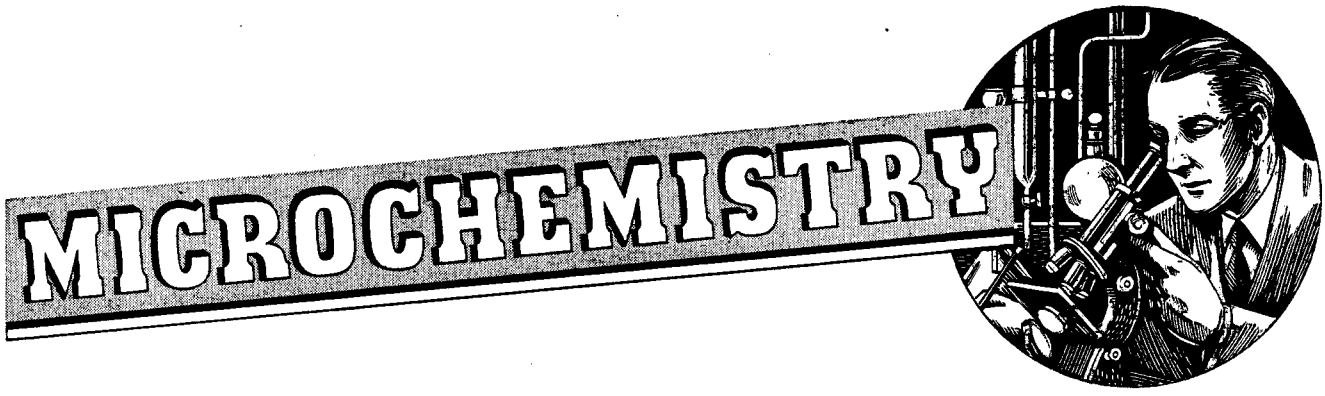

A Review of Organic Microchemistry

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DURING the past thirty years a great part of the research in the different branches of chemistry has dealt with a study of the role of traces of elements and compounds. To isolate, identify, and determine these small quantities has required the development of a new technique called microchemistry. Microchemistry may be broadly defined as that branch which deals with the apparatus and technique of working with small quantities of substances, usually less than a gram. For analysis, the weight of constituent determined is usually 1 to 10 mg., but it may be only a fraction of a milligram.

Organic microchemistry, with which this paper deals, has had a spectacular development, particularly in the field of biochemistry where working with minute quantities is often a necessity. As is so often true with new advances in science, we have the overenthusiastic microchemist who is apt to lose his perspective, and the older chemist who believes the new methods are not practical. This new branch of chemistry has fortunately matured to the point where the pristine glamor and magical trappings which it early acquired have largely disappeared. This has been due in large part to a careful study of the basically important factors by critical chemists in both academic and applied fields. The resulting improvements in apparatus and operating technique have made the methods far more practical than was at first thought possible.

One purpose of this review is to emphasize these later developments and wider applications. While the analysis of organic material is a large and important branch, some attention will be given to those branches which are not strictly analytical. The microanalyst, because of his special skill and knowledge in dealing with small quantities, is often called upon for methods of isolation, purification, and synthesis.

The obvious advantages of saving time and reagents in decomposing and working with small samples have been pointed out repeatedly. In those macromethods where it is necessary to digest or burn a large sample requiring several hours, the time can, in many cases, be reduced to a few minutes when small samples are used. This applies also to filtering large volumes of solutions.

It is not the purpose of this review to give a complete bibliography of the topics discussed. Such data are readily available and are indicated in the text. There does seem to be a need, however, to summarize the general conclusions of the many published papers, so that modifications of methods can be made which will incorporate those improvements established by several independent workers. The mass of published material on the common determinations, such as those in elementary organic analysis, is becoming so great that it is increasingly difficult to separate the important from the less essential matter, much of which is optional, depending on personal preference.

Organic microchemistry has shown important developments in the design and standardization of apparatus; the use of automatic combustion apparatus in routine analysis, which permits full advantage to be taken of the savings in time which are possible with micromethods; successful extension of the methods to the analysis of microgram quantities; application of physical methods to the analysis of small quantities; the design of apparatus for synthesis on a micro scale; and the adaptation of micromethods to systematic organic qualitative analysis.

There are many factors to be considered when an analytical procedure is critically evaluated. These range from the personal prejudices and skill of the analyst to inherent defects in the method which can only be partially controlled, and largely determine its accuracy and precision. In some cases microprocedures will exaggerate these limitations. An analyst who finds macroprocedures difficult will certainly not possess the qualifications required in microtechniques. Likewise, some factors in precipitation, which are not apparent when dealing with 20 to 50 mg. of a constituent, may cause the method to break down when applied to a milligram or less, which is the usual micro range.

It must be constantly borne in mind that all analytical procedures fail to give satisfactory results with certain types of material. Here, judgment is required and the method is not necessarily condemned. Published methods usually apply to a very limited range of materials which are of interest to the particular analyst, although the author often gives the impression in his enthusiastic account that it has unlimited application. In the hands of another, perhaps less skilled worker, or when applied to other materials, the method may yield poor results. This limited study of new procedures naturally leads to much controversy which can best be settled by detailed study and experience in several laboratories. For many micromethods we have, fortunately, a background of published experience over a period of years. In addition, most of the commonly used micromethods are adaptations of standard macroprocedures. The adaptations manifest themselves mostly as equipment designed to weigh and handle small quantities.

The difficulty of obtaining reagent chemicals of the required purity for microprocedures is no longer a problem. In this country the necessary standards have been worked out through the cooperation of the AMERICAN CHEMICAL SOCIETY Committee on Reagents and the Division of Analytical and

Micro Chemistry. Much needed standard specifications on microapparatus are being drawn up by an AMERICAN CHEMICAL SOCIETY committee, in active cooperation with American manufacturers of scientific apparatus. These specifications, as they are finally approved, are being published in the ANALYTICAL EDITION of INDUSTRIAL AND ENGINEERING CHEMISTRY. The first report (*280*) covered apparatus for carbon and hydrogen and Dumas nitrogen; that for sulfur and halogen will be ready soon.

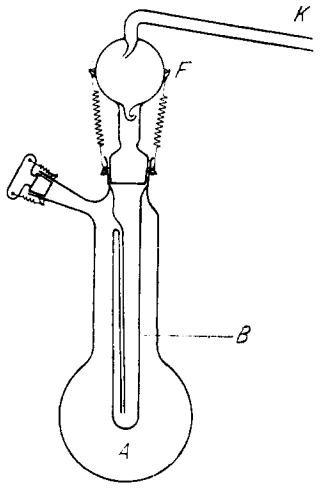


FIGURE 1. STEAM DISTILLATION APPARATUS

The purity of the samples analyzed in published analytical work can sometimes be questioned. The National Bureau of Standards has recently undertaken the preparation of standard samples for organic elementary analysis. These samples have been analyzed by both micro- and precision macromethods and should prove invaluable to all analysts in standardizing procedures for their own laboratories, and as a measure of their precision and accuracy in comparison with others. Two standards are now available—acetanilide and benzoic acid.

Reagents of adequate purity, properly designed apparatus, and standards of unquestioned purity are prime prerequisites in developing and using any analytical procedure. That these three factors are receiving careful study indicates that micromethods are being critically examined as to their requirements and limits of precision and accuracy. So far, the results by micromethods can be considered as accurate within their established limits of precision as those obtained by equivalent macroprocedures.

The quantitative branch of microchemistry initiated by Emich, using a quartz fiber balance, was limited in application, owing to the small load capacity of the balance. With the development of balances of 20-gram capacity and high sensitivity by Kuhlmann and others, the design of equipment and the development of methods for gravimetric procedures have been made practical. Progress in instrument design and the application of new principles to analytical procedures in general have been adapted to microanalysis with a resulting improvement in precision and convenience.

It is the usual practice in analytical procedures to select a sample weight sufficiently large so that the weighing error is small. Then the precision and accuracy of the method are determined by its inherent chemical and manipulative limitations. The weighing precision of standard analytical balances has been improved over a period of years, so that, in addition to the usual balance weighing to ±0.2 mg., most laboratories possess one or more balances weighing to ±0.02 mg. Using these balances and weighing samples of 20 to 50 mg., many laboratories have found that they are able to obtain excellent results without the expense of special balances and equipment. Generally, the apparatus is slightly larger than that used in microprocedures. The skill required with these semimicromethods is not so exacting, and for many academic institutions it is becoming the accepted method. The fact that instruments are now made having greater precision and that microchemistry has demonstrated that smaller samples may be used has had a far-reaching effect on all analytical chemistry. Because of this, even macrosamples are now more often in the range of 50 to 100 mg. This gradual but very definite change has made the dividing line between macro- and micromethods less sharp.

In selecting a suitable method for the determination of a constituent, several factors should be considered:

1. The number of samples to be run. An occasional sample requires a method which avoids the preparation of special solutions and the setting up and conditioning of special apparatus. For the analysis of many samples, apparatus should be designed which permits series runs to be carried out rapidly and with a minimum of effort on the part of the operator. A new branch of chemistry necessarily has many forms of apparatus and modifications of methods. Out of all these, many of which are personal preferences, are evolved essentials which should be retained. Here, again, judgment on the part of the analyst is required, so that he may chose from the many modifications put forward the ones which will best serve his needs.
2. The degree of precision and accuracy desired should be decided upon and the simplest method to achieve this should be adopted. The available instruments for weighing, measuring volumes, color, etc., for general microanalytical work are more than adequate. More often the errors in manipulation or factors of solubility, sharpness of color, etc., determine the error of the method.
3. Where samples of similar composition are to be run, the method can often be simplified. When interfering elements are many and frequently unknown, the procedure must, of course, be modified, and undue simplification is likely to yield unsatisfactory results.
4. It is good technique in any analytical procedure to hold to a minimum operations which involve the transfer of solutions, precipitates, etc. Microprocedures which are not based on this fundamental idea will usually lack precision, and this casts doubt upon the efficiency of an otherwise chemically sound method.

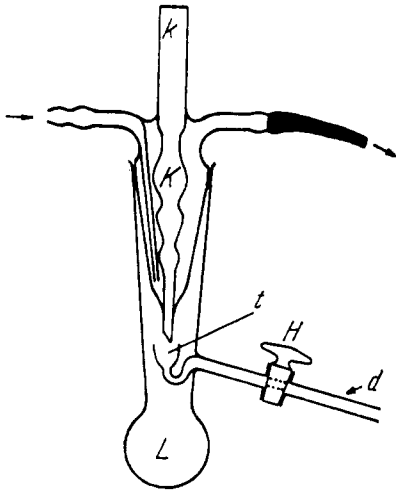


FIGURE 2. COMBINATION REFLUX AND DISTILLATION APPARATUS

An analyst requires all the tools and techniques that are available. He should be flexible in his outlook, so that the limitations and advantages of both macro- and micromethods will be carefully considered. Speed in routine analytical procedures, which has long been a requirement in industry, has been achieved to a remarkable degree. Here the size of the sample is of little importance and the enthusiastic microchemist must show that his procedure is either faster or more convenient, and not too difficult, before the method is even considered. For the solving of special problems, where only small quantities are available, the microchemist is the last hope, and his services are always greatly appreciated.

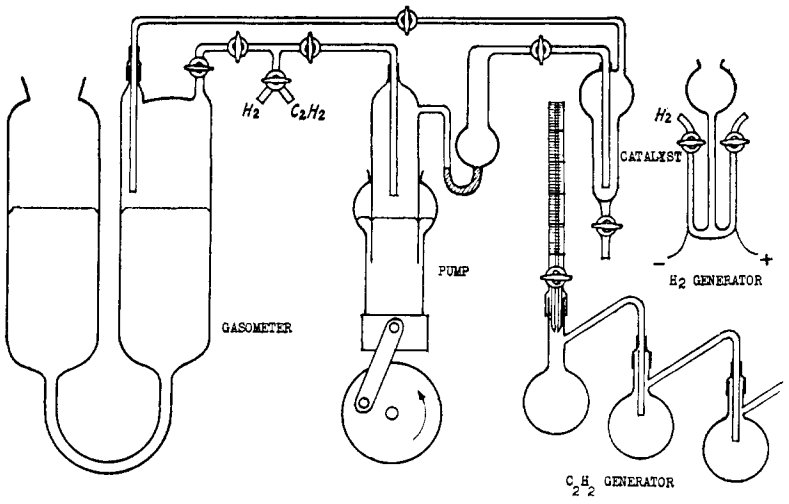


FIGURE 3. CATALYTIC HYDROGENATION APPARATUS

More attention is now being given to a systematic study of the errors in microanalysis. These errors may be grouped as:

1. Weighing
2. Measuring solution volumes (buret errors, etc.)
3. Chemical errors inherent in the procedure
4. Personal

The errors in weighing have been systematically studied (see Microbalance). The errors in volumetric analysis are known and can be controlled by the selection of a solution of suitable normality and measured in a buret so constructed as to permit adequate precision. Titration with solutions 0.001 *N*, with burets reading to 0.001 ml. and error ±1.0 per cent, is now a matter of routine, and for special problems more dilute (0.0002 *N*) solutions and more precise burets (error ±0.1 per cent) can be used. These errors and those of indicators are adequately dealt with by Conway (*70*), Mika (*213*), and Malyarov (*203*).

In a series of determinations on a single sample, most of the values, of course, are close to the mean. Those which appear out of line by inspection are apt to be discarded. This unscientific practice is not justified when mathematical methods are available for calculating when a value exceeds the allowable error of the method and may be classified as an error in manipulation. Likewise, the chances which any one value has of being correct may be determined. In the development of any method, the results should be considered from the standpoint of statistical treatment (*273*). A general text on this subject, of value to the analyst, has been written by Crumpler and Yoe (*73*).

Collaborative studies on the errors involved in the microdetermination of nitrogen, methoxyl, ethoxyl, carbon, and hydrogen and weighing have been completed and are referred to under these determinations in this review. The last three have been subjected to a thorough statistical treatment.

The following example will serve to illustrate the method of expressing precision and accuracy in this review. If the theoretical value for carbon is 50.00 per cent and the found values are 49.7 and 50.3, then the precision and accuracy are ±0.6 per cent. By precision is meant the reproducibility of a method; by accuracy, the deviation from the true or theoretical value.

References to biological micromethods are made only in so far as they illustrate technique and methods which can be applied to general organic microchemistry. Kirk (*149*) has adequately summarized advances in this field.

This review is one of several in a series on analytical topics. In order to keep the present one within reasonable limits, certain methods and instruments are only briefly mentioned as having micromodifications. In such cases, references are given to earlier reviews where sufficient information and illustration will be found.

An attempt has been made, in the description of apparatus and methods, to give a survey of the tools which are at the disposal of the organic microchemist, to show their wide application, to set down their precision and accuracy, and to give a glimpse at advances in design and technique.

SYNTHESIS AND PURIFICATION

ORGANIC SYNTHESIS. Some investigators have found that it is entirely practical to carry out organic syntheses, using micro- or semimicroquantities.

Cheronis (*56*) describes apparatus for refluxing, distilling, extracting, separating, filtering, drying, weighing, and measuring 2 to 4 grams of solids and 2 to 5 ml. of liquid. Detailed directions, using this equipment in teaching organic synthesis, are given for the preparation of nitrobenzene, *m*-dinitrobenzene, cyclohexene, benzoic acid (using Grignard's reagent for introducing the hydroxyl group), ethyl benzene (by the Wurtig-Fittig reaction) aniline (by the reduction of nitrobenzene), and acetylation of aniline. Yields of 0.3 to 1 gram are obtained.

Alber (*4*) synthesized and analyzed organic compounds containing radioactive sulfur, using micromethods.

Erdös and László (*89*) give a summary in English, with nine cuts of apparatus which is useful in carrying synthetic laboratory

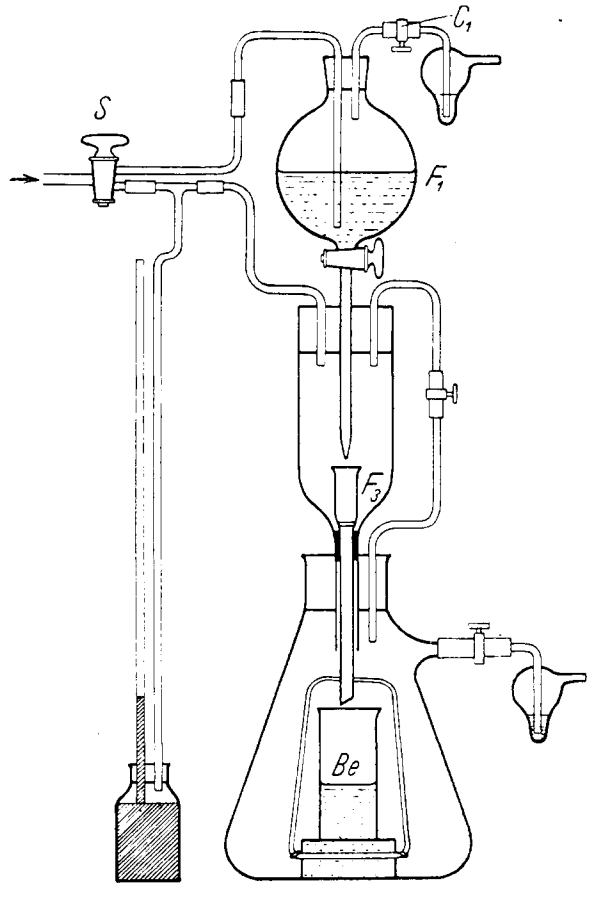


FIGURE 4. APPARATUS FOR REACTIONS OR EXTRACTIONS IN SPECIAL ATMOSPHERES

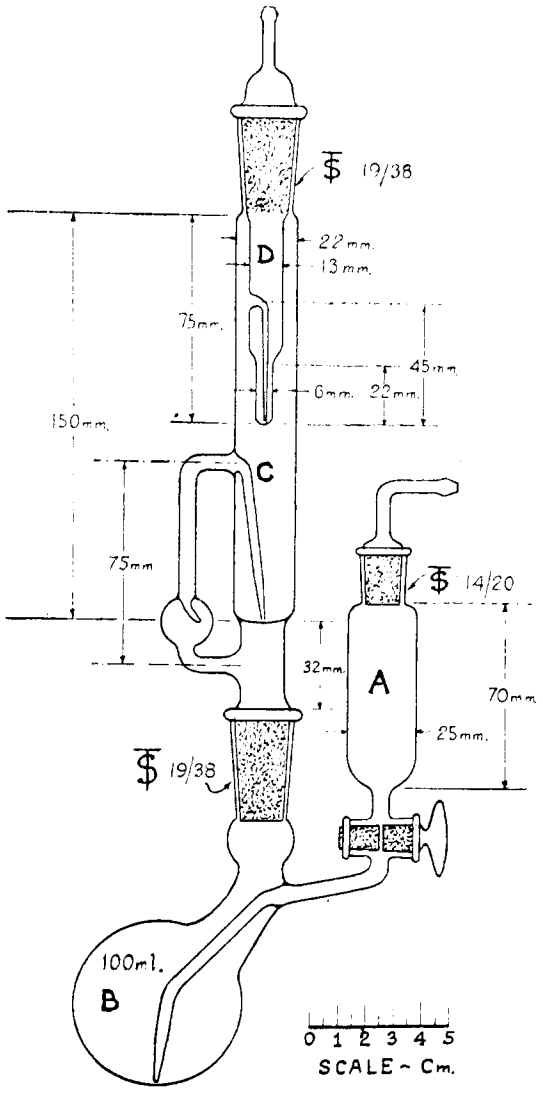


FIGURE 5. APPARATUS FOR QUANTITATIVE LIBERATION AND ABSORPTION OF GASES

work on 50 to 100 mg. or 0.1 ml. of material. An apparatus for distilling under reduced pressure and four types of apparatus for steam-distillation are described. Figure 1 shows one type used for steam-distillation. Steam generated in *A* is led into the sample contained in *B*, and the sample is distilled out through *F* and *K*. A distilling flask, useful for distilling following a reaction involving reflux cooling, is shown in Figure 2. The flask, *L*, 5- to 10-ml. capacity, is the reaction chamber and *K* is a ground-in condenser. The stopcock, *H*, is closed during the reaction. After the reaction is completed by turning *H*, the distilled product is caught in *t* and removed through *d*.

Wright (*371*) summarizes technique with illustrations of apparatus which have been found useful in semimicropreparation and purification of organic substances.

Leĭpunskiĭ and Reĭnov (*183*) describe an apparatus and micromethods for studying chemical reactions of gases, their solubility in liquids, and compressibility at pressures ranging from 10,000 to 12,000 atmospheres and at temperatures up to 450° C.

Dadieu and Kopper (*75*) in a review of micropreparative methods in organic chemistry include a description of apparatus from the unpublished work of O. Kermauner: (1) for the micropreparation by a bomb reaction of $C_2D_2 + DBr \xrightarrow{(AlBr_3\ catalyst)} CD_3CDBr_2$; (2) for reaction of gases in liquids, as, for example, the use of deuteroacetylene and deuterosulfuric acid in the preparation of deuteroacetaldehyde; also (3) an apparatus with a special circulating pump for the catalytic hydrogenation of acetylene to ethylene, using a colloidal palladium solution as the catalyst which is shown in Figure 3. The initial volume of the mixture of acetylene and hydrogen was 500 ml.

A comprehensive summary of microtechnique in synthesis is given by Pfeil (*244*).

Van Straten and Ehret (*352*) propose apparatus (Figure 4) for conducting reactions or extractions in special atmospheres. The reagent or solvent is placed in the 70-ml. separatory funnel, F_1, and the substance that undergoes reaction is placed in the microfilter tube, F_2. The reaction product or leachings are caught in the microbeaker, *Be*. The special gas which fills the apparatus is brought in through *S*.

An all-glass apparatus (Figure 5) for evolving and quantitatively absorbing various gases and vapors is described by Binnington (*34*). The sample is placed in *B*, the reaction mixture in *A*. Air or other gas is drawn through the apparatus and the liberated gas absorbed in the reagent placed in absorbers *C* and *D*. After the reaction is completed, *D* is removed, and its content washed into *C* where the titration is carried out. The apparatus was designed initially for determining 0.5 mg. of bromide by treating it with chromic-sulfuric acid mixture.

RECRYSTALLIZATION. Mechanical loss during crystallization of small quantities must be avoided.

Blount (*38*) describes a simple apparatus, Figure 6 (left), for carrying out this operation. The material is placed in a filter funnel with a G2 porosity sintered-glass plate, which is suspended from the condenser by a platinum wire. The solvent is contained in a flask 3 cm. in diameter and, on refluxing, the solids dissolve. When extraction is complete, the flask is allowed to cool and the substance crystallizes. It may be collected again on the filter and the process repeated as many times as desired.

Craig (*72*) has designed an assembly for the fractional recrystallization, as shown in Figure 6 (center). *H* is the crystallizing vessel and *L* is the filter vessel, which is closed by a glass plug, *M*, not ground in. The fine crystals form the filter mat but, if desired, a sintered-glass plate may be fused in at *L*. *K* and *N* are collars of soft lead or tin. The figure shows the apparatus in the position for the collection of crystals. In the reverse position the material and solvent are contained in the crystallizing vessel, *H*, and for crystallization the apparatus can be heated or cooled by placing in a suitable bath. The apparatus is of such size that it can be readily placed in a small centrifuge. Figure 6 (right) shows a crystallizing assembly which is smaller in size but operates on the same principle. *Q* corresponds to *H*, *R* to *M*, and *S* collects the filtrate. The whole assembly is placed in a stoppered glass tube for centrifuging.

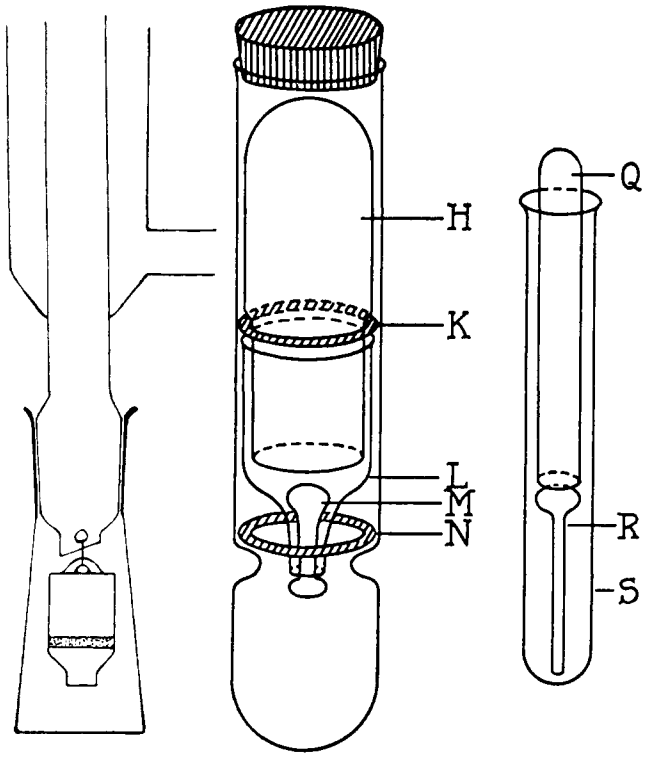


FIGURE 6. RECRYSTALLIZATION APPARATUS

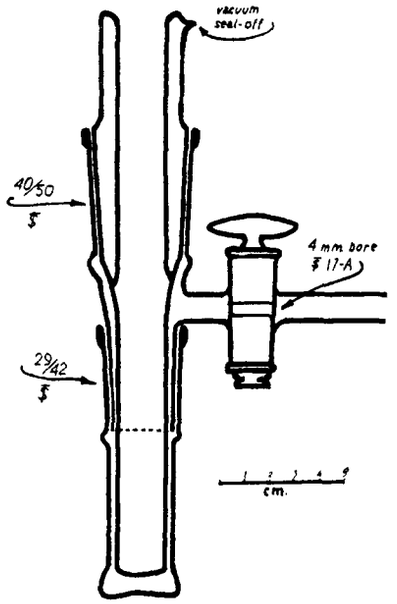


FIGURE 7. HIGH-VACUUM LOW-TEMPERATURE SUBLIMATION APPARATUS

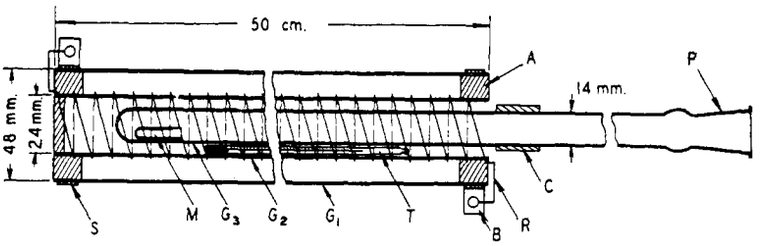


FIGURE 8. FRACTIONAL SUBLIMATION APPARATUS

A. Asbestos tape
B. Binding post and fastener
C. Condenser
G_1, G_2, G_3. Glass tubes 48, 24, and 14 mm. in diameter, respectively
M. Material being sublimed, contained in glass capsule
P. Pump connection
R. Resistance wire
S. Strip of metal to prevent breakage and serve as support for binding posts
T. Thermometer

SUBLIMATION. Purification of solids by sublimation is a simple but effective method, which can be carried out using only a watch glass upon which the material is placed. The watch glass may be heated by a small flame and the sublimate condensed on the under surface of a cool glass plate.

A more elaborate apparatus for quantitative work with traces of material, designed by Clarke and Hermance (*67*), offers advantages. It is electrically heated and thermostatically controlled, and sublimation takes place under a vacuum. Equipment with or without a vacuum has also been designed to fit the microscope stage, so that any significant characteristic of the sublimation process can be more easily followed. Sublimation technique and apparatus, stressing that used with the microscope, are given by Fischer (*100*).

Of interest to those engaged in synthetic work is the high-vacuum semimicrosublimation apparatus of Marberg (*204*). It is constructed of standard-taper joints (Figure 7). The material placed in the lower cup is sublimed by heating and the sublimate collects on the condenser cooled with a low-temperature refrigerant, such as liquid air. The general theory and technique of high-vacuum purification have been described by Hickman and Sanford (*135*), and McDonald (*198*). A novel arrangement (Figure 8) for separating and collecting fractions from a small sample is given by Morton, Mahoney, and Richardson (*218*). After one fraction has been deposited, others may be collected by successively moving the heater jacket back and raising the temperature, whereby a series of bands is formed. The apparatus is also useful in checking the purity of a compound, since the first and last bands should have identical melting points.

