ANALYTICAL · CHEMISTRY

Walter J. Murphy, Editor

The Phenomenal Growth in Instrumentation

Our recent visit to the 22nd Exposition of the Chemical Industries in New York's Grand Central Palace made us recall the old cliché of the vacuum cleaner salesman: "Push the button, and the machine does the rest." Instrumentation for analytical research and process purposes has not only arrived but is rapidly becoming sophisticated. It refuses any longer to be considered an intriguing "gadget," but demands the mature careful consideration and respect that an established technology deserves.

One indication of maturity in a technically based field is a tendency toward subspecialization. If so, instrumentation, using the term in its broadest sense, has reached a readily recognizable state of maturity. Anyone viewing the astonishing variety of instrumentation on display at the exposition would be deeply impressed with the advances in instrumentation made in the past two years. Measuring devices for work on radioactive materials have taken on uniformity of design in a manner reminiscent of the history of pH meters. Another indication was the further widening of the split between laboratory and process instrumentation, a complementary separation that is bound to benefit both fields by allowing them to intensify the development of devices that fill their individual requirements more satisfactorily.

Nature abhors a vacuum and already there are indications that the chasm between laboratory and process instrumentation will be filled by the efforts of a school of pilot plant level instrumentation. This, after all, is a desirable situation, for if raw materials and equipment must run the gamut of laboratory to pilot plant to production scale, why not the instruments that indicate and control the vital steps of a process?

Another development that impressed us at the exposition was the growth of distinction between research and control instruments at the laboratory level. The United States, in its penchant for production line methods, has been too wont to confuse and strive to incorporate the purposes of research and control in one instrument. This was especially true during the war years, when it seemed that everyone concerned himself with the production of instruments so simple that "even a night school art student could operate them." Simplicity of instrument design is not to be decried, but the fact remains that more often than not real instrumentation progress results from studies made on a universal and probably complex research instrument.

We noticed a happy blending of formal training in chemistry, physics, electronics, and mechanics in many of the representatives of the instrument companies who displayed their wares at the exposition. This is a favorable development, for it means that instrumentation will be in a position to anticipate and meet the demands of many branches of science in a highly satisfactory manner.

No objective appraisal of a subject is complete without at least one note of well intended constructive criticism. In this instance we mention the lack of interchangeability of parts as our pertinent complaint. There doesn't seem to be a valid reason why manufacturers of instruments of a similar type should not at least try to tie in the design of their units and auxiliary parts with those of other manufacturers. At present interchangeability is practiced to only a limited extent. If this were increased, all the parties concerned would benefit and much of the friction that faces progress in instrumentation would be erased.

The birth, adolescence, and maturity of instrumentation bring a sigh of regret only to those who have a sentimental attachment to the classical "wet method" type of analysis. The modern analyst, however, will continue to examine, accept, improve, or reject any given instrumental method on the basis of whether or not it helps him in his quest. We do not intend to infer, however, that the classical methods are no longer important. They certainly provide us, in many instances, with the means of knowing the accuracy of faster physicochemical methods.

Conservation of the analyst's time is important, indeed essential for many reasons. One reason, of course, is economy. Of greater significance is the obvious fact that large-scale continuous process operations could not be employed if ways and means for performing fast yet accurate analyses of a repetitive nature were not available.

Our sincere congratulations to the scientific apparatus manufacturers for the progress so much in evidence at the Exposition of Chemical Industries.

Advisory Board Changes

We welcome to our Advisory Board two new members, Philip J. Elving of the Pennsylvania State College, and Wayne A. Kirklin, manager of the analytical division of the Hercules Experiment Station, Wilmington, Del. Edward Wichers, National Bureau of Standards, currently filling the unexpired portion of G. E. F. Lundell's term, has been appointed to the board for a four-year period.

Philip J. Elving and Wayne A. Kirklin need no introduction to the readers of Analytical Chemistry. Both have served the Division of Analytical Chemistry in many capacities, including the chairmanship of the division. Both have attended meetings of the advisory board representing the division. Both have contributed in a major way to the deliberations of the board.

To R. P. Chapman of the American Cyanamid Company, J. R. Churchill of the Aluminum Company of America, and G. E. F. Lundell, retiring from the board, our sincere thanks for their untiring efforts in behalf of the journal.

PRINCIPLES OF PRECISION COLORIMETRY

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A method of precision colorimetry is outlined and examined theoretically. The mode of operation requires the comparison of high absorbance standard solutions with the unknown in a photoelectric colorimeter of variable optical axis. The effects of absorption law deviations, temperature, and refractive index are briefly discussed and data illustrating the method are presented. This approach gives results comparable to those of gravimetry and volumetry.

DURING an investigation of ionic equilibria in aqueous solutions, an attempt was made to set up a differential thermometer scale, based on the color changes occurring as the solutions observed were heated or chilled. To do this with high sensitivity, it was apparent that relative concentration measurements would have to be made with equally high precision. This required an approach differing from conventional colorimetry where a precision within about 1% is usually considered satisfactory. The solution of this problem is, therefore, of some interest to the analyst and is presented here as a general principle of colorimetry.

An examination of the literature of colorimetry showed that attempts had previously been made to accomplish the above objective by proceeding along two mutually supporting lines. In the first case photoelectric instruments of greater sensitivity were designed and, in the second, the errors of colorimetry were studied in an effort to operate existing equipment with maximum precision.

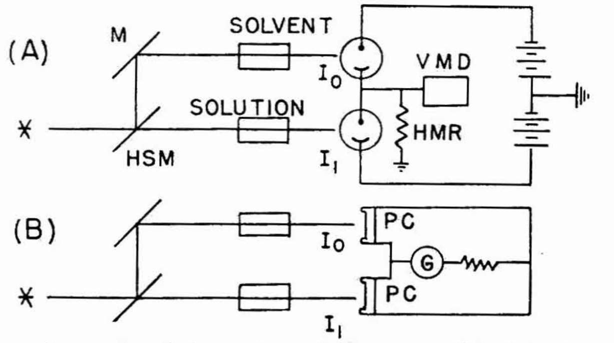

Figure 1. Schematics of Common Electrical Circuits Employed for High Sensitivity

G. Highly sensitive galvanometer ~ 10^{-10} ampere per mm.
M. Mirror
HSM. Half-silvered mirror
HMR. High-megohm resistor
PC. Photocell, barrier type
VMD. Voltage-measuring device

In the field of instrument design the detecting element has usually involved a pair of vacuum or gas-filled phototubes connected in series as shown in Figure 1*A*, with appropriate means for detecting the voltage developed across the high ohmic resistor. These are the types of circuits of such early workers as von Halban and colleagues (*4–7*), Winn (*20*), and Zscheile, Hogness, and Young (*24*), as well as Kortüm (*10, 11*) and Klotz and Dole (*9*).

With circuits of this type it has been found possible to detect differences in transmittance as small as 0.001%, and measurements of the absorbance of a solution with values about 0.43 have been made with a precision of about 0.000004.

The terms used in this paper are in conformity with the list published by the Society for Applied Spectroscopy (*17*):

Transmittance, $t = I/I_0 = P/P_0 = 10^{-abc} = 10^{-A}$

where I/I_0 is the ratio of the light intensity transmitted by sample I to the light intensity incident on sample, I_0; P/P_0 is similar but refers to the radiant power of the light source; a = absorptivity, b = length of optical path, c = concentration, and $abc = A = -\log_{10} t$ = absorbance.

The Beer-Lambert-Bouguer law, $P/P_0 = 10^{-abc}$, is referred to as the absorption law.

Barrier-type photocells in series have also been used by a large number of experimenters (*21–24*), but usually with somewhat lower precision as compared to the previous type of circuit. However, Rabinowitch (*13, 14*), using a pair of barrier-type photoelements in series (Figure 1*B*) and using a sensitive galvanometer as a detecting device for current differences, was able to detect concentration changes as small as 0.002%.

Nevertheless, in spite of these early successes in measuring small concentration differences, these principles have not been extended to routine colorimetric analysis.

Another approach to precision colorimetry was made as a result of the study of errors conducted by van Halban (*5*), Twyman (*18*), Kortüm (*10, 11*), Ringbom (*15, 16*), Hamilton (*8*), Ayers (*1, 19*), and others who examined photometric colorimetry in a wide variety of cases.

From these investigations the authors' present views have been derived. What has not been studied, however, is the type of error function obtained when colorimetric comparison of an unknown solution is made with a standard rather than a solvent. Because this case is of principal interest in measuring differential concentration changes, it is examined here and its application to colorimetry is developed.

THE TRANSMITTANCE RATIO

Consider a situation where three beams of light, of identical frequency and intensity, are permitted to fall on three identical absorption cells which contain, respectively, pure solvent, colored solution of concentration c_1, and colored solution of concentration c_2 (Figure 2).

A comparison of the various exit light intensities of the solutions with that of the pure solvent would give the following relations:

$$I_1 = I_0\, 10^{-abc_1}$$
$$I_2 = I_0\, 10^{-abc_2} \qquad (1)$$

These are, of course, the absorption law expressions for those situations where comparison is made against the solvent being used.

The ratio of the light intensities leaving the solutions of concentrations c_2 and c_1 would be:

$$\frac{I_2}{I_1} = \frac{I_0\, 10^{-abc_2}}{I_0\, 10^{-abc_1}} = 10^{-ab(c_2 - c_1)} = 10^{-ab(\Delta c)} \qquad (2)$$

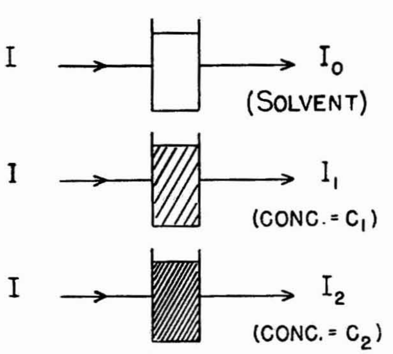

Figure 2. Transmittance Ratio of Three Beams of Light

This may be termed the transmittance ratio. It is apparent that it is the concentration difference between the two solutions which determines the value of I_2/I_1.

If the concentration difference now is expressed in terms of the concentration ratio α—i.e., c_2/c_1—the following expressions apply:

$$\frac{c_2}{c_1} = \frac{c_1 + \Delta c}{c_1} = \alpha \tag{3}$$

$$\Delta c = c_1(\alpha - 1) = c_2\left(\frac{\alpha - 1}{\alpha}\right)$$

$$\frac{I_2}{I_1} = 10^{-abc_1(\alpha - 1)} = 10^{-A_1(\alpha - 1)} \tag{4}$$

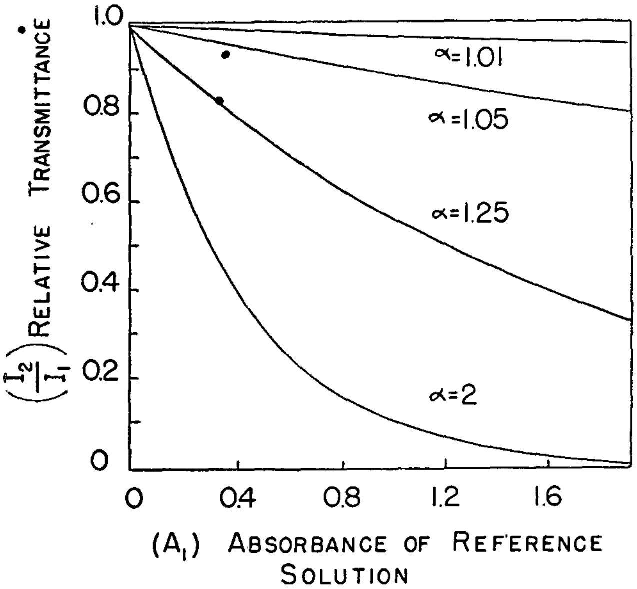

Figure 3. Absorbance of Reference Solution

Equation 4 indicates the relationship that would prevail if the transmittance of an unknown solution were measured by comparison against a solution of the same substance whose concentration was known. This is different from ordinary colorimetric practice. It requires that a standard solution be placed in one cell of a null-type colorimeter with the light intensity adjusted to give full-scale deflection of the galvanometer and in the other cell the unknown solution be placed, followed by balancing of the system by whatever circuit principle is used. Providing a light source of sufficient stability is part of the colorimeter, a single photocell instrument may also be used with substitution of the unknown solution after the instrument is set for maximum deflection by means of the color standard.

From an examination of Equation 4, the important role of the absorbance, A_1, of the standard solution becomes apparent. This is demonstrated in the plot of I_2/I_1 *vs.* A_1 given in Figure 3. The plots are made for different values of the concentration ratio, α.

It is evident that with increasing absorbance in the reference standard the transmittance ratio becomes increasingly sensitive to the ratio of the concentrations being compared. Even when α is only 1.01, it is still possible to demonstrate that fact without much difficulty by working with a reference standard whose absorbance is somewhat more than unity. For a standard whose absorbance is 1.7, I_2/I_1 would be equal to 0.96 in this case and if the colorimeter scale could be read to 0.005, a ratio as low as 1.0012 ought to be detectable.

The physical significance of this type of measurement will become immediately evident by reference to Figure 4.

In *A* the relationships of the various light intensities, I_0, I_1, and I_2, are shown for some particular case. These light intensities relationships are also identical with the corresponding photoelectric current relationships observed when the photoelement gives a linear response with light intensity. When the standard solution is made the basis for a comparison of the unknown, light intensity I_0 must be raised proportionately in order to make the photocurrent due to I_1 equal to that due to I_0. It is seen that raising I_0 to I_0' as in *B* raises I_1 to I_1' and I_2 to I_2'. Thus although the same relative proportions are retained, the difference in the current or instrumental response, I', is increased. Thus, effectively the light intensity scale is being lengthened and it is from this process that the enhanced sensitivity derives.

By comparing the precision of measurement in ordinary colorimetry with that of the relative transmittance function, it will be possible to ascertain the conditions requisite for increased precision.

PRECISION OF ORDINARY COLORIMETRY

In ordinary colorimetric work the light transmittance characteristics of the solvent and of the unknown solution are compared. The relative error is given by the term $\Delta C/C$, which may be evaluated as follows:

$$\log\frac{I}{I_0} = -abc$$

$$d\left(\log\frac{I}{I_0}\right) = \frac{0.04343\,d\left(\frac{I}{I_0}\right)}{\frac{I}{I_0}} = -abdc$$

Therefore:

$$\frac{dc}{c} = \frac{0.4343\,d\left(\frac{I}{I_0}\right)}{\frac{I}{I_0}\log\frac{I}{I_0}} = \frac{-0.4343\,d\left(\frac{I}{I_0}\right)}{A \times 10^{-A}} \tag{5}$$

The value of the error function may be approximated by substituting finite values for the infinitesimals as:

$$\frac{\Delta c}{c} = \frac{0.4343\Delta\left(\frac{I}{I_0}\right)}{\frac{I}{I_0}\log\frac{I}{I_0}} \tag{5a}$$

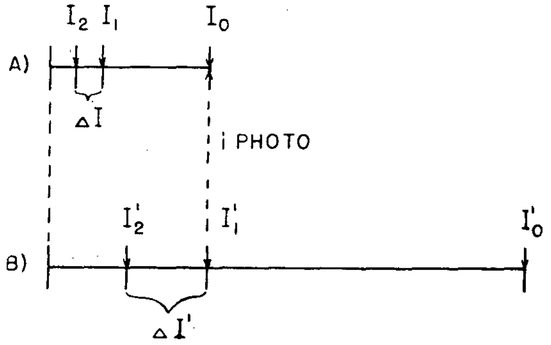

Figure 4. Light Intensity Relations

$\Delta(I/I_0)$ is the uncertainty of reading the exact position on the transmittance scale of the photoelectric colorimeter being used. It arises from the fact that the setting at some value of the transmittance is always uncertain by a constant quantity which in most commercial instruments will be of the order of 0.01 to 0.002 of the total scale. $\Delta(I/I_0)$ is therefore a constant quantity for any particular colorimeter being used. It may be evaluated experimentally by taking some standard solution and measuring its relative transmittance repeatedly. Each measurement should include the operations of emptying, refilling, and repositioning the cuvette. $\Delta(I/I_0)$ may be taken as twice the value of the average deviation.

A plot of the reciprocal of $I/I_0 \log I/I_0$ is given in Figure 5 as a function of the relative transmittance of the solution ($\Delta I/I_0$ is left unspecified). Here it can be seen that the relative error is infinite for 0.0 and 100% transmittances. At intermediate transmittances the relative error decreases to a minimum and then rises again. In the region between 20 and 65% the curve is nearly flat and hence a minimum and nearly constant error is achieved by working in this region.

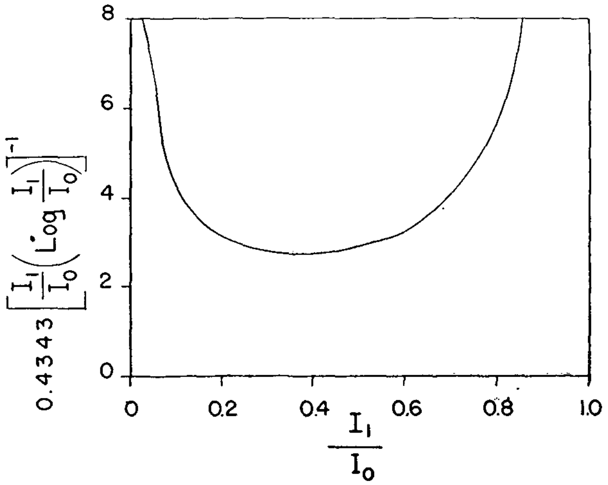

Figure 5. Relative Error as a Function of Transmittance

Differentiating Equation 5a with respect to I/I_0 and setting the function equal to zero gives the point of minimum error.

$$\frac{d\left[\dfrac{0.4343\ \Delta\left(\frac{I}{I_0}\right)}{\frac{I}{I_0}\log\frac{I}{I_0}}\right]}{d\frac{I}{I_0}} = \frac{-0.4343\,\Delta\left(\frac{I}{I_0}\right)\left(0.4343 + \log\frac{I}{I_0}\right)}{\left(\frac{I}{I_0}\log\frac{I}{I_0}\right)^2} \qquad (6)$$

Thus it is seen that minimum error is achieved when the absorbance, $-\log I/I_0$, is 0.4343 or when the transmittance of the solution is about 36.8%.

PRECISION OF TRANSMITTANCE RATIO MEASUREMENTS

The next error functions of interest are those involving the ratio of c_2/c_1. It may be shown that:

$$\frac{d(\alpha - 1)}{(\alpha - 1)} = \frac{d(\Delta c)}{\Delta c} \qquad (7)$$

$$\frac{d\alpha}{\alpha} = \frac{d(\alpha - 1)}{\alpha} = \frac{d(\Delta c)}{c_2} = \frac{dc_2}{c_2} \qquad (8)$$

Now by deriving the error functions in terms of I_2/I_1 ratios determined with a photoelectric colorimeter, expressions comparable to Equation 5 may be obtained:

$$\frac{d(\Delta c)}{\Delta c} = \frac{0.4343\, d\left(\frac{I_2}{I_1}\right)}{\frac{I_2}{I_1}\log\frac{I_2}{I_1}} \qquad (9)$$

$$\frac{dc_2}{c_2} = \frac{0.4343\, d\left(\frac{I_2}{I_1}\right)}{\frac{I_2}{I_1}\left(\log\frac{I_1}{I_0} + \log\frac{I_2}{I_1}\right)} \qquad (10)$$

Equation 10 may be derived easily by remembering that

$$\alpha = \frac{c_2}{c_1}$$

Then

$$d\alpha = \frac{1}{c_1}\,dc_2 \text{ and } \frac{d\alpha}{\alpha} = \frac{dc_2}{c_2}$$

Now

$$\alpha = 1 - \frac{1}{abc_1}\log\frac{I_2}{I_1}$$

Therefore

$$d\alpha = \frac{-0.4343}{abc_1} \times \frac{d\left(\frac{I_2}{I_1}\right)}{\frac{I_2}{I_1}}$$

and thus

$$\frac{d\alpha}{\alpha} = \frac{0.4343\, d\left(\frac{I_2}{I_1}\right)}{\frac{I_2}{I_1}\left(\log\frac{I_1}{I_0} + \log\frac{I_2}{I_1}\right)} = \frac{-0.4343\, d\left(\frac{I_2}{I_1}\right)}{\alpha A_1 \times 10^{-A_1(\alpha - 1)}}$$

From Equation 9 it is now apparent that the relative error of measuring $\alpha - 1$ or the concentration difference between two solutions is dependent on the I_2/I_1 value in exactly the same way as the error of measuring c_1 (Equation 5) was dependent on I_1/I_0. All that has been done in this case is that the standard solution has been considered to be a solvent and the concentration difference between it and the unknown has been evaluated. This measurement, therefore, cannot be made any more accurately than the ordinary colorimetric measurement.

In chemical analysis, however, the prime interest is in the value of the total concentration, and therefore, the main concern is with the error involved in that measurement. This error is seen (from an inspection of Equation 1 and the graphical plot in Figure 6) to be dependent in a pronounced manner on the value of the absorbance of the comparison standard.

In this graph the relative error, dc_2/c_2, is not plotted directly but instead 0.4343 times the reciprocal of $I_2/I_1(\log I_2/I_1 + \log I_1/I_0)$ is plotted on the ordinate and transmittance ratio on the abscissa. In this way the graph is generalized to cover all

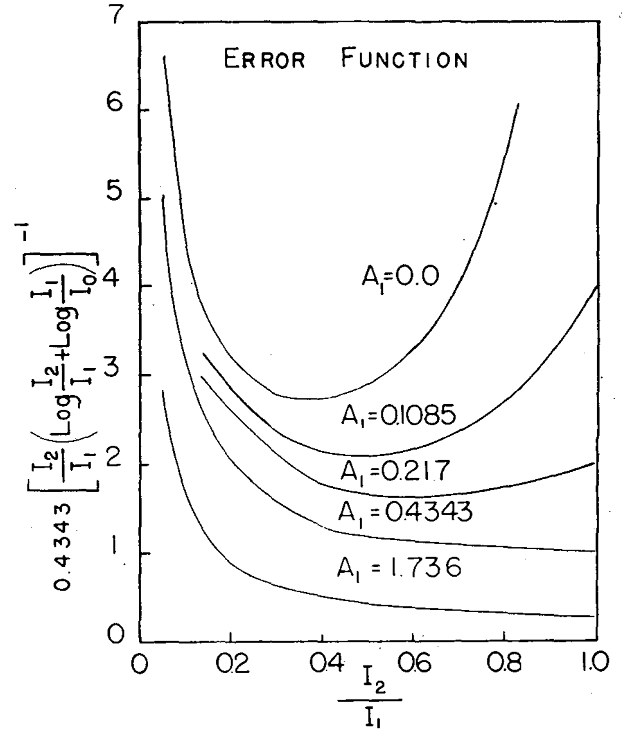

Figure 6. Error of Measurement

photoelectric colorimeters, inasmuch as the value of the term $\Delta(I_2/I_1)$ is not specified. Curves are given for a number of values of the absorbance of the standard solution in which A_1 has the following multiple values of 0.4343: 0.0, 0.25, 0.50, 1.0, and 4.0.

It is seen that the uppermost curve corresponding to an absorbance value of zero is the ordinary colorimetric case given in Figure 5. The ordinate has, however, been expanded in Figure 6 and as a result the curvature appears more pronounced.

As soon as the value of A_1 exceeds zero the value of the relative error becomes finite at the 100% end of the colorimeter scale. At all other values of the scale the error for any given absorbance of the standard is less than is the case with solvent comparison. As the value of the transmittance ratio approaches zero the entire group of curves approaches infinity. Therefore, little gain in precision is to be expected in that region of the scale. Fortunately, that region need not be used in any analytical operation. However, in the 100% region the decrease in error is extremely rapid with increasing absorbance of the standard. In Figure 7 a plot (solid line) is given for the error involved in measuring the concentration of an unknown when its concentration is identical with that of the standard—i.e., $A_2 = A_1$. This plot is made as a function of the relative transmittance of the standard.

Here it can be seen how rapidly the error declines as the absorbance increases. When the standard has a transmittance of as much as 0.69 the relative error of making this particular measurement is identical with minimum error achieved in ordinary colorimetry, and for the other values of I_2/I_1 at the same transmittance for the standard, the error is even less. It remains so until I_2/I_1 decreases to about 0.20, at which point it again becomes equal to the minimum of ordinary colorimetric practice. Nevertheless, there is always a relative gain in precision in working against a standard solution rather than a solvent.

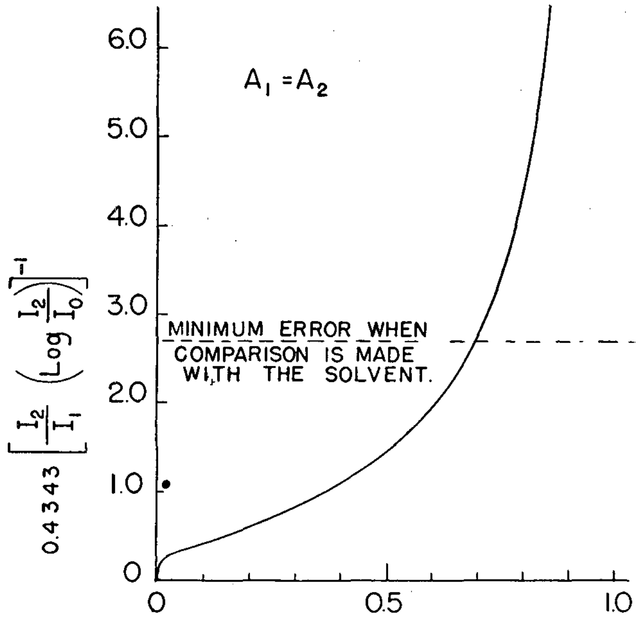

Figure 7. Relative Transmittance of Standard

It is also evident from Figure 6 that the position of minimum error progressively moves from 0.368 on the relative transmittance scale to 1.0 as the absorbance increases. The exact position of this minimum error may be obtained by differentiating the function with respect to I_2/I_1:

$$\frac{d\left(\frac{\Delta c_2}{c_2}\right)}{d\left(\frac{I_2}{I_1}\right)} = \frac{-0.4343\,\Delta\left(\frac{I_2}{I_1}\right)\left(\log\frac{I_2}{I_1} + \log\frac{I_1}{I_0} + 0.4343\right)}{\left(\frac{I_2}{I_1}\right)^2\left(\log\frac{I_2}{I_0}\right)^2} \tag{11}$$

and setting it equal to zero. When this is done

$$\log\frac{I_2}{I_1} + \log\frac{I_1}{I_0} + 0.4343 = 0 \tag{12}$$

also

$$\log\frac{I_2}{I_0} + 0.4343 = 0$$

and

$$\frac{I_2}{I_1} = 10^{(A_1 - 0.4343)} \tag{13}$$

represents the value of the relative transmittance when minimum relative error is to be expected.

Here it is seen that as the absorbance of the standard increases, the value of I_2/I_1 steadily increases and when the absorbance equals 0.4343 the minimum error is obtained when I_2/I_1 equals 1—i.e., at identical transmittances.

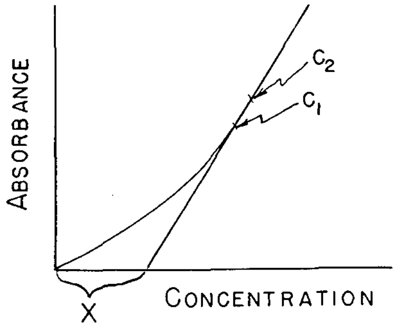

Figure 8. Deviation from Absorption Law Equation

$$I_1/I_0 = 10^{-(abc_1 - X)}$$
$$I_2/I_0 = 10^{-(abc_2 - X)}$$
$$dc/c_2 = \frac{d(I_2/I_1)}{I_2/I_1(\log I_2/I_1 + \log I_1/I_0 - X)}$$

At higher values of A_1, minimum errors are obtained when I_2/I_1 is in excess of 1. The physical meaning of this is that the scale is continually being lengthened as A_1 is increased, but that the value of A_1 is becoming progressively less than A_1. In practice it would mean that after setting I_1 at full-scale reading, on substituting I_2 in place of it, the galvanometer would move off the scale to higher values. Such a method is neither practical nor necessary. Therefore, the practical solution is to achieve at any given absorbance a balance as close to $I_2/I_1 = 1$ as is feasible but without ever having the ratio in excess of 1.

Were it not for the rapid decrease in light transmittance with increasing absorbance, the error could be reduced at will. In practice it will probably be impractical to work with an absorbance in excess of about 1.74 where the light transmittance would be only 1.8%.

With this limitation recognized, however, a considerable gain is still achieved in the precision. Examination of the plot (Figure 6) shows that when A_1 equals zero the minimum error is equal to 2.72. This occurs when the absorbance of the unknown is 0.4343 or has a transmittance of 36.8%. In the case where A_1 has a value of 1.736, the minimum error is 0.25 or about one eleventh that obtained with the solvent as the comparison standard. In this case, however, the absorbance of the unknown and the standard must be made nearly equal to achieve the precision gain.

This general treatment of error applies only to cases where the absorption law holds and where the chemical operations have greater precision than those of the colorimetric measurements. In practice, of course, solutions of high absorbance may often be of relatively high concentration. In such cases deviations from the absorption law may become apparent.

DEVIATIONS FROM THE ABSORPTION LAW

Deviations from the absorption law will be of two broad types. The first case, a positive deviation, is characteristic of systems undergoing complex ion formation. For example, a plot of log I_0/I_1 as a function of concentration of the cobaltous ion in 4 N hydrochloric acid might give the curve indicated in Figure 8 at some wave length in the 650 mμ region. Such a deviation is due to a progressive shift of the equilibrium

$$\underset{\text{pink}}{Co(H_2O)_4^{++}} \rightleftharpoons \underset{\text{blue}}{CoCl_4^{--}}$$

from left to right as the concentration increases. Obviously, the deviation would be eliminated if the ratio of the concentrations of the two forms was kept constant with increasing molarity of the cobalt in the system.

The other type of deviation, a negative one, is shown in Figure 9. This type of deviation is usually, although not always, caused by a white light impurity in the light source or by the broad wave-length interval of the light beam passed by the monochromator. In any case, where there is light impurity coupled with different absorptivities, a, for the different wave lengths passed, it is apparent that those wave lengths whose absorptivities have the highest values will be preferentially absorbed by the solution. Thus the absorptivity observed in a dilute solution will be higher than the coefficient observed in the more concentrated solution. In such a case the calibration curve will have a negative deviation.

In these cases the error functions may be derived in an approximate fashion by the following device.

Consider the curve in Figure 8 and assume that analyses are being made in the range indicated by the $c_1 - c_2$ interval. It may be assumed that the interval is accurately described by a tangent to the mid-point of the interval. If this tangent is then extended so that it intersects the abscissa, it becomes apparent that the relationship between the absorbance and concentrations c_1 and c_2 may be represented by the following equations:

$$\log \frac{I_0}{I_1} \cong abc_1 - X$$

and

$$\log \frac{I_0}{I_2} \cong abc_2 - X$$

[X in this expression represents the product of the distance along the concentration axis (from the point of origin to the intersection of the tangent) and ab. The absorptivity, a, is that value consonant with the slope of tangent and is different from the true absorptivity.]

from which may be derived the relationship

$$\frac{\Delta c_2}{c_2} \cong \frac{\Delta \left(\frac{I_2}{I_1}\right)}{\frac{I_2}{I_1}\left(\log \frac{I_2}{I_1} + \log \frac{I_1}{I_0} - X\right)} \qquad (14)$$

This is the error function for the positive deviation. In the case of the negative deviation the corresponding expressions become:

$$\log \frac{I_0}{I_1} \cong abc_1 + X$$

(X represents the distance along the ordinate from the point of origin to the intersection with the tangent. The absorptivity, a, is the value derived from the slope of the tangent in this case too.)

$$\log \frac{I_0}{I_2} \cong abc_2 + X$$

and

$$\frac{\Delta c_2}{c_2} = \frac{\frac{\Delta I_2}{I_1}}{\frac{I_2}{I_1}\left(\log \frac{I_2}{I_1} + \log \frac{I_1}{I_0} + X\right)} \qquad (15)$$

Thus it is apparent that positive deviations from the absorption law equation give greater precision, whereas negative deviations decrease that precision. To the extent that the positive deviation became increasingly pronounced, to just that extent does our precision increase. Only in rare cases will it be possible to arrange the experimental conditions to achieve a high degree of positive deviation. When this can be done, however, a large gain in precision may be expected.

The more common situation will be that of the negative deviation. If this deviation is pronounced, it may completely vitiate the precision anticipated for the high absorbance systems. It is only a necessary condition that X approach log I_0/I_1 for the precision of the analysis to be as bad as it is for ordinary colorimetric analysis. In fact, Equation 15 approaches the value

$$\frac{\Delta c}{c_2} = \frac{\Delta \frac{I_2}{I_1}}{\frac{I_2}{I_1} \log \frac{I_2}{I_1}}$$

when $X = \log I_0/I_1$. Here it can be seen that the error is approaching infinitely large values. Fortunately, this situation does not occur frequently but when it does rearranging the conditions of the reaction becomes a prerequisite to precise analysis.

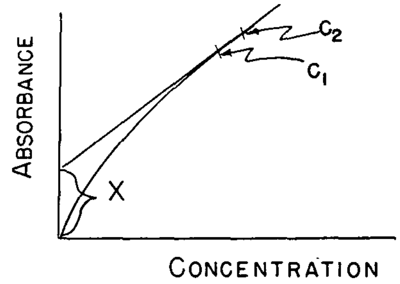

Figure 9. Deviation from Absorption Law Equation

$$I_1/I_0 = 10^{-(abc_1 + X)}$$
$$I_2/I_0 = 10^{-(abc_2 + X)}$$
$$dc/c_2 = \frac{d(I_2/I_1)}{I_2/I_1(\log I_2/I_1 + \log I_1/I_0 + X)}$$

TEMPERATURE CONTROL AND REFRACTIVE INDEX EFFECTS

A brief comment should be made on temperature effects and refractive index effects. If the standard and the unknown differ from each other in temperature and/or refractive index, the value of the transmittance ratio measured will be in error by an amount proportional to the magnitude of the difference. If the difference is a constant one from analysis to analysis, then although the accuracy of the work will suffer, the precision will not be altered.

The only effect of temperature to be considered here is that due to density changes produced in the solvent medium. Although it is an important aspect of the problem of precision analyses to consider the shift of various ionic equilibria with temperature change, this problem is reserved for a forthcoming paper.

For water the temperature coefficient of density at 25° C. is 1.00026 per ° C. Therefore, if the temperature of the standard and the unknown are kept within 2° of each other a precision of 1 part per 2000 may be achieved. For greater precision or with other solvents better temperature control may be needed.

The effect of a refractive index difference may be easily derived. From Fresnel's relation it is seen that the amount of light reflected at an interface is given by the relationship

$$I = I_0 \left[1 - \left(\frac{n - n_1}{n + n_1}\right)^2\right]$$

This expression is derived for an incident light beam normal to the interface and the n terms refer to the refractive indexes of the two phases—i.e., the cuvette wall and the solution. From this it can be shown that the transmittance ratio, I_2/I_1, can be expressed as:

$$\frac{I_2}{I_1} = \left(\frac{n_2}{n_1}\right)^2 \left(\frac{n + n_1}{n + n_2}\right)^4 10^{-A_1(\alpha - 1)}$$

and, consequently, the effect of slight differences in the refractive indexes of the unknown opposed to the standard solution may be evaluated.

It will be simplest to examine this effect by assuming that our two solutions have identical concentrations, so that $\alpha - 1$ is in effect zero. In this case I_2/I_1 will be equal to unity under ordinary circumstances. In the case where a refractive index effect is observed, however, this value will be reduced slightly by the refractive index correction term. For the case $n_2/n_1 = 1.010$ and with values of $n_1 = 1.33287$ and $n_2 = 1.5000$, the corrective term reduces I_2/I_1 to a value equal to 0.9988. Thus, instead of $\alpha - 1$ equaling zero, it will be equal to 0.0012 when the value of A_1 is equal to 0.4343. Thus it is apparent that a constant error of 12 parts per 10,000 has been introduced by neglecting the refractive index terms. Reducing the value of n_2/n_1 by one half will keep the error at no more than 1 part in 2000 or within the precision range for most careful analyses. It is not particularly difficult to do this, because relatively large electrolyte concentration changes are required to produce such refractive index changes. In this regard the papers of Chéveneau (*3*) may be consulted.

Table I. Comparison of Two Methods of Colorimetry on 2,4-Dinitrophenol

	I_1/I_0	I_2/I_0	$A_2 = \log_{10}I_0/I_2$	C_2, Detn. by Transmittance Ratio Method
First day	0.37072	0.36214	0.44112	4.982×10^{-5}
	0.37100	0.36254	0.44064	4.980
	0.37114	0.36280	0.44033	4.979
Av.	0.37095 ± 0.00016	0.36249 ± 0.00024	0.44139 ± 0.00028	
Second day	0.36940	0.36108	0.44239	4.979
	0.36968	0.36130	0.44213	4.980
	0.36994	0.36164	0.44172	4.979
Av.	0.36967 ± 0.00018	0.36134 ± 0.00020	0.44208 ± 0.00024	4.980 ± 0.001 } Av. of all 6

APPLICATION OF TRANSMITTANCE RATIO METHOD

The utility of the transmittance ratio method of colorimetry may be demonstrated from the published work of Kortüm (*10*) and Bastian (*2*). In a series of measurements of the absorptivity of 2,4-dinitrophenol solutions, Kortüm observed that greater precision could be obtained in determining concentrations by making a comparison of an unknown with a standard than in an absolute measurement of the absorbance. His data in part are listed in Table I.

If the average deviations from each of the four sets of transmittance data are averaged it will be found that $\Delta I/I_0 = \pm 0.00020$. The values of $\Delta A_2/A_2$ on the ordinary colorimetric basis and the $\Delta c/c_2$ by the transmittance ratio method now may be calculated and found to be ±0.00055 and ±0.00020, respectively. The observed values averaged for the two sets of absorbance and concentration values are ±0.00059 and ±0.00020 respectively. These are seen to be in good agreement with the experimental evidence.

Between the transmittance data of the two days there is an absolute shift of about 0.0012, due perhaps to some alteration in the optical or electrical circuit. This creates a large spread in the values for A_2 but is virtually without effect on the value of c_2 determined by the transmittance ratio. Thus in another way the advantages of this method are demonstrated.

More recently Bastian has applied the transmittance ratio method to the determination of copper in various copper-base alloys. The instrument used by him is the Beckman spectrophotometer (Model DU). In this instrument the optical scale may be extended by opening the slits so that a larger section of the spectrum is passed through the absorption cell. Working with solutions whose absorbances were about 2.0 he was able to make analyses of copper in lead brasses, phosphor bronzes, and synthetically prepared unknowns with a precision of ±0.001 to ±0.002. There is evidence in this work that the limits of precision are determined not by the colorimetric measurements but rather by the chemical operations. Nevertheless, the precision of his work, as well as the accuracy (assuming the electrolytic values to be correct, the agreement obtained colorimetrically was always within 1 to 2 parts per thousand), far surpasses any other colorimetric process previously reported.

In a future publication data on the performance of a number of commercial photoelectric colorimeters will be published. In a preliminary way it may be said that tests on solutions of a number of salts—i.e., potassium dichromate and copper sulfate—and on various dyes confirm the utility of this approach.

METHOD AS APPLIED IN COMMERCIAL COLORIMETERS

To apply the above principle to commercial photoelectric colorimeters or spectrophotometers it is a requisite condition that the instrument used have a variable optical scale. This variation is achieved in practice in a number of different ways. One method is that of adjusting the slits on a monochromator so that a larger fraction of the spectrum is passed through the solution. A second is to vary the current passing through the light source and thus increase the emission intensity. Another method is to operate the indicating device—i.e., galvanometer, etc.—with a variable sensitivity. Thus, if only a fraction of the total current is fed to a galvanometer during measurements on solutions of low absorbance, then when the shift is made to higher absorbance values the shunt may be adjusted to pass an increasing fraction of the photoelectric current through the galvanometer and thus effectively increase the optical scale.

The transmittance ratio measurement is then made with a pair of optically matched cuvettes, each containing one of the solutions it is desired to compare. Placing the standard solution in the monochromatic (or at least filtered) light beam, adjustment of the optical scale is made until the instrument reads 100% transmittance. The analyst must then make sure at this point that the lower end of the scale is at zero. With the instrument now properly set, the second solution is placed in the light beam, whereupon, if its absorbance is greater than that of the standard solution, the new value read will be less than 100. It will equal $100(I_2/I_1)$. Should the absorbance of the second solution be less than that of the standard, the reading of the I_2/I_1 value will be off the scale, as it will be in excess of 100. To make the comparison, then, it is necessary to reverse the order of the two cells.

There are several precautions to bear in mind in this type of colorimetry. In the first place the optical characteristics of a pair of cuvettes must be known with a precision equal to the best precision anticipated for the work. This will involve comparing the cuvettes when they contain a solution of high but identical absorbance in them. The absorbance of the solution will obviously be the maximum for which full-scale deflection can be achieved. Then the following terms may be applied:

$$\frac{I_2}{I_1} = 10^{-abc_1(\beta - 1)}$$

where

$$\beta = \frac{b_2}{b_1}$$

or the ratio of the apparent path lengths in the two cells. This ratio once evaluated is then applied as a correction term to all other work. It should of course be checked from time to time.

Next it will be necessary to determine the absorbance of the standard solution and to prepare a precise calibration curve for the purpose of seeing the agreement with the absorption law This should be done in the following manner:

Prepare a number of standard solutions covering the range of absorbances desired. If, for example, it is the intention to work from 0.1 to 2.0, a twentyfold concentration range would be needed. Now start with pure solvent in one cell and successively measure the transmittances, I_1/I_0, of the solutions until the value of I_1/I_0 has dropped to about 0.37. Then remove the solvent from the cuvette and substitute in it some of the solution which had the 0.37 value. Compare the two cuvettes to see if I_2/I_1 equals unity. In this way a preliminary value of β will be obtained.

Continue measuring I_2/I_1 values for the more concentrated solutions. Proceed in this way until the value of I_2/I_1 equals 0.37. This means that the transmittance of the solution being examined is only 0.135 with respect to the solvent although it is 0.37 with respect to a solution whose own transmittance is only 0.37. Again β is evaluated by changing our standard so that it is identical with the one already in the other cuvette. Taking this as the second reference point, I_2/I_1 values may be measured for the more concentrated solutions. In this way if the absorption law applies, the precision of measuring the slope relating A to c will be kept at the maximum value possible for the instrument and the absorbance range chosen.

When measurements for the entire concentration range which the instrument can handle have been made, the value of β will be finally evaluated and with maximum precision using the solution of highest concentration.

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Amides of Saturated Aliphatic Acids

An X-Ray Diffraction Study

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X-ray diffraction data for the amides of the saturated aliphatic acids (C_1 to C_{14}) are presented. Isostructuralism among the even members and also among the odd members of the series above C_8 is shown.

SINCE the simultaneous discovery by Debye and Scherrer in Europe and Hull in America of x-ray diffraction powder analysis in 1919, this type of pattern has been found useful in the identification of chemical compounds and minerals. The vast majority of printed data have been limited to inorganic compounds; a very small minority of published patterns are those of organic compounds. Organic compounds, as a rule, have much more complex patterns and exhibit wider diffraction bands than do inorganic compounds, which has made their patterns much more difficult to record. The ease and reliability of this method of identification for organic compounds, however, have increased the amount of research in this field.

Several papers on the identification of closely related organic chemical compounds have been published in recent years, including those by Velick (*6*) and Francis, Collins, and Piper (*1*). X-ray diffraction powder pattern data, published by Matthews and Michell (*4*) for the anilides of the saturated aliphatic acids, demonstrated the ability of this method to distinguish between each member of a homologous series.

For the present study, the authors chose the amides of the saturated aliphatic acids. The required amides were synthesized from the corresponding acids essentially by the method of Fuson and Snyder (*2*). One to 2 grams of the acid were refluxed 5 to 10 minutes with 5 ml. of thionyl chloride. The cooled reaction mixture was then poured into 15 ml. of concentrated ammonia at 0° C., and the resultant amide was filtered. The crude amide was purified by recrystallization from dilute ethanol.

With the exception of myristamide (C_{14}) all melting points agreed well with those recorded in the literature. Three recrystallizations of myristamide gave a constant melting point of 94–95° C., which is considerably lower than the value of 103° given by Kamm (*3*). The melting points of the amides used in this study, as compared with the values in the literature, are given in Table I.

Table I. Melting Points of Amides

Name	Content	M.P. Literature (*5*) ° *C.*	M.P., Experiment ° *C.*
Formamide	1	2	..
Acetamide	2	81	80
Propionamide	3	(79–80)	(78–79)
Butyramide	4	114	(115–116)
Valeramide	5	106	(102–104)
Caproamide	6	101	101
Heptylamide	7	96	96
Caprylamide	8	105	105
Pelargonamide	9	99	97
Decylamide	10	99	99
Undecylamide	11	99	(97–98)
Lauramide	12	100	(97–99)
Tridecylamide	13	100	(97–98.5)
Myristamide	14	103	(94–95)

Table II. Strongest Line of X-Ray Powder Diffraction Patterns (Flat Cassette)

Name	C Content	Strongest Line, Å.
Acetamide	2	3.52
Propionamide	3	8.3
Butyramide	4	10.0
Valeramide	5	11.3
Caproamide	6	14.5
Heptylamide	7	15.4
Caprylamide	8	18.1
Pelargonamide	9	19.6
Decylamide	10	21.2
Undecylamide	11	21.9
Lauramide	12	25.7
Tridecylamide	13	26.6
Myristamide	14	31.0

Table III. X-Ray Powder Diffraction Data

Formamide, C_1 I/I_0	Formamide, C_1 d, Å.	Acetamide, C_2 I/I_0	Acetamide, C_2 d, Å.	Propionamide, C_3 I/I_0	Propionamide, C_3 d, Å.	Butyramide, C_4 I/I_0	Butyramide, C_4 d, Å.	Valeramide, C_5 I/I_0	Valeramide, C_5 d, Å.
1.00	5.4	0.02	6.3	1.00	8.3	1.00	10.1	1.00	11.3
	4.52	0.66	5.7	0.06	6.6	0.01	8.9	0.03	8.7
	3.79	0.01	4.54	0.06	5.2	0.04	8.1	0.04	5.5
	3.41	0.08	4.00	0.74	4.70	0.14	4.98	0.01	5.1
	3.31	1.00	3.52	0.23	4.46	0.15	4.66	0.13	4.78
	3.21	0.16	3.29	0.23	4.14	0.07	4.22	0.01	4.52
	2.92	0.13	3.17		(3.92)[a]	0.11	4.00	0.01	4.26
	2.74	0.21	2.84	0.34	(3.85)[a]	0.53	3.76	0.29	4.06
	2.62	0.05	2.77		(3.80)[a]	0.04	3.64	0.02	3.74
	2.04	0.03	2.67	0.08	3.68	0.05	3.30	0.05	3.58
		0.04	2.53	0.20	3.52	0.04	3.16	0.02	3.33
		0.04	2.49	0.65	3.34	0.02	2.99	0.04	3.12
		0.01	2.39	0.07	3.20	0.09	2.87	0.01	2.92
		0.10	2.31	0.15	2.91	0.03	2.76	0.02	2.82
		0.02	2.23	0.14	2.83	0.05	2.63	0.03	2.73
		0.06	2.17	0.05	2.79	0.05	2.50	0.01	2.63
		0.06	2.14	0.12	2.68	0.01	2.46	0.02	2.51
		0.06	1.99	0.06	2.63	0.02	2.33	0.01	2.39
		0.03	1.94	0.04	2.59	0.02	2.27	0.01	2.25
		0.01	1.85	0.07	2.46	0.01	2.18	0.01	2.19
		0.02	1.78	0.07	2.40	0.03	2.13	0.01	2.07
		0.06	1.75	0.03	2.29	0.02	2.01	0.01	2.01
		0.07	1.71	0.04	2.21	0.01	1.97	0.01	1.95
		0.03	1.57	0.03	2.20	0.05	1.88		
		0.02	1.46	0.04	2.06	0.02	1.77		
		0.01	1.42	0.04	1.98	0.01	1.72		
		0.02	1.38	0.04	1.88	0.01	1.65		
		0.01	1.25						

Caproamide, C_6 I/I_0	Caproamide, C_6 d, Å.	Heptylamide, C_7 I/I_0	Heptylamide, C_7 d, Å.	Caprylamide, C_8 I/I_0	Caprylamide, C_8 d, Å.	Pelargonamide, C_9 I/I_0	Pelargonamide, C_9 d, Å.	Decylamide, C_{10} I/I_0	Decylamide, C_{10} d, Å.
0.37	11.4	0.09	11.9	0.07	6.0	0.10	6.5	0.27	7.1
0.82	4.84	0.37	4.92	0.03	5.44	0.37	5.5	0.05	5.3
1.00	4.50	0.08	4.71	0.62	4.90	0.72	4.94	0.64	4.91
0.33	4.28	0.08	4.50	0.28	4.72	0.08	4.76	0.54	4.48
0.20	3.82	0.27	4.24	0.07	4.56	0.85	4.42	0.14	4.18
0.99	3.64	1.00	3.72	0.52	4.36	0.08	4.18	0.03	4.00
0.22	3.05	0.08	3.51	1.00	4.10	1.00	3.90	1.00	3.79
0.09	2.76	0.07	3.24	0.14	3.88	0.16	3.80	0.16	3.48
0.14	2.35	0.04	3.07	0.23	3.62	0.19	3.54	0.32	3.32
0.09	2.32	0.04	2.90	0.20	3.50	0.50	3.39	0.05	3.21
		0.06	2.78	0.08	3.12	0.18	3.05	0.18	3.00
		0.04	2.67	0.04	2.98	0.13	2.80	0.13	2.76
		0.04	2.61	0.08	2.85	0.06	2.68	0.06	2.67
		0.01	2.54	0.02	2.60	0.05	2.58	0.04	2.59
		0.03	2.45	0.02	2.51	0.14	2.40	0.03	2.51
		0.03	2.38	0.03	2.45			0.05	2.44
		0.02	2.28	0.03	2.37			0.06	2.39
		0.02	2.09	0.03	2.26			0.06	2.32
		0.01	2.02	0.01	2.18			0.05	2.16
		0.03	1.84	0.02	2.11			0.04	2.10
				0.03	2.03			0.05	1.97
				0.04	1.88			0.04	1.88
								0.04	1.83
								0.05	1.80
								0.03	1.76
								0.04	1.71

Undecylamide, C_{11} I/I_0	Undecylamide, C_{11} d, Å.	Lauramide, C_{12} I/I_0	Lauramide, C_{12} d, Å.	Tridecylamide, C_{13} I/I_0	Tridecylamide, C_{13} d, Å.	Myristamide, C_{14} I/I_0	Myristamide, C_{14} d, Å.
0.11	7.3	0.20	8.2	0.35	8.7	0.17	11.0
0.07	5.4	0.56	4.90	0.06	5.2	0.31	9.4
0.71	4.90	0.07	4.74	0.38	4.91	0.38	4.90
0.62	4.38	0.61	4.50	0.24	4.70	0.04	4.74
0.10	4.10	0.43	4.11	0.58	4.52	0.66	4.48
1.00	3.92	0.27	3.87	0.33	4.27	0.26	4.12
0.43	3.66	1.00	3.64	1.00	3.80	1.00	3.94
0.27	3.54	0.17	3.38	0.30	3.60	0.34	3.74
0.21	3.43	0.11	3.20	0.33	3.46	0.36	3.57
0.05	3.24	0.05	2.96	0.08	3.28	0.07	2.97
0.08	3.19	0.11	2.87	0.10	3.07	0.09	2.77
0.13	3.12	0.08	2.72	0.06	2.90	0.06	2.46
0.10	2.99	0.03	2.55	0.06	2.72	0.06	2.30
0.24	2.86	0.07	2.41	0.10	2.35	0.04	2.21
0.19	2.75	0.03	2.37	0.10	2.24	0.04	1.95
0.07	2.45	0.07	2.22	0.06	2.06	0.04	1.86
0.06	2.35	0.03	2.14	0.03	1.98		
0.07	2.22	0.03	1.92	0.05	1.88		
0.06	2.14	0.05	1.84	0.05	1.80		
0.04	2.09	0.05	1.82	0.04	1.71		
0.06	1.99	0.03	1.73				
0.04	1.94						

[a] Not resolved.

Table IV. Three Strongest Lines of X-Ray Powder Diffraction Patterns (Cylindrical Camera)

Name	C Content	Interplanar Spacings● First Line Å.	Interplanar Spacings● Second Line Å.	Interplanar Spacings● Third Line Å.
Formamide	1	3.41	...	...
Acetamide	2	3.52	5.7	2.84
Propionamide	3	8.3	4.70	3.34
Butyramide	4	10.1	3.76	4.66
Valeramide	5	11.3	4.06	4.78
Caproamide	6	4.50	3.64	4.84
Heptylamide	7	3.72	4.92	4.24
Caprylamide	8	4.10	4.90	4.36
Pelargonamide	9	3.90	4.42	4.94
Decylamide	10	3.79	4.91	4.48
Undecylamide	11	3.92	4.90	4.38
Lauramide	12	3.64	4.50	4.90
Tridecylamide	13	3.80	4.52	4.91
Myristamide	14	3.94	4.48	4.90

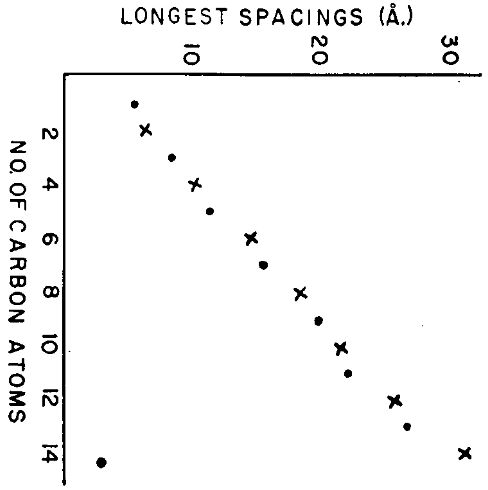

Figure 1. Largest *d* Values of Saturated Aliphatic Acid Amides Plotted against Number of Carbon Atoms

× Even amides
● Odd amides

The x-ray diffraction patterns were made on a General Electric Model XRD Type 9 unit utilizing the standard Debye-Scherrer method. The sample amide was finely ground in an agate mortar, tightly packed into a wedge-type specimen holder, and mounted in the center of a cylindrical camera of 14.32-cm. diameter. Copper radiation filtered through a 0.00035 nickel filter, was used, giving essentially Kα radiation of wave length 1.537 A. The cutoff due to the construction of the cylindrical camera occurred at approximately 12 Å.

In order to record the longer *d*-spacings of the higher amides the film was placed at a greater distance, 10 cm., from the sample and was held in a flat cassette camera. The intensity of the spacings in the flat cassette films was estimated visually by comparison with the cylindrical films. The strongest lines appearing on the flat cassette films of the amides, C_2 to C_{14}, are listed in Table II.

The x-ray powder diffraction data for the amides of the aliphatic acids, C_1 to C_{14}, are presented in Table III. The intensities are recorded relative to the intensity of the strongest line in each pattern, as measured by a Leeds & Northrup recording microphotometer. The three strongest lines of each pattern are listed in Table IV.

A marked similarity is apparent by visual observation among the members of the even series C_8 to C_{14}, showing a similarity of structure. The odd members of the series above C_8 also exhibit this isostructuralism. These findings are in accord with those of Matthews and Michell (*4*) in their study on the anilides and also those of Slagle and Ott (*5*) in their study of the fatty acids.

In Figure 1, the largest *d* values of the saturated aliphatic acid amides are plotted against the number of carbon atoms. Unlike the aliphatic anilides, the amides do not show any evidence of a zigzag structure when the longest spacings are plotted against the number of carbon atoms.

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Determination of Dibasic Acids in Alkyd Resins

Quantitative Methods of Analysis for Phthalic, Sebacic, Fumaric, Maleic, Succinic, and Adipic Acids

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No specific methods of quantitatively determining individual acids in a mixture of dibasic acids in alkyd resins have been previously reported. New methods of analysis, accurate to 0.2% and specific for phthalic, fumaric, maleic, and sebacic acids in alkyd resins, are now available. Under certain conditions, succinic and adipic acids can also be determined by newly developed procedures. A new technique for accurately determining the nonvolatile content of resins, needed for calculating the dibasic acid content, is included.

OF THE synthetic resins, the alkyds probably possess the widest range of applicability, especially for automotive equipment. They are unusually tough and durable and possess excellent solubility, compatibility, adhesion, and gloss-retention properties. Their use, on a volume basis, probably exceeds any other synthetic resin. The properties of the alkyds can be varied by the addition of various constituents to produce modified alkyd resins—the class most frequently encountered in commercial analysis. Originally, alkyd resins were composed entirely of phthalic anhydride, glycerol, and an oil constituent, dissolved in solvent. Among the addition constituents used to vary properties are such other acids as fumaric, maleic, succinic, sebacic, and adipic. The composition of modified alkyds is becoming more and more diverse as constantly increasing new materials are being put to use.

Several methods of determining phthalic anhydride in the unmodified class of alkyds have been proposed and used (*3*). They were designed for use on alkyds that were known to be unmodified and dissolved in solvents that were known not to interfere in the determination. These methods and their many modifications have proved very useful for approximately 15 years, in accomplishing the limited purpose for which they were intended. The best of these methods based their calculations on the weight of the insoluble products of saponification, assumed to be dipotassium phthalate monoethanolate. They are actually a measure of the total insoluble products of saponification including other dibasic acid salts, and frequently contaminated with pigments, metallic hydroxides, potassium carbonate, phenolic condensates, cellulosic materials, etc. During the saponification process small quantities of chlorinated hydrocarbon solvents yield formates that would be weighed and calculated as phthalic anhydride and that resemble dipotassium phthalate in appearance and solubility properties. In addition, results have never been highly reproducible even under controlled conditions and variations of 3% on known samples in the hands of different analysts are common. There is extensive need for a more specific method of analysis for phthalic anhydride as well as the other dibasic acids.

The uses and methods of application of alkyd resins require varied dilutions, so that any quantitative analysis of the dibasic acids present must be based on the nonvolatile content. Surveys have shown that there is even greater variation in the results of determining the solids content of resins of known composition than in the phthalic content. Thus the need for a method of accurately determining the nonvolatile content assumes importance comparable to that for the dibasic acids.

The methods of analysis, detailed herein, enable qualitative identification of phthalic, fumaric, maleic, sebacic, and succinic acids in alkyd resins and are quantitatively accurate to 0.2% for phthalic, fumaric, maleic, and sebacic acids. The nonvolatile determinations are equally accurate. These methods are simple to conduct with standard laboratory equipment and reagents, and are readily adapted to a routine testing procedure. Although complete analysis of some resins may require 3 days, the total man-hours applied are comparatively few.

After saponification by means of a modified Kappelmeier technique, the dibasic acids are separated from the reaction mixture as insoluble potassium salts. The salts are then converted to the acids and purified.

REAGENTS

Alcoholic Potassium Hydroxide, 0.5 *N*, 32.8 grams of c.p. reagent grade potassium hydroxide, assay 85.6%, per liter of absolute ethyl alcohol. This reagent can be prepared rapidly by refluxing 30 minutes, cooling, and filtering through a fritted-glass filter funnel. A fresh supply should be used.

Bromine in Sodium Bromide. Exactly 0.75% solution of liquid bromine in aqueous 50% sodium bromide, c.p., prepared in the following manner:

A quantity of aqueous 50% sodium bromide solution is prepared, using c.p. salt and freshly boiled distilled water. This solution is filtered if not perfectly clear and kept in a glass-stoppered container. When used, the stopper and neck of the flask should be wiped dry to prevent freezing of the stopper. A 125-ml. glass-stoppered flask containing approximately 25 ml. of the 50% sodium bromide solution is weighed and approximately 1 ml. of bromine is added. The exact amount of bromine added is determined by reweighing the flask. It is then transferred to a glass-stoppered graduate and diluted with 50% sodium bromide solution to a volume, calculated from the weight of the bromine used, to give a resulting 0.75% solution. The solution is stored in a dark cabinet. The reagent is stable.

Whenever a new quantity of reagent is prepared it should be checked by withdrawing 2.5 ml. and diluting to 100 ml. The colorimetric reading of this blank should agree closely with the blank readings used to prepare the colorimetric graph. Otherwise, it will be necessary to regraph known quantities or use a correction factor.

Lead Acetate, 25% solution of lead acetate trihydrate (neutral) in glacial acetic acid. Heat may be necessary to dissolve the lead salt. The solution may cloud slightly on standing but is satisfactory for use and need not be filtered.

Mercurous Nitrate, 5% solution of mercurous nitrate monohydrate in 2 N nitric acid, prepared by dissolving 5 grams of the salt in 50 ml. of 4.0 N acid (or equivalent) and diluting to 100 ml. with water. The solution should be agitated during dilution. The reagent is stable.

Zinc Acetate, 2.5% aqueous solution of zinc acetate dihydrate prepared by dissolving 25 grams of the salt in approximately 900 ml. of water, adjusting pH to 6.0 with dilute acetic acid, then diluting to 1 liter. This solution is clear and stable when so prepared.

SEPARATION AND PURIFICATION OF DIBASIC ACIDS

Samples should be run in duplicate or triplicate. The triplicate sample may be useful in determining acids that are present as traces.

A sample of the resin solution, estimated to yield 0.1 to 0.3 gram of potassium salts, is weighed by difference from a closed container into a predried Erlenmeyer flask. Ten milliliters of benzene are added and the sample is dissolved by swirling, warming if necessary. Then 125 ml. of 0.5 N alcoholic potassium hydroxide are added and mixed with the sample. The flask is placed in an oven maintained at 52° ± 2° C., and agitated occasionally for the first few hours. After at least 18 hours in the oven, it is removed and cooled to room temperature and 40 ml. of absolute ethyl ether are added. The flask is tightly stoppered and allowed to stand at least 2 hours. The solution is then filtered through a dry fritted-glass filter crucible, medium porosity, of 30-ml. capacity, prepared with an additional mat of medium-fiber-length filtering asbestos. A 60-ml. fritted-glass Büchner funnel may be substituted for the crucible if desired. The crucible (or funnel) need not be weighed, although an approximate weighing of second decimal accuracy may be of value in estimating aliquot size for subsequent analysis, if anything is known of the nature of the alkyd being analyzed. Absolute ethyl alcohol is used to transfer the precipitate and wash the flask and crucible.

If any difficulty is encountered in scrubbing and quantitatively transferring the contents of the flask, the residual alcohol may be driven off in an oven (110° C.) and the precipitate remaining in the flask dissolved in water. This is combined with the remainder of the sample when the contents of the filter crucible are dissolved in water. The filter crucible is dried at 105° to 110° C. The contents of the crucible are then dissolved in 75 ml. of water. If the Fisher Filtrator is used, the sample is collected in a 250-ml. beaker and an additional transfer is eliminated.

The sample is acidified with nitric acid to exactly pH 2.0, using a pH meter. The solution will normally cloud somewhat on acidifying. If a coarse white precipitate appears, sebacic acid is likely to be present. If this occurs and reasonable dilution fails to redissolve the precipitate, sebacic acid should be determined as in the quantitative procedure for analyzing sebacate alkyds containing more than 2% sebacic acid. The acidified sample is thoroughly stirred and filtered after standing 30 minutes or longer, into a 100 ml. (or other suitable size) volumetric flask. Because the impurities being filtered off are very finely divided it is necessary to filter through a double thickness of the finest filter paper—S. & S. No. 589, Blue Band, gives excellent results. Two folded papers fitted one inside the other, so that the three fold side of one meets the singlefold side of the other, give maximum retention. The paper is washed with water and the sample is diluted to volume. Aliquots are withdrawn for qualitative and quantitative identification as outlined.

QUALITATIVE IDENTIFICATION OF DIBASIC ACIDS

The recommended qualitative identification procedures for phthalic, sebacic, fumaric, and maleic acids are identical with the quantitative procedure for each, so that no separate test is necessary. However, the presence of maleic and fumaric acids is first determined by a test that does not distinguish between the two acids; if a positive test indicates the presence of either or both of these acids, an additional procedure is necessary to determine the fumaric acid. In the absence of fumaric acid, the indications of the first test are calculated as maleic.

The following qualitative test for succinic acid should be made: A 25-ml. aliquot of the sample is withdrawn into a 100-ml. beaker and dried in an oven at 60° C. To obtain the dried acids in fine crystalline form, the dried residue is dissolved in a minimum amount of water and approximately 25 ml. of ethyl alcohol are added. After mixing thoroughly, the sample is redried in an oven at approximately 80° C. Two milliliters of phenylhydrazine are added, and the beaker is covered and heated in an oven at 100° C. for 1 hour with occasional agitation. On cooling, 25 ml. of benzene are added and the sample is stirred occasionally over a period of 1 hour. It is then filtered through a fritted-glass crucible or through paper, transferred, and washed thoroughly with benzene. Whatman black filter paper, No. 29, is advantageous. Upon drying in air or in a low-temperature oven, the residue is washed thoroughly and repeatedly with large quantities of distilled water. If a water-insoluble residue of dianilinosuccinic acid remains, succinic acid is present in the resin sample. Further identification of the water-insoluble residue is not necessary, but if desired, it may be mixed with zinc chloride and moistened with hydrochloric acid; upon fusion, it will form a fuchsin-red mass. The dianilinosuccinic acid melts at about 205° C., and sinters from about 190° C. (*5*).

QUANTITATIVE DETERMINATION OF DIBASIC ACIDS

The behavior of dibasic acids other than those listed is not known, as only the commonly used acids have been investigated.

Phthalic acid is precipitated as lead phthalate in glacial acetic acid—the only specific method developed to determine phthalic in the presence of other dibasic acids. All dibasic acids interfere with the method (*2*) proposed some years ago for determining phthalic acid by precipitating with lead acetate in aqueous solution.

Sebacic acid is determined by precipitating as zinc sebacate. This method is specific and no other dibasic acids interfere.

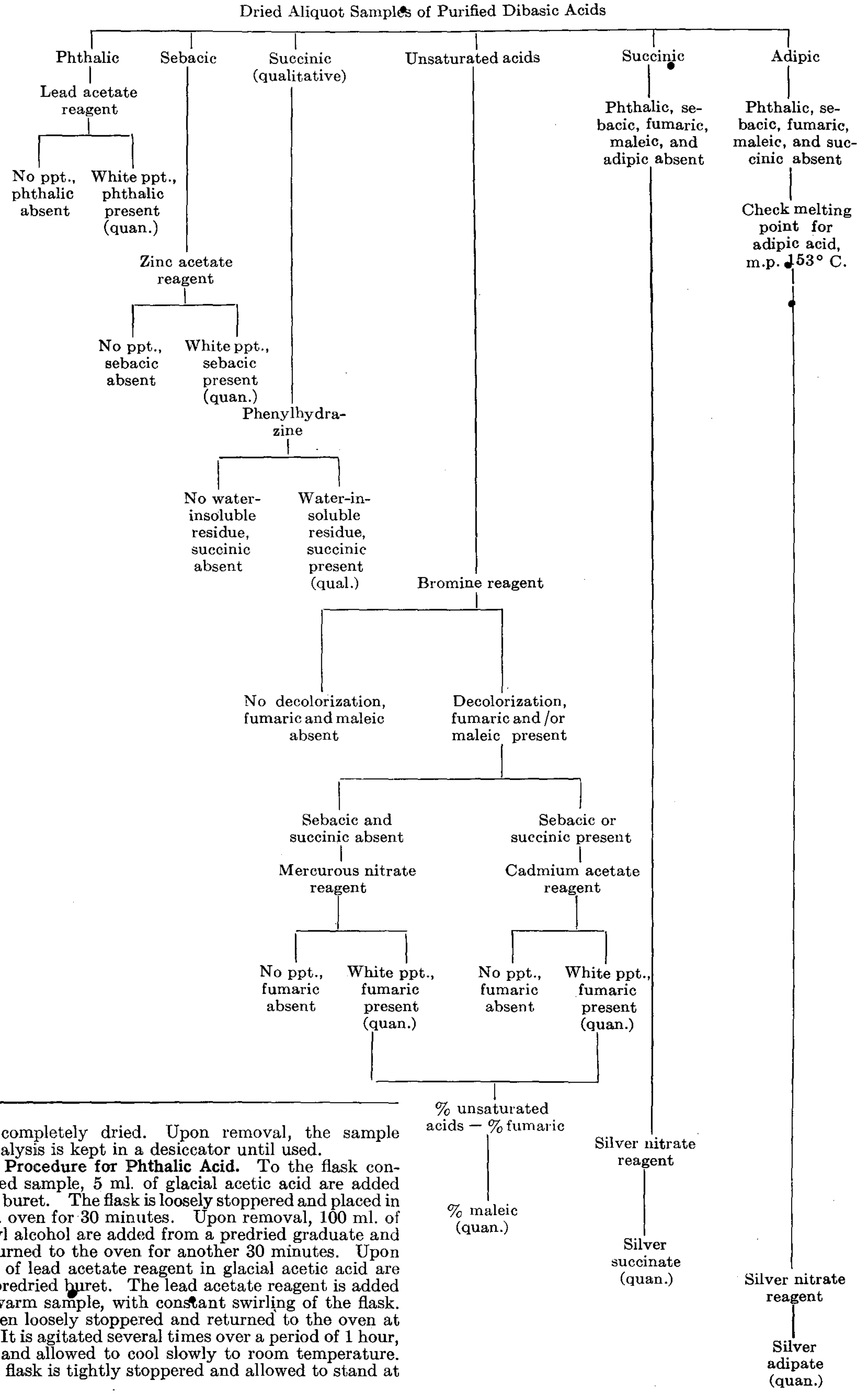
The unsaturated acids, maleic and fumaric, are detected and maleic acid is determined colorimetrically by the degree to which they decolorize a bromine reagent. No other dibasic acids interfere, but this method does not distinguish between fumaric and maleic acids. If the presence of either is indicated by the bromine reagent, specific analysis is then made for fumaric acid by one of the methods listed below. In the absence of fumaric, maleic acid is calculated from the bromine method. If both acids are present, maleic is determined by deducting the fumaric content from the total unsaturated acid content. When the fumaric acid content, as determined by one of the precipitation methods, equals the content as obtained by the bromine method, maleic acid is absent.

Fumaric acid is determined either as mercurous fumarate (when succinic and sebacic acids are absent), or as cadmium fumarate, precipitated in glacial acetic acid. The mercurous nitrate reagent does not precipitate any dibasic acids except fumaric; however, when either succinic or sebacic acid is present, slightly high yields are obtained, necessitating the precipitation of fumaric acid with cadmium acetate. Both methods are given because many samples containing fumaric acid will not contain succinic or sebacic. The mercury method is faster and simpler; the cadmium method is specific.

No specific methods of determining adipic and succinic acids in the presence of other dibasic acids have been developed. However, if either acid occurs alone in an alkyd resin, it may be precipitated quantitatively as the silver salt.

Aliquots of the purified aqueous solution of the acids are withdrawn as follows: 10 ml. into a 250 ml. glass-stoppered Erlenmeyer flask, for phthalic analysis; 10 ml. into a 250-ml. beaker, for sebacic analysis; and 25 ml. into a 250-ml. beaker for fumaric-maleic analysis. All are then placed in an oven at

Figure 1. Graphic Representation of Scheme for Identification and Determination of Dibasic Acids



60° C. until completely dried. Upon removal, the sample for phthalic analysis is kept in a desiccator until used.

Quantitative Procedure for Phthalic Acid. To the flask containing the dried sample, 5 ml. of glacial acetic acid are added from a predried buret. The flask is loosely stoppered and placed in a 50° to 60° C. oven for 30 minutes. Upon removal, 100 ml. of absolute methyl alcohol are added from a predried graduate and the flask is returned to the oven for another 30 minutes. Upon removal, 2 ml. of lead acetate reagent in glacial acetic acid are added from a predried buret. The lead acetate reagent is added slowly to the warm sample, with constant swirling of the flask. The flask is then loosely stoppered and returned to the oven at 50° to 60° C. It is agitated several times over a period of 1 hour, then removed, and allowed to cool slowly to room temperature. When cool, the flask is tightly stoppered and allowed to stand at least 12 hours.

The sample is then filtered through a specially prepared predried and weighed crucible consisting of a medium-porosity, fritted-glass filter crucible to which additional mats of short- and medium-fiber asbestos have been added, so that the short-fiber asbestos forms the top layer. In filtering, the contents of the flask are first agitated, then transferred to the crucible, and the liquid portions are drawn through. The remaining contents of the flask are then transferred with a stream of absolute ethyl alcohol, preferably from a one-hand Smith design wash bottle. Scrubbing is rarely necessary. The sides of the crucible are washed down with the ethyl alcohol and all excess is drained off. The crucible is then redried at 110° C. for one hour, cooled, and weighed. For maximum accuracy, precipitate weight should not be less than 0.0250 gram, nor more than 0.2000 gram.

CALCULATION. Precipitate × 0.33937 = weight of phthalic acid in aliquot.

Precipitate × 0.30254 = weight of phthalic anhydride in aliquot.

Quantitative Procedure for Sebacic Acid. To the 250-ml. beaker containing the dried aliquot, exactly 70 ml. of water are added. The sample is heated to boiling under cover and 30 ml. of zinc acetate reagent are added slowly from a pipet with constant stirring. Boiling is continued for 1 minute and the sample is cooled to room temperature. After 1 hour, it is filtered through a predried and weighed crucible, transferred and washed with absolute ethyl alcohol. The crucible is redried at 110° C. for one hour, cooled, and weighed. For maximum accuracy, the precipitate should weigh not less than 0.015 gram, nor more than 0.100 gram.

In the analysis of sebacic alkyds or resins containing more than 1 or 2% sebacic acid, the process of converting the potassium salts to acids and purifying the sample may have to be modified. Sebacic acid is only slightly soluble in water, about 1.0 gram per liter, and frequently precipitates when solutions of the potassium salt are acidified. To analyze sebacic alkyds, the following procedure is used:

The dipotassium salts are dissolved in water and diluted to volume. An aliquot is withdrawn, diluted to 70 ml., and acidified (pH 3.0) with hydrochloric acid. If sebacic acid precipitates, a smaller aliquot is withdrawn. The sample is then filtered free of resinous matter, if necessary, and heated to boiling; 30 ml. of the zinc acetate reagent are added and the analysis is concluded as outlined above.

CALCULATION. Precipitate (zinc sebacate) × 0.76134 = weight of sebacic acid in aliquot.

Quantitative Procedure for Fumaric-Maleic Acids. The dried sample aliquot contained in a beaker is dissolved in 50 to 75 ml. of freshly boiled and cooled distilled water. It is transferred with water, or filtered if not absolutely clear, into a 100-ml. volumetric flask, and 2.5 ml. of the bromine reagent are added from a clean, dry buret. The sample is diluted to volume and thoroughly mixed. A blank is prepared at the same time with only the sample omitted. If sharp fading of the reagent occurs within 5 minutes, the sample aliquot may be too large. The flasks are placed in a dark cabinet and, after 24 hours, readings are obtained from an electrophotometer, using a blue light filter (approximate wave length, 425 millimicrons).

CALCULATION. The fumaric-maleic acid content is determined by the difference between readings of the sample and the blank by consulting a graph previously prepared in like manner, using known quantities of pure acids or salts of either fumaric or maleic acids. (If maleic anhydride is the only available standard, it should be converted to the acid before use.) The graph is a straight line and should be prepared in terms of fumaric and maleic acids. If desired, the maleic acid content may be changed to maleic anhydride by multiplying by 0.84478. Minimum amount determinable is 1 mg.; maximum, 6 mg.

Quantitative Procedure for Fumaric Acid (succinic and sebacic acids absent). This method is useful either when fumaric acid is known to be present (and sebacic and succinic are absent) or when a fading of the bromine reagent in the procedure for unsaturated acids indicates the presence of either fumaric or maleic acids (sebacic and succinic acids proved absent).

An aliquot of appropriate size is withdrawn from remaining sources of sample into a 250-ml. beaker. The size of aliquot is estimated from the degree of decolorization of bromine reagent obtained by use of the 25-ml. aliquot. This aliquot does not have to be dried. It is diluted to 90 ml. and 10 ml. of mercurous nitrate reagent are added from a pipet, with rapid stirring. If fumaric is present in excess of other acids, a white precipitate forms immediately; if relatively small quantities are present, a stand of several hours or overnight may be necessary. In either case, the sample should be stirred frequently and vigorously, and must not be heated. Several hours after maximum precipitation has apparently been obtained, the sample is filtered through a predried and weighed crucible, transferred, and washed thoroughly with at least 100 ml. of water. The crucible is then dried for 1 hour at 110° C. and weighed. If fumaric acid is present in excess of other dibasic acids, the precipitate weight may range from 0.0100 to 0.1500 gram; if relatively small quantities are present, the minimum precipitate weight should be 0.0500 gram, or larger aliquots should be used.

CALCULATION. Precipitate × 0.22520 = weight of fumaric acid in aliquot.

Quantitative Procedure for Fumaric Acid (succinic or sebacic acid present). An aliquot of appropriate size is withdrawn from the original 100-ml. volumetric flask, into a 125-ml. glass-stoppered Erlenmeyer flask and dried in an oven at 60° C. Using a predried buret, exactly 10 ml. of glacial acetic acid are added. The flask is loosely stoppered and heated until solution is effected. One gram of cadmium acetate crystals, $Cd(CH_3CO_2)_2.2H_2O$, are added and the flask is placed in a 70° C. glycol bath or oven for at least 18 hours. It should be agitated occasionally for the first few hours and may be stoppered tightly after 30 minutes. Upon removal from the oven, 50 ml. of absolute ethyl alcohol are added and the sample is returned to the oven for 30 minutes with periodic agitation. The sample is then filtered through a fritted-glass filter crucible of medium porosity. The precipitate is transferred and thoroughly washed in the crucible with absolute ethyl alcohol. Light scrubbing of the flask may be necessary. For maximum accuracy, the precipitate should weigh not less than 0.0250 gram, nor more than 0.2500 gram.

CALCULATION. Precipitate (cadmium acetate fumarate) × 0.25396 = weight of fumaric acid in aliquot.

Quantitative Procedure for Adipic or Succinic Acid (all other dibasic acids absent). The dipotassium salts, precipitated from alcoholic potassium hydroxide are dissolved in 70 to 80 ml. of water, acidified with dilute acetic acid to pH 5.5, filtered if necessary, and diluted to volume in a 100-ml. volumetric flask. Aliquots are withdrawn into beakers or flasks of suitable size and diluted to 95 ml. for adipic analysis, or to 245 ml. for succinic analysis. Five milliliters of 20% aqueous silver nitrate solution are added with rapid stirring. Samples are then covered or stoppered and placed in a dark cabinet for at least 18 hours. They should be agitated frequently, however, for several hours after precipitation. The precipitated salts are filtered through tared Gooch crucibles, using ethyl alcohol to transfer and wash. Air is drawn through the crucibles until most of the alcohol has evaporated. The crucibles are then dried at 110° C. for 1 hour, cooled, and weighed. For maximum accuracy, maximum precipitate weight should be 0.1000 gram.

CALCULATIONS. For succinates. Precipitate × 0.35579 = weight of succinic acid in aliquot.

For adipates. Precipitate × 0.40598 = weight of adipic acid in aliquot.

Determination of Nonvolatile Content of Resin. This determination is necessary whenever it is desired to express the dibasic acid content of an alkyd resin on the nonvolatile basis.

The method consists of precipitating the resin with water from an alcoholic medium, using proportions that maintain an excess of the precipitating agent as the lower boiling azeotropic mixture is driven off. The resin dries as a thin spongy film which permits the escape of volatile solvents and oils. The container used for the determination is a nonrusting, seamless aluminum box, 3.5 inches (8.75 cm.) in diameter and 2 inches (5 cm.) high. It must have a flat bottom and straight sides and be provided with a tight-fitting cover. The aluminum boxes often used for moisture determination in soil analyses are ideal. The container is provided with a short glass rod 3.75 inches long, with well polished ends. The rod should fit inside the box when the lid is in place, without falling into the bottom.

The aluminum box, dried in an oven at 110° C. and cooled in a desiccator, is tared with rod and cover and 50 ml. of absolute ethyl alcohol are added. A sample of the resin, estimated to yield not more than 0.5 gram of solids, is weighed by difference from a tube into the box. If the resin is dispersed throughout the alcohol by stirring, no warming of the sample is necessary and 10 ml. of water are added from a pipet with stirring to the cold sample. If the resin does not mix with the alcohol, the alcohol is slowly brought to its boiling point by warming the pan gently on a hot plate. It is thoroughly stirred, 10 ml. of water are added at once from a pipet with stirring, and the pan is immediately removed from the hot plate. If the resin is completely soluble in alcohol the ratio of water to alcohol should be 2 to 1, so that it is more convenient to reduce the alcohol volume to 20 ml. and add 40 ml. of water. The box and sample are placed in a drying oven at 105° to 110° C. on a mat of asbestos. The pan cover is placed separately in the oven. After 4 hours of drying time the uncovered pan is transferred to a desiccator. After 1 hour the cover is replaced on the box and the box is weighed.

CALCULATION. % nonvolatile =

$$\frac{(\text{weight of box plus solids} - \text{weight empty}) \times 100}{\text{weight of resin sample}}$$

DISCUSSION

The modifications in the process of saponifying alkyd resins are made to obtain complete precipitation of maleic and fumaric acids. These acids saponify slowly and are not completely precipitated by any of the previously proposed methods of saponifying alkyd resins. The specially prepared crucibles recommended for collecting the potassium salts are particularly valuable whenever fumaric and maleic acids are present in the resin, because these acids form very finely divided crystals which will either clog or pass through ordinary filter crucibles. The appearance of the dried potassium salts resulting from the saponification of an alkyd resin gives some indication of its nature, in that dipotassium phthalate has a characteristic glossy appearance that is usually hidden somewhat by the presence of even a small percentage of the potassium salts of other dibasic acids. All the potassium salts of the dibasic acids are partially soluble in absolute methyl alcohol. If isopropyl alcohol is substituted for ethanol as a saponifying medium and the precipitated salts are weighed, high yields are obtained, due to the coprecipitation of undesirable resinous products, and to the formation of crystals which hold the alcohol even at 110° C. drying temperature. Methanol and isopropyl alcohol are unsuitable media for saponifying alkyd resins.

The technique of acidifying the potassium salts of dibasic acids with nitric acid and drying at low temperature was found satisfactory for converting salts to acids in so far as the analytical procedures for subsequent analysis are concerned. In fact, no completely satisfactory method of extracting the dibasic acids has been found. No decomposition takes place when nitric acid is driven off at the recommended drying temperature of 60° C. The potassium nitrate that is still present in the sample has no effect on the analytical procedures developed.

The methods of analysis were developed by working first with pure dibasic acids. Purity of the acids was determined by titration with standard alkali. The maleic and phthalic acids were prepared from the anhydrides by dissolving in water and redrying. The methods found suitable for analysis of the acids were then applied to solutions prepared from potassium salts by acidifying with nitric acid and drying.

All samples analyzed should be tested qualitatively for rosin. A very convenient and satisfactory rosin test is as follows (*4*):

> A few drops or 1 ml. of the resin solution are dissolved in approximately 3 ml. of chloroform and about 3 ml. of acetic anhydride are added. After thorough mixing, 1 drop of concentrated sulfuric acid is added. If free rosin is present, an intense purple color appears, which quickly changes to intense blue. The presence of modified forms of rosin may produce red or orange colorations in this test. When rosin is present in alkyd resins, there is some doubt that maleic and fumaric acids will be precipitated as potassium salts, or if analysis shows their presence, it is doubtful that quantitative yield is obtained. The presence of rosin does not appear to affect the analysis for other dibasic acids.

One method of analysis for fumarates was developed in the course of the work, but does not appear in the procedures. The ordinary dibasic acids which appear in alkyd resins do not interfere with the method, but succinates and sebacates, if present, cause high results. This quantitative method is based on Steenhauer's qualitative microtest for organic acids (*6*).

