. of concentrated sulfuric acid, and continue the digestion. When decomposition is complete allow the flask and its contents to cool. Through the condenser add Koppeschaar's solution (2.8 grams of potassium bromate and 50 grams of potassium bromide per liter) until excess of bromine persists. Rinse the condenser with water and remove it. If the sample contains iodine some mercuric iodide may sublime into the neck of the flask and the lower part of the condenser. To remove it dip the end of the condenser into a little diluted potassium iodide solution, and pour this into the flask so as to dissolve any mercuric iodide in the neck of the flask. Wash down the surfaces with water and set the condenser aside. The volume at this point should be about 100 ml.

Introduce 5 ml. of 20 per cent potassium iodide solution, or suitably less if some potassium iodide was used previously. Add slowly 20 per cent sodium hydroxide solution until the liquid is alkaline (disappearance of color of iodine) and then 5 ml. in excess. If the liquid becomes turbid owing to separation of mercuric oxide, add 20 per cent potassium iodide solution in 1-ml. portions until a clear solution results. While rotating the flask introduce in a slow stream 10 to 15 ml. of 2 per cent hydrazine sulfate solution, and agitate the mixture occasionally during about 10 minutes. Then add 10 ml. of 5 per cent sodium bicarbonate solution and 5 ml. of 1 per cent magnesium sulfate ($MgSO_4.7H_2O$) solution, both with agitation of the mixture. After about 5 minutes add 5 ml. more of the magnesium sulfate solution, mix well, and allow the flask to stand until the precipitate settles, leaving the supernatant liquid practically clear (10 to 15 minutes).

Decant the solution through a retentive filter paper—e. g., Whatman No. 42, diameter 11 cm.—using a filter cone and light suction. The familiar device of tearing off a corner of the folded paper is not advisable here, as some mercury creeps into the notch. Retain in the flask the greater part of the precipitate, and wash three times with water by decantation. Finally wash the filter three times with water, and transfer the paper and contained precipitate bodily to the flask. Wipe the flange and lip of the flask with a fragment of moistened filter paper, and add this to the contents of the flask.

TABLE III. DETERMINATION OF MERCURY IN ORGANIC COMPOUNDS

Compound	Sample	Mercury Found	Mercury Method of White
	Mg.[a]	%	%
Diacetoxymercuriphenol (*27*)	99.95	64.51	64.54
	99.97	64.58	64.73
	100.07	64.54	
	100.10	64.85	
		Av. 64.62	64.64
Diacetoxymercuriphenol with 50 mg. of NaCl	100.02	64.50	
	100.70	64.88	
	100.60	64.64[b]	(64.64)
		Av. 64.67	
Diacetoxymercuriphenol with 50 mg. of KI	99.98	64.84	
	100.02	64.88	(64.64)
		Av. 64.86	
Mercury di-*p*-tolyl, Eastman No. 1448, Hg calcd., 52.42%	100.28	52.16	
	100.13	52.26	
	99.84	52.01	
	100.72	51.97	51.90
	102.45	51.85	52.02
	105.98	51.92	
	118.37	51.98	
		Av. 52.02	51.96
Mercury di-*p*-tolyl with 100 mg. of *o*-iodobenzoic acid	100.33	51.96	
	100.19	52.06	
		Av. 52.01	(51.96)
Chloromercuribenzoic acid, Eastman No. 1858	100.23	51.40	51.80[c]
	100.43	51.50	51.93[c]
		Av. 51.45	51.87[c]
p-Dimethylaminophenylmercuriacetate (*26*), m. p. 165°, Hg calcd., 52.83%	100.82	53.07	
	99.98	53.24	
		Av. 53.16	
o-Chloromercuriphenol (*17*), m. p. 152.5°, Hg calcd., 60.95%	100.84	60.75	
	110.15	61.14	
	100.16	61.25	
		Av. 61.05	
Mercuric cyanide[d], Hg calcd., 79.4%	100.43	79.32	79.35
	99.96	79.08	79.40
		Av. 79.20	79.38
Mercuric *n*-heptyl mercaptide[e], m. p. 74–76°, Hg calcd., 43.34%	110.40	43.39	
	110.10	43.27	
		Av. 43.33	
Mercuric *p*-tolyl mercaptide[f], m. p. 159–161°, Hg calcd., 44.91%	100.30	44.81	
	101.44	44.73	
		Av. 44.77	

[a] Samples weighed on semimicrobalance.

[b] In this analysis a trap containing water was attached to upper end of condenser and decomposition mixture was heated so vigorously that condenser became hot throughout its length. This resulted in appearance of some mercurous chloride in trap. To complete analysis the trap liquid was treated with several drops each of Koppeschaar's solution and sulfuric acid, and combined with decomposition mixture.

[c] Mercury precipitated as sulfide, dissolved in sodium sulfide solution, and reprecipitated by Volhard method. Results probably somewhat high (*9*).

[d] Decomposition effected in open flask (without reflux) to permit escape of hydrogen cyanide, which otherwise appeared to prevent complete reduction of mercury by hydrazine.

[e] Prepared from *n*-heptyl mercaptan (Eastman No. 2122) and mercuric cyanide, both dissolved in alcohol. Recrystallized from alcohol.

[f] Prepared from *p*-thiocresol and purified as above.

Introduce about 25 ml. of water, and then add from a pipet 25 ml. of 0.1 *N* Koppeschaar's solution. While holding the flask quiet add rapidly 5 ml. of 6 *N* sulfuric acid and at once insert the ground-glass stopper. Shake the flask vigorously until the filter paper is completely disintegrated and no dark particles of mercury are visible (about one minute). Immerse the flask in ice water to produce subnormal pressure within, and after a minute or two pour 10 ml. of 20 per cent potassium iodide solution into the gutter of the flask, and loosen the stopper slightly to allow the potassium iodide solution to pass into the flask. Shake the tightly stoppered flask to absorb in the liquid the vapors of bromine, then remove the stopper and rinse it and the mouth of the flask with water.

Titrate the iodine with 0.05 *N* sodium thiosulfate solution standardized against potassium iodate (*16*). When the iodine color is faint stopper the flask and shake well, to dislodge traces of iodine adherent to the paper. Then add 3 ml. of 0.5 per cent solution of soluble starch and complete the titration. Determine the effective strength of the bromide-bromate solution in a blank titration conducted exactly as described in the preceding paragraph, a filter paper being introduced to compensate any effect due to the filter paper present in the analysis (about 0.07 cc. of 0.05 *N* thiosulfate). Calculate the mercury present from the difference between the two titrations (1 ml. of 0.05 *N* thiosulfate represents 0.005015 gram of mercury).

Analytical Results

The accuracy of the procedure described in the last four paragraphs—i. e., exclusive of the decomposition of organic material—was tested by determinations of mercury in mercuric chloride purified by two recrystallizations from water. Using samples of about 0.1 gram the results were 73.83, 73.74, 73.82, and 73.72 per cent, average 73.78 per cent. The calculated value is 73.88 per cent.

Results obtained in the analyses of organic mercury compounds are presented in Table III. As some of the compounds examined contained other than the theoretical amounts of mercury, and in many cases the purity of organic mercury compounds may not be ascertainable except by analysis, the accuracy of the new procedure was tested by parallel analyses using an umpire method. The method of White (*25*) served for compounds containing no halogen, the mercury being titrated with potassium thiocyanate solution standardized against pure silver. The presence of halogen increases the likelihood of loss of mercury during the White decomposition, and excludes the thiocyanate titration, making necessary the determination of mercury as mercuric sulfide, a procedure affected in this case by relatively large positive errors (*8*, *18*). That the method described is applicable to halogen-containing compounds was shown by the analysis of halogen-free organic compounds in presence of added sodium chloride or potassium iodide. It is an advantage of Rupp's method, and therefore of the new procedure, that the presence of halogen, and especially of iodine (*10*), introduces no obstruction. The method described was tested finally by independent trials made by an analyst previously unfamiliar with it. The two sets of results, included in Table III, show no important differences.

The analysis requires about 3 hours for a single sample. If several decomposition flasks are available, a like number of analyses may be completed in little more time. The method is believed to be widely applicable, and it yields results considerably better than those obtainable by the Rupp procedure. The accuracy of the results appears to be satisfactory, and the variations among duplicates are not greater than is to be expected of a volumetric method in view of the high equivalent weight of mercury. It seems probable that good results need not be attributed to compensatory errors, but to absence of irregularities in the individual operations of the analysis.

Work is now in progress on an analogous method for the determination of arsenic in organic compounds.

Summary

The high results obtained by Rupp's iodometric method for determination of mercury are probably due to reduction of some iodine by formic acid produced from formaldehyde during precipitation of mercury or by Cannizzaro reaction in the initially alkaline liquid. Reducing agents other than formaldehyde also interfere in some way with completion of the analysis by the Rupp procedure. It is therefore necessary to remove mercury by filtration before it is determined iodometrically.

Mercury can be precipitated similarly from alkaline solution of the double bromide, but in presence of dissolved salts in quantity (following decomposition of organic samples) the double bromide becomes too unstable to prevent precipitation of mercuric oxide upon addition of alkali.

Mercury is precipitated in such physical condition as to be both filterable and rapidly dissolved by action of bromine by addition of hydrazine sulfate to the alkaline solution of the double iodide, and it is kept in this condition during the operations of filtration and washing by presence of some precipitated basic magnesium carbonate.

Bromine (from Koppeschaar's bromide-bromate solution) is preferable to iodine for re-solution of the separated mercury, as it acts more rapidly and avoids the visual difficulty in detecting minute particles of undissolved metal in the liquid colored by excess iodine.

Based on Rupp's original method and the findings mentioned above, a new procedure for determination of mercury in organic compounds was elaborated. The organic sample is decomposed by means of persulfate and sulfuric acid. The procedure is convenient, moderately rapid, and applicable in presence of halogens.

Acknowledgment

Grateful acknowledgment is made to Martha Torrey and Charles Judson, who prepared and purified several of the mercury compounds analyzed, and to Ezra Staple, who executed several series of confirmatory analyses.

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THIS paper represents a completed portion of the experimental study to be submitted in partial satisfaction of the requirements for the Ph.D. degree at the University of Pennsylvania.

Photometric Method for Estimation of Minute Amounts of Mercury

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IT HAS been shown by a number of investigators (*4*, *5*, *7*) that mercury resonance radiation of wave length 2537 Å. is absorbed by mercury vapor. If a constant source of this radiation (General Electric 4-watt germicidal lamp) is placed at one end of a tube and a narrow-band photoelectric cell sensitive to this line is placed at the other end, a microammeter, connected through an amplifying system to the photocell, will indicate the amount of light falling on the cell. It is evident that, if the tube contains mercury vapor, some of the light will be absorbed and the microammeter will give a different reading from that given when no mercury vapor is in the light path.

This is the principle of the mercury-vapor detector developed by Woodson (*6*) to determine the amount of mercury vapor in air. Hanson (*2*) has shown that a number of organic solvent vapors absorb this radiation (although to a much lesser degree than an equal weight of mercury vapor), and his Tri-Per-Analyzer is based on the same principle.

These instruments have been designed to estimate mercury or solvent vapors in large volumes of circulating air, and are not applicable to the estimation of minute amounts of mercury present in solution.

The authors have observed that, on heating mercuric sulfide in a closed system in a quartz-ended cell (Figure 1), the amount of absorption of the 2537 Å. radiation is constant and reproducible for a given amount of mercuric sulfide.

It is obvious that if a simple means were available for converting mercuric ions to the sulfide and collecting the mercuric sulfide formed, the photometric method could be used for the determination of mercuric compounds in solution.

Clarke and Hermance (*1*) have shown that minute amounts of metals (the sulfides of which are less soluble than cadmium sulfide) are completely removed by allowing solutions of the metal ions to filter slowly through filter paper impregnated with cadmium sulfide. Some modification of this recovery method is required, since filter paper or other organic matter would be decomposed on subsequent heating, with the formation of smoke, and this would seriously affect the photometric method for the estimation of mercury. The authors have observed that a pad of preignited asbestos fibers impregnated with cadmium sulfide is equally efficient in removing mercury ions from solution and the mercury sulfide so obtained is in an ideal condition for the subsequent thermal treatment. These adaptations of well-known principles are the basis of the method presented.

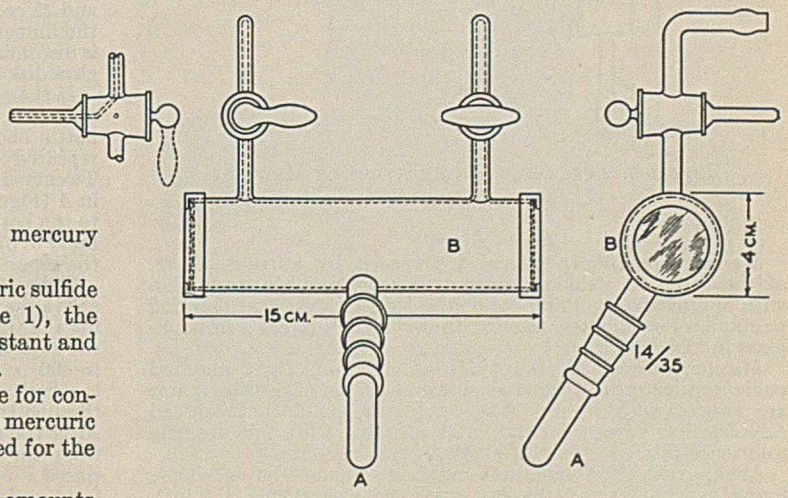

FIGURE 1. ABSORPTION CELL

Recovery of Mercury

REAGENTS. *Cadmium Sulfide–Impregnated Asbestos Fiber.* Acid-washed and ignited long-fiber asbestos is cut into pieces approximately 1.25 cm. (0.5 inch) long and fluffed up in a stream of air (*3*). Five grams of the well-fluffed material (generally freed from any hard pieces) are placed in 100 cc. of 15 per cent cadmium acetate solution and allowed to soak for 5 minutes. The asbestos is drained off through a coarse sintered-glass funnel. The pad, weighing approximately 45 grams, is peeled from the sintered-glass disk, placed in 100 cc. of 5 per cent sodium sulfide solution, and stirred gently for 5 minutes. The impregnated asbestos fibers are separated from the solution again on the coarse sintered-glass funnel and most of the excess sodium sulfide is removed by washing directly on the funnel with distilled water, using suction. The slightly moist pad is peeled off and stirred in 100 cc. of 5 per cent cadmium acetate solution for 5 minutes. The fibers are washed as before, but much more thoroughly, the moist asbestos pad being removed from the filter and dispersed by stirring in 2 liters of water and refiltering. This procedure is repeated 8 to 10 times before the final sucking down to dryness on the filter funnel.

The pad is removed, dried in the oven at 105°, and ignited in a muffle furnace at 550° for 1 hour. After cooling, one half of the sample is placed in a 2000-cc. glass-stoppered flask, 1000 cc. of mercury-free water are placed in the flask, and the flask is well shaken to disperse the fibers. A certain amount of the asbestos will settle to the bottom of the flask, as it was not possible to fluff it enough to give complete dispersion. This should be disregarded and the suspension should be only gently swirled before taking a sample for the final dilution, in which 100 cc. of the above suspension are diluted to approximately 450 cc. with mercury-free water.

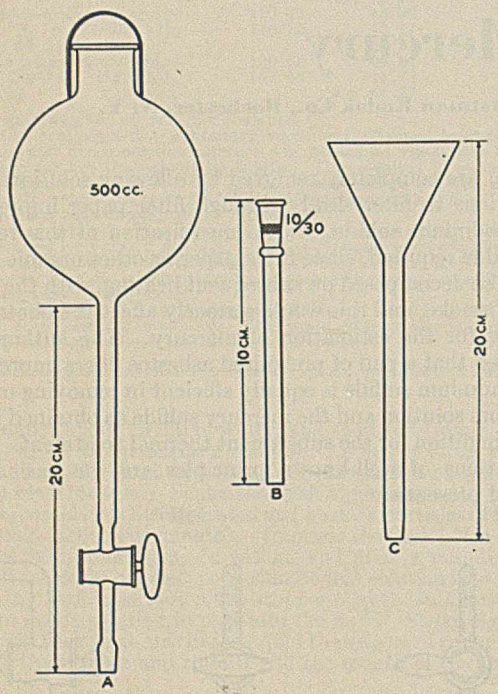

FIGURE 2. APPARATUS FOR RECOVERY OF MERCURY

Mercury-Free Water. This is prepared by stirring 25 cc. of the cadmium sulfide–impregnated asbestos fiber suspension with approximately 2 liters of distilled water and, after allowing to stand a few minutes, filtering the solution through a sintered-glass funnel.

Mercury-Free 80 Per Cent Methyl Alcohol. Eight hundred cubic centimeters of methyl alcohol and 200 cc. of distilled water are treated with 10 cc. of the cadmium sulfide–impregnated asbestos fiber suspension and, after standing a few minutes, the solution is filtered through a clean sintered-glass funnel.

APPARATUS. The apparatus required (Figure 2) is a simple long-stemmed flask, *A* (having at its end a shortened $\overline{5}$ joint), to hold the sample solution, and tube *B* to hold the cadmium sulfide–impregnated pad. (Two short V-shaped slots are made with a triangular file across the bore of the stopcock plug in flask *A*, along the circumference of rotation extending equidistant on either side of the bore. This facilitates regulation of flow.) The pad is prepared in holder *B* by means of the funnel, *C*.

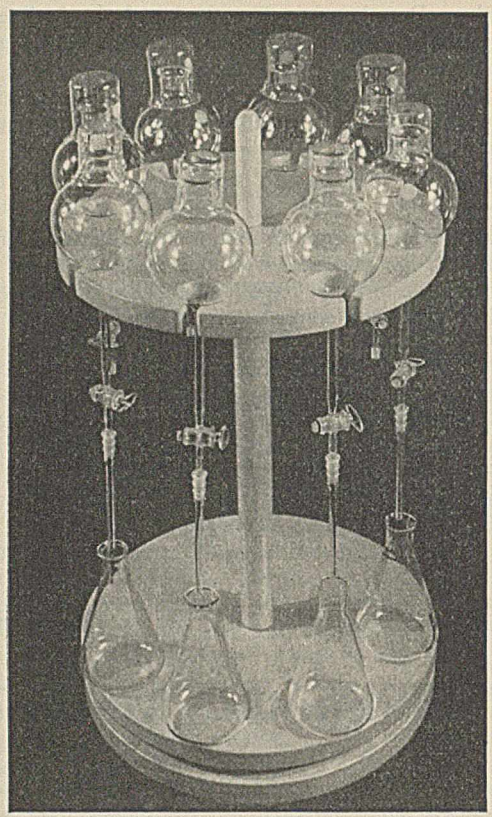

FIGURE 3. BATTERY OF EXTRACTORS

The asbestos pad is held in *B* by means of a piece of coarse sintered glass, approximately 3 mm. thick, ground to fit about half way down the standard taper joint. This is easily prepared by cementing a piece of sintered glass (ground to approximately the right size on a slow-speed wet emery wheel) to a piece of glass tubing with de Khotinsky cement. It is then finally ground into the standard taper joint manually, using a slurry of medium fine emery as abrasive.

PROCEDURE. A sintered-glass disk is introduced into tube *B* by running a glass tube up from the bottom of the tube, placing the glass disk on it, and gently withdrawing the tube; the disk will readily seat itself. The tube is then connected to funnel *C*, and 25 cc. of the well-shaken asbestos suspension are poured into the funnel and allowed to settle slowly onto the disk. The tube is disconnected from the funnel and the pad is drawn down on the glass disk by means of suction applied at the lower end of the tube.

If the pad is not uniformly distributed over the surface of the disk, it is wetted with mercury-free water from an all-glass wash bottle and again drawn down on the disk. This procedure is repeated until the pad appears to be uniformly distributed. Twenty-five cubic centimeters of mercury-free water are placed in *A* (Figure 2) and the stopcock is opened slightly to fill the tube to the bottom of the ground joint, in order to forestall the possibility of bubble formation, either in the stopcock or directly above the asbestos pad.

Tube *B* containing a freshly prepared pad is filled with water above the asbestos pad by holding it under *A* and carefully opening the stopcock; it is then quickly connected to *A* by means of the standard taper joint. The solution under investigation (150 to 400 cc.) is adjusted to pH 5 to 7 and immediately placed in the flask, and the stopcock is turned so that the sample passes through the cadmium sulfide pad at about 2 to 4 cc. per minute. After the solution has passed through the pad, *B* is disconnected from the flask, washed with mercury-free water, and finally rinsed a few times with 80 per cent methyl alcohol (mercury-free) from a glass-stoppered wash bottle. The pad is finally sucked down to a compact pad on the sintered-glass disk, and air is drawn through it for a few minutes by means of a water suction

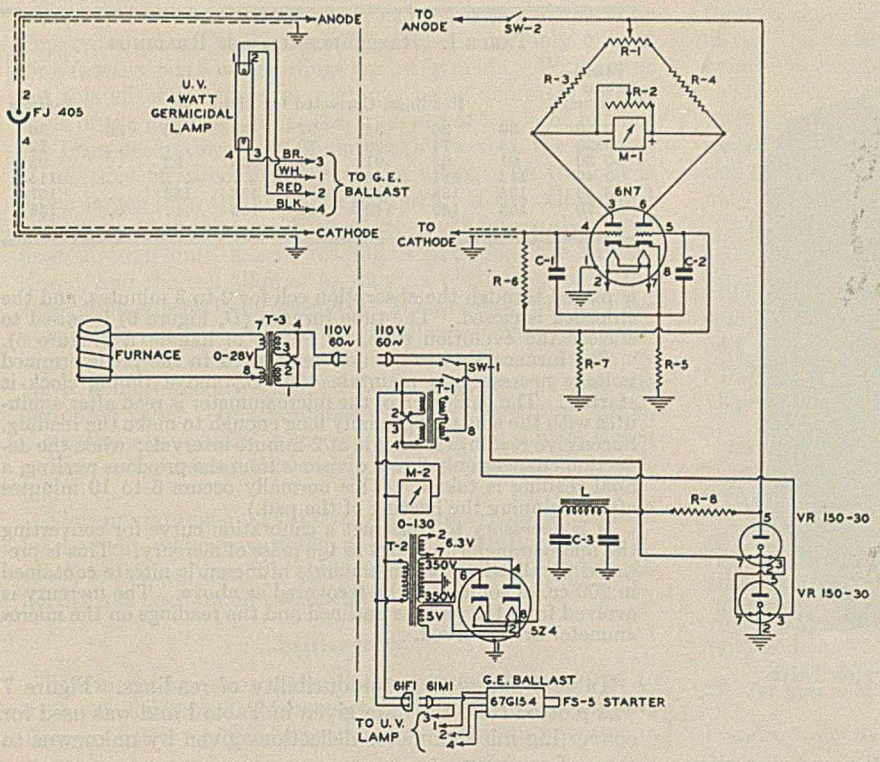

FIGURE 4. SCHEMATIC DIAGRAM OF CIRCUIT

C-1. 0.002 mfd.
C-2. 0.002 mfd.
C-3. 8–8 mfd.
FJ-405. Phototube
L. 15-henry choke
R-1. Bridge balance, 3000 ω.
R-2. Sensitivity control, 1000 ω.
R-3. 1000 ω.
R-4. 1000 ω.
R-5. 15,000 ω.
R-6. 5,000,000 ω.
R-7. 500,000 ω.
R-8. 5000 ω.
M-1. 0–300 D. C. microammeter
M-2. 0–130 A. C. voltmeter
SW-1. D. P. S. T. on-off power supply switch
SW-2. S. P. S. T. bridge balancing and phototube disconnecting switch
T-1. VL-0 Universal Varitran
T-2. Power transformer
T-3. VL-2 Universal Varitran

pump. A glass rod is introduced from the bottom of the tube and the glass disk is gently forced up to the level of the top of the standard taper joint. The asbestos pad is peeled off from the glass disk, using a pair of steel tweezers, and placed in the depression of a spot plate in a vacuum desiccator and the desiccator is evacuated by means of a water pump for approximately 1 hour. The pad is now ready for determination as directed below.

NOTES ON RECOVERY. The cadmium sulfide–asbestos pad should contain no very thin spots through which the solution could pass without completely removing all the mercury present; a simple visual observation will suffice to detect any thin spots. The dried pads weigh approximately 10 mg. Through properly prepared pads the rate of flow of solution has been increased to approximately 10 cc. per minute with complete recovery of mercury. But, using a battery of eight extractors (Figure 3), there is no advantage in speeding up the rate because the pads must be prepared with much greater care and the routine can be so organized that no time is lost at the rate of 4 cc. per minute.

The efficiency of the pads in recovering mercury from solution should be tested by allowing 0.5 microgram of mercury as nitrate in 200 cc. of solution to flow through a pad at the rate selected. The solution is collected in a flask and again passed through a new pad at the same rate. The pads are dried and the mercury is determined in each pad by the method given below. The second pad should not contain over 0.01 microgram of mercury (corrected for blank or untreated pad) as calculated from the calibration curve.

A blank pad, when tested as directed below, should not give a deflection on the microammeter greater than that given by 0.02 microgram of mercury.

The dried pads containing mercury are stable and a number of them may be prepared, using a battery of recovery units and the mercury determined later in a group, or for routine determinations the recovery and determination of groups can be alternated, so that the operations are carried on simultaneously.

If the solution contains suspended material, this must be removed by filtering through a clean sintered-glass filter or a filter paper that has been washed with 1 to 4 nitric acid solution.

Mercuric ions are adsorbed to glass from dilute solutions. The solutions used for calibration and those under investigation should, therefore, be run as soon as prepared. All solutions should be prepared in Pyrex glass.

Tube *B* (Figure 2) containing the sintered-glass disk is rinsed with strong nitric acid, washed, and finally rinsed with mercury-free water just before use.