EXTRACTION. *Solids.* There are two types of extraction apparatus, siphoning and percolating. Extraction may be employed for removing an active ingredient from a heterogeneous mixture as a crude drug mixture, or removing an undesirable material from an impure product, as in the purification of derivatives.

A comparative study has been made by Batt and Alber (*19*) of methods for extracting solid substances by heated solvents, using macro-, semimicro-, and microapparatus. Where the residue, as well as the extracted material, is to be determined, the less commonly used principle of percolation gives better results than when a paper thimble and siphoning (Soxhlet) apparatus are employed. The best apparatus for this purpose is that developed by Gorbach, which uses a platinum filter dish as a container for the material to be extracted. Six types of apparatus are shown (Titus and Meloche, Gorbach, Colegrave, Wasitzky, Slotta, and Hetterich), and the best operating conditions for each are summarized (*19*). Figure 9 shows the first two, which incorporate features which have been demonstrated to be of value. The accuracy of both micro- and macroprocedures was ±2 per cent. The amount of extractable substance used in the microextractors varied from 1 to 100 mg.

Liquids. An apparatus for the extraction of small quantities of liquid, 1 to 20 ml., with organic solvents such as ether, benzene, etc., has been described by Barrenscheen (*17*).

Fabian (*92*) and later Browning (*48*) designed a microseparatory funnel without a stopcock, which is similar to a pipet. A solution of the material is preferably drawn through the capillary by gentle suction. Both the material to be extracted and the solvent are shaken in the usual manner, and after standing, the two phases are separated by applying pressure, whereby the heavier fraction flows out through the capillary. Figure 10 shows the Browning apparatus.

MICROFRACTIONATION. The fractionation of a single drop of liquid by the Emich method has been improved by Morton

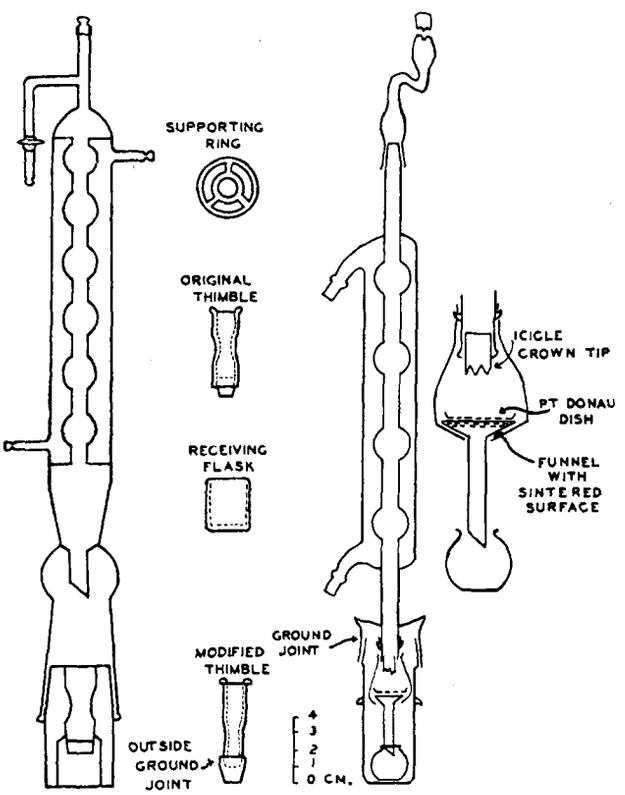


FIGURE 9. EXTRACTION APPARATUS

Left, Titus and Meloche. *Right*, Gorbach

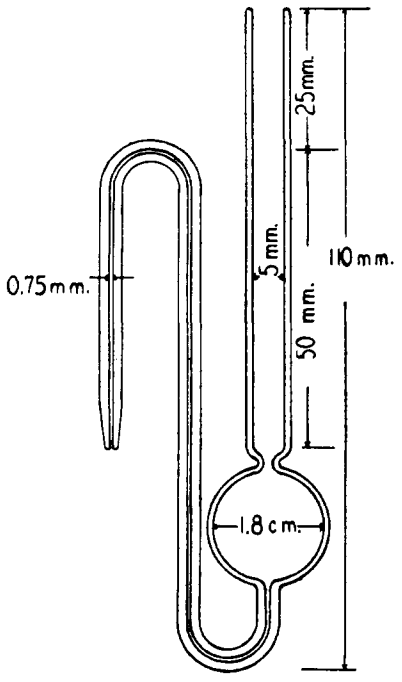


FIGURE 10. APPARATUS FOR EXTRACTION OF LIQUIDS

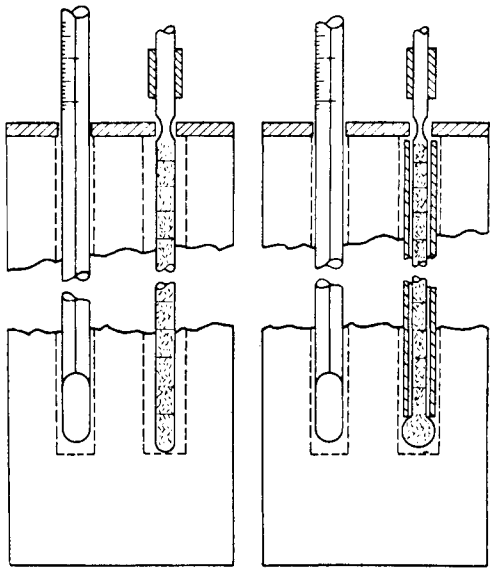


FIGURE 11. FRACTIONATION CAPILLARIES

and Mahoney (*216*), using an electrically heated block (Figure 11).

The capillary is filled with finely ground glass wool instead of being left empty. From 30 to 70 fractions from a single drop (20 to 30 mg.) are possible instead of the usual 6 to 12. It is possible to determine, with reasonable accuracy, the existence of one or more components, as well as the percentage composition.

For the accurate analytical separation of 1 to 10 ml., a spinning metal-band type (*184*) with the band (37.5 cm. long) rotating at 1000 r. p. m. gives a high efficiency (equivalent to 13 plates) with low holdup. It has been shown (*10*) that the holdup on this type of column is 0.12 ml. per theoretical plate, which is approximately 3 times less than any other. A modification by Wyman and Barkenbus (*374*), employing a vacuum, is shown (Figure 12). The analyses reported on samples of methyl esters are accurate within ±5.0 per cent, in the range 10 to 80 per cent. The values for the volatile esters have the greatest error.

An all-glass microdistillation apparatus (Figure 13) for distilling 0.5 to 2.0 grams of liquid into 9 or more receiving cups, each having a capacity of 0.1 ml. under reduced pressure without interruption, has been built by Shrader and Ritzer (*310*). A Vigreux column is used with a holdup of 0.08 to 0.12 gram.

SEPARATION OF MIXTURES OF ORGANIC COMPOUNDS BY CHROMATOGRAPHIC ADSORPTION. Organic chemists have long made use of charcoal and fuller's earth to clarify colored solutions, whereby the colored material is adsorbed and the clear solution remains. It was the botanist, Tswett, who, in 1906, proposed placing the adsorbent in a tube and pouring the colored solution through the packed column. It was demonstrated that some colored organic compounds were selectively adsorbed along the column in bands, depending upon their activity toward the adsorbent medium. This method was first employed to separate plant pigments; hence its name. The amounts of material recovered from the zones by washing out with suitable solvents (eluents) were generally disappointingly small, but with the later development of micromethods of analysis, these small quantities could be more easily analyzed and identified.

About the same time, Goppelsroeder proposed dipping a strip of filter paper in a solution, whereby the components of the solution would rise by capillary attraction and separate out in zones as in the Tswett column. This method, however, has not been found of value in preparatory work for separating organic or inorganic compounds, but for qualitative separations and tests. The filter paper may be treated with reagents which form zones of characteristic color with the components in the solution. The concentration of materials by this method which fluoresce under ultraviolet light is another application in which even some quantitative results have been reported (Germann and Hensley, *112*). The subject of capillary analysis has been reviewed by Rheinboldt (*262*) and by Germann (*111*).

Interest in this subject has revived, and many applications to problems of separating colored and colorless compounds have been made. In certain studies where large quantities of material are not available, or where qualitative runs by the Tswett method are necessary, separations using a small microcolumn may be made. The columns (Figure 14) containing the adsorbent vary from 1 to 5 mm. in bore and are 30 mm. long. At the end of the run, instead of trying to push the

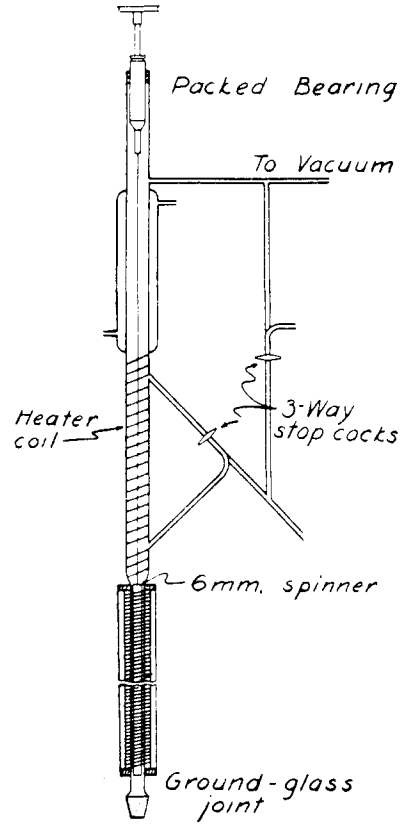


FIGURE 12. SPINNING-BAND VACUUM DISTILLATION APPARATUS

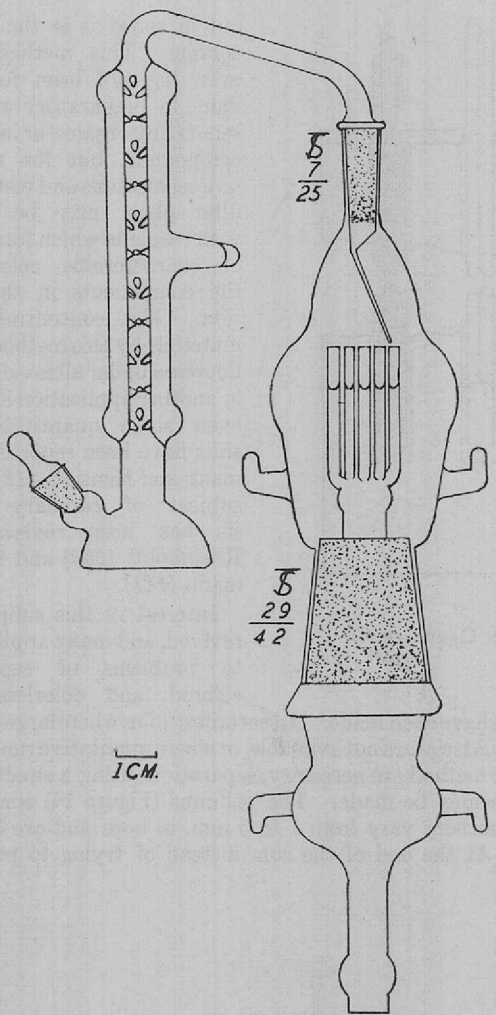


FIGURE 13. FRACTIONAL VACUUM DISTILLATION WITH VIGREUX COLUMN

column of adsorbent out of the tube, the glass may be cut where the zones appear.

Willstaedt and With (*367*) were able to separate semiquantitatively certain carotenoids when the amounts were 30 to 150 micrograms. This method of purification, concentration of traces, and separation of organic compounds has taken its place as one of the useful tools which the organic microchemist has at his disposal for working with relatively small quantities prior to analysis.

Strain (*322*) and Zechmeister and Cholnoky (*375*) give the details of working with this technique, including micromodifications, in its application to the various types of organic compounds. Strain (*323*) has recently summarized the subject from the viewpoint of the analyst. A partial list from Strain will give some idea as to the scope of this method: hydrocarbons, fatty acids, fats, amino acids, phenols, terpenes, benzene derivatives, aromatic-aliphatic compounds, condensed polycyclic compounds, sterols, heterocyclic nitrogen bases, fat-soluble vitamins, water-soluble vitamins, hormones, enzymes, and proteins, anthocyanines and pterins, natural pigments, and coal-tar dyes. Resolution of all mixtures, of course, is not possible, and while some knowledge has been gained as to the best general types of adsorbents, solvents, and eluents, their selection for any particular mixture is largely empirical, and the best conditions must be determined by preliminary tests, using small columns. The best adsorbents found so far are activated aluminum and fuller's earth.

Chromatographic adsorption methods are bound to microanalysis by the fact that, even with improved methods, the amounts obtained in the usual laboratory separations, using a column 6.5 × 42 cm., are rather small—from a few milligrams to a few tenths of a gram. Therefore, for the final identification of these fractions micromethods are essential.

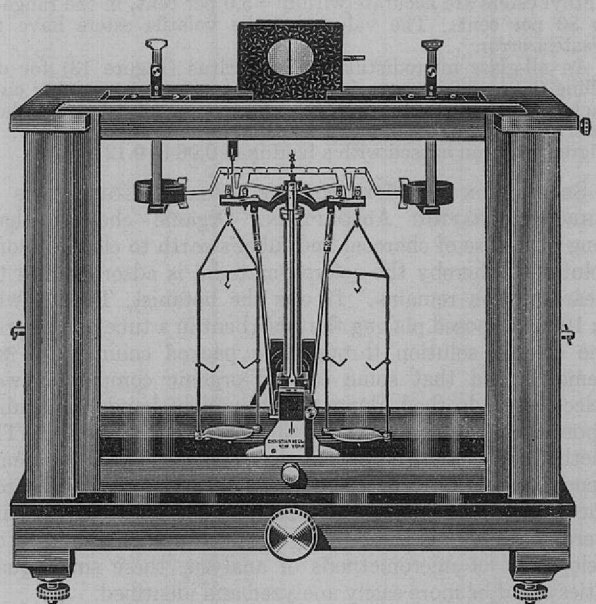
PHYSICAL METHODS

Microbalance

Because of the fundamental importance of the balance in analytical work, a brief summary of later developments and studies on the microbalance and weighing will be given. There are two general classes of balances, one which has a rapid swing period, represented by the Kuhlmann balance, and the other a long-beam balance with a greater swing period which is typical of American-built balances. Where much weighing is done and damping is not used, a short-period balance is to be preferred.

FIGURE 14. MICROCHROMATOGRAPHIC ADSORPTION COLUMN

The precision of the microbalance has been commonly considered in specifications as ±1 microgram. In general practice, however, this precision is not possible. In a recent study (*71*), using six properly working balances, the probable error of any single weighing was found to be ±3 micrograms. In this study, as well as in others (*170*), it has been found that the error in placing the rider accounts for the greater part of this variation. The angle errors cause greater variation than dislocation errors. It has been calculated that a 1° change on the Kuhlmann rider will give a weighing error of 5 micrograms. An attempt was made by Ramberg to lessen the placement error by using a quartz rod rider. However, even with this improvement it was found that an average devia-



Courtesy, Christian Becker

FIGURE 15. MICROBALANCE WITH MAGNETIC DAMPING AND PROJECTION READING

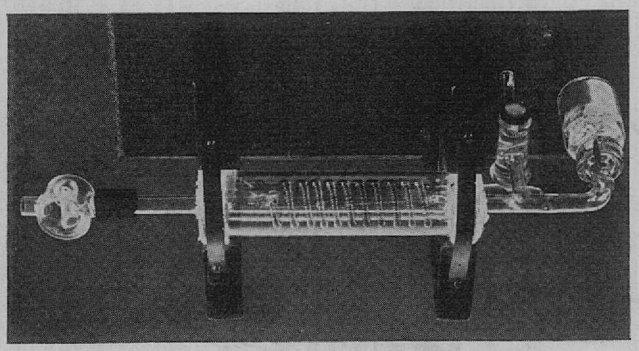


FIGURE 16. DESICCATOR FOR HYGROSCOPIC SAMPLES

tion of 2.6 micrograms was obtained in a series of weighings which is about the same precision as that obtained with other microbalances. For most microprocedures, the error in weighing, 5 to 10 mg., is much smaller than that contributed by chemical manipulation. In this laboratory the weighing variations with standard Kuhlmann balances agree with the above findings. [A cooperative study by several laboratories of microbalance performance has been conducted by the Division of Analytical and Micro Chemistry of the AMERICAN CHEMICAL SOCIETY. A statistical study of the results from twenty-two balances gives the probable error of weighing as 2 to 3 micrograms. A report of these results was made at the meeting in Buffalo, September, 1942 (*269*)].

The improvements which have been made in balances have not increased their sensitivity or precision, but have made them more convenient.

The Ainsworth and Becker balances in this country and the Bunge and Sartorius in Europe use a projection device for easier reading of the scale. Both magnetic and air damping are in use, but must be carefully regulated to prevent overdamping, which will give a premature rest point with a gradual drift to the true rest point. The degree of damping on a magnetically damped balance is easily regulated. Semimicrobalances are also available for the specified sensitivity of ±0.01 mg. Gorbach gives an illustrated review of the different types of European microbalances (*116*). Figure 15 shows a magnetically damped balance. The Ainsworth microbalance may be obtained with an optical lever and an automatic weight carrier (*222, 223*).

Weighing

Changes in temperature, humidity, and pressure all affect weighing, but practically the changes are usually all so gradual that they cause no serious error. Temperature variations of over 0.5° C. cause a shift in the zero point of balances, for which a correction should be applied. A balance room or laboratory of constant temperature and humidity is the best means of controlling these factors. However, the initial expense and upkeep is too great except for laboratories operating under severe conditions of temperature and humidity change, or where air contains dust or corrosive gases. Static on glassware or samples, which may develop under conditions of humidity below 50 per cent, can be readily discharged in a second or so with an ordinary high-frequency spark coil discharge (*351*).

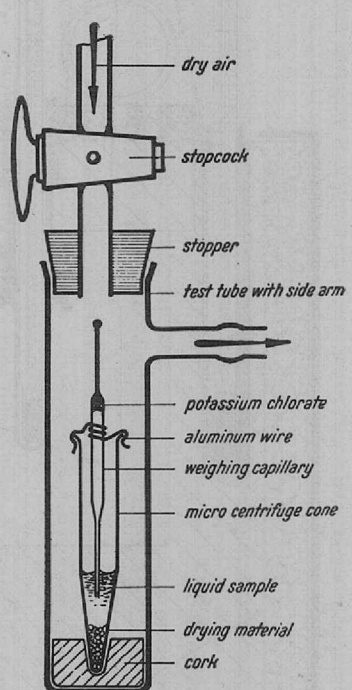
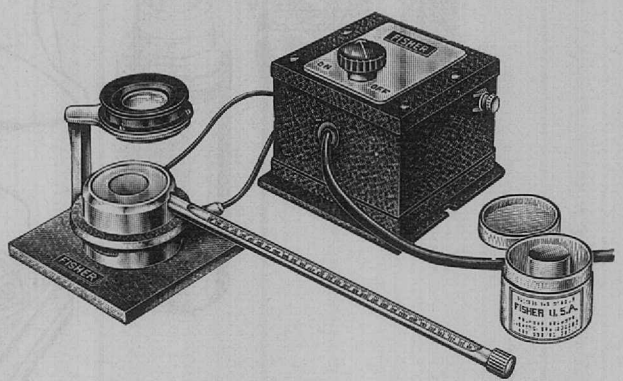


FIGURE 17. APPARATUS FOR FILLING CAPILLARIES WITH LIQUIDS



Courtesy, Fisher Scientific Co.

FIGURE 18. FISHER-JOHNS MELTING POINT APPARATUS

The accurate weighing and handling of hygroscopic substances are difficult and the problem is thoroughly treated by Alber (*5*). The best technique yet developed is that by Rodden (*268*), in which the sample is dried in a boat and transferred to a special weighing stopcock, so arranged that the transfer from the desiccator to the stopcock and then to the combustion tube is accomplished under moisture-free conditions. Figure 16 shows an electrically heated desiccator with the special weighing stopcock attached.

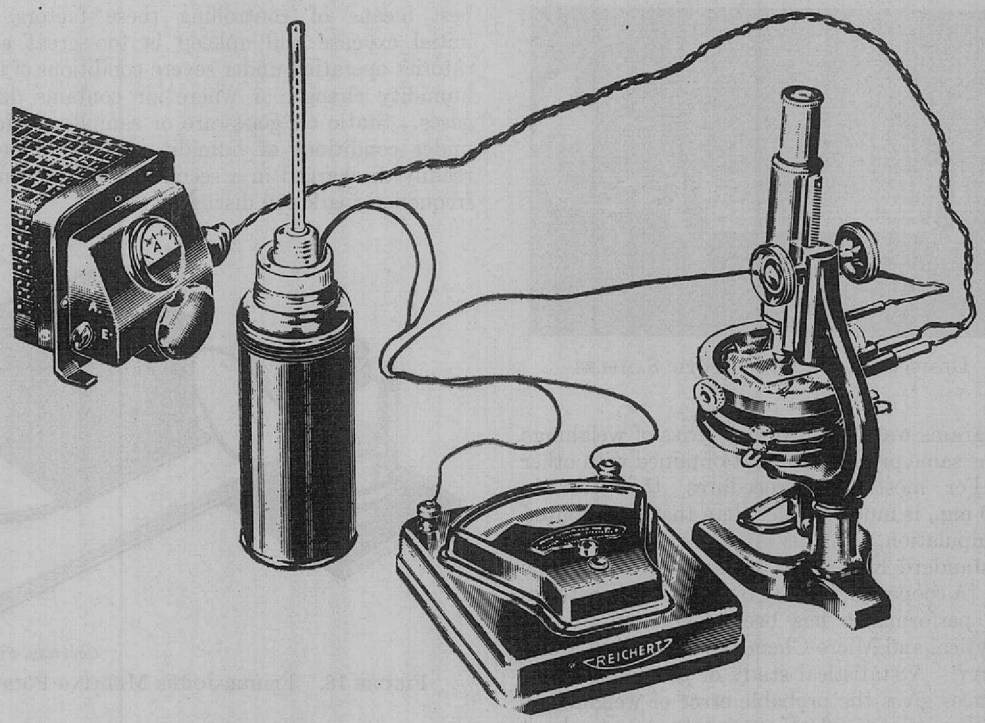
Filling Capillary Tubes

Capillary tubes are best filled by inserting the end of the capillary in the liquid contained in a small vial which is placed in a filter flask (Figure 17) and evacuated momentarily (*5*). This is a great improvement over the usual method of heating and cooling the capillaries.

Melting Point

This determination, even by the standard capillary tube method, does not require more than a few milligrams of sample. For a more rapid and convenient method, an electrically heated copper or aluminum block may be used and the melting point of the sample, placed between cover glasses, is observed through a low-power lens. The commercially available Fisher-Johns apparatus (Figure 18) is a good example of this type. For the determination of the melting point of very small quantities of material down to a single crystal, several special hot stages for microscopes have been designed, some of which allow the melting point to be taken in the presence of an inert atmosphere.

The temperature may be measured with an accurate thermometer or with a sensitive thermocouple. Figure 19 shows a microscope fitted with a Kofler hot stage and thermocouple. The true melting point is most accurately observed under a polarizing microscope for all crystalline compounds except those which are isotropic solids. The temperature at which the color disappears under polarized light is the true melting point, because it represents the point at which the space lattice is ruptured. With such an instrument, Zscheile and White (*378*) obtained a precision of ±0.04° C. on known



Courtesy, Microchemical Service

FIGURE 19. KOFLER-HILBCK HOT STAGE FOR DETERMINING MELTING POINT

compounds. Melting points determined under the microscope are apt to be lower by as much as 1° C. because the point at which melting occurs is more easily seen than by the capillary tube method, which is the one commonly employed for compounds whose melting points are recorded in the organic literature.

A modification by Morton and Mahoney (*217*) of the Emich capillary tube method uses an electrically heated copper block and an optical system to project an image of the capillary on a paper screen (Figure 20). This permits the melting point to be observed more easily than when a liquid heating bath is used. Methods using the electrically heated metal block are not limited in their range of temperature and the rate of heating can be more carefully controlled. While the precise determination of melting point is justified on specially purified compounds, for purposes of identification, a precision of ±0.5° C. is generally satisfactory.

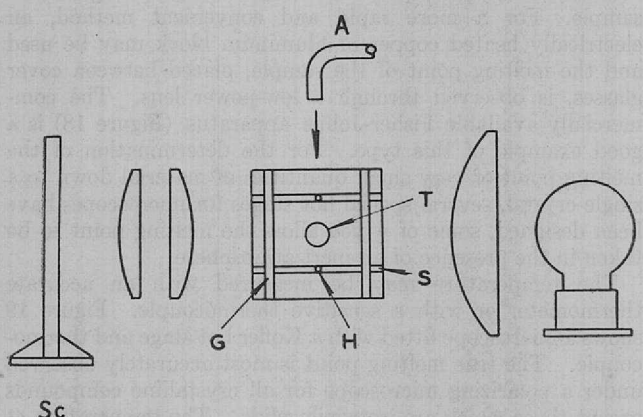


FIGURE 20. APPARATUS FOR OBSERVATION OF MELTING OR BOILING POINT BY PROJECTION

A. Air blast for cooling copper block
G. Pyrex plate
H. Hole for capillary
S. Slit for observation
Sc. Screen
T. Thermometer well

DEPRESSION OF THE MELTING POINT (Determination of Molecular Weight). When a small sample of an organic compound is mixed with camphor or borneol contained in a melting

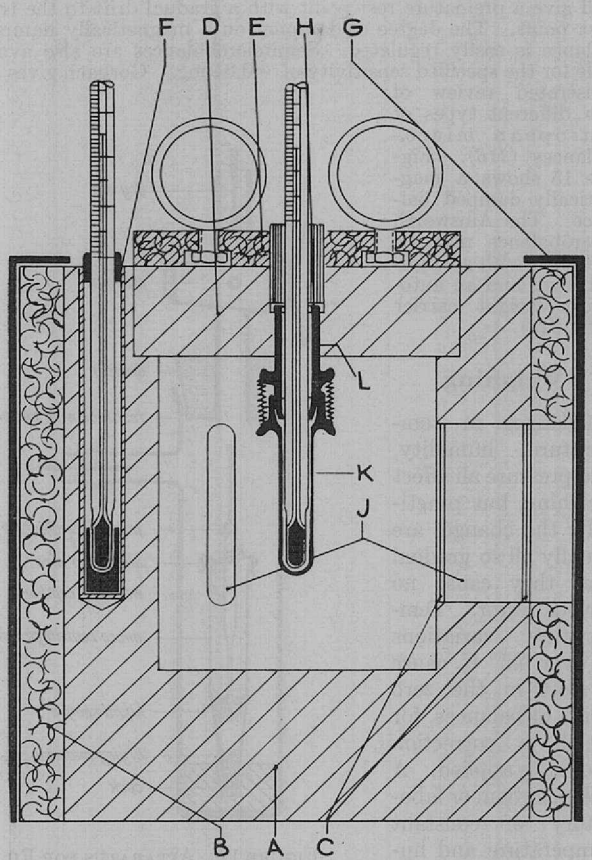


FIGURE 21. COOLING-CURVE APPARATUS

point capillary, a large depression in the melting point is observed. Advantage is taken of this for the determination of the molecular weight of a sample as small as 1 mg. The camphor is first standardized with a pure compound of known molecular weight. The method (*296–305*) is extremely simple and can be carried out by anyone who is familiar with taking a melting point. An ordinary thermometer reading to 0.1° can be used. The method fails with compounds which are not soluble or which decompose.

Cooling Curve. While the determination of a cooling curve is considered the best method of establishing the purity of a solid organic compound, it is seldom used because it requires several grams of the sample. The recent publication of a method (*316*) using only 200 to 300 mg. should make this excellent procedure of more general application. The material contained in a special glass tube is placed in a copper block and heated so that a constant difference in temperature is maintained between the sample and its surroundings. The temperature rise in degrees centigrade is plotted against the time in minutes. The curves which have a characteristic shape when the material is pure are reproducible within 3 per cent. Figure 21 shows the apparatus.