> The sample, containing from 15 to 100 mg. of potassium fumarate, is dissolved in 5 ml. of water. The pH of the solution is adjusted to 3.5 with sulfuric acid and 5 ml. of reagent (4 volumes of 10% copper sulfate solution to 1 volume of pyridine) are added. The sample is agitated occasionally and allowed to stand at least 18 hours. It is then filtered through a fritted-glass crucible containing an additional mat of asbestos, transferred, and washed with alcohol. The precipitate is air-dried and dissolved in 20 ml. of 1 to 1 ammonium hydroxide. It is transferred with water to a 100-ml. volumetric flask and diluted to volume with water. The blue solution is compared at once on an electrophotometer against a tube of water and using a red light filter (approximate wave length 650 millimicrons). The weight of fumarate or fumaric acid in the sample is calculated from a graph previously prepared in similar manner with a series of knowns or from pure copper sulfate standards. Copper standards may be used because the theoretical weights of copper and fumaric acid react, although the precipitate is unstable and cannot be dried and weighed.

The Denigès qualitative reaction for succinic acid and succinates (*1*) is suitable for detecting succinates in the saponification products of resins, but has limited usage because the test is carried out in hot concentrated sulfuric acid in which all dibasic acids and salts, except succinates and phthalates, char and darken the solution, so that the red color formed with succinates cannot be detected.

The alcohol used in the precipitation of lead phthalate serves two purposes. It helps to dissolve the dried acids and it coarsens the crystalline precipitate so that it is filterable.

In the precipitation of fumaric acid with cadmium acetate, each of the replaceable hydrogens is replaced by $Cd.CH_3CO_2$, forming cadmium acetate fumarate.

Table I. Analysis of Pure Dibasic Acids

Sample Weight, Gram			
	Phthalic Acid (Pure Phthalic Acid Standard)		
	Precipitate weight, gram	Factor from yield	Phthalic acid calcd. from av. factor, gram
0.0100	0.0299	0.33445	0.0101
0.0200	0.0591	0.33841	0.0200
0.0500	0.1470	0.34014	0.0499
0.0700	0.2081	0.33638	0.0706
Average for 20 samples		0.33937	
	Sebacic Acid (Dipotassium Sebacate Standard)		
	Equivalent weight as sebacic acid, gram	Zinc sebacate precipitated, gram	Sebacic acid, calcd. from theoretical factor, gram
0.0250	0.01798	0.0238	0.01811
0.0500	0.03595	0.0475	0.03616
0.0750	0.05393	0.0701	0.05337
	Using pure sebacic acid standard		
	0.0125	0.0166	0.01264
	0.0250	0.0331	0.02520
	Fumaric Acid as Mercurous Fumarate (Pure Fumaric Acid Standard)		
	Mercurous fumarate precipitated, gram		Fumaric acid, calcd. from theoretical factor, gram
0.0200	0.0889		0.0200
0.0250	0.1100		0.0247
0.0300	0.1339		0.0302
	Fumaric Acid as Cadmium Acetate Fumarate (Pure Fumaric Acid Standard)		
	Cadmium acetate fumarate precipitated, gram		Fumaric acid, calcd. from theoretical factor, gram
0.0050	0.0192		0.0049
0.0100	0.0395		0.0100
0.0249	0.0983		0.0249
0.0709	0.2796		0.0710
	Succinic Acid (Dipotassium Succinate Standard)		
	Equivalent weight as succinic acid, gram	Silver succinate precipitated, gram	Succinic acid, calcd. from theoretical factor, gram
0.0500	0.03039	0.0860	0.0306
0.0750	0.04558	0.1279	0.0455
0.1000	0.06078	0.1704	0.0606
	Adipic Acid (Dipotassium Adipate Standard)		
	Equivalent weight as adipic acid, gram	Silver adipate precipitated, gram	Adipic acid, calcd. from theoretical factor, gram
0.0250	0.01643	0.0402	0.0[illegible]632
0.0250	0.01643	0.0401	0.01[illegible]28
0.0500	0.03286	0.0813	0.03301
0.0750	0.04929	0.1223	0.04965

Table II. Analysis of Alkyd Resins

Resin No.	Manufacturer's Estimated Composition (Whole Basis), %		A.S.T.M. D 563–45T (Calcd. as Phthalic Anhydride), %	Composition Calcd. from Saponification Yield as Modified, %	Per Cent Composition (as Determined by Detailed Analyses)				
					Phthalic anhydride	Maleic anhydride	Fumaric acid	Sebacic acid	Succinic acid
1	Phthalic anhydride	17.87	18.2, 18.3	18.9, 19.0	17.83	0.6	..	..	..
	Maleic anhydride	0.77			17.81	0.61	..	..	..
	(guaranteed composition)								
2	Phthalic anhydride	30–31	32.4, 33.9	37.5, 37.6	31.59	...	5.4	..	..
	Fumaric acid	4.5–5.5			31.72	...	5.0	..	..
	(on solids basis; solids, 49.75)								
3	Phthalic anhydride	9.5–10.5	18.4, 19.0	19.2, 19.3	9.43	...	0.11	..	7.66[a]
	Succinic acid	7.5–8.5			9.6	...	0.10	..	..
	Fumaric acid	0.1–0.2							
4	Phthalic anhydride	23–25	24.0, 25.1	28.6, 28.66	24.6	1.4	..	..	..
	Maleic anhydride	1.8–2.8			24.8	1.54	..	..	..
5	Maleic anhydride	6–8	3.1, 3.5 (calcd. as maleic anhydride)	6.6, 6.6 (calcd. as maleic anhydride)	...	None	6.1	..	..
					...	...	6.3	..	..
6[b]	Sebacic acid	68.7	68.9, 69.1 (calcd. as sebacic acid)	67.9, 68.0	...	...	..	67.7	..
					...	...	..	67.6	..
7	Specification 3-181 sample submitted for analysis of phthalic anhydride	30–40	32.1, 33.2	30.9, 31.1	28.7	...	..	..	...
					28.45	...	..	..	..

[a] Succinic acid content calculated by difference between acids determined and composition by saponification.

[b] Oil-modified sebacic acid plasticizing-type resin.

Table III. Nonvolatile Determinations

	Resin Solution	Manufacturer's Estimate, %	A.S.T.M. D 154–43, %	Maximum Deviation, %	Average, %	Precipitation Method, %	Maximum Deviation, %	Average, %
1.	Long oil air-dried alkyd	80	81.3, 81.7, 81.2	0.5	81.4	80.3, 80.5, 80.4	0.2	80.4
2.	Short oil nondrying alkyd in xylene	60	58.9, 59.8, 58.4	1.4	59.0	58.2, 58.3, 58.3	0.1	58.27
3.	Oxidizing-type alkyd with linseed oil	33.01% made by dissolving weighed quantity of 100% resin in weighed quantity of toluene	34.1, 33.8, 34.0	0.3	33.97	33.2, 33.3, 33.15	0.15	33.22
4.	Oil-modified sebacic acid, plasticizing-type resin in toluene	60	61.1, 60.9, 61.8	0.9	61.27	59.7, 59.7, 59.6	0.1	59.67
5.	Rosin-modified maleic anhydride	59.2	60.6, 61.3, 63.2	2.6	61.7	59.59, 59.66, 59.7	0.11	59.65
6.	Rosin maleic[a]	Approx. 50.24% made by dissolving 100% resin in toluene	51.4, 51.0, 50.8	0.6	51.1	48.33, 48.37, 48.31	0.06	48.34

[a] Resin solution 6, made by dissolving a weighed sample of dry crystalline solids, contained foreign matter. Solution was not filtered, because of risk of losing solvent and changing calculated solids content.

When lead phthalate precipitates from either water or acetic acid solutions, there is always a certain amount of coprecipitation of the lead acetate reagent. This coprecipitation is constant under the conditions of test as outlined. The factor for calculating phthalic anhydride from the lead phthalate precipitate is empirical rather than theoretical.

Tables I to III present some analytical results. A study of the results of analysis of alkyd resins shows that the yields from the saponification of resins by either the A.S.T.M. or the modified procedure as outlined, agree reasonably well when maleic and fumaric acids are absent. The A.S.T.M. procedure for determining phthalic anhydride consists essentially of refluxing the sample with alcoholic potassium hydroxide for 1.5 hours, cooling quickly, adding ether, and filtering immediately. This technique neither prevents the precipitation of dipotassium fumarate and dipotassium maleate nor accomplishes complete precipitation of these salts. The saponification yields are higher by the modified procedure wherever maleic and fumaric acids are present.

Resin samples 5 and 6 contain no phthalic anhydride, yet if the nature of the samples were unknown and an analysis for phthalic anhydride were made by any of the existing methods based on saponification yields, positive results would be obtained. In the case of sample 6, these results would be very high as well as false. A similar situation exists in the case of resin sample 3, which contains 9.5% phthalic anhydride but analyzes as 18.7% by saponification procedures. In all cases, the sum of the actual dibasic acids present is always less than the indications of the saponification yields. Resin sample 1 is the only sample available with guaranteed exact composition. Formulated to contain 17.87% phthalic anhydride, this sample analyzes 17.82% by the new method.

The results of analysis of resin sample 5 are of special interest. This sample was formulated by the manufacturer with 6 to 8% maleic anhydride, but analyzes 6.2% fumaric acid, with no maleic present. However, maleic acid was found in two other samples in which fumaric was absent. Paint chemists have long surmised that this change or its reverse, fumaric to maleic acid, took place under certain conditions in the making of certain resins, but in the absence of a suitable analytical procedure for determining the two acids, the conditions under which these changes take place have not been surveyed. All resin samples tested containing maleic and fumaric acids seem to be slightly low by comparison in their content of these two acids.

ACKNOWLEDGMENT

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Identification of Dicarboxylic Acids in Polymeric Esters

Preparation and Properties of the Dibenzyl Amides of Eight Typical Dicarboxylic Acids

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American Cyanamid Company, Stamford, Conn.

By refluxing polymeric esters with benzylamine in the presence of a catalyst, characteristic derivatives of the constituent dicarboxylic acids can be obtained. The resulting dibenzyl amides can be separated into saturated and unsaturated fractions by the use of selective solvents. Individual dibenzyl amides can be identified in admixture by comparison of their infrared spectra with those of a standard set of dibenzyl amides prepared from monomeric esters.

IN THE early years of the present century, the original commercial alkyd resins, the reaction products of glycerol and phthalic anhydride, presented a fairly simple analytical problem. The situation became more complicated as these resins were modified by the introduction of additional constituents, such as fatty acids, rosin, natural resins, and phenol and amine aldehyde condensation products. It was found possible, however, to adapt the Kappelmeier procedure (*6, 8*) to a semiquantitative separation method (*7*). Originally designed as a saponification technique for the quantitative estimation of phthalic anhydride content, the Kappelmeier procedure could also be employed as the basis of a qualitative identification scheme (*7*).

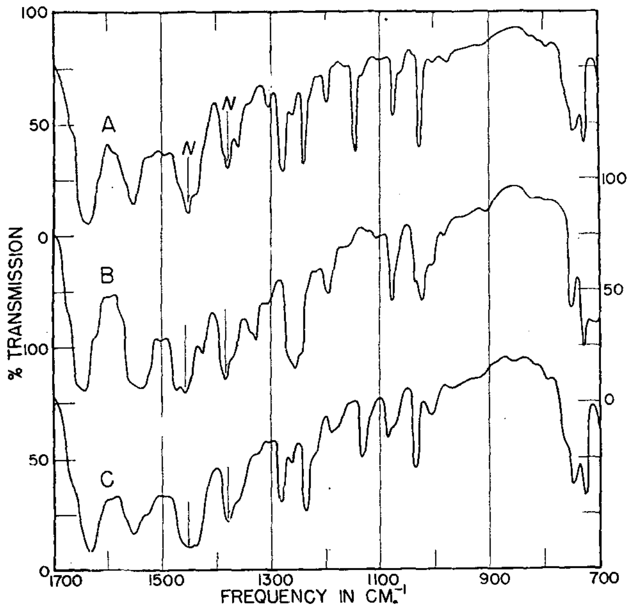


Figure 1. Infrared Spectra of Dibenzyl Amides

A. Adipic acid. *B.* Azelaic acid. *C.* Sebacic acid

A further analytical complication, however, has been introduced by the increasing variety of polycarboxylic acids and glycols. The complexity of the problem is apparent from Wakeman's (*9*) discussion of alkyd resins in general and contact resins in particular. The definite need for improved techniques has been stated by Bradley (*2*) in his survey for the American Society for Testing Materials, and has been emphasized by the experiences of Subcommittee XI of A.S.T.M. Committee D-1.

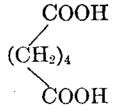
In recognition of this need, a series of investigations has been planned, designed to take advantage of the many war-stimulated developments that have occurred in the field of physical instrumentation. The present report is concerned with the application of one such procedure.

It was decided to select eight commercially important dicarboxylic acids as a basis for investigation. The optimum preliminary step seemed to be the conversion of the selected acids to the diethyl esters. This procedure permits the efficient purification of the acids by the fractional distillation of the respective esters. It also provides a set of liquid derivatives to serve as the source of additional analytical data, as well as stock compounds of known purity for the series of planned alternative analytical procedures. Esters are particularly suitable for the latter purpose, because they can be converted readily to amides, salts, and acids.

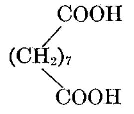
It was originally intended to complete and report on the analytical characterization of the diethyl esters before proceeding further. This phase of the investigation, however, did not appear to offer a quick solution. Because the demand for a method more satisfactory than those currently available was urgent, attention was transferred to a procedure promising usable results in a shorter time. The investigation of the benzyl amide method, which is the subject of this paper, was therefore selected.

EXPERIMENTAL

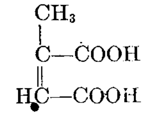
The eight acids chosen for this and subsequent investigations were as follows—four saturated (including phthalic in this sense) and four unsaturated acids are included:


$$\begin{array}{l}\mathrm{COOH}\\ \mathrm{(CH_2)_4}\\ \mathrm{COOH}\end{array}$$

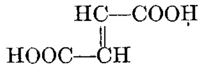
Adipic


$$\begin{array}{l}\mathrm{COOH}\\ \mathrm{(CH_2)_7}\\ \mathrm{COOH}\end{array}$$

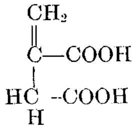
Azelaic


$$\begin{array}{l}\mathrm{CH_3}\\ \mathrm{C{-}COOH}\\ \| \\ \mathrm{HC{-}COOH}\end{array}$$

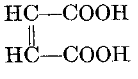
Citraconic


$$\begin{array}{l}\mathrm{HC{-}COOH}\\ \quad\| \\ \mathrm{HOOC{-}CH}\end{array}$$

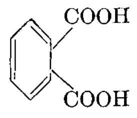
Fumaric


$$\begin{array}{l}\mathrm{CH_2}\\ \| \\ \mathrm{C{-}COOH}\\ \mathrm{HC{-}COOH}\\ \mathrm{H}\end{array}$$

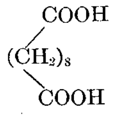
Itaconic


$$\begin{array}{l}\mathrm{HC{-}COOH}\\ \| \\ \mathrm{HC{-}COOH}\end{array}$$

Maleic



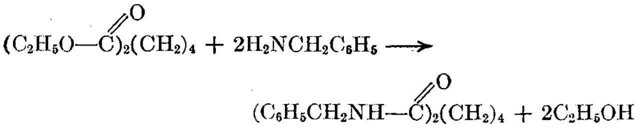
Phthalic


$$\begin{array}{l}\mathrm{COOH}\\ \mathrm{(CH_2)_8}\\ \mathrm{COOH}\end{array}$$

Sebacic

Approximately 1 kg. of the diethyl ester of each of the above acids was prepared and purified by fractional vacuum distillation. Details of the preparation and characteristic physical properties determined on the esters will be reported in a subsequent paper.

Preparation of the Amides. The formation of *N*-benzyl amides proceeds according to the following reaction, using diethyl adipate as an example:



$$(C_2H_5O{-}\overset{O}{\overset{\|}{C}})_2(CH_2)_4 + 2H_2NCH_2C_6H_5 \longrightarrow (C_6H_5CH_2NH{-}\overset{O}{\overset{\|}{C}})_2(CH_2)_4 + 2C_2H_5OH$$

They were chosen as promising derivatives for two reasons: (1) *N*-benzylamine has been reported (*3*) to yield satisfactory products with many of the acids under consideration. (2) *N*-benzylamine is a relatively strong base capable of cleaving monomeric esters. A similar reaction with polymeric esters was therefore considered probable.

In 1943, Dermer and King (*3*) reported the results of a well planned and thorough investigation of the suitability of *N*-benzyl amides as derivatives for 90-odd carboxylic acids. The preparation of the present amides was based on their procedure, which may be briefly summarized as follows:

Reflux 1 ml. of liquid or 1 gram of solid ester (or acid) for 1 hour with 3 ml. of benzylamine and 0.1 gram of ammonium chloride as catalyst. Cool, and wash with water to remove the excess amine. If no solid separates, acidification with hydrochloric acid sometimes precipitates the desired amide. If enough unreacted ester is left to keep the amide in solution, the ester may be expelled by boiling the oily layer with water. Filter off the solid amide, dry, wash with ligroin, and recrystallize, usually from aqueous acetone or ethanol.

Attempts to apply the above procedure to polymeric ester led to the several modifications which are incorporated in the following directions and discussed subsequently in some detail:

Reflux 10 grams of ester for 2 hours with 30 ml. of benzylamine and 1 gram of ammonium chloride. Pour the reaction mixture into 300 ml. of benzene, and allow to cool to room temperature. (Bulky precipitates usually require a double precipitation.) Filter off the precipitated amide, dissolve in a minimum volume of warm ethanol, and reprecipitate by pouring into 300 ml. of dilute hydrochloric acid (approximately 1 *N*). Filter off the precipitated amide, wash with cold 50% ethanol, and dry.

Concentrate the filtrate from the first separation of amide to one half the original volume, cool, and pour into 300 ml. of petroleum ether. Purify the precipitated amide as outlined above.

Table I. Nitrogen Content of the Dibenzyl Amides

Prepared from	% Nitrogen Determined	% Nitrogen Calculated
Adipic ester	8.53	8.64
Azelaic ester	7.47	7.65
Citraconic ester	8.98	9.09
Fumaric ester	9.48	9.53
Itaconic ester	8.96	9.09
Maleic ester	9.44	9.53
Phthalic ester	7.98	8.13
Sebacic ester	7.25	7.37

The amides prepared by the application of the modified procedure to the eight diethyl esters were dried to constant weight in vacuo. A portion of each was dissolved in ethanol, and titrated to a phenolphthalein end point with 0.5 *N* alcoholic potassium hydroxide. No titratable acidity was observed in any instance.

Under the microscope, the amides appeared as homogeneous crystalline products. The determination of the various optical properties will be discussed in a subsequent paper.

Nitrogen Content. The results of Kjeldahl nitrogen determinations run on the eight dibenzyl amides are listed in Table I. The theoretical values are included for comparison.

Melting Points. The melting points of the eight dibenzyl amides were determined by the capillary method, using a Thiele tube equipped with an air bubble stirrer, and were corrected for emergent stem. The results, rounded to the nearest integer, are listed in Table II. The data reported by Dermer and King are included for comparison.

Infrared Examination. The application of infrared examination to the separated benzyl amide fractions permits the qualitative identification of individual acids present in the mixture. Herein lies the principal advantage of the proposed method.

Inasmuch as the use of infrared methods in the solution of chemical problems has been discussed (*1*), no attempt is made here to consider the fundamental aspects of the method.

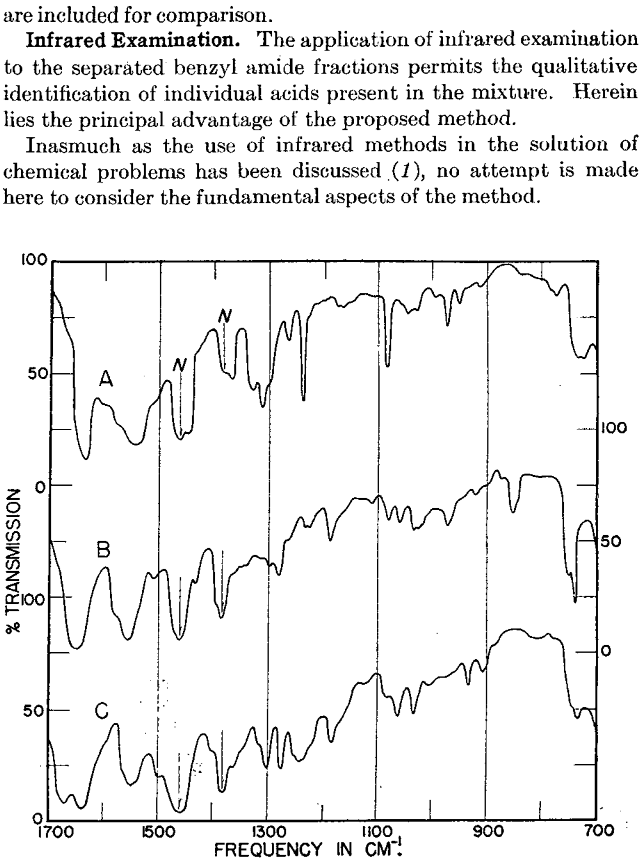


Figure 2. Infrared Spectra of Dibenzyl Amides

A. Phthalic acid. *B*. Fumaric acid. *C*. Itaconic acid

The spectra shown in Figures 1 and 2 were obtained on Nujol oil slurries of the benzyl amides, using a Perkin-Elmer infrared spectrometer Model 12A. The instrument was equipped with a sodium chloride prism, and had been converted to a per cent transmittance spectrophotometer. The spectra are limited to six curves, since, because of isomerization, the amides obtained from maleic and fumaric esters were identical, as were those from citraconic and itaconic esters (see also the determined melting points). To conserve space, only the region from 1700 to 700 cm.$^{-1}$ is shown, although further characteristic differences were apparent in the rest of the spectrum. The absorption contributed by Nujol is designated by the letter N in each figure. It is apparent that the differences illustrated are sufficient to permit the differentiation of the amides even among fairly close members of the same homologous series—e.g., Figure 1.

Qualitative Solubility Data. Inasmuch as the infrared data demonstrated the presence of only six discrete amides, the solubility measurements were confined to these. The solvents

Table II. Melting Points of Dibenzyl Amides

Prepared from	Reported M.P., ° C.	Determined M.P., ° C.
Adipic ester	188–189	189
Azelaic ester	Not reported	147
Citraconic ester	Not reported	106
Fumaric ester	203.5–205	207
Itaconic ester	Not reported	106
Maleic ester	149–150	206
Phthalic ester	178–179	178
Sebacic ester	166–167.5	166

selected were benzene, petroleum ether, and 50% ethanol, all at room temperature. In the case of the saturated amides, the solubility was negligible in all three solvents.

The solubility of the unsaturated amides in petroleum ether was negligible. A low degree of solubility in benzene was observed, as well as a somewhat higher solubility in 50% ethanol. The itaconic amide was the more soluble in both solvents, with a calculated approximate maximum solubility of 750 to 800 mg. per 100 ml. of benzene, and 2000 to 2500 mg. per 100 ml. of 50% ethanol.

DISCUSSION OF EXPERIMENTAL RESULTS

Preparation. In applying the present procedure to polymeric samples, particularly to contact resins, the polyester fraction is first isolated by precipitation with a nonsolvent such as petroleum ether.

The justification and advantages of the modified preparation procedure used in the present investigation may be summarized as follows:

An increase in size of sample was required by the complexity of the polymeric samples, which usually include at least two acids.

An increase in reaction time was required by the polymeric nature of the sample. This point will subsequently be discussed further.

Substitution of benzene for water accomplishes two purposes: (1) Unreacted polymeric esters, being generally soluble in benzene, do not interfere with the filtration by appearing as an immiscible oil. (2) Benzene acts as a selective solvent for the amides of saturated and unsaturated acids, dissolving the latter in the presence of unreacted benzylamine.

Because the unsaturated amides are not precipitated when the reaction mixture is added to benzene, the subsequent use of a nonsolvent such as petroleum ether is required to precipitate the unsaturated amides from the concentrated filtrate.

Although Dermer and King (*3*) recommend the use of hydrochloric acid only when necessary, its invariable inclusion in the washing procedure has been found advantageous. Contaminants found with the precipitated crude amides include: benzylamine hydrochloride resulting from the reaction of the reagent with the ammonium chloride catalyst, unreacted ammonium chloride, benzylamine carbonate resulting from the exposure of benzylamine to air, and unreacted benzylamine.

All these are readily removed by the use of dilute hydrochloric acid, in which the amides are substantially insoluble. The efficiency of the wash is greatly increased by the dissolution of the crude amide fraction in ethanol prior to treatment with dilute acid.

Although no attempt was made to determine the quantitative conversion of the monomeric esters to the corresponding amides, a preliminary study of a polymeric ester of known composition indicated that only a small increase in yield of benzyl amide was obtained on increasing the reflux time from 2 to 4 hours. No further increase was observed when the reflux time was again doubled; doubling the amount of reagent was also without observable effect.

Some attempt was made to determine the sensitivity of the method. It was first evaluated qualitatively, with good results, on binary and ternary mixtures of the monomeric esters. As an instance of extreme practical dilution, it was found possible to detect 1 mole of unsaturated acid in the presence of 9 moles of saturated acid. The method was then evaluated on a series of known polyesters, each containing one saturated and one unsaturated acid. Good results were obtained on four such pairs, with compositions ranging up to 5 moles of saturated acid to 1 mole of unsaturated acid. These sensitivities do not necessarily represent limiting concentrations.

Melting Points and Infrared Spectra. The identical amides obtained from maleic and fumaric esters, and from citraconic and itaconic esters, respectively, deserve further discussion. In the case of maleic and fumaric acids, the latter is known to be the lower energy (more stable) member of this pair of geometrical isomers. Because halogens and halogen acids have been reported (*4*) as effective catalysts for the maleic → fumaric rearrangement, it seemed reasonable to assume that the amide isolated was that of fumaric acid. This assumption was confirmed by reprocessing maleic and fumaric esters by the usual procedure, but omitting the hydrochloric acid wash. The compound obtained from fumaric ester had a melting point and an infrared absorption spectrum identical with that resulting from the application of the recommended procedure. In the case of maleic ester, a compound with a different melting point and infrared spectrum was obtained. This compound contained 10.23% nitrogen, and had a corrected melting point of 150° C., compared with a melting point of 149–150° C., reported by Dermer and King for the dibenzyl amide of maleic acid. Because this compound was apparently not the maleic amide, some further reaction was indicated. The compound was converted to the normal dibenzyl amide of fumaric acid, containing 9.44% nitrogen, by treatment with dilute hydrochloric acid.

The relationship between the citraconic and the itaconic acid was not so evident, as these compounds are structural but not geometric isomers. Examination of the infrared spectrum of a perfluokerosene suspension of the amide obtained by the recommended procedure indicated the absence of methyl absorption. These data suggest that this amide was that of itaconic acid, into which citraconic acid had been converted during the course of the reaction.

Neither hydrochloric acid nor the catalyst was responsible for this rearrangement; subsequent reactions from which these agents were successively omitted produced an amide with the same melting point and spectrum as that already described. It was assumed, therefore, that the benzylamine was responsible for the observed rearrangement. In partial support of this assumption, Hickinbottom's (*5*) brief discussion of the shifting of the double bond in α,β- and β,γ-unsaturated acids under the influence of suitable catalysts may be cited. Aliphatic amines are among the basic reagents listed as generally used to bring about this change.

Solubility. The solubility of the unsaturated amides in benzene was considerably less than would be expected from their behavior during precipitation-separation. It is probable that the greater solubility observed in the latter instance was caused by the presence of unreacted benzylamine.

CONCLUSIONS

The work to date indicates that characteristic dibenzyl amides can be prepared from polymeric as well as from monomeric esters. The saturated amides can be separated from the unsaturated amides. Individual amides can be identified in admixture by examination of the infrared spectra.

Under the reaction conditions used, however, both maleic and fumaric acids yield the same derivative, as do both citraconic and itaconic acids, as a result of isomerization. Modification of the procedure will be necessary to distinguish between individual members of these two pairs of acids.

The scope of the present procedure is not limited to dicarboxylic acid esters. Experimental results not included in this paper have demonstrated the applicability of the procedure to polymeric plasticizers which also include the esters of monocarboxylic acids. The method could probably be further extended to include alkyd resins in general.

ACKNOWLEDGMENT

The opportunity to plan and initiate a general analytical investigation of polycarboxylic acids was made possible by R. Bowling Barnes. The continuance of the program has been encouraged by R. P. Chapman, director of the Analytical and Testing Division. Grateful acknowledgment is also made for the guidance and cooperation of H. Mark, director of the Institute of Polymer Research of the Polytechnic Institute of Brooklyn.

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Coulometric Titration of Iodide

By Electrolytically Generated Bromine and an Amperometric End Point

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A method for the coulometric titration of iodide has been developed in which the iodide is oxidized to the unipositive state by electrolytically generated bromine. The end point is determined amperometrically by measuring the current between two platinum indicator electrodes with an impressed potential difference of approximately 140 millivolts. A study of the behavior of the indicator current during the titration has been made. Confirmatory analyses have shown an accuracy of ±2% for samples from 13 to 50 micrograms, and of ±0.5% for samples of from 65 to 2000 micrograms.

A METHOD and apparatus for the coulometric titration of thiodiglycol have been described by Sease, Niemann, and Swift (*7*), and a further study of this method has been made by Myers and Swift (*6*) in connection with the determination of tripositive arsenic. In these determinations, the thiodiglycol or arsenic is oxidized by means of electrolytically generated bromine, and the end point is determined by observing the current flow between two platinum indicator electrodes which have a small potential difference impressed upon them. By maintaining a constant rate of bromine generation and measuring the time of generation, the number of equivalents of bromine generated can be determined. The advantages of such secondary coulometric processes in which an intermediate reactant—in this case bromide—is provided in relatively large concentration, so that both constancy and efficiency of current generation can be maintained throughout the titration, have been discussed by Meier, Myers, and Swift (*5*). Inasmuch as the coulometric principle possesses certain unique advantages, especially its adaptability to automatic titration procedures, further investigations of its applicability seemed desirable.

Neither thiodiglycol and its sulfoxide nor tripositive and quinquepositive arsenic set up reversible half-cells at the generator or the indicator electrodes under the conditions of the above titrations. Therefore, no significant indicator current flows between the indicator electrodes until the titration end point has been reached. By shielding the generator cathode and by modification of the titration procedure, it should be possible to extend this method to substances involving reversible half-cell reactions.

Iodide ion has been selected as an example of such a substance. A study has been made of the indicator current characteristics during the oxidation of iodide to iodine monobromide, and a method for the titration of small quantities of iodide has been developed.

EXPERIMENTAL

Reagents. Six formal hydrochloric acid solutions were prepared from reagent grade concentrated acid. The acid available commercially was found to contain as much as 3.5×10^{-8} equivalent of reducing agent per milliliter. The amount of reducing agent present was determined by electrolytic oxidation and was removed by boiling the 6 *F* acid with the calculated amount of 3% hydrogen peroxide. Excess peroxide was destroyed by boiling the acid for about 15 minutes.

One formal sodium bromide solutions were prepared from reagent grade salt. These solutions, when tested, were found to contain no extraneous oxidizing or reducing agents.

On occasions, laboratory distilled water was found to contain as much as 10^{-6} equivalent per liter of an oxidizing agent, presumably chlorine. This was removed by bringing the water to a boil and bubbling clean, dry air through it.

Stock 0.1 *F* solutions of potassium iodide were made up by weight from reagent grade salt which had been dried for 1 hour at 110° C. The potassium iodide used was found to contain no iodate. The solutions were made 0.01 *F* in sodium carbonate to minimize air oxidation. Dilutions of the stock solutions, 0.005 *F* in sodium carbonate, were used directly for titrations.

Apparatus. The apparatus used was essentially that employed by Myers and Swift (*6*), except that the generator cathode was contained within a glass tube, open at the top and terminating at the bottom in a sintered-glass disk, and filled above the level of the surrounding solution with 6 *F* hydrochloric acid. This modification removed the danger of reduction of oxidation products at the generator cathode and eliminated any indicator current due to the presence of hydrogen in the titrated solution.

Procedure. When not in use, the electrodes were stored in a solution 2 *F* in hydrochloric acid and 0.1 *F* in sodium bromide. Before use, the indicator electrodes, which were shorted together during storage, were made the generator anode, and then bromine was generated on their surfaces for a period of 5 seconds. This treatment increased the stability of the electrode sensitivity. In some cases, after the electrodes had been used for antimony or arsenic determinations, it was found advisable to generate hydrogen on their surfaces for a short time, and then bromine for periods of up to 50 seconds.

Sensitivity of the indicator electrodes, in microamperes of indicator current per second of bromine generation, at the appropriate rate was determined each day by generating bromine for a certain length of time and recording the indicator current. On the high generation rate (approximately 10 ma.), and with an applied potential difference of approximately 138 mv., 3 seconds of generation gave an indicator current of about 30 μa.; on the low rate (approximately 1 ma.), 30 seconds' generation gave an indicator current of about 26 μa. Indicator current was observed to be a linear function of the bromine concentration at constant values of acid and bromide concentration. For this reason it was necessary to determine the sensitivity under the particular conditions of concentration which were to obtain during the subsequent titrations. Daily determination of sensitivity was necessary, because it was found that the sensitivity dropped somewhat from day to day—from 11.2 to 10.0 μa. per second in 2 weeks, for example. This drop in sensitivity was not constant, and the sensitivity was observed to improve when the electrodes were not used for several days.

To make a determination, a sample of the iodide solution was pipetted into a 40 × 80 mm. weighing bottle and acid, bromide, and water were added to give 50 ml. of a solution 2 *F* in hydrochloric acid and 0.1 *F* in sodium bromide. Neither the acid nor the bromide concentration was found to be critical, if the sensitivity were previously determined at the concentrations used. An acid concentration of 2 *F* and a bromide concentration of 0.1 *F* were used in this work.

The initial indicator current was first noted and recorded; then the indicator circuit was opened and generation started. When the approximate amount of iodide was known, the indicator circuit was closed about 10 seconds before the calculated end point. In the titration of an unknown sample, trial readings of the indicator current were taken during the course of a titration. After the point of maximum current has been reached, two readings will enable one to make an approximate extrapolation forward to the location of the end point.

The point of minimum indicator current was noted, and generation was allowed to proceed until the indicator current had increased to values ranging from 25 to 40 μa. In some cases the generation was stopped at intervals during this current increase and the time and indicator current were recorded. After each titration the indicator electrodes were treated as indicated above, and the generation current was determined. On the high rate, the potential across the standard resistance varied a few millivolts—from 2.036 to 2.040 volts, for example—during the course of an afternoon. Readings were taken after each titration and an average value was used for the series of titrations. On the low rate, the potential across the standard resistance had a constant value from day to day of 0.190 volt. This was verified at the start and finish of each series of titrations. From the values of the initial indicator current and of the current after the minimum point, corrections for the initial current, the blank, and the end point were made by the methods discussed below.

DISCUSSION OF METHOD

Reactions Involved. During the course of a titration in a solution 2 *F* in hydrochloric acid and 0.1 *F* in sodium bromide, bromine is generated at a constant rate at the generator anode and diffuses through the solution. (At the generation rates and concentrations involved the diffusion of iodide to the generator anode limits the anodic oxidation of iodide to a relatively small value.) In the absence of bromide, chlorine is generated at the anode. The values of the molal potentials (*4*) for the half-cell reactions which may be involved are:

$$\begin{array}{ll}
2I^- = I_2 + 2e^- & E_0 = -0.534 \\
I_2 + 4Br^- = 2IBr_2^- + 2e^- & E_0 = -0.87 \\
3Br^- = Br_3^- + 2e^- & E_0 = -1.05 \\
I_2 + 4Cl^- = 2ICl_2^- + 2e^- & E_0 = -1.06 \\
2Cl^- = Cl_2 + 2e^- & E_0 = -1.358
\end{array}$$

From these values and the concentrations involved one would predict that, if equilibrium were attained in the solution, the bromine thus generated would oxidize the iodide first to iodine then to iodine monobromide, and that only then would there be a significant concentration of excess bromine in the solution. In the absence of bromide, the chlorine generated at the anode would oxidize the iodide to iodine, then to iodine monochloride, and finally excess chlorine would be present in the solution, causing an indicator current rise analogous to that caused by excess bromine. The above presentation has been simplified by neglecting the presence of other interhalogen compounds such as Br_2Cl^- and I_2Br^-.

Indicator Current Phenomena. Plots of indicator *current* against time of generation when titrating iodide show that either in solutions containing hydrochloric acid alone (*A*, Figure 1) or hydrochloric acid and bromide (*B*) there is a small initial indicator current before generation is begun, a current increase to a broad maximum somewhat before the calculated time at which all of the iodide should have been oxidized to iodine, a minimum near the calculated equivalence point for the oxidation to unipositive iodine, then finally a current increase. Preliminary experiments by William J. Ramsey have shown that when iodide is oxidized in a perchloric acid solution (by addition of permanganate) the indicator current increases to a maximum close to, if not at, the point at which half the iodide has been oxidized to iodine; it then drops to a very low value (about 1 μa.) at the time corresponding to oxidation of all the iodide to iodine. This system is being investigated further.

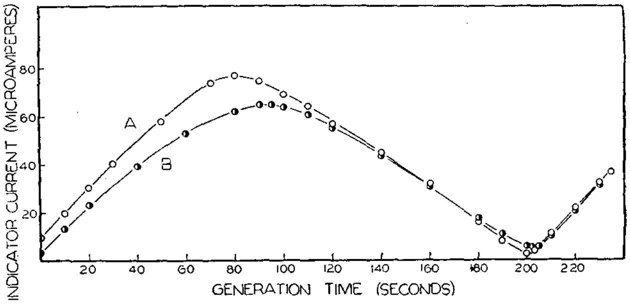

Figure 1. Behavior of Indicator Current during Titrations in Hydrochloric Acid Solutions

A. No bromide present. *B.* Bromide present

The initial indicator current, before starting generation, was found in all iodide solutions upon acidification and is believed to be due to iodine resulting from air oxidation. No iodate could be detected in the potassium iodide used. This initial current was relatively large (5 to 10 μa.) if stock iodide solutions which had not been treated with carbonate were used. Furthermore, if the iodide samples were acidified and allowed to stand, this initial indicator current increased with time of standing. Small initial indicator currents (1 to 3 μa.) were obtained by use of iodide solutions 0.005 to 0.01 *F* in sodium carbonate; these samples were not acidified until immediately before they were to be titrated. Corrections were made for this initial indicator current by the methods explained below.

As stated above, in the absence of bromide or chloride, an indicator current maximum is obtained at approximately the time corresponding to the oxidation of half the iodide to iodine; then the current drops to a very low value at a time corresponding to complete oxidation of the iodide to iodine, presumably being limited during this time by diffusion of iodide to the anode. In the presence of bromide the current continues to rise and reaches a maximum close to the time corresponding to oxidation of all iodide to iodine. This indicates that as the iodide concentration decreases oxidation of iodine to iodine monobromide becomes the controlling factor at the anode. Inasmuch as diffusion of iodine is the controlling factor at both anode and cathode, the point of maximum should occur in the region of maximum iodine concentration, provided the indicator electrodes are of equal size. Thereafter the indicator current drops off from the maximum, and the curve is linear until close to the end point and it is probable that diffusion of iodine to the anode is the limiting factor.

In the vicinity of the equivalence point, the concentration of the iodine becomes very small; after the equivalence point, diffusion

Table I. Potentials of Indicator Electrodes and of Solution during Titration of Iodide with Bromine

$E_{(\text{anode})}$ = potential of indicator anode against calomel half-cell
$E_{(\text{cathode})}$ = potential of indicator cathode against calomel half-cell
$E_{(\text{solution})}$ = potential of solution against calomel half-cell
Iodide titrated, 129.6 micrograms
Calculated time for oxidation of iodide to iodine monobromide, 207 seconds

Time, Sec.	Indicator Current, Ma.	Potentials, Millivolts				
		$E_{(\text{anode})}$	$E_{(\text{cathode})}$	$E_{(\text{solution})}$	$E_{(\text{anode})} - E_{(\text{solution})}$	$E_{(\text{solution})} - E_{(\text{cathode})}$
0	2.0	437	298	400	37	102
40	30.0	501	397	465	36	68
75	39.5	535	441	483	52	42
104	35.5	566	469	497	69	28
140	25.0	605	495	514	91	19
211	4.0	693	558	670	23	112
240	29.5	720	615	705	15	90

of bromine to the indicator cathode governs the magnitude of the indicator current.

It is believed that the indicator current near the equivalence point is controlled by the iodine and bromine in equilibrium with iodine monobromide. Thus, when iodide is titrated in hydrochloric acid solutions but in absence of bromide (see Figure 1, *A*), there is a smaller indicator current at the equivalence point. This may be explained by the small equilibrium constant for the reaction:

$$2ICl_2^- = I_2 + Cl_2 + 2Cl^- \qquad K = 5.8 \times 10^{-15}$$

as compared with the reaction:

$$2IBr_2^- = I_2 + Br_2 + 2Br^- \qquad K = 8.8 \times 10^{-11}$$

The indicator current at the equivalence point was found to be relatively independent of moderate changes of hydrogen ion and bromide ion concentrations, but increased as the quantity of iodide being oxidized was increased. In a study of this minimum current an iodine monochloride solution was prepared by oxidation of iodide in 4 *F* hydrochloric acid with an iodate solution; the iodine monochloride end point was used (*8*). When a sample of this solution, containing iodine monochloride equivalent to the amount of iodine monobromide present at the equivalence point of a 100-second titration on the high rate (669 micrograms of iodide) was placed in the titration cell, an indicator current of less than 1 μa. was observed. Upon addition of 5 ml. of 1 *F* sodium bromide, the current increased immediately to about 8 μa. Titration of an equivalent amount of iodide by electrolytically generated bromine resulted in a final indicator current of 10 μa.

The indicator current was found to be dependent upon the potential impressed across the indicator electrodes. When this potential was increased above about 160 mv., the indicator current increased rapidly with further increase of potential. By use of an applied potential between 120 and 140 mv. (in most cases 138 mv.), the current at the equivalence point was kept at relatively low values (from 0.3 μa. with 12 micrograms of iodide to 20 μa. with 2000 micrograms) and was much less critical upon fluctuations of the applied potential difference.

It was found advisable to open the indicator electrode circuit during the course of titrations on the high rate, as the indicator current was too great to be determined by the microammeter employed. During low rate titrations, the indicator current did not exceed 50 μa. and hence could be read at all times.

Attempts were made to titrate iodide in hydrochloric acid solutions in the absence of bromide. This procedure would have the advantage of giving a very small indicator current at the end point. Two difficulties were encountered. The indicator current attained equilibrium values very slowly, it being necessary to wait for as long as 30 seconds for a steady value to be reached, and positive errors were always obtained.

In the titration curves shown in Figure 1, where 133.9 micrograms of iodide were taken, the calculated time, including corrections for initial current and the blank, was 201.5 seconds for the titration in the absence of bromide; the actual time found was 202.5. It is believed that the error is due to oxidation of the electrodes by chlorine. In the presence of bromide the calculated time, including above corrections, was 202.0; the time found was 201.5.

Indicator Electrode Potentials. In an effort to obtain more evidence regarding the indicator electrode reactions, measurements were made during the course of a titration of the potential against a saturated calomel half-cell of (1) the indicator cathode, (2) the indicator anode, and (3) an independent platinum electrode. The potential of this latter electrode should measure, versus the saturated calomel half-cell, the solution potential—that is, the potential of any and all reversible half-cell couples existing at equilibrium in the solution. The values obtained have been tabulated in Table I.

The indicator cathode used had approximately twice the area of the indicator anode. This should cause a shift of the maximum current toward the beginning of the titration, for during the first part of the titration diffusion of iodine to the cathode is the controlling factor, and therefore increasing the cathode area would result in increased sensitivity and a steeper slope for the current-time curve. Such a shift was observed, the maximum occurring between 70 and 80 seconds instead of approximately 104 seconds.

It is believed that the relative values of the potentials $E_{(\text{anode})} - E_{(\text{solution})}$ and $E_{(\text{solution})} - E_{(\text{cathode})}$ indicate the relative control exercised by each electrode over the indicator current. In Table I there have been tabulated for certain times during the titration the corresponding values of these potentials and of the indicator current. It is seen that during the initial rise the greatest potential drop is at the cathode, where diffusion of iodine would be the controlling factor; in the region of the maximum current the potential drop is of corresponding magnitudes; during the latter part of the titration the diffusion of iodine to the anode is the controlling factor and one observes an increase in potential at this electrode. After passing the point of minimum indicator current, there is a sudden shift of potential drop to the cathode as diffusion of bromine to that electrode becomes the controlling factor.

Determination of End Point. The point of maximum indicator current discussed above cannot be used as an exact point of reference in the titration because, as is seen in Figure 1, it is not a sharp maximum, and its location does not correspond exactly to the calculated time at which all the iodide has been oxidized to iodine. When the titration is run in hydrochloric acid with no bromide present (curve *A*), the maximum is shifted still farther to the left. For these reasons, the end point was taken in the region of minimum indicator current for titrations in either bromide or chloride solutions. As can be seen from Figure 1, in the vicinity of this end point the indicator current reaches a minimum value, then increases rapidly. If, after attainment of this minimum current, a suitable time is allowed to elapse (usually 2 seconds on the high rate, 30 seconds on the low rate), the plot of indicator current versus time of generation is linear and can be safely used for extrapolation to the time of zero current; this time is assumed to represent the equivalence point.

Corrections for Initial Current, Solution Blank, and End Point. Two methods were used for obtaining these corrections.

Method A, approximate in nature, consisted of generating in a solution, prepared as was that to be used for the titration but containing no iodide, until a suitable indicator current reading was obtained, usually from 25 to 40 μa. This current value divided by the generation time in seconds gave a factor, by which

Table II. Comparison of Methods A and B for Calculating End Points

Series	Iodide, Micrograms			
	Taken		Found	
			Method A	Method B
I (indicator current off)	134.4		134.7	134.1
			134.7	134.1
			136.0	134.4
		Av.	135.1	134.2
II (indicator current off)	134.4		135.6	134.9
			135.5	134.3
			135.2	133.9
		Av.	135.4	134.4
III (indicator current on for half of titration)	134.3		133.6	133.8
			134.7	134.1
(Indicator current on continuously)			135.6	134.3
		Av.	134.6	134.1
IV (indicator current on through minimum point only)	134.3		133.3	133.8
			133.4	134.2
			134.6	134.7
		Av.	133.8	134.2
V (indicator current off)	134.3		134.1	133.9
			134.2	134.0
		Av.	134.1	133.9

subsequent indicator current readings could be converted into equivalent generation times. These measurements were repeated until the reproducibility indicated that the desired constancy of electrode sensitivity was being obtained. In the subsequent iodide titrations, the initial current was recorded, then generation was continued until the indicator current had gone through the minimum, and had increased to values of the same magnitude as those obtained for the blank solutions. The initial current and the final current reading were then converted to equivalent times by application of the above factor. The time equivalent to the initial current was added, and the time equivalent to the final current was subtracted from the total generation time.

In Method B, the correction for the initial current was made by recording values of the indicator current during the initial rise, then extrapolating to zero current the linear curve obtained by plotting these values against time. Time equivalent to the distance from zero time to the intercept of this curve was added to the generation time. When generation was started with blank solutions there was a short time interval before the current began to rise, indicating the presence of a small quantity of some substance capable of reducing bromine. A correction was made for this material by continuing generation until the plot of indicator current versus time was linear, then extrapolating this curve to zero current. The time equivalent to the distance from this intercept to zero time was subtracted from the generation time. The end point of the titration was obtained by continuing generation, after the indicator current minimum, until the plot of indicator current versus generation time was linear, then extrapolating this curve to zero current.

Titration values obtained by these two methods are tabulated in Table II. In general, it is believed that Method A can be used without exceeding the other experimental errors, and this method was employed for the confirmatory analyses shown in Table III. There was some indication that small positive errors were obtained if the indicator current was allowed to flow throughout the titration, but this could not be confirmed. As a matter of precaution, in most titrations, the indicator circuit was closed during the titration only as was required to approximate the position of the minimum point.

Comparison of the Coulometric with Other Micromethods for Determining Iodide. Zintl and Betz (*9*), in a potentiometric titration with silver nitrate, determined 126 micrograms of iodide with an accuracy of 1%, and 13 micrograms with an accuracy of 4%.

Endres and Kaufmann (*1*) report a method in which samples containing 1 to 10 micrograms of iodide are oxidized to iodate with bromine water. Upon addition of potassium iodide, iodine is liberated and is distilled off. Treatment of the iodine formed with hydroxylamine results in the formation of nitrous acid, by means of which sulfanilic acid is diazotized. Coupling of the diazonium compound with 1-naphthylamine leads to the formation of a red azo dye, the concentration of which is determined colorimetrically. By this method quantities of iodide from 1 to 10 micrograms are determined with an accuracy of 4%. The complexity of the method, however, excludes it from routine laboratory use.

Kanirath (*2*) determined small quantities of iodide by oxidizing it to iodate, adding potassium iodide, and titrating the liberated iodine with thiosulfate. As in the method of Enders and Kaufmann, six times as much iodide reacts as was originally added. However, this method requires a sample containing 1000 micrograms of iodide.

Laitinen, Jennings, and Parks (*3*), using an amperometric titration with silver nitrate, determined samples containing 635 micrograms with an accuracy of 4 to 13%, and samples containing 254 micrograms with an accuracy of 10 to 11.5%.

Table III. Confirmatory Analyses

Iodide, Micrograms			Error, %	No. of Titrations	Av. Deviation, %
Taken	Found	Error			
12.8	12.7[a]	−0.1	0.8	5	5.5
25.5	25.8	+0.3	1.2	2	...
38.3	38.7	+0.4	1.0	2	...
64.9	65.2[b]	+0.3	0.46	8	0.93
64.9	65.1	+0.2	0.31	2	...
129.6	130.3	+0.7	0.54	3	0.93
129.6	130.0	+0.4	0.31	2	...
194.5	195.3	+0.8	0.31	2	...
194.5	193.7	−0.8	0.31	2	...
648.4	650.0[c]	+1.6	0.25	6	0.11
648.4	650.5	+2.1	0.32	2	...
669.0	671.5	+2.5	0.37	4	0.15
669.0	672.6	+3.6	0.54	3	0.06
1296.8	1295.3	−1.5	0.12	2	...
1296.8	1299.0	+2.2	0.17	2	...
1338.0	1339.8	+1.8	0.13	2	...
1338.0	1339.4	+1.4	0.10	2	...
1625.6	1622.3	−3.3	0.20	2	...
1625.6	1625.0[d]	−0.6	0.04	4	0.04
2007.0	2008.3	+1.3	0.06	2	...
2007.0	2012.7	+5.7	0.29	2	...

[a] 11.8, 12.1, 12.5, 12.8, 14.2.
[b] 64.0, 64.4, 64.6, 65.3, 65.3, 65.6, 65.7, 66.4.
[c] 648.4, 649.4, 650.1, 650.1, 650.4, 651.4.
[d] 1623.6, 1625.0, 1625.7, 1625.7.

In comparison with these methods, the coulometric titration of iodide with electrolytically generated bromine as here described can be conveniently used for routine determination of small amounts of iodide. The data from confirmatory analyses, collected in Table III, show that samples of iodide as small as 13 micrograms can be determined with an accuracy of ±2% and that for quantities from 65 to 2000 micrograms the determination is accurate to ±0.5%. The titration of a 130-microgram sample takes approximately 200 seconds of low rate generation; the titration of a 670-microgram sample takes approximately 100 seconds of high rate generation. The apparatus in its present form is not convenient for the titration of samples containing from 200 to 600 micrograms, or more than 2000 micrograms, because of the excessive time required (greater than 300 seconds). Provision has been made for an intermediate rate of generation in an improved apparatus (*5*).

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Determination of Unsaturation in Organic Compounds by Electrometric Titration

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The mercury-catalyzed addition of bromine to isolated double bonds proceeds so rapidly that they can be determined by direct titration in the homogeneous phase. The end point is indicated electrometrically. As no excess of bromine is used, the risk of substitution or oxidation reactions is minimized or eliminated. The method is particularly suitable for determination of double bonds in the presence of easily substituted compounds. This is important in determining iodine numbers of hydrocarbons such as gasolines, lubricating oils, shale oils, or turpentine. For technical purposes the method is specially suitable because of its simplicity and rapidity. No preliminary determination is necessary and the end-point determination is independent of the color of the solution.

THE bromine or iodine numbers determined by conventional titration methods do not always correspond to the true degree of unsaturation, owing to certain side reactions, especially oxidation and substitution processes which proceed simultaneously with the halogen addition and result in too high iodine or bromine numbers. This is of special importance in the analysis of mineral oils. Holmberg (*7*) has found that the iodine number of Swedish shale oils depends to a great extent on the type and amount of the iodine solution and the time of reaction.

Many attempts have been made to eliminate oxidation and substitution processes in this procedure. McIlhiney (*13, 14*) employed an acidified bromide-bromate solution for determination of the bromine number. After titration of the bromine excess by means of sodium thiosulfate, he determined the hydrogen bromide formed by adding potassium iodide and iodate and titrating the iodine formed. The quantity of hydrogen bromide was considered to be a measure of the substitution. Other investigators [Jordan (*9*) and Lewis and Bradstreet (*11*)] found, however, that considerable amounts of hydrogen bromide were formed upon the bromination of pentene, hexene, octene, and styrene, even if the total amount of bromine absorbed did not surpass the calculated value. The mechanism of the halogen addition thus seems to be considerably more complicated than originally presumed; the 1,2-dibromides primarily formed are probably unstable, and by splitting of a mole of hydrogen bromide are rearranged to stable unsaturated monobromides which do not react with bromine. In any case, the amount of hydrogen bromide formed cannot be considered as a measure of the true substitution.

Another effort to avoid side reactions has been made by using a small, well defined excess of halogen solution and short reaction times, as in the bromine titration methods of Francis (*5*), Johnson and Clark (*8*), and Lewis and Bradstreet (*10*). However, the last-mentioned authors (*11*) have shown that substitution may occur even here, especially if the molecules contain hydrogen bound to a tertiary carbon atom.

Lucas and Pressman (*12*) found that the bromine addition, when catalyzed by mercury salts, can be quantitatively performed within 3 minutes, even in the presence of conjugated bonds or triple bonds, which react rather slowly with bromine.

That these methods, in spite of their obvious advantages, are not yet generally adopted is probably due to the necessity of previously determining the approximate iodine number of the oil sample for calculating the bromine excess which cannot be visually estimated if colored samples are to be analyzed. In most cases it is therefore necessary to make several titrations in order to determine and calculate the exact bromine or iodine number.

APPLICATION OF ELECTROMETRIC END-POINT DETERMINATION FOR BROMINE TITRATION OF OLEFINS

The mercury-catalyzed bromine addition to olefins proceeds so rapidly that the double bonds can be directly titrated with bromine. The solution does not contain any excess of bromine and thus there will be only a slight risk of substitution and oxidation. The end point of the titration was indicated by the electrometric method proposed by Foulk and Bawden (*4*) under the name of "dead-stop end point." It was applied by Wernimont and Hopkinson (*16*) in determining water by titration with the Fischer solution (*3*). It depends on the potential jump occurring between two platinum electrodes when a slight excess of bromine is added to a solution containing bromine ions. The titration device employed in the author's method consists of a rectifier and an amplifier built together and connected with a cathode ray tube magic eye. [The instrument was constructed and built by Bertil Ohlson, Stockholm, to whom the author is indebted for very valuable cooperation. Good results have also been obtained with an ordinary potentiometer circuit (*1*).] The apparatus is similar to that described by McKinney and Hall (*15*). The potential jump at the titration after amplification is made visible by the eye or measured by an ammeter (0 to 2 ma.), thus permitting the determination of the end point of the titration.

The solution must contain bromide ions. Sodium or potassium bromide is not suitable, however, for the end point indication is not sharp and the bromine consumption depends on the amount and concentration of potassium bromide used in the procedure, probably because of the presence of Br_3^- ions. For the same reason a considerable bromine excess can be retitrated with a few drops of sodium thiosulfate solution (of the same normality), owing to the reaction of sodium bromide with the free bromine. When hydrobromic acid was used as a source of bromide ions, no complications could be observed.

The solution should also contain a catalyst, such as a mercury salt, for the addition of bromine. The author has employed mercuric chloride. In neutral solution, however, the bromine combines with the mercury salt, forming a complex salt which prevents the bromine from taking part in the reactions at the electrodes. This can be avoided by using a solution with high concentration of chloride ions—for instance, by adding hydro-

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chloric acid to decompose the complex. Simultaneously the water contained in the hydrochloric acid catalyzes the addition of bromine.

ANALYTICAL PROCEDURE

Methanol Solution. A. Fifteen milliliters of hydrogen bromide, specific gravity 1.38, 170 ml. of concentrated hydrochloric acid, specific gravity 1.19, and 20 grams of mercuric chloride are diluted with pure methanol (free from any oxidizable compounds) to 1 liter. This solution corresponds to 0.1 *N* hydrobromic acid and 2 *N* hydrochloric acid.

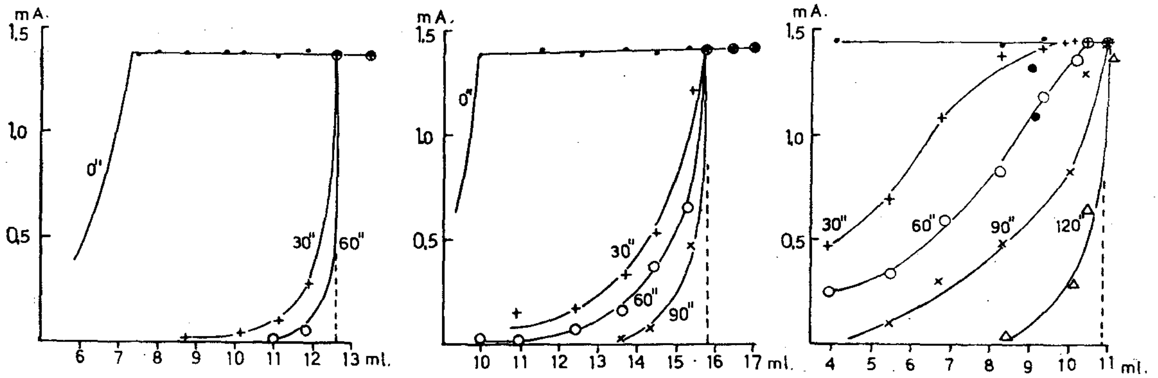

Figure 1. Titration Curves for Electrometric Titration at 20° C.

Left. 0.0642 gram of styrene titrated with 12.57 ml. of 0.0852 *N* bromine solution. Iodine No. 212; according to Hübl 219
Center. 0.2030 gram of oleic acid titrated with 15.80 ml. of 0.0851 *N* bromine solution. Iodine No. 84; according to Hübl 85
Right. 0.1099 gram of stilbene titrated with 10.92 ml. of 0.1112 *N* bromine solution. Iodine No. 140; calculated 141

Bromine Solution. B. About 8 grams, equal to 2.5 ml. of bromine, are diluted with carbon tetrachloride to 1 liter. This solution corresponds to 0.1 *N* bromide and is standardized in the ordinary way by addition of potassium iodide and titration with sodium thiosulfate, starch being used as an indicator. If the carbon tetrachloride contains sulfur the bromine solution is not stable. The solution should be kept in darkness and its normality checked every day.

The titration is carried out as follows: About 1 to 3 milliequivalents of an unsaturated compound are weighed into a 150-ml. beaker. According to Lucas and Pressman (*12*) the catalysis of the bromine addition to the double bond is best when the molar ratio of mercury to bromine is somewhat greater than 1. The proportion of mercury in the methanol solution corresponds to about 35 ml. of bromine solution. The quantity of the unsaturated compound to be weighed should thus be equivalent to an amount of bromine solution not exceeding 35 ml. If the unsaturated compound is not easily dissolved in methanol, it should be brought into solution by adding carbon tetrachloride or chloroform.

By means of a pipet, 50.0 ml. of methanol solution A are now transferred to the beaker. (The solution contains mercuric chloride and is very poisonous.) Thereafter the two platinum electrodes and a mechanical stirrer are placed in the beaker. The electrodes are 0.5-mm. platinum wires and must be completely immersed in the liquid, even if the stirrer is in use. They are connected with the amplifier, and given a suitable potential. The ammeter is adjusted to zero position and the olefin is slowly titrated by stepwise addition of the bromine solution in portions of about 0.5 ml. At the beginning of the titration a stop watch is started and the current between the electrodes is read on the ammeter at the beginning and after 30, 60, 90, and 120 seconds. The titration is continued until the current remains constant during the whole reading period.

A blank titration without unsaturated compound shows that the blank consumption is negligible—only about 0.1 to 0.2 ml. of bromine solution.

The results of this titration are illustrated by Figure 1. As no ion reaction is taking place, the addition of bromine is not instantaneous. After addition of the first drops of bromine the solution will contain an excess of bromine, and a current passes between the electrodes. However, the olefin reacts quickly with the excess of bromine, followed by a decrease of the current to its original value The current curves after 0, 30, 60, 90, and 120 seconds *intersect at a single point,* the end point. On further addition of bromine the current remains constant during and after the whole reading period. As expected, the speed of the reaction is different for different olefins. Styrene absorbs the bromine excess in less than 30 seconds, and oleic acid in about 60 seconds, whereas stilbene, having a double bond between two phenyl groups, requires about 120 seconds for the complete reaction.

In routine analyses 50.0 ml. of the methanol solution are pipetted into a beaker provided with electrodes and stirrer, a potential is applied, and the amplifier is adjusted until the cathode ray tube shows a clear green color. By the addition of a few drops of bromine solution the magic eye will be closed, showing a green cross on a black background, which should be maintained for about 30 to 60 seconds. For this blank titration only 0.1 to 0.2 ml. of bromine solution is required. On the subsequent addition of about 1 to 3 milliequivalents of unsaturated compound from a weighing pipet, the cross in the eye will disappear and turn into a plain green field. The pipet is then weighed and the titration with bromine solution is continued until the cross reappears in the eye. The cross should be visible during 30 to 60 seconds, a time that is determined for the compound in question in the above-mentioned way. The bromine does not react instantaneously, as the addition is a nonionic reaction. To most unsaturated compounds, however, bromine is added fairly rapidly. The bulk of the bromine solution may be added in one jet, after which the last portion is added cautiously—e.g., 20 drops per minute. By this method the complete determination of the bromine number can be accomplished within less than 10 minutes.

The iodine number is calculated in the ordinary way:

$$\text{Iodine number} = \frac{\text{ml. of bromine solution consumed} \times \text{normality of bromine solution} \times 12.69}{\text{weight of sample}}$$

If more than 10 ml. of bromine solution are required, two phases will appear toward the end of the titration due to the limited miscibility of the methanol solution with carbon tetrachloride. However, the speed of reaction between bromine and olefin is not influenced thereby.

If other bromine-consuming reactions are taking place simultaneously with the addition of bromine the curves of titration will be somewhat different from those shown above; thus, the curves in Figure 2 are obtained by titration of pinene and styrene in the presence of thiophene. When the bromine or iodine numbers of pinene are determined under the conventional conditions substitution, reactions occur and anomalous results are obtained. However, by direct titration it is possible to esti-

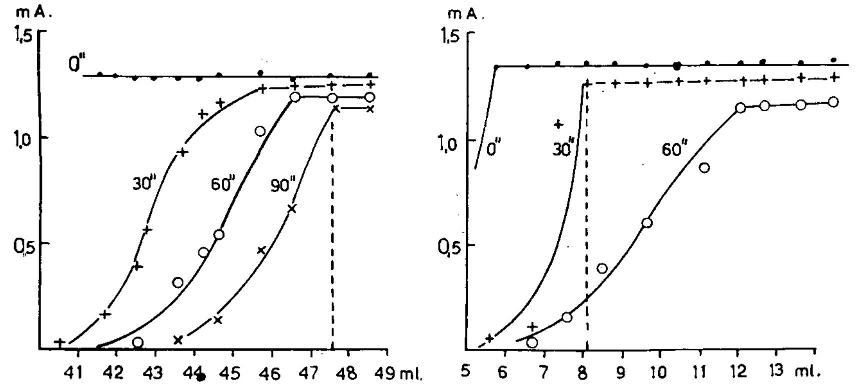

Figure 2. Titration Curve for Electrometric Titration at 20° C.

Left. 0.2158 gram of pinene titrated with 47.5 ml. of 0.0852 *N* bromine solution. Iodine No. 238; according to Hübl 350
Right. 0.0390 gram of styrene plus 0.0604 gram of thiophene titrated with 8.10 ml. of 0.0852 *N* bromine solution. Iodine No. 224; according to Hubl 380

mate the bromine consumption from the titration curves. Even if the current never reaches a completely constant level, because of the bromine consumption by substitution reactions, the difference between the speeds of the two reactions is sufficient to determine the end point of the addition of bromine. Moreover, the shape of the current curve indicates whether the substitution takes place with a speed sufficient to influence the determination of the bromine number. The speed of the substitution depends on the concentration of the compound to be substituted as well as on the reaction temperature.

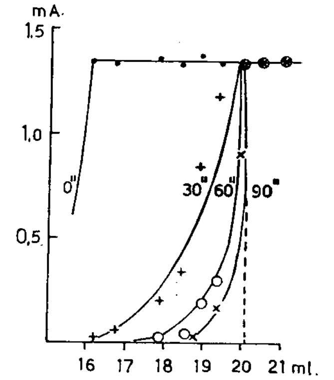

Figure 3. Titration Curve for Electrometric Titration of Shale Oil at 20° C.

0.1995 gram of shale oil titrated with 20.10 ml. of 0.0852 *N* bromine solution. Iodine No. 109; according to Hübl 130

Figure 3 shows a titration curve for Swedish shale oil gasoline containing a certain amount of thiophene and its homologs. As a result, the iodine number determined by electrometric titration is considerably lower than the value obtained by the method of Hübl, and it is clearly seen that the amount of thiophene is too small to influence determination of the iodine number. The above-mentioned titration of pinene and of styrene in the presence of thiophene can be performed in such a way that the substitution reaction is nearly completely repressed. As clearly shown in Figure 4, the end-point determination is sharp at a temperature of −15° C. As regards styrene, the same result is obtained as in the absence of thiophene. For pinene the determined iodine number is 217 corresponding to a calculated value of 186, whereas the determination by the Hübl method gives 350.

These preliminary experiments show clearly the advantage of the direct titration of the double bond as compared with the conventional methods. On the other hand, certain double bonds react only slowly and some compounds are very easily oxidized or substituted. When examining unknown compounds, the titration curves should therefore always be determined as indicated above. In this way the rule of the side reactions can be stated, and the experimental conditions varied as required.

When this work was just brought to an end DuBois and Skoog (*2*) published a similar method using the dead-stop technique for the determination of bromine addition numbers of hydrocarbons and petroleum products. The present author has investigated a considerably larger number of unsaturated compounds as well as the influence of certain sulfur-containing compounds. Moreover, the determination of the titration curve enables a better control of the eventually occurring side reactions.

EXPERIMENTAL RESULTS OF ELECTROMETRIC TITRATION OF OLEFINS

The iodine value of a number of unsaturated compounds has been determined by electrometric titration and by using a cathode ray tube as end-point indicator. All samples were of commercial quality and were not further examined for purity. In Table I the iodine numbers determined are compared with the theoretically calculated ones and those determined by the Hübl (*6*) method (18-hour reaction time). The Hübl method was chosen as a standard, because it is the oldest of the conventional methods for the determination of halogen addition numbers. The reagent gives only relatively little substitution.

As will be seen from Table I, the results are well reproducible, and the iodine numbers are independent of the weight of sample used in the analysis. For some compounds the calculated iodine numbers do not agree well with those determined experimentally, but this is obviously due to impurities in the samples, inasmuch as the electrometrically determined iodine numbers agree fairly well with the Hübl numbers.

For all compounds in Table I the bromine addition proceeds very rapidly and the double bond can easily be determined quantitatively. On the other hand, compounds containing double bonds conjugated to carbonyl groups, such as maleic acid and cinnamic acid, or triple bonds, such as phenylacetylene, react too slowly with bromine and therefore the double bond cannot be determined by direct titration. For such unsaturated com-

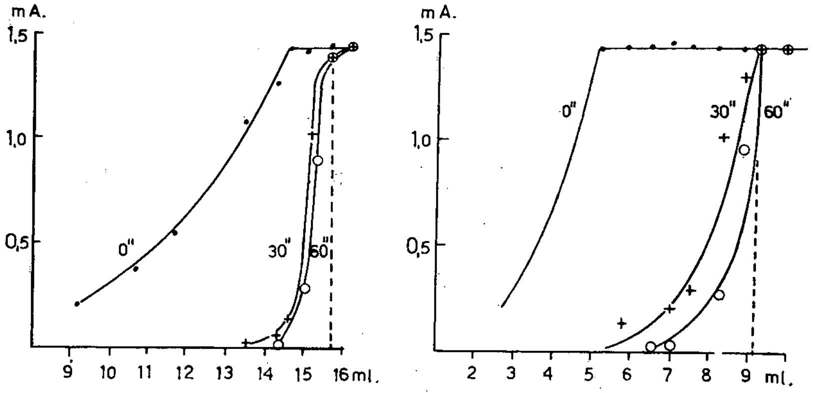

Figure 4. Titration Curves for Electrometric Titrations

Left. 0.0782 gram of pinene titrated with 15.76 ml. of 0.0852 *N* bromine solution. Iodine No. 217; according to Hubl 350. −20° C.
Right. 0.0476 gram of styrene plus 0.0604 gram of thiophene titrated with 9.20 ml. of 0.0852 *N* bromine solution. Iodine No. 209; according to Hübl 380. −15° C.

Table I. Iodine Numbers of Unsaturated Compounds Determined by Electrometric Titration at 20° C.

(End point indication, magic eye)

Material	Weight of Sample, Gram	Bromine Solution, Ml.	Normality of Bromine Solution	Iodine No. Electrometric titration	Iodine No. Hübl	Iodine No. Calculated
Cyclohexene	0.0116	3.06	0.09286	306	...	310
	0.0239	6.09	0.09286	296		
	0.0360	9.22	0.9286	302		
	0.0480	12.18	0.09286	299		
	0.0600	15.91	0.09286	312		
	0.0722	19.14	0.09286	312		
	0.0726	18.85	0.09286	306		
	0.0852	22.66	0.09286	314		
				Mean 306 ± 6		
2-Pentene	0.1505[a]	42.31	0.1000	357	...	363
	0.1508[a]	45.00	0.1000	379		
	0.1806[a]	53.92	0.1000	379		
				Mean 372 ± 9		
1-Octene	0.1704	29.49	0.1014	223	...	226
	0.1705	30.18	0.1014	228		
				Mean 225 ± 3		
2-Octene	0.1798	30.01	0.1000	212	215	226
	0.1900	28.10	0.1112	209		
	0.1902	28.69	0.1112	213		
	0.2006	33.51	0.1000	212		
				Mean 212 ± 2		
1-Decene	0.2003	27.90	0.1014	179	174	181
	0.2005	26.91	0.1014	173		
	0.2103	29.91	0.1014	183		
				Mean 178 ± 4		
1-Hexadecene	0.2003	17.71	0.1014	114	...	113
	0.2197	19.02	0.1014	111		
				Mean 113 ± 2		
1-Octadecene	0.2096	16.20	0.1000	98.2	...	100
	0.2106	16.51	0.1000	99.5		
	0.2198	16.75	0.1000	97.8		
	0.2302	17.83	0.1000	99.2		
				Mean 98.7 ± 0.7		
Styrene	0.0300	5.69	0.09286	224	218	244
	0.0455	5.86	0.08521	209		
	0.0524	9.63	0.09286	217		
	0.0642	12.57	0.08521	212		
	0.0658	12.70	0.08521	208		
	0.0849	15.02	0.09286	218		
	0.1171	21.77	0.09286	220		
				Mean 214 ± 7		
α-Methylstyrene	0.1703	24.61	0.1112	204		
	0.1798	25.38	0.1112	199	204	214
	0.1800	25.79	0.1112	202		
				Mean 202 ± 2		
α-Methyl-*p*-methylstyrene	0.0270	3.93	0.1108	204	185	192
	0.0532	6.65	0.1108	176		
	0.1111	14.79	0.1108	187		
	0.1164	15.50	0.1108	187		
	0.1232	16.38	0.1108	187		
	0.3284	41.91	0.1108	179		
				Mean 186 ± 9		
Stilbene	0.1010	10.19	0.1112	143	...	141
	0.1099	10.91	0.1112	140		
	0.1500	14.63	0.1112	138		
				Mean 140 ± 2		
Pinene	0.0780	13.38	0.1232	268	ca. 350	186
	0.0944	14.82	0.1232	246		
	0.2005	30.00	0.1232	234		
	0.2158	47.50	0.08521	238		
	0.0782[a]	15.70	0.08521	217		
	0.1208[a]	24.45	0.08521	219		
2-Methyl-2,4-pentadiene	0.0959	20.50	0.1108	301	...	310
	0.1161	24.09	0.1108	292		
	0.1444	29.10	0.1108	284		
				Mean 288 ± 6		
Allyl alcohol	0.1210	37.50	0.1108	436	...	437
	0.1234	37.93	0.1108	431		
	0.1769	55.26	0.1108	441		
				Mean 436 ± 2		
Oleic acid	0.1676	10.05	0.1108	84.5	85	90
	0.2791	17.00	0.1108	85.8		
	0.2890	17.39	0.1108	84.8		
	0.2931	18.04	0.1108	86.6		
				Mean 85.4 ± 0.8		
Castor oil	0.1529	9.70	0.1108	88.3	83	...
	0.1548	9.62	0.1108	87.5		
	0.1559	9.80	0.1108	88.5		
	0.1574	9.74	0.1108	87.0		
				Mean 87.8 ± 0.8		
Cinnamic alcohol	0.1191	15.10	0.08521	137	137	189
Eugenol methyl ether	0.0650	6.40	0.1118	140	144	142
	0.0954	13.40	0.08521	151		
				Mean 145 ± 5		
Isoeugenol methyl ether	0.1010	9.60	0.1118	135	134	142
	0.1157	14.26	0.08521	133		
	0.1225	15.08	0.08521	133		
	0.1282	12.02	0.1118	133		
	0.1819	17.10	0.1118	134		
				Mean 133 ± 1		

[a] Titration at −15° C.

Table II. Electrometric Determination of Iodine Number in Presence of Easily Substituted Compounds

(End point indication magic eye)

Material	Weight of Sample, Gram	Easily Substituted Compound, Ml.	Bromine Solution, Ml.	Normality of Bromine Solution	Iodine No. by Electrometric Titration: At 20° C.	At −10° C.	Without easily substituted compound (Table I)
Cyclohexene	0.0590	1 Tetrahydronaphthalene	12.40	0.1154	308	...	306
	0.0590	1 Tetrahydronaphthalene	12.48	0.1154	310	...	...
	0.0590	1 Tetrahydronaphthalene	12.53	0.1154	316	...	
		Mg.					
Styrene	0.0390	50 Thiophene	8.10	0.08521	224	...	214
	0.0476	50 Thiophene	9.20	0.08521	...	209	...
		Ml.					
Oleic acid	0.1350	1 Tetrahydronaphthalene	9.75	0.09775	89.7	...	85.4
	0.1462	1 Tetrahydronaphthalene	10.72	0.09775	91.0	...	...
	0.1809	1 Tetrahydronaphthalene	14.07	0.09775	96.6	...	...
	0.0962	1 Tetrahydronaphthalene	6.72	0.09775	...	86.6	...
	0.1217	1 Tetrahydronaphthalene	8.39	0.09775	...	85.6	...
	0.1602	1 Tetrahydronaphthalene	10.87	0.09775	...	84.3	...
		Mg.					
	0.0990	50 Thiophene	12.10	0.09775	152	...	...
	0.1112	50 Thiophene	17.62	0.09775	197	...	...
	0.1259	50 Thiophene	13.07	0.09775	129	...	...
	0.1252	50 Thiophene	8.49	0.09775	...	84.1	...
	0.1523	50 Thiophene	10.32	0.09775	...	84.1	...
	0.1559	50 Thiophene	11.10	0.09775	...	86.4	...

Table III. Electrometric Iodine Number Determination on Swedish Shale Oil Fractions

(End point indication magic eye)

Type of Gasoline[a]	Boiling Point, ° C.	Iodine No.: Hübl	Electrometric at 20° C.	Electrometric at −10° C.
A	<65	69.3 65.3	... ...	54.7 52.4 55.8
A	65–95	57.1 55.1	... ...	42.2 42.6 44.7
A	95–125	57.8 56.6	53.9 55.5 56.4	54.8 54.4 52.0
A	125–150	60.0 59.3	64.0 62.7 66.1	60.0 59.2 60.6
A	150–200	66.9 65.1	65.6 63.6 65.5	64.7 64.5
B	<65	140.5 139.6	...	115.2 116.0
B	65–95	128.8	...	109.3 109.7 110.5
B	95–125	141.7 138.9	134.8 126.3 123.6	126.7 122.9
B	125–150	130.4 130.3	127.1 123.3 125.4	113.8 117.6 111.0
B	150–200	114.0 112.4	114.8 115.0 115.8	100.7 97.1 101.5

[a] Gasoline from shale oil. A. Obtained by heating in situ (Ljungström method). B. Obtained by coking in retorts (Bergh method).

pounds the conventional methods must be recommended. The only diolefin investigated by the author is 2-methyl-2,4-pentadiene with only one reacting double bond.

Previous experiments with pinene have shown that double bonds can be determined by direct titration even if the material has a tendency for substitution reactions. Table II shows the results of some titrations in the presence of easily substituted substances, such as thiophene and tetrahydronaphthalene. Here the conventional methods give misleading results in contrast to the easily performed electrometric titration. Especially at low temperature—e.g., −15° C.—the speed of the substitution reaction is so low that the determination of the iodine number is not influenced. It is clear that a correct figure for the addition of bromine can be obtained only if side reactions, such as substitution or oxidation, proceed considerably slower than the addition. This is not the case with styryl-*s*-mercaptoacetic acid (C_6H_5-CH=CH—S—CH_2COOH), in which the speed of oxidation of sulfur to sulfone goes on so rapidly that the double bond cannot be determined.

Table III shows the iodine numbers of gasoline fractions from Swedish shale oil, as determined by electrometric titration compared with those obtained by the Hübl method. The shale gasolines are very rich in sulfur and have high iodine numbers. Normally the sulfur content decreases with increasing boiling point of the fractions. Accordingly, the difference between the iodine numbers determined by the two methods decreases in the same order. At low temperature lower iodine numbers are obtained than at normal temperature, and the end point is much sharper and visible for 2 to 3 minutes, as compared with 30 to 60 seconds for titration at room temperature. Finally, the reproducibility is satisfactory even if the low-boiling fractions are rich in mercaptans (thiols) and sulfides.

ACKNOWLEDGMENT

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Polarographic Procedure for Determination of Cobalt as Cobalt(III) Ammine

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A new polarographic procedure for the determination of cobalt in the presence of a large excess of nickel is described. The ammino cobalt(II) ion in ammoniacal solution is quantitatively oxidized to a rose-colored ammino cobalt(III) complex ion with sodium perborate and the excess oxidizing agent is destroyed by boiling. The polarographic wave resulting from the reduction of the ammino cobalt(III) ion to the divalent state is then obtained before the waves of nickel or zinc appear. The height of this wave is directly proportional to the concentration of cobalt.

THE polarographic reduction wave of cobalt(II) ion in aqueous solution is usually obtained at a slightly more negative potential than the nickel(II) ion wave. Consequently, large amounts of nickel and several other elements interfere in the usual polarographic procedures. Applications of the polarographic wave of trivalent cobalt for the determination of cobalt and the use of sodium perborate to obtain quantitative oxidation of cobalt with complete elimination of the excess oxidizing agent are described in this paper.

The cobalt(III) ammine method is especially applicable for the determination of cobalt in electrolytically deposited mixtures of cobalt and nickel, or in impure nickel salts. Nickel and zinc do not interfere, but chromium, as well as iron, manganese, and copper, when present in excess, must be separated. Evans (*5*) determined cobalt colorimetrically as ammino cobalt(III) ion after separating chromium, copper, aluminum, molybdenum, phosphorus, tantalum, titanium, vanadium, tungsten, and zirconium along with an excess of iron by the zinc oxide procedure. The polarographic procedure should be superior, as nickel does not interfere. The method should be applicable after separation of copper, lead, and tin in copper-base alloys by electrolysis at controlled potentials as described by Lingane (*9*).

Metzl (*10*) determined cobalt iodometrically by oxidizing cobalt to the ammino cobalt(III) ion with hydrogen peroxide and then boiling in the presence of potassium hydroxide until no ammonia remained. The resulting black cobaltic oxide was treated with an excess of potassium iodide and acidified. The liberated iodine was titrated with sodium thiosulfate. Tomicek and Freiberger (*11*) determined cobalt potentiometrically by titrating an ammoniacal solution with potassium ferricyanide.

Brdicka (*2*) reported polarograms of ammino cobalt(III) obtained by dissolving solid ammino cobalt(III) salts in ammoniacal ammonium chloride. He observed the first polarographic wave starting at −0.3 volt versus the saturated calomel electrode (S.C.E.) for the reduction to the divalent state, followed by a second wave, twice as high, starting at −1.3 volts (S.C.E.) for the final reduction to the free metal. Willis, Friend, and Mellor (*12*) measured the half-wave potentials of the two waves obtained when various solid ammino cobalt(III) salts were dissolved in potassium sulfate as the supporting electrolyte, and Laitinen, Bailar, Holtzclaw, and Quagliano (*8*) studied the effect of the supporting electrolyte on the hexammino cobalt(III) wave.

The rose color of the complex ion obtained by quantitative oxidation of cobalt in ammoniacal solutions indicates that it consists largely of hydroxopentammino cobalt(III) ion and not the yellow hexammino cobalt(III). Bjerrum (*1*), using the spectrophotometer and glass electrode, proved that hexammino cobalt (III) ion is thermodynamically the more stable. However, the bonding of the complex is so predominantly covalent in nature that the rose-colored pentammino cobalt(III), which is formed in ammoniacal solution, remains essentially unchanged for weeks. A brown hue was always observed in solutions in which the oxidation was incomplete.

APPARATUS

The manual apparatus, described in previous publications (*7*), was used. An outside saturated calomel electrode or a mercury pool in the cell can be used as the anode. A thermostatically controlled water bath was used to maintain a constant temperature of 25.0° ± 0.1° C.

REAGENTS

A stock solution of 0.1 *M* cobalt(II) chloride was prepared by dissolving 23.80 grams of cobalt(II) chloride hexahydrate, reagent grade, in water, adding 10 ml. of 1 *N* hydrochloric acid, and diluting to exactly 1 liter. The molarity was verified by the method of Engle and Gustavson (*4*). Ammonium hydroxide, 6.3 *N*, was prepared by mixing equal volumes of concentrated ammonium hydroxide and water. Ammonium chloride stock solution, 1 *M*, was prepared.

PRELIMINARY SELECTION OF REAGENTS AND CONDITIONS

Oxidation of the cobalt in ammoniacal solutions by free oxygen was incomplete. After oxidation with sodium peroxide or hydrogen peroxide, the excess peroxide was not easily removed. Presumably the difficulty in removal of peroxide is due to its presence in the complex. It was finally found that sodium perborate, $NaBO_3.4H_2O$, rapidly and quantitatively oxidizes cobalt to the trivalent complex ammine in an ammoniacal solution at boiling temperature. One gram of sodium perborate tetrahydrate, theoretically sufficient to oxidize 760 mg. of cobalt(II) ion, was used in all the determinations. The excess perborate was decomposed by boiling the solution 5 to 10 minutes. In order to avoid excessive loss of ammonia, a small 15-cm. (6-inch) Liebig reflux condenser was connected to the Erlenmeyer oxidation flask. The upper orifice of the condenser was connected with a small water trap containing about 5 ml. of water which was finally added to the sample. The nitrogen used to sweep air from samples was passed through two wash bottles containing similar concentrations of ammonia. For routine analysis, a 100-ml. Pyrex volumetric flask fitted with a glass tube to serve as an air condenser can be used during the heating. The nitrogen used to remove oxygen need not be passed through wash bottles containing ammonium hydroxide.