Determination of Recovered Mercury

APPARATUS. Figure 4 is a schematic diagram of the circuit. Figure 1 is a front and side view of the absorption cell, a 4-cm. Pyrex tube ground and polished on the ends, to which two stopcocks and a standard taper male joint are sealed. The stopcocks allow the cell to be swept out with nitrogen and the standard taper joint is used to attach the pad-holding tube, which is heated to evolve mercury vapor. To facilitate cleaning the cell, detachable quartz ends are employed. Male threaded collars are cemented over the outside of the 4-cm. tube, which projects 1 to 2 mm. through the collars. Quartz disks 2 mm. thick are held tightly against the polished glass ends by means of metal screw caps, fitting the male collars. The screw caps are fitted with rubber gaskets similar to those used in polariscope tubes.

Figure 5 (coded to agree with Figure 4) shows the arrangement of essential electric circuit controls and reading dial. Figure 6

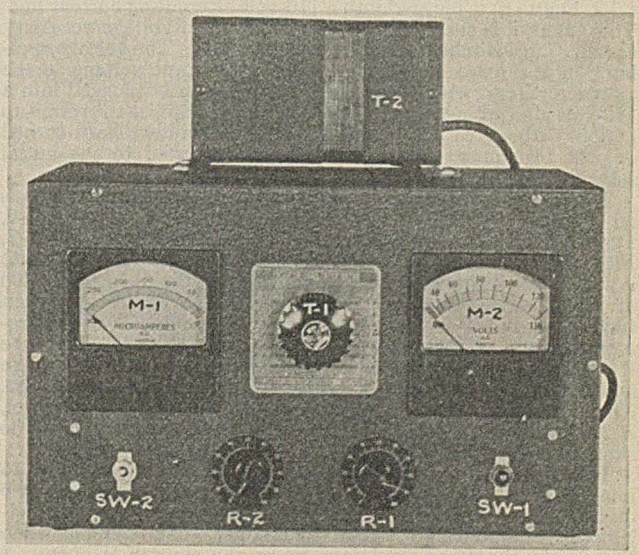

FIGURE 5. ELECTRIC CIRCUIT CONTROLS AND READING DIAL

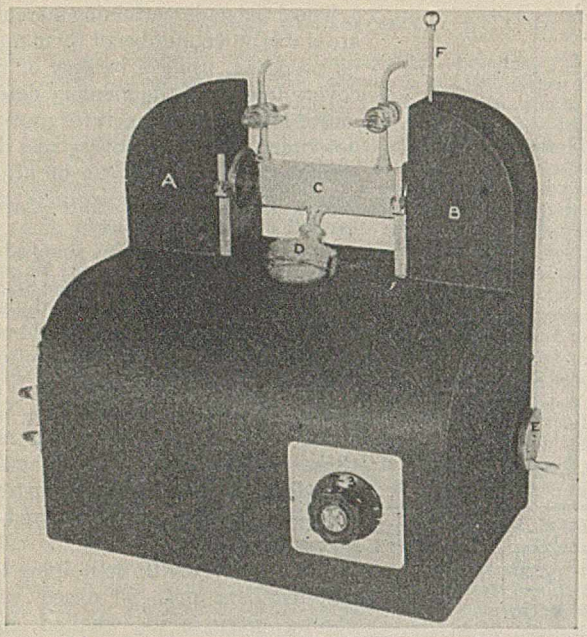

FIGURE 6. EVOLUTION AND ABSORPTION UNIT

shows the arrangement of parts of the evolution and absorption unit. The metal cell holder and the photocell are thermally insulated from other metal parts of the unit, which, although well vented in the rear and bottom, become warm by radiation from the tube furnace. This is made by winding 155 cm. of 22BS 0.0643-cm. (0.0253-inch) Nichrome wire on a 30-mm. diameter 80-mm. high Alundum extraction thimble, and covering the windings and bottom with asbestos cloth. The inner furnace temperature was measured with a thermocouple for various settings of the Varitran and the observed voltages necessary to maintain temperatures of 450° and 550° C., respectively, were used in subsequent mercury determinations. When a series of diaphragms was used to collimate the beam falling on the photocell, after passing through the absorption cell, the amplified electrical response was unsatisfactory because the light energy falling on the photocell was too low. The lamp and photocell were, therefore, mounted close to the quartz ends of the absorption cell to take advantage of reflections from the inner glass wall. The cell was held in a fixed reproducible position.

PROCEDURE. The two operating switches of the instrument are turned on (*SW*-2, Figure 5, the electrical bridge-balancing switch, and *SW*-1, Figure 5, the control switch for the quartz low-pressure mercury-vapor source) and the furnace Varitran (*T*-3, Figure 6) is plugged into a 110-volt alternating current line The Varitran is then set to the predetermined voltage necessary to maintain the tube furnace at about 550° C. The voltage regulator (*T*-2, Figure 5), which supplies a constant voltage to the electrical circuit and light source, is permanently plugged into a 110-volt alternating current line in order to minimize the warm-up period. About 15 minutes after the switches have been turned on, the instrument may be tentatively adjusted to the initial settings as outlined below.

The Varitran controlling the source voltage (*T*-1, Figure 5) is adjusted to deliver 110 volts, as read on the voltmeter (*M*-2, Figure 5). The bridge-balancing adjustment switch (*SW*-2, Figure 5) is turned off and the bridge-balance dial (*R*-1, Figure 5) is turned until the microammeter gives a reading of exactly 300 divisions. The bridge-balance switch (*SW*-2, Figure 5) is then turned on, and if no light is falling on the photocell, the microammeter will maintain the same reading. The sampling tube (*C*, Figure 6) is swept out with nitrogen for a minute to remove any residual vapors, and the shutter (*F*, Figure 6) is then opened. The microammeter shunt adjustment knob (sensitivity control, *R*-2, Figure 5) is adjusted until the meter reads exactly zero. The shutter is then closed.

This routine of tentative adjustment is followed every 10 to 15 minutes until the change in microammeter readings is less than 5 divisions per 15-minute interval. The instrument as finally adjusted should give a microammeter reading of 300 divisions when the shutter is closed, and zero when the shutter is open. A sample pad is placed in the evolution tube (*A*, Figure 1), nitrogen

TABLE I. REPRODUCIBILITY OF READINGS

Micrograms of Mercury	Readings, Corrected for Blank Pad							Average
0.10	39	36	34	37	34	36	36	36
0.20	76	74	68	70	...	...	..	72
0.30	91	93	91	94	95	92	..	93
0.40	113	111	118	114	...	...	..	114
0.50	135	135	143	139	138	133	..	137
0.70	158	159	149	157	156	...	..	156

is passed through the absorption cell for 2 to 3 minutes, and the stopcock is closed. The tube furnace (*D*, Figure 6) is raised to enclose the evolution tube, by means of handle *E* (Figure 6).

The furnace Varitran is now readjusted to the predetermined voltage necessary to maintain 450° C., and a timing clock is started. The deflection of the microammeter is read after 4 minutes with the shutter open only long enough to make the reading. Successive readings are made at 2-minute intervals; when the deflection changes only 2 to 3 divisions from the previous reading, a final reading is taken. (This normally occurs 6 to 10 minutes after beginning the heating of the pad.)

It is necessary to construct a calibration curve for converting the microammeter readings to the mass of mercury. This is prepared by allowing known amounts of mercuric nitrate contained in 200 cc. of solution to be recovered as above. The mercury is evolved from the pads as outlined and the readings on the microammeter are observed.

Table I shows the reproducibility of readings. Figure 7 was plotted from the data given in Table I and was used for converting microammeter deflections given by unknowns to mass of mercury.

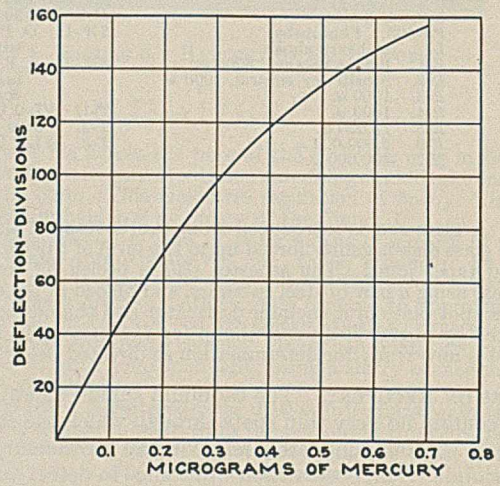

FIGURE 7. DETERMINATION OF MERCURY

For subsequent determinations the furnace is run down, and the Varitran controlling its voltage is altered to the voltage necessary to hold the temperature at 550° C. The mercury vapor is swept out for a few minutes with nitrogen, or until on opening the shutter the instrument again gives a scale sweep of 300 units, showing that the cell has been freed of mercury vapor. Tube *A* is detached at the standard taper joint, a tube containing another sample pad is connected, and, just prior to raising the furnace to heat the pad, the scale sweep of the microammeter is checked.

If necessary, the instrument is adjusted so that a full-scale sweep of 300 divisions is obtained when the shutter is opened. After the instrument has been brought to temperature equilibrium (approximately 1 hour), the instrument is fairly stable and necessary readjustments are slight.

NOTES ON DETERMINATION. If the shutter is kept open and light is allowed to pass continually through the cell, the reading on the microammeter will slowly sink to zero, even when mercury vapor was present in the cell before opening the

shutter. This is not observed, or at least is not of the same time order, when the shutter is kept closed and only opened for a few seconds while readings are being made. Whether or not this effect is caused by mercury being deposited on the walls of the glass cell under the influence of the light has not yet been ascertained. (Calculations show that the amount of mercury required to saturate the nitrogen in the cell is much larger than the amounts present.) If the cell is filled with vapor containing mercury and the light source allowed to pass through until a zero reading is obtained, the shutter closed, and the cell allowed to stand, a partial regain of the absorption effect will be observed. If carried over to a subsequent determination, this in effect will cause erroneous readings, and a probable explanation is that the ionized mercury vapor is more readily adsorbed to the glass surface than the unionized vapor. In accordance with the above observation, the authors have found it necessary to keep the shutter closed except for the shortest time required to make a deflection reading.

The apparatus should not be operated in a room illuminated by daylight, which is variable, unless the evolution unit (Figure 6) is shielded from the light. An inside room lighted artificially has been found satisfactory.

Summary

The method presented allows the determination of 0.02 to 0.60 ± 0.02 microgram of mercury in 150 to 400 cc. of solution. Using a battery of recovery units as shown, an operator in these laboratories has been able to make 25 determinations per day. The instrument described has been in operation for 6 months without noticeable change in operating characteristics.

The method has been used with water-miscible solvent mixtures and with solutions from the digestion of solid organic materials. It would appear to be applicable to the estimation of mercury in biological materials after suitable preliminary digestive treatment.

Acknowledgments

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Separation of Bismuth from Lead with Ammonium Formate

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The sodium formate method, proposed by Benkert and Smith for the quantitative separation of bismuth from lead, shows definite advantages over other procedures, but there are obstacles to its general adoption.

A new procedure is proposed, suited for both the quantitative separation and determination of bismuth and lead and for the quantitative separation of small amounts of bismuth from large amounts of lead.

The nitric acid solution of bismuth and lead is neutralized with ammonia and ammonium carbonate. Ammonium formate is added and the precipitate of basic bismuth formate is filtered off, washed with hot water, reprecipitated, and finally ignited to the oxide. The precipitate can also be dissolved in hydrochloric acid and bismuth determined as oxychloride. If the bismuth formate precipitate is very small it can be dissolved in dilute sulfuric acid and bismuth determined colorimetrically with potassium iodide.

Lead is precipitated in the filtrate of the bismuth formate as chromate with potassium or ammonium dichromate.

MANY laboratories engaged in industrial or research analysis find it necessary to carry out numerous quantitative separations of bismuth from lead, and many cases arise where the time required to perform such a procedure must be kept at a minimum. A comprehensive survey of the literature shows that a large amount of work has been done on methods for the quantitative separation of bismuth from lead, but in general the procedures have not been entirely satisfactory.

The investigation here reported was undertaken with a view to developing a method which would be simple and easy of manipulation and at the same time give results comparable with those obtained by the more complicated methods—in other words, a strictly routine method that could be really depended upon.

Existing Methods

Methods given by standard texts for quantitative separation of bismuth from lead are, with few exceptions, based upon the fact that in weak nitric acid solution the bismuth ion is decomposed upon the addition of water or salts of weak acids, forming basic bismuth compounds.

Probably the best known of these methods was suggested by Loewe (*13*). Briefly, it consists in precipitating bismuth as

basic nitrate of indefinite composition, by evaporating the nitric acid solution to sirupy consistency, adding water, evaporating to dryness, and repeating this operation until further addition of water fails to produce a turbid solution. Lead is finally extracted with a cold 0.2 per cent solution of ammonium nitrate. This method is generally considered very reliable, although Hillebrand and Lundell (*7*) and Herzog (*6*) state that the separation is not entirely satisfactory. Its chief weakness lies in the fact that its application is confined to the separation of bismuth from moderate amounts of lead. Furthermore, chlorides and sulfates, which also form basic compounds with bismuth and cause the retention of some lead, must be absent. The method is time-consuming because of the repeated evaporations.

Various modifications of this method have been proposed since its early use. Luff (*16*) reduces the hydrogen-ion concentration of the solution by adding sodium nitrite to the weak nitric acid solution of bismuth and lead which has previously been neutralized with sodium bicarbonate. According to its author, this method is limited to amounts of bismuth and lead not exceeding 100 mg. Ammonium salts must be absent. The method is not accurate because of the retention of alkali by the precipitate and is not attractive because of the necessity for more or less tedious purification procedures.

Blumenthal (*2*) suggests neutralizing the excess nitric acid with a freshly prepared emulsion of mercuric oxide, and states that as much as 50 mg. of bismuth can thus be separated from 10 grams of lead in one operation. The method is not suited to a simultaneous determination of the lead and bismuth content. The mercuric oxide–bismuth subnitrate precipitate must stand for at least 12 hours to guarantee complete precipitation of the bismuth. The procedure makes it necessary to precipitate and determine bismuth finally as the phosphate, a method that, according to Hillebrand and Lundell (*9*), should not be considered in accurate analyses. In addition, Blumenthal's method is subject to many interfering elements and is hence not practical for commercial analysis. The present writer proposed in a previous paper the use of zinc oxide, in a similar procedure (*12*). One zinc oxide precipitation does not yield a quantitative separation of bismuth from lead, but permits precipitation as bismuth oxychloride.

A separation of bismuth from lead, based on precipitation of the bismuth as basic acetate, is suggested by Herzog (*6*). The method is very time-consuming and has obviously not given satisfactory results in hands other than those of its author (*1*). The separation of bismuth from lead by precipitating the lead as lead sulfate, first suggested by Fresenius (*3*) and recommended by such trustworthy investigators as Scott (*19*) and the Gesellschaft Deutscher Metallhütten- und Bergleute (*5*) is worthless, although often used by many analysts. The lead sulfate, whether thrown out from dilute nitric acid solution by adding an excess of sulfuric acid, or obtained by fuming with sulfuric acid the nitric acid solution of bismuth and lead, invariably occludes bismuth, as stated by Hillebrand and Lundell (*8*), Herzog (*6*), Hills (*10*), Blumenthal (*2*), and Low (*14*). The precipitation of bismuth as bismuth oxychloride, first advocated by Rose (*18*), does not yield a quantitative separation of bismuth from lead, particularly when large amounts of the latter are involved, two reprecipitations being necessary to guarantee complete separation of the two metals. However, a procedure for the preliminary separation of small amounts of bismuth (5 to 50 mg.) from large amounts of lead, as recommended in the well-known Ledoux method (*15*), is based on precipitation of bismuth as bismuth oxychloride. The method is rapid and accurate under certain conditions, but variations in conditions may produce erratic and irregular results. Considerable difficulties are also encountered if only minute quantities of bismuth are involved (*11*).

The only other method which needs to be considered here calls for precipitation of bismuth as oxybromide (*17*), as proposed by Moser and Maxymovicz. It is on a par with the oxychloride method, though not so widely used. Its authors state that the oxybromide furnishes a better separation from lead than does precipitation as oxychloride or basic nitrate. Ammonium salts and chlorides, however, should be absent and not more than 0.35 gram of lead must be present. The method calls for reprecipitation of the bismuth oxybromide precipitate, bismuth finally being determined as phosphate because of the volatility of the oxybromide. This method is not suited for a simultaneous determination of lead and bismuth.

Other methods for the separation of bismuth from lead add nothing of value to those mentioned, although some, such as precipitation of bismuth by means of cinchonine hydrochloride (*4*), are capable of giving good results in special cases.

Of all the methods mentioned, only the one suggested by Loewe (*13*) is suited for the quantitative separation and determination of the two metals. All other methods depend largely on the ratio of the amounts of bismuth and lead and are suited only for the quantitative determination of bismuth.

TABLE I. ACCURACY OF BENKERT AND SMITH'S METHOD

Bismuth Used	Bismuth Found				
	In precipitate	In Filtrates			
		I	II	III	IV
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
10.0	9.1	0.3	0.2	0.3	0.1
20.0	18.6	0.4	0.2	0.5	0.1
50.0	48.3	0.6	0.2	0.6	0.1
50.0	48.5	0.6	0.1	0.5	0.1
75.0	73.2	0.8	0.2	0.7	0.2
100.0	97.5	1.0	0.3	0.9	0.2
100.0	97.1	0.9	0.3	1.3	0.3
200.0	197.0	1.1	0.4	1.3	0.3
300.0	296.8	1.4	0.3	1.2	0.3

Sodium Formate Method

A method which has scarcely been mentioned in the literature was proposed by Benkert and Smith (*1*) who, some 50 years ago, in calling attention to the defects in Herzog's procedure (*6*), reported that they were able to separate bismuth and lead by means of sodium formate, an idea closely related to that of Herzog.

The nitric acid solution of bismuth and lead is "almost neutralized with sodium carbonate, or until the incipient precipitate dissolves slowly, when considerable sodium formate solution of specific gravity 1.084 and a few drops of aqueous formic acid are added". The solution is heated to boiling and held at this point for 5 minutes. The precipitate is allowed to settle and filtered while hot, then dissolved in nitric acid and precipitated with ammonium carbonate. The basic bismuth carbonate contaminated by some lead carbonate is dissolved in nitric acid and the formate precipitation is repeated. The resulting precipitate, which is contaminated by sodium salts, is again dissolved in nitric acid and bismuth is finally precipitated with ammonium carbonate and ignited to the oxide.

The experimental data submitted by Benkert and Smith are extremely meager, and in the investigation here reported the method was tested as regards completeness of precipitation and separation with a view toward possible simplification and improvement. A new procedure incorporating the best features of Benkert and Smith's method has been developed which, in the writer's opinion, has definite advantages over the methods in use at present.

To test the completeness of the bismuth precipitation, varying amounts of bismuth were dissolved in nitric acid and the precipitation of basic formate was carried out, as called for by the authors. The precipitate was filtered off and washed with hot water, retaining the filtrate (I). The precipitate was dissolved in nitric acid and bismuth precipitated as basic carbonate. The filtrate of the basic bismuth carbonate was also retained (II). The precipitate was redissolved in nitric acid and the basic formate and basic carbonate precipitations were repeated, yielding two more filtrates (III, formate, and IV, carbonate). The final basic bismuth carbonate precipitate was ignited to and weighed as bismuth oxide, Bi_2O_3. In addition, the four filtrates were colorimetrically tested for bismuth (Table I).

Table I shows conclusively that bismuth subcarbonate is somewhat soluble in an excess of ammonium carbonate. The precipitation of bismuth as subcarbonate should therefore be avoided, whenever possible. The data also indicate the obstacles to a general adoption of Benkert and Smith's method—namely, the incomplete precipitation of bismuth as basic formate under the conditions called for.

Endeavoring to ascertain why such low results are obtained with this method, comprehensive tests were carried

out and it became apparent that the primary cause of the discrepancies is lack of information concerning the exact acidity at which the precipitation of basic bismuth formate should be carried out. The authors merely state that the nitric acid solution of bismuth and lead should be "almost neutralized with sodium carbonate or until the incipient precipitate dissolved slowly". That these data are too vague and inexact is shown by the following illustration:

Portions of 25 mg. (I) and 200 mg. (II) were dissolved in 8 *N* nitric acid and the solution was carefully neutralized with a 10 per cent (1.89 *N*) solution of sodium carbonate, until the addition of one more drop of sodium carbonate would have caused precipitation of bismuth carbonate. Methyl orange was added at this point, indicating a hydrogen-ion concentration lower than that equivalent to pH 3 (red color). The neutralization with sodium carbonate was continued until the yellow color developed, indicating a hydrogen-ion concentration greater than that equivalent to pH 4.5, using in I 1.4 ml. and in II 1.8 ml. Then 8 *N* nitric acid was added until the precipitate which had formed during the neutralization procedure just dissolved, using in I 0.8 ml. and in II 1.3 ml. The volume of the solution was in I 150 ml. and in II 200 ml. As 1 ml. of 8 *N* nitric acid neutralizes 4.3 ml. of 10 per cent sodium carbonate, the excess nitric acid required to keep the bismuth in solution can thus be calculated.

$$\text{Excess } HNO_3 = 0.8 - \frac{1.4}{4.3} = 0.47 \text{ ml.}$$

$$\text{Excess } HNO_3 = 1.3 - \frac{1.8}{4.3} = 0.88 \text{ ml.}$$

Accordingly the acidity in I amounted to 0.025 *N* and in II to 0.035 *N*.

The foregoing data would indicate that Benkert and Smith by precipitating bismuth formate from a clear nitric acid solution, neutralized with sodium carbonate, carried out the precipitation at an initial acidity of between 0.02 and 0.04 *N* (pH 1.7 to 1.4). It is obvious that the excess nitric acid will react with sodium formate, causing the formation of formic acid. The concentration of the latter is, as suggested by the authors, even increased by a further addition, thus making it necessary to add "considerable" amounts of sodium formate as buffer. But even this precaution does not yield a complete precipitation of bismuth (as shown in Table I), probably because of the formation of complex bismuth formate.

To determine the accurate permissible acidity range, 200-mg. portions of bismuth were dissolved in nitric acid. The solution was neutralized with 10 per cent sodium carbonate, using methyl orange as indicator, until the addition of one more drop of sodium carbonate would have produced the yellow color of methyl orange, indicating a hydrogen-ion concentration equivalent to about pH 4 (at this stage a part of the bismuth had already separated). The solution was acidified with varying amounts of 8 *N* nitric acid, and 40 ml. of 10 per cent sodium formate solution were added, the total volume of the solution being about 250 ml. The precipitate was treated as above and the filtrate tested for bismuth.

The results presented in Table II indicate the importance and necessity of an extremely careful neutralization of the bismuth nitrate solution prior to the addition of sodium formate. The hydrogen-ion concentration should not be below pH 2, and the addition of extra formic acid, suggested by Benkert and Smith, should be omitted. The data in Table II prove that, provided the acidity of the solution is carefully adjusted, fairly good but slightly low results are obtained with Benkert and Smith's method as far as the precipitation of bismuth is concerned.

Table II. Effect of Initial Acidity

(200 mg. of bismuth used)

8 *N* HNO_3 Added	Approximate H^+ Concentration	Bi Found in Filtrate
Ml.		*Mg.*
0.1	2.5	0.2
0.3	2.0	0.2
0.5	1.8	0.5
0.6	1.7	0.6
0.7	1.6	0.8
0.9	1.5	1.0
1.2	1.4	1.2
1.5	1.3	1.3

Table III. Separation of Bismuth from Lead

Bi Used	Bismuth Found: In ppt.	Bismuth Found: Corrected value	Bismuth Found: In 2 Filtrates of $PbSO_4$ Ppt. I	Bismuth Found: In 2 Filtrates of $PbSO_4$ Ppt. II	Pb Used	Lead Found: In 2 Filtrates of Bi Formate I	Lead Found: In 2 Filtrates of Bi Formate II	Lead Found: In Bi_2O_3 ppt.
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
10	9.9	9.8	0.10	0.12	25	22.7	2.2	0.1
20	19.8	19.5	0.19	0.22	50	46.3	3.4	0.3
20	19.9	19.4	0.32	0.26	100	92.3	7.0	0.5
50	50.1	49.1	0.31	0.34	100	90.4	8.7	1.0
50	51.6	49.1	0.31	0.29	200	185.9	11.6	2.4
100	102.0	99.0	0.34	0.34	200	184.0	12.2	3.0
100	104.0	99.1	0.32	0.33	500	462.7	31.8	4.9
300	302.8	298.8	0.46	0.41	200	182.3	13.5	4.0
300	305.2	299.0	0.43	0.40	500	450.0	43.2	6.2
					Grams			
300	309.0	299.1	0.32	0.35	1	907.6	80.9	10.0
25	25.1	24.9	Not determined		20	Not determined		0.2
50	53.7	49.7	Not determined		20	Not determined		4.0
100	107.0	100.2	Not determined		20	Not determined		6.8
200	209.4	199.8	Not determined		20	Not determined		9.6
300	312.8	300.4	Not determined		20	Not determined		12.4
300	311.2	300.1	Not determined		5	Not determined		11.1

To test the separation of bismuth from lead, the writer dissolved varying amounts of the two metals in nitric acid and determined bismuth with the above method, using all precautions during the neutralization process and employing two formate separations. Lead was determined separately in the two filtrates of the bismuth formate, by precipitating and weighing it as the sulfate. The bismuth oxide was tested for lead and the filtrates of the lead sulfate were tested for bismuth.