Mixed Melting Point. When two compounds are mixed together and the melting point does not change from that taken separately, it usually indicates that they are identical. This can be quickly determined in a melting point tube with small amounts of material. Constants, such as melting point, cooling curve, and boiling point, which are obtained by heating the compound, have no value for purposes of identification if, during the process, they undergo decomposition. However, modifications formed during heating, which have a definite composition, can be detected by the cooling curve method.

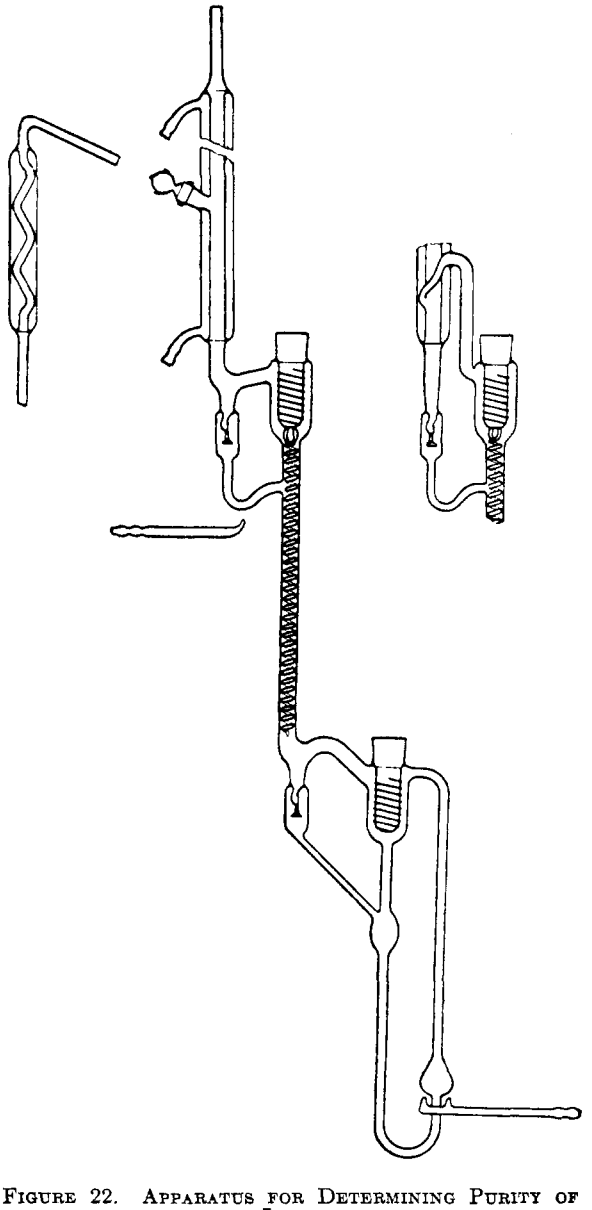


Figure 22. Apparatus for Determining Purity of Liquids

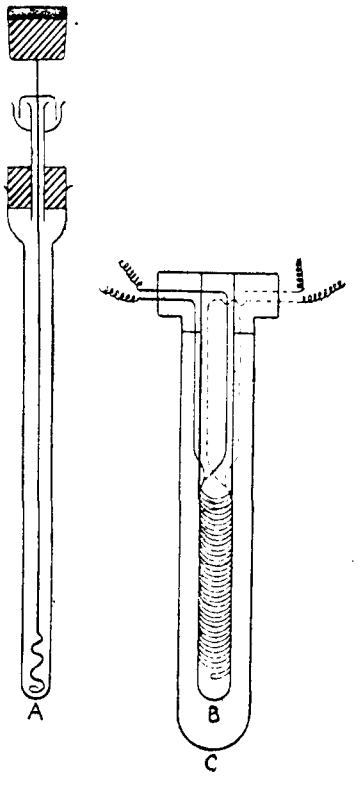


Figure 23. Apparatus for Determining Freezing Point

Boiling Point

The same modification of the Emich method (*217*) described for the melting point (Figure 20) is used to observe the boiling point. The boiling point is reached when the drop of liquid contained in the capillary and heated in a copper block appears at the top of the block. Niederl and Routh (*235*) discuss the determination of the boiling point and the corrections which must be applied.

Purity of Organic Liquids

Swietoslawski (*334*) points out that the purity of an organic liquid can often be determined by the simultaneous measuring of the boiling point and the condensation temperature of the vapor. For this purpose, 25 to 50 ml. are usually required. To allow 8 to 10 ml. of liquid to be used, the design shown in Figure 22, modified from a larger apparatus, has given satisfactory results. While the boiling point of a liquid as normally measured may be off only a few tenths of a degree, on the apparatus shown the difference in temperature between the boiling liquid and the condensation temperature may be as much as 1° C.

An arbitrary scale of purity is proposed. The classification of the liquid according to the exact degree of purity is conditioned by the vapor tension curve of the mixture formed by the impurities and the liquid, and the percentage composition of the impurity present. This method is, therefore, only an aid in the estimation of absolute purity, which must be substantiated by other physical data.

Molecular Weight

By Rise of the Boiling Point. It is very difficult in the Pregl microform of the apparatus, employing a gas burner and a Beckman thermometer immersed in the boiling liquid, to obtain uniform boiling and a constant temperature. This difficulty, of course, is present in some macroforms.

It was Cottrell who first proposed the pumping of liquid and vapor around the thermometer to eliminate superheating.

Riechi (*264*) and later Sucharda and Bobranski (*329*) designed apparatus which incorporate the Cottrell principle and permit constant temperature to be attained more easily. However, these designs do not allow the actual volume of the solvent present to be measured during boiling. To guard against error from changes in pressure, two units should be set up, one with the solvent alone and the other with both solvent and sample. Both apparatus are available commercially.

The micromodification of the Menzies and Wright apparatus, developed by Smith and Milner (*304*), also minimizes the difficulties mentioned above by boiling the liquid with an electrically heated platinum coil placed therein, and using the Cottrell method of pumping liquid and vapor over the bulb of the thermometer, which prevents superheating. The volume of the liquid can be determined accurately by this method. A water-differential thermometer, whose readings are not affected by a change in atmospheric pressure, is used in place of the Beckman. The water differential thermometer, however, limits the choice of solvents to those boiling below 100° C. A sample, 15 to 30 mg. for each addition using 5 ml. of solvent, is required in both methods and the accuracy is within ±5 per cent. The sample, if necessary, can be easily recovered from the solvent.

By Depression of the Freezing Point. The previous methods of determining molecular weight involve heating the material which, in some cases, may decompose the compound or cause it to dissociate. The molecular weight is not commonly determined by measuring the lowering of the freezing point, using microquantities.

A miniature Beckman apparatus (*142*), in which 2 to 3 ml. of solvent and 5 to 10 mg. of sample are used, has given very satisfactory results in this laboratory. A design (*168*) which uses a sensitive thermocouple, instead of the Beckman microthermometer, to measure the lowering of temperature has given good results with 5 to 10 mg. of sample in 1 ml. of solvent (Figure 23). *A* shows the tube with the electromagnetic stirrer for the solvent mixture, which is placed in tube *B*, wound with silver wire for the electro-resistance thermometer. Tube *C* is the usual air jacket which is placed in the freezing mixture.

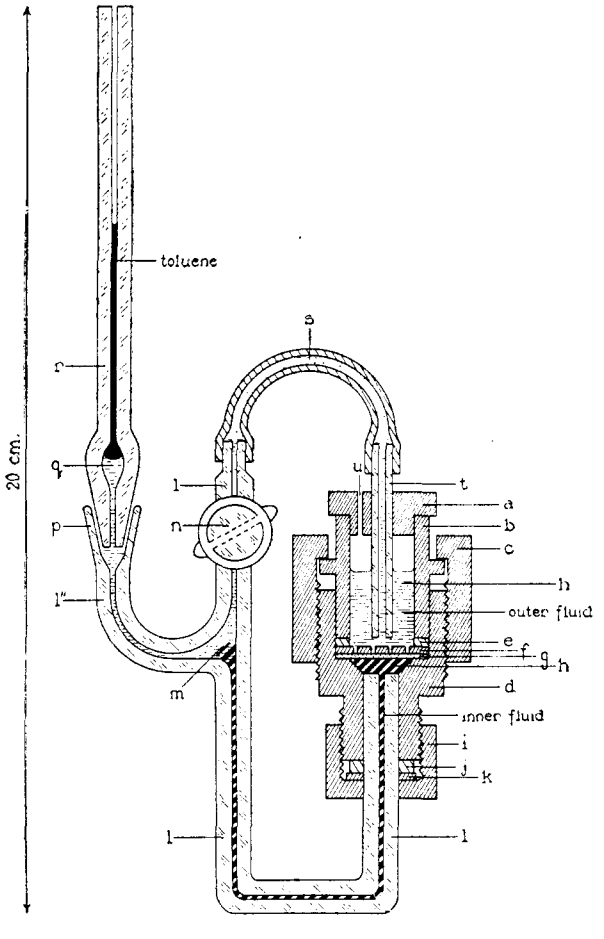


Figure 24. Apparatus for Measuring Osmotic Pressure

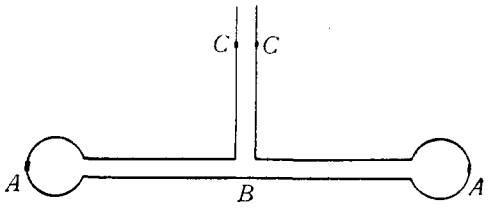


Figure 25. Diagrammatic Sketch of Thermoelectric Method

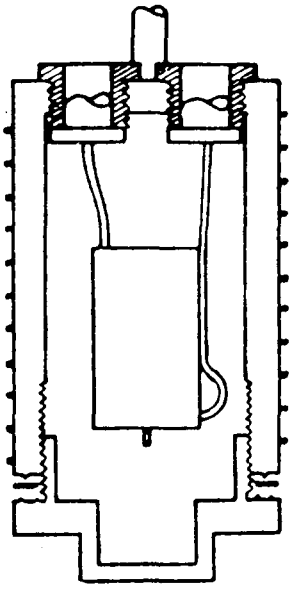


Figure 26. Calorimeter for Heat Capacity

By Isothermal Distillation. The method of determining molecular weights by isothermal distillation, which has received considerable attention, is based upon the fact that if two solutions of different molarity are placed in a common container, solvent from the solution of lower molarity will distill into that of higher molarity until they are equimolar. While the method can yield accurate results, the elapsed time necessary to make a determination requires 1 to 2 weeks. The method is, therefore, suitable only for those investigations in which time is not a factor. It requires about 5 mg. of sample and generally involves the preparation of a series of comparison standards.

Niederl and Levy (*230, 232*, p. 230) have worked out an improved but conventional technique with which a series of known of varying concentrations is paired with the unknown, contained in capillaries. The molarity can be determined within ±0.01 in the range 0.05 to 0.15 molar. Clark (*62*) prefers Singer's method, which involves preparing only one standard. The standard and a weighed amount of unknown contained in 1.5 ml. of solvent (approximately 0.1 molar) are placed in separate but connected bulbs, each having a graduated side arm for measuring the volume of the solution. The system is evacuated and the solutions are allowed to distill isothermally until the volume readings become constant. From these the molecular weight may be calculated. The results reported are all within 2 per cent of theory.

By Victor Meyer Method. For volatile liquids the best method is a micromodification (*306*) of the Victor Meyer apparatus in which 5 to 10 mg. of sample are vaporized in a container of known volume. The volume of the unknown sample is then determined by measuring with a mercury manometer the change in pressure at constant volume from which the molecular weight can be calculated.

Osmotic Pressure

For the Determination of High Molecular Weights. An example of the type of apparatus used for measuring

osmotic pressure of 0.2 ml. of fluid by the membrane method is shown in the design (Figure 24) taken from the work of Bourdillon (*41*).

The unit holding membrane *g* is made from hard rubber. The inner fluid represents the sample. At room temperature the final reading can be made after 8 hours. There is no correction for specific gravity or capillarity. The precision reported on solutions as low as 0.025 *M* is within 5 per cent.

By the Thermoelectric Method. The calculated theoretical difference in temperature that should exist between two thermally insulated surfaces in moist air at 20°, one wet with water and the other with 0.9 per cent sodium chloride, should be about 0.06°.

Baldes (*12, 13*) has modified Hill's thermopile method by measuring this difference in temperature from which the osmotic pressure may be calculated. Thermocouples of manganin and constantan wire, 0.1 to 0.05 mm. in diameter, are used. In Figure 25, *A B A* represent constantan wire about 15 mm. in length, soldered to manganin, the junctions being at *A*, which, in turn, are soldered to copper wire at *C*. Loops containing the thermojunction at *A* are made 1 to 2 mm. in diameter. The thermocouple units are insulated with Bakelite varnish. A drop of the known (0.1 to 2 cu. mm.) is placed in one loop and compared with a drop of the unknown sample. The osmotic pressures of aqueous solutions can be determined accurately within a fraction of 1 per cent on quantities as small as 0.1 mg. A critical discussion of the limitations and factors which affect the accuracy of this method is given by Roepke (*270*).

Calorimeters

Heat Capacity of Organic Compounds. Stull (*328*) has designed a semimicrocalorimeter (Figure 26) for determining the specific heats, together with heats of transition and fusion of organic compounds, over the temperature range 100° to 320° K.

The apparatus does not employ a vacuum and requires only 5 to 6 ml. of sample. Measurements were made with a maximum error of 1.25 per cent.

Swietoslawski *et al.* (*18, 337*) have determined specific heats of solids and liquids, using a microcalorimeter in an apparatus with twin adiabatic calorimeters.

Note. Swietoslawski *et al.* (*336*) report that the smallest calorimeter used for radiological investigations was a silver vessel weighing 0.48 gram and having a thermal capacity of 0.08 calorie, which included the glass containing the sample. This small vessel is placed in a copper block which is evacuated and the whole is placed in a vessel containing water. Temperature is measured by a Beckman thermometer or by means of a thermocouple.

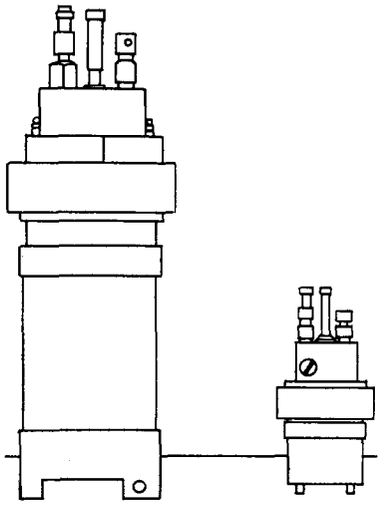


Figure 27. Oxygen Combustion Bomb

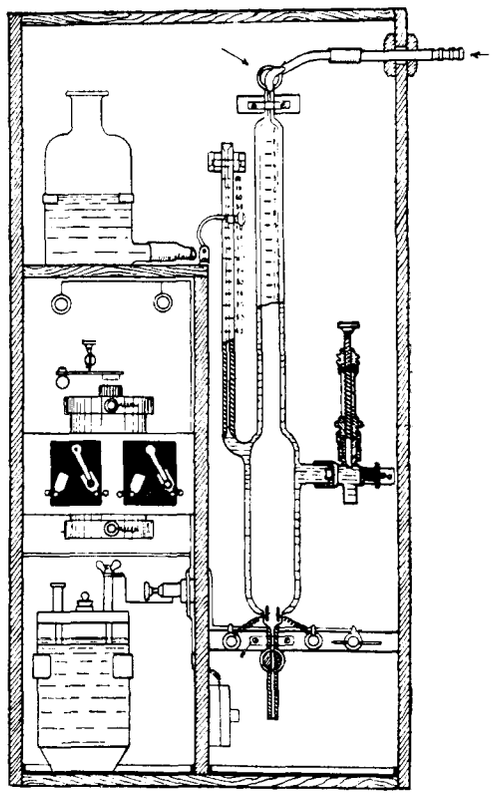


Figure 28. Union Gas Calorimeter

Lipsett, Johnson, and Maas (*192*) designed a calorimeter of the adiabatic type having a heat capacity of 1 calorie and a volume sufficient to hold 4 ml. of solvent. It was employed for measuring heats of solution.

Heats of Adsorption and Vaporization. Swietoslawski and Bartoszewicz (*335*) have designed an adiabatic microcalorimeter requiring only a few centigrams of liquid with which they determined the heats of vaporization of water, benzene, and chloroform with an error not exceeding ±0.03 per cent. The heat of absorption on activated charcoal of benzene, ethyl alcohol, and carbon tetrachloride was measured with an error of 0.6 to 1.7 per cent.

Heat of Combustion. By the Oxygen Bomb. Padoa and Foresti (*241*) developed a microcombustion bomb of 10-ml. capacity for organic material, which was used in a Bunsen ice calorimeter.

When using 6 to 8 mg. of sugar and benzoic acid, they obtained an accuracy of within 1 per cent, and with 30-mg. samples, 0.2 per cent. More recently Papp (*242*), using a similar apparatus, determined the heat of combustion of Acheson graphite accurate to 1 per cent.

Roth, Ginsberg, and Lasse (*278, 279*) used a bomb of 21.5-ml. capacity and a sample of 100 to 200 mg. The results were accurate to ±0.5 per cent. Figure 27 shows the bomb compared to a bomb of regular size.

Vrijling (*358*), working with the Roth-Hugerschoff calorimetric microbomb mentioned above, obtained satisfactory results for the heats of combustion on benzoic acid and petroleum oils.

Gas Calorimeter. This determination with the conventional apparatus uses a large volume of gas.

Blackie (*36*), working on a problem in which only a few milliliters of gas were obtained from the carbonization of small samples of coal, made a study of the Union gas calorimeter and found that, with careful manipulation and standardization of the procedure, as little as 5 ml. of gas could be used. The instrument (Figure 28) consists essentially of a graduated ex-

plosion pipet into which a few milliliters of the gas to be tested are drawn, the rest of the space being filled with air for combustion. The pipet is surrounded by a jacket containing a liquid of a high coefficient of expansion, which jacket is connected to a graduated capillary tube. On explosion the heat evolved is given up to this liquid, which expands, the rise being proportional to the heat absorbed. For purposes of comparison, the pipet is filled with dilute sulfuric acid and electrolytic gas generated. This volume is measured, diluted with air, and exploded, the rise in the capillary tube being noted. As the heat generated in this explosion is known, the heat given out by the gas, whose calorific value is required, can be calculated from the ratio of the rises in the capillary tube. The capillary rise per milliliter of standard electrolytic gas was 4.28 mm. The error with gases up to 550 B. t. u. should not exceed ±0.5 per cent; with gas up to 900 B. t. u., ±2 per cent.

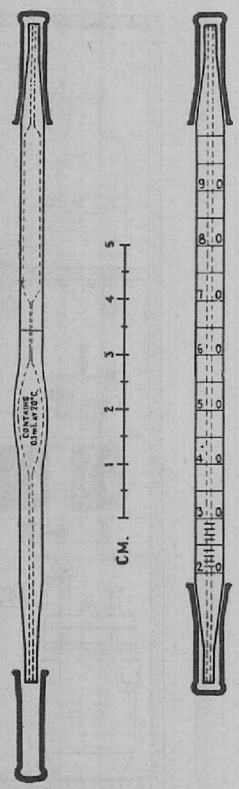


FIGURE 29. PYCNOMETERS

Density

SOLIDS. A general review of the micromethods for the determination of the density of solids and liquids is given by Blank and Willard (*37*) and by Alber (*3*).

Rapid methods for measuring solids using microquantities are not too satisfactory as regards accuracy. Two relatively simple and rapid methods may be used.

1. A capillary tube, 15 to 20 cm. in length and 1 mm. in internal diameter, is calibrated in milliliters and sealed to a larger U-shaped tube. The confining liquid is sealed in the apparatus by mercury. A weighed sample, 10 to 200 mg. (not finely powdered), is placed in a cup on the end of a flexible steel wire. This is inserted under the mercury, so that the sample finally rises to the surface and enters the limb containing the confined liquid. The volume of the sample is read by the rise of the liquid in the capillary tube and from this the density may be calculated. The accuracy is approximately 5 per cent.
2. A well-known method, restricted to a limited range of density, consists in varying the density of a heavy liquid, such as carbon tetrachloride or methylene iodide, with a lighter, miscible solvent until a small particle of the material just floats in the liquid. If the material is finally powdered, the entrapped air carried into the liquid is removed by centrifuging. If a particularly accurate value is required, the final adjustment may be made by controlled cooling or heating of the liquid in a dilatometer, until an exact balance between liquid and solid is obtained. The limiting factor with the method is the accuracy with which the density of the reference liquid is determined. Alber (*3*), measuring the density of the reference liquid with special micropipets, obtained an accuracy of 5 parts per thousand, when using volumes of 0.01 to 0.1 ml. The amount of material required is 50 to 250 micrograms.

DENSITY, USING THE MICROSCOPE. An approximately 1-ml. cell, constructed from glass tubing of known diameter, and mounted on a glass slide, is partially filled with a liquid, such as water, whose height is determined by means of a microscope, the fine adjustment of which has been calibrated. A sample of 10 to 100 mg. is introduced into the cell and the change in height determined, from which the volume and density are calculated. The accuracy is approximately 1 per cent, as determined by the density, using the macropycnometer method (*37*).

LIQUIDS. The determination of the density of liquids, unlike solids, is one of the most important physical constants in organic analysis. The method for this purpose should have an accuracy of 1 per cent or better over a wide range, and it should be possible to recover the liquid for other determinations.

The *Schlieren* method, while it is accurate to 0.0005 and requires little sample, suffers from the fact that the sample is not recoverable under ordinary circumstances, and numerous reference liquids must be available.

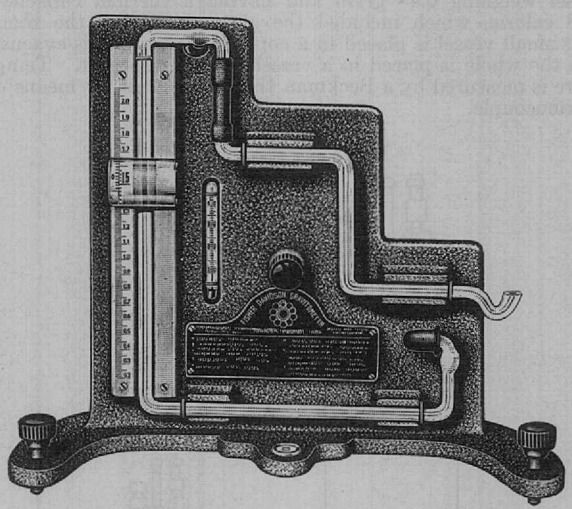
The pycnometer is the most precise method. For microwork the best form is the pipet type (*3*), as shown in Figure 29. The greatest error comes from reading the volume, which is such that weighing to ±0.01 mg. is sufficient when using the 1-mm. pipet. This gives an accuracy within 0.5 part per thousand, when using a volume of 0.1 ml. With the smaller pipet of 1-mm. bore, the accuracy is 10 parts per thousand, and with 0.5-ml. bore, it is within 5 parts per thousand.

A more rapid method for general laboratory work consists in comparing a liquid of known density with that of an unknown, using the balanced-column method. The volume of the sample required is 0.25 to 0.75 ml., depending upon the density of the liquid under test. A known and constant pressure is applied to the unknown liquid in the capillary, and the rise is noted. The point to which water will rise under the same pressure is then determined, and from this the density of the unknown may be calculated. The accuracy is approximately 3 parts per thousand. A commercial apparatus (Figure 30), involving this principle, and using amounts of liquid as low as 0.3 ml. is available. The unknown sample is placed in one column and compared to a standard placed in the other. The accuracy is dependent upon the standard liquid used for comparison. The scale is graduated in 0.01, and one-half a division can be easily estimated.

Fenger-Eriksen, Krogh, and Ussing (*96*) describe a method whereby the specific gravity of liquid is determined by measuring the rate of fall of drops through an immiscible fluid. A precision pipet is used to expel highly uniform drops of any size between 0.01 to 0.1 ml. It has been used for determining the D_2O content, corresponding to density measurements with an accuracy of 1 to 2 in the sixth decimal place, which is about equal to 0.001 per cent D_2O. Figure 31 shows a precision pipet designed by Rosebury and van Heyningen (*272*) for the method just described.

Dielectric Constant

Sufficient data on the dielectric constant of organic liquid compounds are available, so that this measurement can be an aid in the identification and the degree of purity of liquid substances. The contamination of a liquid such as benzene with water can be detected with an accuracy of 0.001 per cent.



Courtesy, Fisher Scientific Co.

FIGURE 30. FISHER-DAVIDSON GRAVITOMETER

Some modifications of the method do not require a large amount of sample, and the cell (*259*) shown in Figure 32 uses about 1 ml. The coatings of the condenser are formed by platinizing the inner tube on the outside and the outer tube on the inside. An excellent review of this subject is given by Pavelka and Kirigin-Mardegani (*243*), who describe a cell and

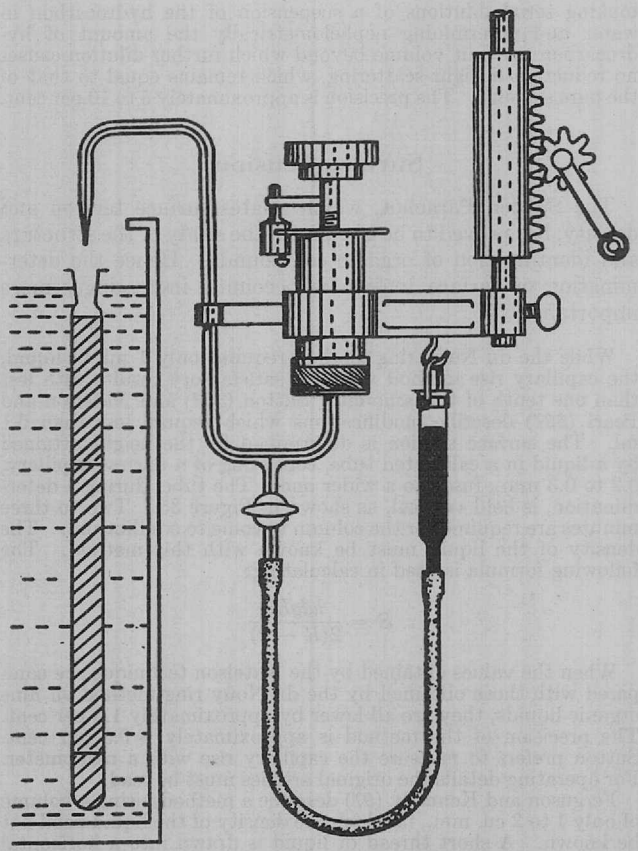


FIGURE 31. APPARATUS FOR DETERMINING DENSITY BY FALLING DROP METHOD

method requiring only a few tenths of a milliliter for carrying out this determination rapidly where the highest accuracy is not required.

Refractive Index

This physical constant is also very useful in the identification of liquids because data are available; but for solids much remains to be done.

The Abbe refractometer, found in most laboratories, requires relatively small amounts of liquid (50 to 100 cu. mm.) and is accurate to ±0.0001. The Zeiss dipping refractometer can be equipped for micro work, which allows a sample of 20 cu. mm. to be measured to within a few units in the fifth decimal place. Methods of less but adequate precision (±0.001) for identification purposes and using less sample (0.1 to 5 cu. mm.) have been developed. Of these, the instrument designed by Jelley (*144*) is rapid and simple, uses only 0.1 cu. mm., and, when properly constructed, is accurate to ±0.001. If the drop of liquid is not homogeneous, this fact may be determined with the microprism, in the case of a mixture of liquids of different volatility, by noting any change in refractive index on standing. In the case of nonvolatile liquids, heating of the prism is necessary and, after cooling, noting any change. Several workers (*6, 152, 228*) have reported on a method which correlates the change of depth of focus with a change in refractive index of the liquid. The volume of sample required is approximately 5 cu. mm. and the accuracy is ±0.001. The instrument is calibrated by liquids of known refractive index.

