Round-Table Discussion

THE Division of Analytical and Micro Chemistry will undertake an interesting experiment at the fall meeting of the AMERICAN CHEMICAL SOCIETY, when it will sponsor round-table discussions in addition to the regular program consisting of symposia and general papers.

Three subjects have been tentatively selected: (1) metallography, or metal analysis, (2) water determination by the Karl Fischer reagent, and (3) polarographic measurements.

We believe that such round-table discussions offer an excellent opportunity for exchange of technical knowledge and experience, and if once placed on a successful basis, can do much to satisfy the varied interests of analytical chemists, especially when it is realized that the division is willing to sponsor the organization of discussions by any interested groups. Another purpose which these discussions can serve is to determine the need for, and aid in the crystallization of plans for, a broader symposium on the selected subject.

Plans are being made to record the round-table discussions and to present them in digested form in ANALYTICAL CHEMISTRY—briefly, progress reports in the various specialized fields that now characterize analytical chemistry.

The success of this new venture depends upon the development of widespread interest in the proposal and active participation in the discussions by individuals engaged in the fields covered. No formal papers will be delivered. A discussion leader, or moderator, will be selected for each subject and in turn he will be asked to select a small panel of experts to cooperate with him, but those attending will be expected to be vocal. Here is a golden opportunity to exchange ideas and to develop more discussion in our programs.

We will report more on the details of the round-table discussions at Atlantic City as they are developed.

Suggestions Wanted

THE Committee for the Standardization of Microchemical Apparatus of the Division of Analytical and Micro Chemistry has now completed the work for the Report on Recommended Specifications for Microchemical Apparatus—carbon-hydrogen, Dumas nitrogen, sulfur, and halogen. In the future the committee will turn its attention to those items for which no recommended specifications have been made, including the following:

1. Kjeldahl apparatus
2. Carius apparatus
3. Wet combustion apparatus, including Van Slyke manometric apparatus
4. Abderhalden (pistol) dryers
5. Pressure regulators
6. Weighing devices
7. Semimicrometers, absorption tubes, etc.
8. Apparatus for group determinations—methoxyl, methylimide, acetyl, etc.

Where a number of different articles are used to do the same function, the committee will recommend specifications for the one most widely used and for one or two of the others, for it is not the function of the committee to recommend apparatus but to recommend specifications for the various pieces so that the article will be identical, regardless of where it is purchased.

This hard-working committee requests recommendations and suggestions of those working in the field of micro, semimicro, or ultramicrochemistry. Suggestions should be addressed to the chairman, A. Steyermark, Microchemical Department, Hoffmann-La Roche, Inc., Nutley 10, N. J.

Basic Data

W. A. KIRKLIN, chairman of the Division of Analytical and Micro Chemistry, has appointed a committee of three—Wallace R. Brode, chairman, Harvey Diehl, and M. R. Fenske—to investigate the broad problem of the compilation and distribution of basic data.

The officers of the division and the members of the special committee are laboring under no illusions that the division single-handed can provide the answers, but it is felt that every day adds to the wealth of basic data being reported and without a more orderly and systematic method of reporting much of the value is being lost or obscured.

The complexity of the problem is such that many organizations must be included in the effort to bring order out of comparative chaos. How to finance such a huge undertaking is but one of the many knotty questions to be discussed. We believe it will be quickly demonstrated that the general subject is one requiring both national and international cooperation of many organizations.

Doctors Brode, Diehl, and Fenske are to be commended for their willingness to undertake an exploratory examination of the problem. It is a difficult assignment but one of prime importance to analysts and, therefore, the division does have a pertinent role.

Summer Symposium

THE Second Summer Symposium, cosponsored by the Division of Analytical and Micro Chemistry and ANALYTICAL CHEMISTRY, is now but a few days off. The central location of Wesleyan University at Middletown, Conn., will make it possible for large numbers to attend. The local committee on arrangements will make every effort to find accommodations for those who have not already signified their intention of attending on June 24 and 25. The opportunity to hear Professor Feigl and other prominent speakers discuss the general topic "Organic Reagents" is one that should not be ignored by analysts in the eastern states.

Evaluation of Accuracy in Photometric Analysis

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In light absorption spectrometry, plotting the data in the form of absorptancy against logarithm of concentration has important advantages over other plotting methods. For many instruments and operating techniques, the optimum range can be defined by inspection of the curve, as it is the concentration range in which it has its steepest slope. The maximum attainable accuracy is easily derived from the slope of the curve; the very flat portions of the curve at low and at high absorptancy emphasize the importance of selection of proper range if best accuracy is to be realized. Various methods of accomplishing measurement in the optimum range are discussed. For systems conforming to Beer's law, maximum accuracy is attained, with the instruments mentioned, when the absorptancy is about 63%, although the errors are not much larger in the absorptancy range 40 to 80%. In the commonly used calibration method, conformity to Beer's law is neither assumed nor required, and the evaluation of optimum range and maximum accuracy from the calibration curve is valid whether Beer's law is followed or not. Numerous instances are cited from the literature, in which the claims for range and accuracy are erroneous. There is a need for repetition of much of the earlier work in colorimetry, using modern photoelectric techniques and evaluating the range and accuracy on a sound basis.

FROM time to time during the past several years, various authors have discussed the errors involved in photometric methods of analysis. Twyman and Lothian (*30*) showed that the minimum error occurs at 36.8% transmittancy. Hogness, Zscheile, and Sidwell (*11*) formulated error curves showing the magnitude of the analysis error for various transmittancies.

In 1939, Ringbom (*24*) presented an excellent treatment of accuracy in colorimetric methods of analysis; in addition to showing how the attainable accuracy is limited by the Bouguer-Beer law, he pointed out that the accuracy ascribed to many determinations recorded in the literature is very arbitrarily established and often fails on the basis of considerations derived from the fundamental (Bouguer-Beer) law of spectrophotometry. Ringbom also emphasized the fact that the use of photoelectric instruments has extended the sphere of photometric analysis for use not only for very small amounts of material, but often for analysis of amounts formerly determined only by gravimetric or titrimetric methods. Schleicher (*26*) in general followed Ringbom's methods for calculating the average analysis error over the useful portion of the "error curve." Barnes, Liddel, and Williams (*3*) emphasized the importance of making analyses in the region of 37% transmittancy, where the error is a minimum. Hamilton (*10*) developed equations and plotted error curves based on the errors in the readings of galvanometer for zero setting, transmittance of blank, and transmittance of sample. Mellon (*19*, p. 56) mentions transmittancy of about 37% as the optimum.

In their well-known textbook, Kolthoff and Sandell (*17*, pp. 667–8) include a brief treatment of error in photometric analysis. Sandell (*25*, p. 49) discusses sensitivity and precision in considerable detail, and shows, as do the authors previously cited, the analysis error as a function of the extinctance of the solution:

$$\frac{dc}{c} = -\frac{dI}{I} \times \frac{0.4343}{E} \qquad (1)$$

in which $E = \log \frac{I_0}{I}$

Minimum error occurs when the extinctance is 0.4343, corresponding to 36.8% transmittancy, at which a 1% absolute photometric error produces a relative analysis of error of 2.72%, although the error is not much greater over the transmittancy range 20 to 60%. The way in which analysis error varies with transmittancy is shown in Table I and in Figure 1, the values of which are calculated from Equation 1.

Hamilton (*10*) states that "most chemists know in a general way that maximum accuracy cannot be achieved when .... readings are either very high or very low." This general principle appears not to have been put into extensive practice. From 1940 through 1947 ANALYTICAL CHEMISTRY has published well over four hundred articles involving photometric analyses; less than 20% of the papers make any mention of the most suitable concentration range for best accuracy in analysis, and even fewer make any evaluation of analysis accuracy on a sound, theoretical basis. The literature on photometric methods is replete with examples in which cognizance is not taken of the large error at low and at high concentrations of substances determined photo-
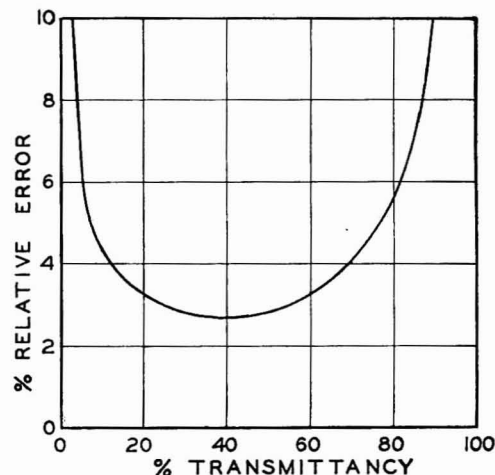

Figure 1. Relative Analysis Error as a Function of Transmittancy

Table I. Analysis Error

Transmittancy, %	% Relative Analysis Error per 1% Photometric Error
95	20.8
90	10.7
80	5.6
70	4.0
60	3.3
50	2.9
40	2.7
30	2.8
20	3.2
10	4.3
5	6.5

metrically. The error at low concentration seems especially to have been ignored, as indicated by the fact that the concentration range of a method is stated in such terms as "0 to 100 micrograms" (29); "up to 15 p.p.m." (15); "precision and accuracy ±0.08 p.p.m. in samples up to about 4.5 p.p.m." (4); "0 to 50 p.p.m." (22); "applies for nickel up to 5%" (9); "accurate to 0.1 microgram in the range of 0 to 10 micrograms per ml." (8); "sensitivity and range can be varied as desired by suitable dilution or variation of sample size" (6). Even the manufacturer of a widely used photoelectric instrument ignores the large analysis error at low concentration (16): "For the majority of colorimetric procedures, the reading will fall between 0 and 200 or 300. Readings above 500 to 600 should not be used as a basis of calculating results, since such readings represent relatively dark solutions. . . .The most satisfactory portion of the scale for colorimetric measurement is in the range 0 to about 400 or so."

Some authors, if not specifically so stating, at least imply that the full range of concentration over which Beer's law is followed is suitable for analysis. Table I and Figure 1, calculated from Beer's law, show that this is far from the truth; Beer's law is followed by most systems that have been applied for photometric analysis, yet in the low concentration range (especially at transmittancies more than 90%) the relative analysis error per unit photometric reading error is very large.

Using a Klett-Summerson instrument, Partridge (23) found that "although the absorption maximum of copper diethylthiocarbamate in organic solutions lies near 440 mμ, so that a blue filter can also be used, greater linear relationship over a wider range of concentration of copper can be maintained with a green filter." For the carbamate method for copper, Sandell (25, p. 223) notes that although a green filter gives a more nearly linear extinction-concentration curve than a blue filter, the latter permits a more accurate determination of small amounts of copper. Another example of an extension of the range by the use of filters is quoted from the Klett-Summerson clinical manual (16): " . . . with the blue color obtained in the Folin-Wu blood sugar method, if a red filter were used the increase in sensitivity would be so great as to make it impossible to read accurately even the moderate color obtained with a normal sample, let alone a high blood sugar, without such a dilution of the color as to impair the accuracy of the procedure. Under these conditions a green or blue filter is used. In this way the most satisfactory relationship between scale reading and a wide range of concentration is obtained."

When calibration data are plotted as per cent absorptancy against logarithm of concentration, as in Figure 2, it becomes obvious that the range cannot be extended over very wide concentrations, solely by the use of a filter, without decreasing the analysis accuracy (represented by the slope of the curve). For some purposes, of course, a wide range of application of a method may
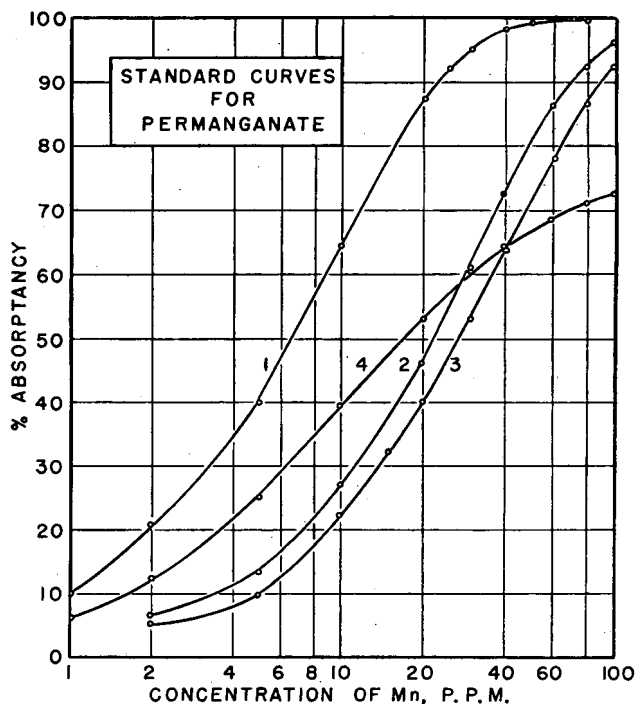

Figure 2.   Standard Curves for Permanganate

1, 2, 3.   Beckman spectrophotometer at 526, 480, and 580 mμ. respectively
4.   Willard and Ayres absorptiometer, Corning filter 430

be of more practical importance than maximum accuracy.

PLOTTING PHOTOMETRIC DATA

The great majority of colorimetric analyses are now made with photoelectric instruments, generally calibrated by the user for the method employed. An excellent critique of the many ways of plotting the data has been made by Mellon (19, pp. 47–49, 79–81). However, all the straight-line calibration graphs (log transmittancy, log absorptancy, optical density, extinctance, etc., against concentration) suffer the disadvantage of failing to show directly the concentration range for best accuracy. The straight-line graph indicates conformity to Beer's law, which is important in certain theoretical studies (dissociations complexation, etc.) and in the analysis of multicomponent systems. However, from the standpoint of utility in analysis of single-component systems by the usual calibration methods, it makes no difference whether the system being measured conforms to Beer's law or not; for application of such a method of analysis, it is more important to select a suitable concentration range and to evaluate the analysis accuracy than to prove conformity to Beer's law.

In order to define a suitable concentration range and evaluate the accuracy in photometric analysis, Ringbom (24) proposed that photometric data be plotted with per cent absorptancy as ordinates against logarithm of concentration as abscissas. If a sufficient concentration range has been investigated, such a curve always passes through an inflection, as shown in Figure 2. The accuracy is greatest when

$$\frac{dI}{\dfrac{dc}{c}} = \frac{dI}{d \ln c} = \frac{dI}{2.303 \, d \log c} \qquad (2)$$

reaches a maximum—that is, at the inflection in the curve, where the curve has its steepest slope. If the system conforms to Beer's law, the general form of the curve is always the same, the inflection occurring at 63.2% absorptancy (36.8% transmittancy); various extinction coefficients and layer depths produce only a displacement of the entire curve along the concentration axis. If the system does not follow Beer's law, the inflection occurs at a different value of absorptancy.

This method of plotting is illustrated in Figure 2. Curve 4 represents manganese as permanganate, determined with the thermoelectric absorptiometer described by Willard and Ayres (32), using a 2-mm. Corning filter No. 430, and round cells 1.8 cm. in inside diameter; curve 1, also for manganese as permanganate, is plotted from data taken by the writer, using a Beckman model DU spectrophotometer at 526 mμ, a square 1.00-cm. cell, selector switch at position 1, and operated at constant sensitivity by setting the blank to 100% transmittance by adjustment of the slit width. Data obtained with a Coleman model 10-S spectrophotometer (526 mμ, square 1.3-cm. cell), with a Lumetron model

Table II. Range and Accuracy

Curve	Instrument	Wave Length, mμ	Optimum Range, P.P.M.	% Relative Analysis Error per 1% Absolute Photometric Error
1	Beckman	526	3 to 20	2.8
2	Beckman	480	10 to 70	2.8
3	Beckman	580	15 to 90	2.8
4	W. and A.	430 filter	3 to 30	4.8

400-A and a Lumetron model 400-G (yellow-green filter 530, round cell 1.5 cm. in inside diameter), and with a Cenco Photelometer, industrial type B-2 (green filter, round cell 1.5 cm. in inside diameter), plot as curves practically coincident with curve 1.

The curves of Figure 2 show the advantage of this method of plotting (absorptancy against log concentration) over the usual Beer's law graph (log transmittancy against concentration, or extinctance against concentration). The considerations below apply to the direct-reading type of instrument, such as the Evelyn photoelectric colorimeter, the Lumetron model 400-A and 400-G, and the Cenco Photelometer, and also to instruments in which a linear potentiometer is used for electrical balancing to read transmittancy against a reference blank set to read 100%, as is the case with the Klett-Summerson instrument, the Fisher Electrophotometer, the Willard and Ayres absorptiometer, and the Beckman spectrophotometer operated at constant sensitivity as described in the previous paragraph; in any of these latter instruments a given transmittancy unbalance produces a constant galvanometer deflection regardless of the absolute value of transmittancy. The following generalizations also assume operation of a given instrument with a constant light source, constant cell thickness, and at a given wave length or with a given filter; the influence of some of these factors is discussed below and reference is made to curves 2 and 3 of Figure 2.

The concentration for greatest accuracy is immediately apparent from the graph, being the concentration corresponding to the steepest slope; there is usually a considerable nearly linear portion around the inflection point of the curve; hence good accuracy can be attained over a moderate concentration range.

The analysis error indicated by Equation 2 can be evaluated readily. Rearrangement of Equation 2 gives

$$\frac{dc}{\frac{c}{dI}} = \frac{2.303}{\frac{dI}{d\log c}}$$

or

$$\frac{\% \text{ relative analysis error}}{1\% \text{ absolute photometric error}} = \frac{230}{\frac{dI}{d\log c}} \quad (3)$$

The relative analysis error for a 1% absolute photometric error is obtained by dividing 230 by the slope of the curve, which is the absorptancy change, in per cent, corresponding to one logarithmic cycle on the concentration axis—i.e., a tenfold change in concentration. Whether the system follows Beer's law or not, the absorptancy–log concentration curve and Equation 3 can be used to evaluate the analysis accuracy.

The very flat shape of the curve at low and at high absorptancies emphasizes the fact that large inaccuracies result if analyses are made in these concentration ranges. Figures 1 and 2 both illustrate this point.

Plotting absorptancy rather than transmittancy has the advantage that low concentrations (abscissas) are associated with low numbers in the photometric data (ordinates); transmittancy, on the other hand, has an inverse relationship to concentration.

From the curves of Figure 2 and by the application of Equation 3, the optimum range and maximum accuracy are readily deduced; the values are given in Table II.

When plotted as log transmittancy against concentration, the data of curves 1, 2, and 3 give straight lines over the range investi-

gated, whereas the data of curve 4 show some deviation from Beer's law (due to the fact that the measurements were made with a filter which was not entirely suitable). Curves 1, 2, and 3 show an inflection in the region demanded by Beer's law (63.2% absorptancy), but curve 4 shows nonconformity to the law by having its inflection point at a much lower absorptancy. The deviation from Beer's law is of no especial consequence in measurements of a single component, and in no way vitiates the considerations outlined above for evaluating the optimum range and maximum attainable accuracy of the method.

MAXIMUM ATTAINABLE ACCURACY

As shown by Equation 1, the accuracy of photometric analyses is limited by Beer's law itself, and, for the instruments mentioned, 36.8% transmittancy (63.2% absorptancy) corresponds to a relative analysis error of 2.72% per 1% absolute photometric error. The accuracy is stated in terms of 1% absolute photometric error; the accuracy can be increased by reducing the photometric error below 1%. For purposes of this discussion, only the reading error (precision in setting and reading the photometer scale) is considered; other photometering errors, such as turbidity of solvent, dirt on absorption cells, cell positioning, stray light, dilution errors, etc., are not considered—not because they are unimportant, but because many of them are difficult to evaluate. A photometric reading error of 0.5% is not uncommon with certain of the instruments mentioned; and although some of the instruments can be read to 0.1% transmittancy, a precision (reproducibility) of 0.2% is probably the practical limit (25, p. 40). The relative analysis error, therefore, can be reduced to about 0.5%, which represents the present performance of many instruments in common use.

A method of reducing the photometric reading error is provided on the Beckman spectrophotometer in the form of a selector switch which "in position 0.1 gives a tenfold increase of sensitivity in range from 0 to 11%, and is used for samples having less than 11% transmission" (21). By the use of this selector switch the transmittancy reading can be made to a precision as good as 0.02% transmittancy, and can thus extend the range and actually increase the accuracy at transmittancies somewhat below 11%.

Curve 1 of Figure 2, from data taken by the writer with a Beckman spectrophotometer, will serve to illustrate this point. Using a 1.00-cm. cell and wave length 526 mμ, the curve indicates an optimum range of 3 to 20 p.p.m. of manganese; evaluating the accuracy in this range by means of Equation 3 gives a relative analysis error of 2.8% per 1% absolute photometric error; if the only consideration were a reading error of 0.2% absolute, then the maximum accuracy is about 0.6% concentration. In the range corresponding to about 90 to 98% absorptancy (10 to 2% transmittancy, concentration up to 40 p.p.m.), the slope of the curve indicates a relative analysis error of about 6% per 1% absolute photometric error; by the use of the 0.1 selector switch in this range, the reading error (precision) is about 0.02% absolute, and hence the maximum accuracy is about 0.1% concentration. The maximum accuracy is therefore attained at about 90% absorptancy (strictly, at 11% transmittancy, where the 0.1 selector switch first becomes operative), and the more accurate range can be extended upward to a point at which the flatness of the curve nullifies the advantage gained by the tenfold increase in the precision of reading the transmittancy. These considerations could be represented graphically, at least as a first approximation, by plotting the curve, above 89% absorptancy, on a tenfold magnified ordinate; inasmuch as a given transmittancy unbalance produces the same meter needle displacement regardless of whether the selector switch is used in position 1 or position 0.1, a tenfold increase in precision of transmittancy reading is not strictly realized.

RANGE

Ringbom's (24) method of plotting the results of photometric analysis has been used for many years by the writer (32) for evaluating optimum range and maximum accuracy, and has been applied to the data of many determinations reported in the litera-

ture. Limiting the discussion to instruments of the types previously mentioned, the general aspects of the change of error with change of concentration may be summarized as follows:

For a given cell thickness and wave length of incident light, the range over which a method has its best accuracy is limited, and is in general about a seven- to tenfold concentration change.

For a given cell thickness and wave length, if a wide range is claimed for a method, the method cannot have high accuracy over this wide range, especially in the low and the high regions. Wide range is often the result of the use of a filter rather than monochromatic light.

As shown previously, the relative analysis error per unit absolute photometric error is theoretically least at a transmittancy of 36.8%, although the error is not much larger at transmittancies between 20 and 60%. Inasmuch as in the Bouguer-Beer law there are three variables that influence transmittancy, there are various ways in which the transmittancy may be brought into this optimum range.

1. **Adjustment of concentration.** With many instruments (especially filter photometers having fixed absorption cell thickness) this is probably the simplest method. By proper selection of sample size or aliquoting, the concentration of the desired constituent is brought into the optimum range.

2. **Use of absorption cells of different thickness,** thin cells for solutions of high absorptance and thick cells for those of low absorptance. In such cases, separate calibration curves for each cell thickness are required if data are plotted as log transmittancy against concentration. For cells of different thickness, a plot of extinction coefficient against concentration can give a single curve cover ng a wide range. This is apparently the basis of the statement by Ashley (1): "Spectrophotometric methods may be applied over an enormous range, sometimes with only a single calibration curve... In the author's laboratory a single calibration curve [for permanganate] has been found usable over a 200-fold range—from about 0.01% to about 2.0% and probably even higher,''although he does not mention specifically the use of cells of different thickness in the construction of this calibration curve. Snyder (28) reports the determination of lead in the range 0 to 450 micrograms, using a Beckman spectrophotometer and cells of thickness 0.35, 1.00, 2.00, and 5.00 cm., and a calibration curve for each cell. His published calibration curves for 0.35- and for 1.00-cm. cells can be combined into a single straight-line calibration by plotting extinction coefficient against concentration. An unknown should be measured in a cell of such thickness that the transmittancy falls in the most accurate range.

3. **Adjustment of specific absorption coefficient,** which varies with the wave length of incident light. This method has been tested by the writer on permanganate solutions, using a Beckman spectrophotometer having 1.00-cm. absorption cells, and operating at constant sensitivity. Permanganate absorbs most strongly at 526 mμ; curve 1 of Figure 2 is a calibration at this wave length. Curves 2 and 3, Figure 2, represent measurements made at 480 and 580 mμ, respectively. These curves are practically parallel with curve 1, hence represent a similar accuracy; but they are displaced toward higher values on the concentration

axis. The use of light filters, rather than monochromatic light, in general gives absorptancy–log concentration curves that cover a wider range (16, 23, 32), but are not so steep (see curve 4, Figure 2) and hence do not give as good accuracy in analysis.

4. **Measurement against solution of known concentration.** Another means of extending the range of determination is to measure against a solution of known concentration, rather than against a blank. Using a standard solution of the desired constituent for reference, the photometer is adjusted to 100% transmittancy, by adjustment of the width of a slit (Beckman) or iris diaphragm (Cenco), or the intensity of the light source (Lumetron, Fisher), or electrically in the balancing circuit (Willard and Ayres), or combinations of these adjustments. A more concentrated solution is then placed in the light beam and its transmittance is measured. A calibration curve can be constructed in the usual way and applied to the measurement of unknowns against the same reference standard; or the concentration of an unknown solution can be calculated from the transmittance ratio, using Beer's law. This method of operation not only permits extension of the working concentration range, but it results in increased accuracy.

Curves 2 and 3 of Figure 3 are plotted from data taken with a Beckman spectrophotometer at 526 mμ, 1.00-cm. cell, and constant sensitivity setting, using reference standards containing 10 and 20 p.p.m. of manganese, respectively; 100% transmittance of the reference standard was accomplished by adjustment of slit width. Curve 1 of Figure 3 is for measurements of the permanganate solutions against a blank solution containing the reagents used for color development; it is identical with curve 1 of Figure 2. The slope of curve 3 corresponds to a relative analysis error of about 0.9% per 1% absolute photometric error, or about 0.2% concentration for a reading error (precision) of 0.2% absolute. When the transmittance ratio is below 11%, the analysis error can be still further reduced by the use of the 0.1 selector switch on the Beckman instrument, as described previously. This method of measuring by transmittance ratios becomes the more accurate—i.e., smaller relative error—the larger the concentration of the solution measured for a given difference between sample and reference standard, and appears to be limited only by the ability to adjust the instrument to 100% transmittance for a concentrated reference standard.

It is not always clear how ranges covering several hundred- to a thousandfold concentration change have been established, by various authors, as limits of usefulness. Many of the limits apparently have been given purely on a basis of convenience or of content of desired constituent in samples available.

Using the Evelyn colorimeter, Dolin (5) reports: "Concentrations from 0.25 to 75% benzene by volume have been determined with a mean error of 0.9%." This range was obtained by the use of aliquots, and not all of it is suitable for accurate measurement; no mention is made of the use of cells of various thickness or other methods of extending an accurate range. For the determination of cobalt with thiocyanate, using visual comparison and also a Spekker instrument with 1-cm. cell, Young and Hall (37) state, in their abstract, that the "useful range for this procedure lies within the limits 0.01 and 4.0% cobalt," although the text of their article shows that this range is obtained by choice of sample size and/or aliquoting, and their calibration curve covers a cobalt concentration range of 0 to 0.5 mg. per 10 ml. Using visual matching (Duboscq colorimeter, and Nessler tubes),
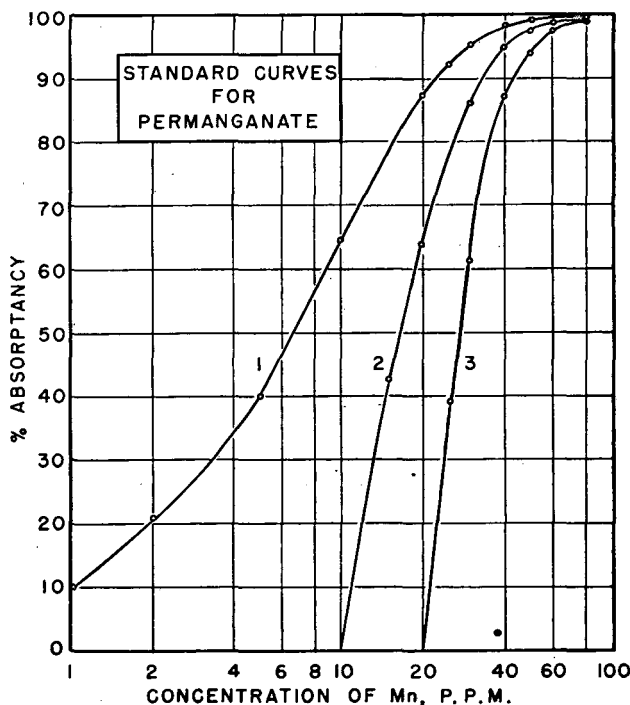

Figure 3.   Standard Curves for Permanganate

1. Solutions measured against reagent blank
2, 3.   Solutions measured against 10 and 20 p.p.m., respectively
All measurements with Beckman spectrophotometer at 526 mμ

Yagoda (34) reports the determination of aliphatic nitrate esters "over a range of 3.0 to 0.005 mg. with an average reproducibility of results of 1 to 3%." Baker, Miller, and Gibbs (2) report the colorimetric determination of tin over a range of 0.0005 to 0.5%, using a Duboscq colorimeter and a Fisher photoelectric filter photometer. In the estimation of oxygen, Winslow and Liebhafsky (33) give the upper limit of usefulness near 0.1%, the lower limit near 0.0001%, and 0.01% perhaps the most favorable concentration; however, they recognize the problem of the low concentration range: "It has not been possible to fix the precision of the method, which will obviously be lowest near 0.0001% oxygen."

In the determination of magnesium with titan yellow, using a Fisher Electrophotometer, Gillam (7) plots per cent "absorption" against concentration of magnesium, and states: "The curve flattens out in the neighborhood of 4 p.p.m. and for this reason the upper limit of concentration is set at 3.0 p.p.m. of magnesium. This flattening of the curve might appear as a possible weakness in the method, because on the level portion one could not judge whether he was dealing with concentrations of magnesium in the neighborhood of 4 p.p.m. or greater than 6.5 p.p.m." After pointing out that solutions containing around 6.5 p.p.m. of magnesium give flocculation of the color lake, whereupon the aliquot can be adjusted to the proper concentration, Gillam goes on: "As the maximum concentration of magnesium that can be present in any aliquot is 3 p.p.m., this method might appear applicable only over a narrow range in concentration—namely, 0.5 to 3.0 p.p.m. of magnesium. However, as the size of the aliquot may be varied from 75 to 1 cc., the concentration range of the method actually lies between 0.5 and 300 p.p.m. of magnesium." The writer sees no a priori reason for defining the range as that obtainable by aliquots "from 75 to 1 cc." if range were to be specified in this manner, so long as dilution errors are kept well below other errors of the method.

In the determination of the solubility of magnesium ammonium phosphate by the titan yellow method for magnesium, Uncles and Smith (31), using a filter photometer of standard design, have followed the same general method of plotting as used by Gillam, and set the upper limit of concentration on the basis of the linear shape and steep slope of a transmittancy-concentration curve in the low concentration range.

The plotting method used by Gillam (7), by Uncles and Smith (31), and by Yagoda (34) is probably the most misleading of all the various methods of handling photometric data; a steep curve at low concentration is taken as indicative of good accuracy, as small differences in concentration are easily detected by comparatively large differences in light absorbed. It must be remembered, however, that these small concentration differences are on small concentrations; in other words, the error may be small, but the accuracy (measured by relative error) may be poor. All colorimetric data plotted as transmittancy or absorptancy against concentration give steep curves at low concentration and flat curves at high concentration, yet the accuracy is highest in the middle range, for in this range a given photometric error produces the least relative analysis error. The stipulation of 3.0 p.p.m. of magnesium as the upper limit in Gillam's determination is not imposed by the flatness of the curve per se, but rather by the precipitation of the lake at a somewhat higher concentration, so that by the cell thickness and filters used by Gillam the method did not cover the transmittancy range where the relative error is the least.

The writer wishes to emphasize that the optimum range for the measurement, at a given wave length and cell thickness, cannot be extended by dilution or concentration, but, rather, that proper dilution or concentration (aliquoting and/or selection of sample size) should be used to bring the concentration of the desired constituent within the range suitable for accurate determination. The same result can be accomplished with some photometers by the use of cells of different thickness, by measuring at different wave lengths, etc. In some cases, maximum accuracy may not always be needed for the purpose at hand, and in trace analysis it may be impossible to obtain a sufficient amount of the desired constituent from a convenient size of sample to permit the measurement to be made in the optimum range.

As indicated previously, the maximum accuracy attainable with many instruments and operating techniques corresponds to a relative analysis error of 2.7% per 1% absolute photometric error when transmittancy is 36.8%, and the error is not much greater at transmittancies of about 20 to 60%, corresponding in many color systems to about a fivefold change in concentration. Below 20% and above 60% transmittancy, the relative error per unit photometric error increases rapidly and becomes enormous below 5% and above 95% transmittancy. Definition of limits of "usefulness" is therefore arbitrary, for the error changes continuously with change in concentration. A statement of useful range could be made more specific by giving the upper and lower limits of concentration between which the relative analysis error, for a given photometric error, will not exceed some specified value. If cells of different thickness, or different wave lengths of incident light or reference standards are used to extend the range, it should be so stated.

Numerous statements in the literature indicate the need for reexamination of many color systems, by modern measuring techniques and methods of evaluating data, in order to correct misleading and erroneous statements and conclusions.

For example, Snell and Snell (27, p. 86), in referring to the determination of chromate by Horn (12), state "that it is easier to distinguish between two colors than between colorless and colored." Sandell (25, p. 40) comments as follows: "It is difficult to see why this should be true, and the experimental evidence on this point is not convincing." As a matter of fact, it can be shown that for a given cell thickness and wave length, the difference between a colorless and a faintly colored substance can be detected readily, although the concentration of colored substance in a faintly colored solution may not be determined with much accuracy. Furthermore, it is difficult to reconcile Horn's report (12) that for chromate the maximum sensitiveness is between 0.004 N and 0.008 N, with the report of Horn and Blake (13) that chromate could be satisfactorily determined from 0.004 N to 0.000007 N. (Incidentally, a concentration expressed in normality has no significance in colorimetry.) Horn and Blake also give the range for copper as 0.6 N to 0.0013 N, and report that the sensitiveness for cupric ion and for cupric ammonia ion is about the same (14). If these reports are valid for visual colorimetry, they are not correct for photoelectric methods by the usual operating techniques.

An outstanding example of erroneous statement of "range" is the following, for the determination of copper by ammonia: "The greatest accuracy is given when the solution contains 0.01274 mg. of copper per cc. The addition of 0.000016 mg. of copper can be detected at that concentration." This statement has appeared for many years in the treatises on colorimetric methods by Snell and Snell (27, p. 143) and by Yoe (35). Mehlig (18) has expressed doubt as to the validity of these claims, especially in view of the fact that Yoe and Crumpler (36) reported that by visual methods, using a roulette comparator, a solution containing 8 p.p.m. of copper could be distinguished only with difficulty from 7 or 9 p.p.m., and with certainty from 6 or 10 p.p.m. On the basis of the considerations previously presented regarding range, a specification of an optimum concentration to four significant figures is absurd.

SCOPE OF LIGHT ABSORPTION SPECTROMETRY

Colorimetric methods have been classified according to the reason for their use (27, p. 72).

Some find their popularity because they are rapid. Accuracy is sacrificed for speed in obtaining the final result.

A second class is used because it furnishes a method of determining small amounts of substances with greater accuracy than is possible by gravimetric or volumetric methods. [Kolthoff and Sandell (17, p. 645) indicate this as the primary advantage of colorimetric methods.]

A third class contains methods where no gravimetric or volumetric method is available.

The utility of the third class of methods is above question. As to the first class, the advantages of rapidity are obvious; however, a sacrifice of accuracy is not a necessary consequence of speed in obtaining the final result, for the maximum attainable accuracy is largely a matter of proper selection of concentration range, cell thickness, wave length of incident light, operating

technique—e.g., measurement of transmittance ratios—and precision of the measuring device. In regard to the second class, the same factors are also important in relation to accuracy. With a modern photoelectric instrument and proper selection of transmittancy range, the error of many colorimetric determinations can be kept within about 0.5%, which does not compare unfavorably with many gravimetric and titrimetric processes. Certain instruments—e.g., the Beckman spectrophotometer, using the 0.1 selector switch—and operating techniques (measurement of transmittance ratios) may reduce the error considerably. The idea that colorimetric methods were particularly useful only for small amounts of material has been the basis for many misleading and erroneous statements, with claims of accuracy in the very low concentration ranges that are impossible with the instruments and techniques employed in the experimental work. Furthermore, relatively large amounts of certain material can be measured photometrically; the writer (32) has measured ferrous sulfate solutions photometrically over a concentration range of 0.4 to 40 mg. of iron per ml., and has shown that in the range of 6 to 30 mg. of iron per ml.—a range entirely suitable for gravimetric or titrimetric determination—the relative analysis error was about 3% per 1% absolute photometric reading error, or 0.6% on the basis of a precision of 0.2% in setting and reading the scale of the instrument used. By employing the method of measuring transmittance ratios, previously described, the range of many methods could be extended upward, with an actual increase in accuracy.

In the interest of stimulating authors to make an evaluation of the range of applicability and the accuracy of a method, the writer proposes the following suggestions:

1. The simplest means of establishing both the range and the accuracy of a method is to plot per cent absorptancy against logarithm of concentration, according to the method of Ringbom. The optimum range, for a given cell thickness and wave length of incident light, is immediately apparent from the curve, and the accuracy is easily evaluated, from the slope of the curve and the precision (reproducibility) of the measurement, by means of Equation 3. Any statement of range that gives zero concentration as the lower limit is in itself evidence that the author has ignored the fundamental principle that at very high transmittancy the analysis error is inordinately large. Although it is often true that the optimum range can be obtained from a log transmittancy-concentration curve by noting the range corresponding to transmittancies from about 60 to 20%, or from an extinctance-concentration curve by noting the range corresponding to extinctancies from about 0.2 to 0.7, these methods of plotting do not show the optimum range in direct fashion, and they provide no simple means of evaluating the analysis accuracy.

2. Cell thickness, wave length of incident light, operating technique (measurement against blank or against reference standards), and the precision with which the scale that indicates transmittancy or absorptancy can be read, should be stated, so that the maximum attainable accuracy can be evaluated by the method discussed earlier in this paper. The problem of evaluation is slightly more involved if the instrument has a scale graduated in arbitrary units, especially if the scale is logarithmic with respect to light transmittancy.

3. Accuracy should be reported in relative rather than absolute values. Kolthoff and Sandell (17, pp. 263–4) point out that the error, E, which indicates the accuracy of a measurement, is not usually expressed in absolute values but in relative values—that is, with respect to the true value, T, because the value of E without regard to the value of T is of no practical importance. When the proposed method involves considerable treatment of the sample in preparation for measuring the desired constituent photometrically, a statement of accuracy on the basis of per cent recovery indicates, of course, the over-all accuracy of the method. The contribution of the error in reading the photometer can be evaluated as in paragraph 1 above, and thus aid in evaluating other sources of error.

4. It is desirable that calibration data (concentrations and photometer readings, preferably in per cent transmittancy or absorptancy) be published for the proposed method. Such data can be given in tabular form, or graphically as transmission-wave length curves for various concentrations, or a "Beer's law" plot, or by Ringbom's method described herein; the writer prefers the latter as being the most useful for the purpose at hand. Space limitations for publication have no doubt contributed to the lack of data in many articles. In discussing Beer's law graphs in

light absorption spectrometry, Mellon (20) mentions that "straight lines show conformity, a fact which ordinarily needs merely to be stated in a paper." However, merely the statement of conformity to Beer's law without any numerical data in any form leaves the reader of such a paper no basis for evaluating the range and accuracy of the method if it was not done by the author of the paper. Many statements or conclusions typified by the examples cited in the present article could be corrected, without a repetition of the experimental work, if calibration data had been published in any form in the original articles.

Although most of the considerations discussed herein were restricted to the operation of certain instruments, such as those in which the indicator unbalance is proportional to the transmittancy unbalance regardless of the absolute value of transmittancy, and for which the optimum is 36.8% transmittancy, it is believed that they will apply in a general way to instruments in which the optimum is not at 36.8% transmittancy, and/or in which the indicator response per unit transmittancy unbalance varies with the magnitude of the transmittancy, or with the wave length as is the case with a Beckman spectrophotometer operated at constant band width. It would seem possible to evaluate analysis accuracy on essentially the same basis as presented in this paper, if the performance of the instrument is known or can be evaluated.

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Chemical Assay of Crystalline Penicillins

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A method for the chemical assay of crystalline penicillins uses potassium ferricyanide as an oxidizing agent and ceric sulfate as a volumetric standard. The method is rapid and sensitive and may be applied to procaine penicillins.

DURING investigations undertaken in this laboratory into chemical methods of analyzing penicillins for total penicillin activity, the use of potassium ferricyanide and ceric sulfate was initiated because it seemed that such a method would possess several advantages: rapidity, sensitivity, and the use of only one standard solution which is stable and may be used for long periods without restandardization. The sensitivity of the method is proved by the fact that 1 mg. of penicillin requires 5.25 ml. of standard ceric sulfate for its titration, while by the iodometric method (*1*) the same weight requires only 2.52 ml. of standard sodium thiosulfate.

REAGENTS REQUIRED

Potassium ferricyanide. Solution A, 15 grams per liter. Solution B, 15 grams of potassium ferrocyanide and 40 grams of sodium hydroxide per liter.
Sulfuric acid, 10 N solution (approximate).
Ceric sulfate, 0.01 N solution.
Setopaline, 0.1% solution.

PROCEDURE

To a 1- to 5-ml. aliquot containing 0.75 to 1.25 mg. of penicillin in a 25 × 200 mm. test tube add 5 ml. of potassium ferricyanide Solution A. To another similar aliquot add 5 ml. of Solution B (not more than one week old). Heat both aliquots in boiling water for 15 minutes. Cool in running water, add 5 ml. of 10 N sulfuric acid, and titrate with 0.01 N ceric sulfate, using setopaline as indicator. Run blank determinations on the ferricyanide solutions daily and subtract these from the penicillin titrations to determine the net titrations. Net titration B minus net titration A measures the amount of penicillin present in the aliquot. Boiling time is not critical and may be extended to 20 minutes. Titrations need not be carried out immediately. Standing for 1 hour has not affected the titration of samples.

For reference a regression line was established using the above procedure. As most crystalline penicillins are largely penicillin G, crystalline sodium penicillin G in amounts varying from 0.10 to 2.25 mg. was used as standard. Titrations varied from 0.40 to 12.25 ml. Both water and a 1% phosphate buffer at pH 6.0 were used as solvents for penicillin. Using 113 points determined over a period of 2 months, the following equation was established

$$Y = 0.0489 + 0.180996X$$

where X represents milliliters of ceric sulfate used and Y milligrams of penicillin. The correlation coefficient of this equation was +0.9975 and the standard error of prediction ±0.04 mg.

Results of the analysis of a number of samples of penicillin by the ferricyanide method are compared with results by the iodometric method and bioassay in Table I. Aliquots containing 0.75 to 1.25 mg. of penicillin were used for the ferricyanide method and aliquots containing 2.5 to 3.0 mg. for the iodometric method. Results by the ferricyanide method approximate those of the bioassay more closely than do results of the iodometric method.

APPLICATION TO PROCAINE PENICILLINS

When the ferricyanide method was used to determine the potency of procaine penicillins, results were approximately double those of the bioassay. To determine the validity of halving the results, a procaine regression line was established using the method outlined above with amounts of procaine hydrochloride varying from 0.25 to 2.5 mg. The factor 0.8663 was used to determine the procaine content. The equation in this case was

Table I. Comparison of Methods of Penicillin Assay

(Results expressed as units per milliliter of solution)

Sample	Bioassay	Iodometric	Ferricyanide
40,206	1200	1096	1090
40,219	1170	1113	1182
40,080	1130	1070	1145
40,379	1050	991	1043
40,380	1160	1178	1240
40,377	1140	1066	1200
40,198	950	881	990
40,105	1090	1046	1107
40,455	1040	933	1028
40,093	1137	1003	1149
41,618	1120	933	1205
41,861	1104	916	1086
40,378	1150	1108	1165
40,394	1210	1108	1254
41,413	1101	846	1198
40,197	1107	1026	1089
41,208	1247	1002	1121
40,304	1148	988	1093
40,196	1132	988	1131
43,572	1155	1114	1294
43,574	1180	1016	1222
44,260	1000	948	1085
Mean	1124	1017	1142

$Y = 0.0054 + 0.145615X$, the correlation coefficient was +0.9966 and the standard error was +0.05 mg. Later, determinations were made with a sample of procaine. These points fell as close to the line as most of those used in its determination; so the line was not changed.

Taking the molecular weight of penicillin G as 334 and procaine as 236, the proportion by weight of penicillin to procaine in 1 mg. of procaine penicillin must be 0.586 to 0.414. The penicillin results used to determine the regression equation were modified by multiplying the titrations by 0.586; the titrations of the procaine regression were multiplied by 0.414. New lines using these values were established. In Figure 1 are plotted the original and the corrected lines. It is readily seen that, for values from 0.35 to 0.60 mg. of penicillin or procaine, there is little differ-
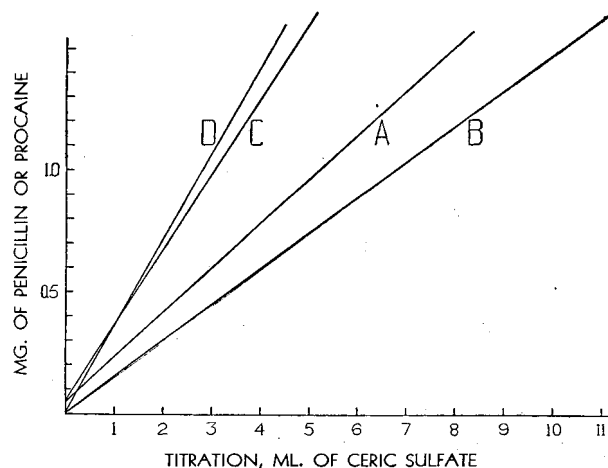

Figure 1. Regression Lines for Ferricyanide Determination of Penicillin and Procaine

A. Penicillin *C.* Penicillin titrations × 0.586
B. Procaine *D.* Procaine titrations × 0.414

Table II. Assay of Procaine Penicillin

(Results in units per milligram)

Sample	Bioassay	Iodometric	Ferricyanide
43,165	995	444	902
43,331	1060	548	872
43,298	1015	942	877
43,300	1000	948	1081
43,321	1030	970	1017
43,322	1040	970	904
43,323	1035	942	964
43,329	1077	942	1028
43,163	942	522	1053
43,164	839	410	959
Mean	1003	764	966

·ence between the corrected lines. Thus, by the ferricyanide method, it is permissible to determine procaine penicillin as penicillin and divide the results by 2 if an aliquot containing approximately 1 mg. of procaine penicillin is used.

Results of the analysis of procaine penicillin by this method are compared in Table II with results by the bioassay and iodometric methods. The mean difference between the bioassay and ferricyanide methods is 37 units, and between the bioassay and iodometric methods it is 239 units.

It is obvious that the ferricyanide method can be used for the analysis of procaine penicillins only while they retain their full potency. If a loss in potency is suspected, the compound should be analyzed before and after the addition of penicillinase. The penicillinase prepared in this laboratory (2) does not interfere with the determination nor affect procaine. Although penicillinase destroys completely the biological activity of penicillin, it does not destroy all its chemical activity as measured by the ferricyanide method. The loss in activity varies with the conditions under which the penicillinase acts but is relatively constant under given conditions. Once this loss has been determined for the given conditions, the difference in the ferricyanide assay before and after the action of penicillinase will give a good estimate of the potency.

CONCLUSIONS

The ferricyanide method possesses several advantages. It is rapid; determinations require less than 30 minutes. It is sensitive, using less than 1 mg. of penicillin per determination. It is simple and needs only a minimum of standard equipment. The number of standard reagents necessary is reduced to one which is stable.

The chief disadvantage of the method is that it may be applied only to crystalline penicillin. Many penicillin preparations contain substances that interfere with the procedure.

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Penicillin in Broths and Finished Products

Chemical Method for Estimation of Types

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THE multiplicity of penicillin types has made necessary a method that permits estimation of the individual penicillins present in mixtures. Various techniques have been developed for this purpose. Applications have been made of ultraviolet and infrared spectroscopy (1, 10, 11), adsorption chromatograms (22), parition procedures involving paper strip (8, 9, 23), as well as packed column chromatography (7, 18) and countercurrent distribution systems (4, 15). Other methods, such as the chemical precipitation of penicillin G as the N-ethylpiperidine derivative (14, 20) and microbiological differential assays, have been useful (12, 19). Each method has certain limitations, such as a requirement of rather pure sample, applicability to only a single penicillin, or lack of clear-cut distinction of types. In the development of the present method advantage was taken of the fact that the various penicillins are characterized by the so-called "R groups," which are in the form of carboxylic acids joined by amide linkages to the penicillin molecule (3). The acids were liberated by boiling in 5 N alkali and extracted into benzene. The individual R acids were separated and identified by the use of partition chromatograms of the type described by Peterson and Johnson (17).

The procedure has been used in the determination of penicillins G, K, F, and dihydro F in fermentation broths as well as in finished products. The method is not applicable to penicillin X because, among other reasons, the hydroxy acid derived from X is relatively insoluble in benzene.

REAGENTS

Benzene. Commercial grade purified by stirring with 10% of its volume of concentrated sulfuric acid for 15 minutes, and separation of the benzene layer, followed by shaking with moistened potassium hydroxide pellets and filtration.

Barium Hydroxide, 0.1 N aqueous solution. Standardization by titration of 0.1 ml. of 0.1 N benzoic acid solution in benzene in the presence of 1 ml. of benzene, 2 ml. of 95% ethanol, and 1 drop of 0.1% phenol red solution.

Celite 545, Johns-Manville, New York, N. Y.

Ethyl Ether, U. S. P. Washed with acidified 1% ferrous sulfate solution and distilled water to remove peroxides and alcohol.

Bromine, 0.4 N solution in carbon tetrachloride.

Other reagents require no explanation as to their preparation.

EXPERIMENTAL

Preparation of Two-Phase Chromatograms (17). Two types of chromatograms were necessary. In one type, designated as the 30 N column, 30 N sulfuric acid absorbed on Celite 545 (a coarse grade of infusorial earth) was used as the stationary phase. The second type was made with Celite and a mixture of concentrated sulfuric and 85% phosphoric acids in an 8 to 11 volume ratio and designated as the SP column.

The general method of preparation of both types of chromatogram was the same. A 14 × 250 mm. glass tube with a constriction at the lower end bearing a layer of glass wool above a wad of cotton was used for supporting the Celite packing. The

A chemical procedure for the estimation of penicillin mixtures is based on the separation and determination of the so-called "R group acids" which characterize the various penicillin types. The penicillins were hydrolyzed by a 3-hour treatment in 5 N sodium hydroxide solution and the liberated acids were taken into 3 ml. of benzene. These acids were separated on two-phase chromatograms which consisted of sulfuric acid or sulfuric and phosphoric acids absorbed on Celite 545 as the stationary phases and benzene as the mobile phase. The effluent fractions were collected in 1-ml. portions and titrated. The conditions for hydrolysis, extraction, and chromatography were based on experiments with pure phenylacetic, caprylic, and caproic acids as well as with the corresponding penicillins. Penicillin F was not available; hence its determination was based on experiments with its R acid, Δ-3-hexenoic acid. Fermentation broths were analyzed after transfers of the penicillins into ether and aqueous alkali, followed by benzene extraction for the removal of interfering materials. The penicillin composition of a *Penicillium chrysogenum* Q176 fermentation in synthetic medium without added precursor was as follows: no G, 30% K, 40% F, and 20% dihydro F. In corn steep medium without precursor, it was 18% G, 20% K, 32% F, and 16% dihydro F. With 0.1% phenylacetic acid added to either medium, the G content was about 80%. Commercial preparations of recent origin were 90% or higher in G content, earlier amorphous products were about 70% G, and some older materials as low as 18% G.

packing material was prepared as follows: To 10 grams of Celite, 5 ml. of 30 N sulfuric acid (or the sulfuric-phosphoric acid mixture) were added, and after rapid and thorough mixing with mortar and pestle, the material was flooded with benzene and stirred into a slurry. Small successive portions of the material were poured into the glass tube and firmly tamped each time with a glass rod. The column was built up to a height of 6 cm. in this manner. Benzene was the mobile phase of the system and the samples were placed on the column dissolved in it.

Under humid conditions it was necessary to use 31 N acid in place of the 30 N to obtain the desired separation of the R acids. Similar adjustments for the SP columns were made by varying the amount of concentrated sulfuric acid in the sulfuric-phosphoric acid mixture. A range of 7 to 8 volumes of sulfuric acid to 11 volumes of 85% phosphoric acid was suitable.

Operation of Two-Phase Chromatograms. The 30 N chromatogram was tested by the separation and recovery of a known mixture of caprylic acid (3.9 micromoles), phenylacetic acid (3.9 micromoles), and caproic acid (5.7 micromoles).

The sample was put on the column in 0.3 ml. of benzene. After the sample had completely entered the packing, enough fresh benzene was put above the column to maintain a flow rate of about 1 ml. per minute. A series of 1-ml. fractions (about 35 in all) was collected in calibrated 10 × 80 mm. test tubes, 2 ml. of 95% ethanol and a drop of 0.1% phenol red solution were added to each tube, and the titrations were made with aqueous 0.1 N barium hydroxide solution. A microburet was used in conjunction with a stirring device consisting of a thin drawn-out glass tube extending to the bottom of the solution bubbling carbon dioxide-free air.

The titrations were usually read to the third decimal place, but because the blank is subtracted from every titration, any error in the blank is magnified when the number of fractions is large. Hence it is desirable to obtain a more accurate estimate (to the fourth decimal place) of the blank titration by averaging a number of consecutive blank titrations. The blank represents the amount of alkali required to titrate the benzene effluent where no R acid is present, and it is subtracted from the individual titrations when summing up the total acidity due to a given component. The data in Figure 1, A, represent the determination of the three acids on the 30 N column. The recoveries of the respective acids were 3.8, 4.0, and 5.5 micromoles for caprylic, phenylacetic, and caproic acids.

The operation of the SP column was essentially the same as described for the 30 N column.

Hydrolysis of Penicillin and Extraction of R Acids. The penicillins (15 to 30 mg.) were hydrolyzed in 25 to 50 ml. of 5 N sodium hydroxide solution. A 100-ml. ground-glass joint flask connected to a reflux condenser was used. A 3-hour period of gentle refluxing on a sand bath was employed on the basis of data to be presented. After the reaction mixture had cooled, it was carefully acidified with 20 N sulfuric acid to pH 1 or less, and solid anhydrous sodium sulfate was added to near saturation. Four successive 25-ml. extractions were then made with benzene and the combined benzene fractions were in turn extracted successively with three portions of 1 N sodium hydroxide solution totaling about 6 ml. The alkaline extracts of the R acids were combined and the accompanying benzene was removed by aeration and evacuation of the sample in a 15-ml. separatory funnel. Thirty drops of concentrated sulfuric acid were added with cooling at the tap to dissipate the heat of reaction. Three grams of sodium sulfate were then added and three successive extractions with 1-ml. portions of benzene were made. The combined extracts were made up to 3 ml. in a graduated test tube, and aliquots of this solution were ready for chromatography. Long-barreled droppers were essential in the quantitative transfers of small amounts of liquids in the above extraction steps.
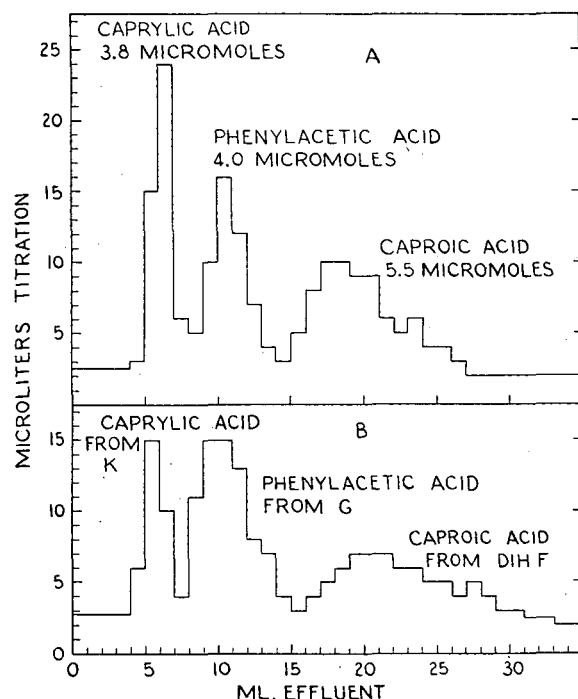

Figure 1.   Separation of R Group Acids on 30 N Chromatograms

A. Known mixture of acids: caprylic 3.9, phenylacetic 3.9, and caproic 5.7 micromoles (titrations expressed in terms of 0.1 N alkali)

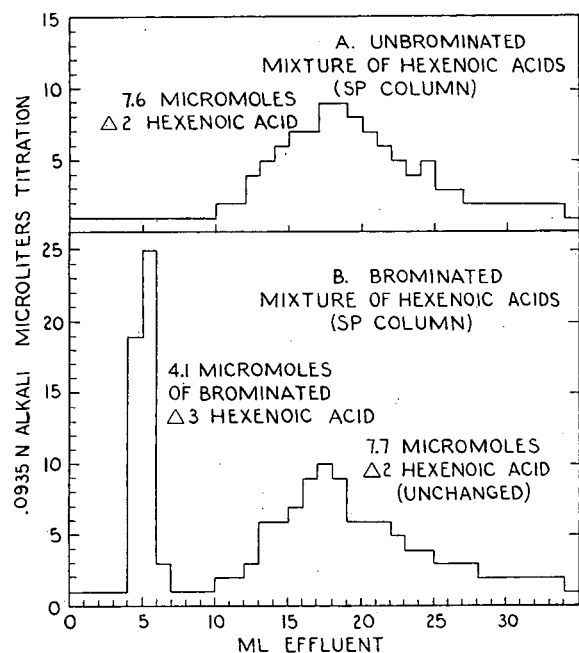*B.* R acids from penicillins G, K, and dihydro F (titrations expressed in terms of 0.087 N alkali)

Figure 2. Determination of Δ-2- and Δ-3-Hexenoic Acids

In mixture obtained from alkaline isomerization and decomposition of 16.2 micromoles of Δ-3-hexenoic acid (SP column)

The procedures outlined for the liberation and extraction of the R group acids of penicillin were based on investigations with crystalline penicillins G, K, and dihydro F, as well as with the corresponding free R acids. The experiments with phenylacetic acid, caprylic acid, and caproic acid showed them to be stable and practically quantitatively recoverable after undergoing the steps of hydrolysis, extraction, and chromatography (Table I). The R acid of penicillin F, Δ-3-hexenoic acid, however, underwent alkaline isomerization and decomposition. The instability of this acid has made necessary the determination of penicillin F based on the partial recovery of the original R acid in the form of the isomeric Δ-2-hexenoic acid (2, 5). As fairly consistent recovery of the Δ-2-hexenoic acid averaging about 44% was obtained in six experiments where the values ranged from 42 to 47% (Table I), the penicillin F contents of samples were calculated accordingly. When a mixture of Δ-3- and Δ-2-hexenoic acids produced by the treatment of Δ-3-hexenoic acid with alkali was put on the SP column, only ·the

Δ-2 acid was eluted (Figure 2, A). However, if the mixture was treated with bromine, both acids appeared (Figure 2, B). The amount of Δ-2 acid recovered in each case was the same; this showed that it was relatively inert to bromine under the conditions used. This technique was used to follow the course of the isomerization and decomposition reaction.

$$CH_3CH_2-CH=CH-CH_2COOH \xrightleftharpoons{5\,N\,NaOH} CH_3-CH_2-CH_2CH=CH-COOH$$

$$\downarrow \text{ Destruction}$$
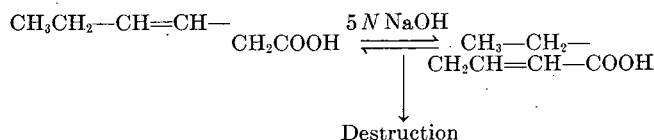
Several samples (9.75 mg. each) of Δ-3-hexenoic acid were treated with boiling 5 N sodium hydroxide solution for varying periods extending up to 3 hours. When the resulting products were analyzed and the data were plotted, it was found that the amount of Δ-2-hexenoic acid attained a fairly constant level in the interval of 2 to 3 hours' treatment (Figure 3).
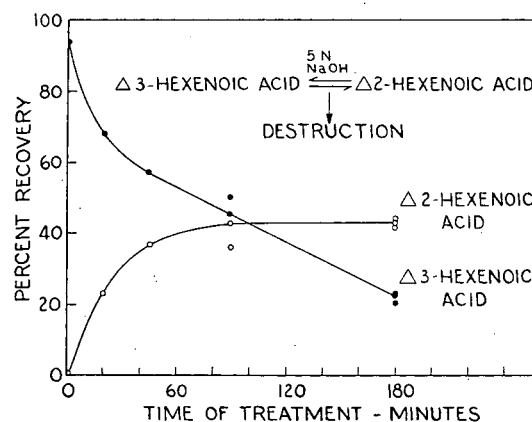

Figure 3. Isomerization and Decomposition of Δ-3-Hexenoic Acid

In refluxing 5 N NaOH solution (determined on SP column)

The bromination was carried out on the acid mixture in a 10 × 80 mm. test tube by the addition of 0.02 to 0.05 ml. of a 0.4 N bromine solution in carbon tetrachloride to 10 micromoles or less of sample in about 0.5 ml. of benzene. The excess bromine was promptly destroyed by the addition of a droplet of mercury with vigorous shaking. The material was then carefully transferred to the SP column with a medicine dropper. About 0.5 ml. of rinsings was used to make the transfer quantitative.

The bromination procedure is a somewhat unspecific method of measuring acids which brominate to give increased titrations in the first peak of the SP column; hence the determination of Δ-3-hexenoic acid was based entirely on the quantity of Δ-2-hexenoic acid obtained. The SP column does not separate mixtures of phenylacetic, caprylic, and caproic acids. These acids come together in a single peak, which includes the brominated Δ-3-hexenoic acid but is distinct from the Δ-2-hexenoic acid peak.

The hydrolysis of benzyl penicillin (G) in boiling 5 N sodium hydroxide was followed by removal of 10-ml. aliquots representing 20.2 mg. of potassium G at varying intervals of time. The liberation of phenylacetic acid was 7% at 10 minutes, 81% at 20 minutes, and 97% at 40 minutes. A 3-hour treatment gave a 98% yield. The amount of caprylic acid obtained from a sample of penicillin K after a 3-hour treatment was about 75% of the theoretical value and remained unchanged after 7 and 20 hours' treatment. The material was presumably not homogeneous; data presented below also support this conclusion. A specimen of dihydro F penicillin stated (18) to contain 85% of the designated type gave a yield of caproic acid corresponding to 94% of the

Table I. Recoveries of Known Amounts of R Group Acids from Chromatograms

·(After 3-hour treatment in boiling 5 N sodium hydroxide)

Sample	Amount Used	Amount Recovered		Recovery
	Mg.	*Mg.*		%
Caprylic acid	7.2	7.0		97
	2.75	2.81		102
Caproic acid	3.32	3.22		97
	11.0	10.65		97
Phenylacetic acid	10.36	10.2		98
	2.34	2.22		95
Δ-3-Hexenoic acid	14.15	4.0	(Δ-3 acid)	28
		6.45	(Δ-2 acid)	45
	4.89	1.45	(Δ-3 acid)	30
		2.24	(Δ-2 acid)	46
	9.75	2.05	(Δ-3 acid)	21
		4.30	(Δ-2 acid)	44
	9.75	2.54	(Δ-3 acid)	26
		4.20	(Δ-2 acid)	43
	9.75	2.44	(Δ-3 acid)	25
		4.10	(Δ-2 acid)	42
	61.6	15.6	(Δ-3 acid)	25
		29.0	(Δ-2 acid)	47

Table II. Separations and Recoveries of Added Free R Acids from Penicillin R Groups in Experimental Test Mixtures

Composition of Mixture	Mg.	Free Acid Recovered		Penicillin R Acid Recovery	
		Mg.	%	Mg.	%
Potassium G	22.0	..	..	7.84	97
Phenylacetic acid	5.75	5.46	95	..	..
Caprylic acid	2.72	2.62	96	..	..
Caproic acid	3.34	3.17	95	..	..
Δ-3-Hexenoic acid	4.88	4.82	99	..	..
Potassium G	23.4	..	..	8.17	95
Phenylacetic acid	19.2	18.5	96	..	..
Fermentation broth plus phenylacetic acid	19.2	18.8	98	..	..
	19.2	19.6	102	..	..
Penicillin only					
Potassium G	8.95	..	..	3.26	99
Sodium K	5.1	..	..	1.44	71.3 (95)ᵃ
Sodium dihydro F	8.2	..	..	2.24	79.1 (93)ᵃ

ᵃ Values in parentheses calculated on assumption that added K and dihydro F were 75 and 85% pure, respectively.

calculated value upon 3 hours' hydrolysis. Penicillin F was not available for this work.

The foregoing experiments were the basis for adopting the 3-hour hydrolysis period. The data indicate that a shorter period may suffice, but the longer treatment was chosen in order to ensure complete hydrolysis.

**Preliminary Treatment of Crude Samples for R Group Analysis.** The widespread use of phenylacetic acid and its derivatives as precursors for penicillin G in fermentation media often results in the presence of the free acid as a contaminant in crude preparations (6, 16, 21). In order to remove such and other interfering materials, the following procedure was devised:

The penicillin preparations were dissolved in 25 ml. of 0.1 N sodium hydroxide solution and placed on a steam bath for 5 minutes. This treatment changes the solubility of the penicillins presumably by the formation of penicilloic acids (3), so that they are not extracted in the subsequent steps. The biological activity was completely destroyed but no measurable release of the penicillin R groups occurred, as this requires more drastic conditions. The solution was cooled and acidified to pH 1.5 with sulfuric acid. Three grams of sodium sulfate were added and three extractions with successive 30-ml. portions of benzene were made. The interfering materials were removed in benzene. The residual material was made 5 N with respect to sodium hydroxide and hydrolyzed in the usual way. As a check on the procedure, known amounts of free R acids were added to various penicillin samples including broth preparations; recoveries of 95% or better were obtained (Table II). The recoveries of penicillin-bound R acids were also determined in these experiments and found satisfactory (93 to 99%) (Table II).

Utilization of phenylacetic acid by *Penicillium chrysogenum* Q176 for penicillin G synthesis accounted for only a small portion of the total disappearing from the medium. The precursor in the form of the free acid and as the R group of G was recovered for several types of broths. The highest recovery obtained was only 25% (Table III). Evidently the organism metabolizes the compound in other ways than in penicillin synthesis.

Table III. Recoveries of Phenylacetic Acid from Penicillin Fermentation Broths (100-Ml. Samples)

Fermentation Medium	Added Precursor in Sample	Recovered Precursor		Total Recovery of Added Precursor
		In G	Free	
	Mg.	Mg.	Mg.	%
Synthetic, no precursor (13)	0	0	0	..
Synthetic + 0.05% phenylacetic acid	50	4.9	0.25	10
Synthetic + 0.1% phenylacetic acid	100	5.2	11.4	17
Corn steep	0	1.66	0	..
Corn steep + 0.1% phenylacetic acid	100	8.5	16	25
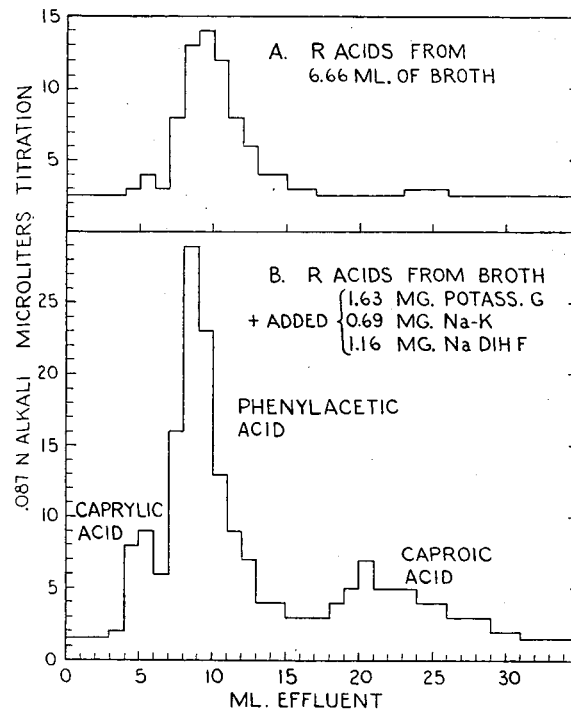
Figure 4. Recoveries of R Acids from Crystalline G, K, and Dihydro F Added to Broth

Extraction of Fermentation Broths for Analysis. Penicillin fermentation broths were prepared for analysis in the following manner:

A 100-ml. sample was acidified to pH 2.0 and extracted with three successive 100-ml. portions of peroxide-free ether. The combined ether extracts were in turn re-extracted with three portions of 0.1 N sodium hydroxide totaling about 25 ml. The aqueous extracts were combined and placed on a steam bath to drive off accompanying ether and to inactivate the penicillins. The succeeding steps involving acidification, extraction of interfering materials, and hydrolysis have been described.

The ether and broth often produced stubborn emulsions. Anhydrous sodium sulfate was sometimes helpful. In certain cases the lower clear aqueous layer was separated and the upper emulsion was decanted into a flask and shaken with enough Celite to form doughlike pellets and leave a clear ether solution. The solid matter was shaken with fresh ether to avoid losses.

The extraction procedure was tested by the addition of known amounts of crystalline penicillins to a sample of broth and recovery of the R group acids. Recoveries of 91, 92, and 95% were obtained on the R groups of penicillin G, K, and dihydro F, respectively (Figure 4). In another similar experiment, the recoveries were 93, 91, and 86% for penicillins G, K, and dihydro F.

RESULTS OF ANALYSES

Analysis of Crystalline Penicillins. Data on the recoveries of R group acids from crystalline penicillins are presented in Table IV. Crystalline penicillin G samples yielded practically the theoretical amount of phenylacetic acid, and this accounted for almost all the acidity found in the benzene extract of the penicillin hydrolyzate. The amount of unsaturated acid present in one sample of G, as determined by the increase in the titration at the first peak upon bromination, was less than 2%.

The crystalline K sample (Pfizer, 4-12-46) gave recoveries of caprylic acid averaging about 75% of calculated value. Longer hydrolysis resulted in no increase of caprylic acid. In one experiment, however, where a 70.5-micromole recovery of caprylic acid was obtained, a further amount of unsaturated acid totaling 9.35 micromoles was detected on the chromatogram by the bromina-

tion procedure previously described. In another experiment where 60 micromoles of caprylic acid were obtained in a sample presumably containing 81.8 micromoles of penicillin K, 8.75 micromoles more of unsaturated acid were found. In each of these experiments the total acidity obtained in the benzene extract of the penicillin hydrolyzate was greatly in excess of that calculated for pure penicillin K. In the first sample 114 micromoles were found instead of 93.5; and in the second sample 103 micromoles were found instead of the calculated 81.8 micromoles (Table IV).

The crystalline dihydro F sample (Pfizer 160,619) was stated to be 85% dihydro F. Assuming this value, the recoveries of caproic acid averaged 94% of the theoretical amount. In one sample 73.5 micromoles of caproic acid were found together with 14.7 micromoles of Δ-3-hexenoic acid. The values correspond to 81% dihydro F and 16% F in the preparation. A total titration of the benzene extract of the dihydro F hydrolyzate amounted to 105 micromoles instead of the 90.5 micromoles expected if the sample were pure dihydro F penicillin (Table IV).

Calculation of Composition of Penicillin Mixtures. Calculation of the percentage composition of various penicillin preparations was based on the biological potency of the original material. The assumption was made that all the R group acids determined were derived from active penicillin molecules, and the amount of each R acid found was then converted into milligrams of the corresponding penicillin. The occurrence of large amounts of inactivated penicillins in a sample was therefore a cause of error. For example, a sample of fermentation broth whose potency had dropped to only 60 units per ml. (at 168 hours) from a peak of over 500 units per ml. (at 120 hours) gave R assay results accounting for 320 units per ml. In order to minimize such discrepancies R group assays should be made before extensive decomposition occurs.

No serious interference, however, could be ascribed to any hypothetical precursor of penicillin; immature fermentation broths whose activity was rapidly increasing and which had attained only 60% of the maximum potency of normal peak yields gave R assay values that corresponded to from 80 to 98% of the bioassays.

The following potencies in units per milligram for the sodium penicillins were employed in the calculations: G 1667, K 2300,

Table V. Analyses of Commercial Penicillin Samples

Sample	G	K	F	Dihydro F
	%	%	%	%
Recent Crystalline Products				
Company A	90	..	..	..
Company B	91	..	..	..
Company C	101	..	..	..
Company D	93	..	..	..
Amorphous Preparations				
Company A	92	..	..	..
Company B	75	11	5	..
Company C	68	4	..	..
	72	2	..	14
Company D	70	18	20	5
Earlier Amorphous Preparations				
Company B	15	24	28	30
Company E	25	34	7	24

Table VI. Analyses of Fermentation Broths

Sample	Bioassay	G	K	F	Dihydro F	Total
	Units/ Ml.	%	%	%	%	%
Synthetic medium, no precursor	188	0	34	46	14	94
	150	0	28	35	24	87
Synthetic medium + 0.05% phenylacetic acid	299	70	13	34	3	120
Synthetic + 0.1% phenylacetic acid	272	83	6	5	2	96
Synthetic + 0.1% β-phenylethylamine	400	67	3	11	2	83
	418	59	4	22	0	85
Corn steep, no added precursor	408	18	20	32	16	86
	320	19	21	45	14	99
	420	15	16	31	18	80
Corn steep + 0.05% phenylacetic acid	410	93	5	15	9	122
Corn steep + 0.1% phenylacetic acid	368	101	6	15	3	125
	556	79	0	16	0	95

F 1550, and dihydro F 1700. The values were chosen from the literature (8) and from data obtained in this laboratory.

Commercial Samples. Analyses of commercial penicillin preparations are presented in Table V. The more recent products, all crystalline substances, were found to contain 90% or more penicillin G. Some amorphous preparations made by the same producers were also largely G (above 70%). Analyses of materials made some years ago, however, showed a very low G content. Undoubtedly, these products were made without the use of G precursors.

Analyses of Fermentation Broths. Various types of fermentation broths of *Penicillium chrysogenum* Q176 were analyzed (Table VI). In the synthetic medium of Jarvis (13), without added precursor, the composition was as follows: no G, 30% K, 40% F, and 20% dihydro F. With added 0.1% phenethylamine about 63% G, 16% F, and small amounts of other types were found. About 83% G was produced when 0.1% phenylacetic acid was used as precursor in the medium. In a corn steep fermentation without added precursor, the percentage composition was about 18% G, 20% K, 36% F, and 15% dihydro F. With added 0.1% phenylacetic acid the penicillin G was increased to 80%. Some of the total

Table IV. Recoveries of R Acids from Crystalline Penicillins

Sample Weight[a]		Micromoles, Calcd.	Total R Acid Fraction by Titration	R Acid by Chromatography		Recovery of Theoretical R Acid	Unsaturated Acid by Bromination
	Mg.		Micromoles	Micromoles	Mg.	%	Micromoles
Penicillin G				Phenylacetic Acid			
Sodium salt	28.2	79.1	...	74.5	10.1	94	..
	26.95	75.6	72.2	71.0	9.66	94	2
	7.73	21.7	21.9	21.7	2.95	100	..
	17.1	48.0	45.6	45.3	6.16	94	..
Potassium salt	27.6	74.2	22.5	70.5	9.6	95	..
	20.2	54.3	53.6	53.6	7.3	98	..
Penicillin K (αD +				Caprylic Acid			
285), sodium salt	34.0	93.5	114	70.5	10.15	75	9.35
	29.8	81.8	103	60.4	8.7	74	8.75
	22.5	61.7	...	47.0	6.76	76	..
	25.6	70.2	...	41.8	6.0	60	..
	22.9	63.0	...	49.1	7.1	78	..
	5.4	14.85	...	10.45	1.51	71	..
	22.45	61.7	...	42.5	6.14	69	..
	27.9	76.7	...	60.7	8.75	79	..
Penicillin dihydro				Caproic Acid			
F (No. 160,619),	27.2	81.0	...	62.8	7.3	78	5.88
sodium salt	30.4	90.5	...	72.3	8.4	80	..
	30.4[b]	90.5	105	73.5	8.5	81	..

[a] Samples of sodium G, potassium G, sodium K, and sodium dihydro F are from single lots.
[b] An amount of Δ-2-hexenoic acid (0.74 mg.) corresponding to 1.68 mg. of initial Δ-3-hexenoic acid was determined. This is equivalent to 16% F in dihydro F sample.

values exceed 100% and probably reflect the presence of inactivated material in the samples.

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Benzylpenicillin Content of Broth

Estimation by a Gravimetric Procedure

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The gravimetric method of Mader *et al.* for the determination of benzylpenicillin in pure salts has been modified for the analysis of broth. The precipitation of penicillin from amyl acetate solution by *N*-ethylpiperidine is somewhat preferential for benzylpenicillin but all types of penicillins present in solution are contained in the precipitate. The total weight of the precipitate is dependent not only on the amount of penicillin contained in the solution but also on the acidity. By applying an empirical correction the amount of benzylpenicillin contained in broth samples can be estimated within 10%.

A VARIETY of techniques have been proposed for the determination of benzylpenicillin in mixtures of penicillins. However, only a few of these are applicable to the analysis of broth: differential assay techniques (6, 12) and methods based on countercurrent distribution (3) or chromatographic separation of the various penicillins followed by microbiological assay of these fractions (4, 7, 14). Although some of the latter methods offer advantages, it was found desirable to have available a direct chemical method for the estimation of the benzylpenicillin content of broth. Because of the opacity of broth, infrared (1) and ultraviolet (2, 5, 8, 11) techniques were inapplicable and therefore the gravimetric method of Sheehan, Mader, et al. (9, 13) was investigated.

SYNTHETIC SAMPLES

Throughout the investigation synthetic samples of fermentation broth and solutions of penicillin in amyl acetate were used. These were prepared as indicated in Table I.