The results in Table III show conclusively that Benkert and Smith's method does not yield a clear-cut separation of bismuth from lead, particularly when large amounts of the latter are involved and only two sodium formate separations are employed. This is, in the writer's opinion, mostly due to the failure to expel by boiling all carbon dioxide (resulting from neutralizing nitric acid with sodium carbonate) prior to the addition of sodium formate. With increasing amounts of lead the influence of formic acid upon the complete precipitation of bismuth diminishes and is negligible when 5 grams of lead are present. This will facilitate considerably the determination of small amounts of bismuth in test or refined lead, or in lead buttons obtained by scorifying or fusing ores.

The major factors which affect the efficiency and accuracy of Benkert and Smith's method pointed out in detail above, make it appear that the method in its original form is incompatible with accuracy. Hence the present writer has worked out a modified scheme of analysis, incorporating the best features of the above method but obviating the attending difficulties.

New Procedure

This method is suited both for the quantitative separation and determination of bismuth and lead, and for the quantitative separation of small amounts of bismuth from large amounts of lead (10 to 50 grams). No other method has this particular advantage.

Extreme care is given to the neutralization of the bismuth and lead nitrate solution when bismuth has to be separated from small or moderate amounts of lead and the hydrogen-ion concentration of the solution should not be lower than that equivalent to pH 1.7. An extremely careful neutraliza-

tion is less important when more than 5 grams of lead are present.

Extensive tests revealed that basic bismuth formate is slightly soluble in sodium salts in general, and in sodium formate in particular (0.1 to 0.4 mg. of bismuth was colorimetrically found in the filtrate of varying amounts of bismuth formate which had been treated with 6 to 10 grams of sodium formate). Therefore, all sodium salts are replaced by ammonium salts. The use of ammonium salts has another important advantage in preventing the contamination of the bismuth formate precipitate by sodium salts. This in turn enables the elimination of the objectionable carbonate precipitation of bismuth with ammonium carbonate. Bismuth formate can be ignited directly to the oxide in the same way as the carbonate. This procedure is new and reduces considerably the time required to perform the separation and determination of bismuth and lead.

If a simultaneous lead determination is not required, the precipitation of the bismuth as basic formate can be connected with a final determination as the oxychloride, only one formate separation being necessary.

If only minute amounts of bismuth are present, the basic formate precipitate can be dissolved in hot dilute sulfuric acid and bismuth determined colorimetrically with potassium iodide.

The final determination of lead with this method is equally simple and easy of manipulation. The two filtrates from the bismuth formate precipitate are combined, nitric acid is added, and the formic acid is expelled by boiling and evaporation. Ammonium acetate is added and lead is finally precipitated as lead chromate with ammonium or potassium dichromate.

Experimental Procedure. Reagents required: nitric acid, 8 *N;* ammonia, 4 *N* and 6 *N;* ammonium carbonate solution, 5 per cent; and ammonium formate solution, about 40 per cent. Dilute 300 ml. of 90 per cent formic acid with 100 ml. of water. Neutralize with 12 *N* ammonia, using litmus paper as indicator (about 650 ml. of ammonia are required).

Neutralize the warm nitric acid solution of bismuth and lead (containing not more than 400 mg. of bismuth) with 4 *N* ammonia until a permanent precipitate develops. Add 8 *N* nitric acid, drop by drop, until the precipitate just dissolves. Neutralize excess nitric acid carefully with 5 per cent ammonium carbonate until further addition would cause precipitation of basic bismuth carbonate. The solution should be definitely clear at this stage.

Heat the solution to boiling and hold at that point for 5 minutes to expel all carbon dioxide. Disregard any precipitate which forms during the heating. Add 7 to 8 ml. of ammonium formate solution, heat to boiling, and allow to stand on a hot plate or water bath for about 15 minutes. (If as little as 1 mg. or less of bismuth is present, allow to stand for about 2 hours.)

Filter through No. 42 Whatman or similar paper and wash precipitate 5 times with hot water. Wash precipitate back into original beaker and dissolve in 5 to 6 ml. of 8 *N* nitric acid.

Repeat the ammonium formate separation and filter through original paper into original filtrate, washing the precipitate 8 times with hot water. Retain filtrates. Wash precipitate once with alcohol. Ignite cautiously in a weighed porcelain crucible. The carbon of the paper is likely to have a reducing action on the precipitate; therefore, after the paper is burned off, cool the crucible and moisten the precipitate with 8 *N* nitric acid, dry on a hot plate, and ignite cautiously to the oxide, Bi_2O_3.

Add 30 ml. of nitric acid to the combined filtrates of the bismuth formate, evaporate to a small volume (20 to 30 ml.) until heavy white fumes escape, and add 5 ml. of 16 *N* nitric acid and 30 ml. of 33 per cent ammonium acetate solution. Dilute to 250 ml., heat to boiling, add an excess of hot ammonium or potassium dichromate solution and boil for 2 to 3 minutes until the precipitate turns to a shade of orange. Filter on a weighed Gooch crucible, wash with hot water, dry at 105° C., and weigh as lead chromate.

Bismuth in Lead. To determine bismuth in lead bullion and refined lead, and in lead buttons, obtained by scorifying or fusing ores, place 10 to 50 grams of lead in a 600-ml. beaker, dissolve in nitric acid (1 to 4), and neutralize the warm solution with 6 *N* ammonia until further addition would cause precipitation of lead and bismuth. The solution should be definitely clear at this point, but the acidity should not exceed 0.05 *N*—that is, 2 ml. of 8 *N* nitric acid in 300 ml. of solution. Add 7 to 8 ml. of ammonium formate solution, allow to stand, and settle on hot plate. As little as 1 mg. of bismuth will separate in one hour. Filter off precipitate and wash 8 times with hot water. Dissolve with warm 4 *N* hydrochloric acid into original beaker, almost neutralize with 6 *N* ammonia, and dilute with hot water to 500 ml. Allow to settle, filter on a weighed Gooch crucible and wash with hot water, dry at 105° C., and weigh as bismuth oxychloride.

Colorimetric Determination of Bismuth (less than 0.7 mg.). Dissolve the bismuth formate precipitate in hot sulfuric acid (1 to 3), transfer the solution (or an aliquot portion) to a 50-ml. Nessler tube, add 2 or 3 drops of dilute sulfur dioxide water, then about 2 grams of potassium iodide, and make up to the 50-ml. mark. Compare in a colorimeter with another tube containing the same amounts of sulfurous acid and potassium iodide and the same volume of solution. Add to this tube a standard bismuth sulfate solution (containing 0.1 gram of bismuth per liter) until the colors of the two solutions are the same depth.

Corroboration and Verification

Varying quantities of bismuth and lead have been determined with the method described above, by adjusting the acidity of the nitric acid solution of the two metals and proceeding as shown in "Experimental Procedure". The accuracy of the method is indicated by Table IV.

Table IV. Accuracy of Proposed Method

Bi Used	Bismuth Found		Lead Used	Lead Found	
	Original	Duplicate		Original	Duplicate
Mg.	*Mg.*	*Mg.*	*Grams*	*Mg.*	*Mg.*
0.5[a]	0.48	0.50	20	Not determined	
1.0[a]	0.99	0.96	20	Not determined	
10.0[b]	9.9	9.8	100 mg.	99.7	99.7
20.0[b]	19.6	20.0	100 mg.	99.8	99.6
50.0[b]	49.7	49.7	100 mg.	99.6	99.7
50.0[c]	49.9	49.8	20	Not determined	
100.0[b]	99.8	99.6	300 mg.	299.3	299.5
100.0[c]	100.0	99.7	20	Not determined	
200.0[b]	199.5	199.9	500 mg.	499.2	500.1
300.0[b]	299.9	300.2	1000 mg.	1000.3	1000.2
300.0[c]	300.3	299.6	20	Not determined	

[a] Bismuth determined colorimetrically, [b] as oxide, [c] as oxychloride.

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Determination of Small Quantities of Fluoride in Water

A Modified Zirconium-Alizarin Method

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METHODS for the determination of fluoride reviewed in a report (*1*) prepared in 1941 by the American Water Works Association Committee on Methods of Determining Fluorides were not found satisfactory for the routine analysis of a large number of samples of natural waters that generally contain less than 0.4 part per million of fluoride. A report (*4*) published in 1941 by the United States Public Health Service, pointing out that small quantities of fluoride in water may inhibit dental caries, has given added significance to the accurate determination of small quantities of fluoride.

Using the zirconium-alizarin indicator of de Boer (*2*), methods of determining fluoride, or modifications, have been developed by Casares and Casares (*3*), Thompson and Taylor (*8*), Elvove (*5*), Sanchis (*6*), and Scott (*7*). In these methods the waters were acidified with hydrochloric acid or with hydrochloric and sulfuric acids. It is known that when the color of the zirconium-alizarin indicator is developed with hydrochloric acid, the effect of sulfate present in the water must be taken into account. When the waters are acidified with both hydrochloric and sulfuric acids the interference of sulfate is less than when hydrochloric acid alone is used, but tests have confirmed the statement of Walker and Finlay (*9*) that sulfate over about 120 parts per million interfered with the colorimetric determination of fluoride by the zirconium-alizarin method when both hydrochloric and sulfuric acids were used. By the procedure reported here the waters are acidified with sulfuric acid, thereby decreasing the interference of sulfate to the extent that waters containing less than 500 parts per million of sulfate may be analyzed for fluoride without resorting to a correction curve. The alkalinity of a sample may interfere with the fluoride determination by its effect on the pH of the solution. This effect is negligible up to about 1000 parts per million of bicarbonate in methods (*5*) using fairly strong hydrochloric acid. The bicarbonate should be neutralized if it is more than about 150 parts per million for methods using hydrochloric and sulfuric acids at a lower concentration (*6*). Neutralization of the alkalinity with nitric acid as prescribed in the proposed method eliminates any uncertainty due to the alkalinity of the sample. The zirconium-alizarin indicator appears to be more stable in sulfuric acid than in hydrochloric acid or in a mixture of hydrochloric and sulfuric acids.

Low or moderate quantities of fluoride in water can be measured conveniently and accurately by selecting the appropriate concentrations of the reagents.

Reagents

PREPARATION OF INDICATOR. (1) Zirconyl nitrate, 1.75 grams of $ZrO(NO_3)_2.2H_2O$ in 250 ml. (2) Alizarin red, 0.35 gram of alizarin monosodium sulfonate in 250 ml. (3) When the indicator is needed add 10 ml. of the zirconyl nitrate solution to approximately 50 ml. of distilled water, then add slowly with constant stirring 10 ml. of the alizarin solution and make up to a volume of 200 ml. with distilled water. The indicator should be stored in the dark and it should be used before it is more than a few days old.

Sulfuric acid, 2.10 *N* (±0.02 *N*).

Nitric acid, 0.1639 *N* (1 ml. will neutralize 10 mg. of HCO_3).

Sodium fluoride. Stock solution, 0.2210 gram of sodium fluoride in 1 liter. Standard solution, dilute 100 ml. of the stock solution to 1 liter (1 ml. equals 0.01 mg. of fluoride).

Procedure

Transfer 100 ml. of the clear samples to be analyzed to comparison tubes, neutralize the alkalinity with the 0.1639 *N* nitric acid (neutralization not necessary for waters containing less than 100 parts per million of bicarbonate), make up to a volume of 105 ml., and add exactly 5 ml. of the 2.1 *N* sulfuric acid. Transfer to a series of comparison tubes 0, 2, 4, 6, 8, 10, and 12 ml. of the standard fluoride solution (1 ml. equals 0.01 mg. of fluoride), make up to a volume of 105 ml., and add exactly 5 ml. of the 2.1 *N* sulfuric acid. Add 5 ml. of the zirconium-alizarin indicator to each sample and standard, mix well, and allow to stand overnight. An inspection made promptly after the indicator has been added will show any samples out of the range of the standards. Take smaller quantities of water for any samples that appear to contain more fluoride than the highest standard. After the samples and standards have stood about 18 hours, mix well, and compare in a 3-hole colorimeter in which each sample is compared with the two nearest standards.

Discussion of Method

When the samples of water to be analyzed are acidified with 5 ml. of 2.1 *N* sulfuric acid a good color range is obtained for amounts of fluoride ranging from 0.0 to 0.12 mg. For waters containing more than about 1.2 parts per million of fluoride, samples smaller than 100 ml. should be used. For waters containing appreciably higher fluoride the samples and standards may be acidified with exactly 5 ml. of 1.8 *N* sulfuric acid. When 1.8 *N* sulfuric acid is used a good color range is obtained for amounts of fluoride ranging from 0.04 to 0.18 mg. In either case the fluoride may be read to 0.01 mg. within the ranges indicated.

Although satisfactory results are sometimes obtained when comparisons are made at considerably less or more than 18 hours, comparisons at about 18 hours are likely to give the best results. For samples and standards that have stood about 18 hours it is found that as much as an hour difference in time of adding the reagents does not appreciably affect the determination. This fact can be used to advantage when any sample is shown by prompt inspection to be out of the range of the standards. A smaller sample can be taken, so that when comparisons are made after about 18 hours all the samples will be within the range of the standards.

Studies of the major ions usually found in natural waters have shown that sulfate causes the most interference. By acidifying the samples with sulfuric acid the interference of sulfate is reduced. On the basis of 100-ml. samples the error that may be introduced by sulfate or chloride is as follows: 500 parts per million of sulfate are equivalent to about +0.01 mg. of fluoride and 1000 parts per million of chloride are equivalent to about −0.01 mg. of fluoride. Since the errors introduced by sulfate and chloride are plus and minus, respectively, the effect of the one will tend to counteract the effect of the other. The effect of nitric acid added to neu-

tralize the alkalinity and of nitrate usually found in natural waters is negligible.

Summary

The zirconium-alizarin method has been modified to facilitate the convenient and accurate determination of small amounts of fluoride in a large number of water samples. Sulfuric acid is used to acidify the samples to reduce the interference of sulfate. The pH is accurately controlled to give the most sensitive comparisons. Most natural waters can be analyzed by the modified procedure without resorting to correction curves. The fluoride content of waters containing less than 500 parts per million of sulfate, 500 parts per million of bicarbonate, and 1000 parts per million of chloride may be determined within a limit of about 0.1 part per million when a 100-ml. sample is used.

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Determination of Melibiase Activity

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IN SUGAR analysis, methods which are based on enzyme hydrolysis are recognized (*6*, *7*) to possess an inherent accuracy superior to customary acid hydrolysis methods. A reason why such invertase methods have not been more generally adopted may be an impression (*6*, *7*) that each laboratory must first laboriously prepare and standardize suitable invertase preparations from yeast. Highly active invertase preparations specifically suited for analytical purposes, however, have been available for a number of years.

In the case of beet sugar refinery control and generally in the determination of sucrose and raffinose, a further difficulty is the lack of a satisfactory procedure for determining the melibiase activity of the enzyme preparations as a basis for their standardization.

Invertase hydrolyzes raffinose to produce levulose and melibiose. The melibiose thus formed may then be hydrolyzed by the enzyme melibiase into its constituent monosaccharides, galactose and glucose. This has been the basis of methods for determining raffinose and sucrose for many years (*3*, *5*, *8*). Bau (*2*) as well as Fischer (*4*) established long ago that bottom-fermenting yeast contains melibiase, whereas top-fermenting yeast does not. Based upon this fact, methods were devised for the determination of raffinose by differential fermentation with the two yeasts.

The literature contains few references to the determination of the activity of the enzyme melibiase.

Reynolds (*9*) was concerned mainly with the preparation of the enzyme; nevertheless, he refers to the unimolecular reaction velocity constant as a measure of the activity of his melibiase preparations. The course of hydrolysis was followed by observing the changes in polarization of a 20° V. melibiose solution. Since the total range of polarization between 0 and 100 per cent hydrolysis is only from +18.2° to +9.1° V., the method is not very sensitive. Pure melibiose being unobtainable at the time, it was necessary to prepare the melibiose from raffinose by fermentation with top-fermenting yeast.

The present A. O. A. C. (*1*) procedure for raffinose determination employs enzyme preparations as prepared from the two types of yeast, invertase with and without melibiase. This is based on the work of Hudson and Harding (*5*) and Reynolds (*9*), and particularly the comprehensive paper by Paine and Balch (*8*).

The method requires that the enzyme solution employed for the hydrolysis of raffinose solutions be capable of hydrolyzing 10 times its volume of 20° V. melibiose solution in 30 minutes at 20° C. to specified degrees of hydrolysis, depending on the amount of raffinose present in the material to be analyzed. The polarizations in degrees Ventzke corresponding to the percentages of hydrolysis which are shown, however, do not allow for dilution of the melibiose by the enzyme. Actually, after such correction, the polarizations equivalent to 35, 50, and 70 per cent hydrolysis would be 15.0, 13.6, and 11.6° V., respectively, instead of 16.4, 14.9, and 12.9° V. as indicated. A considerable difference, amounting to almost 100 per cent of the concentration of enzyme to be employed, is involved in the differences between the two series of polarization values.

In any case, the A. O. A. C. method represents merely a means of ascertaining approximately whether a minimum of enzyme activity will be present to ensure the complete hydrolysis of the raffinose present at each level and is not represented to be a method for expressing by a numerical value the melibiase activity of an enzyme preparation.

Weidenhagen (*11*, *12*) described a method in which the melibiose hydrolysis is followed by determining the changes in reducing action towards Fehling's solution and subsequent titration of the reduced copper with permanganate. This method also has the disadvantage of having only a limited range and hence limited sensitivity (18.7 to 10.5 ml. of potassium permanganate).

In 1932, Tauber and Kleiner (*10*) published a method for determining monosaccharides in the presence of reducing disaccharides, pointing to its probable usefulness in studying saccharases and glycosidases. Their method involves selective reduction of a Barfoed solution, modified by addition of lactic acid,

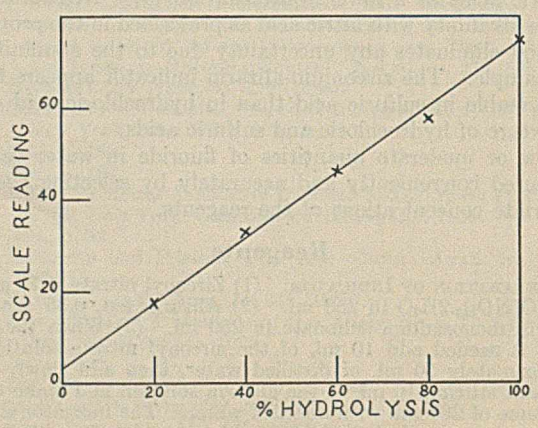

FIGURE 1. RELATIONSHIP BETWEEN PER CENT HYDROLYSIS AND SCALE READING

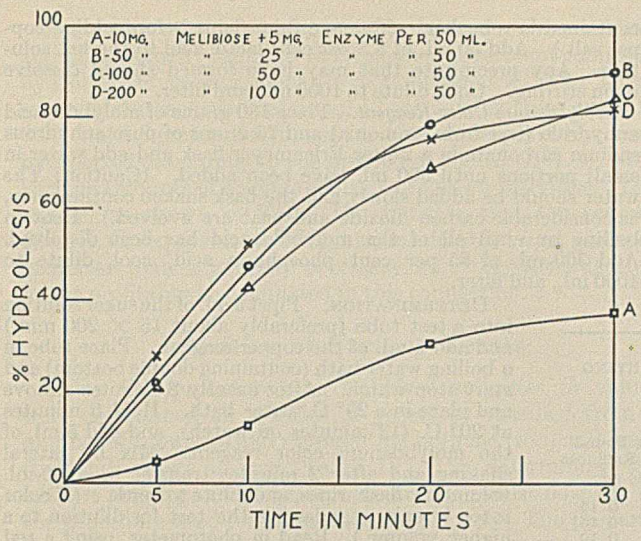

FIGURE 2. EFFECT OF CONCENTRATION ON HYDROLYSIS

and then permitting the cuprous copper formed to reduce an acid molybdate solution, the resulting blue color being measured photometrically. Because of the greatly increased sensitivity possible with relatively minute amounts of material, as compared with existing rotation methods, the procedure appeared excellently suited for following the hydrolysis of melibiose by melibiase. At about this time pure melibiose also became available.

These facts made it possible to investigate the course of the hydrolysis of melibiose as a basis for a method for determining the activity of melibiase preparations used in the analysis of raffinose-containing materials.

Calibration Hydrolysis Curve

Mixtures of melibiose, glucose, and galactose representing the products present in the course of the hydrolysis were prepared to correspond to varying hydrolysis percentages. After the reduction, photometer readings (an American Instrument Co. neutral wedge photometer was used) in a 1.25-cm. (0.5-inch) cell were taken, using a 6100 Å. filter (since maximum absorption is obtained in the red portion of the spectrum). Because of the tendency of the color to fade, readings were made 2 minutes after dilution.

Figure 1 shows the linear relationship between scale readings and per cent hydrolysis, permitting calculation of per cent hydrolysis corresponding to any photometer reading as follows:

$$\% \text{ hydrolysis} = \frac{S_x - S_0}{S_{100} - S_0} \times 100$$

where S_x = scale reading obtained at a given stage in hydrolysis
S_0 = scale reading at 0 per cent hydrolysis, and
S_{100} = scale reading at 100 per cent hydrolysis

Conditions of Hydrolysis

The following test conditions were employed for measuring the hydrolysis:

The substrate, except where otherwise indicated, consisted of 1.104 grams of melibiose hydrate (equivalent to 1.000 gram of anhydrous melibiose) and 5 ml. of Walpole's acetate pH 4.5 buffer diluted to 100 ml. Phosphate buffers may not be used because of subsequent precipitation with the copper reagent.

Ten milliliters of substrate and 25 ml. of water were attemperated at 20° C. and then 5 ml. of the enzyme solution were added. At the end of the specified time of hydrolysis, 2 ml. of a sodium carbonate solution, containing 2 grams per 100 ml., were added to stop the hydrolysis and the volume was adjusted to 50 ml. with water. Ten milliliters of the hydrolysis mixture were diluted to 100 ml., and the monosaccharides were determined on a 5 ml. aliquot as in the method described below.

The enzyme preparation used in the preliminary work was an invertase preparation in scale form similar to the product regularly available for analytical use and obtained by precipitation and purification from bottom yeast autolyzates.

Hydrolysis at Varying Concentrations

A few preliminary tests were conducted to determine the most satisfactory concentration of melibiose to employ to permit a study of the hydrolysis reaction. Melibiose concentrations were varied from 10 mg. per 50 ml. to 200 mg. per 50 ml. (final volume), the ratio of enzyme to substrate remaining constant at 1 to 2. Figure 2 represents the course of hydrolysis for these hydrolysis mixtures.

A concentration of 100 mg. per 50 ml. represented by curve C appears to represent a satisfactory melibiose concentration for such studies and further tests were carried out accordingly on this basis.

TABLE I. HYDROLYSIS AT VARYING ENZYME CONCENTRATIONS

Enzyme Concentration G./100 ml.	Time Min.	Hydrolysis %	k[a]
0.1	15	14.2	0.0044
	30	21.3	0.0035
	45	34.3	0.0041
	60	40.0	0.0037
	90	57.3	0.0041
			Av. 0.0040
0.2	15	21.0	0.0068
	30	42.5	0.0080
	45	57.1	0.0082
	60	69.3	0.0085
	90	82.5	0.0084
			Av. 0.0080
0.4	15	42.2	0.0159
	30	68.6	0.0164
	45	82.5	0.0168
	60	94.6	0.0211
	90	97.5	0.0178
			Av. 0.0176

[a] $k = \frac{1}{t} \log_{10} \frac{a}{a - x}$.

Hydrolysis with Varying Amounts of Enzyme

Reynolds and Weidenhagen have referred to the unimolecular reaction velocity constant, k, as a measure of melibiase activity. Table I contains hydrolysis data including the velocity constants, k, obtained. This is represented also by Figure 3, showing the course of hydrolysis at three

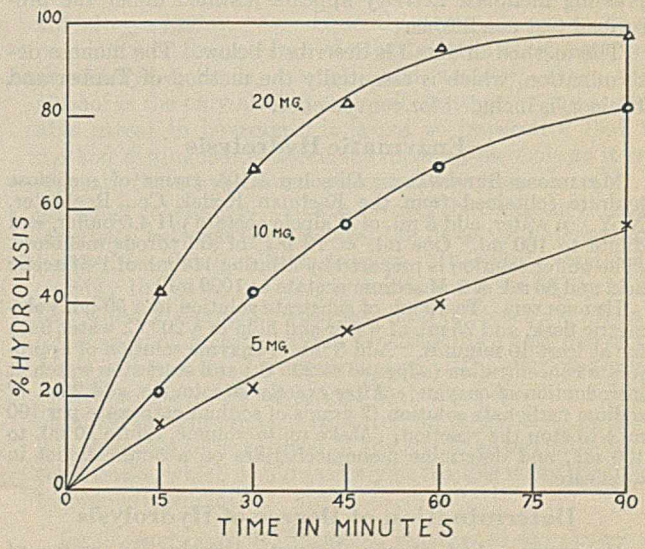

FIGURE 3. HYDROLYSIS AT VARYING ENZYME CONCENTRATIONS

TABLE II. PROPORTIONALITY OF ENZYME CONCENTRATION WITH k VALUES AS OBTAINED FROM 30-MINUTE HYDROLYSIS

Enzyme Concentration	Hydrolysis	Activity of Diluted Enzyme	Activity of Original Enzyme
G./100 ml.	*%*	*k*	*k value*[a]
0.10	14.6	0.0023	2.3
0.20	30.6	0.0053	2.6
0.30	44.1	0.0084	2.8
0.40	55.9	0.0119	3.0
0.50	63.9	0.0147	2.9
0.70	79.0	0.0229	3.3
1.00	89.2	0.0322	3.2

[a] Represents velocity constant, k, multiplied by dilution factor—for enzyme concentration of 1 gram per 100 ml., k value = $k \times 100$.