For solids, an immersion method may be applied, using the above apparatus with the same accuracy. However, the accuracy with which the crystal may be orientated is usually of a lower order, such as to give only ±0.003. Here the refractive index of the liquid is varied until the Becke line disappears, which indicates that the refractive index of solid and liquid is the same. Instead of employing a series of standard liquids for this comparison, the refractive index may be changed by varying the temperature of a single liquid. The refractive index of the liquid at that temperature is then determined by any of the regular methods.

Instead of determining the refractive index of the solid, which is time-consuming, the refractive index of the material at its melting or solidification point may quickly be taken by the method proposed by Jelley, using the instrument designed for liquids. If a crystal of the solid is placed in the microprism and heated until the solid melts, its refractive index can be obtained as readily as that for liquids. Frediani (*102*) shows an instrument incorporating these ideas (Figure 33), using the Fisher modification of the Jelley refractometer with an electrically heated prism and thermometer with which the melting point also may be observed, accurate to within 1° to 2° C. The refractive index of compounds which sublime, decompose, or have a high vapor pressure at the melting point cannot be determined by this method. The results are accurate to ±0.002.

Similar identification methods have been published by Reimers (*260*), using the Kofler method for determining refraction at the melting point.

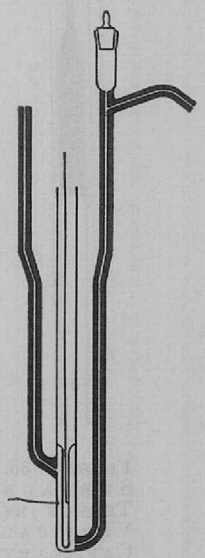
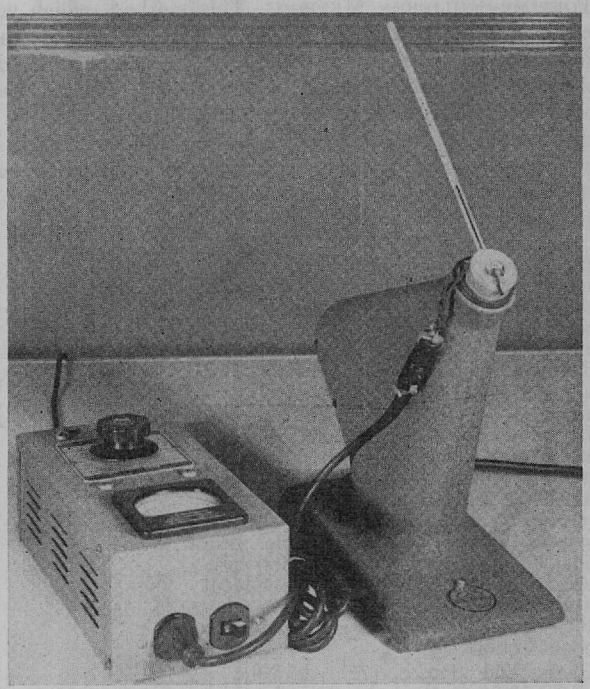


FIGURE 32. CELL FOR MEASURING DIELECTRIC CONSTANT

Specific Rotation

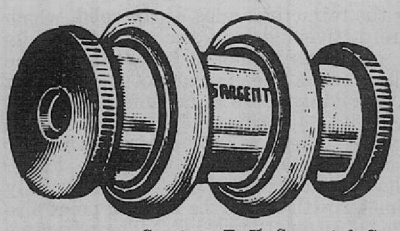
Fischer (*99*) showed that by using capillaries, 5 cm. in length, as polarimeter tubes, results which equaled those obtained with the regular macromethod were possible. Donau (*81*) used tubes of 0.4- to 0.5-mm. bore with satisfactory results. The average of results when compared with capillaries both 5 and 10 cm. long varied approximately ±1.0 from the average of those obtained with the regular-size 10-cm. tube. The variations in successive readings on the same sample are slightly greater than those with the macrotubes.

The Fischer tubes were 1.5 mm. in bore and 5 to 10 cm. in length. The variations on cane sugar, using these tubes, were less (approximately ±0.5 per cent) than the accepted values. It is, therefore, entirely satisfactory (*33*) to measure specific rotation with samples of 5 to 10 mg. and volumes as low as 0.1 ml. with the instruments which are



Courtesy, Fisher Scientific Co.

FIGURE 33. APPARATUS FOR DETERMINING REFRACTIVE INDEX OF SOLIDS AT MELTING POINT



Courtesy, E. H. Sargent & Co.

FIGURE 34. FISCHER POLARIMETER MICROTUBE

used for macropolarimetric measurements. These tubes (Figure 34) are commercially available in 5- to 10-cm. lengths, bore 1.6 to 2.5 mm., and volume 0.1 to 0.5 ml.; semimicrotubes in 10- to 20-cm. lengths, and bore 2 to 4.5 mm. These latter may be had in either metal or glass.

Determination of Solubility

The sample, either liquid (0.1 cu. mm.) or solid (0.1 mg.), is drawn into a capillary tube of 0.5-mm. bore and then about 10 cm. of solvent are added. The end of the capillary is sealed and the two liquids are centrifuged to the closed end. Mixing may be accomplished by means of a thin glass thread with the end fused to form a droplet, or by sealing the other end of the capillary and centrifuging the liquid back and forth. If, after this, the liquids appear clear and homogeneous, they are soluble; if turbid, or if two phases persist, they are insoluble. (For a quantitative study of the phenol-water system, see Smith, *318*.) In the case of solids, it has been found best for qualitative analysis to add the maximum amount of solvent—25 times the weight of the sample.

If a residue remains, the material is termed insoluble. The capillary method of determining solubility is recommended for all semiquantitative work (*291*).

TOEPLER-SCHLIEREN METHOD. This method, which was mentioned under Density, is recommended only for the rapid qualitative detection of the solubility of liquids or solids. The observation was made many years ago by Toepler that when one liquid is added to another, or when a soluble solid is added to a liquid, *Schlieren* or streaks occur, due to regions of changing refraction. These disappear after solution is complete and it again becomes optically homogeneous.

In applying this method to the determination of the solubility of solids and liquids (*291*), the sensitivity must be decreased by using a simple glass cell made from tubing of 4-mm. inside diameter and 60 mm. long, sealed at one end. This holds the static sample, the solvent in this case. The fluid sample whose solubility is under test is added to the static sample from a micropipet with the tip just under the surface of the solvent. The tube is held so that the illumination is obliquely downward. If streaks are observed, the sample is soluble. For solids, a saturated solution is first prepared by adding the solid to the solvent contained in the depression of a spot plate. The clear solution is then used as the test liquid.

Davis and Parke (*76*) describe a nephelometric method capable of measuring solubilities of polycyclic hydrocarbons down to approximately 1 microgram per liter. The method consists in making serial dilutions of a suspension of the hydrocarbon in water and determining nephelometrically the amount of hydrocarbon per unit volume beyond which further dilution causes no reduction in light-scattering, which remains equal to that of the pure solvent. The precision is approximately 5 to 10 per cent.

FIGURE 35. SURFACE TENSION BY VERTICAL CAPILLARY METHOD

Surface Tension

The Sugden Parachor, which relates surface tension and density, has proved to be of value in the study of the structure and identification of organic compounds. Hence the determination of surface tension is becoming increasingly more important.

While the du Noüy ring method requires only 1 ml. of liquid, the capillary rise method will give satisfactory results with less than one tenth of this amount. Sutton (*332*) and Natelson and Pearl (*227*) describe modifications which require less than 0.1 ml. The surface tension is determined by the height attained by a liquid in a calibrated tube, consisting of a narrow capillary, 0.2 to 0.3 mm., fused to a wider one. The tube, during a determination, is held vertical, as shown in Figure 35. Two to three minutes are required for the column to come to equilibrium. The density of the liquid must be known with this method. The following formula is used in calculating:

$$S = \frac{hdgRr}{2(R - r)}$$

When the values obtained by the Natelson technique are compared with those obtained by the du Noüy ring method on nine organic liquids, they are all lower by approximately 1.5 per cent. The precision of the method is approximately ±0.5 per cent. Sutton prefers to measure the capillary rise with a manometer. For operating details the original articles must be read.

Ferguson and Kennedy (*97*) describe a method, using a volume of only 1 to 2 cu. mm., in which the density of the liquid need not be known. A short thread of liquid is drawn into a horizontal capillary, shown at the right in Figure 36. By increasing the pressure, through raising the container at the right, the thread of liquid is moved along the capillary until it is plane with the end. This is judged more easily by using a lower-power microscope and a small lamp, as shown at the left of the capillary. The pressure required to bring the liquid to the "plane" position is measured by a manometer filled with aniline. With tubes of less than 1-mm. bore, operating in a horizontal position, the formula for calculating the surface tension is:

$$\gamma = 1/2\ rhpG$$

where h and p refer to the manometer liquid.

Bowden (*42*) modified Sugden's capillary rise method so that reactive or hygroscopic liquids can be run. Sugden's method involves the measurement of the difference in level to which the liquid rises in two capillary tubes of different radii. Figure 37 shows the two capillaries, B and C, sealed to the tube, A, 7- to 10-mm. bore. The liquid, if necessary, may be vacuum-distilled

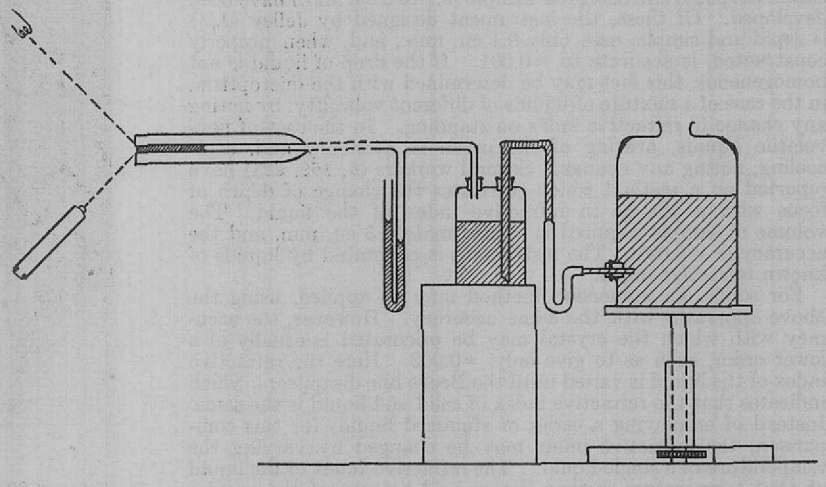


FIGURE 36. SURFACE TENSION MEASUREMENT BY HORIZONTAL CAPILLARY

into A in the absence of air and sealed at S. The surface tension can, therefore, be measured under its own vapor pressure and over moderate ranges of temperature.

The Bowden apparatus, while not developed specifically for micro work, does not require excessive amounts of sample and, since Sugden's apparatus allows working with smaller quantities, the diameter of tube A undoubtedly could be made smaller.

The formula used is:

$$\gamma = \frac{3h + (r_2 - r_1)(D - d) g r_1 r_2}{6(r_1 - r_2)}$$

where h = difference in height in the two capillaries
r_1 and r_2 = radii in the two capillaries
D = density of liquid
d = density of vapor
g = acceleration due to gravity

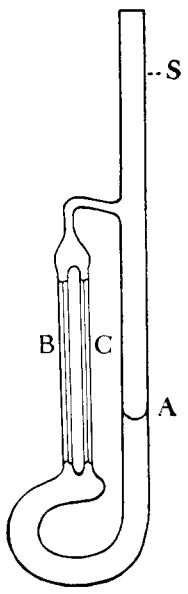


FIGURE 37. SURFACE TENSION BY DOUBLE CAPILLARY METHOD

Sugden's method (*330*), using a cathetometer for measuring the differences in height, is accurate to ±0.3 per cent.

Green (*118*) designed an apparatus which allows the rapid determination of surface tension by the drop-weight method and requires less than 5 drops of liquid. The values are read from a graph scale on the instrument, calibrated in dynes per centimeter.

Viscosity

Since viscosity is associated with structure, constitution, symmetry, polarity, saturation, and molecular size, it can often be an aid in the identification of pure compounds. It has been particularly useful as an aid in the analysis of petroleum fractions.

Cannon and Fenske (*54*) discuss the inherent errors of the capillary-type viscometer. They describe a micromodification (Figure 38, left) which is simple and which, by varying the capillary bore from 0.3 to 2 mm., will cover a range of 0.5 to 800 centistokes. The precision is ±0.2 per cent and the accuracy is approximately ±0.5 per cent.

Bowman (*43*) describes a more complicated apparatus for routine work requiring only 0.03 gram. Its operation, however, is simple and rapid. The method depends on the rate of fall under gravity of a short segment of liquid contained in a longer capillary, which is jacketed as shown in Figure 38 (right). The assembly consists of two jacketed capillaries for different viscosity ranges, together with boiler and controls for maintaining a constant temperature. The precision is 0.1 per cent in the range 2 to 10,000 centistokes, and the absolute accuracy is better than 4 per cent.

MOLECULAR WEIGHT BY VISCOSITY. Through the work of Staudinger (*321*) on the relationship between molecular weight and viscosity, it is possible, by measuring viscosity, to determine the molecular weight of compounds with an accuracy of ±10 per cent in the range 25,000 and higher, using a sample of 5 mg. in 5 ml. of solvent. For making comparisons in a series, this is an excellent method, but for absolute values, careful standardization by osmotic pressure measurements must first be carried out.

The Microscope in Organic Analysis

Inorganic analytical reactions are readily carried out under the microscope, and microscopy, as it is commonly practiced, deals largely with identification by the formation of characteristic crystals on a microscope slide. Most organic reactions, however, are not amenable to this technique. While alkaloid microscopy has developed to a high degree the formation of characteristic crystals, the organic chemist must usually be content to study the crystallographic and optical properties of the crystals of the organic material itself, and of easily formed derivatives. The work of Bryant (*49, 50*) during the past ten years, on the optical crystallography of organic compounds, provides an excellent example of this specialized technique with the polarizing microscope. However, it is not often possible to use microscopical methods as the sole means of identifying organic compounds because optical constants have been determined for relatively few of the enormous number of such known compounds.

The greatest value of microscopy at present consists in comparing the unknown with a selected number of knowns whose properties have been established as similar by chemical tests, melting points, etc. By such means the identity can usually be quickly proved. Then, if necessary, the optical properties and measurement of the crystal angles can be studied carefully and compared with the known, whereby proof of identity often can be more firmly established than by further chemical tests which, even by micromethods, would require considerable sample.

Jelley (*145*) has repeatedly drawn attention to the analytical possibilities of the large influence of wave length on various optical constants of organic compounds. He has described methods of identification by comparing the dispersion of birefringence of different compounds which have some other physical property in common—for example, the same melting point. Although the microspectrographs which he has described are not commercially obtainable at present, and the technique calls for a specialized knowledge of crystal optics, other highly specialized instruments, such as ultraviolet spectrographs and spectrophotometers, have been

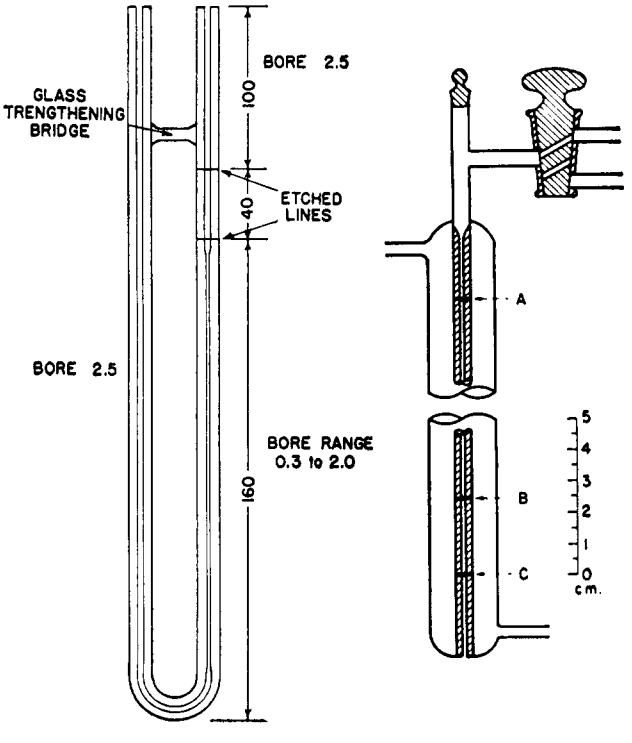
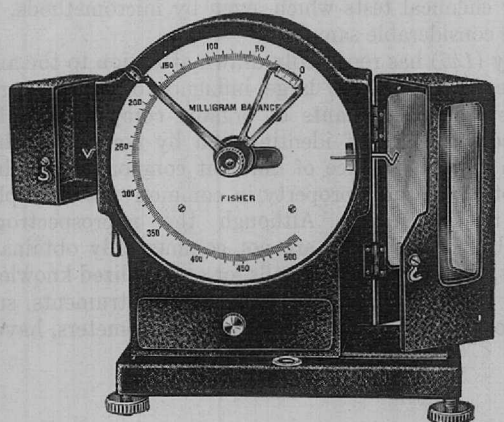


FIGURE 38. VISCOMETERS

developed to the stage where they are commonly used in analytical laboratories.

QUALITATIVE ANALYSIS

A few years ago, if a small amount of an unknown organic material was submitted for identification, unless the analyst was fortunate enough to get an early clue, the material would be used up long before the usual systematic tests had been completed. Organic microchemists until recently have paid little attention to the technique to be applied to the systematic identification of organic compounds. This is in contrast with the wide use in colleges of micro- and semimicrosystems of qualitative inorganic analysis and the publication of numerous books on this subject. The savings in time, material, and reagents, so well established in inorganic microanalysis, have prompted an investigation into the methods which can be applied to organic microanalysis. Most of these tests are adaptations of the standard macroprocedures. Much of the microtechnique used has been published but only recently has any attempt been made to organize it into a systematic, practical scheme of analysis.



Courtesy, Fisher Scientific Co.

FIGURE 39. TORSION BALANCE

The ordinary microscope can be used to advantage in a preliminary examination of the unknown sample as to its homogeneity, crystalline structure, and color. A polarizing microscope can often give additional information on mixtures whose crystals are similar in color and shape, but whose behavior is different under polarized light. If, from this preliminary examination, the substance appears nonhomogeneous, it must be purified before the physical constants can be run. Solids may be recrystallized or sublimed, liquids fractionally distilled. The determination of the cooling curve has long been recognized as the most accurate criterion of purity (see Physical Methods).

Included in the preliminary examination is an ignition test on a few milligrams of the material on a platinum spatula to determine the approximate melting point, the odor given off, the character of burning, and the nature of any residue which, if present, may be further tested for the metals present.

After the preliminary examination, the pure material may be identified by the determination of its physical constants, the optical and crystallographic properties of its crystals, the preparation of derivatives, and the qualitative and quantitative determination of its elements and groups.

In qualitative and preparative work a balance which permits rapid weighing without great accuracy is useful. A torsion-type balance meets these requirements, one of which is shown in Figure 39.

The following section is a summary of a series of papers by Schneider and Foulke (*290*) dealing with micromodifications of methods for qualitative organic analysis based mainly on the scheme of Mulliken and Huntress (*226*).

After the preliminary examination, the compound is tested for the elements most often present other than carbon and hydrogen. These tests are all carried out with not more than 1 mg. of material. The sample is first decomposed by a magnesium powder–potassium carbonate mixture, according to a micromodification of the Barkenbus and Baker (*16*) method. The fusion mixture is leached out with a drop of hot water. Portions of the clear liquid are spot-tested for nitrogen by adding ferrous sulfate and forming Prussian blue, for halogen by adding nitric acid and silver nitrate, and for sulfur by adding acetic acid and a drop of lead acetate.

CARBON, HYDROGEN, AND OXYGEN. Classification reactions are next carried out for compounds of carbon, hydrogen, and oxygen. Micromodifications of the reaction have been developed for:

The fuchsin test for aldehydes
The Molisch test for carbohydrates
The titration of acids
The ferric chloride test for phenols
The saponification of esters, using a diethylene glycol solution of the alkali as proposed by Redeman and Lucas (*257*)
The tests for acid anhydrides and lactones
The phenylhydrazine test for ketones
The alcohol test with sodium

ETHERS AND HYDROCARBONS. If the substance does not give a positive reaction in any of the preceding tests, it is placed in the group of ethers and hydrocarbons. A microtechnique based on the scheme found in the sectional and numbered tests of Mulliken and Huntress has also been worked out for additional tests which limit the field further:

The Fehling and osazone tests for sugar
The acetyl chloride and zinc chloride–hydrochloric acid tests for alcohols
The bromine and phthaline tests for phenols
The bisulfite tests for aldehydes
The Tollens silver mirror test
The bromine addition, iodoform, aluminum chloride, solubility and specific gravity tests for hydrocarbons. (The exact specific gravity need not be known, so that the *Schlieren* procedure with standards chosen for the different ranges can be used to advantage.) For quantitative analysis of hydrocarbons, see Gas Analysis.
The oxidation of side chains
The saponification equivalent of esters (this quantitative determination is run by the Redeman-Lucas method, *257*)

NITROGEN. Garcia and Schneider (*108*), again using the scheme of Mulliken and Huntress, call compounds containing nitrogen those of the second order. These are divided into colored or colorless compounds. The former are small in number and need not be divided further. As the result of microtitrations, the second group is divided into three genera: (1) acid species, (2) basic species, and (3) neutral species. The microtechnique, which involves no special apparatus, has been worked out for the following tests:

Tests for Genus 1. Millon's test, Mulder's reaction, Murexide reaction, Adamkiewicz-Hopkins-Cole reaction.

Tests for Genus 2. Specific tests for ammonia, tests for the salts of the ammonium type, detection of primary amines with nitrous acid, Rimini test for primary aliphatic amines, Simon test for secondary aliphatic amines, and acylation of amines and hydroxyl compounds by: direct acylation, Schotten-Baumann reaction, acylation in pyridine, and formation of picramides.

Separation of amines by formation of aryl sulfonyl derivatives.

Formation of salts of amines: picrates, salts of 3,5-dinitrobenzoic acid, and salts of *p*-toluene sulfonic acid.

Tests for Genus 3. Tests for nitro group; diphenylamine reactions for nitrates, nitrites, aliphatic nitro compounds, and nitrosoamines; biuret reaction; hydrolysis of species of order 2 with hydrochloric acid, sulfuric acid, and potassium hydroxide; alkali decomposition test; silver nitrate treatment of the original substance.

Most of these tests require about 3 to 6 mg. but some only a few micrograms.

ELEMENTARY ANALYSIS

Elementary analysis has received more detailed study than other branches of microchemistry because it serves organic chemistry—a very large and important field—and also be-

Courtesy, Fisher Scientific Co.

FIGURE 40. THERMOSTATIC SLEEVE WITH COMBUSTION FURNACE

cause the methods were taught and popularized by Pregl. This influence is revealed in the many books and monographs on organic elementary microanalysis published both in Europe and in this country. Of those in English, the one by Niederl and Niederl (*232*) is the best for student use. Detailed directions are given for the common determinations by standard procedures and an up-to-date bibliography on the determinations covered is included.

Combustion Furnaces

About 1900 the method of heating combustion tubes with charcoal was being replaced with the far more satisfactory gas-fired unit. Today, electrically heated macrocombustion units are preferred in most laboratories because the heating is more uniform and the furnace temperature can be more easily controlled. For micro laboratories, electric heating has the added advantage that sharp fluctuations in room temperature are avoided, which permits more rapid and precise weighing.

Since, until recently, no electric furnaces for micro work could be purchased, many designs of units built by individual investigators have been published.

Several commercial units are now available, with or without thermocouples. They are of the split type, so that when required they can be pushed away and the combustion tube allowed to cool. The same unit can be used for other determinations, such as halogen, sulfur, and Dumas nitrogen. The gas-heated cymene bath for heating the lead dioxide in the carbon and hydrogen determination is being replaced by an electrically heated, thermostatically controlled unit, which is a great improvement. Figure 40 shows such a thermostatic sleeve with the combustion furnace unit. The sample, however, continues to be heated with a gas burner in all units except one (Figure 41), in which a small movable electric furnace is used.

Sargent (*223*) has designed an apparatus for the determination of carbon and hydrogen, consisting of an electric furnace and a thermostatic sleeve, together with an electrically heated preheater. These, with the rest of the familiar carbon-hydrogen setup, are mounted on a base so that the apparatus is a convenient and integrally designed unit.

While these units are very convenient for laboratories where a few samples are analyzed, some study has been made of designs for laboratories with many samples, so that a minimum of attention is required during the combustion. In contrast to the all-purpose units which are now manufactured, such apparatus must be designed for maximum convenience for the particular determination. The design must be relatively simple, or more time will be spent in keeping the apparatus in working order than is saved by speedier analyses.

One operation that takes up much time in all combustion determinations is the burning of the sample by moving the furnace or gas burner manually along the combustion tube. Consequently, various devices for moving such a furnace have been designed.

Figure 42 shows Reihlen's device (*253*), in which a clockwork arrangement moves the gas burner along the combustion tube. Ten carbon and hydrogen determinations can be run in an 8-hour day.

The author has found satisfactory a design (*123*) in which an electrically heated split-type furnace is moved by means of a screw turned by an electric motor whose speed is varied as the furnace passes over insulated segments. The speed is made slower at the point where the furnace approaches the sample, so that decomposition will proceed smoothly. Figure 43 shows the carbon and hydrogen apparatus with automatic combustion.

Royer (*232*) uses the same principle but prefers to move the furnace at one fixed speed.

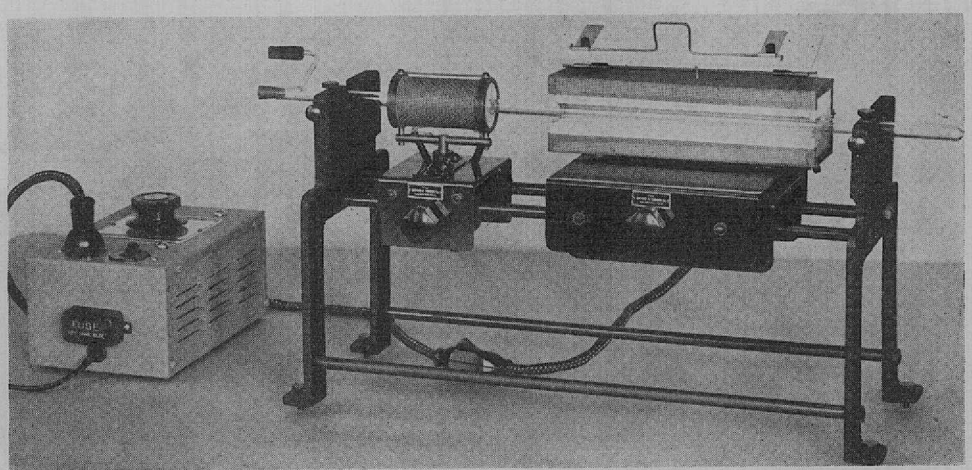
Electric furnaces for heating the sample should be of the split type with lateral movement, so that they need not be turned off at the end of the run to allow cooling of the combustion tube. If they are not so designed, much time is lost in waiting for the furnace to cool or heat.

COMBUSTION TUBES. Side-arm quartz or the new Corning Vycor tubes are much preferred over hard glass because of their freedom from bending under high temperature. Their high cost should be considered prohibitive only by those who have an occasional analysis to run.

Determination of the Elements

By Oxidation Methods

The system commonly employed for the determination of the elements depends upon decomposing the organic com-



Courtesy, Arthur H. Thomas Co.