Large maxima were obtained in the absence of capillary-active substances. Tropeolin 00 formed a precipitate in the solution of cobalt salts and 0.01% thymol, as well as 0.005% methyl red, was ineffective. However, 0.02% gelatin was found to suppress the maximum completely.

PROCEDURE

The sample to be determined may contain from 1 to 120 mg. of cobalt as the nitrate, sulfate, chloride, or perchlorate in about 25

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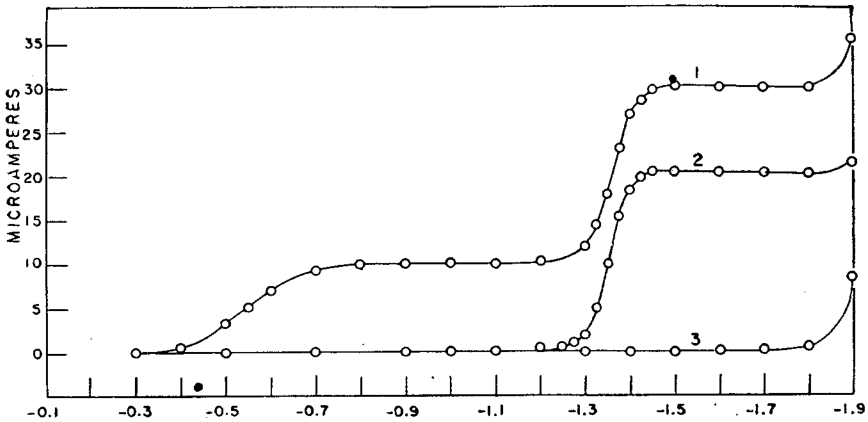

Figure 1. Polarograms of Cobalt(III) and Cobalt(II) in Ammoniacal Solution

1. Cobalt(III) ammine, 5 millimolar
   2.5 *M* ammonium hydroxide
   0.1 *M* ammonium chloride
   0.02% gelatin
2. Like curve 1 but cobalt not oxidized
3. Like curve 1 but no cobalt present

ml. of aqueous solution. Transfer the sample quantitatively to a 250-ml. Erlenmeyer flask and add 10 ml. of 1 *M* ammonium chloride, 40 ml. of 6.25 *M* ammonium hydroxide, and about 1 gram of sodium perborate.

Connect a small reflux condenser and allow the solution to boil gently in a water bath for 10 minutes to decompose the excess perborate. After the solution has cooled, transfer the sample quantitatively, along with a little water used to rinse the condenser, to a 100-ml. volumetric flask. Add 2 ml. of 1% gelatin, dilute to the mark, and mix well.

Transfer a suitable volume of the solution to a polarographic cell and remove oxygen by passing nitrogen through the solution for about 15 minutes.

Obtain the polarogram or merely measure the diffusion current at −0.85 or −0.9 volt *vs.* the saturated calomel electrode, using either an automatic recording or a manual instrument.

CHARACTERISTICS OF POLAROGRAPHIC WAVE

Curve 1, Figure 1, illustrates the polarographic current voltage curve obtained using a solution 5 millimolar in ammino cobalt (III) ion, 2.5 *M* in ammonium hydroxide, 0.1 *M* in ammonium chloride, 0.02% in gelatin, 0.064 *M* in sodium borate, and 10.60 in pH. Curve 2, Figure 1, was obtained using the same concentrations of all constituents without oxidizing the cobalt, whereas curve 3, Figure 1, was obtained in a blank determination using the same constituents and the same procedure as used in the preparation of the solution in curve 1, but without any cobalt present.

Curve 1 verifies Brdicka's (*2*) observation that ammino cobalt (III) ion is reduced in two steps. The first wave, corresponding to the reduction to ammino cobalt(II) ion, was found to have a half-wave potential of −0.534 volt *vs.* the saturated calomel electrode and a constant diffusion current between −0.80 and −1.10 volt *vs.* the saturated calomel electrode. The second wave, due to the reduction of an intermediate ammino cobalt(II) complex directly to metallic cobalt, was found to have a half-wave potential of −1.358 volts *vs.* the saturated calomel electrode and a constant diffusion current between −1.5 and −1.8 volts (S.C.E.). After correcting for the residual current and the small change in the value of $m^{2/3}t^{1/6}$, the total diffusion current at −1.5 volts (S.C.E.) was exactly three times as large as that at −0.9 volt (S.C.E.), proving that the cobalt had been quantitatively oxidized to the trivalent state.

OPTIMUM CONCENTRATION OF SUPPORTING ELECTROLYTE

Because of the volatility of ammonium hydroxide its concentration may vary, especially since an inert gas is bubbled through the solution for 15 minutes to remove dissolved air. The effect of the concentration of ammonium hydroxide on the polarographic wave of the cobalt(III) complex is accordingly of practical importance and was investigated. The concentrations of ammonia and ammonium ion also influence the solubility of other ammino metal ions present in the sample. The effect of the ammonium hydroxide and supporting electrolyte concentrations on the diffusion current is shown in Table I.

From the data in Table I it is evident that the diffusion current is constant within experimental error when the concentration of ammonia is varied from 0.31 to 3.1 *M*. However, when the concentration of ammonium hydroxide was increased to 7.5 *M* the diffusion current decreased by 8%. The oxidation was quantitative in all the solutions containing 10 ml. or more of 6.25 *M* ammonium hydroxide per 100 ml. of final solution. Within experimental error, the diffusion currents measured at −0.9 or −1.6 volts (S.C.E.) were not altered by increasing the ammonium chloride concentration from 0.1 *M* to 0.5 *M* or by replacing the ammonium hydroxide with the indifferent electrolyte, potassium chloride, in a concentration range of 0.1 *M* to 1 *M*. The third, fourth, and fifth samples, which were triplicates, were analyzed by the recommended procedure and yielded the same result within ±1%.

In samples containing 0.6 gram of nickel ion, 10 ml. of 6.25 *M* ammonium hydroxide were insufficient to retain nickel in solution, though as much as 1.2 grams of nickel ion could be retained in solution if 40 ml. of 6.25 *M* ammonium hydroxide were used. For this reason a volume of 40 ml. of 6.25 *M* ammonium hydroxide, corresponding to a final concentration of 2.5 *M*, was employed in the procedure.

Table I. Effect of Concentration of Ammonia and Supporting Electrolyte

Capillary t = 4.12 seconds $m^{2/3}t^{1/6}$ = 1.195 mg.$^{2/3}$sec.$^{-1/2}$ at 0 volt (S.C.E.)
Cobalt(III) concentration 5×10^{-3} *M*, 0.02% gelatin

Final (NH_3) Molarity	Supporting Electrolyte	$i_d - i_r$ at −0.9 Volt (S.C.E.), Microamperes
0.31	0.1 *M* NH_4Cl	(Incomplete oxidation)
0.62		10.14
2.5		10.10
2.5		10.03
2.5		10.10
3.1		10.00
7.5		9.26
2.0	0.5 *M* NH_4Cl	10.04
2.5	0.1 *M* KCl	10.04
2.5	1.0 *M* KCl	10.00

Details concerning the influence of the concentration of ammonia on the various half-wave potentials will be considered in a separate paper. Of practical advantage is the fact that the half-wave potential of this first wave remains nearly constant at −0.534 ± 0.003 volt (S.C.E.) independently of the concentration of ammonia.

PROPORTIONALITY BETWEEN DIFFUSION CURRENT AND CONCENTRATION OF COBALT

The data in Table II show that the diffusion current is strictly proportional to the cobalt ion concentration in the range of 0.2 to 10 millimolar. The accuracy is within the polarographic limit

Table II. Proportionality between Concentration of Cobalt(III) and First Diffusion Current

Regular procedure, 0.1 M NH_4Cl, 2.5 M NH_3, 0.02% gelatin
Capillary t = 4.12 seconds $m^{2/3}t^{1/6}$ = 1.195 mg.$^{2/3}$sec.$^{-1/2}$ at 0 volt (S.C.E.)

Final Cobalt(III) Concentration Millimolar	$i_d - i_r$ at −0.9 volt (S.C.E.), Microamperes	$i_d - i_r$ per Millimolar Concn. of Cobalt(III)
0	0.03	0
0.2	0.40	2.00
0.4	0.81	2.02
1.0	2.01	2.01
2.0	4.02	2.01
5.0	10.10	2.02
7.0	14.10	2.01
10.0	20.20	2.02
	Av.	2.01

of ±1%. The diffusion current constant is the current in microamperes per millimolar concentration of cobalt divided by the capillary characteristic in mg.$^{2/3}$ sec.$^{-1/2}$. The value, I = 1.67, was obtained with two capillaries having the greatly different characteristics of 1.205 and 2.12 mg.$^{2/3}$ sec.$^{-1/2}$, respectively. The concentration of cobalt is readily calculated from the diffusion current by the following equation:

$$c = i/1.67\ K$$

where c is the millimolar concentration of cobalt, i is the diffusion current in microamperes corrected for residual current, and K is the capillary characteristic in mg.$^{2/3}$ sec.$^{-1/2}$.

EFFECT OF OTHER ELEMENTS

Nickel, in amounts as large as 1.2 grams, has no effect on the cobalt(III) wave. Curve 1, Figure 2, is the polarogram obtained using a sample containing 0.59 gram of nickel as nickel nitrate while curve 2 is the polarogram obtained with 0.0059 gram of cobalt and 0.59 gram of nickel in the sample. A steady diffusion current was obtained from −0.8 to −0.9 volt (S.C.E.), but the current increased rapidly after 0.9 volt (S.C.E.) owing to the reduction of nickel. For this reason the diffusion current should be measured at −0.85 volt (S.C.E.) in the presence of large amounts of nickel. In a series of experiments using samples containing 1 to 55 mg. of cobalt and 0.6 to 1.2 grams of nickel, the results were similar to those in Table II. The average diffusion current constant was 1.67 ± 0.03.

The effect of zinc is important, for it is electrodeposited along with cobalt and nickel on a platinum cathode from ammoniacal solution. Zinc, like nickel, does not interfere. The wave due to zinc coalesces with the second cobalt wave, but has no influence on the first wave.

Iron, if present in large excess, causes low results due to the coprecipitation of cobalt with the hydrous ferric oxide formed during the procedure. On a sample containing 0.4 gram of iron as ferric chloride and 29.5 mg. of cobalt as cobalt chloride, only 54% of the cobalt was finally found in solution by the polarographic procedure. When the amount of iron in the sample was reduced to 11.2 mg. with 29.5 mg. of cobalt, 29.3 mg. of cobalt were found, which is within the limits of polarographic accuracy. Thus, iron, if present in relatively large amounts, must be separated, but the separation need not be quantitative. This suggests an extraction by ether. Two successive extractions with equal volumes of isopropyl ether, using conditions described by Dodson, Forney, and Swift (*3*) and essentially the technique described by Hillebrand and Lundell (*6*) followed by an evaporation to dryness and then the present polarographic procedure with samples containing 0.4 gram of iron and 29.5 mg. of cobalt, yielded results for cobalt within experimental error. A single extraction with an equal volume of isopropyl ether resulted in a loss of 2 to 3% of the cobalt, when similar samples were used.

Manganese(II) is converted to manganese dioxide during the oxidation procedure and causes low results due to coprecipitation of cobalt. A sample containing only manganese as manganese sulfate results in a separation of black manganese dioxide when the ammoniacal solution containing the perborate is heated. No diffusion current due to higher valent soluble forms of manganese was detected at −0.9 volt (S.C.E.). The results obtained with several samples containing cobalt and manganese are summarized in Table III. The presence of as much as 1 M calcium ion to compete with cobalt for adsorption reduced, but did not eliminate, the loss of cobalt by adsorption. The procedure can be used for analyzing samples in which the amount of cobalt is much larger than the amount of manganese as in certain cobalt steels, provided sufficient calcium chloride is added before the oxidation to make its final concentration 1 M.

For the separation of cobalt and manganese, 1-nitroso-2-naphthol in 50% acetic acid solution has been found particularly satisfactory. Copper and iron(III) are completely precipitated and some silver, bismuth, chromium, and tin may be precipitated. Generally cobalt has been determined after ashing to Co_3O_4 in the presence of oxalic acid. This classical separation and ignition technique followed by the present polarographic procedure yielded results within polarographic accuracy for samples originally containing 29.5 mg. of cobalt as cobalt chloride and

Table III. Coprecipitation of Cobalt with Manganese Dioxide

Original Cobalt Concn., Millimolar	Original Manganese Concn., Millimolar	Additional Electrolyte	$i_d - i_r$ at −0.9 Volt (S.C.E.), Microamperes	% Cobalt Lost
5	50	None	1.30	87
5	10	0.5 M $Zn(NO_3)_2$	4.89	51
5	10	0.12 M $CaCl_2$	7.71	23
5	10	1.0 M $CaCl_2$	8.66	14
5	1	1.0 M $CaCl_2$	9.95	1
5	0	None	10.06	0

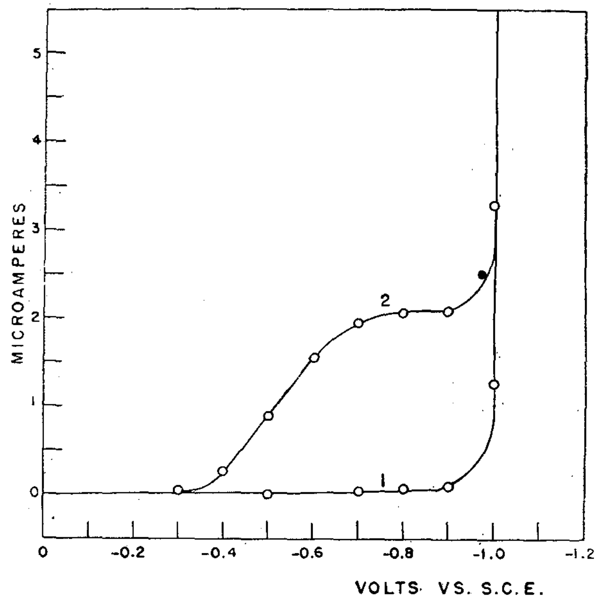

Figure 2. Polarograms of Excess Nickel with and without Cobalt(III)

1. Cobalt absent, nickel(II) ammine 0.1 M
2.5 M ammonium hydroxide
0.1 M ammonium chloride
0.02% gelatin

549 mg. of manganese as manganese(II) chloride. Attempts to shorten the procedure through an extraction of cobalt 1-nitroso-2-naphtholate in chloroform resulted in no improvement, as the very stable cobalt complex was not extracted from the chloroform phase with strong acids and was not completely decomposed through oxidation by hot concentrated sulfuric or nitric acids. Evaporation of the chloroform followed by the above ashing procedure resulted in 100% recovery of cobalt but required more time than the filtration procedure. The chloroform extraction technique may find application when the aqueous phase is also to be analyzed.

Copper in large amounts was found to interfere because of its prior reduction at the dropping mercury electrode. In ammoniacal solution copper(II) ion is reduced stepwise with half-wave potentials of -0.23 and -0.50 volt (S.C.E.). It should be possible to determine small amounts of copper simultaneously with cobalt by the present procedure, or the copper could be separated by classical electroplating procedures. The authors studied a simple procedure of reducing the copper to the free state by zinc.

One gram of zinc dissolved in 5 ml. of mercury was added to a solution of the sample in about 50 ml. of hydrochloric acid having a concentration range of 0.5 to 6 *N* and the solution was heated near 100° C. for 5 minutes after the last trace of blue was observed. The samples contained 29.5 mg. of cobalt and 0.1 to 0.5 gram of copper. As much as 17% of the cobalt was deposited along with the copper in samples containing 0.5 gram of copper, but less than 1% of the cobalt was deposited if the amount of copper was reduced to 0.1 gram. Thus, this simple procedure can be used to remove copper from samples containing less than 0.1 gram of copper.

Chromium interferes, because it is oxidized to chromate ion which is reduced to chromium(III) before ammino cobalt(III). The interference of chromium is readily eliminated by adding a slight excess of 1 *M* barium perchlorate following the oxidation but before diluting to exact volume. Vanadium and molybdenum do not interfere, because the half-wave potentials of vanadate and molybdate in the electrolysis medium are more negative.

SUMMARY

A polarographic procedure for determining cobalt as the cobalt (III) ammine is described. A new method was developed for obtaining rapid and quantitative oxidation of cobalt(II) to cobalt (III) ammine with elimination of the excess oxidizing agent, sodium perborate. The polarographic procedure is particularly useful for determining cobalt in nickel compounds or in the electrolytically deposited mixture of cobalt, nickel, and zinc, which do not interfere.

The amounts of iron or manganese in the final solution must not exceed that of cobalt which is partially coprecipitated with the hydrous ferric oxide and the manganese dioxide, the latter being formed during the oxidation with perborate. The wave for the reduction of copper to the univalent state which precedes the cobalt (III) wave can be used for the simultaneous determination of copper and cobalt but interferes if copper is present in a large excess. Chromium interferes because it is oxidized to chromate, which is polarographically reduced to chromium(III) at nearly the same potential as cobalt(III). Vanadates and molybdates which are formed during the oxidation do not interfere, as their waves occur at more negative potentials. Simple procedures for separating interfering elements which were tested and found satisfactory include the isopropyl ether extraction of ferric iron, the separation of copper with zinc amalgam, and the precipitation of chromate as barium chromate. For the separation of cobalt from manganese, 1-nitroso-2-naphthol is satisfactory.

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Determination of Acetylene and Monosubstituted Acetylenes

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A method has been developed for the determination of those compounds which contain the grouping R—C≡CH, where R can also be a hydrogen atom. The method has definite advantages over others for these compounds, and is rapid and precise.

ACETYLENE has been determined colorimetrically with Ilosvay's solution (*3*, *9*), but the red color of the cuprous acetylide darkens in the presence of oxygen and other impurities through oxidation. The explosive copper acetylide may be dried and weighed (*7*), or the copper may be determined by any standard method and the acetylene estimated.

The use of cuprous ion in acetylenic compound determinations has several disadvantages. The cuprous solutions deteriorate rapidly, the cuprous ion oxidizing to cupric. The precipitate is not conveniently analyzed, because it is explosive, making gravimetric determination difficult though possible. The determination of copper in the precipitate is the preferable method, but this is time-consuming.

Other methods (*1*, *2*) are based on the estimation of acetylene or acetylide by titration of the nitric acid liberated from a neutral silver nitrate solution according to the equations:

$$\mathrm{CH{\equiv}CH + 3AgNO_3 \longrightarrow (CAg{\equiv}CAg).AgNO_3 + 2HNO_3}$$

$$\mathrm{CH{\equiv}C.CH{=}CH_2 + 2AgNO_3 \longrightarrow (CAg{\equiv}C.CH{=}CH_2).AgNO_3 + HNO_3}$$

Novotny (*5*) proposed that the silver in the precipitate be determined.

The disadvantages of using silver ion are mainly due to the interference of any halides, cyanides, sulfides, and traces (down to 0.01%) of aldehydes in the sample. These interferences consume silver ion, the halides and sulfides by precipitation of the silver salts, the cyanides by complex ion formation, and the aldehydes by reduction of the silver ion to metallic silver. Traces of aldehydes affect the determination of the nitric acid liberated by the silver nitrate (*1*, *2*), for the metallic silver formed obscures the end point completely. Small amounts of aldehydes (up to 0.5% as formaldehyde) can be tolerated in the procedure below. However, because aldehydes are oxidized by the potassium mercuric iodide, amounts larger than 0.5% will begin to affect the acetylenic hydrogen results.

Wellers (*10*) absorbed the gas in slightly alkaline solutions of potassium mercuric iodide. Acetylene has also been estimated by determining the mercury in the acetylide precipitate (*4*) by titrating with ammonium thiocyanate by the method of Volhard. For qualitative identification purposes, Shriner and Fuson (*8*) recommend the use of potassium mercuric iodide to form the mercuric derivative of those compounds which contain the grouping $—C{\equiv}CH$, according to the formula:

$$2RC{\equiv}CH + K_2HgI_4 + 2KOH \longrightarrow (R—C{\equiv}C—)_2Hg + 4KI + 2H_2O$$

The procedure described in this paper uses this reaction for the quantitative estimation of acetylenic compounds by the determination of the potassium hydroxide consumed.

REAGENTS AND STANDARD SOLUTIONS

Potassium mercuric iodide reagent, made by dissolving 50 grams of mercuric iodide in 250 ml. of 20% potassium iodide solution. C.P. methyl alcohol. Standard 0.5 *N* sulfuric acid solution. Standard 0.5 *N* sodium hydroxide solution.

PROCEDURE

Fifty milliliters of the potassium mercuric iodide reagent are added to 100 ml. of C.P. methyl alcohol in a 250-ml. Erlenmeyer flask. The sample containing 0.010 to 0.015 mole of acetylenic hydrogen is added to the flask, and 50 ml. of standard 0.5 *N* sodium hydroxide solution are pipetted into the mixture. The excess sodium hydroxide is titrated immediately to the phenolphthalein end point with standard 0.5 *N* sulfuric acid.

Analytical Results

	Found, %	Found by Check Method, %
Propargyl alcohol	100.4 100.5 99.6	100.1[a]
1-Hexyne	98.6 98.1 98.0	97.1[b]
3-Butyn-1-ol	97.9 98.2 97.4	100.1[b]
Acetylene[c]	100.8 99.4	

[a] Value obtained by hydroxyl determination (*6*).
[b] Acetylenic hydrogen determined by method recommended by Altieri (*1*). (This procedure is reproducible to ±1 to 2%.)
[c] Acetylene generated from calcium carbide and water.

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RECORDING REFRACTOMETER

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A continuous recording refractometer, designed and built for industrial control use, is satisfactory for both opaque and clear liquids. The sample stream flows continuously, and may be under pressure. Good stability has been achieved with a maximum sensitivity of ±0.00005 refractive index unit. The action depends on intensity of internal reflection, near the critical angle, in a prism in contact with the sample stream.

REFRACTIVE index has long been recognized as a fundamental property of matter, but in spite of its long usage as a measure of chemical purity it has found little application as a means of process control in chemical industry.

There are basically two types of refractive index measurements in general use: One depends on refraction of light at the boundary between the sample and a reference medium, and the other depends upon the formation of interference fringes to estimate the effective optical path length.

It has been long known and recently emphasized (*3*) that refractive indexes of substances are correlated with the intensity of the reflected component of light impinging on a prism-sample boundary. For a fixed angle of incidence the intensity of the reflected light is dependent upon the ratio of refractive indexes of the prism and the liquid. If the reflected light is converted into an electrical signal, this principle becomes usable in continuously recording instruments.

This method may be exploited by either a single- or multiple-reflection technique. The single-reflection principle may be utilized by a trapezoidal prism as shown in Figure 1 and the multiple-reflection one by a glass rod as shown in Figure 2 (*2*). This paper is concerned with the application of the single-reflection technique to a continuously recording refractometer.

THEORY OF INSTRUMENT

In Figure 1 a parallel beam of light is reflected from a glass-sample boundary. If the angle of incidence is less than the critical, the amplitude of the reflected light will depend on the direction of polarization of the incident light, on the angle of incidence, and on the ratio of refractive indexes of the glass and sample. Use can be made of Fresnel's equations (*1*) where the amplitude of a reflected ray of light, the incident light being polarized normal to the plane of incidence and of unit amplitude, is in the notation of Figure 1

$$A_n = \frac{\sin(\beta - \alpha)}{\sin(\beta + \alpha)}$$

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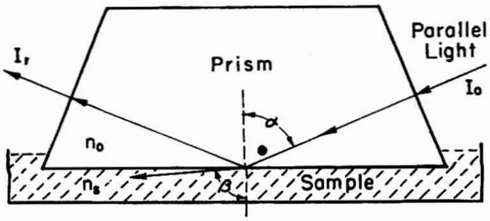

Figure 1. Trapezoidal Prism for Utilization of Single-Reflection System

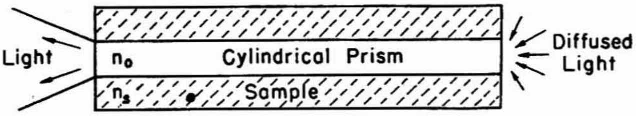

Figure 2. Cylindrical Prism for Utilization of Multiple-Reflection System

Similarly, for light polarized parallel to the plane of incidence

$$A_p = \frac{\tan(\beta - \alpha)}{\tan(\beta + \alpha)}$$

The intensity of the reflected light, however, is proportional to the square of the amplitude. Using unpolarized light and defining the reflectivity, T, as the ratio of the intensities of the reflected and incident light,

$$\frac{I_r}{I_0} = T = {}^1/_2 \frac{\sin^2(\beta - \alpha)}{\sin^2(\beta + \alpha)} + {}^1/_2 \frac{\tan^2(\beta - \alpha)}{\tan^2(\beta + \alpha)} \qquad (1)$$

In addition to Equation 1, angles α and β are related in terms of the ratio of refractive index of the glass to that of the sample. Designating this ratio by n,

$$\sin \beta = n \sin \alpha \qquad (2)$$

It is now possible, by eliminating β between Equations 1 and 2, to express T as a function of n and α. In Figure 3, T *vs.* n is plotted, α being the parameter. In a practical application, α, of course, will be held fixed, so that effective measurements will be limited to a narrow range. For most sensitive operation α should be set slightly below the critical angle. Increased sensitivity is gained if n is small, but the effective range of operation is reduced.

DESIGN OF SINGLE-REFLECTION REFRACTOMETER

Specific conditions imposed on a control instrument were: (1) continuous flow, (2) recording features, (3) pressures up to 150 pounds gage, and (4) operation with opaque liquids; three added prime technical considerations which had to be kept in mind in the design of the plant instrument were (5) sensitivity, (6) stability, and (7) temperature compensation.

An instrument was built utilizing the above principles and meeting these conditions. Sufficient data were obtained with this model to prove the feasibility of such a refractometer for plant control.

Optical System. Because the range of optimal sensitivity of a reflected light refractometer is limited, it is necessary in the design of the optical system to know the range of refractive index to be measured in a specific application. The refractive index and geometry of the prism best suited for that range can then be determined. It is desirable that the light pass into and out of the prism at near normal incidence. This reduced bending of the light at the faces of the prism and simplified the positioning of the detecting element. In order that the light may pass into the prism at nearly normal incidence and still be within the region of most sensitive operation, the refractive index of the liquid to be measured must be known. The prism angle can then be determined in terms of the refractive index of the glass and the liquid.

Referring to Figure 1, at maximum sensitivity $\beta = 90°$, so that from Equation 2 $\sin \alpha = 1/n$. It is apparent, however, that under these conditions the prism angle should be exactly equal to α. The authors' application required the control of a liquid at a refractive index of about 1.39. Using glass of refractive index 1.51, the prism was constructed with a prism angle $= \sin^{-1} 1.39/1.51 = 67°$. By making the angle of incident light and the position of the detector adjustable, it was found possible to make measurement over a range of from 1.36 to 1.42.

The complete optical system employed appears in Figure 4.

The light source consists of a tungsten filament projection lamp and collimator, the collimator being provided with a holder for filters or polarizing elements. Inasmuch as red light is the primary source of drift in barrier layer cells, a light blue filter was made a part of the collimator. A Wratten No. 43 filter was found effective in removing the red end of the spectrum without seriously reducing the intensity at the shorter wave lengths. Although provisions were made for the use of polaroid in the light path, insufficient data have been obtained to date to determine the effect of polarized light on the operation of the instrument. The beam is split into two components, one passing through the sample prism, the other through a reference prism. The reflected light from the prism-liquid boundaries is finally converted to an electrical signal by suitable detectors after it passes out of the prisms.

Detection and Recording. Either vacuum phototubes or barrier layer cells are suitable as the photosensitive elements. Barrier layer cells are easier to use, because they require no power supply, and when properly converted into a null balance circuit, the difference in the output of the two cells may be recorded by any standard recording potentiometer, which has been modified for this purpose.

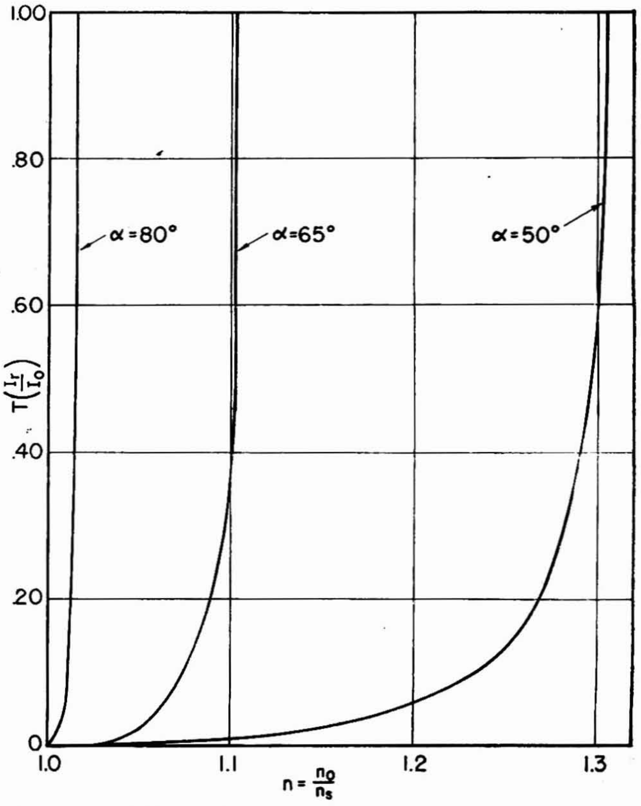

Figure 3. Effects of Refractive Index Ratio and Angle of Incidence on Reflectivity

The circuit shown in Figure 5 was used successfully with a modified Brown Electronik recorder. If vacuum phototubes are used as the detecting elements, a special type of recorder is needed to match the high impedance of the tubes. A recorder manufactured by Leeds & Northrup for pH measurements was found to be well suited for phototube measurements. Essentially it is an electronic millivoltmeter calibrated to record from −200 to +200 millivolts. The null balance circuit of Figure 6 incorporating No. 929 phototubes operated very well when coupled directly to the recorder.

Mechanical Details. One of the difficult problems associated with the construction of the refractometer was that of pressurizing the prisms and making it possible to remove them easily for cleaning. Figure 7 is a photograph of the instrument, and Figure 8 shows the construction details of the prism assembly.

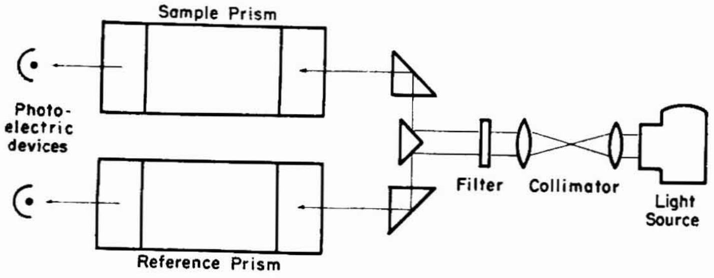

Figure 4. Optical System for Single-Reflection Refractometer

The prism housing is entirely of stainless steel. The four edges of the base of the prism rest on a ledge bordering the liquid compartment. A gasket, cut to fit the ledge, prevents any leakage from the sample or reference chambers. The top of the prism is covered by a strip of gasket material and above that is a metal plate. Three bolts, which can be reached at the top of the housing, seat on the metal plate; when these bolts are tightened the prism is forced against the lower gasket to accomplish the seal. By loosening the bolts and removing the face plate, which supports the detecting element, the prism can be easily removed from the housing.

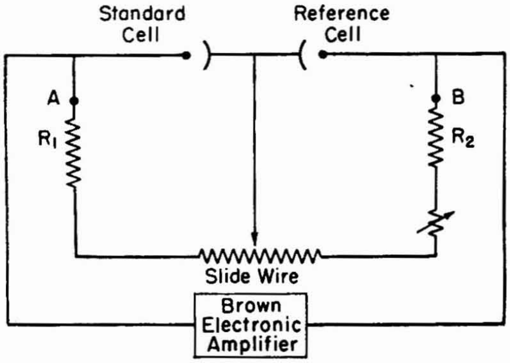

Figure 5. Electrical Circuit Used with Barrier Layer Cells and Brown Recorder

Three adjustments are possible in the optical system: (1) The angle at which the light enters the prism can be adjusted 3° above and below the normal to the prism face, thereby allowing a wider range of operation. (2) The detecting elements can be adjusted vertically along the opposite face of the prism in order to accommodate the setting of the incoming beam. (3) Iris diaphragms are used to adjust the size of the beam, making it possible to balance the sample and reference systems.

The lamp housing and collimator are of aluminum, and the lamp housing is provided with fins for dissipation of heat. Fittings for additional cooling by compressed air may be used with high-wattage lamps.

PERFORMANCE

Sensitivity. Theoretically, the sensitivity in terms of the change in light intensity for a given refractive index change may be found from the curves of Figure 3. The problem becomes one of how small a change in light intensity can be detected and recorded. The output of the photoelectric elements and the minimal response of the recorder are the limiting factors in the degree of precision possible. The sensitivity one can expect for a given set of conditions can be approximated.

For example, using the circuits of Figure 5 let $R_1 = R_2 = 1000$ ohms and the slide-wire = 300 ohms. Initially assuming 15 μa. as the current generated by each cell, *the slide will be at mid-scale*, and the potentials developed at A and B will be 1150 ohms × 15μa. = 17.3 mv. Assuming the reference cell current to remain unchanged with the slide at the extreme left of the scale, the potential at B will be 1300 ohms × 15 μa. = 19.5 mv. For balance the current output of cell A must increase to 19.5 mv. ÷ 1000 ohms = 19.5 μa. Similarly, with the slide at the extreme right the current output of cell A must be reduced to 11.5 μa. for balance, the balance voltage having changed to 15 mv. Hence, for full-scale deflection the current output of A has changed by 8 μa. and the balance voltage by 4.5 mv. Assuming the current output of the cells to be directly proportional to the reflected light intensity, an 8-μa. change in the output of cell A is approximately equivalent to a 50% change in T.

Using this information, the range of refractive index covered by a full-scale deflection can be approximated. Assuming $n_0 = 1.51$ and $n_s = 1.40$, it can be seen from an expanded scale of Figure 3 that in the region of most sensitive operation a change of 50% in T corresponds to a refractive index change of about 0.005. If a recorder with a minimum response of ±0.03 mv. is used, the expected recorder accuracy will be 0.03 mv. ÷ 4.5 mv. = ±0.7% of full scale or, in terms of refractive index, better than ±0.00004.

Figure 9 represents the results obtained when the above conditions were imposed experimentally on blends of benzene and butyl alcohol. The sensitivity is somewhat less than predicted, full-scale deflection representing a refractive index change of about 0.01 or a sensitivity of 0.0001 per chart division. In practical applications it was found that the sensitivity did not change

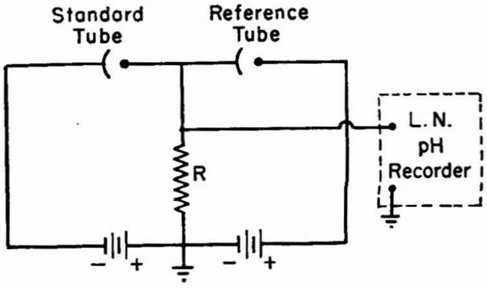

Figure 6. Electrical Circuit Utilizing Vacuum-Type Phototubes and Leeds & Northrup pH Recorder

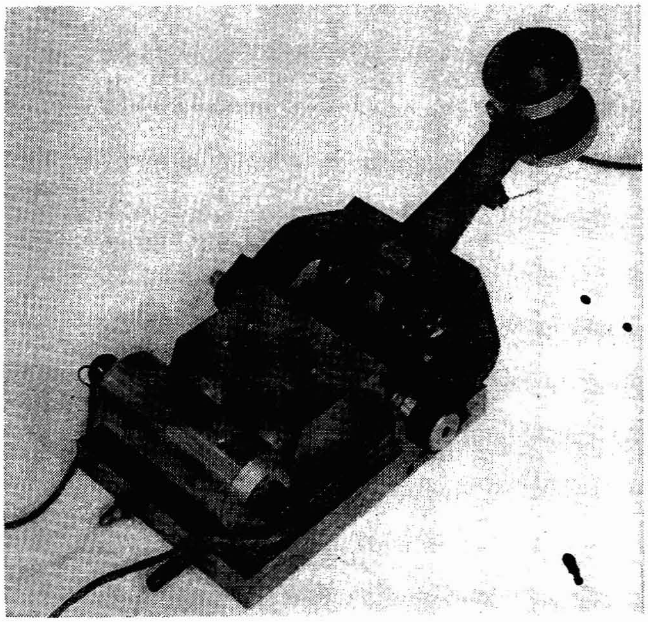

Figure 7. Refractometer, without Recorder and Heat Exchanger

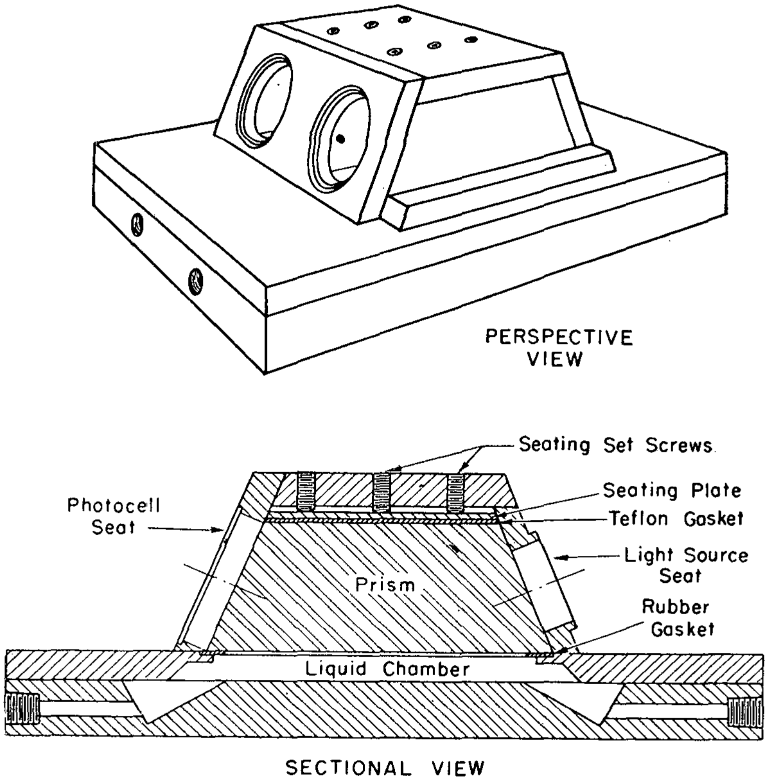

Figure 8. Construction Details of Prism Assembly

appreciably over a range of 0.01 refractive index. In general, the sensitivity can be increased by increasing the intensity of the light source or by increasing the resistors, R_1 and R_2. Unfortunately, however, increasing the light intensity alters the internal characteristics of the cells, so that a high external resistance becomes impractical. It is generally preferable to keep the output of the cells as low as possible, consistent with the minimum response of the recorder, while increasing R_1 and R_2 to obtain the desired sensitivity.

If phototubes are used instead of barrier layer cells, the recording mechanism becomes somewhat more complicated, although much more effective. The maximum sensitivity possible with the circuit shown in Figure 6 is almost unlimited, for several volts can be developed by the tubes from light sources of moderate intensity. For practical purposes a filtered light source of low intensity results in excellent operation and adequate sensitivity. Experimental results are illustrated in Figure 10, obtained on benzene in butyl alcohol solutions, for various load resistors. From these results and from subsequent stability tests an over-all accuracy of ±0.00005 refractive index unit was determined.

Stability. Twenty-four-hour stability runs were made using barrier cells and phototubes as the detecting elements. The sensitivity was set at 0.0001 refractive index unit per chart division in both cases. Runs were made on a continuously circulating sample and also with air in the sample and reference chambers. With the liquid chambers empty, no detectable drift occurred during the 24

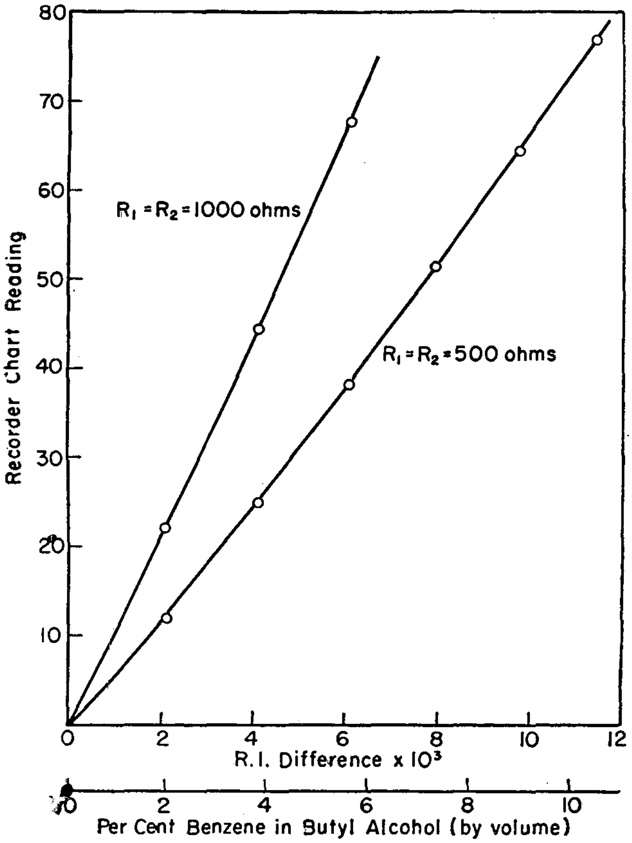

Figure 9. Experimental Data Obtained with Circuit Shown in Figure 5

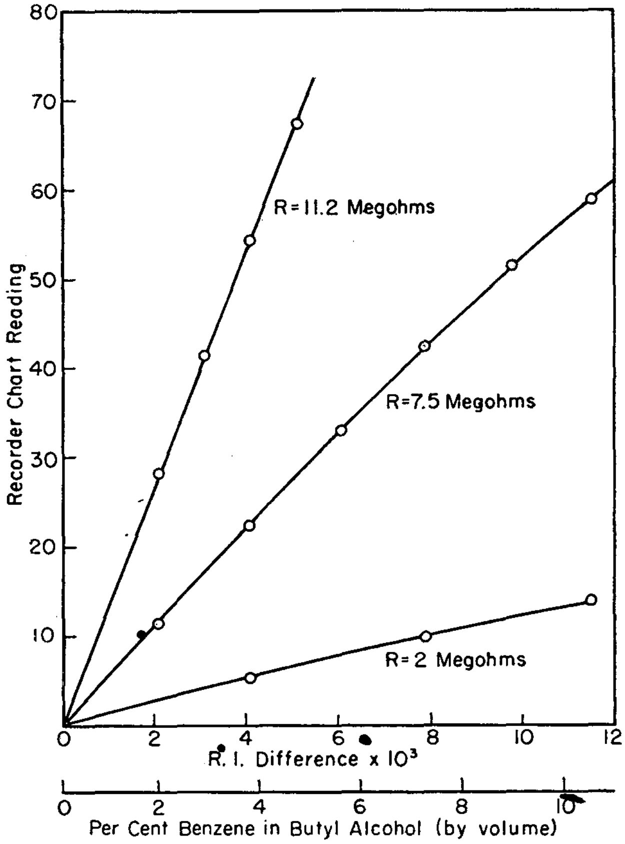

Figure 10. Experimental Data Obtained with Circuit Shown in Figure 6

hours of operation. When liquids were circulated in the sample and reference systems, no drift occurred, but some slight instabilities were caused by small bubbles passing along the interface. At no time, however, were there deviations of more than 0.5 chart division (0.00005 R.I.) from the initial setting.

Temperature Compensation. The main advantage of the null balance system used in this instrument is to eliminate effects due to temperature changes in the sample. By means of a suitable heat exchanger the sample and reference liquid can be maintained at the same temperature, which thereby reduces the degree of temperature control necessary. If n_s and n_r are the refractive indexes of the sample and reference liquids, respectively, then the conditions for good compensation are:

$$n_s - n_r \cong 0$$

$$(Is)_{\text{sample}} - (Ir)_{\text{reference}} \cong 0$$

$$\frac{\partial n_s}{\partial \text{Temp.}} - \frac{\partial n_r}{\partial \text{Temp.}} \cong 0$$

Detector characteristics must be the same.

The greater the deviations from these conditions the poorer the compensation. The third condition can be satisfied closely, and also the first condition, depending upon specific conditions. The second and fourth conditions, however, can only be approximated, so that, in general, absolute temperature variations of more than 1° C. will seriously affect the accuracy of the instrument.

CONCLUSION

The refractometer provides a continuous and accurate method of recording the refractive index of any liquid which does not have a tendency to deposit a film on a glass surface. Even a very thin film will seriously interfere with operation of the instrument. It is independent of any coloration in the liquid. Very little maintenance is required, except for periodic cleaning of prisms and restandardization, and it will operate for long periods of time with an accuracy of better than ±0.0001 refractive index unit.

ACKNOWLEDGMENT

Appreciation is expressed to J. E. Gleeson and Walter Schwartz for operative data; to J. R. Bowman for valuable comments; to W. A. Hamor and G. H. Young for friendly encouragement; to H. B. Stevenson of the Procter and Gamble Company, who simultaneously worked with reflected light type refractometers; and to John Unertl, Jr., of the Unertl Optical Company, Pittsburgh, Pa., for many helpful mechanical suggestions during the construction of the instrument.

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RECEIVED February 16, 1949. Contribution of multiple industrial fellowship of the Reconstruction Finance Corporation, Office of Rubber Reserve.

Pressure-Tight Infrared Absorption Cell for Liquids

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Infrared spectrometric analysis of mixtures of any or all of the C_4 olefins and paraffins in the liquid phase at room temperature has been impracticable because thin absorption cells could not be made tight enough to retain, without leakage, liquids having high vapor pressures. Analysis in the liquid phase has been considered as a means of avoiding inaccuracies resulting from sampling a liquid mixture for analysis in the vapor phase. The design and construction of tight cells are described, as well as the technique used in assembling and testing them. The cells have rock salt windows and were sealed with amalgam. They have been used at pressures up to 35 pounds per square inch gage for recording the spectra, from 1 to 15 microns, of the butenes and 1,3-butadiene. The same cell serves also to prevent loss of volatile solvents from solutions during the recording of spectra.

THE analysis of mixtures of any or all of the C_4 olefins and paraffins in the liquid phase at room temperature by infrared spectrometric methods has heretofore been impracticable because thin absorption cells could not be made tight enough to retain, without leakage, liquids having high vapor pressures. Analysis in the liquid phase is a possible means of avoiding the inaccuracies resulting from the difficulty of securing a sample in the vapor phase which has the same composition as the liquid in the container from which it was withdrawn (*3*).

Difficulties are also encountered in preventing the loss from liquid absorption cells of volatile solvents such as carbon tetrachloride and carbon disulfide with consequent change of concentration of solutions while recording their spectra.

A number of workers (*4, 7, 8*) have designed cells to hold volatile liquids, but of the cells thin enough to permit the study of pure liquids without dilution in a solvent, only those of Gildart and Wright (*5*), Benning, Ebert, and Irwin (*1*), and Kivenson (*6*) are tight at pressures appreciably above atmospheric. Of these, the latter two used quartz windows and sealing methods not applicable to rock salt, and the first did not have connections suitable for filling the cell and confining the sample under pressure.

This paper describes the design, construction, and technique of assembling cells using rock salt windows, based on the principles described by Gildart and Wright (*5*). The cells have thicknesses of from 0.427 to 0.018 mm. and have been used at pressures up to 35 pounds per square inch (2.5 kg. per sq. cm.) gage to record the spectra of the liquid butenes and 1,3-butadiene from 1 to 15 microns at room temperature (*2*).

DESIGN AND CONSTRUCTION

The cell frames were designed to fit the cell holder of Perkin-Elmer spectrometers. They were made of steel and somewhat heavier than normal to minimize possible deformation under pressure.

Preliminary trials indicated that leaks could be made less likely by increasing the length of the potential leak path. To provide for this and also to minimize stresses in the windows, the filling holes were drilled through one of the windows normal to its surface. Because it was desired not to intercept any of the cone of radiation which could pass the usual 21-mm. diameter opening in the base plate, a larger window was necessary. To simplify the procurement of additional windows, one of the standard stock sizes supplied by Perkin-Elmer was chosen (49.5 mm. in diameter and 6.5 mm. thick).

The dimensions and general character of the metal fittings (union-type connections and needle valves developed at the National Bureau of Standards by H. F. Stimson) which it was desired to use to connect sample cylinders to cells were such that connections to the cover plate (communicating through it to the holes in the upper salt window) were made radial rather than normal to its surface to avoid intercepting portions of the beam.

There was insufficient room to connect tubing at the bottom when the cell frame was in position with its connections vertical, but there was ample room for a sealing cap (*N* in Figure 1). It had been determined that cells would fill nearly as well laterally as vertically with liquids having a vapor pressure above 1 atmosphere. Consequently, in this application, the cells were assembled with the connections from side to side (*H* in Figure 1).

Use of the same cells in work with liquids of lower vapor pressure or with solutions (as well as accommodation to a sample-transfer device for vertical filling to be described in a subsequent paper) was made possible by drilling the cover plate with two sets of holes, which permitted use of the cell with the connections in a lateral or vertical position, *J*, as desired.

The possibility of cracking the windows by uneven loading was minimized by a cushion, *B*, on the base plate. The possibility of crushing was minimized by loading the cover plate with a coil spring, *O*, under each of the four nuts.

The construction and parts of the cell are shown in Figure 1 The base plate, *A*, was machined flat and polished on the outer face. The cushion, *B*, was made of rubber, Pliofilm, polyethylene, or blotting paper. None of these materials is as uniform in compressibility or thickness as might be desired. The polyethylene is less absorbent for liquids than the others, but is also less able to deform sufficiently for good load distribution. No windows have been cracked as a result of inadequate support by any of these materials.

The spacer, *D*, between the two windows, *C* and *E*, was made of lead sheet, except the thinnest, which was tin foil 0.0005 inch (0.0127 mm.) thick.

The filling holes in the window, *E*, were drilled by hand with a sharp 1.5-mm. twist drill in a pin vise. By drilling the holes before removing the protecting plastic film, the drill could be run all the way through with very little chipping at the edges of the hole on either side of the window. Any resistance to turning can be felt easily and undue force which might split or chip the window can be avoided.

The gasket, *F*, between the drilled window and the cover plate, *G*, was made of lead sheet thick enough for convenient handling. The two holes were punched with a jeweler's punch and the burr was removed by judicious scraping, followed by pressing between plate glass to ensure flatness and to detect high spots.

The cover plate, *G*, was drilled with two sets of holes for the studs of the base plate, to permit orientation of the filling holes and threaded connections in a lateral, *H*, or in a vertical, *J*, position as desired. The diameter of the window opening between the two straight sides was made large enough to permit use of the full height of the slit with the connections vertical.

Connection to tubing was made by means of brass ells, *K*, machined to fit, and sweated into holes in the cover plate with soft solder, after which the inner face of the cover plate was machined flat and polished, the inner ends of the ells being flush and forming a continuous surface with the face of the plate. The 1-mm. passage through the ell was enlarged to a depth of 3 or 4 mm. at the outer threaded end, *L*, to permit entry of the tip of the stainless steel cone fitting, *M*, which formed the seat for the connection to tubing. The stainless steel cone fitting was silver-soldered to the $^1/_8$-inch outside diameter copper tubing with which the connections were made.

ASSEMBLY

The following description of technique is given at some length in the belief that tightness depends on care with respect to many details.

The cell was sealed by amalgamating the surfaces of the metal spacer which separates the windows, and of the gasket between the outer window and the cover plate. The use of amalgamated lead or tin as described by Gildart and Wright (*5*) provides both an accurate fit of minute surface irregularities and a fair degree of adhesion.

The surfaces of all parts participating in the seals must be flat, smooth, and clean, including the plate glass on which spacer and gasket material is cut, trimmed, and flattened. Even finger marks (on the glass or on the flat surface of the cover plate, *G*), to which particles of dust or lint may adhere and become embedded in, or distort the surface of, spacer or gasket, can cause leaks. Particles resulting from cutting, trimming, or thinning burred edges must be carefully removed before finally flattening the spacer or gasket between two pieces of plate glass. High spots may be made apparent by twisting the upper piece of glass while pressing it against the soft metal.

The spacer, *D*, or gasket, *F*, may be amalgamated on both sides, while lying on the plate glass, by spreading a drop of mercury with a small wad of cotton moistened with carbon tetrachloride. The amalgamation and assembly should be done promptly. The freshly amalgamated second surface, freed from particles of oxide and lint, should have a thin layer of liquid mercury evenly distributed over it. This surface should then be placed face down on the rock salt window, and the newly exposed surface completed in the same way, at the same time orienting and adjusting its position with respect to the window.

In the case of extremely thin spacers it is advisable to apply the mercury after placing the foil on the window. The amalgamated area should not extend to the outer edge, except at the filling holes, and the film of liquid mercury remaining on the surface should be reduced but not wiped dry. Some mercury penetrates between the spacer and the window, amalgamating a narrow band on the underside and often making it unnecessary to treat the underside of the spacer. Excess mercury, oxide, and lint should be carefully removed from the salt window and the position of the spacer adjusted before placing the second window.

After the cover plate, springs, and nuts are in place, the nuts should be turned down until adjacent turns of the springs nearly touch and the amalgam is allowed to harden for about 2 weeks in the case of very thin spacers. Two days were sufficient in those cases where the mercury did not completely penetrate the spacer metal. The mercury was not spread to the outer edge of thin spacers because of the smaller amount of metal available for amalgamation. The longer hardening time was required to permit penetration of the thin sheet radially to an extent sufficient to reduce the mercury content at the edge to the point where a hard amalgam could result.

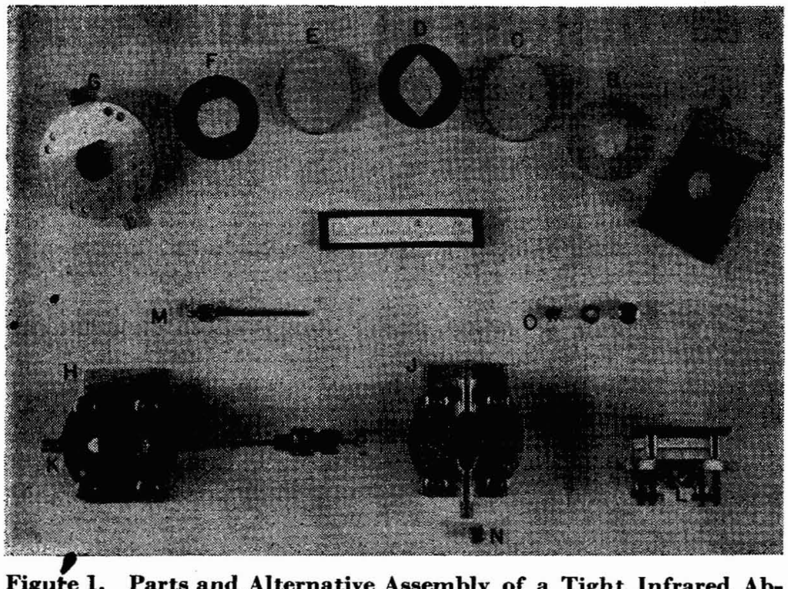

Figure 1. Parts and Alternative Assembly of a Tight Infrared Absorption Cell for Liquids Having Vapor Pressures Up to 35 Pounds per Square Inch Gage

Windows are rock salt sealed with amalgam

TESTING

One of the connections to the cell was capped and the other connected to a cylinder of compressed gas through a pressure regulator.

The cell was immersed in carbon tetrachloride and the pressure gradually increased. Extremely small bubbles are usually formed and rise in a fine stream, which can be detected easily in a good light. When a leak was found the cell was removed from the carbon tetrachloride and the nuts were loosened gradually with the cell under moderate pressure. The spreading leak gradually separated the window from the spacer (or the gasket from the cover plate) as the confining pressure was relieved. The cell was then taken apart only at the layer of separation, the faulty surface reamalgamated as before, the cell reassembled and allowed to set, and retested. This process was repeated until no leaks were detected at 35 pounds per square inch gage. This was the maximum pressure deemed advisable for use with the present loading springs. Stronger springs would permit higher pressures. The maximum safe pressure for salt windows 6.5 mm. thick has not been determined.

DISCUSSION

It is not advisable to attempt to use a cell that leaks even slightly, for the leak is likely to spread and the sharp local chilling resulting from the evaporation of liquid in the leak may crack both windows. The experience of the authors in this respect has convinced them that the crack results from the leak, not the reverse. Such a crack can liberate enough flammable gas to constitute a fire hazard.

After a period of use, the liquid penetrated slightly into cracks in the brittle tin amalgam at the confining edge of the spacer. The expansion accompanying release of pressure during elimination of bubbles while filling, or when emptying or evacuating, has caused small fragments of the confining edge of very thin spacers to separate slightly. This process may eventually progress to the point where leakage will occur. This behavior is not expected to occur with thicker spacers where there is a core of unamalgamated metal left to hold the brittle surfaces intact. It seems advisable, therefore, to test by immersion from time to time cells which show such behavior, thus minimizing the possibility of a leak breaking through while in use. After testing, the thickness of the cells was determined by the interference method described by Smith and Miller (*8*).

The effect of the internal pressure on the thickness of one of the cells was evaluated by determining the thickness before and while the cell was subjected to 25 pounds per square inch gage with oxygen. The increase amounted to 0.0004 mm., about 0.1% of the 0.427-mm. cell or 4% of the thinnest cell.

When it is desired to use the cells for solutions and to fill the cells from the bottom, the change of orientation of the connections from a lateral to a vertical position is readily accomplished by removing the nuts and springs, lifting the cover plate and window assembly as a unit from the studs, rotating through 90°, and replacing them in the desired position without impairing the tightness of the cell.

The cells may be cleaned readily without taking them apart by applying suction to one connection and flushing with solvent from a medicine dropper. When it becomes necessary to repolish the windows, a separation at the least perfect bond may be accomplished as described above in connection with curing leaks. A tightly adhering spacer or gasket, thus exposed on one side, may be removed without damage to the window by disintegration in excess mercury.

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Determination of Carbon Monoxide in Hydrocarbon Gases Containing Olefins

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STUDIES of hydrocarbon synthesis and catalytic cracking made it necessary to determine carbon monoxide in gases that contain large quantities of olefins. Numerous methods have been published for the determination of carbon monoxide, but they are not suitable for samples containing olefins.

Colorimetric methods (*4, 8*) have been used for the determination of carbon monoxide in concentrations less than 1%. Heated cupric oxide (*1*) has been employed for oxidizing carbon monoxide to carbon dioxide. However, hydrogen and some paraffin hydrocarbons are also oxidized at the temperature necessary to oxidize carbon monoxide completely. Teague (*15*) determined carbon monoxide iodometrically in motor exhaust gas, employing iodine pentoxide for the oxidation, with a precision of 0.003% for samples containing 0.01 to 0.1%. Larson and Whittaker (*10*) hydrogenated carbon monoxide over nickel catalyst at a temperature of 290° to 310° C. McCullough *et al.* (*11*) oxidized the gas with red mercuric oxide at elevated temperature.

Some of the reagents more commonly employed for the absorption of carbon monoxide are acid cuprous chloride, ammoniacal cuprous chloride, and a suspension of 2-naphthol in acid cuprous sulfate. These reagents were reported to dissolve olefins (*5*), and Table I illustrates this solubility. They must be removed before the determination of carbon monoxide. All the above methods depend on the absence of unsaturated hydrocarbon gases. For the removal of unsaturates, sulfuric acid and bromine water have been commonly used.

Fuming sulfuric acid and silver sulfate–activated sulfuric acid (*7, 16*) were found unsuccessful in this laboratory for the removal of ethene from ethene-hydrogen mixtures. Results in Table II show that even after 47 passes not all the ethene was absorbed. Incompleteness of absorption was confirmed by hydrogenation of the gases remaining after sulfuric acid treatment of an ethene-hydrogen mixture. Francis and Lukasiewicz (*6*) absorbed ethene in mercuric sulfate–sulfuric acid reagent, but Brooks *et al.* (*2*) found this reagent slowly oxidizes carbon mon-

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A procedure is described for determining carbon monoxide in gases that contain olefinic hydrocarbon gases. All hydrocarbons except methane are removed rapidly by condensation in liquid nitrogen from which the uncondensed gases—carbon monoxide, hydrogen, nitrogen, oxygen, and methane—are removed by a Töpler pump. Orsat apparatus is used for the removal of oxygen from this mixture and for the determination of carbon monoxide. The major part of the carbon monoxide is absorbed in acid cuprous chloride and the remainder in cuprous sulfate-2-naphthol. Carbon monoxide is determined in concentrations from 0.1 to 100% with an average error of less than 0.5% of the carbon monoxide in samples containing 10% carbon monoxide. The accuracy is somewhat less for lower concentrations. The total time required for the determination is less than an hour.

oxide. Savelli (*14*) obtained good results using one fourth saturated bromine water for the absorption of unsaturated hydrocarbons in butane-butene mixtures. However, this procedure, even if it be a satisfactory means of eliminating olefins without affecting carbon monoxide, is longer and requires much more careful control than the procedure described in this paper.

Table I. Absorption of Gases Containing Olefins in Cuprous Reagents[a]

Sample	Composition of Blend, Volume % CO	Hydrogen	Ethene	Propene	Isobutene	2-Butenes	Butane	Olefins + CO	% Gas Absorbed
1	...	71.9	28.1	...	...	...	...	28.1	27.2, 27.7
2	19.9	13.7	...	24.3	...	...	42.1	44.2	46.7, 46.2
3	...	47.2	...	30.5	...	...	22.3	30.5	30.7, 30.5
4	...	67.4	...	...	32.6	...	...	32.6	29.9, 30.3
5	27.7	41.5	...	...	...	30.8	...	58.5	55.1
6	...	69.2	...	...	...	30.8	...	30.8	28.5, 27.7
7	...	39.5	...	...	...	31.6	28.9	31.6	29.9, 29.6
8	...	71.6	...	28.4	...	...	...	28.4	27.3, 26.4

[a] Acid cuprous chloride followed by cuprous sulfate–2-naphthol mixture.

Hopcalite, a mixture of 50% manganese dioxide, 30% copper oxide, 15% cobalt oxide, and 5% silver oxide, is reported (*9*) to oxidize carbon monoxide at ordinary temperatures, which would be a distinct advantage for the present problem. It is reported to be satisfactory on concentrations up to 0.1% (*13*); the oxidation is catalytic and utilizes oxygen from the air or from the sample. Tried in this laboratory over a wide range of concentrations up to 20%, only in the absence of ethene and at low carbon monoxide concentration were fair results obtainable. Oxidation was incomplete on higher concentrations and the presence of ethene causes results to be too high (Table III). This laboratory obtained erratic results for carbon monoxide, generally low, when attempting to hydrogenate the olefins preliminary to the determination of carbon monoxide by cuprous reagents.

Manchot and Scherer (*12*) determined carbon monoxide directly in the presence of ethene by reaction with standard silver hydroxide–pyridine reagent, the silver consumed being a measure of carbon monoxide. However, the authors found that results by this method were high and appreciable amounts of apparent carbon monoxide were found in samples containing none (Table IV). Other investigators (*17*, *18*) also have reported difficulties with this procedure.

Table II. Use of Sulfuric Acid for Removing Ethene from Hydrogen-Ethene Mixtures

Sample	Ethene, Volume % Present	Found	No. of Passes	Reagent Used
1	5.6	3.6	20	Fuming H_2SO_4
2	3.6	2.9	25	Fuming H_2SO_4
3	4.8	1.5	23	Activated H_2SO_4[a]
4	2.4	1.7	30	Activated H_2SO_4
5	7.7	5.8	47	Activated H_2SO_4
6	6.9	5.3	25	Activated H_2SO_4
7	9.0	5.1[b]	20	Activated H_2SO_4

[a] Silver sulfate (0.6%) in 98% H_2SO_4.

[b] Residual gas, after sulfuric acid treatment, of sample 7 contained 3.8% ethene as determined by hydrogenation.

The method described in this paper uses liquid nitrogen to condense and remove all hydrocarbon gases except methane. The noncondensable portion of the sample contains only hydrogen, nitrogen, oxygen, carbon monoxide, and methane. Orsat technique is applied using potassium pyrogallate to remove oxygen and acid cuprous chloride, followed by 2-naphthol in acid cuprous sulfate to absorb the carbon monoxide.

GASES USED

Hydrogen, oxygen, methane, ethane, propane, butane, ethene, propene, and isobutene (2-methylpropene) used in the experimental work were purchased as C.P. materials 99+% pure. Acetylene was purchased as a solution in acetone and was purified by passing successively through a saturated solution of sodium bisulfite and a 20% solution of alkali, and finally dried over anhydrous calcium chloride. The 2-butenes were prepared by dehydration of 2-butanol with sulfuric acid and distilled to a purity of 98+%. Carbon monoxide was prepared by the action of sulfuric acid on formic acid and tested 99+% pure.

REAGENTS AND APPARATUS

The following reagents were used:

Potassium hydroxide solution, 33%.

Potassium pyrogallate solution (*3*) was made from 15 grams of pure pyrogallic acid diluted to 50 ml. with water, and 105 grams of potassium hydroxide diluted to 175 ml. with water. The pyrogallic acid solution was added to the pipet first, followed by the potassium hydroxide solution.

Acid cuprous chloride (*3*) was made from cuprous chloride,

Table III. Determination of Carbon Monoxide by Oxidation with Hopcalite

(50% MnO_2, 30% CuO, 15% Co_2O_3, and 5% Ag_2O)

Sample	Composition of Blend, Volume % CO	Oxygen	Ethene	Hydrogen	CO Found, Vol. %
1	...	100	...	...	0
2	0.9	4.5	...	94.6	0.5
3	1.4	4.2	...	94.4	1.2
4	18.5	81.5	...	...	13.5
5	6.8	93.2	...	...	5.8
6	6.3	93.7	...	...	5.6
7	20.3	10.2	...	69.5	16.0
8	...	5.2	9.5	85.3	1.3
9	1.2	3.3	15.5	80.0	4.7
10	2.2	67.3	30.5	...	16.0
11	2.7	63.5	33.8	...	15.1

Table IV. Determination of Carbon Monoxide with Silver Hydroxide–Pyridine

Sample	Composition of Sample, Volume % Ethene	Hydrogen	CO	CO Found, Vol. %
1	45.8	51.6	2.6	3.8
2	20.4	75.7	3.9	7.5
3	32.3	67.7	0	3.8
4	28.6	71.4	0	3.7
5	20.5	79.5	0	4.8
Blank	...	100.0	...	0.6
Blank	...	100.0	...	1.4

c.p., 75 grams, hydrochloric acid, c.p. (concentrated), 600 ml., and water to make 1000 ml.

Cuprous sulfate–2-naphthol mixture (*3*) is commercially available.

Liquid nitrogen.

Mercury, to fill manometers, burets, and Töpler pump.

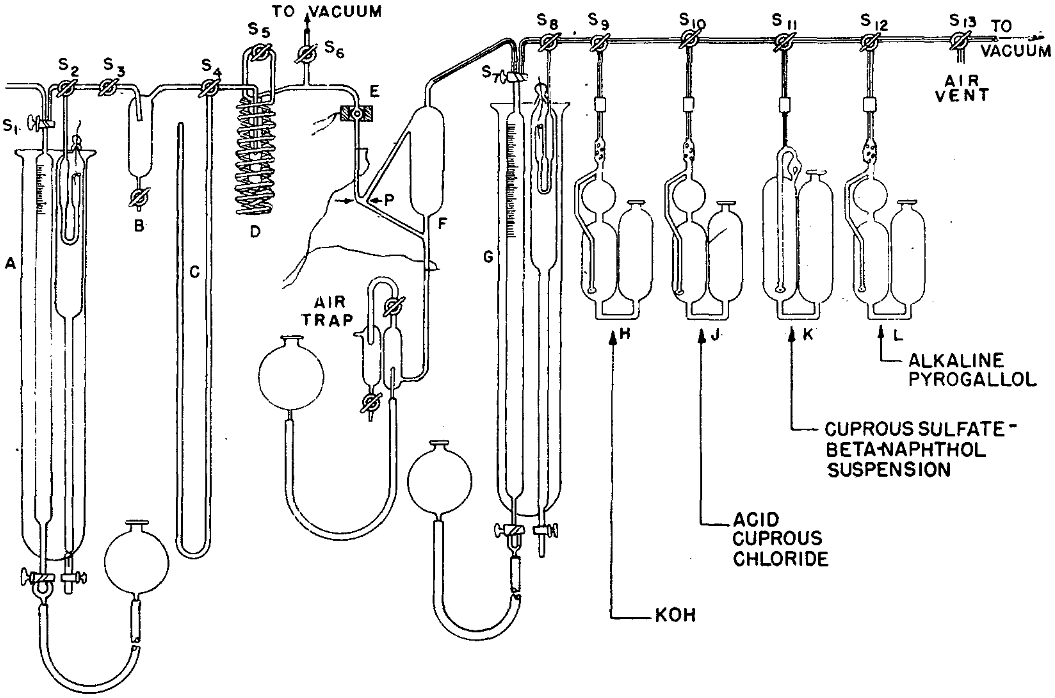

Figure 1. Apparatus for Analysis of Carbon Monoxide in Hydrocarbon Gases

The apparatus employed in the investigation is illustrated in Figure 1. Pipets *H*, *J*, and *L* are Tramm pipets and *K* is a Francis Autobubbler pipet. The Francis Autobubbler pipet is used because cuprous sulfate–2-naphthol does not clog it as badly as a Tramm pipet. Each pipet is charged with 180 ml. of solution. A vacuum pump and Dewar flask, which are needed, are not shown.

PROCEDURE

The apparatus (Figure 1) is prepared for an analysis by drawing the mercury in the compensating manometers up to their respective stopcocks, S_2 and S_8, and the stopcocks are closed to the manometers. Stopcock S_7 is removed and 0.5 ml. of a 1% solution of wetting agent (*19*), such as Tergitol or Aerosol, is introduced into the buret, which serves to saturate the gaseous samples and ensures clean drainage. The wetting solution will need to be replenished only after 10 to 15 runs, as very little is consumed in saturating the gas. The stopcock is greased properly and put back into place. One milliliter of water is added to the compensating manometer jacket of buret *G* to compensate for effect of temperature changes.

The mercury leveling bulb of the Töpler pump, *F*, is set at such a level that mercury will not rise above point *P* when the system is evacuated. The check valve, *E*, is closed by a solenoid when mercury rises high enough to complete the electrical circuit, instead of depending on rising mercury to close it. The apparatus is evacuated from buret *A* to stopcock S_7 while the operations of the next paragraph are conducted.

Table V. Effect of Cuprous Chloride Reagent on Samples of Low Carbon Monoxide Content

		Composition of Blend, Volume %			No. of Passes		
Sample	Run	CO	Methane	Hydrogen	Through Cu_2Cl_2	Through Cu_2SO_4	CO Found, Vol. %
1	1	0.4	77.0	22.6	5	45	1.0
	2				None	45	0.3
	3				None	45	0.4
2	1	2.4	45.9	51.7	None	30	2.2
	2				None	30	2.2
	3				5	60	3.1
3	1	5.2	24.2	70.6	5	30	5.3
	2				None	45	5.2
	3				None	30	5.0
4	1	9.0	71.9	19.1	5	45	9.4
	2				5	45	9.3
	3				None	75	9.1

Because nitrogen is an inert gas and easily obtained from the atmosphere, it is used as diluent for samples in the Orsat part of the analysis. In case a check run is made, a portion of the carbon monoxide–free residue, remaining from the previous run, is used as diluent. The solutions in pipets *H*, *J*, *K*, and *L* are drawn up into the capillary of the pipets to a reference point, just below the rubber connections, and their respective stopcocks, S_9, S_{10}, S_{11}, and S_{12}, are closed. About 20 to 25 ml. of air are taken into buret *G* through stopcock S_{13} and freed of oxygen by scrubbing in the alkaline pyrogallate pipet. The pure nitrogen is saved in *G* for subsequent use as diluent.

The remainder of the system including sampling buret *A*, mercury trap *B*, condensing spiral *D*, and Töpler pump *F* should be completely evacuated by this time. Stopcock S_3 is closed and about 60 ml. of dried sample are introduced into buret *A* through the left bore of stopcock S_1. A calcium chloride tube has been found suitable for drying samples. The sample is measured against the compensating manometer of the sampling buret and stopcock S_2 is closed to this manometer. The volume of the right bore of stopcock S_1 and the manifold as far as stopcock S_3 is added to the volume of the buret.

Stopcocks S_5 and S_6 are closed, but S_4 remains open for gas passage and to manometer *C*. Condensing trap *D* is surrounded with a Dewar flask containing liquid nitrogen and stopcock S_3 is opened. After 5 minutes to allow for maximum condensation of the sample, stopcock S_5 is opened to permit the uncondensed portion to flow through the spiral into the Töpler pump. The mercury in buret *A* is raised to stopcock S_1.

With stopcock S_7 closed, the mercury in buret *G* is lowered so that the diluent (nitrogen) is under reduced pressure, and the uncondensed gas is transferred to it by the Töpler pump. After most of the uncondensed gas has been transferred, stopcock S_5 is closed and the liquid nitrogen removed from the condensing spiral, *D*, so that any occluded noncondensable gas will be liberated. The liquid nitrogen is replaced around spiral *D*, and after 5 minutes stopcock S_5 is opened and the transfer of noncondensable gas is completed without again vaporizing the condensed gas. If methane is present about 30

Table VI. Separation of Condensable and Noncondensable Gases

Sample	Composition of Blend, Volume % 1	2	3	4	5
Carbon monoxide	12.7	54.5	90.3	19.1	38.1
Hydrogen	...	...	...	20.1	17.2
Methane	...	...	...	26.7	20.3
Ethene	43.5	31.9	9.7	34.1	24.4
Ethane	...	13.6	...	...	...
Acetylene	13.9	...	...	...	...
Isobutene	24.9	...	...	...	...
Condensables					
Taken	87.3	45.5	9.7	34.1	24.4
Found	87.3	45.2	10.0	34.3	24.5
Noncondensables					
Taken	12.7	54.5	90.3	65.9	75.6
Found	12.7	54.7	89.9	65.8	75.4

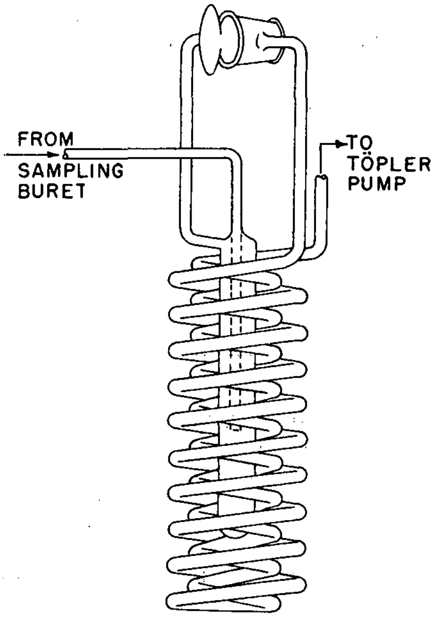

Figure 2. Spiral Condensing Trap

transfers may be required to transfer all of it. However, it is not necessary to transfer all the methane unless it is desired to determine the total noncondensables in the sample. In practice, the completeness of transfer is determined by the volume of gas that appears in the capillary tube which joins the Töpler pump to buret *G*. When the amount of gas in each transfer is less than 0.05 ml. at atmospheric pressure, the removal is considered complete. The time required to transfer all the noncondensable portion is 10 to 20 minutes, depending on the nature of the sample.

The volume of the noncondensed portion of the sample, and the diluent with which it has been mixed in buret *G*, is read after balancing against the compensating manometer. The atmospheric pressure and temperature of the bath surrounding the buret are recorded. Oxygen is removed by passing the mixture through the alkaline pyrogallate solution until constant volume is reached, then carbon monoxide is determined by absorbing the major part of it in acid cuprous chloride and the remainder with cuprous sulfate-2-naphthol mixture. The sample is passed through cuprous chloride five times and through cuprous sulfate-2-naphthol until a constant volume is reached. This preliminary absorption in cuprous chloride has been found sufficient to reduce the carbon monoxide in samples of high concentrations, so that an excessive number of subsequent passes through cuprous sulfate-2-naphthol will not be required. More passes through cuprous chloride result in absorption of measurable quantities of methane. The potassium hydroxide pipet is used to remove acid fumes from the gases before each buret reading.

High results are obtained by the above procedure on samples containing less than 5% carbon monoxide (Table V). Therefore, if the carbon monoxide content is found to be less than 5%, a check run should be made on the sample, using only cuprous sulfate-2-naphthol mixture rather than both cuprous chloride and cuprous sulfate-2-naphthol solutions. For higher concentrations of carbon monoxide the use of cuprous sulfate-2-naphthol alone is much too slow.

CALCULATIONS

$$\text{Total pressure (mm.)} - \text{vapor pressure of water (mm.)} = \text{pressure of dry gas}$$

$$\frac{\text{Pressure of dry gas} \times \text{ml. of CO (wet)}}{\text{total pressure}} = \text{ml. of dry CO}$$

$$\frac{\text{Ml. of dry CO} \times 100}{\text{ml. of original sample}} = \text{volume \% CO}$$

DISCUSSION

The principal advantage of the present method lies in the effective separation of the carbon monoxide from olefins and other hydrocarbons, except methane. The high quality of the separation of noncondensables effected by the segregation step described is apparent from the data in Table VI. The condensables found do not represent determinations by difference but were measured after permitting them to come to room temperature. The total time required to measure the sample and transfer the noncondensed gas to buret *G* is 20 to 30 minutes, depending on the amount of methane present. The vapor pressure of methane at the temperature of liquid nitrogen is approximately 10 mm. Because of this low vapor pressure as much as 20 minutes are required to transfer all the methane and other noncondensed gases in a sample when the methane content is high.

A better view of the trap used to retain the condensable gases is given in Figure 2. The coil is made of 7-mm. glass tubing and the central bulb of 2-cm. tubing. The over-all height, from base of the coil to top of the bulb, is 18 cm. It fits inside a pint Dewar flask, and its spirals present enough cooling surface to prevent danger of condensed gases being carried through the trap by mechanical means.

Table VII shows typical data by the complete method for concentrations of carbon monoxide up to 100%, the effectiveness of the separate steps—segregation of noncondensables and absorption of carbon monoxide—being already established. No corrections for deviations of the gases from the ideal gas law were deemed necessary for the carbon monoxide determination on the type of sample involved in this investigation.

Table VII. Typical Data

Sample	Composition of Blend, Volume % CO	Hydrogen	Methane	Ethene	Ethane	Acetylene	Propane	Isobutene	CO Found, Vol. %
1	12.8	...	...	43.5	...	18.8	...	24.9	12.7 12.9
2	24.2	24.3	26.4	...	...	...	25.1	...	24.3 24.4
3	31.6	37.0	...	...	...	...	31.4	...	31.6 31.5
4	38.7	30.3	31.0	...	...	...	...	...	38.8 38.7
5	54.5	...	...	31.9	13.6	...	...	...	54.1 54.2
6	67.1	4.7	28.2	...	...	...	...	...	66.7 66.8
7	74.0	4.4	21.6	...	...	...	...	...	73.8 73.7
8	89.2	5.4	5.4	...	...	...	...	...	88.9 89.0
9	100	...	...	...	...	...	...	...	99.5 99.[illegible]

The condensables are particularly suitable for subsequent determination of olefins because hydrogen and carbon monoxide, which interfere with most olefin reagents, are already eliminated.

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Separation of Polybasic Acids by Fractional Extraction

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Distribution coefficients of a number of mono- and polybasic acids between water and various organic solvents are reported. The solvents are arranged in order of extracting capacity; this order is valid for most of the acids examined. Several polybasic acids have been subjected to fractional extraction procedures to show that the acids distribute themselves among the funnels in approximately the manner calculated from the known distribution coefficients. Application of the fractional extraction technique to water-soluble acids derived by oxidative cleavage of a butadiene-styrene copolymer results in removal of substantially all the phenyl-containing material from the bulk of the acids as well as in separation of the β-phenyladipic acid that is present.

IN CONNECTION with a series of structure studies being carried out on GR-S and related copolymers of butadiene, it was necessary to develop new techniques for separating the polybasic acids of high molecular weight derived by oxidative cleavage of these polymers. Rabjohn, Bryan, Inskeep, Johnson, and Lawson (*6*) found that distillation of the methyl esters was satisfactory only in the case of the lower members of the dibasic acid series because of the nonvolatility of esters of the acids of higher molecular weight.

The separation of nonvolatile materials by fractional extraction is a method that has been developed in the past few years. Craig (*3*) has presented an excellent review of the literature in this field, and Bush and Densen (*2*) have discussed the principles involved in laboratory-scale fractional extractions. The method has been applied to the separation and identification of the components of mixtures of aliphatic acids by Atchley (*1*) and by Sato, Barry, and Craig (*7*), but in both cases buffered solutions were used and it was difficult to recover the acids in high yields without contamination.

Before attempting to apply the method to the separation of unknown mixtures of acids, the behavior of some known acids in unbuffered extraction systems was investigated. The distribution coefficients of a number of mono- and polybasic acids between water and various organic solvents were determined. After this had been done, fractional extraction procedures were applied to several of these acids to learn whether their distribution among the funnels would check the calculated values.

DISTRIBUTION COEFFICIENTS

Because extremely accurate results were not of critical importance in this work, the distribution coefficients were determined in a simple and rapid fashion. The data obtained, however, are accurate to approximately 3 to 5%.

The acids that were soluble in water to the extent of 1% were made up in distilled water to a concentration of 1 gram per 100 ml. Two milliliters of this solution were pipetted into a test tube, 10 ml. of water were added, the mixture was shaken, and a 2-ml. aliquot was titrated to phenolphthalein end point with 0.010 *N* sodium hydroxide. Ten milliliters of the extracting solvent were added to the test tube, which was stoppered and shaken thoroughly. After the layers had settled, a 2-ml. sample of the water layer was again titrated with the same standard alkali. The distribution coefficient, *K*, was calculated as the quotient of concentration in the water phase (by direct titration) over concentration in the organic phase (by difference). As ratios of less than 1 to 10 or more than 10 to 1 were difficult to estimate accurately, they were reported as 0.1 and 10, respectively.

Acids that were not soluble in water to the extent of 1% could not be handled in this manner. These acids—suberic, azelaic, sebacic, benzoic, and β-phenyladipic—were tested in the following manner. One tenth gram of the acid was dissolved in 100 ml. of distilled water and 2 ml. of this solution were titrated with 0.010 *N* sodium hydroxide to phenolphthalein end point. Ten milliliters of the solution were then extracted with 10 ml. of the extracting solvent and after the layers had separated, a 2-ml. portion of the water layer was titrated. The value of *K* was calculated as before. No special effort was made to keep the temperature of the solutions constant; in all cases the temperature of the room was within 2° of 26° C. When the extracting liquid was soluble in

Table I. Distribution Coefficients

	Citric	β-Carboxyadipic	Succinic	Adipic	Fumaric	Suberic	Sebacic	Benzoic
H_2O–Skellysolve B	10	10	10	10	10	10	6.5	1.4
H_2O–CCl_4	10	10	10	10	10	10	4.5	0.59
H_2O-benzene	10	10	10	10	10	10	3.6	0.42
H_2O–$CHCl_3$	10	10	10	10	10	10	0.62	0.27
H_2O–diisopropyl ketone	10	10	10	4.8	2.8	0.53	0.1	0.1
H_2O–butyl acetate	10	10	10	2.9	2.1	0.38	0.11	0.1
H_2O–ethyl ether	10	10	8.0	2.2	1.1	0.34	0.1	0.1
H_2O–methyl isobutyl ketone	10	6.8	5.4	1.2	0.83	0.21	0.1	0.1
H_2O–ethyl acetate	10	3.2	2.7	0.91	0.59	0.16	0.1	0.1
H_2O–methyl propyl ketone	6.2	2.2	2.3	0.55	0.43	0.12	0.1	0.1
H_2O–methyl ethyl ketone	3.0	1.0	1.0	0.50	0.38	0.21	0.1	0.1
H_2O-cyclohexanone	4.7	0.74	0.91	0.32	0.29	0.14	0.1	0.1
H_2O–*n*-butanol	3.5	0.85	0.83	0.31	0.30	0.12	0.1	0.1

10 signifies equal to or greater than 10; 0.1 signifies equal to or less than 0.1.