Broth filtrate was obtained from essentially completed penicillin fermentations conducted in the usual lactose–corn steep liquor medium, using *Penicillium chrysogenum*-Q-176. This was inactivated so as to yield a solution free from penicillin but containing the impurities normally present in such broth. By one method the penicillin content therein was destroyed by penicillinase. For this purpose, about 50,000 units of penicillinase were added to 1 liter of broth and the mixture was left at room temperature for 24 hours. It was then autoclaved at 14 kg. (30 pounds) pressure for 15 minutes and the destruction of
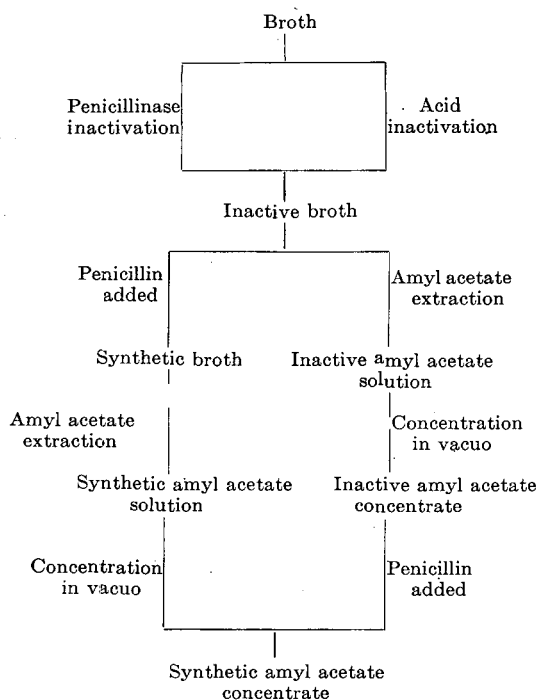
penicillinase was verified by assay. By a second procedure the broth was left at pH 1 to 2 for 24 hours and subsequently neutralized and then assayed to ascertain that no penicillin was present. The inactive broths thus prepared were further treated by either of two methods. By one, known amounts of penicillins were added, yielding a "synthetic broth," which was acidified and extracted with amyl acetate; the extract was subsequently concentrated in vacuo. By the other procedure the inactive broths were acidified and extracted with amyl acetate, and the extracts were concentrated in vacuo. From these inactive solutions "synthetic" amyl acetate concentrates were prepared by transferring into them known amounts of penicillins from acidified aqueous solutions.

PRECIPITATION OF PENICILLIN

When *N*-ethylpiperidine is added to impure amyl acetate solutions of benzylpenicillin the precipitation is sluggish and the crystal formation is markedly different from that in pure solution, in that large clusters of crystals are formed rather than a mat of fine needles. The weight of the precipitate varies with the time of precipitation, as shown in Table II. This table contains some typical examples and it is evident that while equilibrium is reached within 2 hours in pure solutions, a considerably longer time is necessary for equilibrium in impure solutions. It is obvious that the recovery of benzylpenicillin is less in impure solutions.

Because in the precipitation reaction a salt formation is involved, the incomplete precipitation observed in the impure solution was thought to be due to the high acidity of the reaction

Table I. Scheme of Preparation of Synthetic Samples

Broth

Penicillinase inactivation | Acid inactivation

Inactive broth

Penicillin added | Amyl acetate extraction

Synthetic broth   Inactive amyl acetate solution

Amyl acetate extraction | Concentration in vacuo

Synthetic amyl acetate solution   Inactive amyl acetate concentrate

Concentration in vacuo | Penicillin added

Synthetic amyl acetate concentrate

mixture. Investigation showed that there exists a correlation between the "pH" and the amount of benzylpenicillin precipitated (see Figure 1). Because of the difficulty of measuring and evaluating pH in nonaqueous medium, the acidity was evaluated by measuring the pH of an essentially aqueous solution which

was prepared by dissolving the amyl acetate sample diluted with acetone in 10 volumes of 50% ethyl alcohol–water mixture. As shown in Figure 1 the pH can be raised simply by increasing the amount of N-ethylpiperidine in the mixture. This is an important feature of the modified method.

In searching for a medium in which the solubility of the N-ethylpiperidine salt of benzylpenicillin is small and that of the N-ethylpiperidine salts of penicillins K and F is appreciable, various solvent mixtures were investigated. As shown in Table III, a mixture consisting of 2 parts of acetone, 2 parts of amyl acetate, and 3 parts of N-ethylpiperidine has these properties. Therefore, it was chosen as a medium for precipitation as well as for washing of the precipitate.

COMPOSITION OF PRECIPITATE

The precipitate, analyzed by the penicillinase-acidimetric method (10), was found to consist essentially of the N-ethylpiperidine salt of penicillin. In some instances gummy precipitates showed inert material; however, the crystalline N-ethyl-

Table II. Effect of Precipitation Time on Yield of N-Ethylpiperidine Precipitate

Precipitation Time Hours	Recovery of Benzylpenicillin %	
Pure Solutions		
2	100.6	
	97.4	
5–6	97.5	
	98.4	
18–24	100.0	
	99.8	
Impure Solutions		
	I	II
2	95.3	87.5
5–6	97.9	94.6
18–24	98.7	94.5

piperidine salts contained but a negligible amount of inert components. The proportions of the various penicillin entities in the precipitate were determined by the paper chromatographic method (7).

Such data for a typical broth and synthetic broth are shown in Table IV and Figure 2. It is readily apparent that the precipitate consists of N-ethylpiperidine salts of a mixture of penicillins rather than the salt of pure benzylpenicillin. This is discussed in greater detail below.

Recovery experiments with synthetic amyl acetate concentrates in which the total weight of the precipitate was considered as consisting of the N-ethylpiperidine salt of benzylpenicillin, showed an apparent loss of precipitate in the mother liquor (Figure 5). It is apparent that there exists a correlation between the total weight of the precipitate and the benzylpenicillin content of the sample and the two are linked
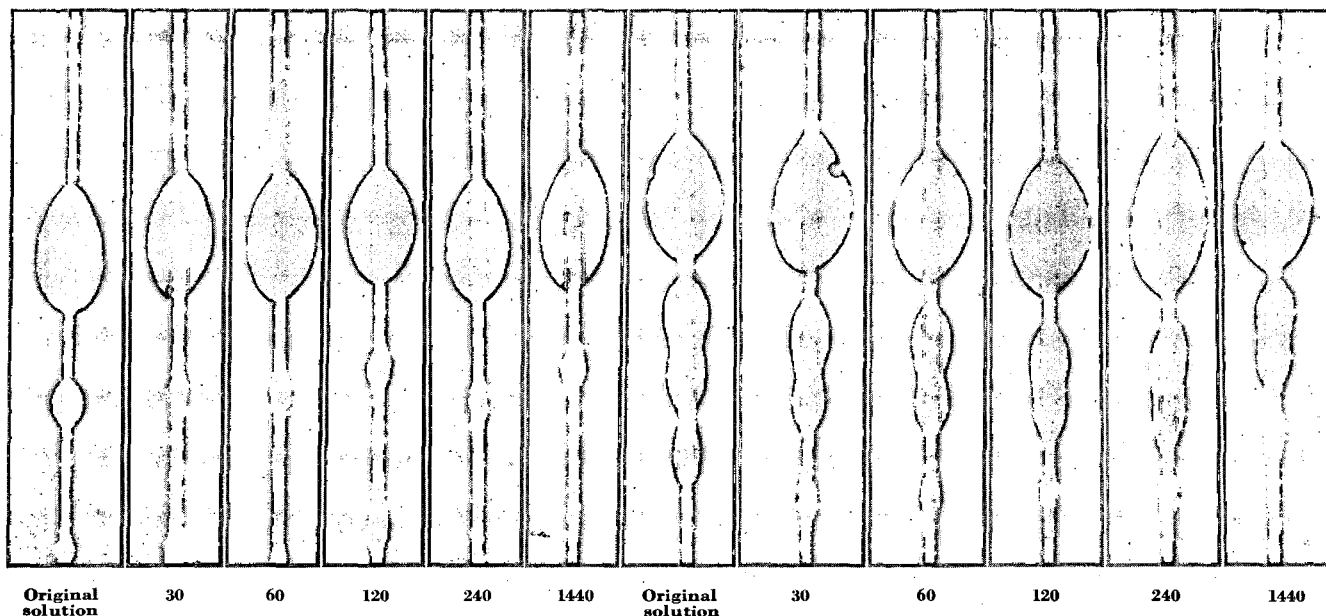

Figure 1. Dependence of Amount of Precipitate and pH Number on Amount of N-Ethylpiperidine Added to Impure Solution of Penicillin

| Original solution | 30 | 60 | 120 | 240 | 1440 | Original solution | 30 | 60 | 120 | 240 | 1440 |

Figure 2.   Shadowgraph of Paper Chromatograms of *N*-Ethylpiperidine Salts Derived from Broth (*Left*) and Synthetic Broth (*Right*)

Precipitation time, minutes

by a correction factor which is dependent on pH. These facts form the basis of the proposed method for the analysis of impure amyl acetate solutions.

In order to complete the technique for the analysis of broth,

Table III.   Solubility of *N*-Ethylpiperidine Salts of Penicillin

Solvent	pH	Solubility Mg./Ml.
Benzylpenicillin		
Acetone	9.2	1.3[b]
Acetone + NEP[a] (4 + 3)	11.7	0.2
Acetone + AA + NEP (2 + 2 + 3)	10.2	0.6[b]
	10.4	0.5[b]
	10.5	0.4[b]
	10.8	0.3[b]
	11.3	0.17[b]
	11.7	0.11
Mixed Penicillins (K, F, Dihydro F)		
Acetone	9.6	24.4
Acetone + NEP (4 + 3)	11.6	3.5
Acetone + AA + NEP (2 + 2 + 3)	10.8	3.8
	11.6	3.1

[a] NEP = *N*-ethylpiperidine.
  AA = amyl acetate.
  Figures under solvent indicate volume proportions.
[b] Based on microbiological assay. Others, gravimetric determinations.

Table IV.   Composition of Precipitate

Precipitation Time Min.	Weight of Precipitate Mg.	Composition of Precipitate			
		G %	F %	Dihydro F %	K %
Broth					
Original solution	...	88.0	5.2	(3.0)[a]	3.8
30	43.3	94.0	3.0	...	3.0
60	58.9	94.0	3.0	...	3.0
120	64.5	91.1	4.6	...	4.3
240	66.6	94.0	(3.0)	...	(3.0)
1440	70.4	94.1	3.9	...	2.0
2880	70.3	...	...	...	...
Synthetic Broth					
Original solution	...	82.1	9.1	6.0	2.8
30	35.8	88.7	5.9	3.6	1.8
60	47.7	88.3	5.8	3.9	2.0
120	61.3	88.3	5.7	3.8	2.1
240	65.8	87.3	6.0	4.4	2.4
1440	68.2	87.4	5.9	4.4	2.4

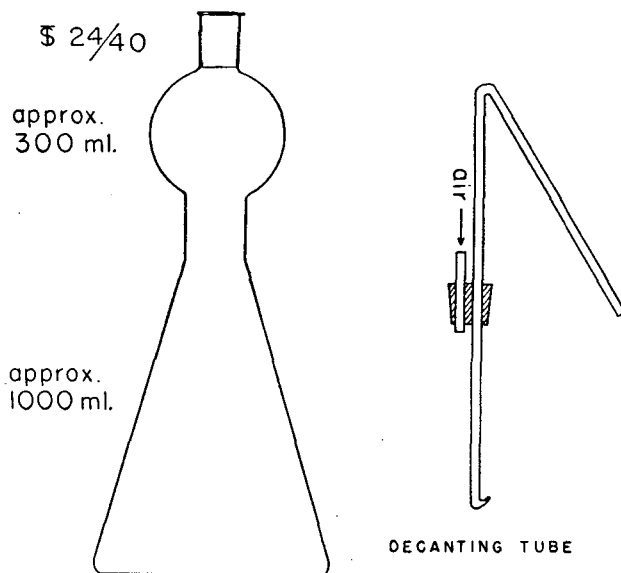[a] Values in parentheses are estimates.

Figure 3.   Broth Extraction Flask

the extraction of penicillin from broth and the concentration of the resulting amyl acetate solution of penicillin were also investigated. A simple standard technique was established as described below. To keep the steps free of losses in benzylpenicillin, the contact time with acid was made short, and the temperature was kept low throughout. The necessary laboratory tools were also designed.

PROCEDURE

Place exactly 1 liter of broth in a 2-liter beaker. Cool to about 4° C. and add about two thirds of exactly 150 ml. of analytical grade amyl acetate. Immerse the electrodes of a pH meter and start adding 42.5% phosphoric acid from a buret. Continue the addition with stirring until pH 2.3 ± 0.05 is reached. Transfer the mixture immediately into the extraction flask (Figure 3) and rinse the beaker with the remaining portion of the 150 ml. of amyl acetate. Repeat the rinsing with a small amount

of distilled water and use a rubber policeman to clean the sides of the beaker.

Stopper the flask and mix the contents by inverting several times. Place the flask in an ice bath, and allow the two phases to separate. If necessary, rotate the flask gently to aid phase separation. Usually within 5 to 10 minutes a clear separation occurs, after which remove the stopper and introduce the decanting tubes. By gentle pressure decant the upper phase, immersing the tube gradually to a point just above the interface.

Collect the amyl acetate layer in a 1-liter Erlenmeyer flask which is immersed in an ice bath. After the amyl acetate is thus separated raise the tube and purge it with air. Remove the mechanism and drain the glass hook into the Erlenmeyer flask by inverting it and touching it to the wall.

Measure exactly 150 ml. of reagent grade amyl acetate and introduce it into the extraction flask. Stopper the flask and mix the contents by repeated inversions. Allow the phases to separate and then decant as above.

Repeat the extraction as above, using 210 ml. of reagent grade amyl acetate. This last portion of amyl acetate should be decanted rather sharply, but the first two decantations need not be so thorough. In all three cases add sufficient water to the contents of the extraction flask to bring the interface to the narrowest part of the flask.

Mix the contents of the Erlenmeyer flask and distribute the solution into 250-ml. centrifuge cups. Add about 25 to 50 grams of anhydrous sodium sulfate per cup. Balance the centrifuge cups accurately and centrifuge for 10 minutes at about 1000 r.p.m. Remove the cups from the centrifuge and carefully decant the supernatant clear amyl acetate solution. For this purpose use first 50-ml. and then 10-ml. pipets or a suitable siphoning arrangement. Recover approximately 450 ml. of this composite clear extract and note the exact volume.
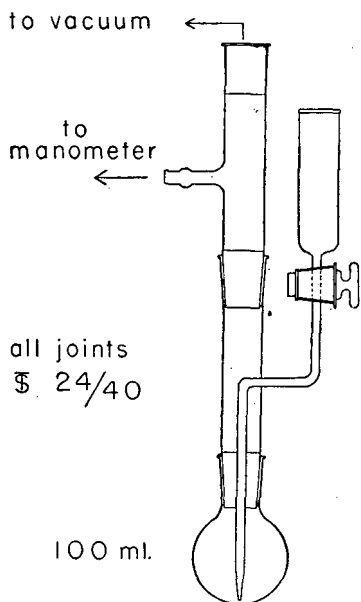
Figure 4. Amyl Acetate Concentrator

Feed this extract into the distilling apparatus (see Figure 4) and distill in a 25° C. water bath under about 5-mm. pressure to a volume of 3 to 5 ml. Transfer the contents of the distilling flask into a 10-ml. graduate. (Prior to use, check the calibration of the graduate.) Disassemble the distilling apparatus and rinse each piece—i.e., the funnel, the adapters, and flask—with reagent grade amyl acetate. For this purpose take up the solvent in a 1-ml. pipet and discharge small portions of the solvent along the glass walls of the apparatus. Finally fill the graduate to a convenient mark—e.g., 8 ml.

Pour the concentrate contained in the 10-ml. graduate through a microfilter containing approximately 1 gram of anhydrous sodium sulfate. Place 2 ml. of anhydrous acetone and 3 ml. of ethylpiperidine base in a preweighed weighing bottle, and add with a pipet exactly 2 ml. of the filtrate. Stopper the weighing bottle and place it in a refrigerator. After 15 to 30 minutes introduce a microcrystal of N-ethylpiperidine salt of penicillin G and return the sample to the refrigerator for 18 to 24 hours.
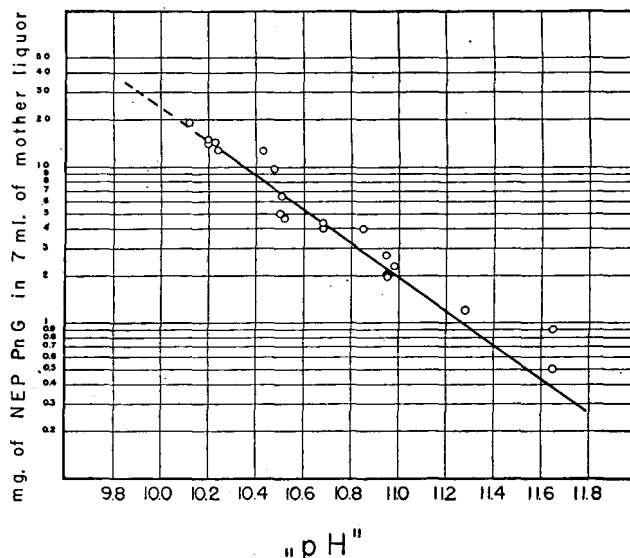

Figure 5. Correction Curve

After this time has elapsed, remove the liquid by suction through a filter stick, which has been preweighed together with the weighing bottle. Receive the filtrate in a 50-ml. Erlenmeyer flask. Wash the tubing with acetone and transfer the contents of the Erlenmeyer flask into a 25-ml. volumetric flask and fill to the mark with acetone. Mix by inverting and pipet a 2-ml. sample into a 50- or 100-ml. beaker. Add 20 ml. of 50% ethyl alcohol and determine the pH with a glass electrode pH meter.

Pipet 2 ml. of a mixture containing 2 volumes of analytical grade amyl acetate, 2 volumes of anhydrous acetone, and 3 volumes of N-ethylpiperidine base into the weighing bottle. Distribute the precipitate by agitating the filter stick and remove the liquid by suction. Repeat this process twice more. Place the open weighing bottle containing the filter stick in a vacuum desiccator and apply vacuum for 1 hour. Remove the weighing bottle, close immediately, and determine the total weight.

Calculation. To the total weight, as determined gravimetrically, add 0.6 mg. (corresponding to the solubility of the salt in the wash amounting to 0.1 mg. per ml.). From Figure 5 read the correction using the pH number determined as above. Add this figure to the measured weight.

Divide this total weight by the volume fractions (in order to correct for the loss of amyl acetate dispersed in the spent broth, instead of 510 ml. use 500 ml. of amyl acetate as a basis of the calculation). Multiply the result by 0.832 to yield mg. of potassium benzylpenicillin per liter of broth.

EXAMPLE. Amyl acetate extract, 450 ml.
  Amyl acetate concentrate, 7 ml. pH, 10.8
  Weight of precipitate, 35.0 mg.

35.0 mg.
+ 0.6 mg. (correction for loss in wash)
+ 3.3 mg. (correction for loss in mother liquor)
─────────
38.9 mg.

$$38.9 \times \frac{500}{450} \times \frac{7}{2} \times 0.832 = 125.9 \text{ mg. per liter}$$

NOTES. Samples containing less than 100 mg. of potassium penicillin G per liter will not yield reliable analysis. If an analysis shows results significantly below that figure, report the results as approximate.

If the pH of the mother liquor falls below 10.2 or if the correction applied exceeds 20 to 30% of that total, report the result as approximate.

If the N-ethylpiperidine precipitate appears as an amorphous brown mass (sometimes gummy) determine the purity of the precipitate. Multiply the total weight found by the per cent purity and proceed with the calculation as outlined in the method, but report the result as approximate.

Occasionally the broth and amyl acetate do not separate clearly. In such cases separate the clear amyl acetate portions and the emulsions from the broth. Recentrifuge the composite several times until a clear amyl acetate solution is obtained.

Experience showed that 1-hour drying time in an efficient apparatus is ample. However, check the completeness of the drying periodically (by drying to constant weight).

Table V. Analysis of Synthetic Broths by Proposed Method

No.	Potassium Penicillin G Added Mg./l.	Potassium Penicillins K and F Added[a] Mg./l.	Analysis Expressed as Potassium Penicillin G Mg./l.	Error %
		June 1947		
1	187.4	47.2	207.0	+10.5
2	150.5	100.3	155.1	+ 3.1
3	Broth of unknown composition		178.0	+ 7.2
4	76.3 (added to 3)		272.7	+ 7.2
5	111.5	71.7	98.2	−11.9
6	145.0	35.1	149.0	+ 2.8
7	145.9	33.8	146.0	0
8	116.7	75.6	116.3	− 0.3
9	144.0	34.2	147.3	+ 2.3
10	115.0	75.3	116.7	+ 1.5
		June 1948		
1	234.8	23.5[b]	245.1	+ 4.4
2	156.9	43.4	164.7	+ 5.0
3	234.2	23.1	250.8	+ 7.1
4	158.6	48.2	173.8	+ 9.6
5	236.1	24.2	232.6	− 1.5
6	154.9	47.1	158.9	+ 2.6
7	234.7	23.9	236.3	+ 0.7
8	156.8	47.0	159.9	+ 2.0
9	318.9	13.3	320.7	+ 0.6
10	268.5	43.6	265.8	− 1.0

[a] Approximate composition, 20% F, 34% dihydro F, 46% K.
[b] Approximate composition, 47% F, 33% dihydro F, 20% K.

PERFORMANCE TEST

To test the performance of the proposed method periodic checks were made using synthetic broths. These results indicate deviations of the order of 5% with no values exceeding 10% significantly. Two of these sets are shown in Table V.

DISCUSSION

As pointed out above, the precipitate of N-ethylpiperidine salts is composed of mixed penicillins. There is a preferential precipitation of penicillin G (and X if present) but this is limited to about 15% at low benzylpenicillin content, while at high ratios of benzylpenicillin the enrichment in the precipitate is undetectable.

The commonly held view that there is coprecipitation of non-G penicillins in the precipitation by N-ethylpiperidine was found to be justified. However, the view that some penicillins "solubilize" the N-ethylpiperidine salt of benzylpenicillin could not be verified. It is felt that this belief has its origin in the fact that in low pH medium less complete precipitation of all penicillins is found.

In the light of these findings the correction (Figure 5) should be interpreted as an empirical correction factor which, when used, will permit the estimation of benzylpenicillin content. It is conceivable that different types of fermentation liquors require different curves (even negative correction). For the types of materials with which the authors have had experience, the curve presented in Figure 5 yields reasonable results within the limits shown by the experimental points.

In summary, the following siutation exists: N-ethylpiperidine precipitates benzylpenicillin preferentially from a mixture of penicillins. An enrichment in penicillin content ranging from 0 to 15% is found, depending on the original composition. In impure solutions the precipitation proceeds slowly and equilibrium is reached after 6 to 48 hours' standing. If the pH of the solution is lowered by acidic impurities, a proportion of penicillins remain in solution. The composition of the precipitate is constant throughout the precipitation. When equilibrium is reached a relation exists between the weight of precipitate, the pH, and the amount of benzyl-penicillin contained in the original solution.

It is evident that the original procedure of Mader and Buck (9) is applicable to the extreme condition of relatively pure solutions containing a high proportion of benzylpenicillin for which it was designed. In such a case the precipitation time is short and 2 hours suffice to reach equilibrium. The pH is high and so the correction is negligible. Thus the two procedures yield essentially identical results with respect to both the composition of the precipitate and the time of precipitation. In Table VI are shown typical data to illustrate this point with two similar amyl acetate concentrates.

Table VI. Synthetic Concentrate in C.P. Amyl Acetate

Precipitation Time Min.	Weight of Precipitate Mg.	Composition of Penicillin Precipitate[a] G %	F %	Dihydro F %	K %
Precipitation with N-Ethylpiperidine by Proposed Method					
Original solution	...	85.2	7.9	4.8	(2.0)
30	77.6	88.2	7.2	4.6	...
60	78.0	90.7	6.3	3.0	...
120	78.0	88.2	7.2	4.6	...
240	78.6	88.5	6.7	4.8	...
1440	78.5	89.2	6.3	4.4	...
Precipitation with N-Ethylpiperidine by Method of Mader and Buck (9)					
Original solution	...	86.6	6.3	4.0	(3.0)
30	39.2	91.5	6.5	2.0	...
60	41.3	88.5	7.2	4.3	...
120	41.8	89.8	6.4	3.8	...
240	41.2	90.5	5.9	3.6	...
1440	41.4	87.2	7.5	5.3	...

[a] By paper chromatograph.
Roman figures in parentheses are estimates.

The proposed method is applicable whenever it is desired to know the amount of benzylpenicillin present in fermentation broth or impure solutions of this penicillin. (If the proportion of benzylpenicillin is to be determined, it is necessary to consider also the results of microbiological assay and so errors due to both of these methods enter into the results. Therefore for this type of analysis the more direct paper chromatographic method may be preferable.) The proposed method is also suitable for the analysis of pure solutions, but in this application it shows no advantage over the official method.

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Infrared Assay of Procaine Salt of Benzylpenicillin

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An infrared method of assay of the procaine salt of benzylpenicillin is based on the measurement of the depth of the β-lactam band of penicillin at 5.6 μ and is performed with fixed cells using chloroform solutions. The method has been applied to a variety of preparations containing procaine penicillin. Comparison of results indicates good agreement with the biological assay.

THE infrared spectra of the sodium salt of benzylpenicillin and of its degradation products have been investigated by several workers in studies on the structure of the penicillin molecule (*3*). Barnes *et al.* (*1*) have published these spectra along with those of the crystalline sodium salts of other types of penicillin and have developed a method for the assay of the crystalline sodium salt of benzylpenicillin making use of the characteristic band at 14.2 μ. It was found in these investigations that all the, different types of penicillin possessed a characteristic band at 5.6 u which was not present in any degradation product of penicillin. This band was attributed to the presence in the penicillin molecule of the β-lactam carbonyl group.

The introduction of the procaine salt of benzylpenicillin has led to an attempt to assay this salt by infrared methods. The fact that chloroform was transparent to infrared rays in the region 5.0 to 5.2 μ (*4*), coupled with the fact that the procaine salt of benzylpenicillin was sufficiently soluble in chloroform to give a concentration adequate to produce a strong absorption band at 5.6 μ, indicated a possible method of assay of this salt. The procaine moiety of the penicillin salt does not interfere with the strength of the 5.6 μ band.

EXPERIMENTAL PROCEDURE AND RESULTS

The instrument used was a Perkin-Elmer infrared spectrometer Model 12B. A fixed cell holder as supplied by the Perkin-Elmer Corporation was fitted with sodium chloride plates and polyethylene spacers and gaskets. The cell was assembled as suggested by Carol *et al.* (*2*); the cell thickness was approximately 0.5 mm. The sample solution was prepared by weighing accurately a 20- to 40-mg. sample of the procaine salt of benzylpenicillin and dissolving it in chloroform, using a 10-ml. volumetric flask. The cell was then filled with chloroform, the wave length drum of the instrument was set at the position of maximum absorption of the 5.6 μ band, and the slit was found for full-scale deflection, using the glass shutter for the density reading of infinity. The gain of the amplifier was kept constant. The chloroform in the cell was then replaced by an aliquot of the procaine penicillin solution in chloroform and the density value read directly from the chart.

To test the validity of Beer's law, concentrations ranging from 0.2 to 1.0% were measured. There is a straight-line relationship between optical density and concentration over a range of concentrations from 0.2 to 0.8%.

Results showing the reproducibility of measurements of any one sample are given in Table I. Each density reading is an average of two aliquots of each concentration. For accuracy the concentrations tested were such as to give density readings in the range 0.15 to 0.40.

Fourteen plant samples of the procaine salt of penicillin were measured as indicated above. Table II lists the results of these tests along with the biological values and calculated conversion factors.

The test was further applied to miscellaneous samples of the procaine salt of benzylpenicillin, including aqueous suspensions and also mixtures in oil gelled with aluminum stearate. In the case of the aqueous suspension, the water was first removed by

Table II. Derivation of Conversion Factor

Sample	$k\left[\dfrac{\text{Optical Density}}{\text{Concn., \%}}\right]$	Biological Test, Units/Mg. Lab. I	Biological Test, Units/Mg. Lab. II	Biological Test, Units/Mg. Av.	Conversion Factor
1	0.76	1003	923	963	1270
2	0.76	999	958	978	1290
3	0.77	997	1000	999	1300
4	0.76	981	989	985	1300
5	0.75	1005	992	998	1330
6	0.77	992	989	991	1290
7	0.77	1009	997	1003	1300
8	0.77	1010	1009	1010	1310
9	0.77	1032	994	1013	1320
10	0.76	929	999	964	1270
11	0.76	936	983	960	1260
12	0.76	988	982	985	1300
13	0.77	977	998	988	1280
14	0.77	985	989	987	1280

Average conversion factor = 1290 ± 2%

Table III. Comparison of Values Obtained by Infrared Method with Biological Assays of Various Samples and Preparations

Sample	Infrared Test[a], μ/Mg.	Biological Test, μ/Mg.	Difference, %
1. Procaine salt of benzylpenicillin with aluminum stearate	880	870	+1
2. Procaine salts of benzylpenicillin with peanut oil containing 2% aluminum stearate	μ/ml.	μ/ml.	
1	320,000	332,000	−4
2	320,000	318,000	+1
3	314,000	322,000	−2
4	329,000	334,000	−1
5	333,000	313,000	+6
6	341,000	351,000	−3
7	340,000	332,000	+2
8	331,000	338,000	−2
3. Aqueous suspensions of procaine salts of benzylpenicillin			
Sample 1			
Initial potency		308,000	
2–5° C. 1-month storage	321,000		
37° C. 1-month storage	240,000	218,000	+10
Sample 2			
Initial potency		303,000	
2–5° C. 3-month storage	321,000		
37° C. 3-month storage	93,000	127,000	−27
Sample 3			
Initial potency		306,000	
2–5° C. 1-month storage	297,000		
24° C. 1-month storage	297,000	291,000	+2
37° C. 1-month storage	286,000	286,000	0
Sample 4			
Initial potency		293,000	
2–5° C. 1-month storage	293,000		
24° C. 1-month storage	288,000	283,000	+2
37° C. 1-month storage	262,000	256,000	+2

[a] k × 1290 (conversion factor).

Table I. Reproducibility of Measurement of a Single Sample

Concentration, %	Optical Density	$k\left[\dfrac{\text{Optical Density}}{\text{Concn., \%}}\right]$
0.393	0.300	0.77
0.198	0.155	0.78
0.234	0.180	0.77
0.312	0.240	0.77
0.410	0.315	0.77

spreading the sample on a watch glass and drying in a vacuum desiccator. For samples containing oil, those compounds which interfered with the infrared absorption at 5.6 μ were removed by treating the sample with petroleum ether and centrifuging. The solvent was decanted and the residue taken up in chloroform and tested. The results of such tests are listed in Table III.

DISCUSSION OF RESULTS

Table II shows that the value of the physical constant, k, is the same, within the error of the method, for all the samples tested. This does not imply a lack of sensitivity on the part of the infrared test as, theoretically, the potency of all the samples listed should be the same. The density readings obtained with any cell vary with the thickness of the liquid in the cell; hence the values of k depend on the cell used and should be established whenever a cell is assembled. Table III establishes the fact that a variety of samples of the procaine salt of benzylpenicillin, includ-

ing certain samples that have broken down on storage, can be tested with accuracy by the infrared method.

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Colorimetric Determination of Benzylpenicillin

Colorimetric Determination of Total Penicillins

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A colorimetric method for the determination of benzylpenicillin in samples of any purity and in fermentation liquors, based on the determination of the phenylacetyl side chain by the Kapeller-Adler reaction, is described. Separation from interfering substances is obtained by solvent extraction of penicillin and by the use of blanks obtained by alkali inactivation. The results are reproducible to ±3%.

Analytical recovery of penicillin G added to fermentation liquors was 97 ± 5%. A hydroxylamine method for the colorimetric determination of total penicillin in samples of any purity and in fermentation liquors is described. Simplification of procedure and improved stability of the final color complex have been obtained. Analytical recovery of penicillin added to fermentation liquors was 97 ± 5%.

VARIOUS methods for the chemical assay of benzylpenicillin have been described in the literature. (The terms benzylpenicillin and penicillin G are used synonymously throughout this paper, as both terms have been employed in the literature cited.) The rather weak absorption of the phenylacetyl side chain at 263 mμ has been used by Philpotts, Thain, and Twigg (13) and by Grenfell, Means, and Brown (6) to determine penicillin G in penicillin preparations. Sheehan, Mader, and Cram (14) and Mader and Buck (11) devised a gravimetric procedure which is based on the fact that the 1-ethylpiperidine salt of benzylpenicillin is considerably less soluble in amyl acetate and acetone than the corresponding salts of the other penicillins. Colorimetric procedures for the determination of the phenylacetyl group of penicillin G, proposed by Page and Robinson (12) and by Del Vecchio and Argenziano (4), are adaptations of the Kapeller-Adler (9) reaction for phenylalanine.

It is stated that all these methods are satisfactory if applied to penicillin G preparations of 50% or higher purity and, in general, best results can be expected with nearly pure preparations. The colorimetric procedures are specific for any phenyl monocarboxylic acid derivatives and do not distinguish the biologically active penicillin G from its biologically inactive degradation product, benzylpenicilloic acid, which will be present to some extent in most isolation intermediates. Particular difficulties must be expected with the assay of fermentation

liquors which contain the various derivatives of phenylacetic acid used for the stimulation of penicillin G formation (2).

The method for the assay of benzylpenicillin described in this paper is based on analytical separation of the active penicillin from the above-mentioned interfering materials, followed by the colorimetric determination of the phenylacetyl side chain of penicillin G by the Kapeller-Adler reaction (9).

Most of the substances used to stimulate the production of penicillin G in fermentation liquors are either basic or neutral phenylacetyl derivatives (2). If the penicillins are extracted from the broth at low pH into an organic solvent and then re-extracted into a neutral aqueous phase, they will be effectively separated from the precursor material. It is apparent that intermediates in the purification following the solvent extraction of penicillin will present different and simpler analytical problems than broth itself.

Two types of phenylacetyl derivatives other than benzylpenicillin are encountered with the various intermediates of penicillin purification: (a) benzylpenicilloic acid, and (b) various further degradation products of penicillin G and any other unrelated phenyl monocarboxylic acid derivatives. The active penicillins are readily and quantitatively separated from the penicilloic acids by extraction with chloroform at pH 2, inasmuch as none of the dicarboxylic acid—i.e., penicilloic acid—is extracted by chloroform. This separation also affords a simple

means of estimating the other types of interfering compounds mentioned under (b). A blank obtained by conversion of the penicillin to penicilloic acid by treatment with either alkali or penicillinase gives a quantitative measure of any phenylacetyl-like substances, other than penicillin G, extractable by chloroform at pH 2. Fortunately, these blanks are usually very small and frequently negligible. In all cases tested by the authors, inactivation by alkali gave the desired result—namely, complete destruction of penicillin without any change in the quantities of the interfering material. The enzyme, penicillinase, if available, can be used in exactly the same manner as the alkali inactivation and is undoubtedly more specific.

The method described first is applicable to penicillin preparations purified by an initial extraction through an organic solvent. A method applicable to fermentation liquors directly incorporates a simple and quantitative pre-extraction to afford the separation from the precursors used in penicillin G production.

DETERMINATION OF BENZYLPENICILLIN

In Preparations Free of Phenylacetyl Group Precursors. REAGENTS. Glycine buffer No. 1, pH about 2.0. Dissolve 9.0 grams of glycine, 7.0 grams of sodium chloride, and 9.0 ml. of concentrated hydrochloric acid in water and dilute to 1000 ml.

Potassium nitrate, 10%, in concentrated sulfuric acid.

Aqueous hydroxylamine hydrochloride solution, 15%. Prepare a fresh solution once a week from reagent grade hydroxylamine hydrochloride.

Chloroform, reagent grade.

Anhydrous sodium sulfate, reagent grade.

Ammonia water, reagent grade, specific gravity 0.90.

Sodium hydroxide, 2 N.

Hydrochloric acid, 0.5 N.

PROCEDURE. The glassware used prior to evaporation of the chloroform is well chilled in the refrigerator. The stopcocks of the separatory funnels are rinsed with chloroform and lubricated just before use by dipping into distilled water. The use of stopcock grease must be avoided, because variable amounts of material reacting as phenylacetyl derivatives are extracted from it. The glycine buffer, chloroform, and any active penicillin solutions are kept in the refrigerator and are used ice cold.

For calibration purposes solutions are prepared containing 100 to 500 micrograms of recrystallized sodium benzylpenicillin per ml. · For each determination 5 ml. of buffer 1 and 25 ml. of chloroform are measured into a 60-ml. separatory funnel; 5 ml. of solution of known penicillin content are added. The funnel is vigorously shaken for exactly 2 minutes, and 2 more minutes are then allowed for phase separation. The chloroform layer is drained into a glass-stoppered 25-ml. graduated cylinder to which about 0.5 gram of anhydrous sodium sulfate has been added by means of a calibrated spoon. The chloroform is superficially dried by shaking with the sodium sulfate for a few seconds.

The dried chloroform is decanted, exactly 20 ml. are pipetted into a 50-ml. glass-stoppered Erlenmeyer flask which contains a glass bead, and the chloroform is slowly evaporated to dryness on a steam bath. One milliliter of the 10% potassium nitrate–sulfuric acid mixture is added to each flask and the flask is rotated in a tilted position to ensure contact of the nitrating agent with any material that might have dried on the walls. The flasks are replaced on the steam bath, after about 1 minute the stoppers are inserted, and the nitration is allowed to proceed at steam bath temperature for 30 minutes. Following nitration, the flasks are placed in an ice bath and chilled thoroughly. Two milliliters of distilled water are added cautiously along the walls of the vessels, and the contents are mixed again and cooled to ice temperature. Two milliliters of ammonia water are added dropwise along the wall from a buret and the contents are mixed. The reaction vessel is removed from the ice bath and allowed to come to room temperature. Two milliliters of 15% aqueous hydroxylamine solution are added, mixed thoroughly, and followed by 5 ml. of ammonia water. After mixing, the flasks are left standing for 45 minutes for color development. The optical density at 580 mμ is determined on a Coleman spectrophotometer in a 13-mm. square cuvette, using distilled water as the blank solution.

A plot of the optical density against the amount of penicillin G used is linear over the entire range passing through the origin, indicating that Beer's law is obeyed and that the blank value is identical with the optical density of distilled water.

For the determination of penicillin G in an unknown sample a solution containing between 200 and 1000 micrograms of penicillin G per ml. is prepared. Five milliliters of this solution are measured into each of two test tubes. To one tube, the blank, is added 1 ml. of 2 N sodium hydroxide and the tube is immersed in a boiling water bath for 1 minute, and cooled, and 4 ml. of 0.5 N hydrochloric acid are added. To the other tube 5 ml. of distilled water are added and the contents are mixed. A 5-ml. aliquot of the contents of each tube is treated in exactly the manner described for the calibration.

It is immaterial whether the optical density of the blank and the "active" solution are both measured against distilled water or whether the blank solution is used to set the instrument to zero optical density and the active solution is measured against this setting. The values obtained from the calibration curve correspond to the penicillin G content of 2.5 ml. of the original solution of the unknown sample.

In Broth. ADDITIONAL REAGENTS. Glycine buffer No. 2, pH about 1.5. Dissolve 45.0 grams of glycine, 35.0 grams of sodium chloride, and 45 ml. of concentrated hydrochloric acid in water and dilute to 1000 ml. with water.

Dibasic potassium phosphate, 0.15 M.

Sodium hydroxide, 1.3 N.

Hydrochloric acid, 2.0 N.

Hydrochloric acid, 0.35 N.

Amyl acetate, reagent grade.

PROCEDURE. The broth is diluted to contain 25 to 150 micrograms of penicillin G per ml. To 120 ml. of amyl acetate and 60 ml. of glycine buffer 2 in a 250-ml. separatory funnel are added 60 ml. of the diluted broth. The funnel is vigorously shaken for exactly 2 minutes and 2 to 3 minutes are then allowed for phase separation. The aqueous phase is drained off and discarded, leaving most of the interface in the funnel with the organic phase. The amyl acetate is decanted into a glass-stoppered 100-ml. graduated cylinder to which about 1 gram of anhydrous sodium sulfate has been added by means of a calibrated spoon. It should be possible to decant from 105 to 115 ml. of amyl acetate free from any interfacial material. The amyl acetate is cleared and dried superficially by shaking for a few seconds with the sodium sulfate. One hundred milliliters of the clear amyl acetate are measured into a cold 125-ml. separatory funnel, 15 ml. of ice-cold 0.15 M dibasic phosphate are added, and the funnel is shaken for 1 minute. The aqueous extract is collected in a test tube and kept cold.

To 6 ml. of this aqueous extract in a test tube are added 3 ml. of 1.3 N sodium hydroxide and the tube is immersed for 1 minute in a boiling water bath. After cooling, 3 ml. of 2 N hydrochloric acid are added and the contents are mixed well. A 10-ml. aliquot is pipetted into a 60-ml. separatory funnel, 25 ml. of chloroform are added, and the funnel is shaken for 2 minutes. The chloroform layer is now treated exactly in the manner described under calibration. This sample represents the blank.

To obtain the "active" solution, 5 ml. of the cold, aqueous phosphate extract are added to a chilled 60-ml. separatory funnel containing 5 ml. of 0.35 N hydrochloric acid and 25 ml. of ice-cold chloroform. After shaking for 2 minutes the chloroform layer is treated according to the procedure outlined under calibration. ·

The colorimetry is performed as previously described. The value obtained from the calibration curve corresponds to the penicillin G content of 16.7 ml. of the diluted broth used for the test.

DISCUSSION. Strict attention must be given to a uniform technique of extraction of the penicillin into chloroform, as the penicillins are unstable at pH 2. The superficial drying of the chloroform is necessary to prevent the presence of any appreciable quantities of moisture in the residue after the evaporation of the chloroform prior to nitration. The chloroform must be evaporated on a steam bath to avoid heating above 100° C. after nearly all the chloroform has been removed. Use of an electric hot plate invariably leads to darkening of the residue and high and unpredictable blanks. The conditions of nitration are critical. In the authors' experience heating on the steam bath for any length of time between 25 and 40 minutes gave identical results and 30 minutes was chosen as intermediate within this interval.

If the heating is insufficient the final color is found to be unstable. Heating for periods longer than stated leads to less intense but stable color development. It is of some practical advantage that the procedure may be interrupted for any length of time at any point after the penicillins have been extracted into chloroform and before the ammonia water has been added.

With solutions of pure penicillin G full color development is obtained within 15 minutes after the addition of the final 5 ml. of ammonia. The color developed considerably more slowly with some impure intermediates, requiring from 25 to 35 minutes. Thus, 45 minutes were allowed in order to ensure maximum color development in all cases. The fully developed color is stable for at least 2 hours.

In Figure 1 an absorption curve on the final colored solution is reproduced. The choice of a wave length of 580 mμ for spectrophotometry is obvious. A light-filter instrument with a filter of maximum transmittance at 540 mμ may be used, as the curve shows a rather flat minimum at 530 mμ. The curve further indicates that a more intense absorption maximum is found below 400 mμ. If ammonia is used in the procedure, this peak is found at 375 mμ. If sodium hydroxide is used, the peak extinction is about twice as intense and shifted to 350 mμ, while the absorption at 580 mμ disappears completely. With pure penicillin G solutions excellent calibration curves and considerably higher sensitivity can be obtained at 375 and 350 mμ, respectively. However, such procedures are of little practical value for impure samples or broth, because the blank readings due to the interfering materials are too high to permit accurate corrections.

The pre-extraction described for broth is designed to give quantitative recovery and a simple arrangement for the preparation of the blank. As most fermentation liquors are strongly buffered, a more concentrated and somewhat more acid buffer was necessary to lower the pH to about 2.0 for the extraction of the penicillin into amyl acetate. The use of an aliquot of the amyl acetate slightly reduces the sensitivity of the method; however, it obviates the necessity for sharp separation of the two phases in the extraction. On extraction of the amyl acetate with the phosphate solution, definite phase separation is, however, always obtained. The amyl acetate contains sufficient acidic material to lower the pH of the phosphate extract to between 7.5 and 8. At this pH penicillin G is quantitatively removed from the organic phase and reasonably stable. Addition of an equal volume of 0.35 N hydrochloric acid to the phosphate extracts lowers the pH to about 2.0 and ensures buffering in this range. On the other hand, addition of half a volume of 1.3 N sodium hydroxide raises the pH above 13. Heating for 1 minute at this pH ensures the complete conversion of the penicillins to the penicilloic acids. Addition of half a volume of 2.0 N hydrochloric acid lowers the pH to about 2.0 and again ensures buffering in this range. These conditions for both the active and the blank solutions are the same as used for extraction in the calibration.

Assays of pure samples of penicillin K and F gave negative results. Interference from penicillins other than penicillin G could be expected only from penicillin X, p-hydroxybenzylpenicillin. Penicillin X of sufficient purity was not available for testing; however, when tyrosine was added to the chloroform extract prior to nitration in amounts equivalent to the penicillin G usually found, a somewhat more intense yellow color was observed (9), which did not interfere when the extinction was measured at 580 mμ (3).

1-Ethyl piperidine does not interfere in the determination and the procedure described for the assay of intermediates can be used to determine the purity of 1-ethylpiperidine salts of penicillin G.

RESULTS. The standard deviation of the slope of a single calibration curve determined in triplicate with five different dilutions of the standard sample was ±1.5%. The slope of the calibration curve could be reproduced in 10 determinations with a standard deviation of ±3.0%.

No other chemical method of sufficient accuracy is available for the assay of penicillin G in fermentation liquor or impure isolation intermediates to permit comparison with this colorimetric procedure. The reliability of the method had to be established by a series of recovery experiments. When varying dilutions of penicillin G corresponding to those of the calibration curve were prepared in broth prior to fermentation but containing precursors for penicillin G formation, a straight line was obtained with a slope of 97.5 ±3.0% of that of the aqueous calibration curve. The value includes the correction for the 5/6 aliquot used for the re-extraction from amyl acetate. This experiment indicates essentially complete analytical recovery through the amyl acetate pre-extraction.
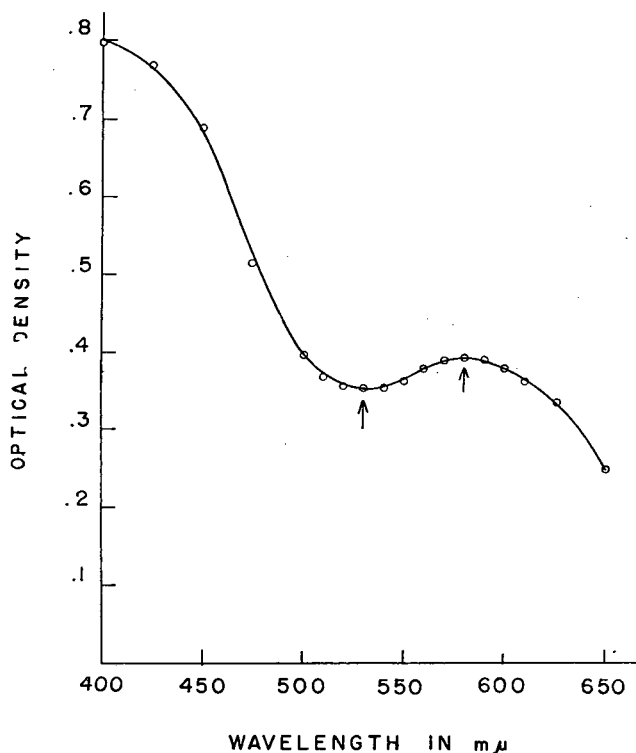

Figure 1. Absorption of Colored Solution

Recovery from unfermented broth is, however, insufficient evidence of the applicability of the method to fermented broth, for various nutrients are removed and a number of metabolic products including pigments and possibly precursor and degradation products of penicillin are formed during the growth of the mycelium. To a series of 25 fermented broths containing penicillin G and representing various stages of fermentation, known amounts of penicillin G at two different levels were added. In 50 determinations the analytical recovery of penicillin G added over and above the amount already present was 97 ±5%. The largest deviations were −13 and +12%.

DETERMINATION OF "TOTAL" PENICILLIN

It is often desirable to obtain the assay for both total penicillin and penicillin G simultaneously. Ford (5) recently described a colorimetric assay for "total" penicillin which is based on the formation of a hydroxamic acid from penicillin and the measurement of the color complex of this hydroxamic acid with ferric ions in acid solution. This procedure has the disadvantage that the color complex obtained is not stable and the timing of the measurement of the color becomes critical. The authors have

independently developed and used an assay based on the same sequence of reactions which they believe to be technically simpler and more reproducible because of a considerably more stable final color complex.

EXPERIMENTAL

Reagents. Hydroxylamine hydrochloride (stock solution), 5 M.

Alkali buffer solution. Dissolve 86.5 grams of sodium hydroxide and 10.3 grams of sodium acetate (anhydrous), in distilled water and make up to 500 ml.

Neutralized alcohol hydroxylamine solution (prepared each day). To one part (by volume) 5 M hydroxylamine hydrochloride, add 1 part of alkali buffer solution and 4 parts of ethyl alcohol (95%).

Ferric ammonium sulfate, 20%, in 3.5 N sulfuric acid. Dissolve 100 grams of ferric ammonium sulfate dodecahydrate (reagent grade) and 46.7 ml. of concentrated sulfuric acid (specific gravity 1.84) in distilled water and dilute to 500 ml.

Penicillinase solution. Dissolve 100,000 "units" of Schenley penicillinase A in 10 ml. distilled water. Keep in refrigerator.

Procedure. Into each of two test tubes is pipetted 1 ml. of broth. To one tube, the blank, one drop (about 0.05 ml.) of the penicillinase solution is added. After 10 minutes at room temperature all the penicillin will be destroyed and the contents of this tube represent the blank. To both tubes, the "active" sample and the blank, 3 ml. of the neutralized hydroxylamine solution are added, followed after 3 minutes by 1 ml. of the ferric ammonium sulfate reagent. The Coleman spectrophotometer is set to zero optical density with the blank solution in the path of the light beam of wave length 515 mμ, and the active solution is read against this setting within 2 to 5 minutes after the addition of the ferric ammonium sulfate reagent. A calibration curve is obtained on dilutions of a recrystallized sample of penicillin G. For the calibration a simple reagent blank is used to set the instrument to zero optical density. Beer's law is obeyed over the entire range.

Discussion. The procedure is essentially the same as the one used by Lipmann and Tuttle (10) for acylphosphates and by Ford (5) for penicillins. Considerable simplification is obtained by combining the hydroxylamine with the buffer and also by combining the strong acid and the ferric ions. The authors found the addition of the ethanol to the hydroxylamine solution necessary, for in some samples material was encountered which precipitated in strongly acid aqueous solutions. The presence of about 30% ethanol in the final solutions not only prevents the formation of such a precipitate but also enhances the color intensity and stability. Hill (7, 8) demonstrated that the instability of the color complex is due to reduction of ferric ions by the excess hydroxylamine and that the color could be stabilized by the use of ferric perchlorate in perchloric acid. As the presence of ethanol was necessary, it seemed inadvisable to use perchloric acid. A considerably more stable ferric ion color complex was obtained by the use of ferric ammonium sulfate instead of ferric chloride. The reagent blank is much smaller when ferric ammonium sulfate is used instead of ferric chloride. The acid concentration recommended (3.5 N) is about optimal; any appreciable increase or decrease leads to decreased color intensity and stability. Further increase in the concentration of the ferric ammonium sulfate slightly increases the color intensity, but the advantage is offset by the increase in the optical density of the blank.

The stability of the color obtained is indicated in Table I. The color is essentially constant within the first 5 minutes after the addition of the ferric ion solution and then decreases at the rate of about 8% every 10 minutes.

The hydroxamic acid formation from penicillin is completed at room temperature within 2 minutes at pH's varying from 6 to 8. Thereafter the hydroxamic acid is stable for at least 2 hours. A large series of determinations, active and blank solutions, may therefore be treated with hydroxylamine and left standing at this stage for a minimum of 3 minutes, but if desirable for as long as 2 hours. This permits the determination of the optical density within the stated time limits by developing the color of one active sample and the corresponding blank at a time.

Phosphate ions reduce the intensity of the color and difficulties would be encountered if the assay of solutions buffered around neutrality were desired. In such cases, the veronal-acetate buffer of Michaelis can be employed without any interference with the color development. The quantity of penicillinase recommended in the procedure is sufficient to destroy completely 4000 units of penicillin in 1 ml. at 20° C. in 7 to 8 minutes. Alkali inactivation cannot be used to obtain the blank for the determination of total penicillin in broth, because interfering material is liberated by heating with alkali. If the assay is applied to samples of penicillin which have been purified by extraction through an organic solvent, the proper blanks can also be obtained by inactivation by alkali as described for the assay of penicillin G.

Table I. Stability of Color Complex

Time after Addition of Ferric Ammonium Sulfate Reagent, Minutes	Relative Intensity of Color, %
2	100.0
3	99.7
4	99.7
5	99.0
6	97.0
7	95.5
8	94.4
9	93.2
10	91.6
20	86.0
30	77.5

Results. The standard deviation of the slope of a single calibration curve determined in triplicate with five different dilutions of a recrystallized sample of penicillin G was ±1.5%. The slope of the calibration curve could be reproduced in five determinations with a standard deviation of ±2.5%. Two different levels of penicillin—in addition to the penicillin already present—were added to 41 fermented broths. The analytical recovery in these 82 determinations was 97 ± 5% and the largest deviations were −13 and +11%. Comparison with the iodometric assay (1) which has similar accuracy was possible when the method was applied to intermediates in the purification of penicillin which had passed through a solvent extraction step. On ten such samples the value obtained by the iodometric assay was found to be 99.4 ± 4.4% of the corresponding colorimetric value. The largest deviations occurring were +6.3 and −8.4%.

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Polarographic Determination of Benzene in Presence of Its Homologs

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Benzene is determined in the presence of toluene and xylene by nitration of the aromatic compound present in the atmosphere with subsequent selective oxidation of the toluene and xylene nitration products and isolation of the dinitrobenzene through differential solubility extraction. The dinitrobenzene is then polarized in the presence of a suitable base, and the amount present in the sample is estimated from a prepared standard curve. Thus the greatest advantages of the polarographic method are realized when both samples and standards are determined under duplicate controlled conditions.

A REVIEW of the literature has revealed many methods for the determination of benzene by colorimetric procedures, fractional distillation, chromatographic adsorption, and other physicochemical approaches, yet very little has been done in the field of polarographic analysis. This line of endeavor has been carried notably by Roubal (8) and Teisinger (10), each of whom is primarily interested in the determination of dinitrobenzene in blood. Lingane's (7) method for the polarographic analysis of dinitrobenzene was specific for dinitrobenzene but the isomeric dinitrotoluenes could not be present, because their waves and those of the dinitrobenzene coalesced.

One of the best methods for the determination of benzene, especially in the presence of its homologs, is a colorimetric one which was developed originally by Schrenk, Pearce, and Yant (9) with modifications proposed and used by Dolin (4) and Baernstein (1). It offered as advantages rapidity of determination, reproducibility of results, and low inherent errors. The last two factors are directly related to adequate instrumentation control. On the negative side are necessity for compression of the composition range and the decidedly rapid deterioration of color complex, especially in the lower portion of the curve; the latter factor normally holds the greater interest for those in the field of industrial hygiene.

This paper outlines a procedure for the polarographic determination of benzene in the presence of its homologs. This is accomplished by nitration of the aromatic compounds present in the atmosphere, subsequent selective oxidation of the toluene and xylene nitration products, and isolation of the dinitrobenzene through differential solubility extraction. The dinitrobenzene is then polarized in the presence of a suitable base, and the amount present in the sample is estimated from a prepared standard curve. Thus, the greatest advantages of a polarographic method are realized when both the samples and standards are determined under duplicate controlled conditions (6).

SAMPLING PROCEDURE

A sample is obtained by aspirating the air at 0.25 liter per minute for a 10-minute period through a U. S. Bureau of Mines type of bubbler for nitrating benzene (also known as the benzol nitrating apparatus), containing 2.0 ml. of nitrating acid, and utilizing an M.S.A. Midget impinger apparatus as the source of vacuum.

Stock Benzene. c.p. benzene was used, and the solution was kept at 20.0° C.

Standard Benzene Solution. A 10% by weight solution of benzene was prepared, using methylcyclohexane as the second component. The composition was checked by refractive index data and found to be 10.06%. This solution was designated as

primary solvent standard V, and polarographic standards were prepared by nitrating weighed amounts according to the information given in Table I.

Stock Toluene. c.p. toluene was used, and the solution was kept at 20.0° C.

Standard Toluene Solution. A 10% by weight solution of toluene was prepared, using methylcyclohexane as the second component. The composition was also checked by refractive index data and found to be 10.00%. This solution was designated as primary solvent standard Q, and polarographic standards were prepared by nitrating weighed amounts according to the information in Table I.

Stock Xylene. c.p. xylene was used, and the solution was kept at 20.0° C.

Standard Xylene Solution. A 10% by weight solution of xylene was prepared, using methylcyclohexane as the second component. The composition was checked as in the other cases by refractive index data and found to be 10.13% as calculated by means of the following formula:

$$\frac{n_m - n_a}{n_b - n_a} \times 100 = \% B$$

where n_m, n_a, and n_b refer to the n_{20}^D of the mixture, methylcyclohexane, and the aromatic in question, respectively, as determined by an Abbe refractometer. This is primary solvent standard Y.

Synthetic Mixture. This mixture was made by weighing out definite amounts of benzene, toluene, xylene, and methylcyclohexane into a tared volumetric flask with the following calculated percentages:

Compound	% by Weight
Benzene	11.80
Toluene	21.08
Xylene	20.73
Methylcyclohexane	46.39

An aliquot portion of this solution was also nitrated (Table I).

Nitrating Acid. Mix equal volumes of fuming nitric acid, specific gravity 1.49, and concentrated sulfuric acid, specific gravity 1.84.

Sodium hydroxide, 10%, prepared from c.p. sodium hydroxide, in distilled water.

Ether, c.p., Merck.

Petroleum Ether, c.p., boiling point 35° to 60° C.

Ethyl Alcohol, 95%.

Ethyl Alcohol, dilute. Dilute 95% alcohol 1 to 1 with distilled water.

Polarographic Base.

Metol	1.0 gram
Sodium sulfite, anhydrous	31.5 gram
Potassium carbonate, anhydrous	6.3 gram
Water, to make	250.0 ml.

This solution also acts as oxygen removal agent, and is similar to Roubal's (8) formula, except that potassium carbonate is used in this case.

Table I. Identification of Primary Solvent Standards

	V	Q	Y	Synthetic Mixture	%
$(n_{20}^D)_m$	1.4283^a	1.4301	1.4301	Benzene	11.80
$(n_{20}^D)_b$	1.4980^a	1.4958	1.4948	Toluene	21.08
$(n_{20}^D)_a$	1.4205^a	1.4228	1.4228	Xylene	20.73
% B	10.06	10.00	10.13	Methylcyclohexane	46.39
B	Benzene	Toluene	Xylene		

V, Nitrating Mixture		Q, Nitrating Mixture		Y, Nitrating Mixture		Synthetic Mixture	
100.0^b	K	100.7^b	L-2	100.2^b	O	100.1^b	N
100.9	K-1	100.5	L-3	99.5	O-2	99.9	N-2
99.8	K-2						
100.3	K-3						
100.7	K-4						

^{a} n_{25}^D. ^{b} Mg./50 ml. of nitrating acid.

Glacial Acetic Acid, c.p.

Chromium Trioxide Solution, a saturated solution of chromium trioxide from c.p. crystals.

Bromothymol Blue Maximum Suppressor. To make 0.1% solution macerate 0.1 gram of dry bromothymol blue powder in a mortar with 16.0 ml. of 0.01 N sodium hydroxide and dilute the mixture to 100.0 ml. with distilled water (5). Use 1.0 ml. per 100.0 ml. of solution to be polarized.

Oxygen Absorbent for Purification of Nitrogen (3). Pass nitrogen through a first scrubbing bath containing a solution of 40 ml. of concentrated ammonium hydroxide and 40 ml. of water saturated with ammonium chloride (approximately 25 grams), and filled with copper gauze, especially in the air spaces; then through a second scrubbing solution of dilute sulfuric acid (5 N).

Mercury Purification (2). Cover the mercury with 5% nitric acid in a filtering flask, aerate for 2 hours by drawing air through a glass tube partially immersed in mercury, wash thoroughly by decantation, and then wash with distilled water. Dry, and distill using Cenco improved vacuum distilling apparatus.

ANALYTICAL PROCEDURE FOR ATMOSPHERIC SAMPLES

Immerse the nitrating apparatus containing the sample in a boiling water bath, and keep at this temperature for exactly
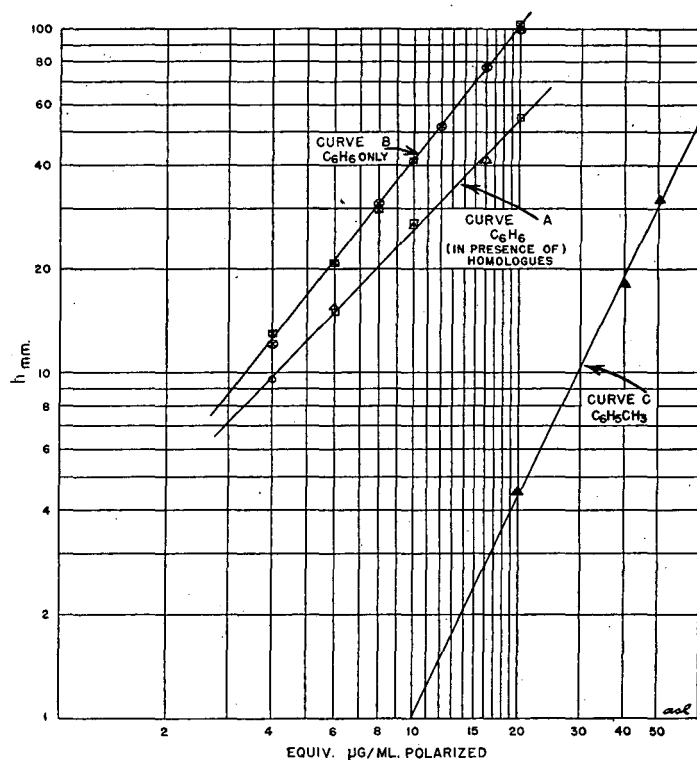
Figure 1. Calibration Curves for Benzene and Toluene

Plotted for wave-step height, $h$mm., against equivalent concentration of compound per ml. polarized

15 minutes. Cool in ice bath, and dilute to approximately 10 ml. with small additions of water, swirling while still in the ice bath. Transfer quantitatively to a 250-ml. Squibb separatory funnel, using 5-ml. portions of distilled water to rinse out the nitrating apparatus twice. Dilute to 35 ml. or to mark previously etched on funnel and extract with 25 ml. of ether, using a 10-ml. aliquot of this ether to rinse out the nitrating apparatus. Continue with two 10-ml. ether extractions and wash ether extract twice with 10 ml. of 10% sodium hydroxide, allowing sufficient time for complete separation of layers. Rinse down sides of funnel with 10 ml. of water, but do not shake. Drain off 5 ml. of water, deliver contents of funnel to a 125-ml. Phillips beaker, add boiling aid, such as ground brick, and evaporate off ether by use of water bath. A heating mantle is convenient at this stage to heat the water bath, and thus eliminate the possibility of igniting ether fumes that are given off.

Cool the beaker and contents in an ice bath to approximately room temperature. Add 1.0 ml. of glacial acetic acid and one drop of chromium trioxide solution, and heat in boiling water bath for exactly 15 minutes. Immerse in ice bath for a few minutes to cool, and add 10 ml. of 10% sodium hydroxide. Transfer to a 250-ml. Squibb funnel, rinse beaker several times with water to final volume of approximately 50 ml., and extract twice with 15-ml. portions of petroleum ether.

Rinse the sides of the funnel containing the extract with 10 ml. of distilled water, discard approximately 8 ml. of the water, and deliver the balance of the contents of the Squibb funnel to a 100-ml. volumetric flask. Add a boiling aid, and evaporate off petroleum ether on a water bath heated by heating mantle. The final remnant of solvent may be removed by immersing the flask in the bath for a minute or so until escaping fumes are no longer visible. Cool, add 50 ml. of polarographic base and 1.0 ml. of bromothymol blue, and make to volume at 20.0° C. with 1 to 1 ethyl alcohol.

Pour a 10-ml. portion into polarographic cell, aerate for exactly 10 minutes, and polarize at 1/5 sensitivity ($S = 5 X$) from 0 to -375×10^{-3} volt, using a water bath to jacket cell at 20.0° C. Plot galvanometer deflections, D, against applied voltage, V. A typical curve obtained under the conditions indicated is illustrated in Figure 2.

If benzene alone is to be determined, not in the presence of toluene, the procedure may be shortened considerably.

Add 1 drop of chromium trioxide to nitrating mixture, and treat the sample as outlined under analytical procedure for atmospheric samples up to the point where 5 ml. of water are drained off. Then transfer contents of Squibb funnel to 100-ml. volumetric flask, add boiling aid, boil off ether on water bath, and prepare for polarizing by adding polarographic base, bromothymol blue, 1 to 1 ethyl alcohol, etc.

A calibration standard curve, B, for the abbreviated ocedure is shown in Figure 1.

ANALYTICAL PROCEDURE FOR POLAROGRAPHIC STANDARDS

Keep all solutions used in the following procedure in a water bath set at 20.0° C. until they are to be used. Transfer a 100.0 ±0.5-mg. sample of the respective primary solvent standard to a special nitrating tube (see Figure 3), using a capillary pipet. Break off the hook on the tube and attach a piece of rubber tubing (with protected mouthpiece as used in glass blowing) to end A. Immerse bulb of nitrating tube under surface of 25.0 ml. of nitrating acid contained in 50-ml. volumetric flask, and break by hitting bottom of flask with simultaneous blowing through rubber tubing. Draw nitrating mixture into nitrating tube several times to rinse, allow to drain completely, and blow out last drop into flask. Shake thoroughly to effect

Table II. Polarographic Standards for Benzene in Presence of Its Homologs

(Sensitivity, X, 5. Calibration standard curve A)

Standard	A-2	D-2	B-3	C-3	D-3	B-4	C-4	E-4
Code No.	39	42	44	45	46	55	56	57
Nitrating mixture	K-2	K-2	K-3	K-3	K-3	K-4	K-4	K-4
Aliquot treated, ml.	2	8	3	5	8	3	5	10
Equivalent polarized, γ/ml.	4	16	6	10	16	6	10	20
E_{dc}, 1 × 10^{-3} volt								
000	-0.5	-0.9	-0.8	-0.9	-0.6	-0.9	-0.9	-0.9
-025	-0.5	-0.7	-0.6	-0.4	-0.3	-0.8	-0.8	-0.5
-050	-0.2	-0.3	-0.2	-0.2	±0	-0.7	-0.3	-0.3
-075	-0.1	±0	±0	±0	+0.3	-0.3	±0	±0
-100	±0	+0.7	+0.7	+0.8	1.2	±0	+0.8	+0.9
-125	±0	1.9	1.6	1.9	2.8	+1.0	2.0	2.3
-150	+0.3	3.9	2.9	3.4	5.0	2.3	3.9	4.8
-175	1.0	7.2	4.8	6.0	8.9	4.3	7.0	9.2
-200	2.0	11.0	5.9	8.2	13.2	5.8	10.0	14.5
-225	3.0	14.7	6.7	9.8	16.3	6.8	11.9	18.9
-250	3.8	16.6	7.0	10.8	18.2	7.1	12.9	21.2
-275	4.2	17.3	7.1	11.1	19.0	7.7	13.3	22.3
-300	4.2	17.7	7.3	11.7	19.3	7.8	13.8	22.8
-325	4.5	17.8	7.3	11.8	19.5	7.9	14.0	22.9
-350	4.5	17.9	7.8	11.9	19.8	8.0	14.2	22.9
-375	4.7	18.1	8.0	12.2	20.6	8.5	15.0	23.9
hmm.	9.5	41.0	16.0	26.7	41.2	15.2	27.1	55.4

complete nitration, and make to volume at 20.0° C. with nitrating acid.

Deliver aliquot portions of the nitrated solution to Folin-Wu digestion tubes (they are conveniently calibrated for dilution purposes, but any large test tube could be used), and treat according to the procedure for the atmospheric samples, but dilute nitrating mixture with distilled water while in the ice bath to a volume of 35 ml., and carry on from there as usual with extraction, evaporation, etc.

Specific examples are noted in Tables II and III, and calibration standard curves are shown in Figure 1.

DISCUSSION

During the investigation which was carried out in the latter part of 1947, it was found that the work of Roubal could be readily duplicated; the only exception was that a maximum developed in the curve where none appeared before. Such a maximum is easily suppressed by using bromothymol blue.

An explanation of this and other phenomenon encountered during the investigation was not attempted, as the main interest was the development of a practical determination of benzene rather than a pure research project.

A typical set of curves for DNB, DNT, DNX is shown in Figure 2. The effect of the selective oxidation procedure is apparent for the treated compound produces a curve that has a very small step in comparison to the untreated nitrated compound. Referring to Figure 1, it may be predicted that concentrations of toluene up to the nitrated equivalent of 1 mg. of toluene under sampling conditions indicated (or 2.5 times the maximum allowable concentration) will not produce a detectable wave step. When the concentration of toluene exceeds that amount, an error is introduced. It should not be large, because of the greater volatility of the benzene in respect to toluene, but work is now in progress to establish a correction factor.

This prediction was observed and estimated according to the information given in Table V. Here the amount of benzene determined from the procedure was found to be in very good agreement with the amount of benzene calculated to be present there.

It was found that the half-wave potential of m-dinitrobenzene and 2,4-dinitrotoluene coalesced. This is in complete agreement with Lingane's (7) statement that polarographic activity displayed is usually characteristic of the reduction of the nitro group or groups contained in them and "apparently there is little or no difference in effect of either methyl or hydroxyl groups on the reducibility of the nitro groups present."

The procedure calls for immersing the nitrating tube in the boiling water bath in order to ensure completion of nitration. Reproducibility of results may be observed in Table II, where three different nitrating mixtures were utilized in pre-
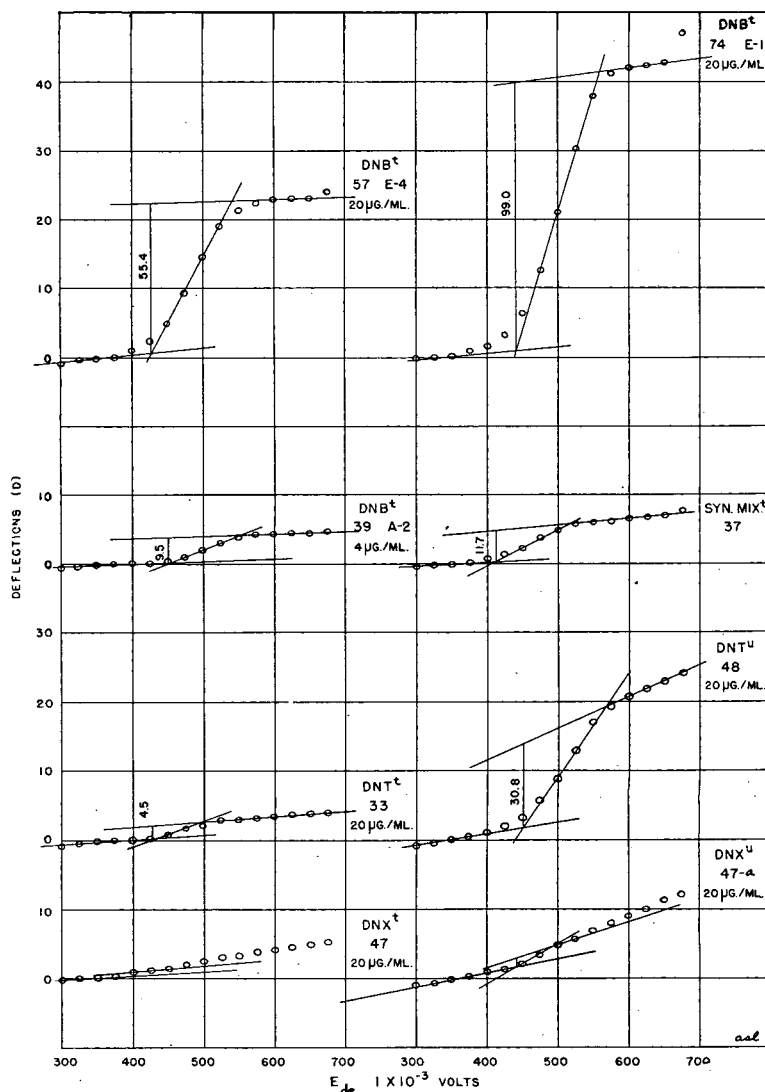

Figure 2. Polarograms

Showing effect of selective oxidation and differential solubility (or treated compound, t) in respect to untreated condition, u
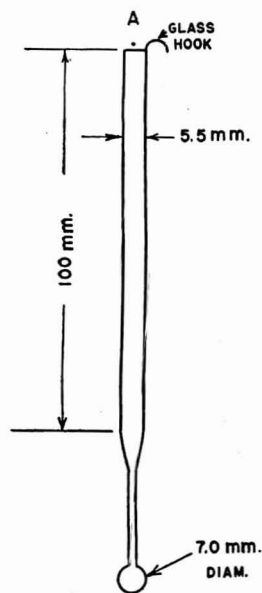
Figure 3. Special Nitration Tube for Standardized Nitrating Conditions

Table III. Polarographic Standards for Benzene

(Sensitivity, X, 5. Calibration standard curve B)

Standard	A	B	C	D	E	A-1	B-1	C-1	D-1	E-1
Code No.	62	63	64	65	66	70	71	72	73	74
Nitrating mixture	K	K	K	K	K	K-1	K-1	K-1	K-1	K-1
Aliquot treated, ml.	2	4	6	8	10	2	3	4	5	10
Equivalent polarized, γ/ml.	4	8	12	16	20	4	6	8	10	20
$h$mm.	12.1	30.9	51.2	77.3	99.0	13.0	20.8	29.9	40.9	100.2

Table IV. Polarographic Standards

(Sensitivity, X, 5)

Code No.	33	48	47	47-a	60	58	49
Nitrating mixture	L-2	L-3	O-2	O-2	L-3	DNT	DNT
Aliquot, ml.	10[a]	10[b]	10[a]	10[b]	25[a]	4.0[a]	5.0[a]
Equivalent polarized, γ/ml.	20	20	20	20	50	40	50
$h$mm.	4.5	30.8	1.0	3.0	31.1	17.9	31.8
Curve	C	...	...	...	C	C	C

[a] Treated to oxidize dinitrotoluene according to procedure.
[b] Untreated.

paring the standard curve. The wave steps obtained produced a curve which was not a straight line in itself, but could be straightened out by plotting on log-log paper. This phenomenon was not interpreted, as a calibration curve was to be prepared.

The color of the solution prior to extraction with petroleum ether but after addition of the sodium hydroxide will be that of dichromate orange if toluene is present, and chrome yellow if toluene is not present.

Finally, the best polarographic technique (6) would require the use of an external calomel electrode, thereby assuring adequate cleaning of the polarographic cell. When this equipment is not immediately available, reproducible results may be obtained by filling the cell with the solution to be tested and using that solution to rinse the dropping mercury electrode, calomel electrode, and nitrogen tube. After thorough draining the aliquot to be polarized is admitted. Under normal drainage conditions, the residual volume will be small and not vary appreciably.

The effect of temperature on half-wave potential and wave step was not determined, but in order to use standardized conditions the polarographic cell was jacketed in a water bath at 20.0° C. and the solutions were polarized immediately after aerating, because they deteriorated rapidly after the addition of the polarographic base.

Table V. Polarographic Standards

Code No.	32	37
Nitrating mixture	N-2	N-2
Aliquot treated, ml.	2.0	2.0
$h$mm.	11.3	11.7
C_6H_6 calculated, γ	4.84	4.84
C_6H_6 from curve A, γ	4.60	4.80
Deviation, %	4.9	0.1

A colorimetric procedure based on the selective oxidation-differential solubility procedure indicated herewith is also being processed.

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Polarographic Determination of Tin in Steel

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THE standard method of Scherrer (6) for the determination of tin in steel is time-consuming, and offers many opportunities for indeterminate errors. It requires a hydrogen sulfide precipitation from a solution obtained from a 10-gram sample; this makes it awkward to use in the case of alloy steels high in molybdenum, because of the difficulty of filtering and washing such large amounts of molybdenum sulfide.

Allsopp felt that this procedure might be shortened, and a smaller sample might suffice, if a polarographic method could be worked out. In a literature search, it was found that Lingane (4) had reported the reduction of stannic ions at the dropping mercury electrode in a supporting electrolyte of 1 N hydrochloric

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acid, resulting in a wave at −0.47 volt vs. the saturated calomel electrode. Lingane also (5) reported a well defined doublet wave for the reduction of chlorostannite ions with a half-wave potential of −0.25 and −0.52 volt vs. the saturated calomel electrode in a supporting electrolyte of 1 N hydrochloric acid and 4 molar ammonium chloride at 25° C. However, no published work on the polarographic determination of tin in steel could be found.

The following research was accordingly carried out with seven Bureau of Standards standard steel samples, six with tin certificate values. It was found that the polarographic analysis of steel is entirely feasible, and saves much time over the earlier method. Results can be obtained on a batch of six or eight steel samples, using the polarographic method described, on the second (8-hour)

A polarographic method for the determination of tin in steel is described. Samples are treated either with sulfuric acid, followed by nitric acid and potassium permanganate, if they are tool steels, or with nitric acid followed by potassium permanganate if they are plain carbon steels. The tin is separated as stannic chloride by successive steps involving hydrogen sulfide, potassium pyrosulfate, hydrochloric acid, ammonia, and hydrochloric acid again. After reduction of any iron with hydroxylamine, the tin is determined by measuring the wave with a half-wave potential of —0.58 volt, using a saturated calomel electrode at 30° C. Percentages of tin are obtained by referring to a standard curve.

day. Scherrer's present method for tin in steel is at least twice as long.

APPARATUS

A Model XXI Sargent polarograph with a dropping mercury electrode adjusted for a drop time of approximately 4 seconds per drop.

H-type polarographic cells with a saturated calomel electrode (3).

Tank nitrogen, after passage over copper turnings at 450° C. to remove oxygen.

A Fisher unitized constant temperature bath, used at 30.0° ± 0.5° C.

A General Electric reflector drying 250-watt type infrared lamp for fuming the samples.

The dropping mercury electrode was held with a holder previously described (1).

Figure 1 shows the entire setup of the equipment used for this work.

PRELIMINARY RESEARCH

A calibration chart was first made using stannic chloride solutions from pure tin, which were also 1 N with respect to hydrochloric acid and 4 molar with respect to ammonium chloride. It was established while using these that hydroxylamine, added in the analysis to reduce iron, did not affect the diffusion current, and that no tin is lost when dilute stannic chloride solution is boiled with hydrochloric acid; this confirms Hillebrand and Lundell (2). A straight-line calibration curve resulted.