FIGURE 41. ELECTRIC COMBUSTION UNIT WITH ELECTRIC BURNING FURNACE

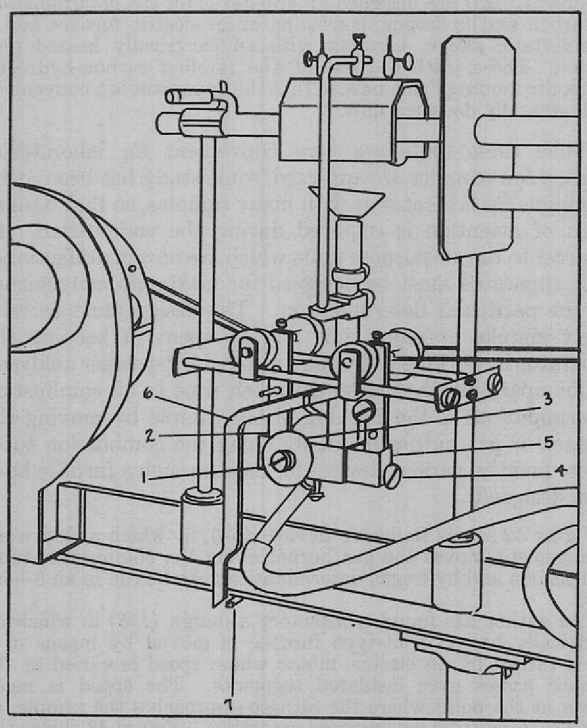


FIGURE 42. REIHLEN AUTOMATIC COMBUSTION FURNACE WITH GAS BURNER

pound by heat in the presence of an oxidizing agent, such as (1) elementary oxygen and a catalyst, (2) wet oxidation, or (3) a dry reagent, such as sodium peroxide or copper oxide. The desired element is retained and determined by appropriate methods. Burning in oxygen gas is preferable because no residue remains and the determination can be carried out with a minimum of interfering ions.

CARBON AND HYDROGEN. *Combustion Tube Filling.* Analysts are divided into two groups, as to the best method of filling combustion tubes for the determination of carbon and hydrogen.

1. Liebig-Pregl. Referring to Figure 44, section *C* is filled with lead dioxide pellets, *B* with silver wool, and *A* with a copper oxide–lead chromate mixture. The fact that metallic silver will take out both halogens and sulfur when heated (preferably by a separate furnace at 450° to 500° C.) has resulted in eliminating the lead chromate from the copper oxide filling (*234*). This results in a much longer life for the combustion tube and the modification should be generally adopted. Substitution of platinized asbestos and platinum foil for part of the copper oxide section has long been recognized as a particularly efficient catalytic mass for substances difficult to burn. Smoother burning, especially of volatile compounds, is obtained if the platinum foil projects 1 cm. beyond the furnace heating section, *A*.

2. Dennstedt-Friedrich (*103*, *105*). This method relies entirely on catalytic combustion over platinum, which is placed in *A* (Figure 44). Lead dioxide contained in two boats is placed in *C* to remove all interfering gases. Some workers prefer to place additional boats containing silver and red lead in *B*, if compounds high in sulfur and halogen are run.

The results obtained by the two methods are the same. Method 1 requires more time in filling the tube. The presence of copper oxide ensures an excess of oxygen if the sample decomposes rapidly. When many samples of varying and unknown composition are analyzed with this filling, less care is necessary in decomposing the sample. The filling will last for 300 to 400 analyses.

Method 2 appeals to those who occasionally analyze only a few compounds. The tube may be rapidly assembled and the boats easily changed at any time to fit the type of compound analyzed. There is no need to add and condition the lead peroxide boat if the compound does not contain nitrogen. Sulfur and halogen can be removed by silver which need not be conditioned. The sample should be heated with a little more care, since there is no secondary source of oxygen.

Absorption Tubes. Absorption tubes, in fulfilling their function as a holder of the absorbent, have been found to cause errors in three ways: (1) the diffusion of oxygen gas and a decrease in weight due to the infusion of air, (2) infusion of moist air and an increase in weight, and (3) static charges accumulating on the glass surfaces. This diffusion and infusion are intensified, of course, when a tube is taken into a balance room of different temperature from that of the combustion room.

This can be prevented by designing a suitable tube or by cutting the diffusion to a minimum by inserting fine wires into the capillaries of the Pregl tube (Figure 45, upper). The sealable type employs mercury (*266*) or a steel ball (*146*) to close the

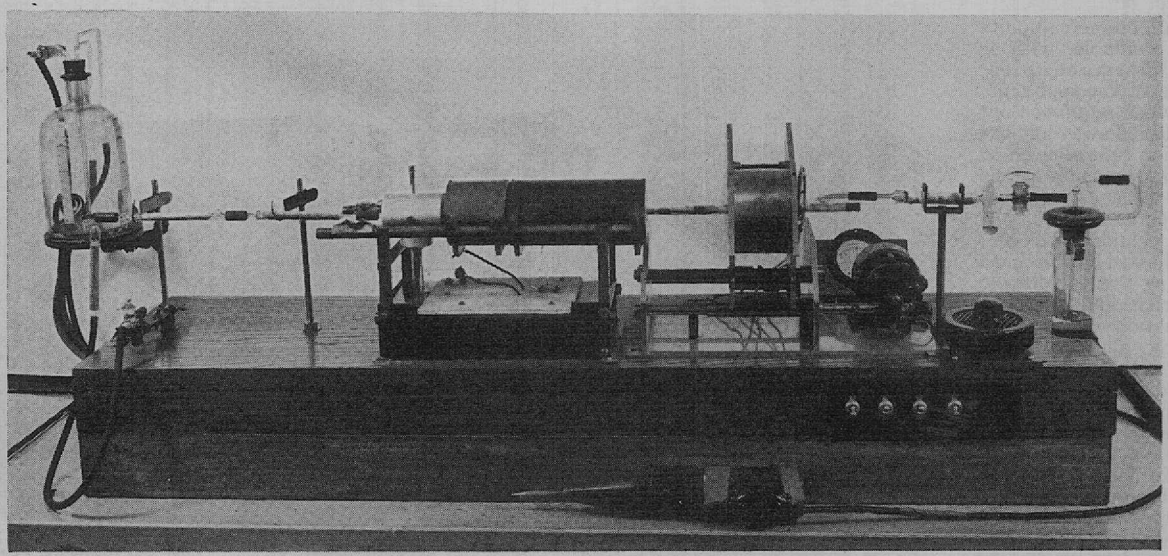


FIGURE 43. HALLETT AUTOMATIC COMBUSTION UNIT WITH ELECTRIC FURNACE

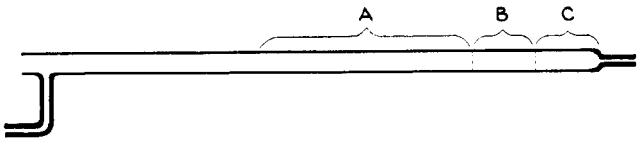


FIGURE 44. COMBUSTION TUBE

capillary opening. The fundamental weakness of the mercury-sealed tube is the possible loss of mercury if the tube is handled carelessly. Another type uses a ground joint (Figure 46) which may be turned to open or close the tube. Many modifications of these sealable tubes have been designed. Still others weigh oxygen-filled tubes with unsealed capillaries and report that satisfactory results are obtained (*282*). The diffusion is so slow that the weighings are not materially affected if the same weighing schedule is adhered to from run to run. In the author's laboratory this method is used with satisfaction. Practically all analysts both in this country and in Europe agree that the best filling for absorbing carbon dioxide is Ascarite. One filling will absorb 500 or 600 mg. Royer (*282*) has found that the drying agent after the Ascarite is not necessary. Of the water-absorbents, indicating-Drierite (anhydrous calcium sulfate) is the most convenient. Other water-absorbents which are as satisfactory but are not indicating are magnesium perchlorate trihydrate and phosphorus pentoxide.

Time of Combustion. The average time taken for burning and sweeping is approximately 45 to 60 minutes, and microanalysts have regarded as critical the rate of combustion and sweeping published in earlier procedures. Laboratory workers with many samples to analyze sometimes wonder how rapidly an analysis could be carried out and still produce acceptable results.

By using a larger combustion tube and packing the filling tightly, Brodie (*46*) cut this time to 25 minutes for 15- to 50-mg. samples. Titov (*344*), using a similar technique, reports 30 minutes for 50- to 60-mg. samples. It has been the experience in this laboratory that a 4- to 6-mg. sample can be burned and swept through with 200 to 300 ml. of oxygen in 15 minutes, using the regular-size microcombustion tube and an automatic combustion furnace (Figure 43). The weighing of both absorption tubes is completed in 8 minutes. As many as twenty samples per 8 hours have been run. (This includes one run over the noon hour unattended.) Belcher (*24*) also uses a high rate of oxygen flow, 12 ml. per minute, whereby a determination requires 35 minutes; using a higher temperature (1350° C.), the combustion time is only 10 minutes.

While the usual weight of sample taken for analysis is 4 to 6 mg., acceptable results with an amount as low as 1 mg. have been reported (*231*).

Procedures have been published for the volumetric determination of water (*187*) and carbon dioxide (*187, 289*) but they are cumbersome when compared to the speed with which the gravimetric determination can be carried out.

Accuracy and Precision. Special studies by Power (*245*) and reports of individual analysts indicate that a skillful operator may expect a precision and accuracy of 2 parts per thousand for carbon and hydrogen, while the average operator will have a variation of 3 parts per thousand. In precision work where a large macrosample (0.2 to 0.5 gram) is used, a precision and accuracy of 2 to 3 parts per ten thousand have been demonstrated (*98, 261*).

Methods which employ wet oxidation are limited to the determination of carbon only and the precision and accuracy are generally not equal to those obtained with combustion in oxygen nor as rapid (*155*). A reliable oxidizing agent has not yet been found. However, recent work by McCready and Hassid (*197*) points to a more satisfactory procedure by using the Van Slyke–Folch combustion mixture, consisting of chromium trioxide, potassium iodate, phosphoric acid, and fuming sulfuric acid. A single determination takes 30 minutes. The carbon dioxide is absorbed and weighed.

NITROGEN. *Dumas* (range 1 to 0.03 mg.). This method depends upon a reliable source of pure carbon dioxide for sweeping the elementary nitrogen after the sample has been decomposed by heat in the presence of copper oxide. Much of the study of this determination has been on methods for the elimination of contamination of the carbon dioxide from the nitrogen of the air. Such contamination will, of course, give a variable blank, which makes it impossible to obtain reliable results.

The common source of carbon dioxide is calcium carbonate (marble) and hydrochloric acid contained in a Kipp generator, so arranged as to prevent contamination from the air. Another method consists in preparing it from a sodium acid carbonate solution and sulfuric acid in a special generator. These methods, once the apparatus is free from air, normally give a satisfactory supply of carbon dioxide. Dry ice contained in a Dewar flask is now being widely used as a source in those laboratories where dry ice is customarily stored. Six pounds placed in a 2-liter flask will last for 3 weeks. The generator is usually freed from air by standing overnight. Carbon dioxide obtained from cylinders (*231*) has been used successfully, but a relatively high pressure of carbon dioxide in the apparatus is necessary.

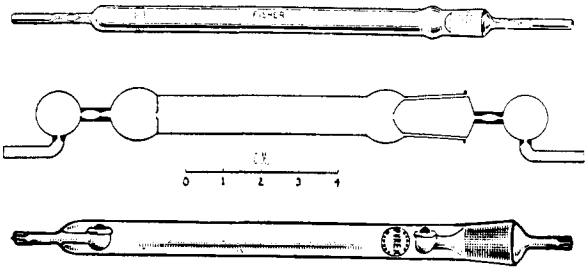


FIGURE 45. ABSORPTION TUBES

Upper. Pregl type
Center. Hallett mercury seal type
Lower. Johns steel ball type

The Dumas method has been generally considered reliable for all forms of nitrogen. However, several reports of compounds giving low results are recorded (*271*). They are usually compounds which form tars or coke on burning—for example, derivatives of pyrimidines, purine, and chlorophyll. However, the nitrogen Kjeldahl values of these compounds were acceptable. Such compounds may often be detected by a slow but continuous flow of bubbles into the nitrometer long after the time a normal compound would have finished burning. The correct nitrogen value for these compounds has been obtained by mixing the sample with copper acetate before adding it to the copper oxide and increasing the temperature (900° to 1000° C.) at which the sample is burned. Passing oxygen generated from potassium chlorate over the heated sample has given good results in some cases. Extra copper must be employed in the combustion tube to take care of the unused oxygen. These modifications are not at present developed to the point where they are reliable and convenient as a general method for such compounds. When it is imperative to determine the nitrogen, these modifications should be tried, but they should be checked by the Kjeldahl method as well as by a carbon and hydrogen determination.

For a more permanent and convenient apparatus the author has found that the capsule method of Clark (*63*) can be used even for microsamples. It allows the combustion tube to remain in place and to be kept at the proper temperature. By means of a special four-way stopcock constructed without dead air spaces, the nitrometers are permanently

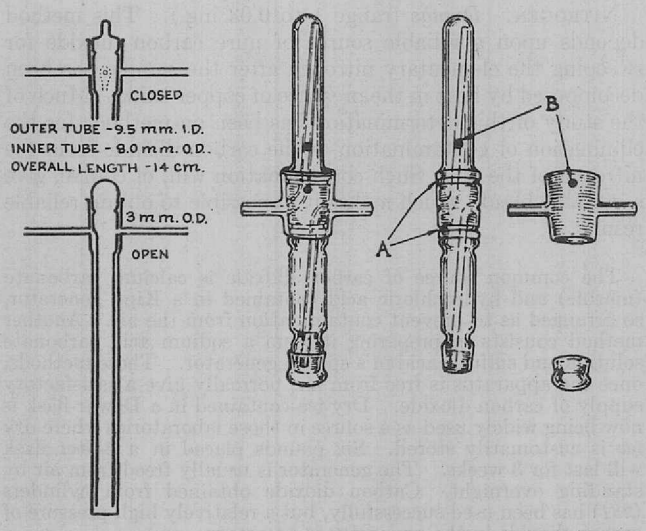


FIGURE 46. ABRAHAMCZIK-TYPE ABSORPTION TUBES
Left, Prater (*246*). *Right*, Clark and Stillson (*66*)

connected to the combustion tube. The section of the combustion tube where the capsule is placed is larger, consisting of a tube 11 mm. in inside diameter. Figure 47 shows the automatic combustion unit with the modification just described.

Because of the variation in operating conditions and in the purity of the carbon dioxide, an increasing number of operators favor considering the vapor pressure of the potassium hydroxide, the film correction of the wall of the nitrometer, and blank separately, in calculating the final nitrogen volume. When operating conditions are uniform, these factors, once determined, are, of course, constant.

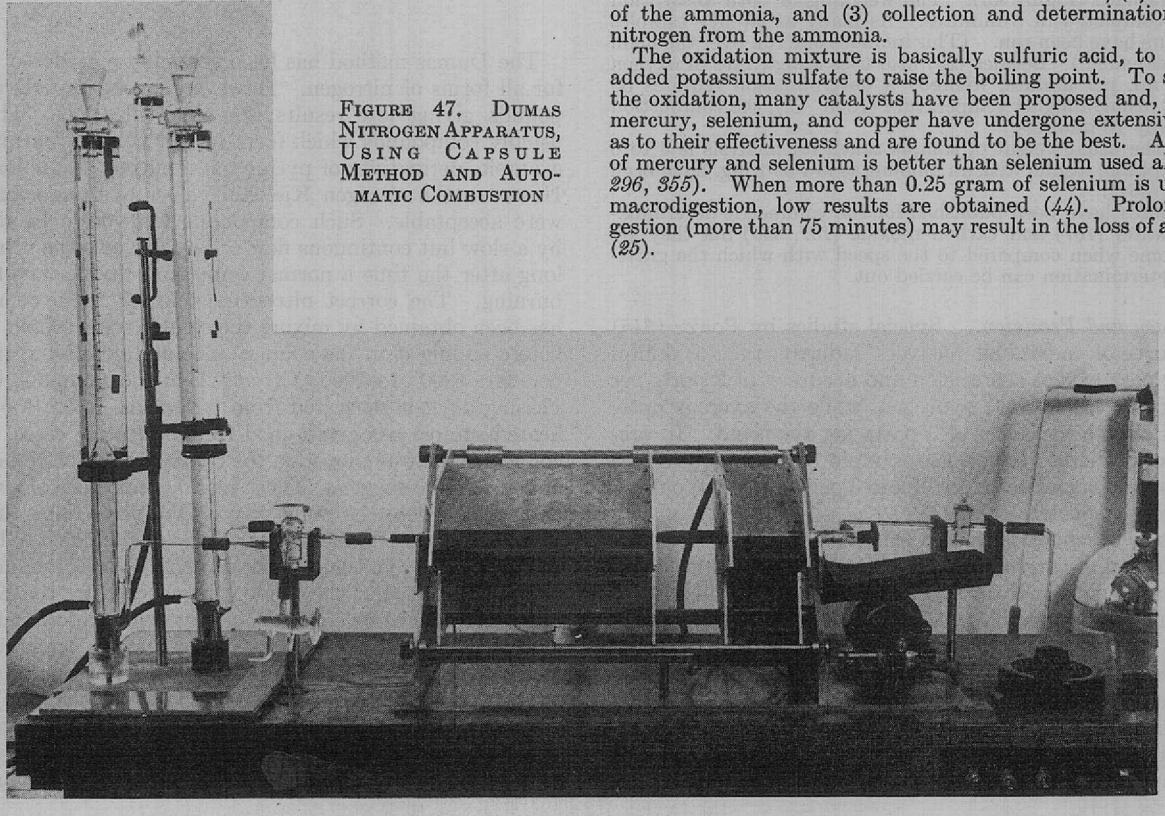


FIGURE 47. DUMAS NITROGEN APPARATUS, USING CAPSULE METHOD AND AUTOMATIC COMBUSTION

Placing the sample in the Pregl shaking tube has been abandoned and the sample is now weighed in a porcelain boat which is added directly to the tube. Static charges developed in a shaking tube make it impossible in many cases to rinse the sample quantitatively into the combustion tube.

Very few references (*166*, *339*, *357*) appear in the literature on the determination of nitrogen below 1 per cent by the Dumas method.

It has been found that samples up to 50 mg. can be burned successfully and the nitrogen collected in a special nitrometer graduated in 0.001 ml. with a total capacity of 2 ml. Using the capsule method, samples as large as 50 mg. have been burned and nitrogen percentages as low as 0.2 per cent determined with an accuracy of ±10 per cent or a variation of ±0.02 in the hundredths place, and using gas volumes as low as 0.03 ml. Vetter (*357*) reports that samples as low as 0.01 per cent can be analyzed, but the author has not had occasion to determine such low percentages. When the regular nitrometer graduated to 0.01 ml. is used, an unknown sample yielding only a few hundredths of a milliliter of nitrogen cannot be measured (*120*). By constructing a nitrometer graduated in thousandths of a milliliter, with a small inverted funnel at the top, the nitrogen can be transferred readily. The small inverted funnel is lowered below the surface of the potassium hydroxide in the funnel of the large nitrometer, and, after the entrapped air is removed by a syringe pipet, the nitrogen is released and caught by the inverted funnel, from whence it is pulled into the nitrometer for measurement. The corrections to be applied appear to be the same as those for the nitrometer of larger bore (3.6-mm.).

As in the carbon and hydrogen determination, it has been found (*21*) that the burning and sweeping time can be decreased, so that only 20 to 30 minutes are required. The time, using the automatic combustion unit (Figure 47), is 25 minutes. It is not necessary to heat the sample twice.

Figure 48 shows the automatic combustion apparatus of Royer (*281*), in which the standard procedure of removing the tube for filling at the end of each run is followed.

Kjeldahl. (0.1 to 3.0 Mg. of Nitrogen.) This method has much to commend it for speedily carrying out routine multiple determinations on compounds of known structure where the proper modification of the oxidizing mixture can be used.

There are three distinct steps: (1) oxidation of the organic material with the formation of ammonium sulfate, (2) liberation of the ammonia, and (3) collection and determination of the nitrogen from the ammonia.

The oxidation mixture is basically sulfuric acid, to which is added potassium sulfate to raise the boiling point. To speed up the oxidation, many catalysts have been proposed and, of these, mercury, selenium, and copper have undergone extensive study as to their effectiveness and are found to be the best. A mixture of mercury and selenium is better than selenium used alone (*23*, *296*, *355*). When more than 0.25 gram of selenium is used in a macrodigestion, low results are obtained (*44*). Prolonged digestion (more than 75 minutes) may result in the loss of ammonia (*25*).

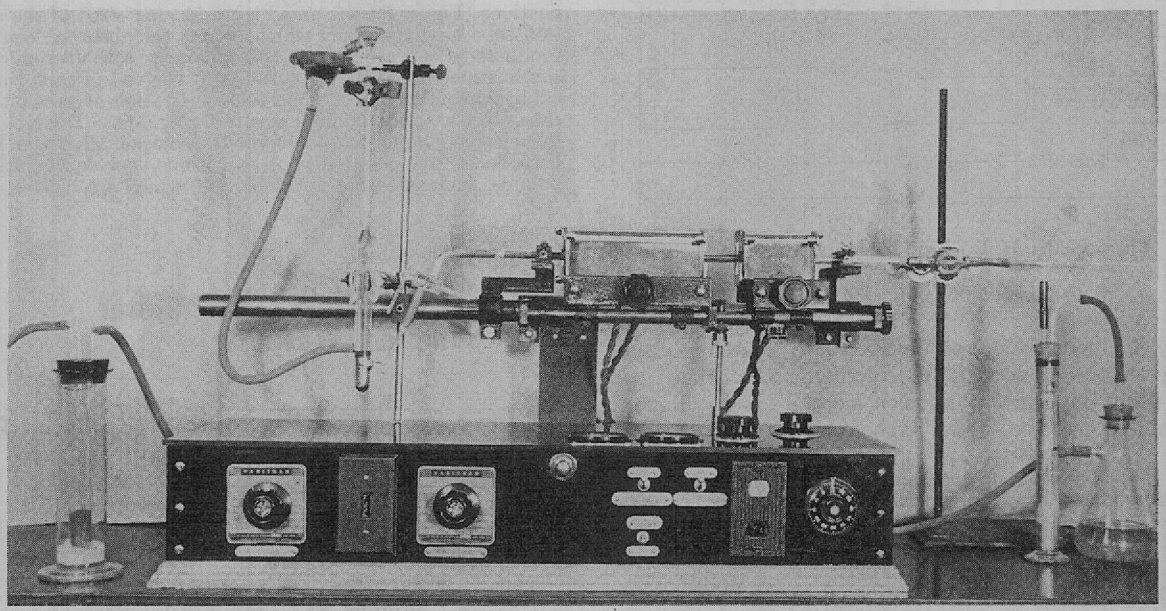


FIGURE 48. ROYER AUTOMATIC DUMAS NITROGEN APPARATUS

Compounds such as hydrazine, nitro, nitroso, and azo must first undergo a preliminary reduction (*287*) prior to digestion, using hydrogen iodide and phosphorus. Diazo compounds should be coupled with phenol to a stable azo derivative before being reduced with hydriodic acid. Volatile compounds must be reduced in sealed tubes at 200° to 300° C. Where a Dumas nitrogen apparatus is available, it will be found more reliable, faster, and convenient to use this method in preference to the modified Kjeldahl procedure just described, for general laboratory analysis of compounds of widely varying composition. For series determinations when the structure is known and the modification has been tested, the Kjeldahl method is much faster. An excellent study of this determination is given by Clark (*65*), using the Gunning-Arnold-Dyer and Friedrich procedures applied to various types of compound. The ammonia is absorbed in boric acid. These methods have been subjected to collaborative study and found satisfactory (*2*).

Many designs of apparatus for the liberation and steam-distillation of ammonia have appeared, the best of which eliminate rubber connections as far as possible, but they are all satisfactory. With the now general use of standard-taper joints, a Kjeldahl digestion flask with a ground-glass joint offers advantages (*349*) in that the sample need not be transferred after digestion to the distillation unit. Figure 49 shows such an apparatus built in this laboratory, using the Nichrome coil heater of Clark (*65*) in the steam generator.

While the collection of the ammonia in standard acid and titration with alkali yields accurate results, considerable work in several laboratories has demonstrated that the alkali may be eliminated and the ammonia collected in boric acid solution, which is then titrated directly with standard acid. The mixed indicator change is not too sharp to an unpracticed eye. However, the method is reliable and simple for routine work when the volume, pH, and end-point color are carefully controlled and standardized (*340*, *359*).

Another widely used method of determining ammonia is by the hypobromite reaction in which nitrogen is evolved. The excess hypobromite is determined by adding potassium iodide and titrating the excess iodine with 0.01 *N* thiosulfate. Here, again, the method to be satisfactory must be carefully standardized (*214*, *253*).

From recent investigations it appears that both the Dumas and the Kjeldahl methods have their limitations but that the latter, with suitable modifications, is reliable for analyzing a much larger variety of compounds than has been considered possible heretofore.

Submicromethods. (10 to 0.3 Mg. of Nitrogen.) In certain biological studies it is necessary to limit the size of the sample and to determine the nitrogen present. The transfer and distillation of such small quantities of nitrogen are not practical if the regular micro-Kjeldahl procedure is used.

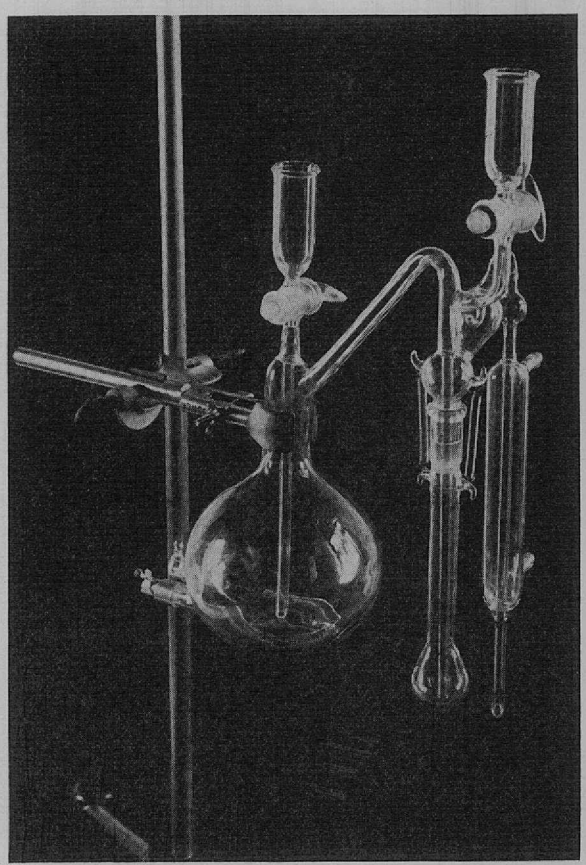


FIGURE 49. KJELDAHL NITROGEN DISTILLATION APPARATUS

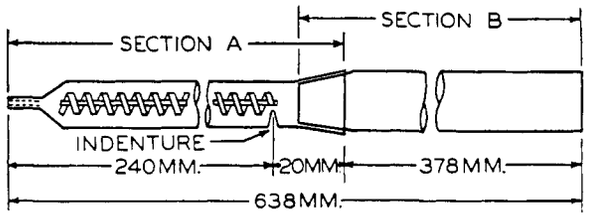


FIGURE 50. REMOVABLE ABSORPTION TUBE

Recently, methods have been developed to determine accurately quantities as low as 0.3 microgram of nitrogen per sample, using the Conway diffusion method for distilling off the ammonia (described under Trace Analysis).

Note. The digestion of the sample by electric heating, as suggested by Clark (*65*), is preferable because bumping is largely eliminated. An electrically heated digestion unit is available from the American Instrument Co.

HALOGENS AND SULFUR. *Oxidation of Organic Matter.*

1. The catalytic combustion method, in which the organic material is burned with oxygen in the presence of platinum catalyst, is reliable for both halogen and sulfur. Two modifications (Figure 50, *22*, and Figure 51, *124*, *125*) of the combustion tube have appeared which permit the absorbed combustion products to be washed from the apparatus without removing the combustion tube from the furnace, at the end of each run. The catalytic combustion method allows titration or precipitation to take place with a minimum of interfering ions. It is not suitable for relatively large samples of volatile compounds, low in halogen, because of the possibility of explosions, nor for volatile compounds, such as carbon tetrachloride, which are high in chlorine (*69*).