Table II. Distribution Coefficients

	H_2O / *n*-Butanol		H_2O / Ethyl Acetate		H_2O / Ether		H_2O / $CHCl_3$		H_2O / Skellysolve B	
Acid	Concn.[a]	Dil.[b]	Concn.	Dil.	Concn.	Dil.	Concn.	Dil.	Concn.	Dil.
Oxalic	4.6	5.8	10	10	10	10	10	10	10	10
Malonic	1.5	1.9	4.0	4.5	8.1	8.5	10	10	10	10
Succinic	0.83	1.0	2.7	4.3	8.0	6.0	10	10	10	10
Glutaric	0.59	0.62	1.5	1.5	4.2	3.9	10	10	10	10
Adipic	0.31	0.36	0.91	0.83	2.2	2.1	10	10	10	10
Pimelic	0.15	0.17	0.38	0.38	0.91	0.91	10	10	10	10
Suberic	0.12	0.12	0.16	0.20	0.34	0.36	10	10	10	10
Azelaic	0.1	0.1	0.1	0.1	0.11	0.14	3.8	4.6	10	10
Sebacic	0.1	0.1	0.1	0.1	0.1	0.1	0.63	0.91	6.5	10
β-Carboxyadipic	0.83	...	3.2	...	10	...	10	...	10	...
β-Phenyladipic	0.1	...	0.1	...	0.1	...	4.0	...	10	...
Fumaric	0.30	...	0.59	...	1.1	...	10	...	10	...
Citric	3.5	...	10	...	10	...	10	...	10	...
Formic	1.2	1.3	1.7	1.7	3.1	2.8	10	10	10	10
Acetic	0.91	0.91	1.5	1.5	2.3	2.4	10	10	10	10
Propionic	0.36	0.36	0.45	0.45	0.67	0.63	5.6	7.6	10	10
Butyric	0.14	0.16	0.18	0.19	0.19	0.22	1.2	1.7	10	10
Benzoic	0.1	0.1	0.1	0.1	0.1	0.1	0.27	0.48	1.4	3.1

[a] Concentration of acid in water before extraction with an equal volume of solvent. For most acids this was 0.16%. For suberic, azelaic, sebacic, benzoic, and phenyladipic it was 0.1%.

[b] One half original concentration was used.

10 signifies equal to or greater than 10; 0.1 signifies equal to or less than 0.1.

water to the extent of more than 2%, the water and organic solvent were mutually saturated at the temperature of the extraction before use.

The results show that the extracting solvents can be arranged in order of increasing effectiveness and that this order can be applied with few exceptions to any of the acids examined. The order in which the solvents may be arranged, proceeding from the weakest to the strongest, is as follows:

Skellysolve B (boiling point 60° to 65° C.)
Carbon tetrachloride
Benzene
Chloroform
Diisopropyl ketone
Butyl acetate
Ethyl ether
Methyl isobutyl ketone
Ethyl acetate
Methyl propyl ketone = methyl ethyl ketone = cyclohexanone = *n*-butanol

The polybasic acids may also be arranged in a definite order of relative affinity for the aqueous phase, and this order holds true for most of the solvents examined. The order in which the polybasic acids may be arranged, beginning with those which favor the water layer the most strongly, is as follows:

Oxalic acid
Citric acid
Malonic acid
β-Carboxyadipic acid
Succinic acid
Glutaric acid
Adipic acid
Fumaric acid
Pimelic acid
Suberic acid
β-Phenyladipic acid
Azelaic acid
Sebacic acid

For the monobasic acids tested, the order runs as follows:

Formic acid
Acetic acid
Propionic acid
Butyric acid
Benzoic acid

The distribution coefficients which were obtained are listed in Tables I and II. Table I includes values for thirteen solvents which were tested with eight acids. In Table II eighteen acids are included and the number of solvents has been reduced to five, which cover the range of extractive power adequately. Coefficients obtained at one half the original concentration are also included in Table II to show the effect of concentration upon the distribution coefficient. All values in Table I are for the more concentrated solutions.

In examining the data in these tables, the polybasic acids must be considered apart from the monobasic acids, for the distribution coefficients of the former change much more with changes in the extracting solvent than do those of the latter. In general, there is little change in distribution coefficient with a twofold change in concentration in extractions using *n*-butanol, ethyl acetate, or ether, but in the cases of chloroform and Skellysolve B the effect of the concentration change is considerable. This is probably due to association of the molecules of acid in the latter solvents, which contain too little water to saturate the hydrogen bonding capacity of the carboxyl groups. The dibasic acids containing an odd number of carbons fit perfectly into the series of even-numbered acids despite the solubility differences between the two groups.

DISTRIBUTION OF ACIDS AMONG THE FUNNELS

The two simplest and most widely used arrangements of funnels in fractional extractions are the "horizontal stage" and the "vertical stage." Bush and Densen (*2*) have discussed the relative merits of these techniques and concluded that the diagonal stage technique is superior. With either arrangement, the operations may be carried out with a series of funnels or with the Craig (*4*) countercurrent distribution machine, if one is available. In this laboratory it has been convenient to replace the series of separatory funnels with one separatory funnel and a series of flasks. By contact of one portion of the lighter solvent with *n* portions of the heavier solvent in succession, then contact of the second lighter layer with the same series of washes, etc., either the diagonal or horizontal type of operation may be carried out with a minimum of contamination with stopcock grease. In cases where large volumes of liquids are used, this technique is especially advantageous, because it eliminates the necessity of handling numerous large separatory funnels.

In order to ascertain whether dibasic acids would distribute themselves among the funnels as calculated from the known distribution coefficients, several acids were subjected to five-plate horizontal pattern fractional extractions between water and ether. The formula which was used in the calculations was derived from the binomial expansion and has since been published in essentially the same form by Williamson and Craig (*8*).

$$\% = \frac{n!k^a}{a!\,(n-a)!\,(k+1)^n} \times 100$$

This expresses the percentage of total material in the *a*th fu[illegible] of an *n*-plate extraction where the distribution coefficient of the material in question is $k/1$. Certain conventions are implied in the use of this formula. The funnels are numbered beginning with 0, so that in an n-plate process there are $n + 1$ funnels. The usual convention that $0! = 1$ must also be observed.

In experiments with succinic and β-carboxyadipic acids, 50-ml. layers of liquid were used, and the amount of acid was 0.05 gram in each case. In the case of β-phenyladipic acid, the volume of each layer was 25 ml. and the amount of acid was 0.02 gram. A series of six 150-ml. separatory funnels was used in each case. The amount of acid in each funnel at the close of the experiment was determined by titration of an aliquot of each layer with standard sodium hydroxide. The results are shown in Table III, together with the calculated values for substances having distribution coefficients of 10/1, 7/1, and 1/10 those of the acids in question.

Succinic and fumaric acids were subjected to the same type of fractional extraction, using 2 volumes of ether to 1 of water. The volume of each water layer was 25 ml. and that of each ether layer was 50 ml. The amount of succinic acid used was 0.05 gram; of fumaric, 0.04 gram. The results are tabulated, together with the calculated values for materials having distribution coefficients of 4/1 and 1/2, in Table IV.

From these results, it may be seen that the acids examined did follow the calculated distributions rather closely.

As a final check on the use of fractional extraction to separate polybasic acids, a known mixture of succinic acid and β-phenyladipic acid was subjected to an 8 × 8 diagonal stage extraction between water and ether. The volume of each layer was 20 ml., and the amount of each acid used was 0.02 gram. The amount of acid in each layer was determined by titration with standard sodium hydroxide. The results, shown in Table V, demonstrate that a quantitative separation was effected, and indicate that fewer funnels could have been used without affecting the completeness of the separation.

SEPARATION OF ACIDS FROM OZONOLYSIS OF BUTADIENE-STYRENE COPOLYMER

A 50/50 butadiene-styrene copolymer was prepared by the Mutual recipe (*5*) at 50° C. for 11 hours.

The emulsion was coagulated with salt and sulfuric acid, and the rubber was washed with distilled water and methanol before being dried in a vacuum desiccator. After two successive reprecipitations in methanol of dilute chloroform solutions of the polymer, a 1.4% solution of 60 grams of the polymer in chloroform was ozonized at room temperature in ten batches, using a 20% excess of 2% ozone. The chloroform was removed from the ozonide at reduced pressure and the ozonide was decomposed with boiling 2% hydrogen peroxide. After separation of the water-soluble and insoluble fractions, the excess hydrogen peroxide was decomposed by heating the water solution on a steam cone with a small amount of Adams platinum oxide catalyst. The nonvolatile acids were then obtained by evaporating the solution to dryness at 40° C. under vacuum. The yield of water-soluble acids, dried in two portions was 0.923 gram per gram of polymer ozonized and that of insoluble acids was 0.534 gram per gram of polymer. The two water-soluble acid fractions had neutralization equivalents of 77 and 83, whereas the neutralization equivalent of the water-insoluble acids was 284. This represents a 96% yield of acids by weight and an 81% yield by titer of carboxyl groups, assuming that the original polymer was exactly 50/50 with respect to styrene and butadiene and contained 20% 1,2-addition of butadiene units.

Table III. Five-Plate Extraction between Water and Ether

	Funnel Number					
	0	1	2	3	4	5
β-Carboxyadipic acid	0	0	1	5	25	69
Calcd. 10/1	0	0	0.6	6.2	31.0	62.1
Succinic acid	0	0	1	9.5	34.5	55
Calcd. 7/1	0	0.1	1.5	10.5	36.6	51.3
β-Phenyladipic acid	56	34	7	3	0	0
Calcd. 1/10	62.1	31.0	6.2	0.6	0	0

Table IV. Five-Plate Extraction between Water and Ether, 1 Volume to 2

	Funnel Number					
	0	1	2	3	4	5
Succinic	1	1.8	5.5	18	39	34.5
Calcd. 4/1	0	0.6	5.1	20.4	41.0	32.8
Fumaric	11	30	32	19	7	2
Calcd. 1/2	13.2	32.9	32.9	16.4	4.1	0.4

Table V. Separation of Polybasic Acids

Fraction No.	% of Total Titer in Fraction	Fraction No.	% of Total Titer in Fraction
1	26	9	0
2	22	10	0
3	11	11	0
4	3	12	1
5	1	13	2
6	0	14	6
7	0	15	12
8	0	16	16

Table VI. Fractional Extraction of 10 Grams of Water-Soluble Acids between Water and Ether

	Water Layers			Ether Layers		
Funnel	Titer of 5 ml.[a]	Wt. in 1 liter	N.E.[b]	Titer of 5 ml.[a]	Wt. in 1 liter	N.E.
	Ml.	*Grams*		*Ml.*	*Grams*	
0	0.11	...	..	1.31	1.1056	150
1	0.13	...	..	0.97	0.7566	139
2	0.11	...	..	0.54	0.4303	139
3	0.08	...	..	0.21	0.2135	181
4	0.08	...	..	0.08	0.1199	268
5	0.10	...	..	0.07	0.0781	202
6	0.28	...	..	0.05	0.0655	234
7	1.04	0.3612	62	0.11	0.0835	135
8	3.03	1.0155	60	0.30	0.1519	90
9	5.85	1.9444	60	0.65	0.2438	67
10	6.01	2.3393	69	0.51	0.2169	76

[a] Titrations with 0.0218 N NaOH.
[b] Neutralization equivalent.

Ten grams of the water-soluble acids having a neutralization equivalent of 77 were subjected to a ten-plate fractional extraction (horizontal stage type) between 1-liter layers of water and ether. The results are shown in Table VI.

The water-soluble acids in fractions 7, 8, and 9 had neutralization equivalents very close to those expected from a mixture made up mainly of succinic acid (neutralization equivalent 59) and β-carboxyadipic acid (neutralization equivalent 63). These fractions were light in color and crystalline. The higher neutralization equivalent of fraction 10 might be caused by the presence of hydroxy acids, as any of those that might have been formed during the breakdown of the polymer would be expected to migrate to this fraction.

The high neutralization equivalents of the small intermediate fractions are probably due to foreign materials introduced during manipulations or to impurities in the large volume of ether which was evaporated to obtain these samples.

Ultraviolet analysis of these fractions indicated that the ether extraction removed essentially all of the phenyl-containing acids from the bulk of the water-soluble acids, for the absorption of these acids in the 2600 Å. region was very weak and there was no sign of the characteristic phenyl peaks in this region. The spectra of fractions 0 to 2 showed strong absorption in the 2400 Å. region.

Inasmuch as β-phenyladipic acid prefers water to chloroform and ether to water, it would be expected to stay with the water-soluble acids in the water fractions when chloroform is used as the extracting solvent and to go with the ether fractions when ether is used. Therefore, the first four ether fractions (0 to 3) were combined and were fractionated between 500-ml. water layers and 250-ml. chloroform layers in a five-plate system (horizontal stage type). The results are shown in Table VII.

Table VII. Fractional Extraction of Fractions 0 to 3 between Water and Chloroform, 2 Volumes to 1

Funnel	Water Layers			Chloroform Layers		
	Titer of layer[a]	Wt. of material	N.E.	Titer of layer	Wt. of material	N.E.
	Ml.	*Gram*		*Ml.*	*Gram*	
0	7.5	...	...	81.1	0.5338	302
1	11.0	...	...	20.2	0.1010	230
2	14.0	...	...	12.9	0.0496	176
3	36.3	0.1054	133	9.5	0.0336	163
4	150.0	0.3757	115	16.8	0.0476	130
5	280.0	0.6917	114	20.7	0.0544	120

[a] Titrations with 0.0218 *N* NaOH.

As had been expected, most of the colored material stayed with the first chloroform layer and most of the acids went with the water fractions. The neutralization equivalent of the material in the water layers of funnels 4 and 5 is very close to the value of 111 expected for β-phenyladipic acid. Crystals which separated during evaporation of the chloroform layer of funnel 5 melted at 147.5–148° C. The melting point found by Rabjohn *et al.* (*6*) for a recrystallized sample of β-phenyladipic acid was 147–148° C. The ultraviolet spectra of fractions 4 and 5 showed characteristic phenyl peaks near 2600 Å.

The composition of the material in funnels 0 and 1 is not known, but the high neutralization equivalents suggest the presence of considerable neutral material. The ultraviolet spectra of these fractions showed very definite maxima at 2400 Å. and no sign of phenyl peaks in the 2600 Å. region, although the background absorption was strong enough to obscure moderate phenyl absorption. Infrared analysis of fraction 1 showed phenyl and carboxyl groups and nothing else of interest.

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Determining the Vapor Pressure of Petroleum Fractions

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A new apparatus has been designed and proved practical for determining the vapor pressure of approximately 5-gram samples of pure or mixed hydrocarbons. A description of the device and its construction is given; its calibration, limitations, and theoretical considerations are discussed. The results obtained are reproducible and in close agreement with those determined by the A.S.T.M. Reid vapor pressure method.

THE apparatus described herein was developed during a research program that necessitated a rapid, reproducible determination of a large range of vapor pressures on relatively small samples of gasoline and blends thereof. The new method was necessary because the A.S.T.M. Reid method (*1*) required considerable time as well as an amount of material greater than that which was available. Various vapor pressure determination procedures involving the isoteniscope (*5, 6, 10*) and other more elaborate apparatus (*4, 7, 8*) were considered, but were not thought applicable to the work at hand.

In the present method, the apparatus is stationary and consists of a water-jacketed glass tube which is one arm of an open-end mercury manometer. The 5-ml. sample is introduced into the top portion of the water-jacketed section through a calibrated funnel. The system is shown in Figure 1. The constant temperature bath and circulation pump, sample trap, and vacuum pump used in the system shown have been omitted for the purpose of simplification.

APPARATUS

Arms *A* and *B* are constructed of 2-mm. inside diameter, thick-walled tubing of uniform bore as shown. The coils at the bottom of *A* are incorporated to absorb the differential temperature expansion. The upper section of *A*, including the spherical sample-receiving chamber, is constructed of ordinary 8- or 10-mm. glass tubing. The outlet tube, however, is made of 2-mm. inside diameter tubing. The upper extension of *A* between *C* and *D* consists of a 75-mm. length of 4-mm. capillary inside diameter tubing for receiving a known amount of sample from the graduated funnel into which the test sample is introduced originally. Water is circulated through the small upper jacket at 32° to 40° F. and at 100° ± 0.1° F. through the larger jacket. Other equipment comprises a calibrated scale to fit over *B*, a mercury reservoir, a vacuum pump capable of evacuating the system to 5 mm. or less mercury pressure prior to each sample inspection, and a trap system located between the outlet tube and the vacuum pump. Dimensions pertinent to the construction of the apparatus are included in the figure.

CALIBRATION OF THE SCALE

With stopcocks *D*, *E*, *F*, and *G* open and stopcock *C* closed and with the mercury level in the reservoir slightly above point *F*, the system is evacuated to 5 mm. of mercury or less. Next, the mercury level in *A* is adjusted by means of the mercury reservoir to position *H* which is several centimeters below the capillary end of *A*. Stopcocks *E* and *G* then are closed. A meter stick (not shown) is set between *A* and *B*, with the zero reading on the stick opposite the mercury level in *B*. This level in *B* is marked and is to serve as the zero reading. The height of mercury in *A* is recorded. By letting air into the system through stopcock *E*, the mercury level in *A* is lowered and that in *B* raised in increments of not more than 10 cm. and the change in levels in *A* and *B* is recorded for each position in *B*.

At any point, the change in height in *A* from the initial reading is designated as ΔH_A, and ΔH_B denotes the corresponding change in *B*. After the levels in *A* and *B* are equal, further readings should be obtained by applying positive air pressure through stopcock *E*. The sum of ΔH_A and ΔH_B at any position of the mercury level in *B* is equal to the increase in pressure in the system in millimeters of mercury. Using the following equation, the pressures are converted from millimeters of mercury to pounds per square inch:

$$\text{Mm. of Hg} \times \frac{14.7}{760} = \text{pounds per square inch}$$

A plot is made of $\Delta H_A + \Delta H_B$. Using this correlation, a scale indicating pressure in 0.1 pound per square inch is constructed and clamped in place over *B*. In making a vapor pressure determination the mercury level in *B* is always adjusted to the zero reading after evacuation of the system and before introduction of the sample, so that the indicated increase in pressure after the sample has been introduced to the evacuated system is a direct reading of the vapor pressure.

HANDLING OF SAMPLE

The sample for test is handled in the same manner as for the Reid method and the same precautions are observed to assure minimum loss.

PROCEDURE

The apparatus is made ready for routine testing by bringing the jacket temperature to 100° ± 0.1° F. When this temperature is obtained, stopcocks *D*, *E*, *F*, and *G* are opened, stopcock *C* is closed, and the system is evacuated to a pressure of less than 5 mm. of mercury. The mercury level in *B* is adjusted to the zero level on the scale and *D*, *E*, and *G* are closed. A 5- to 10-ml. sample of the material being tested is introduced into the charging funnel, which is cooled to 32° to 40° F., and a 3-ml. portion of it is allowed to flow into the apparatus by opening *C*. *C* is closed and *D* is opened, thereby introducing the sample into the thermostatically controlled spherical section of *A*. Partial vaporization of the sample occurs within several seconds and the mercury level falls accordingly. After the mercury levels in *A* and *B* have come to rest, the vapor pressure is read directly from the calibrated scale and reported in pounds per square inch. Apparently agitation is not necessary to bring the vapor and liquid in equilibrium.

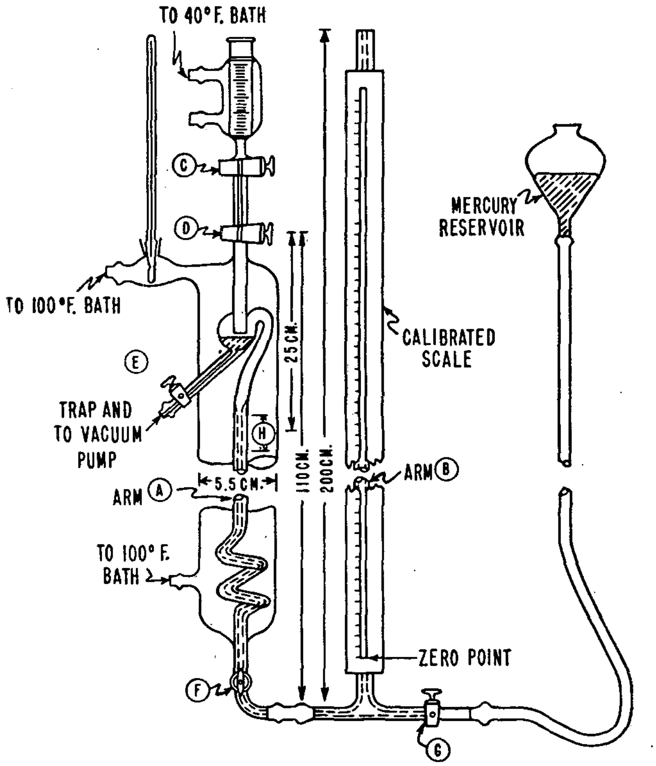

Figure 1. New Manometer-Type Vapor Pressure Apparatus

RESULTS

comparison between the A.S.T.M. Reid method and the present method has been made with data from approximately 1000 samples run in duplicate by both methods and covering a range of pressures from 1 to 25 pounds per square inch. The samples were for the most part hydrocarbon mixtures such as gasolines; a few pure compounds also were tested. The first 50 results obtained by both methods are plotted against each other in Figure 2. The two methods are seen to be in good agreement along the whole range of vapor pressures tested. Duplicate determinations, which are not included individually, indicate slightly better reproducibility for the new method.

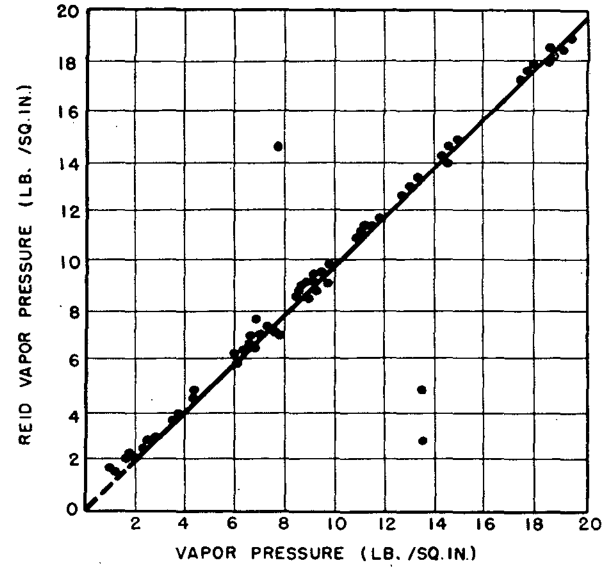

Figure 2. Reid Vapor Pressure *vs.* New Method Vapor Pressure

Representative results on hydrocarbon mixtures obtained by both methods are given in Table I.

Several pure compounds also were tested by means of the new method with the following results:

Compound	Vapor Pressure, Mm. Hg at 100° F. Handbook	Vapor Pressure, Mm. Hg at 100° F. New method
Acetone	384	379
Carbon tetrachloride	202	200
Ethyl alcohol	120	120

ADVANTAGES

Several advantages beside better reproducibility can be realized by means of the new manometer-type apparatus. The new method requires only 3 to 5 ml. of sample, whereas the Reid method requires 130 ml. In some experimental work, this large quantity makes the latter test prohibitive. Approximately 30 minutes are required for an average Reid determination in duplicate, whereas the new method requires only 12 minutes for duplicate determinations, assuming one operator running two units in either case. Furthermore, the Reid gages are subject to frequent mechanical breakdown. The new method avoids this shortcoming by eliminating the gage and substituting the mercury manometer which is the ultimate standard for the Reid gages.

DISCUSSION

Effect of Mercury Expansion. The differential in temperature expansion of the mercury in *A* and *B* introduces a negligible error. The maximum error possible from this source has been calculated to amount to about 0.06 pounds per square inch on a sample of 15 pounds per square inch vapor pressure when the temperature differential between *A* and *B* is 30° F.

Effect of Dissolved Air. Dissolved air in the gasoline evolved when the sample is introduced into the evacuated apparatus causes a slightly positive error in the indicated vapor pressure

Table I. Representative Results

Sample	Vapor Pressure, Lb./Square Inch			
	Reid method		New method	
A	4.3	4.7	4.3	4.4
B	6.0	6.3	5.9	6.1
C	7.6	7.9	7.3	7.5
D	9.6	9.8	9.4	9.5
E	14.0	14.3	14.1	14.3
F	18.2	18.5	18.0	18.0

reading. Inasmuch as air solubility decreases with increasing gasoline vapor pressure, this error decreases accordingly. Increasing the vapor-liquid ratio in the system decreases the error from this source. In the apparatus as shown, in which the vapor-liquid ratio is about 20 to 1, the positive error is estimated to be about 0.1 pound per square inch for a sample of 7 pounds per square inch vapor pressure saturated with air at 40° F. Though for greater precision narrow limits on the vapor-liquid ratio are required (*2*, *3*), the use of the 20 to 1 ratio yielded results which were close enough to those of the American Society for Testing Materials, which uses 4 to 1, to be of practical utility.

Effect of Vapor-Liquid Ratio. A negative error in the indicated reading may be caused by a large vapor-liquid ratio. Such an error is due to change in liquid composition resulting from evaporation effects and does not occur for pure compounds or constant boiling materials. It is greatest for samples showing the greatest slope in the front end of the distillation curve and increases with increasing vapor pressure. The Reid method is subject to this same error, though to a slightly lesser degree.

In the present method the influences of dissolved air and of high vapor-liquid ratio counteract each other to some degree. The experimental evidence to date indicates that results by this method can be considered equivalent to the Reid vapor pressure. Although both are subject to the errors discussed, they are satisfactory for the usual gasoline vapor pressure control.

With the new method it would be possible, if desired, to conduct the test with a zero vapor space and to apply a correction for dissolved air, so that true vapor pressures (*9*) could be obtained.

ACKNOWLEDGMENT

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Spectrophotometric Evaluation of Engine Lubricants

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IN WARTIME investigations (*1*) it was found that the performance of quenching oils for aluminum alloy airplane motor castings could be determined by means of spectrophotometric curves of the oils, which could be correlated accurately with residual strain in the alloy castings. Increasing deterioration, easily detected in the absorption measurements, resulted in increasing strain, which seriously affected load and safety in motors. The method was adopted as a standard test for predicting the performance of new quenching oils, and for working out a procedure for fortifying such oils by use of additives and filtration of old deteriorated oils, resulting in a minimum strain development in castings. It was logical to extend this optical testing method to engine lubricants in the hope of simplifying the complex procedure of ten or a dozen tests, not always satisfactory, adopted as standard in the petroleum industry.

SAMPLES AND TECHNIQUE

Through the cooperation of several oil laboratories it was possible to investigate a number of different oils operating under various conditions. Some of the oils were tested under laboratory conditions by means of an Underwood tester and others were tested under actual operating conditions in automobile and Diesel engines. After a number of the more familiar and accepted tests of the petroleum laboratories, these samples were used for a spectrophotometric study. The transmission data, over the complete visible range of the spectrum, were obtained with one of several spectrophotometers such as the Cenco-Sheard, Coleman, or General Electric recording instruments.

Oils have a large absorption in the near ultraviolet region and often became dark upon use; therefore it is necessary to dilute the samples with a neutral solvent in order to obtain maximum sensi-

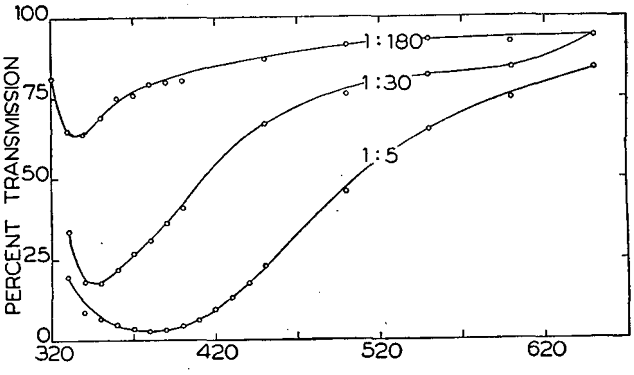

Figure 1. Effect of Dilutions with Chloroform

Wartime investigations showed that the performance of quenching oils for aluminum alloy airplane motor castings could be determined by spectrophotometric curves of the oils which could be correlated accurately with residual strain in the castings. This optical testing method was extended to engine lubricating oils in the hope of simplifying the standard petroleum tests. Some oils were tested under laboratory conditions by an Underwood tester, others under actual operating conditions in automobile and Diesel engines. Performance of new oils may often be predicted from samples prepared by heating in open test tubes in the presence of metal turnings (corresponding to engine walls). The striking similarity between the graphs of optical data and those for such tests as per cent asphaltenes, carbon residues, neutralization number, viscosity, chloroform-solubles and insolubles, etc., suggests a definite relationship between optical properties and chemical and physical data indicating relative amounts of oxidation, polymerization, and other deterioration with use under many conditions of temperature and catalyzing materials. Thus the spectrophotometric method offers a rapid means of rating engine lubricating oils with and in advance of their use. Evidently the change in light absorption is an integrative indication of chemical and physical changes, including development of colloidal sludge, which occur under the conditions of oxidation and heat in an internal combustion engine. The indeterminate variables in engine operation, such as contamination by incompletely burned fuel products, carbon deposits, etc., limit application of this or any other method of used oil analysis.

tivity of the transmission readings. In the present study, chloroform was found to be a satisfactory solvent. It was possible, in cases where the oil remained comparatively clear, to run spectrophotometer curves with no diluent.

Dilution studies were carried out with the same result as with quenching oils—a shift of the absorption peak toward the shorter wave lengths with greater dilution. If the same oil sample is diluted in different proportions with chloroform, the spectrophotometric curves in Figure 1 are obtained. The first requirement of this method of evaluating lubricating oils is to select a desirable diluting ratio.

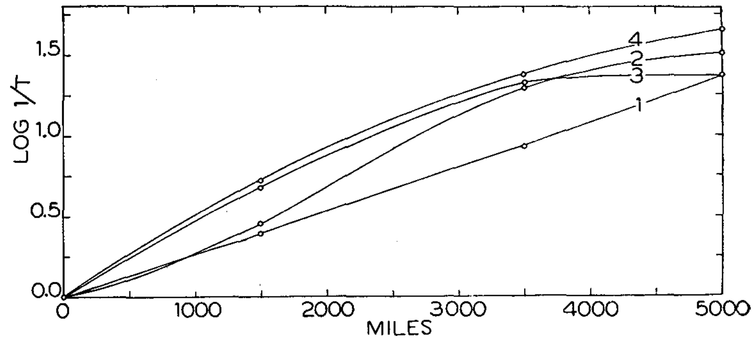

Figure 3. Log of Reciprocal of Minimum Transmission on Spectrophotometric Curves Plotted against Miles of Operation in an Automobile Engine for Four Familiar Commercial Lubricating Oils

EXPERIMENTAL

The results obtained with four different samples of oil tested in an automobile engine are shown in the first series of curves. Transmission curves and the usual petroleum tests were made on samples of the oil after a definite number of miles of service.

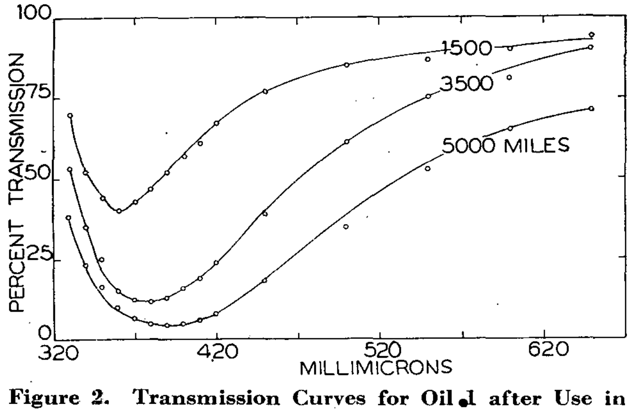

Figure 2. Transmission Curves for Oil 1 after Use in Automobile Engine

Typical curves of the relative transmission versus wave length are shown in Figure 2 for oil 1 after various periods of service. These curves were obtained with a Cenco-Sheard instrument using 1 to 200 dilution. An examination of the curves in this graph reveals several trends after the oil has been in use. There are (1) a definite shift toward higher wave lengths at minimum transmission, (2) a general rounding out of curve at maximum absorption, and (3) a decrease in the relative transmission. Figure 3 illustrates an effective way to show the change in transmittancy by plotting the logarithm of the reciprocal of the minimum transmission read off from the spectrophotometer curves against miles of service in an automobile engine.

Samples of the same four oils were also tested under laboratory conditions in an Underwood tester. In this method the oil is heated at an elevated temperature and sprayed over a metal surface, which produces more rapid deterioration than under service conditions. A comparison of Figures 2 and 4 shows a good

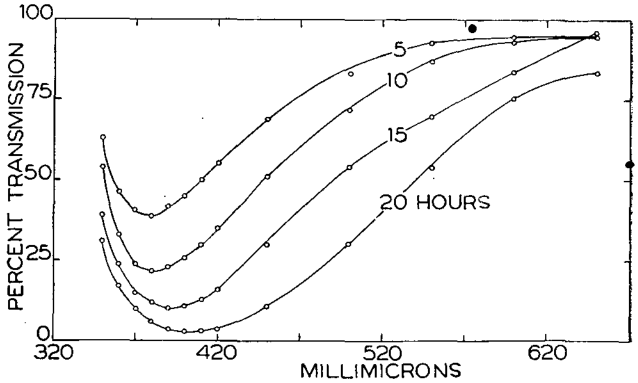

Figure 4. Transmission Curves for Oil 1 after Underwood Test

Compare with Figure 2 for actual engine test

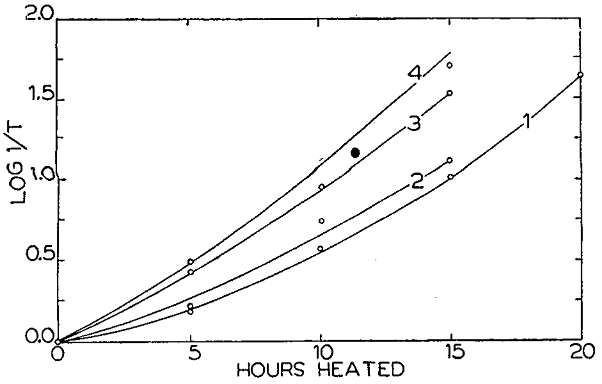

Figure 5. Reciprocal of Minimum Transmission Plotted against Hours in Underwood Test

Compare with Figure 3 for same four oils in actual engine use

correlation between the transmission curves obtained from oil used in an engine and this accelerated method of testing the oil in the laboratory. The data used for the graph in Figure 5 were obtained from the samples of oil used in the Underwood. The same trend is evident as in Figure 3. On the basis of these spectrophotometer tests it may be predicted that these four commercial oils decrease in stability and performance in the order 1 to 4.

Figures 6 to 8 show the results of standard petroleum tests of the four oils after testing in a service car: per cent chloroform-soluble, per cent chloroform-insoluble, per cent naphtha-insoluble, per cent asphaltenes, neutralization number, and viscosity. Similar curves obtained from the oils after testing in the laboratory under accelerated conditions, together with data on carbon residues and corrosion of cadmium-silver and copper-lead bearings, are shown in Figures 9 to 11.

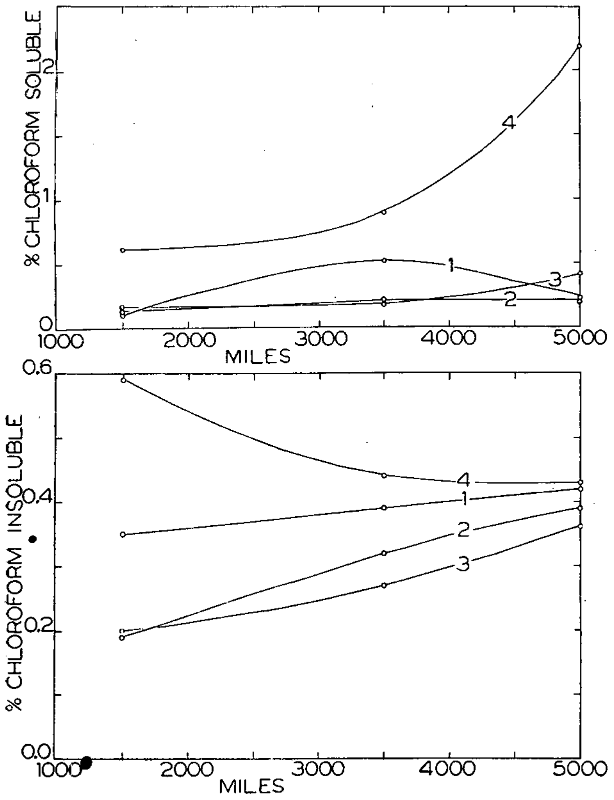

Figure 6. Results of Standard Petroleum Tests on Four Oils

The striking similarity between the graphs of the optical data derived from spectrophotometer curves and those for these familiar tests indicates a definite relationship between optical properties and these other chemical and physical data which are generally accepted as measures of relative amounts of oxidation, polymerization, sludge formation, acid formation, viscosity changes, and other deterioration with use under many conditions of temperature and catalyzing materials. The decreasing rating of the oils from 1 to 4 in order indicated by the spectrophotometer (Figures 3 and 5) is also generally proved by the standard tests (Figures 6 to 11). Where there is confusion in some standard tests, such as the insolubles, the optical data also indicate unre-

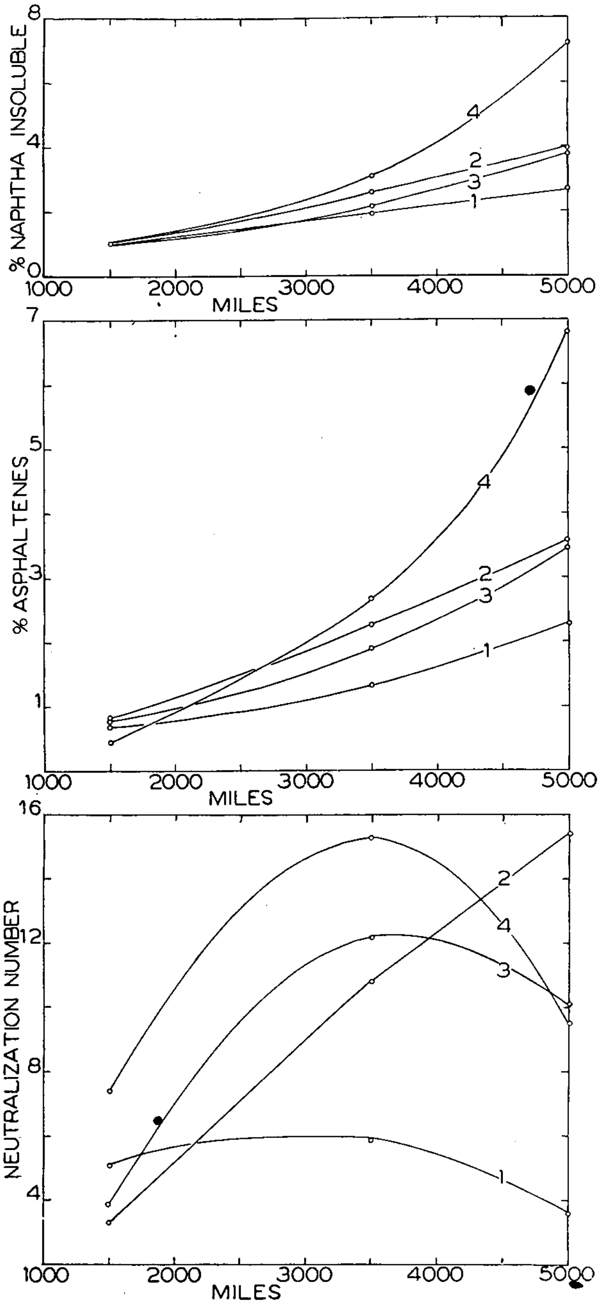

Figure 7. Results of Standard Petroleum Tests on Four Oils

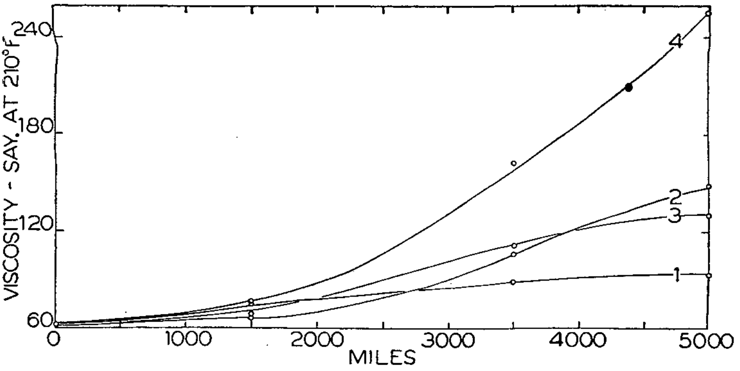

Figure 8. Viscosities Plotted against Miles of Service for Four Oils

liability of these accepted methods, which are generally recognized as troublesome and unreproducible. There is a strong indication that the slope of the optical curves is a remarkably accurate index of the stability of the oil: the greater the slope the less the stability and the greater the deterioration. These curves also contribute added confidence in the reliability of the accelerated Underwood test in indicating performance of lubricating oils in automobiles under controlled conditions. The significance of *development of solid* particles and then growth with aging, as determined by electron micrographs for quenching oils, is substantiated for lubricating oils. Therefore, this simple rapid spectrophotometric technique is recommended as a valuable supplement or even replacement to other test procedures in petroleum laboratories.

Oil used in Diesel engines is subject to more fuel dilution than in gasoline engines. However, the curves in Figure 12, obtained from samples of oil 5, which had been in service for a various number of miles in a Diesel locomotive, show the same general trends which were evident in Figures 2 and 4. The curves on these Diesel oils were obtained on a General Electric recording instrument using a 1 to 20 dilution with chloroform.

A series of tests was made also on stationary Diesel engines.

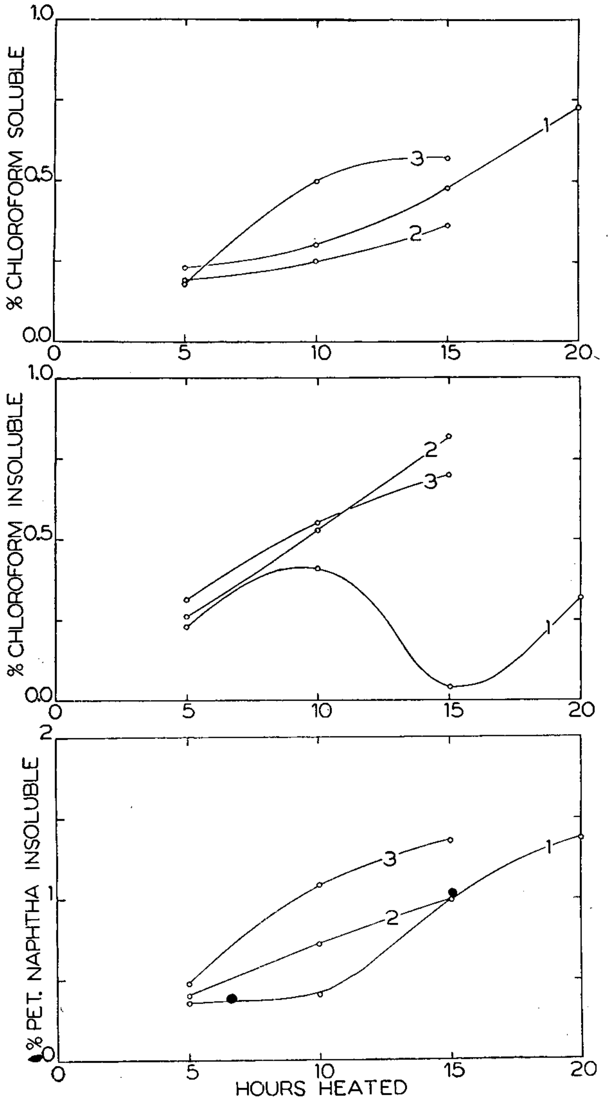

Figure 9. Results of Laboratory Tests of Three Oils

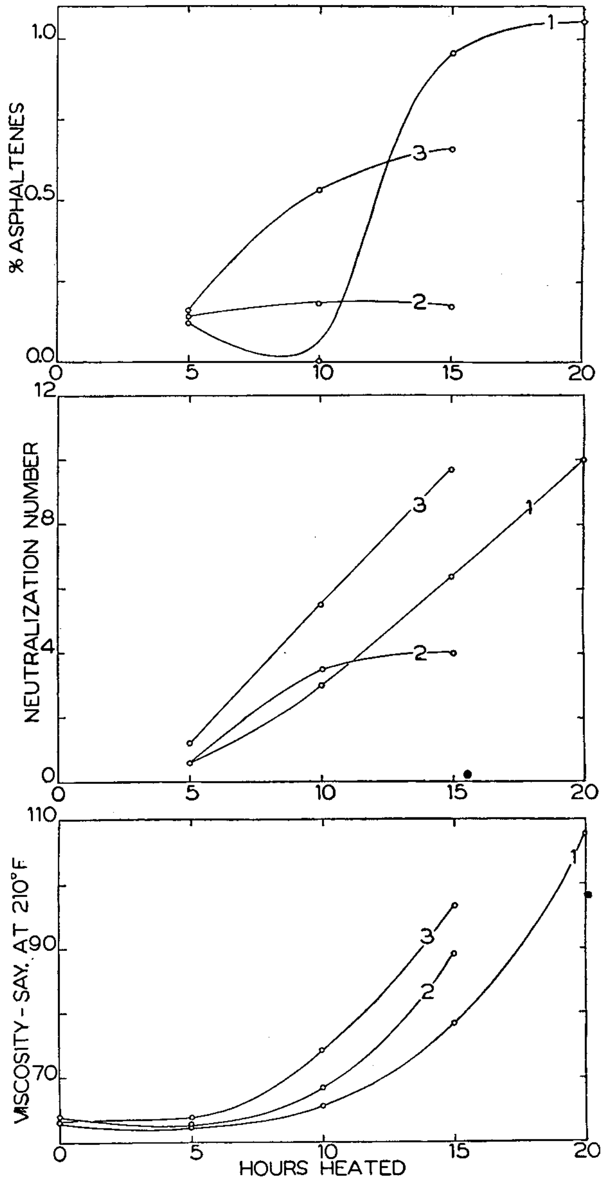

Figure 10. Results of Laboratory Tests of Three Oils

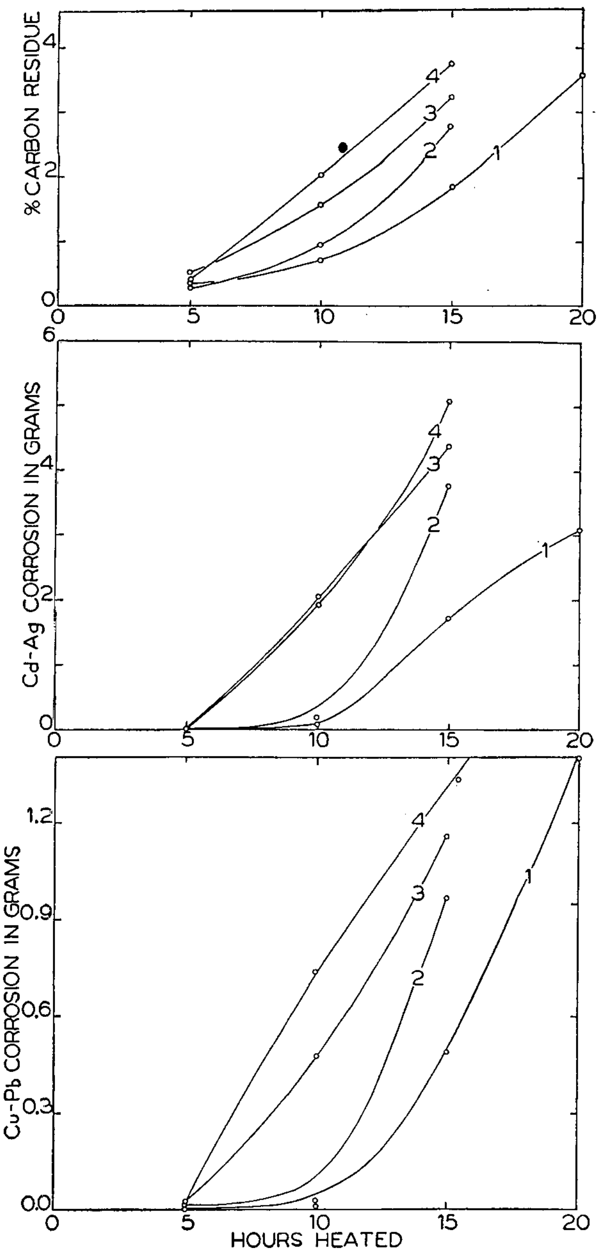

Figure 11. Results of Laboratory Tests on Four Oils

Upper. Carbon residues
Center. Losses by corrosion of cadmium-silver bearings
Lower. Losses by corrosion of copper-lead bearings

Table I. Petroleum Tests on Oil 6

	38 Hours	334 Hours
Conradson carbon, %	0.161	0.173
86 A.P.I. insoluble, %	0.051	0.024
Chloroform-soluble, %	0.051	0.0
Neutralization number	0.049	0.311
Ash, %	0.012	0.001
Viscosity increase		
At 100° F.	None	None
At 212° F., %	None	5

Samples were taken periodically for more than a year in order to follow the condition of the oil. Typical curves obtained from oil sample 6 before use and after a short time of operation are shown in Figure 13 (upper). The fact that the transmission curves have not changed a great deal even after some 300 hours of operation is not too surprising, because of the large oil capacity of the engine. The results of some of the usual petroleum tests made on these samples are given in Table I.

Oil 7 was used in two different stationary Diesel engines. The presence of additives in this oil had no appreciable effect on the transmission curve, as shown by making a curve of the base oil without and then with the additive. The curves shown in Figure 13 (lower), plotted from data obtained with a 1 to 20 chloroform dilution and a Coleman spectrophotometer, are as follows:

Curve No.	Hours of Operation
7	New oil with additive
7A	37, engine 2
7B	333, engine 2
7C	34, engine 3
7D	331, engine 3

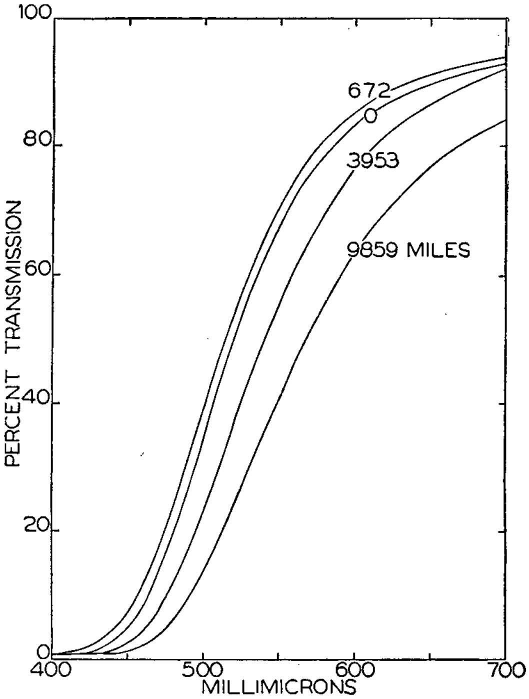

Figure 12. Tests on Diesel Engine Oils

Curves from G.E.-Hardy recording spectrophotometer for oil 5 after test in Diesel locomotive

Table II lists the results of some of the usual petroleum tests on this oil.

In addition to the requirement of a definite diluting ratio in order to make a comparison of the transmission curves of various oil samples, it was also found that there is a considerable change in the curve if the diluted sample has stood for some time. The maximum absorption moves to higher wave lengths upon standing. Curve 6A in Figure 14 was obtained from unused oil 6 immediately after diluting with chloroform to a ratio of 1 to 20. 6B is the curve of the same sample after standing for 3 months.

Table II. Petroleum Tests on Oil 7

	7A	7B	7C	7D
Conradson carbon, %	0.694	0.682	0.966	0.895
86 A.P.I. insoluble, %	0.112	0.184	0.407	0.312
Chloroform-soluble, %	0.065	0.075	0.177	0.106
Neutralization number	0.199	0.248	0.150	0.245
Ash, %	0.199	0.248	0.218	0.238
Viscosity increase				
At 100° F., %	36.0	35.5	39.3	33.7
At 210° F., %	10.6	12.4	11.6	11.4

7E is another new oil diluted to the same ratio, and 7F is the curve after 3 months. Similar shifts in the curves, though not so pronounced as with chloroform-diluted samples, have been observed with new, undiluted oils after standing. This illustrates the difficulty of obtaining and conserving representative samples and of course evaluates relative stabilities of lubricants entirely apart from the severe conditions encountered in the engine.

CONCLUSIONS

Data from these spectrometer curves, obtained from oils at standard dilutions, may be used in a variety of ways. They may be compared from the basis of shift of the minimum transmission, the flattening of the curve at this point, or the logarithm of the reciprocal of the minimum transmission (log $1/T_{\text{min.}}$) plotted against the time of heating during laboratory or Underwood tests, or against miles or hours of service in engines. These curves may be compared with corresponding curves made by standard accepted test procedures such as per cent asphaltenes, carbon residues, neutralization number, viscosity, chloroform-solubles and insolubles, naphtha-insolubles, and bearing corrosion. At the present time transmission curves are being prepared on a routine basis together with the standard tests in an oil laboratory with remarkably good correlation. Thus the curves which may be determined in a few minutes may actually serve in place of the laborious, time-consuming conventional tests in evaluating and predicting the performance of lubricating oils in use.

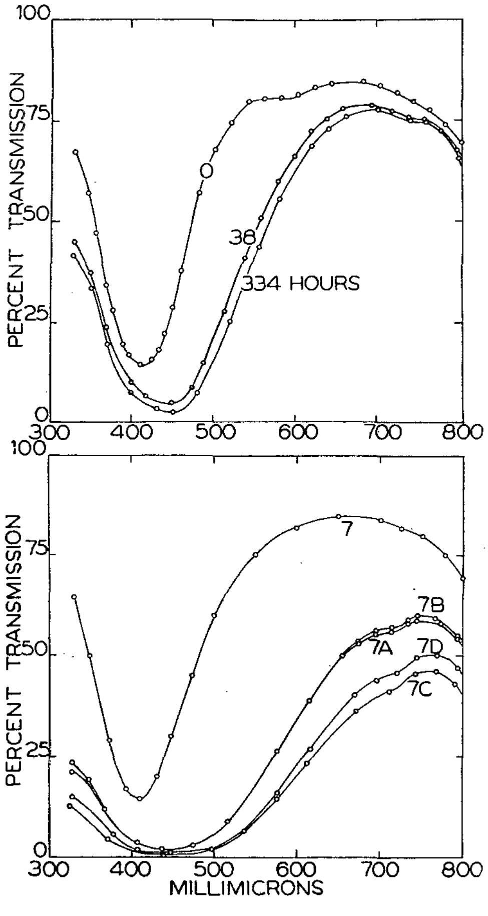

Figure 13. Coleman Spectrophotometer Tests on Oils Used in Stationary Diesel Engines

		Curve No.	Hours of Operation
Upper.	Oil 6	7	New oil with additive
Lower.	Oil 7	7A	37, Diesel 2
		7B	333, Diesel 2
		7C	34, Diesel 3
		7D	331, Diesel 3

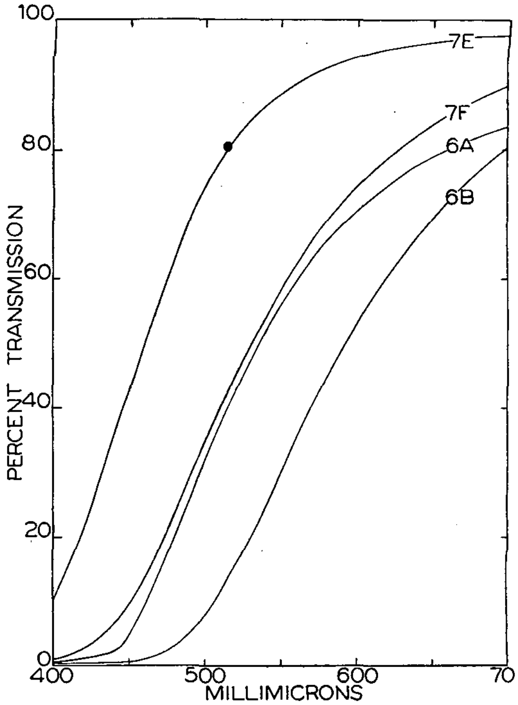

Figure 14. Effect of Standing

Oils diluted 1 to 20 with chloroform
6A, 7E. No standing
6B, 7F. 3 months' standing

Emphasis in this investigation has been on the practical use of the spectrophotometer in evaluating changes and deterioration of engine lubricants. It seems evident that the curves integrate most, if not all, of the chemical changes in oils under oxidizing, catalyzing, and high temperature conditions which characterize operation of internal combustion engines. Specific interpretation, of course, would depend on very complete chemical analyses of oils before and after use. This or any other method of used oil analysis is limited by virtue of the complexity of the total problem of engine and lubricant performances. These methods relate primarily to oxidation, stability with unknown relationship to engine cleanliness and detergency, contamination by incompletely burned fuel products, ring and valve sticking, piston crown and carbon deposits, scratching of cylinder walls and fouling of air-intake ports, protection of bearings by specific additives without affecting oil oxidation, acceleration of oxidation by some detergents, sludge left in the engine, etc. Thus, no single method alone can evaluate an oil unequivocally in the face of such complexity. But the light-absorption method does seem to have the advantages of integrating many changes, including the effects of detergents in producing suspensions of solid products, on oil samples exactly as they are, by a simple, rapid instrumental technique. Because of many indeterminate variables in the use of automotive lubricants, even better results should be expected for turbine and transformer oils and all others used under uniform operating conditions.

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Petroleum Control Testing by X-Ray Absorption

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X-ray absorption techniques have been successfully applied to quality control of petroleum products. Procedures are described for rapid, direct, and nondestructive analytical determinations of sulfur, tetraethyllead, and metallic additives. Measurements were made on an x-ray photometer using petroleum derivatives containing small amounts of elements substantially higher in atomic number than carbon. Application to petroleum products includes the useful range, method of calibrations, and standardization of test conditions. Accuracy is compared with gravimetric or other established procedures. Limitations, including nonspecificity, are covered.

THE economic production of petroleum products has resulted in a great variety of testing to ensure uniformity of product as well as to meet complex specifications. Much of the testing is tedious and complex, and unfortunately does not always give a direct answer. It would be desirable to replace any chemical test with a simple instrument measurement, which might be performed by technicians with a minimum of training.

Aborn and Brown (*1*) first demonstrated the practical importance of the absorption characteristics of tetraethyllead exposed to x-ray radiation. Their work made use of the greater absorption of increasing masses of material at a constant wave length. They pointed out that accurate control of operating conditions was extremely important. Since then improvements in electrical and electronic controls have resulted in greater utilization of x-rays as an analytical tool. It was found that testing could be greatly simplified and standardized by application of photometric methods (*3, 4*).

Liebhafsky, Smith, Tanis, and Winslow (*7*) developed a technique for measuring the x-ray absorption photometrically. A combination of phosphor and multiplier phototube made possible a reasonably precise measurement of x-ray absorption. By recognizing and controlling all the variables in the system it was possible to show that even small amounts of heavier elements were detectable in mixtures. As in any photometric method, constancy of absorption for a given mass of material depends on the nonvarying frequency and intensity of the beam of radiations.

Michel and Rich (*8*) have reported on a highly useful instrument for measuring relative x-ray absorption. The authors have applied this instrument to a wide variety of control testing in a routine petroleum inspection laboratory where a rapid and reasonably accurate evaluation of a blending or additive compound is desired. The procedure involves a differentiation between two samples, which means a comparison between reference and blended stock. The only other requirement is that the additive have one or more elements of relatively higher atomic number than the base stock and that measurable amounts be involved.

X-RAY PHOTOMETER

All measurements were made on a Model 532830G1 x-ray photometer manufactured by the General Electric Company. It is designed to measure the relative x-ray absorption of two samples in terms of the amount of aluminum necessary to add to one path of a divided beam to compensate for a higher absorption in the sample in the other path. The instrument operates on a null method in which the unknown is balanced with a known, by introducing a variable amount of aluminum in the form of a tapered disk controlled by a calibrated dial. The aluminum needed for balance, compared to that required for known blends as determined by a previous calibration, gives a measure of the desired component in the unknown.

The x-ray photometer, shown in Figure 1 in its simplest form, includes a tungsten-target x-ray head with suitable water-cooling facilities and control devices. An applied voltage of about 40 kv. produces a spectrum of x-rays with effective wave length of 0.4 Å. emitted as pulses from the x-ray tube on the positive peak of the 60-cycle alternating current power input. A two-bladed synchronous motor-driven chopper is placed so that it alternately interrupts and divides the beams of x-rays, one half passing through the sample, *S*, and one half through the standard, *X*. The x-rays which pass through the cell impinge alternately on the left and right half of the fluorescent screen where the x-radiation is converted to light. The light from either half passes through a light tunnel, or collector, to a multiplier-type phototube. The electrical output of the phototube then passes through an amplifier to a phase detector. The phase detector is also fed by the output of a small 30-cycle generator which is carried on the same shaft of the synchronous motor driving the two-bladed chopper. The inputs from the phototube and generator are converted by the phase detector into a direct current whose magnitude and direction depend upon the relative intensities of x-rays transmitted through the sample and the standard. This direct current is read on a balance indicator consisting of a zero-centered microammeter.

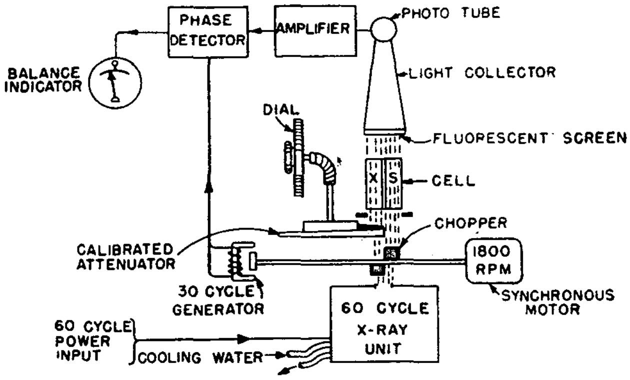

Figure 1. Block Diagram of X-Ray Photometer

A zero reading on this balance indicator means that the intensities of the alternating pulses of x-rays as received on the fluorescent screen are of equal intensities. A dial on the front panel controls the tapered aluminum disk in the beam which passes through the standard in such a fashion as to insert a known absorption. By changing the angular position of this disk, the difference in x-ray absorption between the standard and the sample may be balanced, thus obtaining a direct indication of the difference in absorption of the sample and of the standard. The thickness of aluminum which must be interposed on the standard side to obtain a balance is then a measure of the increased absorption due to the additive. By referring the dial reading to an appropriate calibration, the concentration of the heavier material in the sample may be determined.

USEFUL RANGE

The absorption of the x-rays by various materials in terms of the aluminum disk, was measured to give a comparison of the

Table I. X-Ray Photometer Sensitivities

Element	Atomic No.	Sensitivity %/dial division	Sensitivity Dial division/%
Na	11	0.27	3.7
Mg	12	0.17	5.8
Al	13	0.12	8.2
Si	14	0.087	11.5
P	15	0.065	15.3
S	16	0.051	19.7
Cl	17	0.040	25.0
Ca	20	0.023	44.1
Mn	25	0.014	70.9
Fe	26	0.012	81.3
Co	27	0.010	97.1
Ni	28	0.0097	103.1
Cu	29	0.0089	112.4
Zn	30	0.0083	120.5
Mo	42	0.0057	175.4
Ag	47	0.0044	227.3
Ba	56	0.0039	256.4
Pb	82	0.0033	303.0

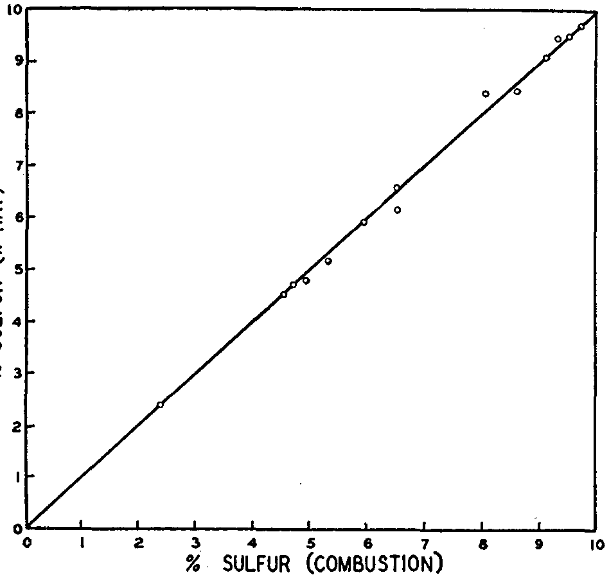

Figure 2. Comparison of Test Results

sensitivity of the instrument toward a number of elements. These measurements were made on liquid samples predominantly hydrocarbons but containing a measurable amount of heavier materials. Under these test conditions substantial variations in the ratio of carbon to hydrogen produce little or no change in x-ray absorption. This simplifies the problem of looking for elements of higher atomic number in petroleum-base stocks.

The data presented in Table I show sensitivities. "Sensitivity" refers to the per cent by weight of the determined element which on the authors' instrument gave 1 dial division change in absorption when a 150-gram sample was used. The repeatability of readings is 0.2 dial division. For sulfur 0.051 weight % in a 150-gram sample gives the same response as 0.0033 weight % of lead, or a difference of more than tenfold. Reference to this table will be helpful in determining application of this technique to any particular analysis. It also indicates the magnitude of possible error in case of contamination of a sample.

PROCEDURE

The primary voltage across the x-ray tube, usually 100 volts, and the voltage determining the level of amplification are kept constant. A sample of unknown is weighed into the right-hand cell compartment, and the same weight of reference stock (standard) into the left. The cell is placed in optical alignment and the x-ray tube is energized. The disk is rotated to vary the thickness of aluminum in the standard side. When the balance indicator reads zero, the reading on the calibrated dial is recorded. This reading is the mils of aluminum equivalent in x-ray absorption under the experimental conditions to all the constituents present in the unknown but not in the standard. Other things equal, the contribution to this equivalent thickness of each such constituent is proportional to its mass.

If the difference in absorption between standard and unknown exceeds the capacity of the calibrated disk, aluminum blocks of known thickness are interposed on the standard side. The instrument is calibrated for a particular element with samples containing known amounts of the desired constituent.

The procedure refers to the use of a base stock which is the most reliable procedure. However, refinery practices may not always permit this because of the frequent doubt or nonavailability of the exact base stock. Refined petroleum stocks contain essentially only carbon and hydrogen in slightly varying proportions from the lightest to the heaviest fractions, and, in addition, a varying percentage of sulfur. Because the x-ray photometer makes no measurable distinction between the small changes in carbon-hydrogen ratio in the range in which the authors worked, it is possible to substitute a highly refined product with a minimum sulfur content for all base stocks concerned. There is, however, an appreciable sensitivity for sulfur in hydrocarbons.

SULFUR

Sulfur in hydrocarbon mixtures can be measured by x-ray absorption. Kehl and Hart (*6*) have recently reported on a procedure using an x-ray spectrometer with a Geiger counter. Zemany, Winslow, Poellmitz, and Liebhafsky (*11*) used the x-ray photometer for evaluation of sulfur in crude oils.

The increased absorption of the x-rays due to sulfur is noticeable throughout the usual range of petroleum hydrocarbons. A straight-line relationship was found between mils of aluminum (dial reading) and per cent sulfur in hydrocarbon materials. This was determined by making a series of blends and comparing the photometer readings with the known sulfur value for each blend. Comparison of test results is shown in Figure 2. The deviations have been within the accepted A.S.T.M. tolerance for accuracy. The petroleum samples used in the above work were, for the most part, mixtures of treated distillates and naturally occurring sulfur compounds present in or recovered from the refining process.

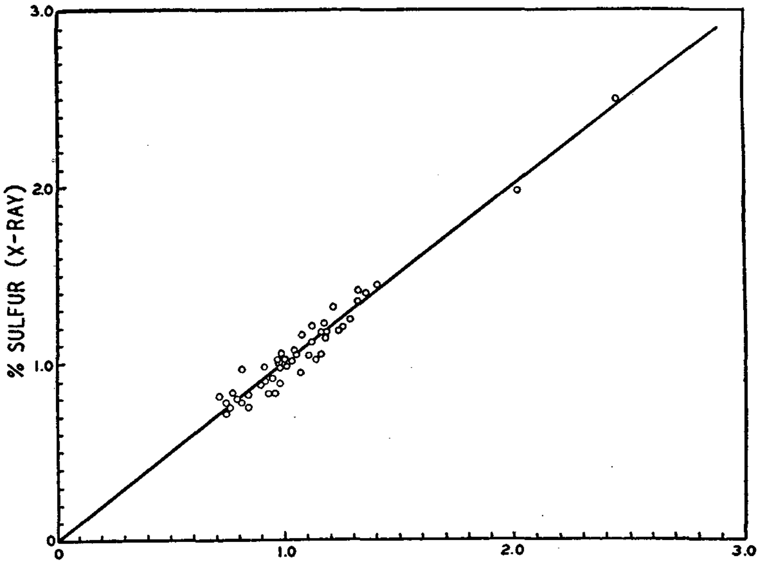

Figure 3. Sulfur by X-Ray and A.S.T.M. Methods

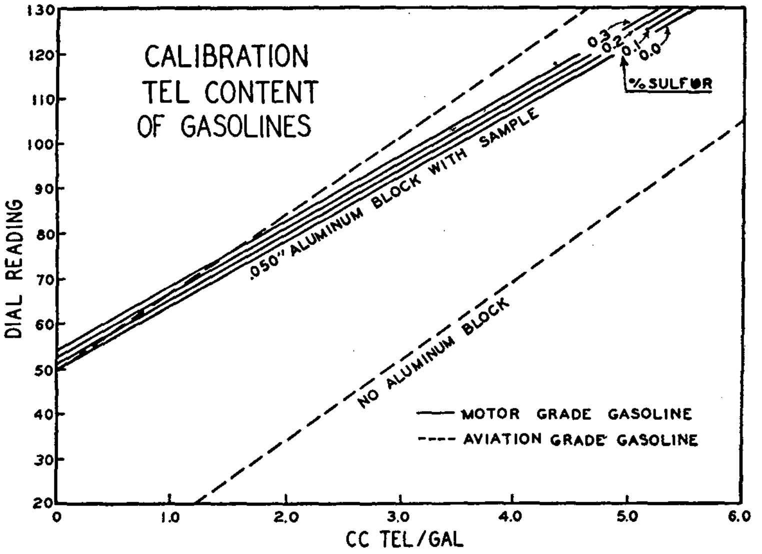

Figure 4. Tetraethyllead in Gasoline

When testing fuel oils the authors noticed a slightly higher absorption for a given sulfur content than their basic curve would lead them to expect. A study of comparative x-ray and analytical determinations showed it was possible to correlate these results. A new curve could be established parallel to and above the basic calibration allowing for slightly increased absorption. This shifting was found to compensate for the low ash content normally present in black fuels. This method of adapting the x-ray photometer will be useful in many instances where calibration can be made for particular stocks even though such calibrations might not be identical with curves prepared using pure compounds.

Figure 3 shows data comparing sulfur by x-ray and by A.S.T.M. Method D 129-44 (*2*).

TETRAETHYLLEAD IN GASOLINE

The determination of tetraethyllead in gasoline was recognized as a possible application of the principle of x-ray absorption technique. As far back as 1929 (*1*) it was demonstrated that it was possible to measure lead content following this basic approach. In 1940 Gross and Staab (*5*) using the improved counter tubes of Trost (*10*) successfully measured this additive in various types of fuels. The recent work of Zemany, Winslow, Poellmitz, and Liebhafsky (*11*) showed excellent precision in determining tetraethyllead by x-ray absorption when the exact base stock is available for comparison. The increased sensitivity of the Gieger counter type of radiation detector for x-ray absorption measurements has been reported by Sullivan and Friedman (*9*).

The refinery control laboratory performing tetraethyllead analyses is faced with certain particular conditions. For many of the samples, including those from outside sources, no base stock is available. Sulfur content, as mentioned above, will influence x-ray absorption when sulfur percentage is several times the lead concentration. For example, a gasoline containing 0.25% sulfur and 2 ml. of tetraethyllead per gallon actually contains only about 0.05% lead or about one fifth as much lead as sulfur. Variations in chlorine and bromine content of the tetraethyllead fluids can introduce a further slight inaccuracy. For purposes of uniformity it is desirable to weigh all samples while tetraethyllead content is expressed on a volume basis. Calibration is made with a representative gasoline of known density. A correction may be applied to results by multiplying by the ratio of the density of the sample to that of the calibrating stock. The magnitude of such a correction amounts to 0.02 ml. of tetraethyllead per gallon for 1° A.P.I.

The authors' solution was to prepare two sets of calibration curves. For motor gasolines normally blended with motor grade tetraethyllead fluid a family of curves is prepared with sulfur content from 0 to 0.30%. A single curve is adequate for aviation gasolines blended with aviation grade fluid, as sulfur content is always low on aviation gasolines (Figure 4).

A comparison of x-ray with analytical determinations by A.S.T.M. Method D 526 (*2*) is shown in Figure 5.

COMPOUNDING IN OILS

The variety and number of compounding materials added to petroleum products are legion. Such additives may improve oiliness, detergency, viscosity index, low temperature characteristics, load-carrying capacity, or other qualities. It is desirable to determine whether the proper amount of such compounding has been added. Although tests such as viscosities or pours will sometimes satisfy this need, in many instances an analytical chemical determination has been felt necessary as a control on quality.

Many of these additives contain one or more elements of higher atomic number than carbon and hydrogen. If only one such element is present, as in the case of a metal soap, we may calibrate the photometer directly in terms of this metal. Frequently additives are complex derivatives whose formulation includes more than one element of higher atomic number.

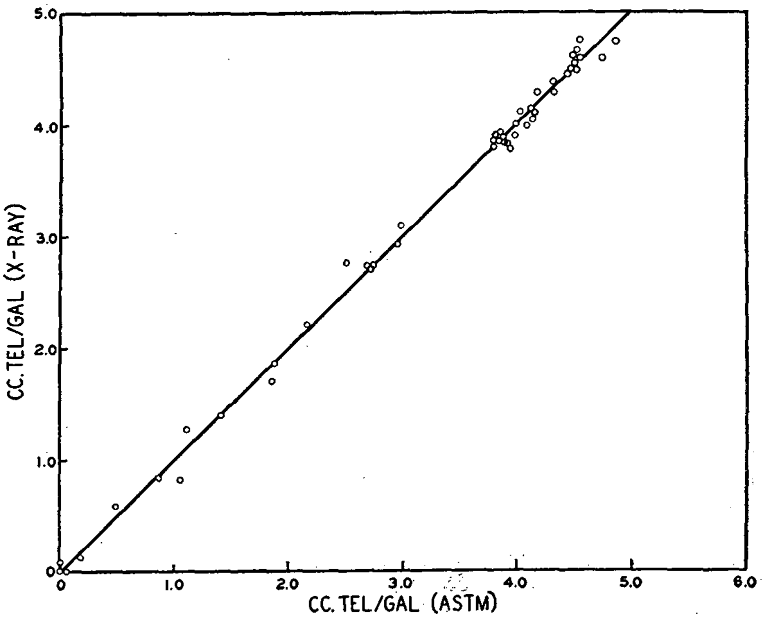

Figure 5. Tetraethyllead in Gasoline

This is best illustrated by a fairly simple example involving a gear lubricant additive containing substantial percentages of sulfur and chlorine. Suppose we are blending 10% of this additive with 90% mineral oil. We take a series of readings on the base stock and several blends. Results may be reported in per cent compounding, or in terms of one or more of its active ingredients. This procedure has been applied to lubricating oil compounding, where the actual amount of active metals was only a fraction of that present in the example chosen.

METAL ANALYSES

The manufacture of metal-organic derivatives requires a great deal of inorganic analytical testing to arrive at the desired metal content. All the metals involved are of considerably higher atomic number than carbon and are frequently present in comparatively large amounts. It is possible to prepare standards of varying metal content and calibrate to a sensitivity as close as the usual routine analytical accuracy. The proper choice of sample weight is an important factor on samples of high metal content, for beyond a certain mass the x-rays are so completely absorbed that insufficient impulse remains to activate the amplifier.

ACKNOWLEDGMENT

The authors wish to express appreciation for the helpful criticisms and suggestions received from John Y. Beach of the California Research Corporation.

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Preparation of Sugar Liquors and Sirups for Color Determinations

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Definite procedures for clarification and pH and density adjustments, essential for accurate determination of colors of sugar solutions, have been investigated in some detail in connection with a program of replacing visual color methods with photoelectric determinations. The results of this investigation led to the selection of an optimum method of preparing sugar liquors and sirups for routine color determinations. The sugar products to be tested usually require adjustment in density, to permit filtration and to give a color reading that will fall within the effective range of the color instrument. A schedule of standard dilutions has been established for determining the color of liquor and sirup samples normally encountered in refinery practice. Based on experimental work, a standard filtration procedure has been established to provide a clear sample and yet avoid removal of color by the diatomaceous filter medium employed. A standard pH of 7.0 has been selected and all samples are adjusted to this pH value in order to avoid the effect of variations in color due to the original acidity or alkalinity of the sample. Color readings are determined for solutions prepared in the prescribed manner and calculated to a standard reference basis (100% solids). This calculation is briefly discussed.

THE accurate measurement of the colors of sugar liquors and sirups is of considerable importance in the sugar industry for controlling operations and maintaining product quality. This measurement depends primarily upon two factors: an accurate and reliable instrument for the determination of color, and a standardized procedure for the preparation of solutions for color determination. The first factor has been investigated in considerable detail in this laboratory and several photoelectric instruments have been developed for measuring colors of process liquors (*8*), refined white sugars (*7*), and soft sugars (*6*). The development of these instruments has resulted in the replacement of all the visual color methods formerly used in this laboratory with photoelectric methods which are much more accurate and reliable.

The second problem of preparing solutions properly for color determinations arises principally in connection with the routine analysis of sugar sirups and liquors. The colors of a wide variety of refinery products are measured in the photoelectric colorimeter developed for this purpose (*8*). These products vary from the light-colored washed raw sugar liquor, on the one hand, to the dark-colored affination green sirup on the other hand. There is considerable difference in the densities, pH values, and clarities of the products. Obviously, a standardized method for preparing these materials for color determination is essential if comparable results are to be obtained. The present paper describes the results of an investigation which was carried on to determine the optimum procedure.

PREVIOUS WORK IN THIS FIELD

A considerable body of literature has been developed relative to the proper preparation of sugar solutions for color determinations. The most detailed of these procedures have generally involved rather technical and complex techniques which are difficult to apply to routine laboratory analytical work.

Most previous investigators agree that color determinations on sugar solutions should be made at the highest possible density, preferably 55° to 65° Brix (*3, 9, 12*). In diluting sugar solutions below these high densities, colloidal material is apt to be precipitated, which, in some cases, can be removed only with great difficulty. Consequently, the sugar solutions must be maintained at the higher densities to avoid these problems. How-

Table I. Comparison of Colors of a Sugar Liquor

(Water and colorless sugar solution used as diluents)

Sample	Dark Liquor	Colorless Sugar Solution	Distilled Water	Solids of Final Solution	Color Reading	Color Calcd. to 100% Solids of Dark Liquor
	Ml.	*Ml.*	*Ml.*	%	° *Stammer*	° *Stammer*
1A	10	90	..	43.7	1.55	29.5
2A	20	80	..	43.7	3.11	29.6
3A	30	70	..	43.7	4.76	30.2
4A	40	60	..	43.6	6.39	30.8
5A	50	50	..	43.4	8.08	31.0
6A	60	40	..	43.5	9.8	31.3
1B	10	..	90	4.95	1.58	31.3
2B	20	..	80	9.80	3.30	32.4
3B	30	..	70	14.5	4.95	32.3
4B	40	..	60	19.1	6.59	32.0
5B	50	..	50	23.4	8.29	32.2

ever, Zerban and Sattler (*12*) have pointed out that precipitation of colloidal matter may occur even in the high density solutions.

Generally, the use of these high density solutions necessitates filtering while hot. Brewster and Phelps developed a procedure wherein the solutions were heated to 80° to 90° C. (*3*). More recently, the Bureau of Standards has suggested a temperature of 50° C. (*2*). Obviously, the use of high temperatures is undesirable in a procedure for color determination because of the development of color in heating. This comment is equally applicable to the recommended practice of dissolving solid sugars in boiling hot water (*4*).

With regard to the filtration of sugar solutions for the removal of suspended matter, there have been some differences of opinion on the proper filter medium to be used. Peters and Phelps (*9*) and, later, Brewster and Phelps (*3*) established a procedure involving the filtration of the solution through a mat of specially prepared asbestos which they indicated gave a very clear solution without any removal of color. In their opinion, the use of other materials caused a loss of some color. Balch (*1*), on the other hand, filtered the solution through kieselguhr, which he found did not absorb color. However, it is likely that no medium can remove all the turbid matter without some effect on the color of the solution.

Zerban and Sattler (*12, 13*) after an extended investigation found that results obtained when asbestos was used as the filtering medium varied considerably, depending upon how the asbestos pad was packed, whereas results with kieselguhr were much more uniform. Their conclusions were that, of the filtering media tested, kieselguhr was probably the most desirable, but that selection was a matter of personal preference. This subject of filtration is well summed up by Spencer and Meade (*10*): "In view of the divergence of opinion on asbestos and the tediousness of the procedure, it is probable that kieselguhr is preferable for routine work and, possibly, for all types of work."

pH has a considerable effect on color; the higher the pH the darker the appearance of the sugar solution. Consequently, there is considerable recognition that the pH of sugar solutions should be standardized before color determinations may be made (*4*), although in some instances, it may be desirable to determine color at the original pH of the sample.

DESCRIPTION OF INVESTIGATION

There are various objections to the methods for preparation of sugar products for color determination, as described in the literature, particularly from the standpoint of use for routine control. The present work has been directed toward providing a simplified, rapid, and accurate procedure. All the factors that influence the color readings of sugar solutions have been considered during the course of this investigation: dilution, clarification, pH adjustment, and, finally, calculation of the resulting color to a uniform basis.

Dilution. In order that color readings will fall within the most sensitive range of the instrument, adsorption vessels at different thicknesses may be employed or the samples may be diluted. Some investigators have suggested the use of a heavy density colorless sugar solution in order to accomplish the required dilution. Obviously, the use of such a product makes it very difficult to filter the solutions. Consequently, distilled water was considered preferable in this laboratory, as it not only provided the necessary dilution but also greatly simplified filtration.

In view of the fact that the color of a given product does not vary greatly from time to time, it has been found possible to dilute each sample to some predetermined point to bring color readings within the desired instrument range. Such dilutions give solutions varying from 30° to 40° Brix for washed raw sugar liquors to 2° to 5° Brix for affination sirup samples. Dilutions are made prior to filtration, so that possible precipitated colloidal material is removed. This procedure has been satisfactory and there has been no evidence of precipitation following filtration.