After tin was in solution, it could readily be separated and made ready for the polarograph in a series of steps involving hydrogen sulfide, potassium pyrosulfate, hydrochloric acid, ammonia, and hydrochloric acid again. The chief problem, apparently, was to have all the tin from the steel precipitated by hydrogen sulfide at the start of the series. Treatment of the original sample with sulfuric acid followed by nitric acid and potassium permanganate worked with all tool steels, but low results were obtained with one plain carbon steel. To determine tin in the latter sample an alternative procedure was used starting with nitric acid.

DETERMINATION OF TIN IN TOOL STEEL

Dissolve a 1.5-gram sample with 100 ml. of 1 to 4 sulfuric acid in a 800-ml. beaker with the aid of heat. A larger sample may be used

if the molybdenum and copper concentration will permit the sulfide precipitate to be easily handled. (If the steel contains less than 0.5% molybdenum, add a solution of molybdic oxide in 1 to 4 sulfuric acid to bring the molybdenum concentration to that of a 0.5% molybdenum steel. The molybdenum sulfide acts as a collector precipitate during the hydrogen sulfide precipitation.) After the sample has dissolved, add 15 ml. of 1 to 1 nitric acid; after the reaction has subsided add 10 ml. of 1.5% solution of potassium permanganate or add until manganese dioxide appears, boil for 10 minutes, and add 8% potassium nitrite dropwise until the precipitate disappears. Boil the solution until salts appear and then carefully fume with the aid of gentle heat and an infrared lamp.

Cool and dilute to approximately 100 ml. with distilled water and add 10 grams of tartaric acid. Make the resulting solution ammoniacal and warm until all the tungstic acid is in solution.

Dilute to 400 ml., add 24 ml. of 1 to 1 sulfuric acid, and heat to boiling. Any insoluble chromium salts will go into solution at this point. Pass hydrogen sulfide through the solution for 45 minutes. Let the precipitate digest in a warm place for another 45 minutes, then filter and wash it with a 1% sulfuric acid solution saturated with hydrogen sulfide.

Ignite the precipitate in a 35-ml. porcelain crucible and fuse with 3 grams of potassium pyrosulfate. Cool and leach the fused mass with 2 N hydrochloric acid, keeping the volume less than 100 ml.

Transfer to a 250-ml. beaker and add a slight excess of ammonium hydroxide. Boil for a few minutes to coagulate the precipitate and filter. If less than 30 mg. of ferric hydroxide is present,
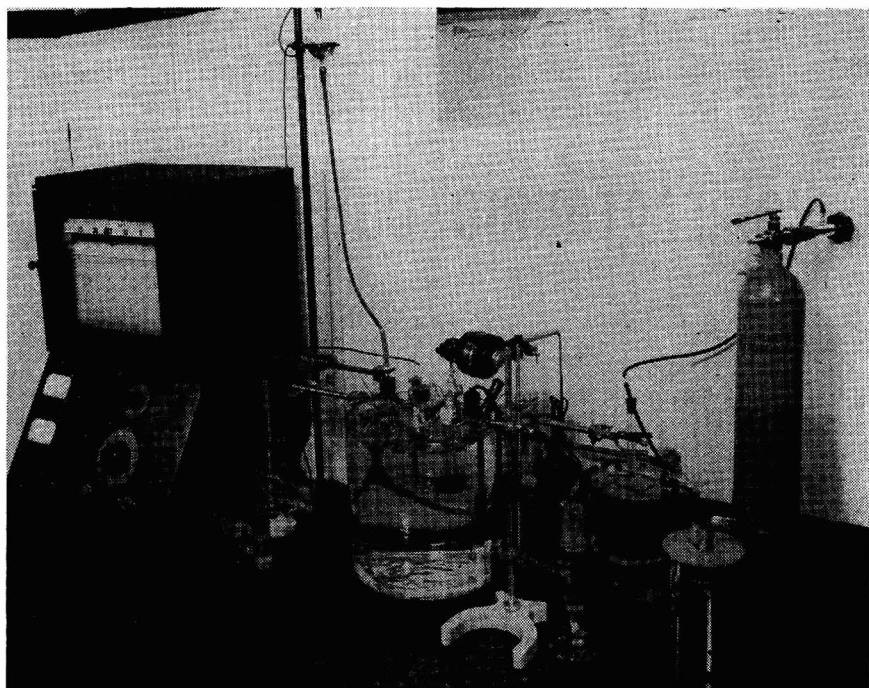

Figure 1. Setup of Equipment for Tin Determination

Table I. Determination of Tin in Steel

Sample[a]	Type of Steel	Sn Certificate Value %	Sn Detected %	Av. Deviation of Mean %
50a	Chrome-tungsten-vanadium	0.025	0.027[b]	0.0017
50b	Chrome-tungsten-vanadium	0.025	0.028[b]	0.0020
51a	Electric steel, 1.2% C	0.011	0.009[b]	0.0012
55a	Open-hearth iron	0.007	0.007[b]	0.0005
65c	Basic electric, 0.3% C	0.008	0.007[b]	0.0008
152	Basic open-hearth, 0.4% C	0.036	0.028[b]	0.0060
152	Basic open-hearth, 0.4% C	0.036	0.035[b,c]	0.0009
134	Molybdenum-tungsten-chrome-vanadium	0.013[d]	0.015[e]	0.0012

[a] National Bureau of Standards.
[b] Average of three determinations.
[c] Alternative procedure.
[d] No certificate value reported. Determined with method of Scherrer.
[e] Average of six determinations.

add ferric ion as a collector. Wash the precipitate well with 2% ammonium hydroxide and twice with water.

Dissolve the precipitate from the paper with 50 ml. of 2 N hydrochloric acid and catch the filtrate in the original 250-ml. beaker used in the ammonia precipitation. Wash the paper twice with water. Add 0.75 gram of hydroxylamine hydrochloride and boil gently for 5 minutes.

Transfer the warm solution to a 100-ml. volumetric flask that contains 21 grams of ammonium chloride. Add 2 ml. of a 0.5% gelatin solution and dilute to the mark with water.

Remove the dissolved oxygen from the solution with pure nitrogen and obtain a polarogram over the range 0 to -1.4 volts vs. the saturated calomel electrode.

Measure the height of the polarograph wave occurring at a half-wave potential of -0.58 volt vs. the saturated calomel electrode at 30° C., using the method of Taylor (7), and relate this to concentration of tin.

ALTERNATIVE PROCEDURE FOR DETERMINATION OF TIN IN PLAIN CARBON STEEL

In an effort to make the proposed procedure more versatile, and so include a greater number of types of steel, National Bureau of Standards standard sample No. 152 was dissolved in nitric acid as recommended by Scherrer (6) and the tin was precipitated from the resulting nitric acid solution with hydrogen sulfide. It was found necessary to add a solution of molybdic ion to the dissolved sample before precipitation to act as a collector during the sulfide precipitation and to ensure the complete recovery of tin.

Dissolve a 1.5-gram sample in a 600-ml. beaker with 60 ml. of 1 to 4 nitric acid with the aid of heat. If the steel contains less than 0.5% molybdenum, which is likely, add a solution of molybdic oxide in nitric acid to bring the molybdenum concentration to that of at least a 0.5% molybdenum steel. Warm the mixture to facilitate solution and then boil to remove oxides of nitrogen. Add 10 ml. of 1.5% potassium permanganate solution, boil the mixture for 10 minutes, and add 8% potassium nitrite solution dropwise until the permanganate color disappears. Continue boiling for a few more minutes to remove oxides of nitrogen again. Dilute the resulting solution to 300 ml., heat to boiling, and then pass hydrogen sulfide through the solution for 45 minutes. Filter and wash with 1% sulfuric acid solution saturated with hydrogen sulfide. Handle the resulting precipitate, consisting of the group II sulfides and a large quantity of sulfur, as in the procedure for tool steels.

The results are shown in Table I.

DISCUSSION OF RESULTS

Results obtained with Bureau of Standards steels fall within, or very close to, the results accepted by the bureau for certification of the steels, and never differ more than 0.001% from the extreme values used for calculating certification. The authors feel that the proposed polarographic method will yield satisfactory results for tin in steel.

ACKNOWLEDGMENT

The authors are deeply grateful to the Cleveland Twist Drill Company of Cleveland for the equipment and laboratory space used in this research, and to chief metallurgist J. V. Emmons and chief chemist W. L. Emerson of that company for their helpful interest.

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Improvement in Precision of Polarographic Analysis

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SINCE the photorecording system was introduced in polarography, numerous papers have interpreted photorecorded polarograms, but no definite conclusion has been reached as yet with respect to the degree of accuracy that can be expected in measuring these curves.

Buckley and Taylor (2) estimated that the value of i_d (diffusion current) is reproducible within the limits of 0.5 and 4%, whereas Mueller (11) believes that an accuracy of $\pm 1\%$ can be reached by using averages of several polarograms of the same solution. To minimize these errors Jablonski and Moritz (6) suggest the use of an accurate measuring microscope for determining the wave height, and Baumberger and Bardwell (1) recommend the drawing of abscissas on the photographic paper by the help of an additional lamp, in order to facilitate a direct reading of the applied voltage.

Probably as a consequence of these uncertainties, Kolthoff and Lingane (7), Zlotowski and Kolthoff (14), and Lingane and Meites (10) depart from the use of the more convenient polarograph and prefer manual measurements, in order to attain maximum precision.

It appears that sufficient emphasis has not been placed on errors arising from the photorecording of polarograms and their measurement. Continuous dimensional changes of the photographic paper itself under the influence of changes in the relative

humidity of the surrounding air (R.H.) have been reported (3-5) to amount to as much as 2.5% of its width and 0.5% of its length.

Investigations were therefore undertaken to determine the extent of dimensional changes occurring at different degrees of relative humidity for the particular type of photographic paper recommended for polarographic work, to study the influence of these variations upon the accuracy of measurements on polarograms, and to devise methods to minimize these errors.

EXPERIMENTAL

A Model XII Heyrovský polarograph and Kodak bromide paper F-1, 15.2 × 25.4 cm. were used for the investigations. Strips of the paper 3 cm. in width were cut parallel to the smaller side of the sheets. Each strip was placed in a glass cylinder 60 mm. in diameter and 130 mm. in height, and a slow stream of air with a known moisture content was passed over it. The air was analyzed for its moisture content by a Serdex Boston hygrometer, Model 201, with a reported precision of ±1%. The rate of flow was measured by a calibrated flowmeter. The flow was kept at a low value in order to approach conditions to which polarograms actually may be exposed. The dimensional changes were registered. The accompanying changes in moisture content were measured by weighing in a small (15-cc.) covered weighing bottle. All transfers were made as rapidly as possible.
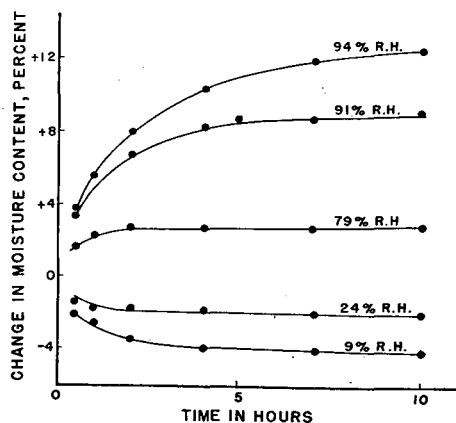
Figure 1.   Change in Moisture Content of Undeveloped Photographic Paper at Different Degrees of Relative Humidity

Paper seasoned at 52% R.H. prior to determination. Flow of air, 300 cc. per minute.   Temperature 28° ± 0.5° C.

The shrinkage and expansion of a recorded polarogram on a full size sheet of photographic paper were determined under similar conditions. No temperature control was used, but the temperature did not vary more than 1° C. during a series of experiments.

Dimensional changes as well as the change in moisture content of the paper were also determined over a number of water–sulfuric acid mixtures in desiccators.

In all cases the paper under investigation was conditioned with air of known relative humidity prior to the experiment. The length of the strips was measured always at the same place, as slight deviations in dimension were observed at different sections of the paper.

DIMENSIONAL CHANGES IN PAPER

Changes between Dry and Wet State under Extreme Conditions. A developed sheet of photographic paper was dried in a calcium chloride desiccator for a week. The width and length of the paper were measured; then the paper was thoroughly moistened by immersing in water at room temperature for 15 minutes and freed from excessive moisture by means of a blotter. The width and length were again measured. The expansion observed from the dry to the wet state was 4.3% in width and 1.6% in length.

The results show that under these conditions dimensional variations of a considerable magnitude occur in either direction.

It follows that under similar conditions the accuracy of measurements on polarograms will be seriously affected with regard to both wave height and half-wave potential.

As the change in width was found to be nearly three times the change in length, the effect of variations in relative humidity upon the width and the accuracy of wave height measurements was selected for a detailed study.

INFLUENCE OF HUMIDITY OF AIR

Influence upon Moisture Content and Width of Undeveloped Photographic Paper. The rate of adsorption and desorption as well as expansion and shrinkage in the width of undeveloped photographic paper was determined under a slow stream of air with known moisture content. Figures 1 and 2 show the results obtained.

The values of width in Figure 2 reached after 10 hours are plotted in Figure 3 compared with those obtained from determinations on developed paper, using the same experimental procedure.

Figure 4 shows the rate of adsorption and expansion in width of undeveloped photographic paper in a quiet atmosphere (desiccator) of 84% relative humidity.

The changes in width on samples of undeveloped paper were then measured at different degrees of relative humidity in the same way, at the end of 51 hours. A maximum deviation of 2.8% was found between the width of the paper at 14 and at 92.5% relative humidity; the points in between formed an S-shaped curve similar to the ones given in Figure 3.

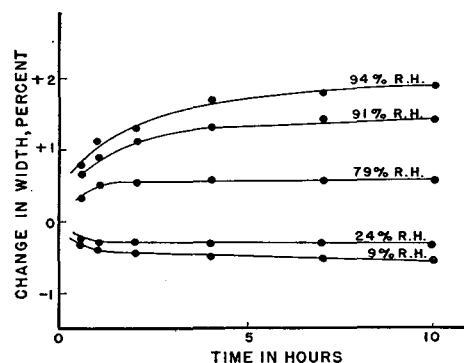Results of the following qualitative test clearly show that these

Figure 2.   Change in Width of Undeveloped Photographic Paper at Different Degrees of Relative Humidity

Paper seasoned at 52% R.H. prior to determination. Flow of air, 300 cc. per minute.   Temperature 28° ± 0.5° C.
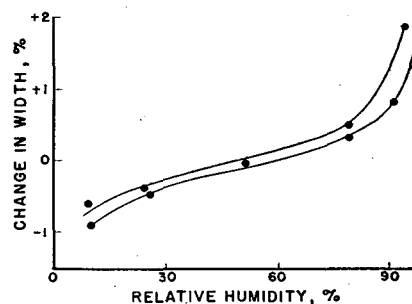

Figure 3.   Change in Width of 3-Cm. Strips of Photographic Paper with Changing Relative Humidity

Flow of air, 300 cc. per minute.   Seasoned at 52% R.H. in desiccator. *Upper.*   Undeveloped paper,   temperature during experiment 28° ± 0.5° C. *Lower.*   Developed paper, temperature 25° ± 0.5° C.

The effect of relative humidity of air upon the dimensional changes of photore-
corded polarograms has been investigated. A method is described for mini-
mizing this error. The procedure consists of photographing two parallel lines,
which serve as a comparative scale, on the photographic paper at the time the
polarogram is taken. This method increases the precision of wave height
measurements about 25-fold.
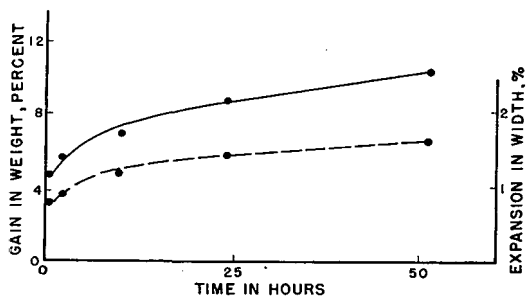
**Figure 4. Rate of Adsorption and Expansion in
Width of Undeveloped Photographic Paper**

Quiet .atmosphere at 84% R.H. and 26° ± 1° C. (desic-
cator)
Pretreatment of paper, 4 days over calcium chloride in
desiccator
Solid line, gain in weight
Broken line, expansion

variations in width of undeveloped paper actually influence the
accuracy of polarograms when taken at different degrees of rela-
tive humidity.

The camera was loaded and inserted into the polarograph.
Without removing the camera, a stream of air (95% relative
humidity) was passed through it for 17 hours by means of a rubber
tubing leading into the interior of the inner cylinder through the
small space left free at the end of the paper-retaining bar. The
rubber tubing was then detached. The camera was made ready
for exposure as usual. Lines were drawn photographically at set-
tings of 5, 75, and 145 mm., respectively, of the galvanometer
light beam on the visual scale. The lines were run from settings 3
to 4.5 on the camera drum. Then the shutter was closed. The
procedure described above was repeated, using air of 15% rela-
tive humidity, and passing this air through the camera for 5
hours. Lines were drawn from settings 4.5 to 6 on the camera
drum, again at settings of 5, 75, and 145 mm., respectively, of the
light beam on the visual scale. The temperature during the ex-
periment was 25° ± 0.5° C. Figure 5 shows the photograph ob-
tained.

**Influence upon Moisture Content and Width of Developed
Photographic Paper.** Under the same experimental conditions
as in the determinations with undeveloped paper, the type of ad-
sorption and desorption as well as the type of expansion and
shrinkage of the developed paper was found to be identical with
that observed with the undeveloped paper under a slow stream of
air. Figure 3 shows the changes in width of the developed paper
after a 10-hour exposure to air of different degrees of relative
humidity compared to the behavior of undeveloped paper.

Aside from variations in moisture content and width at differ-
ent degrees of relative humidity, the width of developed paper
was found to be smaller by 0.33% than at the same degree of
relative humidity in the undeveloped state. The shrinkage was
measured on five samples after seasoning at 52% relative humid-
ity.

The change in width of developed paper in a quiet atmosphere
was determined, using similar experimental conditions as with
undeveloped paper. The maximum deviation in width between
paper treated at 21 and 90% relative humidity, respectively, was
found to be 1.7% after 51 hours of exposure.

To observe the behavior of developed paper under conditions
to which a photorecorded polarogram might be exposed in normal

practice, five samples of developed photographic paper were
placed at a spot in the laboratory. The width was measured after
24 hours and, from then on, once a day for a week. In samples
seasoned under a flow of air at 9.5, 26, 79, 91, and 97% relative
humidity, respectively, for 10 hours prior to the test the devia-
tion in width was found to be less than ±0.1% among the five
samples at any date of measurement.

Figure 6 shows the range in which the relative humidity of the
laboratory air changed during the one week's test, and the ac-
companying changes in width of the paper.

**Influence upon Calculation of Diffusion Current from a Polaro-
gram. MINIMALIZATION OF ERROR.** A polarogram of copper in
ammoniacal medium was prepared. Figure 7 shows the copper
wave, FC, and the residual current, GD. At the same time this
polarogram was taken, two ad-
ditional ordinates were drawn
on the photographic paper at
constant settings of the light
beam on the visual scale of the
instrument, with the cell circuit
open. The lines were at a dis-
tance of 140 mm. on this scale.

The polarograph had been
calibrated previously according
to the procedure given by Kolt-
hoff and Lingane (8), using two
precision resistances. The dif-
fusion current was calculated
from this polarogram according
to the "graphical method" as
well as the "exact method,"
suggested by Taylor (13) for
absolute work in polarography
(12). The exact method cor-
responds to the one suggested
earlier by Kolthoff and Lingane
(8).

Table I shows the values ob-
tained according to the conven-
tional methods, in Table II the
values for the diffusion current
are corrected. In this case the
distance between the two ordi-
nates was measured first and
compared with the theoretical

**Figure 5. Displace-
ment of Photographed
Ordinates by Change in
Relative Humidity**

Lines obtained in either case
at settings of 5, 75, and 145
mm. of light beam on visual
scale. Lines on left after
passing air of 95% R.H.
through camera for 17
hours. Lines on right after
passing air of 15% R.H.
through camera for 5 hours

**Table I. Dependence of Calculated Diffusion Current
upon Degree of Relative Humidity at Which Polarogram
Was Seasoned** [a]

Seasoning, % R.H.	Graphical Method Copper Wave Height, FC		Residual Current Wave Height, GD		Exact Method FC − GD
	Mm. obsd.	μamp.	Mm. obsd.	μamp.	μamp.
97	91.1	45.55	5.4	0.14	45.41
86	90.2	45.10	5.3	0.13	44.97
59	89.4	44.70	5.3	0.13	44.57
40	89.2	44.60	5.3	0.13	44.47
12	88.7	44.35	5.2	0.13	44.22

[a] See Figure 7. Shunt copper wave = 100, shunt residual current = 5,
$s° = 0.0050$ μ amp./mm. on visual scale.

Table II. Elimination of Deviations in Size of Diffusion Current Caused by Changing Relative Humidity[a]
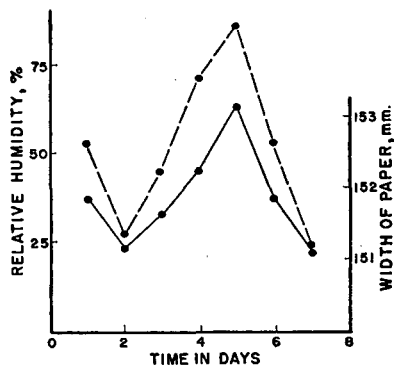
Seasoning, % R.H.	Distance between Ordinates, Mm.	$F = \dfrac{140 \text{ Mm.}}{\text{Distance between Ordinates, Mm.}}$	Diffusion current (FC to GD, μ amp.) $\times F$
97	138.5	1.0108	45.90
86	137.2	1.0204	45.89
59	136.3	1.0271	45.78
40	135.8	1.0309	45.84
12	134.9	1.0378	45.89

Av. 45.86 ± 0.06 μ amp.

[a] See curve Figure 7. Shunt copper wave = 100, shunt residual current = 5. Two ordinates were drawn at a distance corresponding to 140 mm. on visual scale. $s° = 0.0050 \mu$ amp./mm. on visual scale.

distance of 140 mm. A factor, F, was thus obtained, which served to correct the diffusion currents calculated from the polarogram according to the exact method.

Inaccuracies of Photorecorded Polarograms Caused by Factors Other Than Humidity of Air. Lingane (9) reports a deviation of about 3.5% for the galvanometer deflection on the visual scale from the measurement on the photographic paper. An inaccuracy of the same order was observed in the present study, although the precise amount of this deviation was not determined.

Although the source of this error if overlooked would affect the accuracy of wave height measurements considerably, a similar inaccuracy in the determination of the half-wave potential may arise from improper loading of the camera. It was noticed during the present investigation that lines drawn parallel to the width of the paper by moving the light beam over the visual scale at constant settings of the camera drum, were not recorded as parallels. Several sheets of paper were treated this way and examined. The perpendicular distance between any two lines was as much as 0.7% greater at the points corresponding to the zero value of the visual scale than at the opposite end. A change of only 0.25 mm. in distance of the surface of the photographic paper from the center of the camera would result in a deviation of this magnitude. Therefore, it is more than likely that the deviation originates in the construction of the paper-retaining bar, which probably tightens the photographic paper to the inner cylinder of the camera to a better degree at its hinged end than at the part containing the spring clip.

DISCUSSION

The data in Table I reflect the deviation in diffusion current calculated from one and the same polarogram, treated at different degrees of relative humidity. The error between 12 and 97%, respectively, is roughly 2.5%.

Table II shows that the influence of the relative humidity has been minimized to a great extent. In this case the slight deviations are mainly due to subjective errors in measurement. The deviation is only ±0.13%. Table II shows, furthermore, that not only the influence of the changing relative humidity but also the inaccuracies caused by instrumental shortcomings have been

Figure 6. Daily Variation in Relative Humidity of Air in Laboratory

Concurrent variations in width of openly exposed developed photographic paper. Measurements of width (solid line) are average values from 5 samples each. Samples pretreated at 9.5, 26, 79, 91, and 97% R.H. for 10 hours
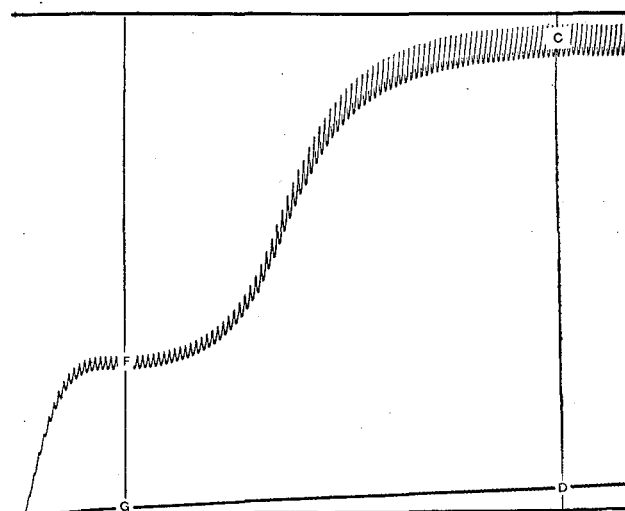
Figure 7. Polarogram of Copper in Ammoniacal Solution

Used for determining influence of R.H. on wave height and accuracy that can be obtained by drawing two parallel lines representing known current (Tables I and II)

reduced to a minimum. These latter inaccuracies would increase the error to 3.5% when wave heights were measured according to conventional methods and no correction was made. The combined error for influence of relative humidity and instrumental inaccuracy can be obtained by comparing the average value of the last column in Table II with those in columns 3 and 6 of Table I.

SUMMARY

The influence of the relative humidity of air ranging from 12 to 100% upon dimensions and moisture content of photographic paper used in polarographic work has been studied. The paper investigated responds quickly to sudden changes in relative humidity and approaches equilibrium values in width in about 4 hours under conditions similar to those to which polarograms are exposed in research and routine work. The dimensional changes of the paper are correlated with changes in moisture content, though not in a linear fashion.

The precision of wave height measurements is ±0.7% in the range between 30 and 75% relative humidity. Errors of greater magnitude up to 2.5% occur at humidities beyond these limits. An instrumental inaccuracy has been observed with the particular commercial polarograph used for this investigation increasing the over-all error to ±3.5% under unfavorable conditions. The inaccuracies observed in measuring the wave heights under defined experimental conditions have been verified in a series of tests corresponding to conditions which are encountered in the normal practice of polarographic work.

A simple method has been developed for minimizing the combined errors in measuring wave heights caused by varying relative humidity and instrumental inaccuracy. It consists of drawing two additional ordinates on the photographic paper at a known distance as a scale for calibration. The possible over-all error has been reduced thus from ±3.5 to ±0.13%.

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DIFFERENTIAL THERMAL ANALYSIS

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Equations are derived which make possible the calculation of heats of transformation from differential heating curves, independent of external calibrations. The rate of restoration of a thermal steady state after a transformation is employed to establish the relation between the differential temperature and the heat absorption producing it. Valid results are obtained for such widely divergent processes as the melting of stearic acid and the vaporization of water, using exactly the same rapid and convenient experimental procedure. A fully automatic self-recording differential calorimeter is described, and the factors influencing the design finally adopted are discussed.

DIFFERENTIAL thermal analysis is essentially a refinement of the classical procedure of studying phase transformations by means of time-temperature records during uniform heating or cooling of the system. It appears to have been first employed by Le Chatelier (6) in 1887 but has become popular again only recently. Examples of its employment are given by Kracek (5) for sodium sulfate, Partridge, Hicks, and Smith (8) for sodium polyphosphates, Hendricks, Nelson, and Alexander (4), Grim and Rowland (3), Speil *et al.* (10) and Norton (7) for clays, and Vold (11–13) for soaps.

The experimental procedure consists of heating or cooling the sample side by side with an inert reference material in the same furnace, and measuring both the sample temperature and the temperature difference between sample and reference material as a function of time. When a phase change occurs involving absorption or evolution of heat, the temperature difference between reference and sample begins to increase; after the transformation is complete the temperature difference declines again. Thus each transformation produces a peak in the curve of temperature difference against time, from which it should be possible to derive information about the transformation temperature, heat of transformation, and rate of transformation.

This paper describes the construction and operation of a fully automatic differential calorimeter and presents an analysis of the course of the curve of differential temperature against time from which heats of transformation may be calculated.

DIFFERENTIAL HEATING CURVES

In order to analyze the differential heating curve, it is convenient to write down a formal expression for the rate at which heat is transferred into and out of the sample or reference cell.

$$\frac{dq_s}{dt} = K_s(T_w - T_s) + \sigma(T_r - T_s) + \alpha_s(T_o - T_s) \tag{1a}$$

$$\frac{dq_r}{dt} = K_r(T_w - T_r) + \sigma(T_s - T_r) + \alpha_r(T_o - T_r) \tag{1b}$$

Here dq/dt is the rate at which heat is received by the reference material (subscript r) and sample material (subscript s), respectively. K_r and K_s are heat transfer coefficients between the materials and the furnace wall. They are made as nearly identical as possible by choice of reference material and design of cell and furnace. Sigma (σ) is the heat transfer coefficient between the cells, and alpha (α_r and α_s) is the heat loss (chiefly along the thermocouple wires) to the outside environment. T_w, T_r, T_s, and T_o are the temperatures of the furnace wall, reference and sample materials, and external environment, respectively.

For experimental arrangements in which the furnace is a metal block with the materials contained in wells drilled in the block, a linear dependence of the rate of heat transfer on the temperature difference as given in the equations is certainly justified. When the materials are contained in metal capsules or cells suspended in air, and a portion of the transfer is by convection currents in the air, the validity of this assumption is open to question. White (15) has shown that heat transfer by convection, when the air flow in cylindrical space (up the walls and down the center of the cylinder) is not turbulent, is proportional to the square of the temperature difference between the walls and the center of the cylinder, but when the diameter of the cylinder is not small compared to its length he shows experimentally that the rate of heat transfer increases less rapidly with temperature difference, and approaches a first-power law.

Next use can be made of the identity

$$\frac{dq}{dt} = \frac{dH}{dt} = \frac{dH}{dT}\frac{dT}{dt} \tag{2}$$

In the case of the reference cell, dH/dt is simple C_r, the heat capacity of the cell plus that of its contents. For the sample it is convenient to segregate the portion of the increased heat content arising from phase change, writing

$$\frac{dq_s}{dt} = C_s\frac{dT_s}{dt} + \Delta H\frac{df}{dt} \tag{3}$$

Here C_s is the heat capacity of the cell plus its contents, while ΔH is the heat of the transformation and df/dt is its time rate of occurrence under the conditions of the experiment, f being the fraction of the sample transformed at any time t.

Every effort is made to have the two materials located symmetrically within the furnace, so that one can write

$$K_s = K_r - \delta K \tag{4a}$$

$$\alpha_s = \alpha_r - \delta\alpha \tag{4b}$$

with the assurance that δK and $\delta \alpha$ are small. The various equations can then be combined to yield an expression for the rate of change of the differential temperature $(T_r - T_s)$ with time which is

$$\frac{d(T_r - T_s)}{dt} = -\frac{K_r + \alpha_r + 2\sigma}{C_s}(T_w - T_s) +$$
$$\left(1 - \frac{C_r}{C_s}\right)\frac{dT_r}{dt} + \frac{\Delta H}{C_s}\frac{df}{dt} - \frac{1}{C_s}[\delta K(T_w - T_s) -$$
$$\delta\alpha(T_s - T_o)] \quad (5)$$

At times when $df/dt = 0$—i.e., when the sample is not undergoing a transformation—Equation 5 can be integrated directly, subject to the assumptions that dT_r/dt, $(T_w - T_s)$, $(T_s - T_o)$, and C_r and C_s are independent of time and temperature. In the apparatus here described dT_r/dt is controlled at a constant value. $(T_w - T_s)$ is at least slowly varying. $(T_s - T_o)$ appears with the coefficient $\delta\alpha$ (small), so that its rise with increasing time can be safely neglected.

It is convenient at this point to introduce a more compact nomenclature—i.e., $y = T_r - T_s$; $A = (K_r + \alpha_r + 2\sigma)/C_s$; y_1 a given value of y at $t = t_1$, serving as a boundary condition, and

$$y_s = \left[(C_s - C_r)\frac{dT_r}{dt} - \delta K(T_w - T_s) + \delta\alpha(T_s - T_o)\right]\Big/$$
$$(K_r + \alpha_r + 2\sigma) \quad (6)$$

With these changes the integrated form of Equation 5 for $df/dt = 0$ is

$$y = y_s(1 - e^{-A(t-t_1)}) + y_1 e^{-A(t-t_1)} \quad (7)$$

It is apparent that y_s is a steady state value of the differential temperature achieved at a sufficiently long time after the initial condition $y = y_1$ at $t = t_1$. At the outset of an experiment $y_1 = 0$ at $t = t_1 = 0$. The differential temperature rises to a value y_s, dependent primarily on the difference in heat capacity of the sample and reference materials, the heating rate, and the heat transfer coefficients. After a transformation is complete, the differential temperature again approaches y_s according to the same equation. The constant, A, can thus be evaluated from a plot of $\log (y - y_s)$ versus t. In the new nomenclature, Equation 5 may be written

$$\frac{\Delta H}{C_s}\frac{df}{dt} = \frac{dy}{dt} + A(y - y_s) \quad (8)$$

Graphical or numerical integration of Equation 8 over the period of time during which the transformation is occurring then yields a value for the heat of the transformation.

Any time interval wide enough to include the whole transformation may be taken. When $df/dt = 0$ before the transformation has begun $dy/dt = 0$ and $y = y_s$. When $df/dt = 0$ after the transformation, dy/dt is equal in value and opposite in sign to $A(y - y_s)$. The integral of the second term on the right-hand side of Equation 8 is simply the area under the peak, while that of the first term is $(y_2 - y_1)$ where y_1 and y_2 are the values of y at the beginning and end of the time period chosen.

In practice, in analyzing a differential heating curve, it is convenient to plot $(y - y_s)$ against time, beginning at the top of a peak. The points lie on a curve which becomes linear at the end of the transformation and thus yields a value of the time at which the transformation is over. The temperature of the sample at
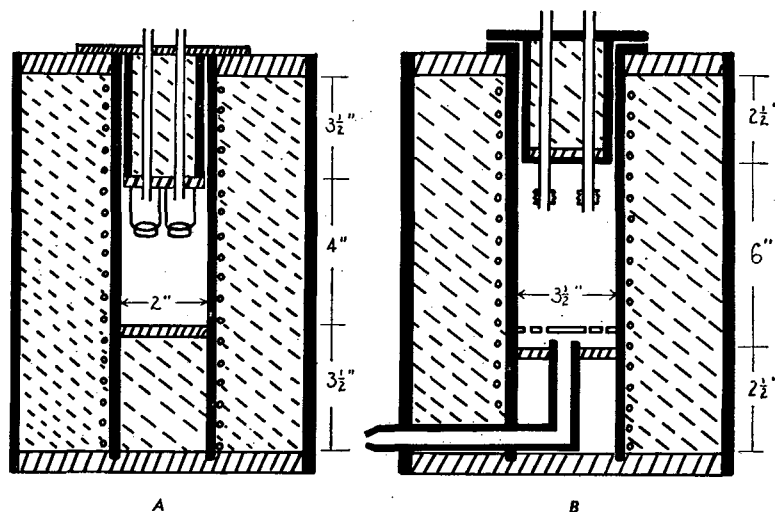

Figure 1. Differential Calorimeter Furnaces

Metal (mild steel for A, brass for B)
Transite
Asbestos magnesia packing
C, C'. Glass tubes for entry of thermocouple leads
D. Glass cell support for sample and reference cells
O. Heating element

this time is of phase significance as the "liquidus" point in a binary system if it can be shown by experiment to be independent of the heating rate.

DIFFERENTIAL COOLING CURVES

If the rate of cooling is controlled, the analysis of differential cooling curves is identical to that given for differential heating curves. If the calorimeter is allowed to cool at its natural rate which is measured experimentally, an entirely similar, though numerically much more complex, analysis can be carried through. The complexity arises from the fact that y_s is now time-dependent. In many cases the rate of transformation on cooling is very small, so that the differential cooling curve exhibits a wide shallow dip whose area beneath the varying base line, y_s, is hard to determine with any accuracy. For these reasons, differential heating curves are much more convenient in the study of phase transformations in most cases.

RATE OF TRANSFORMATION

Equation 8 can be used to calculate df/dt at any time (and hence any T_s) after ΔH has been obtained. At low heating rates, the values probably have little significance, as they are determined primarily by the rate at which heat is made available to the sample. From differential cooling curves, or at high heating rates, values of df/dt may be an interesting property of the system under study.

INHERENT LIMITATIONS OF DIFFERENTIAL THERMAL ANALYSIS

Although sensitive and convenient, this method contains two factors militating against its development into a technique of high precision. One is the assumption of a constant value of the heat capacity of the sample. The second is the assumption that the sample temperature is uniform throughout at each time instant.

The heat capacity of the sample is that of the cell plus that of the transformed portion of the sample plus that of the untransformed portion. The relative proportions of transformed and untransformed sample change during the heating. In practice, if the heat capacity of the cell is deliberately made large, this fluctuation is minor, but sensitivity is sacrificed, because a given

heat effect then produces a smaller differential temperature. A solution of Equation 8, taking the variation of C_s into account, has been worked out by successive approximations, but it is too cumbersome a procedure for routine use.

Precise analysis of the effect of the existing temperature gradient in the sample on the calculated heat effect has not been accomplished. Judging from the results obtained, it is not serious enough to vitiate the method. Its chief effect is in the determination of the transformation temperatures rather than on the calculation of heat effects. The differential temperature begins to rise when the outside of the cylindrical sample reaches its transformation point. The center of the cylinder has been shown to be as much as 3 to 4° cooler at a heating rate of 1.5° per minute and dependent on the heating rate and the thermal conductivity of the sample. Reduction of the heating rate (with accompanying loss of sensitivity), measurement of the sample temperature at its outside surface nearest the furnace wall, and various extrapolation procedures all reduce the error involved but can never render it entirely negligible.

Figure 2. Differential Calorimeter Cells

Made of 18-8 stainless steel

Figure 3. Control Apparatus for Differential Calorimeter

APPARATUS

The essential parts of a differential calorimeter are the furnace, devices for containing the sample and reference materials, the accessories for controlling and measuring the heating rate, and the accessories for measuring the temperature difference between sample and reference material.

Considerations underlying the design of a furnace are adequate insulation to prevent undue dissipation of the heat input to the room and erratic temperature gradients within the active space, adequately sized heating elements arranged to minimize the gradients within the active space, and a heat capacity low enough for rapid response in the furnace temperature to changes in heat input, but high enough so that fluctuations in the heat input do not cause rapid fluctuations of the rate of temperature rise about its mean value. In addition, the heat transfer coefficient (K + 2σ) has to be considered. The smaller this quantity, the larger will be the peak obtained for a given transformation, but the temperature difference will decline more slowly to its steady value, so that successive transformations will be more difficult to resolve.

On the other hand the rate of the transformations probably depends to a certain extent on the rate at which heat can be supplied, so large values of $K(T_w - T_s)$ are desirable. If a metal block is used, the high value of (K + 2σ) must be compensated by using relatively larger samples and a high heating rate to achieve the same sensitivity. The most desirable type of furnace is believed therefore to consist of an air oven, suitably insulated. The designs given in Figure 1 have proved very satisfactory.

Furnace A consists of two concentric steel tubes 12 inches tall of 0.125-inch wall thickness, the outer 8 inches in diameter, the inner 2 inches. The inner is wrapped with a single layer of 0.125-inch asbestos cloth upon which are wound 20 feet of chromel A resistance wire (No. 22). The coil is more closely wound (4.75 turns per inch) around the upper and lower portions than around the central space (3.125 turns per inch). The space between the two tubes is packed with asbestos magnesia insulation, as is the lower third of the inner tube. The base of the furnace is 0.5-inch Transite board and the top is $^3/_{16}$ inch Transite board. The central space is closed by a sliding plug of 0.125-inch steel tubing closed at top and bottom by 0.125-inch Transite and filled with asbestos magnesia insulation. Pyrex tubes 6 mm. in outside diameter carry the thermocouple leads through this plug to the measuring instruments, while the glass supports for the sample containers are suspended from its bottom. The lead wires are covered with glass fiber sleeving and completely fill the inlet tubes, so that air currents through these tubes are minimized.

The most desirable shape for the sample is that of a thin cylindrical shell whose inside diameter is just large enough to accommodate the necessary thermocouples. In the present application it was desired also to keep air out and vapor in; consequently a closed cell is necessary. Furthermore, the cell should have as small a heat capacity as possible. Figure 2 shows two designs, both of which have proved satisfactory. Cell A proved very difficult to machine, and the small diameter of the thermocouple well made it difficult to insert and remove the thermocouples without mechanical damage to the junctions. Furthermore, the glass supports are awkward to handle and crack frequently when samples are quenched by immersing the cells in freezing mixtures. Cell B, though larger and heavier, has an improved design of support. The first cells were constructed
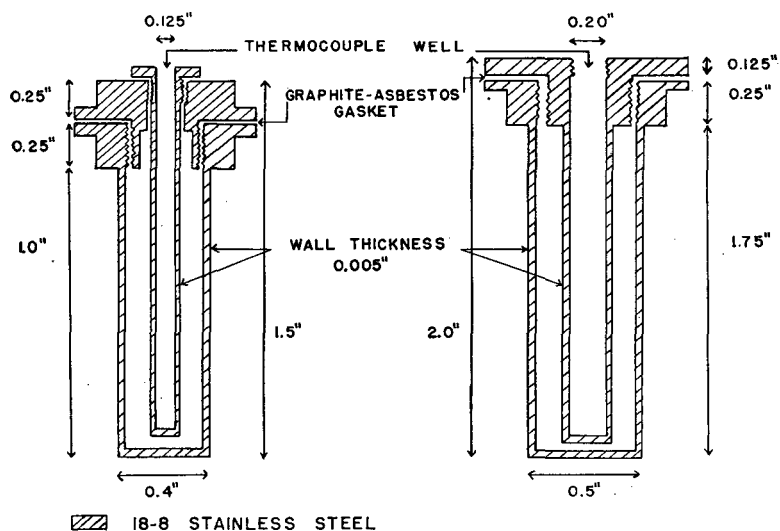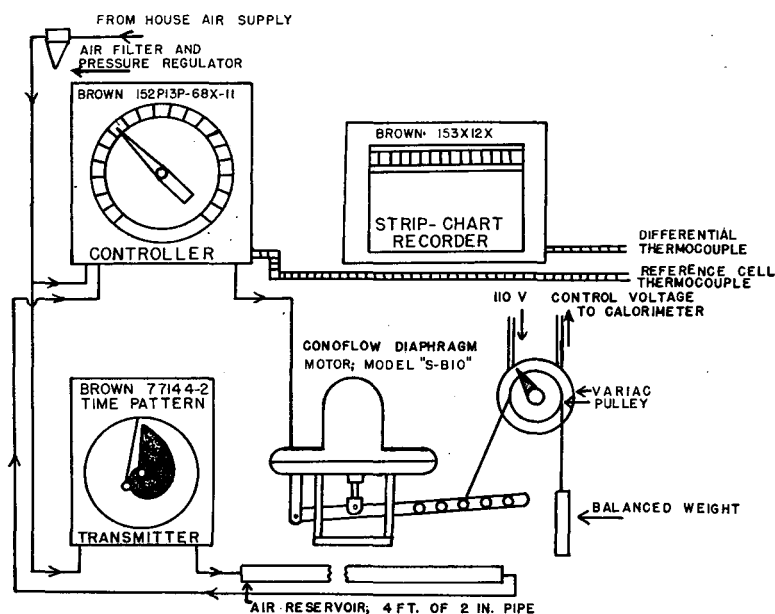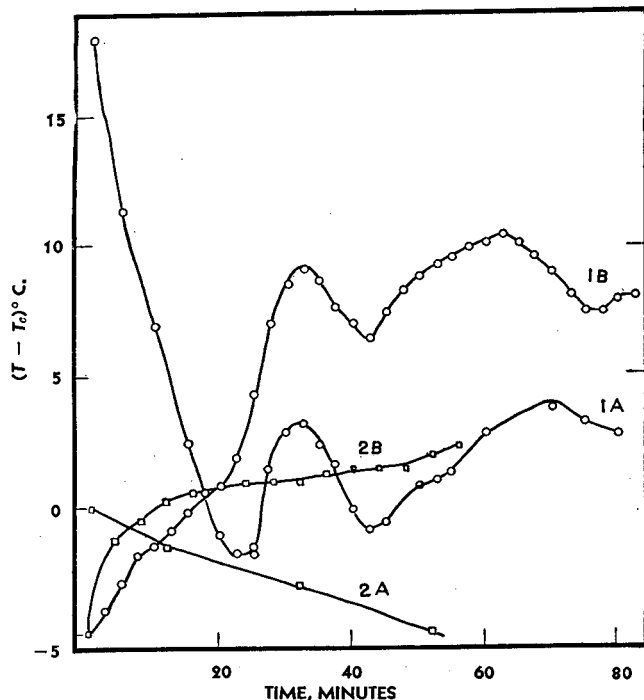
Figure 4. Effect of Fluctuating Heating Rate on Differential Heating Curves

1A. Fluctuating difference between actual and desired temperature during heating $(T - T_c)$
1B. Corresponding curve of temperature difference between reference and sample cells $(T_r - T_s)$ versus time. In both cases average heating rate is 1.5°/min.

Figure 5. Wiring Diagram of Strip Chart Potentiometer

R_A (3), 253.4 ohms; R_C (4), 509.5 ohms; R_H, 5.00 ohms; slide wire unit, 20 ohms. R_0 (1) was originally 2.474 ohms but was made variable by placing a 20- to 150-ohm resistance in parallel with it. R_t (2) was originally 1.474 ohms but was made variable at from 0.3 to 1.5 ohms, the lower figure giving maximum sensitivity.

of aluminum alloy 24ST, but later stainless steel was employed because the threads machined in the aluminum alloy wore out very rapidly.

The control and measuring assembly is shown in Figure 3.

The heating rate is measured and controlled by means of the Brown Instrument Co. pneumatic time-temperature pattern controller. The desired time-temperature pattern is plotted on an aluminum disk in polar coordinates and the disk is then cut evenly along the curve to give a control cam. This is mounted on the shaft of an electrically driven clock motor. A wheel rolling along the curved surface of the cam controls the position of a pointer on the scale of the circular chart temperature recorder. The furnace temperature is measured by means of a single-junction iron-constantan thermocouple. When this temperature differs from the control temperature as indicated by the pointer, an e.m.f. proportional to the difference is applied to open a valve by which air pressure is transmitted to a Conoflow air-operated motor, which drives the control spindle of an autotransformer (Variac model VMT5, 0 to 130 volts) governing the current passing through the heating elements of the furnace. The drive is adjusted so that 30 volts are applied to the heating element when the applied air pressure is zero, and 130 volts when the applied air pressure reaches its maximum (14 pounds). The range of this model is from 0° to 400° C.

A control knob enables the experimenter to apply a given fraction of the total available air pressure for a given percentage deviation between recording pen and control pointer, based on the full scale. This is known as "per cent throttling." Thus 20% throttling applies full air pressure for an 80° deviation, 10% for 8°, etc. Owing to lag in the furnace, low throttling gives considerable fluctuation of the temperature about its controlled value. With high throttling, the applied current is generally not enough to maintain the desired rate of heating, so that a further control is necessary. This is designated "automatic reset," and provides a continuous, independent increase in the applied air pressure, again proportional to the deviation between actual and control temperature but applied at a controllable rate to overcome the fluctuations that would result from a sudden increase in heating current. The operation of these instruments has been described in greater detail (14).

Selection of the proper control settings for the given furnace is essential. Figure 4 shows two sets of curves for the deviation of
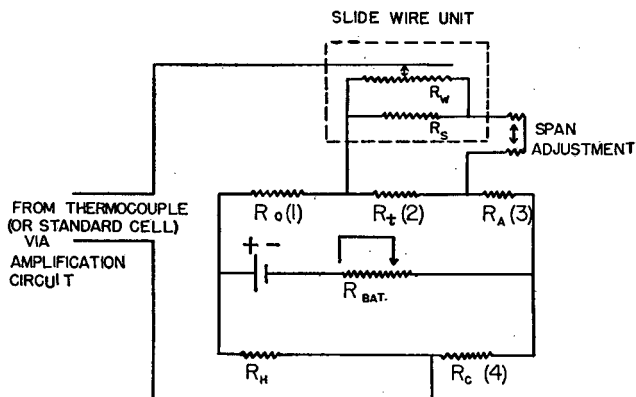
actual and control temperatures, and for fluctuation of the differential temperature between sample and reference cells (in the absence of phase changes) for different values of throttling range and reset control. In set 1 the heating rate, dT_r/dt, oscillates, and the differential temperature, y, oscillates also (as it should according to Equation 7). The corresponding peaks in the curve of differential temperature against time simulate peaks due to phase changes. The better choice of control variable gives rise to the curves in set 2, where the $y - t$ curve is almost a straight line, after time enough has elapsed for the temperature difference to reach its steady state value.

The temperature difference between sample and reference cells is measured by means of a three-junction iron-constantan thermopile, and recorded continuously on the Brown strip-chart potentiometer. The thermocouple junctions are formed of No. 30 wire, spot welded, and insulated from each other by fiber glass sleeving further impregnated with Dow Corning silicone varnish 996. They have been found durable over periods of 2 to 3 months' almost continuous use, and fail ultimately because of breakdown in the insulation.

The potentiometer as supplied has a pen travel of 12 inches (50 scale divisions) per 5 millivolts. As the rated sensitivity is such that full voltage is applied to the balancing motor for an e.m.f. across the thermocouples of 20 microvolts, it is practicable to increase the sensitivity about fivefold by altering the resistance ratio in the arms of the bridge. The wiring diagram for the instrument is given in Figure 5, showing this change and the addition of a further auxiliary variable resistance to vary the position of zero e.m.f. on the scale. With this arrangement both positive and negative values of the temperature difference can be recorded; high values of the sensitivity are used for transformations having small heat effect and lower values for transformations in the same sample having larger heat effects.

Experimental Results. To test both the instrument and the equations described above, five runs were made on a specially pure sample of stearic acid (9) and one on Baker's c.p. analyzed benzoic acid.

The powdered sample was tamped firmly into its calorimeter cell and hung in position from the plug of the colorimeter furnace. The reference cell was then filled with sufficient white mineral oil (Nujol) to give nearly the same calculated heat capacity, and assembly of the calorimeter was completed. After about 20 minutes the initial temperature difference between the cells (due to handling) had disappeared, and the automatic heating and recording had begun. For these relatively large heats of fusion the instrument was operated at a sensitivity of 0.131° per scale
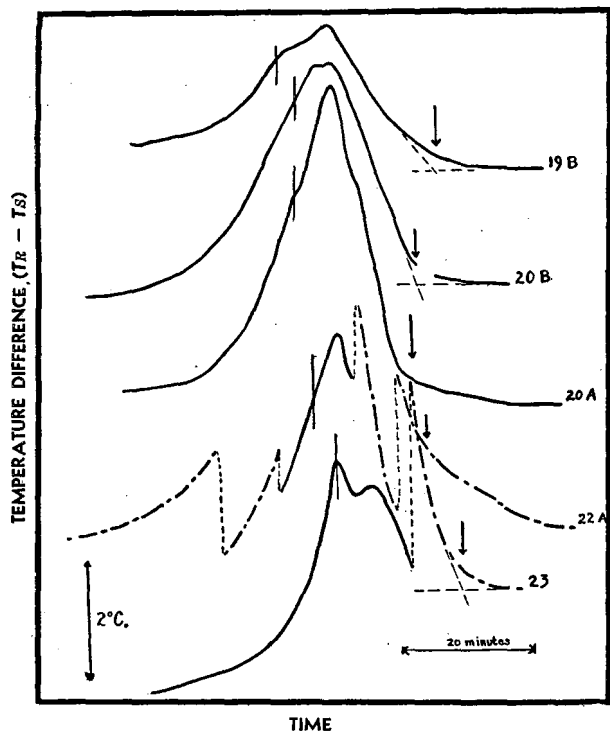
TIME

Figure 6. Differential Heating Curves of Stearic and Benzoic Acids

Pantograph reductions of automatic records. Arrows show time at which melting begins. Vertical full lines show time at which calculation indicates that transformation is complete. Vertical dotted lines correspond to breaks in automatic record produced by deliberate test changes in sensitivity of recorder.

division for the measurement of temperature difference as compared with its maximum of 0.089° per scale division. However, in two cases the highest sensitivity was employed at the beginning of the run, and decreased sensitivity later as required to keep the recording pen on the scale.

Pantograph reductions of some of the automatically recorded curves of temperature difference vs. time are given in Figure 6.

Temperatures of Transition. The differential temperature, y, begins to rise as soon as the outside of the sample reaches a transition point, (T_m). The inside of the sample at the same instant, T_s, however, is below T_m by an amount dependent on the rate of heating and the thermal conductivity of the sample. The difference has been measured for the reference cell filled with Nujol and found to be of the order of 5° for a heating rate of 1.5° per minute. The thermal conductivity of the powdered sample, however, is not necessarily even approximately equal to that of Nujol nor reproducible from run to run, so no reliable estimate of the difference $(T_s - T_m)$ can be formed to serve as a systematic correction. By the time that the differential temperature is rising rapidly the sample temperature must be somewhat above T_m in order to keep up a steady flow of heat to the transforming portion. The best estimate of T_m is therefore obtained by extrapolating the steeply rising portion of the curve backward to its intersection with the initial base line. As there is a degree of arbitrary choice involved in such an extrapolation, the values obtained are uncertain to about 3° to 4°, with a tendency to be low rather than high. The arrows on Figure 6 show the times at which the sample temperature was taken to be equal to T_m. The values obtained average 4° lower than the known melting points of the samples, as seen in Table I.

Heats of Transition. Heats of transformation were calculated from Equation 8.

Table I. Temperatures and Heats of Fusion of Stearic and Benzoic Acids[a]

Run	Sample Weight, G.	dT_r/dt, °/Min.	T_m, (Extr.) Obsd., °	ΔHcalcd., Cal./G.
20 A	1.523	1.95	68	50.5
20 B	1.523	1.45	64	49.4
22	1.300	1.90	67	49.6
23	1.300	1.75	63	47.3
44b	1.077	1.70	67	48.0
Mean			65	49.2
19 C	1.306	1.40	118	31.4

[a] M.P. of stearic acid, determined directly, was 69°. ΔH_f (2) is 47.6 cal./g. For benzoic acid accepted m.p. is 122° and ΔH_f is 33.9 cal./g. (1).
b Run made using model B furnace and cells of Figures 1 and 2. All others with model A furnace and cells.

The value of A was first determined by plotting log $(y - y_s)$ against time. Two such plots are shown in Figure 7. The points fall on good straight lines after the transformation is over, at the times indicated on the figure. At the end of the transformation, so determined, the base line y_s, was assumed to have its final value—i.e., c_s equal to the heat capacity of the cell plus that of the melted sample. At the beginning of the transformation—i.e., the point selected as T_m—y_s was assumed to have its initial value (c_s equal to that of the cell plus that of the unmelted sample). The base line under the peak was taken as a straight line between these two points.

Figure 8 shows a typical run replotted to show the actual magnitude of T_r, T_s, and y. The base line and area taken in evaluating ΔH are shown. The results, given in Table I, are obviously not of high precision, but when it is remembered that they are absolutely independent of any empirical calibrations, they give an assurance of validity to the theoretical analysis.

In one experiment, employing technical stearic acid which melted visually over a range from 56° to 60° C., two runs were made at heating rates of 0.5° and 1.5° per minute. Heat effects of 48.5 and 47.4 calories per gram were obtained, respectively.

To guard against the remote possibility that the agreement between observed and theoretical values might be fortuitous, the same method was applied to an experiment in which powdered,
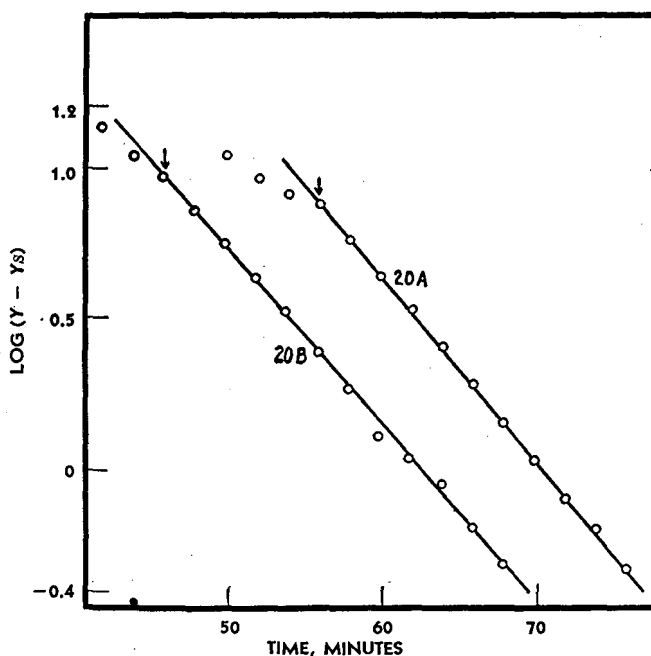

Figure 7. Plot for Determining Constant A

A governs rate at which a thermal steady state is re-established after a transformation occurs. Arrows mark time at which transformation is finished; subsequent points lie on straight lines
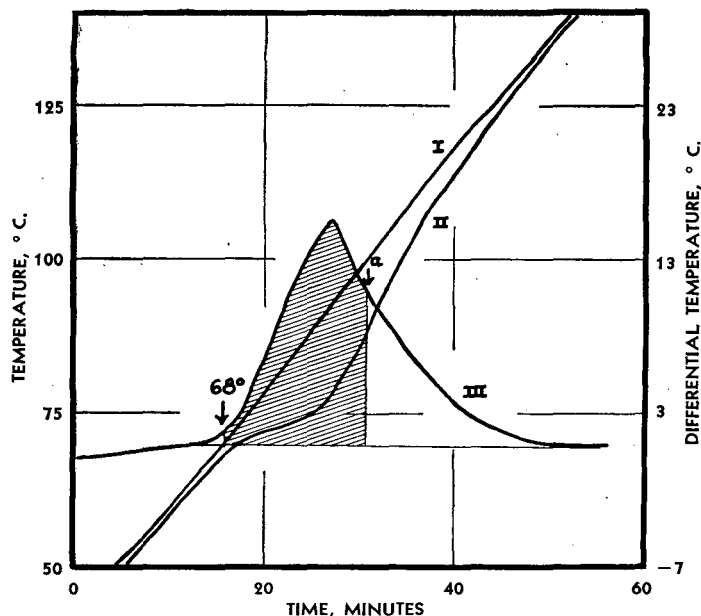
Figure 8. Total and Differential Heating Curves for Stearic Acid and Nujol

I. Heating curve for Nujol cell. II. Heating curve for stearic acid cell. III. Differential heating curve. At 68° melting begins. *a* is time at which calculation shows that melting is complete. Shaded area is that taken into account in calculating Δ*H*.

of the cell as it was heated at a rate of 1.5° per minute. A value of 500 calories per gram for the heat of vaporization of water was obtained. This type of experiment is of potential value in studying the thermal dehydration of clay minerals and similar problems.

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oven-dried (150°) silica was used as reference material and the sample cell was packed with powdered glass and filled with water, which was allowed to vaporize through a hole drilled in the side

Analysis of Recycle Styrene

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Samples of "recycle" styrene, selected to be representative of stock from commercial processing, were analyzed with respect to their major components. The material was analyzed by a combination of procedure involving separation by distillation of the C_4 portion of this material, high-efficiency azeotropic distillation of the C_8 portion, and precision measurements of freezing points on the original material and appropriate portions of the distillates. The amounts of the various components in the recycle styrene samples, Rubber Reserve standard blends 1, 2, and 4, respectively, were found to be as follows, in percentage by weight: 1,3-butadiene, plus other C_4 hydrocarbons, 1.93, 4.50, 1.77; 4-vinyl-1-cyclohexene, 1.78, 3.84, 4.89; ethylbenzene, 1.36, 2.21, 3.20; styrene, 94.52, 88.79, 88.63; C_9 and higher hydrocarbons, 0.41, 0.66, 1.51.

IN CONNECTION with the government synthetic rubber program, in the copolymer plants, the Office of Rubber Reserve requested the National Bureau of Standards to make analyses of selected samples of "recycle" styrene. The present report gives the method and other pertinent details of these analyses of recycle styrene.

SAMPLES ANALYZED

The samples of recycle styrene analyzed are identified as follows:

Rubber Reserve reference blend No. 1, from the B. F. Goodrich Company, Agent for the Office of Rubber Reserve at Port

Neches, Tex., was received on January 9, 1946, in a 2-gallon screw-cap can. The analysis was reported to the Office of Rubber Reserve on September 27, 1946.

Rubber Reserve reference blend No. 2, from the B. F. Goodrich Company, Agent for the Office of Rubber Reserve at Port Neches, Tex., was received on January 4, 1946, in a 2-gallon screw-cap can. The analysis was reported to the Office of Rubber Reserve on September 26, 1946.

Rubber Reserve standard blend No. 4, from the Copolymer Corporation, Agent for the Office of Rubber Reserve at Baton Rouge, La., was received on June 12, 1946, in three 28-ounce bottles sealed with bottling caps. The analysis was reported on August 26, 1946.
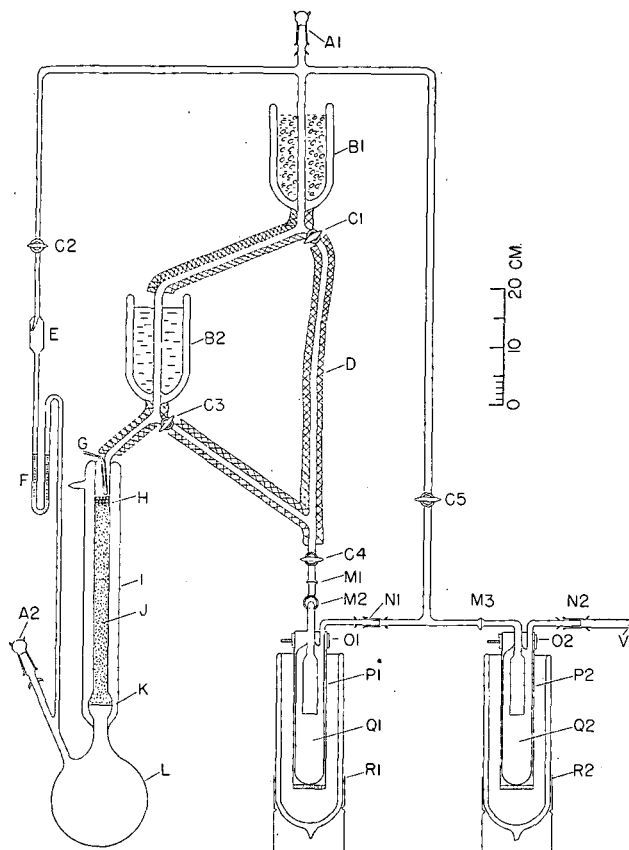
Figure 1. Distilling Column Used for Removal of C₄ Components

A1, A2. Standard-taper, 14/35, Pyrex joint
B1, B2. Vacuum-jacketed, silvered, Pyrex condenser
C1, C2, C3, C4, C5. Pyrex stopcock, ground for high vacuum
D. Lagging, asbestos rope covered with aluminum foil
E. Pyrex surge trap
F. Oil manometer
G. Thermocouple
H. Iron chain
I. Evacuated, unsilvered, Pyrex jacket
J. Rectifying section, 2 × 36 cm., packed with 1/8 inch glass helices
K. Packing support, Pyrex rod "spider"
L. Round-bottomed, 3-liter, Pyrex still pot
M1, M2, M3. Spherical ground-glass joint, 18/7, Pyrex
N1, N2. Standard-taper 12/30 Pyrex joint
O1, O2. Clamps
P1, P2. Brass cylinder with asbestos pad. (When liquid nitrogen is used, metal shield must be provided with suitable openings in sides and bottom or omitted. For use with liquid air, metal shield should be constructed to keep hydrocarbon from contact with liquid air.)
Q1, Q2. Pyrex condensing tube
R1, R2. Pyrex Dewar flasks, 1-quart capacity
V. Connection to vacuum system

METHOD OF ANALYSIS

The original sample was separated by fractional distillation into a "C₄" portion and a "C₈" portion. For standard blend 4, the amount of 1,3-butadiene in the C₄ portion was determined by measurement of freezing points. The C₈ portion was subjected to an analytical distillation at high efficiency and high reflux ratio (*6*). Data on the boiling points and refractive indexes of the distillate were used to calculate the amounts of the individual components (*4*). The amount of styrene in the original sample, in selected fractions of the distillate, and in the polymer-free residue from the analytical distillation of the C₈ portion, was determined by measurement of freezing points. Final values of the composition of the original sample were calculated from the foregoing data.

EXPERIMENTAL PROCEDURE

Measurements of Freezing Points. The method, apparatus, and experimental procedure for the determination of the freezing points of 1,3-butadiene and styrene together with the cryoscopic

constants necessary for the calculation of the amount are given in (*1*) and (*3*). The amount of material used for the freezing point measurements was 60 ml. of liquid at about −78° C. (temperature of solid carbon dioxide) for the 1,3-butadiene, 50 ml. at room temperature for the C₈ material. The original material was cooled to about 0° C., to minimize preferential loss of the C₄ components in the mixture by vaporization, and appropriate samples were removed for examination.

Distillation. A simple distillation at a pressure of 57 mm. of mercury was used to separate the original material effectively into two portions, a low boiling portion consisting essentially of C₄ hydrocarbons with 1,3-butadiene as the major component, and a higher boiling portion consisting essentially of C₈ hydrocarbons with styrene as the major component. The low pressure was used to reduce the temperature and hence retard the polymerization of the styrene.

The details of the distilling column used are shown in Figure 1.

A weighed amount (about 1900 grams) of the original sample was cooled to −10° C. and introduced into the refrigerated still pot, *L*. The lower condenser, *B2*, was maintained at a temperature near −20° C. in order to prevent solidification of styrene (which occurs near −31° C.). The upper condenser, *B1*, was maintained at a temperature near −78° C., with a refrigerating mixture of solid carbon dioxide in a 50–50 mixture of carbon tetrachloride and chloroform. The stopcocks throughout were lubricated with Apiezon grease. The condensing tubes *Q1* and *Q2*, were cooled to about −185° C. by liquid air. The former served as a receiver for the C₄ fraction and the latter as a trap. After introduction of the sample, the pressure was reduced to 57 mm. of mercury and the material was allowed to warm from −10° C. to room temperature.

Initially in the distillation, the C₄ components passed as vapors from the still pot without wetting the packed section, *J*, being condensed at *B1* and revaporized by the warmer condenser, *B2*; some material was collected in the receiver and trap as overhead from condenser *B1*. After refluxing, which served to remove any C₈ hydrocarbons that were entrained in the C₄ vapors (the upper portion of the packed section gradually becoming wetted), stopcock *C1* was adjusted for a slow withdrawal of the C₄ distillate into the receiver. The pot was finally heated to produce a reflux of the C₈ hydrocarbons from the lower condenser with *J* acting now as a rectifying section. The C₄ components escaped from this lower condenser by vaporization, and the C₈ components were retained as liquid under total reflux.

The distillation was halted when the reflux from the lower condenser as determined by the thermocouple, *G*, showed a steady temperature (about 74°, 67°, and 67.5° C. for blends, 1, 2, and 4, respectively). Stopcocks *C1*, *C2*, *C4*, and *C5* (stopcock *C3* was closed throughout the entire distillation) were closed and the material in the pot was allowed to cool to room temperature. Any material in the trap was transferred to the receiver, the entire contents of which were transferred to a bomb and weighed as described in (*1*). (For blend 4, this C₄ portion was used for precision measurements of freezing points to determine the amount of 1,3-butadiene.) The residue was withdrawn after it had cooled to room temperature. The column and pot were washed by several portions of ethylene glycol monomethyl ether (methyl Cellosolve) introduced at *A1*. These washings, together with the above residue, were included as part of the charge for the analytical distillation of the C₈ portion. (For blend 4, a sample of the hydrocarbon part of this material was used for precision measurements of freezing points to determine the amount of styrene.)

The analytical distillations of the C₈ portion were performed in columns of high efficiency (125 theoretical plates at total reflux) operated at high reflux ratio (*6*).

Prior to the analytical distillation of the C₈ portions of Rubber Reserve standard blends 1, 2, and 4, an extended analytical distillation had been performed on a previous sample of recycle styrene, from the Copolymer Corporation at Baton Rouge, La. In this distillation, the recycle styrene was completely distilled by azeotropic distillation with methyl Cellosolve at 57-mm. pressure without any noticeable polymerization. Polymerization of the styrene in such a distilling operation was minimized by reduction of the pressure, which lowered the temperature of distillation; performing the distillation with an added azeotrope-forming sub-
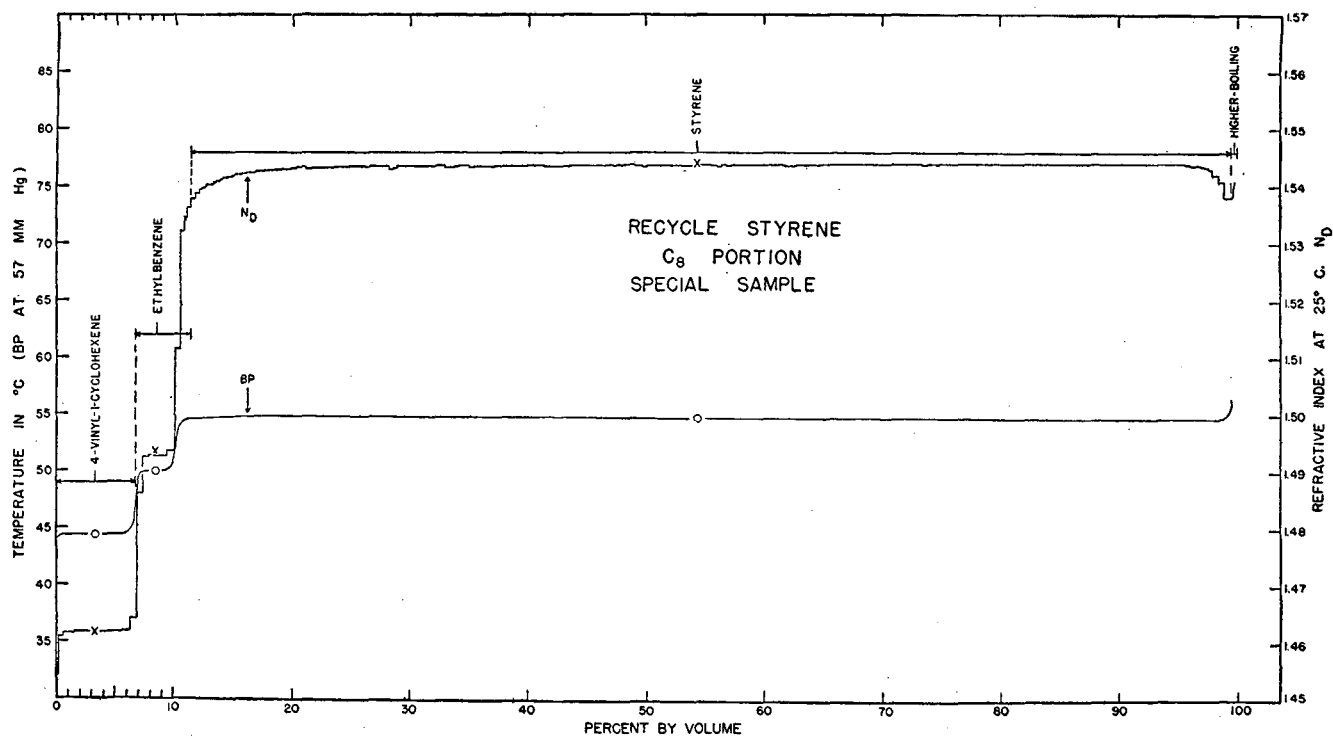
Figure 2.   Results of High-Efficiency Azeotropic Distillation of C₈ Portion of Recycle Styrene

The ordinate scale on right gives the refractive indexes of the hydrocarbon portion of the fractions of azeotropic distillate, and the ordinate scale on left gives the boiling point of the azeotropic distillate at 57 mm.   The scale of abscissa gives the per cent by volume of the hydrocarbon portion of the distillate.   The amounts by volume of the various components are indicated in the upper portion.   The circles denote the boiling point at 57-mm. pressure of the binary azeotropes of the hydrocarbons with ethylene glycol monomethyl ether.   The crosses denote the refractive index of the pure hydrocarbons without the azeotrope-forming agent.

stance, which not only served to reduce the temperature of distillation still more but also diluted the styrene through the system; and addition of a suitable inhibitor.   This distillation was therefore selected as a pattern and reference for the distillation of the other blends.

The charge consisted of 2700 ml. of the C₈ portion plus 6400 ml. of methyl Cellosolve, together with 25 grams of hydroquinone; the azeotropic distillation at 57 mm. ran continuously for about 60 days, and distillate was removed at the rate of 4.5 ml. per hour.   The fractions were freed of the azeotropic agent (methyl Cellosolve) by three water extractions in a separatory funnel and the refractive index was read to 0.0001 unit on a Valentine refractometer, Abbe type.

A plot of the boiling point of the azeotropic distillate and the refractive index of the hydrocarbon portion of the distillate as a function of the volume of hydrocarbon in the distillate is shown in Figure 2.

For the analytical distillations of blends 1 and 2, the charge consisted of 1000 ml. of the C₈ portion, plus 3000 ml. of methyl Cellosolve, together with 25 grams of hydroquinone.   For blend 4, the charge consisted of 2000 ml. of the C₈ portion, plus 4000 ml. of methyl Cellosolve, and 25 grams of hydroquinone.   The distillations were performed at 57-mm. pressure and were stopped when 0.2 liter of styrene was obtained as distillate.   This procedure, which was made possible by having performed the one complete distillation, saved considerable time in the analysis.   These distillations were plotted in the manner shown in Figure 2.   The residue from these distillations was given a simple distillation in vacuum to remove any polymer before the freezing point measurements.

RESULTS

The weight per cent of C₄ hydrocarbons in the sample was obtained from the weight of the C₄ portion and the weight of the sample from which it was obtained in the fractional distillation.

For blend 4, an independent value of the weight per cent of C₄ hydrocarbons was determined from precision measurements of the freezing point of styrene in the original and in the C₈ portion.   This value (1.96 ± 0.30) was in substantial accord with the former (1.77 ± 0.08), although less precise because there are required values of density and molecular weight and because of possible loss of C₄ components in the determination of the freezing point of the original sample.

The amount of 1,3-butadiene in Rubber Reserve standard blend 4 was determined from measurement of the freezing point of the C₄ portion, which contained 1,3-butadiene as the major component (1).   All of the most probable impurities in commercial 1,3-butadiene and in recycle 1,3-butadiene have been shown to produce a lowering of the freezing point which is in substantial accord with the ideal lowering (1).   The conversion of mole per cent into weight per cent was made with little uncertainty, as the components other than 1,3-butadiene are essentially all butenes, and 1-butene and trans-2-butene comprise about 90% of the total impurity (2).   For a detailed discussion of the impurities in recycle 1,3-butadiene which would be typical of this portion see (2).   Part of the C₄ portion was submitted for mass spectrometer analysis (5) for details of the C₄ impurities associated with 1,3-butadiene.

The reported weight per cent of styrene in the original sample was determined from the analytical distillation of the C₈ portion, using precision measurements of freezing points on distillate fractions containing styrene including the undistilled portions (residue), together with the amounts of 4-vinyl-1-cyclohexene and ethylbenzene as determined from the boiling point and relationships of refractive index to volume.   These values were cross-checked by precision measurements of freezing points for styrene on the original samples and also for blend 4 on the entire C₈ portion.   These latter values, which were in accord within their respective uncertainties with the reported values, required a knowl-

Table I. Composition of Recycle Styrene, Rubber Reserve Standard Blends 1, 2, and 4

Component	Boiling Point at 1 Atm., ° C.	Amount Based on Original Sample					
		Blend 1		Blend 2		Blend 4	
		Weight %	Mole %	Weight %	Mole %	Weight %	Mole %
1,3-Butadiene	-4.4	}1.93 ± 0.15	}3.64 ± 0.30	}4.50 ± 0.15	}8.29 ± 0.30	1.53 ± 0.08	2.90 ± 0.16
Other C₄ hydrocarbons						0.24 ± 0.08	0.45 ± 0.15
4-Vinyl-1-cyclohexene	128.9	1.78 ± 0.50	1.69 ± 0.50	3.84 ± 0.50	3.56 ± 0.50	4.89 ± 0.50	4.66 ± 0.50
Ethylbenzene	136.2	1.36 ± 0.50	1.31 ± 0.50	2.21 ± 0.50	2.09 ± 0.50	3.20 ± 0.50	3.10 ± 0.50
Styrene	145.2	94.52 ± 0.30	93.01 ± 0.30	88.79 ± 0.30	85.51 ± 0.30	88.63 ± 0.30	87.60 ± 0.30
C₉ and higher hydrocarbons	> 145.2	0.41 ± 0.25	0.35 ± 0.20	0.66 ± 0.30	0.55 ± 0.25	1.51 ± 0.50	1.29 ± 0.50
Total		100.0	100.0	100.0	100.0	100.0	100.0

edge of the relative amounts of the impurities present (which was manifested by the analytical distillation) for selection of the appropriate cryoscopic constants for conversion of the freezing point into the amount of styrene (1).

The composition in volume per cent of the hydrocarbon and the boiling points in degrees Centigrade, at 57-mm. pressure, of the binary azeotropes of the main C₈ hydrocarbon components with ethylene glycol monomethyl ether (methyl Cellosolve), as determined from the data of the azeotropic distillation shown in Figure 2, were as follows: 4-vinyl-1-cyclohexene, 70%, 44.4° C.; ethylbenzene, 58%, 50.0° C.; and styrene, 38%, 54.8° C.

The amounts of the various components in the Rubber Reserve standard blends 1, 2, and 4 are given in Table I. A comparison of the C₈ components associated with styrene in blends 1, 2, and 4 gave a ratio for 4-vinyl-1-cyclohexene (cyclic dimer of 1,3-butadiene) to ethylbenzene of 1.29, 1.70, and 1.51, respectively.

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Fatty and Rosin Acids, Soaps, and Their Mixtures

Conductometric and Potentiometric Analysis

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A study has been made to ascertain whether conductometric and potentiometric titration methods can be applied to the analysis of fatty and rosin acids, soaps, and acid-soap mixtures. Utilizing a solution of isopropyl alcohol–water as solvent, it has been found that, although potentiometric titrations are of limited applicability, conductometric methods lend themselves very readily to such determinations. Direct titration with base can be used to determine fatty and rosin acids, and titration with acid can be used for analysis of soaps. By an indirect titration method, the soap and acid contents, or the soap and alkali contents, of soaps and soap solutions can be determined by a single titration.