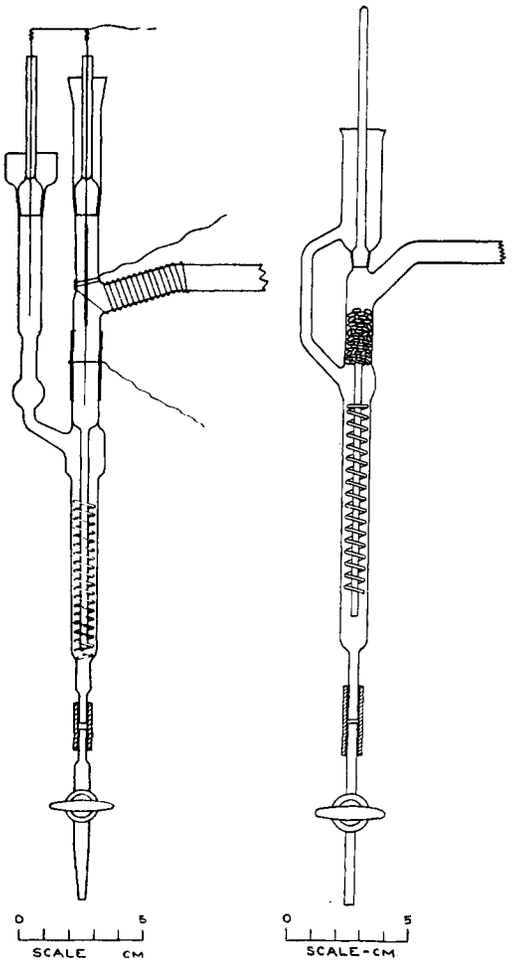


FIGURE 51. ABSORPTION APPARATUS
Left. For sulfur
Right. For halogens

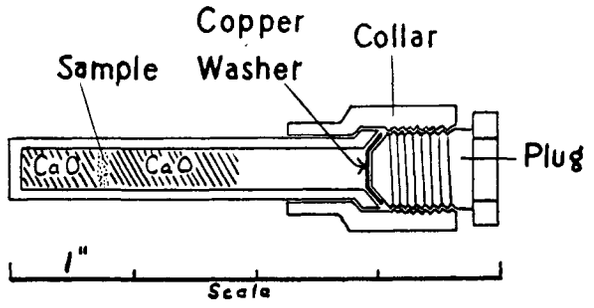


FIGURE 52. LIME FUSION BOMB

2. For such chlorine compounds the well-known lime fusion method is best. MacNevin and Baxley (*199*) use the iron bomb shown in Figure 52, in which to heat the sample. The method has been used in this laboratory for traces of chlorine (0.1 to 0.2 mg.) in volatile solvent compounds (50 to 100 mg.) with satisfactory results. Iodine and bromine cannot be run by this procedure. By using the same method, but placing the mixture in a sealed glass tube and heating to 425°, Weber (*360*) has successfully analyzed chlorine, bromine, and iodine.

3. The Carius method of decomposing a sample in concentrated nitric acid in a sealed tube at 300° C. has been a standard procedure for years. Even in the micromodification it still suffers from the disadvantages of an occasional explosion, the long heating period, and the opening of tubes under pressure with a possible loss of material and contamination from glass splinters. A recent paper (*229*) on these defects shows how they may be minimized. The heating period is reduced to 30 minutes. An electrically heated bomb furnace is available from the American Instrument Co. Details for constructing a furnace of this type are given by Kuck and Griffel (*169*).

4. Procedures using a micro- and semimicro nickel Parr bomb have been carefully studied (*297*). Because of the relatively large quantity of sodium nitrate, sugar, and sodium peroxide, a blank on new reagents is especially necessary. Because of this high salt concentration, the conditions of precipitation in the case of sulfate—e. g., barium sulfate—must be carefully controlled to eliminate coprecipitation. The method requires careful measuring of the reagents and thorough mixing with the sample to ensure proper ignition. The method is usually reliable, and, when several bomb cups and a titration procedure are used, it is rapid for series runs. The average time for a determination is 30 minutes. When salts, such as sodium, are analyzed, there is no danger of loss of sulfur or halogen, due to holding back part of the sulfur as sulfate in the ash—a weakness of the catalytic combustion method.

It is equally satisfactory for chlorine, bromine, or iodine, although Colson (*69*) reports poor results for chlorine in carbon tetrachloride.

A disadvantage of the bomb procedures for gravimetric determination in the case of liquids, which must be weighed in a capillary, is the solution and, later, separation of silica with the precipitate. Modifications (*185*) overcome this but when possible it is more convenient to use a titration procedure.

Estimation of Halogen. The gravimetric determination of halogen as the silver salt is time-consuming and is useful only for the occasional analysis when the preparation of standard solutions is not warranted. The gravimetric factor for silver iodide is so unfavorable that in micro work very careful technique is required on the part of the analyst. While the end point in the Volhard method (*299*) does not appear sharp to those who use it only occasionally, precise results (±0.01 ml.

of 0.02 *N* silver nitrate) are possible when the eye becomes accustomed to it. In the case of chloride, the silver chloride must be removed by filtering or centrifuging to prevent fading of the end point.

The method (*298*) of oxidizing iodide to iodate with bromine water and subsequently liberating six iodines from potassium iodide, which are titrated with sodium thiosulfate, is preferred for iodine because of the excellent factor. The method can also be used for the determination of iodine in the presence of chlorine and bromine (for review see Leipert, *181*).

Similarly, bromine may be oxidized to bromate with chlorine water and the same procedure followed. It is especially useful for small amounts of bromine in the presence of chlorine (*164*). A review of one hundred and fifty papers on the microanalytical determination of bromine is given by Leipert (*182*).

Adsorption indicators are used in the titration of chlorine and bromine with silver nitrate but the end-point color change is not too sharp. The addition of acetone (*51*) or a measured amount of the halogen will give a more satisfactory titration. The color change is easily observed when more silver halide is present.

Volumetric Sulfur. A good indicator for the titration of sulfate with barium chlorides has been sought for some time. Studies in Europe (*1*) and in this country (*126*) on the use of tetrahydroxyquinone have shown that while the end point is not strikingly sharp, any chosen color point is reproducible under standardized conditions with adequate precision. For routine work it is to be preferred over the gravimetric procedure. Its use with the Parr-bomb procedure has been advocated but the end-point change is difficult to detect (*201*).

When the gravimetric procedure is used, time can be saved by adding picric acid to speed up the precipitation of the barium sulfate. It can be safely filtered after one hour (*185*). Ignition of the precipitate is not necessary, and drying at 150° C. gives equally accurate results (*286*).

By Hydrogenation Methods

The precision and accuracy for the determination of halogen and sulfur are within 1 per cent.

Another system introduced by ter Meulen (*174, 209, 210*) for semimicroanalysis is based upon decomposition of the organic material in a combustion tube in the presence of hydrogen and the passing of the gases over a suitable catalyst, whereby:

Nitrogen (thoriated nickel) gives ammonia, which is titrated (*119, 136, 176, 208, 312*). Soda lime is added to remove halogen and sulfur.

Halogen (ammonia and nickel) gives ammonium halide or (oxidized chromite) (*175*) gives halogen acids (*52, 313*).

Sulfur (platinum or platinized asbestos) gives hydrogen sulfide (*80, 110, 117, 195, 342, 364*).

Oxygen [nickel chromite (*114*), nickel (*188*), nickel thoria (*53, 157, 350*), platinum in silica gel (*215*)] gives water.

Note. Zimmermann (*376*), Unterzaucher (*348*), and Korshun (*165*) have successfully used Schutze's method, in which the decomposition products are passed over hot carbon in the presence of nitrogen. The carbon dioxide is oxidized with iodine pentoxide, absorbed, and weighed.

Arsenic, cadmium, and mercury (*210*) have been determined in organic compounds by passing the gases with hydrogen over heated asbestos into a detachable cooled tube in which the metal is deposited and weighed.

Carbon and hydrogen (*119, 153*) are determined by burning the sample in oxygen in a combustion tube and passing the gases first over a manganese dioxide catalyst at 400°, then over heated lead dioxide, where any sulfur or nitrogen oxides and halogen are removed. This simple tube filling, together with a low-temperature catalyst, makes the method an improvement over that of Liebig-Pregl. A modification, whereby the lead dioxide is replaced by potassium chromate, and Pb_3O_4 and platinum are added to the manganese dioxide, is given by Hepner and Pojas (*131*). Overheating may lead to destruction of the catalyst, so that it is advisable to use electric heating.

The results obtained by these procedures are as accurate and rapid as those by other methods. They have been tested by a sufficient number of investigators to warrant further study and more general adoption. The nitrogen and halogen determination by this method is a simple titration. In the case of sulfur, the hydrogen sulfide can be titrated iodometrically, and the author has obtained excellent results with this method. Greater interest has centered around the determination of oxygen by hydrogenation because this element has always been determined by difference in the oxidation system. Most of the factors which contribute to successful results have been studied, so that the method should soon take its place as a reliable procedure.

Because the oxidation methods are the ones commonly taught and used, they have been exhaustively studied with all types of compounds; this is not true, of course, of the ter Meulen system. When decomposition takes place in hydrogen, the coke and hydrogenation products must be burned out periodically with oxygen. If this is not done, low results may be obtained, owing to retention of part of the constituent by the carbon. Most of the references to the ter Meulen system are recent and present the latest developments as well as past studies.

LESS COMMON ELEMENTS IN ORGANIC COMPOUNDS. Organic compounds containing elements, other than those ordinarily present, are often prepared and a determination for such constituents may be necessary. The determination of metals by burning to the sulfate, oxide, or the metal itself, requires careful manipulation and considerable sample because of the low factor. It is always assumed that other interfering constituents are not present.

Much study has been given, particularly in biochemistry, to the estimation of traces, but the microdetermination of such constituents of organic compounds, present in amounts of several per cent, has not received as much attention. The following outline summarizes methods which can be applied to these determinations.

Meyer and Hoehne (*211*) report on methods for the estimation of metals in complex organic salts by combustion in oxygen of a 5- to 30-mg. sample for 30 minutes and weighing the metal or its oxide. Hydrogen is used for reduction when required. From 1 to 8 mg. of residue is weighed and the results are accurate to approximately ±1 per cent. Cobalt and nickel are weighed as the metal; iron, chromium, and vanadium, as the oxide. Calcium, barium, sodium, and potassium are usually determined as the sulfate by adding sulfuric acid to the sample in a platinum boat, placed in a platinum cylinder to prevent loss by creeping. Roth (*276*) has determined rubidium and cesium in organic compounds by this method.

Tabern and Shelberg (*333*) discuss the general problem of the decomposition of organometal compounds for macrosamples and prefer a sulfuric–hydrogen peroxide digestion.

Arsenic. Sloviter, McNabb, and Wagner (*315*) have obtained satisfactory results in determining arsenic in organic compounds by decomposing with nitric-sulfuric acid and precipitating the arsenic with hypophosphorous acid. The arsenic is collected on a filter and dissolved in a measured excess of bromine (bromate-bromide solution). The excess bromine is determined iodometrically by titrating with 0.1 *N* sodium arsenite. The precision of the titration and the accuracy of the method with compounds containing 30 per cent arsenic, when using samples of 30 to 50 mg., are ±1 per cent. Halogens do not interfere. The time required to carry out a single determination is 2 hours.

Kolthoff and Amdur (*163*) reduce the combined arsenic to elementary arsenic with hypophosphite reagent, dissolve in an excess of standard ceric sulfate, and back-titrate the excess with arsenic trioxide with an accuracy of 0.5 per cent for 1 mg. and within 2 per cent for 0.1 mg.

Wintersteiner (*301*) uses a sample of 5 to 10 mg., which is decomposed with sulfuric acid and hydrogen peroxide. Potassium iodide is added and the liberated iodide titrated with 0.01 *N* sodium thiosulfate. The sample may be decomposed with

sodium peroxide in a Parr microbomb and the arsenic titrated, as just described (*20*). Bromide and iodide interfere and must be removed.

Satisfactory results may also be obtained by precipitating the arsenic as magnesium ammonium pyroarsenate.

For quantities of arsenic up to 1 mg., Cassil (*55*) prefers to take the digested sample and evolve the arsenic as arsine, which is absorbed in mercuric chloride–gum arabic solution. It is then titrated with 0.05 *N* iodine solution. The accuracy is ±1 per cent.

Jacobs and Nagler (*143*) and How (*139*) present excellent summaries with references to the analytical microdetermination of arsenic.

Boron. Roth (*275*) fuses the organic material with sodium carbonate in a platinum crucible and titrates the boric acid with mannitol; or the boric acid may be distilled as the methyl ester absorbed in alkali and titrated. The results reported are accurate to within 1 per cent.

Calcium. Calcium is usually precipitated as the oxalate and may be weighed in this form (*255*) or the calcium oxalate may be titrated, using potassium permanganate (*293*). Ceric sulfate, added in excess and back-titrated with thiosulfate, has given satisfactory results. Lindner and Kirk (*189*) have found that, with small quantities of calcium, the potassium permanganate method is accurate, owing to compensating errors, and they, therefore, prefer to titrate directly with ceric sulfate, using phenanthroline–ferrous sulfate as the indicator.

Cobalt. Cobalt may be precipitated with anthranilic acid as $Co(C_{17}H_6O_2N)_2$ (*361*). Nickel and copper interfere.

Copper. For amounts of copper above 1 mg. the electrolytic method with direct weighing is satisfactory. MacNevin and Bournique (*200*) have shown that weighing errors are greater than those of manipulation. Hecht and Reissner (*130*) have obtained accurate results with amounts of 1 mg. or less by precipitating with the following organic reagents: (1) 5,7-dibromo-8-hydroxyquinoline, $Cu(C_9H_4ONBr_2)_2$; (2) benzoinoxime (*178*), $Cu(C_{14}H_{11}O_2N)$; and (3) salicylaldoxime, $Cu(C_7H_6O_2N)_2$.

Fluorine. The decomposition of organic fluorine compounds is sometimes attended with great difficulty. Hubbard and Henne (*140*) used a microcombustion procedure of burning the sample in oxygen and passing the gases over silicon dioxide at 900° C. to form silicon fluoride, which was absorbed in water and titrated with cerous nitrate. However, some compounds, especially those with high fluorine content, are not decomposed by this treatment. Methods using an alkali-metal fusion have given satisfactory results on a variety of such compounds. Elving and Ligett (*86*) have made a study of the alkali-metal fusion method for decomposition of macrosamples and found it satisfactory for all types of fluorine compounds—solid, liquid, and gas. Vaugh and Nieuwland (*353*) used metallic sodium in ammonia for decomposing organic halogen, including fluorine compounds. In connection with this work, they showed that a micromodification could be used. It seems likely, therefore, that a microprocedure, as suggested by Elving and Ligett, using the alkali fusion method, could be worked out employing thorium nitrate. Considerable work on this titration for amounts below 50 micrograms has been done, but for satisfactory results, rigid standardization is necessary. For larger quantities of 1 mg. or more, the method should not present difficulties (*68*).

Iron. 1. For 0.5 to 5 mg., reduction and titration with dichromate, using diphenylamine, have given good results (*303*). Titration with potassium permanganate may also be used (*160*).

2. By reduction with silver (*236*), Fe^{++} is formed, which can be titrated with ceric sulfate (0.002 to 0.015 *N*) but with results low by approximately 1 per cent of the amount present. For drop analysis, Kirk and Bentley (*151*) use a cadmium-mercury liquid amalgam for reduction. An excess of ceric sulfate is added, which is back-titrated with standard ferrous ammonium sulfate solution, using phenanthroline–ferrous sulfate indicator.

3. Straub (*324*) and Rappaport and Hohenberg (*254*) have used the iodometric method for the determination of iron. Results accurate to within 1 to 2 per cent may be expected.

Lead. The separation of lead from impurities by electrolytic deposition is practical but, unless great precaution is taken, the lead deposit may be of variable composition. Therefore, some workers prefer to titrate the lead iodometrically (*302*), or the lead after electrolysis may be changed to lead sulfate and weighed. By this method, Brantner and Hecht (*45*), with 0.5 to 5.0 mg. of lead, succeeded in obtaining results accurate to within ±1 per cent.

Magnesium. For 0.5 to 1 mg. of magnesium, precipitation and weighing as magnesium ammonium phosphate ($MgNH_4PO_4 \cdot 6H_2O$) is satisfactory. There is no need to ignite to the pyrophosphate (*368*). Benedetti-Pichler and Schneider (*27*) used the method and obtained results on 0.5 mg. of magnesium accurate to ±0.5 per cent.

Strebinger and Reif (*326*) precipitate the magnesium with 8-hydroxyquinoline [$Mg(C_9H_6ON)_2.2H_2O$]. The error is approximately ±1 per cent. Calcium interferes when it is present in concentrations greater than 0.04 mg. with 5 mg. of magnesium.

Mercury. The organic material containing 2 to 5 mg. of mercury is destroyed with concentrated nitric acid by the Carius method and the metal is electrolytically deposited on a gold cathode. The accuracy and precision of the method as shown by the results of eight compounds, reported by Verdino (*356*), are within ±0.4 per cent.

Rutgers (*283*) prefers to decompose the organic compound in a tube with oxygen, charged with the vapors of aqua regia. The mercuric chloride formed is electrolyzed as above.

Boetius (*39*) burns the organic substance in oxygen and passes the gases over heated lead oxide and silver to remove the halogen. With compounds containing nitrogen the material is burned in the presence of carbon dioxide, with a tube filling of lead chromate, copper, and silver-covered pumice. The mercury is caught in a cooled tube filled with gold, which is weighed after the combustion.

Decomposition in a flask of the organic material, using concentrated nitric acid, gives satisfactory results but a reflux condenser must be attached to prevent loss of mercury during digestion.

For organic salts the method of Rauscher (*256*) is both accurate and rapid. The sample containing 2 to 50 mg. of mercury is reduced by ethanolamine in 5 to 15 minutes and the metallic mercury centrifuged (*311*) and weighed or dissolved in nitric acid and titrated with thiocyanate. Results are accurate to ±0.2 per cent. Where the mercury is directly bound to carbon, this method is not generally applicable, but refluxing with sodium in ethanolamine and dioxane has been used with some success for such compounds.

Sloviter, McNabb, and Wagner (*314*) have modified Rupp's volumetric method for determining semimicroquantities of mercury in organic compounds. The sample is decomposed with potassium persulfate and concentrated sulfuric acid. The mercury is precipitated as the metal by reduction with hydrazine sulfate of the double iodide. The metal is dissolved in 0.1 *N* potassium bromate–potassium bromide solution, potassium iodide is added, and the liberated iodine is titrated with 0.05 *N* sodium thiosulfate. A single analysis requires 3 hours and halogens do not interfere.

Phosphorus. The well-known Lorenz (*193, 300*) method of precipitating the phosphorus as ammonium phosphomolybdate has given uniformly good results in microprocedures. The precipitate may be weighed or titrated. The organic material is best decomposed by nitric acid and hydrogen peroxide or by fusion with sodium peroxide in a Parr microbomb. The Carius method (*239*) may give low results, through combination of the phosphoric acid with the glass.

Potassium. 1. The determination of potassium by precipitating as potassium sodium cobaltinitrite has received much study (*267*) and the conclusions drawn point to a lack of reproducibility by this method among different analysts, owing to the variable composition of the precipitate. If conditions are carefully standardized, however, satisfactory results accurate to within 1 per cent are obtained.

2. The most accurate and reliable method for amounts of 0.5 to 1 mg. consists in precipitating as potassium chloroplatinate and weighing (*60*). Sodium up to four times that of the potassium present does not interfere. Results accurate to 0.5 per cent are obtained. Bullock and Kirk (*51*) prefer to reduce the potassium chloroplatinate in a neutral water solution with metallic magnesium and titrate the chloride with silver nitrate. The results reported for this procedure on 0.5 to 1 mg. of potassium are accurate to 0.5 per cent.

A volumetric method based upon the equations:

$$K_2PtCl_6 + 6KI = K_2PtI_6 + 6KCl$$

$$K_2PtI_6 + 2Na_2S_2O_3 = K_2PtI_4 + 2NaI + Na_2S_4O_6$$

has given good results according to Shohl and Bennett (*309*). Results on amounts of potassium, 0.4 mg. and above, are accurate to ±2 per cent.

A bibliography on the determination of potassium is given by Cimerman and Rzymowska (*57*).

Selenium. The compound containing 0.5 to 2 mg. of selenium is burned in oxygen and the gases are passed over platinum contacts. The selenium dioxide formed is absorbed in water. It can be estimated by reducing from metallic selenium with sulfur dioxide and weighing as the metal if the amount is 2 mg. or above (*7*). Smaller amounts (0.5 to 1.0 mg.) can be determined accurately by treating the selenious acid with bromide–hydrobromic acid mixture and titrating the selenium iodometrically

(*362*) with 0.02 *N* thiosulfate. The accuracy and precision are ±1 per cent.

Silica. For amounts below 5 per cent, the well-known colorimetric method (*79*) of forming yellow silicomolybdic acid with permanent standards made from picric acid is best. Phosphorus and iron interfere. For larger amounts, ashing and determining the silica by loss of silicon tetrafluoride must be used. When the residue is treated with hydrogen fluoride satisfactory results with samples containing 1 mg. or more of silica and accurate to ±1 per cent are reported (*292*, *343*).

Silver. For 0.5 to 5 mg. of silver, the Volhard method may be used, in which the silver is dissolved in nitric acid and titrated with 0.01 to 0.02 *N* ammonium thiocyanate, using ferric alum as the indicator.

Schulek (*294*) proposes a method which can be used in the presence of chlorides, bromides, and cyanides. The organic matter is first destroyed by sulfuric acid and hydrogen peroxide. To the neutral solution is added excess potassium cyanide. The excess is then destroyed by formic acid; 2 per cent phosphoric acid and an excess of bromine are added. Phenol is added to destroy the excess bromine and the cyanogen bromide is determined by adding potassium iodide and titrating with 0.01 *N* sodium thiosulfate. Results agree with the Volhard microprocedure:

$$AgCN + HCN + 2Br_2 = AgBr + HBr + 2CNBr$$
$$CNBr + 2HI = HCN + HBr + I_2$$

Sodium. The organic material is ashed and the sodium, 0.5 to 1 mg., is determined as sodium zinc uranyl acetate by the method of Barber and Kolthoff (*15*). In a drop-scale method, Holmes and Kirk (*137*) have succeeded in reducing the uranium salt with cadmium and titrating with ceric sulfate. Results accurate to ±1 per cent are reported when using 0.13 to 4.13 micrograms of sodium. Phosphorus interferes and must be removed.

Strontium. Strontium, 4 to 5 mg., has been successfully determined as the carbonate or sulfate (*325*, *377*).

Tin. One milligram or less may be determined iodometrically with results accurate to ±0.005 mg. (*138*).

Zinc. 1. The organic material containing 1 mg. of zinc is ashed and then determined iodometrically by a modified Lang procedure (*134*, *177*) according to the equation

$$2K_3Fe(CN)_6 + 2KI + 3ZnSO_4 = K_2Zn_3[Fe(CN)_6]_2 + 3K_2SO_4 + I_2$$

2. If the zinc is precipitated as the sulfide, it may be determined by liberating the sulfur as hydrogen sulfide and titrating iodometrically.

3. It may be precipitated with 8-hydroxyquinoline and weighed. The error is ±0.005 mg (*58*).

4. It may be precipitated by oxine [$Zn(C_9H_6ON)_2$] and titrated with a measured excess of potassium bromate mixture:

$$C_9H_7ON + 2Br_2 = C_9H_5ONBr_2 + 2HBr$$

Four moles of bromine react with the oxine, which is equivalent to one of zinc. The excess bromine is titrated with sodium thiosulfate. The error is ±0.005 mg (*59*).

DETERMINATION OF GROUPS

ACETYL. The determination of acetyl has been carefully studied The procedures can be classified in two groups:

1. Those in which aqueous acid hydrolysis is followed by distillation and estimation of the acetic acid formed. The method of Elek and Harte (*84*) is an example in which aqueous *p*-toluene sulfonic acid is used. The acetic acid is vacuum-distilled and determined iodometrically.

2. Those employing alcoholic acid hydrolysis which is followed by distillation of the resulting ethyl acetate. The acetyl in the acetate is estimated by hydrolysis with an excess of standard alkali and by titrating the excess. Strong alkali must be employed and for this reason it has been difficult to obtain accurate results on titrating alkali of this concentration with a microprocedure. Clark (*61*) has modified this method for semimicroquantities by steam-distillation of the acetic acid, followed by titrating with 0.02 *N* alkali. Matchett and Levine (*206*), in a method which can be modified for semimicroquantities, fractionally distill the ethyl acetate into 0.1 *N* alkali using a packed

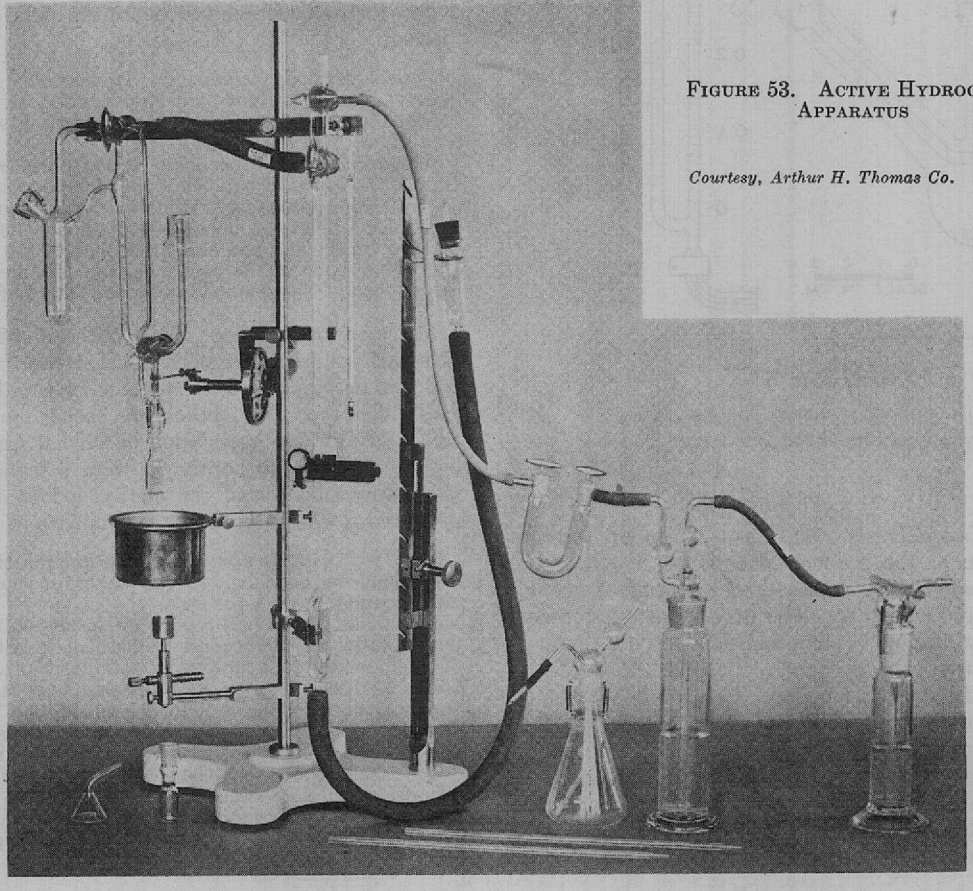


FIGURE 53. ACTIVE HYDROGEN APPARATUS

Courtesy, Arthur H. Thomas Co.

column. The column is arranged for operation under total reflux with intermittent, small-volume take-offs.

CARBOXYL. In addition to the usual method of determining carboxyl by titration with alkali (*233*), Tsurumi and Sasaki (*346*) have developed a micromodification of the method of Fuchs (*104*) and Hunter and Edwards (*141*), which depends upon the fact that even weak organic acids will immediately liberate hydrogen sulfide from solutions of potassium hydrosulfide, saturated with hydrogen sulfide, one molecule of hydrogen sulfide being evolved for each carboxyl group. The method possesses the advantage over the titration procedure that, since the reagent is a solution, acid with hydrogen sulfide, with few exceptions lactones and alcohols or phenol hydroxyl have no effect.