In Table I is shown a comparison between the colors of various samples of 44° Brix sugar liquor, obtained by diluting in one case with distilled water and in the other case with a 44° Brix solution of confectioners' sugar. In this comparison, there is better agreement between the solutions which were diluted with water. In view of these and similar results, distilled water was adopted as the diluent in the procedure developed.

Clarification. A number of filtering media have been tested in this laboratory in order to find a suitable method for clarifying the diluted sugar solutions. Some time was spent in attempting to develop the asbestos filtration method as recommended by Peters and Phelps and improved by Brewster and Phelps for the purpose of routine color determinations. However, this method was very time-consuming, did not give clear solutions, and therefore did not seem suitable for routine analytical control. Consequently, it appeared that a filter aid such as one of the diatomaceous earths would be required.

In order to ascertain the most satisfactory material, a number of filter aids of different types were tested. Table II summarizes the results of one test series in which a number of diatomaceous filter aids were employed in different quantities, to clarify solutions for color determinations. The filter aids used were as follows:

Filter Aid No.	Description
1	Uncalcined (natural) kieselguhr
2	Calcined kieselguhr
3 4 5 6	Kieselguhr calcined with alkaline earth compounds; with increasing flow rate characteristics
7	Analytical grade kieselguhr
8	Factory regenerated kieselguhr

Table II. Clarification of Washed Raw Sugar Solution with Filter Aids[a]

Filter Aid	Turbidity				Color, ° Stammer per 100% Solids			
	0.5 g./ 250 ml.	1 g./ 250 ml.	2 g./ 250 ml.	3 g./ 250 ml.	0.5 g./ 250 ml.	1 g./ 250 ml.	2 g./ 250 ml.	3 g./ 250 ml.
1	1.8	1.3[b]	1.1	1.0	8.07	8.00[b]	7.60	7.23
2	2.5	2.5	2.8	3.0	8.42	8.29	8.27	8.22
3	2.0	1.9	1.7	1.5	8.29	8.18	8.04	7.79
4	2.4	2.1	2.0	2.0	8.52	8.45	8.35	8.25
5	2.6	2.5	2.4	2.3	8.79	8.85	9.07	9.07
6	2.8	2.9	3.1	3.3	8.75	8.80	9.03	9.14
7	2.3	2.3	2.3	2.2	8.47	8.35	8.22	7.95
8	2.7	3.1	3.2	3.4	8.67	8.67	8.67	8.67
Through filter paper		2.9				8.59		
Original sample		4.4				8.85		

[a] Washed raw liquor diluted to 35° Brix, filtered, and then adjusted to pH 7.0. First 50 ml. of filtrate discarded and next 100 ml. collected for test.

[b] Present standard method.

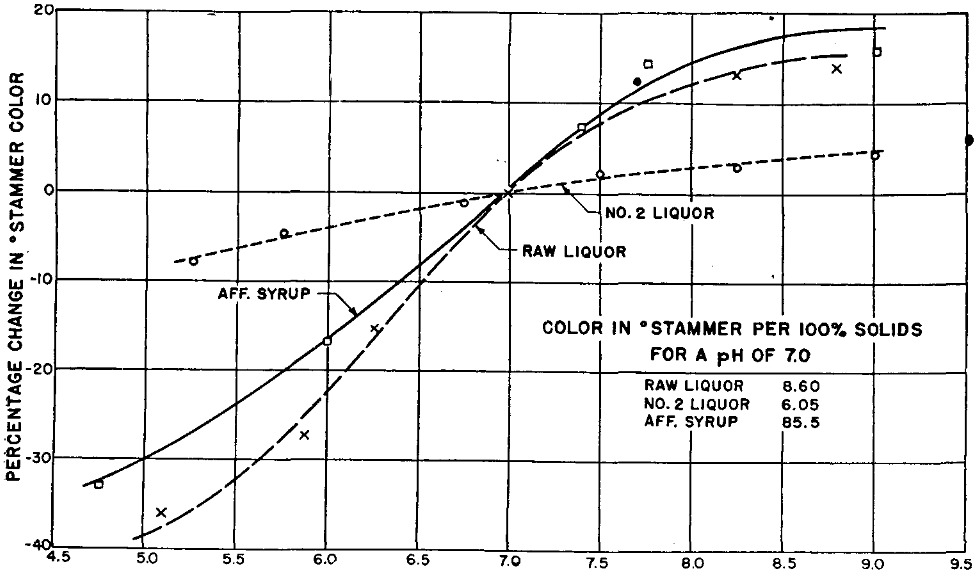

Figure 1. Effect of pH on Color of Typical Refinery Products

On the basis of these tests, uncalcined kieselguhr appeared to be the only filtering medium that would give a clear filtrate as determined by a photoelectric turbidity indicator, based on measurement of the Tyndall effect (*5*). Some kieselguhrs, particularly the calcined materials actually gave increasing turbidities as the quantity of filter aid was increased. Other materials added color as the amount of filter aid was increased, although the clarity was somewhat improved.

The selection of a particular filter aid and the quantity of that filter aid to be used represents, of course, a compromise among a number of factors, including the resulting clarity which is obtained, the apparent removal of color, and the length of time required to obtain a suitable quantity of filtered material. In this laboratory, it has been determined that the use of 1 gram of natural kieselguhr per 250 ml. of diluted liquor offers the optimum filtration characteristics. Tests have indicated that this quantity of kieselguhr absorbs very little color and gives satisfactory clarity. In practice, the first 50 ml. of filtrate are discarded, inasmuch as usually this material may be somewhat cloudy. The next 100 ml. are collected for color determination.

pH Adjustment. The color of a sugar solution changes with a change in alkalinity or acidity. The extent of this variation in color with the pH of three sugar products is shown in Figure 1. It is obvious from this figure that some standard pH value must be selected if color results are to have any meaning or are to be suitable for comparative purposes. A pH value of 7.0 has been selected in this laboratory. After the samples have been filtered, the pH is adjusted to the neutral point by titration with either 0.0357 N sodium hydroxide or sulfuric acid as required.

Calculation of Color. The colorimeter used in this investigation is the photoelectric device described previously (*8*). This instrument was designed to read transmittance directly, and was therefore calibrated in terms of $-\log T$.

The photoelectric colorimeter was also calibrated in Stammer units (*11*), inasmuch as the Stammer colorimeter had been in prior use in this laboratory. This secondary calibration was possible because the Stammer scale is directly proportional to $-\log T$. Numerous comparisons between visual Stammer readings and photoelectric color determinations developed the following relationship: Degrees Stammer = 11.0 ($-\log T$) where T is in transmittance of light of 535 millimicrons dominant wave length through an absorption vessel of 3-cm. thickness.

This relationship holds only for the particular Stammer glasses used; however, these glasses were carefully selected from among a large number and represented median characteristics. In view of the arbitrary nature of the Stammer unit, it is ultimately planned to adopt the more basic $-\log T$ unit, when this receives more general usage in the sugar industry.

The color readings obtained on the photoelectric colorimeter, as described above, are calculated to a 100% solids basis. This involves dividing the color reading by the solids content and specific gravity and multiplying by 100. Inclusion of the specific gravity is necessary in the calculation to obtain comparable colors regardless of density. This is indicated by Table III, which shows the colors of a series of samples at different densities calculated with and without the specific gravity factor. There is considerable variation in the colors calculated without taking specific gravity into account.

Table III. Colors of Washed Raw Sugar Liquor Determined at Various Densities

% Solids	Color Reading, ° Stammer	Color, ° Stammer per 100% Solids: With sp. gr. factor	Color, ° Stammer per 100% Solids: Without sp. gr. factor
47.0	5.23	9.2	11.1
41.3	4.48	9.2	10.8
34.3	3.64	9.2	10.6
28.8	2.97	9.2	10.3
24.0	2.41	9.2	10.1

PROCEDURE

On the basis of the foregoing, the procedure established is as follows:

The sample is diluted to a predetermined point to give a suitable reading within the range of the instrument.

One gram of filter aid is mixed with 250 ml. of diluted sample.

The sample is filtered through a coarse filter paper (rapid gray sugar paper No. 124$^1/_2$, Geo. D. Feidt & Co., Philadelphia, Pa.) in a stemless funnel; the first 50 ml. are discarded and the next 100 ml. saved for color determination.

The filtered sample is adjusted to pH 7.0, using 0.0357 N sulfuric acid or sodium hydroxide as the adjusting reagent.

The color is read in the colorimeter.

The solids content of the sample is determined with the refractometer.

The color is calculated in accordance with the following formula:

Color in degrees Stammer per 100% solids =

$$\frac{\text{color reading, ° Stammer} \times 100\%}{\% \text{ solids} \times \text{specific gravity}}.$$

SUMMARY

The procedure described has been in satisfactory use in this laboratory for a number of years. Reliable and consistent results have been obtained even by nontechnically trained laboratory personnel. The method is relatively simple and rapid, and consequently has proved very suitable for process color control.

It is of especial value for investigative purposes. Previous methods did not give consistent or reliable color data and, consequently, much time and effort were uselessly expended in attempting to interpret color information. The situation has been completely changed and color results are now accepted without question. This has saved a great deal of time and manpower in plant and laboratory investigations.

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Rapid Measurement of Cellulose Viscosity by the Nitration Method

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A simplified technique is described for rapidly determining the nitrate viscosity of cellulose. Convenience in procedure is achieved by forming the pulp into soft, thin disks which may be easily handled in the various stages of nitration and stabilization. Degradation is kept to a minimum by using a small sample of open structure and further by using an optimum acid composition under favorable conditions of time and temperature. Data are presented to show the effect of various changes in method on the level of calculated degree of polymerization.

THE nitration method for measuring cellulose viscosity has in recent years been widely used in both fundamental and development research dealing with the structure and properties of cellulosic materials. It has not, however, been accepted by the pulp and paper industry as a control method for mill operations. Although Berl (*1*) has suggested such use and has outlined a procedure for obtaining rapid measurement of viscosity, it has usually been thought, and rightly so, that the method was highly complicated and would yield precise data only in the hands of a highly skilled analyst.

In adapting the measurement of nitrate viscosity for more general and practical use, the present method provides a much simplified technique which gives highly reproducible data and eliminates many of the difficult, tedious, or dangerous steps that have formed the basis for most objections to previous methods.

The nitrate viscosity method offers certain advantages which other viscosity methods do not afford: Because the solution is made in a simple organic solvent, a representative sample may be readily dissolved, aliquoted, and diluted to any suitably low concentration for viscometric determination of degree of polymerization. The degree of polymerization of the carbohydrate portion of unbleached pulps containing large quantities of lignin, or even of wood itself, may be reliably measured, inasmuch as with suitable nitration conditions, the lignin is substantially removed in the nitration step and therefore does not interfere with the viscosity measurement on the nitrated cellulosic constituents (*6*).

The nitration method is based on conversion of the cellulose sample into cellulose nitrate by a nitration step which does not appreciably degrade the cellulose (*2*, *8*), measurement of the viscosity of a dilute ethyl acetate solution of the nitrate, and calculation of the average chain length (*3*, *4*).

The method in its present status is an accurate and fairly rapid means for measuring cellulose intrinsic viscosity and may well replace the very slow cuprammonium method or serve as a check and means of calibration for the very fast cupriethylenediamine method. The time requirement is about 2 to 3 hours and the reproducibility of degree of polymerization values is within 1%.

The values as calculated are not presumed to be absolute. They serve, nevertheless, as a convenient means for expressing the relative significance of measured differences in solution viscosity.

METHOD

Preparation of Cellulose. The cellulose sample is prepared by remaking it into a thin soft sheet that will permit ready penetration of the nitrating acid. For wood cellulose this is best accomplished by dispersing the pulp fibers in water and flowing the slurry onto a sheet mold. About 20 grams of pulp are used for a 13 × 13 inch (32.5 × 32.5 cm.) sheet which is lifted from the sheet mold and, without pressing, dried at 50° C. on a stainless wire screen support. Enough disks, 40 mm. in diameter, are cut from the dried sheet to give about 1.0 gram of pulp (three or four disks). The disks are further softened by flexing between the fingers, placed in a weighing bottle, and redried to a constant weight at 50° C. (about 99% bone dry) in a mechanical convection oven.

Preparation of Nitrating Mixture. The nitrating acid mixture is prepared by adding cautiously, with a spatula, 404 grams of phosphorus pentoxide very slowly to 1000 grams of cold 90% nitric acid (90% fuming acid but not red fuming) contained in a 2-liter Erlenmeyer flask. The acid is kept ice-cold by immersion in an ice water bath and is swirled continuously during addition of the phosphorus pentoxide. This produces a mixture with the composition: nitric acid, 64%; phosphoric acid, 26%; phosphoric pentoxide, 10%. With occasional gentle shaking, solution is complete in a few hours and the acid mixture is then filtered through glass wool into a glass-stoppered bottle and stored in a cool dark place.

Nitration. In the actual nitration, a 40-gram portion of the prepared nitrating acid mixture is weighed out into a tall weighing bottle of about 100-ml. capacity, and placed in a constant temperature bath at 20° ± 0.1° C. With the use of stainless tweezers, the cellulose sample comprising several disks of pulp totaling approximately 1 gram is introduced quickly into the nitrating acid, a single disk at a time. The nitration is allowed to proceed for 20 minutes, the sample being swirled at about 5-minute intervals, and the disks are then removed with the tweezers and stacked in a sintered-glass Büchner-type filter funnel which is about 40 mm. in diameter. Through an appropriately trapped filter apparatus, suction is applied and at the same time the disks are gently pressed into the slightly constricted bottom of the funnel with a flattened stirring rod.

After a few seconds of suction, which suffices to remove the excess acid, the disks are removed with tweezers and drowned in cold (10° C.) distilled water, stirred for a moment, and neutralized by adding a small amount of powdered sodium carbonate to the water. The nitrate is washed with about three changes of water, the disks being transferred with tweezers to the fresh water. The nitrate is then boiled for 20 minutes in distilled water, drained, soaked for 10 minutes in 50 ml. of methyl alcohol, and drained again, preferably with suction. The disks are placed in small 200-mesh stainless wire baskets, dried for about 1 hour at 50° C. in a mechanical convection oven, weighed, and stored.

Measurement of Viscosity. For the measurement of viscosity a 0.250-gram sample of nitrate is weighed out and dissolved in a 50-ml. portion of absolute ethyl acetate, and an aliquot is diluted to yield a final solution containing 0.05 gram of cellulose nitrate per 100 ml. of ethyl acetate. The viscosities of the solvent and the solution are measured in a suitable Cannon-Fenske (*3*) type viscometer at 20° ± 0.1° C.

Calculation of Degree of Polymerization. The following formula is used:

$$\mathrm{DP} = K[\eta]$$

where $$[\eta] = \frac{\eta_{sp}/C}{1 + k'\,\eta_{sp}}$$

$$\eta_{sp} = \eta_{r-1}$$

$$\eta_r = \frac{\text{viscosity of solution}}{\text{viscosity of solvent}}$$

$$K = 75$$

$$k' = 0.35$$

$$C = \text{grams of nitrate per 100 ml. of solvent}$$

Table I. Effect of Sample Form on Nitrate Degree of Polymerization

Sample Standard Pulp	$[\eta]$	DP Using $K = 75$	K to Give DP of Std. Method
Disks unpressed, flexed, 1 gram, 20 min.	15.2	1140	75
Carded fluff, 1 gram, 20 min.	15.7	1180	72.5
5 × 5 mm. pulp square, 10 grams, 5 hours	11.4	855	100

DISCUSSION OF ANALYTICAL PROCEDURE

Conditions of this standardized procedure have been selected to provide a nitration technique which, in addition to being rapid, convenient, and easy to follow, gives a minimum of degradation of the cellulose. Convenience in operation is achieved through the use of pulp disks, and degradation is minimized by the use of a sample of small size and open structure. In addition, an acid of optimum composition is used under favorable conditions of time and temperature.

For these small scale nitrations, fluffed pulp is, of course, ideal for obtaining immediate and instantaneous wetting of each individual fiber by the acid at the start of nitration and by the water on drowning, but is less convenient than disks in several respects: Fluff is rather difficult to prepare. If a fluffing card is used, one must either laboriously clean it after each sample or have a different card for each type of pulp. The sample may be slurried, dewatered with acetone, and fluffed with a jet of air, but cleaned air must be provided and suitable precautions taken to prevent loss of fines. Fluff is much less easily handled than the disks. The acid slurry containing the fluff must be transferred to the filter at the end of nitration (danger of acid splash) and filtered at each change of wash water, whereas the disks may be handled with tweezers in a much simplified operation. In Table I it is shown that the degree of polymerization values obtained with soft, thin disks are only very slightly lower than with fluff.

Table II. Effect of Moisture Content of Pulp on Nitrate Degree of Polymerization

Moisture Content of Standard Pulp, %	DP Using $K = 75$
0 (100% B.D.)	1150
1 (99% B.D.)	1150
6 (94% B.D.)	1140
10 (90% B.D.)	1120
16 (84% B.D.)	1110
18 (82% B.D.)	1085

The standard procedure specifies that the pulp be dried to about 99% bone dry prior to nitration. However, Table II indicates that relatively small variations in the moisture content have little effect on the measured degree of polymerization.

The preparation of the nitrating acid is a rather disagreeable task; the one redeeming feature is that one batch of 1404 grams will suffice for 35 samples of 1 gram of cellulose. If desired, an alternative method of acid make-up may be used; the required amount of phosphoric anhydride may be added to 85% phosphoric acid and the warm mixture poured into cooled 98 to 99% nitric acid. The acid mixture should be stored in a cool dark place and preferably used only after 72 hours and within a few weeks after make-up (see Table III). Deterioration of the acid is indicated by a marked increase in color due to the oxides of nitrogen.

The particular nitrating mixture chosen gives a minimum of degradation with a high degree of nitration (13.95 to 14.10% nitrogen) and is easy to handle because it is not sirupy. The acetic acid and acetic anhydride mixtures also give a high degree of nitration and result in high viscosities, but are more dangerous and deteriorate more rapidly than do phosphoric and phosphoric anhydride mixtures, and do not effect sufficient delignification of wood or of high TAPPI K number (*10*) unbleached pulps to give satisfactory nitrate solubility. The conventional sulfuric acid mixtures give a lower degree of nitration (12 to 13% nitrogen) and an appreciably higher degree of degradation (Table IV).

Table III. Effect of Age of Nitrating Mixture on Nitrate Degree of Polymerization

Age of Mixture Used on Standard Pulp	DP Using $K = 75$
2 hours at 20° C.	845
Days	
1	1010
2	1085
3	1120
4	1150
8	1140
16	1160
23	1160
30	1160
37	1150
44	1150
51	1160
65	1120
73	1130
83	1115
90	1085
100	1080
107	960

The nitration temperature of 20° C. has been chosen as the most convenient. A temperature of 0° gives slightly less degradation but requires an ice bath, makes it necessary to use longer nitration times, particularly with unbleached pulps, and results in a more sirupy mixture which is slower to penetrate and more difficult to remove from the cellulose sample (Table V). Although 50% acetic acid is preferred by some workers (*8*), water has been found in these experiments to be a convenient and satisfactory drowning medium.

The nitration time of 20 minutes is used for bleached pulps (Table VI). Unbleached pulps should be nitrated long enough to ensure sufficient attack on the lignin so that it may be solubilized and removed in the stabilization process. A workable rule appears to be to nitrate for a time in minutes equivalent to about 10 times the TAPPI K number (*10*) of the unbleached pulp. Samples of properly prepared raw wood shavings usually require about 24 hours' nitration time (*6*). The nitrate from unbleached pulps, and particularly wood, should be thoroughly washed and boiled with many changes of distilled water, then carefully leached with methanol. Good lignin removal is essential in obtaining a nitrated carbohydrate fraction which will be completely soluble in ethyl acetate or acetone.

Table IV. Effect of Nitrating Acid Composition on Nitrate Degree of Polymerization

Nitrating Mixture Used for Std. Pulp			Temp., ° C.	Time, Min.	DP Using $K = 75$
H_3PO_4, %	HNO_3, %	P_2O_5, %			
16	74	10[a]	20	20	1095
26	64	10[a]	20	20	1140
36	54	10[a]	20	20	1135
46	44	10[a]	20	20	1105
56	34	10[a]	20	20	1085
51% H_2SO_4	33	16% H_2O	35	150	500
10% $(CH_3CO)_2O$	52	38% CH_3-COOH[b]	0	60	1260

[a] Sirupy.
[b] Dangerous mixture.

Table V. Effect of Nitration Temperature on Nitrate Degree of Polymerization

Temperature Used for Standard Pulp, ° C.	[η]	DP Using $K = 75$	K to Give DP of Std. Method
0	16.1	1210	71
20	15.2	1140	75
40	13.9	1045	82

In the case of unbleached pulps, the yield of the nitrated cellulosic constituents is especially valuable, as it may be used to estimate the lignin content. Knowing the yield of nitrate from a pure cellulose, one may divide the difference in yield between a lignified cellulose and a pure cellulose by the yield from the pure cellulose and multiply by 100 to obtain the percentage lignin content. A rayon grade wood cellulose usually gives, by this method, a nitrate containing 13.9 to 14.0% nitrogen with a yield of about 180%. Unbleached pulps give lower yields, and, corresponding to lignin content, may range down, for example, to 120% or so for wood itself. Western hemlock gives a yield of about 126%, which represents a lignin content in the wood of about 30% on a solvent-extracted moisture-free basis; the other 70% comprises 50% α-cellulose with average degree of polymerization of about 2500 and 20% hemicellulose with average degree of polymerization of about 70.

The time needed to obtain complete solution depends on the degree of polymerization and degree of subdivision of the sample, usually ranging from a few minutes for rayon or cellophane pulps to several hours for very high viscosity paper pulp or raw cotton. If speed is desirable, it is best to pick the nitrate disk into small pieces before weighing out for the dissolving step.

Absolute ethyl acetate gives high intrinsic viscosity values and is the preferred solvent. Butyl acetate, being more viscous, requires too much time in the dissolving step and in viscosity measurements. Acetone is much more susceptible to pickup of moisture than ethyl acetate, gives appreciably lower intrinsic viscosity values, and, furthermore, gives nitrate solutions that show a greater tendency toward viscosity decrease on standing (Table VII).

The preferred temperature for measurement of viscosity is arbitrarily 20° C. Solvent temperature has a noticeable effect on measured intrinsic viscosity, as shown in Table VIII.

Table VI. Effect of Nitration Time on Nitrate Degree of Polymerization

Minutes at 20° C. Used for Standard Pulp	DP Using $K = 75$
5	1110
10	1130
20	*1140*
60	1145
300	1120

Table VII. Effect of Solvent on Nitrate Degree of Polymerization

	[η][a]	DP Using $K = 75$	K to Give DP of Std. Method
Acetone, highest purity	12.1	905	94
Ethyl acetate, absolute	15.2	1140	75
Butyl acetate, C.P.	15.4	1155	74

[a] Value of $k' = 0.35$ used for each solvent in calculating intrinsic viscosity.

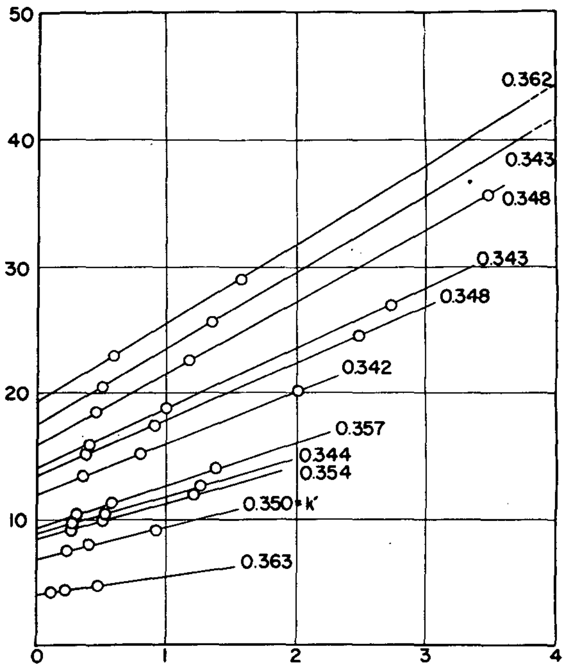

Figure 1. Typical Curves Used in Evaluation of Huggins k' (*4*) for Solutions of Cellulose Nitrate in Ethyl Acetate

In making viscosity measurements, it is desirable to vary the concentration of the cellulose nitrate to suit the level of degree of polymerization, inasmuch as the flow time and specific viscosity are more constant if one uses, for example, a concentration of 0.10 gram per ml. in the case of rayon or very low viscosity pulps and 0.025 gram per 100 ml. in the case of very high viscosity paper pulps, native cotton, or rayon grade wood cellulose. In line with obtaining a high degree of reproducibility, a good procedure to follow in making up the 0.05% solution from the 0.50% solution is to pipet 2 ml. of the 0.5% nitrate solution into a 2-ounce bottle; with the same 2-ml. pipet, pipet four 2-ml. portions of ethyl acetate into the bottle; and with a 10-ml. pipet add 10 ml. of ethyl acetate to the bottle.

The viscometer now in use in this laboratory, Cannon-Fenske type for nonviscous liquids (*3*), has a flow time of 72.3 seconds per ml. for ethyl acetate at 20° C. and requires no kinetic energy correction. The capillary was made to specification, 140 × 0.4 mm. For any given viscometer a curve may be plotted which gives degree of polymerization directly from seconds of flow time for 0.10, 0.05, and 0.025 gram per 100 ml. of solutions.

The value 0.35 for k' was determined according to Huggins (*4*) by plotting η_{sp}/C vs. η_{sp} for selected cellulose nitrates, covering a wide range in η, at concentrations of 0.025, 0.05, and 0.10 gram per ml. of ethyl acetate. The family of curves was found to be a series of straight lines having a ratio of slope to intercept falling with no apparent order within the range 0.335 to 0.365 (see Figure 1 for typical curves). Some data obtained on the value of k' for acetone and butyl acetate solutions indicate that 0.35 is a good average for these solvents also.

Table VIII. Effect of Solvent Temperature on Nitrate Degree of Polymerization

Ethyl Acetate, Solvent Temperature, ° C.	[η]	DP Using K = 75	K to Give DP of Std. Method
10	16.3	1220	70.5
20	15.3	1150	75
30	14.2	1065	80.5
40	13.15	985	87

With the present nitration procedure utilizing a sample of cellulose of 1 gram or less, and with ethyl acetate as the solvent, the value of 75 for K gives values of degree of polymerization which appear to be consistent with the accepted level for rayon, wood pulp, and cotton (*5, 7–9*). In fractionation studies involving larger scale nitrations under slightly different conditions, a value of K equal to 100 for calculating the degree of polymerization of separated fractions has been used (*6*), but the present technique results in higher viscosities and therefore gives the same level of degree of polymerization with a lower value of K. The value of K for butyl acetate is little different than for ethyl acetate, about 74 as compared with 75; the value for acetone is appreciably higher, about 94 (Table VII).

This method with K equal to 75 yields degree of polymerization values of the same order of magnitude as values calculated by Staudinger's formula using $K_m = 11 \times 10^{-4}$ from viscosities obtained in acetone solution at suitably low concentrations. The formula used is, however, much to be preferred to that of Staudinger, mainly because it eliminates the concentration effect.

Table IX. Degree of Polymerization for Cellulosic Materials Nitrated by Standard Method

	DP Using K = 75
Lint cotton (chlorite bleached)	3800
Wood pulp (very mildly cooked)	2200
Rayon grade wood pulp	1150
Cellophane grade wood pulp	780
Commercial rayon	350

Some typical nitrate degree of polymerization values for various celluloses listed in Table IX range from 3800 for lint cotton to 350 for rayon.

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Estimation of the Color of Tomato Paste

Application of a Color Index

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A simple, objective, and quickly prepared color index, obtained by measuring the color of extracts by a photoelectric colorimeter instead of by reflectance measurements, has been applied to tomato juice and to tomato paste prepared in the laboratory and to commercial samples of paste collected from plants in California. The color index method described is not significantly less accurate than existing methods and is more quickly and conveniently applied.

THE growth of the tomato paste industry in California has been rapid in recent years, but improvements in chemical methods for controlling the quality of the paste have not kept pace with this rapid development. Suitable objective methods for measuring the color of processed food products are needed by the industry, and because the color of tomato products is regarded as a good criterion of quality, a convenient method for measuring the color would aid in setting up and maintaining control measures. This paper reports results of an attempt to apply such a method of color measurement, referred to as the color index, to commercial samples of paste collected in California, to pastes prepared in the laboratory, before and after storage, and to the juices from which the pastes were prepared. The color index is defined as a series of graduated numerical readings obtained by measuring the light absorption of the acetone extracts of tomato pigments in a Klett-Summerson colorimeter with a No. 44 blue filter. The scale readings of this instrument are proportional to light absorption.

The importance of color in the production and marketing of tomato products is too well known to require emphasis. The need for a simple and rapid test for gaging the color not only of tomato products but of other fruits and vegetables has been made clear from previous studies by other workers (*9, 10*). As early as 1918, efforts were made by Howard (*6*) to determine the effect of heat on the color of tomato pulp and paste. There is evidence that the Munsell system (*11*) and the color chart procedure (*3*), which are not entirely objective, do not always give satisfactory results (*12*).

Although the estimation of color of tomato products by visual means or by spectral reflectance curves has been developed (*9, 15*), little attention has been given to the measurement of the color of extracts from these products and its use as an index of

Table I. Comparison of Color Index Values with Quantity of Pigments Determined by Different Chemical Methods

(Based on a total solids content of 25%)

			Binary Spectrophotometric Method[b]			Chromatographic Analysis					
						Spectrophotometric[b]			Colorimetric[b]		
Variety	Color Index[a]	Yellow Color[a]	Total carotenes	Lycopene	Carotene	Total carotenes	Lycopene	Carotene	Total carotenes	Lycopene	Carotene
					Juice						
Pearson[c]	184	..	443	409	34	372	348	24	419	390	29
San Marzano[d]	211	..	480	461	19	411	396	15	422	402	20
Average[e]	193	..	455	426	29	385	364	21	420	394	26
					Paste, before Storage						
Pearson	174	44	398	369	28	351	321	30	373	342	31
San Marzano	182	48	415	393	23	366	348	18	337	321	16
Average	176	46	403	377	26	356	330	26	361	335	26
					Paste, after Storage						
Pearson	173	38	416	382	35	380	355	25	366	339	25
San Marzano	185	45	456	438	18	432	408	24	414	395	23
Average	177	41	429	401	29	397	373	25	382	358	24

[a] Colorimetric reading.
[b] Expressed as micrograms per gram.
[c] Pearson variety, average of 8 lots.
[d] San Marzano variety, average of 4 lots.
[e] Weighted averages.

color. In this work, the light absorption of extracted pigments was measured to estimate the color of tomato juice and paste.

GENERAL PROCEDURE

In a series of experiments designed to study the effects, on the color of the pastes, of concentrating tomato juice at various temperature, it was obvious that a suitable objective method would be needed to evaluate the differences in the various samples. Juice from locally grown San Marzano and Pearson varieties of tomatoes was concentrated to approximately 25% total solids in a small laboratory evaporator. In order to make comparisons of the color index values of the extracts with the actual pigments present in samples from the same lots, the total carotenes, the lycopene, and the carotene fractions of the carotenoid pigments were determined in the juice before concentration to the paste, in the paste soon after preparation and canning, and in the canned paste after one year's storage at room temperature. As soon as possible after their preparation, samples were ranked visually on the basis of color by a panel of nine observers at the Western Regional Research Laboratory, Albany, Calif. Commercial samples collected from various plants in central and southern California were also evaluated on the basis of their color index values and analyzed for their carotenoid pigment content.

DETERMINATION OF COLOR INDEX

One gram of the well-mixed paste is quickly weighed on a balanced watch glass by means of a chain weight or other suitable balance, transferred to the bowl of a Waring Blender or similar mixer, and mixed for a few minutes with sufficient metaphosphoric acid (approximately 1%) to cover the revolving blades. Two level teaspoonfuls of filter aid are stirred into the mixture in a beaker and the suspension of paste is filtered on a filter paper thinly precoated with filter aid in a Hirsch-type funnel having a filtering surface about (1 inch) 2.5 cm. in diameter. A vacuum pump not affected by volatile solvents, such as the Gast-type rotary pump, is convenient for maintaining a high and even pressure. The filter cake is washed with water and with rinsings from the mixer and the beaker, to remove all traces of the water-soluble yellow pigment and the metaphosphoric acid. Then the pigments held in the filter cake are extracted by additional small charges of acetone until approximately 45 ml. have been added. To counteract the lowering of the temperature resulting from the rapid evaporation of the acetone due to the reduced pressure, the acetone is warmed to 25° to 30° C. before it is added.

When the extract is made to a volume of 50 ml., care should be taken that large variations from room temperature do not occur, and that any crystals of lycopene formed are redissolved. A 5-ml. aliquot of this solution is diluted to 25 ml. with acetone, and the color is measured in the colorimeter with a No. 44 blue filter, which transmits light at a range of 410 to 480 millimicrons. The readings of the colorimeter are recorded as the color index. About 15 minutes are required from the weighing of the sample to the reading of the index number.

After removal of the water from the filter cake with acetone, solvents other than acetone can be used to extract the pigments to test the completeness of the extraction. Acetone was selected because it completely extracts a wide range of pigments and the extract is free from turbidity. Freedom from turbidity is important in the colorimetric readings. Because of these qualities of acetone, and because of other factors mentioned subsequently, the method is applicable to many diverse types of fruits and vegetables and their products.

The water-soluble yellow color present in small quantities in tomato paste is not included in the index but may be measured separately. Twenty-five grams of the paste are mixed with filter aid, extracted with sufficient 4 to 5% metaphosphoric acid, filtered, and diluted to 225 ml. with water. This extract gives a clear solution of sufficient strength to be measured in the colorimeter with a No. 44 filter.

A few miscellaneous items of importance in the extraction of the pigments should be emphasized. Filter aid was used because it disperses the particles of paste and prevents clumping, which ordinarily occurs when tomato paste or pulp is shaken with such solvents as chloroform, benzene, acetone, or hexane-acetone, without filter aid. The quantity of water present is also an important factor in extraction with certain solvents (*1*, *2*). Consideration was given to inflammability and toxicity in selecting a solvent (*7*). Metaphosphoric acid aids materially in hastening the speed of filtration. Pigments prepared by mixing the paste with metaphosphoric acid were compared with pigments prepared by mixing with water. There was no indication that the presence of the acid reduced the quantity of the extracted pigments.

METHODS OF DETERMINING PIGMENTS

The lycopene and carotene fractions of the carotenoid pigments were determined by three chemical methods: the quick and convenient binary spectrophotometric method of Zscheile and Porter (*17*), chromatographic analysis followed by spectrophotometric measurement (*14*, *17*), and chromatographic analysis followed by colorimetric measurement. A comparison of results obtained by two types of instruments was thus permitted. Lycopene and carotene in the hexane solution remaining after the determination of pigments by the binary method were separated by chromatography on a 50–50 magnesia–filter aid column. After their separation, these pigments were determined in aliquots from the same solutions, by a Beckman spectrophotometer, designated as a spectrophotometric method, and by a Klett-Summerson colorimeter with a No. 44 blue filter, designated as a colorimetric method. All determinations were made at least in duplicate.

Table II. Carotene and Lycopene Expressed as Percentage of Total Carotenes Found by Three Methods

	Lycopene			Carotene		
	Binary spectro-photo-metric	Chromatographic		Binary spectro-photo-metric	Chromatographic	
		Spectro-photo-metric	Colori-metric		Spectro-photo-metric	Colori-metric
			Juice			
Pearson[a]	92.4	93.7	93.1	7.6	6.3	6.9
San Marzano[b]	96.0	96.4	95.3	4.0	3.6	4.7
Average[c]	93.6	94.6	93.8	6.4	5.4	5.8
			Paste, before Storage			
Pearson	92.9	91.4	91.7	7.1	8.6	8.3
San Marzano	94.6	95.0	95.3	5.4	5.0	4.7
Average	93.5	92.6	92.9	6.3	7.4	7.1
			Paste, after Storage			
Pearson	91.6	92.5	93.5	8.3	7.5	6.5
San Marzano	95.9	95.4	94.6	4.1	4.6	5.4
Average	93.1	93.5	93.8	6.9	6.5	6.1

[a] Pearson variety, average of 8 lots.
[b] San Marzano variety, average of 4 lots.
[c] Weighted average.

RESULTS

As shown in Table I, in which the results of the determinations of color by means of the color index and of the pigments by chemical determination are summarized and compared, the color index readily differentiates between Pearson tomatoes (eight lots) and San Marzano tomatoes (four lots) in both paste and juice. In all cases except two, the higher values of the color index parallel the higher content of total carotenes and lycopene of the San Marzano tomatoes. Carotene values are lower in the San Marzano than in the Pearson tomatoes. There is also a correlation between the higher average values of the color index for the juice as compared to average values of the paste before storage. The chemical determinations show an apparent increase in pigments of the paste after storage. However, the difference between the indexes of paste before and after storage is slight.

The pigment values obtained by the spectrophotometric and colorimetric methods are somewhat lower than the values obtained by the binary spectrophotometric method, because of the loss of pigment when the extract goes through the adsorption column used in separating lycopene and carotene. However, the three methods of chemical determination give similar comparative results.

The agreement of relative values for total carotenes is of interest because total carotenes are determined by the quick binary method by substitution of spectrophotometric readings in a formula (*17*), whereas total carotenes are determined in both the spectrophotometric and colorimetric methods by addition of carotene and lycopene values.

Because extracts of water-soluble yellow color gave low readings in comparison with extracts of carotene approximately 30 times more dilute, the water-soluble yellow color would have had little influence on the values of the color indexes had they been included.

In Table II, the percentage composition of the chemically determined pigments is brought out more clearly, as is the general agreement in results by different methods for the two varieties of tomatoes whether in the form of juice or paste. More than 90% of the pigment measured is in the lycopene fraction.

Figure 1 gives a comparison of the color indexes of seventeen commercial samples of tomato paste with reflectance values obtained by means of a recording Hardy spectrophotometer (*5*) and with values of yellow color obtained by use of the Hunter reflectometer (*5*). The evaluation of the color of tomato paste from the three reflectance curves by a person untrained in such evaluations is difficult. On the other hand, the location of a colorimeter reading on a graduated scale is a simple process in applying the color index method. In Figures 2 and 3, the color index values arranged in order of increasing magnitude are compared with lycopene values obtained by chemical analysis, and also with values obtained by visual ranking before storage. Inspection alone is not sufficient to determine precisely the extent of correlation of color indexes with chemical determinations, although correlation is evident in Table I and is shown more clearly in Figures 1, 2, and 3. For this reason, the significance of correlation was estimated from tables of significance of correlation coefficients (*13*). In most cases, as indicated in Table III, significant correlation was found between color indexes and the values found by the different chemical methods. The value of the correlation coefficients necessary for significance is 0.6. Although the number of values available for calculation of the correlation coefficients was not large, the correlation was sufficient to show agreement with the comparisons described in Table I and Figures 2 and 3, in most cases.

DISCUSSION

In all comparisons involving color values, attention is directed to the narrow range of color both in the commercial lots (ex-

Table III. Correlation Coefficients

(Correlation between color determined by color index and color evaluated by pigment analysis. To be significant, a correlation value of 0.6 must be reached)

	Lycopene			Total Carotene		
	Binary spectro-photo-metric	Chromatographic		Binary spectro-photo-metric	Chromatographic	
		Spectro-photo-metric	Colori-metric		Spectro-photo-metric	Colori-metric
Pigments assayed by chemical analysis						
Commercial lots	...	0.8	...	...	...	...
Laboratory lots						
Juice	0.8	0.9	0.7	0.8	0.8	0.9
Paste, before storage	0.7	0.9	0.6	0.6	0.3	0.4
Paste, after storage	0.9	0.8	0.9	0.9	0.8	0.7
Visual ranking of commercial paste, 0.7						

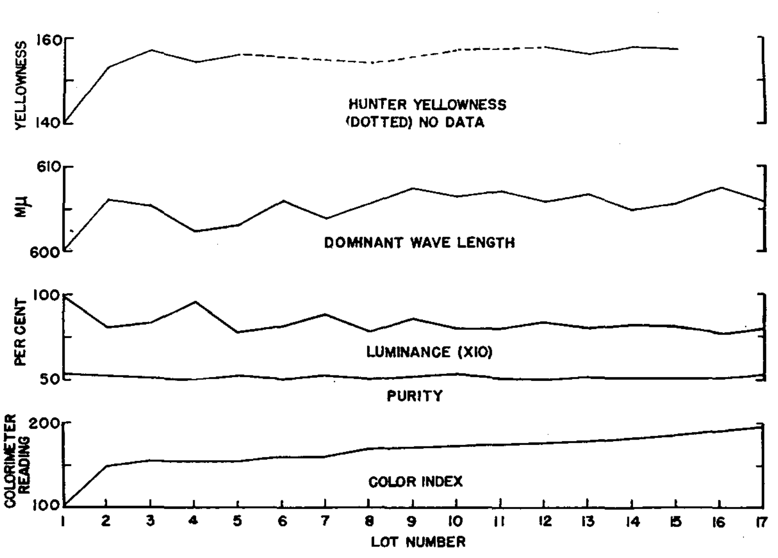

Figure 1. Commercial Tomato Paste Lots

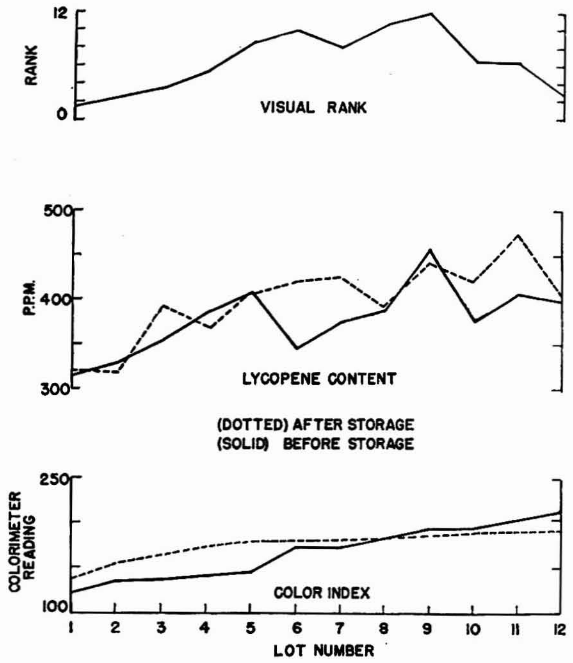

Figure 2. Color Index and Lycopene Content of Tomato Paste

25% total solids basis except visual

cluding one very low value) and lots prepared in the laboratory. Overlapping of color values due to variations in the method probably occurs and small variations due to unknown factors may have an exaggerated effect.

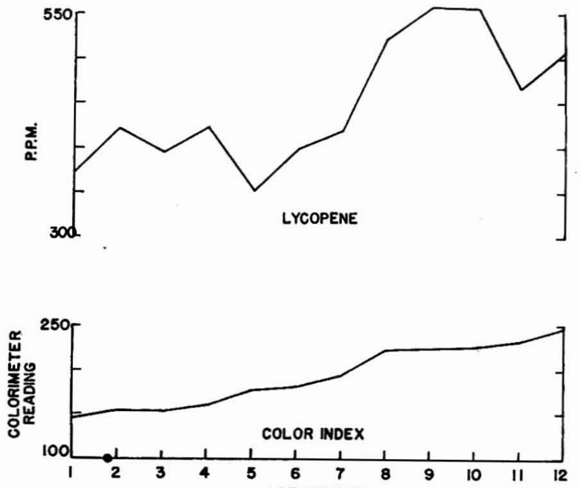

Figure 3. Color Index and Lycopene Content of Tomato Juice

25% total solids basis

One of the unknown factors is the change in isomers of lycopene and carotene. Neither the binary method (*17*), in the use of which 20% neo-lycopene A and 18% neo-β-carotene are assumed to be present, nor the chromatographic method gives opportunity for determining these isomers. Reversion of neo-lycopene A to lycopene, which causes a change of color from yellow-red to red, has been reported to occur (*16*). If this isomer were present in sufficient amount, the apparent gain in pigments of stored lots might be explained.

Variations in the quantity of xanthophylls and other oxygen derivatives of carotenes included in the extracts measured by the color index, but not in the extracts of the pigments determined chemically, may interfere with the correlation. However, spectrophotometric readings of hexane solutions of the extracted pigments can serve as color indexes for extracts from which xanthophyll and related substances soluble in methyl alcohol have been removed. The correlation coefficients calculated from these readings are but slightly different from those for which the acetone-extracted color was used as a basis for calculation. Therefore it seems unlikely that oxygen derivatives of the carotene seriously interfere with correlation.

Although the specification of color of fruits, vegetables, and their processed products has been developed for the most part from results obtained by reflectance measurements, significant preliminary studies of specification of color by measurements of color of the extracts have been made (*1, 2, 7, 8, 10*). The development of the photoelectric colorimeter (*4*) and low-priced spectrophotometers is accelerating the studies of light transmittance by extracts, but hitherto the extraction method itself has been neither convenient nor rapid. This difficulty has been overcome in the present method of extraction of tomato paste, a material difficult to extract completely.

In view of the difficulties which beset attempts to obtain color measurements of fruits, vegetables, and their products, it is pertinent to point out that the color index method described, although giving approximate values, is not significantly less accurate than existing chemical methods which are tedious and time-consuming; that it is much simpler in operation; and that the results may be simply expressed. It has been applied in a preliminary way to pepper powders (not included in this report) and gives promise of broad application to many kinds of food products including products from citrus fruits, avocados, and dates.

ACKNOWLEDGMENT

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RECEIVED April 17, 1949. Presented before the Division of Agricultural and Food Chemistry at the 115th Meeting of the AMERICAN CHEMICAL SOCIETY, San Francisco, Calif. The mention of special instruments throughout this paper does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned

Device and Technique for Rapid Determination of Effluent Fluorides

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A rapid field test for the determination of fluoride ion is described. Semiquantitative results have been obtained in tests with fluorine, hydrofluoric acid, and soluble inorganic fluorides. Preliminary work shows that any fluorine compound that can be broken down on paper wetted with various reagents and placed ahead of the test paper will give semiquantitative determinations of fluoride ion.

NUMEROUS tests have been run on various types of methods for the past 5 years in an effort to find a rapid field method for the determination of fluorides as an atmospheric contaminant, particularly as fluorine might be connected with welding. The standard analytical method of Willard and Winter (*6*) is used for laboratory analytical procedures, but is unsatisfactory as a field test, in part because of the time involved in the distillation to remove impurities. A modification of the alizarin sodium sulfonate and zirconium nitrate method was evolved which eliminates acetate interference, but this also was time-consuming. The range of greatest significance from the health standpoint involves concentrations from 1 to 10 p.p.m. and requires a more rapid analytical determination in the area where operations are being performed than can be obtained by any method that involves carrying solutions to the laboratory for analysis after spending from 15 minutes to 2 hours in collecting the air sample.

Some inorganic compounds of fluorine are gaseous and require an estimation of the concentration within a few minutes, so that safety and health requirements may be met.

Because methods involving the collection of a sample and the subsequent transport of the sample to a laboratory do not meet this time requirement, methods for collecting the sample in a liquid medium in the presence of reagents that would immediately indicate the relative amounts of fluoride were tried. While working on this approach, it was decided that the fastest determination would be through the use of an indicating paper. Semiquantitative to quantitative results may often be obtained by pumping known amounts of contaminated air through the chemically treated paper (*3*).

Experiments were conducted with papers treated with sodium alizarin sulfonate and zirconium chloride, but these were not as satisfactory even under ultraviolet light as papers treated with zirconium salts and azoarsenic acids. The paper finally selected was treated with *p*-dimethylaminoazobenzenearsonic acid and zirconium oxychloride (*4*). In dilute acid solution, an insoluble salt of zirconium azobenzenearsenate, brown in color, is deposited in the pores of the paper. The excess of *p*-dimethylaminoazobenzenearsonic acid is washed out with dilute acid. When fluorides are drawn through the prepared paper, a colorless (ZrF_6) ion and the free azobenzenearsonic acid, which is red, are formed. Thus, there is a distinct color change from the brown zirconium compound to the red of the free azo acid, and this has been made the basis for a semiquantitative field method for the determination of the fluorine ion.

In very small quantities, such are dealt with in determining concentrations injurious to health, fluorine itself is hydrolyzed to hydrofluoric acid and this is assisted by moistening the paper with hydrochloric acid. Soluble fluorides, such as sodium fluoride, react on the hydrochloric acid–moistened paper to give an immediate fluoride ion reaction. The insoluble inorganic fluorides, such as calcium fluoride, are retained by the moist filter paper and react to give the typical fluoride reaction, but not until a longer period of time has elapsed. The method is roughly quantitative in that values in the range from 6 to 10 p.p.m. can be determined within ±1 p.p.m. and from 10 to 40 p.p.m. with decreasing sensitivity as the concentration of fluoride ion increases. In concentrations below 6 p.p.m., the method becomes increasingly less sensitive to each stroke of the pump, and at 2 p.p.m. may be estimated within ±0.2 p.p.m. The accuracy at the given concentrations is largely determined by the number of strokes of the calibrated pump. These concentrations were chosen because they were the most important to health and safety.

PREPARATION OF CHEMICALLY TREATED PAPER

The treated paper is made as directed by Feigl and Rajmann (*4*), except that the paper is washed without the use of ether. Properly prepared, these papers are a light canary yellow when dry. Prior to insertion in the pump attachment head, they are wetted with 2 *N* hydrochloric acid. The color changes to a light brown. After exposure to the fluoride ion, a faint red-pink color appears which is taken as the end point of the test. Larger concentrations of the fluoride ion cause the exposed paper to turn bright red.

INTERFERENCES

Theoretically, those anions interfere which give insoluble or stable soluble complex compounds with zirconium. Organic hydroxy acids, arsenates, phosphates, sulfates, and thiosulfates are included in this group. However, under the conditions of the test in the ranges set up, sulfates and arsenates do not interfere in amounts below 50 p.p.m. Using the calibration procedure described below, and testing for fluoride ion in concentrations from 2 to 10 p.p.m., no deviation was noted where sulfate ion of 10 times the concentration of the fluoride ion was introduced into the test atmosphere. If sulfates are present in very high concentrations and no fluoride ion is present, there is a slight

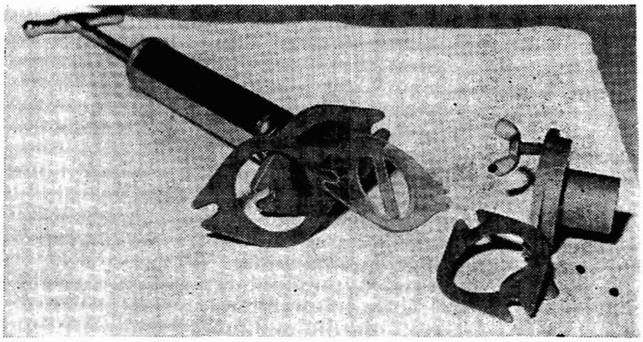

Figure 1. Preliminary Pump and Paper Holder

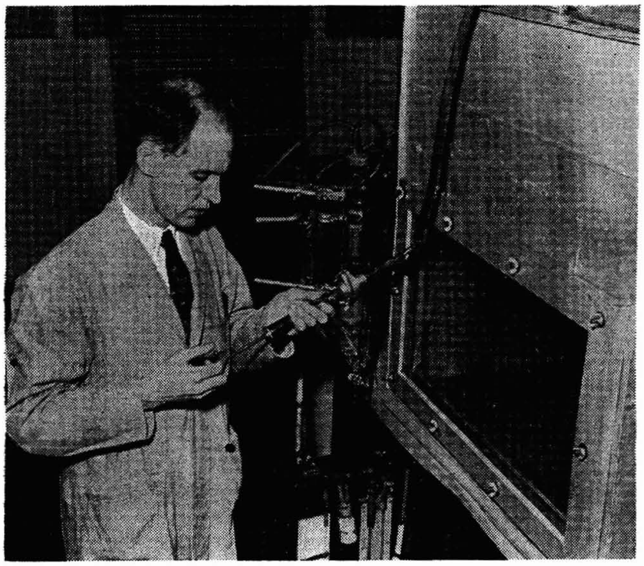

Figure 2. Calibrating Paper with Known Quantities of Hydrofluoric Acid

reaction, but the resultant color is a light salmon pink instead of a red pink. Under usual conditions, sulfates do not constitute a serious interference, but phosphates will interfere and will give approximately the same color effect as the fluoride ion when in higher concentrations than those fluoride concentrations of from 1 to 40 p.p.m. with which we are largely concerned.

PUMP

A pump of 50-ml. capacity was chosen, as this volume of air on preliminary crude calibration tests apparently would allow the greatest accuracy for the concentrations from 1 to 40 p.p.m. of fluoride ion which was the concentration of greatest interest from a health and safety viewpoint. A head to hold the test papers was built as shown in Figure 1. (Complete improved test set and calibrated test papers are manufactured by the Production Equipment Company, 6432 Cass Ave., Detroit, Mich.) A Plastacele insert holder 1.25 inches (3.1 cm.) in diameter with a 0.25-inch strip across the center provided an opening that permitted the air to flow through the paper at a rate that permitted standardization of the reaction of the fluoride ion with the test paper.

CALIBRATION

Some of the early attempts at calibration were unsuccessful but are briefly mentioned here because they are a logical and rapid means of obtaining an answer and, as such, have been used by a number of investigators in several universities in verifying the accuracy of the fluoride field test.

A 46.2-liter bottle was coated with a fine film of paraffin and volumetrically measured amounts of 48% hydrofluoric acid were introduced in a paraffin block placed on the bottom of the bottle. Air was circulated over the drop of hydrofluoric acid until it was diffused throughout the jar. A number of preliminary tests indicated that about 50 strokes of the pump were needed for 1.1 p.p.m., 17 strokes for 3.5 p.p.m., and 10 strokes for 5.6 p.p.m. No quantitative significance was attached to these results because of the inequalities of distribution and doubts as to the concentration of the concentrated hydrofluoric acid in the authors' possession.

In order to determine the fluorine more accurately, potassium fluoride dihydrate was weighed on a microbalance in amounts calculated to give values in the range from 0.5 to 10 p.p.m. in the 46.2-liter bottle employed. This fluoride salt was placed in the hollow of a paraffin block and a drop of concentrated sulfuric acid was dropped into the depression in the block after the bottle was closed. The evolved hydrogen fluoride was recirculated throughout the bottle by means of a rubber bulb. Two separate determinations at 3 p.p.m. resulted in a very light pink coloration at from 4.5 to 5 strokes of the pump in both instances. Later tests showed that distribution was not uniform, and that this method of calibration would not give accurate results. Part of the fluoride was rapidly removed on the wall surfaces and some formed a complex ion.

The following system (Figures 2 and 3) was then set up for the calibration of the treated paper:

To establish an atmosphere that would contain a definite value in parts per million of hydrofluoric acid, a dilute hydrofluoric acid solution was atomized into a 46.2-liter bottle at a constant rate. The air-acid mixture was drawn from this bottle into a 630-liter chamber with an electrically driven air pump calibrated at 0.85 cubic foot per minute. Two combination impinger and fritted-glass mist collectors were placed between the pump and large chamber with a set of T-tubes and pinchcocks. After pumping at a constant rate on one bubbler until the rate of flow and the parts per million of hydrofluoric acid became constant, the flow was switched to the second bubbler and timed with a stop watch. With the rate known in cubic feet per minute and with known time, the parts per million of hydrofluoric acid were established by titrating the acid solution in the second bubbler.

The titration was made with standard sodium hydroxide and potassium nitrate according to Scott (*5*). Without hydrofluoric acid, the system shows a blank of 0.5 p.p.m. A comparison of the method with the thorium nitrate–alizarin (*1*, *2*) titration shows a check within 0.5 p.p.m.

To establish a value in terms of the number of strokes on the hand pump, the sample was taken from a T-tube by-pass attached just above the bubbler intake. While the air was passing at a constant rate, sufficient sample was drawn to give the first perceptible pink on the standard paper. The number of strokes required to do this is established before the titration is made; thus, there is a definite relation between this number and the parts per million of hydrofluoric acid determined later.

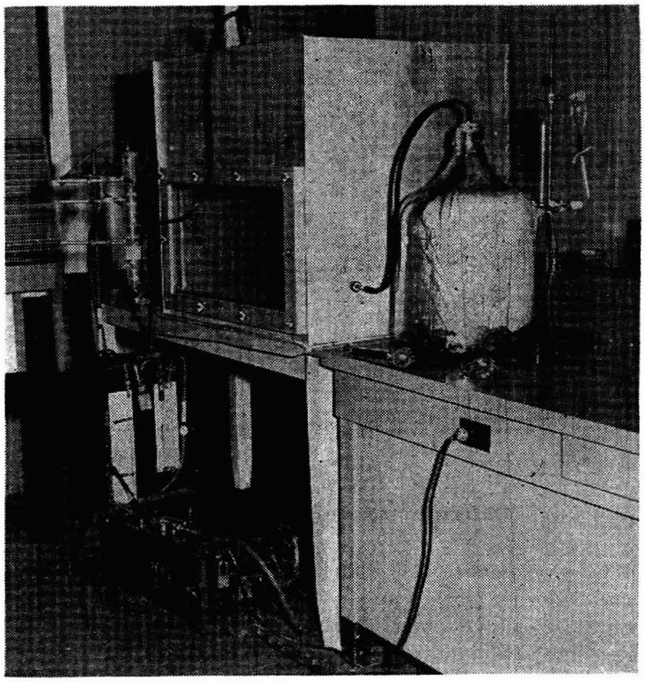

Figure 3. Calibration Apparatus

A series of 39 points was determined, some of which show wide distribution (Figure 4). However, it is known that a portion of this is due to failure to establish a constant flow before switching to the second bubbler. Care also must be taken to avoid interfering gases in the laboratory, such as ammonium hydroxide or hydrochloric acid. The extreme deviations found, including values with known errors, are shown in Figure 4.

For the calibration curve (Figure 5), all values in parts per million at a given number of strokes on the pump were averaged. Any values that varied 1 p.p.m. from this average were discarded and the average of the remaining factors was used to establish the final curve. The calibration curve represents

61.5% of all points determined. All discarded values were in the less sensitive part of the curve above 6 p.p.m.

PRECAUTIONS

In preliminary work, it was observed that uniformity of treatment of the paper employed is essential to good results. The authors found that a Whatman No. 41-H paper is most suitable for the test and the results specified were obtained when this paper was used. Uniformity of deposition of chemicals upon the paper is important, and it is essential to wash out completely the excess *p*-dimethylaminoazobenzenearsonic acid with great care before washing with alcohol and drying in vacuo. The paper was kept in a sealed vacuum chamber but there was, apparently, no great decomposition when it was kept in sealed brown bottles. Paper 3, 6, and 12 months old proved to be as sensitive as freshly made paper. Paper has been kept under carefully controlled conditions for 5 years with no detectable loss in sensitivity. Safety considerations, in actual field use, dictate replacement after 3 months to one year because treated paper exposed to light and oxidizing influences is not as sensitive as paper not so exposed. Exposed paper will give indications of concentrations of fluoride in the range of 6 p.p.m. but with a considerable loss in accuracy after one week's exposure. Such exposed papers are not dependable.

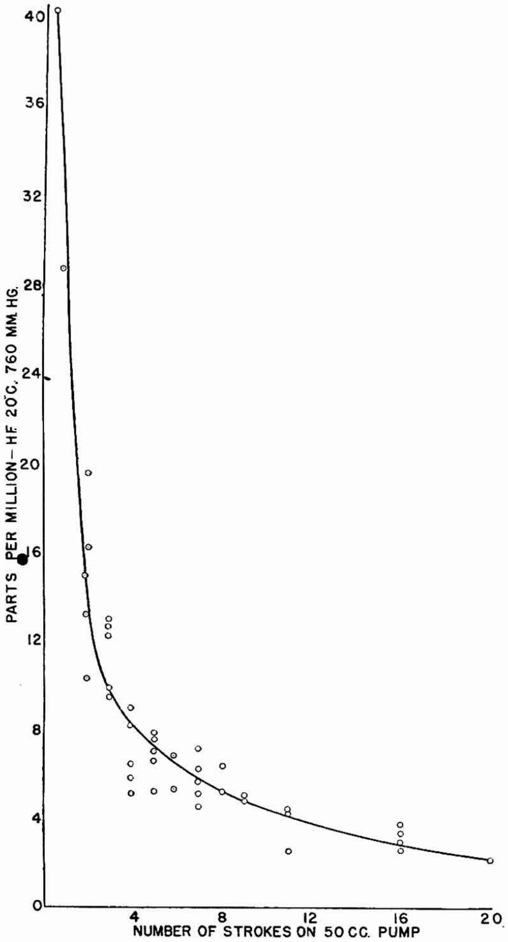

Figure 4. All Points Obtained in Determination of Hydrofluoric Acid

FIELD TEST PROCEDURE

Prepared zirconium azoarsenate paper is placed in the special fixture attached to a 50-cc. pump. The paper is dipped into 2 *N* hydrochloric acid immediately before being placed in the apparatus; in the field, the paper may be placed in the holder dry and then wetted with the acid added with a dropper just before use. Air is drawn slowly through the filter paper for a given number of strokes of the pump. It is recommended that a definite number of strokes of the pump be taken, so that quantities of fluoride that would be immediately hazardous may be determined quickly. For example, if after 3 strokes of the pump in which 150 cc. of contaminated air have been drawn through the paper a reaction of greater or less intensity has occurred, dangerous concentrations exist and the necessity for immediate evacuation is indicated. If, on removal from the apparatus, the paper shows two very faint lips of pink coloration against the brown background, it will indicate the concentration to be 11 p.p.m. The first appearance of pink on the brown background is taken as the end point of the reactions.

Should no pink appear, the same paper may be replaced and another five strokes of the pump taken. By taking these strokes in groups of five, it may be roughly determined after 18 strokes that no great danger exists, as the indicated concentration will be less than the 3 p.p.m. stated to be safe by the United States Public Health Service. After the first examination of three strokes, when it is determined that danger does not exist and that immediate evacuation of the area is not imperative, fresh papers should be used and about 17 strokes should be taken to see whether the suggested 3 p.p.m. value has been approached. Any method useful in a particular situation may be used with the calibration chart.

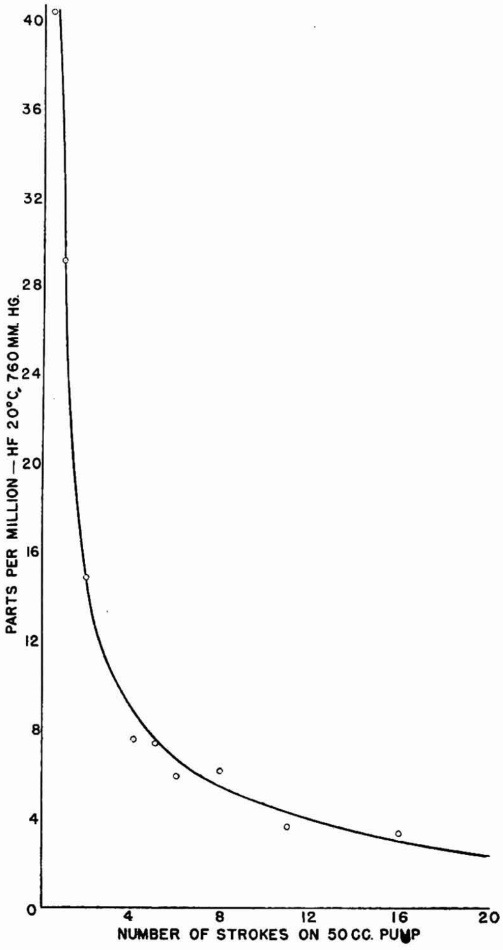

Figure 5. Average Calibration Curve

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Spectrophotometric Method for Determining Streptomycin

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A chemical method for determining streptomycin is presented. A distillation procedure is used to separate the maltol derived from the streptomycin. This method has been applied very successfully to fermentation broths, concentrates, and clinical samples.

THE analysis of streptomycin by chemical means has been the subject of several publications. Marshall, Blanchard, and Buhle (*4*) developed a colorimetric method for streptomycin based on the reaction of the carbonyl group in this compound with a highly colored semicarbazide. Boxer and Jelinek (*1*) suggested a fluorometric method based on the formation of a hydrazone of streptomycin with the fluorescent 9-hydrazinoacridine hydrochloride. The excess of the reagent and acidic or neutral hydrazones were extracted from acidified solution with benzyl alcohol. The remaining basic hydrazone from streptomycin was left in solution and its fluorescence was measured. Obviously, any basic substance capable of forming a hydrazone would interfere with this method.

Schenck and Spielman (*6*) observed that streptomycin when heated in dilute alkaline solution formed maltol, 2-methyl-3-hydroxy-γ-pyrone. This fact was used by Boxer, Jelinek, and Leghorn (*2*) as the basis of a fairly specific method for this antibiotic. For clinical preparations, the maltol was formed by heating an alkaline solution of streptomycin in a boiling water bath for 3 minutes. After the solution was cooled, an acidified solution of ferric ammonium sulfate or some phenol reagent (*3*) was added to develop a visible color from the maltol produced. Titus and Fried (*7*) in their countercurrent distribution studies on streptomycin merely used the ultraviolet absorption at 320 mμ of the alkaline solution after hydrolysis for their determinations.

Boxer and co-workers (*2*) extended their maltol method to determine streptomycin in broths and urine. In order to separate the maltol from possible interfering compounds and various pigments, a double extraction with chloroform from acid medium to remove the maltol was suggested. The maltol in the chloroform was then removed with dilute aqueous alkali and the color of the maltol in the water layer was developed with ferric ammonium sulfate reagent.

Although this procedure for assaying broths was found to be fairly satisfactory, a time-consuming difficulty with emulsions was frequently encountered. A better method for removing maltol from its reaction mixture prior to its actual determination is therefore desirable. This paper describes another method for removing maltol quickly from possible interferences.

REAGENTS AND APPARATUS

Sodium hydroxide, 5 *N* aqueous solution.

Sulfuric acid, 5 *N*.

Ferric chloride solution, 2 grams of ferric chloride hexahydrate dissolved in 100 ml. of 2 *N* hydrochloric acid. Phenol reagent, according to Folin and Ciocalteu (*3*). Sodium carbonate, 20% aqueous solution. Ammonium sulfate, reagent grade crystals.

The distillation apparatus is shown in Figure 1.

PROCEDURE

Pipet a suitable sized sample of streptomycin (not over 4 ml. in volume and containing between 50 and 2500 units of streptomycin) into a standard-taper glass-stoppered test tube. If the volume of solution taken is less than 4 ml., dilute the sample with water to this volume. Add to the solution 1 ml. of 5 *N* sodium hydroxide and then heat the tube and its contents in a boiling water bath for 3 minutes. Allow the solution to cool to room temperature and add 2 ml. of 5 *N* sulfuric acid and 1 gram of ammonium sulfate. Distill the resulting solution (an apparatus similar to that shown in Figure 1 is very convenient) and collect a total of 10.0 ml. of distillate.

Robert P. Popino of the Heyden Laboratories has modified the distillation apparatus shown in Figure 1 to make it more convenient for routine determinations. In his modification, a bubble counter is substituted for the steam generating flask. In order to achieve the same effect as the continuous flow of steam, a slow stream of air from a compressed air line is passed through the apparatus during the distillation. After the reaction mixture has been distilled to near dryness, about 5 ml. of water are added to the test tube and the distillation is then continued until 10.0 ml. of distillate are collected. This modified apparatus and procedure have been used continuously on a routine basis with very satisfactory results.

If the sample contains over 500 units of streptomycin, add 1.0 ml. of the ferric chloride solution to the distillate. Measure at 550 mμ the color produced versus 1.0 ml. of the same reagent added to 10.0 ml. of distilled water.

If the sample contains less than 500 units, add 1.0 ml. of the phenol reagent to the distillate. Allow this solution to stand 1 to 2 minutes and then add 3.0 ml. of 20% sodium carbonate solution. Measure the optical density of this solution at 775 mμ versus a reagent blank.

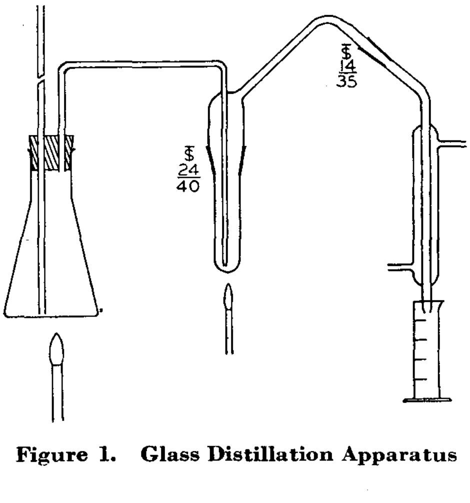

Figure 1. Glass Distillation Apparatus

From calibration curves prepared from known amounts of streptomycin and with both reagents, the number of units of streptomycin in the sample is readily deduced.

DISCUSSION AND RESULTS

All spectrophotometric measurements described in this paper were made with a Beckman DU quartz spectrophotometer. A slit width of 0.040 mm. was used throughout.

Table I. Analyses of Finished Samples

Boxer's Procedure			Distillation Procedure		
Aliquot taken, ml.	Streptomycin found, units	Average, units/ml.	Aliquot taken, ml.	Streptomycin found, units	Average, units/ml.
0.5 1.0	1260 2800	2660	2.0 2.0	5660 5670	2835
0.5 1.0	1200 2620	2510	2.0 2.0	5250 5300	2637
0.5 1.0	940 2010	1945	2.0 2.0	3940 3940	1970
0.5 1.0	935 2110	1990	2.0	4180	2090
0.5	892	1784	2.0	3940	1970

Table II. Analyses of Impure Solutions

Sample No.	Aliquot Taken, Ml.	Streptomycin Found, Units/Ml.	Biological Assay, Units/Ml.
1	4.0 4.0	355 358	346[a]
2	4.0 4.0 0.5[b]	349 346 350	240[a]
3	4.0 4.0	346 349	320[a]
4	4.0	390	375, 363 250, 433 Av. 355
5	4.0	296	295, 295 275, 275 Av. 285
6	4.0	253	245, 245 250, 275 Av. 254

[a] Single biological assays.

[b] Phenol reagent used in this determination. Ferric chloride reagent used in all other determinations.

Because it was known that maltol sublimes readily, it seemed reasonable to assume that this compound should distill with steam. About 5000 units of streptomycin in 4 ml. of water were treated according to the recommended procedure, except that four 3-ml. portions of distillate were collected. Inasmuch as over 94% of the maltol was found in the first three portions of distillate, it was decided to standardize on 10.0 ml. of distillate in the actual procedure.

The ferric chloride reagent combined with the distillation procedure was used to develop the color produced from various amounts of pure streptomycin varying from 500 to 10,000 units. A linear calibration line that intersected the origin when extrapolated to zero units was obtained. Another calibration line, using the phenol reagent, was run with amounts of streptomycin varying from 50 to 500 units in the 4.0 ml. of sample. Again, a linear line was obtained. Thus, it would appear that the distillation procedure must remove all the maltol formed.

Ammonium sulfate is added to increase the boiling point of the solution being distilled. The optimum effect is produced by the addition of 1 gram of this salt. It is important to add the ammonium sulfate after the sulfuric acid has been added. On several occasions when the salt was added before the solution was acidified, the recovery of maltol was low.

It is important to distill the acidified solution almost to dryness at least once before the 10.0 ml. of distillate are collected. This can be done easily by regulating the two burners shown in Figure 1 so that the distillation from the reaction mixture proceeds much faster than that in the steam-generating flask. A continuous flow of steam through the test tube prevents the reaction mixture from bumping during the distillation and also helps to sweep the volatile maltol into the distillate.

Several determinations were made to see if the color produced by the ferric chloride reagent and the distilled maltol was stable. Solutions exhibiting widely different amounts of color were found to have exactly the same optical densities after 12 hours of standing as they had 10 minutes after the reagents were mixed. Therefore, it is not necessary to read the solutions immediately after adding the reagent.

The results of some determinations which were run on finished samples are shown in Table I. Vials containing about 1,000,000 units of streptomycin were diluted to 500 ml. with water. Various sized aliquots of these solutions were analyzed by the procedure of Boxer *et al.* (*2*) and by the distillation procedure. It is obvious from the results that the simple maltol procedure, involving no separation of the maltol from the reaction mixture, does not give the same results for different sized samples. However, the agreement between the two procedures is remarkably good if the results of the larger aliquots are used for comparison.

The results obtained with several impure solutions which were analyzed by the maltol distillation method and by a biological assay method are shown in Table II. These solutions differed widely in composition and were believed to contain relatively small amounts of mannosidostreptomycin. With one exception, the values from the chemical procedure agree to within less than 10% with those from the biological method. The slightly higher values from the chemical method can be explained either by the presence of a small amount of mannosidostreptomycin or by inaccuracies of the biological method.

One limitation of all chemical methods so far reported for determining streptomycin is that they will not distinguish between streptomycin and mannosidostreptomycin. Although this latter compound is only 15 to 20% as biologically active as pure streptomycin against the usual assay organisms, it will produce 82% as much maltol on a weight basis. Therefore, the chemical and biological assays on broths containing appreciable amounts of the less active streptomycin will not agree. Schenck, Shaw, and Hargie (*5*) made use of this difference between the chemical and biological assays of broths to determine the amounts of the two streptomycins present.

A calibration curve by the distillation procedure was run with various amounts of some pure streptomycin trihydrochloride. The slope of this line corresponded to an optical density of 0.111 per 1000 units of pure streptomycin. When samples of the master standard of streptomycin sulfate from the Food and Drug Administration were used, an average optical density of 0.113 per 1000 units was obtained. These results demonstrate the reproducibility of the distillation procedure.

Several broths of comparatively low activity were analyzed by the recommended procedure, using both the ferric chloride and phenol reagents. The results with these two reagents agreed to within 3% in all cases. This would indicate that no harmful interferences, even for the sensitive phenol reagent, are distilled from acidified broth samples. Consequently, if the phenol reagent is used, samples having an activity as low as 10 units of streptomycin per ml. can be analyzed by the recommended procedure.

The time required for each analysis, including all the pipetting, is probably about 30 minutes. The distillation operation, which takes less than 10 minutes, is considerably shorter than that required by any other suggested method of separating maltol from broth samples.

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Use of Glycolic Acid Derivatives in Determination of Zirconium

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The zirconium-precipitating action of mandelic acid is also shown by other glycolic acid derivatives. By use of appropriate derivatives, larger volumes of precipitate per unit weight of zirconium can be obtained than with mandelic acid. *p*-Chloromandelic acid and *p*-bromomandelic acid were successfully applied to the determination of zirconium in commercial zirconyl chloride, an iron-zirconium-aluminum alloy, and a silicate ore. Sulfuric acid up to 5% by weight does not prevent quantitative precipitation. The possibility of determining zirconium by direct weighing of the mandelate precipitates was investigated. The preferable reagent for the determination of zirconium by the mandelate method was found to be *p*-bromomandelic acid, then *p*-chloromandelic acid, and finally mandelic acid.

THE methods available for the gravimetric determination of zirconium are not satisfactory. They employ reagents which are lacking in selectivity or involve difficult and tedious procedures (*10*).

The discovery by Kumins (*10*) that mandelic acid precipitates zirconium quantitatively from strongly acidified solutions which may also contain titanium, aluminum, iron, vanadium, chromium, tin, bismuth, antimony, cerium, calcium, thorium, copper, cadmium, and barium suggests that the glycolic acid group (—CHOH—COOH) may be specific for zirconium. Consequently, a number of glycolic acid derivatives were prepared and their reaction with zirconium was studied. Because the effect of various substituting groups on the nature of the precipitate produced with zirconium (weighting effect) is of great interest, these reactions were carried out in graduated cylinders under identical experimental conditions. The procedure was that described below for the quantitative tests, except that half the quantities given were used here. An approximate idea of the weighting effect of the various groups was thus obtained (Table I).

A notable increase in volume of precipitate per unit weight of zirconium is obtained, especially in the aromatic series of derivatives. A number of the latter were tested for possible quantitative application.

Five milliliters of a standard solution of zirconyl chloride containing the equivalent of 0.1 gram of zirconium oxide are diluted with 15 ml. of concentrated hydrochloric acid and 30 ml. of water. The manner of addition of reagent is varied according to the precipitant used. For mandelic acid, 50 ml. of a 1.0 *M* solution of this acid are added to the cold zirconium solution and the mixture is then heated to 85° C. For the other water-soluble reagents, 50 ml. of a 0.1 *M* solution of reagent are added slowly from a buret to the hot solution. Water-insoluble reagents are added as 0.1 *M* solutions of the sodium salt. The precipitate which forms is digested at 85° C. for 15 to 20 minutes and then filtered in a Selas crucible. The mandelic acid precipitate is washed with a hot solution containing 2% hydrochloric acid and 5% mandelic acid. The other precipitates are washed with distilled water. The precipitates are then dried, ignited to the dioxide, and weighed.

The results (Table II) indicate that any of these derivatives of glycolic acid might be suitable as a zirconium precipitant. Benzilic acid, 2-naphthylglycolic acid, and *p*-iodomandelic acid exhibit large weighting effects but are not water-soluble. The use of water-insoluble reagents somewhat complicates the analytical procedure and was considered undesirable. *p*-Methyl-

Table I. Weighting Effect of Glycolic Acid Derivatives on Precipitation of Zirconium

Reagent	Formula	Reference to Synthesis	Test	Color of Ppt.	Volume of Ppt. in Cylinder, Ml. (Weighting Effect) 4 hours	2 days
Mandelic acid	C_6H_5—CHOH—COOH	...	Pos.	White	1	1
p-Methylmandelic acid	C_6H_4Me—CHOH—COOH	(*1*)	Pos.	White	2	2
p-Nitromandelic acid	$C_6H_4(NO_2)$—CHOH—COOH	(*3, 6*)	Pos.	Yellow	3	2
p-Bromomandelic acid	C_6H_4Br—CHOH—COOH	(*1*)	Pos.	White	3	3
p-Chloromandelic acid	C_6H_4Cl—CHOH—COOH	(*1*)	Pos.	White	5	4
p-Iodomandelic acid	C_6H_4I—CHOH—COOH	(*12*)	Pos.	White	12	6
α-Hydroxydecanoic acid	C_8H_{17}—CHOH—COOH	(*8*)	Pos.	White	11	7
Benzilic acid	$(C_6H_5)_2$—COH—COOH	(*11*)	Pos.	White	25	23
2-Naphthylglycolic acid	$C_{10}H_9$—CHOH—COOH	(*12*)	Pos.	White	30	29
o-Nitromandelic acid	$C_6H_4(NO_2)$—CHOH—COOH	(*4*)	Neg.[a]	...	...	...
m-Nitromandelic acid	$C_6H_4(NO_2)$—CHOH—COOH	(*5*)	Neg.[a]	...	...	...
Lactic acid	CH_3—CHOH—COOH	...	Neg.[a]	...	...	...
Glycolic acid	CH_2OH—COOH	...	Neg.[a]	...	...	...

[a] Precipitates are obtained under conditions of lower acid concentration and greater zirconium concentration.

Table II. Quantitative Precipitation Zirconium by Glycolic Acid Derivatives

Reagent	Weighting Effect (from Table I)	Zirconium Oxide Present	Zirconium Oxide Found	Difference	Deviation
		Gram	*Gram*	*Gram*	*%*
2-Naphthylglycolic acid (sodium salt)	29	0.0782	0.0771	−0.0011	−1.4
			0.0791	0.0009	1.1
Benzilic acid (sodium salt)	23	0.0782	0.0767	−0.0015	−1.9
			0.0710	−0.0072	−9.2
p-Iodomandelic acid (sodium salt)	6	0.0782	0.0787	0.0005	0.6
p-Chloromandelic acid	4	0.0782	0.0785	0.0003	0.4
			0.0769	−0.0013	−1.7
p-Bromomandelic acid	3	0.0782	0.0787	0.0005	0.6
			0.0784	0.0002	0.3
			0.0788	0.0006	0.8
p-Methylmandelic acid	2	0.0782	0.0786	0.0004	0.5
			0.0764	−0.0018	−2.3
Mandelic acid	1	0.0782	0.0783	0.0001	0.1
			0.0781	−0.0001	−0.1
			0.0782	0.0	0.0

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Table III. Determination of Zirconium by Various Methods

Reagent	Zirconium Oxide
	Gram
Cupferron	0.0782
Ammonia	0.0784
Mandelic acid	0.0782
p-Chloromandelic acid	0.0782
p-Bromomandelic acid	0.0786

mandelic acid offers no appreciable advantage over mandelic acid. Consequently, *p*-chloromandelic acid and *p*-bromomandelic acid were selected for further testing. The results, using the procedure given above, showed satisfactory agreement with determinations by the cupferron and ammonia methods (Table III).

To determine whether or not any loss in selectivity occurs through the use of the halomandelic acids, the zirconium in a standard zirconyl chloride solution was determined in the presence of the cosolutes listed by Kumins. These findings (*9*) confirm the results obtained by Kumins with mandelic acid, and also show that *p*-chloromandelic acid and *p*-bromomandelic acid retain the selectivity exhibited by the parent compound.

As with mandelic acid, it was found that up to 5% free sulfuric acid by weight may be present without preventing quantitative precipitation of zirconium by *p*-chloro- and *p*-bromomandelic acids.

Table IV. Analysis of Zirconium-Bearing Materials

Reagent	Zirconium Present	Zirconium Found	Difference
	Gram	*Gram*	*Gram*
Zirconyl chloride			
Mandelic acid	0.0611[a]	0.0612	0.0001
p-Chloromandelic acid	0.0611	0.0609	−0.0002
p-Bromomandelic acid	0.0611	0.0616	0.0005
	%	%	%
Fe-Zr-Al Alloy			
Mandelic acid	46.44[a]	47.59	1.15
p-Chloromandelic acid	46.44	46.67	0.23
p-Bromomandelic acid	46.44	47.06	0.62
Zirconium silicate			
Mandelic acid	64.68[a]	64.14	−0.54
p-Chloromandelic acid	64.68	64.55	−0.13
p-Bromomandelic acid	64.68	64.66	−0.02

[a] Cupferron determinations.

APPLICATION TO COMMERCIAL PRODUCTS

Samples of commercial zirconyl chloride, an iron-zirconium-aluminum alloy, and a siliceous zirconium silicate were analyzed by use of the three mandelic acid reagents.

The zirconyl chloride was analyzed by dissolving a known weight in water and diluting to 500 ml. Aliquots of this solution were then analyzed by the procedure previously described.

The alloy sample was treated as follows:

Approximately 0.15 gram of the powdered alloy is placed in a 150-ml. beaker and treated with 5 ml. of water, 2 ml. of concentrated sulfuric acid, and 3 drops of hydrofluoric acid (more, if necessary to effect solution). The beaker is kept covered with a watch glass. Toward the end of the reaction 1 ml. of nitric acid is added. The solution is evaporated to dryness on a sand bath. The residue is dissolved in 50 ml. of water containing 1.0 ml. of concentrated hydrochloric acid. Then 50 ml. of the particular reagents are added as previously described. A white precipitate readily develops. This is digested at 85° C. for 10 to 20 minutes, cooled, filtered through porcelain filtering crucibles, dried, and ignited.

The zirconium in the siliceous ore was determined as recommended by Kumins, using a borax fusion followed by solution of the melt in 1 to 5 hydrochloric acid (*7*). Direct treatment of this solution with the halomandelic acid reagents resulted in considerable interference from the silica present. When the silica was removed by making the solution alkaline, decanting the sodium silicate solution, and dissolving the residual zirconyl hydroxide in acid, satisfactory results were obtained. This is not a serious interference, as in a complete analysis the silica would be previously removed as part of the regular procedure.

The results obtained by the above procedures are compared with those using the cupferron method in Table IV.