TABLE III. HYDROLYSIS OF SUCROSE AND RAFFINOSE WITH VARYING AMOUNTS OF MELIBIASE

Melibiase Activity	Amounts Taken: Sucrose	Amounts Taken: Raffinose (anhydrous)	Invert Polarizations: Direct Polarization	Invert Polarizations: Invertase	Invert Polarizations: Invertase + melibiase	Amounts Found: Sucrose	Amounts Found: Raffinose
k	*G./100 ml.*		*° V.*	*° V.*	*° V.*	*G./100 ml.*	
0.01	13.00	0.20	+51.38	−15.20	−15.62	13.00	0.15
0.05					−15.65	12.98	0.16
0.10					−15.75	12.96	0.19
0.20[a]					−15.68	12.98	0.17
0.02	13.00	0.65	+54.34	−13.60	−14.23	13.20[b]	0.22[b]
0.10					−15.27	12.98	0.59
0.20					−15.16	12.99	0.55
0.35[a]					−15.26	12.98	0.59
0.04	13.00	1.30	+59.20	−11.30	−12.75	13.52[b]	0.51[b]
0.20					−14.27	13.17[b]	1.05[b]
0.40					−14.77	13.05	1.23
0.60[a]					−14.75	13.05	1.22

[a] Enzyme activities recommended by A. O. A. C.
[b] Incomplete hydrolysis.
Invertase activity more than 0.1 (k value) in all tests.

enzyme concentrations under the conditions described. As in the case of the application of such k values to invertase the reaction appears to follow a unimolecular course within limits.

For simplicity, it would be desirable for the determination of melibiase activity of unknown preparations to involve determination of per cent hydrolysis after a single hydrolysis period. Accordingly, Table II shows calculated k values obtained when varying amounts, from 5 to 50 mg., of a particular enzyme preparation were employed in the hydrolysis of a melibiose solution for exactly 30 minutes and the per cent hydrolysis and corresponding k values obtained. A satisfactory linear relationship between enzyme concentration and k value is shown to exist between the limits of 30 and 70 per cent hydrolysis.

Based on these data, the use of calculated k values for expressing melibiase activity appears justified under the prescribed test conditions.

The method in detail is described below. The monose determination, which is essentially the method of Tauber and Kleiner, is included for convenience.

Enzymatic Hydrolysis

MELIBIOSE SUBSTRATE. Dissolve 1.104 grams of melibiose hydrate (obtained from the Eastman Kodak Co., Rochester, N. Y.) in water, add 5 ml. of Walpole acetate pH 4.5 buffer, and dilute to 100 ml. One ml. = 10 mg. of anhydrous melibiose. (The buffer solution is prepared by diluting 114 ml. of 1 M acetic acid and 86 ml. of 1 M sodium acetate to 1000 ml.)

HYDROLYSIS. To 10 ml. of substrate solution in a 50-ml. volumetric flask, add 25 ml. of water and hold in a 20° C. water bath for at least 10 minutes. Add 5 ml. of enzyme solution of appropriate concentration (adjusted to 20° C.) and start stop watch at introduction of enzyme. After exactly 30 minutes, add 2 ml. of sodium carbonate solution (2 grams of sodium carbonate per 100 ml.) to stop the reaction. Make up to volume, dilute 10 ml. to 100 ml., and determine monosaccharides on a 5-ml. aliquot in duplicate.

Determination of Degree of Hydrolysis

REAGENTS. *Acid Copper Reagent.* Dissolve 48 grams of copper acetate (Merck, normal, C. P.) in 850 ml. of boiling water contained in a beaker. (Remove from flame before adding copper salt.) Add 50 ml. of 8.5 per cent lactic acid to the hot solution. Any precipitate that may have formed should dissolve upon stirring. Cool, dilute to 1000 ml., and filter.

Molybdenum Color Reagent. Place 150 grams of molybdic acid anhydride (free from ammonia) and 75 grams of pure anhydrous sodium carbonate in a 2-liter Erlenmeyer flask and add water in small portions until 500 ml. have been added. (Caution: The water should be added slowly and the flask shaken continuously, as considerable carbon dioxide and heat are evolved.) Heat to boiling or until all of the molybdic acid has been dissolved. Add 300 ml. of 85 per cent phosphoric acid, cool, dilute to 1000 ml., and filter.

DETERMINATION. Pipet 5 ml. of the sugar solution into a test tube (preferably about 15 × 200 mm.) and add 5 ml. of the copper reagent. Place tube in a boiling water bath (containing double bottom) and start stop watch. After exactly 8 minutes remove and place in a 20° C. water bath. Hold 5 minutes at 20° C. (13 minutes on watch), and add 5 ml. of the molybdenum color reagent. Mix by lateral shaking and after 2 minutes transfer to a 50-ml. volumetric flask, rinse, and dilute to mark. (If color is too intense, standardize the test for dilution to a higher volume.) Read in photometer, using a red filter, within 2 to 3 minutes after dilution (17 to 18 minutes on stop watch), as the color fades fairly rapidly. Since the method is empirical, conditions must be closely adhered to.

CALCULATION OF k VALUE. Determine the photometer reading for the 0 and 100 per cent hydrolysis. The 0 per cent hydrolysis is represented by the solution of the enzyme added to the substrate after addition of the sodium carbonate. The 100 per cent hydrolysis is obtained by hydrolyzing the substrate with sufficient enzyme overnight at room temperature.

Calculate the per cent hydrolysis as indicated above and the equivalent unimolecular constant:

$$k = \frac{1}{t} \log_{10} \frac{100}{100 - x}$$

where x = % hydrolysis

Example. 0.2% enzyme solution used in hydrolysis

$S_x = 37.9$
$S_0 = 10.6$
$S_{100} = 74.9$

$$\% \text{ hydrolysis} = \frac{37.9 - 10.6}{74.9 - 10.6} \times 100 = 42.5$$

$$k = \frac{1}{30} \log_{10} \frac{100}{100 - 42.5} = 0.0080 \text{ (activity of enzyme solution as used for hydrolysis)}$$

$$k \text{ value of original enzyme} = \frac{100}{0.2} \times 0.008 = 4.0$$

TABLE IV. MELIBIASE ACTIVITIES OF BREWERS' YEASTS

Number	Type of Yeast	k Value (Dry Yeast)
1	Ale	Nil
2	Lager	0.70
3	Lager	0.24
4	Ale	Nil
5	Lager	0.85
6	Lager	0.76
7	Lager	0.25
8	Lager	0.95

Application to Raffinose Determinations

The difficulty encountered in the proper interpretation of the A. O. A. C. procedure for determining the amount of enzyme to use at varying expected raffinose levels has been referred to.

It would, no doubt, be of advantage to establish the activity requirements of melibiase in terms of k values. Experiments which were made to establish the relationship between k values by the proposed method and per cent hydrolysis as determined by the A. O. A. C. procedure indicate that enzyme solutions of k values of 0.2, 0.35, and 0.6 are required to yield respectively, 35, 50, and 70 per cent hydrolysis under the A. O. A. C. conditions.

It was also considered of interest to determine the melibiase activities actually required to bring about overnight complete hydrolysis of various amounts of raffinose at 20° C. Three sugar mixtures, each containing 13 grams of sucrose and 0.2, 0.65, and 1.3 grams of raffinose per 100 ml., respectively, were hydrolyzed according to the method of the A. O. A. C. with various amounts of enzyme solution of known activities. The results, as shown in Table III, indicate that the present A. O. A. C. requirements involve a considerably larger excess of melibiase than is actually necessary. Further investigation might profitably be conducted in this connection.

Activities of Various Yeasts

Using this procedure, the melibiase activities (k value) were determined on a series of yeasts as obtained from a number of breweries and representing both ale (top) and lager (bottom) yeasts in use (Table IV). In determining the enzyme activity of live yeast, consideration must be given to the procedure used for extracting the enzyme. The preparation of invertase from yeast usually involves some means of autolysis of the yeast, toluene being the most common reagent referred to. Weidenhagen, in investigating melibiase activities as obtained with different autolyzing solvents, found that ethyl acetate appeared to give the most satisfactory result. This has been confirmed, and accordingly ethyl acetate was used in these tests.

Determination of Melibiase Activity of Yeast

Drain the liquid yeast as received, on a Büchner funnel, and wash several times with cold water. Plasmolyze 5 grams of the yeast cake by adding 1 ml. of ethyl acetate and stir intermittently for 10 minutes. Add 5 ml. of water, stir well, and after 5 minutes dilute to 100 ml. Use 5 ml. of this suspension directly for the hydrolysis as described above. Filter immediately after addition of sodium carbonate at end of hydrolysis and proceed as directed. Make a blank determination on another portion of substrate by adding sodium carbonate prior to the yeast and then continuing as above.

The procedure represents also a convenient rapid chemical method for distinguishing between ale and lager yeasts.

Summary

A new method for the determination of the activity of the enzyme melibiase utilizes the Tauber and Kleiner procedure for determining monosaccharides in the presence of disaccharides as a means of following the course of hydrolysis of melibiose. The hydrolysis has been found to follow a unimolecular course within limits and hence the activities have been expressed as k values as determined after a 30-minute hydrolysis period. Experiments made on various sucrose-raffinose mixtures indicate that the amounts of enzymes specified by the A. O. A. C. for hydrolysis are probably unnecessarily high. A method for determining the melibiase activity of yeast is described and the values for a number of brewery yeasts are given; the method has also been found suitable for distinguishing between top- and bottom-fermenting yeasts.

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Method for Determination of Ethyl Alcohol for Medicolegal Purposes

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CONSIDERABLE interest exists regarding methods for the determination of ethyl alcohol in body fluids, since the alcohol content of these fluids is used to determine the degree of inebriety. Most of the methods proposed depend upon the direct oxidation of the volatile substances separated from the body fluids by aeration or distillation from an acid medium. Direct aeration or distillation from an acid medium must necessarily assume the presence of only relatively small amounts of other volatile substances which might reduce the oxidizing agent and be interpreted as alcohol.

Hine (7) reports that other reducing substances are not present in sufficient quantity to increase the alcohol reading significantly. However, sufficient formaldehyde or acetone may be present to increase the alcohol reading to a level considered intoxicating. Methenamine is commonly used as an urinary antiseptic and significant quantities of formaldehyde appear in the urine after its administration. While acetone is not oxidized quantitatively under the conditions commonly employed, its presence does increase the readings for alcohol.

In medicolegal work, the state of health of the individual or whether or not some kind of medication had been taken is not always known. Consequently, there always exists the possibility of the presence of other volatile substances, such as aldehydes, acids, phenols, and acetone. Since ethyl alcohol is the only alcohol ordinarily present to any appreciable extent in beverages, it is not so imperative that the method distinguish between the different alcohols as it is to separate the ethyl alcohol quantitatively from the other interfering substances. Where it may be necessary to differentiate between the alcohols, the distillate obtained may be used for this particular purpose. It is more essential to simplify the procedure and to increase the specificity of the test without sacrificing accuracy than it is to have available a less specific microtechnique which requires only one or two drops of blood for the determination. The proposed microtechniques have the advantage that blood specimens can be obtained by skin pricks. However, for medicolegal purposes, it is desirable to have some of the specimen available for future checks, which would scarcely be feasible when the microtechnique is employed.

Gettler and Siegel (5) suggested isolation of the alcohol and determination of its physical constants; however, this technique

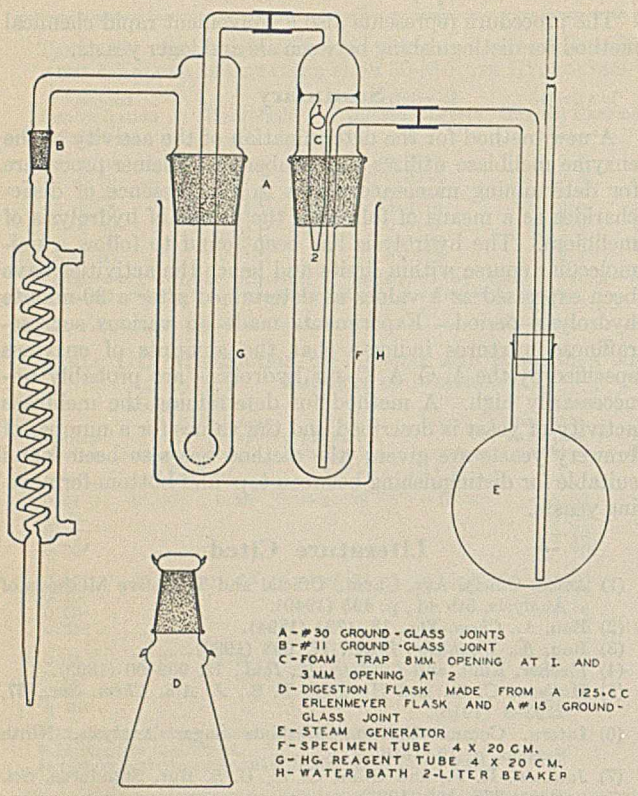

FIGURE 1. DISTILLATION APPARATUS

is intended for the determination of alcohol in tissues and relatively large quantities of material are required. Gorr and Wagner (*6*) studied the removal of aldehydes and ketones from alcohol solutions by refluxing the mixture with mercuric chloride in 1 *N* or 2 *N* sodium hydroxide for 2 to 5 hours and then distilling the alcohol. They found that the acetaldehyde and acetone were removed completely with recoveries of 94 to 96 per cent of the added alcohol.

A further study of the efficiency of mercuric oxide to remove aldehydes and ketones was made by Friedemann and Klaas (*4*), who substituted calcium hydroxide for sodium hydroxide. These authors suggest distilling the alcohol from an acid medium after precipitating the proteins with sodium tungstate and mercuric sulfate, and a second distillation from calcium hydroxide containing mercuric oxide to remove any volatile substances, other than alcohol, which were distilled from the acid medium. This technique requires a second transfer and distillation of the specimen, which is quantitatively undesirable for routine purposes.

The method described in this paper has been developed for routine analysis of specimens for medicolegal purposes. The authors found that the interfering substances are removed quantitatively by passing the distilled vapor from the acid medium through a solution of concentrated sodium hydroxide containing mercuric oxide. The alcohol is distilled and the interfering substances are removed in one operation, considerably simplifying the technique. It provides a simple means of separating the alcohol quantitatively from the other substances which may be present in biological material and dispenses with the necessity of testing for the presence of these contaminants. The acid medium removes any basic volatile substances, while the concentrated sodium hydroxide containing mercuric oxide removes the acids, aldehydes, acetone, and phenols. The method has the added advantage of distilling and digesting the specimen in an all-glass apparatus and hence prevents contamination from cork or rubber stoppers.

The authors have also observed that low recoveries are obtained by oxidizing the alcohol in an open container, undoubtedly owing to loss of between 10 and 20 per cent or even more acetaldehyde during the digestion. To prevent this loss, the alcohol is oxidized in a closed flask. The method of oxidizing the alcohol, or the determination of the excess oxidizing agent, is a matter of preference. Apparently all the reagents suggested for this phase of the determination give accurate results. However, the authors prefer to use potassium dichromate because the solution will keep indefinitely, which obviates the necessity of periodic restandardization. The excess dichromate is reduced with potassium iodide and the liberated iodine titrated with a 0.1 *N* sodium thiosulfate solution.

Apparatus

The distillation apparatus and the digestion flask are shown in Figure 1. The two tubes, *F* and *G*, are held in place during the distillation by springs attached to the top of the tubes and the side arms. The foam trap, *C*, as constructed will prevent the mechanical carrying over of material, even when considerable foaming occurs. The vapor passes through the opening at 1, and the condensate, together with any fluid forced through the opening, returns to the distillation tube through the opening at 2. The alcohol is collected from the condenser in flask *D*.

The male portion of the joint of tube *G* must be covered with graphite to prevent sticking. The graphite is best applied by using a carpenter's pencil, although an ordinary pencil will serve the purpose.

A 2-liter beaker may be used for the boiling water bath, *H*. The digestion flask, *D*, is constructed from a 125-cc. Erlenmeyer flask and a No. 15 interchangeable ground-glass joint. The cap is kept in place with two coil springs, 0.6 cm. (0.25 inch) in diameter, made from 20-gage spring steel wire. Considerable tension must be maintained on the springs to prevent the cap from being raised by the vapor pressure and breaking the cap or the flask when it snaps back into position. Two 10-cc. all-automatic burets are most convenient for the 0.1 *N* potassium dichromate and 0.1 *N* sodium thiosulfate solutions.

Reagents

Potassium dichromate solution, 0.1 *N*, 4.903 grams per liter, reagent quality. A solution prepared from reagent quality potassium dichromate agrees with the theoretical yield of alcohol by direct digestion of standard alcohol solutions. One cubic centimeter of the solution is equivalent to 1.15 mg. of alcohol. (A 0.1 *N* solution is preferred to more dilute solutions because it covers the range of the quantities of alcohol commonly found in the blood or urine specimens, and if reasonable care is exercised, excellent checks can be obtained even with as small quantities as 0.005 per cent.)

Sodium thiosulfate solution, 0.1 *N*, 25 grams of sodium thiosulfate plus 0.1 gram of sodium bicarbonate per liter. This solution is standardized against the 0.1 *N* potassium dichromate solution.

Potassium iodide crystals, analytical reagent, must be free from iodates.

Arrowroot starch, 1 per cent solution.

Sulfuric acid, concentrated, reagent quality.

TABLE I. RECOVERIES OF ALCOHOL FROM URINE AND BLOOD

Material	Sample	Alcohol Added	Acetone Added	Formaldehyde Present	Alcohol Recovered	
	Cc.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*%*
Water	2.5	2.35	..	..	2.32	98.7
Water	5	4.70	..	..	4.70	100.0
Water	5	4.70	10	..	4.70	100.0
Water	5	4.70	15	..	4.69	99.7
Water	5	5.00	7.5	7.5	4.92	98.4
Urine	2	4.70	..	..	4.68	99.5
Urine	5	4.72	15	..	4.72	100.0
Urine	5	4.72	15	..	4.77	101.1
Urine	5	4.72	20	..	4.77	101.0
Urine	5	4.72	25	..	4.60	97.5
Urine[a]	2	1.90	10	1.2	1.89	99.4
Urine[a]	2	1.90	10	1.2	1.91	100.5
Urine[a]	5	1.90	10	3.0	1.90	100.0
Urine[a]	5	5.00	20	19.2	4.91	98.2
Urine[a]	5	5.00	20	19.2	4.88	97.6
Blood	4	7.60	..	..	7.62	100.2
Blood	2	3.80	..	..	3.76	99.0
Blood	1	1.90	5	..	1.88	98.9
Blood	2	0.95	10	..	0.96	101.0
Blood	1	0.47	..	..	0.47	100.0

[a] Specimen from patients after administration of 4 grams of methenamine. Urine was steam-distilled from acid solution and formaldehyde approximated colorimetrically.

Mercuric chloride, saturated aqueous solution.
Sodium hydroxide, saturated solution.
Sodium tungstate, 10 per cent solution.
Sulfuric acid, 1 *N* solution.

Procedure

The blood or urine specimen (1 or 2 cc.) is measured into the distillation tube, *F*, and the protein is precipitated by the addition of 5 cc. of 10 per cent sodium tungstate and 5 cc. of 1 *N* sulfuric acid. These quantities of tungstate and sulfuric acid for a 2-cc. blood specimen effect a complete precipitation of the proteins, and little, if any, foaming occurs during the distillation. Since urine specimens do not ordinarily contain protein, it is usually unnecessary to add the tungstate. The analyses can be made on coagulated blood; however, care must be exercised when taking a sample for the determination to obtain proportionate amounts of the serum and clot. Whenever possible, an anticoagulant, such as sodium citrate, oxalate, or fluoride, should be employed.

Ten cubic centimeters of a saturated solution of mercuric chloride and 10 cc. of a saturated solution of sodium hydroxide are measured into tube *G*. The tubes are then connected to the distillation apparatus and immersed in the hot water bath, which should be kept at the boiling temperature during the distillation process. The alcohol is steam-distilled directly into the digestion flask, *D*. After 25 to 30 cc. are distilled, 10 cc. of the 0.1 *N* potassium dichromate solution and 5 cc. of concentrated sulfuric acid are added to the distillate. The sulfuric acid should be allowed to run down the side of the flask to prevent it from mixing with the aqueous solution and causing the heat of solution to raise the temperature before the flask is closed. After the flask is closed and the cap fastened with the two springs, the solution is mixed. The flask is then placed in a boiling water bath for 20 minutes. The solution is cooled, and the dichromate solution is washed down the sides of the flask with a stream of water from a wash bottle. Enough water should be added to dilute the acid to a 10 per cent solution or less. The excess dichromate is determined by adding approximately 0.2 gram of potassium iodide crystals and titrating the liberated iodine with the standardized solution of 0.1 *N* sodium thiosulfate. Addition of the starch indicator should be delayed until the iodine color is nearly removed. Five drops of the 1 per cent starch solution are then added and the titration is completed.

Experimental

Standard alcohol solutions were made from absolute alcohol which was prepared according to the technique suggested by Noyes (*8*) and Castille and Henri (*2*). Pure acetone was prepared according to the method suggested by Frankforter and Cohen (*3*). A U. S. P. solution of formaldehyde was assumed to contain 37 per cent of formaldehyde.

Alyea and Bäckström's method (*1*) was used to test for the presence of acetone and the modified Schiff's reagent (*9*) was used to test for the presence of formaldehyde in the distillates.

Alcohol was recovered from pure solutions, blood, and urine, according to the procedure outlined above. Table I shows that quantitative recoveries of the alcohol are obtainable and that the acetone and formaldehyde are completely removed.

The efficiency of the method in removing acetone and formaldehyde was determined by distilling known quantities of these compounds and testing the distillates. It was found that the method will remove 75 mg. of formaldehyde and 30 mg. of acetone, quantities considerably in excess of those which would be present in blood or urine specimens under the most extreme conditions.

Table II shows the effect of distilling urine specimens through concentrated sodium hydroxide containing mercuric oxide. In the absence of the mercuric oxide–sodium hydroxide reagent the alcohol readings are erroneously high, especially on materials obtained from individuals receiving methenamine. On these specimens the commonly employed methods would indicate that the individual was under the influence of alcohol. While acetone does not present a very serious problem, its presence in borderline cases may increase the alcohol reading to a level considered intoxicating, especially where certain levels of alcohol in the blood or urine are being accepted for defining intoxication. It will be noted in Table II that the mercuric oxide–sodium hydroxide reagent reduces these readings to insignificant quantities.

Table II. Difference in Apparent Alcohol Reading with and without Sodium Hydroxide–Mercuric Oxide Reagent

Type of Urine	Apparent Alcohol Reading Without reagent	Apparent Alcohol Reading With reagent
	Mg./cc. of urine	
Diabetic[a]	0.115	0.036
	0.159	0.040
	0.050	0.010
	0.152	0.038
	0.060	0.010
Methenamine[b]	0.960	0.010
	0.920	0.010
	2.250	0.020
	1.770	0.030
	2.890	0.030
	1.040	0.020
	1.950	0.020
	1.060	0.030
	1.090	0.010
	1.180	0.010

[a] Diabetic patients were emergency cases, either in coma or approaching comatose condition when urine specimens were taken.
[b] Patients received 2.5 to 4.0 grams (one daily therapeutic dose) of methenamine in 8-hour period in four divided doses. Urine specimens were collected at intervals after first dose and up to 14 hours after last dose.

It has been suggested that the accumulation of intermediate metabolites in the system during various forms of anoxia may be a source of error in the alcohol determination. To test the possibility of this error, rabbits were asphyxiated in an airtight chamber until they were maximally dyspneic and cyanotic; others were rendered anoxic by exposure to carbon monoxide or to oxygen at a partial pressure of 60 mm. of mercury (equivalent to 8 per cent of oxygen at 760 mm. of mercury). These conditions failed to produce any reducing substance which would pass over with the distillate and be read as alcohol.

Summary

A method for the determination of alcohol in biological material is described. Its advantages over previously described methods are: (1) the alcohol can be separated from interfering substances, such as acids, bases, aldehydes, and ketones, in one operation and it is not necessary to test for the presence of these contaminants; (2) the distillation and digestion of the specimen are done in an all-glass apparatus, preventing contamination from stoppers. Alcohol can be recovered quantitatively when acetone or formaldehyde is present in greater concentration than would be present under the most extreme conditions. Although Table I shows that alcohol was quantitatively recovered in the presence of acetone and formaldehyde, a few samples are included in Table II to illustrate the operation of this principle on natural samples: urine from diabetic patients containing acetone bodies and from patients receiving methenamine containing formaldehyde. Accumulation of intermediate metabolites due to various types of anoxia has no effect on the alcohol reading. The method largely overcomes the criticism of nonspecificity of the methods commonly employed.

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Two Improved Pressure-Regulation Devices

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THE regulators described in this article resulted from a need for a sensitive and positive vacuum control in experiments on vapor pressures of binary mixtures.

The first instrument (Figure 1) possessed certain features and characteristics which may be useful for other purposes. No rubber tubing is necessary, for the regulator may be attached directly to the system to be controlled. Articles ordinarily found in the laboratory were used in its construction. It may be used either with weights or with the mechanism shown in Figure 2.