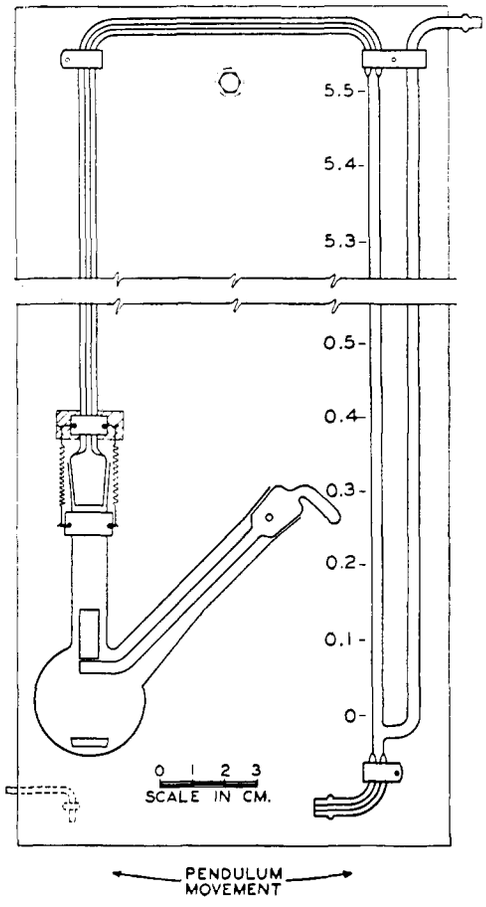


FIGURE 54. SINGLE-UNIT NONCOMPENSATING-TYPE HYDROGENATION APPARATUS

CARBONYL. Falkenhausen (*93*) has successfully used a micromodification of the method of Strache, in which the compound is treated with a measured quantity of phenylhydrazine and sodium acetate. The excess phenylhydrazine is determined by adding Fehling solution and the evolved nitrogen is measured.

SH GROUPS. In glacial acetic acid, compounds containing SH groups can be dehydrogenated to disulfide with iodine in stoichiometric proportions (*172*).

DOUBLE BONDS. Unsaturation is measured by (1) quantitative hydrogenation (see under Microhydrogenation), and (2) bromine addition by the Becker gravimetric method, using a 1- to 10-mg. sample (*274*).

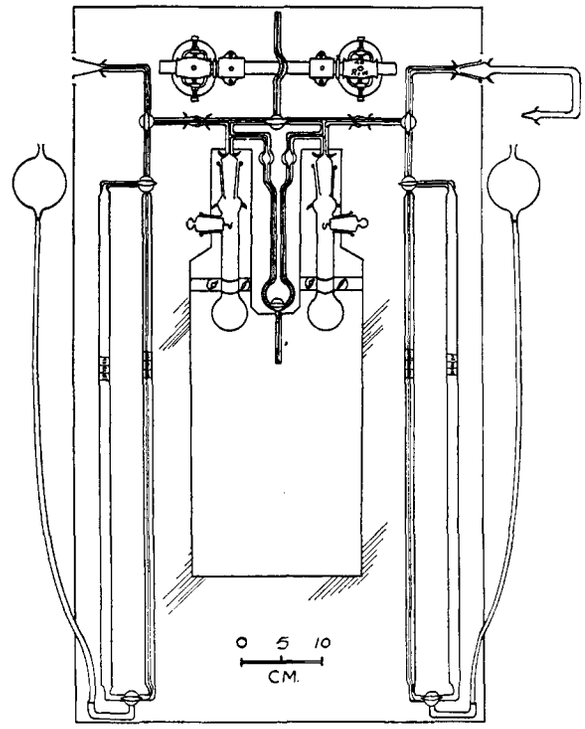


FIGURE 55. DOUBLE-UNIT COMPENSATING-TYPE HYDROGENATION APPARATUS

EXCHANGEABLE OR ACTIVE HYDROGEN. By the micromodification of the method of Chugaev and Zerevitinov, a known amount of Grignard reagent is added to the sample and methane is formed, which is measured (*319*) or burned (*91*). Figure 53 shows the apparatus used for the volumetric method.

Special Methods Limited in Application. The substance is dissolved in deuterium oxide, evaporated to dryness, and the increase in weight caused by replacement of active hydrogen by deuterium (*366*) is determined.

A modification of the above method has been developed by Hamill (*127*), which is much slower but serves to distinguish between active hydrogen and labile, or slowly exchanging, hydrogen, as in the methylene group of malonic acid. The determination depends upon the decrease in density of 15 to 100 mg. of 98 per cent deuterium oxide due to exchange, which is measured by a small quartz float. The method is accurate to ±3 per cent.

METHOXYL-ETHOXYL. This determination has become standard and reliable among microprocedures. Occasionally compounds which are not soluble in the digestion mixture give difficulty. The method has been subjected to collaborative study by the Association of Official Agricultural Chemists, and the results obtained were satisfactory (*64*). The iodometric titration of the methyl iodide or ethyl iodide is preferred to the gravimetric determination.

Elek (*83*) discusses the different modifications and the best conditions for carrying out this determination. The precision and accuracy are ±1 per cent.

Kolka and Vogt (*162*) report low results on the macro scale for pentamethyl anisole because of distillation of the compound out of the reaction mixture, with solidification in the condenser. This was overcome by sealing the sample with the reaction mixture in a tube and digesting for 2 hours at 135° C. The tube was cooled, opened, and quickly placed in the Zeisel apparatus, and the determination was completed in the usual manner.

The factors affecting the accurate determination of alkoxyl groups in cellulose ethers have been studied over a period of several years by Samsel and McHard (*284*). By using a slight modification of the usual method and apparatus, more consistent results were obtained.

Microhydrogenation

During recent years it has been found that by the quantitative determination of the amount of hydrogen absorbed by certain unsaturated compounds, information as to their structure may be obtained.

The sample, 5 to 10 mg., placed in 5 ml. of a solvent such as alcohol or acetic acid, together with a catalyst of platinum black, platinic oxide, or palladium black is shaken in an apparatus in the presence of hydrogen. The volume of hydrogen consumed is measured, and from this the degree of unsaturation can be calculated. While the time required to carry out a determination depends on the type of compound, the actual time required for hydrogenation is usually not over an hour. The accuracy is 1 to 2 per cent.

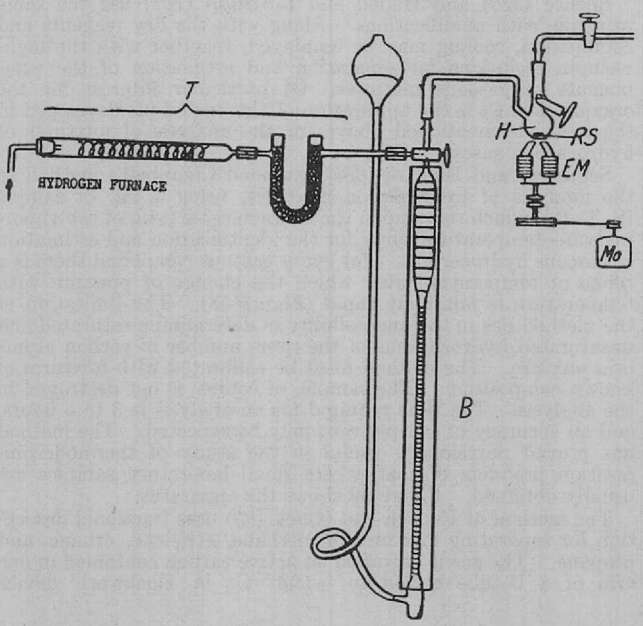


FIGURE 56. HYDROGENATION APPARATUS WITH MAGNETIC STIRRER

Inherent difficulties of this procedure are the need for shaking the apparatus and for accurate measurement of the hydrogen, which involves corrections or compensations for changes in pressure and temperature. Many designs have been published to accomplish this. Figure 54 shows a relatively simple form of apparatus by Johns and Sieferle (*147*), who give the method of calculating corrections for changes in temperature and pressure. The accuracy with this unit is ±2 per cent.

Prater and Haagen-Smit (*248*) have designed (Figure 55) a more complicated apparatus, typical of its class, in which two identical hydrogenation units are assembled, one of which may be used when temperature compensation is required; where this is not necessary, two determinations may be run. Both units carry a 4-ml. and a 50-ml. buret, which allows the apparatus to be used for small-scale preparative work. The maximum error on standards reported was 2 per cent when using 10-mg. samples. This apparatus may be purchased from the National Technical Laboratories, South Pasadena, Calif.

The two forms of apparatus just described are rigidly mounted and the shaking of the contents of the flask is accomplished by oscillating the complete unit with an electric motor.

Shaking a relatively large complicated and fragile unit, in order to agitate the contents of a small flask, leaves room for much improvement in design. To overcome this weakness so that only the flask need be shaken, a glass coil or oscillation around a ground joint has been employed.

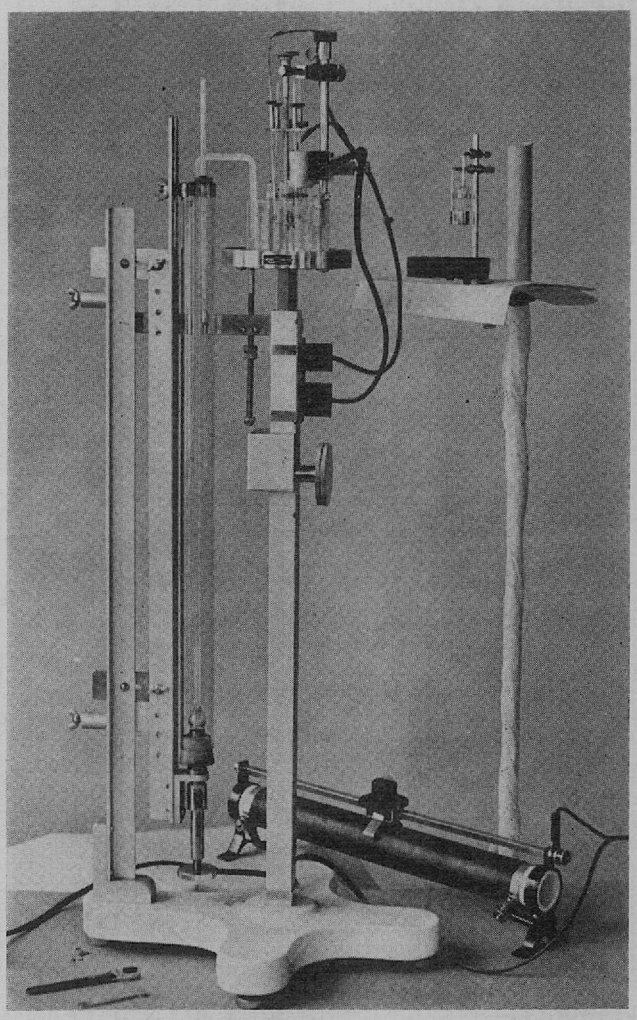
The novel arrangement of Weygand and Werner (*363*), where the contents of the flask are agitated with an electromagnetic stirrer, seems to be more logical and permits a more stable and convenient setup of the apparatus. Figure 56 shows the hydrogen purification train, *T*, the jacketed measuring buret, *B*, the hydrogenation flask, *H*, with the glass-covered iron stirrer, *RS*, which is rotated by the motor, *MO*, and the electromagnet, *EM*. The authors report an accuracy of ±1 per cent when using 3 to 5 mg. of sample.

Gas Analysis

CONSTANT-PRESSURE, VARIABLE-VOLUME METHOD. The technique for the analysis of a gas mixture, 25 to 100 cu. mm., consisting of constituents such as the carbon gases, water, hydrogen, nitrogen, and oxygen, has been studied in some detail. Krogh (*167*) obtained satisfactory results using the liquid absorbing agents commonly employed in standard gas analysis. The method employing liquid reagents has not found favor in precision work involving several components because it is difficult to prevent and eliminate errors of absorption of gases other than the one sought, and the rate at which the bubble rises or falls in the capillary affects the volume reading. While it is true that only differences are measured in gas analysis, it is more difficult with the wet method to keep these factors of vapor pressure and wall-film thickness constant in passing from one solution to another.

Krogh used a capillary of 0.25-mm. bore and volumes of 0.01 to 20 cu. mm. Schmit-Jensen (*288*) extended the system to combustible gases and perfected technique to the point where accuracy of ±0.1 per cent was possible, which was chiefly caused by the error in reading the buret to ±0.1 mm.

Blacet *et al.* (*35, 307, 317*) prefer dry reagents which are applied to the sample in a small container. The gas is trans-



Courtesy, Arthur H. Thomas Co.

FIGURE 57. BLACET-LEIGHTON GAS ANALYSIS APPARATUS

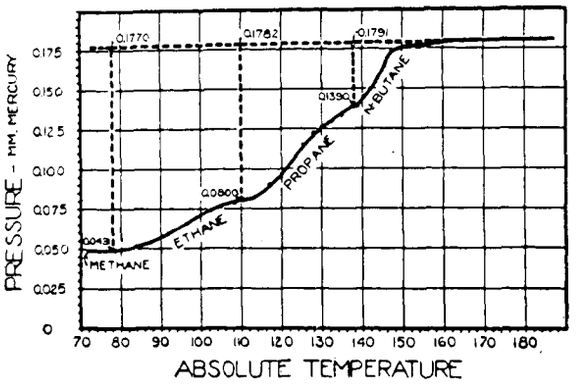


FIGURE 58

ferred and measured in a vertical gas buret of 0.5-mm. bore over mercury, as required. A sample of 50 to 100 cu. mm. is generally used in this method. Figure 57 shows a new assembly of this apparatus which has just been completed.

Specific reagents for such compounds as acetylene, benzene, and ethylene are available, which, with Blacet's combustion technique, allow the separation and determination of simple hydrocarbon mixtures. Complex mixtures must be first separated by other methods, described below, after which the combustion method can be applied for final identification and determination.

Sutton (*331*) uses the same method but prefers the buret in the horizontal position and adds a compensating buret which allows a change in temperature and pressure to be measured and the corresponding change made in the sample-measuring buret. A larger sample is used, 0.1 to 0.3 ml. at standard temperature and pressure, for easier manipulation in routine work. A precision of 0.2 per cent is obtained when using a 0.2-ml. sample, which is that commonly obtained in macro work. Swearingen, Gerbes, and Ellis (*333*) also prefer the horizontal buret.

CONSTANT-VOLUME, VARIABLE-PRESSURE METHOD. Many designs of this type of apparatus, first developed by Langmuir (*179*), have been made and this method was widely used before measurement in capillary tubes was considered accurate. They are usually elaborate, are built for a specific problem, and require some skill to operate and maintain. The gases are handled and passed over solid reagents at low pressures with the conventional vacuum technique, and the volume is usually measured by differences in pressure with some form of a McLeod gage.

Prescott and Morrison (*249*) have demonstrated that by this method it is possible to analyze samples of 5 to 25 cu. mm. at standard temperature and pressure with an accuracy of 2 per cent of the total sample; 5 per cent, with 1 cu. mm.; 0.025 cu. mm. may be detected.

Spence (*320*) and Haden and Luttropp (*121*) use the same principle with modifications. Along with the dry reagents and combustion, cooling may be employed, together with the high-vacuum technique for separation and estimation of the components of gaseous mixtures. Of particular interest for the organic chemist is the application of this technique developed in the methods mentioned above for the analysis of mixtures of hydrocarbon gases.

Sebastian and Howard (*308*) extended Campbell's method to the analysis of hydrocarbon mixtures, using 1 ml. of sample (S. T. P.), which relies upon the characteristic form of the vapor-pressure–temperature curve for the identification and estimation of gaseous hydrocarbon. For every gaseous compound there is a range of temperature over which the change of pressure with temperature is relatively rapid (Figure 58). The limitation of the method lies in the impossibility of determining saturated and unsaturated hydrocarbons of the same number of carbon atoms in a mixture. The method must be calibrated with mixtures of known composition. The sample, of course, is not destroyed in the analysis. The time required for an analysis is 3 to 5 hours, and an accuracy of ±1 per cent may be expected. The method has proved particularly useful in the study of thermodecomposition products of coal, where small laboratory samples are usually obtained. Figure 59 shows the apparatus.

The method of Euchen and Knick (*90*) uses fractional desorption for separating mixtures of methane, ethylene, ethane, and propane. The gas is absorbed on active carbon contained in one arm of a U-tube cooled to −103° C. A clockwork device

A. Dewar flask with vacuum-jacketed tube connection
B. Copper-constantan thermocouple in direct contact with freezing bulb
C. Copper-jacketed freezing tube placed in copper shield 2 mm. thick
D. Lower Dewar flask containing liquid nitrogen
E. Electric heating coil in series with rheostat and ammeter
F. McLeod gage
G, H, I. Mercury reservoirs
J, K, O. Mercury cut-off tubes with mark provided on *J*
L. Liquid nitrogen trap
M. Mercury vapor pump backed by Cenco Hyvac oil pump
N. Electric heater
P. Connecting U-tube for introduction of sample
Q, S. Mercury-filled U-tubes in connection with leveling bottles
R. Expansion bulb
T. Sampling reservoir
U, V, Z. Two-way stopcocks for connection with atmosphere or suction
X. Screw attachment connected with stopper for closing communication between reservoir *G* and McLeod gage
Y. Ground-glass stopper provided with cup for holding weights

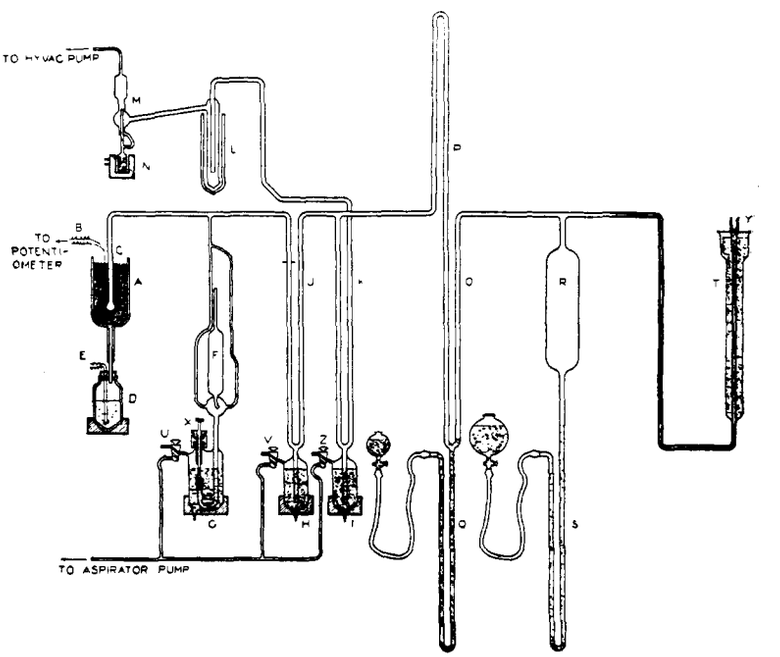


FIGURE 59. APPARATUS FOR MICROANALYSIS OF GASEOUS MIXTURES BY PRESSURE-TEMPERATURE CURVES

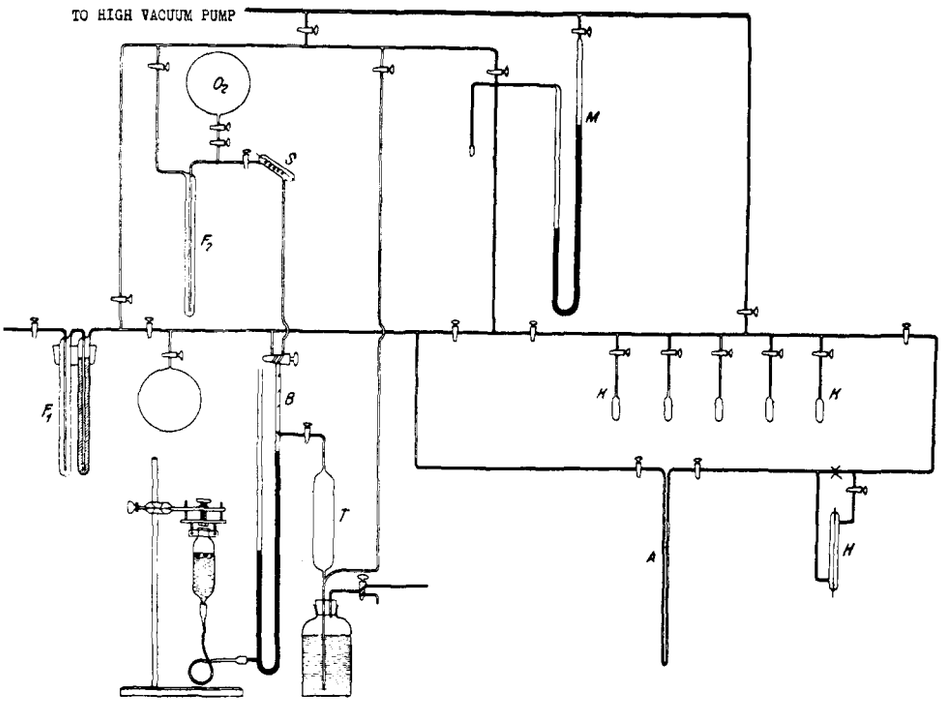


FIGURE 60. APPARATUS FOR ANALYSIS OF HYDROCARBONS BY FRACTIONAL DESORPTION

A. Fractional desorption tube
B. Buret
F_1. Liquid air trap
F_2. Tube for CO_2, H_2O, and O_2 after combustion
H. Hot-wire manometer
K. Receiver
M. Manometer
O_2. Oxygen
S. Platinum wire combustion tube
T. Toepler pump

slowly raises the U-tube into an electrically heated furnace at 250° C. and the liberated gases are delivered by a mercury vapor pump to a collecting vessel. The pressures, p in the U-tube and P in the vessel, are recorded as functions of time. Minimum values of p are found at the end of the desorption of each constituent and the proportions of the constituents are determined from the corresponding values of P.

Küchler and Weller (*171*) have modified the method of Euchen and Knick for mixtures of saturated and unsaturated hydrocarbon. The gases are first passed through a liquid air trap (Figure 60, F_1) and the uncondensable hydrogen and methane are measured and analyzed. The remaining hydrocarbons are fractionally desorbed from active carbon, each fraction is measured, and the pressure is plotted against time, as before. The final identification of each fraction is made by a microcombustion in oxygen over a heated platinum coil, *S*. The products of combustion are collected in F_2, separated by cooling, and measured. When these values are compared with calculated values, the hydrocarbon may be finally identified. A binary mixture such as ethylene and ethane may also be identified and its composition established by this combustion method. About 1 ml. of gas at normal temperature and pressure is required for an analysis, and the accuracy is approximately 1 per cent.

Benson (*28*) has designed an apparatus for condensing and separating volatile hydrocarbons by fractional distillation (Figure 61). The vapor pressure, heats of vaporization, density, and molecular weight are determined on the separated fractions and, from these physical constants, together with the gas analysis micromethods of Blacet *et al.*, amounts of hydrocarbons as small as 5 mg. can be identified. The physical constants can be measured with an error of approximately 1 per cent. Later refinements allow vapor pressure measurements of smaller samples (3 ml. at normal temperature and pressure) and at temperatures up to the boiling point. As an aid in the identification of liquids, an adaptation of the bromination titration method of Uhrig and Levin (*347*) to determine the olefin content, has proved satisfactory.

Aristarkhova (*9*) also uses a fractionation procedure and then analyzes each fraction to obtain a carbon or hydrogen number by which the gas is finally identified. Gas mixtures of 0.03 to 5 ml. are used.

Special Techniques

VOLUMETRIC ANALYSIS USING SMALL VOLUMES. When dealing with quantities below 1 mg., gravimetric procedures are not applicable because a balance of sufficient precision is not available. (Balances weighing 0.01 microgram, or less,

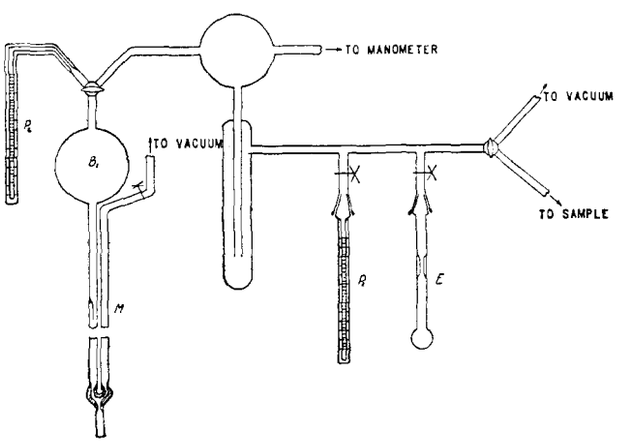


FIGURE 61. APPARATUS FOR ANALYSIS OF HYDROCARBON BY FRACTIONAL DISTILLATION

B_1. Toepler pump
E. Weighed bulb
M. Manometer
P_1, P_2. Micropycnometers

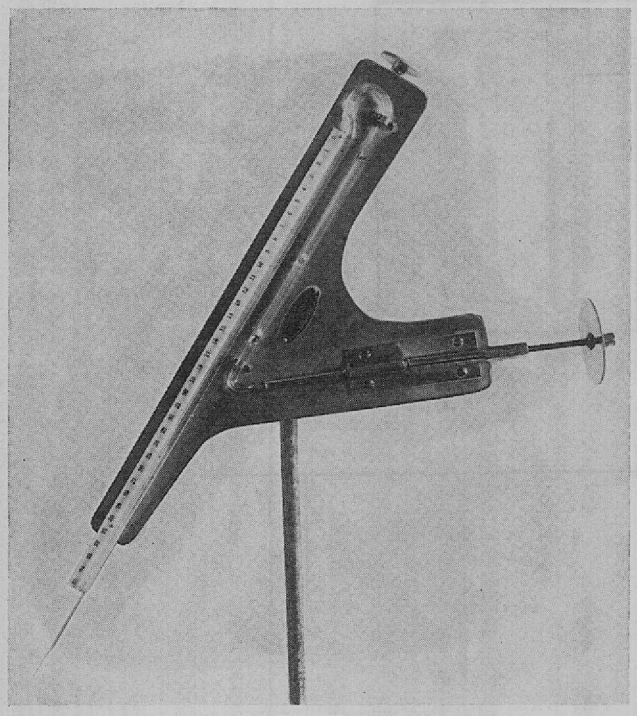


FIGURE 62. KIRK BURET

have been constructed, but their low capacity, need for careful manipulation, and rigid control of atmospheric conditions prohibit their use except for special research problems.) For such work, therefore, volumetric or colorimetric methods are best suited. In recent years the accuracy and precision of these methods have been improved, so that they are now a useful analytical tool. While they have been developed principally for biological studies, they are mentioned here for their application in the solution of special analytical problems.

The disadvantages of dealing with a trace of constituent in a large volume are many, among which factors of solubility, lack of sharpness in indicator change, and faintness of color make even ordinary microprocedures of gravimetric, volumetric, and colorimetric analysis impossible.

Kirk and co-workers (*150*) and Linderstrøm-Lang and Holter (*186*) in a series of papers deal with the technique, apparatus, and methods for determinations using volumes of 0.025 to 0.1 ml. Here the solutions are relatively more concentrated, and by using the standard volumetric methods with special precision pipets (0.01 to 0.2 ml.) and burets (0.5-mm. bore, accurate to ±0.02 cu. mm.), it has been found possible to achieve an accuracy of 1 to 2 per cent in determinations involving a few micrograms of constituent.

Figure 62 shows the buret used by Kirk. It is of the air-interface type, in which the solution does not come into contact with the actuating mercury thread.

Figure 63 shows the Rehberg buret with mercury plunger-type valve and reservoir at the top for filling. These burets and others with modifications, such as interchangeable tips, etc., are now available commercially.