Table V. Comparison Weights of Various Zirconium Compounds

Compound	Molecular Weight	Ratio to M.W. of ZrO_2
Zirconium oxide	123	1
Zirconium tetramandelate	6[illegible]6	5.7
Zirconium tetra-*p*-chloromandelate	834	6.8
Zirconium tetra-*p*-bromomandelate	1011	8.2

POSSIBILITY OF DIRECT WEIGHING

The investigations of Kumins indicate that the precipitate produced by zirconium and mandelic acid is zirconium tetramandelate: $Zr(—OOC—HOHC—C_6H_5)_4$. The possible inner-complex nature of this compound has been discussed by Feigl (*2*). The work on the halomandelates indicates that they are similar in nature of the zirconium mandelate. Consequently, the possibility of determining zirconium by "direct weighing" of these mandelate precipitates is worth considering, from the standpoint of both the saving in time effected in not having to conduct the ignition to zirconium dioxide and the increased weight of the molecule per unit weight of zirconium (Table V).

The possibility of direct weighing with mandelic acid is slight because a high concentration of reagent is used for the precipitation and the excess reagent cannot be effectively removed, especially in view of the significant solubility of zirconium mandelate in water. The zirconium salts of the halomandelic acids can be washed with water without loss of zirconium. Accordingly, a known weight of zirconium was precipitated by the procedure previously described, and the precipitate obtained was dried at 130° C., weighed, and ignited to the oxide, and the ignition residue was weighed. Inasmuch as favorable results were obtained with pure zirconyl chloride solutions, the same procedure was followed in samples containing added cosolutes. The findings also were favorable (Table VI).

When attempts were made to apply the direct weighing technique to alloy and ore samples, the results were erratic and unreliable, even though the ignited results were correct. It is

Table VI. Direct Weighing *vs.* Ignition

Reagent	Direct Weighing	Ignition	Deviation
	Gram of ZrO_2		
Pure zirconyl chloride[a]			
Mandelic acid	0.0769	0.0764	−0.0005
	0.0784	0.0760	−0.0024
	0.0787	0.0762	−0.0025
p-Chloromandelic acid	0.0774	0.0768	−0.0006
	0.0764	0.0766	0.0002
	0.0776	0.0773	0.0003
	0.0767	0.0772	0.0005
p-Bromomandelic acid	0.0778	0.0779	0.0001
	0.0769	0.0765	−0.0004
	0.0774	0.0774	0.0000
Zirconyl chloride[a] with added cations			
Mandelic acid[b]	0.0780	0.0775	−0.0005
p-Chloromandelic acid[c]	0.0770	0.0774	0.0004
p-Bromomandelic acid[d]	0.0773	0.0775	0.0002

[a] Contained 0.0775 gram of zirconium dioxide.
[b] Average of 13 determinations.
[c] Average of 20 determinations.
[d] Average of 15 determinations.

planned to study the direct weighing method further in an effort to determine the factors which militate against its use in alloy and ore samples.

ACTION WITH ALKALI

Kumins found that zirconium mandelate dissolves in sodium hydroxide with the production of zirconyl hydroxide, and in ammonium hydroxide without the production of a precipitate. The halomandelates show this same action. Feigl (*2*) has pointed out the unusual nature of the reaction of ammonia with zirconium mandelate. This interesting masking action will be studied further from the standpoint both of possible analytical applications and of the isolation of a new class of inner-complex compounds.

ACKNOWLEDGMENT

The authors wish to thank the Titanium Alloy Division of the National Lead Company for furnishing the zirconium samples used in this research.

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Self-Absorption Curves of C^{14}-Labeled Barium Carbonate, Glucose, and Fatty Acids

Influence of Physical Form of Sample

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Comparative self-absorption curves of C^{14}-labeled barium carbonate, glucose, and fatty acids are given. It is shown that these curves are dependent on the technique employed in the sample preparation. In counting solid samples the physical form and density should be comparable in order to obtain reproducible results.

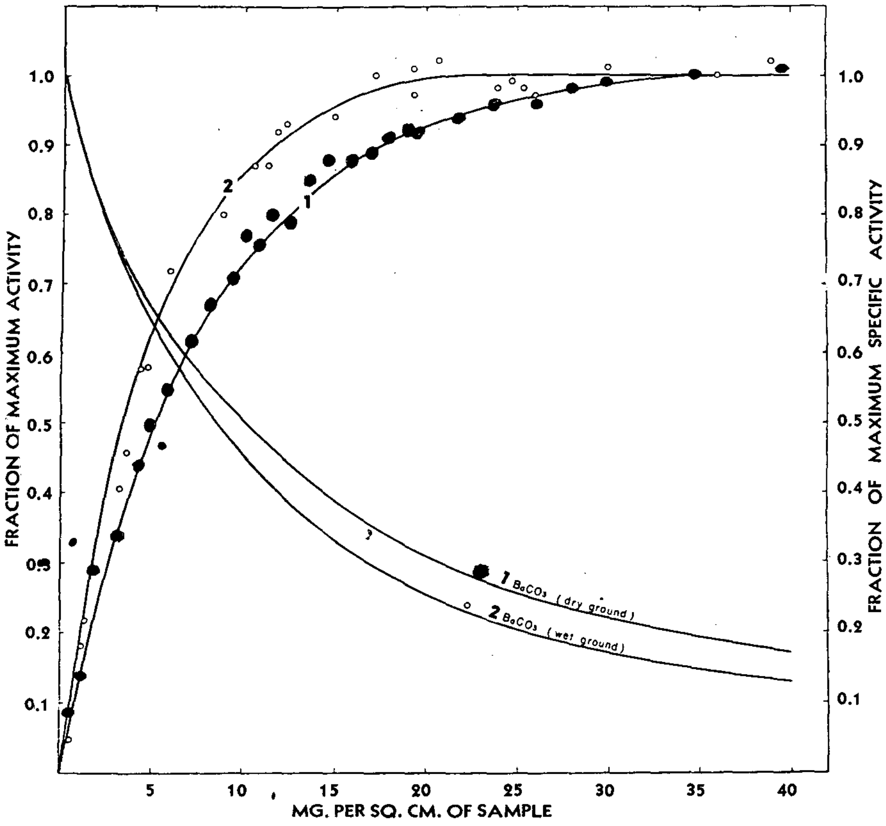

Figure 1. Relative Absorption Curves for Dry- and Wet-Ground $BaC^{14}O_3$
1. Barium carbonate, dry ground. 2. Barium carbonate, wet ground

SELF-absorption is of considerable importance in the measurement of C^{14} in solid samples. In biological experiments where only relative activity is desired and if sufficient material is available, it is possible to minimize the self-absorption factor by counting a sample of material so thick that the radiation emitted from the bottom layer of the sample cannot penetrate to the counter. It has been frequently stated that the only requirement for the measurement of thick samples is that the material be well mixed and the top of the precipitate be flat and smooth. When an insufficient amount of material is available for the so-called "saturation thickness" measurement, a calibration curve is used for obtaining comparative results by calculating to infinite thickness, zero thickness, or a reference density. Absorption curves for $BaC^{14}O_3$ have been reported by Libby (*3*) and others (*1*, *4*, *6*). Except for the work of Yankwich and Weigl (*7*), who compared the relationship of back-scattering to self-absorption of samples of $BaC^{14}O_3$ and C^{14}-labeled *p*-phenylphenacyl acetate dissolved in wax and mounted on aluminum, data are lacking for other substances. Direct counting of various compounds is often desirable in dealing with low count samples.

In this report the importance of technique in the preparation of samples using C^{14}-labeled barium carbonate, glucose, and fatty acids is shown.

The glucose was obtained by plant leaf synthesis and the fatty acids were isolated from rats that had received radioactive glucose. The counting was carried out with a mica end window counter (Tracerlab TG C-2) of 1.86 mg. per sq. cm. The sample mounts were made from 0.032-inch gage sheet aluminum. The counting area, 3.78 sq. cm. in a 0.3-mm. depression, was 3 mm. from the counter window. The C^{14}-labeled barium carbonate, glucose, and fatty acid samples gave 5000, 5000, and 1000 total counts per minute, respectively, at infinite thickness. Approximately 20 planchets were used for the fatty acid calibration curve. For each of the barium carbonate and glucose curves approximately 50 samples of different densities were prepared.

The data obtained with these samples are shown in Figures 1 and 2, in which the fraction of maximum activity and the fraction of maximum specific activity are plotted against sample thickness.

The technique used in preparing the samples from which $BaC^{14}O_3$ (No. 2, wet ground) and radioglucose (No. 2, crystalline) were obtained is the same as that employed by Dauben, Reid, and Yankwich (*2*). The carbonate (or glucose) was ground in an agate mortar under 95% ethanol. After a few minutes' grinding, the slurry formed was allowed to stand for a few seconds to permit the coarse particles to settle out and the suspension of fine particles was added to the planchets. The samples were dried under an infrared lamp. The result for the $BaC^{14}O_3$ curve (Figure 1) prepared under these conditions is essentially that reported by Yankwich (*5*) and Armstrong (*1*). The absorption curve for radioglucose prepared under identical conditions gave a somewhat similar curve, although the point of "saturation thickness" was attained at a slightly greater density.

Of particular interest were the results obtained by preparing the radioglucose and carbonate planchets under different conditions.

Glucose curve 1 (amorphous) was obtained by dissolving the radioglucose in water and drying in an oven at low temperature followed by a 1-week drying time in a desiccator over fresh Drierite. This procedure, which gave amorphous glucose samples with a glossy finish, had the effect of a general lowering of the entire curve and a shifting of the point of infinite thickness from 20 to 25 mg. per sq. cm. The $BaC^{14}O_3$ curve 1 (dry ground and same material as used in 2) was made by grinding the dry powder in an agate mortar and placing the ground material on the planchets. Ethanol (95%) was added to make a slurry of the coarse and fine particles which was spread out uniformly with a stirring rod. After settling, the material was dried with the infrared lamp as before. The samples prepared by this method appeared uniform and similar to those made for curve 2, although a trained observer could detect a difference in the smoothness of the samples prepared by the two techniques. It is seen in Figure 1 that this procedure gave an absorption curve of considerable variance from curve 2 with the point of infinite thickness at approximately 35 mg. per sq. cm.

The above determinations for $BaC^{14}O_3$ were repeated using copper planchets of the same design. The self-absorption curves resulting from the two methods of sample preparation were, for all practical purposes, the same as those obtained with the aluminum background.

The solid C^{14}-labeled fatty acids were spread on the planchets and softened with an infrared lamp to give a smooth top surface upon cooling. Under the same condition of counting, the fatty acid samples gave a curve similar to the wet-ground $BaC^{14}O_3$.

The most reasonable explanation of the variable results obtained with the C^{14}-labeled barium carbonate and glucose samples prepared under different conditions of preparation is the effect of packing due to physical form and density of the solid substance. This appears to be especially pertinent, because the self-absorption curves of C^{14} radiation are functions, among other things, of the scattering and absorbing powers of the sample. Extreme care must be exercised in obtaining uniform sample preparation, not only when dealing with the thin samples but also when dealing with samples in the range of infinite thickness.

In view of the fact that two distinctly different correction curves can be obtained for either C^{14}-labeled barium carbonate or glucose by an alteration of the sample preparation technique, it is not surprising that some of the points in Figures

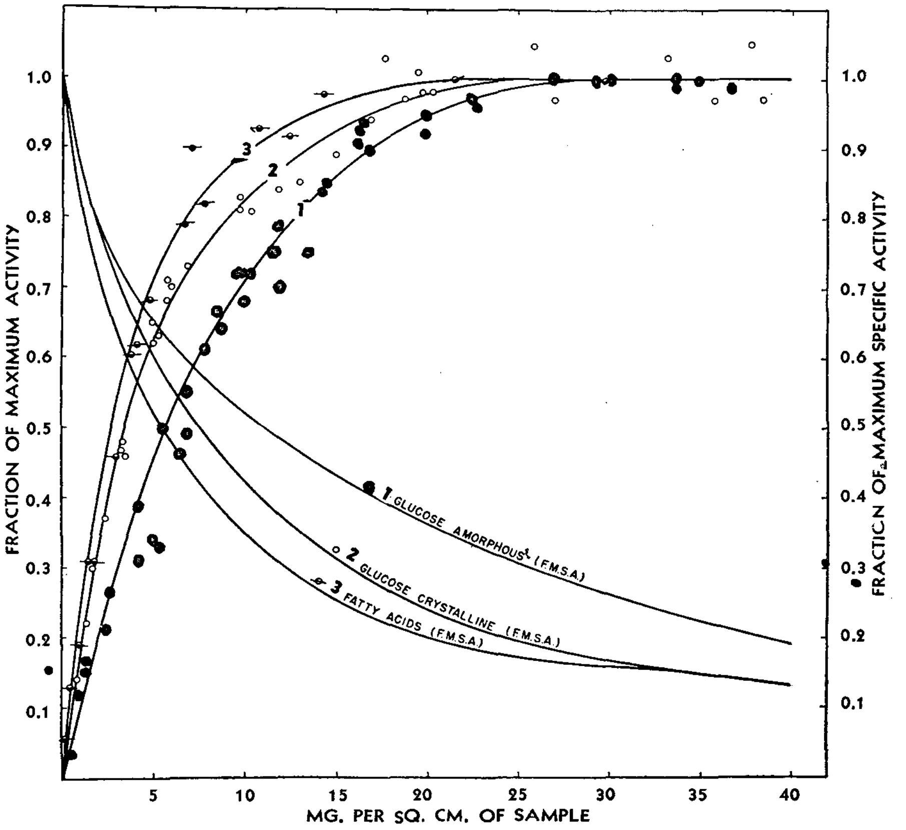

Figure 2. Relative Absorption Curves for C^{14}-Labeled Glucose (Amorphous), Glucose (Crystalline), and Fatty Acids

1. Glucose, amorphous. 2. Glucose, crystalline. 3. Fatty acids

1 and 2 deviate considerably from the designated curve. That this deviation of individual samples from the curve is a characteristic of the sample and not due to counting error was established by recounting, which always gave the same results.

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Determination of Aluminum Nitride Nitrogen in Steel

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A method for determining aluminum nitride nitrogen in steel utilizes solutions of the halogens in an anhydrous aliphatic ester to dissolve the iron matrix without decomposing the aluminum nitride in the steel. The residue containing the aluminum nitride is recovered from the ester-halogen solution and reaction products by filtration. The nitrogen content of the recovered aluminum nitride is obtained by the method for determining nitrogen in steel described by the author in an earlier paper. The ester-halogen method requires inexpensive apparatus, is simple to use, and yields highly reproducible results. It is not subject to interference from compounds of the elements commonly used in steel. The procedure is economical enough for routine use and sufficiently accurate and precise for research investigations. It has been used successfully for a large number of analyses in establishing the changes in the aluminum nitride content of steel caused by different thermal treatments and fabricating operations and in investigating deoxidation and steelmaking processes.

NITROGEN and its compounds exercise a significant influence on the properties of steel and on its response to thermal treatment. Development of analytical methods which enable the total amount of nitrogen or the amount of a given nitrogen compound to be determined with a minimum of time and labor is a prerequisite to most efficient research upon the influence of nitrogen in steel.

Much interest in austenitic grain size of steel was aroused when McQuaid and Ehn (*27*) published an account of their work on the relation of grain size and heat treatment in 1922. Since then, the use of aluminum in steel making for modifying such characteristics as grain size, strain sensitivity, and aging has become an accepted commercial practice. The role of aluminum in controlling these characteristics has been investigated extensively (*2*, *4–17*, *19–25*, *30*, *31*) and it has been postulated that they are governed by the presence of free aluminum, aluminum oxide, and aluminum nitride. Considerable indirect evidence has been accumulated regarding each of these hypotheses, but direct experimental data are meager (*18*). Recent investigations support the premise that aluminum nitride is perhaps the most important factor in controlling the properties of aluminum-killed steel. Despite the well recognized need for a method of determining this compound quantitatively, a satisfactory procedure has been lacking. The fractional vacuum fusion method, investigated for this purpose, involves the assumptions that only aluminum nitride nitrogen will be liberated in the high temperature fractions and that the steel will lose none of its aluminum nitride nitrogen at lower temperatures. Results so obtained may be misleading.

The writer has described a simple, economical method for determining combined nitrogen in steel (*3*). The present paper describes convenient methods and reagents for determining its aluminum nitride nitrogen content quantitatively. The aluminum nitride is separated from the steel matrix and its nitrogen content is determined by the usual procedure involving steam distillation in micro-Kjeldahl apparatus and the Nessler color reaction. The reagents used for separating aluminum nitride from steel are solutions of halogens in anhydrous aliphatic esters of organic acids; bromine dissolved in methyl acetate has been used most extensively.

The ester-halogen method is useful for separating certain of the nonmetallic compounds such as aluminum nitride from steel in a form that enables them to be analyzed by microchemical or spectroscopic methods and identified by x-ray diffraction techniques. The reagents and procedure are suitable both for research on the behavior of nonmetallic compounds in steel and for quantitative routine control analyses.

Considerable research in chemical metallurgy and steelmaking was necessary in securing material to establish the validity of the ester-halogen method for the determination of aluminum nitride in carbon steel. A detailed description of the work is not within the scope of this paper. Limited amounts of the data so obtained have been published (*32*) and those secured in other phases of the investigation will be presented in papers now in process of publication.

REAGENTS

The reagents required for the extraction of aluminum nitride from steel are bromine and methyl acetate of analytical grade. Those necessary for determining the nitrogen content of the aluminum nitride are given in an earlier paper (*3*).

APPARATUS

Aluminum nitride is most conveniently separated from steel with reflux extraction units such as are illustrated in Figure 1.

They consist of 200-ml. beakers connected to indented West-type condensers by means of 55/35 standard-taper joints. A drying tube may be attached to the top of the condenser by means of a 24/40 joint when required. Loss of the volatile reagents from the apparatus is negligible when a 300-mm. barrel condenser is used. An electric hot plate adjustable through a range of temperatures is convenient as a source of heat. The usual arrangement for filtering with a Gooch crucible may be used. A sketch of the necessary apparatus for determining the nitrogen content of the residue is shown in Figure 2.

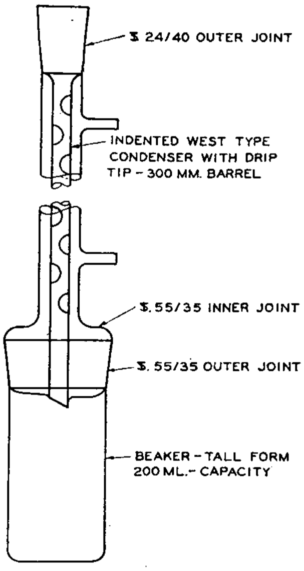

Figure 1. Ester-Halogen Extraction Apparatus

PROCEDURE

A weighed sample is placed in the beaker, bromine is added, and the apparatus is assembled as shown in Figure 3. In general, a 1-gram sample will be convenient for aluminum-killed Bessemer steels and a 3-gram sample for scrap practice open-hearth grades. The quantity of sample should be varied, depending on the amount of aluminum nitride present; 3 ml. (8.94 grams) of bromine for each gram of steel generally will be satisfactory. The flow of water is started through the condenser and 2 ml. of methyl acetate (for a 1-gram sample) are added to the distilling flask in the assembled apparatus. The reaction initiated by the methyl acetate is exothermic and vigorous; when larger samples than 1 gram are used, proportionately less methyl acetate should be added initially and the first 6 to 10 ml. added in three or four increments.

When the initial reaction subsides, 13 ml. of methyl acetate (or proportionally more if a sample larger than 1 gram is used) are added through the condenser to the sample, heat is applied to the flask, and the solvent is refluxed until the sample is completely dissolved. The resulting solution is filtered through asbestos in a Gooch crucible, the residue is washed free from reaction products with methyl acetate, dried at 105° C., and transferred to the digestion flask of the micro-Kjeldahl unit, and its nitrogen content is determined by the same method used for steel (*3*).

The time necessary for solution of the iron matrix in the ester-halogen reagent varies with the composition and particle size of the specimens, but usually compares favorably to that of steel in the usual inorganic acids.

The method of separating the residue containing aluminum nitride from the ester-halogen solution may be varied to suit the purpose for which the residue is to be used. For example, the total amount of residue may be determined by using a weighed Gooch crucible; the carbon content of the residue may be determined by inserting the crucible and contents into the combustion train and determining carbon in the usual way. If the residue is to be examined spectroscopically or by x-ray diffraction methods, it may be collected on a filter paper or by means of a centrifuge.

PRECAUTIONS

Bromine in the absence of the ester does not react with the steel so far as is evident from visual observation, even on prolonged contact with the steel at the boiling temperature of bromine. A few drops of the ester will initiate the reaction, however, even at room temperature and because the reaction is exothermic the temperature will rise to the boiling point of the solvents. Initial dilution of the bromine with ester is not a feasible means for controlling the rate of the reaction. But the rate can be controlled conveniently and effectively by adding the ester in small increments and thus restricting the amount in contact with the bromine in the initial stages of the reaction. Later the amount of ester added is not critical and the reaction will proceed smoothly over a wide range in the amount of ester present. The amount of bromine used is not critical, provided a definite excess is present.

The reaction products are readily soluble in the ester, and the residue can therefore be washed free from solvent quickly and easily. Water must be avoided during solution of the iron matrix and during the washing operation following filtration, or low results will be obtained. The residues may be stored in a desiccator or in an oven at 105° C. Heating the residue in air to temperatures much in excess of 430° C. will cause loss of nitrogen.

A blank is determined on the reagents in exactly the same manner as for the steel, except for omission of the sample. Inasmuch as standard steels with cooperatively established values for aluminum nitride content are not available, the practice of simultaneously checking the extraction procedure and the nitrogen determination may be followed. Two samples of known uniformity, one of which is virtually free from aluminum and therefore free from aluminum nitride and another which is aluminum-killed and known to contain an appreciable amount of aluminum nitride nitrogen, may be used. Spectroscopically pure iron (containing less than 0.05% total impurities) is readily available and is a good aluminum-free sample to use. Values in excess of 0.0005% nitrogen in the ester-halogen residue of this or any other aluminum-free sample, regardless of the original nitrogen content, are evidence of contamination of the residue. Failure to recover the usual amount of nitrogen in the ester-halogen residue from the aluminum-killed steel known to contain aluminum nitride is generally indicative of water in the ester or of exposure of the residue to moisture at some stage in processing. With the usual grade of reagents the blank correction is negligible and recovery of the nitrogen in the ester-halogen residue is very consistent. The determination of a reagent blank should be made a daily practice, nevertheless.

Consistent results cannot be obtained unless all apparatus is free from traces of ammonia.

The amount of nitrogen in the residue from the ester-halogen extraction of commercial aluminum-killed carbon steels can be changed by subjecting the steel to suitable thermal treatments. Specimens with thermal histories identical with those of the physical test specimens must be selected therefore when it is desired to correlate the amount of nitrogen in the ester-halogen residue with physical properties.

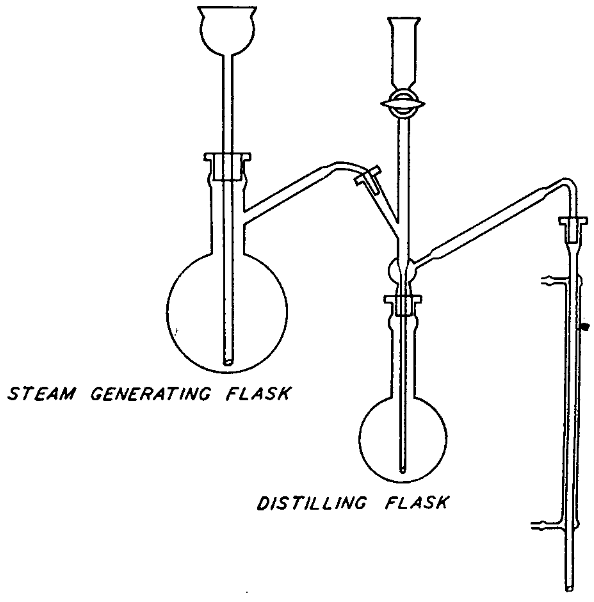

Figure 2. Micro-Kjeldahl Apparatus for Determining Nitrogen in Ester-Halogen Residue

Table I. Recovery of Nitrogen with Different Steel Solvents

Solvent	Nitrogen in Residue, %[a] Aluminum-killed steel	Rimmed steel
Hydrochloric acid (1:1)	0.0005	0.0004
	0.0006	0.0006
Sulfuric acid (1:3)	0.0007	0.0005
	0.0007	0.0006
Phosphoric acid (1:3)	0.0002	0.0004
	0.0002	0.0003
Hydriodic acid (1:1)	0.0002	0.0003
	0.0002	0.0002
Hydrobromic acid (1:1)	0.0002	0.0003
Bromine–methyl acetate	0.0051	0.0002
	0.0049	0.0003

Calculated on basis of weight of original steel sample.

EXPERIMENTAL

Aluminum nitride in contact with water is known to hydrolyze and liberate ammonia. Primarily, the problem in isolating this compound from steel was one of finding reagents that would dissolve the iron matrix without at the same time decomposing any aluminum nitride present. A secondary problem was to find out what other elements or compounds would be isolated along with the aluminum nitride. Finally, it was necessary to establish the accuracy of the method and to determine the behavior pattern of aluminum nitride in steel in order to set up sampling techniques that would enable the analytical results to be correlated with fabricating processes and physical test data in a significant manner.

Reagents for Solution of Iron Matrix. Early in the work while studying synthetic aluminum nitride (AlN), prepared by the reaction of high-purity aluminum with nitrogen, it became apparent that, from a practical standpoint, aqueous solvents would not be feasible for use as reagents in isolating this component from steel. A search was made for reagents that would react with steel in the absence of water, and for solvents of these reagents and of their reaction products with steel. Of the reagents investigated, the halogens and certain of their compounds appeared most promising; the elements bromine and chlorine and their anhydrous acids were known to react directly with steel in an anhydrous atmosphere at elevated temperatures (*26*) but to be without significant effect at room temperatures. From the standpoint of experimental technique, reactions at high temperature are not attractive and results so obtained would not be necessarily indicative of the aluminum nitride content of the unheated steel.

Two or more representatives each of the families of alcohols, ethers, ketones, esters, and organic acids were investigated as solvents for the halogens, for certain of their compounds, and for other inorganic compounds known to react with steel; carbon disulfide and carbon tetrachloride also were tried. Reagents investigated for dissolving the steel included the halogens, their acids, acid anhydrides, compounds such as mercuric chloride which dissolve iron by displacement, and various electrolytes. These substances were evaluated on the basis of: (1) rate of attack on steel, (2) mutual solubility, (3) solubility of the reaction products in the organic solvent, (4) compatibility of the reagent and solvent at room and at elevated temperatures, and (5) absence of undesired side reactions.

The aliphatic organic esters were superior to all others as solvents when the experimental data were evaluated on the basis of the above criteria. The halogens were most promising as reagents for dissolving steel, for they met all the above requirements in a satisfactory way. Bromine and iodine were selected for further study. Fortunately, these elements in the presence of esters reacted readily with steel at temperatures which were convenient to use, and the reaction products were readily soluble in the ester. A consideration of the chemical and physical properties of these materials suggested that methyl acetate or ethyl formate would be a good solvent for bromine, whereas butyl acetate appeared well suited as a solvent for iodine. The vapor pressure curves of bromine and methyl acetate almost coincide, and this fact was of practical value in using these reagents. Semiquantitative trials on rimmed steel (aluminum-free) and aluminum-killed steels indicated that the nitrogen contents of the residues from the aluminum-killed steels were significantly higher than those from the rimmed steels.

Data are shown in Table I for later trials under controlled conditions with different reagents. These are all on samples from single sets of millings from an aluminum-killed and a rimmed steel. The results when bromine was used with methyl acetate were strikingly different from those obtained when aqueous solutions were used. This difference between aluminum-killed and rimmed steels could not be distinguished by ordinary methods of analysis and strongly indicated that ester-halogen reagents would be suitable for isolating a nitrogen-containing constituent believed to be aluminum nitride. Subsequent trials showed that other esters could be used in place of methyl acetate if desired. Iodine may be used in place of bromine but does not react as rapidly.

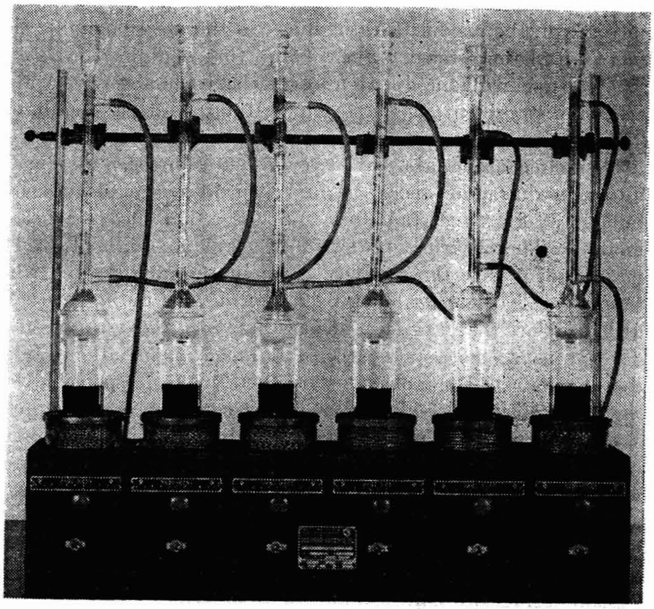

Figure 3. Ester-Halogen Apparatus Assembled for Extracting Aluminum Nitride from Steel

Identity of Nitrogen Compound in Ester-Halogen Residues. The spectrograph provided a simple means for determining what metals present in the original steel were retained in the ester-halogen residue.

Residues separated from steels with the ester-halogen solution were collected on a 4.25-cm. filter paper in a Büchner funnel by use of suction, washed thoroughly with methyl acetate, and dried in air at 105° C. The residue was removed carefully from the filter paper to a cigaret paper with a platinum microspatula and transferred into a cup in a high-purity graphite electrode for spectroscopic analysis. Care was exercised to minimize the quantity of filter paper removed with the residue in order to reduce contamination of the residue from this source. The residues were volatilized with a 220-volt direct current arc and their spectra photographed with a large quartz prism, Littrow-type spectrograph.

The elements in the residues were determined by comparison of their spectra with a labeled spectrum of spectrographic iron and with the spectra of high-purity elements whose presence was suspected in the residue. The results obtained were considered to be indicative of the amounts of a given metal in the residue, as the residues were all from the same initial quantity of steel.

Table II. X-Ray Diffraction Data for Aluminum Nitride

$a = b = 3.127$ Å. $c = 4.995$ Å.

Observed Lines	
$d(kX)$	I/I_0
2.70	1.00
2.38	0.89
2.50	0.71
1.56	0.65
1.417	0.57
1.322	0.48
1.83	0.43
1.303	0.20
1.350	0.12
1.188	0.09
1.247	0.04
1.133	0.02

Aluminum was present in the residues from all steels that contained nitrogen in the ester-halogen residue. The aluminum contents were very low in the residues from the aluminum-killed steels which did not contain nitrogen in the ester-halogen residue. The amounts appeared to be about the same as those present in the residues from steels to which no aluminum had been added. The amount of aluminum present in the residue had no relation to the total amount of "acid-soluble" aluminum or nitrogen in the steel but showed a consistent correlation with the amount of nitrogen in the residue. Aluminum was the major metallic element in all the ester-halogen residues that contained nitrogen. The iron and manganese contents of all the residues were very low. Silicon was present in the residues from all steels containing silicon.

Residues containing ester-halogen insoluble nitrogen were made into x-ray diffraction specimens and their diffraction patterns obtained using iron K-radiation and the usual Debye-Scherrer method. The patterns of the synthetic nitride gave data (Table II) in agreement with those of Ott (*29*), but the initial trials with residues from the ester-halogen treatment gave dark films with little evidence that a crystalline material was present. Carbon in the residue, which appeared to be retained quantitatively from the original steel sample, was believed to be responsible for the unsatisfactory results. When this carbon was eliminated, satisfactory patterns were obtained yielding data in good agreement with those on the synthetic aluminum nitride. This later was verified on residues from low-carbon steels. In these cases, the aluminum nitride pattern could be obtained without eliminating carbon. Thus the chemical, spectroscopic, and x-ray diffraction data all indicated that a definite compound, aluminum nitride, is a constituent of some aluminum-killed steels.

Factors Influencing Recovery of Nitrogen in Ester-Halogen Residues. The preliminary work was carried out on steels known to contain only carbon, manganese, phosphorus, sulfur, aluminum (in some cases), the gases oxygen and nitrogen, and traces (0.01% or less) of other elements. It was necessary to determine whether variation in the proportions or purity of the reagents could be tolerated, and whether amounts of the various alloying elements and deoxidizers commonly used in commercial steels would interfere with recovery of the aluminum nitride in the ester-halogen residue. Initial efforts were devoted to working out details of the method, so that reproducible results could be obtained. It was assumed that a halogen in the presence of an ester would react with the iron and other elements in steel in accordance with Equations 1 and 2.

$$\mathrm{Fe} + \mathrm{X_2} \xrightarrow{\text{ester}} \mathrm{FeX_2} \tag{1}$$

$$2\mathrm{Fe} + 3\mathrm{X_2} \xrightarrow{\text{ester}} 2\mathrm{FeX_3} \tag{2}$$

but would not react with compounds having chemical properties like those of aluminum nitride. Early in the work it was discovered that the thermal history of the sample had a pronounced influence on the amount of aluminum nitride in the ester-halogen residue. This additional variable was investigated.

Purity of Reagents. The effect of changing the proportions of the various reagents on the recovery of nitrogen in the ester-halogen residue was investigated by treating a 1-gram sample of steel with different amounts of reagents and determining the nitrogen contents of the residue recovered. It was found that double the required amount of bromine may be used without significantly changing the amounts of nitrogen recovered in the residue.

The effect of varying the amount of methyl acetate used may be seen in Table III; a very large excess of the ester may be used without appreciable effect on the recovery of nitrogen. Quantities of less than 15 ml. per gram of steel are inconvenient to work with, however, and mechanical loss of the sample may occur.

Different quantities of water were added to methyl acetate to determine how seriously the presence of this compound in the reagents would affect the results. The effect of water was to decrease the amount of nitrogen recovered. The time of contact of the water, as well as the amount of water present, has a bearing on the amount of aluminum nitride decomposed. Table IV shows the serious effect of water on the recovery of nitrogen in the ester-halogen residue. The same steel used in the earlier experiments was used in securing these values and the extraction procedure was as described previously except that the residue was washed with hot water or sodium bicarbonate as indicated in Table IV instead of with methyl acetate. The residue was washed by filling the Gooch crucible with wash solution while the vacuum line was open, allowing it to drain empty, and then refilling. The wash with water decomposed more than half of the original nitrogen in the residue and the loss was even greater with sodium bicarbonate.

Methyl alcohol in the methyl acetate lowers the amount of aluminum nitride nitrogen recovered. The loss of nitrogen may become greater as the time of contact is prolonged; there is visual evidence of side reactions (the condensate becomes water-white) which use up a portion of the halogen and an insoluble black deposit contaminates the residue from the steel.

Table III. Recovery of Nitrogen with Different Methyl Acetate Concentrations

Reagents Used, Ml.		Nitrogen Recovered, %[a]
Br_2	Methyl acetate	
3	5	0.0042
3	10	0.0049
3	15	0.0053
3	30	0.0056
3	60	0.0052
3	90	0.0058

[a] Calculated on basis of weight of original steel sample.

Table IV. Recovery of Nitrogen after Wash with Water or Sodium Bicarbonate

Wash Solution	No. of Washes	Total Volume of Wash, Ml.	Nitrogen Recovered, %[a]
Hot water	10	330	0.0028
	15	500	0.0026
$NaHCO_3$, 15%	10	320	0.0012
	15	580	0.0011
Methyl acetate	10	320	0.0056

[a] Calculated on basis of weight of the original steel sample.

Variation in the amount or proportion of either the bromine or the ester or in the time of contact is without significant effect on recovery of nitrogen in the residue.

Composition of Sample. The use of complex deoxidizers (ferroalloys containing selected proportions of two or more elements) is relatively common in steelmaking practice. It was necessary to determine whether the elements in the more commonly used of these alloys would react with the nitrogen in steel to form compounds that would appear in the residue from the ester-halogen treatment.

Table V. Effect of Alloying and Deoxidizing Elements on Acid-Soluble Nitrogen Content of Ester-Halogen Residues

Sample No.	C	Mn	P	S	Si	Cu	Ni	Cr	V	Mo	B	Al	Ti	Zr	Acid-Soluble Nitrogen, % Steel	Acid-Soluble Nitrogen, % Residue
12	0.04	0.43	0.012	0.030	0.01	....	....	.....	.....	....	...	0.21	....	..	0.027	0.027
13	0.08	0.34	0.020	0.030	0.01	....	....	.....	.....	....	...	0.63	....	..	0.008	0.0075
14	0.08	0.31	0.017	0.031	0.01	....	....	.....	.....	....	...	1.45	....	..	0.015	0.014
15	0.25	1.06	0.028	0.040	0.08	....	....	.....	.....	....	...	0.26	....	..	0.010	0.0092
16	0.19	1.03	0.027	0.036	0.05	....	....	.....	.....	....	...	0.17	....	..	0.017	0.016
53	0.13	0.37	0.007	0.004	0.47	....	....	.....	.....	....	...	.....	0.89	..	0.001	0.0012
46	0.44	1.87	0.008	0.004	0.64	....	....	.....	.....	....	...	.....	0.60	..	0.001	0.0011
119	0.06	0.32	0.004	0.025	0.06	....	....	.....	0.75	....	...	.....	....	..	0.001	0.0007
50	0.04	0.15	0.006	0.032	0.03	....	....	.....	0.60	....	...	.....	....	..	0.002	0.0017
80	0.04	0.39	0.003	0.022	0.15	....	....	.....	.....	....	...	.....	....	0.72	0.025	0.023
32	0.46	1.78	0.023	0.041	0.23	....	....	.....	.....	....	...	.....	....	0.19	0.019	0.015
76	0.11	0.88	0.088	0.394	0.03	1.06	....	.....	.....	....	...	.....	....	..	0.015	0.0005
77	0.14	0.97	0.102	0.400	0.05	....	....	.....	.....	1.05	...	.....	....	..	0.015	0.0005
B.S. 101a	0.05	0.47	0.017	0.009	0.34	0.05	8.99	18.33	0.030	0.01	...	.....	....	..	0.044	0.0009
B.S. 106	0.34	0.48	0.020	0.019	0.25	0.14	0.13	1.29	0.008	0.16	..	1.06	....	..	0.009	0.0087
B.S. 8f	0.07	0.42	0.097	0.080	0.01	0.009	0.003	0.004	0.003	0.001	...	.....	....	..	0.015	0.0005
61	0.09	0.36	0.021	0.020	0.01	....	....	.....	.....	....	0.039	0.023	....	..	0.009	0.0062
62	0.08	0.32	0.019	0.018	0.01	....	....	.....	.....	....	0.024	0.023	....	..	0.010	0.0070
Spectroscopic iron	0.005	0.00001	0.001−	0.003	0.001−	0.0008	0.0015	0.00005	0.00005	0.0001	...	0.00005	0.0001	..	0.004	0.0002

Table VI. Nitrogen Contents of Ester-Halogen Residues after Heating in Air at Various Temperatures

Temperature of Heating, ° C.	Time at Temperature, Min.	Nitrogen before Heating, %[a]	Nitrogen after Heating, %[a]
340	10	0.0047	0.0044[b]
430	10	0.0047	0.0041
540	10	0.0047	0.0038
650	10	0.0047	0.0036
900	10	0.0047	0.0018 0.0010

[a] Calculated on basis of weight of original steel sample.
[b] Carbon not all eliminated.

Several series of heats were made in the induction furnace using scrap from a single heat selected for its low content of residual alloying elements. Some of these heats were of approximately the same composition as commercial grades of carbon steels, but nitrogen in the form of ammonia and a different alloying or "deoxidizing" element was added to each. These elements were added in a relatively pure form, rather than as commercial ferroalloys which generally contain appreciable amounts of one or more deoxidizing elements in addition to the principal one. These heats were rolled into bars in the laboratory mill, millings from the cross section of these bars in the as-rolled condition were extracted by the ester-halogen method, and the acid-soluble nitrogen in the ester-halogen residue was determined.

Some typical results for these steels in the as-rolled condition are shown in Table V, which also gives the amount of acid-soluble nitrogen and of alloying element in the steel, and the amount of acid soluble nitrogen in the ester-halogen residue. Similar information is shown in this table for National Bureau of Standards samples 106 (Nitralloy G), 101a (18-8 stainless), 8f (Bessemer), and high-purity (spectroscopically inspected) iron.

The acid-soluble nitrogen in the steels treated with such elements as titanium and vanadium is low. Elements, of which these are examples, may form "acid-insoluble" nitrogen compounds which are not decomposed during the ester-halogen extraction and may appear in the ester-halogen residue along with the aluminum nitride. The acid-soluble nitrogen of ester-halogen residues from such steels can be determined in the usual way, while the acid-insoluble nitrogen may be determined on a second sample following a digestion treatment to decompose the compounds in which it is held; or the acid-insoluble nitrogen may be separated from the acid-soluble (which will include aluminum nitride) by use of dilute aqueous solutions of inorganic acids. A digestion treatment for decomposing these acid-insoluble nitrogen compounds has been described (*1*). [Since preparation of this manuscript an additional method for decomposing acid-insoluble nitrogen compounds in steel has been described (*16A*).]

Of all the elements added—aluminum, boron, vanadium, titanium, zirconium, copper, molybdenum, chromium, and nickel—only the steels treated with aluminum and zirconium contained acid-soluble nitrogen compounds that appeared in the residue from the ester-halogen treatment. If both these elements are present in the same sample, it would be necessary to determine the acid-soluble nitrogen and either the acid-soluble zirconium or aluminum in the ester-halogen residue; from the information so obtained the amount of nitrogen combined with each could be determined.

The relation between various thermal treatments and the elements that are recovered in the ester-halogen residue from a given grade of steel can best be determined for each grade of steel; a description of such investigations is not within the scope of this paper. The ester-halogen reagents appear applicable for many types of alloy steels and have interesting possibilities for use as etchants; their application to a specific problem should be preceded by some exploratory work to establish the most favorable conditions for their utilization. The ester-halogen method is not subject to interference from the elements commonly present in carbon steels, although two samples of identical composition may give widely differing values for acid-soluble nitrogen and for aluminum nitride as a result of a difference in thermal treatment or processing condition. This fact must be taken into consideration in evaluating the reproducibility and accuracy of the method and in correlating results obtained with it with physical properties.

THERMAL HISTORY OF SAMPLE. Aluminum nitride has been reported to have a decomposition temperature of 1400° C. or greater (*28*) whereas free carbon oxidizes at a substantially lower temperature. It appeared that carbon could be removed from the ester-halogen residue by direct oxidation without decomposing the aluminum nitride. Several samples of a steel having a known amount of ester-halogen insoluble nitrogen were extracted, their residues were heated in air by means of an ordinary muffle furnace for 10 minutes at various temperatures, and their nitrogen contents were determined. They are shown in Table VI along with the temperature of the muffle. An appreciable loss of nitrogen from the residues occurred in air at the higher temperatures but only a slight loss resulted in 10 minutes at a muffle temperature of 430° C. These conditions were used to eliminate carbon from the residues when preparing samples for x-ray diffraction work. In all cases where this was required, duplicate samples were extracted and heated under identical conditions. One was retained for x-ray work, while the nitrogen content of the other was determined in order to make certain that a large loss of nitrogen was not caused by the heating operation.

Data such as are given in Table VI suggested that the thermal history of the steel sample would have an important bearing on the amount of nitrogen that was recovered in the ester-halogen residue. This assumption was consistent with other experimental data indicating that some aluminum-killed steels contained aluminum nitride in substantial quantity, whereas others con-

Table VII. Relation between Temperature, Time, and Ester-Halogen Residue Nitrogen Contents of Killed Steel

Temperature, ° C.	Time at Temperature, Hour	Residue Nitrogen, %[a]
Hot rolled	...	0.0019
100	1	0.0023
260	1	0.0019
490	1	0.0027
620	1	0.0052
730	1	0.0122
820	1	0.0117
960	1	0.0119
1150	1	0.0068
1290	1	0.0013

[a] Calculated on basis of weight of original steel sample.

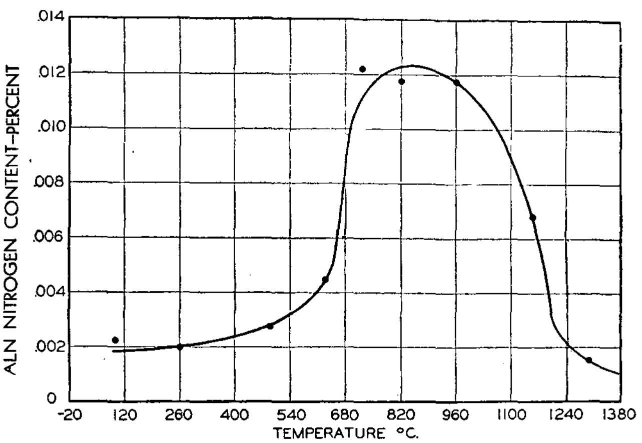

Figure 4. Aluminum Nitride Nitrogen Content of Ester-Halogen Residues from Steel Heated for 1 Hour at Different Temperatures

taining identical amounts of aluminum and nitrogen contained virtually no aluminum nitride in the ester-halogen residue. The validity of this assumption was supported by experimental evidence, of which that in Table VII for a commercial aluminum-killed steel is typical. The analyses for aluminum nitride nitrogen shown in this table are on different portions of the same piece of steel after they had been heated for 1 hour at different temperatures and then cooled quickly in water. Figure 4 was constructed from these values. A very pronounced increase in aluminum nitride content of the residue occurred on the specimens heated in the range of about 620° to 1150° C. Table VIII shows aluminum nitride nitrogen values on heats of commercial steel sampled at different stages in processing. It is evident from a study of this table that a marked difference in the amount of nitrogen in the ester-halogen residue existed at different stages in processing.

It is apparent from the foregoing that the amount of nitrogen in the ester-halogen residue can be made to vary by suitable thermal treatment and that it changes during commercial fabricating operations. These facts must be taken into consideration by the chemist when selecting samples for aluminum nitride analyses, as well as by the metallurgist who wishes to correlate physical properties with analytical results and by operating personnel who wish to produce a uniform product. A change in the aluminum nitride content in the ester-halogen residue of carbon steel is not necessarily accompanied by any change in either the total or the "acid-soluble" nitrogen or aluminum contents of the steel as determined by conventional methods. The ester-halogen method provides a feasible and economical procedure not available heretofore for investigating the effect of different thermal treatments and processing operations on the behavior of the compound aluminum nitride in steel, and for relating the amount of this compound in steel to the physical properties of the metal. The simplicity of the method recommends it as an economical research tool.

REPRODUCIBILITY AND ACCURACY OF METHOD

Ten 1-gram samples from a single set of millings were treated with the ester-halogen solution under routine conditions and the nitrogen contents of the residues were determined; 3 ml. of bromine and 15 ml. of methyl acetate were used in every case, but no two extractions were made on the same day. The maximum value obtained was 0.0057% and the minimum was 0.0050% nitrogen. The average for the series of ten runs was 0.0054%.

A series of samples from commercial steels and from experimental heats made from commercial scrap selected for its low content of residual alloys was extracted in the same manner.

The acid-soluble aluminum, total nitrogen, and nitrogen in the ester-halogen residue of these samples in the as-rolled condition are shown in Table IX. The reproducibility of the results on these tests was excellent, but there is no consistent correlation between either the acid-soluble aluminum content of the steel and residue nitrogen content, or the total nitrogen content of the original steel and that of the ester-halogen residue. This lack of correlation is very evident in comparing values on samples A16 and A107. The steels for which data are shown in Table IX were selected to preclude the possibility that results might be influenced by the presence of alloying elements in different amounts or of a deoxidizer other than aluminum. Spectroscopic examination of additional residues extracted from these steels showed a close correlation between the aluminum content of the residues and their nitrogen contents. Samples A26 and A107, for example, had about the same amount of aluminum in their residues; this amount was appreciably greater than that in the residues from samples such as A28 and A4. Residual alloying elements commonly present in carbon steels were virtually absent from the spectra of these residues.

The typical values in Table IX show that the reproducibility of the ester-halogen method under routine conditions is superior to that of many of the commonly used methods of analyzing metals for other constituents. In no case was there a high recovery of the nitrogen in the ester-halogen residue from steels that were virtually free from aluminum. Although the reproducibility of the results was good, not all the steels containing aluminum contained all or even a major portion of their original nitrogen in the ester-halogen residue.

Table VIII. Ester-Halogen Residue Nitrogen Contents[a] of Killed Steel at Different Stages in Processing

Sample	Hot Rolled, % Slab	Hot Rolled, % Sheet	Annealed Sheet, %
1	0.0046	0.0008	0.0057
2	0.0036	0.0005	0.0028
3	0.0032	0.0004	0.0034
4	0.0044	0.0007	0.0055
5	0.0028	0.0006	0.0031
6	0.0027	0.0005	0.0036

[a] Calculated on basis of weight of original steel sample.

The quantitative recovery of aluminum nitride from steels containing known amounts of the compound could not be checked in the conventional manner, because standard samples or other methods of analyzing for this compound were not available to use as a basis for comparison with the ester-halogen method. Substantially all the nitrogen in a number of steels was recovered in the ester-halogen residue, however (note samples 12 to 16, inclusive, and B.S. 106 in Table V). The excellent reproducibility of the method on duplicate analyses from the same set of millings and the recovery of all the nitrogen in some steels as aluminum nitride indicate the method is quantitative, as does the fact that when investigating thermal treatments, a given treatment which

Table IX. Reproducibility of Nitrogen Recovery in Residue from Ester-Halogen Treatment of Carbon Steels

Sample		Composition, %		
		Steel		
Identity	Weight, g.	Al	N	Nitrogen in residue[a]
A 10	1.0000	0.013	0.011	0.0011
	3.0000			0.0007
	3.0000			0.0009
A 11	1.0000	0.014	0.023	0.0012
	3.0000			0.0012
	3.0000			0.0014
A 12	1.0000	0.003	0.003	0.0006
	3.0000			0.0003
	3.0000			0.0003
A 13	1.0000	0.004	0.019	0.0005
	3.0000			0.0004
	3.0000			0.0002
A 14	1.0000	0.006	0.004	0.0005
	3.0000			0.0002
	3.0000			0.0003
A 15	1.0000	0.006	0.023	0.0011
	3.0000			0.0008
	3.0000			0.0008
A 16	1.0000	0.023	0.019	0.0019
	1.0000			0.0023
	3.0000			0.0014
A 27	1.0000	0.012	0.015	0.0005
	1.0000			0.0003
A 28	1.0000	0.075	0.016	0.0023
	1.0000			0.0022
	1.0000			0.0026
A 3	3.0000	0.031	0.002	0.0008
	3.0000			0.0012
	3.0000			0.0007
A 4	3.0000	0.044	0.003	0.0008
	3.0000			0.0009
	3.0000			0.0009
A 26	1.0000	0.11	0.019	0.012
	1.0000			0.013
	1.0000			0.013
	1.0000			0.012
A 107	1.0000	0.025	0.020	0.012
	1.0000			0.012

[a] Calculated on basis of weight of original steel sample.

can be carefully controlled will, when repeated on material from the same lot, form the same amount of aluminum nitride recoverable by the ester-halogen method.

SUMMARY

A unique method for determining the amount of nitrogen present in steel as aluminum nitride utilizes a halogen dissolved in an anhydrous organic solvent for solution of the iron matrix without decomposing the aluminum nitride; the aluminum nitride is then recovered from the soluble reaction products by filtering. The residue, containing the aluminum nitride and the asbestos mat on which it is collected, is transferred to a simple micro-Kjeldahl apparatus and the nitrogen content of the residue is determined in the usual manner. The reproducibility of results is superior to that obtained with many of the commonly used methods of analyzing ferrous metals for other constituents.

The ester-halogen method is not subject to interference from compounds of the elements commonly present in carbon steels. The amount of nitrogen that can be recovered as aluminum nitride from a given aluminum-killed steel may be increased or decreased by use of suitable thermal treatments. This last fact must be taken into consideration in utilizing the method and data obtained with it. The procedure has been used for several thousand analyses on carbon steels made mainly by the open-hearth and Bessemer processes. The apparatus and reagents can be handled with facilities available in most industrial and academic laboratories. The technique of the method is simple. The procedure is no more exacting than for an ordinary nitrogen determination and the time required for making an analysis is approximately the same in either case. The method is sufficiently economical for routine use and is precise enough for research investigations.

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Use of Perchloric Acid in Determination of Copper in Sulfide Ores

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The iodometric determination of copper gives good results if the copper, arsenic, antimony, and iron are in their higher valence states, the iron is complexed with fluoride, the pH is maintained near 4.8, and thiocyanate is added just before the end point. Considerable time is required to dissolve sulfide ores with the common acids. In hot, concentrated perchloric acid such ores are dissolved in less than 5 minutes and all the metals are taken to their higher valence states. Results on pure copper and on sulfide ore demonstrate that the method is extremely precise and accurate as well as rapid.

THE iodometric method for determining copper has been studied by Mott (*6*), Parks (*7*), Foote (*4*, *5*), and Crowell and co-workers (*1–3*). Mott pointed out that interference by ferric iron could be eliminated by using fluoride. Parks showed that proper pH control was necessary to prevent interference by arsenic and antimony. Foote and Vance further improved the method by adding thiocyanate near the end point. Crowell improved on Parks's work by eliminating the use of biphthalate as additional buffer.

Nitric acid interferes and the method of dissolving the sample and preparing it for neutralization with ammonium hydroxide is involved and time-consuming. Several hours are required to dissolve copper sulfide ores using mixtures of nitric, hydrochloric, and finally sulfuric acid. The use of perchloric acid as the solvent is the subject matter of the present paper.

The method has been used in the sophomore quantitative analysis course at Iowa State College with good results.

PROCEDURE

Place the sample in a small-mouthed 500-ml. Erlenmeyer Pyrex or Vycor flask; a dry flask is preferable. Use one or two glass beads to serve as boiling chips. Add 15 ml. of 70 to 72% perchloric acid in such a manner that the walls of the flask are washed down by the acid. Insert a refluxing still head (*8*) in the neck of the flask, and heat rapidly to boiling temperature. Adjust the burner so that perchloric acid refluxes on the walls of the flask but acid fumes do not escape into the room. Boil gently for about 5 minutes. Remove from burner, allow to cool for about 2 minutes, and add 50 ml. of water. Mix and boil gently for about 5 minutes to remove chlorine. Cool, wash, and remove stillhead. Neutralize with 1 to 1 ammonium hydroxide until the brown ferric hydroxide that first precipitates turns brown-green owing to the appearance of some blue cupric ammonium complex. If too much ammonium hydroxide is added a deep blue color results. Should this happen, add enough dilute sulfuric acid to restore the brown-green color. Cool the slightly basic solution in tap water and add 2 grams of ammonium bifluoride crystals. Mix and wash down side walls of flask. The total volume should be about 150 ml. at this stage. Add 2 grams of potassium iodide (may be added in 40% solution form) and titrate immediately, with constant swirling, using 0.1 *N* thiosulfate solution. During the first part of the titration the coffee color will gradually fade to a cream color. At this point add 2 ml. of 2% starch solution and continue the titration until the return of the starch-iodine color after each addition is slow. Add 2 grams of potassium thiocyanate (may be added in 40% solution form) and titrate, splitting drops, to the final disappearance of all blue color. No starch-iodine color should reappear on standing for several minutes.

ORE DISSOLVED STUDIES

Various mixtures of nitric, sulfuric, and hydrochloric acids were used to dissolve a series of copper sulfide ores. Several hours were required in all cases. A sulfur bead formed during the early stages of the dissolving process and was extremely slow in dissolving with nitric acid or nitric in combination with hydrochloric and/or sulfuric acid. The addition of perchloric acid made it possible to dissolve the sulfur bead more rapidly, but even this was slow. By using perchloric acid alone the samples could be completely dissolved in less than 5 minutes without the formation of a sulfur bead.

The material not dissolved by the perchloric acid was white and appeared to be silica. To check the perchloric acid dissolving process further, the insoluble residue was filtered off and decomposed in a platinum crucible with hydrofluoric and perchloric acids. The nonvolatile material did not liberate iodine when the analytical procedure was applied, showing that all the copper was dissolved by perchloric acid alone in the first treatment.

Another check on perchloric acid as the solvent was accomplished by adding sulfuric and nitric acids following the perchloric

Table I. Analysis of No. 800 Copper Sulfide Ore

No.	Sample	$Na_2S_2O_3$[a]	Copper	Deviation from Average
	Gram	*Ml.*	%	%
Perchloric Followed by Sulfuric and Nitric Acids as Solvent				
1	0.4537	44.59	46.14	−0.01
2	0.4127	40.52	46.10	−0.05
3	0.4238	41.68	46.18	+0.03
4	0.4080	40.12	46.17	+0.02
			Av. 46.15	0.03
Perchloric Acid as Solvent				
1	0.4498	44.19	46.13	+0.00
2	0.4529	44.49	46.12	−0.01
3	0.4434	43.55	46.12	−0.01
4	0.4251	41.77	46.14	+0.01
5[b]	0.4478	44.00	46.14	+0.01
6[b]	0.4619	45.39	46.14	+0.01
7[b]	0.4193	41.19	46.13	±0.00
8[b]	0.4368	42.90	46.12	−0.01
			Av. 46.13	0.01

[a] 1 ml. of $Na_2S_2O_3$ equivalent to 0.004695 gram of copper.

[b] 10 ml. of Br_2 water added after dissolving, 50 ml. of H_2O added, boiled 10 minutes.

Table II. Electrodeposition Analysis of Metallic Copper Used in Standardizing Thiosulfate Solution

No.	Copper Taken	Copper Found	Copper	Deviation from Average
	Grams	*Grams*	%	%
1	1.0075	1.0071	99.96	−0.046
2	1.0044	1.0050	100.06	+0.054
3	1.0004	1.0003	99.99	−0.016
			Av. 100.003	0.039

Table III. Standardization of Thiosulfate

No.	Cu *Gram*	$Na_2S_2O_3$ *Ml.*	Cu *G./l.*	Deviation from Average *%*
	Metallic Copper[a] Dissolved in Perchloric Acid			
1	0.1618	34.48	4.693	−0.002
2	0.1711	36.44	4.695	±0.000
3	0.1773	37.75	4.697	+0.002
4	0.1684	35.85	4.697	+0.002
5[b]	0.1732	36.90	4.694	−0.001
6[b]	0.1630	34.69	4.699	+0.004
7[b]	0.1725	36.81	4.697	+0.002
8[b]	0.1675	36.65	4.698	+0.003
			Av. 4.695	0.002
	Resublimed Iodine			
	Iodine *Gram*			
1	0.4167	44.50	4.690	−0.005
2	0.4194	44.81	4.688	+0.003
3	0.3966	42.33	4.693	−0.002
4	0.4054	43.25	4.694	−0.001
5	0.4112	43.80	4.702	+0.007
			Av. 4.695	0.004

[a] Average of three electrodeposition analyses 100.003% (see Table II).

[b] 10 ml. of Br_2 water added after dissolving, 50 ml. of H_2O added, boiled 10 minutes.

acid treatment and digesting for 1.5 hours. During this period the nitric and perchloric acids were driven off; thus the later portion of the digestion took place with boiling concentrated sulfuric acid. After cooling, water and bromine-water were added and the solution was boiled for 10 minutes and titrated (Table I). The average of four determinations varied only 2 parts in 4613 from the average of eight determinations using perchloric acid only as the solvent. The addition of bromine water is unnecessary when perchloric acid is used.

APPLICATION OF METHOD TO PURE COPPER

The thiosulfate was standardized against metallic copper dissolved with perchloric acid and also resublimed iodine to show that the perchloric acid method gives stoichiometric results. The copper metal was analyzed for purity by electrodeposition (Table II). In the thiosulfate standardization process metallic copper was dissolved and titrated under the same conditions as the ore sample. The metal dissolves somewhat more slowly than copper ore. The data presented in Table III include four solutions that were treated with bromine water and four that were not. Unusually precise results were obtained. It is apparent that the copper is in the cupric state after the perchloric acid digestion, inasmuch as the bromine water treatment did not alter the results. The standardization of the thiosulfate solution was checked against iodine and the data given in Table III show that both methods give the same results.

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Iron in Aluminum Alloys

Colorimetric Determination Using 1,10-Phenanthroline

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A study of several factors influencing the determination of iron in aluminum alloys containing copper, nickel, and zinc by means of 1,10-phenanthroline is presented. A precise and accurate method which avoids interference by the above elements is detailed.

THE colorimetric determination of iron in aluminum alloys is usually carried out by employing the thiocyanate reaction. Recently, however, two methods have been published (*2*, *6*), based upon the color reaction of ferrous iron with 1,10-phenanthroline. Such a method has been in use in this laboratory for some time.

Although 1,10-phenanthroline has greater sensitivity and greater stability of color than thiocyanate (*7*), and it follows Beer's law more accurately, the reagent is expensive and its use renders analysis considerably slower than the thiocyanate method in some cases. For routine use the 1,10-phenanthroline method has proved inferior in the authors' hands to the thiocyanate method, but it may be used to advantage when a check on the latter is desirable, or in special cases such as the estimation of iron in pure aluminum.

PRELIMINARY ATTACK ON ALLOY

To incorporate the use of 1,10-phenanthroline for the determination of iron in a concise scheme of analysis, the method of attack upon the alloy must produce a final solution which has the maximum freedom from elements known to interfere in the 1,10-phenanthroline reaction and at the same time contains as many of the other elements to be determined as possible. The concentration of elements present in commonly occurring aluminum alloys likely to interfere has been determined by Fortune and Mellon (*4*).

Alkaline Digestion to Remove Aluminum, Silicon, and Zinc. Although this method succeeds in removing silicon and zinc, it is nevertheless very unsatisfactory. Variable proportions of iron dissolve in the caustic alkali; this is particularly marked for aluminum alloys containing high proportions of silicon, doubtless because of the high concentration of alkali needed in such cases. Thus, one alloy of 0.41% iron and 11.5% silicon content gave only 0.02, 0.03, 0.06, 0.03, and 0.05% iron after digestion in sodium hydroxide. The hydrazine hydrate method of Bartram and Kent (*3*) was tried, but not only was appreciable iron dissolved by the strong caustic solution but it was extremely difficult to obtain complete recovery of manganese. The solubility of these elements in sodium hydroxide has been noted (*1*).

Solution in 1 to 1 Hydrochloric Acid. This method was used by Pepi (*6*), who claims that hydrochloric acid attack prevents the solution of copper, bismuth, and silicon, the elements he had found to interfere with the iron determination. Such a method of attack has several disadvantages. It precludes the possibility of determining manganese and copper in a composite scheme, and it introduces all the zinc present in the sample into the solution. Furthermore, the authors are unable to agree with Pepi that no copper is taken into solution. When this method of attack was used, large and variable amounts of copper were found in the acid solution—in one case as much as 16 p.p.m. of copper in the solution in which the iron color was to be developed.

Digestion with Mixed Acids. Digestion of the alloy in a mixture of sulfuric, nitric, and hydrochloric acids completely decomposes the alloys and on fuming produces final solutions that are free from silicon and chloride. All the zinc is taken into solution, but the effect of zinc can be successfully overcome. It was feared that this method would lead to production of final solutions having an erratic pH, but experience showed that solutions were produced lying well within the prescribed pH limits given below.

The procedure adopted to bring an alloy into solution is given under the heading Complete Method. The instrument used throughout this work was Hilger's Spekker absorptiometer.

FACTORS INFLUENCING THE DETERMINATION

Choice of Filter. In view of the finding of Fortune and Mellon (*4*) that the nickel ion produces a change in hue of the iron–1,10-phenanthroline complex, the filter chosen for use in this determination was the Ilford green (604), for which the maximum absorption is at 520 mμ.

Effect of pH. Pepi (*6*) has stated that in the analysis of aluminum alloys the pH of the color solution need not be regulated too closely, but color development is slow below pH 2 and reduction slow above pH 3. It seemed necessary, therefore, to explore the influence of pH on color development. For this purpose a standard alloy was digested and a stock solution obtained. Details of the standard alloy, stock solution, and color solution are given in Table I.

Five-milliliter aliquots were taken from the stock solution, at which point it is 1 *N* in acid, reducing agent was added, and the solutions were buffered to various pH's by the addition of varying quantities of sodium acetate solution. The volumes were made up to 90 ml. with distilled water, 5 ml. of 1,10-phenanthroline solution (0.2%) were added, and the volumes were adjusted to 100 ml. with distilled water. All pH measurements were made by the glass electrode. Table II records the percentage iron recovered at stated intervals of time and the influence of pH upon recovery, at 18° C.

At pH values below 2.4 and above 3.5, color development was slow. Above pH 3.8 aluminum hydroxide tends to precipitate from the solution. For complete color development the optimum pH therefore lies between 2.9 and 3.5, and a reading can be taken after 10 minutes' standing.

Slow color development can be corrected by the addition of uneconomical amounts of 1,10-phenanthroline.

As a solution of hydroxylamine hydrochloride deteriorates on standing, it must be freshly made up or its acidity adjusted before use.

Table I. Composition of Stock Solution of Alloy

Element	Cu	Fe	Ni	Zn	Mg	Mn	Ti	Si
% in alloy	3.20	0.80	0.22	0.16	0.05	0.38	0.02	4.85
Mg./liter in stock soln.	..	80	22	16	5	38	2	..
P.p.m. in color solution, 5-ml. aliquot	..	4	1.1	0.8	0.25	1.9	0.1	..

Table II. Effect of pH on Rate of Color Development

pH	5 Min.	10 Min.	15 Min.	30 Min.
2.10	50	61	72	83
2.40	64	89	94	100
2.66	96	98	100	100
2.88	97	100	100	100
3.10	98	100	100	100
3.52	98	100	100	100
3.85	88	88	90	92

Table III. Effect of Nickel Concentration on Rate of Fading

Time, Min.	Fe and Ni Concentrations in Color Solution, P.P.M.					
	1 Fe 1.13 Ni 10.20	2 Fe 2.13 Ni 10.20	3 Fe 3.13 Ni 10.20	4 Fe 4.13 Ni 10.20	5 Fe 5.13 Ni 10.20	6 Fe 6.13 Ni 10.20
15	100	100	100	100	100	100
45	100	100	100	94	97	97
180	93	91	95	87	91	87
15 hour	40	35	36	41	37	40

pH = 2.95. Temp. = 16° C.

Table IV. Effect of pH and Temperature on Rate of Fading

Solution	pH	Temp., ° C.	Time, Minutes 3	7	15	25	30	45
1	2.15	15	78	94	96	100	96	90
		31	79	61	55	30	..	..
		15	97	98	100	100	100	96
	2.90	31	90	83	72	49	47	40
6	2.15	15	87	91	98	100	96	88
		31	30	24	15	..	..	..
		15	96	97	100	97	95	93
	2.90	31	45	33	..	..	..	..

Effect of Temperature. Experiments carried out at pH 2.15 showed that increase of temperature has little effect upon color development in this particular case, and is unable to accelerate the complete formation of the complex at pH well below the optimum.

Effect of Nickel. During the work the present authors observed repeatedly that solutions containing a high nickel content faded in color upon standing.

This phenomenon is illustrated by data obtained from a Light Metal Founders' Association standard Y alloy having the following composition: copper 4.25, nickel 2.04, manganese <0.01, magnesium 1.775, iron 0.225, silicon 0.30, lead 0.07, titanium 0.07.

To the final color solution increasing amounts of iron were added before color development, and the percentage recoveries shown in Table III were obtained after the addition of 5 ml. of 1,10-phenanthroline solution (0.2%—i.e., sufficient to provide an excess greater than 1.5 times the stoichiometric ratio of 1,10-phenanthroline to iron for highest range of iron content (No. 6). This is the ratio of 1,10-phenanthroline to iron that has been found necessary to produce rapid, full color development.

Furthermore, the rate of fading at normal temperatures was markedly accelerated by pH values lower than 2.9 and increase of temperature had the same effect at pH values between 2 and 3.5. Table IV records the effect of pH and temperature on the recoveries obtained for solutions 1 and 6.

It seemed probable that in order to achieve stability of the iron complex in the presence of nickel, the total amount of 1,10-phenanthroline to be added might be the sum of the separate quantities required to satisfy iron and nickel separately.

Stock solutions of iron and nickel were made containing 0.1 mg. per ml. of each metal. The iron solution was obtained from iron wire (99.7%) and the stock solution was adjusted to 1 *N* with respect to sulfuric acid. The nickel solution was produced by dissolving nickel sulfate (analytical reagent grade) in water and adjusting to the required concentration of nickel. A range of iron and nickel concentrations was taken, the reducing agent was added, the solutions were buffered to pH 2.9 with sodium acetate, and varying quantities of 1,10-phenanthroline (0.2%) were added; the lowest quantity of reagent used was that required to produce rapid, full development of the iron color. Table V records the percentage recoveries obtained in these experiments at 15° C.

If the conditions of pH and temperature used in obtaining the results given in Table IV are maintained, full recovery of iron in the presence of comparatively large amounts of nickel is obtained if 1.5 times the stoichiometric amount of 1,10-phenanthroline required by iron alone is added and the absorption is measured no later than 20 minutes after addition of 1,10-phenanthroline. However, if the reading is taken later than 20 minutes after color development, the recovery may be low. pH values lower than 2.9 and increased temperature accentuate these defects, especially when 1,10-phenanthroline is present in amounts less than are given in the formula below.

Examination of these results shows that to obtain rapid, full, and stable color development of iron in the presence of nickel at pH 2.9 to 3.5 the minimum amount of 1,10-phenanthroline to be added, expressed in parts per million in final 100 ml. of color solution, is given by the formula

$$([\mathrm{Fe}] \times 1.5 \times 9.6) + ([\mathrm{Ni}] \times 9.2)$$

The same conclusion is reached from experiments in which the iron was kept constant and the nickel varied. In all cases, except those in which sufficient reagent was added to produce a stable color, serious fading took place given sufficient time, and the greater the deficiency of reagent the more rapidly the fading occurred.

Effect of Zinc. Goodman (*5*) has shown that the interfering effect of zinc may be overcome by the addition of larger quantities of 1,10-phenanthroline than are normally required to produce full color development. This interference was shown originally by Fortune and Mellon (*4*) to give rise to incomplete recovery of iron. It was calculated from Goodman's data that for full color development of iron in the presence of zinc the amount of 1,10-phenanthroline to be added in excess of that required by the iron alone lay between 1.5 and 2.0 times the zinc content. As the zinc content of an aluminum alloy can be as high as 14.5%, the concentration of zinc in the color solution can reach 73 p.p.m., and from the point of view of the cost of the procedure the difference between 1.5 and 2.0 times the zinc concentration is considerable. Experiments carried out over a range of iron and zinc contents showed 2 p.p.m. of 1,10-phenanthroline for each 1 p.p.m. of zinc to be necessary to produce complete color development in 10 minutes at 18° C. At 31° C. the lower ratio is sufficient.

Order of Addition of Reagents. In the analysis of aluminum alloys the order of addition of reagents has no significant effect on the determination.

An effort was made to save time by using a mixed buffer–reducing agent solution, but the mixed solution was unstable upon standing. A feature of this instability is the development of a red-violet color when added to the iron solution in the absence of 1,10-phenanthroline. Experiments indicate that this is due to the slow formation of acetohydroxamic acid.

COMPLETE METHOD

Digest 1 gram of alloy with 20 ml. of 10.0 *N* sulfuric acid, 5 ml. of nitric acid (specific gravity 1.42), 5 ml. of hydrochloric acid (specific gravity 1.16), and 20 ml. of distilled water in a 400-ml. Pyrex beaker. Boil to complete solution, and then take down to fuming. Fume gently for 5 minutes, cool, wash down the sides of the beaker, and dilute to about 60 ml. with distilled water. Boil until salts are dissolved, and filter off the bulk of the insoluble material using suction. Wash the filter with hot water and pass hydrogen sulfide through the filtrate for 5 minutes. Filter off copper sulfide on a Whatman No. 31 paper, and wash well with hydrogen sulfide water slightly acidified with sulfuric acid. Reserve the residue for copper determination. Boil the filtrate down to about 80 ml., cool, make up to 100 ml. in a graduated flask, and mix well. Pipet a 5-ml. aliquot into a 100-ml. graduated flask, and add 5-ml. aliquots of hydroxylamine hydrochloride (20 grams per liter) and sodium acetate (84 grams of anhydrous salt per liter). Make up to 80 ml. with distilled water and either (*a*) add sufficient 1,10-phenanthroline (0.2%) to satisfy the anticipated requirements of iron and zinc—i.e., ([Fe]) × 9.6 × 1.5) + ([Zn] × 2.0)—and read at the end of 10 minutes at 18° C., or (*b*) add sufficient reagent to satisfy the requirements of iron, nickel, and zinc—i.e., ([Fe] × 9.6 × 1.5) + ([Zn] × 2.0) + ([Ni] × 9.2). In this case a reading can be taken at any time after 10 minutes' standing at any room temperature up to 31° C.

Table V. Effect of Excess Reagent and Reaction Time on Interference by Nickel

Solution		1,10-Phenanthroline, P.P.M.		Time, Minutes						
Fe, p.p.m.	Ni, p.p.m.	Added	(Fe × 9.6 × 1.5) + (Ni × 9.2)[a]	15	20	30	40	60	90	900
4	3	60	85.2	100	100	100	98	98	95	72
		80		100	100	100	100	100	97	83
		85		100	100	100	100	100	100	100
6	3	90	114.0	100	100	100	98	96	94	73
		110		100	100	100	100	100	98	82
		115		100	100	100	100	100	100	100
7	3	100	128.4	100	100	100	98	96	93	70
		120		100	100	100	100	98	95	85
		125		100	100	100	100	100	100	100
5	5	80	118.0	100	100	100	96	93	89	48
		110		100	100	100	100	100	96	73
		120		100	100	100	100	100	100	100
5	15	80	210.0	100	100	97	93	90	83	41
		200		100	100	100	100	100	96	76
		210		100	100	100	100	100	100	100

[a] Minimum for stable color.

ACCURACY OF THE METHOD

To demonstrate the accuracy and reproducibility of the method, standard alloys were analyzed by two unskilled assistants who had no previous experience with the method. Assistant 1 was given a sample of DTD 424, which had been standardized by two independent analysts, and assistant 2 was given a Light Metal Founders' Association standard Y alloy. Each assistant carried out six determinations:

DTD 424 Analysis

	Fe, %			Fe, %
Firm A	0.80	Assistant 1		0.802
Firm B	0.80			0.802
				0.805
				0.797
				0.800
				0.802
			Av.	0.801
			ä =	±0.003

Y Alloy Analysis

L.M.F.A.	0.225 ± 0.01	Assistant 2		0.224
				0.223
				0.231
				0.228
				0.225
				0.223
			Av.	0.226
			ä =	±0.003

In both cases all the results fall within a range about the average of less than 2ä, so the precision is high. Compared with independent analyses which are assumed to be true figures, the results obtained show the accuracy of the method is also high.

CONCLUSIONS

If certain requirements are fulfilled, 1,10-phenanthroline is a satisfactory reagent for the determination of iron in aluminum alloys.

The optimum pH range is 2.9 to 3.5.

At pH less than 2.9, color development is slow and increase of temperature to 31° C. causes no acceleration. A large excess of 1,10-phenanthroline corrects this slow development.

For stable color development sufficient reagent must be added to allow for the formation of both nickel and iron complexes. Providing pH and temperature are correct, only sufficient 1,10-phenanthroline need be added to satisfy the requirements of iron if a reading is taken at the end of 10 minutes and not later.

The effect of zinc can be overcome by adding an additional amount of reagent equivalent to 2.0 times the zinc concentration at 18° C., or 1.5 times at 31° C.

The order of addition of reagents has no significant effect.

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Spectrophotometric Determination of Sulfaquinoxaline

Its Application to Poultry Feeds and Feed Premixes

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The suitability of the Bratton-Marshall color reaction as the basis for a spectrophotometric assay of sulfaquinoxaline was investigated using *N*-(1-naphthyl)-ethylenediamine dihydrochloride as the coupling agent. Because a nonlinear relationship between concentration and color intensity obtains in the adopted procedure, extinction coefficients were determined at several concentrations. The effects of acidity and salt concentrations on the color intensity were also investigated, and optimum conditions for color formation recommended. The method was applied to sulfaquinoxaline samples of known purity, to certain of its salts, to mixtures with inorganic diluents, and to medicated poultry feeds and feed premixes. In the use of the feed mixtures, convenient sample sizes and dilution schedules are suggested for handling mixtures containing up to 15% of sulfaquinoxaline.

SULFAQUINOXALINE (*15*) (2-sulfanilamidoquinoxaline) has been found (*2, 3, 5, 12*) to be an effective chemotherapeutic agent for the prevention and control of certain poultry diseases. Being a sulfonamide in which sulfanilic acid is condensed with 2-aminoquinoxaline, it shows the typical color reaction that is the basis of the assay method proposed by Marshall (*1, 8–11*) and colleagues for sulfanilamide and related compounds. The color is generated by diazotization of the free arylamino group and coupling with a suitable agent.

Of the large number of coupling agents examined (*1*), *N*-(1-naphthyl)-ethylenediamine dihydrochloride was cited by Bratton and Marshall as the most satisfactory. The present authors have studied this reaction spectrophotometrically in the case of sulfaquinoxaline and find that, despite the many factors that influence the color intensity, it may be made the basis of an analytical method. The resulting colored compound has the same visible absorption spectrum as the azo dye obtained from sulfanilamide (*1*), exhibiting a characteristic band at 545 mμ. The method was tested with a number of sulfaquinoxaline samples of known purity and was applied to mixtures with inorganic diluents. An extension to mixtures of sulfaquinoxaline with an organic diluent, such as poultry feed, which requires extraction of the sulfonamide is also described.

SPECTROPHOTOMETRIC STUDY

Reagents. The following aqueous reagents were employed in this investigation: 0.02 *N* and 0.50 *N* solutions of hydrochloric acid, 0.5 *N* solution of sodium hydroxide, 0.1% solution of sodium nitrite, 0.5% solution of ammonium sulfamate (La Motte "pure-standardized"), 0.1% solution of *N*-(1-naphthyl)-ethylenediamine dihydrochloride (Eastman Kodak), and sodium chloride. Unless otherwise stated, Merck reagent grade chemicals were employed. The coupling agent was stored in a dark-colored glass bottle to avoid photodeterioration. Fresh sodium nitrite solution was prepared daily. The concentration of the 0.50 *N* acid had to be regulated fairly accurately because pH control was necessary for reproducible color production.

Procedure. The conditions for color formation were studied with a sulfaquinoxaline preparation, the purity of which was 99.8% as determined by solubility analysis (*7, 14*). Elementary analysis of this standard compound showed 56.29% carbon, 4.29% hydrogen, and 18.86% nitrogen compared to the calculated 55.96% carbon, 4.03% hydrogen, and 18.66% nitrogen; the melting point, with decomposition, of the standard was 248.2–248.5° C. compared to a reported value (*15*) of 247–248° C.

Extinction coefficients and the effect of factors that might influence the color formation were studied at a series of sulfaquinoxaline concentrations ranging from 0.05 to 0.35 mg. per 50 ml. In a typical series of experiments, about 25 mg. were weighed (microbalance) into a 250-ml. volumetric flask containing 50 ml. of 0.5 *N* sodium hydroxide, and the resulting solution of the sodium salt of sulfaquinoxaline was diluted to volume. Appropriate portions of this stock preparation were diluted with water to yield solutions containing 0.5, 1.0, 1.5, 2.0, 2.5, and 3.5 mg. of sulfaquinoxaline per 50 ml., and 5-ml. aliquots of each were transferred to individual 50-ml. volumetric flasks for color formation. These aliquots, containing ≈0.05 to 0.35 me. of alkali and 0.05 to 0.35 mg. of sulfaquinoxaline, were just neutralized to phenolphthalein by addition of 0.02 *N* hydrochloric acid, after partial neutralization of the more alkaline solutions of a series with 0.5 *N* acid. Although the procedure finally adopted for routine analyses utilized less alkali, thereby obviating any need for this neutralization step, it was retained throughout the spectrophotometric investigation to permit proper acidity control at the diazotization and coupling stages of the color development.

To each of the neutral solutions were added in succession 5 ml. each of 0.50 *N* hydrochloric acid and 0.1% sodium nitrite solutions. After standing for 3 minutes, 5 ml. of 0.5% ammonium sulfamate solution were added. Finally, after an additional lapse of 2 minutes, 5 ml. of the 0.1% solution of coupling agent were added, and the flask contents were diluted to volume. The contents of the flasks were swirled to ensure mixing after each addition of reagent. The final pH of these diazotized and coupled sulfaquinoxaline solutions was 1.3 to 1.4. The red color developed rapidly to maximum intensity and could be measured 30 seconds after coupling. Except at the highest concentrations involved, the color was stable for several hours. At the highest concentrations (0.25 to 0.35 mg. per 50 ml.) the color tended to fade slowly owing to precipitation of the azo dye, at a rate which the authors believe to be influenced by acidity. Under the conditions recommended herein, this effect amounted to 2 to 3%

in the first hour, and could be circumvented by determining the color intensity within 15 minutes after color development. At lower concentrations, however, this effect was negligible. Optical densities were measured at 545 mμ in 0.5- or 1.0-cm. cells, using a model DU Beckman spectrophotometer. A reagent blank was used as a reference liquid, despite the fact that these blanks exhibited a negligible color.

Effect of Sulfaquinoxaline Concentration. Extinction coefficients (calculated on the basis of sulfaquinoxaline weight), $E^{1\%}_{1\,\text{cm.}}$, at sulfaquinoxaline concentrations ranging from 0.05 to 0.35 mg. per 50 ml. were determined by the standard procedure described above. Average values of about 15 to 20 standardizations at each level, together with mean deviations, are reported in Table I. The standard deviations (*4*) amount to ±2.1%, so that an average of three sulfaquinoxaline determinations would have a "probable" error of ±0.8% and a 95% probability of being within 2.4% of the proper value.

It is clear from Table I that the relationship between color intensity and concentration is not linear under the conditions imposed by the above procedure, inasmuch as extinction coefficients decrease with increasing concentration. For this reason, the evaluation of an unknown for its sulfaquinoxaline content necessitates interpolation of the extinction coefficient data. With this end in view, optical densities (1-cm. cells) are recorded in the second column to facilitate choosing a proper $E^{1\%}_{1\,\text{cm.}}$.

The molecular extinction coefficient, ε, for the colored sulfaquinoxaline derivative is very close to that obtained with sulfanilamide (*1*). For example, the $E^{1\%}_{1\,\text{cm.}}$ value of 1750 for sulfaquinoxaline corresponds to an ε of 52,500, as compared to the sulfanilamide (concentration unspecified) value of 47,500 reported by Bratton and Marshall (*1*). Actually the authors find an ε of 51,500 for the azo dye of sulfanilamide (Merck production material), at concentrations of 0.1 to 0.3 mg. per 100 ml. The discrepancy is probably due to nonproportionality between color intensity and concentration which the authors have observed with higher sulfanilamide concentrations, just as with sulfaquinoxaline, under conditions prevailing in their procedure for color formation. This similarity of spectra and ε's accentuates the inability of the color reaction to distinguish between individual sulfonamides.

Table I. Extinction Coefficients ($E^{1\%}_{1\,\text{cm.}}$ at 545 mμ) *vs.* Concentration and Optical Density (at pH 1.3)

Concentration, Mg./50 Ml.	Average Optical Density	Average $E^{1\%}_{1\,\text{cm.}}$	Mean Deviation
0.05	0.175	1750	±30
0.10	0.348	1740	±30
0.15	0.513	1710	±30
0.20	0.676	1690	±30
0.25	0.830	1660	±25
0.35	1.127	1610	±30

Effect of Acidity. The influence of final solution acidity on the color intensity was studied by omitting the neutralization step, and varying the volume of 0.50 *N* hydrochloric acid added prior to diazotization between ≈1.5 and 30 ml. Diazotization and coupling were then performed in the prescribed manner, and the color intensities determined after dilution to volume. In this way, final pH's ranging from ≈2.0 to 0.5 were obtained. The concomitant acidity interval at the diazotization step was ≈0.04 to 1 *N*. Actually the acidities studied extended beyond these limits, but only results within this pertinent range are presented. All final pH's were measured with a Cambridge Research Model pH meter, except as otherwise indicated below.