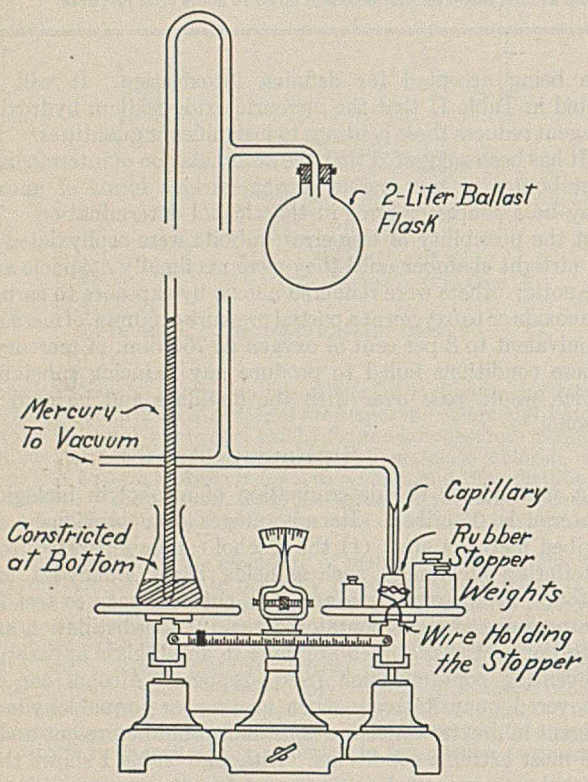

FIGURE 1

Using weights, any pressure in a range from 1 to 200 mm. is quickly obtained and kept, but above 400 mm. the pressure fluctuates and the instrument "spills", letting in large amounts of air. Control in the range from 200 to 400 mm. requires some care. The precision attainable with both regulators is 0.1 mm. in the pressure range above 100 mm. Below 100 mm. the variation in pressure is inappreciable.

The operation is shown by Figure 1. A decrease in pressure in the system decreases the force exerted on the left balance pan, indicated by the rise of the height of mercury, and permits the right-hand balance pan to lower and let air in through the capillary. The dynamic equilibrium set up in this way may be altered to raise or lower the pressure in the system by the simple addition or subtraction of weights on the right-hand pan. A table of weights corresponding to various pressures may be made, but these are subject to slight changes in the atmospheric pressure.

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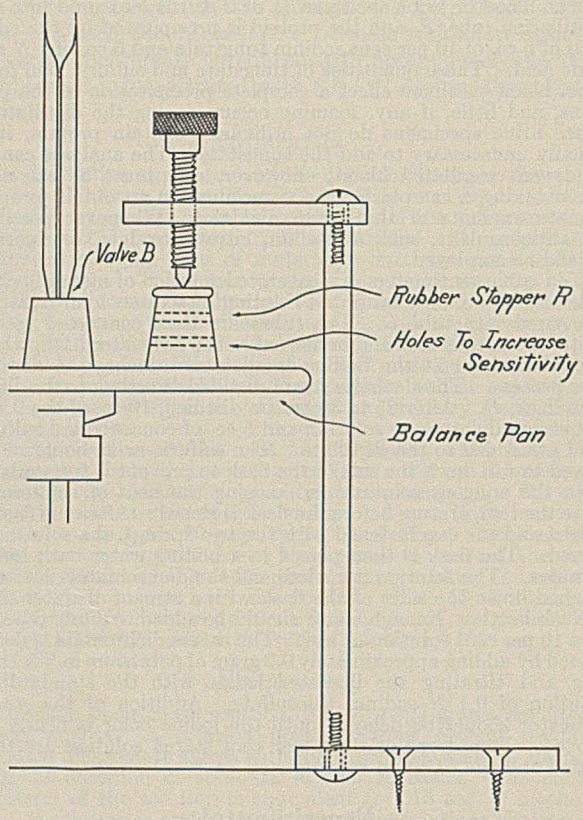

FIGURE 2

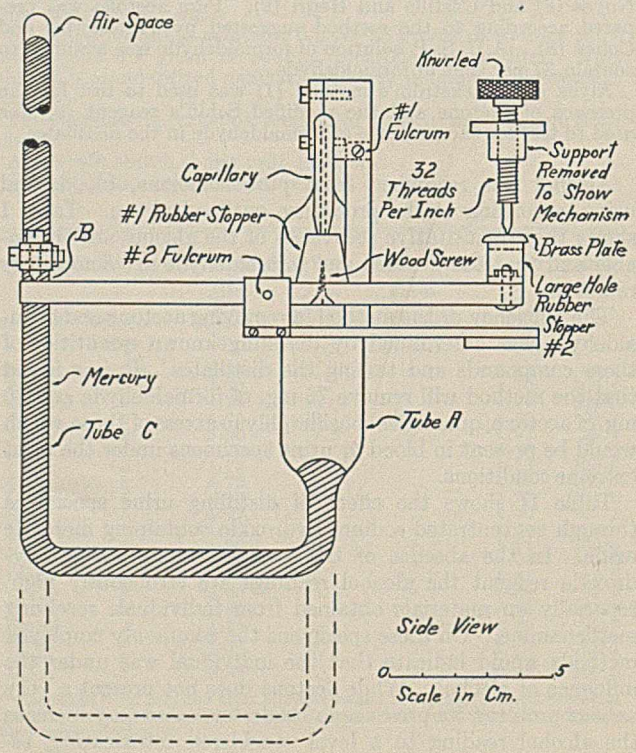

FIGURE 3

Using the mechanism shown in Figure 2, any pressure from atmospheric down to 1 mm., with definite control at any point, may be obtained. This mechanism eliminates the disadvantages of the weights. When pressure on the balance pan is increased by turning the screw down on the perforated rubber stopper, *R*, more air is admitted to the system until the increase of air pressure in the system closes valve *B* against the pressure of *R*. Heavy sponge rubber can be used in *R*. For pressures in the system close to 760 mm. it is best not to have the source of vacuum running at full capacity.

The balance was bolted to a ring stand, which in turn was bolted to a wooden base, so that the regulator could be carried from place to place as a unit. To ensure smooth operation a ballast flask was included in the system and the bottom of the tube dipping into the mercury was constricted. About 70 ml. of mercury were used, but this could be reduced by using a smaller flask. The glass tubing was 6 mm. and the capillary was 1.5 mm. in inside diameter.

The rubber stopper used as a valve seat was an ordinary stopper boiled in sodium hydroxide solution for approximately 0.5 hour. The capillary was ground down on an emery wheel and the flat portion which presses against the rubber stopper was ground down, using first a coarse and then a fine grade of emery powder in glycerol as a grinding medium. The grinding was facilitated by clamping a cork borer in a vertical position and using it as a bearing for the capillary tube as it was rotated. In this way the plane end of the capillary is made perpendicular to the axis of the capillary.

In order to have a more compact unit and to avoid exposure of the mercury to the air, a lever arm was substituted for the balance and a closed manometer was used. An excellent instrument has been designed by Schierholtz (*1*).

Figures 3 and 4 show the construction in some detail. The screw which constitutes fulcrum 1 is not tightened up completely so that the entire glass portion moves with it as a center. As the mercury falls in the small glass arm and rises in the large portion, owing to lowering of pressure in the system, less weight is supported by the clamp pressing on the lever arm at *B* and more by fulcrum 1. The more firmly the screw mechanism presses on rubber stopper 2, the higher will the pressure rise in the system and the higher will the mercury rise in the small side arm to force rubber stopper 1 against the capillary and shut off the supply of air.

About 0.75 ml. of air left in tube *C* increases the range of pressure over which the instrument is sensitive. With the air pocket,

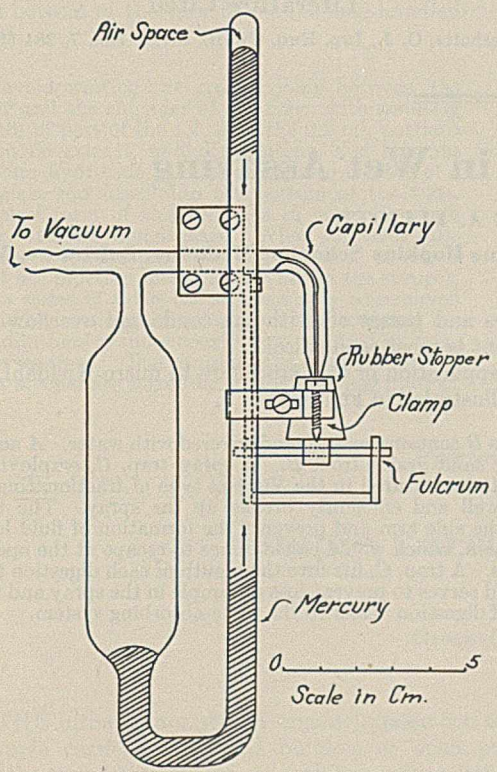

FIGURE 4

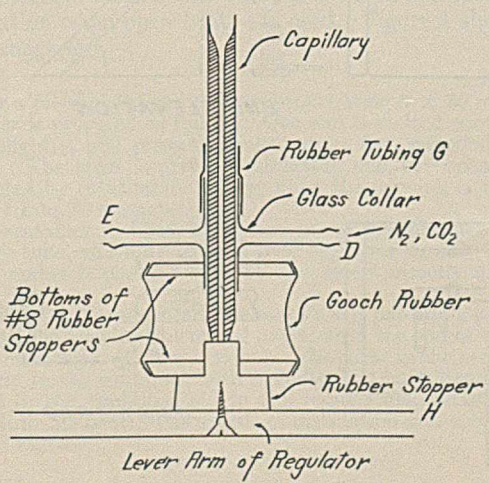

FIGURE 5

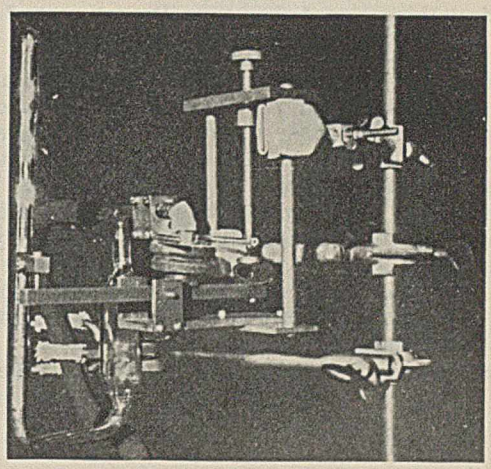

FIGURE 6

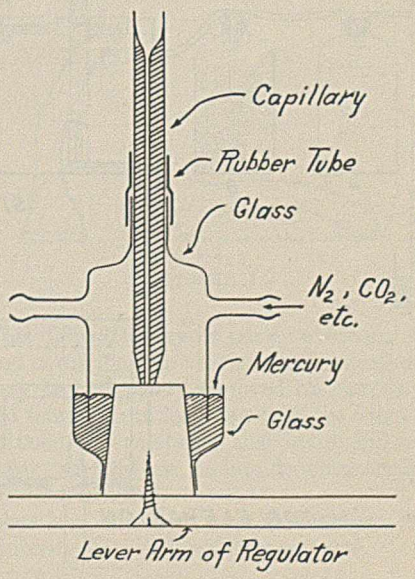

FIGURE 7

the range of control due to the movement of mercury extended from 1 to 350 mm.; without the air pocket the range was from 1 to 220 mm. Besides increasing the effective range of the regulator 100 mm., the air pocket helps to prevent the breaking of the glass arm by the mercury if the pressure in the system suddenly increases.

No rubber tubing is used in the regulator itself. Since connection to the system is made close to the fulcrum, heavy pressure tubing may be used without interfering with the action of the regulator.

If gases other than air are desired in the system during a distillation, the apparatus shown in Figures 5 and 6 may be added. This addition is facilitated by the relatively little movement between the capillary and the rubber stopper. If side arms *D* and *E* are left open to the air, air will be drawn into the system, but if nitrogen or carbon dioxide is passed through *D* and out *E*, these gases will be drawn into the system instead. Before starting a distillation the system should be flushed out with the gas to be used by applying a vacuum and having the capillary open as much as possible. The glass collar is fastened to the capillary by a short length of rubber tubing, *G*. A glass-to-glass seal is better, since no flexibility is desired at this point. Rubber stopper *H*, forming the valve seat, was cut halfway through with a cork borer and then the outside portion was cut away with a razor as shown in Figure 5. Two No. 8 rubber stoppers were bored to fit *G* and *H*, respectively, and a 4-mm. section was taken from the bottom of each. They were fastened together with a short length of Gooch rubber tubing to provide free movement between the capillary and rubber valve seat.

In Figure 7 the same effect is accomplished using a mercury seal. This is much more rugged and easily handled, but a sudden increase of nitrogen or carbon dioxide pressure might blow out the mercury.

Acknowledgment

The author wishes to acknowledge the helpful encouragement of P. A. van der Meulen and D. L. Cottle. Appreciation is also due F. G. Horstmann, Belleville, N. J., for the unlimited use of his machine shop.

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Disposal of Acid Fumes in Wet Assaying

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WORKERS in old or improvised laboratories are frequently confronted with the problem of disposal of acid fumes from wet-assay digestions. If the fume-laden gases are passed through zeolite sand covered with water, the fumes are effectively removed. The surface afforded by the grains of sodium aluminum silicate adsorbs and reacts with the fumes rapidly and completely and allows relatively large volumes of inert gas to be passed without the passage of the acid fumes. The fumes are removed so completely that any type of evacuating pump can be employed with the exhaust open to the laboratory.

The commercial grade of synthetic zeolite sand gradually dissolves and passes off with the condensed overflow. The absorbent is most economical.

The application of this procedure to micro-Kjeldahl digestion is illustrated in Figure 1.

Bottle *B* contains zeolite sand covered with water. *A* acts as a trap for sand grains from *B*. A spray trap, *C*, employing the indentations featured in the Vigreux type of fractionating head, drains well and efficiently breaks up the spray. The tip, *D*, drains the side arm and prevents the formation of fluid locks in the system, which would cause fumes to escape at the open connections. A trap, *C*, fits into the mouth of each digestion tube or flask and serves to prevent loss of sample in the spray and to connect the digestion vessel to the fume-absorbing system.

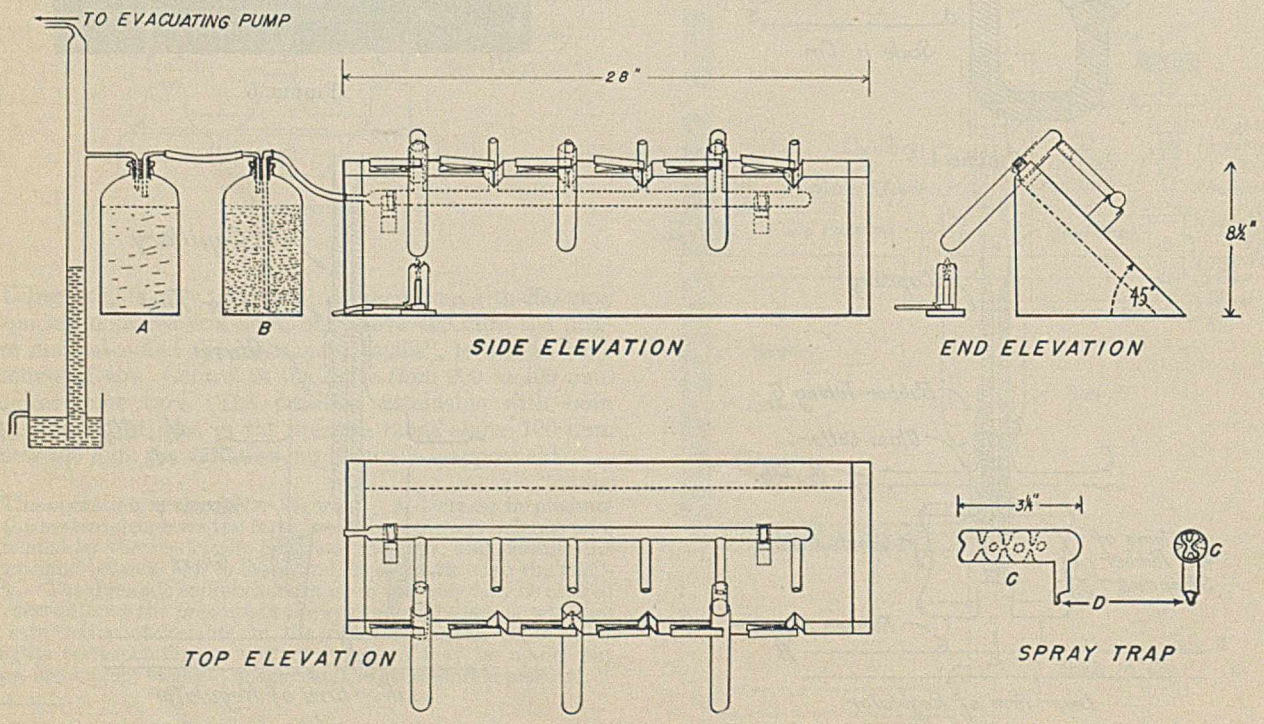

FIGURE 1. DIGESTION RACK FOR MICRO-KJELDAHL DETERMINATIONS

A Reaction Tube for Determination of 17-Ketosteroids

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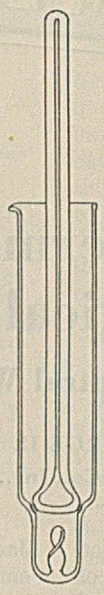
IN THE method of Holtorff and Koch (*2*) for the determination of 17-ketosteroids, the presence of more than a trace of carbonate in the reaction mixture results in turbidity in the final diluted alcoholic solution. Absorption of carbon dioxide during the reaction may be prevented by use of the tube shown in the accompanying figure, in which the concentrated alkaline solution is protected from contact with the air in the small space at the bottom of the tube below the long-handled stopper.



To avoid wetting the ground joint between the stopper and the shoulder of the tube, with resulting exposure of part of the solution, the 0.2-ml. portions of alcoholic extract, *m*-dinitrobenzene, and aqueous potassium hydroxide are delivered onto the top of a short glass rod fused into the bottom of the tube. The spiral form of this rod aids in rapid thorough mixing with the diluent later. When sufficient time has elapsed for completion of the reaction, 10 ml. of alcohol are pipetted into the tube above the stopper. From a series of tubes the stoppers may be removed in rapid succession, thus decreasing the interval between dilutions to more nearly that required in making the readings. The concentrated solution and the diluent alcohol are mixed by a rapid swirling motion, and are transferred to the Evelyn colorimeter tubes as required. By the use of this reaction tube, long contact of strongly alkaline solutions with the colorimeter tubes is avoided.

The small bottom part is of 15-mm. tubing (outside diameter) and has a depth below the ground joint of approximately 20 mm. The upper part is of 25-mm. tubing of any convenient length (an over-all length of 115 mm. was used). The stopper is easily made of 1-mm. capillary tubing which gives it sufficient weight to sink in bichromate–sulfuric acid cleaning solution. The stopper is ground in with a simultaneously lateral and rotary motion of the handle to produce spherical ground surfaces which fit in any position of the handle.

In this laboratory, the potassium hydroxide solution is stored in and delivered from a small mercury-sealed buret (*1*, Figure 3) having a delivery tip sufficiently long to reach to the top of the spiral glass rod. The tip of the Koch buret (*3*) is also long enough for this purpose. Many ordinary burets require an extension of the tip, preferably fused on.

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Sample Carrier for Organic Liquids

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IN THE ultimate analysis of organic liquids the common sample carriers are sealed bulblets or open porcelain boats, the sealed bulblets for use with low-boiling liquids and the open boats for use with high-boiling liquids.

A carrier device suggested for use with liquids of intermediate boiling temperatures does away at once with the time consumed when sealed bulblets are used and the loss by evaporation when open boats are used. Figure 1 shows the device suggested.

Using a piece of Pyrex tubing approximately 6 × 30 mm., the container is prepared as follows: One end is melted completely, blown slightly, and pressed firmly onto a flat, noncombustible surface. The stem is given a curvature and the open end is constricted by rotating in a flame until the opening is approximately 1 mm. in diameter.

The container is filled by means of an eye dropper with a capillary tube, and may be used over and over again. The flat bottom makes it possible to stand the object directly upon the balance pan.

For the actual combustion the container is put into a porcelain boat with the opening downward and toward the end of the boat nearer the catalyst (Figure 1, right). The curve in the neck keeps the liquid in the container until it is distilled out. Small pieces of glass in the container aid in the regular distillation of the sample into the boat. The speeds of distillation and combustion are thus controlled.

Just as some liquids do not volatilize completely when distilled from a bulblet carrier, so some liquids will leave a small carbonaceous deposit when distilled from the suggested

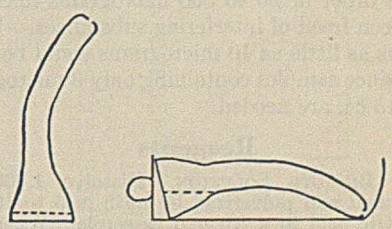

FIGURE 1

carrier. This difficulty may be partly overcome by decreasing the speed of distillation. However, if a small deposit does occur, it may be completely removed by heating the carrier to a dull red temperature for a few minutes while the oxygen flow is continued. Nevertheless this device will undoubtedly be limited, for practical use, to liquids which char very little or not at all when distilled.

The author has obtained very satisfactory results with liquids of intermediate boiling temperatures.

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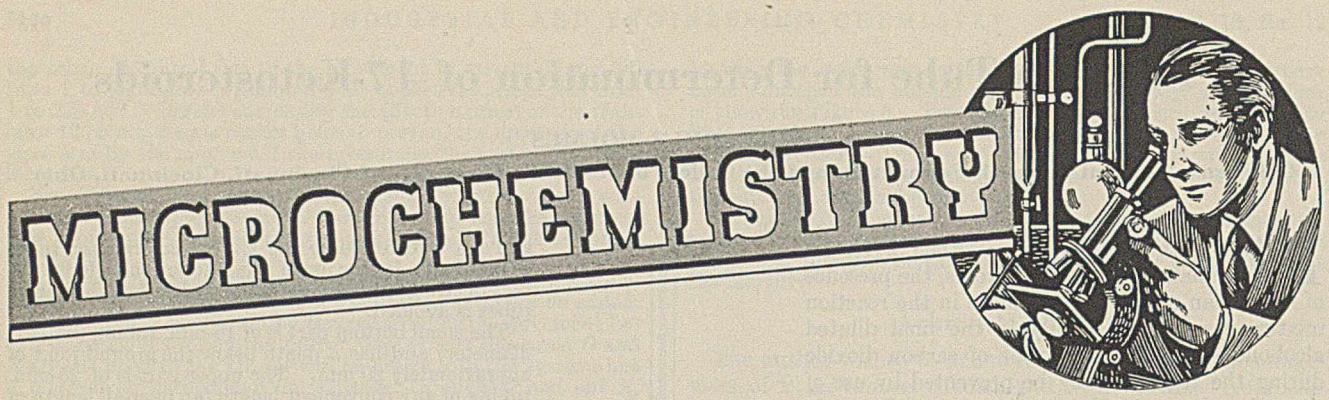

Iodobismuthite Determination of Bismuth in Biological Samples

Application of Neutral Wedge Photometer

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THE estimation of bismuth by the iodobismuthite method was originated by Thresh in 1880 (*6*). Application of this method to the determination of bismuth in biological samples was attempted by various investigators and their efforts were consummated in a method published by Leonard (*3*) in 1926. Several modifications (*1*, *2*) of the Leonard procedure have been employed since that time. A review of the several methods and their modifications has been compiled by von Oettingen (*4*).

In the early nephelometric methods it was necessary to employ small quantities of reagents and in most cases to employ the entire analytical sample in making a determination, so that any error due to faulty manipulation in the color developing stage could not be readily corrected. The precision of the determinations was limited by the visual interpolation of the operator.

The method which has been developed for use with a neutral wedge photometer overcomes these difficulties. This method is applicable to the estimation of quantities of bismuth of the order of 50 to 500 micrograms in any solution which has been freed of interfering substances. With proper modifications as little as 10 micrograms could be determined accurately, since samples containing only 3 micrograms of the element in 15 cc. are needed.

Reagents

STANDARD BISMUTH SOLUTION. Dissolve 1.0000 gram of freshly fractured and pulverized bismuth in 5 to 10 cc. of concentrated nitric acid in a covered casserole. When solution is complete dilute with 100 cc. of distilled water. Wash the cover glass thoroughly and add the washings to the solution in the casserole. Transfer the solution quantitatively to a 1-liter volumetric flask and dilute to the mark with distilled water. This solution contains 1.00 mg. of bismuth per cc.

SULFURIC ACID, 20 PER CENT. Slowly add 200 grams of concentrated sulfuric acid to 800 grams of distilled water.

POTASSIUM IODIDE, 5 PER CENT. Dissolve 50 grams of potassium iodide (U. S. P.) in 950 grams of distilled water.

HYPOPHOSPHOROUS ACID, 10 PER CENT. Dilute 200 grams of hypophosphorous acid (purified, 50 per cent) with 800 grams of distilled water.

Method

The yellow potassium iodobismuthite solution which constitutes the basis of the method is stated by Rasmussen, Jackerott, and Schou (*5*) to absorb light up to 4500 Å. They employed the mercury line at 4359 Å. in a photometric investigation of the method of Leonard.

To determine the most satisfactory wave band for use in the determinations with the neutral wedge photometer, absorption curves for solutions containing different concentrations of bismuth were made. The data appearing in Table I and plotted in Figure 1 show that concentrations of bismuth up to 300 micrograms per 50 cc. can be effectively determined, using a 100-mm. cell and a filter transmitting light of 460-millimicron wave length.