THIS paper presents the results of an extended study made to determine the applicability of conductometric and potentiometric procedures to the determination of acid, soap, and free alkali contents of fatty and rosin acids, soaps, and their mixtures. The aim in this work was to see whether these rapid and simple techniques could be substituted for the well-known (1), but more cumbersome and time-consuming, methods.

There are very few references in the literature to the application of potentiometric or conductometric methods to the analysis of soaps and their acids.

Potentiometric titrations for the estimation of fatty acid materials have been used by Vishnyakov and Radicheva (8), Ekwall and Juup (3), Jarrett (5), and Bishop, Kittredge, and Hildebrand (2). Vishnyakov and Radicheva showed that fatty acid soaps can be titrated potentiometrically with acid by use of either glass or antimony electrodes. Ekwall and Juup utilized titration with silver nitrate to determine the sodium salts of lauric, myristic, palmitic, and stearic acids. Jarrett, working with dark oils and varnishes, employed potentiometric titration of these materials with base to ascertain their acid contents, while Bishop, Kittredge, and Hildebrand used a hydrogen electrode to determine palmitic acid in presence of its glyceride by titration with sodium ethylate in alcoholic solution. The only reference found to use of conductometry is that to Jander and Weitendorf (4), who used conductance measurements for estimating small amounts of fatty acids in soap. They first extracted the acids with low boiling solvents, removed the solvents by evaporation, and then titrated conductometrically the residual acid dissolved in 75% ethyl alcohol with sodium hydroxide.

In the early stages of this research all conductometric and potentiometric titrations were run in aqueous medium. This medium was not too satisfactory, however, and much better and reliable results can be obtained in a solvent mixture of equal volumes of

water and 99% isopropyl alcohol. In the latter solvent certain types of potentiometric titrations worked very well, but they were not as rapid, simple, reproducible, or versatile as the conductometric titrations. Consequently most of the data reported here were obtained by conductometry, and potentiometry was used only where possible as a check on the former technique.

APPARATUS, REAGENTS, AND GENERAL PROCEDURE

All conductometric titrations were run with a Leeds & Northrup portable conductivity bridge No. 4866. The cell consisted of a 600-ml. beaker in which were immersed a pair of dip-type platinized platinum electrodes and a mechanically driven glass stirrer. Generally a 10-ml. calibrated buret completed the conductance titration assembly. For the potentiometric titrations the same arrangement was used, except that the conductance electrodes were replaced with external type Beckman glass and calomel electrodes, and the bridge with a Beckman Model G pH meter.

The lauric, palmitic, and stearic acids were obtained from the Amend Drug and Chemical Company, New York, N. Y., and were designated as c.p. grade. The dehydroabietic acid was specially purified by D. A. Shepherd of the University of Illinois, and made available through his courtesy. The Resin 731 and K-wood rosin were Hercules commercial products, from which the potassium soap of the latter acid was made by saponification with potassium hydroxide. Finally, the sodium oleate was J. T. Baker's neutral powder, and this soap was used either directly or as an aqueous solution.

The 0.1 N solutions of sodium hydroxide used for titration were prepared by dilution of carbonate-free base with doubly distilled water. They were standardized against Bureau of Standards potassium acid phthalate using phenolphthalein as indicator. The corresponding 0.1 N hydrochloric acid solutions were standardized then against the base solutions using indicator, conductance, and potentiometric titrations. All three methods gave concordant results.

The general procedure in making conductance titrations was to prepare the appropriate sample in the isopropyl alcohol–water medium, and to titrate it with acid or base, as required, by addition of reagent in steps until an appreciable excess was introduced. The conductance readings taken after each addition of reagent were plotted then against volume of reagent added, and the end points were ascertained from the plots. The potentiometric titrations were run in a similar manner, and the end points again were determined graphically from plots of $\Delta pH/\Delta V$ vs. volume, V, of added reagent.

All titrations reported here were run in a medium consisting of 200 ml. of doubly distilled water and 200 ml. of 99% isopropyl alcohol, which was used without any purification. Investigation has shown that such a mixture of water and isopropyl alcohol shows no blank consumption of hydrochloric acid on potentiometric or conductometric titration. On the other hand, both types of titrations show a blank consumption of 0.0230 to 0.0310 milliequivalent of sodium hydroxide. This blank is constant for a given sample of isopropyl alcohol, and cannot be eliminated by redistillation of the alcohol.

Extensive study has shown that this blank enters only when a weak acid is titrated in the solvent mixture with sodium hydroxide, but not on titration of a strong acidlike hydrochloric acid. Consequently, this correction is to be applied to all titrations of fatty or rosin acids with base. The blank must also be applied to titrations where any preliminary reaction of the solvent with present or added base takes place.

DIRECT TITRATION OF FATTY AND ROSIN ACIDS WITH BASE

Weighed samples of the vacuum-dried fatty or rosin acids were dissolved in the solvent mixture, and the solutions were titrated conductometrically or potentiometrically with 0.1 N sodium hydroxide. For determination of the acid contents of soap solutions the same procedure was used, except that the samples taken were larger and were titrated with 0.05 N base.

Figure 1 shows typical conductance curves obtained in the titration of fatty or rosin acids with base. Inasmuch as both branches of the curves are very good straight lines, there is no difficulty in ascertaining the position of the end points. Figure 2 shows similar curves for soap solutions when titrated with base for any free fatty or rosin acid present. Here the conductance curves are generally found to consist of three straight lines intersecting at two points, as pictured. Study with solutions of known acid contents has established that the end points sought are given
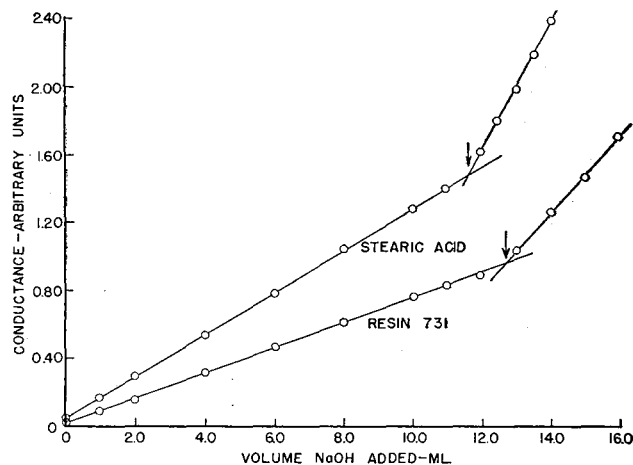

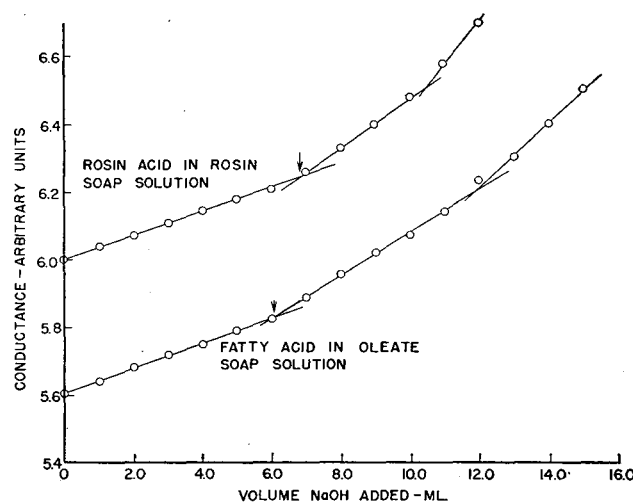Figure 1. Titration of Rosin and Fatty Acids with Base

Figure 2. Titration of Fatty or Rosin Acids with Base in Soap Solutions

by the first of these discontinuities (marked by arrows). The significance of the second discontinuity is not clear. In the case of rosin acids, this break could well be due to the presence of phenolic materials, but such an explanation would not apply to the fatty acid titrations. In any case, the authors have definitely established that the occurrence of a second break is in no way related to the purity of the isopropyl alcohol.

The potentiometric titration curves observed with rosin and fatty acids were of the usual form, and were very satisfactory for all materials except Resin 731 and the soap solutions. For Resin 731 the titration curves could only be characterized as "fair" for

Table I. Direct Titration of Fatty and Rosin Acids with Base

Substance Titrated	No. of Detns.		NaOH, Me. Consumed/Gram Sample	
	Cond.	Poten.	Cond.	Poten.
Lauric acid	4	3	4.963 ± 0.22%	4.952 ± 0.14%
Palmitic acid	4	3	3.923 ± 0.23%	3.899 ± 0.15%
Stearic acid	5	4	3.501 ± 0.51%	3.469 ± 0.29%
Dehydroabietic acid	4	2	3.347 ± 0.06%	3.350 ± 0.03%
Resin 731	10	2	2.787 ± 0.21%	2.791 ± 0.04%
Fatty acid in potassium oleate soln. II	3	...	0.00455 ± 0.22%	...
Rosin acid in rosin soap soln. II	6	...	0.0295 ± 1.69%	...

end-point estimation, and the soap titration curves were so flat as to be totally unsatisfactory.

In Table I are summarized the results obtained for the conductometric titration with base of samples of lauric, palmitic, stearic, and dehydroabietic acids, Resin 731, and solutions of potassium oleate and K-wood rosinate. Also given are the results obtained potentiometrically for all these materials except the soap solutions. In all cases the data have been corrected for the solvent base consumption blank. For each acid the concordance in the results obtained by a given method is very good, and the agreement between the conductometric and potentiometric results is in all cases within less than 1%.

The data given in Table I for dehydroabietic acid are in excellent agreement with results obtained by Laitinen and Jennings (6) on the same sample of the acid by titration with base using indicators—namely, 3.340 milliequivalents per gram. This agreement and the concordance shown by the data in Table I indicate that potentiometric titrations can be applied to the analysis of rosin and fatty acids, and that conductance titrations in particular are very satisfactory not only for the determination of the acids themselves, but also for the estimation of such acids in soap solutions. Another advantage of conductometric titrations is this fact. If in the samples being titrated any free strong acid is present, then the titration curves in Figures 1 or 2, instead of exhibiting a continual rise in conductance on initial addition of base, show first a decrease in conductance followed by an upswing. As a result the presence of such acid can be detected and determined. Titration curves of this type are discussed in greater detail below.
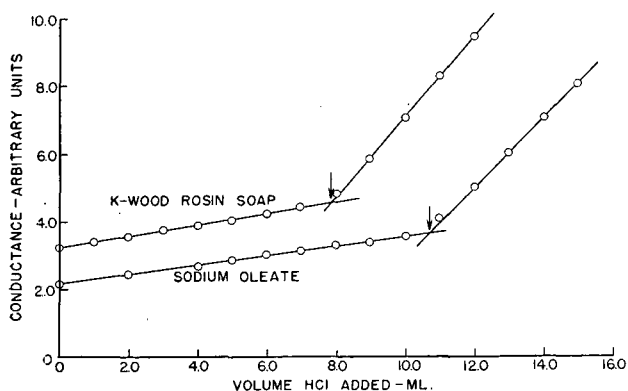

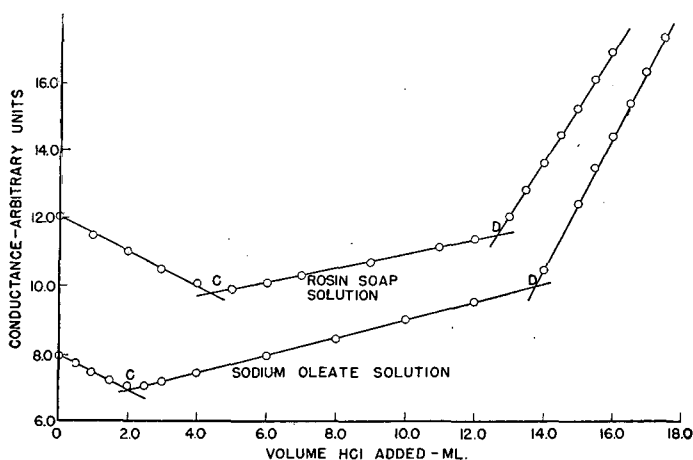Figure 3. Titration of Fatty or Rosin Soaps with Acid

Figure 4. Indirect Titration of Soaps with Acid in Presence of Excess Sodium Hydroxide

Table II. Direct Titration of Fatty and Rosin Soaps with Acid

Substance Titrated	No. of Detns.		HCl, Me. Consumed/Gram Sample	
	Cond.	Poten.	Cond.	Poten.
Solid Na oleate	4	3	3.496 ± 0.06%	3.502 ± 0.29%
Na oleate soln. II	3	...	0.1306 ± 0.08%	...
Rosin soap soln. I	4	...	0.1014 ± 0.29%	...
Rosin soap soln. II	5	...	0.5002 ± 0.32%	...

DIRECT TITRATION OF FATTY AND ROSIN SOAPS WITH ACID

For determination of the soap content of solid soaps and their solutions appropriate samples were weighed out and added to the solvent mixture employed, and the solutions were titrated with standard hydrochloric acid conductometrically or potentiometrically.

The conductance curves were found to be excellent in all cases, as may be seen from the typical plots shown in Figure 3. On the other hand, the potentiometric titration curves were only fair in distinctness for fatty acid soaps, and totally unsatisfactory for rosin soap solutions.

In Table II are presented results obtained for solid sodium oleate and aqueous solutions of sodium oleate and potassium soaps of K-wood rosin. Here again the concordance in the conductometric results is very good, and the agreement between the potentiometric and conductometric results for solid sodium oleate is excellent.

Conductance curves such as those in Figure 3 indicate absence of free alkali in the titration samples. If free alkali were present the curves would show an initial decrease in conductance on addition of acid, followed by a subsequent increase. However, although the presence of free alkali can be detected in this manner, its content cannot be determined with accuracy.

INDIRECT CONDUCTOMETRIC DETERMINATION OF ACID AND SOAP

By Titration with Hydrochloric Acid. In the above-described conductometric titrations the free fatty or rosin acid and soap contents of, say, a soap solution have to be determined by two titrations on two separate samples of the substance. Theoretically it should be possible to determine both soap and acid contents conductometrically in a single titration by first adding a known excess of alkali to neutralize any free acid present, and then titrating the solution with acid to ascertain the excess alkali remaining and the total amount of soap present. Figure 4 shows conductance curves obtained for such titrations in the isopropyl alcohol–water mixture. In these plots point C should represent the volume of acid required to neutralize any excess alkali present and D the total alkalinity of the sample. Hence $(D-C)$ should be the volume of hydrochloric acid required to react with all of the soap—i.e., soap originally in the sample and that formed from the fatty or rosin acid as a result of alkali addition. From these volumes and the normalities it should be possible to calculate the soap and free fatty or rosin acid contents of the samples.

Table III shows results obtained in this manner for analysis of a sodium oleate solution, Resin 731, and dehydroabietic acid. The columns marked "Taken" refer to the analytical results found by direct titrations for soap and acid contents. Inspection of this table reveals that in all instances there is no agreement between the quantities taken and found; the latter are invariably too high. These discrepancies are not due to absorption of carbon dioxide, for identical results were obtained in an atmosphere of nitrogen. It must be concluded, therefore, that conductometric titrations of this

Table III. Indirect Conductometric Titration of Acid and Soap with Hydrochloric Acid

Substance Titrated	No. of Detns.	Rosin or Fatty Acid Taken	Rosin or Fatty Acid Found	Soap + Acid Taken	Soap + Acid Found
		Milliequivalents per Gram of Sample			
Na oleate soln. I	5	0.000	0.0021 ± 0.0004	0.1093	0.1114 ± 0.0006
Resin 731	9	2.787	2.858 ± 0.012	2.787	2.857 ± 0.015
Dehydroabietic acid	4	3.347	3.434 ± 0.042	3.347	3.445 ± 0.047

Table IV. Indirect Conductometric Titration of Acid and Soap Contents with Base

Substance Titrated	No. of Detns.	Rosin or Fatty Soap Taken	Soap Found, S	Soap + Acid Taken	Soap + Acid Found, Y	Rosin or Fatty Acid Taken	Rosin or Fatty Acid Found, X
		Milliequivalents per Gram of Sample					
Na oleate soln. I	4	0.1093	0.1085 ±0.0005	0.1093	0.1091 ±0.0004	0.0000	0.0006 ±0.0004
Rosin soap soln. II	5	0.5002	0.5005 ±0.0024	0.5297	0.5291 ±0.0030	0.0295	0.0286 ±0.0025

nature are not reliable. The same conclusion was reached by Poetke (7).

By Titration with Sodium Hydroxide. An alternative procedure for determination of the soap and fatty or rosin acid contents of a sample is to add standard hydrochloric acid in excess and then to titrate conductometrically with sodium hydroxide with the excess hydrochloric acid and all of the free fatty or rosin acid. Typical conductance curves obtained by this method with soap solutions is 50% isopropyl alcohol–water are shown in Figure 5.

Here point E should give the volume of sodium hydroxide required to react with any free hydrochloric acid still present, F the total acidity of the solution, and (F−E) should be the volume of base required to react with all of the free fatty or rosin acid present. Then

$$S = \frac{V_A N_A - V_E N_B}{W} \quad (1)$$

$$Y = \frac{(V_F - V_E) N_B - B}{W} \quad (2)$$

$$X = Y - S \quad (3)$$

where S = milliequivalents of soap, Y = milliequivalents of soap plus fatty or rosin acid, and X = milliequivalents of free fatty or rosin acid, all per gram of sample; V_A = volume of hydrochloric acid added, N_A = its normality, B = blank correction in milliequivalents, W = weight of sample taken, N_B = normality of the base, and V_F and V_E = volumes corresponding to points F and E, respectively, in Figure 5.

The results thus obtained with solutions of sodium oleate and the potassium soap of K-wood rosin are summarized in Table IV. Here again the columns marked "Taken" refer to results of direct analysis. The concordance in the data for soap and total soap plus acid contents is excellent, being in all instances less than 0.6% while the data for the free acid contents are as good as can be anticipated in view of the small quantities to be determined as the difference of two relatively large numbers. Again, the agreement between the indirect and direct averages is also good, being 0.73 and 0.06% for the soaps, 0.18 and 0.11% for the total soap plus acid contents. This procedure can be used, therefore, as a single titration method for determination of the soap and free fatty or rosin acid contents of soaps and soap solutions, or for the determination of the total fatty or rosin acid content of

such substances. Furthermore, titrations of this type, by giving the requisite recovery of excess hydrochloric acid, establish also the fact that, if during the direct titration of fatty or rosin acids with base presence of free strong acids is detected, the amount of such acids present can be determined reliably from the initial break in the conductance curves (point E in Figure 5).

DETERMINATION OF SOAP AND FREE ALKALI

By Titration with Sodium Hydroxide. When a soap or soap solution contains free fatty or rosin acid, the quantity S, as calculated by Equation 4, is less than the quantity Y given by Equation 5. However, should S be found from a titration result to be greater than Y, then instead of free acid an excess of free alkali is present. Equality of S and Y denotes absence of both free acid or alkali. In case of S>Y, Equations 1 to 3 no longer hold, and we have now instead
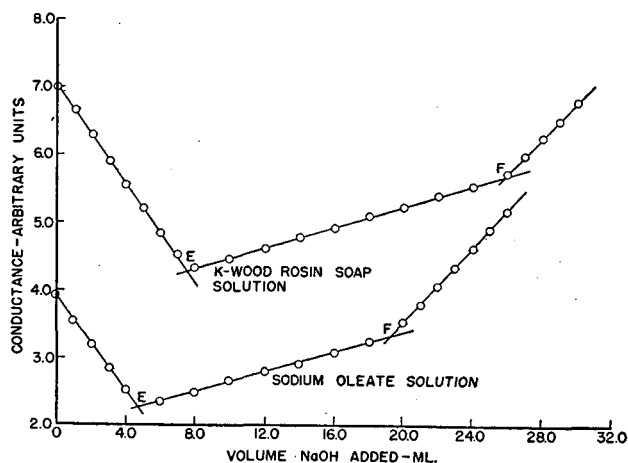
$$Z = \frac{(V_A N_A - V_E N_B)}{W} \quad (4)$$

$$S = \frac{(V_F - V_E) N_B - B}{W} \quad (5)$$

$$R = Z - S \quad (6)$$

where all the symbols used before have the same significance, Z = total alkalinity in milliequivalents, and R = milliequivalents of free alkali present, all per gram of sample.

To test this possibility of determining the total alkalinity, soap, and free alkali contents of soaps by a single titration, analyzed

Figure 5. Indirect Titration of Soaps with Base in Presence of Excess Hydrochloric Acid

Table V. Indirect Determination of Soap and Free Alkali with Base

Weight of Sample, Grams	Total Alkali, Me. per Gram of Sample Taken	Total Alkali, Me. per Gram of Sample Found, Z	Total Alkali, Me. per Gram of Sample Dev., %	Soap, Me. per Gram of Sample Taken	Soap, Me. per Gram of Sample Found, S	Soap, Me. per Gram of Sample Dev., %	Free Alkali, Me. per Gram of Sample Taken	Free Alkali, Me. per Gram of Sample Found, R	Free Alkali, Me. per Gram of Sample Dev., %
			Sodium Oleate Solution II						
10.2431	0.1499	0.1493	−0.20	0.1352	0.1349	−0.22	0.0147	0.0144	−2.04
10.4901	0.1494	0.1504	+0.66	0.1352	0.1355	+0.22	0.0142	0.0149	+4.93
11.3489	0.1480	0.1492	+0.81	0.1352	0.1360	+0.59	0.0128	0.0132	+3.13
			Potassium K-Wood Rosinate Solution II						
4.5329	0.6308	0.6334	+0.41	0.5297	0.5393	+1.81	0.1011	0.0941	−6.92
5.1204	0.5966	0.5964	−0.03	0.5297	0.5297	±0.00	0.0669	0.0667	−0.30
5.1546	0.5959	0.5984	+0.42	0.5297	0.5279	−0.34	0.0662	0.0705	+6.50
5.5926	0.6061	0.6078	+0.28	0.5297	0.5323	+0.49	0.0764	0.0755	−1.18

samples of solutions of sodium oleate and of potassium K-wood rosinate with known excess quantities of free alkali were weighed out and dissolved in isopropyl alcohol–water, excess hydrochloric acid was added, and the samples were titrated conductometrically with sodium hydroxide. The results, evaluated by Equations 4 to 6 from titration curves identical in form with those shown in Figure 5, are given in Table V. These data definitely establish the feasibility of such a procedure. In all cases but one the total alkalinity and soap found were within 1% of those taken, while the recovery of free alkali was within about 7%. These results are as good as can be anticipated when it is considered that the free alkali contents are obtained by difference, that in no case did the free alkali constitute more than about 13% of the total alkalinity of the samples, and that we are referring to single determinations on each sample.

DISCUSSION AND CONCLUSIONS

The results of this paper and experience of several years with soap titrations of various kinds indicate that potentiometric methods of analysis in the isopropyl alcohol–water medium have only limited applicability. They may be employed for the determination of the acid contents of fatty or rosin acids, and possibly for the analysis of fatty acid soaps, but they are either unsatisfactory or unsuitable for the determination of the acid and soap contents of commercial rosin acids, soaps, and soap solutions. Furthermore, the potentiometric titrations are slower, more tedious, and less versatile than the corresponding conductometric procedures.

From the data presented in this paper it is evident that conductometry is readily applicable to the direct determination by titration with base of the acid contents of fatty and rosin acids, soaps, and soap solutions. Again, the soap contents of fatty and rosin soaps and of their solutions can readily be obtained by direct titration with acid. In the course of the former titrations the

presence of any free strong acid can be detected and estimated from the shape of the titration curves, while during the latter titrations presence of free alkali can be detected but not determined. Furthermore, by employing conductometry, and by using the technique of addition of excess acid followed by titration with base, it is possible to obtain from a single titration either the soap and acid contents of a sample, or of the soap and any free alkali that may be present. Because the single titration procedure based on the addition of excess base followed by titration with acid is unreliable for the determination of the soap, acid, or alkali contents of a sample, the indirect titration method based on addition of excess hydrochloric acid is the only one that can be employed to analyze any soap or soap solution containing free alkali.

The authors' experience indicates that in general the direct titration methods with acid or base are somewhat more precise than the corresponding indirect ones. However, the latter titrations possess sufficient accuracy and reproducibility for routine analysis of fatty and rosin acid materials, and it is primarily for such purposes that these procedures are intended.

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Determination of Cellulose in Cotton and Cordage Fiber

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The effect of alcoholic amines in ethylene glycol has been explored as a means of determining cellulose in cotton and cordage fibers. Results using 1 part of monoethanolamine in 3 parts of ethylene glycol compared favorably with other methods in determining cellulose in cotton fiber. The action of the reagent on hard and soft fibers shows promise as a method for determining cellulose, but in some cases the fiber may require some bleaching. Comparisons of the action of various cellulose reagents on cordage fiber are given.

THE determination of cellulose in plant fibers used in textiles and cordage involves very much the same difficulties as those encountered in other plant materials such as wood and straw—in cotton fiber to a lesser extent than the leaf and bast fibers. For the cordage fibers, reagents must be found which will leave the cellulose and at the same time remove the extraneous materials, made up chiefly of pentosans and lignin.

The methods that make use of chlorine—those of Cross and Bevan (2) and Norman (10), and more recently the chlorite method of Sarkar and Chatterjee (13)—are the most popular; however, they are lengthy procedures and require more or less constant attention. The method of Kurschner and Hoffer (9) using 20% nitric acid in ethanol is fairly rapid, but indications are that it may go too far and remove some of the

cellulose itself. Monoethanolamine (2-aminoethanol) in ethanol was proposed by Van Beckum and Ritter (14) as having possibilities for the determination of holocellulose. Wise, Peterson, and Harlow (15) suggested the use of concentrated monoethanolamine as a reagent for cellulose. This method has been used and modified by others (12) with success, but some reason, perhaps cost of the reagent or, if the reagent is reclaimed, the additional labor required for reclamation, has made the method less attractive.

Observations while working with cordage fibers have indicated that the hard or leaf fiber requires less contact with the reagent than the soft or bast fiber. Material of higher pentosan contents may account for this difference, but probably it is due to a combination of substances and a difference in the physical and

Table I. Effect of Time on Action of Monoethanolamine in Ethylene Glycol in Determination of Cellulose in Kenaf Fiber

			Cellulose[a] Found	
Fiber	Concen-tration of Monoethanol-amine	Boiling Time	Monoethanol-amine in ethylene glycol	Monoethanol-amine in ethylene glycol plus bleach[b]
	%	Hours	%	%
Cuban kenaf	25	1	72.3	70.7
		2	71.6	70.1
		3	70.7	69.4
		4	70.1	68.8

[a] Based on oven-dry weight.
[b] 3.5% NaClO₂ in 3% acetic acid for 0.5 hour at 60° to 70° C.

Table II. Effect of Change in Concentration of Monoethanolamine in Ethylene Glycol on Determination of Cellulose without Variation of Time

			Cellulose[a] Found	
Fiber	Concen-tration of Monoethanol-amine in Ethylene Glycol	Boiling Time	Monoethanol-amine in ethylene glycol	Monoethanol-amine in ethylene glycol plus bleach[b]
	%	Hours	%	%
Cuban kenaf	10	2	71.30	69.0
	25	2	71.20	68.6
	50	2	71.50	69.3
				Av. 69.0

[a] Based on oven-dry weight.
[b] 3.5% NaClO₂ in 3% acetic acid for 0.5 hour at 70° C.

chemical make-up of the two groups of fibers. An analysis shows that lignin is in about the same concentration in both groups of fibers; however, the amount of lignin remaining after extraction is invariably greater in the soft fiber. Because methods of processing vary, it therefore follows that the amount of extraneous or secondary constituents will also vary, and this makes it almost impossible to predict how a certain fiber will act under treatment by an extraction reagent. The cellulose of cotton fiber does not require so vigorous a treatment to purify it as do the other plant fibers. A 1% sodium hydroxide boil for 2 hours after wax removal as proposed by Kettering and Conrad (6) will give reasonably pure cellulose.

Aronovsky and Gortner (1) found that some alcohols and glycols had merit when used as pulping agents on wood. Later Hessler (3) showed that monoethanolamine in concentrations of 2.5 to 5.0% in ethylene glycol and glycerol removed encrustants from hemp fiber with a minimum of cellulose degradation. This work has led to the trial of additional amines in ethylene glycol, which is reported here, together with other information on the determination of cellulose in cotton and cordage fibers.

REAGENTS AND METHODS

Monoethanolamine Reagent. A solution of 1 part of monoethanolamine (2-aminoethanol, redistilled technical grade) in 3 parts of ethylene glycol (technical grade).

Chlorite Bleach Solution. A solution of 3.5% sodium chlorite (technical grade) in 3.0% acetic acid.

The lignin and pentosans were determined by methods described by one of the authors (4) and used in the analysis of dew-retted hemp fiber.

Kurschner and Hoffer (9) Method for Cellulose. Weigh 100 mg. of finely ground fiber (60-mesh) into a test tube (32 × 200 mm.), add 20 ml. of reagent (1 part of nitric acid to 4 parts of 95% ethanol), and boil gently 1 hour, using a "cold finger" to condense the liquid. Allow to settle and decant solution into a tared medium fritted-glass crucible, retaining as much fiber as possible. Repeat the process by renewing the reagent twice and at the end of the final boiling wash the cellulose into the crucible with hot alcohol. Wash four times with hot alcohol and each time retain the wash solution in the crucible a few minutes. Dry at 105° C. for 4 hours and report as per cent cellulose. If a fiber has extremely high ash, a correction may be necessary.

Kettering and Conrad Method (6) for Determination of Cellulose in Cotton Fiber. Weigh 100 mg. of alcohol-extracted cotton into a weighing bottle and dry 4 hours at 105° C. to determine dry weight.

Transfer the sample into a test tube (32 × 200 mm.), add 50 ml. of 1% sodium hydroxide (carbonate-free), and then reflux with the aid of a cold finger for 2 hours. Filter through a tared fritted-glass crucible and wash thoroughly with hot water. Dry at 105° C. for 4 hours, correct for ash, and report as per cent cellulose.

Chlorite Method of Sarkar and Chatterjee (13) for Determination of Cellulose. Weigh 100 mg. of finely ground fiber into a test tube (25 × 200 mm.), and add 35 ml. of chlorite solution (3.5% sodium chlorite in a 3.0% acetic acid solution). Place a smaller test tube in the larger test tube to act as an air condenser, then place the tubes in a water bath maintained at 60° to 70° C. for 4 hours, with occasional stirring. Filter the contents through a tared medium fritted-glass filter, and wash with hot water, 3% sodium bisulfite solution, next with 5% sulfuric acid, and finally with hot water until free from sulfate. Dry at 105° C. for 4 hours, correct for ash, and report as per cent cellulose.

EXPERIMENTAL

Tests made using Cuban kenaf (*Hibiscus cannabinus*) fiber to study the boiling time and the most effective concentration of monoethanolamine in ethylene glycol are reported in Tables I and II.

Kenaf is a bast fiber closely resembling jute; an average analysis of the green processed fiber is shown in Table VIII. Concentration of amine has very little effect on the removal of extraneous materials from the fiber, whereas 2 hours' boiling removed within 2.5% of the total removable material, if the bleached determination in Table II may be taken as the actual percentage of cellulose in the fiber. A large amount of the additional material removed after 2 hours was shown by tests to be largely ash. Therefore 2 hours were believed sufficient boiling in the reagents for most fibers and 10 to 25% monoethanolamine will give optimum extraction. Bleaching is necessary in most cordage fibers to remove the last traces of lignin; however, some of the bleached samples showed very little loss in weight, and for certain types of fiber bleaching may not be necessary—for example, in Table VIII flax and abaca showed very little additional loss on bleaching.

In Table III are shown a number of amines in ethylene glycol and ethylene glycol alone for extraction purposes on kenaf fiber. The amines are equally effective, with the exception of butyl urea (Buramine). Ethylene glycol when used alone removes 24.4% of the total 31% extractable material. The effectiveness of the alcoholic amines as extracting agents for cellulose is believed due to their strong reducing action (7, 8). Reagents which are effective as extractants for cordage fiber cellulose should be very effective in determining the cellulose in cotton fiber; therefore, very little experimental work was necessary on cotton. Tables IV and V show very good agreement between duplicates and methods on cotton fiber.

Table III. Effect of Organic Amines in Ethylene Glycol and Ethylene Glycol Alone on Removal of Encrustants from Kenaf Fiber

Fiber	Concentration of Amine in Ethylene Glycol, %	Amine	Boiling Time, Hours	Cellulose
Cuban kenaf	25	Monoethanolamine	2	70.5
	25	1-Amino-2-propanol	2	70.6
	25	Diisopropanol amine	2	70.5
	25	Butyl urea (Buramine)	2	71.9
	100	Ethylene glycol alone	2	75.6

PROPOSED METHOD OF CELLULOSE DETERMINATION IN PLANT FIBERS

Weigh 100 mg. of finely ground, dried fiber into a 32 × 200 mm. test tube, add 25 ml. of monoethanolamine reagent eliminate, and reflux with the aid of a cold finger for 2 hours. Partially cool and add 25 ml. of ethanol to aid filtering, then filter through a tared medium fritted-glass crucible and wash with hot water. This treatment is sufficient for cotton fiber. Dry for 4 hours at 105° C. Correct for ash and report as per cent cellulose. (Heating may be done by using an electric micro-Kjeldahl digestion heater or an oven type of heater made from a

Table IV. Effect of 25% Monoethanolamine in Ethylene Glycol on Cotton Fiber Cellulose Determination

			Cellulose[a] Found	
No.	Fiber	Boiling Time	Monoethanolamine in ethylene glycol	Monoethanolamine in ethylene glycol plus bleach[b]
		Hours	%	%
26	Cotton fiber	2	95.7	95.5
			96.0	95.8
26	Cotton fiber dewaxed	2	96.8	96.6
			96.6	96.4
29	Whatman No. 2 filter paper	2	99.8	99.8

[a] Based on oven-dry weight.
[b] 3.5% NaClO₂ in 3% acetic acid for 0.5 hour at 70° C.

Table V. Comparison of Monoethanolamine in Ethylene Glycol Method on Cotton Fiber with Chlorite and Kier Boil Methods

		Cellulose[a] Found		
No.	Fiber	Monoethanolamine in ethylene glycol, %	Chlorite, %	Kier boil, %
23	Cotton fiber dewaxed	94.5	94.8	94.8
24	Cotton fiber dewaxed	95.9	96.0	95.8

[a] Based on oven-dry weight.

Table VI. Precision of Monoethanolamine in Ethylene Glycol Method in Determination of Cellulose in Cotton Fiber

Determination	1	2	3	4	5	6	Mean
Cellulose, %	91.0	91.2	91.0	91.4	91.6	91.5	91.2
Deviation from mean, %	0.2	0.0	0.2	0.2	0.4	0.3	
Average % deviation of cellulose from mean	0.2						

Transite box employing a resistance coil. The temperature may be controlled by means of a Variac.)

With other fibers, it may be necessary to bleach for 0.5 hour with a chlorite solution at 60° to 70° C. This may be done in the crucible by placing it in a dish and maintaining the temperature on a hot plate or steam bath. On fibers of very high lignin content the bleach treatment may be repeated until no further loss in weight is obtained or no further test for lignin results from the use of sulfite solution. After the bleach is removed, the cellulose is washed with hot 3.0% sodium bisulfite solution followed by 5% sulfuric acid, and finally with hot water until free from sulfate. The cellulose is then dried by alcohol and ether or in an oven, corrected for ash, and reported as per cent cellulose. Fibers of like character may have an ash factor determined, eliminating the ash determination on each sample.

RESULTS AND DISCUSSION

In Tables IV and V are shown the cellulose values for dewaxed and undewaxed cotton fiber. The bleach has removed very little additional material in either sample. Consequently, unless extremely accurate results are required, it may be dispensed with for cotton. The action of the reagent on Whatman filter paper is shown in Table IV. This material must be considered a highly degraded cellulose when compared with plant fibers, yet a 2-hour boil with the monoethanolamine reagent removed only 0.2% of material. Further bleaching did not show any decrease in the amount of cellulose. Table V shows a comparison of the proposed method with the kier boil and chlorite determinations. There is little to choose among the three methods; however, for ease of operation and the length of time to complete a determination, the advantage is in favor of the monoethanolamine method. Time is saved because it is not necessary to dewax the fiber. Table IV shows a difference of 0.85% between the two cotton samples, which is approximately the alcoholic extractable of this variety of cotton.

The precision of the method for cellulose in cotton fiber is given in Table VI.

The average deviation of cellulose from the mean is approximately 0.2%. This precision may be considered good for a biochemical determination of this magnitude.

The difficulty in analyzing cordage fiber for cellulose is indicated in Table VIII, where the various methods show considerable variation. The results are given for lignin and total pentosans, for cellulose by the chlorite cellulose method, the Kurschner and Hoffer method, and the proposed method with and without bleach, and the pentosan and lignin remaining after monoethanolamine reagent extraction for four hard, three soft, and one hull fiber. The fibers listed with the exception of flax are high in pentosans and consequently high in associated pentosans. Because this fraction is believed to be an integral part of the cellulose molecule, its removal in determining cellulose has proved difficult (10, 11). Most cellulose methods will not remove this pentosan fraction. The true cellulose may be had by subtracting the pentosan in the crude-cellulose fraction from the crude cellulose. (The term "true cellulose" does not apply to cotton in the same sense that it does to the cordage fibers. Cotton fiber after purification results in a relatively pure cellulose. Cordage fibers differ in this respect. They contain varying percentages of pentosans which are difficult to remove and which should be corrected to give a cellulose approaching the standard of purity obtained from cotton fiber.) If the true cellulose is determined by the monoethanolamine method, the Kurschner and Hoffer method approaches the true cellulose value; both average 60.1% for the eight samples analyzed. The proposed method after bleach agrees very well with the Norman-Jenkins method for the hard fibers and coirs, as shown in Table VIII, but the great variation in processing, which produces fiber of variable cellulose content, has prevented any agreement in the soft fiber values.

The chlorite method gave the highest cellulose values, which indicates incomplete removal of pentosans, as the chlorite reagent has good bleaching properties and should remove lignin from most plant fibers.

The total pentosans reported in Tables VII and VIII include the pentose units from hemicellulose and the polyuronides from the pectic substances. These latter materials are present in small quantity in most fibers and for comparative purposes no attempt was made at a separation.

Table VII. Chemical Analysis of Cordage Fibers (10, 11)

Fiber	No. of Samples	Cellulose %	Lignin %	Total Pentosans %	Pentosans in Cellulose %
Abaca (leaf)	1	74.1	8.51	14.05	14.0
Henequen (leaf)	None	...	...	...	...
Sansevieria (leaf)	1	75.1	7.31	19.13	12.3
Sisal (leaf)	2	77.2	6.30	20.50	17.5
Flax (bast)	2	91.7	4.66	3.24	2.2
Jute (bast)	21	72.8	11.99	15.85	11.8
Kenaf (leaf)	1	76.6	5.95	17.52	13.8
Coir (hull)	6	53.9	30.41	21.82	12.7

A volumetric determination as proposed by Kettering and Conrad (6) can be used for cotton, but for fibers with high associated pentosans a factor must be determined to compensate for the five carbon sugars; obviously, the factor will vary with each type of fiber. If the fiber is sufficiently uniform, so that an ash factor can be determined, the time saved in the volumetric method hardly offsets the loss in accuracy.

The main reasons for using the monoethanolamine in ethylene glycol are the saving in time and labor. The monoethanolamine reagent requires no elaborate means of temperature control,

Table VIII. Comparison of Chlorite, Kurschner and Hoffer, and Monoethanolamine Methods

Fiber	Total Lignin %	Total Pentosan %	Chlorite method %	Kurschner and Hoffer method %	Mono-ethanol-amine in ethylene glycol %	Mono-ethanol-amine in ethylene glycol plus bleach[b] %	Pentosans in cellulose %	Lignin in cellulose %
Abaca	8.6	15.5	86.1	66.0	81.0	80.3	15.7	0.28
Henequen	5.4	16.7	88.3	64.6	87.4	84.2	17.3	0.38
Sansevieria	6.1	16.2	88.0	59.3	78.1	76.4	17.0	0.56
Sisal	5.8	16.4	89.1	59.8	80.0	77.2	17.1	0.55
Flax	8.6	8.8	87.7	73.1	80.5	80.5	2.2	1.95
Jute	10.8	15.4	87.6	62.2	80.5	78.2	15.8	1.25
Kenaf	8.0	15.8	82.1	57.7	70.5	69.1	16.4	2.06
Coir	33.1	16.0	61.3	37.8	55.6	53.9	17.5	10.35

Cellulose[a] Found. Determination on Cellulose from Monoethanolamine Reagent.

[a] Based on oven-dry basis.
[b] 3.5% NaClO₂ in 3% acetic acid solution.

for the glycol boils at 197° C. or far enough above the amine to make use of its maximum extraction ability. Furthermore, the concentrated amine, being a reducing agent, has a tendency to deteriorate on boiling and consequently loses its extraction properties, whereas in the glycol the amine shows no tendency to break down. The cost of the monoethanolamine reagent as proposed is considerably less (approximately one half) than the monoethanolamine as used alone, one third less reagent is used. It is possible to reclaim both reagents, but as the cost of the reagent is not excessive, no attempt is made to reuse it.

The effect of monoethanolamine on cellulose has been shown to be mild (12) and there is every reason to believe the amine in ethylene glycol would be less degrading. Recent experiments (3, 5) on the degree of polymerization of plant fiber after boiling

in higher boiling alcohols and glycols have shown a minimum of cellulose chain degradation.

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Determination of Titanium and Iron

Rapid Control Determination of Both in Same Sample

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The determination of titanium and iron in a single sample by titration of a reduced solution with standard potassium permanganate is described. Methylene blue is employed for the titanium end point and o-phenanthroline ferrous complex is used for the iron. Comparison of potential and indicator breaks is shown.

AS ALL commercial titanium ores contain iron, it is frequently desirable to determine these two metals in a single titration. Various methods—colorimetric (1, 4, 8, 12, 15, 16), gravimetric (2, 10, 13), and volumetric (3, 6, 9, 11, 14)—have been employed for the determination of titanium. The volumetric methods include titration of the reduced titanium with an oxidizing agent such as potassium permanganate, ferric ion, or methylene blue; all involve a separate determination of iron.

Because the potentials of titanous and ferrous ions are over 500 millivolts apart, it has been possible to titrate first the titanium and then the iron with potassium permanganate. The end points may be determined potentiometrically (7) or by means of methylene blue and o-phenanthroline ferrous complex.

Figure 1 shows the course of the potentiometric titrations (potential against saturated calomel electrode) of two ore solutions which contain different amounts of iron. The agreement of the potential and indicator breaks is indicated.

REAGENTS

Potassium permanganate, 0.1 N.
Methylene blue, 0.5% aqueous solution.
o-Phenanthroline ferrous complex, 0.025 M.
Sulfuric acid, 1 to 1.
Sulfuric acid, 5% by volume.
Amalgamated zinc, +25-mesh zinc (high grade), is prepared in the usual manner.

APPARATUS

Figure 2 illustrates the apparatus used to accomplish the reduction of the titanium and iron and the titration of the reduced solution. The Jones reductor is inserted in one opening in the flask. The rubber stopper in the center supports the inlet and outlet tubes for carbon dioxide and the thermometer. The buret fits into the other opening in the three-necked flask.

DECOMPOSITION OF SAMPLE

Titaniferous material—e.g., a titanium ore (0.25 to 0.35 gram)—is decomposed by fusion in a platinum crucible with fused potas-
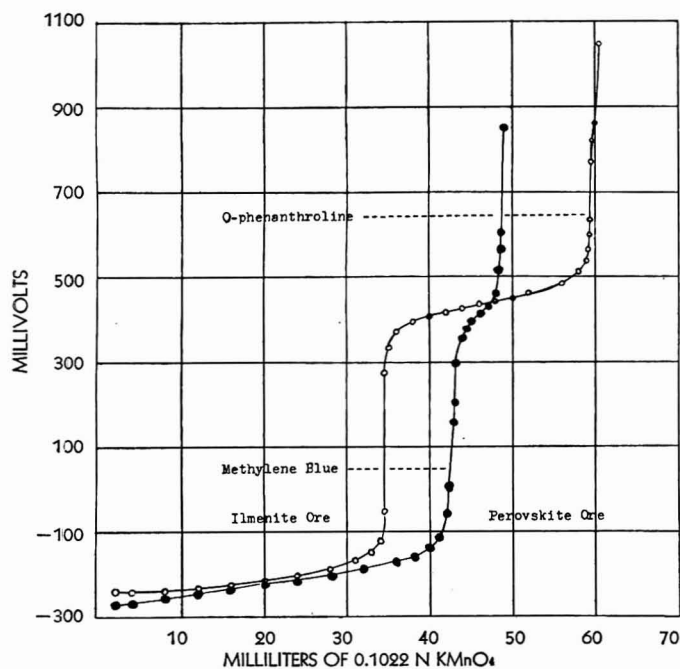
Figure 1. Potentiometric Titration of Ore Solutions

Table I. Analysis of Titaniferous Materials

Material	Proposed Method		Other Methods	
	TiO$_2$	Fe	TiO$_2$[a]	Fe[b]
Titanium ore	%	%	%	%
1	38.1	41.0	38.4	41.2
2	35.5	42.3	35.9	42.5
3	36.5	42.7	36.6	42.9
4	36.3	43.7	36.3	44.1
5	36.4	43.6	36.3	43.7
6	37.0	42.7	36.9	43.0
7	36.0	43.5	36.0	43.7
8	36.5	43.0	36.6	43.2
9	37.6	39.8	37.7	40.1
10	36.9	42.3	37.0[c]	42.2
			36.8[d]	42.3[d]
Bureau of Standards sample 154, numerous determinations	98.5–99.0	0.0	98.7	0.0

[a] Ferric alum titration, after Jones reduction.
[b] Hydrogen sulfide reduction, permanganate titration.
[c] Gravimetric, cupferron precipitation.
[d] Consulting laboratory.

sium bisulfate (7 grams) or by digestion with concentrated sulfuric acid and ammonium sulfate. The decomposition of materials that contain silica is aided by the addition of sodium fluoride in the fusion. The melt is extracted in 25 ml. of sulfuric acid and about 50 ml. of hot distilled water. Solution should be complete before it is transferred to the reductor.

In the analysis of titanium solutions, a suitable aliquot is transferred to a 200-ml. tall-form beaker, treated with 25 ml. of sulfuric acid (1 to 1), and diluted to approximately 100 ml. with distilled water.

PROCEDURE

The zinc in the reductor is washed with hot sulfuric acid (5%) and hot water, then the liquid level is drawn down to approximately 5 cm. (2 inches) above the level of the glass wool. [In starting a new column, it should be washed well with hot sulfuric acid (5%) to dissolve any contaminating free iron and a standard sample should be run to determine whether the column is operating satisfactorily before employing it for routine analyses.] The receiving flask containing 50 ml. of sulfuric acid (1 to 1) is connected to the reductor. If sodium fluoride was employed in the fusion, about 1 gram of boric acid (5) should be added. A slow stream of carbon dioxide is started to flush air from the receiving flask. The nearly boiling solution to which 4 drops of methylene blue solution have been added is transferred to the reductor and reduced by contact with the zinc for about 15 minutes. It is then withdrawn at approximately 100 ml. per minute and the column is washed with four 100- to 125-ml. portions of hot sulfuric acid (5%), then with hot, distilled water to a total volume of about 900 ml. Care is exercised during washing to prevent entrance of air into the zinc column. The reduced solution has a temperature of about 60° C. At lower temperatures, the indicator reaction becomes sluggish.

The reduced solution is titrated with the standard potassium permanganate solution until 1 drop of potassium permanganate produces a light blue color which represents the titanium end point. During titration, it may be necessary to increase the carbon dioxide flow to provide ample agitation.

The titrated solution is now cooled by means of a cooling water bath to about 25° C., because the indicator is unstable at higher temperatures. Eight drops of o-phenanthroline ferrous complex solution are added and the titration with potassium permanganate is continued until a bluish gray color is obtained. One drop of permanganate should produce a clear blue color at the iron end point. The iron titration is the difference between the total and the titanium titration. An indicator correction of 0.25 ml. should be made on the iron titration because of the consumption of permanganate by the o-phenanthroline ferrous complex.

Figure 2. Apparatus

A. Jones reductor
C. Solution level
E. Zinc
G. Glass wool
H. Thermometer
L. Buret
N. Carbon dioxide exit
S. Three-necked 1-liter flask
T. Carbon dioxide entrance

RESULTS

The results shown in Table I have been obtained on titaniferous materials.

DISCUSSION

This method, like other methods that employ potassium permanganate as the oxidant, is subject to interference by vanadium, chromium, molybdenum, or other elements whose ions are reducible in the Jones reductor. If desired, corrections for these elements may be made. However, the total amount of these interfering elements is generally less than 0.3% on most high grade titanium ores.

This method avoids the separate determination of iron and has been found very useful in the routine analysis of titanium ores and solutions.

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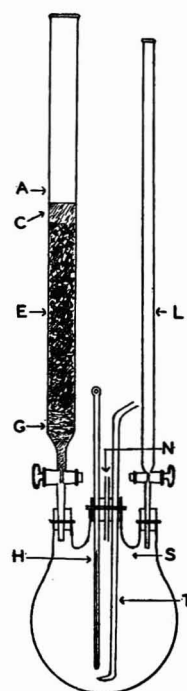(16) Wells, R. C., *J. Am. Chem. Soc.*, 33, 504 (1911).

Volatilization of Elements from Perchloric and Hydrofluoric Acid Solutions

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Mixed perchloric and hydrofluoric acid solutions containing compounds of 37 elements were evaporated at 200° C. Analysis of the residues showed that appreciable quantities of boron, silicon, germanium, arsenic, antimony, chromium, selenium, manganese, and rhenium are lost during such treatment. The loss is due, in most cases, to the volatilization of the fluorides of the elements. No loss was observed on similar treatment of compounds of sodium, potassium, copper, silver, gold, beryllium, magnesium, calcium, strontium, barium, zinc, cadmium, mercury, lanthanum, cerium, titanium, thorium, tin, lead, vanadium, bismuth, molybdenum, tungsten, uranium, iron, cobalt, and nickel.

THE preparation of a solution of a sample for analysis is not always handled so carefully as are the succeeding analytical operations. Lundell and Hoffman (5) have pointed out many solvents and fluxes for preparation of solution, and discussed the difficulties often encountered in this operation. They state that, depending on the solvent, many elements are volatilized during the preparation of solution. Mixed sulfuric and hydrofluoric acids have long been used as such a solvent. More recently, perchloric acid has been substituted for the sulfuric acid in many cases (13). Many silicate minerals are dissolved

Figure 1. Apparatus

a. Electric hot plate at 75° to 80° C.
b. Air blast to increase rate of evaporation
c. Resistor in series with overhead heating element
d. Heating element (Arthur H. Thomas Co., 5790 heating unit)

by treatment with mixed perchloric and hydrofluoric acids, with the coincident loss of silicon as the volatile tetrafluoride (8). No systematic study has been made of the possible volatility of other elements from such an acid mixture. Hoffman and Lundell (4) have studied the volatility of the more common elements from six different mixed acid solutions, by analyzing the distillate obtained from mixed acid solutions of the various elements. Such a procedure, involving hydrofluoric acid, would require a platinum still.

The procedure used in the present work was less direct. Samples were fumed with perchloric and hydrofluoric acids in platinum dishes, just as in the course of analytical procedures. The resulting solutions were analyzed and losses determined by

difference from the known weight of sample taken. In every case, blanks were run without hydrofluoric acid.

PROCEDURE

An appropriate weight of a high purity compound of the element was dissolved in a solution (usually perchloric acid) which introduced no interfering constituents into the subsequent analysis. This solution was filtered and diluted to 500 ml. and 25-ml. portions were taken for analysis. The portions were evaporated to 3 to 5 ml. in platinum dishes, and 10 to 15 ml. of 70% perchloric acid and 8 to 10 ml. of 30% hydrofluoric acid were added. The solutions were evaporated to strong fumes of perchloric acid by surface evaporation (Figure 1). The overhead heating element was adjusted to obtain a temperature of 200° C. at the surface of the liquid, without the air blast in operation. In operation, this easily assembled apparatus gave rapid evaporation with no determinable losses due to spattering. Following the first fuming, 5 to 10 ml. more of 30% hydrofluoric acid were added, and the solution was again evaporated to strong fumes of perchloric acid. The residue was analyzed for the element under consideration. Umpire methods of analysis (2) were used in all cases.

It was necessary to use gravimetric sampling procedures for titanium, germanium, and tin.

RESULTS

The above procedure was used with compounds of 37 elements. Analyses of the resulting solutions gave low results in the case of nine elements (Table I).

The figures in Table I stating the amounts lost are approximate only, and represent the actual losses determined in the course of the standardized procedure. More prolonged fuming with the mixed acid solution did not affect the elements showing no loss, but increased the losses in the other nine elements. For example, it was possible to volatilize all the chromium from solutions by repeated fuming with perchloric and hydrofluoric acids.

DISCUSSION

It was established that fuming perchloric acid oxidizes all elements tested (except manganese and lead) to their maximum valence states. Therefore the use of only one valence state of each element was justified. Lundell and Hoffman (6) give an excellent discussion of the effect of digestion with perchloric acid on the several elements.

The volatility of some compound of aluminum cannot be the cause of low results in the case of aluminum (not included in Table I). According to Zernicke (16), aluminum fluoride sublimes at 1290° C. Marvin and Woolaver (7) have shown that aluminum perchlorate decomposes to yield the nonvolatile oxide of aluminum at 200° to 300° C. The procedure used in the
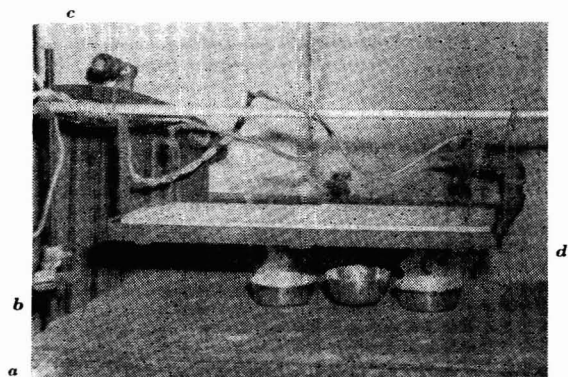
Table I. Effect of Treatment with Perchloric and Hydro-fluoric Acids

No Loss	Apparent Loss
Sodium, potassium	Boron, 100%
Copper, silver, gold	Silicon, 100%
Beryllium, magnesium	Germanium, up to 10%
Calcium, strontium, barium	Arsenic, 100%
Zinc, cadmium, mercury	Antimony, up to 10%
Lanthanum, cerium	Chromium, varies greatly
Titanium, thorium	Selenium, varies greatly
Tin, lead	Manganese, up to 3%
Vanadium	Rhenium, varies greatly
Bismuth	
Molybdenum, tungsten, uranium	
Iron, cobalt, nickel	

analysis for aluminum was gravimetric, and the apparent loss was due to the inability of fuming perchloric acid to remove all fluoride associated with the aluminum as a complex ion. This complex prevents the precipitation of hydrous aluminum oxide in the usual manner (*3*). No attempt is made to explain the loss of manganese; but Hoffman and Lundell (*4*) observed that manganese compounds were slightly volatile from mixed perchloric and hydrochloric acid solutions.

The eight other elements which showed loss on treatment with the acid mixture were expected to do so. Boron and silicon fluorides are very volatile (*8*). Selenium forms at least two volatile fluorides (*14*). Antimony and arsenic form volatile fluorides (*11*), as do chromium (*10*), germanium (*1*), and rhenium (*12*).

According to Ruff (*11*), molybdenum and tungsten form volatile fluorides and oxyfluorides. No such fluorides are formed, however, when salts of these elements are fumed with the acid mixture, despite the vigorous dehydrating action of concentrated perchloric acid. In contrast chromium is easily volatilized as the oxyfluoride from the acid mixture. This indicates that it is virtually impossible to predict the volatility of many elements from mixed acid solutions.

Selenium and rhenium are appreciably volatile from fuming perchloric acid solutions alone, as might be expected from the physical properties of the oxides of these acid-forming elements (*9, 15*). The volatility of rhenium, under these conditions, has been suggested (*6*).

The results of this investigation show that nine elements, if present in a material that is subjected to routine analytical treatment with perchloric and hydrofluoric acids, will be lost in varying amounts. Consequently, determination of any of these elements in the resulting solution would give low values based on the original material.

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Determination of Alumina in Steel

A Spectrochemical Method

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THE extensive use of aluminum in the steel-making industry as a deoxidizer, for control of grain size, etc., has created a need for an accurate and rapid means of determining aluminum in steel. The aluminum may be present in steel in solid solution and as compounds such as alumina, Al_2O_3, and aluminum nitride, Al_2N_2. Aluminum present in solid solution is soluble in hydrochloric acid. The aluminum compounds are insoluble. When alumina is determined in steel, the insoluble residue is washed with a solution of sodium carbonate to ensure the removal of any nitrides left undissolved by washing with dilute acid (*1*).

In this paper the term "alumina" designates the combined aluminum that remains in the residue after the sodium carbonate wash.

Gravimetric and volumetric methods of analysis for alumina, because of the many separations and reprecipitations involved, have proved too tedious and time-consuming when a relatively

large number of samples are to be analyzed. Colorimetric and photometric procedures also are too involved, because they require the complete removal of iron and other interfering elements before comparison can be made.

No particular problems were encountered in the spectrographic determination of the total aluminum which is carried out in this laboratory by means of pressed pellets made from drillings or millings of the steel using a high voltage condensed spark for excitation. Total aluminum results obtained in this manner are, for practical purposes, independent of the concentration of alumina. Results on several samples analyzed spectrographically for total aluminum containing alumina in concentrations as high as 0.06% were in good agreement with the chemical results obtained on the same samples.

Because spectrographic analysis does not provide a direct means for distinguishing between acid-insoluble and acid-

A chemical separation of the acid-soluble and acid-insoluble *components* of the steel is made. The aluminum present in the acid-insoluble portion, after removal of aluminum nitride, is considered as being the aluminum oxide in the steel. The acid-insoluble portion is fused and taken up in a weak acid solution. Iron is added as an internal standard. Spectrographic examination of the solution is made by means of a dropping electrode whereby fresh solution is continuously supplied to the spark gap during exposure. Calibration is based on synthetic standards, covering approximately 0.001 to 0.08% alumina.

soluble aluminum, the only recourse seems to be a combination of chemical and spectrographic methods. In this procedure the two are separated chemically. A solution of the alumina is prepared and the determination is made spectrographically.

Two articles that describe a spectrochemical method for alumina in steel are by Schliessmann (4) and Carlsson (2), who separate chemically the acid-soluble and acid-insoluble aluminum. Carbon electrodes are impregnated with the solution to be analyzed. Schliessmann uses a strong controlled spark for excitation, and Carlsson employs a Pfeilsticker arc.
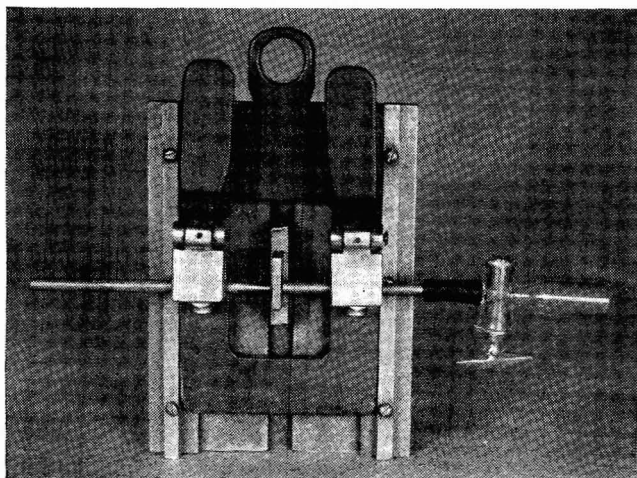
Figure 1. Spark Stand

In this laboratory the soluble and insoluble components of the steel are separated by dissolving the sample in dilute hydrochloric acid and filtering to remove the insoluble residue. The residue is fused with an appropriate reagent, and the melt is dissolved in dilute acid. Iron is added to this solution as an internal standard. Excitation of the solution is carried out in such a way that fresh solution is continuously supplied to the spark gap during the exposure. The working curves are based on synthetic standard solutions.

CHEMICAL PROCEDURE

Preparation of Samples. Ten grams of millings or drillings are transferred to a 600-ml. Pyrex beaker and treated with 400 ml. of 1 to 3 hydrochloric acid. The beaker is covered with a cover glass, placed on a steam plate, and heated to a temperature just below 100° C. until all the soluble matter is in solution. The beaker is removed from the steam plate and the walls are washed. The cover glass is scrubbed with a rubber-tipped glass rod and carefully rinsed with a jet of hot water. The solution is filtered upon a very close textured 11-cm. filter paper containing a small amount of ashless paper pulp. The filter is then washed several times with warm dilute 1 to 20 hydrochloric acid and with hot water until free from iron salts. The residue and paper are washed with 150 ml. of a 3% solution of sodium carbonate (80° to 90° C.), then successively with hot water, a solution of 1 to 10 hydrochloric acid, and finally hot water. The paper and residue are transferred to a platinum crucible and heated gently to char the paper without allowing it to flame. Finally, the

temperature is increased and the paper ignited under good oxidizing conditions until all carbon is removed.

The resulting ash is fused with 2 grams of potassium pyrosulfate in a covered platinum crucible. The melt is cooled and dissolved in a minimum volume of hot water and 5 ml. of concentrated hydrochloric acid. The solution is transferred to a 100-ml. beaker, covered, and heated to boiling to ensure complete solution. At this point, 1 gram of iron is introduced as an internal spectrographic standard by adding 10 ml. of a concentrated hydrochloric acid solution containing 0.10 gram of iron per ml. The solution is then diluted to approximately 50 ml. and cooled. Portions of this solution, which now contains the aluminum formerly present in the steel sample as alumina, are used for the spectrographic examination.

No separations are carried out after the residue is dissolved, as none of the elements other than the iron left in the residue could offer any interference in the applied spectrographic method. Careful investigation proved that the quantity of iron left in the solution relative to the amount added as an internal standard is negligible and may be disregarded.

Preparation of Standards. Ten-gram samples of pure iron are transferred to 600-ml. beakers (Westinghouse pure iron was found to be aluminum-free and was used for this purpose). The procedure for the preparation of steel samples is followed to the point where the internal reference standard iron solution is added. Then aliquot portions of a standard aluminum solution, 1 ml. of which is equivalent to 0.02% alumina, are added to give a range of standards from 0.005% to 0.08% alumina. This standard aluminum solution is prepared by dissolving 0.5295 gram of pure aluminum foil in 100 ml. of 1 to 1 hydrochloric acid. When solution is complete, 450 ml. of concentrated hydrochloric
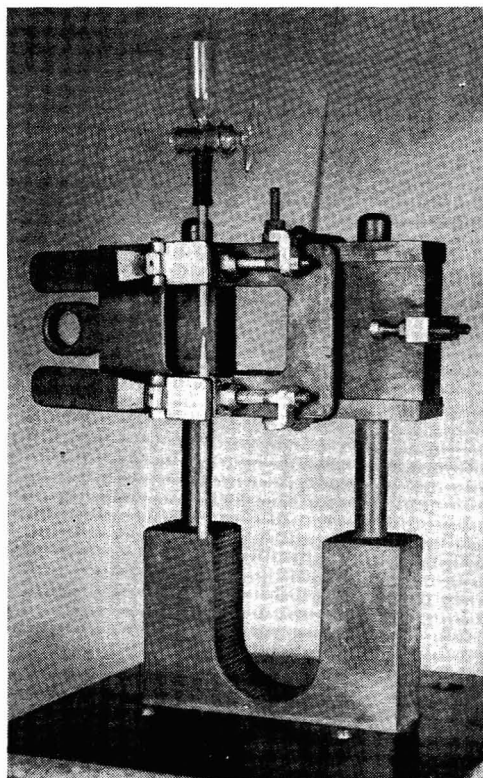

Figure 2. Spark Stand

acid are added and diluted to 5 liters with a 2% solution of ammonium chloride.

To check the aluminum content of this standard solution, several aliquot portions were taken and the aluminum was determined by established chemical procedures.

SPECTROGRAPHIC PROCEDURE

Solution Apparatus. Because of the low concentrations of alumina usually encountered (0.003 to 0.05%), various methods of excitation were tried to find one that would give the necessary sensitivity, combined with adequate reproducibility. Flat graphite electrodes, impregnated with a drop of the solution and vaporized in the high voltage condensed spark, did not give the sensitivity necessary for the lower concentrations. The direct current arc with electrodes treated in the same manner gave adequate sensitivity, but failed in reproducibility tests.

Attention was then turned to the solution method used in Twyman's laboratory (5), whereby fresh solution is continuously supplied at the spark gap during the exposure. In his arrangement, the spark gap is formed by two graphite electrodes 5 mm. in diameter. The solution to be analyzed enters the spark gap through a channel in the lower electrode from a glass container equipped with a special stopcock for controlling the rate of flow of the solution. Twyman's claim of very high intensities and constant sparking conditions with this arrangement was born out in the authors' investigation.

However, in order to adapt the method of excitation to the special spark stand used in this laboratory, and at the same time make it more convenient to change from the solution method of analysis to the routine analysis of solid steel samples carried out with the same spectrograph, it was found desirable to direct the flow of the solution through the upper electrode by means of a dropping electrode similar to that of Keirs and Englis (3).

Any spark stand, suitable for the analysis of pins, can be used for the method of analysis of solutions here suggested. The spark stand used in this laboratory is composed essentially of two parts: a removable frame with clamps for holding the electrodes, and a fixed frame attached to the optical bar of the spectrograph.

Figure 1 shows the electrodes assembled in the removable frame of the stand. The steel spacing block on which the frame is placed is used to position the electrodes.

Figure 2 is a view of the frame and electrodes partially inserted in the fixed part of the stand. Electrical contacts between the two parts are made by means of the banana-type plugs visible in the photograph. The horizontal movement of the frame is regulated by a metal stop, the vertical movement by two set-screws. The stand is machined from Micarta and the clamps are made of stainless steel. The plugs and receptacles are commercially available. For routine analysis of steel, two of the removable frames are used; one set of pins is sparked, while others are prepared and positioned in the extra frame.

The solution apparatus finally adopted in this laboratory is shown in Figure 3.

A is a glass reservoir graduated in milliliters. Its capacity should be about 20 ml. *B* is a stopcock for regulating the rate of flow of the solution. *C* is a rubber coupling, and *D* is a 0.6-cm. (0.25-inch) special graphite electrode (high purity) about 10 cm. (4 inches) long with a 0.15-cm. ($^1/_{16}$-inch) channel along the axis. The lower electrode, *E*, is a 0.25-inch high purity electrode pointed with a pencil sharpener. (A suitable reservoir for the apparatus was made by cutting down the tube of a small buret, and removing most of the delivery tip, leaving only enough for fastening the rubber coupling.)

Excitation. To prepare the apparatus for use, a graphite electrode is coupled to the glass reservoir and the system is flushed with approximately 5 ml. of the solution to be analyzed.
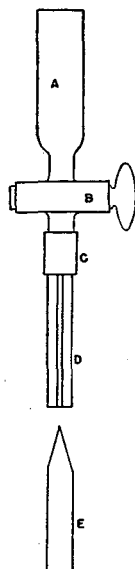
Figure 3. Solution Apparatus

Table I. Effect of Rate of Flow

Exposure	Drops per Minute	Al_2O_3,%
1	7	0.014
2	7	0.013
3	7	*0.013*
4[a]	7	0.011
5	9	0.014
6	12	0.014
7	12	0.014
8	27	0.014
9	27	0.014
10	30	0.014
11	30	0.014
12	33	0.015

[a] Flow of solution interrupted during exposure by obstruction in channel of graphite.
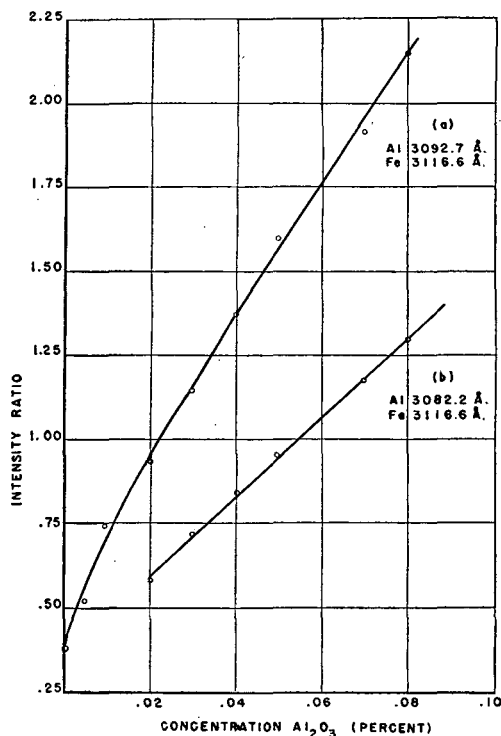
Figure 4. Working Curves Prepared with Synthetic Standards

After proper alignment of the electrodes, the stopcock is closed and the reservoir filled with several milliliters of the solution to be analyzed. The stopcock is then adjusted so that the rate of flow is 10 to 20 drops per minute. For most samples an exposure of 80 seconds is made. Where the concentration is expected to be very low, exposures up to 100 seconds are made.

Rigid control of the rate of flow, as well as the exposure time (see Table II), is unimportant, provided fresh solution is continuously supplied at the spark gap at a rate exceeding approximately 7 drops per minute. Results in Table I were obtained by varying the rate of flow from 7 to 33 drops per minute.

The low result obtained on the fourth exposure is characteristic of those resulting when the rate of flow is very low (less than 7 drops per minute) or if for some reason the flow is interrupted. In such cases, lower intensity ratios are obtained together with greater scattering of the results. Although results are unaffected by a relatively rapid flow, it is desirable in practice to keep the rate of flow at a minimum to reduce the spray that results from an excessive flow of solution into the spark gap.

Table II is a tabulation of results obtained on four successive 80-second exposures. The spark was started and continued for 320 seconds without interruption. The plate was racked

Table II. Results of Four 80-Second Exposures

Time, Seconds	Al_2O_3, %
80	0.019
160	0.020
240	0.020
320	0.020

Plate racked after each 80-second interval.

Table III. Reproducibility Obtained on Four Samples of Steel

Sample	$Al_2O_3{}^a$, %	Deviation from Average, Al_2O_3, %	Sample	Al_2O_3, %	Deviation from Average, Al_2O_3, %
1	0.009	0.000	3	0.053	0.000
	0.008	−0.001		0.053	0.000
	0.009	0.000		0.054	+0.001
	0.008	−0.001		0.055	+0.002
	0.011	+0.002		0.050	−0.003
	0.010	+0.001		0.050	−0.003
Av.	0.009		Av.	0.053	
2	0.063	−0.003	4	0.044	−0.002
	0.070	+0.004		0.046	0.000
	0.063	−0.003		0.045	−0.001
	0.068	+0.002		0.047	+0.001
	0.066	0.000		0.049	+0.003
				0.046	0.000
Av.	0.066		Av.	0.046	

^{a} Results are single determinations made on different portions of the same solution.

Table IV. Reproducibility Obtained with Different Solutions of Same Sample

Solution No.	$Al_2O_3{}^a$, %	Deviation from Average, Al_2O_3, %
1	0.024	0.000
2	0.023	−0.001
3	0.027	+0.003
4	0.027	+0.003
5	0.026	+0.002
6	0.018	−0.006
Av.	0.024	

^{a} Each result is average of two determinations.

Table V. Comparison of Chemical and Spectrographic Results

	Per Cent Alumina	
Sample	% Al_2O_3 by chemistry	% $Al_2O_3{}^a$ by spectrograph
A	0.015	0.016
		0.016
		0.018
		0.016
	Av.	0.0165
B	0.009	0.008
		0.009
		0.008
		0.007
	Av.	0.008
N.B.S. 14C	0.006	0.008
		0.006
		0.007
		0.006
	Av.	0.007

^{a} Results are single determinations.

after each 80-second interval. The consistency of the results indicates that several checks may be obtained on a solution without changing electrodes between exposures or interrupting the source of excitation.

A high voltage condensed spark source of the Feussner type is used for excitation. The transformer (approximately 20,000 volts root mean square) charges a 0.021-microfarad capacitor. The added inductance included in the circuit is 0.08 mh.

The spectrograph is a large quartz Littrow type (Hilger) with a dispersion of approximately 5 Å. per mm. in the range used for this investigation. The slit width is 0.02 mm. The spark stand is placed 50 cm. (20 inches) from the slit, and no condensing lens is included in the optical system.

The spectrum is recorded on Eastman spectrum analysis No. 1 plates which are developed for 3 minutes in Eastman D-19 developer at 70° F. and fixed for approximately 1 minute, followed by the usual process of washing and drying.

Working Curves. To cover the range of concentrations usually encountered, two aluminum lines were chosen. Line 3092.7 Å. was found to have the necessary sensitivity for concentrations as low as 0.001% alumina. For higher concentrations (0.03 to 0.08% alumina) it was found convenient to use the weaker aluminum line, 3082.2 Å. As an internal standard, iron line 3116.6 Å. (actually two iron lines not separated with the available dispersion) was found suitable for both aluminum lines.

The only interference to be considered is the superposition of the aluminum line, 3092.7 Å. by an iron line, 3092.79 Å. However, its influence on the sensitivity at even the lower concentrations was found to be negligible.

Figure 4 is a plot of the working curves obtained with the synthetic standards prepared as described in the chemical procedure.

Curve (a) was obtained by plotting the intensity ratio of the stronger aluminum line, 3092.7 Å., and the reference iron line, 3116.6 Å., against concentration of alumina. Curve (b) was similarly constructed using the weaker aluminum line, 3082.2 Å., and the same iron reference line. Each point represents the average of five measurements of each standard solution.

The point at zero concentration for curve (a) was obtained with a sample prepared following the procedure for the standards, except that no aluminum was added. By means of such a solution (aluminum-free), the overlapping iron line, 3092.79 Å., was measured and the intensity ratio at zero concentration determined.

Reproducibility. Shown in Table III are some typical results obtained on a number of samples analyzed. The results given for each sample are single determinations of the same solution. This tabulation, however, does not show the possible error that may be caused by the chemical preparation or by the non-uniformity of the sample. Table IV is a tabulation of the results obtained on several solutions of the same sample prepared over a period of several weeks. Each result reported in this case is an average of two determinations of each sample solution prepared.

The results obtained on these and similar series indicate that the mean deviation of a single determination from the average is approximately ±0.002% of alumina for the range of concentrations covered.

A comparison of chemical and spectrographic results is shown in Table V. The chemical values given for samples A and B are averages of results obtained by ten laboratories using gravimetric methods. The third sample is the National Bureau of Standards sample 14C.

ACKNOWLEDGMENT

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SILICA REFRACTORIES

Spectrographic Analysis Using a Controlled Multisource Power Unit

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In a search for a more reliable and less time-consuming method for the analysis of silica refractories containing approximately 95% silica, several methods were studied which made use of the spectrograph, using different techniques for preparing the sample for excitation. A method was developed which utilized a sample of the pulverized refractory directly for the determination of calcium, magnesium, titanium, aluminum, iron, sodium, and potassium, and determined silicon by difference. Excitation conditions are described and data obtained in determining the precision of the method are presented.

IN CONNECTION with an investigation of the roof life of open hearth furnaces, it became necessary to make fairly complete analyses of a rather large number of silica bricks. Because wet chemistry analyses consume a large number of man-hours, and past experiences indicated that even trained chemists frequently disagreed among themselves on the results, spectrographic methods of making the analyses were investigated.

A number of methods have been described for the quantitative spectrographic analysis of powders and ceramic materials, including silica. Most of them make use of a buffer material which furnishes the internal standard lines, and include carbon to render the mixture conducting, as well as to obtain smoother operation of the source. It is also claimed that the addition of carbon causes elements of widely different volatility to evaporate simultaneously (3).

Fitz and Murray(1) analyzed small samples of powders by mixing 1 mg. of the unknown with 20 mg. of barium nitrate and ammonium nitrate as a buffer, making a pellet 2 mm. in diameter which they burned completely in a carbon holder with a direct current arc, a process requiring about 2 minutes.

Zander and Terry (6) made control analyses of powdered ceramic materials by mixing with a suitable flux, making 1.25-cm. (0.5-inch) briquets of the mixture, and sparking with a critically damped discharge from a controlled Multisource power unit. They observed that different working curves were necessary for samples having different average particle size and even for samples having the same average particle size but received from different suppliers.

Helz and Scribner (2) described a similar method for the analysis of portland cement, but used a high capacity overdamped type of discharge from the Multisource unit.

Smith and Hoagbin (4) also analyzed ceramic materials spectrographically by mixing 2 parts of the 100-mesh sample with 1 part of a suitable internal standard and carbon powder as a carrier. This mixture they placed in a hollow carbon crater and burned for 30 seconds with a 10-ampere direct current arc.

The method herein described for silica refractories having a silica content of approximately 95% has the advantage of permitting the direct use of a sample pulverized to pass a 100-mesh sieve; it thus materially increases reproducibility and makes possible a saving of time otherwise devoted to preparing the mixture and making pellets. This method consists of using, as the lower electrode, a center-post crater type carbon, containing the sample, and a pointed upper electrode. Thus the carbon center post furnishes the necessary conductivity, and the major constituent, silica, furnishes the internal standard lines.

EQUIPMENT

All the equipment used is commercially available. Excitation is obtained from a controlled Multisource power unit. The spectrograph is a standard 1.5-meter type with a 24,000 line per inch grating have a uniform dispersion of 7 Å. per mm. The spectrum is recorded on Eastman spectrum analysis No. 1 film. Spectral line densities are determined by reading their transmittances on a projection type densitometer employing a photocell and galvanometer.

PROCEDURE

The dried sample which has been pulverized to pass through a 100-mesh sieve is used directly. The lower positive electrode consists of a center-post crater type carbon made from 0.25-inch spectrographic carbons (Figure 1). The sample is forced into the crater by gently pressing the crater into the sample and twisting slightly, repeating if necessary until the crater is level full. The upper negative electrode is hemispherically tipped and also made from 0.25-inch spectrographic carbons. An analytical gap of 3 mm. is used. It is then sparked with a high capacity overdamped condenser discharge.