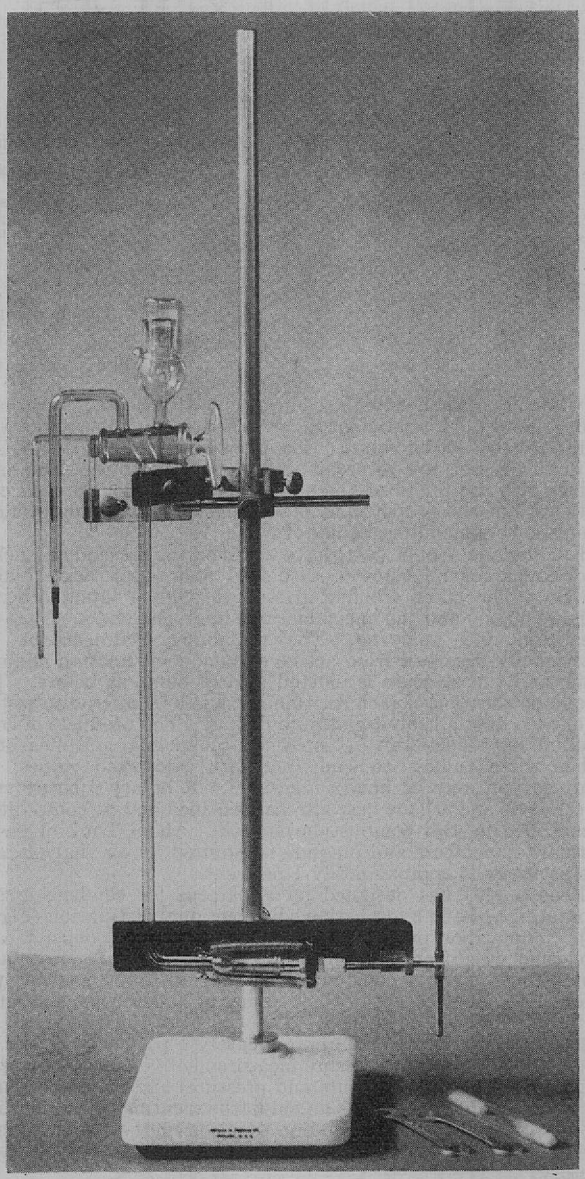
Two examples will serve to show the concentration of solutions used:

1. Calcium (0.5 to 12 micrograms) may be precipitated as the oxalate and determined by adding a measured amount of 0.01 *N* ceric sulfate in excess of the oxalate. The excess oxalate is titrated with 0.01 *N* ferrous ammonium sulfate, using phenanthroline–ferrous sulfate, as indicator (*154*, *189*).
2. Phosphorus (0.5 to 9 micrograms) may be precipitated as ammonium phosphomolybdate and a measured excess of 0.1 *N* sodium hydroxide added and the mixture heated. Then a volume of 0.1 *N* hydrochloric acid is added and the excess acid is titrated with 0.1 *N* sodium hydroxide to the pink phenolphthalein end point (*190*).

Drop-scale procedures have also been developed for chloride, using a potentiometric titration (*74*, *295*), iron (*151*), nitrogen (*345*), and sodium (*191*). (For a review of potentiometric titrations see Furman, *106*.)

MICRODIFFUSION METHODS. Another system of small-volume analysis is the diffusion method (*70*).

If, for example, a substance is placed in a vessel with a reagent which liberates a gas phase, and in another vessel is placed a reagent which continually removes this gas phase, the reaction, contrary to expectation, goes to completion (Figure 64) under suitable conditions in a surprisingly short time (0.5 to 2 hours). The errors in all these determinations can be kept within ±0.5 to 1 per cent. The simplest diffusion unit is shown in Figure 65. The liquid sample for the determination of ammonia (0.1 to 2 ml.) is placed in the outer chamber and 2 ml. of standard acid are run into the inner chamber. The greased glass lid is put in place over the cell, the lid is pushed back, and 1 ml. of potassium carbonate is added. The chamber is now sealed with the glass plate and, after a suitable time for absorption, the lid is detached and



Courtesy, Arthur H. Thomas Co.

FIGURE 63. REHBERG BURET

the contents of the inner chamber are titrated. To speed up the determination, cells have been made which may be evacuated, heated, or shaken. Nitrogen, 0.1 to 100 micrograms, may be determined with an accuracy of ±0.5 per cent. Nitrogen may be satisfactorily determined from material which liberates ammonia on treating with alkali, as well as from Kjeldahl digests. Prater, Cowles, and Straka (*247*) employ an ordinary Petri dish and absorb the liberated ammonia in drops of glycerol–boric acid mixture, suspended from the glass cover. Excellent results were obtained with amounts of ammonia nitrogen varying from 0.1 to 9 mg.

Other determinations by this technique include volatile amines, such as trimethylamine and halogens—these are oxidized in the outer chamber by acid permanganate or dichromate, and absorbed in 1 ml. of 20 per cent potassium iodide. This liberates an equivalent of free iodine which is titrated with sodium thiosulfate when the amount is above 35 micrograms. For 7 to 35 micrograms of chlorine, the liberated iodine is run directly into a microcolorimeter. Below 7 micrograms, starch is added and the color measured. The chlorine procedure may also be used for the determination of bromine. By this method carbon dioxide from carbonates may be run on samples liberating as little as 0.2 to 1.0 mg. with acid. The gas is absorbed in barium hydroxide, which is titrated with acid using an indicator of thymolphthalein.

Kirk (*345*) has modified these procedures for 1 to 20 micrograms of nitrogen for the drop-scale technique. Figure 66 shows the diffusion cups for Kjeldahl nitrogen designed so that the digestion and diffusion can be carried out in the same unit. The central cell in this apparatus is the small cup, and contains the measured amount of acid to absorb the ammonia which is titrated with 0.025 *N* sodium hydroxide. The probable error is 1 per cent with a precision of 0.3 per cent. Unit *B* has a ground-glass joint, so that the diffusion may be carried out in a vacuum.

In a method for nitrogen developed by Borsook and Dubnoff (*40*) the sample is digested by the Kjeldahl method using special digestion tubes holding 0.5 ml., and an aliquot of the digest is added to the regular diffusion cell.

The final titration on 0.1 ml. containing 0.3 to 10 micrograms of nitrogen is carried out electrometrically, using a glass electrode (Figure 67) and a Beckman pH meter. The precision is ±2.0 per cent and the accuracy within 1 per cent. Figure 68 shows the assembled titration apparatus, in which *g* is a precision microburet and *h* is standard sodium hydroxide.

Determination of alcohol in blood by diffusion has been proposed by Winnick (*369*). This is an adaptation of the method of Widmark. The alcohol diffuses from the sample in the outer chamber of the unit into the central chamber where it is oxidized by a solution of potassium dichromate. The excess dichromate is determined iodometrically. The precision is ±2 per cent and the accuracy ±3 per cent.

The same author (*370*) determines acetone by diffusion into sodium acid sulfite, which forms a complex with the acetone. This is later dissociated and the acetone determined by titrating the liberated sulfite with iodine.

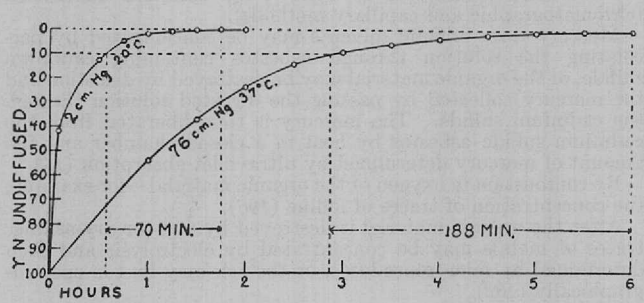


FIGURE 64

Diffusion methods in general require a minimum of mechanical manipulation and the transfer of the end product for the final operation is avoided. The disadvantages are possible interferences from the sample which diffuse and react similarly to the constituent desired. While diffusion methods have been developed primarily for biological chemistry, the method is described here because such procedures can, by modification, be applied to many types of problems in general microanalytical chemistry.

TRACE ANALYSIS. The determination of traces of constituents usually involves the separation and concentration of the trace from a large bulk of material in sufficient quantity for estimation. When macroprocedures are employed the size of the initial sample is often discouragingly large. By employing sensitive micromethods the sample size can usually be held within reasonable limits. The problem may be divided into two parts:

1. The constituent is present in relatively high concentration but the quantity of the sample is small. The technique for these determinations has been worked out by the drop-scale or ultramicromethods already mentioned.
2. The constituents are present in a large volume of material. If the system is a simple one where the trace material is the only one present which varies in quantity, a physical method can often be applied.

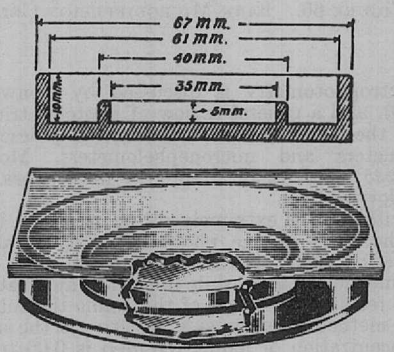


FIGURE 65. DIFFUSION CELL

Methods used include the following:

1. Ultraviolet absorption may be used with gases, liquids, and solids to measure the concentration of the impurity. The method of detecting and estimating trace impurities by their fluorescence under ultraviolet light is widely used, although it is limited to those materials which fluoresce or can be made to fluoresce by combination with reagents. Traces below the range for fluorescence can often be concentrated by capillary or chromatographic methods. Haitinger (*122*) reviews the application of fluorescence to microanalysis and Radley and Grant (*251*, *252*) cover the general subject.
2. Infrared absorption may be used for the detection of traces of organic constituents in an organic liquid. The detection and estimation of small quantities of impurities in organic gases, liquids, and solids by infrared spectroscopy have been made practical (*372*). The method requires standardization with knowns for quantitative work or it may be used to compare one or more samples as to their similarity. A sample of only 0.1 ml. is required and this is not destroyed by the analysis. The method is sensitive, 0.1 to 0.05 per cent by volume.
3. The difference in refractive index may be measured by an interference-interferometer (*224*). This method may be applied to liquids or gases. It is very accurate and sensitive—e. g., an accuracy for benzene vapors in air of 0.0008 per cent is reported by Brodskiĭ using a large Haber and Lowe instrument (*47*).
4. Determination of the dielectric constant of an organic liquid—for example, the determination of traces of water in benzene.
5. The detection, by the polarograph, of traces of certain organic compounds which undergo oxidation or reduction at the dropping mercury electrode. The polarograph is becoming a very useful instrument in organic analysis for determining mixtures of organic compounds, as well as for impurities present in trace amounts. While the method normally requires only 10 to 15 ml., special microcells (*202*) holding only a few drops of liquid are available. An excellent review on polarography as applied to organic chemistry is given by Müller (*219*).
6. The development of color on the addition of a suitable reagent and determination of the quantity of impurity by means

of a simple color comparison colorimeter or spectrophotometer. The measurement of color by spectrophotometric absorption is becoming a preferred method because, once standardized with knowns, the calibration requires only periodic checking.

A more important reason is that by the use of color filters or a diffraction grating a desired constituent may be selectively absorbed from a mixture of colors, and its concentration accurately measured. This new development is of great importance to those interested in trace analysis where the development of a desired color without interference is sometimes a difficult problem. Vaughan (*354*) has made a detailed study of the method as applied to metallurgical analysis, Knudson, Meloche, and Juday (*161*) to the determination of aluminum in the presence of iron with the aluminum-iron-hematoxylin system, and Owens (*240*) to organic compounds.

FIGURE 66. KIRK MICRODIFFUSION CELLS

Microspectrophotometry is discussed by Conway (*70*) and Müller (*220*), and a general review of photoelectric colorimetry is given by the latter (*221*). Kul'berg (*173*) describes various microcolorimeters and micronephelometers. Most macroinstruments (*225*) can now be adapted to micro work with cells of volumes as low as 1 ml.

7. The titration of extremely dilute solutions (0.001 *N*) by observing the color change photoelectrically is an interesting application (*194, 221*). Titration of such solutions by the dead-stop electrometric method, while limited in application, can give very precise results in the case of the iodine thiosulfate titration (*362*). The method is ten times as sensitive as the starch method and the concentration of iodine required is 0.06 to 0.09 microgram per ml.

8. Flagg and Wiig (*101*) summarize the application of tracer isotopes to analytical problems. For the organic chemist the stable isotopes can be of service in the analysis of mixtures of closely related compounds. When using N_{15}, a change of 3 to 5

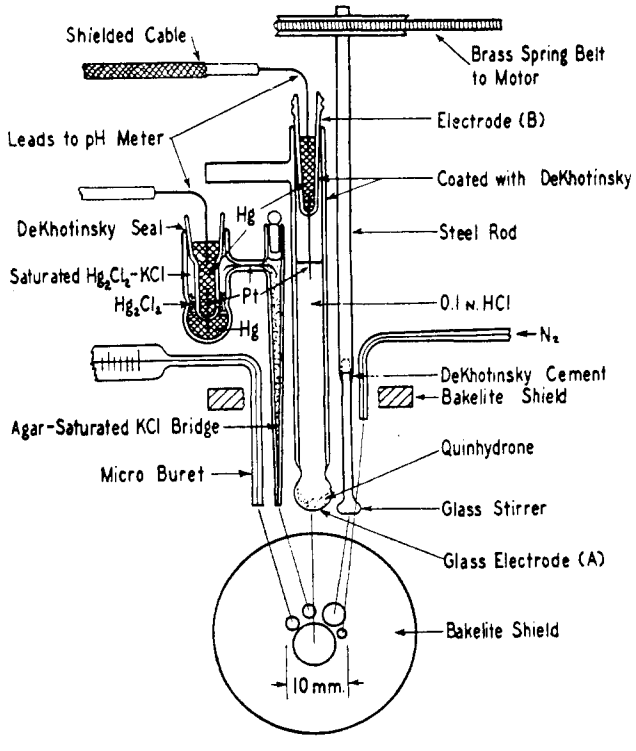


FIGURE 67. GLASS ELECTRODE SETUP

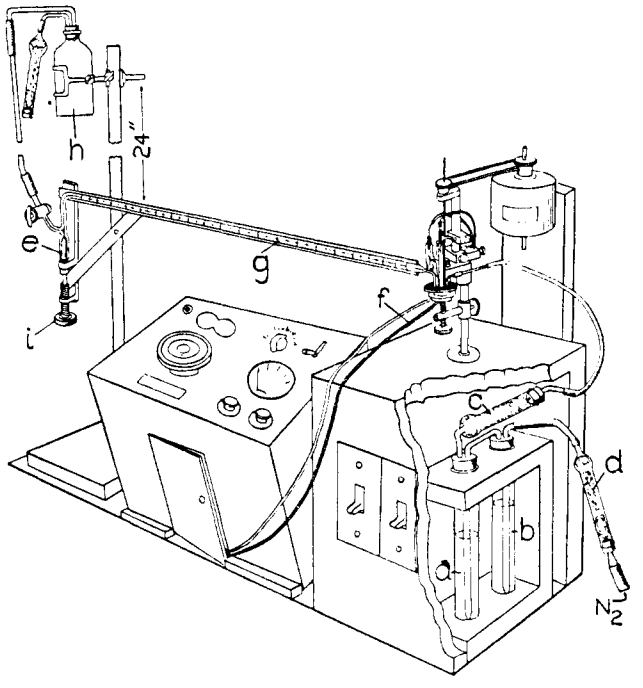


FIGURE 68. ASSEMBLED TITRATION APPARATUS

per cent of the amount normally present can be readily detected. The determination (accurate to 1.5 per cent) of the isotope by the mass spectrometer requires 2 to 25 mg. of the element which has been "enriched". The technique employed for the analysis of mixtures of amino acids is given by Rittenberg and Foster (*265*). The method is limited for most workers to deuterium, which has been available for some time, and to N_{15} which can now be supplied by the Eastman Kodak Company.

Where the trace amount is too low to be determined by the foregoing methods, some form of concentration can be applied until an amount is collected which is sufficient for a determination by micromethods.

For Gases. By freezing out the trace constituent at a suitable temperature; by passing the gas through a reagent, such as activated charcoal, whereby the trace is absorbed; and by concentration of the trace by passing the gas through liquid reagents.

For Traces in Liquids or Solids. By selective adsorption (chromatographic and capillary methods).

Mercury from organic solvents may be concentrated by percolating the solution through asbestos containing cadmium sulfide, or the organic material may be destroyed by digestion and the mercury collected by passing the digested solution through the cadmium sulfide. The mercury is then liberated from the cadmium sulfide–asbestos by heat in a closed chamber and the amount of mercury determined by ultraviolet absorption (*14*).

By combustion in oxygen of the organic material—for example, the concentration of traces of iodine (*196*).

After the organic material is destroyed by wet- or dry-ashing, traces of metals may be concentrated by electrolysis and then determined by microprocedures, or the ash may be run spectrographically (*132*).

For the determination of traces of ash in organic compounds, Norton, Royer, and Koegel (*238*) have shown that results, as accurate as those obtained by the macroashing method, are possible by burning in a combustion tube a 150-mg. sample with oxygen at 800° C. A special automatic combustion furnace (Figure 69) is used and the total elapsed time required is 30 minutes, as compared to 7 hours required for large samples burned in a muffle furnace. The amount of ash weighed was 0.03 to 0.6 mg.

Applications in Organic Fields

The application of microanalysis to certain organic fields is noteworthy and a very brief summary of the more important applications follows.

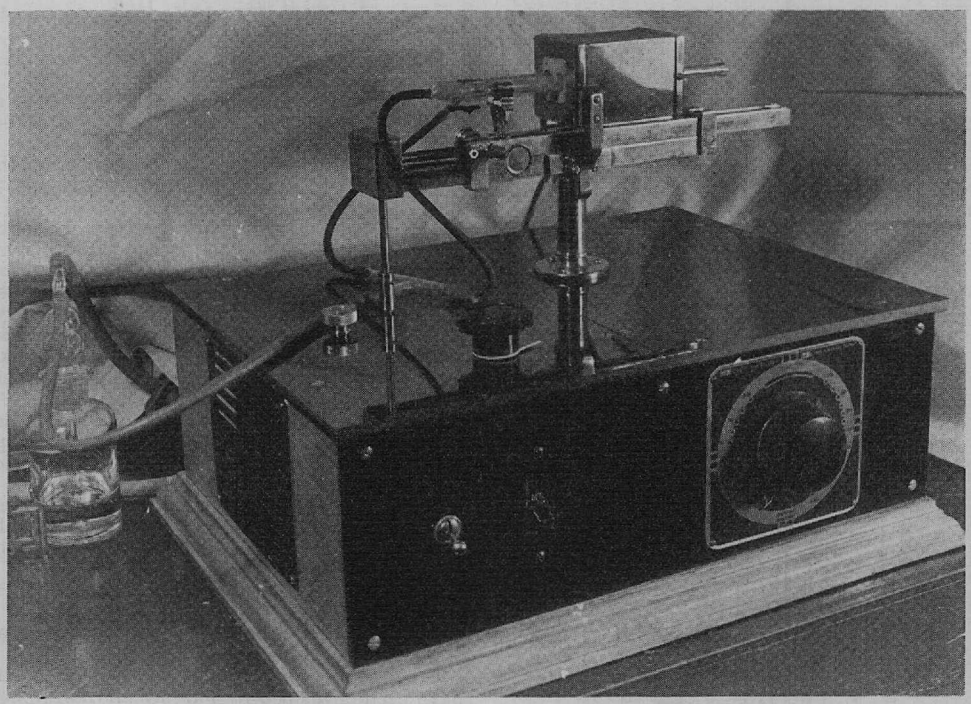


FIGURE 69. ROYER AUTOMATIC ASHING FURNACE

COAL AND SOLID FUELS. Kirner (*156*) has successfully applied micromethods to the determination in coal of carbon and hydrogen, nitrogen (by Kjeldahl or Dumas), sulfur (by Carius), ash, molecular weight, and methoxyl. The determination of moisture, using small amounts, while reproducible, is not representative of a mine-run sample, so that a large macrosample must be used. A critical discussion of the methods is given. The analysis of coal, whether by micromethods or by macroprocedures, shows the same inherent difficulties.

Belcher and Spooner (*26*) prefer semimicromethods, using 50- to 100-mg. samples, in order to eliminate possible sampling errors. Determination of nitrogen in coke, by the Kjeldahl procedure, is relatively slow but may be speeded up by mixing the sample with soda lime and passing steam through the mixture, whereby ammonia is freed and absorbed in dilute sulfuric acid. This ammonia is then liberated by sodium hydroxide, steam-distilled, and determined in the usual manner. The total time for one determination is 25 minutes. Carbon and hydrogen, using the Friedrich modification with platinum contacts and lead dioxide in a boat, has been found more satisfactory than the Liebig-Pregl method. A 50-mg. sample is used, over which oxygen flows at the rate of 12 ml. per minute. The time for one determination is 35 minutes.

It has been shown that the determination of nitrogen in certain coals by the Dumas or Kjeldahl method can yield values which are incorrect, owing to the incomplete destruction of the organic material or to loss of nitrogen from the digest. High results may be obtained in the Dumas procedure, because of the formation of methane.

Sulfur and chlorine are determined simultaneously by burning a 0.5- to 1-gram sample in oxygen at 1350° C. and absorbing the gases in hydrogen peroxide. At this elevated temperature no oxides of nitrogen are formed, so that a mixture of hydrochloric acid and sulfuric acid is obtained. The total acidity is determined by titration with alkali and the chloride is then run on this same solution by adding mercuric oxycyanide which reacts according to the equation:

$$Hg(OH)CN + NaCl \longrightarrow NaOH + HgCl.CN$$

This liberated sodium hydroxide represents the chloride, and the sulfate is obtained by difference. The combustion time is 10 minutes. Using this high-temperature, rapid-combustion technique, Belcher and Spooner (*26*) have succeeded in determining simultaneously carbon, hydrogen, sulfur, and chlorine. In this case, however, the sulfur and chlorine are collected on a weighed and heated silver gauze. The silver sulfate is dissolved off with water and from the loss in weight the sulfur is determined. The remaining silver chloride is dissolved in ammonium hydroxide and by again determining the weight loss the chlorine is estimated.

Gillet, Grandry, and Delaude (*113*) describe a modified Endell-Berl microfurnace for observing, in an inert atmosphere with a microscope, the melting point and decomposition characteristics of coal.

For studying the hydrogenation products of coal, Berl and Koerber (*30*) describe a semimicromethod using 2.5 to 5 ml. of sample for the determination of saturated hydrocarbons by nitrating with sulfuric-nitric acid mixture by which the aromatic hydrocarbons are made soluble. The original mixture is then catalytically dehydrogenated into aromatic compounds which are subsequently nitrated, giving a measure of the sum of the two types of compounds. The material unattacked in the second nitration represents the aliphatic compounds present in the original mixture. Results are accurate to ±1.0 per cent.

EXPLOSIVES. The advantages of using small samples of explosives for tests are obvious. The analysis of explosives for carbon and hydrogen has been successfully accomplished by mixing the sample with an inert material, such as fine sand or ignited kieselguhr (*8*, *107*).

Michel-Levy and Muraour (*212*) have used the microscope in the study of detonation.

Roth (*277*) has studied the same problem, using columns of explosives 15 to 30 mm. long, with an electric method for determining a time interval as small as 3 to 7×10^{-6} second. Results are accurate to ±3 per cent.

Berl and Kunze (*31*) modified the apparatus of Will so that the stability of cellulose nitrate may be determined with a 0.5-gram sample. Later, Berl, Rueff, and Carpenter (*32*) improved the test by keeping the products of decomposition in contact with the sample in a sealed tube and measuring changes of pressure within the tube by means of a "feather manometer". The size of the sample required is 5 to 50 mg. The method gives very reproducible results.

Klemenc and Hayek (*159*) describe a Lunge-type nitrometer for the determination of nitrate nitrogen, using 6 ml. of gas which is measured to ±0.02 ml.

Berl, Hoffmann, and Bremmann (*29*) also used a Lunge-type nitrometer with which they analyzed samples of 30 to 100 mg. to within 1 per cent of the truth.

Elving (*87*) has built a mechanically shaken compensating-type du Pont semimicronitrometer, (Figure 70) one tenth the capacity of the macromodel, requiring only 5 pounds of mercury and 100-mg. samples. The precision with samples of 10 to 13 per cent nitrogen is ±0.2 per cent. The results on cellulose nitrate when compared to those by the standard nitrometer are lower by approximately 1 per cent.

HYDROCARBON ANALYSIS (for analysis of hydrocarbon mixtures see Gas Analysis). *Aniline Points.* Wilkinson (*365*) used a semimicroapparatus for the determination of aniline points employing 1 ml. of olefin.

In the Mellon Institute (*207*) the fellowship of the Gulf Research and Development Co. has worked out methods for the aniline procedure, using 0.5 ml., as well as the titration for organic acidity where 0.05 ml. of sample can be examined. The final estimation is accurate to ±10 per cent, using a photoelectric colorimeter.

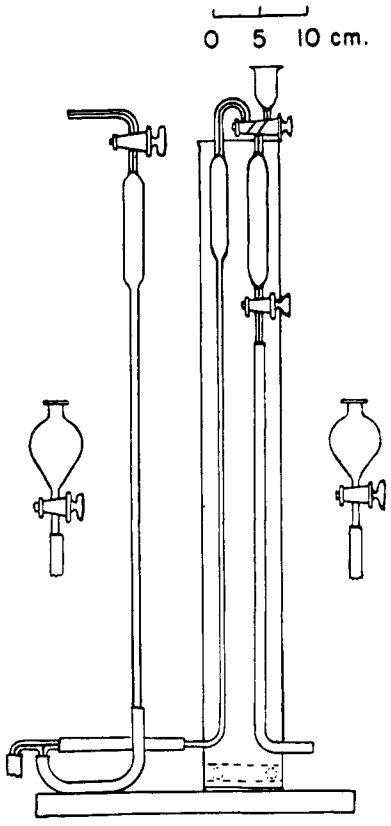


FIGURE 70. DU PONT SEMIMICRONITROMETER

Marion and Ledingham (*205*) have designed a convenient apparatus for measuring and introducing hydrocarbon gases into the combustion tube for the determination of carbon and hydrogen by the regular combustion procedure.

MILK. Several papers have been published on the analysis of milk, employing small samples. Typical among these is Elser's work (*85*) on semimicromethods for the analysis of condensed milk. Eight to 9 grams of milk are used to determine total solids, lactose, sucrose, protein and albumin globulin by the micro-Kjeldahl method, fat, chloride, and ash. The ash is used to determine calcium, potassium, iron, and phosphorus.

RUBBER. Wyatt (*373*) has analyzed soft vulcanized rubber compounds by microanalysis for the determination of acetone, chloroform, and alcohol-potassium hydroxide extracts, mineral fillers, total and free sulfur, and sulfur in fillers.

Proske (*250*) has applied the polarograph to the determination of organic compounds, such as accelerators and inorganic constituents. The precision is ±2 to 3 per cent.

FOODS. Diemair and Herrmann-Tross (*78*) summarize the micromethods which have been applied to the analysis of foods. The following determinations are covered: proteins and amino acids, fats, lipoid phosphorus, cholesterol, carbohydrates, organic acids, sulfurous acid, metals and non-metals, and miscellaneous references, including vitamins.

The concentration and identification of food colors, especially artificial dyes, offer a difficult problem to which new technique has been applied (1) by capillary analysis (*77*); (2) by chromatographic adsorption (*11*, *341*); and (3) by electrocataphoresis (*285*).

The determination of selenium has been the subject of recent investigations (*158*, *362*).

The testing of grain and flour using small samples has been investigated (*88*), and the results in most cases agree with those employing larger quantities (*128*, *129*).

FATS. The analysis of fats by micromethods has received sufficient investigation to establish the procedures as having adequate precision and accuracy. Gorbach (*115*) outlines the methods and apparatus for the estimation of total fats and lipoids by microextraction, unsaponifiable matter, phosphatide phosphorus, sterols, acid number, saponification number, iodine number, hydroxyl number, thiocyanate, and peroxide values.

Faure and Pallu (*95*) have applied capillary analysis for detecting and studying the rancidity of oils and fats.

Dyer, Taylor, and Hamence (*82*) have developed a semimicromodification for the determination of Reichert, Polenske, and Kirschner values in butter fat.

PULP AND TEXTILE FIBERS. *Cellulose.* Geiger and Müller (*109*) have studied the reducing values of cellulose, oxycellulose, and hydrocellulose, using the micromodification of the copper, ferricyanide, and iodine number procedures.

Heyes (*133*) has determined the copper number, using a 0.25-gram sample. Kettering and Conrad (*148*) have developed a rapid semimicromethod for the determination of cellulose in raw cotton fiber (*327*).

Larocque (*180*) describes a small single-rod mill for beating 1 gram of pulp.

The solubility number of cellulose has been determined by Nodder (*237*), using a 0.1-gram sample.

Micromethods for raw cotton for the determination of wax have been developed by Fargher and Higginbotham (*94*).

Ridge, Corner, and Cliff (*263*) describe the microdetermination of copper, iron, and zinc in textile fibers.

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