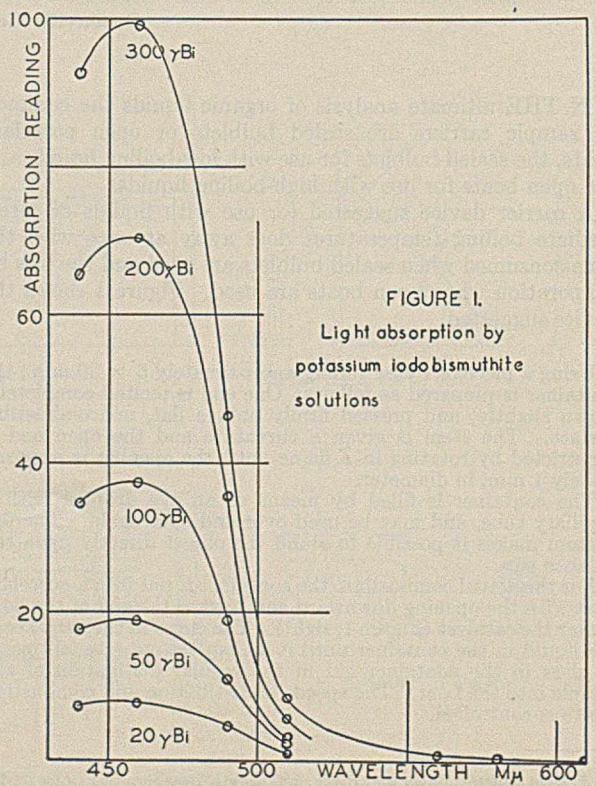


FIGURE I.
Light absorption by potassium iodobismuthite solutions

TABLE I. ABSORPTION DATA FOR POTASSIUM IODOBISMUTHITE SOLUTIONS[a]

Bismuth	Absorption Readings at Wave Length[b]: 440	460	490	510	560	580	610
Micrograms/ 50 cc.							
20	7.5	7.6	4.7	2.3	0.5	0.3	0.2
50	17.6	18.4	10.6	2.5	0.7	0.3	0.2
100	34.5	37.3	18.8	2.7	0.4	0.3	0.2
200	65.2	70.5	35.5	5.5	0.3	0.5	0.2
300	92.5	99.3	46.2	8.5	0.3	0.4	0.2

[a] Using 100-mm. absorption tube.
[b] Effective wave length in millimicrons of eyepiece filters supplied by American Instrument Co.

Adding 10 cc. of 5 per cent potassium iodide and 10 cc. of 10 per cent hypophosphorous acid to a sulfuric acid solution of bismuth and making up to 50-cc. volume with water provide a satisfactory medium for conducting the estimation. Since the instrument cell has a volume of about 15 cc., not all of this solution is needed; part of it provides a rinse for the cell before making the reading. These solutions may be prepared in any 50-cc. volumetric container.

The data in Table II, which have been plotted in Figure 2, present the evidence for the choice of the above components of the solution. Potassium sulfate and ammonium sulfate

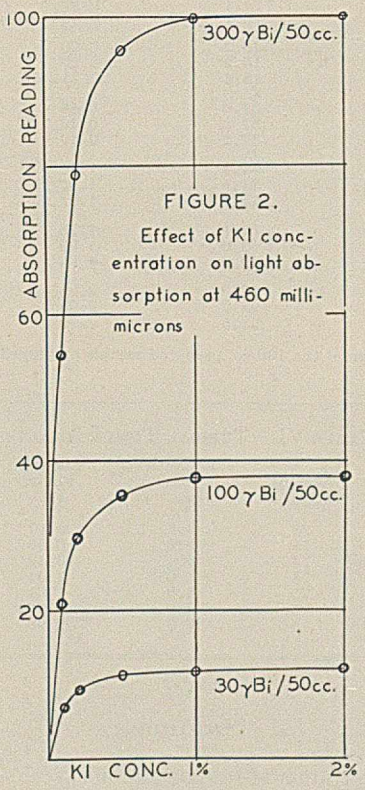


FIGURE 2. Effect of KI concentration on light absorption at 460 millimicrons

have been included in the experiment because they are usually present in samples prepared by the digestion of organic materials. Inspection of the data shows that only the potassium iodide affects the color intensity to any appreciable extent. Therefore it is the only reagent included in Figure 2.

The choice of hypophosphorous acid was made after the sulfites and sulfurous acid which are usually employed as reducing agents were found to contain an impurity that developed an interfering yellow color with potassium iodide in acid solution. Samples from different manufacturers showed the presence of the same impurity. Sulfurous acid prepared from sodium thiosulfate crystals also produced an interfering color. It was found that both photographic glycin (*p*-hydroxyphenyl aminoacetic acid) and hypophosphorous acid were free of such impurities. Glycin was abandoned because it develops an interfering color in the presence of ferric iron. Hypophosphorous acid proved suitable in every respect.

The calibration curve for the instrument was made by determining the absorption at 460 millimicrons with known quantities of bismuth. A blank solution of the reagents was used to establish the zero point of the instrument. These data are shown in Table III and Figure 3. The slight deviation from Beer's law apparent in the curves is probably due to the potassium iodide–bismuth ratio in the solution. The deviation is not of sufficient magnitude to warrant an increase in the potassium iodide content in the solution from which color absorption readings are to be made.

Estimation of Bismuth in Urine

For the estimation of bismuth in biological samples the organic matter is first destroyed by wet-digestion of the sample in a sulfuric acid mixture. The ultimate product is a solution of bismuth sulfate in 100 cc. of dilute sulfuric acid. Aliquot portions of this solution are taken for the preparation of the iodobismuthite solution from which the final estimation is made.

The presence of small quantities of elements other than bismuth in the prepared urine samples causes considerable

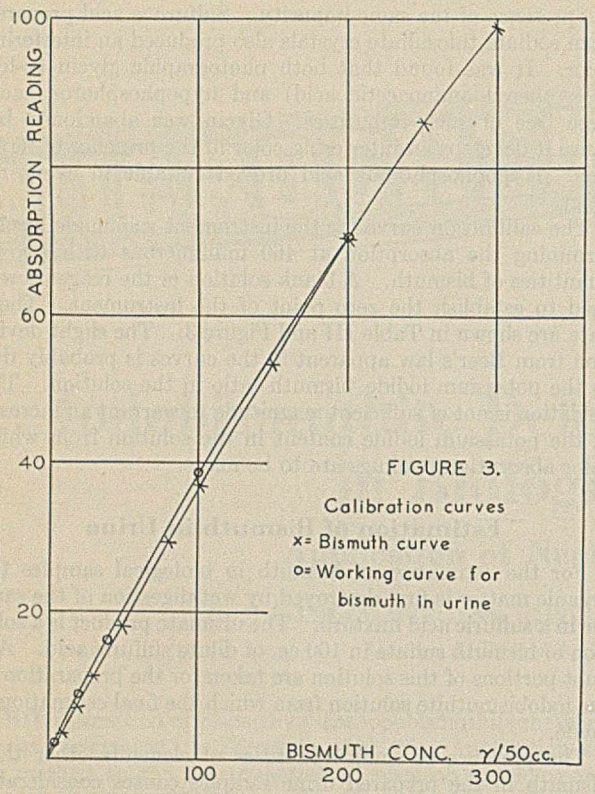
TABLE II. EFFECT OF SOLUTION COMPONENTS ON DEVELOPMENT OF POTASSIUM IODOBISMUTHITE COLOR

Bi Added	20% H_2SO_4	5% KI	10% H_3PO_2	10% K_2SO_4	10% $(NH_4)_2SO_4$	Absorption Reading for 100-Mm. Tube at 460 Millimicrons
Micrograms	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	
300	10	1	10	..	..	54.2
300	10	2	10	..	..	78.6
300	10	5	10	..	..	95.5
300	10	10	10	..	..	99.9
300	10	20	10	..	..	100.0
100	10	1	10	..	..	20.7
100	10	2	10	..	..	29.6
100	10	5	10	..	..	35.2
100	10	10	10	..	..	37.7
100	10	20	10	..	..	37.7
30	10	1	10	..	..	6.7
30	10	2	10	..	..	9.4
30	10	5	10	..	..	11.3
30	10	10	10	..	..	11.9
30	10	20	10	..	..	12.0
100	2	10	10	..	..	36.4
100	20	10	10	..	..	38.5
100	10	10	10	..	..	39.6
100	10	10	5	..	..	38.3
100	10	10	20	..	..	37.5
100	10	10	10	5	..	38.7
100	10	10	10	20	..	38.8
100	10	10	10	..	5	38.2
100	10	10	10	..	20	38.8

TABLE III. BISMUTH CALIBRATION CURVE

(100-mm. absorption cell with 460-millimicron filter. Solutions made to contain in 50 cc., 10 cc. of 20% H_2SO_4, 10 cc. of 5% KI, and 10 cc. of 10% H_3PO_2)

Bismuth Added	Absorption Reading
Micrograms	
10	3.6
20	7.2
30	11.4
50	17.9
80	29.2
100	36.9
150	53.4
200	70.1
250	86.0
300	98.9



error when determinations are made in the lower range. This is due to the deviation of the curve from the bismuth calibration curve (Figure 3). However, by establishing a working curve for urine samples using samples containing known amounts of bismuth (Table IV, Figure 3) determinations can be made with less than 5 per cent error in the range of 20 to 3000 micrograms of bismuth per sample (Table V). Operating similarly, the method may be employed for tissue analysis. Some selected results are shown in Table VI.

Procedure

Place 100 cc. of urine in a 500-cc. Kjeldahl flask with 10 cc. of concentrated sulfuric acid, 10 grams of potassium sulfate, and 25 cc. of concentrated nitric acid. Add two glass beads and concentrate the urine to a small volume by rapid boiling. Digestion of the organic matter follows and proceeds smoothly. When the solution is clear and usually colorless (about 1 hour) allow it to cool and add 40 to 50 cc. water. Filter the solution into a 100-cc. volumetric flask, wash the Kjeldahl flask and filter with successive small portions of water, and add the washings to the main portion of the filtrate. Finally adjust the volume to the mark with water and mix well. This constitutes the prepared sample from which the bismuth determination is made.

Table IV. Calibration Curve for Bismuth in Urine

(100-mm. absorption cell with 460-millimicron filter. Solutions made to contain in 50 cc. 10 cc. of 5% KI, 10 cc. of 10% H_3PO_2, and stated quantity of 100-cc. prepared samples. 100 cc. of urine digested)

Bismuth Added	Aliquot	Bismuth in Aliquot	Absorption Reading				Deviation from Mean
			I	II	III	Av.	
Mg.	*Cc.*	*Micrograms*					%
0.02	20	4	..	2.0	2.4	2.2	10
0.10	20	20	8.9	8.8	8.3	8.7	5
0.2	20	40	15.9	16.6	15.5	16.0	3
0.5	20	100	38.8	38.5	38.5	38.6	1
1.0	20	200	70.5	70.3	70.4	70.4	0.2
2.0	10	200	70.2	70.3	69.8	70.1	0.4

Pipet an aliquot portion (20 cc.) of the prepared sample into a 50-cc. volumetric flask and add 10 cc. of 5 per cent potassium iodide from a delivery pipet. Mix the two by shaking. To this add 10 cc. of 10 per cent hypophosphorous acid and sufficient distilled water to make up the volume. Mix the sample and rinse the absorption cell with a portion of it. Fill the cell with the solution and wipe the polished ends with a clean dry cloth before making the absorption reading. The micrograms of bismuth corresponding to the absorption readings are read off from the working curve and the bismuth content of the prepared sample is calculated.

The above sequence of reagent addition must be followed because potassium iodide is oxidized to iodine by ferric iron and other oxidizing impurities. This is capable of reduction by hypophosphorous acid, whereas the original oxidizing agents are not. This procedure yields a solution which does not change in color for several hours.

Table V. Determination of Bismuth in Urine

Bismuth Added to 100 Cc. of Urine	Absorption Reading	Bismuth Found	Error
Mg.		*Mg.*	%
0.02	2.0	0.02	0
	2.0	0.02	0
	2.3	0.02	0
0.10	8.5	0.10	0
	8.1	0.095	5
	8.1	0.095	5
0.20	16.2	0.20	0
	16.2	0.20	0
	16.4	0.21	5
0.50	37.0	0.48	4
	37.3	0.49	2
	37.2	0.49	2
1.0	71.3	1.01	1
	71.5	1.01	1
	71.4	1.01	1
2.0[a]	69.8	1.98	1
	69.0	1.95	3
	69.3	1.96	2
3.0[a]	98.0	2.98	0
	98.2	2.98	0
	98.5	2.99	0

[a] 10-cc. portion of the 100-cc. prepared sample employed instead of usual 20-cc. portion.

Table VI. Typical Tissue Analyses

Tissue	Weight	Bismuth Added	Bismuth Found	Error
	Grams	*Mg.*	*Mg.*	%
Liver	75	0.1	0.10	0
Muscle	75	0.5	0.50	0
	75	0.5	0.49	2
	75	1.0	1.01	1
	75	1.0	1.01	1
	75	4.0	3.96	1
	75	4.0	3.96	1

Summary

The concentration of bismuth in biological samples by the iodobismuthite method may be rapidly and accurately determined with the neutral wedge photometer. The method is not recommended when less than 20 micrograms of the element are available in the analytical sample. For analyses of various materials, working curves may be prepared and employed, as in the example for bismuth in urine.

The major factor in analyses by this method is the concentration of potassium iodide. This factor has not been previously considered of critical importance and different investigators have employed varying concentrations of this reagent in developing the iodobismuthite color. The effect of small variations in the volume of 5 per cent potassium iodide solu-

tion is shown in Figure 2. In determining bismuth in the range 20 to 3000 micrograms, the concentration of potassium iodide should be at least 1 per cent. The Leonard method employs about 0.3 per cent potassium iodide. In this range of potassium iodide concentration a small error in measurement of the potassium iodide could result in an appreciable error in the bismuth content. The technique here described, if followed precisely, will result in the minimum error due to measurement of reagents.

This method has been employed routinely in the investigation of the bismuth content of biological samples in conjunction with studies on the absorption and excretion of bismuth by the animal organism originating in these laboratories (*7*).

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PRESENTED before the Division of Analytical and Micro Chemistry at the 101st Meeting of the American Chemical Society, St. Louis, Mo.

Determination of Arsenic in Biological Material

A Photometric Method

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STUDIES necessitating the accurate quantitative determination of arsenic in biological material made apparent the need for a rapid and flexible method of analysis by which large numbers of samples, containing arsenic in amounts ranging from less than a microgram to several milligrams, could be handled simultaneously. Recent literature describes a number of methods (*1*, *2*, *4*, *5*, *6*) of acceptable accuracy but none of them filled the author's needs completely. Combination of the better features of these methods plus some slight modifications resulted in the development of a procedure which met all requirements. This modified method consists essentially of the wet-ashing procedure suggested by Kahane and Pourtoy (*4*) and later used by Morris and Calvery (*6*), the evolution of arsenic trichloride from the prepared sample in the manner described by Scherrer (*8*) and Rodden (*7*), and a final photometric measurement similar to that described by Morris and Calvery (*6*).

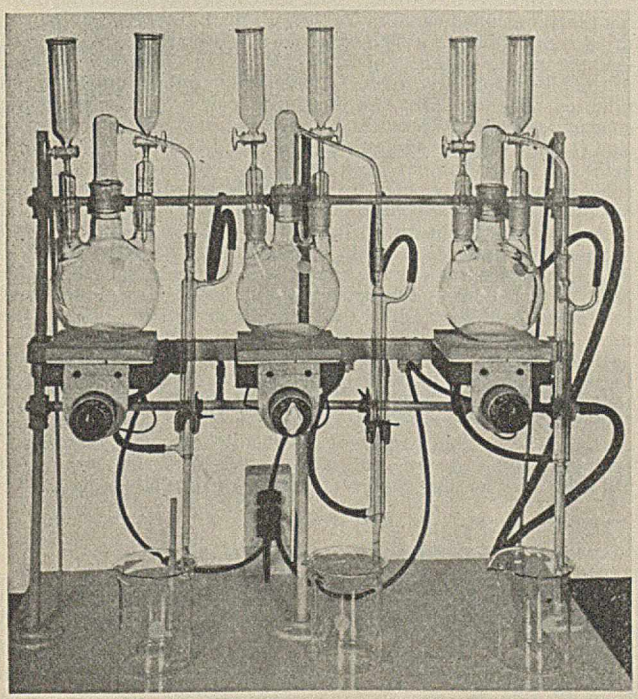

FIGURE 1. WET-ASHING APPARATUS

Reagents and Apparatus

High-grade chemicals are used throughout the analysis to ensure a low arsenic blank. Grasselli C. P. reagent nitric, sulfuric, and hydrochloric, and Mallinckrodt analytical reagent perchloric and hydrobromic acids and ammonium molybdate have been found suitable.

Before use all glassware (Pyrex) is washed thoroughly with hot dilute nitric acid (50 ml. of nitric acid, specific gravity 1.42, per 100 ml.) and rinsed with distilled water to remove arsenic present as surface contamination from previous use.

The photoelectric spectrophotometer, previously described (*3*), is employed for density measurements. Readings are taken with the monochromator set at 620 mμ. Aminco, Style D, class 3, high-precision matched cells, holding approximately 8 ml. and with an internal length of 100 mm., are used.

Samples are wet-ashed in 1-liter distilling flasks provided with three necks carrying ⅘ interchangeable ground-glass connections, into which are fitted the distilling head and two separatory funnels, as described by Morris and Calvery (*6*). Heat is furnished by electric heaters connected with Variacs for voltage control and subsequent temperature regulation. The essential portions of the complete setup are illustrated in Figure 1, which also shows the stand holding a battery of three flasks.

The apparatus for isolation of arsenic from the ashed sample is shown in Figure 2. Two units were found to be sufficient. The device is similar to that described by Scherrer (*8*) and Rodden (*7*), but has been modified by the introduction of three ⅘ interchangeable ground-glass connections which greatly facilitate charging and cleaning the apparatus. The thermometer used (range 0° to 250° C.) has a 7.5-cm. (3-inch) stem below the ⅘ joint which fits into a female connection in the side of the flask. No trouble has been encountered with sticking joints, especially if the apparatus is dismantled while warm. The distilling head is connected with a downward water-cooled Allihn condenser, the lower end of which dips below the surface of the distilled water contained in a tall cylinder surrounded by an ice-water bath. A Cenco adjustable electric heater is used as the source of heat.

Procedure

ASHING THE SAMPLE. Place the sample (100 ml. or less of urine, or 10 grams or less of other biological material such as blood or tissue) in the 1-liter distillation flask (Figure 1). Add 20

ml. of concentrated sulfuric acid (specific gravity 1.84), 20 ml. of concentrated nitric acid (specific gravity 1.42), and several pieces of Carborundum, and mix well. Then insert the flask with contents, connect with the apparatus, and gently heat. One separatory funnel contains 5 ml. of perchloric acid (70 to 72 per cent) and the other, 30 ml. of concentrated nitric acid (specific gravity 1.42). Raise the temperature by Variac adjustment just to boiling and start the addition of the nitric acid drop by drop, catching the distillate in an open beaker. When approximately 15 ml. have been added, the major portion of the organic matter will have been oxidized, and the color of the solution will have become lighter; at this point, add the perchloric acid, drop by drop, until the entire 5 ml. have been introduced; meanwhile continue to add the remainder of the nitric acid, reserving 1 ml. in the funnel. Continue heating until dense white fumes of perchloric acid begin to appear. If charring occurs at this point, dispel by drop-by-drop addition of the remaining 1 ml. of nitric acid. In any case, add the remainder of the nitric acid and continue heating for 30 minutes after the initial formation of copious fumes of sulfur trioxide. (Excessive charring should be avoided by the immediate addition of nitric acid when charring occurs. Excess perchloric acid in the presence of sulfuric acid as used here has not been found dangerous.) Allow the distillation flask with contents to cool, disconnect, and transfer the entire contents to a 50-ml. glass-stoppered cylinder, rinsing several times with small portions of distilled water. Mix well and when cool dilute to the mark at 20° C.

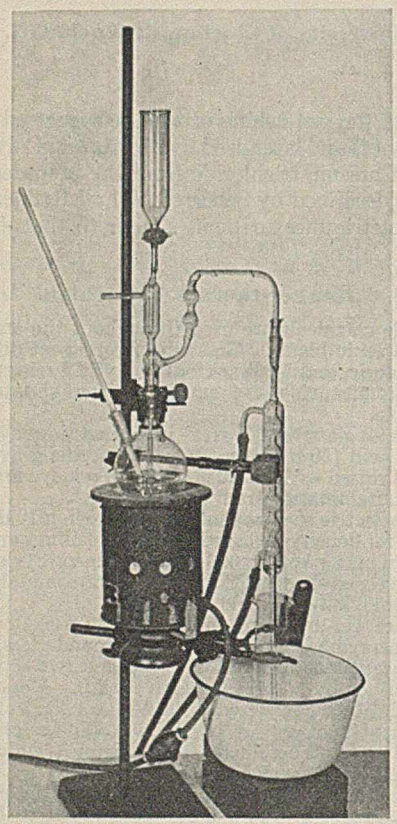

FIGURE 2. DISTILLATION APPARATUS

ISOLATION OF ARSENIC AS TRICHLORIDE. Transfer one half of the prepared sample (25 ml.) by means of the separatory funnel to the distillation flask (200-ml. capacity) used for volatilization (Figure 2). In the same manner add 5 ml. of hydrobromic acid (40 per cent) and 40 ml. of concentrated hydrochloric acid (specific gravity 1.19). Mix 1 gram of hydrazine sulfate with 30 ml. of concentrated hydrochloric acid (specific gravity 1.19), crushing lumps with the flattened end of a glass stirring rod, and add this also through the separatory funnel, sealing the stopcock with a few milliliters of distilled water. Connect a carbon dioxide cylinder to the side opening and allow gas to bubble through the liquid in the distillation flask at a rate of approximately 4 bubbles per second. Heat by means of the Cenco adjustable heater and allow the temperature to rise gradually to 111° C., catching the distillate in 40 ml. of distilled water in a tall glass cylinder immersed in ice water (about 20 ml. of distillate come over during a heating period of approximately 35 minutes). Transfer the distillate plus a few milliliters of distilled water used to rinse down the condenser walls to a 100-ml. flask containing 25 ml. of concentrated nitric acid (specific gravity 1.42) and make up the volume to the mark with distilled water. Transfer all or a suitable aliquot to a 100-ml. beaker, cover with a Fisher Speedyvap beaker cover, and evaporate to dryness. Remove beaker cover and heat the beaker with contents in an electric oven at approximately 120° C. for one hour to remove all trace of oxidizing agents.

TABLE I. ARSENIC RANGE, VOLUMES OF DEVELOPING SOLUTION, AND FINAL DILUTIONS OF DEVELOPED COLOR

Range *Micrograms*	Volume Used *Ml.*	Final Dilution *Ml.*
0– 10	5	10
0– 50	25	50
0–100	50	100

TABLE II. RECOVERY OF KNOWN AMOUNTS OF ARSENIC ADDED TO URINE

(50-ml. samples)

Range Used *Micrograms*	Arsenic Added *Micrograms*	Arsenic Found *Micrograms*	Arsenic Recovered *Micrograms*
0– 10	Nil	2.0[a]	....
0– 10	Nil	2.1[a]	....
0– 10	Nil	3.9	....
0– 10	Nil	3.9	....
0– 10	1.0	4.9	1.0
0– 10	1.0	5.1	1.2
0– 10	5.0	9.0	5.1
0– 10	5.0	9.1	5.2
0– 50	10.0	14.0	10.0
0– 50	10.0	14.5	10.5
0– 50	15.0	19.5	15.5
0– 50	15.0	19.0	15.0
0– 50	25.0	29.0	25.0
0– 50	25.0	28.5	24.5
0– 50	50.0	53.5	49.5
0– 50	50.0	53.5	49.5
0–100	75.0	79	75
0–100	75.0	78	74
0–100	100.0	103	99
0–100	100.0	104	100
0–100	2000.0	1980	1980
0–100	2000.0	1980	1980

[a] Calculated as a reagent blank, 50 ml. of distilled water being substituted for 50 ml. of urine.

DEVELOPMENT OF COLOR. Add the correct amount of developing solution, as indicated in Table I, to the beaker containing the dried residue; cover with a cover glass and place in a water bath (temperature maintained at 70° to 75° C.), until the development of the blue color is complete—generally 30 minutes suffice but 45 minutes will not harm the developed color. [The developing solution contains 10 per cent by volume of acid molybdate solution (1 gram of ammonium molybdate per 100 ml. of 5 *N* sulfuric acid) not more than one week old, 89 per cent by volume of distilled water, and 1 per cent by volume of a freshly prepared aqueous hydrazine sulfate solution (0.15 gram per 100 ml.), added and mixed together in the order given. The solution should be mixed in necessary amounts as used.] When the color has developed to its full intensity, remove the beaker with contents from the water bath, allow to cool, and transfer quantitatively to the proper-sized graduated cylinder (Table I), mixing well and diluting to the mark at 20° C.

PHOTOMETRIC MEASUREMENT OF COLOR DENSITY. Density measurements may be made immediately or 24 hours later. The same pair of cells is used for all ranges, one containing the solvent (distilled water), and the other the solution. The readings are evaluated from working curves obtained with known amounts of arsenic (as an aqueous solution of arsenic pentoxide) treated in the same way as the evaporated and dried distillate residues.

Analytical Results

In Table II are listed results obtained by the analysis (in duplicate) of 50-ml. samples of urine containing known added amounts of arsenic, and in Table III, results from 5-gram samples of whole blood. The first two results in the "Arsenic Found" column in each table represent the actual reagent blanks obtained by substituting equivalent amounts of dis-

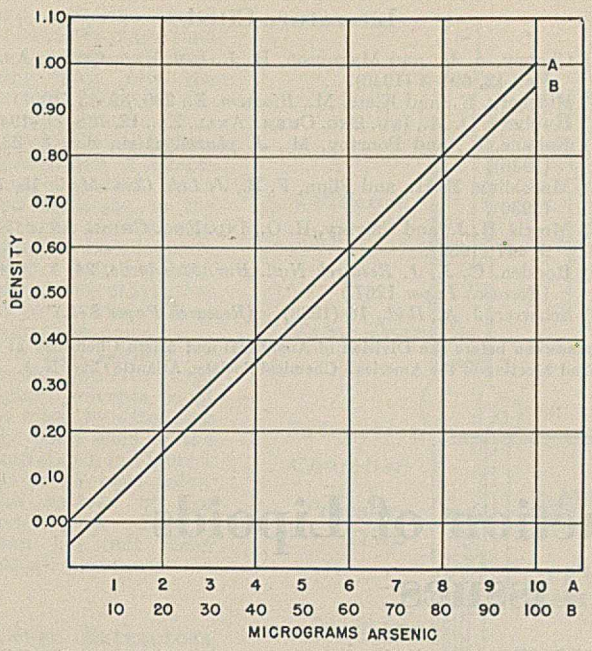

FIGURE 3. ARSENIC CURVE

tilled water for 50 ml. of urine and for 5 grams of blood, respectively.