Two sets of excitation conditions are used as listed in Table I, one for calcium oxide, alumina, ferric oxide, magnesia, and titania; the other for the alkalies sodium oxide and potassium oxide. No prespark is used for either. In the case of the alkalies, a 30-second exposure with 40 mfd. does not produce lines of sufficient intensity. Because at the end of this time, most of the material is blown out of the crater, a longer exposure has no effect. This is over-
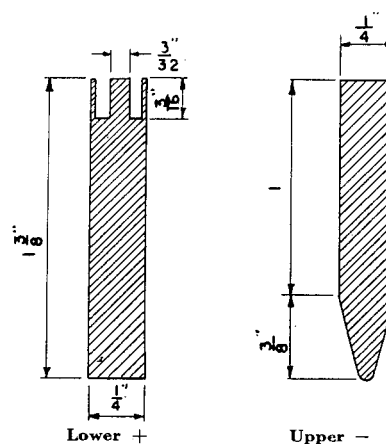

Lower +   Upper −

Figure 1. Longitudinal Section of Electrodes

Table I. Excitation Conditions

	Alkalies	Others
Output potential, volts	940	940
Capacitance, mfd.	60	40
Inductance, mh.	480	480
Resistance, ohms	25	25
Phase angle, °	70	70
Charge vs. discharge, °	180	180
Exposure period, sec.	20	30
Filter	None	Screen

come by increasing the capacitance to 60 mfd. and exciting two samples for 20 seconds each, the spectra from the two separate sets of electrodes being superimposed for each determination.

The film is developed in Eastman D-19 developer for 3 minutes at 70° F., hardened in a 5% solution of Eastman Kodak liquid hardener for 15 seconds, fixed in Eastman concentrated x-ray fixer for 1 minute, washed 1 minute, and dried for 1.5 minutes on a film dryer.

PHOTOMETRY

Analytical line pairs are listed in Table II. In general, transmittance readings should be between 10 and 90%. The average of duplicate determinations is reported. A control standard is run with each set of samples.

Intensity ratios of selected analytical lines compared to a silicon line as internal standard are determined and the various concentrations are read from analytical curves calibrated to read directly as the oxides of the elements determined.

Table II. Analytical Line Pairs

Element	Wave Length, Element Line, Å.	Wave Length, Silicon Line, Å.	Index[a] (Oxide Curves)	Range (Oxide), %
Ca	3158.9	2532.4	2.17	1.00 to 4.00
Al	3961.5	2532.4	1.14	0.30 to 1.75
Fe	3020.6	2532.4	0.45	0.20 to 1.00
Mg	2852.1	2532.4	0.13	0.05 to 0.30
Ti	3349.0	2532.4	0.12	0.05 to 0.20
K	4044.1	3991.6	0.005	0.01 to 0.50
Na	3302.3	3991.6	0.06	0.03 to 0.35

[a] Defined as concentration at which intensity ratio of analytical line pair is unity.

In attempting to apply this method to other types of refractories having higher ferric oxide content, some interference of Fe 2532.5 with Si 2532.4 was noted, especially above about 2% ferric oxide. Most of the above ranges could undoubtedly be extended either way if desired.

ACCURACY AND PRECISION

The accuracy of any spectrographic result depends altogether on the accuracy of the working curves used, provided that the sample being analyzed is similar in composition to the samples used in constructing the working curves. The accuracy of the working curves depends on the accuracy of the values used as standards in establishing the working curves, as determined by wet chemical methods. Assuming that dependable standards have been used to construct the working curves, and that the curves are being used in the analysis of materials whose composition is essentially similar to that of material used in establishing the curves, the essential point to consider is the precision or reproducibility of the method. Comparison of the results obtained on a number of samples with that obtained by wet chemical methods is not always a fair criterion, for not only are the accuracy and precision of the spectrographic method involved, but also those of the wet chemical methods.

Table III. Composition of Samples

No.	SiO₂, %	Fe₂O₃, %	Al₂O₃, %	TiO₂, %	CaO, %	MgO, %	Na₂O, %	K₂O, %
1	93.94	0.66	1.96	0.16	2.29	0.21	0.06	0.29
2	95.64	0.55	1.02	0.09	2.10	0.17	0.04	0.18
3	...	0.43	0.43	0.07	1.58	...	0.10	0.05
4	...	...	...	⊺...	3.58	...	...	...

In this work, the following standards were used to establish working curves: (1) National Bureau of Standards silica brick, No. 102; (2) a sample of silica brick prepared by a Carnegie-Illinois Steel Corporation laboratory and analyzed cooperatively by eight laboratories; and (3) from the analyses of several samples of

Table IV. Analysis of Cooperative Sample

No.	Fe₂O₃, %	Al₂O₃, %	CaO, %	MgO, %	TiO₂, %	Na₂O, %	K₂O, %	SiO₂ (Diff.), %
1	0.58	1.02	2.07	0.17	0.09	0.05	0.22	95.80
2	0.51	1.02	2.04	0.16	0.08	0.05	0.19	95.95
3	0.58	1.04	2.34	0.18	0.10	0.05	0.23	95.48
4	0.54	1.01	2.06	0.17	0.09	0.04	0.16	95.93
5	0.62	1.03	1.92	0.18	0.09	0.04	0.24	95.88
6	0.57	1.05	2.11	0.16	0.09	0.05	0.18	95.79

silica brick, certain values were selected which corresponded to values needed to construct working curves to cover a definite range. These determinations were carefully checked, so as to arrive at the most accurate values obtainable.

The composition of these samples is tabulated in Table III.

In order to establish the precision of the method, 29 analyses were made of the above-mentioned Carnegie-Illinois cooperative sample, determining seven elements on each. Because it was the intention' to report silica by difference when this method was used, calculations were made for silica on all the analyses. A few results selected at random from these analyses are shown in Table IV.

From the complete data there have been calculated the standard deviation and per cent standard deviation for the eight elements determined. In doing so, the A.S.T.M. manual on presentation of data was followed. This method uses the formula $s = \sqrt{\frac{\Sigma d^2}{n}}$ where d is difference between individual results and the average, and n is the total number of determinations. If $n-1$ is used in this formula as recommended by Snedecor (5) and others, the value for s will be slightly greater. Results are summarized in Table V.

Table V. Standard Deviation

Element	Average, %	Standard Deviation, %	% Standard Deviation	No. of Determinations
SiO₂	95.80	0.15	0.16	27
CaO	2.07	0.12	5.8	29
Al₂O₃	1.03	0.035	3.4	29
Fe₂O₃	0.58	0.035	6.0	29
MgO	0.17	0.007	4.1	29
TiO₂	0.09	0.005	5.5	29
Na₂O	0.05	0.006	12.0	27
K₂O	0.21	0.03	14.3	27

DISCUSSION

It is possible to analyze spectrographically a powdered sample of ceramic or refractory material without the addition of graphite or a buffer when it contains 95% or more of a major constituent, by volatilizing the sample from a center-post crater type carbon. For elements giving faint lines, this operation may be repeated, and the images superimposed and integrated on the film or plate. Although precision is not so high as in the case of metals, it is very satisfactory for the routine analysis of this type of material.

Some error is undoubtedly introduced into the method by using an internal standard line so widely separated from certain of the analytical lines, notably the aluminum 3961.5 line. However, the number of silicon lines available is limited and the aluminum line used gave better precision than any of the others that might have been chosen.

During the exposure, the refractory material is blown out of the crater into the gap, but this is an advantage rather than a disadvantage as fresh material is drawn up into the gap continuously. There is only a small amount left in the crater at the end of the exposure. The presence of the center post tends to slow up the rate at which the material leaves the crater as well as to center the discharge, thus materially increasing the reproducibility.

A few variations have been tried in an effort to improve repro-

ducibility, with varying success. A 2-mm. analytical gap was tried with the 40-mfd. exposure, but showed very little improvement. Pulverizing the sample to pass through a 200-mesh sieve improves the reproducibility somewhat, but it is questionable whether the improvement is sufficient to justify this additional expenditure of time.

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Ascorbic Acid, Dehydroascorbic Acid, and Diketogulonic Acid

In Fresh and Processed Foods

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Foods in various states of preservation were analyzed by the method of Roe, Mills, Damron, and Oesterling for L-ascorbic acid, dehydro-L-ascorbic acid, and diketo-L-gulonic acid. Values obtained are reported. The majority of fresh foods assayed showed less than 5% of the total vitamin C-like compounds present as the antiscorbutically inactive diketogulonic acid. Processed foods contained more of the oxidized forms of ascorbic acid, and dehydrated foods showed the greatest amount of the inactive diketogulonic acid. The rate of change of ascorbic acid into dehydroascorbic acid and of dehydroascorbic acid into diketogulonic acid in orange juice and potato slurry during storage at 2° C. was followed.

THE primary oxidation product of L-ascorbic acid in plant materials is dehydro-L-ascorbic acid. The latter is not a stable compound; it undergoes spontaneous conversion to a second product which has been fairly well characterized as 2,3-diketo-L-gulonic acid, formed by the opening of the lactone ring of dehydroascorbic acid (1, 3).

Dehydroascorbic acid has considerable antiscorbutic potency because it is readily reduced to ascorbic acid in the animal body (4, 6, 10). The exact value for its antiscorbutic activity has not been satisfactorily determined, but it is probably about 75% of that of ascorbic acid. Diketogulonic acid, on the other hand, has no demonstrable antiscorbutic activity (4).

The actual antiscorbutic potency of a foodstuff depends on the amount of both ascorbic acid and dehydroascorbic acid present. The oxidation-reduction methods for the determination of vitamin C are reliable only if all the antiscorbutic material present is ascorbic acid, and other reducing substances are not present in large enough concentrations to interfere. This ideal condition is usually not encountered.

The Roe-Kuether (7) and Roe-Oesterling (9) methods, which use 2,4-dinitrophenylhydrazine to couple with the oxidized forms of ascorbic acid, do not differentiate between dehydroascorbic acid and diketogulonic acid, both of which react with the reagent to give an identical derivative. The original dinitrophenylhydrazine methods are accurate for antiscorbutic assay only when ascorbic acid and dehydroascorbic acid occur in the food and diketogulonic acid has not been formed in appreciable amounts. This is some improvement over the oxidation-reduction methods by virtue of increased specificity and the inclusion of the dehydroascorbic acid portion, but these methods lose their usefulness when diketogulonic acid is present in the foodstuff, unless they are used as Guild, Lockhart, and Harris suggest (2) to show what the original ascorbic acid content of the foodstuff was at the time of harvesting.

The new method developed by Roe, Mills, Damron, and Oesterling (8) permits the simultaneous determination of ascorbic acid, dehydroascorbic acid, and diketogulonic acid in the same tissue filtrate. It is based upon the following principles. Diketogulonic acid is the oxidation product of ascorbic acid in a metaphosphoric acid filtrate that is not reducible by hydrogen sulfide and couples with 2,4-dinitrophenylhydrazine. Dehydroascorbic acid is the fraction in the filtrate that is reducible by hydrogen sulfide and couples with the reagent only before reduction. The ascorbic acid in the filtrate does not couple with 2,4-dinitrophenylhydrazine under the conditions of the method until after it is oxidized by bromine. The analytical steps involved theoretically confer a high degree of specificity upon the method used. This procedure should show the closest correlation possible up to the present time between the antiscorbutic biological assay and the actual chemical analysis of a foodstuff.

There are many reports in the literature of the discrepancies between biological and chemical assays for vitamin C. There are also instances of comparative analyses of stored foodstuffs wherein the indophenol value decreased and the dinitrophenylhydrazine value remained constant (5) or increased (7) with storage. These results are consistent with the known chemistry of ascorbic acid, dehydroascorbic acid, and diketogulonic acid. Storage with the resultant oxidation of ascorbic acid to dehydroascorbic acid would decrease the indophenol value; however, the biological potency would change but little until diketogulonic acid was formed from the dehydroascorbic acid. As soon as diketogulonic acid was produced by "mutarotation" of the dehydroascorbic acid, the biological assay value would begin to decrease, but the dinitrophenylhydrazine assay value by the original methods (7, 9) would remain the same, or show an increase because of the more rapid rate of reaction of diketogulonic acid with 2,4-dinitrophenylhydrazine in the 3-hour coupling period employed.

Table I. Fresh Fruits and Vegetables

Food	AsA[a]	DHA[a]	DKA[a]	$\frac{DKA}{Total} \times 100$
		Mg. per 100 grams		
Apple	3.0	0	0	0
Banana	17.0	0	0	0
Cantaloupe	50.0	5.0	1.2	2.2
Grapefruit	36.8	0.6	0.6	1.6
Lemon	50.7	0	0.5	1.0
Lime	25.0	0	1.0	3.9
Orange	41.3	0	0	0
Peach	7.75	0	0.5	6.4
Watermelon	7.7	0	0.8	10.4
Beans, green	17.0	1.2	0.0	0
Beans, lima	33.0	0.0	0.0	0
Broccoli	84.5	10.5	3.5	3.6
Cabbage	42.5	3.7	0.0	0
Cauliflower	79.5	6.2	1.4	1.6
Celery	1.0	2.0	0.0	0
Chard	26.2	3.0	0	0
Cucumber	2.3	0	0	0
Kale	188.0	11.9	1.9	0.9
Lettuce, leaf	32.8	3.2	0.7	2.0
Lettuce, head	6.0	2.0	0	0
Parsley	276.0	16.0	1.5	0.5
Peas	21.8	1.2	0.5	2.1
Pepper, green	156.0	5.2	0.0	0
Potato, white	30.0	0.0	0.2	0.6
Spinach	26.0	2.9	0.0	0
Tomato	5.75	1.9	0.6	7.6
Turnip	18.7	6.0	0.0	0

[a] AsA. Ascorbic acid.
DHA. Dehydroascorbic acid.
DKA. Diketogulonic acid.

FOOD ANALYSES

Using the new method, with a 6-hour coupling period, the authors have analyzed representative fresh and processed fruits and vegetables for their content of ascorbic acid, dehydroascorbic acid, and diketogulonic acid, and have followed the changes of these three components in several stored foodstuffs.

Foods in four general states of preservation were studied: fresh fruits and vegetables from city markets, dehydrated foods, canned foods, and frozen foods. The first series of foods analyzed was fresh fruits and vegetables (Table I). No attempt was made to select only fresh samples; some specimens were in good condition, while others were older and more withered. The data obtained indicate that fresh foods as purchased in markets contain a certain amount of dehydroascorbic acid and diketogulonic acid, but this amount is small unless the foods have been allowed to deteriorate considerably.

The diketogulonic acid values in Table I are small. The analyses of peach, tomato, and watermelon yielded data that represent 6.4, 7.6, and 10.4% of the total vitamin C compounds, respectively. The values for diketogulonic acid in 24 other foods in this table fall below 5% of the total of the compounds with vitamin C-like structure; and in 13 foods there was no diketogulonic acid present. The values for diketogulonic acid in this table include all substances in metaphosphoric acid filtrates not reducible by hydrogen sulfide that will couple with 2,4-dinitrophenylhydrazine. Granting that the diketogulonic acid fraction would include interfering substances, if present, the observation that this fraction is very small, or is not present at all in many fresh foods, is good evidence for the specificity of the dinitrophenylhydrazine methods for the determination of vitamin C.

Other foods studied consisted of processed foods, purchased at the markets, or supplied by the American Can Company and the Continental Can Company. Table II shows the results obtained with the frozen foods analyzed; Table III, the results from canned foods; and Table IV, the results from dehydrated foods. Some of the dehydrated and canned foods were of old stock, which had been stored for 5 or 6 years, and the dehydrating conditions were extreme in some instances, so that these results are more a measure of how bad values can be than of the degree of deterioration to be found in foods that usually reach the consumer.

Results show that frozen and canned foods may be as high in ascorbic acid and as low in dehydroascorbic acid and diketo-

gulonic acid as fresh market foods. Frozen foods which have been poorly preserved can reach the highly oxidized state represented by the strawberries in Table II. Dehydrated foods as a rule contain more dehydroascorbic acid and diketogulonic acid than do the other types of processed foods, and they may show marked destruction of the ascorbic acid in materials dehydrated at elevated temperatures, as the cabbage in Table IV.

In several instances no dehydroascorbic acid was found, but values for diketogulonic acid were obtained. The amounts of diketogulonic acid observed in the absence of dehydroascorbic were small. These findings are interpreted as being due either to interfering substances or to diketogulonic acid resulting from the complete transformation of dehydroascorbic acid by the irreversible reaction. This is feasible, for conditions can be produced which protect ascorbic acid but catalyze the transformation of dehydroascorbic acid into diketogulonic acid. It has been demonstrated that ascorbic acid and diketogulonic acid may coexist in pure solutions at low pH ranges when no dehydroascorbic acid is present, owing to the greater instability of the latter substance.

STORAGE CHANGES

Experiments were performed to test the keeping qualities of ascorbic acid in two different types of stored foodstuffs.
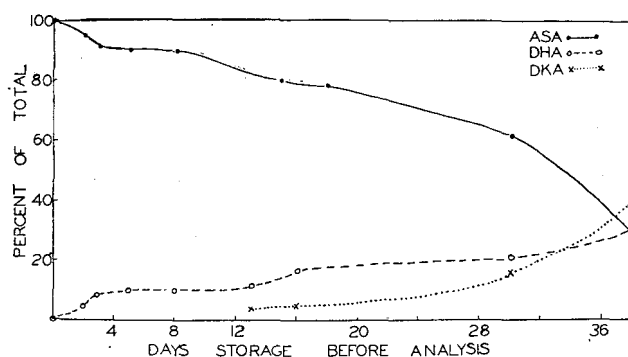

Figure 1. Changes in Distribution of Ascorbic Acid, Dehydroascorbic Acid, and Diketogulonic Acid in Orange Juice at 2° C.

Table II. Frozen Foodstuffs

Food	AsA	DHA	DKA	$\frac{DKA}{Total} \times 100$
		Mg. per 100 grams		
Beans, green	7.6	1.3	3.1	26.0
Beans, lima	4.97	0.92	0.74	11.2
Broccoli	105.0	7.3	1.0	0.89
Orange juice	41.0	0.5	2.75	6.2
Peas	4.2	0	2.3	35.4
Spinach	30.0	8.5	7.0	15.4
Spinach	26.9	2.9	0.0	0
Strawberries[a]	0.0	12.7	37.5	75.0
Strawberries	80.0	12.0	3.0	3.2

[a] Poor state of preservation.

Table III. Canned Foodstuffs

Food	AsA	DHA	DKA	$\frac{DKA}{Total} \times 100$	Remarks
		Mg. per 100 grams			
Beans, green	5.0	0	0	0	6 years old
Beans, lima	8.5	0.5	0.25	27.0	Market purchased
Grapefruit juice	1.8	4.0	1.0	14.8	5 years old
Grapefruit	0.4	4.4	0.0	0	5 years old
Orange juice	37.5	1.0	0	0	Market purchased
Peas	10.0	1.0	0	0	5 years old
Spinach	17.5	1.9	0.6	3.0	5 years old
Sweet potato	13.7	2.0	0.5	3.1	5 years old
Tomato juice	11	0.7	0	0	5 years old
Tomato juice	17.0	0	0	0	5 years old

Table IV. Dehydrated Foodstuffs

Food	AsA	DHA	DKA	$\frac{DKA}{Total} \times 100$	Remarks
		Mg. per 100 grams			
Blueberries	7.9	8.6	0	0	5 years old
Carrots	0.0	26.2	6.1	18.9	5 years old
Cabbage	186.0	39.0	11.0	4.7	Less than 1 year old
Cabbage	308	27.5	1.25	0.37	Less than 1 year old
Cabbage	33.9	22.2	0	0	Dehydrated under CO_2, 4 years old
Cabbage	37.5	6.0	9.0	17.1	Dehydrated under air, 4 years old
Cabbage	29.6	1.5	14.8	32.2	Dehydrated under N_2, 4 years old
Cabbage	33.4	16.1	4.95	11.1	4 years old
Potatoes	18.8	2.0	0.0	0	Less than 1 year old
Tomato juice cocktail	8.0	10.0	6.0	25.0	Dehydrated under CO_2, 4 years old
Tomato flakes	1.23	28.0	12.3	29.6	Dehydrated under air, 4 years old

Orange juice and a slurry of white potato in distilled water were stored in the refrigerator at 2° C. for a prolonged period and aliquots were removed for analysis at varying intervals. The orange juice was prepared on a glass juicer and filtered through cheesecloth and a portion was analyzed immediately. The remainder was placed in the refrigerator in a stoppered glass flask and samples for analysis were removed by pipet.

The percentages of the total vitamin C–like compounds represented by ascorbic acid, dehydroascorbic acid, and diketogulonic acid present at certain times during the experiment are shown in Figure 1. Originally all the ascorbic acid was present in the reduced form as shown in the first analysis. The second day's analysis showed that 5% dehydroascorbic acid had been formed. The amount of dehydroascorbic acid gradually increased through the storage period. On the 13th day some diketogulonic acid (4%) was found to be present for the first time. The amount of diketogulonic acid slowly increased until at the last analysis (38th day) it comprised the largest percentage of the total. The total amount of the three components remained constant for the first 30 days, then slowly decreased to about 83% of the original total by the 38th day (Figure 2).

These data are of special interest in regard to the question of deterioration of orange juice upon standing in the icebox. Results show that 72 hours' storage allowed only 8% oxidation of the ascorbic acid to dehydroascorbic acid, which would hardly alter the antiscorbutic potency; and after 13 days' standing there still remained 82% of the vitamin in the form of ascorbic acid and 11% as dehydroascorbic acid. These data show that orange juice stored at 2° C. retained approximately 90% of its antiscorbutic potency, as measured by chemical methods, for 13 days.
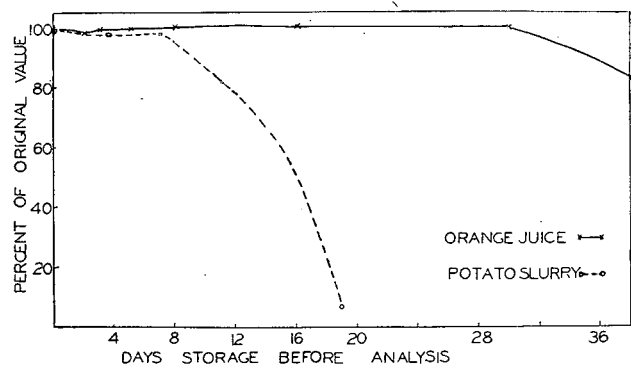
Figure 2. Changes in Total Amount of Ascorbic Acid, Dehydroascorbic Acid, and Diketogulonic Acid in Orange Juice and Potato Slurry at 2° C.

The potato slurry was made up in a dilution of 1 to 50 with distilled water in the Waring Blendor. A portion was analyzed immediately and the remainder was stored in the refrigerator at 2° C., samples being removed for analysis at varying intervals by pipet. The original slurry showed almost no ascorbic acid (Figure 3) but about 90% of dehydroascorbic acid and 10% of diketogulonic acid. Within 6 hours these values had reversed and diketogulonic acid comprised over half of the total. The remaining analyses gave values falling on smooth curves. By the 19th day, 90% of the total was in the form of diketogulonic acid. The values for the total amount present, shown in Figure

2, did not diminish much during the first week, but later rapidly decreased until at the last analysis only 5% of the original total remained. These results are consistent with the known behavior of diketogulonic acid, which has been shown to undergo fragmentation of its carbon chain at the hydrogen ion concentration of the slurry (pH 6.7).

The experiments of Figures 1 and 3 are of interest in that they show the early stages in the degradation of ascorbic acid in plant tissues. In Figure 1 the decreases in ascorbic acid are paralleled by corresponding increases in dehydroascorbic acid and the appearance of diketogulonic acid follows the formation of dehydroascorbic acid. In Figure 3 the curves showing changes
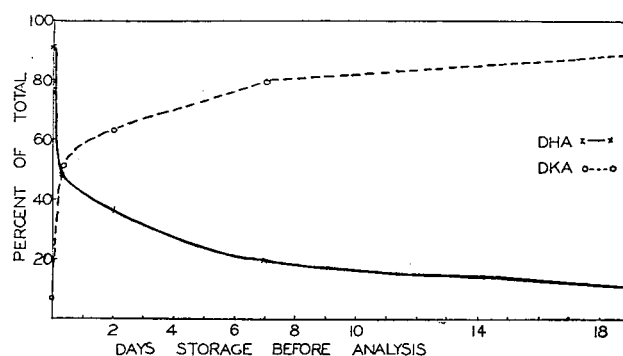

Figure 3. Changes in Distribution of Dehydroascorbic Acid and Diketogulonic Acid in Potato Slurry at 2° C.

in dehydroascorbic acid and diketogulonic acid bear a close reciprocal relationship to each other; these curves appear to show that the disappearance of dehydroascorbic acid gives rise to the formation of equivalent amounts of diketogulonic acid.

The fact that the total amount of ascorbic acid and its oxidation products in stored orange juice remains the same for 30 days, while the relative percentages of the three gradually change from a complete absence of dehydroascorbic acid and diketogulonic acid to the preponderance of these latter substances, affords excellent evidence that the methods used are highly specific and serve to separate and identify these three compounds, qualitatively and quantitatively.

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Determination of Aluminum in Presence of Iron

Spectrophotometric Method Using Ferron

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Aluminum and ferric iron in a buffered acetate–acetic acid solution form complexes with the reagent ferron, 8-hydroxy-7-iodo-5-quinolinesulfonic acid. The absorption maxima of the two complexes are sufficiently far apart to permit spectrophotometric measurements of the ferric-ferron complex at 600 mμ and the ferric-ferron complex plus the aluminum-ferron complex at 370 mμ. The complexes are stable for a reasonably long period of time. The method is suitable for the accurate determination of 0 to 50 micrograms of aluminum in the presence of 0 to 100 micrograms of iron.

FERRON was first proposed as a reagent for iron by Yoe (*5*). Later Swank and Mellon (*4*) reported that aluminum in large concentrations (2 mg. of aluminum to 0.1 mg. of iron in 100 ml.) interfered in the ferron method for iron, although no visible colored complex was formed.

The author has found that this aluminum-ferron complex has a maximum absorption at 370 mμ and that at this wave length, using small concentrations of aluminum at a constant pH, the complex obeys Beer's law. The iron-ferron complex also absorbed at this wave length but the amount of absorption due to the iron complex can be accurately determined. One method using hematoxylin (*1–3*) makes use of the mixed colors obtained from the aluminum and iron lakes. However, the absorption curves for the two lakes overlap each other, which means that the optical density at each absorption maximum is dependent upon the concentration of both cations. The color of the aluminum-hematoxylin lake changes rapidly on standing.

SOLUTIONS AND METHODS

Redistilled water was used in the preparation of all solutions.

Standard aluminum solution, 1.000 mg. per ml., was prepared by dissolving 1.000 gram of aluminum metal turnings (J. T. Baker Chemical Co.) in 100 ml. of 1 to 9 hydrochloric acid and diluting to 1 liter. The final solution was standardized gravimetrically using ammonium hydroxide. A solution of 10.0 micrograms per ml. was prepared by dilution of an aliquot of the standard.

Standard iron solution, 1.000 mg. per ml., was prepared by dissolving 0.100 gram of iron wire (J. T. Baker Chemical Co. No. 36 iron wire for standardizing) in 50 ml. of 1 to 20 hydrochloric acid and diluting to 100 ml. A solution of 10.0 micrograms per ml. was prepared by diluting an aliquot of the standard.

Ferron, 0.2%, obtained from Eastman Kodak Company was used without further purification. An aqueous solution was prepared by dissolving 1.000 gram of reagent in 500 ml. of redistilled water.

Ammonium acetate, 10%. Fifty grams of reagent grade salt were dissolved in 400 ml. of redistilled water and diluted to 500 ml.

Hydrochloric acid, 1 to 9, C.P. analytical grade.

Nitric acid, 1 to 9, C.P. analytical grade.

Buffers. The desired pH values were obtained by varying the concentration of either hydrochloric acid or nitric acid prior to the addition of 5 ml. of ammonium acetate. This procedure was followed in order to ensure complete solution of aluminum and iron. In every case 5 ml. of the ammonium acetate solution and 2 ml. of the ferron solution were added. Solutions were made up to a final volume of 25 ml. and read in a Beckman spectrophotometer using 1-cm. cells.

SPECTRAL ABSORPTION AND EFFECT OF pH

It can be seen from Figures 1 and 2 that absorption maxima for the iron and aluminum complexes are obtained at a wave length of about 370 mμ and that at a wave length of about 600 mμ only the colored complex of iron shows absorption. The
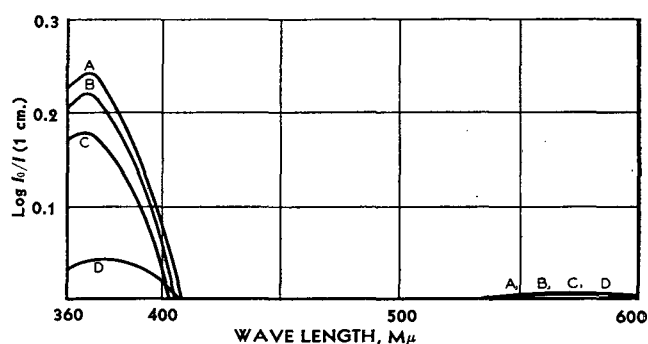

Figure 1. Effect of pH on Spectral Absorption of Aluminum-Ferron Complex

In acetic acid–acetate solution. 20γ of aluminum plus 2 ml. of ferron (0.2%) in 25 ml.

A. pH 5.5 *B.* pH 5.0 *C.* pH 4.0 *D.* pH 3.0

aluminum-ferron complex transmits more light than the blank between 410 and 540 mμ. Maximum color development for each of the complexes was found at approximately pH 5. Absorption by the aluminum-ferron complex decreases rapidly at pH values higher than 5.5. At pH 6.4 the absorption is less than that shown by the blank.

Table I. Effect of Common Cations on Aluminum-Ferron Complex

(20.0 γ of aluminum in 25 ml.)

Ion	Present in 25 Ml., γ	Increase in Absorption at 370 mμ, %
Ca^{++a}	20.0	0
Cr^{++++++}	20.0	14
Cu^{++}	20.0	100
Mg^{++}	20.0	0
Mn^{++}	20.0	5
Mo^{++++++}	20.0	24
Ni^{++}	20.0	100
Th^{++++}	14.5	50
U^{++++++}	20.0	19
Zn^{++}	20.0	65
Zr^{++++}	20.0	35

[a] As much as 1 mg. of calcium in 25 ml. did not affect absorption.

VALIDITY OF BEER'S LAW

Calibration data for varying iron concentration *vs.* extinction at 370 and 600 mμ and for varying aluminum concentration *vs.* extinction at 370 mμ give the curves obtained in Figures 3 and 4. The aluminum-ferron complex does not obey Beer's law at con-

centrations higher than 40 micrograms per 25 ml. but the curve may be used to at least a concentration of 60 micrograms per 25 ml. The plot of iron concentration against extinction data at 600 mμ gives a straight line under the above conditions, whereas Swank and Mellon (2) reported that Beer's law was not obeyed under their conditions (pH 2 to 3) when the ferron concentration was kept constant.

EFFECT OF FOREIGN IONS

The effect of anions was not investigated. A number of cations give positive interference. Uranium, thorium, copper, nickel, chromium, molybdenum, manganese, zirconium, and zinc interfered when present in a 1 to 1 ratio with aluminum. The degree of interference encountered when these cations are present appears in Table I. In general, cations that form hydroxyquinolates in the pH range of the colorimetric method will interfere. Thus, magnesium, which completely precipitates with 8-hydroxyquinoline in the pH range 9.4 to 12.7, does not interfere when present in moderate amounts. Thorium, which precipitates at pH 4.4 to 8.8, gives a definite positive interference in the colorimetric method.

PROCEDURE

Calibration curves for aluminum and iron should be prepared as in Figures 3 and 4.

Transfer to a 25-ml. volumetric flask 5 to 10 ml. of a nearly neutral sample solution containing aluminum, iron, or both, having a total concentration of 10 to 60 micrograms. Add, in the following order, 1 ml. of hydrochloric acid (1 to 9), 1 ml. of nitric acid (1 to 9) (to convert all iron to the ferric state), 5 ml. of ammonium acetate (10%), and 2 ml. of ferron (0.2%). Dilute to
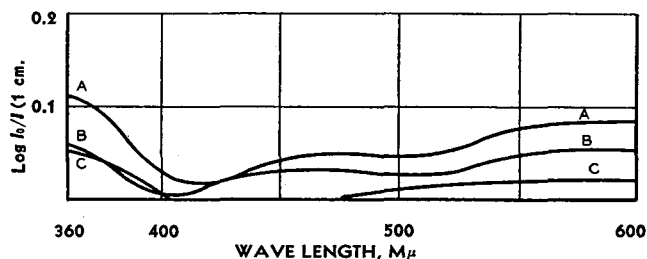
Figure 2.   Effect of pH on Spectral Absorption of Iron-Ferron Complex

In acetic acid–acetate solution.   20γ of iron plus 2 ml. of ferron (0.2 %) in 25 ml.

A. pH 5.0 to 5.5   *B.* pH 4.0   *C.* pH 3.0
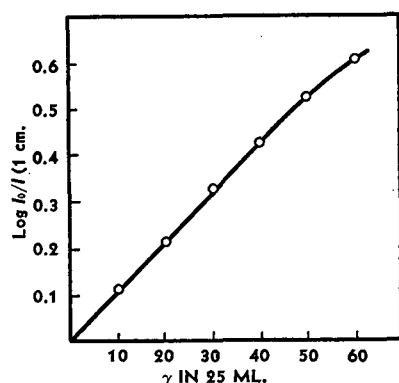
Figure 3.   Aluminum Calibration Curve

370 mμ, pH 5.0.   2 ml. of ferron (0.2 %)
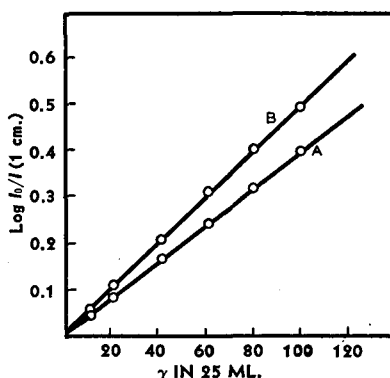
Table II.   Analysis of Aluminum-Iron Solutions by Ferron Method

Sample No.	Present Al, γ/25 ml.	Present Fe, γ/25 ml.	Found Al, γ/25 ml.	Found Fe, γ/25 ml.
1	10.0	20.0	9.5	19.5
2	20.0	20.0	20.0	20.5
3	30.0	20.0	29.0	20.5
4	20.0	10.0	19.2	10.0
5	20.0	20.0	20.0	20.0
6	20.0	30.0	19.2	30.5
7	10.0	10.0	9.6	10.0
8[a]	10.0	80.0	9.2	78.0
9[a]	40.0	10.0	39.0	10.0
10[a]	20.0	40.0	19.5	40.0

[a] Absorption did not change after standing 24 hours.

25 ml. and read the extinction in Beckman 1-cm. cells at wave lengths 600 and 370 mμ. Determine the total iron present from curve *A*, Figure 4, the calibration curve for iron at 600 mμ. The contribution of this concentration of iron to the total extinction measurement at 370 mμ may be determined from curve *B*, Figure 4. Subtract this value from the total extinction. Calculate from Figure 3 the concentration of aluminum present which is responsible for the remainder of the total extinction at 370 mμ.

RESULTS

A number of mixed solutions containing known amounts of aluminum and iron in varying ratios were prepared and analyzed by the ferron method (Table II).

SUMMARY

Aluminum in a buffered acetate solution at a pH 5 forms a complex with ferron which obeys Beer's law for amounts of aluminum in the range 0 to 40 micrograms in 25 ml. The interference due to iron may be accurately determined by spectrophotometric measurements of the same solution at another wave length.

The complexes of aluminum and iron are stable for at least 24 hours. Cations which form hydroxyquinolates in slightly acid media give positive interference in the colorimetric method. In the presence of these interfering cations, a preliminary separation of aluminum and iron is necessary. The method is applicable to the direct determination of traces of aluminum in water solutions and soil extracts.

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Figure 4.   Iron Calibration Curve

pH 5.0.   2 ml. of ferron (0.2%)

A. 600 mμ *B.* 370 mμ

Determination of Cellulose by Acid-Dichromate Oxidation

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It was shown on the basis of numerous determinations on alkali- and ethanolamine-purified samples of cotton cellulose, that acid-dichromate oxidation accounted for only about 97% of the theoretical weight of the oven-dried material. A detailed study was therefore made of the application of the method to highly purified cotton cellulose, to National Bureau of Standards samples of glucose and sodium oxalate, and to recrystallized sucrose. The principal source of deficiency was found to be the formation and escape of carbon monoxide equivalent to 1.66% cellulose. The remaining deficiency was accounted for by the presence of 0.5 to 0.6% moisture which is not removed by conventional drying in an air oven

at 103° to 110° C., and by residues of 0.10 to 0.15% ash which were not completely removed from the cellulose in the conventional purification techniques. No acetic acid was produced during oxidation of the cellulose. From the percentages of cellulose on the ash- and moisture-free basis oxidized to carbon monoxide and dioxide and the milliequivalent weights of cellulose corresponding to each, a new "experimental" milliequivalent weight of 6.861 mg. has been computed for routine use. When the experimental milliequivalent weight of cellulose was used on six samples of purified cellulose selected at random, the cellulose found averaged 99.91%, as compared with 98.33% by the former method.

I N THE routine quantitative determination of cellulose in cotton by the technique described by Kettering and Conrad (14), which involves solution of the purified cellulose in 12 M sulfuric acid, oxidation with standard dichromate solution, and back-titration with ferrous ammonium sulfate, only about 97% of the theoretical amount of cellulose could be accounted for. Thorough investigation showed that experimental error or faulty technique could be ruled out. Other possible explanations of the difference were therefore investigated.

Because the method of Kettering and Conrad had been developed on the basis of the findings of Launer (16), it seemed pertinent to re-examine Launer's work. The re-examination revealed that Launer, using the theoretical milliequivalent weight of cellulose, 6.75 mg. per ml. of 1 N dichromate solution, had realized 98.7 to 100.7%, or a mean of 99.7%, of theory. Birtwell and Ridge (1) give data which show a mean of 100.0% realization of the theoretical milliequivalent weight, in the range 98.5 to 101.5%.

But failure to realize as closely as this the theoretical values for cellulose with acid-dichromate oxidation has been reported by other investigators.

Thus, Boivin (2), Christensen, Williams, and King (3), and Houghton (12) have dealt with the formation of carbon monoxide through incomplete oxidation. Houghton also reported the formation of acetic acid and acetaldehyde when certain substituted celluloses and some other carbohydrates were oxidized.

Considering the effect of reagents on the amount of oxidation, Birtwell and Ridge (1) held that dichromate is lost by decomposition unless the acid-dichromate ratio is held to not more than 1 volume of concentrated sulfuric acid to 1 volume of 1 N dichromate solution. Cross and Bevan (4) claimed that the dichromate must be present in 30% excess of that theoretically required in order to assure complete oxidation of the cellulose. Phelps (20) noticed that potassium dichromate as well as chromic oxide was affected by temperature and acid strength and that keeping both the temperature and the acid strength relatively low prevented the decomposition of dichromate with liberation of free oxygen.

A small but definite source of error which undoubtedly enters indirectly into the ordinary determination of cellulose is that of the moisture in the oven-dried material at the time of weighing. This moisture cannot be removed entirely by ordinary drying at 103° to 105° C. in the air oven, and it is known that various methods of oven drying in air produce different results.

Nelson and Hulett (19) found 5.49% moisture in some absorbent cotton by drying at 115° C. with a pressure of 0.1 micron. They computed by an extrapolation process which corrected for decomposition that an additional 0.41% moisture would be removed at 250° C., a temperature at which they claim on the basis of vapor pressure considerations no adsorbed moisture could be retained. These results were confirmed by Davidson and Shorter (6) and by Hermans (9). Nelson and Hulett show no figures comparing drying by air oven and vacuum oven. Hermans (9), however, found that after constant weight had been attained by oven drying in air at 110° C., the 0.4 to 0.5% moisture still remaining could be removed either by a stream of well-dried nitrogen at 110° C. or by drying in vacuo over phosphorus pentoxide. Keating and Scott (13), comparing the moisture obtained by the Karl Fischer technique on various textile samples with that obtained by drying in an air oven at 105° to 110° C., found that the Karl Fischer technique gave 0.34 to 0.36% more moisture. This percentage was confirmed by determining in the samples, oven-dried as described above, the remaining moisture, which was found to average 0.34%. It was entirely expected, of course, that the Karl Fischer technique would not be affected by factors such as the relative humidity of an oven atmosphere, and would thus give somewhat higher moisture contents than the air oven.

It is desired, in the present investigation, in view of the discrepancies between the actual and theoretical values for cellulose determined by acid-dichromate oxidation, to investigate fully each of the possible variables or sources of error, indicated in the previous studies, with the idea of determining the extent to which each factor is involved in the incomplete oxidation.

MATERIALS AND METHODS

Purification of Samples. In the preliminary experiments described below 0.1-gram samples of various raw and purified cottons, cotton linters, α-cellulose, and rayons were subjected to the routine purification treatments described by Kettering and Conrad (14).

These consisted of either heating the sample under reflux for 2 hours in boiling 1% sodium hydroxide solution, or heating with monoethanolamine under reflux for 2 hours at its boiling point (about 170° C.). However, instead of being dissolved directly in 12 M sulfuric acid as in the volumetric procedure, the thoroughly washed, moist residues were then transferred to tared, fritted-glass crucibles, dried overnight in an air oven at 103° to 105° C., cooled in a desiccator, and weighed. They were subsequently dissolved in 12 M sulfuric acid and the cellulose was determined as described by Kettering and Conrad (14).

The findings in the preliminary studies indicated the need for a cotton cellulose of the highest purity, prepared in such a manner as to keep degradation to a minimum.

For this purpose, 100 grams of a clean, white, untreated cotton were placed in a large Soxhlet extractor with 3 liters of 95% ethanol, and the extraction was carried out for a total of 16 hours. The extracted cotton was washed with three changes of distilled water, each of 6 liters, and hand-squeezed. The moist material was placed in the extraction compartment of a large extractor especially adapted so that a continuous unidirectional flow of fresh, hot, 1% sodium hydroxide solution—prepared by adding the proper amount of 48% carbonate-free sodium hydroxide solution to 5 liters of boiled distilled water—could pass through the cotton at the rate of 0.6 liter per hour simultaneously with the passage of nitrogen freed of oxygen by passage through alkaline pyrogallol. The solution was kept boiling by means of a burner placed under the extractor. To free the cotton of sodium hydroxide, about 20 liters of boiling distilled water were passed through until the washings were neutral to litmus. Then about 5 liters of 1% acetic acid were slowly passed through the cotton, followed by a passage of distilled water. The thoroughly washed cotton was hand-squeezed and then spread out to dry under a layer of clean cheesecloth over which air was circulated by an electric fan. The final preparation consisted in grinding the cotton in a large Wiley mill to pass a 2-mm. sieve.

On a moisture-free basis this cotton was found to contain 0.15% ash, leaving what was assumed to be 99.85% cellulose. Even if this was not literally true, the effect of small traces of oxidized cellulose or of traces of pectic substances could be of little practical significance, because of the nearly identical equivalence of these substances with that of pure cellulose as far as oxidation with dichromate is concerned.

Also used in the present experiments were dextrose and sodium oxalate furnished by the National Bureau of Standards for standardization, recrystallized sucrose, and highly purified sweet potato starch. The latter was a high viscosity mill-run sample which had been carefully washed free of chloride ion. It contained 0.32% ash and 0.015% soluble matter.

Preparation of Solutions. The potassium dichromate solutions were prepared in 20-liter bottles, using only finely crystalline c.p. dichromate, in either 0.6 or 0.8 N concentrations. They were standardized against Bureau of Standards' ingot iron (sample 55b), and some were checked further against especially purified reagent grade potassium iodate.

The ferrous ammonium sulfate solutions, approximately 0.5 N, were prepared just before use from fine crystal special salt containing better than 99.95% pure salt. They were made up in 1.0 N sulfuric acid and were standardized against the potassium dichromate solution just before use.

The 12 M sulfuric acid was made up from c.p. concentrated acid according to calculated proportions. The strength of the cooled acid was checked with a precision hydrometer and specific gravity tables.

Moisture Determination. Except where otherwise described, moisture determinations were made on 0.5-gram samples in tared aluminum weighing boxes. The samples were dried at 103° C. in an air oven of the de Khotinsky cylindrical chamber type. The samples were ordinarily left overnight (about 16 hours), to ensure constancy of weights.

Carbon Dioxide Determinations. For determination of carbon dioxide resulting from oxidation, the absorption train shown diagrammatically in Figure 1 was used.

Nitrogen, freed from carbon dioxide and oxygen, was used as the carrier gas. Prior to each determination the walls of the condenser were dried with alcohol and acetone to remove the retained condensate. To assure accuracy a blank run was made ahead of each sample. The procedure was to place the sample, 25 ml. of dichromate solution, and 10 ml. of water in the oxidation flask, and to sweep the system for 30 minutes through the guard tube, L, at a rate of flow of 10 liters per hour. Then the condenser end was capped with a sealed-off female joint while the flask was removed for adding 25 ml. of 12 M acid. The flask was replaced, and the gas flow was directed through the absorption tubes and adjusted to a rate of 5 liters per hour. Heat was applied to the oxidation mixture, causing it to reflux for 1 hour,
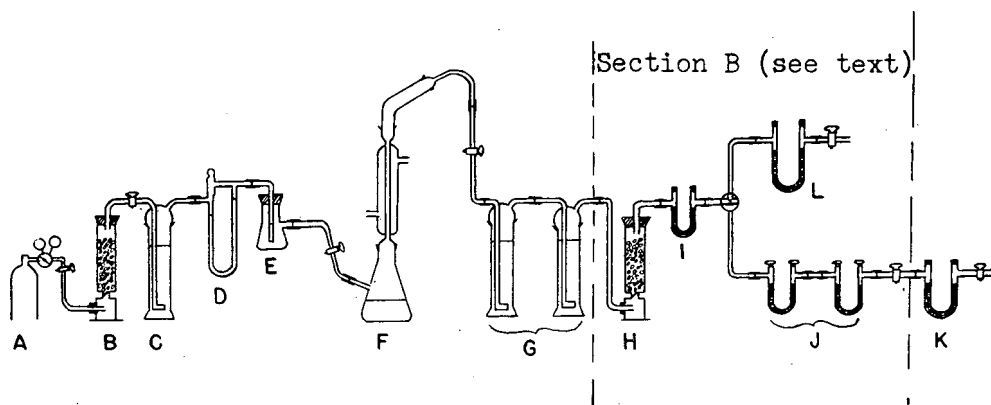

Section B (see text)

Figure 1.   Carbon Dioxide Absorption Train

A. Nitrogen tank with pressure reducing valve
B. Ascarite
C. Oxygen absorbent
D. Flowmeter
E. Bunsen valve
F. Oxidation flask with reflux condenser
G. Concentrated sulfuric acid
H. Drierite
I. Magnesium perchlorate
J. Carbon dioxide absorption tubes (Ascarite)
K, L. Guard tubes

Section A (see text)
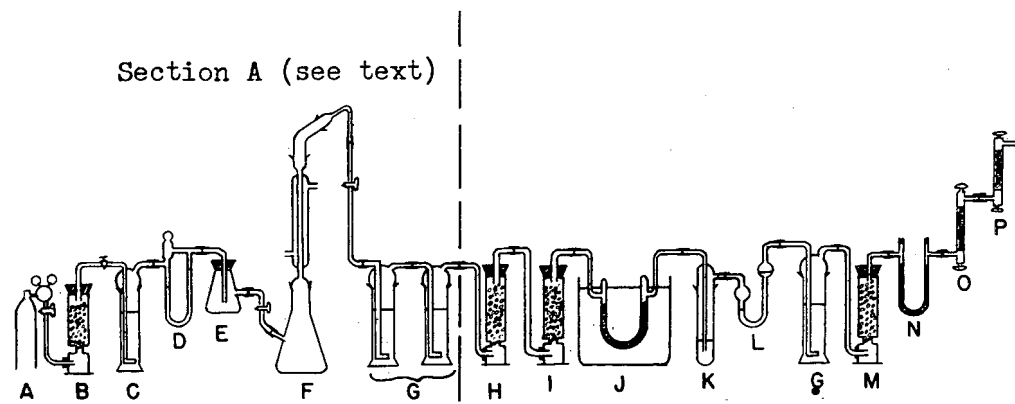
Figure 2.   Carbon Monoxide Absorption Train

A. Nitrogen tank with pressure-reducing valve
B. Ascarite
C. Oxygen absorbent
D. Flowmeter
E. Bunsen valve
F. Oxidation flask and reflux condenser
G. Concentrated sulfuric acid
H. KOH pellets
I. Soda-lime
J. Oil bath with U-tube containing I_2O_5
K. Iodine absorber (KI)
L. KI bubbler
M. Drierite
N. Magnesium perchlorate
O. Carbon dioxide absorption tube (Ascarite)
P. Guard tube

Table I. Volume of 1 N Dichromate Solution Required to Oxidize One Milliequivalent (6.75 Mg.) of Raw and Purified Cotton Celluloses

Volume of 1 N Dichromate Reduced		No. of
Class range	Midpoint value	Observations
$Ml.$	$Ml.$	
0.918–0.932	0.925	1
0.933–0.947	0.940	3
0.948–0.962	0.955	15
0.963–0.977	0.970	19
0.978–0.992	0.985	8
0.993–1.007	1.000 (theory)	4
1.008–1.022	1.015	1
		Total 51
Mean	0.969 $\pm$ 0.003	
Standard deviation	0.018	

after which the heat was removed and the system swept for an additional hour at a gas flow rate of 10 liters per hour. Because of the large amount of carbon dioxide expected from the cellulose sample taken, two Ascarite absorption tubes were used to assure complete absorption. The absorption tubes after removal from the train were handled with the usual precautions required by gravimetric technique.

A tare tube was used and received exactly the same treatment as the absorption tubes.

Carbon Monoxide Determination. For determination of carbon monoxide, the iodine pentoxide method described by Dennis (7) was selected as the most suitable, and the gas train shown in Figure 2 was set up. The equation of the reaction

$$5CO + I_2O_5 \xrightarrow{150°C.} 5CO_2 + I_2$$
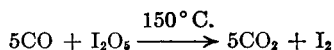
indicates that carbon monoxide should be determinable from either the carbon dioxide formed or the iodine liberated. The procedure of Livingston, Morgan, and McWhorter (17) was followed with only the slight changes evident in the diagram. That the carbon dioxide resulting from the oxidation of the cellulose was completely absorbed was proved when Ascarite absorption tubes, placed immediately after the soda-lime tower, did not gain in weight during a run. The train was swept for 30 minutes after the finish of reflux to ensure complete removal of the oxidation products. A blank run preceded each determination to assure accuracy.

While the liberated iodine was titrated it consistently gave low results. It was subsequently found that when the thiosulfate solution was standardized against dichromate, as was done here, low results invariably occurred (11, 15, 24). Therefore, only values from carbon dioxide formation were used.

Acetic Acid Determination. Acetic acid was determined in steam distillates with the aid of an electronic titrimeter, calibrated to read directly in pH values.

Calibration of the titrimeter was accomplished by means of accurate buffers above and below the pH range of interest against which the scale was adjusted; the calibration was checked before each titration. The approximately 500-ml. volumes of the distillates were titrated with 0.01 N sodium hydroxide solution, added in small portions, and the pH value was read and recorded after each addition. The pH values were then plotted against volumes of sodium hydroxide solution used and the volume at pH 7.5 was read off the chart. Similar quantities of distillate from distilled water alone were titrated to this pH value in the same way and the volume of sodium hydroxide solution required was subtracted from that required for the acetic acid distillates. The difference was recorded as milliliters of 0.01 N acetic acid present.

The end points in titrating residual dichromate with ferrous ammonium sulfate were determined with the titrimeter, using platinum and tungsten electrodes.

EXPERIMENTS AND RESULTS

Volume of Dichromate Reduced by Cellulose. PRELIMINARY EXPERIMENTS. The results in the preliminary analysis of raw and purified cottons, cotton linters, and α-cellulose were computed in terms of volume of 1 N dichromate solution required to oxidize

1 milliequivalent (6.75 mg.) of cellulose. The results were examined separately for raw cotton, and for cotton purified by either boiling 1% sodium hydroxide or boiling monoethanolamine. As the volume of 1 N dichromate per milliequivalent of cellulose was found to be the same within the limits of experimental error, the data were combined into a single group and classified, as shown in Table I. It was evident that the volume required varied over a considerable range. It was also evident that as 1 milliequivalent of cellulose should require 1 ml. of 1 N dichromate solution for complete oxidation, about 3.1% of the cellulose was unaccounted for.

Because small amounts of impurities in these materials might conceivably account for the incomplete oxidation, the same oxidimetric method was applied to other materials, consisting of the highly purified cotton cellulose and certain compounds of known purity. The results, computed on the assumption of complete oxidation to carbon dioxide and water by the appropriate oxidation equivalents, and expressed in terms of the original sample, are presented in Table II. Sodium oxalate gave the full theoretical quantity of reduction; all the other substances failed to a greater or lesser degree to reach the theoretical expectation. Although the purity of the cotton cellulose and starch might be questioned because of their amorphous nature, such an objection could scarcely be leveled against the standard dextrose (only 0.02% moisture) and the highly pure sucrose. It seemed more than ever evident that a small discrepancy was occurring in the technique, or elsewhere, which prevented full realization of the theoretical oxidation of these substances.

Table II. Extent of Oxidation of Certain Organic Substances

(Based on assumption of complete oxidation to carbon dioxide and water)

Substance	No. of Observations	Milliequivalent Weights of Substance	Dichromate Reduced, % of Theory
		$Mg.$	%
Cotton, highly purified	16	6.750	97.61 $\pm$ 0.18
Sodium oxalate, N.B.S.[a]	30	67.000	100.14 $\pm$ 0.06
Dextrose, N.B.S.[a]	59	7.506	98.87 $\pm$ 0.11
Sucrose, recrystallized	3	7.128	98.83 $\pm$ 0.01
Starch, purified sweet potato	6	6.750	96.52 $\pm$ 0.20

[a] National Bureau of Standards chemicals for standardization purposes.

OTHER POSSIBLE VARIABLES AFFECTING VOLUMETRIC DETERMINATION. The empirical formula for cellulose—namely, $C_6H_{10}O_5$—which is used in computing the equivalent oxidation value is, of course, only strictly applicable to an infinite degree of polymerization, there being the equivalent of 1 additional water molecule for any finite degree of polymerization. However, computations readily show that with degrees of polymerization of 1000 or more this effect could cause errors in the amount of cellulose accounted for of no more than 1 in the second decimal of the percentage.

The possibility that cellulose was slightly oxidized during the preliminary 2-hour alkali extraction was considered, in view of the knowledge as reported by Davidson (5) that the presence of alkali greatly promotes air oxidation of cellulose. Partial preliminary oxidation of the cellulose during the alkali extraction would obviously give rise to deficiencies in the amount of cellulose accounted for by titration. Weighed samples were extracted under reflux with sodium hydroxide solution or ethanolamine, using as carrier gases either air or nitrogen gas. The cellulose accounted for in all cases was identical within the limits of experimental error, irrespective of whether the carrier gas was air or pure nitrogen.

The possibility of resin formation by 72% sulfuric acid, used to dissolve the cellulose, was considered but found not to be a factor.

The total dilution of the oxidation mixture used was found not

to be a factor unless the volume was increased beyond 75 to 100 ml. when the error due to incomplete oxidation within the 1-hour heating period amounted to about 1.3%. Therefore, slight fluctuations from the normal total volume of 60 ml. could not be held responsible for erroneous results.

Moisture in Oven-Dried Cotton Cellulose. In order to obtain more definite evidence on the amount of moisture held in cotton, 0.5-gram portions of the highly purified sample were weighed out.

Half of the samples were dried in aluminum boxes in the air oven in the customary way, while the other half were dried at 110° C. in a stream of previously dried air at a reduced pressure of 12 mm. of mercury. The air for this oven was dried by passage through a gas scrubber freshly filled with concentrated sulfuric acid. After 16 hours both sets of samples were cooled and weighed. The vacuum-dried samples were then returned to the oven and dried 23.5 hours longer at 110° C. In addition to the purified cotton, samples of raw 24/4/3 cotton cord and 24/1 cotton yarn were dried at 103° C. in the same way as the purified cotton cellulose and in parallel experiments the moisture was determined by the Karl Fischer method. The results are presented in Table III.

It is evident that the vacuum oven removed 0.33% more moisture than the air oven. It is not evident whether additional moisture still remained adsorbed to the cellulose after 39.5 hours in the vacuum oven. With the Karl Fischer technique, on the other hand, it is evident that from 0.4 to 0.6% additional moisture was removed as compared with the air oven at 103° C.

In another line of attack, the Whitwell and Toner (23) extrapolation technique was applied to the data of Neale and Stringfellow (18). It was assumed that the conditions under which the ovens were operated in the laboratory could be sufficiently well represented by an average relative humidity of 50% at a temperature of 80° F. (26.7° C.). The data of Neale and Stringfellow are undoubtedly the most accurate yet obtained at very low moisture contents, but their work was not conducted at a temperature above 80° C. Nevertheless, as Whitwell and Toner point out, constant regain values on an Othmer plot may be extrapolated to any temperature (assuming no decomposition of the material, of course). It was readily computed that laboratory air of the above temperature and humidity would, after appropriate adjustments for temperature and pressure, represent a relative humidity of 1.70% at 103° C., 1.70% at 105° C., and

1.69% at 110° C. It is thus evident that at these temperatures a variation from 103° to 110° C. has practically negligible effect on the relative humidity.

Taking account of Whitwell and Toner's (23) findings that the Othmer plots are parallel in the region of Neale and Stringfellow's data and using the average computed slope for moisture content between 0.234 and 0.795%, it was computed that the equilibrium moisture content of the fibers in the same air at 103° C. would be 0.62%. The moisture content corresponding to relative humidity of 1.69% at 110° C. is also 0.62%, and therefore indistinguishable from that at 103° C.

From these studies, it appears that 0.5 to 0.6% additional moisture must be allowed for in cotton cellulose dried at 103° C. overnight under ordinary laboratory conditions.

Influence of the Dichromate Excess. Cross and Bevan (4) considered a 30% excess of dichromate over that required by the theory necessary for complete oxidation, whereas Kettering and Conrad (14) ordinarily provided only about 6% excess. It seemed desirable, therefore, to examine further the influence of amount of excess.

Maintaining a constant volume of 60 ml. of oxidation mixture and constant acid concentration, the dichromate excess over theory was varied from a few per cent to 45 to 50%. The results from the oxidation of purified cotton linters, National Bureau of Standards dextrose, and recrystallized sucrose are shown in Table IV. It is evident that within the range studied, the amount of excess has little if any influence on the amount of substance accounted for. It does not appear, therefore, that the low excess employed in the Kettering and Conrad technique can account for the incomplete realization of theory.

Formation of Carbon Dioxide. A consideration of the cellulose oxidation equation

$$C_6H_{10}O_5 + 4K_2Cr_2O_7 + 16H_2SO_4 \longrightarrow 6CO_2 + 4K_2SO_4 + 4Cr_2(SO_4)_3 + 21H_2O$$
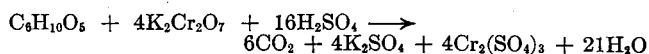
provides another method, based on the carbon dioxide formed, of studying the cause for the low cellulose values found. It is evident that each anhydroglucose unit of the cellulose should furnish, on complete oxidation, 6 moles of carbon dioxide. Anything under this amount would point to incomplete oxidation.

The above equation was studied in two ways—namely, by holding the composition and volume of the oxidation solution constant and varying the sample weight, and by holding the sample weight constant and varying the volume of 12 M sulfuric acid, 0.8 N dichromate solution, and water. The results obtained by the two methods are shown in Tables V and VI, respectively.

It is evident from Table V that variation of the sample weight has no significant effect upon the carbon dioxide produced. The result obtained with the smallest sample weight is slightly higher than the others; but owing to the small weight of sample taken, too great accuracy in this result cannot be expected. The average of all determinations is 98.04% of theory, and confirms the general findings, based on titration.

The results shown in Table VI are in fairly good agreement among themselves, though somewhat lower, on the average, than those of Table V. The result with the composition of oxidation mixture routinely used— i.e., that shown in the second row from the top—gave the highest recovery, based on the expected yield.

Both sets of results confirm the titration findings in showing

Table III. Results of Drying Experiments under Different Conditions

Method Used for Moisture	In purified cotton cellulose %	In raw 24/4/3 cotton cord %	In raw 24/1 cotton yarn %
		Moisture	
Air oven at 103° C. for 16 hours	5.73	6.15	6.32
Vacuum oven at 110° C. for 16 hours	6.00	...	...
Vacuum oven at 110° C. for 39.5 hours	6.06	...	...
Karl Fischer method	...	6.74	6.73
Increase over air oven at 103° C.	0.33	0.59	0.41

Table IV. Cellulose, Glucose, and Sucrose Accounted for with Different Percentage Excesses of Dichromate Taken

Material Taken	Sample Used G.	0.8098 N Dichromate Added Ml.	Theoretical Milliequivalent Weight of Sample G.	Excess Dichromate %	Carbohydrate Found, Based on Theoretical Milliequivalent Weight %
Linters (high viscosity)	0.0949	25.06	0.00675	44.55	98.06 ± 0.03
		20.00		15.25	97.83 ± 0.06
		18.00		3.74	97.98 ± 0.04
		17.77		2.47	97.52 ± 0.18
Dextrose (National Bureau of Standards)	0.1000	25.00	0.007506	51.75	99.35 ± 0.13
		20.00		21.42	99.31 ± 0.04
		18.00		9.31	99.24 ± 0.09
		16.90		2.65	98.99 ± 0.13
Sucrose (recrystallized)	0.1000	25.00	0.007128	44.34	98.59 ± 0.22
		20.00		15.41	98.75 ± 0.06
		18.00		3.97	98.78 ± 0.13

Table V. Comparison of Carbon Dioxide Produced from Different Amounts of Purified Sample with Theoretical Amounts Expected

Sample Taken, Oven-Dried at 103° C.	Carbon Dioxide Formed	Carbon Dioxide Expected	Carbon Dioxide Found, Based on Theory
$Mg.$	$Mg.$	$Mg.$	%
94.0	150.0	153.16	97.94
70.5	112.3	114.87	97.76
61.1	97.3	99.56	97.73
47.0	75.6	76.58	98.72
Average proportion of theoretical carbon dioxide found		98.04 ± 0.23	

Table VI. Carbon Dioxide Produced by Oxidation of Purified Sample with Variations in Volumes of Components of Hydrolytic Mixture

Sample Dried at 103° C.	0.8 N Dichromate Solution	12 M Sulfuric Acid	Water Added	Carbon Dioxide Found	Carbon Dioxide Found, Based on Theory
$Mg.$	$Ml.$	$Ml.$	$Ml.$	$Mg.$	%
94.68	25.00	25.00	5.00	149.6	96.95
94.68	25.00	25.00	10.00	150.4	97.47
94.68	25.00	25.00	15.00	149.7	97.02
94.68	25.00	20.00	10.00	149.4	96.82
94.68	25.00	30.00	10.00	149.9	97.15
Average proportion of theoretical carbon dioxide found				97.08 ± 0.11	

that incomplete oxidation must be assumed. This could be due to the formation of carbon monoxide, which would not be absorbed in the oxidation train used for the carbon dioxide; or it could be due to residual acetic acid, escaping oxidation.

Determination of Carbon Monoxide. Carbon monoxide would obviously be a half-way step in the complete oxidation of cellulose with acid dichromate solution. If carbon monoxide were formed in any appreciable quantity, being a gas it could readily pass out of the oxidation mixture before it would be further oxidized to carbon dioxide.

A test was made for carbon monoxide using the method described above (Figure 2). Approximately 0.2-gram triplicate samples of purified cotton, commercially purified linters, National Bureau of Standards dextrose, and recrystallized sucrose were oxidized. In all cases, iodine was liberated, as indication of carbon monoxide formation. Carbon dioxide was absorbed at the same time in the Ascarite tube and was weighed. The results, together with the percentages of original sample that may be computed to be "lost" in the usual acid dichromate oxidation through carbon monoxide formation, are shown in Table VII.

Table VII. Carbon Monoxide Formed from Oxidation of Carbohydrates, and Equivalents in Terms of Original Substance

Substance	Sample Dried at 103° C.	Carbon Monoxide Found	Theoretical Equivalent of Substance	Substance Lost as CO
	$Mg.$	$Mg.$	$Mg.$	%
Purified cotton	188.6	2.99 ± 0.09	2.88	1.53
Linters (commercial purified)	188.9	2.88 ± 0.10	2.78	1.47
Dextrose	200.0	1.91 ± 0.10	2.09	1.04
Sucrose	200.0	2.08 ± 0.06	2.12	1.06

The mean of the percentages of purified cotton lint and linters presumed as described above to be lost in the usual acid dichromate oxidation is 1.50. From the data in Tables V and VI, it is evident that the percentages of cellulose accounted for under routine oxidation conditions, based on carbon dioxide formed, are 97.94 and 97.47, respectively, or an average of 97.70%. If to this average are added 1.50% ordinarily lost through escape of carbon monoxide and 0.5 to 0.6% due to moisture retention, we have accounted for a total of 99.7 to 99.8% of the original sample taken.

Combined Determination of Carbon Dioxide and Monoxide. In order to verify, in a single determination, the percentage of cellulose accounted for by carbon dioxide and carbon monoxide, the train shown in Figure 2 was modified.

In place of the potassium hydroxide and soda-lime towers, H and I, the equipment in Section B, Figure 1, was substituted, except for the guard tube, K. After the preliminary trials showed that the carbon dioxide blank was small and reproducible, only Section A of Figure 2 was swept free to remove carbon dioxide before beginning a determination.

The sample, with 25 ml. of dichromate solution and 10 ml. of water, was placed in the oxidation flask and the system was swept with nitrogen through guard tube, L (Figure 1), at a rate of 5 liters per hour for 30 minutes. Twenty-five milliliters of 12 M sulfuric acid were then added to the oxidation flask and the gas flow was adjusted to 0.5 liter per hour, and directed through the entire train. The sample was now heated to boiling for 1 hour and, after removal of the heat, the system was swept for a total of 7 additional hours at the same rate of 0.5 liter per hour. The results of nine consecutive determinations are presented in Table VIII.

It is seen that carbon dioxide was formed directly from the dichromate oxidation, and also secondarily from the carbon monoxide with the aid of the iodine pentoxide.

Table VIII shows that, on the average, 97.60% of the expected carbon dioxide is obtained from the acid dichromate oxidation and 1.65% from the carbon monoxide which would ordinarily escape. These two values together account for 99.25% of the expected carbon dioxide. If 0.15% is allowed for ash and 0.50 to 0.60% for additional moisture, it is evident that 99.90 to 100.00% of the original cellulose present has been accounted for.

Acetic Acid. Although, according to Houghton (12), there was little reason to believe that acetic acid is a product of the incomplete oxidation of cellulose, there remained the possibility that small traces of acetic acid could be formed in some side reaction. If acetic acid were formed it would be incompletely oxidized, as shown, for example, by the work of Simon (21, 22); and though somewhat volatile, would be retained in the oxidation residue due to the reflux condenser. It was therefore thought desirable to examine the oxidation residues for the presence of any unoxidized acetic acid.

For this purpose a steam distillation apparatus was set up. It consisted of a steam generator and a 2-necked round-bottomed oxidation flask; one neck was connected to the steam generator, and the other supported a 3-ball Snyder column, whose top supported a thermometer. The Snyder column contained a side arm fitted with a condenser which could be turned up for refluxing or down for collection of distillate. All joints were of ground glass.

Approximately 1-gram samples of purified cotton cellulose were used in the oxidation, thus providing 10 times the quantity used in the preliminary routine volumetric determination. The mixture of sulfuric acid, dichromate, and water, in the proper proportions for the increased sample weight, was first heated to boiling and swept out with steam for some time before the cellulose sample was added. The sample added, oxidation was conducted at the boil for 1 hour with the condenser in the reflux position. The condenser was then swung down and distillate collected from the mixture. This distillate was examined from time to time on a spot plate with bromothymol blue, La Motte yellow, and bromocresol green. Although it seemed to have a slightly acid reaction, some refinement of techniques and checking of blanks revealed that the reaction was no more acid than that obtained without the cellulose but with only the oxidation mixture present.

In order to make sure that acetic acid if present could be detected, experiments were conducted by electrometric titration, as described above under methods. Different amounts of acetic acid were placed in the oxidation flask together with the oxidation mixture and steam-distilled, and the distillate was collected in successive 500-ml. volumes. In like manner, 1 gram of purified cotton cellulose was oxidized for 1 hour, and steam-distilled. The results are presented in Table IX.

It is apparent that the acetic acid formed in the oxidation of any cellulosic material would be readily detected if present in

Table VIII. Total Carbon Dioxide Obtained from Purified Cotton Cellulose by Combined Acid Dichromate and Iodine Pentoxide Oxidation

Cellulose Sample Dried at 103° C. Equivalent CO_2 expected		Carbon Dioxide from Dichromate		Carbon Dioxide from Carbon Monoxide		Total Carbon Dioxide Observed	
Weight Mg.	expected Mg.	Weight Mg.	Portion of expected %	Weight Mg.	Portion of expected %	Weight Mg.	Portion of expected %
94.1	153.27	149.0	97.21	2.8	1.83	151.8	99.04
94.0	153.11	149.8	97.84	2.5	1.63	152.3	99.47
94.0	153.11	149.9	97.90	2.4	1.57	152.3	99.47
93.8	152.78	148.3	97.07	2.4	1.57	150.7	98.64
93.8	152.78	149.0	97.53	2.4	1.57	151.4	99.10
94.0	153.11	149.0	97.32	2.5	1.63	151.5	98.95
94.0	153.11	149.7	97.77	2.8	1.83	152.5	99.60
93.7	152.62	148.5	97.30	2.2	1.44	150.7	98.74
93.7	152.62	150.3	98.48	2.7	1.77	153.0	100.25
Mean			97.60 ± 0.15		1.65 ± 0.05		99.25 ± 0.17

Table IX. Acetic Acid by Electrometric Titration of Steam Distillates from Different Mixtures

Weight of Sample Placed in	Acetic Acid Recovered			
	First 500 ml. Mg.	Second 500 ml. Mg.	Third 500 ml. Mg.	Total Mg.
Oxidizing mixture— 10 mg. acetic acid	8.7	1.4	0.2	10.3
4 mg. acetic acid	2.8	0.7	0.0	3.5
1 g. cotton	0.4	0.2	0.0	0.6
500 ml. distilled water— 5 mg. acetic acid (not steam-distilled)	5.3	...	...	5.3

quantities of 4 mg. or less. Actually 0.6 mg. was indicated by the electrometric titration, which, if computed back on the original sample, would amount to only 0.06%. It seems evident, thus, the acetic acid, if formed in the oxidation, cannot be a significant factor affecting the completeness of oxidation, based on theory.

"Experimental" Milliequivalent Weight. It is evident from the foregoing that two sources of error practically account for the discrepancy between experiment and theory: the moisture remaining in the sample after the usual drying procedure, and the carbon monoxide that escapes from the oxidizing mixture before conversion into carbon dioxide.

The moisture discrepancy can be overcome in a practical way by arbitrary addition of 0.5 to 0.6% to the moisture content found by drying in an air oven at 103° to 105° C. (Such an arbitrary correction has been used in the work reported by Frilette, Hanle, and Mark, 8).

The adjustment for carbon monoxide escape is accomplished as follows:

From the data of Table VIII the average cellulose lost through carbon monoxide formation was seen to be 1.65%. On the other hand the cellulose accounted for by acid dichromate oxidation was 97.6%. Together, the two values account for 99.25%. If we allow 0.75% for the combined ash and moisture we shall need to revise slightly the above percentages of cellulose accounted for by acid dichromate and carbon monoxide oxidation to 98.33 and 1.66, respectively, on the moisture- and ash-free basis. If we accept 6.750 mg. of cellulose as the milliequivalent weight when carbon dioxide and water are the only products, and 13.51 mg. as the milliequivalent weight when carbon monoxide and water are the only products of oxidation, then we can compute an "experimental" milliequivalent weight when both carbon monoxide and carbon dioxide are formed in the above ratio, as

$$0.0166 \times 13.51 + 0.9833 \times 6.750 = 6.861 \text{ mg.}$$

This experimentally determined milliequivalent weight was applied to a series of samples corrected to a moisture-free basis, but not corrected for ash (Table X). The cellulose found on the basis of the theoretical milliequivalent weight is seen to average 98.33%, whereas that computed on the basis of the "experimental" milliequivalent weight averages 99.91%. The ash probably accounts for the remainder, although the precision of the

experiments does not warrant absolute certainty.

DISCUSSION

It is concluded that two principal sources of error account for the discrepancies between experiment and theory in the acid dichromate volumetric determination of cellulose: the amount of moisture adsorbed on the sample dried in the air oven at 103° to 105° C., and the amount of carbon monoxide formed during the oxidation step. A third minor source of error is found in traces of ash which are retained in spite of careful purification.

The moisture cannot be removed from samples dried in an air oven, even though the temperature is raised to 110° C., if normal laboratory air is used as the drying medium; neither can the moisture be fully removed by means of a vacuum oven.

According to Heuser (10), it is probable that carbon monoxide originates from either or both formic and oxalic acids, which are intermediates in the oxidation of glucose, and which break up in the presence of heat and sulfuric acid to give carbon monoxide as well as carbon dioxide. Christensen, Williams, and King (3) found it impossible to prevent the formation of carbon monoxide during the oxidation of certain compounds, notably carbohydrates. It is somewhat surprising that neither Birtwell and Ridge (1) nor Launer (16) observed the loss of carbon monoxide in connection with their volumetric determinations.

No variation of technique was discovered in the present studies for preventing the formation of carbon monoxide. Although carbon monoxide can be readily oxidized with iodine pentoxide, as was done in the present studies, for routine determinations the technique would be cumbersome and unsatisfactory. On the other hand the use of an "experimental" milliequivalent weight of cellulose seems to be entirely practical and convenient.

Table X. Moisture-Free Cellulose

Sample Designation	Moisture-Free Cellulose Found	
	Using theoretical milliequivalent weight (6.750 mg.) %	Using experimentally determined milliequivalent weight (6.861 mg.) %
A	98.53	100.11
B	98.30	99.87
C	98.10	99.67
D	97.85	99.42
E	98.88	100.46
Mean	98.33	99.91 ± 0.18

CONCLUSIONS

A study of the technique of Kettering and Conrad for the volumetric acid-dichromate determination of cellulose in cotton fiber, made with a view to accounting for the lower than theoretical cellulose values obtained, has permitted the following conclusions:

From 0.5 to 0.6% moisture, depending on the atmospheric condition of the room, is retained in cotton samples dried to constant weight in an air oven at 103° to 110° C.

Acid-dichromate oxidation fails to convert all the cellulose (or dextrose or sucrose) into carbon dioxide and water. The amount of ash- and moisture-free cellulose accounted for is only 98.33% of the theory

Carbon monoxide is formed during the acid-dichromate oxida-

tion of cellulose, and in the usual technique escapes into the air. The amount of cellulose "lost" in this way amounts to 1.66%.

Acetic acid is not a product of the acid-dichromate oxidation of cellulose.

Accurate cellulose analysis can be accomplished by the use of an experimental milliequivalent weight, 6.861 mg., based on the observed relative amounts of carbon monoxide and carbon dioxide formed during the acid-dichromate oxidation.

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Estimation of Moisture in Sweet Potato Starch

Specific Gravity Method

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A simple and rapid specific gravity method for the estimation of moisture in sweet potato starch at moisture contents ranging from 15 to 50% is described, by which a determination accurate to within about 0.5% moisture may be completed in approximately 7 minutes. The method, which is based in principle on the conventional pycnometer procedure for determining particle density, allows the use of a torsion balance, large starch samples, and volumetric flasks, and thus is convenient for either laboratory or plant operations. Weight data are converted graphically to terms of moisture content and temperature compensation data are applied to eliminate the necessity for careful control of working temperature. Directions are given for adapting the method to the estimation of moisture in other starches and in other suitable materials. The method may also prove applicable to chalk, sands, soils, certain abrasive powders, and resins, as well as to ceramic materials.

INCIDENTAL to investigations on the production and processing of sweet potato starch, and to application of the findings in factory operation, it was frequently necessary to know, with minimum delay, the approximate moisture content of wet starch from settling tanks, tables, centrifugals, and filters. Such information was particularly needed in adjustment and control of centrifugal and dryer operation. The moisture contents encountered ranged from about 30 to 45% and higher. Conventional methods for determining moisture on such starches by drying are too slow for practical manufacturing plant control, because moisture in excess of about 15% must be removed at a relatively low temperature to avoid gelatinization of the starch. Accordingly, consideration was given a specific gravity procedure, as highly accurate results were not essential and, in most cases, the determinations were to be made on starch relatively free of nonstarch solids. Inasmuch as starch is insoluble in, and denser than, water the specific gravity of a suspension of starch in water can be used to calculate the original moisture content of the starch.

Specific gravity procedures for determination of moisture in starch have previously been reported.

Sarre (7) described a method for measuring the moisture content of white potato starch in which a weighed sample of the starch was transferred to a volumetric flask and made up nearly to volume with water. The suspension was brought to a temperature of 17.5° C. by placing the flask in a water bath for 30 minutes before final adjustment of the volume. The weight of the suspension was converted to terms of starch moisture content by means of tabular data calculated from a previously established starch density value. This method was time-consuming and, moreover, the values given in the tables were based on a starch density of 1.65, which is appreciably different from that of sweet potato starch. Various other moisture methods applicable to white potato starch have been described and evaluated by Porter and Willits' (5). For many years the hydrometer has been used for estimating the dry substance content of starch suspensions or slurries. Tables of improved accuracy for Baumé-starch content of cornstarch suspensions have been published by Cleland, Fauser, and Fetzer (3) and similar tables specifically for sweet potato starch have been developed in this laboratory. However, such procedures are not applicable when the solids content exceeds about 45% or the moisture content is lower than about 55%.

Papadakis (4) reported a rapid procedure for determining moisture in soils which was somewhat similar to Sarre's method for starch, and Waters (8) used the same principle for determining moisture in sand. Bauer (2) also described a specific

gravity method for the determination of moisture in sand and developed a formula for calculating moisture content which proved useful in the present study.

EXPERIMENTAL

Starch Density. In order to develop a suitable specific gravity moisture method it was necessary first to establish the density (grams per milliliter) of sweet potato starch. This was done by means of the conventional pycnometer procedure, except that calibrated 500-ml. volumetric flasks and 100-gram starch samples were used instead of pycnometers and small samples. Because of the hygroscopic nature of dry starch, air-dry starch of known moisture content was utilized. Moisture was determined by a vacuum-oven procedure essentially similar to the A.O.A.C. method for wheat flour (1), but with the following modifications:

The samples were dried to constant weight at $100° \pm 0.5°$ C. at a pressure of 1 mm. or less. Pressure in the vacuum oven was restored through a desiccant train consisting successively of two 19-liter (5-gallon) bottles in series, each filled to a depth of 2.5 cm. (1 inch) with concentrated sulfuric acid; a large tower filled with ceramic packing frequently wetted with concentrated sulfuric acid; a tower filled with glass wool; two towers of activated alumina; and a final tower of phosphorus pentoxide on glass wool. The samples were cooled 30 minutes over activated alumina. These modifications are essentially similar to those recommended by Sair and Fetzer for a reference method for moisture in starch (6).