The effect of pH on the color intensity at two sulfaquinoxaline concentrations is shown in Table II. It is clear that the greatest optical density and minimum pH sensitivity are realized in the final pH range 1.0 to 1.6. It is for this reason that in the standard procedure adopted, a pH of 1.3 to 1.4 is specified.

The lower color intensities obtained at higher pH's are believed primarily due to an effect of final acidity on the color of the dye itself rather than to the influence of acid on preliminary stages of color development, since, for example, readjusting the pH's of the first two solutions listed in Table II (concentration ≈0.101 mg. per 50 ml.) from 2.06 and 1.87 to 1.49 raises the respective densities (corrected for dilution upon reacidification) to 0.343 and 0.350. On the other hand, the density (and extinction coefficient) drop in very acid medium is attributable, at least in part, to the diminished solubility of the azo dye, which actually precipitates out at very high sulfaquinoxaline concentrations.

Table II. Effect of pH on Color Intensity

Optical density (1-cm. cells)

Concentration 0.101 Mg./50 Ml.		Concentration 0.253 Mg./50 Ml.	
Final pH	Density	Final pH	Density
2.06	0.317	2.04	0.792
1.87	0.327	1.94	0.829
1.75	0.337	1.80	0.842
1.58	0.351	1.65	0.853
1.45	0.350	1.59	0.853
1.36	0.350	1.48	0.853
1.25	0.353	1.42	0.853
1.11	0.345	1.34	0.858
0.70[a]	0.350	1.26	0.855
0.50[a]	0.340	1.08	0.855
		0.70[a]	0.837
		0.50[a]	0.810

[a] Calculated values.

Effect of Salt Concentration. In view of the necessity for controlling acidity, the feasibility of using a buffer was investigated. This approach was abandoned, however, when it was discovered that increasing the salt concentration tended to diminish the color intensity. This effect is illustrated by the data in Table III obtained at two concentrations by the standard procedure, except for the addition of varying amounts of sodium chloride after diazotization and coupling, but prior to dilution to volume. The final pH was ≈1.4 in these experiments.

It is clear that salt (sodium chloride) concentrations in excess of 100 mg. per 50 ml. must be avoided. This restriction is satisfied in the assay procedure, where the salt content is several milligrams each of sodium chloride, ammonium sulfamate, and coupling agent, as well as in the experiments performed to determine extinction coefficients, where the maximum sodium chloride content was ≈23 mg. per 50 ml. at the highest sulfaquinoxaline level investigated. The existence of the salt effect contributed to the decision to add hydrochloric acid of fixed (0.50 *N*) concentration to a neutral or slightly alkaline solution, in order to ensure the desired pH range and to minimize the salt content. By so doing, the electrolyte content and resulting ionic effects (*6*) were maintained at a low and relatively constant level.

APPLICATIONS TO SULFAQUINOXALINE, WITH AND WITHOUT INORGANIC DILUENTS

The procedure adopted for routine evaluation of commercial preparations of the drug with and without added inorganic dilu-

Table III. Effect of Salt Concentration on Color Intensity

	Optical Density (1-Cm. Cells)	
NaCl, Mg./50 Ml.	Concentration, 0.101 mg./50 ml.	Concentration, 0.253 mg./50 ml.
50	0.355	0.855
100	0.350	0.850
300	0.349	0.810
600	0.334	0.800
1200	0.321	0.750
2400	0.281	0.500[a]

[a] Density variable owing to precipitation of azo dye.

ents was slightly modified by diminishing the quantity of alkali employed to dissolve the sample. This obviated the necessity for neutralizing the sulfaquinoxaline solution prior to acid addition, inasmuch as dilution of the stock solution of sample, which preceded acidification, was in itself sufficient to reduce the pH almost to 10. Accordingly, the reagents required for actual assay purposes are identical with those listed for the spectrophotometric study except for the 0.02 *N* hydrochloric acid which was eliminated.

Procedure. A 0.1-gram sample (*w*) of sulfaquinoxaline is weighed and dissolved in 100 ml. (volumetric flask) of water containing 2 to 10 ml. of 0.5 *N* sodium hydroxide. A 4-ml. aliquot is diluted to 50 ml. and a 2-ml. aliquot of this diluted solution is pipetted into a 50-ml. volumetric flask for color formation and measurement as directed in the spectrophotometric study. In the case of sodium and magnesium salts of sulfaquinoxaline, which are extremely water-soluble, alkali is entirely unnecessary. Accordingly, a 0.1-gram sample of salt is dissolved directly in water in a 100-ml. volumetric flask. Thenceforth, dilutions and color formation are effected exactly as described above for sulfaquinoxaline. As regards mixtures of sulfaquinoxaline with inorganic components, the size of aliquots was varied with the sulfonamide content. For example, the 25% mixtures reported below were treated in the normal fashion except that 5-ml. aliquots were used for both the dilution and color formation stages instead of the 4-ml. and 2-ml. aliquots, respectively, specified above for undiluted sulfaquinoxaline. The only effect is to diminish the numerical factor required to convert density to percentage sulfaquinoxaline.

Calculations. Deviations from a linear color intensity *vs.* concentration relationship are serious enough to warrant the use of a graph or tabulation of extinction coefficients versus optical density (Table I).

The sulfaquinoxaline content (% SQ) of a sulfaquinoxaline sample can be calculated from the expression

$$\% \text{ SQ} = \frac{\text{d}}{w \times E^{1\%}_{1\text{ cm.}}} \times 31{,}250$$

if the recommended procedure is followed. Modification will alter the value of the numerical factor. The particular value of $E^{1\%}_{1\text{cm.}}$ depends on the sulfaquinoxaline content, hence on the optical density, d, of a given solution and is obtained by graphical interpolation of the data in Table I. In the case of salts, the % NaSQ or % Mg(SQ)_2 is obtained by multiplying per cent sulfaquinoxaline by the factors 322.2/300.2 = 1.073 and 311.2/300.2 = 1.037, respectively, to correct for molecular weight differences. For the 25% sulfaquinoxaline mixtures, the numerical factor is 10,000. If an instrument of lower resolving power is employed, the extinction coefficients should be checked with a sample of pure sulfaquinoxaline.

Results. The applicability of the method was tested with a number of sulfaquinoxaline preparations, the purity of which had been determined by solubility analysis in acetone. Two salts of relatively high purity, one a sodium and the other a magnesium salt, as well as a number of mixtures of sulfaquinoxaline (25.0% level) with various inorganic diluents were also analyzed.

All results are compiled in Table IV.

The sulfaquinoxaline samples taken for this comparison are not necessarily typical of the current commercially available product; they were chosen merely because their purity had been evaluated by an independent means. The salts were laboratory preparations of fairly high purity, although no independent test of purity was made. The somewhat low (by ≈3%) values are due in part to the difficulty of removing quantitatively the volatile matter present in these salts. The mixtures contained 25.0% sulfaquinoxaline besides the following additional ingredients: mixture A, 75.0% limestone; B, 74.9% limestone and 0.1% potassium iodide; C, 73.9% limestone and 1.1% manganous sulfate; D, 73.8% limestone, 1.1% manganous sulfate, and 0.1% potassium iodide. Mixtures E, F, G, and H were similar to A, B, C, and D, respectively, except that feed grade rock salt was substituted for the limestone. The limestone presents no problem because it does not hinder the solution of sulfaquinoxaline in the alkaline stock; and whatever fraction is transferred with the aliquot dissolves in acid. Furthermore, the total concentration of added salt is too slight, because of extensive dilution prior to color development, to affect the color intensity significantly. This effect may become serious in mixtures containing ≈0.1% or less of sulfaquinoxaline.

Table IV. Test of Spectrophotometric Method

Sample No.	Substance	% Sulfaquinoxaline: Spectrophotometric	% Sulfaquinoxaline: Solubility analysis	Deviation[a], % SQ
1	Sulfaquinoxaline	99.7	96.8	+2.9
2		98.2	96	+2.2
3		93.9	93.2	+0.7
4		92.3	93.7	−1.4
5		92.7	95.7	−3.0
6		85.6	86	−0.4
7		90.5	88	+2.5
8		99.6	97	+2.6
9	Sodium salt	96.7[b]	..	...
10	Magnesium salt	97.6[b]	..	...
A	25% sulfaquinoxaline in limestone, etc., mixtures	25.6	..	+0.6
B		24.4	..	−0.6
C		25.2	..	+0.2
D		24.9	..	−0.1
E	25.0% sulfaquinoxaline in rock salt, etc., mixtures	25.5	..	+0.5
F		25.1	..	+0.1
G		25.2	..	+0.2
H		24.0	..	−1.0

[a] Difference between spectrophotometric and solubility analysis values for undiluted sulfaquinoxaline; difference between spectrophotometric and make-up (25.0%) values for mixtures.

[b] Corrected for volatiles content of preparation. Values represent % NaSQ and % Mg(SQ)_2 in respective preparations.

APPLICATIONS TO POULTRY FEEDS AND FEED PREMIXES

The spectrophotometric method was also applied to mixtures of sulfaquinoxaline with poultry feeds. Analysis of these mixtures requires extraction of the sulfaquinoxaline prior to color formation. Tyler, Brooke, and Baker (*13*) have successfully extracted sulfaquinoxaline from poultry feed with alkali, and removed dispersed protein by addition of trichloroacetic acid. Because of the limited solubility of sulfaquinoxaline in acid solution, the application of their procedure to concentrated feed premixes requires the use of small samples, or a considerable dilution of the alkaline extract. This difficulty is not encountered if zinc sulfate is employed instead of trichloroacetic acid. Accordingly, in the authors' procedure, zinc sulfate was added to dilute alkali extracts to precipitate protein material. After filtration of the coagulated precipitate, the color was developed as directed above for alkali-soluble preparations. Thus, the only additional reagent employed in assaying for sulfaquinoxaline in feeds is a 1% solution of zinc sulfate heptahydrate.

Recovery tests were made by adding varying amounts of sulfaquinoxaline (in alkaline solution) to a typical commercial poultry feed, and subjecting these test mixtures to the assay procedure described below. Such tests were performed on preparations equivalent to 0.01, 0.1, 1, 5, and 15% sulfaquinoxaline. In every case, recovery was within 2% of the make-up. Assay results reported below show how successful the method is when applied to commercially prepared feeds and premixes containing sulfaquinoxaline in concentrations ranging from 0.0125 to 15%.

Procedure. An appropriate weight (*w* grams; see Table V) of sample is weighed out into a 250-ml. beaker; 100 ml. of water and 2 to 3 ml. of 0.5 *N* sodium hydroxide are added, and the mixture is stirred and tested for alkalinity with litmus paper. If the mixture is not alkaline, 1 ml. more of 0.5 *N* alkali is added, and the mixture is heated to boiling, and boiled gently for 1 to 2 minutes. It is cooled to room temperature and the beaker contents are transferred quantitatively to a 250-ml. volumetric flask, diluted to volume, and mixed well. Then an aliquot, *V* ml. (see Table V), is pipetted into a 100-ml. volumetric flask, and 10 ml. of the 1% zinc sulfate solution are added, diluted to mark, and mixed well. It is filtered through a suitable filter paper such as S. & S. No. 597 paper (9-cm.), and the first 10 ml. are discarded. If filtration is too slow, as is likely when 2- to 5-gram samples are used, the following procedure will prove helpful.

The 100 ml. of solution and protein precipitate are transferred to a 100-ml. graduate, which is placed in a beaker of hot water (≈60° to 70° C.). The graduate is removed after 1 to 2 minutes and let stand an additional 5 to 10 minutes. The protein precipitate will settle rapidly and the relatively clear supernatant liquid may be pipetted off and filtered. Again the first 10 ml. of filtrate are discarded. The volumetric flask itself may be immersed in the hot water bath if desired. The graduate is suggested because removal of the relatively clear supernatant liquid is somewhat facilitated. Pipetting of this supernatant liquid is recommended instead of simple decantation because the coagulated solid is easily redispersed by agitation.

An aliquot, *v* ml. (see Table V), of the filtrate is pipetted into a 25-ml. volumetric flask, and 2.5 ml. of 0.50 *N* hydrochloric acid and 2 ml. of 0.1% sodium nitrite solution are added and let stand for 3 minutes. Then 2 ml. of 0.5% ammonium sulfamate solution are added and let stand an additional 2 minutes. Finally 2 ml. of the *N*-(1-naphthyl)-ethylenediamine dihydrochloride solution are added and diluted to mark. The contents of the flask are swirled after addition of each reagent.

The optical density, d, of the colored solution is measured in a Beckman Model DU spectrophotometer at 545 mμ, using a reagent blank as reference. A very slight turbidity will be noticed occasionally in the final colored solution, particularly when dealing with very dilute mashes (0.01 to 0.1% sulfaquinoxaline), for which large samples are required. This turbidity may be corrected for by measuring the optical density of a blank prepared in the following manner. Another aliquot, *v* ml. of the filtrate, is pipetted into a 25-ml. volumetric flask, 2.5 ml. of 0.50 *N* hydrochloric acid and 2 ml. of the *N*-(1-naphthyl)-ethylenediamine solution are added, and diluted to the mark. The optical density of this solution is subtracted from the optical density of the colored sample solution to yield the corrected optical density of the latter.

Recommended Dilution Schedule. The above method is valid for sulfaquinoxaline contents varying from 0.01 to 15%, which involves a wide range of sample sizes and aliquots for dilution. In order to facilitate the production of a conveniently measured color intensity, a recommended schedule of sample weights and dilutions is listed in Table V.

Table V. Dilution Schedule

% SQ in Feed	Sample Weight, *w* Grams	*V*, Ml.	*v*, Ml.
0.01–0.1	5	50	10–15
0.1–0.5	2	25	10
0.5–1.0	1	25	10
1.0–5.0	1	10	4
5.0–15.0	1	10	2

Calculations. If the above procedure is followed, the sulfaquinoxaline content (in per cent) of the feed or premix is given by the equation:

$$\%\ \mathrm{SQ} = \frac{\mathrm{d} \times 625{,}000}{E^{1\%}_{1\,\mathrm{cm.}} \times w \times V \times v}$$

where $E^{1\%}_{1\,\mathrm{cm.}}$ is again the extinction coefficient corresponding to the measured optical density, and is obtained by graphical interpolation of the values listed in Table II.

Results. The spectrophotometric method was applied to the analysis of a large number of commercial poultry feeds and premixes containing sulfaquinoxaline. All samples were prepared in commercial mixing equipment and are representative of the levels being marketed. Average results of typical analyses (in triplicate) of nineteen different mixes furnished by eleven different manufacturers (referred to as A to K) are reported in Table VI.

Agreement between make-up reported by the manufacturer and assay values is very satisfactory; the maximum deviation observed is 9.6% for one of the 0.0125% feeds. Reproducibility of replicate assays is excellent, the maximum average deviation noted amounting to ±0.019% in a total sulfaquinoxaline content of 0.497%.

Table VI. Sulfaquinoxaline Assay of Commercial Feeds

Feed Base	% Sulfaquinoxaline Reported make-up	 Assay	%[a] Deviation
A	0.0125	0.0113 ± 0.0000	−9.6
B	0.0125	0.0125 ± 0.0002	0.0
C	0.0125	0.0116 ± 0.0002	−7.2
D	0.0125	0.0130 ± 0.0001	+4.0
K	0.0125	0.0137 ± 0.0002	+9.6
J[b]	0.0125	0.0130 ± 0.0003	+4.0
E	0.50	0.497 ± 0.019	−0.6
F	0.625	0.634 ± 0.015	+1.4
F	0.625	0.609 ± 0.015	−2.6
F	0.625	0.604 ± 0.009	−3.4
F	0.625	0.641 ± 0.011	+2.6
F	0.625	0.625 ± 0.002	0.0
F	0.625	0.632 ± 0.007	+1.1
J	1.0	0.961 ± 0.027	−3.9
G	2.50	2.60 ± 0.01	+4.0
A	4.68	4.31 ± 0.03	−7.9
I	10.0	10.4 ± 0.2	+4.0
G	10.0	9.65 ± 0.01	−3.5
H	15	15.2 ± 0.1	+1.3

[a] $\%\ \text{deviation} = \dfrac{\text{make-up}\ \% - \text{assay}\ \%}{\text{make-up}\ \%} \times 100.$

[b] Pelleted feed; run in duplicate only.

SUMMARY

A spectrophotometric method of analyzing sulfaquinoxaline preparations has been developed, and extinction coefficients have been established at a number of concentrations because color intensity is not strictly proportional to concentration under the conditions of color formation here recommended.

The effects of pH and salt concentration on the color intensity have been investigated and optimum conditions selected.

The method has been tested with sulfaquinoxaline preparations of approximately known purity, as well as with 25% sulfaquinoxaline mixtures with inorganic diluents.

Further application was made to the analysis of mixtures with poultry feeds and feed premixes. Such preparations required prior extraction with alkali and precipitation of protein with zinc sulfate. The concentration range covered successfully was 0.01 to 15% sulfaquinoxaline.

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Colorimetric Determination of Indene

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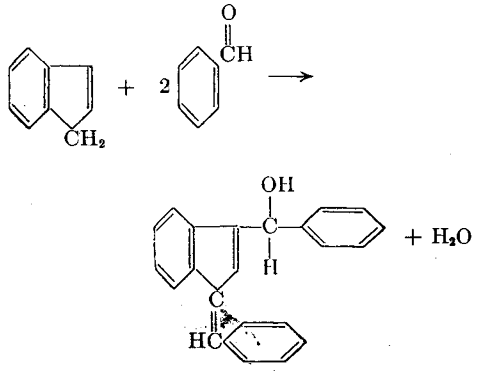
A method for the analysis of indene in hydrocarbon mixtures is described. The procedure involves reaction of the indene with benzaldehyde in the presence of alkali to produce a highly colored condensation product which is suitable for the colorimetric estimation of the indene. The proposed method is rapid, accurate, and reasonably specific.

IN CONNECTION with the preparation and investigation of certain aromatic compounds it became necessary to develop a method for the analysis of indene in the presence of other hydrocarbons. Attempts to utilize the ultraviolet absorption of indene for such an analysis were not successful because the absorption of other aromatic compounds present in the samples interfered. Hammick and Langrish (*1*) have suggested the use of bromine addition for the determination of indene, and indeed, excellent results were obtained when an electrometric method for the determination of bromine numbers was applied to some of the samples. However, in other cases, the analyses were not satisfactory because olefinic compounds in the samples also absorbed bromine, and for this reason a more specific method was sought.

Uhrig, Lynch, and Becker (*4*) have shown that the reaction between cyclopentadiene and carbonyl compounds in the presence of alkali to produce highly colored fulvenes can be used for the colorimetric determination of cyclopentadiene. Thiele (*3*) has observed that indene undergoes a somewhat analogous reaction although at considerably slower rates, and Mighill (*2*) has employed such a reaction with acetone as a qualitative test for indene in light oils.

Because this reaction appeared to offer a reasonably specific means for the desired analysis, the condensation of indene with a number of carbonyl compounds was investigated and, from the standpoint of reproducibility of color, benzaldehyde was found to to be the most satisfactory reagent. According to Thiele (*3*) the reaction produces 1-(α-hydroxybenzyl)-3-benzalindene, as is shown by the equation



The product imparts an intense yellow color to the solution, which is well suited for the colorimetric estimation of indene.

REAGENTS

Indene. Colorless; boiling point 180–181° C.; $n_D^{20} = 1.5768$.

Benzaldehyde Solution. Dissolve 5 ml. of U.S.P. grade benzaldehyde in 95% ethyl alcohol and dilute to 250 ml.

Alcoholic Potassium Hydroxide. Dissolve 3.0 grams of C.P. potassium hydroxide in 100 ml. of methanol. Heat to hasten solution. Allow to stand 2 hours and filter through a fine-fritted glass crucible.

APPARATUS

All measurements were made with a Beckman Model D.U. spectrophotometer using 1-cm. cells.

RECOMMENDED PROCEDURE

Preparation of Calibration Curve. Prepare a standard indene solution by weighing approximately 5 grams of indene to the nearest 0.02 gram into a 250-ml. volumetric flask and diluting to the mark with benzene. Transfer a 10-ml. aliquot to a 200-ml. flask and dilute to the mark with petroleum ether. This solution will contain approximately 1 mg. of indene per ml.

With a buret measure 1-, 3-, 5-, 7-, 9-, and 11-ml. portions of the standard indene solution into 50-ml. Erlenmeyer flasks. Add 20 ml. of the benzaldehyde solution and 5 ml. of the alcoholic potassium hydroxide. Prepare a blank with the same quantities of benzaldehyde and potassium hydroxide solutions. Bring the solutions to a slow boil and hold them at this temperature for 10 to 12 minutes. Cool to room temperature, add 1 to 2 ml. of glacial acetic acid, transfer to 50-ml. volumetric flasks, and dilute to the mark with 95% ethyl alcohol. Determine the optical density with 1-cm. cells at 420 mμ, correct the optical density readings for the blank, and plot the corrected values against the concentration of indene. A straight-line curve should result.

As an alternative procedure, the color may be developed at room temperature. In this case transfer aliquots of the standard indene solution to 50-ml. volumetric flasks, add the benzaldehyde and potassium hydroxide reagents, and allow the solutions to stand from 8 to 24 hours. Add 1 to 2 ml. of glacial acetic acid and dilute to the mark. The slope of the calibration curve ob-

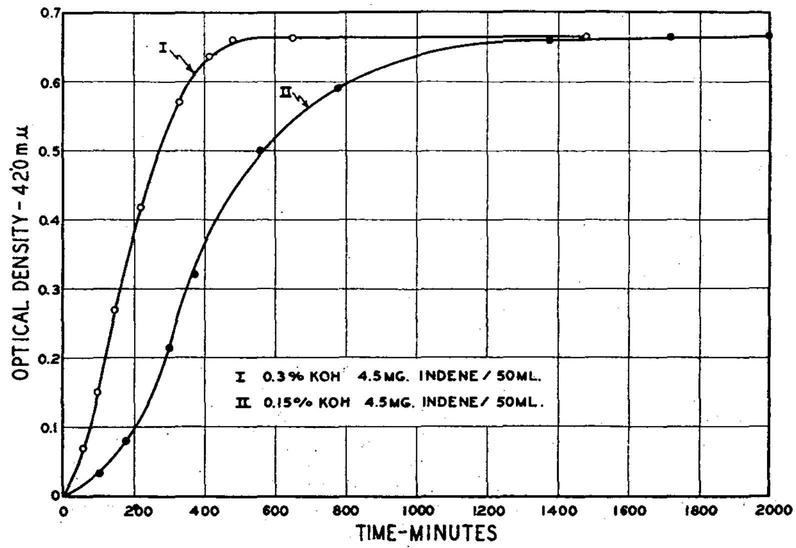


Figure 1. Rate of Color Development at Room Temperature

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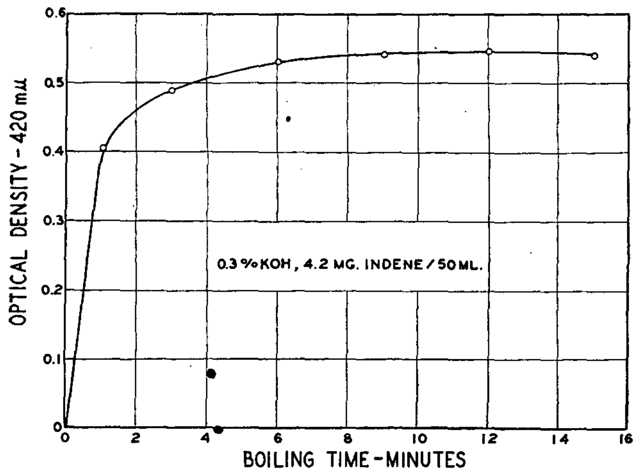

Figure 2. Rate of Color Development at Boiling Temperature

tained by this method will be slightly greater than that obtained by heating the solutions.

Analysis of Samples. Weigh out samples and dilute with benzene or petroleum ether to such a volume that the concentration of indene is between 0.2 and 2 mg. per ml. Take 5-ml. aliquots and treat in exactly the same way as the standards. A reagent blank should be run with each series of samples. Reference to the calibration curve gives the milligrams of indene in the aliquot.

DISCUSSION

The color obtained upon mixing benzaldehyde with indene in the presence of alkali is an intense yellow which has an absorption maximum in the ultraviolet region of the spectrum. However, the quantitative measurements described herein were made at 420 mμ because it was desired to have a method that would be adaptable to the use of visual photometers or colorimeters.

The rate of color development is relatively slow at room temperatures. For example, in the presence of 0.3% potassium hydroxide at least 8 hours are required to reach the maximum color, and 24 hours are required to reach the same color with 0.15% potassium hydroxide (Figure 1). Higher concentrations of alkali give more rapid reactions; however, these are to be avoided because excessive values for the blank are obtained as a result of side reactions of the benzaldehyde. The reaction rate can be markedly increased by raising the temperature. Thus at 40° C. 2 hours are required for maximum color, while at reflux temperatures full color is obtained in less than 10 minutes when the alkali concentration is 0.3%. This is illustrated in Figure 2.

The color produced by the reaction was found to follow Beer's law. However, the slope of the straight line relating optical density and concentration was somewhat less when the color was developed at the boiling temperatures of the solutions than when the reaction was carried on at room temperatures (Figure 3). From the standpoint of accuracy and reproducibility, the two methods of color development are equally satisfactory and both have been used for analyses.

The stability of the color was found to be excellent, particularly if the excess potassium hydroxide was neutralized with a little acetic acid after the reaction was complete. For example, solutions measured immediately after color development and after 72 hours showed no change in optical density.

In order to test the proposed method of analysis, a series of synthetic samples containing known quantities of indene was prepared and analyzed by the recommended procedure. There were included in some of the samples various hydrocarbons which it was thought might interfere with the analysis. The results from these tests are given in Table I; excellent results were obtained in each case. The only hydrocarbons which have been encountered which interfere with the procedure are cyclopentadiene and some of the methyl derivatives of indene. The presence of the former in a sample is indicated by the development of a yellow color within 3 minutes after adding the reagents and at room temperature. The methyl derivatives give a color which is nearly identical with the color formed with indene, and the proposed method has been used also for the analysis of these compounds in certain mixtures.

Table I. Analysis of Indene in Synthetic Samples

Approximate Composition of Diluent	Indene, % Present	Indene, % Found	Error, %
100% toluene	5.69	5.73	0.7
90% toluene, 10% naphthalene	2.58	2.61	1.2
32% toluene, 5% styrene, 63% benzene	1.89	1.87	1.1
74% toluene, 26% Tetralin	4.26	4.27	0.2
73% toluene, 27% cyclohexene	1.96	1.99	1.5
93% toluene, 7% fluorene	2.14	2.19	2.3

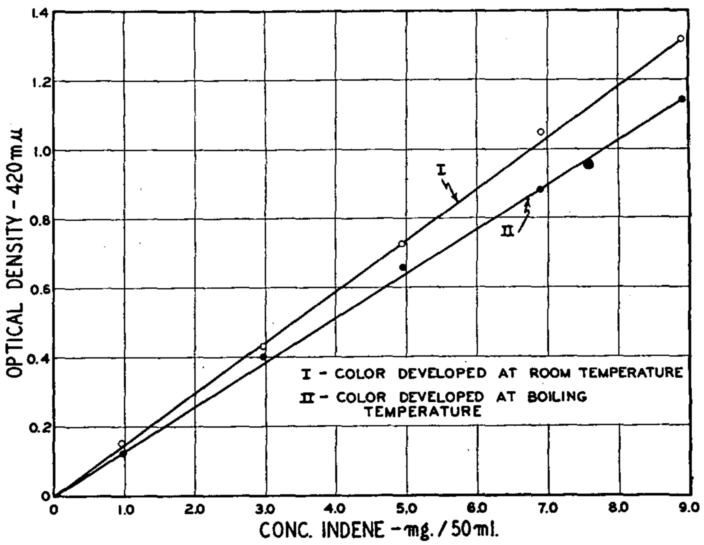

Figure 3. Calibration Curves for Indene Analysis

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Correction

In the article on "Determination of Cottonseed Oil on Tin Plate" [Donelson, J. G., and Neish, R. A., Anal. Chem., **21**, 1102 (1949)], Figure 2 on page 1104 was printed upside down. The numbers on the horizontal axis should have been 10, 20, 30, 40, 50, and not 10, 20, 40, 60, 80 as printed.

Determination of Vitamin A in Whale Liver Oils

Activated Glycerol Dichlorohydrin

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Activated glycerol dichlorohydrin has been applied to the determination of vitamin A in whale liver oils. The absorption curves of the color reaction were found to be identical in shape to those developed by pure vitamin A with the same reagent, which indicates a high degree of specificity of the reaction. This has been demonstrated by analyses of mixtures of vitamin A and purified kitol.

SEVERAL investigators have shown that whale liver oils give ultraviolet absorption curves with the maximum displaced toward wave lengths shorter than 325 mμ, which is characteristic of pure vitamin A (*1, 4, 6, 7, 10, 11, 13*). It has been demonstrated that this is caused by the presence of kitol (*5, 8*), which has an absorption maximum at approximately 285 mμ. Therefore, vitamin A cannot be determined directly in these oils by the commonly used ultraviolet spectrophotometric method (*3, 9*). Sobel and Werbin (*12*) have applied glycerol dichlorohydrin to the determination of vitamin A. Their results were completely verified by the author.

Studies are reported here in which activated glycerol dichlorohydrin (activated GDH, J. B. Shohan Laboratories, Newark, N. J.) has been applied to the determination of vitamin A in whale liver oils. All investigated samples gave colors with absorption curves identical with those developed by pure vitamin A and the same reagent (Figure 1). This indicates that activated glycerol dichlorohydrin reacts specifically with the vitamin A in these oils.

For this study a standard curve for vitamin A was made using vitamin A natural ester concentrates, Control Nos. PC-3 and PC-4, from Distillation Products, Inc. Vitamin A content is expressed in micrograms, calculated on the following basis: pure vitamin A alcohol (Distillation Products, Inc., Control No. K-842) gave an average $E^{1\%}_{1\text{cm.}}$ (325 mμ) value of 1756 in absolute ethanol. In the same solvent PC-3 and PC-4 gave, respectively, 100.75 and 158.4, which confirmed data supplied by the producers. Using the assumption that the absorption measured is entirely attributed to vitamin A, this gives a content of 5.74% vitamin A in PC-3 and 9.07% in PC-4. Figure 2 shows the standard curve of the color reaction. Six different weighings were made and diluted in chloroform (U.S.P. grade), and measurements were taken on the Beckman spectrophotometer at wave length 555 mμ. All dilutions were made, and all colors developed and measured at 25° C.

As kitol causes the principal interference in the ultraviolet absorption curves of whale liver oils, special studies were carried out on purified kitol (Distillation Products, Inc., Control No. B641). It gave an absorption curve in the ultraviolet (Figure 3) with absorption maximum at approximately 285 mμ, and corresponding $E^{1\%}_{1\text{cm.}}$ value of 465 in absolute ethanol, and 469 in isopropyl alcohol. In cyclohexane the absorption maximum was found at 287.5 mμ, and an $E^{1\%}_{1\text{cm.}}$ value of 461. (Each value is the average of three different weighings.) As pure kitol is reported (*2*) to have an $E^{1\%}_{1\text{cm.}}$ (290 mμ) value of 707, it indicates approximately 60% purity of the analyzed sample. Only in strong concentration could a color be obtained with activated glycerol dichlorohydrin, giving an $E^{1\%}_{1\text{cm.}}$ (555 mμ) value of approximately 3. That no consistent curve could be plotted between 400 and 700 mμ suggests that the developed color was caused by small amounts of impurities, rather than by kitol itself. For further test on noninterference with the color reaction, mixtures of vitamin A and kitol were analyzed. Table I shows that kitol does not interfere with the color reaction.

It has been confirmed that cholesterol, which is present in fairly large amounts in whale liver oils, does not interfere with the color reaction (*10*).

Analyses were carried out on eight samples of whale liver oils, some of them concentrates. Weighed portions of the oils were

Table I. Analyses of Mixtures of Vitamin A and Kitol with Activated Glycerol Dichlorohydrin

Mixture No.	Vitamin A	Kitol	Total	Vitamin A Found	% of Theoretical
	Micrograms per ml.				
1	6.45	0	6.45	6.45	100.0
2	6.45	13.95	20.40	6.40	99.3
3	6.45	27.90	34.35	6.45	100.0
4	6.45	55.80	62.25	6.42	99.6

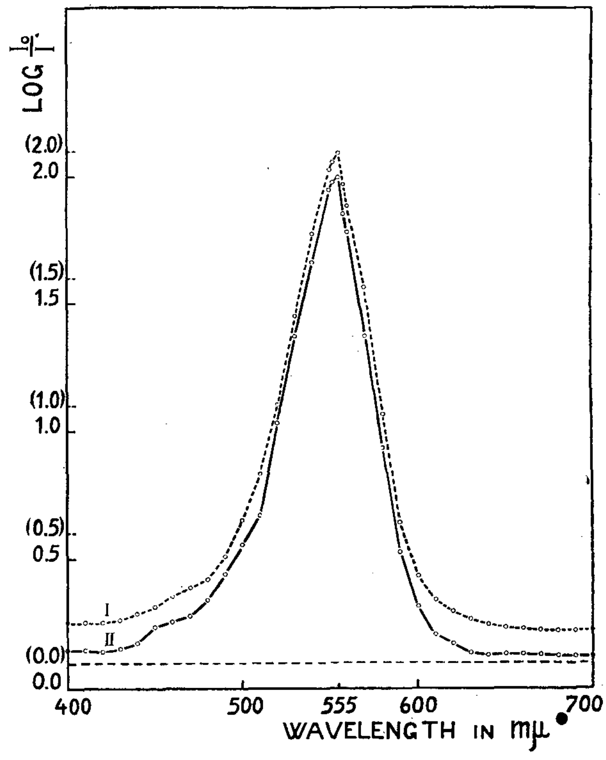

Figure 1. Absorption Curves

I. Pure vitamin A
II. Whale liver oil

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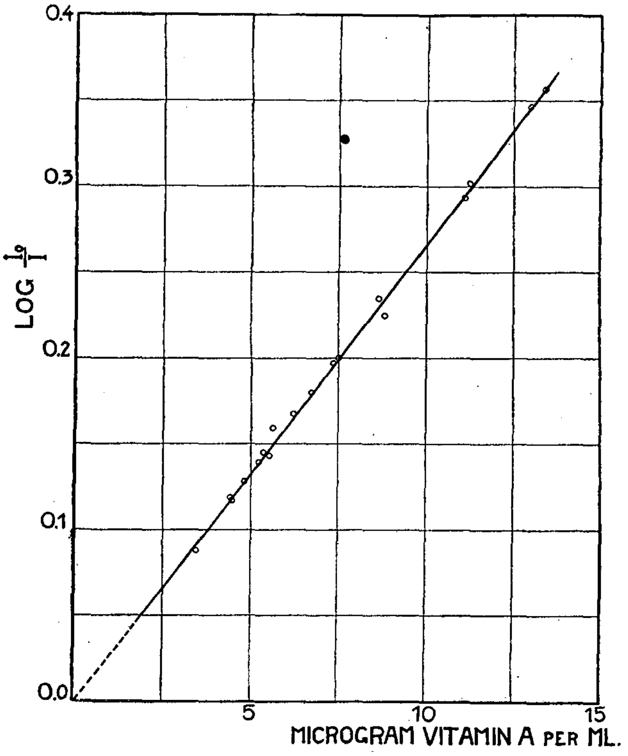

Figure 2. Standard Curve for Vitamin A

Table II. Determination of Vitamin A in Whale Liver Oils

(Activated glycerol dichlorohydrin compared with ultraviolet measurements)

	Spectrophotometric Detns.					
Oil No.	Absorption maxima, mμ	$E^{1\%}_{1cm.}$ (325 mμ)	*Mg./g.*	Color Detns. *Mg./g.*	Differences *Mg.*	Differences %
1	315	99.0	56.74	49.93	6.81	11.7
2	315	100.5	57.23	48.84	8.39	14.7
3	310	297.0	169.13	128.21	40.92	24.2
4	315	173.0	98.52	81.76	16.76	17.1
5	315	170.5	97.10	84.96	16.14	12.5
6	308	29.0	16.52	11.56	4.96	29.9
7	314	61.75	35.17	28.02	7.15	20.3
8	315	75.5	42.99	36.65	6.34	14.7

dissolved in ether, two equal portions were pipetted out, the ether was evaporated in vacuum, and suitable dilutions were made up in absolute ethanol and chloroform. Dilutions containing between 5 and 8 micrograms were used, allowing easy checking of the absorption curves of the developed colors. All samples were run in duplicate. Table II summarizes the results of ultraviolet and color readings from the investigations.

Table II shows that the values obtained by using activated glycerol dichlorohydrin are 10 to 30% lower than those obtained by the spectrophotometric method based on $E^{1\%}_{1cm.}$ (325 mμ). In the same table are also shown the absorption maxima of the different samples of whale liver oils. The displacements range from 10 to 17 mμ below 325 mμ, which is the characteristic wave length for the absorption maximum of vitamin A. If kitol were the only irrelevant absorbing substance, the displacements should give good criteria for the correction of the ultraviolet absorption readings at 325 mμ. The relation between absorption maximum and correction could be worked out either experimentally by analyzing mixtures of pure vitamin A and kitol, or theoretically based on the known absorption curves and constants for both substances. The differences obtained between the methods are higher in the present analyses than expected theoretically. This indicates a considerable irrelevant absorption in addition to that caused by kitol, but this irrelevant absorption in the ultraviolet spectrum does not seem to interfere with the color reaction. This should justify the conclusion that the determinations by the use of activated glycerol dichlorohydrin give the true values of the vitamin A content of whale liver oils.

The results will be further studied by biological experiments.

SUMMARY

The use of activated glycerol dichlorohydrin provides a valid method for the determination of vitamin A in whale liver oils. Kitol and some closely related compounds do not interfere with the reaction.

ACKNOWLEDGMENT

The author wishes to express his thanks to J. G. Baxter for the samples of kitol provided for this work, and to E. M. Nelson, Vitamin Division, Food and Drug Administration, for allowing checking analyses to be carried out in his laboratories.

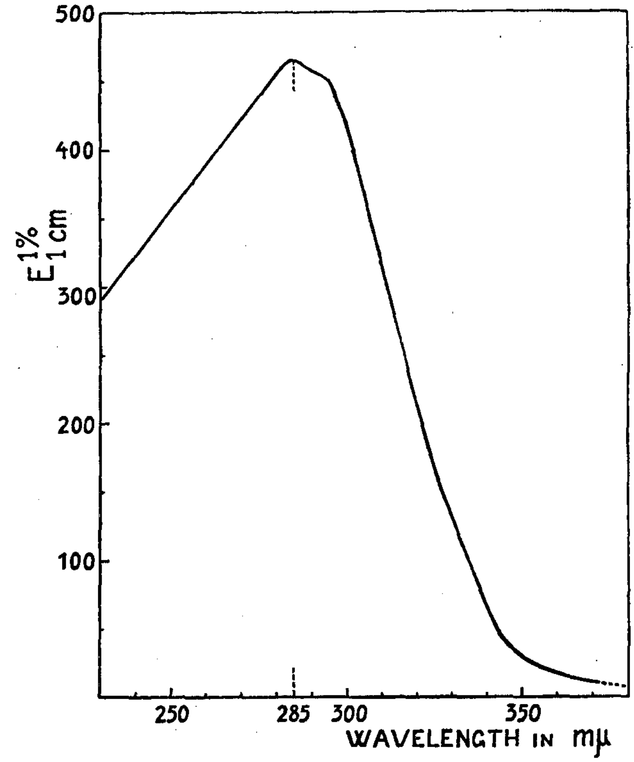

Figure 3. Absorption Curve of Purified Kitol

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Magneto-optical Rotatory Power of Hydrocarbons and Their Mixtures

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An apparatus for determination of magneto-optical rotatory power is described. The Verdet constants for Na 5893 Å. and Hg 5461 Å. and the dispersion in the visible region are given for a number of hydrocarbons. The apparatus is used to investigate whether magnetic rotation can be used for the analysis of hydrocarbon mixtures. It is shown that in hydrocarbon mixtures magnetic rotation is an additive property when change in density on mixing is small. A method involving rotation and refractive index is proposed for analysis of ternary mixtures.

SINCE the discovery of magneto-optical rotatory power by Faraday it has been established that all transparent isotropic substances show this effect. Magnetic rotation differs from natural optical rotation in that if a beam of plane polarized light is passed through an optically active medium and then is reflected back through the material, the rotations of the outgoing and returning light beams will oppose and cancel each other. Magnetic rotation, on the other hand, is dependent only on the direction of the magnetic field and not on the direction of the light; therefore if the light is reflected back to retrace its path through the substance, the resultant rotation will have twice the original value. In hydrocarbons the direction of rotation is positive—i.e., in the same direction as the amperian current producing the field. The angle of rotation, α, is given by Verdet's law, which states that

$$\alpha = VHl \cos \theta$$

where V = the Verdet constant, H = the strength of the magnetic field, l = the length of the light path in the medium, and θ = the angle between the direction of the light path and the direction of the lines of force.

The Verdet constant, V, usually expressed in minutes per gauss centimeter, is a characteristic of the substance for a particular temperature and wave length.

The effect of temperature on rotation of diamagnetic molecules is small and a rise in temperature is invariably accompanied by a fall in rotation. Rodger and Watson (*10*) have shown that for water, between 0° and 98° C.

$$V_D^t = 0.01311\ (1 - 3.05 \times 10^{-5}t - 3.05 \times 10^{-6}t^2)$$

and for carbon disulfide between 0° C. and its boiling point

$$V_D^t = 0.04347\ (1 - 0.001696t)$$

where V_D^t is the Verdet constant for Na 5893 Å. at t° C.

In the homologous series the effect of temperature usually increases with the molecular weight and is more pronounced in aromatics than in aliphatics (*7*).

The first systematic investigations on magnetic rotation of hydrocarbons were carried out by Perkin (*8*), later by Scherer (*11*), and Gabiano (*4*), and recently by Waterman and his collaborators (*1*) and Foehr and Fenske (*3*). Hydrocarbons have been studied primarily in connection with constitution and the contributing magnetic rotation of various hydrocarbon groups; such observations have been almost entirely restricted to a single wave length and thus relatively little information on the rotatory dispersion of hydrocarbons has appeared in the literature. The magnetic rotation of multicomponent hydrocarbon mixtures has not been subjected to a systematic investigation, although Waterman and his co-workers (*1*) have examined the rotation of binary hydrocarbon mixtures for Na 5893 Å.

It was therefore decided to determine the magnetic rotatory dispersion of hydrocarbons over a wide range of wave lengths including the ultraviolet, using a uniform magnetic field, and to investigate the possibility of using magnetic rotation as a method of analysis of hydrocarbon mixtures. In the present paper the rotatory dispersion—i.e., the change in Verdet constant with wave length—of a number of paraffins and naphthenes in the visible region is reported together with the Verdet constants of a number of paraffins, naphthenes, and aromatics for Hg 5461 Å. In addition, the magnetic rotations of a number of two-, three-, and four-component hydrocarbon mixtures have been examined and a scheme is proposed for the analysis of such mixtures.

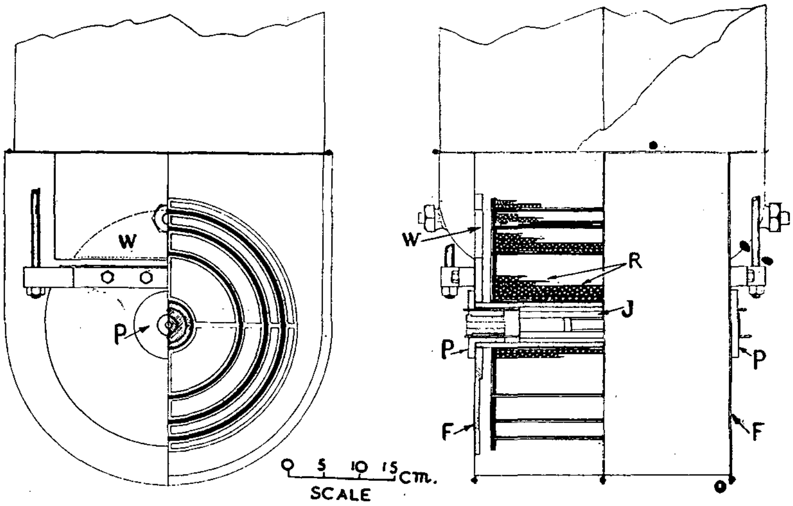

Figure 1. Solenoid

F. **Flange**
J. **Water jacket**
P. **Mild steel plug**
R. **Tufnol spacing rods**
W. **Flange window**

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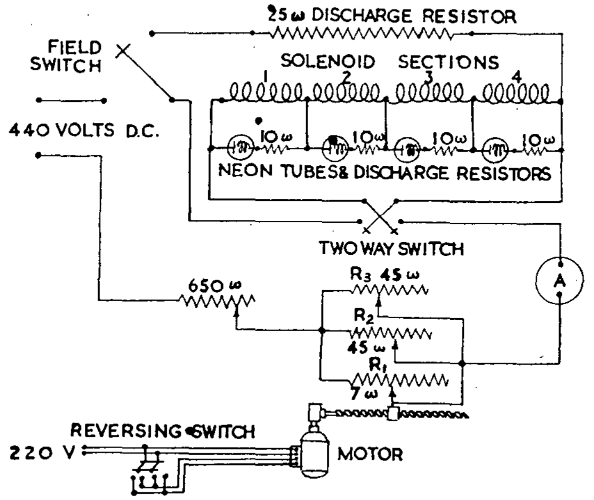

Figure 2. Electrical Circuit

THE SOLENOID

The solenoid, a cross section of which is shown in Figure 1, is wound with No. 14 S.W.G. (0.080-inch diameter) enameled copper wire on four concentric brass bobbins. The wire has a length of 3 miles and is shared among the bobbins so that they may be put in parallel, if need be, to increase the field strength. Furthermore, in case of a breakdown in the insulations the entire windings need not be unwound.

Prior to winding, each bobbin was given two coats of insulating varnish, baked at 150° C. for 1 hour, followed by two thicknesses of 0.005-inch (0.0127 cm.) oiled linen and a further coat of varnish, and baked again. The bobbin was then mounted on wooden formers and held in a heavy lathe which was geared down to 10 r.p.m. Every layer was given an adequate coat of insulating varnish and wrapped with one thickness of oiled linen, except that the linen was not applied where the layer forms part of a cooling duct. The cooling ducts, numbering about 1100, are formed by placing Tufnol rods (insulating rods of fiber impregnated with a thermosetting resin), *R*, of cross section 0.2 × 0.4 cm. on every other layer at a distance of 1 cm. from one another as shown in Figure 1. Mild steel flanges *FF*, 0.5 inch thick, have a diameter of 16 inches and are screwed on the brass cylinder core of the solenoid. This brass cylinder is 15.25 inches long and 0.25 inch thick and has an inside diameter of 2.25 inches. A window, *W*, is cut in the upper part of each flange, while the lower part is partially milled to assist the inflow of air. Plugs, *PP*, one at each end of the solenoid, render the magnetic field uniform and were specially designed as described below. Five iron-constantan thermocouples insulated by empire sleevings are placed in suitable parts of the solenoid inside the air ducts. Other characteristics of the solenoid are:

Length of windings	32 cm.
Diameter of 1st layer	7.5 cm.
Diameter of last layer	38 cm.
Number of layers	45
Total number of turns	6713
Resistance at room temp.	24.3 ohms
Number of air ducts	1118

The solenoid is suspended from the ceiling of the laboratory by four 0.5-inch steel bars and the main inlet and outlet air ducts are bolted to the sheet metal covering of the solenoid. The air, provided by a blower, circulates through the coil at the rate of 670 cubic feet per minute at 17° C. with a pressure drop of 10 cm. of water across the coil. As the value of the average friction factor, $(R/\rho v^2)$, and other characteristic constants are not known for ducts of the kind used in the solenoid, some experimentally determined values may be found of interest. From the expression for the pressure drop (*13*)

$$P = K\left(\frac{R}{\rho v^2}\right)\frac{L}{d}\frac{\rho v^2}{2g}$$

where P = pressure drop in pounds per square inch
K = 0.0538
R = frictional force per unit area of wetted pipe surface
ρ = specific gravity relative to air
v = velocity in feet per second
L = length of cooling duct, inches
d = diameter of duct in inches
g = acceleration due to gravity in feet per second squared

the average friction factor for a single duct inside the solenoid was calculated to be 0.00923. The average Reynolds' number in each duct obtained from the results of a number of tests was over 2300, indicating that the flow is conveniently within the turbulent régime. The average temperature of the wire at 15 amperes, with air entering at 17° C., was calculated from the results of resistivity measurements, and was found to be 37° as against 40° C., the highest recorded by the thermocouples. The value of over-all coefficient of heat transfer for the air ducts obtained from thermal tests on the solenoid was found to have an average of 10 B.t.u./hr./ft.^{2}/° F. mean temperature difference.

Electrical Circuits. In Figure 2 the coil is shown in four separate windings and in series with the ammeter, *A*, and parallel resistances R_1, R_2, and R_3. The coarse resistances, R_2 and R_3, are of 18 S.W.G. resistance wire, 45 ohms each, and are roughly set by hand, while R_1, made of 13 S.W.G. resistance wire and of 7 ohms' resistance, is used for fine control and its heavy contact is driven on a threaded shaft (12 threads per inch) by means of a reversible motor geared down to 58 r.p.m. and operated by hand. The ammeter, a precision instrument, is equipped with a 6-inch mirrored scale reading from 0 to 20 amperes and divided into 100 divisions. The scale is viewed under a large magnifying lens, so that it may be read to 0.01 ampere.

The solenoid is provided with a suitable field switch and a discharge resistor of about the same resistance as the coil. On disconnection, the discharge circuit is completed by auxiliary contacts when the switch is nearing the off position. Furthermore, prior to disconnection the current is always reduced to a low value by means of a variable resistance. For protection against an accidental break in the supply lines, each of the bobbins is put in parallel with a neon tube (with the base resistance removed) and in series with a 10-ohm resistor.

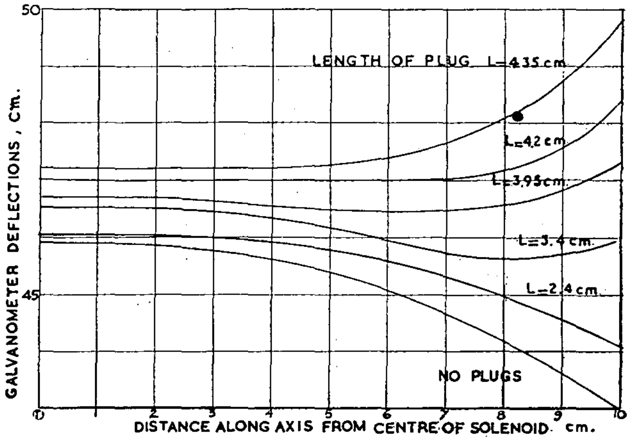

Figure 3. Uniformity of Magnetic Field in Solenoid

Calibration of Magnetic Field. The mild steel plugs, *PP*, were placed at the ends of the solenoid as shown in Figure 1. The center portion of each plug can be withdrawn for the insertion of the polarimeter tube into the water jacket. A $^5/_{16}$-inch hole was drilled in the center of each plug to admit passage of light. The magnetic field was scanned with plugs and water jacket in position, by means of a specially made search coil and a ballistic galvanometer. The search coil was capable of rotating through 180° about an axis perpendicular to the lines of force. Repeated scanning was followed by machining of the plugs until the magnetic field was uniform over a length of 12.5 cm. along the axis of the solenoid.

The effect of length of the plugs upon the uniformity of the field is shown in Figure 3. For the calibration of the strength of the magnetic field, it was checked against that of a permanent magnet, which was in turn calibrated against a standard solenoid. The number of line linkages for the permanent magnet and its search coil were thus measured to be $(223 \pm 1.5)10^3$ Maxwell turns as against $(227 \pm 1)10^3$ Maxwell turns obtained by the National Physical Laboratories for the same magnet and search coil. Giving these two results their respective weights, the intensity of the magnetic field along its uniform portion was found to be 245.1 ± 1.9 gauss per ampere. The rotations of conductivity water and carbon disulfide were measured using this value

Table I. Verdet Constants for Water and Carbon Bisulfide

Source	Temp., ° C.	V_D^t	$V_D^{\circ a}$
		Water	
This work	20	0.01308	0.01309
Aron	23	0.01295	0.01298
Rodger and Watson	0	...	0.01311
Siertsema	13.4	0.01302	0.01303
Agerer	18	0.01309	0.01310
Roberts, Wallace, and Pierce (*9*)	20	0.01308	0.01309
		Carbon Disulfide	
This work	20	0.04202	0.04340
Becquerel	0	0.04341	0.04341
Rayleigh	18	0.04202	0.04335
Koespel	18	0.04199	0.04332
Rodger and Watson	0	...	0.04347
Stoyanoff	14	0.04239	0.04344

[a] Values of V_D° are compiled from equations of Rodger and Watson (*10*).

of the magnetic field intensity. The results of the determinations are compared with the literature values (*12*) in Table I.

The Polarimeter. This is a Hilger instrument, the circle of which can be read to 0.01° and estimated to 0.005° by means of verniers.

The optical system is a three-field Lippich type with Foucault prisms and a variable 0° to 15° half-shadow angle. The polarimeter tubes are straight heavy-walled Pyrex tubings, on the ends of which disks of good quality microscope cover slips are sealed. The end plates are protected by brass caps which fit inside the water jacket and act as guides to keep the tube concentric with the solenoid. The tubes are placed in the uniform portion of the field and always in the same position by means of suitable stops. In preliminary experiments with polarimeter tubes having loose end plates it was found that a slight strain due to the screw-on brass caps produced birefringence and increased the rotation of the end plates to such an extent that reproducible results could not be obtained; variations due to the end plates were avoided in the arrangement adopted.

Sources of Light. All major visual observations have been made for Hg 5461 Å., which was adopted because of the higher rotations, and the greater sensitivity of the eye, for green than for yellow Na 5893 Å., which has been used extensively in the literature.

The lamps used were a Hanovia mercury vapor lamp and a sodium Osira lamp. For photographic method of dispersion measurements, a high-tension spark operating at 15,000 volts was used with a 0.005 µf. condenser in parallel with the electrodes and a radio interference suppressor directly between the mains and the transformer. The spark, although of lower intensity, was preferred to the arc on account of its ease of control and low electrode consumption which made frequent resetting and realigning of the electrodes unnecessary. To obtain a spark of favorable intensity in the visible region, a number of easily obtainable metals were examined and it was found that lead electrodes cast from lead water pipes gave good results. Some of the lines due to impurities such as iron were found of advantage.

Other apparatus used consisted of a Hilger constant-deviation spectrometer which could be fitted with a $^1/_4$-plate camera. A Pulfrich refractometer and a pycnometer were used for determination of refractive index and density respectively.

Temperature Control. The water jacket, *J*, consists of three concentric brass pipes fitted together as shown in Figure 1. The outer pipe is machined to fit tightly in the solenoid, while the inner pipe has a bore of about 2 cm. to accommodate the observation tube. Cold water from a tap circulates, when required, in the outer jacket carrying away the excess heat of the solenoid which was maintained by air cooling at a safe temperature not exceeding 50° C., depending on the temperature of the inlet air. Water from a thermostatically controlled bath circulates countercurrently in the inner jacket which surrounds the observation tube. The inlet and outlet pipes are brought out through holes drilled in the mild steel plugs.

All experiments were carried out at 20° ± 0.2° C., the temperature being read upon entering and leaving the water jacket.

Experimental Procedure. The polarimeter tube, prior to each experiment, was left in the water jacket for at least 10 minutes to reach the temperature of the jacket. The tube was always placed in exactly the same position and in the center of the uniform portion of the magnetic field, care being taken that the tube was not changed end for end and that it was not rotated about its axis throughout the experiments. To facilitate calculations the value of the current was kept constant throughout each set of experiments, at either 15 or 16 amperes, depending on the value of main voltage prevailing at the time of the experiment. The current was read from the ammeter by means of a large magnifying lens and controlled by hand as already described to ±0.02 ampere.

The light source, *S*, Figure 4 is placed in alignment with the optical axis of the polarimeter and is focused by the condenser lens, *C*, on the objective, *O*, of the observing telescope, *OE* (the usual condition in polarimetry). Condenser *C* is followed by the three-field Lippich polarizing system, *P*. The diaphragm, *D*, limits the beam of light entering the polarimeter tube, *T*, and this prevents stray illumination by internal reflections, while diaphragm *G* limits the undesirable effects of such reflections and prevents them from entering the eyepiece, *E*. The low-power telescope, *OE*, follows the analyzer, *A*, and by adjusting *E* the edges of the Lippich prisms are brought into focus. In visual and photographic work using the constant-deviation spectrometer, *E* was replaced by a quartz-fluorite lens by means of which an image of the three-field system was thrown on the slit of the spectrometer. A half shadow angle of 3° and wide spectrometer slit were used for the visual determination of rotations for mercury and sodium lines.

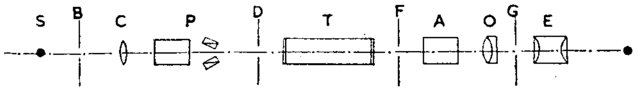

Figure 4. Optical System

A.	Analyzer	*P.*	Polarizing system
BDFG.	Diaphragms	*S.*	Light source
C.	Condensing lens	*T.*	Polarimeter tube
OE.	Telescope		

In photographic determination of rotatory dispersion the spectrometer telescope was replaced by a camera, a narrow slit and large half-shadow angle were used, and a number of photographs for various settings of the polarimeter circle, usually at 0.05° intervals, were taken. Ilford HP3 Panchromatic plates were used and processed as prescribed by the makers. The rotations have been determined for both senses of the field, whereby the magnitude of rotations is doubled. Because of proximity of optical parts of the polarimeter to the solenoid it was found that the stray magnetic lines of force produced in these parts a rotation of about 0.1° for Hg 5461 when the field inside the solenoid was 3800 gauss. This rotation and that due to end plates were, however, measured together at all wave lengths and used as zero correction. No corrections for pole pieces were necessary, as the solenoid was calibrated with them in position.

ERRORS OF DETERMINATION

Verdet's law may be written as

$$V = \frac{\alpha}{Hl} \times \frac{1}{\cos\theta}$$

Angle α may be read to 0.01° and the current maintained at 15 or 16 amperes and controlled to ±0.02 ampere. The length of the polarimeter tube is measured with a micrometer to better than 0.0025 cm. There is no significant error in the measurements due to θ with the present arrangement of apparatus, for the position of the polarimeter tube with respect to the axis of the solenoid is rigidly fixed and a lateral displacement of the optical axis of the polarimeter cannot be undetected owing to the presence of fixed diaphragms. The error due to the variation of ±0.2° C. in the temperature is negligible.

Partially differentiating the above expression and neglecting the effect of cos θ,

$$dV = \frac{d\alpha}{Hl} - \alpha\frac{dH}{H^2l} - \frac{\alpha dl}{Hl^2}$$

Dividing both sides by V and giving all terms similar signs,

$$\frac{dV}{V} = \frac{d\alpha}{\alpha} + \frac{dH}{H} + \frac{dl}{l}$$

since $VHl = \alpha$.

If l be taken as 10 cm. and α as 7° for an average hydrocarbon at 4000 gauss for Hg 5461 Å., because H is directly proportional with current,

$$\frac{dV}{V} = \frac{0.01}{7} + \frac{0.02}{15} + \frac{0.0025}{10} = \pm 0.27\%$$

As the rotations increase, the errors will tend toward a limit which is almost entirely due to variations in the current. The errors discussed above are the errors of a single determination. However, in calculation of absolute Verdet constant for theoretical studies the absolute value of the field intensity as well as its fluctuations plays an important part. Therefore, taking into account the errors of measurements as well as the deviation in the absolute value of the field intensity, the Verdet constants reported in this work may have a deviation of ±1.1% from their absolute values.

EXPERIMENTAL RESULTS

The magnetic rotatory dispersions for the range 4000 to 6000 Å. have been determined by the spark photographic method for ten hydrocarbons. The properties of these hydrocarbons are given in Table II.

The dispersions of these hydrocarbons, with the exception of *n*-hexane (*6*) and *n*-heptane (*2*), do not appear to have been previously reported in the literature. The Verdet constant–wave-length dispersion curves are given in Figures 5 and 6 and the values of Verdet constant for a number of wave lengths are given in Table III.

Table II. Physical Constants of Paraffins and Naphthenes Used in Dispersion Measurements

Compound	Boiling Point, ° C.	Freezing Point, ° C.	d_4^{20}	n_D^{20}
n-Pentane	36.15	...	0.6259	1.3576
n-Hexane	68.75	−95.45	0.6593	1.3751
n-Heptane	98.55	−90.66	0.6837	1.3878
2,3-Dimethylpentane	89.90	...	0.6944	1.3919
n-Octane	125.55	−57.25	0.7025	1.3976
2-Methylheptane	117.70	...	0.6977	1.3950
2,5-Dimethylhexane	109.20	...	0.6938	1.3926
2,2,4-Trimethylpentane	99.33	−107.32	0.6917	1.3914
Methylcyclopentane	71.85	...	0.7489	1.4099
Methylcyclohexane	101.05	−126.34	0.7695	1.4229

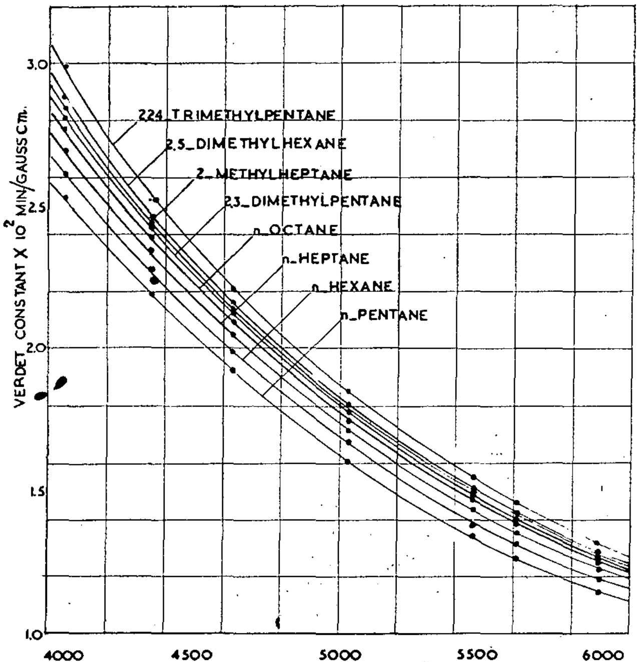

Figure 5. Magnetic Rotatory Dispersion in Visible Region of Eight C_5 to C_8 Paraffins

Table III. Verdet Constants of Paraffins and Naphthenes at Some Wave Lengths Used in Dispersion Measurements

Compound	$V_D^{20} \times 10^2$				
	5893 Å.	5608 Å.	5461 Å.	5027 Å.	435[illegible] Å.
n-Pentane	1.144	1.279	1.343	1.621	2.193
n-Hexane	1.184	1.315	1.391	1.675	2.283
n-Heptane	1.221	1.360	1.432	1.728	2.348
2,3-Dimethylpentane	1.262	1.400	1.482	1.778	2.434
n-Octane	1.249	1.395	1.474	1.760	2.397
2-Methylheptane	1.273	1.418	1.495	1.823	2.450
2,5-Dimethylhexane	1.284	1.430	1.503	1.830	2.461
2,2,4-Trimethylpentane	1.321	1.460	1.552	1.882	2.530
Methylcyclopentane	1.262	1.400	1.478	1.776	2.427
Methylcyclohexane	1.283	1.434	1.504	1.842	2.470

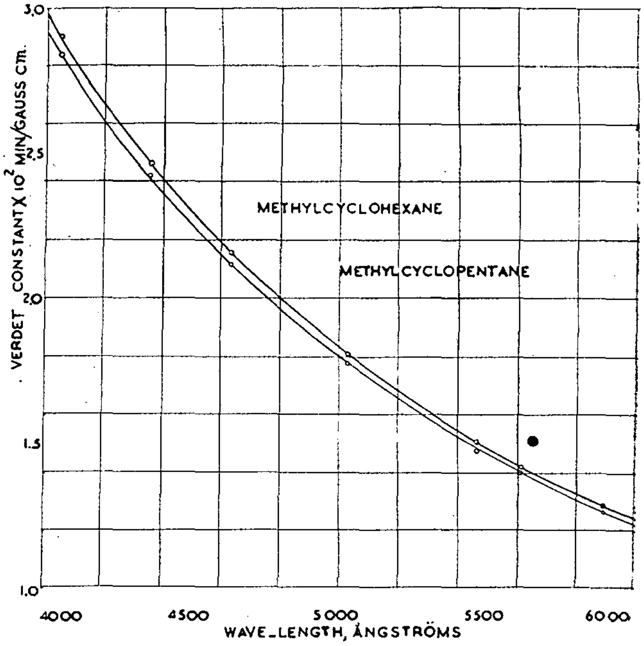

Figure 6. Magnetic Rotatory Dispersion in Visible Region of Methylcyclopentane and Methylcyclohexane

Table IV. Verdet Constants for Na 5893 Å. and Hg 5461 Å., and Other Physical Constants for Paraffins, Naphthenes, and Aromatics

Material	$V_D^{20} \times 10^2$	$V_{5461}^{20} \times 10^2$	$M_2 = \frac{MV_{5461}^{20}}{\alpha}$	d_4^{20}	n_D^{20}
n-Pentane	1.144	1.343	1.545	0.6259	1.3576
n-Hexane	1.184	1.391	1.814	0.6593	1.3751
n-Heptane	1.221	1.432	2.094	0.6837	1.3878
2,3-Dimethylpentane	1.261	1.482	2.134	0.6944	1.3919
n-Octane	1.249	1.474	2.392	0.7025	1.3976
2-Methylheptane	1.275	1.495	2.443	0.6977	1.3950
2,5-Dimethylhexane	1.284	1.503	2.470	0.6938	1.3926
2,2,4-Trimethylpentane	1.321	1.552	2.558	0.6917	1.3914
Cyclopentane	1.214	1.426	1.338	0.7458	1.4063
Methylcyclopentane	1.262	1.478	1.613	0.7489	1.4099
Methylcyclohexane	1.283	1.504	1.915	0.7695	1.4229
Ethylcyclohexane	1.297	1.519	2.158	0.7884	1.4331
Benzene	2.964	3.536	3.151	0.8754	1.5008
Toluene	2.653	3.158	3.354	0.8663	1.4965
Ethylbenzene	2.558	3.063	3.744	0.8672	1.4961
o-Xylene	2.604	3.097	4.222	0.8803	1.5054
m-Xylene	2.450	2.912	4.042	0.8644	1.4971
n-Propylbenzene	...	2.893	4.499	0.8616	1.4917
Pseudocumene	2.379	2.827	4.325	0.8758	1.5045
Hemimelletene	2.523	2.998	4.493	0.8942	1.5137
p-Cymene	2.272	2.703	4.675	0.8557	1.4907
tert-Butylbenzene	...	2.832	4.837	0.8665	1.4923

From the dispersion curves it may be noted that the Verdet constant increases with decreasing wave length and to a marked extent as the ultraviolet is approached. The divergence between the curves for different hydrocarbons gradually increases with decreasing wave length and much larger differences may exist between some hydrocarbons for rotations at wave lengths in the neighborhood of an absorption band. It was found that the simple dispersion formula:

$$V = \frac{K}{\lambda_2 - \lambda_0^2} \quad (K \text{ and } \lambda_0 = \text{constants})$$

which expresses the natural rotatory dispersion in a wide range of compounds, can be used sufficiently well for magnetic rotatory dispersion of hydrocarbons in the major portion of this region of wave lengths. This is shown in Figure 7 where reciprocals of the Verdet constant are linear with the square of wave length.

The Verdet constants for Hg 5461 Å. and in most cases for Na 5893 Å. have been determined for an additional number of naphthenes and a number of C_6 to C_{10} aromatics. All results for the above two wave lengths are listed in Table IV. The natural optical rotation of these hydrocarbons, if any, for the above wave length was not much larger than the error of experiment and was eliminated by taking measurements for both senses of the magnetic field.

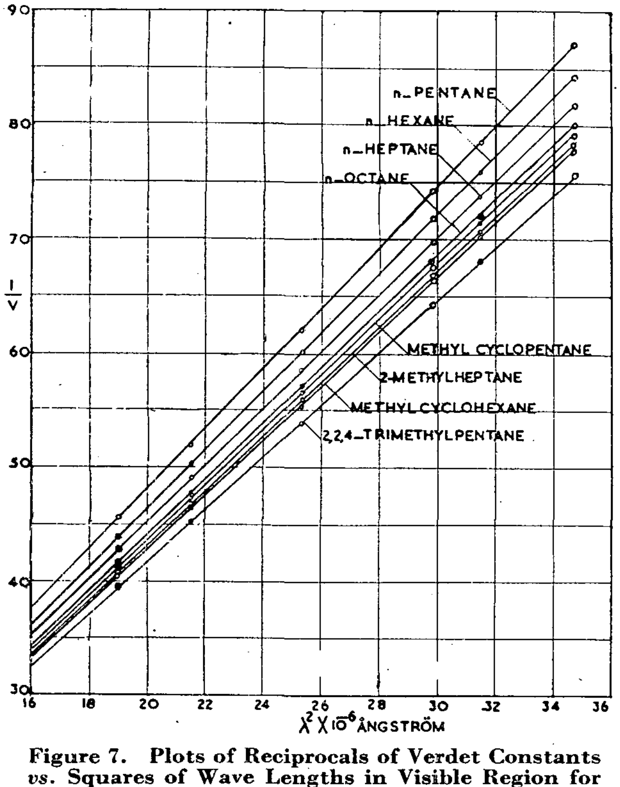

Figure 7. Plots of Reciprocals of Verdet Constants *vs.* Squares of Wave Lengths in Visible Region for Paraffins and Naphthenes

Comparison of results for the sodium line with those of Waterman and his co-workers (*1*), Table V, shows that generally their data are about 1% higher than the present authors', but it is difficult to account for the difference because the above investigators apparently did not check their magnetic field against a standard, or report the magnetic rotation of such liquids as conductivity water or carbon disulfide which have been most frequently reported in the literature. The systematic differences between the two sets of data are significant and may be due to differences between the magnetic field intensities.

Table V. Comparison of V_D^{20} Determined in Present Work with Those of Waterman and Co-workers (*1*)

Material	$V_D^{20} \times 10^2$ Waterman	$V_D^{20} \times 10^2$ Present work
n-Pentane	1.149	1.144
n-Hexane	1.197	1.185
n-Heptane	1.231	1.221
2,3-Dimethylpentane	1.271	1.261
n-Octane	1.261	1.249
2,5-Dimethylhexane	1.296	1.284
2,2,4-Trimethylpentane	1.330	1.321
Cyclopentane	1.228	1.214
Methylcyclopentane	1.271	1.262
Methylcyclohexane	1.295	1.283
Ethylcyclohexane	1.301	1.297

Magnetic Rotation of Hydrocarbon Mixtures. The analysis of any mixture is dependent on the employment of a satisfactory mixture law, for the rotation of a mixture may not always of necessity be equal to the sum of the rotations of its pure components. Waring (*14*) and his co-workers have studied the rotation of carbon disulfide in a number of organic solvents including toluene and cyclohexane, while Waterman (*1*) and his collaborators have investigated the rotation of a number of binary hydrocarbon mixtures. For calculating the rotation of mixtures they used the following expression:

$$D = \Sigma_i D_i W_i$$

where $D_i = \frac{9n_i}{(n_i^2 + 2)^2} \times \frac{M_i V_i}{d_i}$

and W_i is the weight fraction of component *i*. In the above expression, *n* is the refractive index, *M* the molecular weight, *V* the Verdet constant, and d the density of the material under investigation. They confirmed this expression for a number of binary hydrocarbon mixtures. When an appreciable change in density on mixing occurs and association is present, some deviation from the normal is observed.

For the analysis of hydrocarbon mixtures, however, a much simpler rule may be used if Verdet's law is modified as given below:

$$\alpha = [M]CHl \qquad (1)$$

where *C* is the gram mole per cubic centimeter of the substance and $[M]$ is its molecular magnetic rotatory power. The parallelism between the direction of the light and that of the lines of force has been assumed. It may be noted from Equation 1 that when the rotation of a compound in its pure state is measured $[M]\ C = V$ and $[M] = MV/\text{d}$. In hydrocarbon mixtures it has been generally observed that

$$\frac{\alpha}{Hl} = \Sigma_i [M]_i C_i \qquad (2)$$

where α is the rotation due to the mixture and $[M]_i$ and C_i are the molecular magnetic rotatory powers and gram mole per cubic centimeter of the *i*th component in the total volume. Considering that $\text{d} = \Sigma_i M_i C_i$ where d is the density of the mixture, Expression 2 may also be written in the form

$$\frac{V}{\text{d}} = \Sigma_i \frac{V_i}{\text{d}_i} W_i \qquad (3)$$

where W_i is the weight fraction of component *i*. Expression 3 has been confirmed in the following binary mixtures and Figure 8 indicates the linearity of magnetic rotation when plotted against weight fraction of one of the constituents: benzene in benzene-cyclohexane, 2,3,3-trimethylpentane in 2,3,3-trimethylpentane–*n*-heptane, 2,3,3-trimethylpentane in 2,3,3-trimethylpentane–methylcyclohexane, and cyclohexene in cyclohexene-cyclohexane.

The compositions of these mixtures are given in Table X.

This rule is also obeyed in hydrocarbon mixtures containing more than two components, and therefore in such mixtures the rotation of the mixture may be computed by means of Expression 3 from those of its components. This is shown in Tables VI and

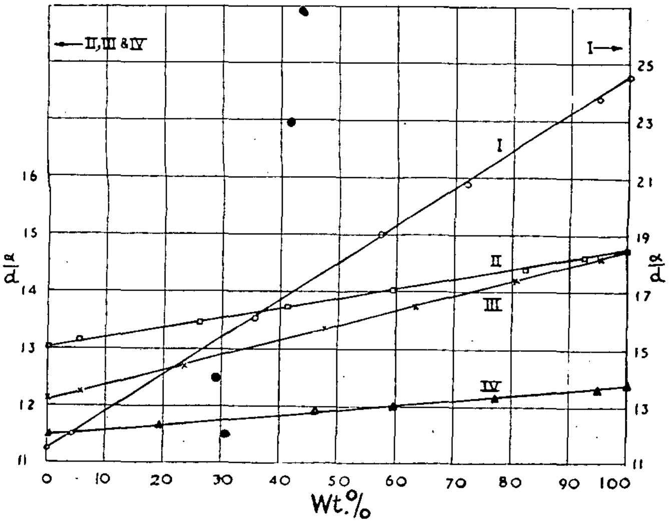

Figure 8. Plots of α/d *vs*. Weight Per Cent Composition

- I. Benzene in benzene-cyclohexane blends
- II. 2,3,3-Trimethylpentane in 2,3,3-trimethylpentane–*n*-heptane blends
- III. 2,3,3-Trimethylpentane in 2,3,3-trimethylpentane–methylcyclohexane blends
- IV. Cyclohexene in cyclohexene-cyclohexane blends

Table VI. Calculated and Observed Values of α^{20}_{5461}

Blend	α^{20}_{5461} Calculated	α^{20}_{5461} Observed
Blends of Toluene, 2,3,3-Trimethylpentane, and Methylcyclohexane		
1	12.46	12.46
2	11.16	11.09
3	10.12	10.11
4	14.15	14.13
5	10.07	10.10
Blends of *n*-Heptane, 2,2,4-Trimethylpentane, and Cyclohexane		
1	9.14	9.08
2	9.09	9.10
3	9.19	9.24
4	8.91	8.93

VII. The observed and calculated rotations α^{20}_{5461} for blends of toluene, 2,3,3-trimethylpentane, and methylcyclohexane, and *n*-heptane, 2,2,4-trimethylpentane and cyclohexane are given in Table VI, and their compositions are given in Tables XIII and XV.

In Table VII, similar results together with the compositions are given for the following four-component mixtures: A and B; methylcyclohexane, *n*-heptane, 2,2,4-trimethylpentane, and toluene; C,2,2,4-trimethylpentane, 2,3,3-trimethylpentane, benzene, and toluene; and D,*n*-heptane, 2,3,3-trimethylpentane, benzene, and toluene.

The rotations of the constituents of the above blends are given in Table VIII.

Table VII. Magneto-optical Properties of Quaternary Mixtures of Hydrocarbons

Blend No.	A	B	C	D
Composition, wt. %				
n-Heptane	28.0	28.1	...	27.7
2,2,4-Trimethylpentane	16.3	38.2	26.4	...
2,3,3-Trimethylpentane	...	...	18.1	28.6
Methylcyclohexane	26.5	10.7	...	...
Benzene	...	...	11.8	22.4
Toluene	29.2[a]	23.0[a]	43.7[b]	21.3[a]
Observed magneto-optic rotation, α^{20}_{5461}	11.60	11.12	14.88	13.74
Specific gravity, d^{20}_{20}	0.752	0.731	0.785	0.766
Specific magneto-optic rotation, α/d	15.42	15.22	18.96	17.94
Calculated magneto-optic rotation, α^{20}_{5461}	11.62	11.11	14.86	13.70

[a] Technical grade toluene $n^{20}_D = 1.4928$.

[b] Pure toluene $n^{20}_D = 1.4965$.

Analysis of Hydrocarbon Mixtures. In each set of experiments reported here the rotations of the components, as well as those of their mixtures, have been determined in the same tube and for the same strength of the magnetic field. To simplify calculations the rotation, α, instead of the Verdet constant has been used. Furthermore, the specific gravity, d^{20}_{20}, has been used in place of density. The majority of hydrocarbons used in preparation of mixtures have been of technical grade.

Binary Mixtures. In Tables IX and X are given the rotations α^{20}_{5461}, specific gravity d^{20}_{20}, specific rotations $\frac{\alpha}{d}$, and observed and actual compositions of the following blends: benzene-cyclohexane, 2,3,3-trimethylpentane–*n*-heptane, methylcyclohexane–2,3,3-trimethylpentane, and cyclohexene-cyclohexane.

Ternary Mixtures. Five blends of toluene, 2,3,3-trimethylpentane, and methylcyclohexane were prepared and their rotations were measured visually for Na 5893 Å., Hg 5461 Å., and Hg 4358 Å. at 20° C. From results given in Table XI it may be observed that it is not practicable to carry out analysis by solving a set of simultaneous equations corresponding to rotations for the three wave lengths given.

This is demonstrated in the following equations, where W_1, W_2, and W_3 denote weight fractions of the respective constituents.

Table VIII. Magneto-optical Properties of Hydrocarbons Used in Quaternary Blends

Blend	α^{20}_{5461}	d^{20}_{20}	α/d
Methylcyclohexane	9.23	0.769	12.00
n-Heptane	8.87	0.685	12.96
2,2,4-Trimethylpentane	9.54	0.693	13.77
2,3,3-Trimethylpentane	10.38	0.716	14.49
Benzene	21.60	0.879	24.57
Toluene[a]	19.37	0.8675	22.33
Toluene[b]	18.88	0.8625	21.89

[a] Pure toluene, $n^{20}_D = 1.4965$.

[b] Technical grade toluene, $n^{20}_D = 1.4928$.

Table IX. α^{20}_{5461}, d^{20}_{20}, and α/d

Blend	α^{20}_{5461}	d^{20}_{20}	α/d
Benzene-cyclohexane Blends			
Benzene	21.49	0.876	24.48
1	20.67	0.869	23.79
2	17.47	0.838	20.84
2	15.68	0.826	18.99
4	12.88	0.805	16.01
5	9.36	0.781	11.99
Cyclohexane	8.93	0.779	11.47
2,3,3-Trimethylpentane–*n*-heptane Blends			
2,3,3-Trimethylpentane	10.53	0.716	14.71
1	10.39	0.712	14.60
2	10.24	0.710	14.41
3	9.86	0.702	14.04
4	9.54	0.696	13.72
5	9.34	0.694	13.46
6	9.03	0.686	13.15
n-Heptane	8.92	0.685	13.04
Methylcyclohexane–2,3,3-trimethylpentane Blends			
Methylcyclohexane	9.33	0.769	12.14
1	9.395	0.768	12.22
3	9.60	0.755	12.71
3	9.85	0.737	13.37
4	10.08	0.734	13.73
5	10.31	0.725	14.21
6	10.46	0.717	14.58
2,3,3-Trimethylpentane	10.53	0.716	14.71
Cyclohexene-cyclohexane Blends			
Cyclohexene	10.77	0.873	12.34
1	10.62	0.865	12.28
2	10.28	0.848	12.13
3	9.955	0.832	11.97
4	9.79	0.822	11.90
5	9.275	0.798	11.62
Cyclohexane	8.93	0.779	11.47

Table X. Actual and Observed Compositions of Blends

Blend	Weight Per Cent Actual	Observed	Actual	Observed
	Benzene		Cyclohexane	
1	94.9	94.7	5.1	5.3
2	72.3	72.0	27.7	28.0
3	57.5	57.5	42.5	42.5
4	35.4	34.9	64.6	65.1
5	4.1	3.9	95.9	96.1
	2,3,3-Trimethylpentane		*n*-Heptane	
1	92.7	93.3	7.3	6.7
2	82.2	81.8	17.8	16.2
3	59.4	59.7	40.6	40.3
4	41.0	41.6	59.0	58.4
5	26.0	25.1	74.0	74.9
6	5.5	6.6	94.5	93.4
	Methylcyclohexane		2,3,3-Trimethylpentane	
1	94.6	96.9	5.4	3.1
2	76.8	77.8	23.3	22.2
3	52.3	52.1	47.7	47.9
4	36.6	38.1	63.4	61.9
5	19.3	19.5	80.7	80.5
6	4.9	4.7	95.1	95.3
	Cyclohexene		Cyclohexane	
1	95.0	93.1	5.0	6.9
2	77.2	75.6	22.8	24.4
3	59.8	57.0	40.2	43.0
4	46.0	48.8	54.0	51.2
5	19.3	16.9	80.7	83.1

Table XI. d_{20}^{20}, α_{5893}^{20}, α_{5461}^{20}, α_{4358}^{20}, and α/d for Toluene, 2,3,3-Trimethylpentane and Methylcyclohexane Used in Ternary Blends

Compound	d_{20}^{20}	Na 5893 α	α/d	Hg 5461 α	α/d	Hg 4358 α	α/d
Toluene	0.8625	15.04	17.43	17.88	20.72	31.20	36.16
2,3,3-Trimethylpentane	0.716	8.33	11.64	9.81	13.71	16.31	22.8
Methylcyclohexane	0.769	7.45	9.69	8.69	11.30	14.33	18.62

$$17.43\ W_1 + 11.64\ W_2 + 9.69\ W_3 = \alpha/\mathrm{d} \text{ at } 5893\ \text{Å}.$$

$$20.72\ W_1 + 13.71\ W_2 + 11.30\ W_3 = \alpha/\mathrm{d} \text{ at } 5461\ \text{Å}.$$

$$36.16\ W_1 + 22.8\ \ W_2 + 18.62\ W_3 = \alpha/\mathrm{d} \text{ at } 4358\ \text{Å}.$$

These equations are very nearly identical, and therefore it would follow that the calculated values of W_1, W_2, and W_3 must vary within wide limits. This is due to the fact that the dispersion curves of hydrocarbons, although somewhat divergent as they approach the shorter wave lengths, do so in such a proportionate way that they cannot lend themselves to analysis of multicomponent mixtures. However, rotations in the ultraviolet might be successfully used for the analysis of such mixtures, especially those containing unsaturated constituents with absorption bands in this region of the spectrum. In view of the difficulties mentioned above, the possibility of using refractive index and specific gravity in conjunction with magnetic rotation was investigated. MacFarlane and Wright (*5*) have shown that the refractive indexes and densities of binary hydrocarbon mixtures, when change in volume on mixing does not occur, are additive on a volumetric basis. It appears, therefore, that analysis of ternary mixtures in the visual part of the spectrum may be possible if rotations together with refractive indexes measured at a convenient wave length are used. The assumption is made that the change in volume upon mixing hydrocarbons is negligible. From values of rotation and refractive indexes of a set of ternary blends given in Table XII an analysis was carried out by solving a set of simultaneous equations of the following types:

$$\alpha = \Sigma_i \frac{M_i\alpha_i}{\mathrm{d}_i} C_i$$

$$n = \Sigma_i \frac{M_i n_i}{\mathrm{d}_i} C_i$$

$$\mathrm{d} = \Sigma_i M_i C_i$$

where C_i is the gram mole per cubic centimeter total volume of component i. The above equations, however, would reduce to the following equations:

$$\frac{\alpha}{\mathrm{d}} = \Sigma_i \frac{\alpha_i}{\mathrm{d}_i} W_i$$

$$\frac{n}{\mathrm{d}} = \Sigma_i \frac{n_i}{\mathrm{d}_i} W_i$$

$$1 = \Sigma_i W_i$$

where W_i is the weight fraction of component i. The results of the analysis are given in Table XIII.