Discussion

The size of the aliquots of prepared samples chosen for distillation and color development is dictated by experience. In most cases one half of the prepared sample may be used if the quantity of arsenic present in the aliquot is not greater than 1 mg., the maximum quantity ordinarily distilled under the conditions outlined above. Aliquots of the distillates also may be used for color development, but the amount of arsenic in the aliquot should not exceed 100 micrograms. If more than this amount is present, the color development should be repeated with a smaller aliquot of the distillate; if this is not possible, the distillation step must be repeated with a smaller portion of ashed sample. In the latter event, care must be taken to see that 10 ml. of sulfuric acid (specific gravity 1.84) are present during the distillation. In the case of certain materials, particularly blood and spinal fluid, usually low in arsenic, and when only very small samples are available, the entire sample is used for the analysis.

The technique of wet-ashing employed reduces to a minimum the amount of arsenic lost by entrainment in the distillate, in confirmation of Morris and Calvery's (*6*) work. A definite excess of nitric and perchloric acids is maintained at all times during the destruction of organic matter, and entrainment losses are negligible as compared with the sensitivity of the final photometric measurement. A separate analysis of the distillate from the wet-ashing of 100 ml. of urine containing 4000 micrograms of arsenic showed a loss of only 12 micrograms or 0.3 per cent of the original content.

The blue color produced in the conversion of arsenic acid to an arsenic-molybdenum compound by treatment with a solution containing hydrazine sulfate and ammonium molybdate has been found to be very stable; density measurements have remained constant over a period of 24 hours, obeying Beer's law; this confirms the findings of Rodden (*7*). The temperature of the water bath used in color development may range from 70° to 75° C. Constant temperature has proved to be unnecessary. The distillation step separates the arsenic from silica and phosphorus, which also give blue-colored molybdenum compounds, while other volatile chlorides of antimony, selenium, and germanium do not interfere in the subsequent color development.

It is essential to run a reagent blank for each lot of reagents used, because of their variability in arsenic content (Tables II and III). Dust and other particulate matter such as cigaret ashes contain small amounts of arsenic; therefore it is necesary to guard carefully against dust contamination, particularly when dealing with small quantities of arsenic.

The use of battery-type equipment with adjustable electrical heating facilities makes possible the uniform, rapid, and convenient handling of a large number of samples daily in a small working space. What is more, after the digestion process has started, it requires little attention. The use of interchangeable joints at strategic places in the distillation apparatus also makes the operation more convenient, particularly in respect to rapid cleaning. The accuracy of recovery by this method is superior to that of the method described by Chaney and Magnuson (*1*), and equal to that obtained by Morris and Calvery (*6*), but in the author's hands, at least, the procedure seems to be more rapid and the substitution of the distillation step for the arsine evolution and combustion technique makes for simplicity as well as compactness of apparatus. A further advantage lies in the fact that a single pure color is employed, instead of the "mixed color" obtained by Chaney and Magnuson (*1*).

The method has been found applicable to a wide variety of biological materials and has been especially useful in the analysis of the normal daily fecal and urinary excretions of rabbits, which entails an accurate determination of arsenic in amounts of less than 10 micrograms, and in the analysis of small samples of blood and spinal fluid. Several modifications would aid in accelerating the analyses in certain cases—for example, when extremely large amounts of arsenic are present, the distillate may be titrated directly by Scherrer's method (*8*). Again, the possibility of determining arsenic polarographically is being investigated in this laboratory, and the preliminary work promises a sensitivity comparable to that of the method here reported, with the advantage of increased simplicity. It is hoped that the data on this development will be available shortly.

TABLE III. RECOVERY OF KNOWN AMOUNTS OF ARSENIC ADDED TO WHOLE BLOOD

(5-gram samples)

Range Used *Micrograms*	Arsenic Added *Micrograms*	Arsenic Found *Micrograms*	Arsenic Recovered *Micrograms*
0– 10	Nil	0.5[a]	...
0– 10	Nil	0.7[a]	...
0– 10	Nil	0.6	...
0– 10	Nil	0.7	...
0– 10	1.0	1.7	1.1
0– 10	1.0	1.5	0.9
0– 10	5.0	5.5	4.9
0– 10	5.0	5.7	5.1
0– 50	15.0	16.0	15.5
0– 50	15.0	15.5	15.0
0– 50	50.0	50.5	50.0
0– 50	50.0	50.0	49.5
0–100	100.0	100	100
0–100	100.0	99	99

[a] Calculated as reagent blank, 5 grams of distilled water being substituted for 5 grams of whole blood.

Summary

A photometric method has been applied to the microdetermination of arsenic in small samples of biological material—for example, 100 ml. or less of urine and 10 grams or less of blood or other tissue.

The sample is prepared for analysis by the wet-ashing method, employing perchloric, nitric, and sulfuric acids. The arsenic contained in the ashed sample is isolated completely

from other interfering constituents, such as silica and phosphorus, by distillation as the trichloride, in a distillation apparatus designed to ensure ease of cleaning and charging. The arsenic is oxidized to arsenic acid and the density of the blue color obtained by formation of the stable arsenic-molybdenum compound is measured photometrically.

Three ranges are used for the quantitative estimation of the arsenic—namely, 0 to 10, 0 to 50, and 0 to 100 micrograms—the accuracies for the above ranges being, respectively, ±0.1, ±0.5, and ±1.0 micrograms. A reagent blank which varies from 0.5 to 2.0 micrograms is run simultaneously with each set of analyses and it is determined with an accuracy of ±0.1 microgram, thus securing an accuracy of ±0.2 microgram for the lowest range.

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Apparatus for Extraction of Lipoids from Wet Tissues

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THE small apparatus shown in Figure 1 has been used in this laboratory since 1932 for the extraction of lipoids from blood in the determination of cholesterol. The method used has been published only in the abstract (*8*) without a sketch of the apparatus. The essential modification in this extractor from previous ones of similar type, such as those of Leiboff (*12*, *13*), Ling (*14*), and numerous others, is the trap fixed to the reflux condenser at its lower end. Moisture from the sample distills with the solvent and is retained in the trap. A notable improvement in consistency of results has been obtained, and there are reasons for concluding that direct extraction with chloroform under these conditions results in complete, or nearly complete, removal of cholesterol from the sample. However, although most of the author's work has been done with chloroform as the solvent, this extractor, and the larger ones shown in Figures 2 and 3, are adaptable to use with other solvents.

Microextractor

The apparatus (Figure 1) consists of an extraction tube having a bulb at the bottom calibrated to contain 5 ml. of extract and four lugs to support the sample, and a separate one-piece assembly of condenser and trap which fits into the top part of the tube. The body of the tube above the constriction is of 24- to 25-mm. tubing and has an approximate length of 230 mm. The four lugs, shown laterally and in section, are approximately 50 mm. above the top of the constriction. The constricted part is of 11-mm. tubing and has a length of 13 to 15 mm., these dimensions having been found best to give sufficient accuracy in making up to volume and still to permit rapid and thorough mixing of the extract with the reagents in the determination of cholesterol. The condenser has a long skirt which serves to hold it and the trap in the center of the extraction tube and also to deflect any moisture from the air down the outside of the apparatus. The extraction tubes and condenser skirts are kept within close enough tolerances so that a large number of tubes and several condensers are interchangeable. The outer shell of the trap and the part of the condenser hanging in the extraction tube are of 16-mm. tubing. The inner tube of the trap is of 11-mm. tubing extending at least 12 mm. below the ring seal joining it to the shell, and the shell extends like a collar at least 12 mm. above the seal. The openings through the supporting member connecting the trap to the condenser have a diameter of not less than 5 mm. The holes to the outside through the shell below the ring seal have approximately 3 mm. diameter. The bottom of the trap should be between 15 and 30 mm. above the lugs.

For operation, 5 to 6 ml. of solvent are placed in the bulb, a piece of fat-free filter paper 20 × 20 × 1 mm. with its corners bent at right angles is dropped "ears up" onto the lugs, 0.25 ml. of the specimen (serum, heparin plasma, whole blood, "saline" suspension of cells, or other fluid) is delivered onto the paper from a graduated 1-ml. pipet or from a special pipet (*7*), and the extraction tube with the condenser in place is immersed to the level of the sample in a boiling water bath. (A tall-form 400-ml. beaker makes a satisfactory bath for two tubes.) The extraction is continued for 1 hour.

Vapor of the boiling solvent passes around and bathes the sample, carrying moisture from the sample to the condenser. The condensate flows into the trap through the openings in the supporting member. Solvent and water separate in the inner tube of the trap, the water being retained and the solvent flowing around the bottom of the inner tube

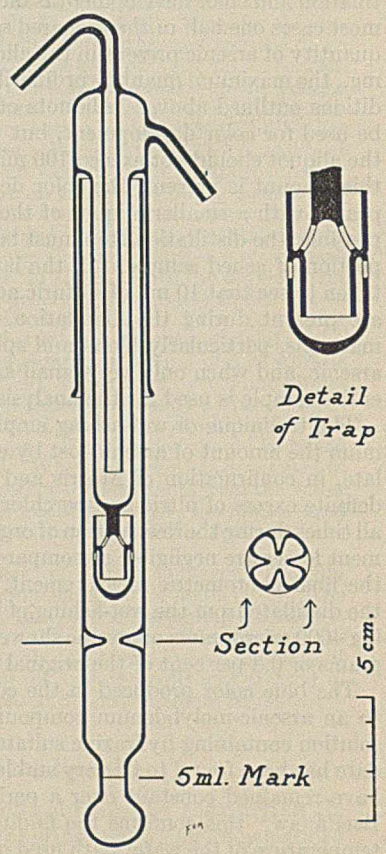

FIGURE 1. MICROEXTRACTOR FOR EXTRACTION OF LIPOIDS FROM BLOOD
Condenser and trap in position in tube

and out the small holes in the outer shell of the trap and dripping back onto the sample (heavy solvents).

For solvents lighter than water, a simple cup supported by a rod extending to the bottom of the cup seems to be adequate. Such a trap has been tested with ethyl ether in two or three determinations.

A trap suitable for both heavier and lighter solvents could be made by extending the outer shell of the trap shown in Figure 1 about 15 mm. below the bottom of the inner tube. Such a trap has not been tested.

Large Extractors with Water Traps

The performance of the microextractor (discussed below) suggested the use of similar apparatus for extractions on a larger scale.

In 1940, Kaye and co-workers (*9*) described an apparatus for drying and extracting specimens in a practically continuous operation without transfer from the one apparatus. The water trap in Kaye's apparatus could easily have been redesigned to retain water above a heavier solvent. Instead, the entire apparatus was redesigned to accomplish several objectives: (1) to eliminate the large ground joints at the top of the extraction chamber and below the reflux condenser, (2) to eliminate fragile outside tubes, and (3) to make the parts of the apparatus coaxial in order to save space and to eliminate the extra water bath. Whether or not these objectives are also advantages depends upon the personal preference of the person using the apparatus.

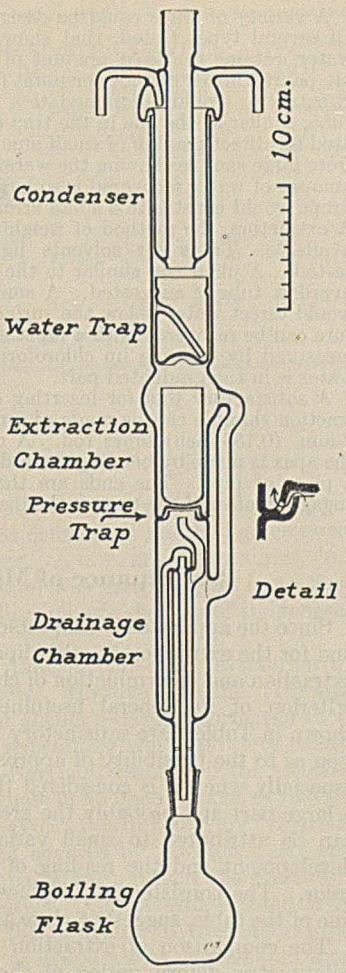

FIGURE 2. TYPE A LARGE EXTRACTOR FOR USE WITH THIMBLE

Left half of pressure trap in detail at right; arrow shows path of vapor

The first extractor tested is shown in Figure 2. This was again redesigned with the object of forcing vapor through the sample to produce vigorous agitation of the sample in the solvent, and the extractor shown in Figure 3 was built.

These two extractors have been tested only to the point of performing extractions in them. It was intended to compare extracts of wet material with those of dried material in respect to total lipoid extracted, volatile constituents, and possibly the changes in labile constituents such as unsaturated fatty acids, vitamins, etc., but this work has been indefinitely postponed. It seems advisable to make the apparatus available now, rather than to wait until work can be resumed.

LARGE EXTRACTOR FOR USE WITH A THIMBLE. In the extractor shown in Figure 2, Type A, solvent vapor from the boiling flask passes upward through the large center tube (containing the siphon) into the drainage chamber, through the vapor tube (side tube, not labeled) to the extraction chamber, and upward to the condenser. The reflux fills the water trap and flows down the walls of the extraction chamber and back into the drainage chamber. When the drainage chamber becomes full enough to cover the lower end of the vapor tube, solvent is forced up into this tube, and the pressure of the vapor is increased, forcing it through the small amount of reflux collected in the pressure trap. The purpose of the pressure trap is to prevent drainage of all but a small portion of the liquid solvent from the extraction chamber. Small increments of refluxed solvent draining into the lower chamber raise the level in the vapor tube and hence the pressure of the vapor below the trap, so that the vapor can support, and is forced through, an increasing depth of liquid in the extraction chamber.

When solvent in the extraction chamber reaches sufficient depth to fill the thimble to within about 8 mm. of the top edge, it begins to splash over into the vapor tube. The bulblike expansion in the top of the extraction chamber allows this splash to be directed outward toward the wall instead of into the top of the thimble. Continued increase of solvent in the extraction chamber results in overflow into the vapor tube, and this excess flows into the drainage chamber until it overflows through the siphon. Drainage of solvent from the thimble can then continue during a considerable period of time until the level of liquid in the lower chamber again reaches the bottom of the vapor tube.

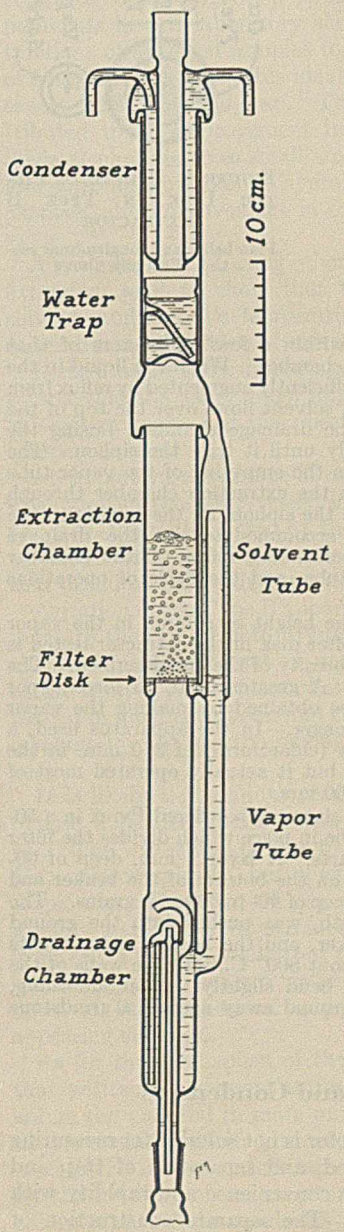

FIGURE 3. TYPE B LARGE EXTRACTOR PRODUCING AGITATION OF SAMPLE

The pressure of the vapor against the liquid in the drainage chamber is practically equal to that exerted through the siphon from the boiling flask, and consequently the siphon operates like an ordinary siphon at atmospheric pressure. It is essential that the top of the siphon be correctly designed to prevent dripping and failure to trip. The design shown here has worked well. The arm of the siphon in the drainage chamber should have a slightly larger inside diameter than that of the arm descending into the boiling flask (inside the large center tube), and should be of a size that will not entirely damp the small surges of liquid which aid in tripping the siphon. Inside diameters of 4 and 3 mm., respectively, were found to be satisfactory. To prevent its tripping too soon, the top of the descending limb of the siphon should be between 15 and 20 mm. above the level of the top of the opening into the vapor tube.

The extractor may be built in two parts, the upper diaphragm of the trap being formed on the lower end of the extraction chamber and the lower half of the trap on the top of the drainage chamber. The upper and lower halves of the vapor tube may then be joined, and while the glass is still soft, the halves of the

trap may be brought together and fused, a fine glass rod being used to encircle the junction, filling any cracks and forming a smooth ring seal. Immediate thorough annealing is necessary.

LARGE EXTRACTOR PRODUCING AGITATION OF SAMPLE. In the extractor shown in Figure 3, Type B, the operation of the siphon and solvent vapor ducts is the same as in the Type A apparatus. The solvent backs up in the vapor tube and produces pressure on the vapor in the chamber below the filter disk in the same manner. However, the reflux, instead of flowing down the walls of the extraction chamber, is caught in the annular channel at the top of the chamber and directed down the solvent tube. It passes down this tube, up through the outer portion of the filter, over the filter, and down through the center part of the disk (Figure 4), flushing any residual extract from the preceding cycle out of the bottom of the mass being extracted. When the pressure of the vapor below the disk becomes sufficiently great to stop this flow, bubbles of vapor begin to pass upward into the extraction chamber. As soon as the hot vapor has warmed the solvent in the extraction chamber to its boiling point, the bubbles pass through the chamber, producing an almost violent agitation of the solvent and of the solid material suspended in it.

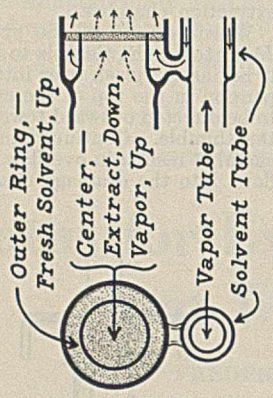

FIGURE 4. DETAIL OF FILTER DISK IN TYPE B EXTRACTOR

Plan below and longitudinal section of column above

The pressure required to force liquid solvent up through the outer part of the filter is so low that the level of the top of the liquid in the solvent tube remains within a few millimeters of that of the liquid in the extraction chamber. When the liquid in the extraction chamber has been sufficiently augmented by reflux from the condenser and water trap, solvent flows over the top of the vapor tube and down into the drainage chamber, raising the level in this chamber gradually until it trips the siphon. The drop in pressure resulting from the emptying of the vapor tube permits solvent to drain from the extraction chamber through the filter. After flow stops in the siphon, all the solvent in the extraction chamber can be accommodated in the drainage chamber. Extract and the continuing reflux fill the chamber to the bottom of the vapor tube, and the cycle of operations begins to repeat itself.

The relationship between the height of column in the vapor tube and the porosity of the filter disk in the extractor tested is satisfactory for a disk of the porosity of the one described. The disk used was rather coarse. A greater head, to force vapor through a finer filter, could be obtained by making the vapor tube and drainage chamber longer. In the apparatus used, a maximum elevation of solvent (chloroform) of 370 mm. in the vapor tube could be reached, but it actually operated most of the time at between 230 and 300 mm.

The filter disk was made by sintering powdered Pyrex in a 50-ml. beaker, with the center tube in place which divides the filter into central and peripheral parts. A layer 1 mm. deep of 60- to 80-mesh grains was spread on the bottom of the beaker and over it was laid a layer 2 mm. deep of 40- to 60-mesh grains. The center tube, cut to final length, was pushed into the ground Pyrex to the floor of the beaker, and the beaker and contents were heated in a furnace at about 800° C. until the walls of the beaker began to thicken and bend slightly. After annealing, the glass of the beaker was ground away against a grindstone (Norton, No. 37150-1).

Water Traps and Condensers

The trap of the microextractor is not suitable for measuring the volume of water collected, and separation of trap and condenser would sacrifice the convenience and rapidity with which they can be handled. The separate construction of trap and condenser in the large extractors facilitates interchange of parts and removal of the water collected. The condenser shown has been adequate.

A variety of traps could be designed for the large extractors. Of several types tested, that shown, for solvents heavier than water, retains the least amount of moisture when its contents are poured into a cylinder or buret for measurement. The Type B extractor would accommodate a deeper trap having an inner tube, similar to the one in the trap of the microextractor, graduated for direct reading of small amounts of moisture. However, from large samples having the water content of fresh tissues, the amount of water recovered is so large that the loss of one or two drops would constitute a small error. For the micro- and Type A extractors, the method of weighing the dry residue is always available. Traps for solvents lighter than water were not tested. A plain cup similar to the trap shown but without the overflow tube is suggested. A small powder funnel in the top would direct water below the surface. Small amounts of moisture can be recovered with a pipet with capillary tip, and can be measured by drawing up chloroform below the water until the water is in the graduated part.

A satisfactory tool for inserting or removing the trap or extraction thimble can be made of 3-mm. (0.125-inch) drill rod or 4-mm. (0.156-inch) brass rod. A deep V with a short coil at the apex is made by bending the rod one and a half times around a piece of pipe. The ends are then bent outward slightly to engage the top of the trap or the inside surface of the thimble.

Performance of Microextractor

Since the apparatus is adaptable for use with other solvents and for the extraction of other lipoids, its performance in the extraction and determination of cholesterol is a rather narrow criterion of its general usefulness. However, the results shown in Table I are satisfactory in significance and implication as to the possibility of approaching complete extraction, especially when it is considered that, up to 6 or 8 per cent, a large part and probably the greater part of the differences can be attributed to small variations in both the rate of development and the reading of the Liebermann-Burchard color. The consistency of values of duplicates, in the first line of the table, suggests a close approach to a limit.

The comparison of extraction in the microextractor and colorimetric determination of cholesterol with alcohol-ether extraction and determination by the digitonide method of Kirk, Page, and Van Slyke (*11*) must be interpreted with caution. Excess color due to esters (Kelsey, *10*) could be thought to have compensated for and concealed a deficiency in the extraction, particularly a failure to extract a definite portion associated with the proteins (*5*, *6*). That the deficiency, if any, in the extraction itself is much smaller than might be inferred, is shown by the small amount of residual cholesterol found after saponification in previously extracted samples (saponified, acidified, and re-extracted). The explanation of the small effect of the esters and of other substances producing excess color (*2*, *4*, *15*, *16*) has been learned only in part. "Recovery" of added cholesterol, since it is so easily extracted, has only the negative value of showing that no gross errors occurred in the colorimetry.

TABLE I. PERFORMANCE OF MICROEXTRACTOR

Nature of Data[a]	Number of Trials	Differences Found: Maximum %	In 95% of cases %	Average %
Difference between duplicate colorimetric determinations on chloroform extracts	603	13	<6.2	0.7
Comparison of values in same sample by above method and by Kirk's method	65	15	$<\pm 9.2$	+1.3
Recovery of added cholesterol by above method (per cent of total, that in sample + that added)	9	−4.3	$<\pm 4.1$	−1.0
Re-extraction after saponification of extracted sample (24 samples combined, yield = 22 mg. %, Wrattan No. 71-A red filter in eyepiece, extract, brown)	(Color, olive green)			<5.3

[a] First-named method against second as standard.

TABLE II. COLORIMETRIC VALUES

Nature of Data[a]	Number of Trials	Differences Found: Maximum %	In 95% of cases %	Average %
Comparison of readings on same extracts through red filter with direct readings of green color after extraction in microextractor	63	− 7.4	<− 4.2	1.0
Similar comparison after alcohol-ether extraction and careful drying in open beaker	11	−35	<−20	−15
Comparison on same specimens of color from alcohol-ether extract with that from microextractor	11	+23	<±18	+11

[a] First-named method against second as standard.

Use of Chloroform as Solvent

Chloroform was used in the large extractors merely as a starting point, because it was convenient and had given good results in the small apparatus. A. C. P. grade containing "up to 1 per cent" absolute alcohol was used. There is no purpose implied here of an indiscriminate defense of its general use as a lipoid solvent.

It is generally accepted that alcohol-ether extraction of blood, serum, etc., is complete. However, Mueller (*16*) and others have found, and the author's data confirm the fact, that such extracts contain substances which produce excess of color in the Liebermann-Burchard reaction if the alcohol-ether extract is taken to dryness in an open beaker, even with the utmost care. Bloor (*3*) avoids this difficulty by evaporating off the solvent in a flask covered by a watch glass, and others interpose an additional step in which the residue is extracted with petroleum ether. Chloroform extraction in the microextractor solved the difficulty in this laboratory. Not only is it not necessary to evaporate to dryness, but if an extract accidentally goes dry, the residue appears to be much less sensitive to these color-producing changes. A comparison of colorimetric values obtained after chloroform extraction in the microextractor with those found after careful evaporation of alcohol-ether extracts in open beakers is given in Table II. The light filter (Wrattan No. 71-A), transmitting above 620 mμ, decreases or eliminates the effect of yellowish tints on the readings. Since esters are equally present in both kinds of extract, it is probable that other substances are involved in the discrepancy.