To determine starch density, a weighed sample of the air-dry starch was dispersed in 300 ml. of distilled water, transferred to a volumetric flask, and diluted nearly to volume. The contents were evacuated to remove entrapped air, brought to a temperature of 25° C., made to volume, and weighed. Weighings were made in air to the nearest milligram on a large analytical balance having a capacity of 1 kg. and a sensitivity of 1 mg. All weight data were corrected to vacuum. Starch density was calculated on the basis of moisture-free starch taken. Determinations in quadruplicate on each of three commercial sweet potato starches yielded density values ranging from 1.631 to 1.634. The arithmetic mean of these values was 1.633, which is used herein as the apparent density of sweet potato starch at 25° C. Subsequent density determinations on more than 50 samples including bleached, unbleached, and modified sweet potato starches yielded results that agreed closely with this density value.

With the apparent density of sweet potato starch thus established, the procedure for determination of moisture content was based on the difference between the weight, at 25° C., of a 500-ml. aqueous suspension containing a 100-gram starch sample and the weight of an equal volume of water. If the temperature of the suspension was other than 25° C. a correction was applied to the tare weight of the water. From this difference in weight, called the "difference value," and the densities of starch and water, the percentage moisture content of the starch was obtained. A graph was prepared to facilitate conversion of the difference value to terms of per cent moisture.

Preparation of Graph. Bauer (2) reported a basic formula for computing per cent moisture from experimental data of the type obtained by the foregoing moisture and density determinations.

The formula given by Bauer for computing the moisture per cent on the bais of the wet weight of the sample is

$$w = \frac{\left(W - \frac{W}{G}\right) - (W_b - W_a)}{\left(W - \frac{W}{G}\right)} 100 \qquad (1)$$

where w = % moisture on wet basis
  W = weight of moist sample, in grams
  W_a = weight of pycnometer full of water, in grams
  W_b = weight of pycnometer, material, and water
  G = specific gravity of material

Formula 1 may be modified for use in the present starch moisture method as follows:

Letting w = % moisture on wet basis
  W = weight of moist starch sample in grams = 100
  W_a = weight of flask plus water at 25° C.
  W_b = weight of flask plus suspension (starch sample and water) at 25° C.
  G = 1.633, density of sweet potato starch
  $D.V.$ = difference value, equivalent to $(W_b - W_a)$ from Formula 1
  D_w = density of water

and simplifying Formula 1 we have

$$w = \left(1 - \frac{(W_b - W_a)}{W\left(1 - \frac{1}{G}\right)}\right) \times 100$$

or

$$w = 100 - \frac{100\,(W_b - W_a)}{100\left(1 - \frac{1}{G}\right)}$$

or

$$100 - w = \frac{W_b - W_a}{\left(1 - \frac{1}{G}\right)}$$

Then, as $W_b - W_a = D.V.$

$$100 - w = \frac{D.V.}{\left(1 - \frac{1}{G}\right)}$$

or

$$D.V. = (100 - w)\left(1 - \frac{1}{G}\right)$$

where 1 in the ratio $1/G$ represents the density of water. Substitution of the symbol D_w for unity in this formula allows consideration to be given the influence of temperature on the density of water, and yields the modified formula

$$D.V. = (100 - w)\left(1 - \frac{D_w}{G}\right) \qquad (2)$$

Inasmuch as the relation between $D.V.$ and w is linear, two points which become the axis intercepts of a straight-line graph may be computed by letting $w = 100$ and $w = 0$.

Using Formula 2 and the foregoing symbol equivalents it was found that at 25° C., where $D_w = 0.99707$,

For $w = 0$, $D.V. = 38.94$

For $w = 100$, $D.V. = 0$

By plotting these values for $D.V.$ on the ordinate and their corresponding equivalents for w on the abscissa, and connecting the points by a straight line, a graph is obtained. Per cent moisture, on a wet basis, equivalent to $D.V.$ is read directly from the graph.

The ordinate intercept for a temperature other than 25° C. may be found by substituting for D_w in Formula 2 the absolute density of water at that temperature. Thus, the ordinate intercepts will be $D.V. = 38.87$ at 20° C., and $D.V. = 39.03$ at 30° C. It is obvious that the abscissa intercept, representing a $D.V.$ of 0 and a moisture content of 100%, will remain unchanged irrespective of temperature. For practical purposes a graph bearing a single 25° C. temperature line is sufficiently accurate for converting difference values obtained at working temperatures between 20° and 30° C. to terms of per cent moisture.

DETAILS OF METHOD

Apparatus. Torsion balance with a capacity of 1 kg. and an accuracy of 0.1 gram, 500-ml. volumetric flasks, and tared 400-ml. beakers.

Procedure. First obtain the tare weight of a 500-ml. volumetric flask filled to the mark with water at 25° C. Make this and sub-

sequent weighings to the nearest 0.1 gram. Weigh 100 grams of the starch sample into a tared 400-ml. beaker and add 250 to 300 ml. of water at a temperature of 20° to 30° C. Stir with a spoon until all lumps are broken up, and with the aid of a funnel and a jet of water quantitatively transfer the mixture to the empty volumetric flask. Adjust to final volume by slowly adding water from a pipet or dropper without unduly mixing the water with the starch suspension. Remove surplus water from the neck of the flask with a roll of filter paper, and wipe the outside of the flask dry. Weigh, and finally measure the temperature of the suspension.

If the final temperature of the suspension is 25° C., subtract the tare weight of the flask and water at 25° C. from the observed weight of the flask and suspension to obtain the difference value. If the temperature of the suspension is other than 25° C., use the data in Table I to correct the tare value to what it would be if determined at the temperature of the suspension; then make the subtraction. Convert the difference value to per cent moisture with the aid of the graph.

Notes. The computed data in Table I represent the difference between the weight of 500 ml. of water at 25° C. and at each of the other temperatures specified. Although this procedure is an indirect method of compensating for deviations in the suspension temperature, the error induced thereby is insignificant. If it is necessary to make moisture determinations at working temperatures outside the 20° to 30° C. range, the tare weight of the flask plus water may be determined at a temperature near the middle of the required temperature range and a new series of suitable correction factors computed.

If the starch or similar materials to which the method is applied are dirty or contaminated with proteinaceous matter, slight foaming may tend to distort or obscure the meniscus in the

volumetric flask. In such a case the flask is allowed to stand a moment after the contents are made to volume and, with a pipet, the foam and upper 2 to 3 ml. of liquid above the suspension are drawn off and discarded. The flask is refilled to the mark with water and the regular procedure continued.

In determining the density of sweet potato starch the weight data were corrected to an in vacuo basis for computing density. It was noted, however, that density values computed from both corrected and uncorrected weight data differed only in the fourth decimal place. The difference is so insignificant that in both density and moisture determinations corrections for air buoyancy may be safely disregarded.

Tests of the effect of evacuating the starch-water suspension during moisture determinations showed that, if the starch sample is thoroughly mixed with water before being transferred to the volumetric flask, evacuation is unnecessary.

Where large numbers of moisture determinations are to be made in routine work it is desirable to mark permanently and tare several flasks and beakers, so that repeated tare weighings may be avoided.

RESULTS

Table II presents the comparative results of moisture determinations by the specific gravity method and by the reference vacuum-oven method on a number of sweet potato starches ranging in moisture content from 12 to 75%. Included were moist starch cakes from factory centrifugals, air-dry bleached commercial starches, and both bleached and unbleached starches of pilot plant origin.

The maximum deviation of the individual moisture values obtained by the specific gravity method from those by the reference method is approximately 0.7% moisture, and the maximum deviation between the averages of replicates is 0.6% moisture. In every case except one the mean deviation is less than 0.5% moisture. A similar order of agreement between individual moisture values obtained by the specific gravity method indicates that this method yields fairly reproducible results. The results are equally satisfactory with commercially dry starches of 12 to 13% moisture content and with wet starches of 20 to 50% moisture. Although sweet potato starch becomes the dispersed phase at moisture contents above 50%, moisture determinations on suspensions proved satisfactory up to a moisture content of

Table I. Data for Correcting Established Tare Value for Weight of Flask Plus Water at 25° C. to Tare Value at Temperatures Indicated

Temperature of Suspension When Weighed	Weight Correction to Apply to Tare Weight of Flask Plus Water at 25° C.
° C.	Gram
20	+0.6
21	+0.5
22	+0.4
23	+0.3
24	+0.1
25	0.0
26	-0.1
27	-0.3
28	-0.4
29	-0.6
30	-0.7

Table II. Comparative Results of Moisture Determinations on Representative Sweet Potato Starches

Sample No.	Sweet Potato Starch	Moisture Content		Deviation of Specific Gravity Method from Reference Method Averages	Deviation of Individual Moisture Values by Specific Gravity Method from Reference Moisture Content		
		Reference, vacuum-oven method, av. of duplicate determinations	Specific gravity method, av. of replicate determinations		Minimum	Maximum	Mean
		%	%	%	%	%	%
1	Commercial product	12.22	12.30	+0.08	-0.02	+0.28	±0.15
2	Commercial product	13.00	13.20	+0.20	+0.10	+0.40	±0.25
3	Commercial product	13.05	13.00	-0.05	+0.05	-0.25	±0.15
4	Commercial remoistened	43.35	43.73	+0.38	+0.15	+0.65	±0.40
5	Commercial remoistened	45.74	45.90	+0.16	-0.04	+0.26	±0.15
6	Commercial remoistened	45.94	45.90	-0.04	+0.06	-0.24	±0.15
7	Commercial remoistened	29.17	29.20	+0.03	+0.03	+0.03	±0.03
8	Commercial remoistened	20.73	21.15	+0.42	+0.17	+0.67	±0.42
9	Pilot-plant product, moist, never dried	45.05	45.07	+0.02	-0.05	+0.15	±0.10
10	Pilot-plant product, partially dried	26.34	26.73	+0.39	+0.16	+0.66	±0.41
11	Cake from centrifugal	37.26	37.60	+0.34	+0.24	+0.44	±0.34
12	Cake from centrifugal	37.32	37.35	+0.03	+0.12	+0.18	±0.15
13	Cake from centrifugal	36.60	36.75	+0.15	-0.10	+0.40	±0.25
14	Cake from centrifugal	37.93	37.75	-0.18	+0.07	-0.43	±0.25
15	Cake from centrifugal	37.50	37.35	-0.15	+0.20	-0.50	±0.35
16	Cake from centrifugal	34.44	34.10	-0.34	-0.24	-0.44	±0.34
17	Cake from centrifugal	33.10	32.50	-0.60	-0.60	-0.60	±0.60
18	Cake from centrifugal	33.25	32.85	-0.40	-0.55	-0.25	±0.38
19	Cake from centrifugal	33.85	33.75	-0.10	-0.35	+0.15	±0.25
20	Cake from centrifugal	33.25	33.15	-0.10	-0.45	+0.25	±0.35
21	Starch-water mixture[a]	47.80[b]	48.10	+0.30	+0.20	+0.40	±0.30
22	Starch-water suspension[a]	56.50[b]	56.70	+0.20	+0.20	+0.20	±0.20
23	Starch-water suspension[a]	65.20[b]	65.10	-0.10	-0.20	0.00	±0.10
24	Starch-water suspension[a]	73.90[b]	74.20	+0.30	+0.30	+0.30	±0.30

[a] Mixtures of weighed quantities of starch (of known moisture content) and water.
[b] Theoretical moisture content.

75%. Above 75% the difference value becomes so small that weighing errors are magnified. However, the specific gravity method was developed for and is recommended for application to starches containing approximately 15 to 50% moisture. For starches of under 15% moisture content another rapid method of greater accuracy has been developed in this laboratory (publication pending). For fluid suspensions hydrometer procedures are more rapid and are more accurate at the higher dilutions.

The time required to complete a moisture determination by the specific gravity method averaged 7 minutes. By following the prescribed procedure other operators have also obtained results of the concordance shown in Table II.

ESTIMATION OF MOISTURE IN OTHER MATERIALS

The specific gravity method may be adapted to the determination of moisture in other root, tuber, and cereal starches. It may be adaptable to a variety of other materials—for example, chalk, sands, soils, ion-exchange resins, certain types of abrasive powders, and some ceramic materials. Suitable materials would of course be insoluble in, wettable by, and markedly denser than water. They probably would also have to be of a non-swelling and nonhydrating nature, at least to a large degree; either homogeneous, or of constant density; and noncolloidal in particle size, to permit fairly rapid settling and to prevent oriented absorption of water molecules on particle surfaces.

To adapt the method to a suitable material, determine the density of the material on a moisture-free basis, substitute this density value in Formula 2, and prepare a graph in the manner described for sweet potato starch. Follow the same procedure as before for determining moisture and correcting for temperature effects. If, however, the material is not readily wetted by water, so that air bubbles are displaced from the particles, evacuate the suspension just before making to final volume.

Formula 1 and its modified form, Formula 2, yield per cent moisture on a wet-sample basis. If it is desired to compute per cent moisture on a dry-sample basis, another formula given by Bauer (2) may be utilized. This formula, expressed in terms of the same symbol equivalents cited for Formula 1, except that here w = per cent moisture on dry basis, is

$$w = \frac{\left(W - \frac{W}{G}\right) - (W_b - W_a)}{(W_b - W_a)} \cdot 100 \tag{3}$$

By using the same symbols as in Formula 2 but with w representing per cent moisture on a dry basis. Formula 4 for computing starch moisture may be derived from Formula 3 as follows:

$$\frac{w}{100}(W_b - W_a) = W\left(1 - \frac{1}{G}\right) - (W_b - W_a)$$

or

$$\frac{w}{100}(W_b - W_a) + (W_b - W_a) = W\left(1 - \frac{1}{G}\right)$$

and since

$$(W_b - W_a) = D.V.$$

$$D.V. = \frac{W\left(1 - \frac{1}{G}\right)}{\left(\frac{w}{100} + 1\right)}$$

which, for any density D_w is written in the form

$$D.V. = \frac{W\left(1 - \frac{D_w}{G}\right)}{\left(\frac{w}{100} + 1\right)} \tag{4}$$

Formula 4 may be solved for w, or, more conveniently, for $D.V.$ with a number of assumed values substituted for w to obtain sufficient $D.V. - w$ equivalents for preparing a graph.

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Elemental Analysis by Lamp Combustion

Application of Modified Burners to Special Problems

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THIS paper describes the construction of several modified burners which have been used in ultimate analysis of organic materials by lamp combustion. They have been applied to the determination of sulfur in aromatics, halogens (chlorine and bromine) in gasolines, and carbon and hydrogen in certain classes of organic substances. It has been found possible to carry out the combustion of nonvolatile as well as volatile liquids with the aid of suitable modified burners.

The determination of sulfur in volatile organic materials by the lamp method is a technique well known in the petroleum industry and extensively used in control of refinery operations and in the evaluation of finished gasolines. The volatile hydrocarbon is allowed to burn from a wick. Any sulfur present is converted to sulfur dioxide or trioxide and is retained by scrubbing the exhaust gases through a suitable medium. If the products of combustion are scrubbed with a known volume of standard sodium carbonate solution, the hydrogen ion, equivalent to the sulfur present, may be estimated by back-titration with standard acid. If hydrogen peroxide is used as the absorbent, sulfur will be completely oxidized and retained as sulfuric acid, which may be titrated directly with standard base. Other refinements include the use of closed systems supplying purified air to reduce the blank; the substitution of a synthetic carbon dioxide-oxygen atmosphere to eliminate an inherent error in the use of air due to slight oxidation of nitrogen; and the use of all-glass lamps with standard-taper interchangeable joints. The procedure

This paper describes several modified burners which are superior to the A.S.T.M. standard burner for lamp combustion. Burners for both volatile and nonvolatile liquids are illustrated. Application to the following problems is discussed: determination of sulfur in aromatics without recourse to blending; determination of halogens (chlorine and bromine) in gasoline—essentially complete combustion is obtained as contrasted to results 10 to 30% low using the standard burner; determination of carbon and hydrogen in petroleum hydrocarbons. Although satisfactory results for hydrogen only may be obtained with the A.S.T.M. burner, it is known to yield low results for carbon.

described by Zahn (8), comprising the use of exhaustively purified air and the turbidimetric determination of sulfur as barium sulfate, permits the detection and estimation of as little as 0.0001% sulfur.

This latter procedure is used in this laboratory. It has been found eminently suitable to all ranges of sulfur content, particularly when the gravimetric process is employed for the higher concentrations. Moreover, it is free of error inherent in acidimetric measurement due to inclusion of hydrogen ion from other sources such as halogens or nitrogen in the sample or oxidation of atmospheric nitrogen.

DESCRIPTION OF MODIFIED BURNERS

The standard apparatus used in many laboratories for burning liquid hydrocarbons is the A.S.T.M. lamp (1). The burner supplied with this lamp is suitable for most materials, but this paper describes several modified burners which have proved useful in the burning of unusual samples.

Burners for Volatile Materials. A common problem encountered in petroleum and solvents laboratories is the determination of sulfur in aromatic compounds such as benzene, toluene, and xylene or in mixtures rich in aromatics. It is impossible to burn these materials in the normal manner, because they burn with a very smoky flame. Low results will be caused by incomplete combustion of the sample, and the soot formed may clog the absorber. Some finished gasolines containing a moderate proportion of aromatics do not smoke but may produce an off color in the absorber liquid or may impart a characteristic odor to the exit air.

It is possible to burn aromatic hydrocarbons by diluting them sufficiently with sulfur-free alcohol or paraffinic solvent, but this technique introduces errors in blending, restricts the amount of sample that may be taken, and lengthens the time of analysis. Combustion may also be improved by enriching the air stream with oxygen to bring the oxygen concentration up to 35 or 40%, but this is a hazardous practice not suited to routine operation.

Figure 1, B, illustrates a modified burner that has been found very useful in the combustion of highly aromatic materials. A burner utilizing the carburetion principle was originally described by the Anglo-Iranian Oil Company (2).

The novel feature which makes possible the burning of aromatics is the introduction of the primary air stream directly into the wick tube, so that an intimate mixture of fuel and air is produced. This carburetor type of burner is in contrast to the conventional type in which the primary air stream is concentric to, but not in direct contact with, the fuel except at the flame.

The burner in Figure 1, B, was modified from this Anglo-Iranian burner. The carburetion principle was retained but was incorporated with the A.S.T.M. burner (shown for comparison in Figure 1, A) to give a burner that could be used interchangeably in the A.S.T.M. lamp. A description of the original Anglo-Iranian apparatus is now available (5).

The modified burner is easily made by shortening an A.S.T.M. burner 1.25 cm. (0.5 inch) at the top and adding a new top as illustrated, leaving a 1.56-mm. (1/16 inch) gap in the wick tube. The hole in the base of the burner should also be filled in by the glass blower.
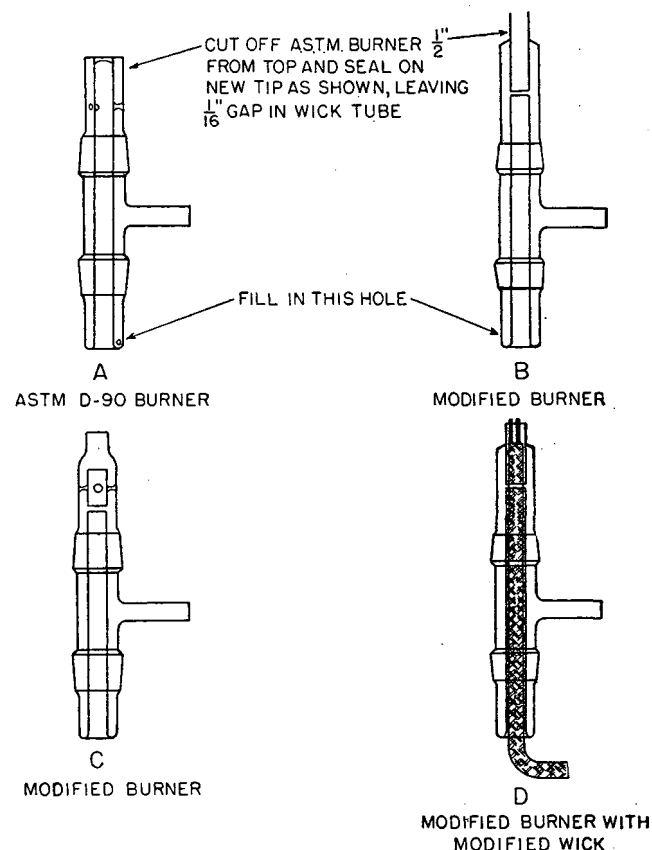

Figure 1. Modified Burners for Lamp Combustion

The operating conditions using this modified burner are as follows:

The wick should be packed loosely (2 or 3 strands). If it is packed too tightly, slugs of liquid sample are lifted out of the burner by the air stream.

The wick should be drawn down about 0.6 cm. (0.25 inch) below the burner tip.

The assembled lamp should be allowed to stand until the wick has reached equilibrium saturation before weighing and lighting.

Very little primary air is needed as compared to the conventional lamp, less than 0.5 cubic foot per hour for the average sample.

Figure 1, C, illustrates a second modified burner which has also proved useful for the combustion of aromatic materials.

This burner is a compromise between the A.S.T.M. and Anglo-Iranian burners. The wick tube is split to allow entry of the primary air, but the annular path for the air around the wick tube has not been sealed off. Therefore, a portion of the primary air may pass along each route. This burner is used in a manner similar to that described above, except that more primary air may be used, because only a portion of this stream enters the wick tube. The proportion diverted will depend upon the tightness of packing of the wick.

Some samples, such as gasolines that cover a wide boiling range, exhibit a tendency to flood when burned as described above. This condition may be remedied by using a modified wick as shown in Figure 1, D. The wick sheath rather than the wick itself is used to pack the burner, the hollow sheath allowing less retention of free liquid. The sheath is drawn down to a level about 0.25 inch above the air inlet and immediately over it is placed a loosely rolled cylinder of platinum gauze about 0.375 inch in length or just long enough to reach to the top of the burner.

Burners for Nonvolatile Materials. Another type of sample encountered in the petroleum industry is the heavy nonvolatile material which will not travel up the wick of the burners described above. A novel burner for burning such heavy stocks, described by Hindin and Grosse (3), is so constructed that the sample reservoir is at a higher level than the tip of the burner, so that the sample is delivered to the flame by gravity flow rather than by upward capillary movement.

The burner of Hindin and Grosse has been modified by the authors to incorporate the features contained in the burners shown in Figure 1. Moreover, it has been found possible to decrease the weight and size of the burner by eliminating one reservoir and the intervening stopcocks.

To equalize air pressure in the reservoir and chimney and ensure smooth burning, a tie line is sealed between the reservoir and burner. These burners also have the advantage of being interchangeable with the A.S.T.M. lamp and burner, as the glass joints of the A.S.T.M. burner are used in fabrication of the lamp. The construction of the burner for nonvolatile materials is illustrated in Figure 2. Suggested dimensions are indicated, but they are not critical so long as the finished burner is convenient to use. It should not be too bulky or too heavy, when filled, to weigh on an analytical balance. Care should be taken to carry the tie line as close as possible to the sample reservoir in order to avoid interference with the chimney when the lamp is assembled.

This burner may be made up with any style tip as shown in Figure 1, A, B, or C. For general use, the A.S.T.M. tip (Figure 1, A) is suggested. For carbon-hydrogen determination or for aromatic materials, the intermediate tip, C, is preferred. Although B may also be used, it is not recommended, as it shows a tendency to sputter with heavy materials.
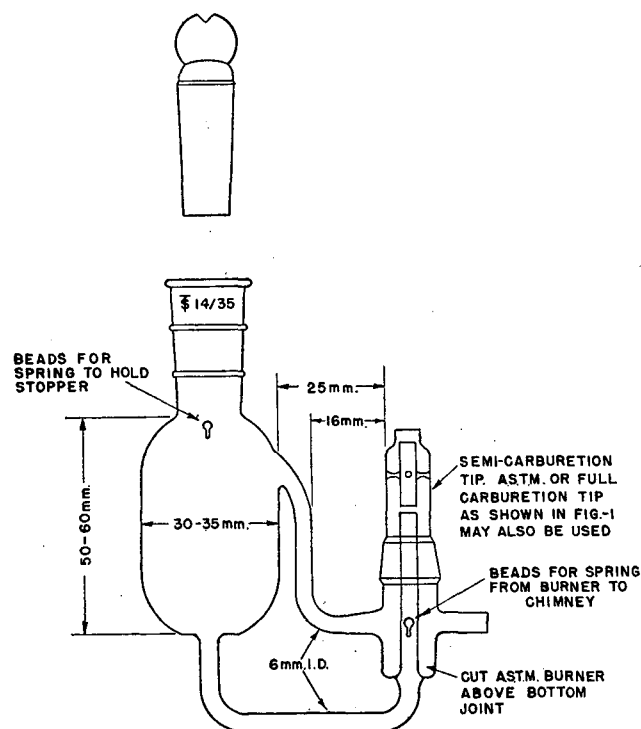
Figure 2. Modified Burner with Semicarburetion Tip for Nonvolatile Materials

Table I. Determination of Combined Sulfur[a] by Lamp Combustion Using Modified Burner B

Material	Run No.	Per Cent Sulfur Calculated	Per Cent Sulfur Experimental
Toluene plus isopropyl trisulfide	1	0.100	0.100
	2		0.099
	3		0.102
	4		0.099
	5		0.103
			Av. 0.101

[a] Free sulfur dissolved in hydrocarbons cannot satisfactorily be determined by the lamp method. Although the modified burners are primarily used to give more efficient combustion, tests with samples containing free sulfur indicate no improvement in burning using the modified burner. A sample of desulfurized kerosene blended to contain 0.0917% free sulfur showed 0.087% sulfur when analyzed using burner B and 0.088% when using A.S.T.M. burner.

In operating the burner, the wick cannot be threaded in the usual manner, owing to the U-shape of the burner. Instead, the wick (2 strands) may be inserted by starting it into the burner end and then drawing it through by applying vacuum to the reservoir, meanwhile holding a finger over the primary air inlet. The sample level in the reservoir should be kept below the level of the side air tube; and, if the C tip is used, the sample level should also be kept below the break in the wick tube to avoid flooding. If the sample is very heavy, the last inch of wick above the break in the tube may require a long time to become saturated. In this case, time may be saved by drawing out the wick and snipping off the dry portion with a pair of scissors.

The above notes on use of the modified burners are not intended as a specific set of directions to cover all types of samples. The use of the modified burners is a subjective art that requires a great deal of manipulative skill and practice on the part of the analyst. It has been demonstrated in connection with other analyses involving lamp combustion that these modified burners give essentially complete combustion, whereas the A.S.T.M. burner does not. The difference is very slight, however, and introduces no error in the estimation of small amounts of sulfur (less than 1%). In view of the ease with which the A.S.T.M. burner can be handled, it is recommended that it be used wherever possible and that the modified burners be reserved for samples that are high in aromatics or otherwise difficult to burn.

APPLICATIONS OF MODIFIED BURNERS

Determination of Sulfur in Aromatics. The modified burners shown in Figure 1, B and C, were originally designed to enable the burning of highly aromatic samples without recourse to dilution. They have proved very satisfactory for this purpose.

Table I presents results obtained on a synthetic blend containing a known amount of sulfur in toluene.

Determination of Halogens. The lamp method has also been used for the estimation of chlorine and bromine in gasoline (present as organic lead scavenging agents in tetraethyllead fluids). However, low results are obtained due to incomplete conversion of the organic chloride or bromide to halide ion when the ordinary burner is used. The error is small for chlorine, 2 to 5% of the amount present, but may approach 10 to 20% in the case of bromine.

Experimental work has verified that the modified burners will give essentially stoichiometric conversion of organic halogen to halide ion. Because the procedure used for halogen determination is not well known and has not been published elsewhere, it is given below in some detail.

Use modified burner B and burn the sample in the usual manner. In the absorber, place 50 ml. of 0.15 N sodium carbonate to which has been added hydrazine sulfate in a concentration of 0.5 gram per 50 ml. After 5 to 10 grams of sample have been burned, proceed as follows if the sample contains tetraethyllead. Remove the wick from the burner, place the burner in inverted position in a 400-ml. electrolytic beaker, and add a few milliliters of 8 N nitric

Table II. Determination of Halogens in Gasoline

Material	TEL, Ml./Gal.	Per Cent Halogen Calculated	Modified burner
n-Heptane	None	Br, 0.110	0.109
			0.110
			0.108
			0.108
Aviation gasoline	None	Br, 0.110	0.112
			0.112
			0.111
			0.112
Aviation gasoline	None	Cl, 0.109	0.109
			0.112
Aviation gasoline	4	Cl, 0.109	0.112
			0.111
Aviation gasoline	None	Br, 0.082	0.083
			0.083
			0.085
		Cl, 0.027	0.028
			0.029
			0.027
Aviation gasoline	4	Br, 0.082	0.078
			0.079
		Cl, 0.027	0.026
			0.028

acid. Let stand until the lead crust at the tip of the burner has dissolved. Remove the burner and rinse with distilled water. Rinse the spray trap, adding the washings to the beaker. Now add 5 to 10 ml. of 8 N nitric acid to the chimney. Rotate the chimney in such a manner that all traces of lead deposit are dissolved. Transfer the washings to the beaker and rinse with distilled water. In a similar manner, dissolve out any deposit from the fritted disk of the absorber.

The amount of nitric acid used will have been more than sufficient to neutralize the carbonate and the solution can be titrated directly with 0.02 N silver nitrate solution after diluting, if necessary, to a volume of 300 to 350 ml. Use a potentiometer (Beckman Industrial Model M pH meter) and silver-glass electrode system to detect the equivalence point. The potentiometric method is especially suitable when both halogens are present, as both can be determined directly by the appearance of a double inflection in the titration curve. When both are known to be present, the addition of 10 ml. of 10% potassium alum solution will aid in resolution of the bromide-chloride inflection point.

The careful washing of all lead deposit out of the lamp assembly is essential to the success of the determination, because some halogen is retained in this deposit. In other words, low results may be due to two sources: incomplete combustion of the organic halide if combustion is faulty or retention of halogen in the lead deposits. This is particularly true when bromine is the halogen sought. Chlorine is not retained in the deposit to as large an extent, or is more readily removed by a water washing only. Other solvents for the deposit have been tried, such as potassium hydroxide and ammonium acetate, but the 8 N nitric acid has proved most successful.

Table II gives illustrative data on synthetic samples containing known amounts of chlorine and/or bromine.

Examples given in Table III demonstrate low results due to use of the regular A.S.T.M. burner and to failure to wash out the lead oxide from the chimney with nitric acid.

Determination of Carbon and Hydrogen. In addition to the determination of sulfur in aromatic materials and halogens in gasoline, the modified burners have also been applied to the determination of carbon and hydrogen in liquid organic compounds.

The determination of hydrogen by a lamp method was originally described by Hindin and Grosse (4), who used the standard A.S.T.M. burner and chimney followed by an absorption bulb containing calcium chloride and phosphorus pentoxide to collect the water formed upon combustion. They reported that aromatics could be burned after 50% dilution with a pure saturate. Calculation of hydrogen content would require correction for hydrogen content of the diluent. Hindin and Grosse also indicated that, while hydrogen could be determined quantitatively, conversion of carbon to carbon dioxide was only about 98% complete.

The authors have found that carbon may be quantitatively determined with the modified burners and that even aromatic

materials may be analyzed for carbon and hydrogen without blending. Aside from the substitution of the modified burners, the apparatus used is similar to that described by Hindin and Grosse.

The air used for combustion is ordinary compressed air passed through Ascarite, calcium chloride, and Dehydrite. The combustion products are caught in two absorbers. The first, containing calcium chloride and Dehydrite, retains the water and the second, containing Ascarite and Dehydrite, retains carbon dioxide. Nesbit or Turner bulbs fitted with ground-glass joints make suitable absorbers.

Considerable heat will be developed in the tubes during the absorption of water and carbon dioxide and provision should be made for immersing them in an ice water bath.

Depending upon the diameter of the inlet and outlet orifices of the absorption bulbs and the tightness of packing of the absorbents, excessive back pressure may make it difficult to burn the sample. In this case, a slight vacuum may be applied to the exit end of the system, although normally it is not necessary.

A blank determination carried out over a period of 10 minutes, the average burning time of the sample, showed a gain in weight of 3.0 mg. due to water absorption. There was a negligible gain due to absorption of carbon dioxide. The blank is therefore subtracted from the weight of water recovered during the analysis.

In any determination involving the use of the lamp for combustion, an appreciable loss is encountered before and after burning. The loss before burning occurs during the time it takes to light the burner and insert it into the chimney. The loss after burning is due to evaporation of the sample from the hot burner after removal from the chimney before the final weighing can be made.

Table III. Determination of Bromine

Material	TEL, Ml./Gal.	Per Cent Bromine Calcd.	A.S.T.M. burner	Modified burner
Effect of Burner on Combustion of Halogens				
n-Heptane	None	0.121	0.107	0.122
			0.109	0.118
Aviation gasoline	4	0.130[a]	0.082	0.114
			0.086	0.118

Effect of Dissolving Lead Film from Chimney			Chimney Washed with:	
			Water	8 N nitric acid
Aviation gasoline	4	0.110	0.099	0.109
			0.097	0.110
				0.105

[a] Determined independently by decomposition with sodium.

The first source of error may be eliminated in either of two ways:

An internal ignition system may be used to eliminate external lighting of the burner with the pilot flame. The ignition system consists of two tungsten electrodes sealed through the lamp chimney and connected externally to a sparking coil. The electrodes should be inserted at such a height that the spark passing between them will ignite the burner. The alternative method of eliminating the initial error is accurately to measure the time required to

Table IV. Carbon and Hydrogen by Lamp and Liebig Combustion

Material	Found by Lamp Method, % Carbon	Hydrogen	Found by Liebig Combustion, % Carbon	Hydrogen
Oxidized hydrocarbon 1	83.90	16.03	83.98	16.09
Oxidized hydrocarbon 2	83.96	16.08	83.91	15.99
Oxidized hydrocarbon 3	83.88	16.06	83.91	15.99
Oxidized hydrocarbon 4	84.02	15.71	83.41	15.78
30% benzene in reference fuel	86.85	13.08	86.96	13.17
Aviation gasoline	85.34	14.37	...	...

Table V. Carbon and Hydrogen Results on Pure Materials

Sample	Found, % Carbon	Found, % Hydrogen	Theoretical Values, % Carbon	Theoretical Values, % Hydrogen	Deviations, % Carbon	Deviations, % Hydrogen
Toluene	91.11	8.78	91.25	8.75	−0.14	+0.03
Iso-octane	85.04	15.86	84.12	15.88	−0.04	−0.02
	84.20	15.86	84.12	15.88	+0.08	−0.02
n-Heptane	83.88	16.12	83.90	16.10	−0.02	+0.02
Absolute ethanol[a]	51.75	13.09	51.98	13.12	−0.23	−0.03
Methylcyclohexane	85.58	14.40	85.63	14.37	−0.05	+0.03
	85.64	14.36	85.63	14.37	+0.01	−0.01
	85.54	14.39	85.63	14.37	−0.09	+0.02
				Average deviation	±0.08	±0.01

[a] Estimated to contain 0.25% water, theory calculated for 100% purity.

Table VI. Determination of Carbon and Hydrogen in White Oils

Material	% Carbon-Hydrogen Lamp	% Carbon-Hydrogen Liebig combustion
White oil 1		
H	13.93	13.97
C	86.15	86.10
	100.08	100.07
White oil 2		
H	13.85	13.48
C	86.22	86.50
	100.07	99.98
White oil 3		
H	13.85	13.87
C	86.22	85.80
	100.07	99.67
White oil 4		
H	13.78	13.56
C	86.25	86.02
	100.03	99.58
White oil 5		
H	14.26	14.31
C	85.84	85.86
	100.10	100.17
White oil 6		
H	13.90	
C	86.10	
	100.00	
White oil 7		
H	13.84	
C	86.16	
	100.00	

light the burner and insert it into the chimney and the total time during which the sample is burning and then to correct the weight of products accordingly—for example, if the burner is lit for a total of 500 seconds and 10 seconds are lost in inserting the burner, then the weight of products collected should be corrected by multiplying by the factor 500/490.

The evaporation error encountered after-burning may be minimized by capping the wick tube with a rubber policeman or glass cap.

All the samples reported in Tables IV to VI were hydrocarbons or oxygenated materials substantially free of foreign materials such as halogen, sulfur, or nitrogen. No provision has been made so far for the removal of these impurities.

Some difficulty may be experienced in burning hydrocarbon mixtures such as gasolines or reaction products containing extremely light ends when the modified burners are used with regular cotton wicking. Better results will be obtained in such cases by using the wick sheath and placing a small roll of platinum gauze on top of the wick, so that it just protrudes above the burner tip.

Table IV presents data obtained on a variety of materials of wide boiling range which were burned using the burner and wick sheath illustrated in Figure 1, D. The results are compared with the conventional Liebig combustion technique.

Table V shows results obtained on a variety of pure compounds.

The determination of carbon and hydrogen in nonvolatile materials is illustrated in Table VI. In this case, the samples are white oils with negligible amounts of sulfur or other impurities, so that addition to 100% should be a criterion of reliability of the analysis. For some samples, a comparison with the Liebig combustion is available. This work was done using the burner shown in Figure 2.

As a final illustration of the application of the lamp method to the determination of carbon and hydrogen, the authors have devised a technique for the estimation of carbon-hydrogen ratio. Such a procedure is useful for the extremely accurate analysis of materials which are known to contain only the atomic species, carbon and hydrogen.

To determine the carbon-hydrogen ratio, it is only necessary to insert a T stopcock in the chimney line between the lamp and the absorbers, so that one leg exhausts to the atmosphere. With the stopcock set in the exhaust position, the operator may light the burner, adjust the flame, and close the system. No sample weight is recorded, and the setting of the flame may be done leisurely and carefully to obtain the best possible adjustment. Then, when the sample is burning nicely, the T stopcock is quickly turned so that the combustion products pass through the absorbers. After a suitable length of time (1 to 2 grams of sample are usually burned), the stopcock is switched back to the atmosphere. The absorbers are removed, dried, allowed to reach equilibrium in the balance case, and weighed. From the relative weights of water and carbon dioxide, the carbon-hydrogen ratio of the sample may be calculated. Assuming that carbon plus hydrogen totals 100% for the sample, the per cent carbon and hydrogen may be calculated from the ratio. No sample weight is recorded in this procedure. Thus, the errors due to loss of sample during lighting and after extinguishing the flame are eliminated.

Table VII. Comparison of Carbon and Hydrogen Determination by Ratio and Direct Method

(Sample 300–370° F. petroleum fraction)

	C/H	% Hydrogen	% Carbon	Total
Direct experimental determination		13.94	86.02	99.96
		13.98	86.22	100.20
		13.98	85.89	99.87
		13.97	86.04	100.01
	6.154	13.98	86.02	100.00
Analysis calculated from	6.154	13.98	86.02	100.00
ratio assuming 100% total	6.145	14.00	86.00	100.00
for carbon plus hydrogen	6.158	13.97	86.03	100.00
	6.153			

Table VII gives a comparison of results obtained by the carbon-hydrogen ratio method vs. the direct determination. The sample used for this work was distributed by Subcommittee XXV-G of A.S.T.M. Committee D-2, which is engaged in the study of the lamp method for the determination of hydrogen. The burner used was type B.

ACKNOWLEDGMENT

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CORRECTION: In the article on "Microscope Optics" [Foster, L. V., ANAL. CHEM., 21, 432 (1949)], the notation "made by Miriam S. Flower, Polaroid Corp." should have appeared under Figure 4 on page 434 and not under Figure 3 on page 433.

Determination of Color of Turbid Waters

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A convenient procedure for determining the color of turbid waters, using the principle of precipitation of turbidity by the electrolyte calcium chloride, is described. Because the stable turbidity of many surface waters cannot be completely precipitated by conventional centrifuging alone, this procedure presents a means of flocculating the turbidity without affecting the true color of the water. In the determination of true color of turbid samples one of the most prevalent errors is caused by the reading of color on samples not completely free of turbidity. Pertinent data are presented on color and turbidity of waters as related to the principles involved in the determination of color.

IN WATER analysis the "color" or "true color" of water is that color due only to substances in solution—that is, the color of the water after the suspended matter has been removed. Many surface waters and occasionally some ground waters are turbid and remain so almost indefinitely. In order to determine the color of water samples it is necessary to remove the turbidity without affecting the true color, as the turbidity gives an apparent color which may be considerably higher than the true color.

It is reported that the suspended matter may be removed by centrifuging the samples (1). However, this apparently is in regard to water samples on which increasing the force of gravity is sufficient to overcome the critical mobility of the sol. Many surface waters low in mineral content and having very fine or colloidal suspended material remain turbid almost indefinitely. Conventional centrifuging alone will not precipitate or remove this turbidity.

A turbid sample of water of low mineral content was settled for 6 days and then centrifuged for 1 hour. After centrifuging, the sample still remained turbid and only the apparent color could be determined, which was 134. Another portion of this same sample was treated with 1 ml. of a calcium chloride solution and centrifuged for 1 hour. All turbidity was precipitated and the color was readily determined as 48.

The nature and size of particles of suspended matter carried by streams may vary widely, but a usual turbidity which is stable in waters of low mineral content presumably forms a negatively charged sol. Several electrolytes were studied which might be used in overcoming the critical mobility of this turbidity without affecting the true color of the water. As there are exceptions to the Schulze-Hardy rule of coagulation of sols by electrolytes, it can be considered only as a useful approximation in selecting the electrolyte for the flocculation of turbidity. Calcium chloride ($CaCl_2.2H_2O$) was selected as being effective in precipitating the stable turbidity of water samples.

The procedure reported here covers the flocculation of turbidity in natural water samples. It does not cover the inadequacy of the platinum-cobalt standard when used for comparisons with polluted waters which may have colors noticeably different from that of the standards. In this connection spectrophotometric procedures may be worthy of consideration in the development of a standard method which would give satisfactory results.

REAGENTS

Calcium chloride, 300 grams of calcium chloride dihydrate made up to 500 ml. with distilled water (filter). 1 ml. = 0.6 gram of $CaCl_2.2H_2O$.

PROCEDURE

Transfer 100 ml. of the samples (or smaller aliquots diluted to 100 ml. with distilled water when the color exceeds approximately 50 units) to suitable tubes and add 1 ml. of the calcium chloride solution. Mix well and centrifuge until all turbidity has been completely precipitated. The time of centrifuging is dependent upon the nature of the samples, but up to 1 hour should be sufficient. When the results are not needed immediately, and in laboratories not having a centrifuge, the samples may be treated with the calcium chloride and allowed to stand in Nessler tubes until all turbidity has precipitated. The time required for flocculation on standing will depend on the nature of the samples and will require about 2 to 6 days. Regardless of whether centrifuging or standing undisturbed is employed, the samples should be compared with a blank of distilled water to be sure that all turbidity has been completely precipitated. One common error in the determination of color of turbid waters is the reading of color of samples not completely free of turbidity. A slight turbidity may give an apparent color noticeably higher than the true color.

Dilutions should be made when the color of the water exceeds approximately 50 units before adding the calcium chloride solution; the calcium chloride may precipitate a little of the color when it is added to the more highly colored waters. The color in some of the more highly colored waters may have a tendency to fade and/or precipitate on standing, irrespective of the treatment with calcium chloride. For these waters it is advisable to determine the color as soon as possible and to centrifuge after the treatment with calcium chloride.

The color is measured by the commonly adopted platinum-cobalt standard using the usual procedure (2). The samples may be compared with prepared standards or with previously checked slides or disks. The comparison should employ sufficient depth to give accurate results. The treatment of 100-ml. samples is suggested, because this gives sufficient volume to decant off the portion of sample needed without disturbing the precipitated sediment.

The addition of 1 ml. of calcium chloride increases the volume by 1 ml. and for a 100-ml. sample a 1% error is introduced, but this is well within the limit of accuracy of the determination of color. However, when the turbidity is precipitated on standing in the Nessler tubes this small error may be corrected by stoppering the tubes when the natural evaporation has reduced the volume to the original 100 ml.

DISCUSSION OF METHOD

The determination of color of stable turbid waters has been a problem, for conventional centrifuging will not remove the turbidity and there was always some question of loss of color when it was determined on the filtered water. Some of the most stable turbid waters are those of low mineral content from clay type soils. Calcium chloride was found to be effective in flocculating the turbidity of water samples for the determination of color in conjunction with centrifuging or through settling alone over a longer period of time. Other electrolytes will also flocculate the stable turbidity of waters. Ten grams of sodium chloride were found to be slightly less effective than 0.6 gram of calcium chloride. However, an exact comparison of the precipitating action of calcium chloride versus sodium chloride cannot be made, as the tests were made on different samples and not under identical conditions.

The precipitation of turbidity by electrolytes involves the concentration of electrolyte, nature of sol, adsorption, valence of effective or precipitating ion which is opposite in charge to that

on the colloidal particles, and valence of the stabilizing ion which is of the same sign of charge as that on the colloidal particles.

In order to determine whether the calcium chloride had any effect on the true color, tests were made on water samples which were free of all turbidity or from which all turbidity had been removed by diatomaceous filtration. Each sample was divided into two portions and the calcium chloride was added to one portion which was allowed to stand up to 6 days. The color was then determined on the treated and untreated aliquots and was found to be the same. However, when the calcium chloride was added to the more highly colored waters before dilution, a little of the color in some of these waters was precipitated on standing. To prevent this, the color of the aliquot to which the calcium chloride is added should not exceed approximately 50 units.

When dilutions are made the color reading is multiplied by the factor involved.

In Table I results of tests on water samples are shown for (1) color after precipitation of all turbidity by calcium chloride or sodium chloride, (2) color after filtration through diatomaceous filter cylinders, and (3) suspended matter. The results in this table were selected from a number of tests to show a range of conditions including high and low color and suspended matter, different types of water and suspended matter, and some data on the removal of color by diatomaceous filter cylinders. The tests show that although the removal of color by the diatomaceous filters was not so extensive as has been customarily assumed, nevertheless these filters, as well as others, will exert decolorizing action on some waters and there is possible loss of color when it is determined on the filtered samples. The decolorizing action of diatomaceous filter cylinders is dependent upon the fineness of the filters, the nature and extent of mud coating the filters, and the color of the water. When the filters exerted decolorizing action the color of the filtered aliquot was less than the color of the electrolyte-treated aliquot and when filters did not exert decolorizing action the color of filtered and electrolyte-treated aliquots was the same, as shown by examples in the table.

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Analytical Procedures for Control of Saccharification Operations

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Plant control methods for the rapid analysis of pentosans and cellulose in agricultural residues and of pentoses, furfural, and dextrose in saccharification liquors are presented. Equations for calculating yields of products are outlined.

THE Synthetic Liquid Fuels Project under the Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, is a part of a broad national program for large-scale research on the production of synthetic liquid fuels from non-petroleum sources. Its purpose is to determine the manufacturing steps and costs of a process (2) for the hydrolysis of agricultural residues to sugars for conversion into liquid fuels.

The main difference between the saccharification process under investigation and other saccharification processes lies in the fact that a preferential hydrolysis is used to separate the pentosans almost quantitatively from the cellulose. The primary reactions involved in this saccharification process may be characterized by the following equations:

$$\text{Holocellulose} + \text{H}_2\text{O} \xrightarrow[\Delta]{\text{H}_2\text{SO}_4} \text{pentoses}_{\text{(soln.)}} + \quad (1)$$
$$\text{furfural}_{\text{(soln.)}} + \text{cellulose}_{\text{(solid)}}$$
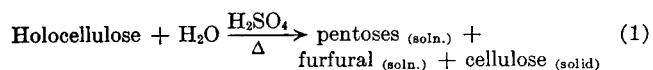
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$$\text{Cellulose} + \text{H}_2\text{O} \xrightarrow[\Delta]{\text{H}_2\text{SO}_4} \text{dextrose}_{\text{(soln.)}} \quad (2)$$
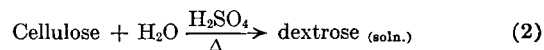
In order to calculate yields of products from the above equations, it is necessary to determine pentosans and cellulose in the raw materials and to determine pentoses, dextrose, and furfural in the hydrolysis liquors. Investigations (1, 4, 5) have resulted in methods of analysis which are, in general, sufficiently precise and accurate for the present work, but more rapid methods were needed for analytical control purposes.

QUANTITATIVE SACCHARIFICATION

Previous laboratory development work indicated that agricultural residues could be almost quantitatively saccharified when exposed to 72% sulfuric acid at 50° to 60° C. for relatively short periods of time.

To investigate quantitative saccharification under these conditions, a relatively large sample of corncobs was ground to pass a 20-mesh screen. After 2-gram samples of these cobs had been dried to constant weight at 105° C., they were mixed with 10-ml. portions of 72% sulfuric acid which had been preheated to five different temperatures ranging from 45° to 65° C. Duplicate samples were placed in a water bath maintained at the respective temperatures, and digested for 5 minutes with constant stirring. The digested samples were immediately diluted with 275 ml. of water to give a concentration of 4% sulfuric acid and then autoclaved at 15 pounds per square inch for 45 minutes. Total reducing sugars were then determined by the Shaffer-Hartmann method (6).
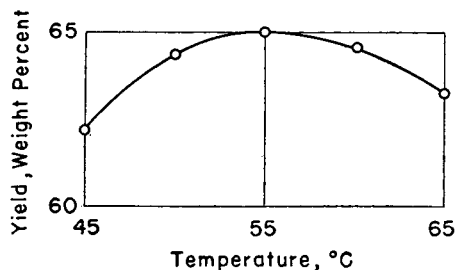
Figure 1.   Effect of Temperature on Yield of Total Reducing Sugars

5-minute digestion period

The effect of digestion temperature on saccharification yields is shown in Figure 1, where the weight per cent of reducing sugars from oven-dry cobs is plotted against temperature.

The same procedure was followed in a second series of experiments wherein the length of time of digestion instead of temperature was varied, and the temperature was held constant at 55° C. The effect of time on the saccharification reaction is shown in Figure 2.

Experiments were next conducted in which samples were digested in 72% sulfuric acid at 55° C. for 5 minutes and 6 minutes, and the time and temperature of autoclaving were varied. Figures 3 and 4 show that a digestion period of 5 minutes and an autoclaving at 30 pounds per square inch for 25 minutes give the maximum yield of products in the minimum time.

As a result of these studies, the following procedure is recommended for quantitative saccharification of agricultural residues for plant control:

The moisture is first determined on a 100-gram sample of the coarsely ground material being processed in the plant. A portion of this dried material is ground through a 20-mesh screen, after which a sample is taken for another moisture determination, so that calculations to dry weight will not be affected by loss or gain of moisture during grinding. A 2-gram sample of the ground material is mixed in a 50-ml. beaker with 10 ml. of 72% sulfuric acid preheated to 55° C., then placed in a water bath at 55° C. for 5 minutes with constant stirring. At the end of the 5 minutes, the sample is washed from the beaker into a 500-ml. Erlenmeyer flask with 275 ml. of water and autoclaved at 30 pounds per square inch for 25 minutes. It is cooled, and made up to 300 ml. with water, and 10 ml. are analyzed for sugars by the Shaffer-Hartmann method (6).

Another sample of corncobs was saccharified by the methods of Peterson et al. (4) and Saeman et al. (5), and by the method outlined above. Seven determinations by each method were made. The average of total reducing substances reported as glucose, and the deviations in the seven different determinations were:

	% and Average Mean Deviation
Peterson et al.	62.6 ± 0.94
Saeman et al.	64.4 ± 1.6
Present method	63.4 ± 0.90

To determine the accuracy of the method under investigation, purified cellulose was saccharified; 95.2% of the theoretical yield of dextrose was obtained. The value, 0.952, is used, therefore, as the cellulose saccharification correction factor. The procedure under investigation thus appears to be as precise and accurate as other known procedures and, in addition, is considerably more rapid.

FURFURAL

It is now necessary to determine the respective quantities of furfural, pentoses, and dextrose in the hydrolyzates produced in the plant, so that yields of these products may be calculated. The method of Hughes and Acree (3) is used for the determination of furfural in 360 ml. of distillate from a 25-ml. sample of hydrolyzate. Because furfural interferes in the Shaffer-Hartmann (6) sugar determination, this determination must be corrected for the furfural present. It has been found that 75% of the furfural in a sample must be subtracted from the total reducing sugar value as obtained by the Shaffer-Hartmann method in order to correct the total reducing sugar value for the amount of furfural present.

PENTOSES AND DEXTROSE

In general, the method of Saeman et al. (5) was used with slight variations for the determination of pentoses in the presence of dextrose.

The sugar sample is diluted to a concentration of approximately 7 mg. of sugar per ml.; 10 ml. of this sample are pipetted into a 25 × 200 mm. test tube and the sample is neutralized to pH 5.0 with sodium hydroxide; 2 ml. of potassium citrate buffer are added to give a final pH of 5.2 to 5.4; 2 grams of baker's yeast suspended in distilled water are added, and the final volume is adjusted to 25 ml. The sample is placed in an incubator at 30° C. for 1 hour with no agitation. After sorption of the sugars by the yeast, the total volume of the sample is made up to 100 ml., the yeast cells are centrifuged, and the supernatant liquor is analyzed for reducing sugar.

Preliminary yeast-sorption experiments in which mixtures of pure xylose and dextrose as substrates were used indicated that xylose recoveries were variable under the aerobic conditions obtained by conducting the fermentation in an Erlenmeyer flask. Under the partial anaerobic conditions obtained in the test tubes, however, 100% (±2%) yields of xylose could be obtained in fermentations lasting only 1 hour.

Pentosans (and cellulose) in agricultural residues may now be determined by saccharifying a sample of the residue followed by yeast sorption of the dextrose. When this method was com-
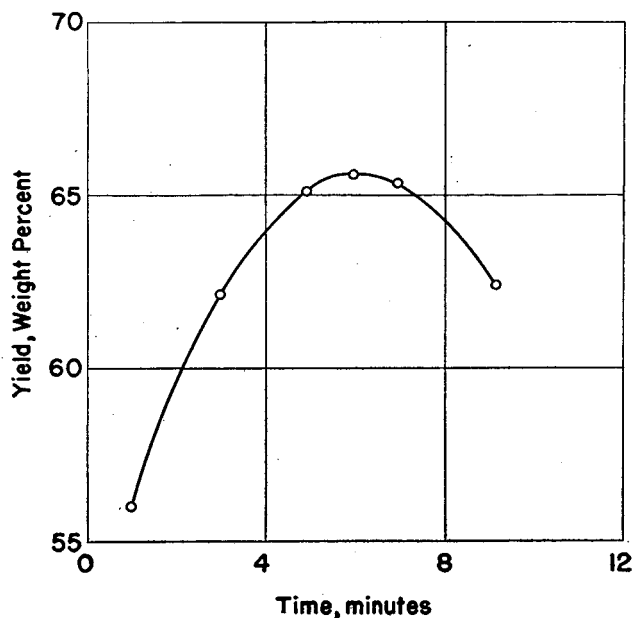

Figure 2.   Effect of Time at 55° C. on Yield of Total Reducing Sugars
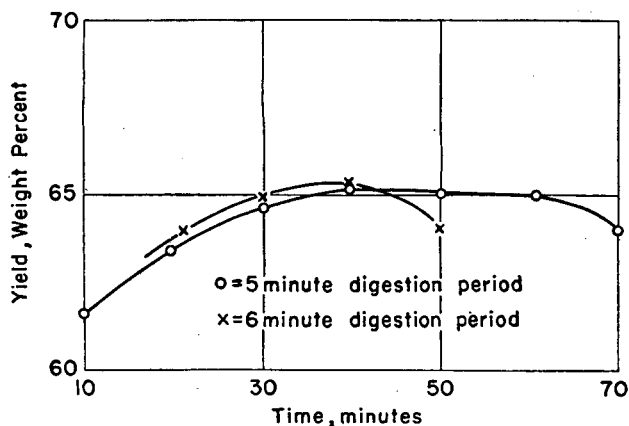
Figure 3.. Effect of Autoclaving Time on Yield of Total
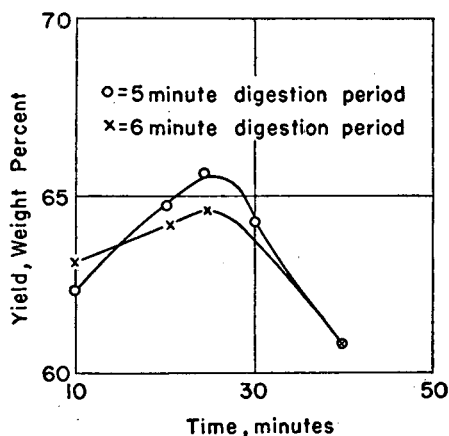Reducing Sugars

15 pounds per square inch

Figure 4. Effect of Autoclaving Time on
Yield of Total Reducing Sugars

30 pounds per square inch

pared with the method of the Association of Official Agricultural
Chemists (1) for the determination of pentosans, the analyses
showed that the analytical saccharification–yeast sorption pro-
cedure gave an amount of pentosans equivalent to 0.94 of that
indicated by the A.O.A.C. method. A factor of 0.94 is used,
therefore, to correct the yeast-sorption pentosan value to the
A.O.A.C. value when determining pentosans in agricultural resi-
dues.

Another series of comparative determinations was made on
pentosan hydrolyzates containing approximately 15% of total
reducing sugars, only a negligible amount of which was dextrose..
When analyzing hydrolyzates, the saccharification procedure is
omitted; only the furfural determination and yeast-sorption pro-
cedures are necessary. To samples of a pentosan hydrolyzate
were added amounts of dextrose ranging from 0 to 70% of the pen-
toses, and amounts of furfural ranging from 0 to 25% of the pen-
toses. Assuming that the A.O.A.C. method for the determina-
tion of pentoses accounts, on the average, for 100% of the pen-
toses present, the two methods gave the following data:

| A.O.A.C. method | 100 ± 7.1 |
| Present method | 103.6 ± 2.8 |

Under conditions of variable amounts of xylose, dextrose, fur-
fural, it appears that the yeast-sorption method gives more pre-
cise results than the A.O.A.C. method, even though the yeast
sorption method indicates slightly higher amounts of pentoses.
The value 1.036 is used, therefore, to correct the yeast-sorption

pentose values on hydrolyzates to the A.O.A.C. values. About
70 different analyses were made to determine this factor of 1.036.

CALCULATIONS

By the methods thus described, pentosans and cellulose in raw
materials and furfural, pentoses, and dextrose in hydrolyzates
can be rapidly and rather accurately determined. Yields of prod-
ucts can thus be calculated by a series of equations. As an ex-
ample, yields from one plant run were calculated as follows:

The corncobs used in the plant run analyzed 33.26% pentosans
and 38.13% cellulose on the dry-weight basis. A total of 218
gallons of pentosan hydrolyzate was obtained for each 1000 pounds
of dry-weight cobs processed.

Samples of the pentosan hydrolyzate were analyzed for fur-
fural, total reducing sugars, and pentoses. The determinations
indicated that 218 gallons of pentosan hydrolyzate contained
5.08 pounds of furfural, 330.4 pounds of total reducing sub-
stances, and 318.6 pounds of reducing sugar after yeast sorption.
The pounds of pentosans equivalent to the furfural in the 218
gallons are:

$$(5.08)\,(1.375) = 6.98$$

The pounds of pentosans equivalent to the pentoses in the 218
gallons are:

$$\frac{(318.6)}{(1.036)\,(1.136)} = 270.7$$

wherein 1.036 corrects the yeast sorption pentose value on hy-
drolyzates to the A.O.A.C. method. The pounds of cellulose
equivalent to the dextrose in the 218 gallons are:

$$\frac{(330.4)\,-\,(5.08)\,(0.75)\,-\,\left(\frac{318.6}{1.036}\right)}{(1.11)} = 17.2$$

wherein (5.08) (0.75) corrects the total reducing substance value
for furfural.

The yields of products then are:

$$\frac{(6.98)\,(100)}{(0.3326)\,(1000)} = 2.1\%\ \text{of pentosans converted to furfural}$$

$$\frac{(270.7)\,(100)}{(0.3326)\,(1000)} = 81.4\%\ \text{of pentosans converted to pentoses}$$

$$\frac{(17.2)\,(100)}{(0.3813)\,(1000)} = 4.5\%\ \text{of cellulose converted to dextrose}$$

The yields of products from the cellulose hydrolysis may be
calculated in the same manner.

During one year's experience, these procedures have proved
reliable and suitable for routine control analysis of semiworks
plant operations involving corncobs. Laboratory saccharification
experiments indicate that these methods are generally applicable
to bagasse, cottonseed hulls, oat hulls, and other agricultural resi-
dues.

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SILICA REFRACTORIES

Spectrographic Analysis Using the Direct Current Arc and High Voltage Spark

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A method is described for the quantitative spectrographic analysis of silica brick for alumina, titania, potassium oxide, and sodium oxide. The accuracy is comparable with the best chemical methods.

SILICA brick is one of the most widely used high temperature refractory materials. The quality of the brick is adversely affected by the presence of undue amounts of alumina, titania, potassium oxide, and sodium oxide, an accurate chemical determination of these compounds is exceedingly difficult and requires many hours. The spread or range of the results reported by the National Bureau of Standards and the cooperating laboratories on the Bureau of Standards silica brick 102 sample is shown in Table I.

Table I. Silica Brick 102

Constituent	Range, %	Assigned Value, %
Al_2O_3	1.84–2.04	1.96
TiO_2	0.15–0.17	0.16
K_2O	0.26–0.31	0.29
Na_2O	0.04–0.09	0.06

The spectrographic determination of these constituents by the following method requires from 1 to 1.25 hours for a single sample. The direct current arc is used as the excitation source for the alkalies and a high voltage spark is used as the excitation source for the alumina and titania.

EQUIPMENT AND PROCEDURE

The direct current arc is a commercial unit with full wave rectification. The spark unit consists of a high voltage commercial spark unit of 2 kv.-amp. with an added inductance of 0.045 millihenry and a capacitance of 0.021 microfarad. This unit has a Fuessner synchronous rotating auxiliary gap. These electrical conditions provide a very short period condensed spark.

Table II. Exposure Conditions

Subject	For Alkalies	For Al_2O_3 and TiO_2
Power source	D.C. arc	Spark
Power	6 amp.	2 kv.-amp.
Preburn	None	None
Exposure	18 sec.	11 sec.
Rotating sector	50%	None
Spectrographic slit width	15 microns	30 microns
Analytical gap	1 mm.	3 mm.

The spectrograph is a 1.5-meter instrument with a 24,000 lines per inch grating, providing a uniform dispersion of 7 Å. per mm. The camera holds a 100-foot roll of film. Spectrum analysis No. 1 film is used.

In analyzing for the alkalies 1.000 gram of silica brick (ground to pass an 80-mesh sieve), 0.600 gram of pure graphite powder, and 0.100 gram of pure barium carbonate are weighed and ground together in an agate mortar. The mixture is then compressed into briquets, each weighing approximately 0.5 to 0.6 gram. The briquet is shaped like a cupcake, being 0.6 cm. (0.25 inch) in diameter at the bottom with sides slightly tapered outward to the dome-shaped top. The compressing operation is carried out in a hydraulic press under a pressure of 250,000 pounds per square inch. The finished pellet is placed in the end of a tubular steel holder whose sides are slit to provide a little spring grip-

ping action. The pellet in its holder is used as the lower (+) electrode. A graphite rod 0.6 cm. (0.25 inch) in diameter with a conical tip of 120° included angle is used as the counterelectrode (−).

The spark technique described by Steinberg and Belic (1) is used for the determination of alumina and titania. The powdered silica brick (ground to pass an 80-mesh sieve) is packed into a crater drilled in the end of a graphite rod 0.6 cm. (0.25 inch) in diameter and cemented with 2 drops of a 2% solution of Ethocel (an ethylcellulose ether) dissolved in butyl acetate. The butyl acetate is evaporated in a stream of air. The dried, loaded graphite rod is then used as the lower electrode. The counterelectrode is a graphite rod 0.6 cm. in diameter with a conical tip of 120° included angle.

Both the powdered graphite used in briquetting and the graphite rods are of special high purity grade.

Exposure conditions are shown in Table II.

Table III. Analytical Curve Data

Oxide	Element Line		Internal Standard Line	Index[a] %	Range, %	Excitation
TiO_2	3088.03	Si	2987.65	0.18	0.07–0.20	Spark
Al_2O_3	3082.16	Si	2987.65	1.66	0.95–2.10	Spark
K_2O	4044.14	Ba	3611.00	0.23	0.08–0.33	Arc
K_2O	4047.20	Ba	3611.00	0.30	0.10–0.35	Arc
Na_2O	3302.32	Ba	2771.36	0.13	0.02–0.30	Arc
Na_2O	3302.32	Ba	3315.80	0.07	0.02–0.30	Arc

[a] Defined as concentration at which intensity ratio of analytical line pair (unknown and internal standard) is unity.

DEVELOPMENT AND PHOTOMETRY

The film is developed for 2 minutes in Eastman D-19 developer, placed in a 2.5% acetic acid stop bath for 10 seconds. and fixed in Kodak rapid liquid fixer for 45 seconds. After washing for 1 minute, the film is dried by infrared radiation in a stream of warm air.

The film is stretched taut in a film holder and the whole is placed in a commercial microphotometer in order to measure the density of the spectral lines.

WORKING CURVES

Two film calibration curves are used, one for the 3600 to 4300 Å. region and the other for the 2500 to 3600 Å. region of the spectrum.

The analytical curves are prepared by plotting the logarithm of the relative intensity ratios against the logarithm of the concentrations of the various oxides. Thus the percentage of an oxide constituent is taken directly from the analytical curve, using the proper film calibration curve. The analytical curves were prepared by using National Bureau of Standards silica brick 102 and several samples of silica brick which were analyzed

Table IV. Replicate Determinations on Same Sample

TiO_2, % 0.11, 0.09, 0.10, 0.11, 0.10, 0.10, 0.11
Al_2O_3, % 1.00, 1.07, 1.09, 1.00, 1.09, 1.05, 1.04, 1.04, 1.07
Na_2O[a], % 0.045, 0.045, 0.05, 0.055, 0.055
K_2O[a], % 0.29, 0.29, 0.29, 0.28, 0.28, 0.25, 0.305, 0.27

[a] Only 2 decimals are normally reported; the third decimal is shown for demonstration of precision.

chemically in the South Works Chemical Laboratory. Analytical curve data are shown in Table III.

PRECISION

The general reproducibility of results on a single sample, which is at present being used as a routine reference, is shown in Table IV. The results shown are for single determinations with no corrections for curve shifting, which fortunately appears to be of a very minor character. The figures, taken from routine determinations, are typical of the checks to be expected and appear to be of the same order of accuracy as those originally reported by the National Bureau of Standards and the cooperating laboratories on their silica brick 102 sample.

Routine samples are generally run either in duplicate or triplicate and the average result is reported.

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X-RAY DIFFRACTION PATTERNS

For the Identification of Surface-Active Agents

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SURFACE-active agents have acquired a wide field of usefulness as detergents, emulsifying agents, dispersants, and wetting agents. Considerable investigation and development of commercial synthetic detergents are conducted at this laboratory, and a rapid method for the identification of active ingredients was desirable. A rapid method was also necessary as a basis for specifications requiring certain compositions in lieu of performance tests.

The authors have prepared a file of x-ray data for 19 common surface-active agents for use in the synthetic detergent work of the laboratory (*2*).

EXPERIMENTAL PROCEDURE

The surface-active agents were separated from commercial products by two extractions with 95% ethyl alcohol (*1*). X-ray diffraction transmission patterns on film were made using a General Electric XRD unit and a flat cassette. The distance from the film to the sample holder was set at 100 mm. Two-hour exposures were made with unfiltered copper radiation at 40 kv. and 15 milliamperes. Photographs were taken using both the 0.25-mm. (0.010-inch) and 0.025-inch collimating systems. The distance from the film to the sample was calibrated with sodium chloride.

EXPERIMENTAL DATA AND DISCUSSION

Interplanar spacings calculated from photographs are shown in Table I. The scale of intensities is an arbitrary one on which 10 represents the most dense line and 1 the faintest line.

As a supplementary study, measurements of interplanar spacings and intensities were also made, using a North American Philips x-ray spectrometer, Type 12021. It was found that more lines and halos were recorded photographically than on the spectrometer chart and that the spectrometric measurements of 2θ were in general less reproducible. However, more reproducible values of 2θ were obtained for very high interplanar spacings using the spectrometer. At the high interplanar spacings, the width of the incident beam was limited by an extra slit in front of the regular slit and the 2θ values were obtained by setting the goniometer manually for the greatest number of counts.

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Table I. Diffraction Data for Nineteen Crystalline Surface-Active Agents

Spacing, Å.	Intensity	Spacing, Å.	Intensity	Spacing, Å.	Intensity
1. Sorbitan Monostearate		**8. Sodium n-Octyl Sulfate**		**15. Diamyl Sodium Sulfosuccinate**	
61.6	10	29.6	10	20.0	10
15.7	1	4.32	5	9.60	1
4.18	6	4.08	4	8.85	1
2. Sodium Salt of Sulfonated Ethyl Methyl Oleyl Amide		**9. Dodecyl Benzene Sodium Sulfonate**		4.34	1
				Halos	
				39.5	1
51.5	10	26.5	10	4.91	2
25.0	4	21.6	1		
16.8	3	18.5	1	**16. Dihexyl Ester of Sodium Sulfosuccinate**	
5.03	3	13.4	2		
4.44	2	Halo		19.7	10
3.96	4	4.97	3	18.4	9
3.78	2			4.96	3
3. Sodium Oleyl Sulfate		**10. Monobutyl Phenyl Phenol Sodium Monosulfonate**		4.70	1
				Halo	
50.0	10	26.2	10	40.7	3
36.7	5	13.0	2		
24.5	4	10.4	2	**17. Sodium Tetrahydronaphthalene Sulfonate**	
16.6	2	7.02	2		
4.34	4	Halo		19.7	10
4.10	3	4.85	1	15.7	3
4. Stearyl Amide of Sulfonated Sodium Succinate		**11. Decyl Benzene Sodium Sulfonate**		5.72	4
				5.56	1
				4.87	5
48.6	10	25.7	10	4.53	1
22.6	3	20.5	1	4.31	2
14.9	2	17.2	1	4.11	1
4.20	2	12.6	2	3.84	2
Halo		Halo		3.68	1
40.7	4	4.83	3	3.48	1
5. Sodium Salt of Sulfonated Ethyl Oleate		**12. Sodium Di-3,7-dimethyl Octyl Sulfosuccinate**		**18. Dibutyl Sodium Sulfosuccinate**	
48.4	10	25.2	10	16.4	10
24.5	4	14.4	1	9.19	4
15.7	3	Halos		7.79	2
4.08	4	48.1	4	6.55	1
		4.98	1	5.39	1
6. Sodium Lauryl Sulfate				5.08	4
		13. Monobutyl Diphenyl Sodium Monosulfonate		4.66	1
42.7	10			3.97	4
21.7	5	25.0	10	3.47	4
13.8	4	15.5	2	3.28	2
4.34	1	13.0	2	3.14	1
4.06	2	10.4	2	3.05	1
		Halo			
		4.85	1	**19. Sodium m-Nitrobenzene Sulfonate**	
7. Sodium Lauryl Sulfoacetate		**14. Dioctyl Ester of Sodium Sulfosuccinate**		20.3	4
				15.5	10
41.4	10	21.7	10	8.25	1
20.8	4	6.17	2	5.84	1
13.7	3	4.32	2	5.07	3
5.17	1	3.27	2	4.18	4
4.59	2	Halo		4.01	3
4.14	4	4.14	3	3.38	2
				3.27	4

The views expressed by the authors are their own and are not to be construed as representing the official views of the Navy Department.

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Spectrophotometric Determination of Hydrogen Sulfide

Methylene Blue Method

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Hydrogen sulfide is absorbed from gases and precipitated as zinc sulfide. The precipitate is then redissolved and allowed to react with *p*-aminodimethylaniline in the presence of ferric chloride. The optical density of the resulting methylene blue solution is measured at 670-millimicron wave length and the corresponding quantity of sulfide is read from a previously prepared calibration curve. The method is sensitive to about 3 micrograms and the range up to about 500 micrograms. The procedure is convenient for occasional as well as frequent use.

THE determination of hydrogen sulfide in gases has usually been accomplished by iodometric methods (*2, 6*). These give accurate results on appropriate samples but are often too insensitive for samples containing very little hydrogen sulfide. A much more sensitive method is that of Field and Oldach (*3*), in which the sulfide is converted to bismuth sulfide which is determined photometrically while in suspension. This method, although very sensitive (1.4 micrograms), is not well suited for the occasional user, because very rigid control of technique is said to be necessary and all solutions must be protected against oxygen.

The method described herein is a refinement of the methylene blue method (*1, 5, 7*). The technique has been improved by use of optimum conditions for the principal reaction and by applying modern spectrophotometry to the measurement of concentration. The manipulation is simple and the results are not affected by minor variations. The method has been in successful use in the form given for several years.

The hydrogen sulfide is absorbed from a stream of gas in a suspension formed by adding sodium hydroxide to a solution of zinc acetate. The stripped gas is then suitably metered. The suspension then containing the absorbed sulfide as zinc sulfide is treated with an acid solution of *p*-aminodimethylaniline, followed by the addition of a small amount of ferric chloride solution. After time has been allowed for the formation of the methylene blue, the solution is diluted in a volumetric flask and an aliquot is transferred to the spectrophotometer for measurement. The corresponding quantity of sulfide is then determined from a previously prepared calibration curve, plotted from similar measurements on methylene blue solutions prepared in the same manner with known amounts of sodium sulfide or hydrogen sulfide.

The method is sensitive to about 3.5 micrograms of sulfide when used as given. Greater sensitivity could be obtained fairly easily by appropriate reductions in the volumes of solutions used. The upper limit of the method as given is about 500 micrograms. The

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precision at such high concentration is somewhat poorer than at about 100 to 200 micrograms, where it is ±3%.

APPARATUS

The list of apparatus includes the items necessary for taking two samples simultaneously and thereafter treating them consecutively.

Two 250-ml. coarse sintered-glass type gas washing bottles. (Those made by Corning Glass Works are suggested.)
Two test meters, either wet or dry type.
One pipet, 25-ml.
Two pipets, 5-ml.
One graduated cylinder, 250-ml.
One glass tubing cross, 8-mm.
Three tubing clamps, screw type.
Ten meters of 7-mm. Tygon tubing.
Three volumetric flasks, 250-ml.
One spectrophotometer or filter photometer.

REAGENTS

No especial care need be taken in the preparation of the reagents. Deviations up to 5% in the concentrations given are allowable. If the diamine used produces a dark colored solution, a fresh supply should be obtained.

Zinc acetate, c.p., 1% solution in distilled water.
Sodium hydroxide, c.p., 12% solution in distilled water.
Ferric chloride, c.p., 0.023 molar solution in 1.2 molar hydrochloric acid.
p-Aminodimethylaniline sulfate, Eastman white label, 0.5 gram in 500 ml. of 5.5 molar hydrochloric acid.

SAMPLING

A dual sampling procedure in which two samples are obtained simultaneously is recommended. The absorption should be done if possible directly at the source. Gas samples brought into the laboratory in metal or rubber vessels usually give low results due to the reaction of hydrogen sulfide with the metal or its oxide or to its solubility in rubber. The pressure at the source must be at

least 50 mm. of mercury above atmospheric or a pump must be used to draw the sample through the absorption bottle.

One arm of a glass cross is connected to the source with Tygon tubing. A 5-cm. length of tubing is attached to another arm and a screw clamp is placed on the tubing. Each absorber is then charged with 130 ml. of 1% zinc acetate solution and 5 ml. of 12% sodium hydroxide solution, and the solutions are mixed by swirling. The parts of the bottles are assembled with petrolatum and fastened with rubber bands. The inlets of the bottles are connected to the remaining arms of the cross and screw clamps are placed on the connecting tubing. A test meter is then connected to the outlet of each absorber.

With all screw clamps open, the gas is turned on at the source at a rate considerably in excess of the sampling rate. Then the clamp on the bleeder arm is slowly closed until gas passes through the gas washing bottles at a rate of about 170 liters per hour (6 cubic feet per hour). The rates through the two bottles may be equalized by adjusting the screw clamps on the connecting tubing. The amount of sample to be taken should be that which will contain between 35 and 350 micrograms. Where it is necessary to use samples smaller than 50 liters, the sampling rate should be reduced correspondingly.

If the method is being used to determine the amount of hydrogen sulfide resulting from the conversion of other sulfur compounds to hydrogen sulfide for analysis (4), only one gas washing bottle is used and the test meter can be replaced by a simple flowmeter.

PROCEDURE

After the sample has been passed through the gas-washing bottle, the inlet and outlet of the bottle are closed by slipping the ends of a 25-cm. length of tubing over them. Just before beginning the methylene blue reaction the temperature of the bottle and contents is adjusted to $24° \pm 3° C.$; the temperature of the diamine reagent should be similarly adjusted. Then the top of the gas-washing bottle is raised and 25 ml. of diamine reagent are pipetted into the bottle. The bottle is closed quickly and the contents are swirled until all the precipitate is dissolved. Then by alternately applying slight pressure and suction on the inlet, a small amount of the solution is forced back and forth through the sinter in order to dissolve any zinc sulfide that may have concentrated there. When all the sulfide is dissolved, the top is again raised and 5 ml. of ferric chloride reagent are pipetted into the bottle, followed by mixing as before. The use of pipets designed for short delivery time rather than great accuracy is recommended.
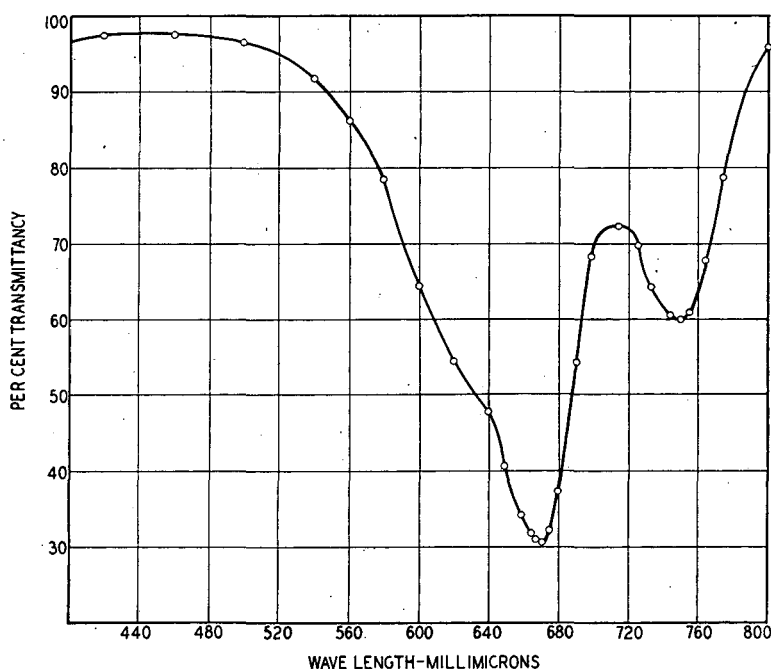
Figure 1. Transmittancy of Methylene Blue Solution

**157 micrograms of sulfur in 250 ml. Spectral band width, 2 to 3 millimicrons.
Cell thickness, 1.00 cm.**

Table I. Specimen Calibration Data

(Coleman Universal spectrophotometer, wave length 650 millimicrons)

Sulfide Inserted, Micrograms/250 Ml.	Optical Density
64.8	0.21
64.8	0.22
129.6	0.41
129.6	0.43
259	0.80
259	0.82
389	1.12
389	1.16

After the closed bottle is allowed to stand for 10 minutes the blue solution is transferred to a 250-ml. volumetric flask and diluted to the mark with distilled water. Before the optical density is measured, the solution should be allowed to stand at least 20 minutes but not more than 20 hours in a place out of direct sunlight.