Performance of Large Extractors

TYPE A. The operation of the extractor was reliable and satisfactory except as noted. Considerable latitude was permissible in the rate of refluxing and boiling of the solvent. Chloroform was used. The rate of drying of the specimen was disappointing; about three times as much time was required to drive off moisture as with the Type B extractor. This extractor would be useful when ample time could be allowed, and under such circumstances its fully automatic operation would effect a saving in actual working time.

TYPE B. A mash of canned peas containing 83 per cent of moisture required about 20 hours to dry to constant weight in an oven at 90° C. No significant amount of weight was lost on further drying at 105° C. From 62 grams of the original wet mash in the chamber of the extractor shown in Figure 3, water equal to 77 per cent was recovered in the trap in 12 hours, using chloroform as the solvent. At this time the extraction seemed to become accelerated as gaged by the increase of green color in the extract. After 8 hours more, 83 per cent had been recovered as water in the trap. These rough measurements serve sufficiently well as a basis for comparison. The drying and extraction appear to be somewhat faster than the conventional drying and extraction of the same material separately.

It was planned to use the apparatus for the extraction of animal tissues, chopped, rather than ground, to avoid loss of watery constituents. The conditions of the extraction were, therefore, made intentionally more than ordinarily rigorous. Pieces of the seeds ranging from 2 to 5 mm. across and large pieces of seed coat were seen in the extracted residue.

Discussion

It is hardly necessary to present data or cite references in support of the assertion that the extraction of wet samples by chloroform in extractors of the Soxhlet or Leiboff types is incomplete. Extraction by chloroform or ethyl ether from carefully dried samples may be nearly complete or far from complete, depending upon circumstances. Preliminary experiments in this laboratory showed three out of seven extractions of air-dried samples to lack more than 10 per cent of completion in apparatus identical with the microextractor except that it had no trap. The water trap definitely contributed to an improvement in the chloroform extraction. Since alcohol dissolves small amounts of nonlipoid material, and since acid or alkaline hydrolysis may produce undesired changes, any improvement in extraction may be of definite value.

A few experiments, using other alkyl chlorides in the microextractor, tend to show that the chemical nature of the solvent, and not the temperature of the extraction, determines the completeness of extraction (*5*) in one hour's time.

It may be of some importance in the rate of drying the sample that the water vapor appears to be layered above the chloroform vapor, but this observation was not subjected to comparative test.

An important advantage of conducting the drying and the extraction in the same vessel, in the presence of the solvent and with the outlet closed by the condenser, was pointed out by Kaye and associates—volatile fractions were retained that would be lost by drying with heat in an open vessel.

Agitation in the Type B apparatus undoubtedly contributes much to the rate of both drying and extraction; there is no chance for imprisonment of moisture in lumps of particles of the sample, all surfaces of every particle are continuously bathed in moving solvent, and the temperature of the solvent is maintained at near its boiling point throughout the chamber.

It is likely that minute coagula or varnishlike films may oppose a barrier to the extraction of portions of material (*1*). The writer was unable to demonstrate such coagula or films microscopically, but they might well be of submicroscopic size. However, the pea mash dried in the oven showed a shiny varnishlike appearance and adherence of particles in mosslike masses, while that dried in the solvent in the extractor (Type B) below a water trap showed discrete particles down to the fineness of a powder with "flat" powdery-appearing surfaces.

As for the mechanism of the extraction, it is conceivable that, with solvent present as the material dries out, the solvent enters the material in more minute division and in more intimate contact with its ultimate particles. In a hypothetical picture the solvent enters interspaces before they are closed by the receding of the moisture. The marked tendency of chloroform to form emulsions with water may be a factor, to which the trace of alcohol may contribute. Since the material goes through all degrees of moisture content, it must at some point pass through the optimum for extraction.

Summary

Three extractors are described in which the moisture of the specimen or material to be extracted is driven off in the presence of the solvent and its vapor. In the micromethod, at least, specimens are extracted more nearly completely than when they have been previously dried or when they remain wet throughout the extraction. The moisture is removed in all three extractors by means of a trap interposed in the reflux between the condenser and the sample. One extractor provides for vigorous agitation by a stream of bubbles of solvent vapor flowing upward through the solvent and sample in the extraction chamber. In all three, the specimen is dried and extracted in the same apparatus without interruption of its automatic operation. The parts are coaxial to save space.

Acknowledgment

Nearly all the determinations by the method of Kirk, Page, and Van Slyke were made by Mildred Kaucher.

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Estimation of Small Amounts of Antimony with Rhodamine B

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Rhodamine B reacts with Sb^V in hydrochloric acid solution or sulfuric acid solution containing chloride ion to form a red, water-insoluble stable compound, soluble in organic solvents. The excess reagent is destroyed with bromine, the rhodamine B complex put into solution by addition of alcohol, and the amount present estimated colorimetrically. Practically all acid radicals except chloride and sulfate interfere, as do arsenic, iron, and certain metals of the dithizone group. The latter interference can be avoided by preliminary extraction with dithizone. The method will detect as little as 0.1 microgram of Sb^V in 5 ml. of solution, and is rapid, inexpensive, and specific. The exact procedure is described for the concentration range 0.1 to 300 micrograms of antimony.

THE determination of small amounts of antimony, such as are encountered in tissue and air hygiene analysis, is extremely difficult and in some instances impossible with the usual methods for determining this metal. The chemistry of antimony and its compounds has been inadequately investigated and frequent cases of anomalous behavior are encountered in ordinary analytical experience. The difficulty becomes more marked when the concentration of the metal in the sample under study falls below 1 mg. The industrial hygienist and toxicologist have need for a method which will function in the range 1 to 1000 micrograms.

This paper reports the result of an investigation of the reaction between Sb^V and rhodamine B and describes a colorimetric procedure based upon this reaction.

Reagents

Sulfuric acid, 25 per cent solution, 125 ml. of sulfuric acid, specific gravity 1.84, made up to 500 ml. with distilled water.

Ceric sulfate, 0.1 *N* solution, 3.3 grams of anhydrous ceric sulfate made up to 100 ml. with 3 per cent sulfuric acid.

Sodium thiosulfate, 0.1 *N* solution, 2.5 grams of sodium thiosulfate pentahydrate made up to 100 ml. with distilled water.

Lithium chloride solution, 25.5 grams of lithium chloride made up to 100 ml. with distilled water.

Saturated bromine solution, an excess of liquid bromine in approximately 100 ml. of distilled water.

Rhodamine B, 0.2 per cent solution, 1 gram of rhodamine B dissolved in 500 ml. of distilled water and filtered. The rhodamine B used in this work was a practical grade obtained from the Eastman Kodak Company.

Hydroxylamine hydrochloride, 1 per cent solution, 1 gram of hydroxylamine hydrochloride made up to 100 ml. with distilled water.

Ethanol, 95 per cent, or absolute methanol.

Standard Solutions

Dissolve 0.5 gram of reagent grade antimony metal in 125 ml. of hot concentrated sulfuric acid, cool, and dilute to 500 ml. Dilute 50 ml. of this solution to 500 ml. with 25 per cent sulfuric acid solution; 1 ml. of the dilute standard contains 100 micrograms of antimony. Experience in this laboratory indicates that a solution of the theoretical amount of undried tartar emetic will serve as a satisfactory standard. Samples obtained from three manufacturers (Mallinckrodt, Merck, and J. T. Baker) were found to be identical in antimony content. A given lot of tartar emetic should be checked against an antimony metal standard before use.

To prepare a standard for color comparison, add an appropriate amount of the dilute standard solution from a 5- or 10-ml. microburet to a 50-ml. glass-stoppered graduated cylinder and make the total volume up to 5 ml. with 25 per cent sulfuric acid, using this solution to rinse down the sides of the cylinder. Then develop the color as described below. Standards should be made in duplicate and for colorimeter work should be within 25 micrograms of the unknown for best accuracy. The color is stable and the standard may be kept for several days without showing color change.

Procedure for Colorimeter

At the start of the analysis, the antimony must be in an approximately 25 per cent sulfuric acid solution. Place from 1 to 5 ml.

of the sample (keeping the total antimony content between 50 and 250 micrograms) in a 50-ml. glass-stoppered graduated cylinder. Bring the total volume to 5 ml. with 25 per cent sulfuric acid solution, adding the solution down the sides of the cylinder by means of a buret. Add 1 ml. of lithium chloride solution, mix, add 0.1 *N* ceric sulfate solution (approximately 2 drops) until a pale yellow color persists, and then add sufficient 1 per cent hydroxylamine solution (approximately 2 drops) to discharge the yellow color. Cool in an ice bath for 15 minutes. Remove, add 1 ml. of 0.2 per cent rhodamine B solution from a 25-ml. buret, mix, and cool in an ice bath for 10 minutes. Add 10 ml. of water and cool in the ice bath for 10 minutes. Remove, add 2 drops of saturated bromine water, quickly mix, immediately add a similar volume of 0.1 *N* sodium thiosulfate solution, and mix. Then immediately add 20 ml. of alcohol, shake, bring the volume to 50 ml. with distilled water, and shake. Let stand 20 to 30 minutes and compare with similar standards in a Duboscq colorimeter. Add all reagents directly to the solution and not down the side of the cylinder. Mixing implies gentle swirling in contrast to shaking. Run blanks on the reagents.

When the antimony content of the sample taken for analysis is below 25 micrograms, use only 0.5 ml. of rhodamine B and make the comparisons in Nessler tubes using the standard series procedure.

Table I. Reagent Required

Antimony Content of Sample *Micrograms*	Rhodamine B Reagent Required *Ml.*
0–50	0.5
50–150	1.0
150–200	1.5
200–300	2.0

Precautions

The bleaching operation is the most critical step in the procedure. The temperature of the solution must be kept below 10° C. to ensure complete insolubility of the complex. Exposure to bromine must be no longer than necessary to produce a bleach. Only a moderate excess of reagent is permissible, and this excess must be promptly destroyed with thiosulfate. If the acidity is too high or too low the bleach is not satisfactory. As bromide ion forms a colored complex with the reagent, the presence of too large an excess of reagent in the sample will give a positive blank. Blank reactions conducted with 1 ml. of rhodamine B reagent should result in no more than a pale pink color and with 0.5 ml. of reagent should be water-white. The proper amount of reagent to use in respect to the antimony content of the sample is shown in Table I.

The colorimetric method described is sensitive to the manipulative procedure used. Most analysts become proficient with the method after 2 or 3 days of practice. Samples containing no antimony should be run until satisfactory blanks can be obtained, then samples containing 100 micrograms of antimony should be run until reproducible results can be obtained. In this laboratory about 4 per cent of the analyses made by this procedure fail for one reason or another. Accordingly, samples should always be analyzed in duplicate. All precautions common to trace analysis, such as scrupulous cleanliness, tested reagents, use of Pyrex glassware, and accurate volumetric glassware, must be observed.

In order to obtain a high sensitivity the volume of solution at the time of reaction must be kept as small as possible. Sensitivity could probably be increased by reducing the final working volume to 10 ml. instead of 50 ml.

Although care has been taken to select quantities of reagents in such amounts as to make their measurement noncritical, reasonable accuracy must be employed in their measurement. The depth of color is dependent upon the alcohol concentration up to about 36 per cent. The sulfuric acid content must be within five per cent of the indicated amount. The volume of water added prior to bleaching should be held to within 2 ml. of the indicated value.

As the color intensity is influenced by temperature, standards and samples should be at the same temperature when compared. The apparent color may be influenced by the deposition of air bubbles on the bottom of the colorimeter plungers; alcoholic solutions are especially troublesome in this respect.

If too large an excess of sodium thiosulfate is added, a turbidity forms, due probably to colloidal sulfur, which interferes with the determination.

Discussion of Method

In a qualitative test utilizing the reaction mentioned in this paper (*1*, *2*), the rhodamine B solution is treated on a spot plate with the test solution strongly acidified with hydrochloric acid and previously oxidized with sodium nitrite. In the presence of antimony, the light red dye solution changes to violet. A quantitative reaction could not be developed from this procedure, because of interference of excess sodium nitrite and excess reagent in the solution at the completion of the test.

Sb^{V} reacts with an aqueous solution of rhodamine B in a solution strongly acid with hydrochloric acid or one strongly acid with sulfuric acid and containing chloride ion, to form a dark red compound, extremely insoluble in aqueous solvents. In fine suspension it has a bluish purple fluorescence and when precipitated in the presence of a protective colloid such as gum ghatti, the suspension appears blue in a colorimeter. Under proper conditions the reaction appears to be quantitative and upon filtering no antimony can be detected in the filtrate. The compound formed is soluble in many organic solvents, filters readily, and is unusually stable. Attempts at analysis have not met with success, but apparently one atom of antimony is associated with one molecule of rhodamine, possibly as a complex salt. The presence of adequate chloride ion is absolutely essential to the reaction, and chloride is present in considerable amount in the final compound. Although several attempts were made to determine the chloride content, using the Parr bomb and the Willard-Thompson method, consistent results were not obtained, perhaps because of variable composition of the compound or failure to obtain satisfactory decomposition of the complex, which is exceedingly stable. It is most conveniently decomposed a few tenths of a gram at a time by digesting with hot 30 per cent fuming sulfuric acid until well in solution and then cautiously adding 30 per cent hydrogen peroxide until the mixture is colorless.

The compound may also be formed in 50 per cent hydrochloric acid solution but a 25 per cent sulfuric acid solution is preferable for colorimetric determination.

The choice of oxidizing agents for converting Sb^{III} to Sb^{V} is limited. If sodium nitrite is used, the excess nitrite interferes with the reaction and must be destroyed with urea, and under some circumstances the nitrate formed by the reaction interferes. Sodium chlorite is satisfactory and preferable if the reaction is carried out in hydrochloric acid solution and the excess reagent is destroyed with hydroxylamine hydrochloride. The reagent of choice is ceric sulfate, when a sulfuric acid solution is used. It serves as its own indicator and the excess is readily removed with hydroxylamine hydrochloride. A slight excess of ceric or cerous ion influences the final color.

It is important that the complex be formed at high acidities if a quantitative reaction is to be obtained. The acidity is not critical except in the colorimetric method, where the shade of the final color is influenced by the acidity and the range in which bromine will function as a bleach is somewhat limited.

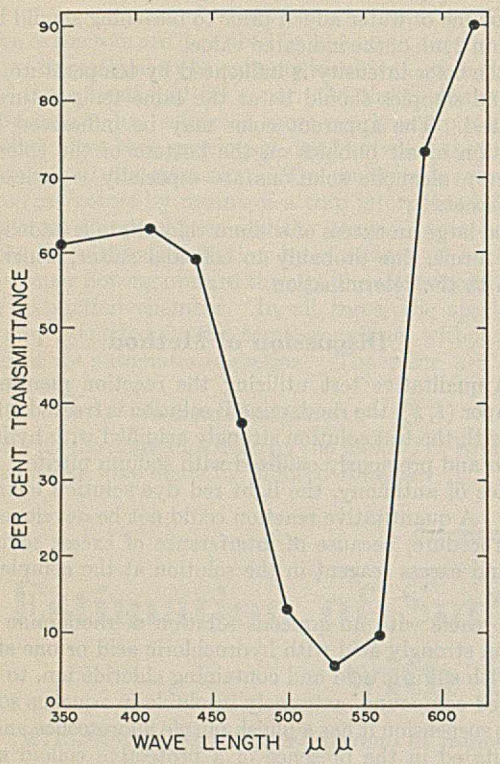

FIGURE 1

Many bleaching agents were tried, but only bromine water is satisfactory. Rhodamine B itself is not easily decolorized. On the other hand, a too active reagent will attack the solid rhodamine B–antimony complex.

The color produced when the complex is dissolved in alcohol appears to follow the Beer-Lambert law in all concentration ranges studied. The ethyl alcohol solution showed a marked absorption to light of about 530 millimicrons wave length as determined by the Coleman R. S. photometer (Figure 1).

Lithium chloride must be used as a source of chloride ion, since it is the only readily available inorganic chloride salt which will not interfere with the reaction and still be sufficiently soluble in 40 per cent alcohol to avoid precipitate formation.

The procedure here described is rapid and requires little unusual or expensive equipment or reagents. In routine, an analyst can conveniently prepare standards and analyze 30 samples in the usual working day if samples are handled in groups of 12 or 24.

Interfering Substances

The effect of several metallic ions, salts, and acids is shown in Table II. Nearly all acid radicals except chloride and sulfate will cause trouble. When the sample contains 10 micrograms or less of antimony, the effect of many interfering substances is greatly diminished. Metals of the dithizone group, such as mercury, copper, nickel, cadmium, and zinc, may be conveniently removed by preliminary extraction of the sample at approximately pH 8.5 with 100 mg. of dithizone per liter of carbon tetrachloride solution, using the usual dithizone technique. Antimony does not appear to react with the dithizone or be lost in the extraction process.

Results of Analysis

Typical analytical results based on synthetic samples of known antimony content are shown in Table III. These data were obtained by five different analysts and represent single determinations on single samples. Some samples have permissible amounts of other compounds present, some represent antimony added to beef tissue and ashed, and some represent results obtained by making the analysis in 50 per cent hydrochloric acid solution, employing sodium chlorite as the oxidizing agent. No attempt has been made to classify the data further, inasmuch as more than 1000 separate analyses of all kinds showed no significant deviation or trends among themselves.

Analysts using this method only occasionally may expect to obtain results within ±5 per cent of the truth for antimony concentrations in the range of 25 to 200 micrograms, and within 25 per cent of the truth for concentrations below 10 micrograms. In routine use somewhat better accuracy may be obtained. Deviations greater than ±10 per cent are rarely encountered in the range of 25 to 300 micrograms of antimony, even though the technique may be faulty. The accuracy and precision have about the same order of magnitude.

TABLE II. INTERFERENCE TABLE

(100 micrograms of SbV in sample)

Substance	Amount	Interference Effect	Substance	Amount	Interference Effect
	Mg.			*Mg.*	
H_3PO_4	200.0	None	Ce	1.0[a]	Off color
Al	7.5	None	Hg	0.5[a]	Deepens color
Mn	7.5	None	Cd	3.0[a]	Deepens color
Bi	7.5	None	Zn	3.5[a]	Deepens color
$CaSO_4$	Saturated solution	None	$KClO_4$	Trace[a]	Deepens color
Mg	7.5	None	Sn	3.5[a]	Deepens color
$PbSO_4$	Saturated solution	None	$HClO_4$	Trace[a]	Deepens color
Fe	4.0[a]	Off color	Cu	Trace[a]	Weakens color
AsIII, V	Trace[a]	Off color	Ni	7.5[a]	Weakens color
$NaNO_3$	1.0[a]	Off color	$NaNO_2$	Trace[a]	Weakens color
NaF	1.0[a]	Off color	NaBr	0.5[a]	Weakens color
Tl	Trace[a]	Off color	H_2O_2	Trace[a]	Weakens color

[a] Maximum permissible amounts for samples containing 100 micrograms of antimony.

TABLE III. ANTIMONY RECOVERY

Added	Found	Error	Added	Found	Error
	Micrograms			*Micrograms*	
200	195.7	−4.3	50	50.2	+0.2
	199.6	−0.4		50.2	+0.2
	200.5	+0.5		47.5	−2.5
	197.1	−2.9		50.1	+0.1
	198.0	−2.0		49.4	−0.6
	194.3	−5.7		51.2	+1.2
	200.5	+0.5		50.2	+0.2
100	99.0	−1.0	100 (dried beef)	102.8	+2.8
	99.0	−1.0		96.6	−3.4
	98.5	−1.5		98.0	−2.0
	92.2	−7.8		100.5	+0.5
	98.5	−1.5		96.1	−3.9
	101.0	+1.0		101.5	+1.5
	102.6	+2.6		99.6	−0.4

Acknowledgment

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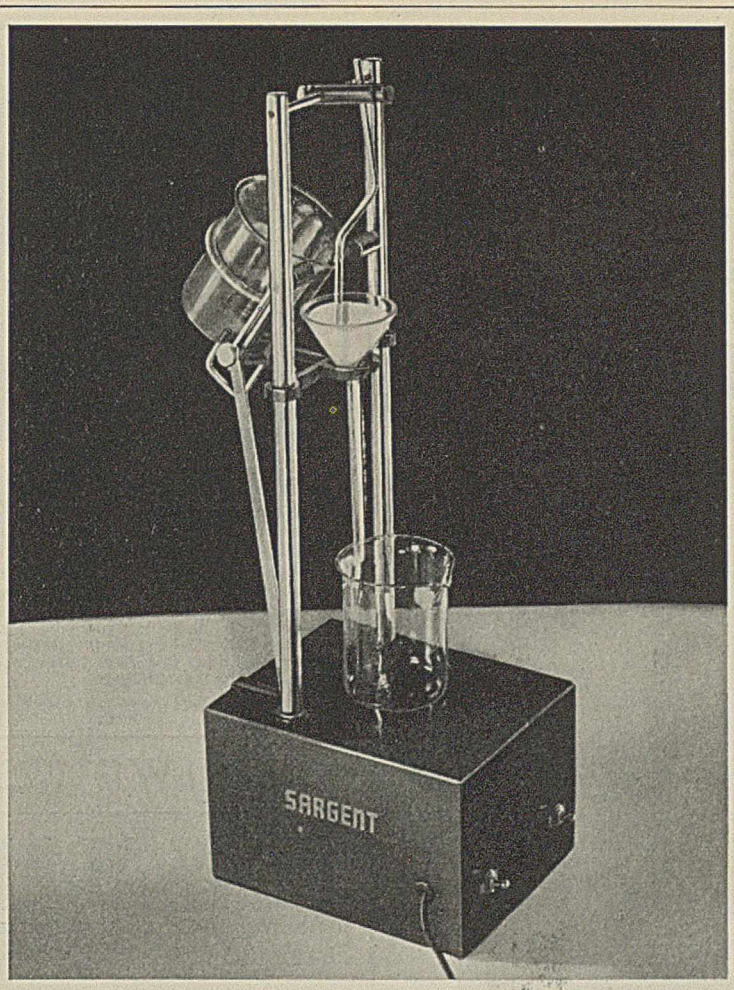

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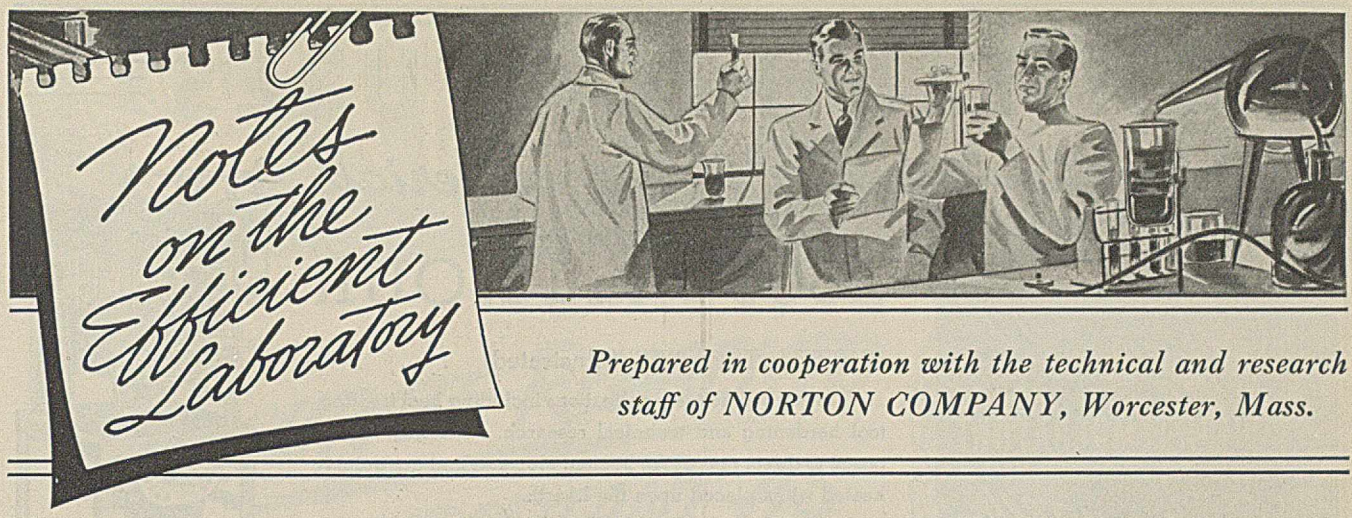

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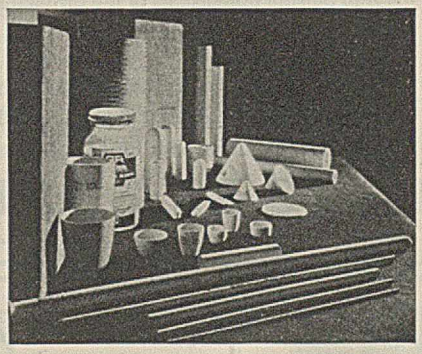

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For washing and drying crystals and washing precipitates.

THIMBLES
For extraction of feeds, tar, chocolate, rubber and fats. Copper and fatty acid determinations.

CRUCIBLES
For fine (Barium Sulphate) and coarse (Silver Chloride) precipitates. Used also in Mn, Pb, Sn and Si determinations.

Important Footnote: for Crude Fibre determinations, you may obtain the special mixture RA 766, of greater porosity than RA 98. Perhaps *you* have a specific problem in laboratory ware requiring the more-than-ordinary attention which we would give it.

Getting the Right Refractory Cements for Resistor-Wound Electric Furnaces

The picture shows an externally wound ALUNDUM Conical Core with cement partially applied. Occasionally, the cement will react with and destroy the resistor element under operating conditions. It's something to look out for, if you're designing or constructing electric furnaces for your laboratory. Write for our booklet, "The Construction of Electric Furnaces for the Laboratory." It gives the complete story.