A blank solution is made by mixing the same amounts of the four solutions used above in a 250-ml. volumetric flask and diluting to 250 ml. with distilled water. This solution should be allowed to age for about 30 minutes before use in the spectrophotometer; the solution may be stored for several days in a dark or dimly lighted place.

The optical density or transmittance of the test solution is determined by making the initial adjustment of the instrument while the cell is filled with the blank solution. Normally, and for highest sensitivity, the measurements are made with light of 670-millimicron wave length. Light of 750-millimicron wave length may be used if the solution is unusually opaque.

CALIBRATION

If the measurement of the optical density of the test solution is to be useful, a calibration curve must be prepared by making up several standards in the manner described above but using carefully measured quantities of sodium sulfide solution or hydrogen sulfide in place of the sample. A solution of sodium sulfide containing about 20 micrograms of sulfur per milliliter is satisfactory. The lumps of sodium sulfide should be thoroughly washed immediately before making the solution, in order to remove any sodium sulfite. Oxygen-free distilled water should be used in making the solution. The solution is standardized iodometrically. Care must be taken throughout the preparation of the standards to protect the sodium sulfide solution from more than a minimum amount of contact with oxygen.

The calibration is completed by measuring the optical densities of the standard methylene blue solutions and plotting the values obtained against the corresponding mass of sulfide used in preparing the 250-ml. solution. The resulting curve should be nearly linear in the lower half of the useful range of concentrations. Specimen calibration data are given in Table I. Once made the calibration may be used indefinitely. Data should be obtained at 670 millimicrons and also if possible at 710 and 750 millimicrons. The apparent peak absorption wave-length may vary somewhat from 670 millimicrons when instruments of low spectral purity are used. For example, with the Coleman Universal spectrophotometer the apparent peak is at 650 millimicrons; this is apparently due to this instrument's band width of about 35 millimicrons. The absorption spectrum for a methylene blue solution compared to a blank solution with a Beckman Model DU spectrophotometer using a 2 to 3 millimicron band width is shown in Figure 1.

EXPERIMENTAL

The amount of methylene blue finally formed in the reactions involved is a function of the tem-

Table II. Effect of Temperature on Yield of Methylene Blue

Temperature, ° C.	5	20	25	30	40	55	75
Relative yield, %	76	99	100	98	79	64	43

Table III. Effect of Acid Concentration* of Diamine Reagent on Optical Density of Methylene Blue Solutions[a]

Molarity (HCl) of diamine reagent	2	4	5	5.5	6	7	8	10
Optical density at 650 millimicrons	0.33	0.66	0.69	0.71	0.70	0.66	0.63	0.57

[a] All solutions contained 222 micrograms of sulfide per 250 ml.

perature and other variables. At higher temperatures the reaction is rapid but greater amounts of hydrogen sulfide escape from the acid solution into the vapor space of the gas-washing bottle before reacting; at low temperatures little hydrogen sulfide escapes but the methylene blue reaction becomes so slow that side reactions occur to a greater extent. The over-all effect of temperature on the relative yields of methylene blue from identical reaction mixtures is shown in Table II Fortunately, the maximum yield occurs at about 24° C. and a reasonable tolerance may be allowed.

The effect of final acid concentration on the optical density of a methylene blue solution formed from a given amount of hydrogen sulfide was investigated by preparing the solutions as described above but with diamine reagents of various acid concentrations. All the solutions contained 222 micrograms of sulfide per 250 ml.; the results are given in Table III. The effect is believed to be due largely to the influence of acidity on the absorption spectrum of methylene blue rather than to influence on the yield of the reaction.

When the diamine reagent is added to the suspension containing zinc sulfide, hydrogen sulfide is formed. Some of it escapes into the vapor space of the bottle and is lost. The amount which escapes is a function of the solubility and the total amount present. When only small amounts of sulfide were present no hydrogen sulfide was detectable over the solution and this was arbitrarily assumed to indicate complete conversion to methylene blue.

Then solutions were prepared with greater amounts of sulfide and these solutions were diluted with blank solution sufficiently so that the diluted solution should have corresponded to the one in which complete conversion was assumed. Invariably the optical densities of the diluted solutions were found to be less than that of the reference solution, indicating a loss. The results of these experiments are given in Table V. Because corresponding losses occur in preparing the calibration curve, this effect is not considered to be important for methylene blue solutions representing less than 470 micrograms of sulfide in 250 ml. of solution. This effect accounts for the deviation of the calibration curve from Beer's law.

Table IV. Recovery of Hydrogen Sulfide as Methylene Blue

Sulfide inserted, micrograms	35	122	243	366	487	610	730	855
Recovery, %	100	99	98	97	96	94	88	80

The reaction time of 30 minutes allowed in the procedure includes a considerable safety factor. Periodic determinations of the optical density of a solution during the reaction period indicated that the reaction was just completed after 10 minutes—that is, no further increase in the optical density was detected after 10 minutes. After about 20 hours a decrease due to fading may begin to be measurable.

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Conversion of Sulfur Compounds to Hydrogen Sulfide

In Air, Fuel Gas, or Mixtures

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THE sulfur content of fuel gases is usually determined either by oxidation or by hydrogenation. Oxidation methods (5, 8, 10, 11) are capable of accurate results on gases containing as little as 114 micrograms of sulfur per cubic meter (0.005 grain per 100 cubic feet), but the technique is cumbersome and the apparatus is likely to be capricious. Hydrogenation methods

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(2, 4, 6, 9) have some advantages but are limited in scope by the interference of oxygen, which is plentiful in certain types of gases. Even the oxidation methods cannot be applied to explosive mixtures or nonflammable gases. A method that can be used on any mixture of air and fuel gas became necessary for this laboratory in order to determine whether the natural gas present in soil gases was that normally present in the soil of certain areas or leakage from gas distribution lines which carry natural gas

Organic sulfur compounds are quantitatively removed from gases by adsorption on silica gel at about 25° C. and subsequently desorbed at 500° C. and hydrogenated over a quartz catalyst to hydrogen sulfide, which may be determined by any suitably sensitive method. Any hydrogen sulfide or carbon oxysulfide present in the gas sampled passes through the silica gel. The principal feature of the method is the silica gel adsorption technique, which permits analysis of gases that would otherwise explode in the hydrogenation tube.

odorized with Calodorant [a mixture of alkyl sulfides, disulfides, and mercaptans (thiols) dissolved in gasoline].

In order to overcome the difficulty encountered with explosive mixtures and to provide for convenient sampling at the source of gas, a technique was developed in which the sample is dried and passed through a bed of silica gel which adsorbs the sulfur compounds. At any convenient time thereafter the latter are desorbed at about 500° C. and passed with hydrogen through a bed of quartz chips at approximately 1000° C., resulting in complete conversion of all sulfur present to hydrogen sulfide. The hydrogen sulfide so formed should be determined by a sensitive method so that only small sample volumes are required, thus avoiding overloading the silica gel with consequent danger of loss of volatile sulfur compounds. The methylene blue method for hydrogen sulfide (3) seems most suitable. Hydrogen sulfide and carbon oxysulfide are not appreciably adsorbed by silica gel, but probably could be simultaneously determined in sampling by means of the methylene blue method and Brady's method (1) for carbon oxysulfide.

SCALE IN CM.
0 1 2 3 4

SILICA GEL   ALUMINUM

GLASS WOOL   ALUNDUM

TRANSITE   ASBESTOS

Figure 1. Adsorption Tube and Desorption Furnace

The over-all accuracy of the method is partially limited by the accuracy of the hydrogen sulfide determination. Consequently no exact statement can be made concerning the technique dealt with in this paper alone. It is believed that with average size samples of, say, 250 micrograms of sulfur the uncertainty introduced by the sampling and hydrogenation procedure is not greater than ±15 micrograms and is ordinarily about ±7 micrograms of sulfur.

APPARATUS

The sampling train consists of the following items connected in series with 7-mm. Tygon tubing: a calcium chloride drying tube, one or two adsorption tubes (Figure 1, A) containing purified 8- to 14-mesh silica gel, and a suitable gas meter for determining the volume of the sample. If it is desired to remove any constituent selectively before adsorption, the necessary reagents should be used in a scrubber ahead of the drying tube. Thiols may be excluded by scrubbing with an alkaline solution of cadmium sulfate.

The complete hydrogenation train is shown diagrammatically in Figure 2. A cylinder of commercial hydrogen, A, equipped with a pressure regulator (or needle valve), B, is connected to the adsorption tube, E, with 7-mm. Tygon tubing. The adsorption tube rests horizontally in the desorption furnace, D, which is shown in diagrammatic detail in Figure 1, B (the old style Burrell copper oxide tube heater is suggested). This furnace has a 150-watt wire-wound heating element controlled by a rheostat, C, of 0 to 300 ohms. The outlet of the adsorption tube is connected with 7-mm. Tygon tubing to the quartz hydrogenation tube, F, by means of a glass tube through a rubber stopper that has been thoroughly boiled in concentrated sodium hydroxide solution. The central section of the hydrogenation tube containing the catalyst, G, is heated by the 750-watt electric furnace, H, which is controlled by a 0- to 130-volt, 2 kv.-amp. autotransformer, I. The outlet of the hydrogenation tube, which is drawn down to about 8 mm., is connected with Tygon tubing to the hydrogen sulfide absorber, J. The outlet of the absorber is connected to a flowmeter, K, which indicates the rate of flow of hydrogen. The used hydrogen is vented into a safe place.

PREPARATION OF APPARATUS

Adsorption Tubes. Sufficient 8- to 14-mesh silica gel to fill one or more adsorption tubes is placed in the center section of the hydrogenation tube (normally occupied by the catalyst) and parts F, H, I, and K are assembled as in Figure 2. The silica gel is heated to 760° C. and the tube is purged with nitrogen. Hydrogen is then passed through it at a rate near 85 liters (3 cubic feet) per hour. While the hydrogen is flowing, occasional checks for hydrogen sulfide are made on the effluent from the hydrogenation tube with a Mine Safety Appliance Co. hydrogen sulfide detector tube (7) which may be inserted just ahead of flowmeter K. When a fresh detector tube shows practically no discoloration after a 5-minute exposure the silica gel may be considered free from sulfur and the purification procedure stopped. After cooling, the silica gel is sealed into glass tubes such as shown in Figure 1, A. Before use the adsorption tube should be heated to about 250° C. while air, nitrogen, or hydrogen is passed through it in order to remove any water vapor it may have adsorbed. The ends of the tube should be capped immediately after this treatment to prevent further access of water vapor.

After some use the silica gel may become coated with carbon; this can be safely removed with air at about 540° C.

Hydrogenation Tube. To prepare the hydrogenation tube about 100 grams of clean 4- to 8-mesh quartz chips are placed in the central part of the quartz tube. Alternatively the tube may be completely filled with chips. It is important that no
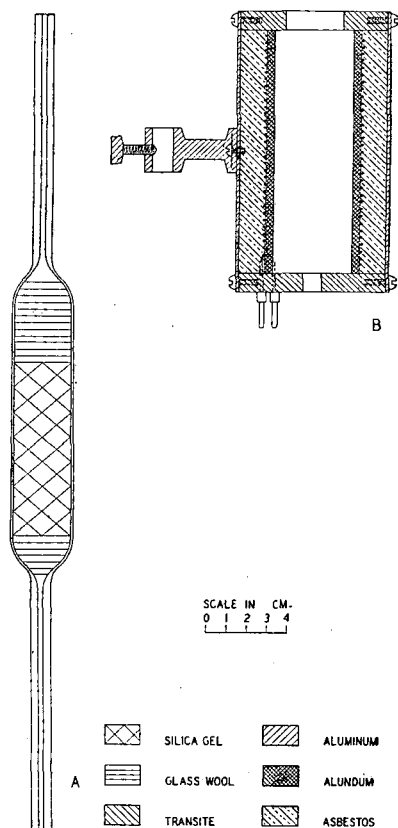
open channel be left along the upper side of the tube. If the quartz becomes coated with carbon after use, this should be burned off with air, as low results may otherwise be obtained.

SAMPLING

The sampling train up to but not including the adsorption tube is purged with the gas to be sampled; a sulfur-free adsorption tube and the meter are then attached. The sample is passed through the train at a rate of about 150 liters (5.4 cubic feet) per hour. The temperature of the metered gas and the barometric pressure should be noted. Only enough sample to provide about 150 micrograms of sulfur need be taken if the methylene blue method for analysis is used; for other methods appropriate amounts may be taken, but for optimum results it is best to use a method that does not require more than 1 mg. The limit is determined by the adsorptive capacity of the silica gel tube.

If the gas sampled is dry the drier may be omitted from the sampling train. The temperature of the adsorption tube should ordinarily not be allowed to rise above about 25° C. during the sampling period, although it may exceed this temperature after sampling, if the ends of the tube are tightly capped.

One adsorption tube usually provides sufficient retentivity for most sulfur compounds, but in some cases—for example where the sample contains methanethiol—unless the sample contains no higher boiling compounds two adsorption tubes in series may be required.

PROCEDURE

The hydrogenation tube furnace is adjusted to about 1000° C. with the hydrogenation tube and catalyst in place; the desorption furnace is adjusted to about 500° C. with a spare adsorption tube in place. A small thermocouple type pyrometer is convenient for making the temperature adjustments. The hydrogenation tube is purged with nitrogen, and hydrogen is then passed through it at about 85 liters per hour until a 5-minute check with a M.S.A. hydrogen sulfide detector tube indicates freedom from hydrogen sulfide. The hydrogen sulfide absorber is charged with the appropriate reagents and attached to the outlet of the hydrogenation tube and to the flowmeter.

The cool adsorption tube holding the sample is purged with

Table I. Recovery of Sulfur Compounds from Gases on Silica Gel

(Conversion of hydrogen sulfide over quartz at 1000° C.)

Material Tested	Sulfur Inserted, Micrograms	Sulfur Recovered, Micrograms	Deviation, Micrograms
Blank	0.0	6.5	+6.5
	0.0	6.5	+6.5
	0.0	3.5	+3.5
Calodorant	71.3	71.3	0.0
	149.	143.	−6.0
	292.	285.	−7.0
	363.	363.	0.0
	9080	9080.	0.0
Carbon disulfide	104.	104.	0.0
	318.	311.	−7.0
	58.3	64.8	+6.5
	324.	330.	+6.0
Sulfur dioxide	350.	356.	+6.0
	350.	362.	+12.0
Methanethiol	408.	395.	−13.0
Ethanethiol	168.	181.	+13.0
	434.	434.	0.0
Butanethiol	90.7	90.7	0.0
	304.	304.	0.0
Dimethyl sulfide	129.	117.	−12.0
	434.	434.	0.0
Diethyl sulfide	84.3	90.7	+6.4
	298.	317.	+19.0
Diamyl sulfide	227.	220.	−7.0
	117.	130.	+13.0
	324.	330.	+6.0
Dimethyl disulfide	356.	349.	−7.0
	110.	116.	+6.0
Diethyl disulfide	407.	394.	−13.0
	129.	136.	+7.0
	259.	246.	−13.0
Diethyl sulfate	233.	233.	0.0
	117.	123.	+6.0
Hydrogen sulfide	648.	6.5	...
Carbon oxysulfide	388.	13.0	...

about 7 liters of nitrogen or hydrogen (this serves to remove residual amounts of hydrogen sulfide and carbon oxysulfide as well as to displace any air that might cause an explosion in the hydrogenation tube). The adsorption tube is then inserted into the desorption furnace and immediately connected to the hydro-
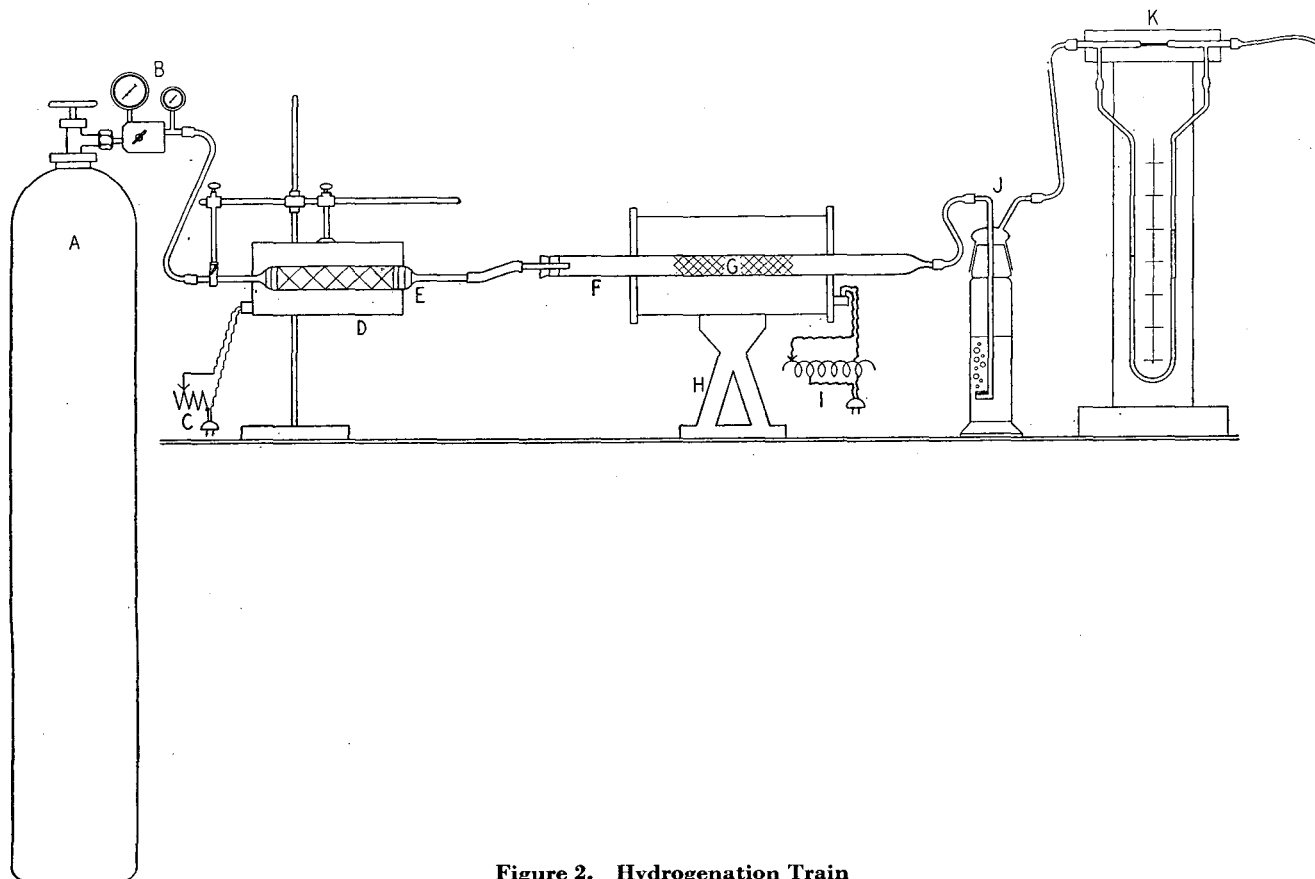
Figure 2. Hydrogenation Train

gen cylinder and to the hydrogenation tube and flow of hydrogen is begun at once at a rate of about 85 liters per hour. This is continued for 15 minutes, which is usually ample time for the complete removal of the sample from the silica gel; however, it is best to check the completeness of removal by detaching the hydrogen sulfide absorber after 15 minutes and checking the hydrogen stream for hydrogen sulfide by inserting a M.S.A. detector tube. If a positive indication is obtained in 1 minute the absorber is reattached for 5 minutes, followed by another check with a M.S.A. tube. This is continued until a negative indication is obtained on a 1-minute trial. If experience shows that more than 15 minutes are required for complete desorption, the duplicate sample is run for the full time necessary before any checks are made. Only samples containing very strongly adsorbed sulfur compounds require a desorption temperature as high as 500° C., but it is best to run all samples at this temperature in order to avoid low results and imperfectly cleaned adsorption tubes. When the hydrogenation is completed the hydrogen sulfide absorber is removed and its sulfur content is determined.

For highest accuracy a blank run should be made by following the procedure using an identical adsorption tube holding no sample, and the result subtracted from the sample determination. Blanks usually are less than 10 micrograms of sulfur.

EXPERIMENTAL

Three adsorbents were tried as sample collectors and several materials as hydrogenation catalysts. It was found that silica gel is the most satisfactory absorbent because it reversibly adsorbs small amounts of sulfur compounds. Activated alumina usually contains alkaline substances which react with thiols and other acidic compounds, making it difficult to get quantitative desorption; activated charcoal (coconut) adsorbs small amounts of sulfur compounds so strongly that almost none can be desorbed. A catalyst other than the quartz tube is not absolutely required, but better contact is obtained with the quartz chips and flow rates may be increased. Catalysts such as cobalt, molybdenum, copper, and nickel are unsatisfactory because they retain some of the sulfur. Iron reduced on quartz was found fairly satisfactory but gave some erratic results. Activated alumina was also fairly good but tends to retain sulfur and release

it slowly. Quartz is fast and nonabsorptive; it gives consistently good results and so was adopted.

To test the method, dilutions of various organic sulfur compounds and Calodorant in petroleum ether were vaporized with air or natural gas and passed through the adsorbents and then the procedure given above was followed. Tests were also made with measured volumes of sulfur dioxide, hydrogen sulfide, carbon oxysulfide, and methanethiol. Of all the compounds tested only hydrogen sulfide and carbon oxysulfide could not be quantitatively adsorbed. In fact, they were so slightly adsorbed that following the sampling by a purge of 7 liters of nitrogen or hydrogen entirely removed (within experimental error) these two substances from the adsorption tube without affecting others present. A summary of some tests made with silica gel adsorbent and quartz catalyst is given in Table I.

A single adsorption tube was found able to hold quantitatively up to 35 mg. of sulfur present in Calodorant (6% sulfur content) but it is considered poor practice to take large samples unless no low boiling sulfur compounds are present. In sampling gases containing methanethiol, sulfur dioxide, or carbon disulfide together with higher boiling compounds (not necessarily sulfur compounds) it was found advisable to use two adsorption tubes in series; the presence of little or no sulfur in the second tube then offers proof of complete retention of all but hydrogen sulfide and carbon oxysulfide.

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Bearing Corrosion Test for Lubricating Oils

Correlation with Engine Performance

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THE use of "detergent" type additives in motor oils resulted in an increase in the bearing corrosion problem and it became necessary to develop a laboratory test that would predict the corrosive tendencies of these additives. The present study was undertaken with the objective of developing a laboratory corrosion test that could be correlated with the standard Chevrolet laboratory engine test (7). An attempt was made to incorporate into the test as many as possible of the factors that are responsible for the corrosion of bearings in engines. Much of the information that had been gained during the development of the Sohio oxidation test (6) was applied to the proposed test procedure. The Sohio oxidation test yields correlative data concerning oxidation and detergency. It also differentiates between noncorrosive and extremely corrosive oils, but does not evaluate examples of mild corrosion.

A survey of the literature indicated that a number of methods for testing bearing corrosion had been developed. A description

of the tests may be found in a recent review of the subject by Larsen (8). Of the existing tests, the Underwood (13), the MacCoull (9), and the corrosion and stability apparatus (14) have shown correlation with the standard laboratory engine tests. These tests have in common a high rate of shear in the oil adjacent to the bearing. A serious disadvantage of the Underwood test is that a large oil sample is required and the apparatus is difficult to clean. The corrosion and stability apparatus is complex and not applicable to multiple operation. The MacCoull test possesses no objectionable features, but the original publication was not clear as to the engine test procedure or the nature of the oils used in the correlation study. None of these tests was considered to meet the objective of the present study.

As a result, a modification of the Sohio oxidation test method was developed in which satisfactory bearing corrosion correlation was obtained by employing the principle used in the above tests—producing a high rate of shear in the oil surrounding the bearing.

A description is given of a thrust bearing apparatus adaptable to the Sohio oxidation test for lubricating oils. The operating conditions and catalyst components have been determined so that the test at 10 hours correlates for copper-lead corrosion with the 36-hour, L-4 Chevrolet (A.S.T.M.) test for varied groups of inhibitors and oils. At the same time correlative information is obtained on the oxidation characteristics of the lubricating oils. The test is shown to be correlative for 76 oil-inhibitor combinations comprising four inhibitor types and two commercial oils. The test is useful both in the field of additive development as a weeding-out tool to indicate inhibitor-oil combinations meriting further study in the engine, and as a quality control procedure for plant batches of inhibited motor oils.
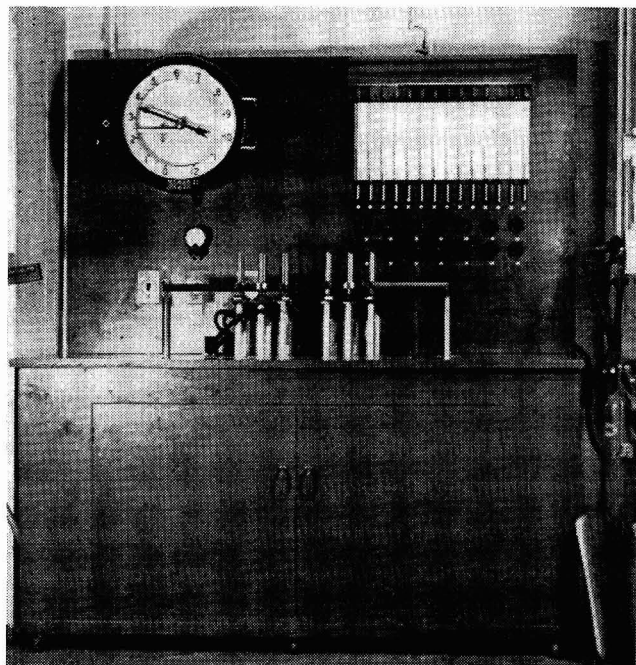
Figure 1. Sohio Corrosion Test Apparatus

The importance of shear in a laboratory bearing corrosion test has been discussed by Talley, Larsen, and Webb (12).

The design of the corrosion apparatus is described and the choice of the optimum test conditions is discussed here. With the developed test conditions the evaluation of a varied group of oils, typical of modern commercial products in method of refining and additive content, is demonstrated. A correlation study has been made of the laboratory and engine test data obtained on these oils.

DESCRIPTION OF APPARATUS

A photograph of the apparatus is shown in Figure 1. The aluminum block, flowmeter, and air train are those used in the Sohio oxidation test. The arrangement of the test setup, except for the corrosion apparatus, is shown in an earlier paper (6).

A 100-gram sample of oil is placed in a glass tube, A, Figure 2, 45 mm. in outside diameter and 42 cm. long. A glass bubbler, B, attached to the air inlet tube, C, is placed in the glass test tube. B is spaced from the bottom of A by 2-mm. glass tabs to allow passage of oil and suspended solids into the support and up the air lift. The corrosion test unit, D, fits into the glass tube above the bubbler and is held in place by a steel support arm, E, which fits into a support holder, thereby positioning it in A. Two test units are attached to each support arm.

In the Sohio corrosion apparatus a hardened steel drill rod rotates against a weighed copper-lead bearing metal

test piece. The shear produced in the oil layer between the two surfaces is intended to be comparable to the shear resulting from the motion of the steel crankshaft of an engine with respect to the connecting rod bearing.

The corrosion test unit, as shown in a cutaway view (Figure 3) consists of the following parts:

A section of steel tubing, A, 37.5 cm. (15 inches) long and 2.58 cm. ($1^1/_{32}$ inches) in outside diameter, attached to the support arm by two hexagonal nuts, B.

A steel cup, C, 2.5 cm. (1 inch) long, 2.58 cm. ($1^1/_{32}$ inches) in outside diameter, and 2 cm. ($13/_{16}$ inch) in inside diameter which is threaded into the steel tubing and contains a hole 9.4 cm. (0.375 inch) in diameter in the bottom for pumping oil upward through the corrosion chamber.

A circular, relatively fine grained copper-lead test piece, D, of $13/_{16}$ inch outside diameter is used. The test piece fits snugly into the steel cup and has a hole 0.25 inch in diameter in its center which fits over the hole in the cup. (Commercial bearing strip flats of the Clevite 35 type were obtained from the Cleveland Graphite Bronze Company.) The test piece has an exposed copper-lead surface of 3.00 sq. cm. Of this surface area, 1.85 sq. cm. act as a loaded bearing and are faced against a hardened steel drill rod.

A section of steel rod, E, 0.375 inch in diameter and 19 inches long, serves as a shaft and is positioned by two bearings. The lower bearing, F, is made of steel and is pressed into the outer tubing, A. The upper bearing, S, is Oilite and is pressed into the upper brass screw cap, R. Holes ($3/_{16}$ inch) are drilled in the
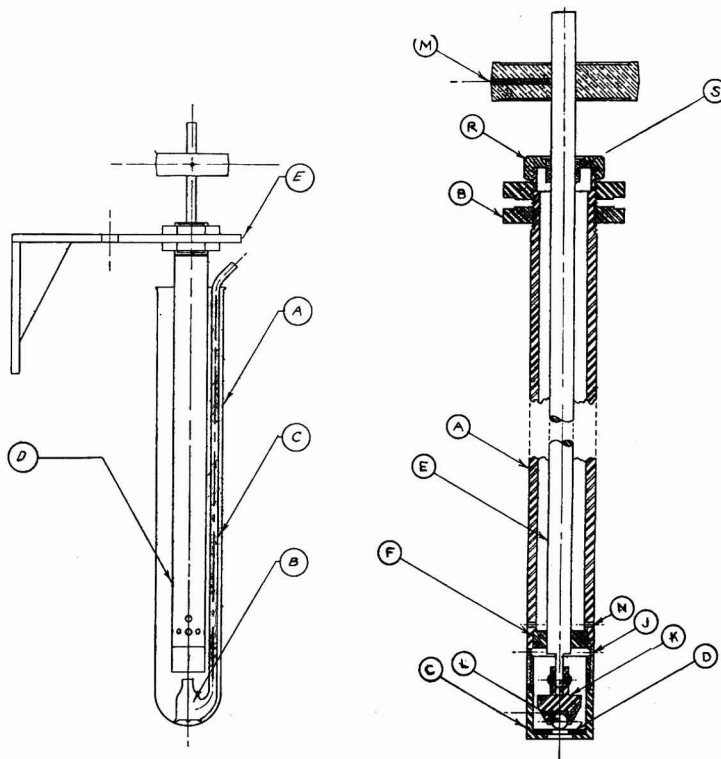
Figure 2. Arrangement of Corrosion Apparatus and Oil Test Tube

Figure 3. Corrosion Apparatus

steel tubing above N and below J, the lower bearing. The two holes, N, above the bearing facilitate the cleaning of the apparatus, while the eight holes, J, below the bearing make possible the circulation of oil through the corrosion chamber. Cleaning the inside of tube A is also facilitated by the removal of screw cap R.

A steel, swivel drill rod holder, K, is connected to shaft E by a self-aligning yoke and pin coupling. A section of hardened, steel drill rod, L, $^{21}/_{32}$ inch long and 0.25 inch in diameter and of 51–57 Rockwell hardness is fitted into K and held in place by a setscrew. The yoke-type swivel assures instantaneous and continuous alignment of the drill rod bearing member against the bearing surface at all times during the test. A new drill rod is used in each test.

A brass pulley, M, 2 inches in diameter, is fitted to the top of the steel shaft and held in place by a setscrew. Six such pulleys are connected by a belt made from friction tape which is driven by a brass pulley 0.75-inch in diameter, connected to 0.25-hp. motor. The shafts are operated at 625 ($\pm$10) r.p.m. Two banks of six units may be operated simultaneously.

The parts of the unit consisting of the brass pulley, steel shaft, steel swivel, and drill rod weigh 600 ($\pm$5) grams and the gravitational force of this unit represents the thrust load on the bearing. The air lift action from the bubbler and the rotation of swivel K, which acts like a centrifugal pump, forces the oil up through the chamber containing the test piece and out through the holes in the outer steel tubing at a high rate.

DEVELOPMENT OF TEST

The first studies devoted to an investigation of the mechanical causes of corrosion were conducted by construction of various mechanisms for flexing, vibrating, and rubbing the bearing surface or creating a shear in the oil layer adjacent to the bearing under conditions conducive to corrosion. Flexing and vibration were found to have little effect on surface films, whereas actual rubbing in many examples removed the films formed by noncorrosive oils. The effect of shear, as applied to a loaded bearing, showed promise of correlation and was tried with both felt and hardened steel surfaces facing the test pieces. The use of a felt surface gave erratic results and was discarded in favor of steel because of its better reproducibility and ease of operation. When a glass rod was used in place of steel it was found that much lower bearing metal weight losses resulted, particularly with corrosive oils.

After standardization upon the ultimate style of apparatus and the use of a steel thrust member, studies were conducted upon the effect of speed, load, and hardness of the steel member. Rotational speed of the shaft was found to have little effect in the range from 400 to 1300 r.p.m.; a speed of about 625 r.p.m. was finally selected merely for the sake of convenience in pulley diameter. Variations in load from 400 to 800 grams showed minor variations in corrosion results. Substantially larger loads resulted in a wear effect on the test piece, particularly in the case of oils containing little or no additive. This effect presumably was due to a change from hydrodynamic to boundary lubrication. Oils containing additives which were rich in sulfur and/or phosphorus were found capable of carrying much heavier loads. Similar results concerning speed and load and their theoretical background have been described by Talley, Larsen, and Webb (12). The steel drill rod was used in two different degrees of hardness, 17 and 53 Rockwell C. During the usual test procedure the surface of the steel drill rod showed wear even though the bearing metal underwent exceedingly low corrosion. With the softer steel, the wear on the steel rod was noticeably increased and test results were somewhat less reproducible. For this reason the harder steel was adopted for use.

OPERATION OF TEST

A new copper-lead bearing metal test piece is used for every test. It is ground on a surface grinder before use until the overall flatness is within 0.0005 inch, and the surface imperfection is no greater than 25 root mean square. Superfinishes of greater perfection have no effect on the test result. The test piece is weighed before and after the test to evaluate corrosion. At the conclusion of the test the piece is cleaned by successive washing with pentane and chloroform to remove oil and lacquer deposits.

Table I.

Temperature, 325° F.
Oil sample, 100 grams
Air flow rate, 70 liters per hour
Time, 10 hours
Catalysts, steel
   Copper-lead bearings, 3-sq. cm. area; 1.85-sq. cm. bearing surface
   Ferric 2-ethylhexoate, 0.05% as Fe_2O_3 in c.p. benzene
   Lead bromide, 0.1% as precipitated
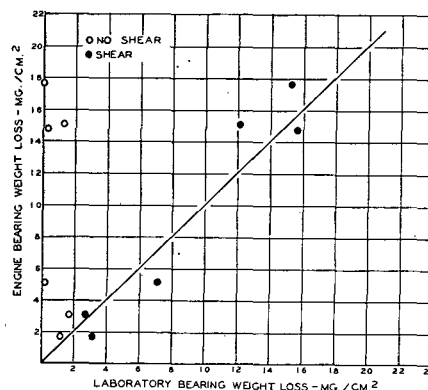Bearing assembly load, 600 grams
Speed, 625 r.p.m.

Figure 4. Effect of Shear on Laboratory Test Corrosion Results

Before each test the exterior of the steel units is cleaned with emery paper to remove lacquer deposits, then washed with carbon disulfide and pentane. If sludge has accumulated on the inside of the units, the screw caps, R (Figure 3), are removed and the inside of the tubes, A, is cleaned. For this purpose steel wool is employed.

The laboratory test conditions that were found to correlate best with the 36-hour Chevrolet procedure are shown in Table I.

By extending the laboratory test to 20 hours it was found that correlation with 72-hour engine procedure could be obtained.

The lacquering properties of an oil are evaluated by observing the outer surface of the steel tube of the corrosion apparatus in much the same visual manner that the piston skirt, cylinder walls, etc., of an engine are rated for varnish.

A test temperature of 325° F. was selected in order to simulate the temperature of the bearing surfaces in the engine. Estimates of bearing surface temperatures in the literature (10, 11) have indicated an increase of 50° to 75° F. over bulk oil temperatures due to frictional effects.

A sufficient volume of used oil is obtained from the test for the determination of the usual used oil properties such as pentane-insolubles, benzene-solubles, viscosity change, and neutralization and saponification numbers.

Experimental data indicated that a test time of 10 hours gave satisfactory correlation with the 36-hour Chevrolet engine procedure. By extending the laboratory test to 20 hours correlation with the 72-hour engine procedure was obtained.

The catalysts used were 0.05% ferric oxide as ferric 2-ethylhexoate and 0.1% lead bromide, similar to the concentrations used in the Sohio oxidation test.

Effect of Shear. To show the importance of the shear in the laboratory test, six oils (which had shown correlation with the engine under the standard laboratory test conditions) were retested; the only change in conditions was that the steel drill rod was not rotated against the bearing metal surface. The results from these experiments, presented graphically in Figure 4, show the much lower corrosion results and the failure to correlate in the absence of rotation.

Table II. Reproducibility of Laboratory Corrosion Test Data

Weight Loss of Bearing Metal Test Piece, Mg.	% Deviation
23.1, 22.9	0.4
12.2, 14.8	9.6
20.8, 22.3	3.3
12.4, 13.5	3.8
24.0, 27.8, 25.1	5.5
19.0, 22.0	7.3
42.0, 45.0, 48.9	5.3
43.7, 40.2	4.1
77.7, 69.1	5.9
29.9, 30.3	0.7
45.2, 51.4	6.4
39.5, 41.2	2.0
Average deviation	4.5

REPRODUCIBILITY

The reproducibility of the corrosion weight losses between check experiments was of the order of 5%. A group of randomly selected results obtained using different units and by different operators (Table II) from tests with various oils and additives illustrates the range of test piece weight losses and their variations. The test data are considered more reproducible and accurate than the corresponding engine data, although the test has significance only in so far as the relationship between engine and laboratory data can be established.

CORRELATION WITH ENGINE DATA

Correlation between Chevrolet engine results and the laboratory test was studied with oils containing both detergent and nondetergent additives. Two distinctly different groups of additives of each type were investigated. A general description of the different additives is shown in Table III.

Detergent Oils. GROUP I. In the first of these studies most of the data were obtained on various experimental modifications and concentrations of a metal-phosphorus pentasulfide-amine reaction product additive (2, 3) in acid- and solvent-refined base oils. Two reference oils, REO-7 and REO-8, a combination of these two oils, and a commercial oil were included in this group. Almost all these oils (Group I) were characterized by a high resistance to oxidation and varnish formation, resulting in excellent used oil properties and engine ratings. Because these values give insignificant differences, only corrosion correlation is shown on this group of oils.

The results obtained by plotting 36-hour engine bearing corrosion against 10-hour laboratory corrosion, both expressed in milligrams per square centimeter, are shown in Figure 5. These data, which are based on a basis of weight loss per unit area, gave a fairly good straight-line correlation relationship. At a first glance it might appear that the points deviated to a marked extent from the theoretical line, representing perfect correlation. However, the actual magnitude of deviation is only 2 to 2.5 mg. To obtain exact agreement in weight loss of test piece between the laboratory and the engine would be extremely difficult and not necessary in predicting the corrosivity of an oil.

The three reference oils—REO-7, REO-8, and the blend of these two oils—correlated particularly well and covered a wide corrosion range.

The practical value of the laboratory test in predicting the engine performance of an oil is illustrated by the points falling in the shaded area of Figure 5. In using the Chevrolet L-4 procedure as a specification test for motor oils, 350 mg. per whole bearing have been considered the maximum allowable bearing corrosion (4). Thus, points falling below 6.7 mg. per sq. cm. on the engine scale would be considered satisfactory. The use of a qualifying value of 6.7 mg. per sq. cm. weight loss for the laboratory test also resulted in an area describing oils considered satisfactory by both test procedures. Of the 29 oils tested, 13 fell within this acceptable region and 4 others which might be con-

sidered borderline oils were very close to it. Severe corrosion values were shown by both test methods for the remaining 12 oils. The greater discrepancy in correlation results at higher corrosion values was partially explained by the poor engine reproducibility from test to test, and even from bearing to bearing in the same test, when severe corrosion was encountered.

72-Hour Engine Tests. Several of the better oils (Group I) described above were subjected to a 72-hour engine test using L-4 conditions, except for the time variable. In testing these oils by the laboratory corrosion test, the time variable was doubled to a value of 20 hours, just as it had been doubled in the engine. As shown in Figure 6, corrosion correlation again was found between the two test procedures when the oils were compared on a basis of weight loss per unit area. In this comparison the experimental corrosion curve showed the engine to be slightly more severe than the laboratory test procedure.

The ability of the laboratory test to correlate with the engine at two intervals during the evaluation of these oils was considered to indicate that the rate of corrosion produced by the two methods was approximately equivalent. The close agreement in test piece weight loss per unit area was further evidence of this.

GROUP II. The second study of detergent type additives was applied to a metal-phosphorus pentasulfide-hydrocarbon type additive and its modifications. Oils (Group II) containing this additive failed to show good correlation between engine and laboratory test, as shown in Figure 7. Of eighteen oils tested, eight were rated noncorrosive and two were rated borderline by the two procedures. Seven of the other oils did not show correlation. Five of them gave high engine corrosion values and the other two showed high laboratory corrosion results. The only ready explanation for this failure to correlate is the borderline nature which several of the oils exhibited. In the few instances in which check engine tests were conducted on these oils very poor corrosion reproducibility was found.

Nondetergent Oils. The next series of correlation studies was devoted to oils containing nondetergent additives of the phosphorus sulfide type. The two additives that were studied
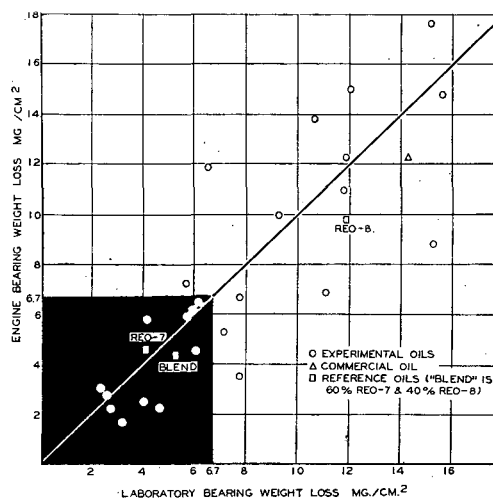
Figure 5. Corrosion Correlation between Engine and Laboratory Tests for Group I Oils

Table III. Additives

Group No.	Type of Additive	Additive
I	Detergent	Metal-P_2S_5-amine
II	Detergent	Metal-P_2S_5-hydrocarbon
III	Nondetergent	P_2S_5-olefin
IV	Nondetergent	P_4S_3-reaction products
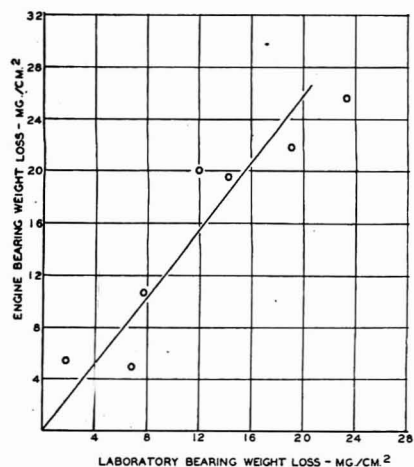
Figure 6. Corrosion Correlation between 72-Hour Engine and 20-Hour Laboratory Test for Group I Oils
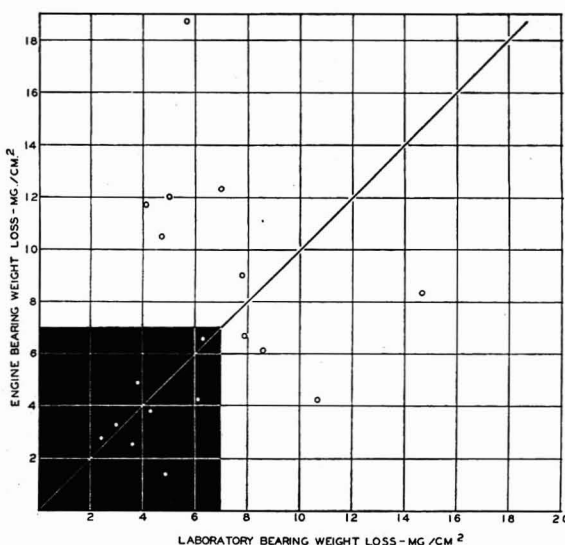
Figure 7. Corrosion Correlation between Engine and Laboratory Tests for Group II Oils
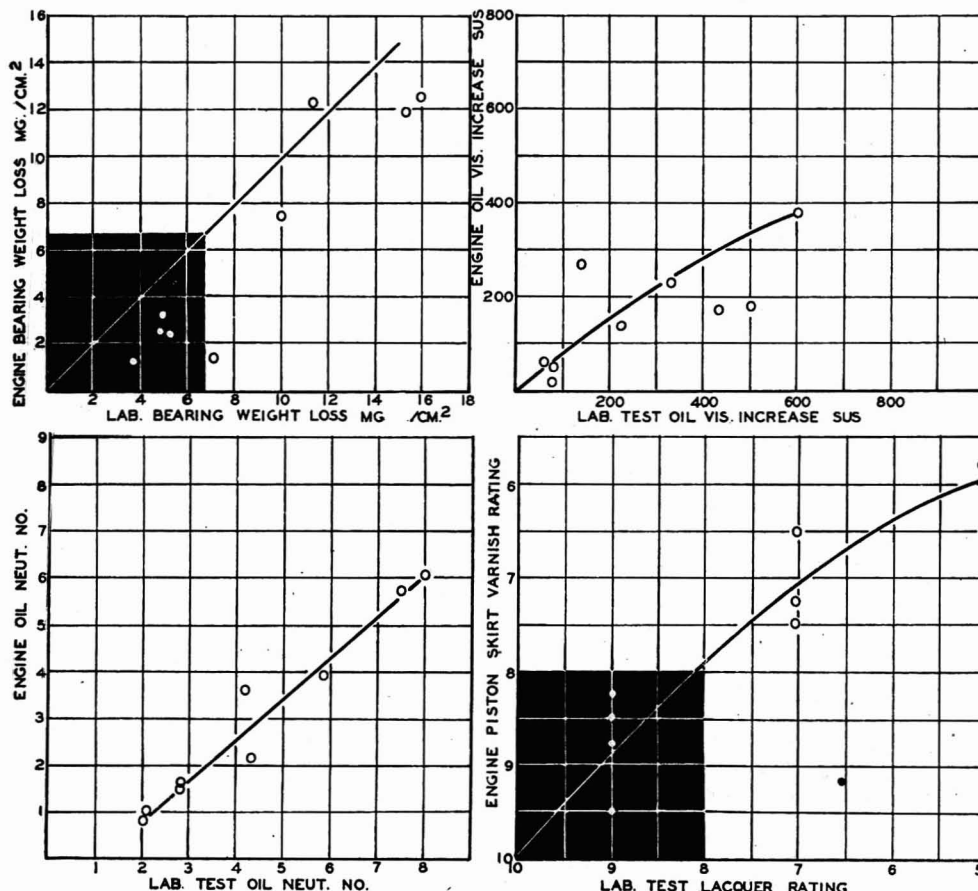
As shown in Figure 8 (upper left) the comparison between laboratory and 36-hour L-4 engine data gave fairly good corrosion correlation. Using the technique described above of dividing the test oils into "satisfactory" and "unsatisfactory" classifications from the engine corrosion viewpoint, it was found that accurate predictions were made by the laboratory test. Of the nine oils tested, four fell well within the shaded noncorrosive region and one was borderline. The remaining four oils were corrosive by both procedures and showed the large varia-

differed in the phosphorus sulfide which was used and in the reactant from which the additive was prepared.

Group III. The first of these nondetergent additives was prepared by the reaction of phosphorus pentasulfide and olefins. Some of the additive modifications and concentrations which were used allowed the oils (Group III) to undergo extensive oxidation and varnish formation. This relatively wide range of results enabled oxidation and varnish correlation in addition to corrosion.

tion in results characteristic of severe corrosion.

On these same oils fairly good correlation of the common used oil properties, neutralization number and viscosity increase, was observed between laboratory test and engine (Figure 8, upper right and lower left). The laboratory test was successful in predicting the approximate extent of oxidation which the oil underwent in the engine test. That the correlation line was nearer the laboratory test axis indicated the laboratory procedure to be slightly more severe than the engine test on these oils.

A third used oil property, sludge content or pentane-insolubles, showed poor correlation. This was due principally to the presence of appreciable amounts of lead compounds in the used engine oils. These lead compounds caused engine oils to show about 1.0% more pentane-insolubles than the corresponding used oil from the laboratory test.

LACQUER CORRELATION. Another correlation which was attempted on this series of oils was the engine piston skirt rating versus the visual estimate of the extent of lacquer formation on the outer surface of the corrosion test unit. A set of colored standards similar to those used in the L-4 method was developed for purposes of comparison. In order to simplify the rating system only four ratings, corresponding to 9, 7, 5, and 3 on the L-4 scale, were used. A photograph illustrating typical tests having these ratings is shown in Figure 9.

The correlation between the laboratory lacquer rating and the L-4 piston skirt

Figure 8. Correlation of Corrosion and Oxidation between Engine and Laboratory Tests for Group III Oils

values on the above nondetergent oils (Figure 8, lower right) indicated good agreement. Four of the test oils which were rated in the range 8.25 to 9.50 by the L-4 procedure gave ratings of 9 in the laboratory test. From the engine viewpoint this group of oils would probably fall in the general classification of satisfactory on this particular property. The satisfactory region again is shown as a shaded area to indicate its significance. Three other oils which were rated in the range 6.5 to 7.5 by the engine gave ratings of 7.0 in the laboratory test. The two remaining test oils were very poor in both the laboratory and the engine tests.
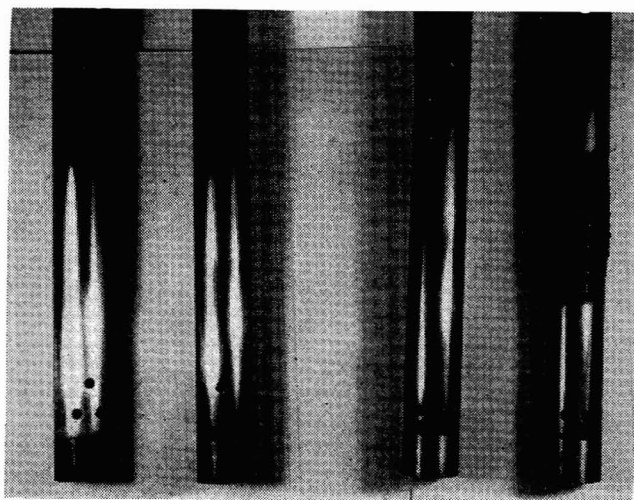
9   7   5   3
Figure 9.   Typical Laboratory Lacquer Ratings

The above data show that the laboratory corrosion test predicted the piston skirt lacquering properties of these test oils. Examples were found with other additives and base oils in which similar agreement was observed. Lacquering in these instances was believed to be due to severe oil oxidation or to temperature-sensitive additives which caused lacquer formation in the hot zones of the engine.

In other examples, with certain additive types, very poor lacquer correlation was observed. In some of these cases it was demonstrated that fuel components or their oxidation products were the dominant factors in lacquer formation in the engine. Recent literature references (1, 5) have pointed out the importance of fuel effects on the formation of engine deposits.

Effective correlation on varnish formation for fuel-sensitive additives or oils would probably have to include a means of simulating the fuel factor.

GROUP IV. In the second study of nondetergent additives a product employing phosphorus sesquisulfide in its preparation was used. As indicated in Figure 10, oils (Group IV) containing modifications of this additive were relatively noncorrosive. Of the 28 oils tested 26 were rated noncorrosive by both the engine and the laboratory test. Of the two remaining oils, one was corrosive and correlated well; the other was rated noncorrosive by the engine test but gave a very high laboratory corrosion result.

STATISTICAL SUMMARY OF CORROSION CORRELATION

In order to show a clearer picture of the corrosion correlations described above, the results are summarized on a statistical basis (Table IV). In this evaluation the data obtained with each additive type are listed separately and then all the results are totaled. The criterion of successful correlation is that described above—i.e., the prediction of the laboratory test as to whether or not an oil was acceptable by engine test results and specifications.
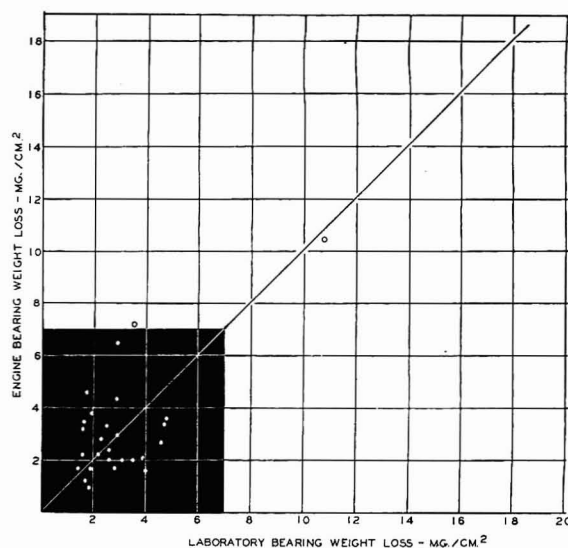
Figure 10.   Corrosion Correlation between Engine and Laboratory Tests for Group IV Oils

A combination of the above results showed that 76 of the 84 oils, or a percentage figure of 90.5, gave a satisfactory correlation. Of the four different additive types investigated, the laboratory test procedure predicted the engine corrosion of over 95% of the oils tested for three of the groups.

USEFULNESS OF TEST

A laboratory bearing corrosion apparatus and test procedure have been developed which give satisfactory correlation with the standard Chevrolet engine test. The apparatus is easy to clean and to assemble. Using this test procedure, twelve samples may be rated for corrosion behavior and oxidation stability in about one fourth the time required to rate one sample in the engine. The laboratory test procedure serves to eliminate the extremely corrosive or mildly corrosive oils and to indicate which oils warrant further testing by the full-scale Chevrolet engine procedure.

In the field of additive development the laboratory corrosion test procedure can be used to study:

The experimental variations in additive composition.
The effect of additive concentration in a chosen base oil.
Additive response in base oils of different types.
The use of corrosion inhibitors in combination with corrosive detergent type additives.

Another important application of the laboratory corrosion test is its use as a quality control procedure for plant batches of commercial motor oil. The test can be used to check the uniformity of different batches of an oil and to ensure that each batch conforms to the specifications listed for that particular oil.

The laboratory test can also be used to study the effect of test variables upon oils and additive-oil combinations. Examples of these are variations in time and temperature and response to lead bromide and soluble iron concentrations.

Table IV.   Statistical Summary of Corrosion Correlation

Additive Type	No. of Oils Tested	Correlation Result No. correct	No. incorrect	% Correct Predictions
Metal-P_2S_5-amine and reference oils	29	29	0	100
KOH-P_2S_5-hydrocarbon	18	11	7	61.2
P_2S_5-olefin	9	9	0	100
P_4S_3-containing	28	27	1	96.5
Total	84	76	8	90.5

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Recording Differential Refractometer

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An improved and simplified recording differential refractometer has been constructed. Its sensitivity is of the same order of magnitude as obtained with an interferometer. The previously used beam-splitting method, as described by Claesson, has been simplified by the use of a twin-cathode phototube. Pen and ink recording is used.

REFRACTIVE index measurements are of great value in following the results of a fractionation process such as a fractional distillation or an adsorption fractionation. They are of particular value in the latter process, where thermometry is not applicable.

Claesson (1, 2) has described an automatically recording differential refractometer for adsorption analysis and with its aid has been successful in applying chromatographic methods to colorless solutes and in putting these methods on a more quantitative basis. His technique involves the deflection of a beam of light as it traverses a diagonal boundary between a solution and the corresponding solvent. The beam of light is split by a hexagonal prism; the two separate beams fall on separate barrier-layer

photocells which are connected in opposition, so that the galvanometer deflection is proportional to the deflection of the beam in the refractometer cell but greatly amplified. Such differential refractometer cells for visual observation have also been described recently by Dutton (4), Debye (3), and Kegeles (5).

The purpose of the present work is to improve or simplify the Claesson apparatus in the following ways: to use pen and ink recording in place of the less convenient photographic recording, to use a simple twin-cathode photocell in place of the more complicated prism and photocell arrangement for splitting the beam, to use a photoemissive type of cell in place of the barrier-layer cell so as to avoid possible fatigue effects, and to increase the sensitivity by using a suitable electronic circuit. The volume or weight of effluent is not recorded in this apparatus.

DESCRIPTION OF APPARATUS

The Refractometer Cell is shown in detail in Figure 1, which is a top view.

The cell is composed of four brass parts 5 × 5 cm. (2 × 2 inches) in cross section, bolted together and holding between them microscope slide cover glasses, C, to serve as end windows and diagonal windows of the cell. The two main blocks of the cell with the diagonal interface have holes A and B drilled through them to form the half cells for solvent and solution, respectively, and for passage of the beam of light from right to left. The opening, B, of 0.125-inch diameter is smaller than A, which has a 3/16 inch diameter, so as to keep the volume of the solution half cell as small as possible and also so that the beam of light after being deflected at the diagonal boundary will not be interrupted by the side of opening A. The length of each half of the cell averages 0.5 inch. The volume of B is 0.10 ml. which is somewhat less than Claesson's volume of 0.23 ml.

Inlets and outlets of 1/16 inch diameter for the solution and solvent are at top and sides, respectively, and so positioned lengthwise of each half cell that the fluid in flowing through the half cell enters at one end and goes out at the other with very little dead space. The solvent for reference is conveniently held stationary in its half cell, and, although the glass separator is not sealed in any way, no difficulty has arisen due to possible leakage of solution into the solvent compartment.

The two end plates, which hold the outer windows in place, are soldered to brass tubes of 1 inch inside diameter which serve as
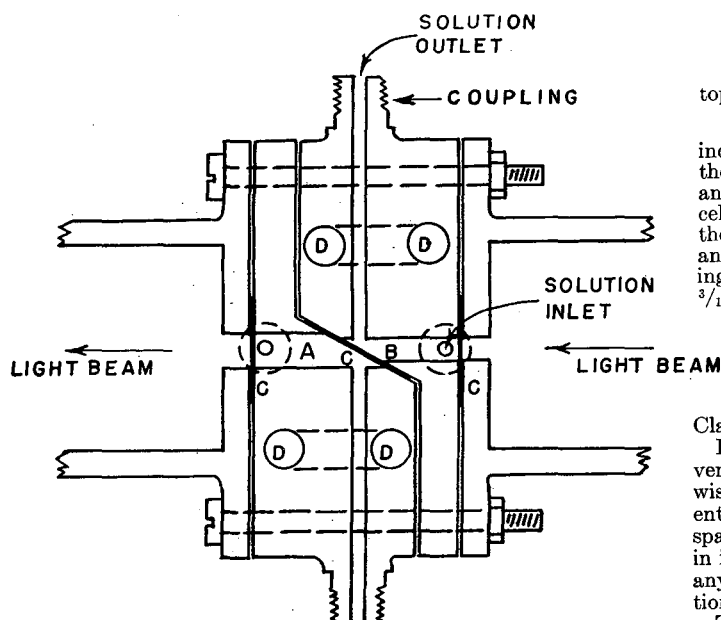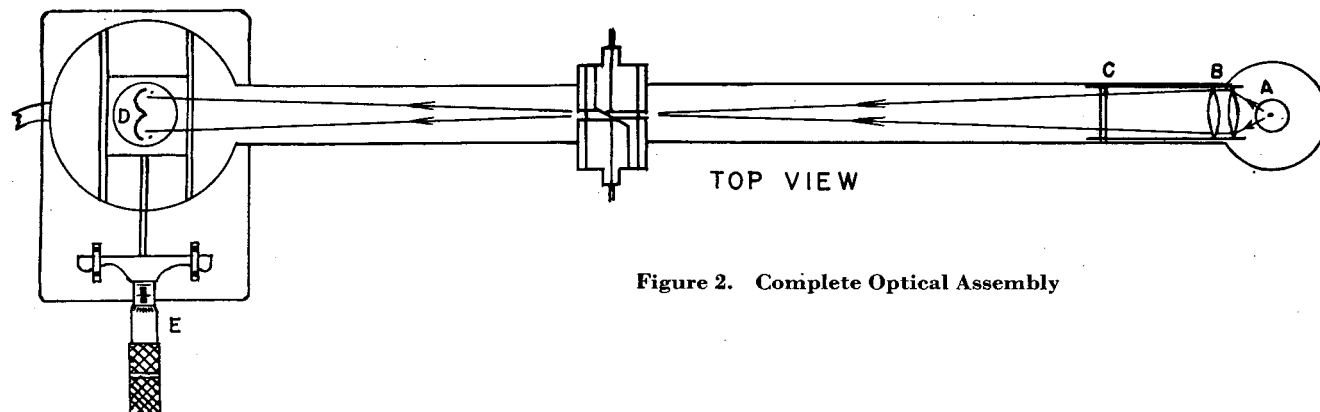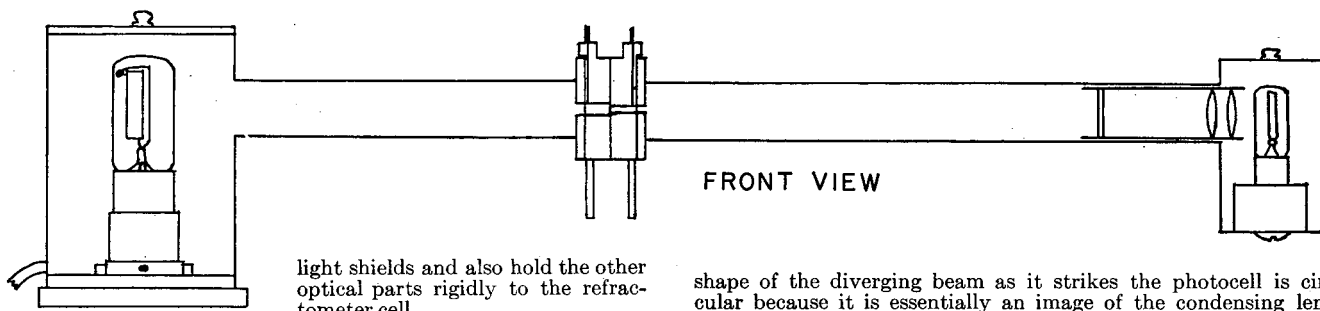

Figure 1. Refractometer Cell

Figure 2. Complete Optical Assembly

TOP VIEW

FRONT VIEW

light shields and also hold the other optical parts rigidly to the refractometer cell.

The diagonal boundary is at an angle of 30° from the axis of the light beam, giving an angle of incidence of 60°. This angle was chosen greater than Claesson's angle of 45° so as to increase the sensitivity which is proportional to the tangent of this angle (5).

The cell is thermostated by passing thermostat water through $3/16$ inch holes, D, drilled in each main half of the cell block. The water entrance and exit are at the bottom of each part and the same water can be circulated through a jacket on the adsorption column or other device with which the refractometer is used.

The Optical System as well as the general layout is shown in Figure 2.

White light from a lamp, A, is focused by the condensing lenses, B, on the entrance to the refractometer cell. A plate of heat-absorbing glass, C, decreases the heating of the solution in the cell. After passage through the cell the light diverges and partially fills the two cathodes of the photocell at D. The deflection of the beam due to the difference in refractive index between the solution and solvent usually will be small enough so that the full beam remains on the photocell cathodes. However, a large deflection can be handled through a lateral motion of the photocell by means of the micrometer screw, E, which may also be used to center the photocell for zero response when the same fluid is in both halves of the refractometer cell.

The Lamp is a 6-watt "exciter" tungsten lamp operating on 6 volts from a storage battery. The filament is tightly coiled, to give a nearly uniform intensity when imaged on the refractometer cell. Because of the relatively large image distance the exact position of the lamp is critical. However, adjustment of the lamp by hand with a screw for clamping in the desired position has been found satisfactory. An opening in the tube near the refractometer entrance and ordinarily covered by a sliding sleeve makes it possible to observe the image when making adjustments of focus and position of lamp. A current of air for cooling is passed through the lamp housing. Monochromatic light might be desirable, but for the applications in mind at present, white light is just as satisfactory; the response of the instrument is dependent on an average refractive index difference for the wave lengths involved.

The Photocell is an R.C.A. No. 920. The use of such a twin-cathode photocell is an important feature in the simplification of the apparatus. Not only does this replace two cells by one but it also eliminates a prism or mirror arrangement for splitting the beam. The

shape of the diverging beam as it strikes the photocell is circular because it is essentially an image of the condensing lens formed by the refractometer cell acting as a pinhole camera. This beam falls on both cathodes with only a slight loss of light due to the approximately $1/16$ inch separation between them. A deflection of the beam due to a change in refractive index causes an increased photocurrent from one cathode and a decreased current from the other. The amount of light lost between the cathodes may change and cause a slight nonlinearity between the response and the refractive index. Nonlinearities may also occur through nonuniformity of the beam.

The apparatus is held together rigidly by the two brass tubes on either side, the distance between centers being 12.75 inches from lamp to refractometer cell and 8.75 inches from the latter to the photocell. Despite the great sensitivity, no special precau-
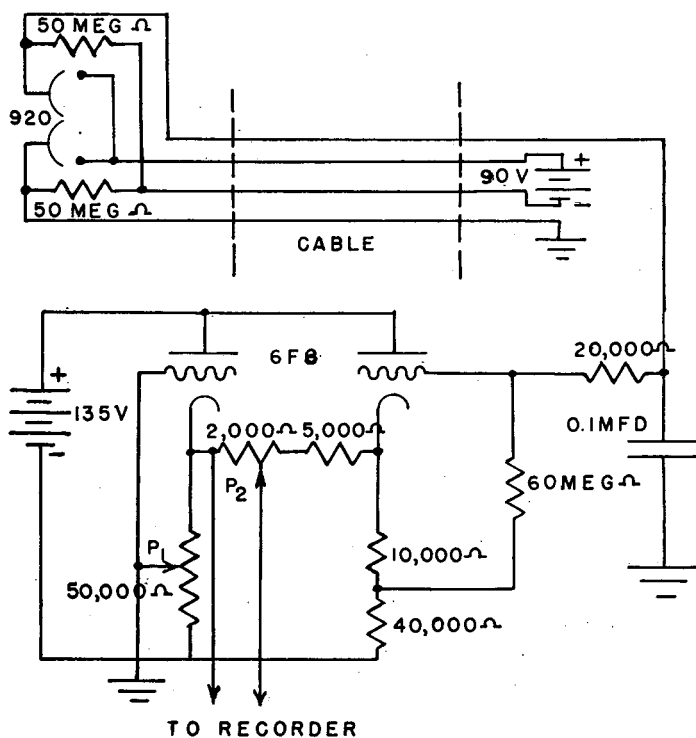

Figure 3. Electronic Circuit

tions to avoid strain are needed, and the apparatus is clamped by Bunsen clamps to an ordinary frame support.

The Electrical Circuit is shown in Figure 3. The two halves of the photocell are connected in a bridge circuit with 50-megohm resistors mounted in the photocell compartment. The differential output is applied to one grid of 6F8 twin triode, and the other triode grid is held at constant potential as determined by the setting of the potential divider, P_1. The differential output of the two triodes is tapped by the potential divider, P_2, and this voltage is applied to a Brown strip-chart potentiometric recorder of 5-mv. range. The vacuum tube is used here to match the high impedance of the photocell to the low impedance of the recorder. Batteries are used to supply all voltages so as to be as free as possible from fluctuations. P_1 is useful in changing the zero setting on the recorder while P_2 varies the sensitivity.

OPERATION AND TYPICAL RESULTS

The sensitivity is limited by the instability of the apparatus. The recorder fluctuates by an amount which corresponds to about 2×10^{-6} in refractive index change. A drift also takes place during the warm-up period of 0.5 to 1 hour.

The behavior of the apparatus was tested by introducing solutions of known refractive index and observing the response of the recorder. The effect of shifting the position of the photocell by a given amount was observed and compared with that to be expected on the basis of the optical theory (5). The theory predicts that for small deviations of the light beam, θ, the angle in radians is

$$\theta = \Delta n \tan \alpha$$

where Δn is the change in refractive index and α is the angle of incidence of the beam of light on the diagonal separator.
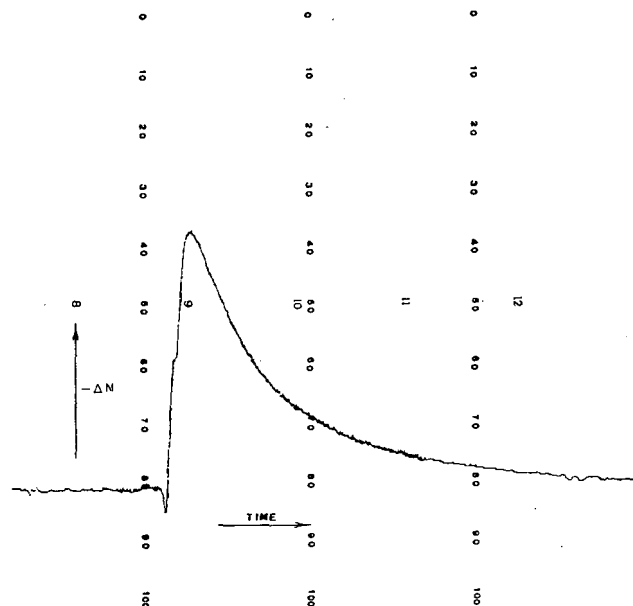
Figure 4. Elution Curve

As an example, a solution of ethanol in carbon tetrachloride 1.00% by volume and differing in refractive index for sodium light from pure carbon tetrachloride by 1.01×10^{-3} was found at a particular recorder sensitivity setting to give a recorder deflection of 111 recorder units. This amounted to 0.91×10^{-5} change in refractive index per recorder unit. It was found that shifting the photocell 0.010 inch caused the recorder to move 68.5 units. This shift amounts to a θ of 1.14×10^{-3} radian and according to the equation Δn then would be $\theta/\tan 60°$ or 6.6×10^{-4}. This would be a sensitivity of 0.96×10^{-5} expressed as Δn per recorder unit as compared with 0.91×10^{-5} found above. The agreement is all that could be expected, considering nonlinearities in response and the use of white light with a different average refractive index.

In actual use the recorder response must be calibrated against known solutions to obtain quantitative results. Such a calibra-

tion is made directly in terms of the relation between recorder response and concentration of solutions being used and so avoids the problem of just exactly what kind of an average refractive index is involved.

Because the minimum detectable Δn is about 2×10^{-6}, the greatest sensitivity that could be used for 1% accuracy of a full-scale deflection would be 2×10^{-6} per recorder unit or 2×10^{-4} for full scale deflection, as there are 100 divisions across the chart. The minimum sensitivity is determined by the size of the spot of light in relation to the photocell cathodes. This results in a Δn of about 1×10^{-2} for full scale deflection.

The minimum detectable refractive index change of 2×10^{-6} is about one fifth as great as with Claesson's recording differential refractometer and about twice as great as with Tiselius and Claesson's (1, 7) nonrecording interferometric method.

Figures 4 and 5 show typical recorder tracings obtained in connection with adsorption columns.
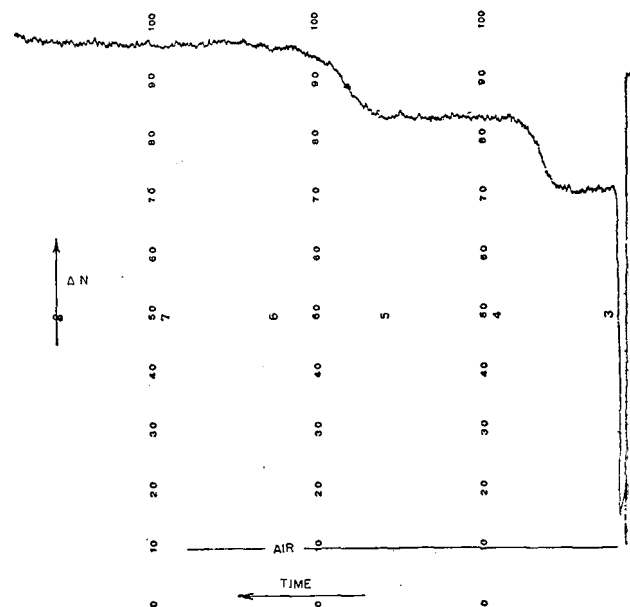

Figure 5. Frontal Curve

Figure 4 is for a 0.5-ml. sample of a 10% ethanol in carbon tetrachloride solution adsorbed on an alumina column and then eluted with carbon tetrachloride. Full scale deflection of 0.91×10^{-3} for Δn corresponded to 0.90% ethanol and the wave as it came through had a peak concentration of 0.40%. Figure 5 shows how the differential refractometer can be used in the study of gas mixtures. A sample of natural gas as distributed in gas mains was slowly passed through a bed of charcoal previously saturated with air. The successive steps in the curve are typical of the "frontal analysis" method. The full scale deflection was 2.4×10^{-4} for Δn.

ACKNOWLEDGMENT

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High-Temperature Gas Burners

For Microcombustion Methods of Ultimate Analysis

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Coil and ring gas burners are described which fulfill the requirements of the thermal decomposition method for the direct determination of oxygen in organic compounds. The coil burner is designed for pyrolyzing the sample at 900° to 1000° C.; however, it is capable of producing a temperature of 1300° to 1350° C. inside the reaction tube. The ring burner is designed to provide a temperature of 1100° C. near the end of the furnace used for conversion of the pyrolysis products to carbon monoxide. These gas burners have also been found useful in other microcombustion methods of ultimate analysis. The burners, which utilize a mixture of propane gas and air, are inexpensive, heat and cool the reaction tube rapidly, and provide a uniform distribution of heat around the tube.

IN THE thermal decomposition method for the direct determination of oxygen in organic compounds, first proposed by Schütze (2), the substance is pyrolyzed in a clear quartz tube in a stream of pure nitrogen at a temperature of 900° to 1000° C. In a recent investigation of this method (1) a Fisher high-temperature burner was used to provide this temperature. In order to maintain this temperature uniformly around the tube the flame was confined by mounting a U-shaped Nichrome wire gauze hood over the top of the burner, in the manner described by Schütze and Unterzaucher (2, 3). However, even with a hood over the burner, a considerable amount of heat was wasted and the temperature of the heated portion of the reaction tube was not uniform.

In the course of an investigation for a more efficient device for pyrolyzing the substance, electric combustion furnaces were considered. Electric furnaces wound with base metal resistance wire ordinarily do not have a long life when operated continuously at 900° to 1000° C., and furnaces wound with noble metal resistance wire are expensive, especially as the type needed for microcombustion methods is not available as a stock item. In view of these factors, attention was directed to more efficient gas burners.

After the difficulties of preliminary models had been overcome, two satisfactory high-temperature gas burners were designed by the author and made in this laboratory: coil and ring burners. Each has a specific use. The burners utilize a mixture of propane gas and air, are inexpensive, heat and cool the reaction tube rapidly, and can be made in various lengths and coil diameters.

Although originally designed for use in the thermal decomposition method for the direct determination of oxygen in organic compounds (1), these burners have been found very useful in other microcombustion methods of ultimate analysis, such as the determination of carbon and hydrogen and Dumas nitrogen.

RING BURNER

In the direct determination of oxygen by the thermal decomposition method it is necessary to have a sufficiently high temperature to ensure complete removal of all oxygen from the pyrolysis products. Because of the thick wall insulation necessary in a high-temperature electric furnace, a zone of lower temperature always exists in the reaction tube in this insulated area. This low-temperature zone introduces the problem of the condensation of oxygen-containing pyrolysis products, which are difficult to remove and necessitate special provision for lateral movement of the furnace (1).

This objectionable feature was overcome by installing a ring burner adjacent to the wall of the furnace, as shown in Figure 1. In making this burner, holes, 0.05 to 0.06 inch in diameter and 0.25 inch apart, are drilled in a straight line through one wall of a suitable length of seamless nickel tubing 0.25 inch in diameter. The drilled section is then bent into a ring so that the holes in the ring are directed away from the center of an angle of 45°, in order to direct the flame of the burner toward the interior of the furnace.

To provide maximum conductance of heat and at the same time to protect the reaction tube, a sheet of platinum rolled into cylindrical shape, about 1.5 inches long, is slid over the reaction tube to a point where it projects about 0.5 inch from the furnace
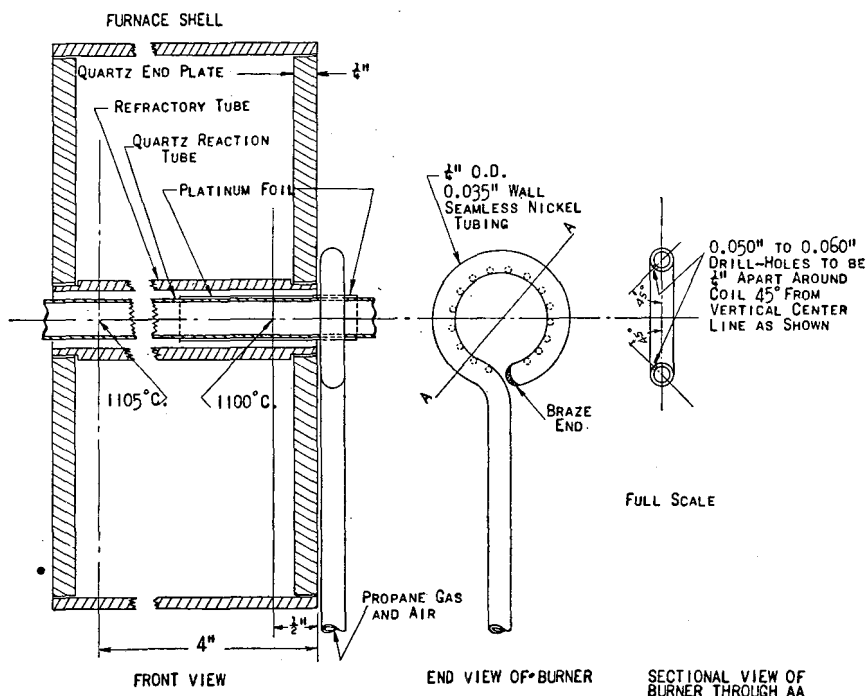

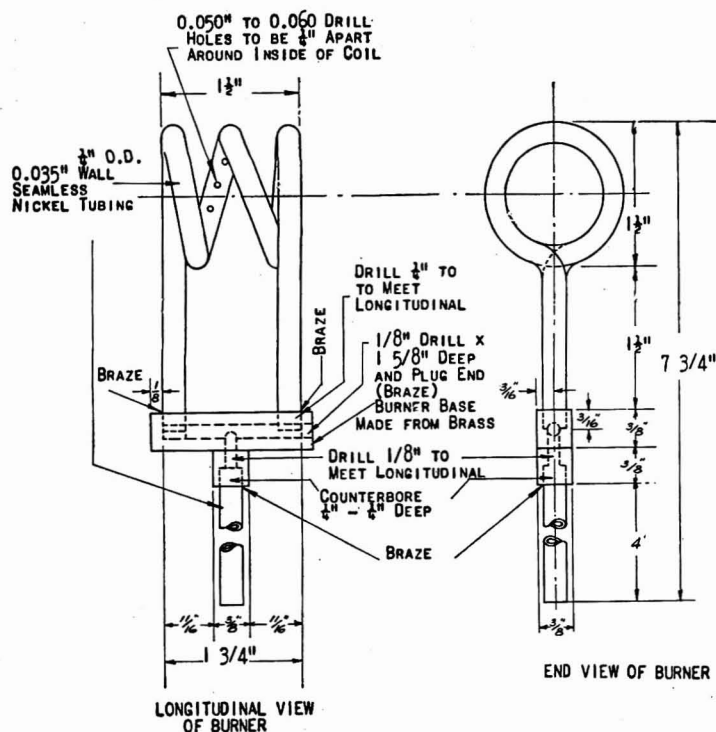**Figure 1. Sectional View of Furnace with Ring Burner in Position**

Figure 2. High-Temperature Coil Gas Burner

wall. Tests showed that prior to the installation of this burner, the temperature in the reaction tube at a point 0.5 inch inside the furnace (Figure 1) was only 650° to 700° C.; after installation of the burner the temperature was raised to 1100° C.

COIL BURNER

The essential features of construction of the multiple-coil burner are shown in Figure 2.

In making this burner, holes, 0.05 to 0.06 inch in diameter and approximately 0.25 inch apart, are drilled in a straight line through one wall of a suitable length of seamless nickel tubing 0.25-inch in diameter. (Gases other than propane will require holes of different diameter.) The drilled section is bent into a three-spiral coil approximately 1.5 inches in diameter. The open ends of the coil are then inserted into holes drilled in the horizontal arm of a T-shaped brass base and brazed. A second suitable length of the same nickel tubing, to provide a gas feed, is
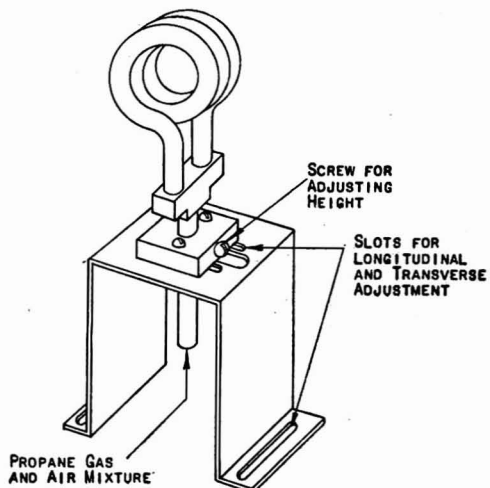
inserted into a hole drilled in the bottom of the vertical arm of the T-shaped brass base, and also brazed. Iron pipe or any other metal tubing may be used instead of nickel tubing for the gas feed. The drilled T-shaped brass base supplies gas-air mixture to both ends of the coil, thereby providing a uniform flame. One method of mounting this burner to allow for close adjustment around the reaction tube is shown in Figure 3.

In order to obtain maximum efficiency with this burner, the ends of the coil are closed by two silica (Vitreosil) disks, having dimensions shown in Figure 4. A roll of Nichrome wire gauze, approximately 0.625 inch in diameter, is inserted in the coil, through the holes in the silica disks, and the ends of the gauze are flared to hold it in place. A larger roll of gauze is placed around the outside of the coil and the edges are bent down over the silica disks (Figure 4). In addition to holding the silica disks in place, both rolls of gauze aid in confining the heat to that section of the reaction tube covered by the burner.

Operation of the burner is simple. After the gas is turned on by means of a needle-type valve and lighting, the air is turned on until a nonluminous flame is produced. The intensity of the flame can be varied as in any burner by regulating the gas and air supply. At the completion of the combustion the gas is shut off and the air flow is increased to cool the reaction tube rapidly. In this way the waiting period before the introduction of the next sample is greatly reduced.

In laboratory tests the coil burner produced a maximum temperature of 1300° to 1350° C., as measured inside the reaction tube, using a mixture of propane gas and air. These burners have been in daily use at 900° to 1000° C. for one year and are still in excellent condition.

No tests were made in this laboratory to determine the maximum number of spirals that could be effectively incorporated into a coil burner of this type.

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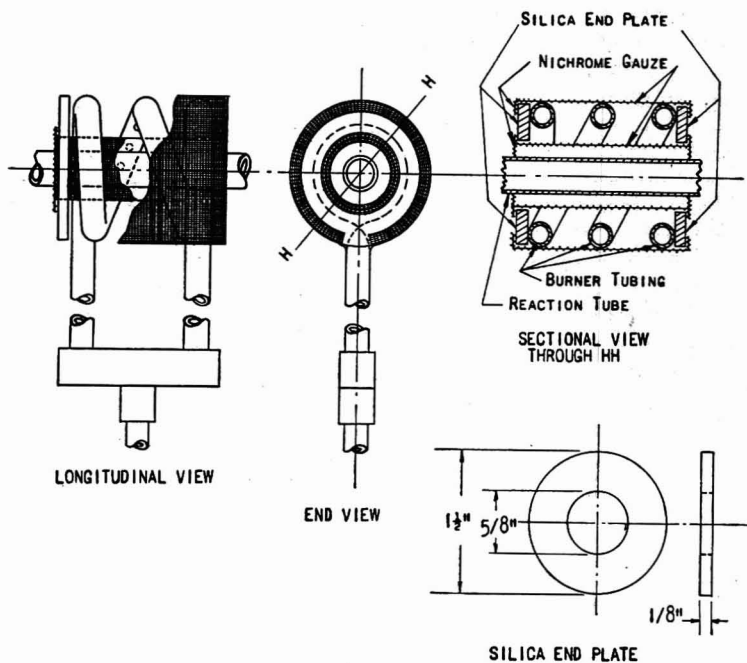
Figure 3. High-Temperature Coil Gas Burner with Mounting

Figure 4. High-Temperature Coil Gas Burner Shown in Assembly

Apparatus for Mincing Biological Materials

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FREQUENTLY in laboratory and other work there is need for a mechanical device that will mince or subdivide moist or fresh biological material into a form suitable for subsequent exhaustion or other extraction of desired ingredients. The Waring Blendor and similar machines serve this purpose admirably, but in general are limited, except for micro work, by their small volume, and by the undesirable incorporation of gasket materials and lubricants in their design when one wishes to employ nonaqueous solvents. It would seem to be a definite advantage to be able to mince the material in the presence of the extracting solvent (1).

Although Davis (3) mentions what is apparently the first laboratory application of the Waring Blendor, Benne (1) extended the idea and reported the preparation and use of three different kinds of small blending vessels which possessed advantages over the commercial containers for certain purposes. In addition, Benne (1) summarized very well the limitations of existing typical laboratory practices for reducing plant material to pieces of small size—e.g., food chopper, hand shears, grinding with sand, meat grinder, scissors, and vegetable shredder. Subsequently, Comar *et al.* (2) presented an adaptation of the Waring Blendor for preparing emulsions by a continuous process.

Figure 1. Mincing Apparatus Mounted in Back-Draft Hood

The necessity for mincing thousands of 0.9- to 1.8-kg. (2- to 4-pound) samples of fruits, vegetables, and other plant tissues in insecticide residue studies led to the development and routine application of the device described herein which circumvents satisfactorily the above limiting conditions. Further adaptation of this mincer is that it may be used directly and safely with flammable solvents as the fluid media.

APPARATUS

Mincer. A standard-make, 35-cm. (14-inch), bench drill press was mounted in front of a 0.25-hp. explosion-proof electric motor fitted with a 16-inch cloverleaf fan, with outside exhaust. In order to increase draft efficiency, a wooden hood was constructed around the drill press. Another 0.25-hp. explosion-proof electric motor with a vertical thrust bearing was mounted on the drill press, with V-belt and suitable pulleys so as to afford about 4000 r.p.m. at the chuck. The actual mincing blade was constructed of 0.5-inch stainless steel rod with 20-gage stainless steel splash guard and cloverleaf cutter welded on, and balanced in a high speed lathe. After being filed to near-razor sharpness, the cutting blades were bent uniformly so as to thrust down (Figure 1).
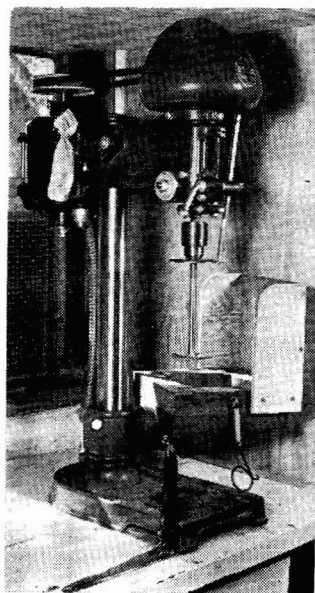
Containers. Glass containers were not deemed suitable for the mincing operations from safety considerations. Instead, the 20-gage stainless steel cans (sample in Figure 2) were utilized. These cans were of outside-welded construction, and were 9.5 inches high and 5.625 inches square with 1-inch bevels on all corners. The reinforced neck openings were 4 inches in diameter. Because the author's operations subsequent to the mincing operation in benzene involved sealing and rapid end-over-end tumbling for 30 or more minutes, the cans shown were fitted with cork shock gaskets and 0.125-inch thick iron lids that could be bolted to six lugs projecting from the reinforced neck of each can. A double layer of No. 300MST cellophane served admirably as the actual contact gasket for most of the organic solvents encountered.

It is important that all seams in such cans be outside welded, for many organic solvents—such as benzene and dioxane—will slowly attack silver-soldered unions. A cradle to hold one of these cans was mounted on the swivel table of the drill press, with strong spring clamps to hold the can firmly seated while mincing operations were carried out (Figure 1).

Switch. A treadle-type, foot-control switch, made explosion-proof with rubber gaskets, was fastened to the floor under the drill press assembly so that the hands of the operator would be free at all times, and, even more important, so that the machine could not be left running while unattended. This device must not be entrusted to the hands of careless or unwary persons, but must be regarded with the same wholesome respect that one accords a buzz saw.

OPERATION

The can containing the material to be minced plus an appropriate quantity of the desired solvent was inserted into the cradle, swung into position under the cutting blades, and clamped tightly into place. After being centered, the swivel table was also locked firmly in place by means of the vice clamp provided by the manufacturer of the drill press. While the treadle switch was pumped slowly, the cutting blades were eased gently down through the mixture to maximum depth whereupon the motor was allowed to attain full speed. Subsequent operations are obvious, with the eventual particle size being in large part dependent upon the duration of mincing.

Figure 2. Stainless Steel Container for Mincing Operations

Efficiency of Mincing. Ordinarily, a 0.125-inch clearance between the inside bottom of the can and the lower edges of the cutting blades affords good mincing in reasonable time. Figure 3

Figure 3. Navel Orange Peel before and after a 1-Minute Treatment on Mincing Apparatus

shows the "before and after" aspects of 2 pounds of fresh mature navel orange peel segments subjected to a 1-minute treatment in 1800 ml. of benzene, then filtered. It was found empirically that under the author's conditions 2 ml. of solvent per gram of fresh material sufficed for most plant parts—e.g., alfalfa, bark,

citrus fruits, corn on the cob, deciduous fruits, grapes, melons, olives, string beans, sugar-beet leaves, twigs, whole walnuts, etc.

OTHER APPLICATIONS

There are many potential uses for this apparatus. With minor changes in the design of the cutter assembly and of the stainless steel containers, it can readily be adapted for blending, chopping, cutting, emulsifying, and shredding of almost any material in the presence of almost any solvent.

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Trap for Determination of Water by the Distillation Method

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THE types of traps commercially available for the determination of water by distillation of samples with an immiscible liquid of low density, such as toluene, are in some respects unsatisfactory. With a trap such as that of Bidwell and Sterling (2), having a graduated portion with a bore sufficiently small to obtain reasonably precise readings of small volumes, sharp separation of the immiscible liquid and the water often does not occur even when the trap has been carefully cleaned. Droplets

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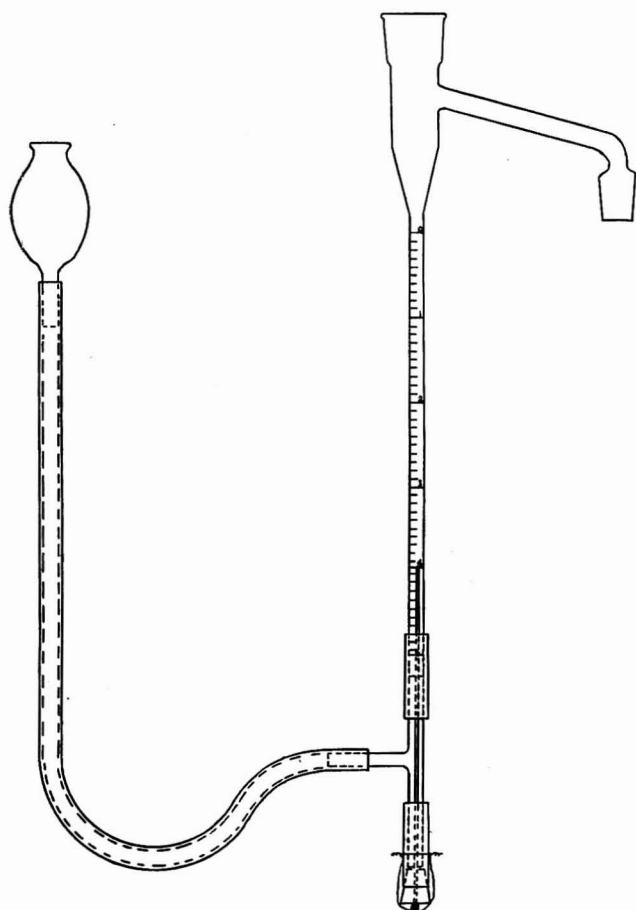
Figure 1. Improved Trap for Collecting and Measuring Water

of water may adhere to the glass and fail to coalesce, or alternate slugs of immiscible liquid and water fill the graduated tube so that sharp separation must be brought about by some mechanical means. Sharp separation usually occurs in traps that have graduated tubes of larger bore (3), but this involves a sacrifice of precision in reading the volume of water.

A trap of the design shown in Figure 1 combines the advantages of both types of traps. The lower portion of the graduated part is open and is attached to a rubber tube connected to a leveling bottle or funnel containing mercury. In operation the level of the mercury is brought up into the wider part of the trap while the water is distilling over. As soon as all the water has distilled over and sharp separation has occurred, the mercury level is lowered so as to draw the water down into the graduated part for measurement. The water is almost invariably drawn down at once into the narrow graduated tube sharply and cleanly, but if this does not occur because the tube is not clean, two or three raisings, or lowerings of the mercury level will by mechanical action bring about a sharp separation.

This principle of operation is not entirely new; the same idea was employed by Beckel, Sharp, and Milner (1) in an apparatus for the determination of water by distillation. However, these workers do not appear to have realized the full potentialities of this principle of operation, nor did they test it critically, or mention the existence of one difficulty connected with its practical application.

By means of this principle of operation, traps with graduated tubes of considerably smaller bore may be used than is possible otherwise. It was found possible to draw down collected water from a wide tube into a tube having a bore of only 1 mm. However, the use of a graduated tube of such small diameter is not very satisfactory for the separation of water and an immiscible liquid such as toluene over a mercury surface. In spite of the fact that water dropping onto a mercury surface covered with toluene appears to displace the toluene completely, so that only water remains in contact with the mercury, a very thin film of toluene adheres tenaciously to the mercury surface. If the mercury level is then lowered so as to bring the water layer completely into the narrow tube, the film of toluene contracts as the surface area of the mercury decreases, and it finally forms a droplet of varying size at the water-mercury interface. This causes an uncertainty in reading the volume of confined water.

If the tube is not too small in diameter this droplet may be displaced from the mercury surface and made to rise into the toluene layer by means of the device shown at the bottom of the graduated tube in Figure 1: a thin rod of glass or stainless steel centrally placed in the lower part of the graduated tube, and mounted

Table I. Apparent Change in Volume of Given Quantity of Water Passed Five Times from Upper Part to Graduated Part of Trap

Initial Reading	Intermediate Readings		Final Reading	Difference between Initial and Final Reading	Greatest Observed Difference
	Highest	Lowest			
Ml.	Ml.	Ml.	Ml.	Ml.	Ml.
0.20	0.21	0.20	0.20	0.00	0.01
0.44	0.43	0.42	0.42	0.02	0.02
0.95	0.94	0.93	0.94	0.01	0.02
1.91	1.91	1.91	1.91	0.00	0.00
2.89	2.91	2.89	2.89	0.00	0.02
3.88	3.88	3.88	3.88	0.00	0.00

Table II. Agreement of Duplicate Determinations

Water Taken	Tube Reading	Correction	Water Recovered	Difference between Duplicates
Ml.	Ml.	Ml.	Ml.	Ml.
2.00	1.91	0.09	2.00	..
2.00	1.90	0.09	1.99	0.01
3.99	3.90	0.11	4.01	..
3.99	3.88	0.11	3.99	0.02

Table III. Determination of Water in Hydrated Barium Chloride

(Determined gravimetrically and by distillation)

Samples taken, g.	1.5301	6.4955	13.72	17.55
Tube readings, ml.	.....	.....	1.91	2.48
Corrected volumes of water, ml.	.....	.....	2.00	2.58
Derived weights of water, g.	.....	.....	1.99	2.57
Loss of weight on heating, g.	0.2232	0.9490	...	...
Water found, %	14.59	14.61	14.5	14.6
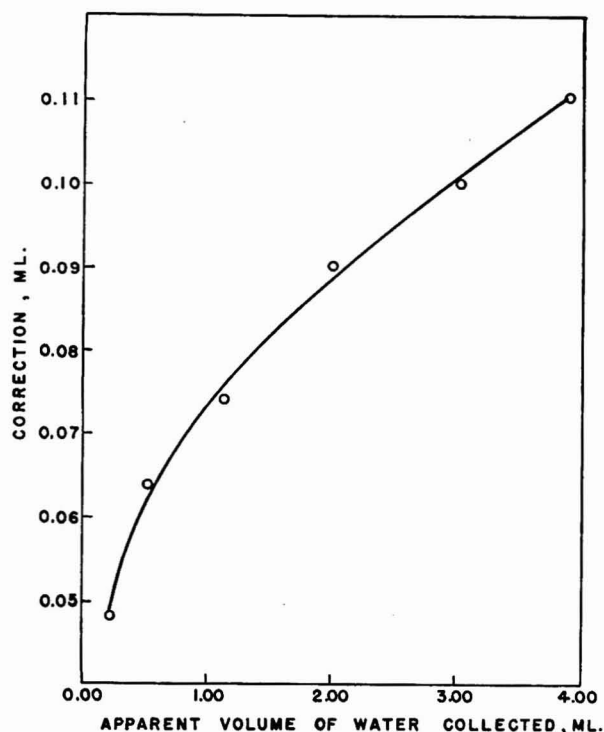
Figure 2. Calibration Graph

with rubber tubing so that its flat tip may be displaced horizontally. When the mercury with such a droplet on its surface is lowered past this obstruction it is knocked off at once and rises to the top, or a slight adjustment of the position of the top of the rod will cause its removal when the mercury is again lowered. There is also a lower limit to the volume of water that may be successfully drawn down from a wide tube into a narrow tube. If the water is so small in volume that it does not form an intact layer be-

tween the toluene layer and the mercury surface in the wide tube, the toluene tends to be drawn past the water when the mercury is lowered into the narrow tube. Because of this difficulty the volume of water collected should not be less than about 0.20 ml.

The trap used for the test experiments described here had as its basis a 5-ml. Pyrex measuring pipet with an internal bore of 5.5 mm. As shown in Figure 1, it is important for successful operation that the connection between the wide tube and the narrow graduated tube be gradually tapered. The capacity of that part of the trap between the inlet tube and the top of the graduated tube should be at least twice and preferably three times that of the graduated tube itself. In calibrating traps of this type for accurate work it is not sufficient to determine the capacity by volumetric calibration with water. In actual operation the volume of the collected water is most conveniently read between the bottom of a water meniscus at the toluene-water interface and the top of a mercury meniscus at the water-mercury interface, thus involving a considerable meniscus error if the usual method of calibration is taken as the basis. Moreover, in a given apparatus there is a slight but appreciable loss of water due to imperfect condensation or to loss at the ground-glass joints that increases with increase in the amount of water distilled over. It is far better, therefore, to calibrate the graduated tube on the basis of actual runs in which accurately measured or weighed portions of water are distilled in the apparatus and compared with the apparent volumes read in the trap. The data thus obtained are used to construct a convenient calibration or correction graph (Figure 2) for the apparatus used for the experiments here reported.

The results in Table I indicate that no appreciable losses are caused by repeated transfer of the collected water from the upper to the lower part of the trap. Table II shows the agreement obtained in duplicate runs, in which the stated volumes of water were measured into the distilling flask with a calibrated pipet. The small differences found in both these sets of experiments probably represent observational error in reading volumes rather than error of method. Table III shows the outcome of test determinations on a uniform lot of hydrated barium chloride. The water content found by distillation agrees well with the accurate gravimetric results found by loss on oven drying.

It is inconvenient and unnecessary to dismantle traps of the type described here after each determination for treatment with cleaning solution, as is the common practice with the usual type of trap.

It is sufficient first to disconnect the distilling flask, place a small beaker under the inlet tube, and raise the mercury so as to expel nearly all the water and toluene or other immiscible solvent. Any residual toluene may be largely removed by flushing out with distilled water. Grease-free acetone is used to flush out the water without changing the level of the mercury. Then the mercury is lowered to the bottom of the graduated tube and this is filled with acetone. By alternately raising and lowering the mercury from the bottom to the top of the graduated tube three or four times the walls are freed of water and thoroughly cleaned. The mercury is then raised so as to expel most of the acetone into the collecting beaker. Finally, the residual acetone is allowed to evaporate spontaneously, or the inlet tube is stoppered and suction is applied to the top of the trap so as to evaporate the acetone rapidly. Any mercury accidentally spilled in the cleaning operation will be caught in the beaker and may be returned to the leveling apparatus.

This method of cleaning lends itself well to the operation of a series of setups for the determination of water by the distillation method.

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Direct Titration of cis-Glycols with Lead Tetraacetate

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THE rapid oxidative cleavage by lead tetraacetate of cis-glycol groups located on five-membered rings has been known from the work of Criegee (*2, 3*) and Hockett and co-workers (*6, 7*). It has now been found that this reaction is sufficiently rapid to allow direct titration of such groups. The titration can be followed potentiometrically with lead and platinum electrodes.

The reactivity with lead tetraacetate of true cis-glycols is so much greater than that of the other types of glycols that the end point of the titration is not appreciably displaced by the presence of the other glycols. Aliphatic glycols, trans-glycols on five-membered rings, and cis- and trans-glycols on ordinary pyranoside rings have failed to interfere with the titration. In the titration of methyl α-D-mannofuranoside (Figure 1, *A*), the end point corresponded to the addition of 1 mole of lead tetraacetate, although the glycoside contained an aliphatic glycol in addition
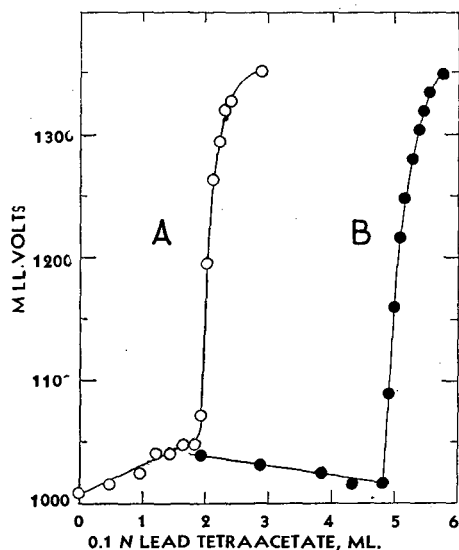

Figure 1. Potentiometric Titration of cis-Glycols

Lead electrode negative with respect to platinum
A. Methyl α-D-mannofuranoside (0.1 mmole) in 25% acetic acid solution *B.* Erythritol anhydride (0.25 mmole cis-glycol) and 1,6-dibenzoyl-2,5-anhydrosorbitol (0.27 mmole trans-glycol) in 50% acetic acid solution

to the cis-glycol on the five-membered ring. Erythritol anhydride (cis-3,4-dihydroxytetrahydrofurane) and 1,4-anhydromannitol also each consumed exactly 1 mole of lead tetraacetate in the titrations. In separate experiments it was found that ethylene glycol, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, or 1,6-dibenzoyl-2,5-anhydrosorbitol did not interfere with the titration of erythritol anhydride (Table I and Figure 1, *B*). Reducing sugars, on the other hand, do displace the end point, presumably because of their ability to form furanose rings containing cis-glycols.

It now appears that cis-hydroxyl groups on a pyranoside ring can also be titrated directly with lead tetraacetate, provided they are oriented in the true cis position, a condition requiring an unusual "boat" conformation of the ring instead of the ordinary trans or "chair" form. Thus the α-glycol at position 3,4- of methyl 2,6-anhydro-α-D-altropyranoside (*8*) can be titrated, whereas in methyl α-D-altropyranoside the same group cannot be so titrated.

EXPERIMENTAL

Titrations were carried out on 0.1 to 0.25 millimole of cis-glycol in 20 to 30 ml. of purified acetic acid or acetic acid–water solution. The lead tetraacetate reagent was prepared with purified acetic acid and standardized against thiosulfate in the manner described by Hockett, Dienes, and Ramsden (*5*). The lead electrode was cleaned before each titration in 0.5 N nitric acid, and rinsed with distilled water. A titrimeter of the type described by Buras and Reid (*1*) was employed after being calibrated in millivolts with a potentiometer. The lead tetraacetate reagent may be added rapidly at the beginning, but as the end point is approached should be added at approximately 0.2 to 0.4 ml. per minute.

Although there was no doubt about the position of the end point, the observed potentials were not closely reproducible from one titration to another. They were affected by the rate of stirring when pure acetic acid was the solvent; and the initial values depended, apparently, upon the condition of the lead electrode. The potentials immediately following the end point may be unstable because of secondary reactions.

The erythritol anhydride was prepared by refluxing inactive erythritol with 50% sulfuric acid by the method of Henninger (*4*). It was a colorless sirup which distilled at 120° (bath temperature) at 0.8 mm. and gave correct carbon and hydrogen analyses. The physical properties of the other substances employed were in close agreement with those recorded in the literature.

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Table I. Potentiometric Titration of cis-Glycols with Lead Tetraacetate, with and without Added Substances

cis-Glycol Name	Mg.	Added Substance Name	Mg.	Solvent	Moles of Reagent per Mole of cis-Glycol[a]
Erythritol anhydride	26	None		HOAc	0.98
Erythritol anhydride	26	Methyl α-glucoside	194	HOAc	1.01
Erythritol anhydride	26	Ethylene glycol	1100	25% HOAc	1.02
Erythritol anhydride	26	Methyl α-D-mannopyranoside	100	25% HOAc	1.01
Erythritol anhydride	26	1,6-Dibenzoyl-2,5-anhydrosorbitol	100	50% HOAc	1.00
Methyl α-D-mannofuranoside	19.5	None	..	25% HOAc	1.00
1,4-Anhydromannitol	16.8	None	..	HOAc	1.03
Methyl 2,6-anhydro-α-D-altropyranoside	13.3	None	..	50% HOAc	1.03

[a] Values determined graphically from plot of Δ mv./Δ ml. versus ml. added.

Method for Complete Deoxygenation of Water

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THE methods ordinarily employed for removal of oxygen from water, such as boiling, agitation under reduced pressure, or bubbling of an inert gas through the water to be freed of oxygen, are not capable of complete oxygen removal. Certain other purely physical methods give what is probably complete oxygen removal but are cumbersome and time-consuming.

Taylor (*1*) obtained very complete oxygen removal by fractional distillation under vacuum. His apparatus required, however, a week's time for the production of a small quantity of oxygen-free water.

On the other hand, the convenient chemical methods, although giving sufficiently complete oxygen removal, invariably leave the water contaminated with either the reagents added or the products of the reaction or both, so that a distillation is always required.

In the method described here a combination of physical and chemical methods is used; both reactants and oxidation products are insoluble in water. A number of chemical substances, such as finely divided ferrous or manganous hydroxides, fulfill this solubility requirement, but these have practical disadvantages. They are readily acted upon by the air, and even if introduced into the water to be treated while still in an active condition, would have their surfaces quickly oxidized, and further access of oxygen to the interior of the solid particle would be greatly retarded.

Both these difficulties are overcome by using as the oxidizable material a suitable substance in liquid form. This allows a minimum of surface to be exposed when the material is handled in air, and yet by agitation a large and continuously renewed surface could be presented to the water, or solution, to be deoxygenated.

In the first promising experiments amalgams of cadmium and tin were shaken vigorously with water saturated with air at room temperature. Turbidity is observed almost immediately, denoting a reaction between the metal and the dissolved oxygen. There are, however, several objections to the use of amalgams. First, the active deoxygenating metal (here tin or cadmium) may be amalgamated only to the extent of a few weight per cent before the amalgam begins to become viscous and difficult to agitate properly. Secondly, the reaction at room temperature does not go quickly enough to completion. Finally, under these conditions a small amount of hydrogen peroxide may be formed.

A better choice for a deoxygenating material was found to be Wood's metal alloy (50% bismuth, 25% lead, 12.5% tin, and 12.5% cadmium). The particular sample used in these experiments had a melting point of 73° C. Thus the temperature employed must be higher than this, but higher temperature favors greater speed for the reaction. Furthermore, all the four components are metals whose oxidation products are practically insoluble in water.

The most satisfactory of a number of apparatus for employing this scheme is shown in Figure 1. All-glass apparatus is used.

About 200 grams of solid Wood's metal, corresponding to a volume of approximately 20 cc., and 500 ml. of the water are put into the three-necked flask. The system is then gradually evacuated, at room temperature, to perhaps 10 to 20 mm. of mercury pressure by applying a pump at point *D*. Stopcocks *A* and *E* (open to manometer) are closed to the atmosphere during this time. Within the flask air bubbles rapidly form within the liquid and rise to the surface; the flask is occasionally gently tapped or shaken to loosen any bubbles adhering to the sides of the flask. About 10 or 15 minutes after the vacuum has been applied the liquid will have been cooled to such an extent that no more oxygen will be evolved. It is not advisable to heat the flask at this point, however, as violent bumping usually occurs if this is done.

Next, stopcock *D* is closed and line *A* which is attached to a nitrogen supply (free of any traces of oxygen) is opened to allow the slow entry of this gas into the flask. The time at which the

pressure inside the flask reaches atmospheric is noted on the manometer, and at this point valve *E* is opened to the atmosphere to allow the nitrogen to leave the system from then on at the same rate at which it enters at *A*. The steady stream of nitrogen is then allowed to bubble through the liquid in the flask for about 0.5 hour, during which time additional oxygen is thereby removed. During this period the flask is slowly heated, so that at the end of this time the Wood's metal is molten and boiling of the water about to begin.

When gentle boiling commences stopcock *A* is closed and the flow of nitrogen stopped. In order to prevent leakage of any air back into the system a positive pressure of about 20 mm. of mercury above atmospheric is maintained at all times by operating stopcock *E*. *E* is opened only when the internal pressure becomes greater than 20 mm. of mercury above atmospheric, as read on the attached manometer, never to let air into the flask. If the pressure tends to drop below this value it is raised by slightly increasing the rate of boiling or, better, by slowing down somewhat the rate of flow of cooling water through the reflux condenser.

By boiling the water good convection is obtained, and the Wood's metal liquid is continuously agitated, presenting always fresh droplets to the water. A gradually increasing turbidity is observed as time goes on and it is probable that the stannous tin which composes a part of this suspension is also effective in deoxygenating within the body of the solution.

The refluxing is stopped after about 3 hours, *E* is closed, and some nitrogen is let in through *A* to keep the pressure constant, and still slightly above atmospheric, during cooling. Some time is allowed also for the suspended material to settle to the bottom of the flask if the liquid is to be used without distillation. The water in the flask, when clear, may then be siphoned out through *A* into the properly attached apparatus in which it is to be used. If water free of even the slightest trace of tin or cadmium is required it is distilled off through *D*.

Water prepared in this way showed no trace of oxygen when subjected to the Winkler test (*2, 3*), after distillation from the apparatus, although this is claimed to be sensitive to a concentration of oxygen as low as 0.01 cc. per liter.

An even more sensitive test for oxygen was made as follows.

A cell which may be represented as Hg/1*F* KCl/Hg was constructed and a galvanometer sensitive to about 10^{-6} ampere connected across the terminals. If neither of the mercury surfaces is disturbed the cell is symmetrical, no electromotive force is de-
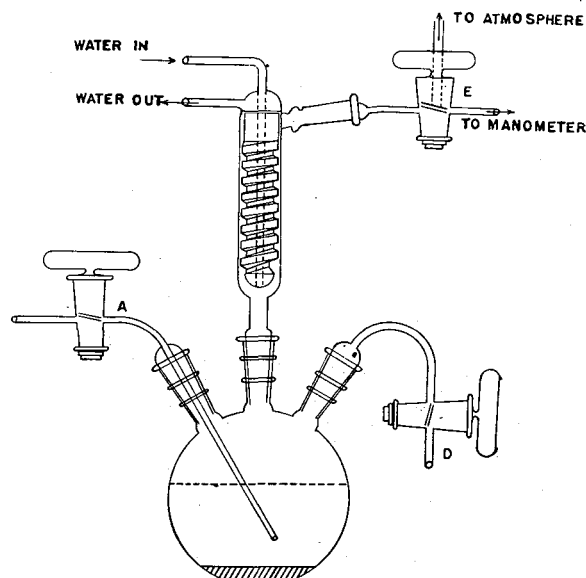

Figure 1. Apparatus

veloped, and no deflection is observed on the galvanometer. If one of the mercury surfaces is disturbed by gentle shaking or tapping, while the other is kept quiescent, an electromotive force is developed and a current flows in the galvanometer, provided that there is a trace of oxygen present in the electrolyte to be reduced at the still mercury surface. If, however, an electrolyte free of any trace of oxygen is used, no current is observed to flow. When a solution of potassium chloride, prepared from water deoxygenated according to the above procedure, was used as the electrolyte in this cell, no current was observed to flow until air was admitted to the cell and then the usual deflection of the galvanometer was obtained.

It is believed that this electrical method provides an even more

sensitive qualitative test than the Winkler method and confirms the efficacy of this technique for complete removal of oxygen from water.

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Polarographic Determination of Pentavalent Antimony in the Presence of Pentavalent Arsenic

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THIS work was started in 1940, but interrupted during the war. Since 1940 two publications on the reduction of pentavalent antimony have appeared. Kraus and Novak (2) found that pentavalent antimony is reduced in two steps in relatively concentrated hydrochloric acid. The first wave corresponds to the reduction of Sb^V to Sb^{III} and the second to the reduction of Sb^{III} to antimony in an amalgam. The two waves are not distinguishable until the concentration of the acid is greater than 6 N. At concentrations above 7 N the waves are well developed and the reduction is complete. Without being acquainted with this study, Lingane and Nishida (4) reported that a small reduction wave of pentavalent antimony is observed when the hydrochloric acid concentration is 0.5 N, that in 4 N hydrochloric acid two waves are observed, and that in 6 N acid the height of the first wave ($Sb^V \longrightarrow Sb^{III}$) is exactly 0.4 of the total diffusion current.

The authors' work carried out before 1942 is in general agreement with the above studies. They were interested in making use of the polarographic reduction of Sb^V to Sb^0 for the determination of antimony in the presence of arsenate. Although arsenate has been found not to be reducible at the dropping mercury electrode, they found small reduction waves in relatively concentrated (6 N) hydrochloric acid solutions. Large amounts of arsenate, therefore, interfere with the polarographic determination of pentavalent antimony in 6 N hydrochloric acid. By changing the supporting electrolyte they have succeeded in eliminating this interference.

EXPERIMENTAL

Standard solutions of trivalent arsenic and antimony were prepared and standardized in the usual way. By oxidation with bromine, standard solutions of arsenate and pentavalent antimony were prepared.

The polarographic measurements were carried out in the absence of oxygen at 25° ± 0.1° C. The characteristics of the three capillaries used were determined at various potentials. In order to intercompare diffusion currents obtained with different capillaries the values of the diffusion current constant (3) $I = i_d/cm^{2/3}t^{1/6}$, in which i_d is the diffusion current, c the millimolar concentration, m the mass of mercury in milligrams flowing out per second, and t the drop time, are reported in the tables. The values of $m^{2/3}t^{1/6}$ in a potential range between −0.4 and −1.0 volt [vs. saturated calomel electrode (S.C.E.)] of the three capillaries were 1.78, 1.82, and 2.07, respectively.

REDUCTION OF PENTAVALENT ANTIMONY

In agreement with Kraus and Novak (2) and with Lingane and Nishida (4) two waves were found at hydrochloric acid concentrations equal to or greater than 6 N hydrochloric acid. The first wave corresponds to a reduction of Sb^V to Sb^{III} and the second to that of Sb^{III} to Sb amalgam. At acidities smaller than 4 N

only one wave is observed, the height of which decreases with decreasing acidity. Even in 4 N hydrochloric acid the diffusion current is smaller than the value calculated for complete reduction of Sb^V to Sb^0. No reduction wave was observed in alkaline medium.

The results are summarized in Table I. The second diffusion current i_{d_2} refers to the total current (sum of the two wave heights). All values are corrected for the residual current. The half-wave potential $\pi_{1/2}$ refers to the saturated calomel electrode.

The value of the diffusion current constant of 7.50 in 6 N hydrochloric acid is the same as the value reported by Lingane and Nishida (4). Both diffusion currents in 6 N hydrochloric acid were found to be proportional to the concentration of pentavalent antimony in a range between 0.17 and 2 × 10⁻³ M. The diffusion current of trivalent antimony was determined in the same concentration range in 6 N hydrochloric acid. The ratio of the total diffusion current (i_{d_2}) of Sb^V to that of Sb^{III} was found to be 5/3 × 0.91. If the diffusion coefficients of Sb^V and Sb^{III} in 6 N hydrochloric acid were the same the ratio would have been 5/3. The above results indicate that the diffusion coefficient of Sb^V (in 6 N hydrochloric acid) is smaller than that of Sb^{III}. In 8 N hydrochloric acid the ratio was found equal to 5/3 × 0.94.

Further investigations were made of the reduction of pentavalent antimony in 1 N hydrochloric acid in the presence of varying amounts of alkali bromides and chlorides. As is evident from the

Table I. Reduction of 1.53 Millimolar Pentavalent Antimony Solution at 25° ± 0.1° C. at Varying Acidities

Concentration of Hydrochloric Acid, N	i_{d_1}, μa.	i_{d_2}, μa.	I_1[a]	I_2[a]	$\pi_{1/2}$ (vs. S.C.E.)
8[b]	8.1	20.5	3.03	7.45	c
6[b]	8.3	20.8	3.03	7.50	−0.11
4[d]	...	20.0	..	7.2	−0.18
2[d]	...	15.0	..	5.4	−0.24
1[d]	...	5.2	..	1.9	−0.35
0.2[d]	...	<0.5	..	..	....

[a] I. Diffusion current constant.
[b] Capillary 3 used.
[c] Anodic chloride wave interferes.
[d] Capillary 2 used.

Table II. Reduction of 1.5 × 10⁻³ M Sb^V at 25° C. in 1 N Hydrochloric Acid Containing Varying Concentrations of Potassium Bromide or Lithium Chloride

(Capillary 2)

Concentration and Added Electrolyte	i_d	I	$\pi_{1/2}$ (vs. S.C.E.)
0	5.2	1.9	−0.35
1 N KBr	14.8	5.32	−0.43
2 N KBr	18.1	6.50	−0.37
4 N KBr	20.8	7.48	−0.35
4 N LiCl	14.7	5.28	−0.21

results in Table II, the reduction of Sb^V to Sb^0 is complete in a medium composed of 1 N hydrochloric acid and 4.0 M potassium bromide. This medium is saturated with the bromide. Only one wave is observed, because the anodic bromide wave occurs at a more negative potential than that of the first reduction wave of Sb^V to Sb^{III}. The half-wave potential in the bromide-containing medium does not correspond to the true half-wave potential of Sb^V to Sb^0 because this potential is also shifted to a more negative value by the anodic bromide current when the bromide concentration is greater than 2.0 M. The diffusion current constant, I, in the presence of 4 M bromide was found to be 7.48 while in 6 N hydrochloric acid it was equal to 7.50. This indicates that the reduction is complete in the 4.0 M bromide medium.

The results in Table III show that the diffusion current is proportional to the antimony concentration in a medium 1.0 N in hydrochloric acid and 4.0 M in potassium bromide.

No reduction waves of pentavalent arsenic are observed in solutions containing 4 N or less hydrochloric acid, and no reduction wave is found in solutions which are 4 N in potassium bromide and 1 N in hydrochloric acid. On the other hand, waves of small height are found in 6 to 8 N hydrochloric acid. Therefore, it is to be expected that a medium 4 N in potassium bromide and 1 N in hydrochloric acid is much more suitable than a solution of 6 N hydrochloric acid for the polarographic determination of pentavalent antimony in the presence of pentavalent arsenic. This is shown to be true by results presented in Table IV.

Table III. Diffusion Currents of Sb^V at Varying Concentrations in Solutions 1 M in Hydrochloric Acid and 4 N in Potassium Bromide at 25° C.

(Capillary 3)

Concentration $Sb^V \times 10^3$ M	0.443	0.649	0.845	1.033	1.213	1.53
id	6.80	10.10	13.20	16.00	18.80	20.8
I	7.42	7.52	7.54	7.48	7.49	7.45

Average value of I = 7.48 ± 0.03.

### Table IV. Diffusion Current Constant I Calculated from Diffusion Current Obtained with 0.001 M Sb^V Solutions

Ratio As^V/Sb^V	I in 6 N HCl	I in 4 N KBr + 1 N HCl
0	7.50	7.48
3	7.6	7.46
10	7.95	7.45
30	9.5	7.40

TRIVALENT ANTIMONY AND ARSENIC IN ALKALINE MEDIUM

Trivalent antimony can be determined in the presence of trivalent arsenic by well known methods (1). Trivalent antimony in a supporting electrolyte composed of 0.1 to 1 N potassium hydroxide gives a well defined reduction wave, whereas arsenite does not give a wave (2). The diffusion current constant, I, for Sb^{III} was found to be 4.95 in 6 N hydrochloric acid, 5.36 in 4 N acid, 5.61 in 1 N acid, 5.9 in 0.1 N hydrochloric acid, and 6.0 in 1 N alkali. In 0.1 N hydroxide the half-wave potential was found equal to -1.07 volts, and in 1 N hydroxide to -1.15 volts (vs. S.C.E.). Addition of tartrate or citrate to the alkaline solution decreases the diffusion current constant. For example, in a solution 0.5 N in potassium hydroxide and 0.25 M in tartrate the constant was found equal to 4.8 (6.0 in the absence of tartrate). Similarly, in 0.1 N hydroxide and 0.5 M citrate the constant was found equal to 4.4. The tartrate and citrate have little effect on the half-wave potential, tartrate shifting it about 0.2 volt to more negative values, whereas the effect of citrate is hardly noticeable.

Trivalent antimony in the presence of an excess of alkali hydroxide gives a typical anodic wave. The cathodic wave corresponds to the reduction Sb^{III} to Sb^0, while the anodic wave corresponds to the oxidation Sb^{III} to Sb^V. A typical example is given in Figure 1.
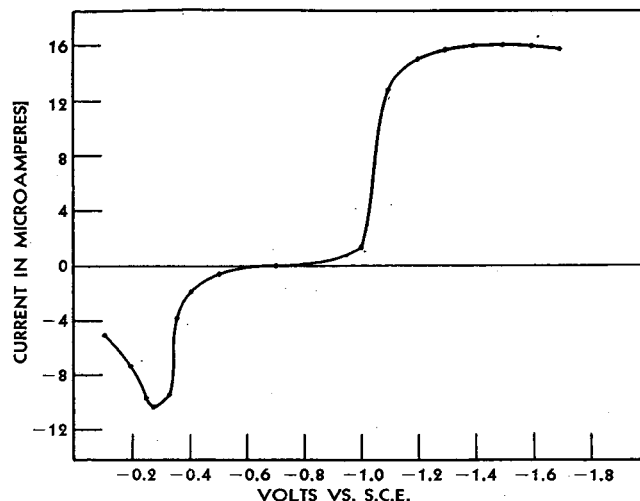
Figure 1. Anodic and Cathodic Waves for Trivalent Antimony

1.53 millimolar antimony in a solution of 0.1 N potassium hydroxide; capillary 2 was used

The anodic wave portrays a pronounced maximum which is not eliminated by either gelatin or peptone. The maximum is not of the ordinary type; it occurs when the current has attained the value of the diffusion current. In 0.5 N potassium hydroxide the maximum anodic current could not be determined with accuracy because of the maximum. The half-wave potential was found to be -0.34 volt in 0.1 N and -0.45 volt in 1 N potassium hydroxide.

Trivalent arsenic also yields an anodic wave which, again, is characterized by a pronounced maximum occurring when the current becomes equal to the diffusion current. The maximum is suppressed but not eliminated by addition of gelatin or peptone. In the presence of 0.025% gelatin the anodic diffusion current was found to be proportional to the concentration of arsenite in the range between 0.9 and 4 $\times$ 10^{-3} M. The diffusion current constant in 0.5 M potassium hydroxide in the above concentration range was 3.82 ± 0.02 at 25.0° C. The half-wave potential was found to be -0.26 volt (vs. S.C.E.) in 0.5 N potassium hydroxide and independent of the concentration. This half-wave potential is about 0.1 volt more positive than that of antimony at the same alkalinity.

SUMMARY

Pentavalent antimony gives a well defined diffusion current (Sb^V to Sb^0) in a supporting electrolyte which is saturated with potassium bromide and is 1 N in hydrochloric acid. In this medium arsenate does not give a reduction current. Sb^V can be determined polarographically in this medium in the presence of a large excess of As^V. In 6 to 8 N hydrochloric acid As^V gives slight reduction currents. This medium is not suitable for the determination of Sb^V in the presence of large amounts of As^V.

Trivalent antimony and arsenic both give typical anodic waves in 0.1 to 0.5 N potassium hydroxide solutions. The characteristics of the waves are briefly discussed.

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Iodometric Microdetermination of Bromine

Application to Analysis of Organic Compounds

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THE volumetric microdetermination of bromine in organic compounds using silver nitrate (6), although more convenient than the gravimetric procedure, leaves something to be desired. Owing to the unfavorable factor involved, a fairly large sample weight is required especially when the bromine content is low. The presence of oxidizing or reducing agents in the solution to be titrated can interfere seriously with the results of the analysis.

Because the catalytic combustion of organic compounds containing bromine yields an indeterminate amount of bromate ions (5), a procedure which would ensure complete conversion to bromate seemed to offer much promise. Such a method for bromine would yield a factor six times greater than that used for silver nitrate, enabling one to use, in some cases, less than the sample weight required for most microchemical analyses.

Van der Meulen (4), in an orginal method, used sodium hypochlorite to oxidize bromide to bromate followed by sodium formate at elevated temperatures to remove excess hypochlorite. Kolthoff and Yutzy (3), in a method for determining bromide in brines, improved the procedure, giving experimental details which proved various points in the procedure. The buffer in the latter method consisted of sodium dihydrogen phosphate, while in a subsequent paper by Willard and Heyn (7) a citrate buffer was employed so as to avoid possible interferences with certain metals present in brines. As such metals were not to be encountered in the present study, the phosphate buffer system was retained.

EXPERIMENTAL

A 20% by weight sodium dihydrogen phosphate buffer solution was used throughout in this work. For greater convenience the strength of the sodium formate solution was increased to 50% while the sodium hypochlorite solution used by Kolthoff was retained. The combustion of the sample was carried out according to the Pregl catalytic method (5) with certain modifications as used for the determination of sulfur (1). An approximately 1 N sodium hydroxide solution was selected as a suitable absorber for the spiral, as this would absorb a greater amount of acids produced by combustion. The use of this alkali necessitated checking the pH, since Willard and Heyn (7) found that the reaction is complete at a pH of 5 to 7. With a variable amount of

1 N sodium hydroxide (0 to 2 ml.), 5 ml. of 20% phosphate buffer and 5 ml. of the hypochlorite solution, the pH was found to be 6.2.

The prepared hypochlorite solution deteriorated after several weeks even on standing in the refrigerator, whereas a Chlorox commercial bleach (with a concentration of 5.25% sodium hypochlorite) did not lose its oxidizing capacity even after several months. Five milliliters of this solution were found adequate for the oxidation of all compounds tested. The commercial bleaching solution was adopted for its stability and convenience and, therefore, was used in all subsequent determinations. No difference was observed if the solution is heated to boiling for several minutes or just to boiling as Kolthoff observed. A rather vigorous effervescence takes place on the addition of a 50% sodium formate solution to this hot solution and serves as a good indication of the hypochlorite strength. A weak or mild effervescence indicates a deterioration of the oxidizing capacity of the bleach. After cooling to room temperature, the amount of sulfuric acid necessary was found to be at least 10 ml. of 9 N, which is more convenient than the amount described by Kolthoff.

The question of whether large amounts of sodium chloride are necessary was investigated and it was found that no chloride ion was necessary even when less than normal amounts of bromine were to be determined.

Several determinations in which the Parr microbomb technique (2) was substituted for the Pregl tube indicated that this combustion method can be used satisfactorily (Table I). The convenient combustion method of Sundberg and Royer (6) can also be employed for this purpose, as the combustion is carried out under similar conditions.

With compounds containing metals, the residue in the boat consisting of the metal bromide, can be dissolved and added to the spiral rinsings without any loss in accuracy.

A blank determination on the complete procedure should be run with each new batch of bleaching solution. The blank correction usually is about 0.2 to 0.4 ml. of thiosulfate.

PROCEDURE

A sample of 3 to 6 mg. was taken for analysis and required 20 to 30 minutes for combustion. The spiral contents were rinsed into

Table I. Results of Iodometric Microdetermination of Bromine

(Comparison of experimental with calculated values)

Compound	Per Cent Bromine		Compound	Per Cent Bromine	
	Found	Calculated		Found	Calculated
o-Bromobenzoic acid	39.71[a]	39.79	$C_{12}H_{10}ONBr$	30.55 / 30.18	30.30
Tribromophenol	72.48[b] / 72.29	72.46	$C_6H_3O_2NBr_2$	53.66 / 53.82	53.84
Cholesteryl bromide	17.91[b] / 17.66	17.82	$C_8H_{18}NBr$	39.84 / 40.22	39.98
6-Bromo-7-ketocholestanyl acetate	15.26[b] / 14.98	15.26	$C_8H_{19}NBr_2$	55.59 / 55.44	55.34
2-Bromocholestanone	17.17 / 16.94	17.20	Research compound CHONSBr	23.89 / 24.22	24.06
Stigmasteryl acetate dibromide	26.44 / 26.22	26.06	Research compound CHOBr	29.22 / 29.56	29.42
C_5H_4ONBr	46.11 / 46.05	45.98	Research compound CHOBr	28.60 / 28.85	28.67

[a] Average of 20 analyses, highest 40.18, lowest 39.54.
[b] Analysis according to Parr microbomb.

a 250-ml. Pyrex iodine-titration flask with about 20 to 30 ml. of water. After the addition of 5 ml. of a 20% sodium dihydrogen phosphate solution, 5 ml. of Chlorox commercial bleaching solution were added and the solution was heated just to boiling. The addition of 5 ml. of a 50% sodium formate solution to this should be accompanied by vigorous effervescence. After cooling to room temperature, 10 ml. of 9 N sulfuric acid, 1 drop of 0.5 N ammonium molybdate, and approximately 1 gram of potassium iodide were added, and the liberated iodine was titrated within 1 to 2 minutes. The calculations are as follows:

$$\% \text{ Br} = \frac{(\text{ml. of } 0.01 \text{ } N \text{ thiosulfate} - \text{blank correction}) \times \dfrac{79.97}{6}}{\text{weight of sample in mg.}}$$

The data in Table I show that satisfactory agreement with the calculated values was obtained with a wide variety of bromine compounds. Although no improvement in accuracy and precision over existing methods is noted, there is an advantage in time consumed for analysis. The average analysis requires only 1 hour. The convenient and usually preferred iodometric titration procedure might also be cited as an advantage.

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Electrolysis with a New Type of Mercury Cathode Cell

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THE removal of various cations by electrolysis with a mercury cathode has been discussed (1, 2). In such a procedure, the cation is deposited in the mercury cathode, usually from a dilute sulfuric acid solution. The amalgam is then separated from the electrolyte without interrupting the flow of current, lest the cation be returned to solution. Melaven (3) reviewed the operation of various mercury cathode cells and concluded that their disadvantage was in the amalgam-electrolyte separation. Using a mercury-leveling bulb attachment to this cell, he was able to effect a clean and easy separation of the mercury and the electrolyte.
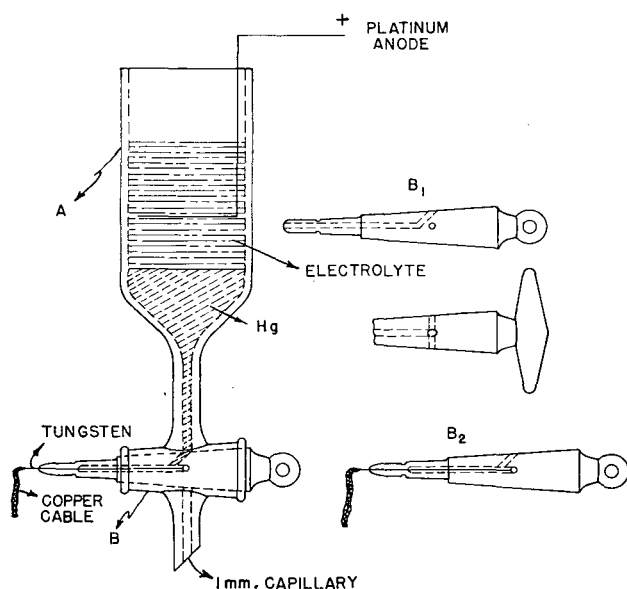

Figure 1.   Mercury Cathode Cell

The cell illustrated in Figure 1 was designed for electrolytic removal of iron and other heavy metals. It facilitates the amalgam-electrolyte separation without resorting to a mercury-leveling bulb attachment.

The cell consists of vessel A with a rounded base attached to a special capillary stopcock, B, which permits continuous current flow (except for a fraction of a second) during electrolysis and while the mercury is being drawn off. The stopcock was made from a three-way, solid stopper (plug) capillary stopcock. A tungsten wire (platinum may be used) was sealed into the longitudinal bore of plug B_1 which was drilled out to the transverse opening, B_2. A copper cable was welded to the tungsten wire to permit free rotation of the plug when the negative element was connected to the cathode of storage battery.

Operation.   While the plug is in position B_2, mercury is added to the cell until the level is about 2 cm. from the bottom. The solution to be electrolyzed is introduced and a platinum anode is immersed in the electrolyte. The electrodes are connected to a 6-volt storage battery and the solution is electrolyzed with a current of about 2 amperes (0.24 ampere per sq. cm.). The mercury and solution are stirred with an electric stirrer, and the cell is cooled with a stream of air (a water-cooled jacket also may be used). After a spot test is made for complete removal of the cation, the stopcock is rotated through 90° and the mercury is drawn off. Fresh mercury is added to the cell and removed as before, thus washing out any amalgam that may have adhered to the stem. A platinum wire may be used to dislodge the mercury left in the capillary. The cell is drained of the electrolyte and readily washed.

When a solution containing 0.100 gram of iron was electrolyzed for 50 minutes using the cell, all the iron was removed from the electrolyte as indicated by the α,α-bipyridyl colorimetric test. Although this cell was used for micro and semimicro separations, it may be adapted to macrotechniques by increasing the capacity of the vessel and the diameter of the capillary stopcock.

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Determination of Nitrous Oxide

KELSO B. MORRIS AND ETHEL M. DAVIDSON

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IN THE course of oxidation studies under an Office of Naval Research contract, it became necessary to determine nitrous oxide, a product of the reaction. The authors elected to use two well-known methods—slow combustion and catalytic reduction—in obtaining data on nitrous oxide and nitrous oxide–nitrogen mixtures. The chemical equation that represents the reaction in both methods is

$$N_2O + H_2 \longrightarrow N_2 + H_2O$$
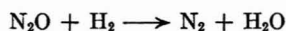
in which the total contraction is equal to the volume of nitrous oxide. Kobe and MacDonald (1) employed with success a commercial silica gel catalyst containing 0.125% of platinum and reported that nitrous oxide may be reduced over the catalyst by a limited excess of hydrogen at 515° C.

A special Burrell Build-Up gas analysis unit containing slow-combustion and catalytic oxidation (or reduction) assemblies was used in the work. The latter assembly provides for heating of the catalyst tube by means of a Perma Therm heater (Burrell Technical Supply Co., Pittsburgh, Pa.). The manufacturer states that the heater is adjusted to operate at any point between 500° and 525° C. and the maximum variation over a range of 105 to 125 volts is ±2.5° C. The manufacturer, though not willing to release data on the catalyst, asserts that its activity differs from that of Kobe and other previous catalysts.

The volume of hydrogen used was from 2 to 2.5 times the volume of nitrous oxide in the sample. One double pass at the rate of 4 to 5 ml. per minute was the procedure adopted for the slow-combustion pipet. For the catalyst tube, the method involved three double passes at 25 ml. per minute.

Data show that the commercially available catalyst tube and heater are convenient and satisfactory for the determination of nitrous oxide, and indicate practically the same order of accuracy for the two methods. One and three double passes, respectively, at the flow rates indicated, are satisfactory for the slow-combustion technique and the catalyst tube. For the same volume of gas passed, the catalyst tube is faster. Thus, the minimum handling time for 50 ml. of gas would be 20 to 25 minutes in the slow-combustion analysis as compared with 12 minutes with the catalyst tube. Traces of ammonia were noticed occasionally where the slow-combustion pipet had been used, but never where use had been made of the catalyst tube.
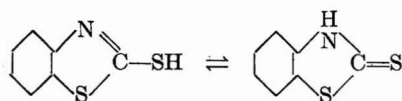
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CRYSTALLOGRAPHIC DATA

Contributed by Armour Research Foundation of Illinois Institute of Technology

19.   2-Mercaptobenzothiazole

Tautomeric Forms of 2-Mercaptobenzothiazole

2-Mercaptobenzothiazole is very difficult to crystallize and no crystals except from the melt apparently ever show reproducible and definite interfacial angles. The best crystals obtained for this 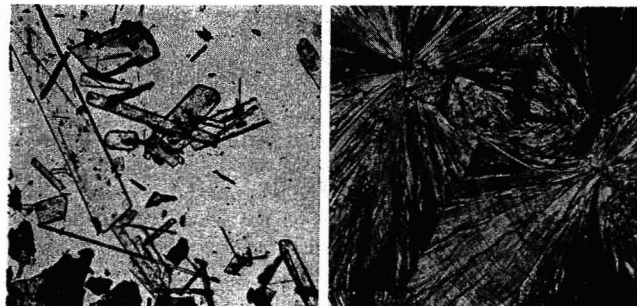study were obtained by slow evaporation of a chloroform or a 50:50 chloroform-xylene solution. The commercial samples usually contain considerable ammonium chloride, which should be removed by extraction using very slightly alkaline solutions.

CRYSTAL MORPHOLOGY (determined by W. C. McCrone).

Crystal System. Monoclinic.

Form and Habit. Crystals obtained from chloroform were long flat rods showing basal pinacoid {001}; orthopinacoid {100}; and prisms {210}. The interfacial angles vary by as much as 10° from crystal to crystal and even on the same crystal; many faces are curved.

Axial Ratio. $a:b:c = 2.655:1:1.335$.

Interfacial Angles (Polar). $210 \wedge \overline{2}10 = 77°$.

Beta Angle. 109°.

Cleavage. 010, 100.

Figure 1.   2-Mercaptobenzothiazole

Left. Crystals from chloroform and xylene
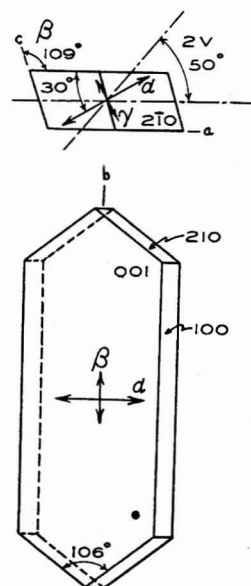*Right.* Fusion preparation showing characteristic shrinkage cracks

Figure 2. Orthographic Projection of Typical Crystal of 2-Mercaptobenzothiazole

X-Ray Diffraction Data (determined by J. Whitney and I. Corvin).

Cell Dimensions. $a = 15.899$; $b = 5.989$; $c = 7.995$.
Formula Weights per Cell. 4.
Formula Weight. 167.24.
Density. 1.42 (buoyancy; x-ray).

Principal Lines

d	I/I_1	d	I/I_1
7.61	1.00	2.579	Very weak
6.51	0.40	2.509	0.19
5.57	0.26	2.418	0.28
4.75	0.46	2.398	0.26
4.42	0.39	2.336	Very weak
4.26	0.39	2.204	0.20
3.97	0.62	2.172	0.16
3.87	0.62	2.038	0.16
3.78	0.97	1.983	0.24
3.67	0.74	1.942	Very weak
3.34	0.27	1.902	0.26
3.21	0.70	1.837	0.18
3.14	0.86	1.769	0.31
2.984	Very weak	1.739	Very weak
2.815	0.48	1.697	Very weak
2.719	Very weak	1.667	Very weak

Optical Properties (determined by W. C. McCrone).

Refractive Indexes (5893 Å.; 25° C.). $\alpha = 1.665 \pm 0.005$; 1.667 (1). $\beta = 1.96 \pm 0.01$; 1.965 (1). $\gamma = 2.04$ (calcd.); 2.06 (1). α' (projection on 001) = 1.688. (This refractive index

is *not* correct but is the Cargille liquid which, when saturated with mercaptobenzothiazole, causes the crystal to disappear.)

Optic Axial Angles (5893 Å.; 25° C.). $2\,V = 52°$; 50° (1). $2H = 70°$; 67° (1).

Dispersion. Inclined $r > v$. (Note. Conoscopic observation parallel to BXa shows both brushes in the field with N.A. = 1.25; one optic axis shows $v > r$, and the other, $r > v$.)

Sign of Double Refraction. Negative.

Acute Bisectrix. α.

Extinction. $||a$.

Molecular Refraction (R) (5893 Å.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.881$. R (calcd.) = 49.4. R (obsd.) = 53.9.

Fusion Data (determined by W. C. McCrone).

2-Mercaptobenzothiazole melts at 181° C. and solidifies spontaneously on cooling. There is a slight tendency for sublimation but usually only droplets or long needles (elongated parallel to *b*) are obtained. The crystals grow rapidly from a small number of nuclei as spherites made up of large rods and needles growing elongated parallel to *b*. These crystals show all possible orientations normal to the *b* axis. Some views using oil immersion show both optic axes within the field with $2H = 70°$. The interference figure is unusual in that one optic axis shows strong dispersion, $r > v$, and the other shows less dispersion, $v > r$. The sign of double refraction is negative.

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CORRESPONDENCE

Polarograms by an "Undamped" Polarograph

Sir: In a recent paper by Lingane (*1*) the following statement is made concerning the 25% sensitivity increase obtained by Schulman, Battey, and Jelatis with "undamped" operation over that obtained by conventional damped operation of their new polarograph (*2*): "The alleged increase in sensitivity that results from measurement of the maximum rather than the average of the undamped recorder oscillations is more or less illusory, because the residual current in terms of maximum oscillation is also larger."

Lingane's statement implies that the sensitivity is determined by the ratios $\dfrac{I_{\text{diffusion}}}{I_{\text{residual}}}$ or $\dfrac{I_{\text{total}}}{I_{\text{residual}}}$, which do not change with change in damping. Neither these ratios nor the residual current are involved, however, in the expression for the sensitivity. This quantity is defined as K in the equation: $I_{\text{diffusion}} = KC$, where $I_{\text{diffusion}}$ is the diffusion current and C is the concentration of electro-oxidizable or electroreducible substance. "Undamped" operation increases both the residual current and the total current by 25%. Therefore their difference—the diffusion current—is likewise increased by 25%, and sensitivity K is increased by this amount. The data of Schulman, Battey, and Jelatis unequivocally demonstrate the reality of this increase in sensitivity.

It is granted that a sensitivity gain of 50 or 100%—had this been obtainable by changing from damped to undamped operation—would be of greater practical value than the observed 25% gain. It appears to the writer that a sensitivity increase of this magnitude is nevertheless worth having.

Lingane has correctly stated that the principal advantage of the undamped instrument lies in the speed with which polarograms can be run without distortion of half-wave potentials.

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James H. Schulman

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Sir: Schulman is correct in his criticism of the reason which I stated for my opinion that the increased sensitivity which results from measurement of undamped maximum polarographic recorder oscillations is more or less illusory—viz., "because the residual current in terms of maximum oscillations is also larger." I am glad to accept correction on this point. The thought in mind, which I did not express correctly, is that increasing the magnitude of a measured quantity by only 25% does not produce any very significant increase in the sensitivity or precision of the measurement.

James J. Lingane

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Cambridge, Mass.

Polarographic Method for Copper, Lead, and Iron

Sir: We have read with great interest the paper entitled "Polarographic Method for Copper, Lead, and Iron" [Anal. Chem., **21**, 176 (1949)] by Reynolds and Rogers. However, we feel that two topics of considerable importance have been unduly slighted in their discussion:

1. Directions are given for the preparation of the solution for analysis, but the optimum pH is not stated. This matter is likely to be of vital interest to a user of the method.

2. The reported effect of gelatin on the lead wave is strikingly similar to the effects we have found with a number of micelle-forming agents in other systems. From a study of the effects of gelatin on several copper tartrate systems, we have concluded that the critical concentration for micelle formation of gelatin is

close to 3.5 × 10^{-3} %. It would be of interest to know whether the region in which the effect was found by Reynolds and Rogers is in agreement with our data.

LOUIS MEITES
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SIR: The optimum pH for the analysis was found to be between 9 and 11. Below pH 9, zinc pyrophosphate begins to precipitate; above pH 11, the various hydrous oxides begin to precipitate.

It was found that 0.01% gelatin was not sufficient to suppress the copper maximum completely, but that it depressed the lead wave to about 10% of its true height. Therefore, higher concentrations of gelatin were not tried. The copper maximum was not sufficiently suppressed by 0.001% gelatin to permit the determination of the lead diffusion current, owing to overlapping of the two waves. By using pyrophosphate solutions containing only lead at pH 10, it was found that 0.001% gelatin was not enough to depress the wave height appreciably. Concentrations of gelatin intermediate between 0.001 and 0.01% were tried in copper-lead mixtures, but either the overlapping was too great or the lead diffusion current was cut down too much. No quantitative studies were made to correlate the decrease in the lead diffusion current with the gelatin concentration, but even these data are in good qualitative agreement with the results on other systems reported by Meites and Colichman.

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Book Reviews

Absorption Spectrophotometry. *G. F. Lothian.* 196 pages. 76 illustrations and diagrams. Hilger and Watts, Ltd., 98 St. Pancras Way, Camden Road, London, N.W. 1, England, 1949. Jarrell-Ash Company, 165 Newbury St., Boston, Mass. Price, $7.60, postage prepaid.

This book was originally intended to be a third revised edition of Twyman and Alsopp's "The Practice of Absorption Spectrophotometry with Hilger Instruments," but the tremendous advances in this field since the appearance of the second edition of that book in 1934 makes the present edition virtually a new work. With each opening of the book, the reviewer's respect for it has ncreased, and he is now convinced that the author has made a worthy contribution by condensing so clearly and concisely in less than 200 pages the essentials of the principles and practice of absorption spectrophotometry. This volume might well perform the dual role of orienting the newcomer in the field and serving as a valuable handbook to the experienced worker.

The subject matter is well organized and treated in three parts: principles, applications, and techniques. The discussion of theoretical principles is sound and is about as complete as one could expect in 62 pages. One might take exception to some of the terminology employed—e.g., density instead of the somewhat more specific expression optical density, or better still absorbancy—but on the whole there is nothing that should cause any confusion. Obviously the practical applications can only be indicated in the 35 pages allotted to this subject, especially in view of the wide spectral range covered, roughly 2000 to 250,000 Å. (25μ). To one whose principal interest is in, say, spectrophoto-

metric methods for the analysis of metallurgical specimens, this section may be a disappointment until it is recalled that the title of the book is "Absorption Spectrophotometry," a physical phenomenon for which the analytical chemist finds useful application in various ways. In the section on techniques (77 pages) the author describes in some detail the instrumentation available for visual, photographic, photoelectric, and thermal spectrophotometry, with most emphasis on those particular instruments with which he has had personal experience. The worker whose equipment is not described may feel some disappointment, but as the author points out, instrumentation is developing so rapidly that no treatment of the subject can long remain up to date.

The author lists 219 literature references in the text, and suggests 21 other books and papers as valuable sources of additional information on spectrophotometry.

PAUL K. WINTER

Elastomers and Plastomers. Their Chemistry, Physics, and Technology. Volume III. Testing and Analysis: Tabulation of Properties. *R. Houwink,* editor. 174 pages. Elsevier Publishing Co., Inc., 215 Fourth Ave., New York, N. Y. Price, $4.50.

This volume is a compilation of physical, chemical, mechanical, optical, and electrical test methods for many applications of the plastics using the principal types of resins. Typical values obtained by these methods are tabulated in two chapters, subdivided into elastomers and plastomers. American, British, and German standard methods, as well as isolated examples from other countries, are incorporated in obtaining these data. Methods of determining the presence of typical classifications of resins are included. No test methods or results on the behavior of resins in solution have been included—e.g., solution viscosity.

This book should serve as a reference on plastics for the over-all comparison of test methods and values obtained therefrom.

R. C. BACON

Industrial Uses of Radioactive Materials

A selected bibliography on industrial uses of radioactive materials has been prepared by Arthur D. Little, Inc., Cambridge, Mass., as an 18-page pamphlet. Following a 2-page introduction, six general background references are cited. Survey articles of industrial applications are then listed and the bibliography continues with references dealing with specific fields: petroleum industry, mining and metallurgy, textiles, instruments, radiography, analysis, pharmaceutical, glass, radioisotope preparation, and miscellaneous industrial applications. Copies of the bibliography are available on request from Arthur D. Little, Inc., Memorial Drive, Cambridge 42, Mass.

The Analyst's Calendar

American Council of Commerical Laboratories. Curtis Hotel, Minneapolis, Minn., June 23 and 24

American Society for X-Ray and Electron Diffraction. Cornell University, Ithaca, N. Y., June 23 to 25

Second Annual Summer Symposium on Analytical Chemistry. Wesleyan University, Middletown, Conn., June 24 and 25

Fourth Instrument Conference and Exhibit. Municipal Auditorium, St. Louis, Mo., September 12 to 16

Third Symposium on Analytical Chemistry. Louisiana State University, Baton Rouge, La., January 30 to February 2, 1950

AIDS FOR THE ANALYST . . . . . .

Air Displacement with Dry Ice in Iodometric Titrations. Frederick G. Strong, Bryn Mawr College, Bryn Mawr, Pa.

THE performing of frequent iodometric analyses on hot summer days suggested that air displacement and simultaneous cooling by use of dry ice might be substituted for chemical generation of carbon dioxide in the reaction flask, if sodium bicarbonate or sodium carbonate was added to excess acid before addition of iodide ion.

Scott ("Standard Methods of Chemical Analysis," Furman, N. H., ed., 5th ed., Vol. I, p. 1211, New York, D. Van Nostrand Co., 1939) says that in standardizing sodium thiosulfate with potassium dichromate, "the acid solution should be freed of dissolved air (previous boiling or treatment with carbon dioxide)." Pierce and Haenisch ("Quantitative Analysis," 3rd ed., p. 244, New York, John Wiley & Sons, 1948) suggest the use of excess sulfuric acid and sodium carbonate. Willard and Furman ("Elementary Quantitative Analysis," 3rd ed., pp. 267–8, New York, D. Van Nostrand Co., 1940) advise the use of excess hydrochloric acid and sodium bicarbonate.

Experiments were carried out with both boiled and air-saturated solutions, using dry ice to effect air displacement and cooling by the procedure of Pierce and Haenisch.

I. Standard Method. One hundred milliliters of freshly boiled 2 N sulfuric acid and 25.00 ml. of 0.02538 N potassium dichromate were placed in a 500-ml. Erlenmeyer flask, 2.0 grams of sodium carbonate were added in portions while the contents were swirled, 5.0 grams of sodium iodide dissolved in 10 ml. of freshly boiled water were then added, and the flask was stoppered and allowed to stand for 10 minutes in subdued light. The temperature of the solution was 28° C. Two hundred milliliters of freshly boiled water were then added to reduce the acidity and the solution was titrated with sodium thiosulfate.

II. No Air Displacement. In a 500-ml. Erlenmeyer flask were placed 75 ml. of distilled water at 28° C., saturated with air (by drawing air through it for several hours), 25.00 ml. of 0.02538 N potassium dichromate, 5 ml. of 6 N hydrochloric acid, and 5.0 grams of sodium iodide in 10 ml. of water. After being stoppered and allowed to stand for 10 minutes in subdued light, the solution was titrated with sodium thiosulfate solution.

III. Dry Ice. In a 500-ml. Erlenmeyer flask were placed 75 ml. of distilled water at 28° C., saturated with air as in II, 25.00 ml. of 0.02538 N potassium dichromate, and 5 ml. of 6 N hydrochloric acid. Dry ice was added (30 grams each for solutions 1 and 2, 20 grams each for 3 and 4) and allowed to sublime (1 and 2 took rather long, 3 and 4 about 5 minutes). The temperature was taken at this point (9° C. for 1 and 2, 14° for 3 and 4), 5.0 grams of sodium iodide in 10 ml. of water were added, and the flask was stoppered, allowed to stand (10 minutes for 1, 2, and 4; 5 minutes for 3), and titrated.

|	I	II	III
		Ml. of Na₂S₂O₃	
1	32.83	33.28	32.87
2	32.85	33.23	32.84
3	32.88	33.20	32.83
4	32.87	33.26	32.85
Av.	32.86	33.24	32.85
		Normality of Na₂S₂O₃	
	0.01931	. . . . . .	0.01931

Results I and III check well, showing that dry ice can replace chemical evolution of carbon dioxide as a method of air displacement in iodometry. Neglect of this factor gives high results (II). That the lowered temperature does not seriously decrease the rate of reaction between dichromate and iodide is shown by the general agreement and the fact that solution 2 (III) stood only 5 minutes before being titrated.

The use of dry ice is suggested as an alternative method of iodometry, rather than as a significant improvement. If dry ice is readily available and a routine is established in a series of titrations to use the time required for its sublimation, the total time and effort required are no more than for preparing acid of the right concentration and weighing out the proper amount of carbonate (which must be done fairly accurately). Occasions may arise when it would be undesirable to increase the acidity of a solution in order to perform an iodometric titration. The amount of solid carbon dioxide can soon be estimated without weighing; about 15 grams per 100 ml. are suggested, but less may be sufficient. Bringing iodide and oxidizing agent into contact with each other before all carbon dioxide has sublimed seemed to give equally accurate results. Use of too much solid carbon dioxide caused the pieces to stick to the bottom and become covered with ice, and slowed down the rate of sublimation considerably.

A Sintered-Glass Manostatic Valve for Maintaining an Inert Atmosphere. J. C. Cavagnol[1], Johns Hopkins University, Baltimore, Md.

THE all-Pyrex apparatus shown in the accompanying sketch was designed to remedy the nuisance of blowing mercury out of a manostat or sucking it back into the attached system. It has also served to maintain an inert atmosphere during a reaction or distillation and subsequently to keep the isolated product under the same conditions. The problem of replacing air with other gases in an apparatus of large volume is simplified by having this valve link the gas and vacuum lines with the system.
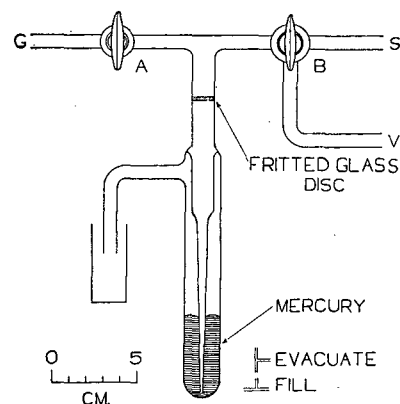
Only four regular seals and one ring seal are required to assemble the 13-mm. tube with medium porosity fritted disk, 22 × 175 mm. test tube, 2-mm. straight-bore stopcock, and 2-mm. three-way T stopcock. An 8-mm. side arm on the body functions as a mercury overflow. With stopcock A open and stopcock B in the "fill" position, gas is allowed to enter slowly at G. Now S is connected to the system to be filled and V is attached to a vacuum pump. By turning B back and forth through a 90° arc between the two positions it is possible alternately to evacuate and fill any apparatus as often as necessary. With reaction mixtures evolving gas, the air is first replaced with an inert atmosphere, A is closed, and B is placed in the fill position with the gas and vacuum lines disconnected, so that the manostat functions as a one-way valve.

The utilization of the impermeability of sintered or porous glass plugs to mercury has resulted in several elegant designs [Lewis, F. M., IND. ENG. CHEM., ANAL. ED., **13**, 418 (1941); Taylor, R. C., and Young, W. S., *Ibid.*, **17**, 811 (1945)]. Although no originality is claimed for any of the principles involved, this multipurpose arrangement may provide simplification or additional flexibility to existing setups.

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