A further set of ternary blends of *n*-heptane, 2,2,4-trimethylpentane, and cyclohexane was studied. The results of measurements and analysis are given in Tables XIV and XV.

It appears, therefore, that magnetic rotations may be utilized for the analysis of binary hydrocarbon mixtures with an accuracy depending upon the experimental error and the magnitude of the difference in rotations of the pure constituents. This method alone is not particularly sensitive for analysis of ternary mixtures but in conjunction with refractive index and specific gravity in the manner shown, may yield reasonable results.

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Table XII. α_{5461}^{20}, n_{5461}^{20}, α/d, and n/d for Ternary Blends of Toluene, 2,3,3-Trimethylpentane, and Methylcyclohexane

Blends	α_{5461}^{20}	n_{5461}^{20}	α/d	n/d
Toluene	17.88	1.4970	20.72	1.7346
2,3,3-Trimethylpentane	9.81	1.4109	13.70	1.9705
Methylcyclohexane	8.69	1.4249	11.30	1.8529
1	12.46	1.4500	15.65	1.8216
2	11.09	1.4316	14.55	1.8787
3	10.11	1.4199	13.81	1.9397
4	14.13	1.4617	17.44	1.8046
5	10.10	1.4341	12.93	1 8362

Table XIII. Actual and Observed Compositions of Ternary Blends of Toluene, 2,3,3-Trimethylpentane, and Methylcyclohexane

Blend	Wt. % Toluene Actual	Observed	Wt. % 2,3,3-Trimethylpentane Actual	Observed	Wt. % Methylcyclohexane Actual	Observed
1	42.8	42.1	13.4	16.1	43.8	41.8
2	23.2	23.0	48.7	45.1	28.1	31.9
3	7.9	6.2	74.3	79.9	17.8	13.9
4	60.4	60.2	20.1	19.5	19.5	20.3
5	16.0	16.6	4.0	2.8	80.0	80.6

Table XIV. α_{5461}^{20}, n_{5461}^{20}, α/d, and n/d for Ternary Blends of *n*-Heptane, 2,2,4-Trimethylpentane, and Cyclohexane

Blends	α_{5461}^{20}	n_{5461}^{20}	α/d	n/d
n-Heptane	8.87	1.3896	12.95	2.0286
2,2,4-Trimethylpentane	9.54	1.3934	13.77	2.0107
Cyclohexane	8.88	1.4842	11.40	1.8334
1	9.08	1.4134	12.27	1.9100
2	9.10	1.4028	12.71	1.9592
3	9.24	1.3987	13.03	1.9728
4	8.93	1.4160	11.91	1.8880

Table XV. Actual and Observed Compositions of Ternary Blends of *n*-Heptane, 2,2,4-Trimethylpentane, and Cyclohexane

Blend	Wt. % *n*-Heptane Actual	Observed	Wt. % 2,2,4-Trimethylpentane Actual	Observed	Wt. % Cyclohexane Actual	Observed
1	9.8	14.5	28.5	27.2	62.8	58.3
2	32.3	35.0	32.7	32.4	35.2	32.6
3	28.2	22.1	47.6	54.3	24.2	23.6
4	18.0	20.7	9.5	8.0	72.3	71.3

mingham for the loan of various electrical measuring apparatus, the Anglo-Iranian Oil Co., Ltd., for the loan of the spark equipment, and the Institute of Petroleum for supplying the majority of hydrocarbons used in this work.

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Analysis of Thallium Halide Crystals

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IN THE preparation and investigation of the properties of thallium iodide–thallium bromide crystals (*2, 3*) for use in experimental studies in long wave-length spectrometry a relatively simple analytical procedure that permits a determination of the composition of the crystals is desirable. Little published information is available concerning either the details of the various methods used in the preparation of the crystals or suitable methods by which a reliable chemical analysis can be effected. If it is assumed that the pure materials used in the preparation of the crystal undergo no decomposition or change—that the final product is composed only of thallium iodide and thallium bromide—a determination of any of the three components permits the composition of the crystal to the computed. A determination of either the iodide or the bromide appears most attractive. If subsequent investigation indicates the presence of iodine and bromine in forms other than iodide and bromide, the foregoing assumption fails and the problem becomes more complex.

A preliminary study at the National Bureau of Standards indicated that, when a sample of the crystal is treated with dilute acid and zinc, satisfactory decomposition results in the precipitation of metallic thallium (*1*) and the liberation of hydriodic and hydrobromic acids. Decomposition of the sample is best effected when the sample is in a fine state of subdivision. Following filtration and washing of the insoluble metallic residue, a potentiometric titration of the halides with silver nitrate gave satisfactory results for the iodide. On continued titration under the prevailing conditions, less satisfactory results for the bromide were obtained because of a weak and uncertain end point.

Experiments in which known mixtures of potassium iodide and potassium bromide were titrated potentiometrically with silver nitrate indicate that when the solution contains approximately 2% by volume of sulfuric acid, a positive error that averages from 1 to 2 parts per thousand results. At lower concentrations of acid unsatisfactory results were obtained because of an excessive consumption of silver nitrate.

A procedure has been developed, based on decomposition of the sample as described, followed by titration of the iodide with silver nitrate and an indirect determination of the bromide.

Accurately weigh a 1-gram sample of the crystal, obtained by filing the material with a No. 2 file (rejecting any chips or coarse material) in a 500-ml. glass-stoppered wash bottle. Add 20 grams of granulated zinc (30-mesh) and 100 ml. of sulfuric acid (3 + 97) and digest the mixture at room temperature, with occasional agitation, until there is no evidence of unattacked material. Add 5 grams of granulated zinc and 5 ml. of diluted sulfuric acid (1 + 1) to the wash bottle and digest for 30 to 45 minutes with occasional agitation. Filter the solution through a small filter containing a little zinc, and wash both flask and filter with small portions of diluted sulfuric acid (1 + 99). Reserve the filter and residue of zinc and thallium. Titrate the iodide potentiometrically, using silver iodide and calomel electrodes, with standard 0.1 *N* silver nitrate added dropwise while the solution is stirred vigorously by mechanical means. The titration should be performed without delay to minimize oxidation of the iodide by air. Transfer the reserved residue of zinc and thallium with filter to the wash bottle and treat with 100 ml. of sulfuric acid (2 + 98). Digest for a few minutes, filter, and titrate potentiometrically with standard 0.01 *N* silver nitrate. The recovery, approximating 0.04 to 0.06 ml. of 0.1 *N* silver nitrate, is added to the amount already found and the result is calculated as thallium iodide.

Confirmatory evidence of composition is afforded by a determination of the thallium bromide.

Decompose a weighed sample of approximately 0.5 gram in the manner just described, precipitate the iodide and bromide with silver nitrate, and filter, wash, dry, and weigh in the conventional manner. Deduct from the weight of the combined silver halides the silver iodide equivalent of the thallous iodide already found. Calculate the thallous bromide equivalent of the remaining silver bromide.

A few experiments on two samples of thallous iodide–thallous bromide crystals by the foregoing procedures gave:

	Sample A %	Sample B %
Thallous iodide	57.82	61.16
	57.90	61.10
	57.80	...
Av.	57.84	61.13
Thallous bromide	42.18	38.74
	42.20	38.76
Av.	42.19	38.75

The method in practice presents but few difficulties aside from the end point that denotes complete precipitation of the silver iodide. This end point must be approached slowly, and adequate time afforded for the establishment of equilibrium.

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VITAMIN A IN MILK

Microestimation with Activated 1,3-Dichloro-2-propanol

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Activated 1,3-dichloro-2-propanol (glycerol α,γ-dichlorohydrin) has been applied to the determination of vitamin A and carotene and milk. This reagent eliminated the need for anhydrous precautions and permitted the simultaneous reading of both vitamin A and carotene absorption at 455 and 800 mμ, respectively. Sample volumes for each determination were reduced from the 10 to 100 ml. normally required to as little as 0.25 to 1.0 ml. by saponifying and extracting the milk sample in a single test tube, and by employing horizontal cuvettes with a 5.0-cm. light path. The results with the new method were close to those obtained when vitamin A was determined by antimony trichloride and carotene by light absorption at 440 mμ.

THE purpose of this study was to develop a simple, reliable method for the estimation of vitamin A and carotene in small milk samples. The need for such a method arose in the course of an investigation of changes in the vitamin A levels of human milk at frequent short intervals. Two aspects of this problem were investigated:

A method of extraction of vitamin A and carotene from small milk samples. To date, all procedures require milk sample volumes ranging from 10 to as many as 100 ml. (*1, 4, 5, 7, 8*).

Applicability of the 1,3-dichloro-2-propanol [activated α,γ-glycerol dichlorohydrin, (GDH)] reaction (*11–15, 17*) in place of the Carr-Price reaction (*2*) which is used exclusively in hitherto available methods for milk.

The GDH reaction has the following advantages:

The color is stable for 8 minutes.
The reagent is noncorrosive.
The reagent is not affected by extreme humidity.
Use of specially purified chloroform and petroleum ether is not required.

Table I. Comparison of 1,3-Dichloro-2-propanol and Antimony Trichloride Methods

Milk No.	Antimony Trichloride		Activated GDH	
	Vitamin A	Carotene	Vitamin A	Carotene
	γ/ml.	*γ/ml.*	*γ/ml.*	*γ/ml.*
1	3.22	3.00	3.12	3.30
2	2.02	3.00	2.20	3.33
3	4.82	5.02	4.93	5.00
4	1.90	3.30	1.81	3.10
5	3.36	3.42	3.37	3.49
6	1.20	1.92	1.26	1.84
7	2.30	4.06	2.22	3.95
Av.	2.69	3.39	2.70	3.43

COMPARISON OF ANTIMONY TRICHLORIDE AND GDH METHODS OF ANALYSIS

In this experiment, saponification and extraction of the vitamin A–containing milk lipides were effected by heating a milk aliquot with an equal volume of 1 *N* potassium hydroxide in 90% ethanol and shaking with an equal volume of petroleum ether. One-milliliter portions of this extract were taken for each method of analysis. The results are given in Table I. In the antimony trichloride method, carotene content was evaluated by reading the petroleum ether extract first at 440 mμ by the method of Dann and Evelyn (*3*), then evaporating, dissolving the lipide residue in anhydrous chloroform, adding the Carr-Price reagent (*2*), and reading absorption of the blue color at 615 mμ. With 1,3-dichloro-2-propanol, carotene content was evaluated directly by reading the color formed by adding 4 ml. of the reagent to 1 ml. of the extract in chloroform solution at a dial setting of 800 mμ and at 555 mμ for vitamin A (*13*). The close values shown in Table I indicate the essential identity of results obtainable by either method.

Investigation of the Method of Extraction. The method of extraction employed is based on that described for the estimation of vitamin A in saponified serum with the aid of 1,3-dichloro-2-propanol (*11*), except that three extractions were found necessary for the complete removal of the vitamin A–containing lipid. Both saponification and extraction take place in the same test tube, using 0.25- to 1.0-ml. samples of milk. The reference method, which employs 10 ml. of milk, was that of Hrubetz *et al.* (*5*). In this method the sample, after saponification with refluxing, is transferred to a separatory funnel and extracted with six separate aliquots of petroleum ether. The extract is then washed three times with 1% hydrochloric acid and evaporated to dryness on a water bath. The anhydrous conditions which the original paper required were eliminated, because the vitamin A and carotene content was evaluated with 1,3-dichloro-2-propanol. It has been shown in this study that 1,3-dichloro-2-propanol values are comparable to antimony trichloride values (see Table I). It is readily seen that the reference method of extraction requires individual attention for each sample, whereas by the method of the authors as many as forty simultaneous extractions were carried out. Typical results are shown in Table II. With the new method, either the same or slightly elevated values are obtained. This is not unexpected, for with the reference method there is a greater possibility of mechanical loss as well as loss through oxidation. The new method of extraction is equally applicable to use of the 1,3-dichloro-2-propanol or antimony trichloride reagents for the final colorimetric measurements.

Investigation of the Need for Saponification. The next point studied was whether saponification is absolutely necessary as a preliminary step. In the estimation of serum vitamin A with 1,3-

Table II. Comparison of Macro and New Method of Extraction

No.	Sample	Reference Method		New Method	
		Vol. used	Vitamin A	Vol. used	Vitamin A
		Ml.	*γ/ml.*	*Ml.*	*γ/ml.*
1	Bovine cream plus water	20	0.54	1.0	0.55
2	Human milk	5.0	1.41	1.0	1.48
3	Human milk	5.0	0.24	1.0	0.24
4	Human milk	4.0	0.46	1.0	0.57

Table III. Extractability of Saponified and Whole Milk Samples

No.	Vitamin A Extracted	
	From saponified milk sample	From whole milk sample
	γ/ml.	*γ/ml.*
1	1.12	0.00
2	2.45	0.00
3	2.42	0.07
4	1.60	0.52
5	1.15	0.42

dichloro-2-propanol (*11*), approximately the same results are obtained with saponification as without. As seen from the data in Table III, however, saponification of the milk sample is a necessary step. Without saponification, none or practically none of the vitamin A is extractable with petroleum ether. This suggests that vitamin A–containing lipids, in fresh milk at least, are bound more firmly to proteins than in blood. Alcohol denaturation is not sufficient to break this bond.

Recovery of Added Vitamin A. To investigate further the validity of the new method, known amounts of vitamin A were added to samples of milk prior to saponification. Values obtained after 1,3-dichloro-2-propanol analysis of these enriched samples were compared to those found for the initial unenriched samples. The results are presented in Table IV. Precision of the recovery was 100 ± 3.5% of the calculated value. The mean error of the recovery estimation is −0.02 microgram, while the average error is ±0.04 microgram. The deviation of duplicates from the mean is ±0.05 microgram, which is ±2.4% of the total value. Thus the new method appears valid not only in a comparison with an antimony trichloride analysis but by a more rigid test of running a recovery sample through the whole procedure.

Table IV. Recovery of Vitamin A Acetate Added to Milk

Sample No.	Vitamin A Content	Vit. A Acetate Added	Calcd. Recovery[a]	Found Recovery	Recovered	Mean Error
	γ/ml.	*γ*	*γ/ml.*	*γ/ml.*	%	*γ/ml.*
1	1.47 ± 0.02	2.50	3.97	3.95	99.5	−0.02
2	0.10 ± 0.03	0.48	0.58	0.63	109.	+0.05
3	4.26 ± 0.03	1.00	5.26	5.28	100.	+0.02
4	1.04 ± 0.04	0.50	1.54	1.54	100.	0.00
5	0.87 ± 0.03	2.00	2.87	2.83	98.6	−0.04
6	2.52 ± 0.06	2.00	4.52	4.45	98.4	−0.07
7	7.20 ± 0.20	2.00	9.20	9.18	99.8	−0.02
8	1.03 ± 0.02	3.00	4.03	3.95	98.0	−0.08
Av.	2.31 ± 0.05	1.69	4.00	3.98	100 ± 3.5	−0.02

[a] Column 2 plus column 3.

The finally developed method shows numerous advantages over procedures available until now.

Small amounts of milk are required (sample volumes from 0.25 to 1.0 ml. instead of from 10 to 100 ml.).

As many as forty simultaneous determinations may be carried out with less effort on the part of the analyst than four to six analyses by previous methods.

By extracting with petroleum ether instead of ethyl ether, destruction due to possible peroxide formation is avoided.

By eliminating the separatory funnel and washing of the extract, mechanical losses are avoided.

By using 1,3-dichloro-2-propanol in place of antimony trichloride, anhydrous measures are eliminated.

No difficulty in the handling of reagents is encountered on humid days.

Instrument deterioration due to antimony trichloride is avoided and the special problem of measuring out the Carr-Price reagent does not occur (*9, 10, 16*).

REAGENTS

Ethanol, 95%, refluxed with solid potassium hydroxide and distilled; first and last portions discarded.

Potassium hydroxide, 1 *N* in 90% ethanol, freshly prepared (from 1 part of a stock solution of 11 *N* aqueous potassium hydroxide and 10 parts of absolute ethanol. The stock aqueous potassium hydroxide solution may be used for several months).

Petroleum ether, boiling point 30° to 60° C., analytical reagent grade.

Nitrogen, Ohio Chemical Company.

Sodium sulfate, anhydrous, analytical reagent grade.

Chloroform, analytical reagent grade. Analytical reagent grade washed with water, distilled, first and last portions discarded, and stored over anhydrous sodium sulfate.

Antimony trichloride, analytical reagent grade.

Carr-Price reagent, 30 grams of antimony trichloride in 100 ml. of anhydrous chloroform.

Carotene, 90% β-10% α, from General Biochemicals Company, Inc.

Standard Vitamin A. Vitamin A acetate in cottonseed oil, gelatin capsules from U.S.P. reference standards.

Activated 1,3-dichloro-2-propanol. Glycerol dichlorohydrin (Eastman Kodak, practical grade; a mixture of 1,3-, and 2,3-dichlorohydrin from the Shell Chemical Company) is distilled with approximately 1% by weight of antimony trichloride at from 10 to 40 mm. pressure, and first and last fractions are discarded (*11*).

APPARATUS

Coleman Universal spectrometer, Model 11, calibrated according to directions given by the manufacturer. This instrument has a 35-mμ light band.

Standard perpendicular 1.3-cm. light path cuvettes and carrier from Coleman Instruments, Inc.

Horizontal 5.0-cm. light path cuvettes from Pyrocell Manufacturing Company, 207 East 84th St., New York 28, N. Y. Carrier from Coleman Instruments, Inc.

555-mμ filter (combination 3482,M608, 1.0 mm. and 5300,M954, 4.0 mm.) from Corning Glass Works, Corning, N. Y.

PROCEDURE FOR VITAMIN A ANALYSIS OF MILK

One milliliter of milk is pipetted into a 0.375 × 4 inch test tube, 1 ml. of 1 *N* potassium hydroxide in 90% ethanol is added, the contents of the tube are mixed, and the tube is placed in a 60° C. water bath (or in a 60° oven in a vessel of water) for 35 minutes. The tube is removed and allowed to cool to room temperature. Two milliliters of reagent grade petroleum ether are added, and tube is stoppered with a size 00 stopper pre-extracted with petroleum ether and shaken for 10 minutes. (A shaking machine is normally used.) After shaking, the tube is centrifuged for approximately 30 seconds to separate the phases sharply. The supernatant petroleum ether extract is aspirated with a fine-tipped rubber bulbed dropper and placed in a 0.5 × 4 inch test tube. The saponified milk sample is shaken with two succeeding 1-ml. aliquots of petroleum ether, allowing 5 minutes for each shaking. The supernatant petroleum ether extract is collected as previously.

The extract is evaporated to dryness by placing the tube in a 40° to 50° C. water bath and running a slow stream of nitrogen into it. One milliliter of reagent grade chloroform is added to bring the dried extract into solution. This solution is placed in a 25° C. water bath, and 4 ml. of 1,3-dichloro-2-propanol are added. The contents of the tube are mixed with a flat-tipped stirring rod. Two minutes after the initial mixing the solution is transferred to the 5.0-cm. light path cuvette. The absorption of the solution is read at a wave-length dial setting of 550 mμ (with the 555 mμ filter in the filter housing) against a blank consisting of 1 ml. of reagent grade chloroform and 4 ml. of activated 1,3-dichloro-2-propanol. The wave-length dial is then set at 800 mμ; the 555 mμ filter is replaced with the Coleman PC-5 filter and the absorption is read 4 minutes after the initial mixture of the reagents.

The absorption at 800 mμ will give the carotene content from a carotene calibration chart. The carotene content permits evaluation of the interference due to carotene at 555 mμ from a carotene interference chart. The optical density due to carotene interference at 555 mμ is subtracted from the total optical density at this wave length, and the resultant optical density permits evaluation of vitamin A content per milliliter from a vitamin A calibration chart.

The volume of the 1,3-dichloro-2-propanol-chloroform mixture is 5.0 ml. Because the horizontal cuvette has a capacity of less than 3 ml., sample and reagent aliquots may be reduced to six tenths of the above volumes. Where the analysis of milk samples of unusually low vitamin A content is not expected, it is possible to use the short light path (1.3-cm.) cuvettes or else reduce the amount of milk sample taken to as little as 0.25 ml.

PREPARATION OF CALIBRATION CHARTS

Vitamin A. The vitamin A reference graph is prepared with standards in chloroform solution ranging from 0.2 to 5.0 micrograms of vitamin A per ml. All reagents are preheated to 25° C. before use. To 1 ml. of standard in a glass-stoppered cylinder are added 4 ml. of 1,3-dichloro-2-propanol. The contents are mixed by inversion and placed in a 25° C. water bath for 2 minutes. The absorption of the pink color produced is read in the 5.0-cm. cuvette against a blank consisting of 1 ml. of chloroform and 4 ml. of 1,3-dichloro-2-propanol. The wavelength dial of the spectrophotometer is set at 550 mμ with the 555 mμ filter in the filter housing. Optical densities are plotted against vitamin A concentration per milliliter. If 1 ml. of milk is used as a starting sample, the chart may be used to evaluate directly the vitamin A content of 1 ml. of milk.

Carotene. This chart is prepared in the same manner as the vitamin A chart. The wave-length dial is set at 800 mμ with the Coleman filter PC-5 in the filter housing. Optical densities due to concentrations of 1.0 to 10.0 micrograms of carotene per ml. of chloroform are read 4 minutes after reagent mixture.

Carotene Interference. This chart is prepared in the same manner as the vitamin A chart. The carotene concentrations prepared above for the carotene calibration chart are read at the 550 mμ setting with the 555 mμ filter in the filter housing. This graph is used to correct the vitamin A reading for carotene interference as follows:

Carotene content is evaluated with the carotene calibration chart.

The optical density which this amount of carotene gives at 555 mμ is found on the carotene interference graph. This optical density is subtracted from the total optical density read at 555 mμ.

With the corrected optical density, vitamin A content is evaluated from the vitamin A calibration chart.

For convenience, all three graphs are drawn with common axes on the same sheet of coordinate paper.

EXPERIMENTAL

Comparison of Antimony Trichloride and 1,3-Dichloro-2-propanol. A series of 3-ml. milk samples was saponified by warming in a water bath at 60° C. for 35 minutes with an equal volume of 1 *N* potassium hydroxide in 90% ethanol. The contents of each tube were shaken with 3 ml. of redistilled petroleum ether. One milliliter of the supernatant extract was pipetted into a test tube and evaporated, and vitamin A and carotene content were evaluated with 1,3-dichloro-2-propanol as described above. Another milliliter of the same extract was made up to 4 ml. with redistilled petroleum ether. Absorption due to carotene was read at 440 mμ in the 1.3-cm. light path cuvettes against a blank of redistilled petroleum ether (*3*). Carotene content was evaluated from a calibration chart prepared by plotting the optical densities against concentrations of from 1.0 to 10.0 micrograms of carotene in petroleum ether. Approximately 20 mg. (1 pinch) of anhydrous sodium sulfate were added to the petroleum ether solution, which was stoppered and placed in a dark container in the refrigerator for an hour. At the end of this time, the petroleum ether solution was transferred quantitatively to another tube and evaporated to dryness in a 40° to 50° C. water bath under a stream of nitrogen. The dried extract was dissolved in 1 ml. of anhydrous chloroform and quantitatively transferred to the 1.3-cm. light path cuvette. Three milliliters of the Carr-Price reagent (*2, 13*) were added and the optical density of the blue color formed was read after 4 seconds at a dial setting of 615 mμ with a Coleman PC-4 filter in the filter housing. Vitamin A content was evaluated from a vitamin A calibration chart and a carotene interference chart (*6*), in the same manner as described for the 1,3-dichloro-2-propanol calibration charts above.

Investigation of Method of Extraction. To several 1-ml. milk aliquots in 0.375 × 4 inch test tubes were added 1-ml. portions of 1 *N* potassium hydroxide in 90% ethanol. The samples were shaken and placed in a 60° C. water bath. After 30 minutes of heating, the precipitated milk protein was sufficiently broken down to give a homogeneous mixture on shaking. The saponified samples where then shaken with 2 ml. of petroleum ether, the tubes being stoppered with size 00 rubber stoppers which had been pre-extracted with petroleum ether. The supernatant petroleum ether was then transferred to 0.5 × 4 inch test tubes and the milk sample was shaken with succeeding 1-ml. portions of petroleum ether. After a total of three shakings, the petroleum ether extract was found to contain no more vitamin A or carotene. The collected extracts were evaporated and analyzed for vitamin A and carotene as described above.

As a reference method, the extraction procedure of Hrubetz, Duel, and Hanley (*5*) was employed.

Five milliliters of the milk were pipetted into a small distillation flask. To this were added 5 ml. of 95% ethanol and 1 ml. of aqueous 40% potassium hydroxide. The mixture was saponified by refluxing for 30 minutes, then made up to 40 ml. with 95% ethanol and extracted with six 20-ml. portions of petroleum ether. The combined extract was washed three times with 1% hydrochloric acid. It was then evaporated to dryness in a 40° to 50° C. water bath under a stream of nitrogen. The residue was dissolved in 5 ml. of chloroform. One milliliter of this chloroform solution was analyzed for vitamin A and carotene content with 1,3-dichloro-2-propanol as described above.

Necessity of Saponification. A series of duplicate milk samples was pipetted into 0.375 × 4 inch test tubes. To one set of duplicates was added 1 ml. of 1 *N* potassium hydroxide in 90% ethanol. These tubes were warmed for 35 minutes in a 60° C. water bath. To the other set of duplicates was added 1 ml. of 95% ethanol and the contents of the tubes were mixed by tapping. The contents of all tubes were extracted and analyzed with 1,3-dichloro-2-propanol as described above.

Preparation of Recovery Samples. A solution of 1.0 to 3.0 micrograms of vitamin A per ml. was prepared in petroleum ether, and 1 ml. of this solution was added in a calibrated centrifuge tube to 1 ml. of the milk to be analyzed. The milk level before addition of the petroleum ether was carefully noted. A stream of nitrogen was played into the tube through a capillary held firmly in position 2.5 cm. above the surface of the sample. The tube was then immersed in a 50° C. water bath and agitated constantly until the meniscus in the tube had returned to the previously noted level. This technique adds a finely dispersed supplement of vitamin A acetate to the milk. The enriched sample was saponified and extracted in the same manner as the original unenriched sample. Values obtained were compared with values found for the original sample plus the value of the supplement to give a recovery percentage.

SUMMARY

Activated 1,3-dichloro-2-propanol (glycerol α,γ-dichlorohydrin) was used as a colorimetric reagent in analyzing milk for vitamin A and carotene. This reagent eliminated the need for anhydrous precautions and permitted the simultaneous reading of both vitamin A and carotene absorption at 555 and 800 mμ, respectively. Sample volumes for each determination were reduced from the normally required 10 to 100 ml. to as little as 0.25 to 1.0 ml. by saponifying and extracting the milk sample in a single test tube, and by employing horizontal cuvettes with a 5.0-cm. light path.

A comparative study of this and a method using larger volumes of milk showed higher values for this method. Added vitamin A was quantitatively recovered. Comparing the GDH with an antimony trichloride method of analysis showed similar values. Saponification of small milk samples was found to be absolutely necessary in order to extract the vitamin A–containing lipid completely from fresh milk.

ACKNOWLEDGMENT

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Determination of Traces of Iron, Nickel, and Vanadium in Petroleum Oils

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Methods have been developed for the determination of iron, nickel, and vanadium in petroleum oils. The sample is ashed and taken up with potassium bisulfate and the aqueous solution of the bisulfate cake is analyzed by spectrophotometric procedures. The measurements are based on the development of colored solutions by reagents specific for one element under the analytical conditions. Iron is determined as the iron–2,2′-bipyridine complex, nickel as colloidal nickel dimethylglyoxime, and vanadium by reaction with diphenylbenzidine. The analyses can be made with an accuracy of ±10%, or better, in the presence of the many elements likely to occur in petroleum oils. Interferences are minimized or eliminated without recourse to separation techniques. The detection limits are approximately 0.005 mg. of vanadium pentoxide of nickel oxide, 0.007 mg. of nickel oxide, and 0.01 mg. of ferric oxide.

The present trend in catalytic cracking is in the direction of utilizing heavier oils as feed stocks. It has been found, however, that as deeper cuts are taken into the crude the concentration of metal contaminants increases rapidly. These metals, which are in the main deposited on the cracking catalyst, may adversely affect both the selectivity and activity of the catalyst. This is understandable when it is realized that a catalyst replacement rate of 0.5 pound (0.23 kg.) of catalyst per barrel of oil containing only 1 p.p.m. of metal could result in an equilibrium catalyst containing about 0.06 weight % of the metal.

The major metal contaminants in petroleum oils are usually aluminum, sodium, iron, nickel, and vanadium with, frequently, lesser amounts of silica, magnesium, calcium, copper, manganese, lead, tin, barium, zinc, molybdenum, chromium, and titanium. The form in which the metals occur in the crude is not known; in all probability they are present both in suspension as inorganic salts and in solution as metallo-organic compounds. Although the metals concentrate in the residual fraction when the crude is processed by either distillation or propane extraction, small quantities of the metals appear in the lighter fractions from either method of processing.

The concentrations of iron, nickel, and vanadium have been determined on a number of cracking feed stocks in order to obtain an index as to the probable degree of contamination of the cracking catalyst. It has been found necessary to devise methods for determining as little as 0.5 p.p.m. of each of these metals with a probable accuracy of ±10%.

In order to provide an analytical procedure that would be applicable to crudes from a variety of fields, it was necessary to develop methods that would be subject to a minimum of interference from a large number of possible elements. In the interests of speed and economy it was also desirable to avoid the use of separation techniques in eliminating the interferences. The spectrophotometric procedure reported here fills these requirements when applied to the residue left from the complete ashing of the oil.

SAMPLE PREPARATION

The oil to be analyzed is completely ashed by the following technique. The sample is thoroughly agitated and a weighed amount is poured into a 200-ml. platinum evaporating dish. It is then warmed to the flash point and allowed to burn quietly without further heating. The residual coke is ignited by heating with a Meker burner. Fresh charges of oil are added until an observable amount of ash, which may be evidenced as a stain on the dish, is obtained. It is not necessary to ash completely between successive additions of oil. For concentrations of the order of 1 p.p.m., 100 to 200 grams of oil are usually sufficient.

When sufficient oil has been burned the residue is ignited to constant weight. The silica is then removed in the usual manner by treatment with hydrofluoric acid. Following this the residue is fused with 5 grams of analytical grade potassium bisulfate and, after cooling, the bisulfate cake is dissolved in water containing not more than 1 ml. of 12 *N* hydrochloric acid. If much acid is used, too great a dilution occurs in subsequent neutralizations. The solution is diluted to 100 ml. in a volumetric flask.

A bisulfate fusion is used, rather than a carbonate fusion or an acid, to ensure the complete dissolution of aluminum oxide which may occlude the other elements. It has been found desirable to use 5 grams of potassium bisulfate in order to ensure adequate wetting of the platinum dish.

DEVELOPMENT OF METHODS

All colors are developed in 25-ml. volumetric flasks to minimize the volumes of the solutions to be tested. Color densities are measured with a Beckman spectrophotometer (Model DU) using 1-cm. Corex cuvettes with distilled water in the reference cell. Greater sensitivity could be obtained by the use of longer cuvettes; in this case a reagent blank should be employed in the reference cell, whereas in the procedures used here the blank correction was negligible. All reagents employed are of analytical grade, except as noted. Reference solutions of the metal ions are prepared from ammonium metavanadate, nickel ammonium sulfate hexahydrate, and electrolytic iron.

Iron. Of the many methods available for the colorimetric determination of iron, the method of Moss and Mellon (*8*) has been selected as being relatively simple and free of interferences from the other metals likely to be present. This procedure is based on the red complex formed between 2,2′-bipyridine and ferrous ion. Tests have shown that comparable concentrations of nickel, vanadium, and zinc, present simultaneously, lead to an interference of about 3%, which is within tolerance. Of the interferences listed by Moss and Mellon, these three metals are the only ones that have been found to occur in petroleum in high enough concentrations to be troublesome. Aluminum will interfere if present in 100-fold excess, owing to the formation of the hydroxide.

Table I. Duplicate Analyses

	Fe_2O_3 *P.p.m.*	NiO *P.p.m.*	V_2O_5 *P.p.m.*
Venezuelan distillate No. 1	1.1 1.0	0.30 0.30	3.0 2.9
Venezuelan distillate No. 2	0.4 1.1	0.9 1.1	16 14

Table II. Determination of Iron, Nickel, and Vanadium in Known Solutions

(All quantities are expressed as mg. in 100 ml.)

	Fe_2O_3	NiO	V_2O_5	MoO_3	MnO_2	Cr_2O_3	Al_2O_3	MgO
Synthesis	74.3	10.2	14.3	4.8	0.0	0.0	15.1	0.0
Analysis	74.2	10.1	13.7	..	..	..	...	...
Synthesis	17.2	25.5	85.7	2.4	0.0	0.0	15.1	0.0
Analysis	17.2	24.3	80.3	..	..	..	...	...
Synthesis	10.0	9.93	200	0.0	1.58	0.0	200	8.3
Analysis	10.1	9.51	195	..	..	..	...	...
Synthesis[a]	21.4	3.8	4.07	0.0	0.0	0.0	7.6	0.0
Analysis	..	..	3.98	..	..	..	...	...
Synthesis[a]	0.0	0.0	3.62	0.0	31.7	29.2	0.0	0.0
Analysis	..	..	3.77	..	..	..	...	...

[a] Samples ignited and subjected to bisulfate fusion to simulate sample preparation step.

Only two revisions in the procedure of Moss and Mellon are required. The pH of the solution is held between 5 and 8, and the color development solution is allowed to stand overnight before readings are taken. Moss and Mellon indicate that the color development reaches a maximum almost immediately. In this application the color forms slowly, probably because of the high salt concentration arising from the bisulfate fusion.

The absorption due to the 2,2′-dipyridine–iron complex is measured at 5200 Å. The range is from 1.0 to 7.0 micrograms of ferric oxide (Fe_2O_3) per ml., corresponding to transmittances of between 80 and 20%. Beer's law is obeyed over the indicated range.

Nickel. Most colorimetric methods for nickel employ the development of the wine-red color of colloidal nickelic dimethylglyoxime. This method, although the most reliable, suffers from interferences and it has been recommended (*2, 4*) that a separation be carried out prior to color development. The procedure developed here is satisfactory without prior separation. Iron interference, for concentrations less than 100 micrograms per ml., is suppressed by the addition of tartrate ion. Aluminum, vanadium, molybdenum, and manganese cause less than 2% error when present in 50-fold excess. The major interferences, copper and cobalt, are not important in this case, as it has been shown by qualitative spectrographic analyses of a number of oils that they occur in negligible amounts. Alcohol is not added to stabilize the colloid, as in the procedure of Mitchell and Mellon (*2*), because this would precipitate potassium sulfate.

The solution to be tested is neutralized with 0.5 *N* ammonia, and tartaric acid is added, then bromine water, followed by concentrated ammonia; dimethylglyoxime is added before the solution is made up to volume. The color develops gradually, reaching maximum intensity in about 1 hour, and then fades slowly. Because the time to attain maximum intensity depends on the other constituents present, several readings are taken to determine the maximum value. The transmittance is measured at 4660 Å. The range is from 0.5 to 5 micrograms of nickel oxide per ml. Beer's law is obeyed over the indicated range.

Vanadium. The two most thoroughly investigated methods for vanadium are the hydrogen peroxide (*7*) and the phosphotungstic acid (*6*) procedures. These methods are not applicable, because the peroxide method is not sufficiently sensitive and the formation of vanadophosphotungstate is prevented by the precipitation of potassium tungstate. An attempt to use the vanadophosphomolybdate complex, as in phosphorus analysis, has not been successful. A method based on diphenylbenzidene has been developed. Oxidation indicators such as aniline, diphenylamine, phenylenediamine, benzidine (*1, 5*), and diphenylbenzidine (*4*) have frequently been suggested as colorimetric agents. Their chief fault is that they are not specific for any particular reducible ion. It is possible to consider them in the present application, inasmuch as the coking, ashing, and bisulfate fusion used in sample preparation preclude the presence of most metals in their highest oxidation state. Diphenylbenzidine has been chosen as giving the most stable color and apparently having the highest oxidation potential of the indicators tested. Ferric iron is the element most likely to have an oxidation potential high enough to be considered as an interference. In acid solution ferric ion concentration of 4 micrograms per ml. causes no color formation. The blue color due to the partially oxidized form of diphenylbenzidine is most stable in acid solution, phosphoric acid being particularly favorable; this is a fortunate circumstance, for phosphoric acid removes ferric ion by complex formation. Other active oxidizing agents, including nitric acid, must be absent.

In the determination of vanadium a slightly acid aliquot of sample is treated with a few drops of bromine water, which is then expelled by gentle heating; this step is necessary to ensure that all the vanadium is in the +5 state of oxidation. The acidic bromine solution does not oxidize other metallic ions to valence states capable of forming the blue oxidation product of diphenylbenzidine. Concentrated phosphoric acid and water are then added and after the solution has been cooled the diphenylbenzidine reagent is added. The transmittance is read at 5750 Å., 15 minutes after color development. The colored solutions obey Beer's law over the working range of 0.4 to 3 micrograms of vanadium pentoxide per ml.

RECOMMENDED COLORIMETRIC PROCEDURES

Iron. Reagents. Hydroxylamine hydrochloride, 5% in water. Ammonium hydroxide, 0.5 *N* and 2 *N*. 2,2′-Bipyridine, 0.05% in water.

Procedure. Titrate an aliquot of the sample with 0.5 *N* or 2 *N* ammonium hydroxide solution to the methyl orange and phenolphthalein end points; the choice of normality depends on the acidity of the sample. Transfer another aliquot of the sample containing 0.02 to 0.18 mg. of iron (as Fe_2O_3) to a 25-ml. volumetric flask, and add 1 ml. of hydroxylamine hydrochloride solution. Adjust the pH to between 5 and 8 with a volume of ammonium hydroxide equivalent to the mean of the values determined by the aforementioned titration. Add 5 ml. of 2,2′-bipyridine solution, dilute to 25 ml. with water, mix, and allow the solution to stand overnight. Read the transmittance at 5200 Å. (0.05-mm. slit width) using distilled water as a blank. Determine the ferric oxide content from a calibration curve and calculate the total iron in the original sample from the dilutions used. An optical density of 0.1 in a 1-cm. cell is equivalent to 0.89 microgram of ferric oxide per ml.

Nickel. Reagents. Tartaric acid, 20% in water. Saturated bromine water. Ammonium hydroxide solution, concentrated, 0.5 *N* and 2 *N*. Dimethylglyoxime, 1% in ethanol.

Procedure. Transfer an aliquot of the sample containing 0.01 to 0.12 mg. of nickel oxide to a 25-ml. volumetric flask. Adjust the pH, using the same relative amount of ammonium hydroxide as in the procedure for iron. Add 1.25 ml. of tartaric

Table III. Analyses of Processed Crude Oils

Oil	° A.P.I	Fe_2O_3 *P.p.m.*	NiO *P.p.m.*	V_2O_5 *P.p.m.*
Arabian residuum	16.3	139	2.0	6.5
Arabian vacuum distillate	21.4	7.7	0.0	1.8
California residuum	16.7	25	46	59
California vacuum distillate	26.1	0.1	0.5	0.8
Colorado residuum	17.8	12.0	1.3	4.3
Colorado propane decarbonized oil	24.5	0.81	0.14	0.11
Hastings propane decarbonized oil	22.4	0.68	0.86	0.20
Juesepin vacuum distillate	20.2	4.6	0.70	0.60
Light Mercedes vacuum distillate	23.8	1.1	0.30	3.0
Mid-continent vacuum distillate	33.5	1.3	0.60	2.4

acid solution, followed by 1.25 ml. of saturated bromine water and 2.5 ml. of concentrated ammonium hydroxide. Mix the solutions and then add 1.25 ml. of dimethylglyoxime solution. Dilute to 25 ml. with water and mix. Read the transmittance at 4660 Å. (0.05-mm. slit width) at the time of maximum color development (approximately 1 hour after mixing), using distilled water as the blank. Determine the nickel oxide content from a calibration curve and, from this the nickel content of the original sample. An optical density of 0.1 in a 1-cm. cell is equivalent to 0.57 microgram of nickel oxide per ml.

Vanadium. REAGENTS. Diphenylbenzidine. Prepare a saturated solution of diphenylbenzidine in 95% ethanol. Filter and dilute 1 to 1 with phosphoric acid, specific gravity 1.71. This procedure was desirable in order to provide a clear, stable solution that does not produce cloudiness during color development.

Saturated bromine water.

Phosphoric acid, specific gravity 1.71.

PROCEDURE. Transfer an aliquot of the sample containing 0.01 to 0.08 mg. of vanadium pentoxide to a 25-ml. volumetric flask. Add 1 drop of saturated bromine water and then heat the flask gently over a microburner until all the color due to the bromine is removed; this takes about 5 minutes. After cooling the flask, add 10 ml. of phosphoric acid and cool again. Dilute with water to almost 20 ml. and cool thoroughly after mixing. Add 5 ml. of the diphenylbenzidine solution and dilute with water to the mark. Read the transmittance at 5750 Å. (0.05-mm. slit width) 15 minutes after color development, using distilled water as the blank. Determine the vanadium pentoxide content from a calibration curve. An optical density of 0.1 in a 1-cm. cell is equivalent to 0.38 microgram of vanadium pentoxide per ml.

Table IV. Metal Content Versus Extent of Distillation

Yield of distillate, vol. %	100 (feed)	66.2	54.2
Fe_2O_3, p.p.m.	55	0.40	0.30
NiO, p.p.m.	38	0.85	0.35
V_2O_5, p.p.m.	435	15.8	6.2

The reagent solution is very dilute and its strength should be determined by following the above procedure, but using an excess of standard vanadate solution and 5 ml. of reagent. From this the maximum optical density that can be relied upon may be determined. If, in an analysis, this maximum optical density is approached, the unknown solution should be diluted.

PRECISION AND ACCURACY

The precision of the method can be considered as dependent on three separate stages of the analysis: photometry, color development, and sample preparation. The accuracy of measurement with a Beckman spectrophotometer is considered to be within about 1%. The color development on standard samples of nickel, vanadium, and iron is accurate to within 2%, as deviations from the best straight line through the calibration points (optical density *vs.* metal content) were all within this value. The slope of the curve was the same on two independent calibrations more than a year apart for all three determinations. The ashing procedure may have inherent errors, principally due to volatilization and incomplete fusion. However, check analyses indicate that this is not large.

Table I shows two examples of the reproducibility to be expected. The two analyses of distillate No. 2 were run on samples taken several months apart; the discrepancy in iron content is possibly due to contamination from the container.

The accuracy depends on the size of the sample prepared from a reasonable amount of oil and on interferences. The colorimetric procedures can determine 0.01 mg., or less, of each of ferric oxide, nickel oxide, and vanadium pentoxide. Hence, the original 100-ml. solution should contain at least 0.1 mg. of the metal oxides. Smaller quantities of the three elements can be detected and determined when it is possible to prepare the test solution in a smaller volume; however, in some instances the unused part of the 100-ml. solution has been used for other tests or determinations. The interferences are the most important and least known cause of error. Interferences due to a likely amount of any one of the metals known to be in the oils should be less than 2%. When several of these metals are present together, the error may be greater than this. It is advisable to precede any series of analyses of oils from a given region by a semiquantitative spectrographic analysis, in order to assess the possibility of major interferences.

The results obtained on a number of synthetics are shown in Table II. In addition to the interference tests on individual elements, the synthetic solutions were prepared to determine the effect of widely varying compositions with respect to both the nature of the metals present and the relative concentration of the specific elements. The representative mixtures listed in the table show good agreement between synthesis and analysis in all cases.

APPLICATION

The procedures described above have been applied to a variety of oils. Analyses of several oils from various fields and at various depths of cut are given in Table III. These data indicate the wide variations in composition that may be expected.

The principal application has been to feed stocks for catalytic cracking obtained from reduced crudes. It has been observed that as the percentage overhead obtained by vacuum distillation of a reduced crude is increased, the amount of metal contaminants in the overhead oil is also increased. A typical set of analyses illustrating this effect is given in Table IV for a Venezuelan reduced crude feed and for 54 and 66% fractions from this feed by vacuum distillation.

The concentrations vary in a regular manner with the yield of distillate. From such data it is possible to predict the effect of depth of cut into a crude on the characteristics of the catalyst employed in the cracking operation.

The analytical procedures have also been applied, with minor modifications, to the analysis of cracking catalysts contaminated by the feed stocks.

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Photoelectric Colorimeter for Use in Microanalysis

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A photoelectric colorimeter is described which may be used with a variety of cuvettes, including those with an absorption path of 10 cm. and a capacity of 0.4 ml. Two amplifier circuits—one battery-operated, the other line-operated—are described. In either case, per cent transmittance is read directly from a meter.

IN ATTEMPTING to determine the concentration of cobalt in the tissues of laboratory animals, the need arose for a colorimeter requiring only small volumes of liquid and yet maintaining an absorption path of 5 or 10 cm. The colorimeter described here has been found satisfactory for the determination of not only cobalt (*1*) but other elements as well. It is particularly suited for methods in which the final colored complex may be concentrated in a small volume of an organic solvent, although aqueous solutions may be used with all but the smallest cuvettes.

To illustrate the advantage gained by the use of small volumes and a long absorption path, the determination of copper by the carbamate method may be considered. Assume that the blank is set at 100 on a 0–100 scale. In the usual microcolorimeter a volume of 2 ml. is required for a 1-cm. absorption path. Approximately 0.25 microgram of copper as the carbamate per ml. of carbon tetrachloride or a total of 0.5 microgram of copper will be required to give a reading of 90. With the smallest cuvette described below, 1 ml. of solution is adequate for an absorption path of 10 cm. or a total of 0.025 microgram of copper to give the same reading. Thus the sample required is reduced by a factor of 0.05.

In the case of copper in plant and animal tissues where ashing is necessary, 0.025 microgram of copper is about the minimum that can be dealt with because unavoidable contamination and losses become limiting factors. In other methods, the point at which these and other factors become limiting is reached with larger volumes and shorter absorption paths, and the authors feel that in the determination of the trace elements, the colorimeter described will be found adequate in a large majority of the methods now available.

The early work of Müller (*5*) demonstrating the value of a photocell-amplifier combination led to its use in the present colorimeter.

DESCRIPTION OF COLORIMETER

The arrangement of the components of the colorimeter and the general constructional details are shown in Figure 1. Figure 2 shows the arrangement within the cabinet and the position of the light shields.

All components, except light shields, are mounted on an optical bench consisting of two lengths of 1 × 1 inch (2.5 × 2.5 cm.) angle iron positioned as shown in Figure 1. The bench rests on mounting blocks and is secured to the base of the cabinet. The bases of the components are grooved to fit the lips of the bench and are secured by bolts through the slot in the bench.

The lamp, a 50-cp. prefocus auto headlight lamp (Mazda No. 2531), is mounted on a support which permits adjustment of the lamp in a plane normal to the optical axis of the instrument and with the axis of the filament coil about 60° from the optical axis. The lamp is operated from either a 6-volt storage battery or a suitable transformer.

The lens is mounted so that adjustments in the plane normal to the optical axis and on a line parallel to the axis are possible. A lens board mounted in a frame as shown in Figure 1 permits the adjustments normal to the axis. The lens is mounted in a barrel which screws into the lens board, thus permitting adjustment along the optical axis for accurate focusing. The barrel also accommodates fixed diaphragms. The American Optical Company 116-mm. doublet No. 11161-701 has proved satisfactory in this service.

The filter holder consists of a simple frame with spring clips to hold 2 × 2 inch glass filters. The holder fits into an opening in the first light shield, which is an integral part of the cabinet.

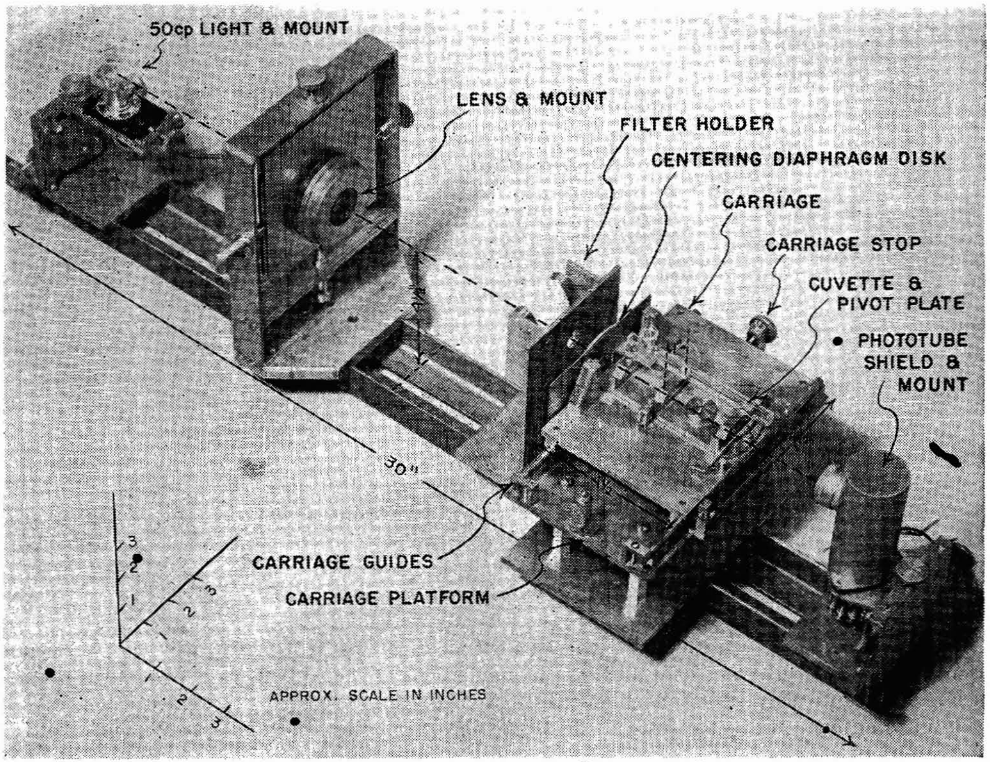

Figure 1. Exposed View of Colorimeter

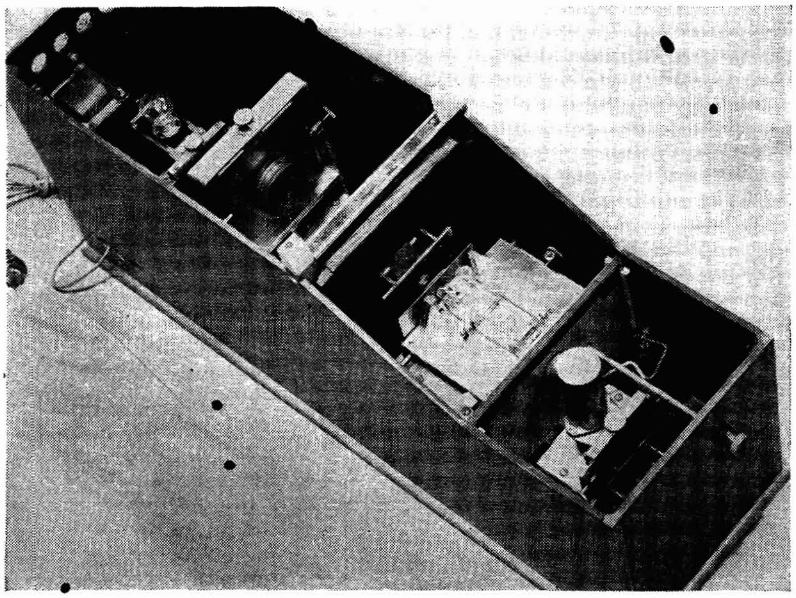

Figure 2. Colorimeter Arrangement in Cabinet

The cuvette carriage assembly consists of three parts: a platform, the carriage, and the cuvette support. The top plate of the platform (the bottom plate of which rests on the optical bench) carries the guides, carriage stop bosses, and adjustable diaphragm. The carriage slides on two parallel guides made of $^1/_4$ inch steel rod, the contact being two brass strips in one of which is cut a 90° V to hold the carriage in alignment. Screws running through the carriage stop bosses permit adjustment of the lateral travel of the carriage. The adjustable diaphragm consists of a brass disk pivoted at the center to another brass plate, which in turn is fastened to the platform. Several sets of holes of different diameters located at various radii from the center of rotation are drilled through the disk. The diameter of the cuvette used and the amount of light needed will determine the hole used. V-ways may be used to raise cuvettes of small diameter into the light beam. Wire drills No. 30, 40, 50, and 60 give a satisfactory selection of size, and steps of $^1/_{16}$ inch are satisfactory for height adjustment.

The carriage is moved either by a small rod threaded into either side of the carriage and extending through the side of the cabinet, or by a pinion which engages a rack mounted on the carriage as shown in Figure 1.

The support for the cylindrical cuvettes consists of three pairs of carefully machined V-ways mounted on a plate, pivoted on the carriage so that it may be swung out of the way when the holder for square cuvettes and test tubes is slid onto the two tongued bosses shown. The plate is positioned by means of two bosses fitted with adjusting screws which press against the ends of the two V-ways. A spring clip is used to hold the plate firmly in position.

A simple lever-type light shutter is provided to cover the hole in the light shield located between the carriage and phototube compartments. The lid covering the carriage compartment is hinged at the top, and, as the lid closes, it pushes against the short arm of the lever, raising the shutter arm. As the lid is raised, the shutter arm drops by gravity, closing the opening into the phototube housing.

The photocell is mounted on a support similar to the light support, permitting adjustments in the plane normal to the optical axis of the instrument. A brass shield fitted with a thin glass window (microscope cover slips are satisfactory) fits over the phototube. The shield serves to protect the tube from dust and stray light.

The cuvettes are cylindrical, with plane, parallel ends of Corex or Pyrex glass 1 mm. thick. They are filled and emptied through a tubular opening approximately 1.5 cm. from the front end. On the smaller cuvettes this opening is approximately 4 mm. in diameter and 1 cm. in length. A small tab of Lucite or similar material $^1/_8$ inch thick is cemented on the cuvettes for convenience in handling and to avoid heating during the filling operation. If the cuvettes are held in the fingers, the heat causes the formation of tiny bubbles in many organic liquids, and a wait of a minute or two is needed to get full light transmission. This difficulty is minimized if handled only by the tab. (Satisfactory cuvettes may be obtained from the American Instrument Company, Silver Springs, Md., or they may easily be made by slowly fusing the end pieces to Pyrex tubing ground to a flat surface. A small oxygen-gas flame is used to accomplish the fusion. The end disks may be cut from flat polished glass obtainable from the Corning Glass Company, Corning, N. Y. The glass is embedded in plaster of Paris, and the cutting is done on a drill press using copper tubing and a paste of No. 120 Carborundum and turpentine.)

The dimensions of the cuvettes that have proved convenient are given in Table I. The 0.4-ml. cuvette is readily filled with organic solvents by means of a medicine dropper drawn to a fine tip, although with some liquids a little tilting and tapping may be necessary. The 2-ml. size may be used with aqueous solutions, provided it has been thoroughly cleaned. The 5-cm. cuvettes described by Kirk, Rosenfels, and Hanahan (*3*) or the 1-cm. cuvettes described by Lowry and Bessey (*4*), both of which are particularly adapted for use with small volumes of aqueous solutions, could be used in place of the cuvettes described here.

The amount of light energy to be measured in this colorimeter is rather small, but the high sensitivity of either of the amplifiers described below makes possible the use of light filters transmitting a relatively narrow portion of the spectrum.

DESCRIPTION OF AMPLIFIERS

Two amplifier circuits have been used with the colorimeter described above. One, a battery-operated Vance amplifier of high sensitivity, is shown schematically in Figure 3. The other, line-

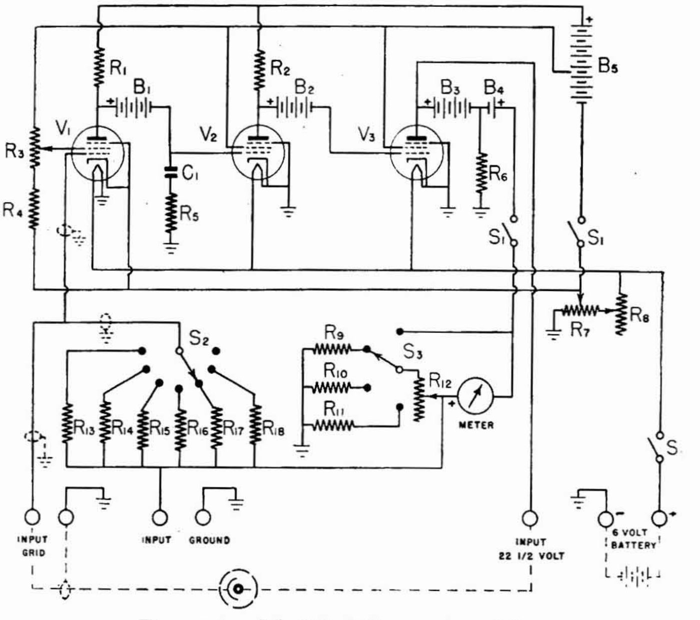

Figure 3. Modified Vance Amplifier

C_1.	0.01 µf. paper	
R_1, R_2.	2 megohms, 2 watts	
R_3.	25 K/W W potentiometer	
R_4.	30 K/2 watts	
R_5.	9 K/2 watts	
R_6.	100 K/2 watts	
R_7.	100/W W potentiometer	
R_8.	30/W W potentiometer	
R_9.	1 K/2 watts	
R_{10}.	3 K/2 watts	
R_{11}.	10 K/2 watts	
R_{12}.	10 K/W W potentiometer	
R_{13}.	1 megohm	High grade, high resistance resistors
R_{14}.	10 megohms	
R_{15}.	100 megohms	
R_{16}.	300 megohms	
R_{17}.	1000 megohms	
R_{18}.	3000 megohms	

S_1.	3-pole single-throw switch
S_2.	7-position ceramic low-loss switch
S_3.	4-position ceramic low-loss switch
B_1, B_2, B_3.	22$^1/_2$ volt B battery
B_4.	1$^1/_2$ volt A battery
B_5.	90 B battery, 28.5-volt tap
Meter.	0–100 µa. (Weston 622 or equivalent)

All resistors in ohms; all capacitors in microfarads operated, is shown in Figure 4, with a built-in power supply for a photomultiplier tube.

Neither of these circuits possesses complete stability over a period of several hours, but such stability was not considered essential because a rapid interchange of reference blank and sample is provided for in the colorimeter. The battery-operated amplifier is essentially that described by Vance (*7*). Because portability is not of prime importance, the 1B4 tubes have been replaced with 6C6 tubes, and a 6-volt storage battery is used to supply the heater current of approximately 1 ampere. A Weston Model 622 0–100 microammeter is used instead of the 0.1-volt meter of the original design. Except for the filament battery which must be recharged regularly, the dry battery life is about one year when used 2 to 4 hours daily.

The amplifier consists of a simple three-stage cascade of sharp cut-off pentodes with a high degree of negative feed-back. Resistors R_9, R_{10}, R_{11}, and R_{12} form a meter-sensitivity network for adjusting the meter to 100. Resistors R_7 and R_8 will zero the instrument and are sufficient to handle dark currents normally found with the RCA 929 phototube used. The potentiometer, R_3, permits adjustment of the first stage screen potential. The input resistor bank, R_{13} through R_{18}, serves as the primary range control. The meter sensitivity network overlaps the range between steps in the input resistor bank. Full-scale meter deflection will be obtained with approximately a 100-millivolt signal. This is equivalent to an approximate full-scale current sensitivity of 10^{-10} to 10^{-11} ampere, using the 3000-megohm input resistor.

Two inputs are provided. The input terminals marked zero and grid are used for potentiometric titrations or for a phototube with external driving potential. The terminals marked 22$^1/_2$ volt and grid provide a 22.5-volt driving potential sufficient for direct connection of the RCA 929 phototube as shown.

In the line-operated amplifier (Figure 4), some stability and amplifier-sensitivity have been sacrificed for the convenience of line operation. However, if the 931-A photomultiplier tube is used the over-all sensitivity for photometric purposes is greater than the battery-operated system described above.

The amplifier proper is a simple vacuum tube bridge circuit, similar in most respects to the mu-bridge circuits described by Turner (*6*) and Garman and Droz (*2*). The mu-bridge conditions have not been strictly satisfied, and the meter is used for direct reading of in-balance rather than as a null point indicator.

The two halves of the 6SN7 form two arms of a resistance bridge, while the resistors R_{15}, R_{16}, and R_{17} form the other two arms. One half of the tube acts as a fixed-reference resistor, the other half as a variable resistor controlled by the signal voltage developed across the input resistor, R_6 plus R_7. Potentiometer R_{17} serves to balance the meter to zero for zero signal. Full-scale adjustment is made with the input resistor. A full-wave rectifier 5W4 and a simple RC filter circuit supplies the plate potential to the bridge. This potential is stepped down by the bleeder-voltage divider, R_4, and regulated by the VR-75 voltage regulator tube. The tap on R_4 is adjusted so that the VR tube will fire under all line conditions and draw about 15 ma. on open circuit. A high-voltage power supply using a half-wave 2X2 rectifier is built into the unit to supply the 931-A photomultiplier tube.

The amplifier requires a signal of approximately 200 millivolts for full scale. The input resistor is limited to about 2 megohms, which is equivalent to a current sensitivity of about 10^{-7} ampere for full scale of meter. With an input resistance of up to 0.05 to 0.1 megohm, the grid return resistance of the reference tube is not too critical. With higher input resistances, the resistance in the two grid circuits should be matched.

Although photocurrents of 100 to 150 microamperes are easily obtained with the 931-A, operation of the tube at this level is not satisfactory. Best operation, from the standpoint of fatigue and stability, is obtained with current drains of less than 50 microamperes. Resistor R_7 is so chosen that the amplifier cannot be adjusted for photocurrents in excess of 40 μa., except by reducing the light intensity. Resistor R_6 is then chosen to cover the desired operating range.

The stability of the two measuring devices as indicated by reproducibility of readings taken on standard solutions or neutral wedges in the colorimeter is less than 0.5% full scale for the battery-operated circuit. The stability of the bridge-type line-operated circuit is about 1% full scale when erratic values due to line transients are ignored and drift corrections made. The response of both circuits with their photocells is strictly linear when tested with varying concentrations of colored solutions known to follow the Lambert-Beer law.

The authors gratefully acknowledge the help given by F. M. McNall in adapting the Vance amplifier to the present application.

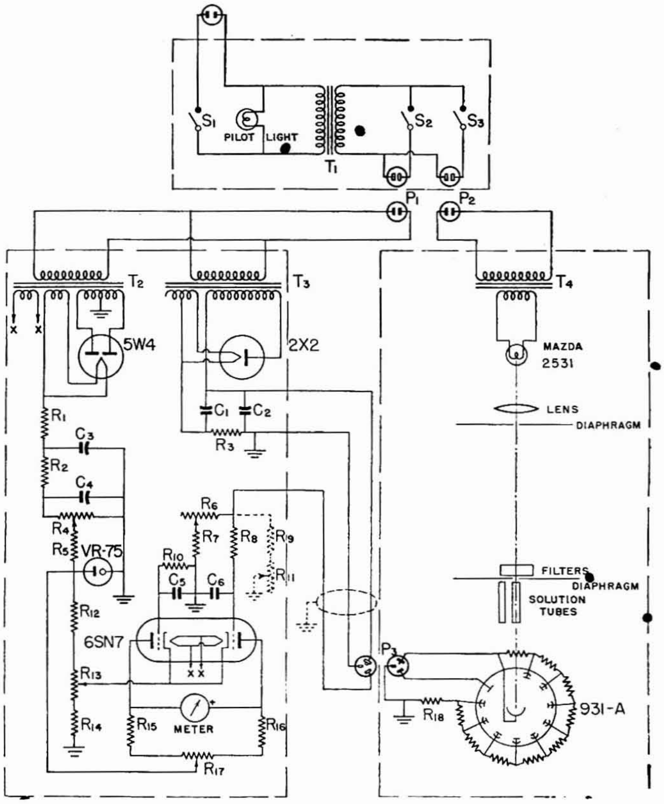

Figure 4. Schematic Diagram of Line-Operated Amplifier

C_1, C_2.	2/1000 volt electrolytic
C_3, C_4.	25/400 volt electrolytic
C_5, C_6.	0.05 μf. paper
R_1, R_2.	400/10 watts
R_3, R_8, R_9.	220 K/2 watts
R_4.	50 K/50 watts
R_5.	300/2 watts
R_6.	50 K/W W potentiometer
R_7.	5 K/2 watts
R_{10}.	270 K/2 watts
R_{11}.	250 K/potentiometer
R_{12}, R_{15}, R_{16}.	10 K/2 watts
R_{13}.	300/W W potentiometer
R_{14}.	200/2 watts
R_{17}.	10 K/W W potentiometer
R_{18}.	1 megohm(10–100 K/2 watts)
T_1.	Constant voltage transformer or supply. Approx. 100 V A required
T_2.	Power transformer, 650 VCT/40MA (Stancor P-6010 or equivalent)
T_3.	Power transformer, 700 V/100 M (Stancor P-6013 or equivalent)
T_4.	Filament transformer 6.3 V/10 a (Stancor P-3064 or equivalent)
S_1, S_2, S_3.	Single-pole single-throw toggle switches
P_1, P_2.	Standard power plug and receptacle
P_3.	Low loss shielded plug and receptacle
Meter.	0–100 μa. meter (Weston 622 or equivalent)

All resistors in ohms; all capacitors in microfarads

Table I. Dimensions of Cuvettes

Type	Length	Diameter		Capacity
		Inside	Outside	
	Cm.	*Mm.*	*Mm.*	*Ml.*
Cylindrical	5	8.5	10.5	3.0
	10	2.2	6.5	0.4
	10	5.0	6.5	2.0
	10	8.5	10.5	6.0
Square, test tube[a]		13.0	16.0	3.0 min.

[a] In one colorimeter, V-ways can be rotated 90°, leaving space near front of carriage where a detachable holder for cuvettes of this type is placed. Light beam passes through these cuvettes near bottom, so that only 3 ml. of liquid are required.

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Spectrophotometric Determination of Total Nitrogen in Oils

A Semimicro-Kjeldahl Method

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It is frequently necessary to analyze petroleum feed stocks and products for small amounts (less than 0.1%) of combined nitrogen. The conventional macro-Kjeldahl method requires large samples, prolonged acid digestion, and large amounts of reagents. A semimicro-Kjeldahl procedure has been devised to use small samples and to allow the determination of very low concentrations of nitrogen. The oil samples are digested with acid in special Kjeldahl flasks which fit onto an all-glass apparatus for steam distillation of the ammonia. The ammonia is determined by spectrophotometric measurements of the color developed with Nessler's reagent. Nitrogen present as amino, nitro, nitroso, azo, and ring compounds can be determined on an average to ±10% over a concentration range of 0.002 to 1.0%.

IT IS frequently necessary to analyze petroleum feed stocks and products for nitrogen content; small amounts of nitrogen are known to have deleterious effects upon some types of catalysts and also lead to the formation of deposits of solid ammonium or cyanide salts in lines. The conventional Kjeldahl method (*2*) is satisfactory for the analysis of oils that contain as much as 0.1% nitrogen. When the nitrogen content is less than 0.1%, however, large samples of oil are necessary to obtain sufficient ammonia to titrate satisfactorily. With samples of oil larger than 1 gram, increased amounts of sulfuric acid are required and excessive foaming occurs during the early period of digestion. Furthermore, with some types of oils, when samples large enough to give adequate titrations are used, several days are required to complete the digestion. In such cases the blanks are often larger than the actual sample titrations and are very erratic. It is desirable, therefore, in the analysis of oil to use small samples in order to obtain rapid digestion with minimum amounts of sulfuric acid. This requires the measurement of very small amounts of ammonia for samples low in nitrogen content.

Beeghly (*1*) described a method for the determination of small amounts of combined nitrogen in steels without the use of large samples. His procedure involves dissolution of the steel in hydrochloric acid, steam distillation of the ammonia, and spectrophotometric measurement of color developed by ammonia with Nessler's reagent. This procedure has been applied, with a few modifications, to the analysis of petroleum. A small sample is digested with sulfuric acid in a special Kjeldahl flask. The ammonia is liberated by steam distillation and reacts with Nessler's reagent, and the color is measured with a spectrophotometer.

APPARATUS

The samples are digested in 100-ml. Kjeldahl flasks (over-all length, 25 cm.) equipped with 28/15 spherical joints to fit on the distillation apparatus.

The apparatus for steam distillation of the ammonia is similar to that described by Beeghly (*1*) for the determination of nitrogen in steel, with the following modifications:

Spherical joints and clamps are used instead of tapered joints, except on the neck of the steam generator. With tapered joints the Kjeldahl flasks frequently froze to the apparatus and had to be broken loose; with spherical joints, the apparatus is more flexible and less likely to be broken by ordinary use.

The joint that connects to the condenser is placed midway between the condenser and the Kjeldahl flask to make the apparatus more flexible.

The delivery tube extending to the bottom of the Kjeldahl flask for the introduction of sodium hydroxide is flared considerably on the end. This prevents plugging of the tip by sodium sulfate precipitated during the neutralization of the sulfuric acid.

Fifty-milliliter volumetric flasks are used to collect the distillate under the condenser.

A Coleman Model 11, Universal spectrophotometer and, for a few analyses, a Beckman Model DU spectrophotometer were used to measure the concentrations of ammonia in the distillates.

REAGENTS

Sulfuric acid, C.P., 96%.

Catalyst Mixture. Mix 5 parts of anhydrous potassium sulfate, 2 parts of mercury (II) oxide, 1 part of anhydrous copper (II) sulfate, and 1 part of selenium.

Salicylic acid.

Sodium hydroxide, 50% solution, containing 1% of sodium sulfide.

Nessler's Reagent (*4*). Dissolve 50 grams of potassium iodide in a minimum volume of cold water (approximately 35 ml.). Slowly add a saturated solution of mercury (II) chloride until the first slight red precipitate persists, and then add 400 ml. of 9 *M* sodium hydroxide. Dilute to 1 liter with ammonia-free water and allow to stand until the supernatant liquid is clear.

Standard ammonium chloride solution, 100 mg. of nitrogen per liter.

Organic nitrogen compounds used for the preparation of synthetic samples were obtained from the Eastman Kodak Company.

PROCEDURE

Preparation of Calibration Curves. Pipet 5-, 10-, 15-, 20-, and 25-ml. aliquots of the standard ammonium chloride solution into 50-ml. volumetric flasks. Dilute almost to the necks of the flasks with ammonia-free water and pipet into each flask 1 ml. of Nessler's reagent. Swirl the flasks during the addition of the Nessler's reagent to prevent precipitation. Fill to the marks with ammonia-free water, mix, and allow to stand for 5 minutes. Measure the absorbancies at 450, 475, and 500 mμ, with distilled water in the reference cell. Prepare calibration curves for the three wave lengths by plotting the concentrations of nitrogen per 50 ml. against the corresponding absorbancies for each wave length.

Analysis of Sample. Weigh a 0.05- to 1.0-gram sample, from a weight buret if the material is a liquid, into a 100-ml. Kjeldahl flask equipped with a 28/15 spherical joint (to fit on the distillation apparatus). Add approximately 0.1 gram of salicylic acid and 0.1 to 0.2 gram of catalyst mixture. Take care that the sample and other materials added do not stick on the neck of the flask. Add 7 to 8 ml. of concentrated sulfuric acid and allow to stand 5 to 10 minutes. Heat the mixture under a hood until the solution is clear. This usually requires 0.5 to 2 hours. Care must be exercised at the beginning of the digestion to prevent excessive foaming. For certain types of oils, it is sometimes necessary to add 2 to 3 ml. more of acid during the digestion when the volume of the solution becomes low. After the solution clears, continue the digestion for 1 hour and allow to cool. Dilute with 5 to 10 ml. of ammonia-free water, again cool, and add several drops of phenolphthalein.

While the sample is digesting, the steam-generating flask of the distillation apparatus should be half filled with distilled water and heat applied. The steam-generating flask should contain 1 to 2 ml. of sulfuric acid to prevent the distillation of any ammonia in the water. Connect an empty Kjeldahl flask to the unit and allow the steam to pass through it into the condenser to free the apparatus of traces of ammonia.

With the steam-generating flask maintained at a temperature just below the boiling point, connect the Kjeldahl flask containing the digested sample to the unit. Fill the dropping funnel above the Kjeldahl flask with 50% sodium hydroxide. Cautiously neutralize the acid to phenolphthalein and add about 1 ml. of excess sodium hydroxide. Increase the temperature in the steam-generating flask sufficiently to cause a steady evolution of steam. Place a 50-ml. volumetric flask under the condenser to collect the water and ammonia. Allow the flask to fill exactly to the mark with the condensate; remove it from the condenser, and mix the solution thoroughly. The temperature of the steam-generating flask can be lowered by the addition of more water, and the apparatus is immediately ready for the substitution and distillation of a second sample.

Table I. Determination of Nitrogen in Synthetic Samples

Compound Added to Gas Oil	Weight % Nitrogen Added	Weight % Nitrogen Found	Average Difference	% Error
Aniline	0.0030	0.0032 0.0039	+0.0006	+20
	0.0066	0.0067 0.0070	+0.0003	+5
	0.061	0.048 0.051	−0.011	−18
Dimethylaniline	0.0030	0.0039 0.0038	+0.0009	+30
	0.0066	0.0068 0.0063	0.0000	0
	0.0126	0.0116 0.0116	−0.001	−7
Diethylaniline	.09	1.13 1.08	+0.025	+2
2-Naphthylamine	0.0028	0.0025 0.0033	+0.0001	+4
	0.0061	0.0066 0.0057	+0.0001	+2
	0.0117	0.0119 0.0114	0.0000	0
Nitrotoluene	0.0112	0.0107 0.0111 0.0113	−0.0002	−2
2-Nitroso-1-naphthol	0.020	0.025 0.024	+0.0045	+22
Azobenzene	0.0145	0.0138 0.0144	−0.0004	−3
	0.150	0.146 0.142	−0.006	−4
Dimethylquinoline	0.0028	0.0034 0.0034	+0.0006	+21
	0.0061	0.0061	0.0000	0
8-Hydroxyquinoline	0.055	0.049	−0.006	−11

Transfer a 10-ml. aliquot of the 50 ml. of distillate to another 50-ml. volumetric flask and fill almost to the neck with ammonia-free water. Add 1 ml. of Nessler's reagent, with swirling, and dilute to the mark with ammonia-free water. Allow to stand for 5 minutes but not over 1 hour, and measure the absorbancies at 450, 475, and 500 mμ. From the previously prepared calibration curves, read and average the concentrations of nitrogen corresponding to the absorbancies at the three wave lengths. If the color of the solution is too dark or if a precipitate is formed, take a smaller aliquot of the distillate. If the color is not dark enough to give an accurate measurement, take a larger aliquot or add the Nessler's reagent directly to the original distillate. If the latter is done, the solution is not diluted to volume with water, for the effect of dilution by addition of Nessler's reagent is negligible for this low concentration of nitrogen.

A blank determination must be carried through the entire procedure with the same amounts of reagents as are used in the analysis of a sample. The concentration of nitrogen found in the final distillate from a sample is corrected for this blank value. A blank usually corresponds to 0.01 mg. of nitrogen or less.

Table II. Determination of Nitrogen in Miscellaneous Plant Samples

Sample	Type of Material	Wt. % Nitrogen
A	Gas oil	0.002 0.004
B	Gas oil	0.005 0.005
C	Gas oil	0.011 0.012
D	Gas oil	0.025 0.025
E	Gas oil	0.064 0.063
F	Gas oil	0.094 0.098
G	Gas oil	0.16 0.16
H	Gas oil	0.30 0.30
I	Catalyst	0.18 0.21
J	Catalyst	0.56 0.56
K	Catalyst	1.10 1.13
L	Synthetic rubber	7.13 7.11

RESULTS AND DISCUSSION

A number of synthetic samples, chosen to represent a variety of forms of combined nitrogen, have been analyzed by this procedure. The samples were prepared by the addition of known amounts of nitrogen-containing compounds to a gas oil which contained less than 0.001% nitrogen. All analyses were corrected for a blank on the gas oil. The results, shown in Table I, indicate that by this method small amounts of amino, nitro, nitroso, azo, and ring nitrogen in oil can be determined on an average to within ±10% over a concentration range of 0.002 to 1.0% nitrogen. In a few cases the error was in the range of 20 to 30%, and for concentrations below 0.005% the error was usually positive, indicating slight contamination. An average of eight analyses can be made in one day. Ring nitrogen compounds of higher molecular weight than pyridine are successfully attacked; this was also observed by Shirley and Becker (*3*). However, attempts to determine nitrogen in samples containing pyridine were always unsuccessful.

The application of the method to the analysis of actual samples submitted to the laboratory is illustrated by the results in Table II. An average deviation of less than ±5% was obtained.

The relationship between absorbancy and concentration of nitrogen is a straight line with the Beckman spectrophotometer, but curves slightly at higher concentrations with the Coleman instrument. It was found to be more satisfactory, when using the Coleman spectrophotometer, to measure the absorbancies at three wave lengths and average the concentrations to reduce the errors of measurement. The calibration curves should be checked occasionally to detect slight changes that might occur in the Nessler's reagent or other steps in the measurement of the color.

As reported by Beeghly (*1*), the ammonia was found to be completely removed with 50 ml. of distillate. The amount of ammonia is so low that no loss of ammonia from the steam condensate occurs, unless relatively large amounts of ammonia are liberated. For samples containing more than 0.5% nitrogen, it is desirable to add 2 ml. of 0.1 *N* hydrochloric acid to the receiving flask before the distillation.

Although the method was devised for the determination of small amounts of nitrogen in oil, it has been applied to other types of samples—for example, catalysts, synthetic rubbers, and other solid materials that contained as high as 7% nitrogen have been successfully analyzed by this method. Some typical analyses of such materials are included in Table II. An advantage of this method for the analysis of such materials is that very small samples (less than 0.1 gram) are required.

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Microdetermination of Chlorine and Bromine

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A method is described for determining halogen in organic compounds by fusing with potassium and titrating the resulting potassium halide with silver nitrate, using dichlorofluorescein as an indicator. An attempt was made, with limited success, to determine halide with solid silver iodate by Sendroy's method.

OF THE many reported methods for the determination of halogen in organic compounds, few are suitable for use with very volatile liquids. The Carius method is the simplest and most suitable, but is tedious, as three weighings of the filter stick are necessary for the first analysis and two weighings are necessary for each succeeding analysis that day (*6*). Therefore, a volumetric method for determining halogens which could be used for all types of compounds, including volatile liquids, would be of distinct value.

The most promising of the methods reported in the literature for decomposing the organic halogen compound was judged to be fusion with an alkali metal in a sealed and evacuated tube. On a macro scale, using this method of decomposition, excellent gravimetric results were obtained by Elving and Ligett in 1942 for all halogens, including fluorine compounds (*4*). They announced then their intention of extending their work to a semimicro and micro scale, but have not yet done so.

At about the same time, Bürger (*2*) used a similar fusion in a nitrogen atmosphere to decompose the organic compound, determining chlorine and bromine by the Volhard method. His prime interest, however, was in the determination of sulfur, and his description of the method for halogen determination is somewhat vague and perfunctory. His procedure for the preparation of the potassium and decomposition of samples also seemed unduly complicated. In view of this, the author undertook to develop a more satisfactory microvolumetric procedure.

A review of methods for estimating the resulting halide ion indicated that a trial might profitably be made of the reaction between the ion and solid silver iodate, whereby the silver halide is precipitated and the filtrate analyzed for potassium iodate (*5, 7*). This method was investigated; although known solutions of potassium chloride and potassium bromide gave good results, organic compounds did not.

In view of recent favorable reports on the use of dichlorofluorescein in the titration of halide obtained by decomposition of organic compounds in the Grote combustion apparatus (*8*), and its reported use in titrating barium chloride in the presence of dispersed barium carbonate (*1*)—conditions similar to the author's—it was decided next to try a direct titration with silver nitrate using that indicator. Early experiments using this method produced low and inconstant results, which were traced to the difficulty of washing the reaction tube quantitatively. The use of hot acidified water for washing eliminated that difficulty and good results were then obtained with chloro compounds on a micro scale. However, semimicro samples were necessary to get satisfactory results with bromo compounds.

Another attempt was then made to develop the iodate procedure, using hot acidified washes for the reaction tube, and with this modification somewhat better results were obtained. However, although bromo compounds gave acceptable values, the results with chloro compounds were still not very satisfactory.

APPARATUS AND REAGENTS

Reaction tubes were 25-cm. lengths of 10- to 11-mm. Pyrex tubing, on which test tube ends were blown. Tubes can be reused twice before they become too short for use.

A gas-fired Carius furnace was used and an additional Bunsen burner was found necessary to attain the proper temperature.

The funnel was of the Hirsch type with a fritted 20-mm. disk M porosity. The disk surface was protected by a Whatman No. 540 2.1-cm. filter paper disk. After about six to eight filtrations, the paper was lifted out and the funnel washed by reverse suction with about 200 ml. of water, after which it was again ready for use. When necessary, it could be cleaned in warm dichromate cleaning solution overnight.

Phosphoric acid was made by diluting 100 ml. of 85% reagent to 1 liter.

Dichlorofluorescein (*8*), 10 mg. dissolved in 100 ml. of 60% ethanol to which 2.5 ml. of 0.01 *N* sodium hydroxide were added.

Silver nitrate, 0.01 *N* was used as primary standard.

Potassium chloride, 0.01 *N*, was standardized against silver nitrate.

Sodium thiosulfate, 0.04 *N*, was standardized against potassium dichromate.

All other reagents were best commercial grade.

EXPERIMENTAL

Preliminary Experiments on Determination of Inorganic Halide by Silver Iodate Method. Destroy several slivers of potassium in a 50-ml. beaker with ethanol and add a measured volume of 0.01 *N* potassium chloride or potassium bromide to each solution. Acidify the resulting solution with phosphoric acid to an alizarin red end point and add one drop in excess. Then dilute to about 25 ml. and concentrate on the hot plate to the proper volume. Add a spatula tip (about 50 mg.) of silver iodate, heat the mixture with occasional stirring for about 2 minutes, then allow it to cool, filter, and titrate as below.

Procedure for Decomposition of Organic Compounds. Weigh the liquid sample (4 to 8 mg. for micro and 10 to 20 mg. for semimicro) in a weighing capillary, the handle of which is a glass rod 20 to 25 mm. long, and place the capillary end down in the reaction tube. Weigh the solid sample with a long-handled weighing stick and place as far down in the reaction tube as possible. Cut under ether about three shavings of potassium each about 1 cu. mm., dry rapidly with a clean tissue, and add to the microsample. Use two to five times as much for semimicrosamples or in the presence of nitrogen or sulfur. Heat with rotation at a point about 3 cm. below the open end of the tube and allow glass to gather there, then draw to a thick-walled capillary. When cool, connect the open end to a water aspirator protected by a safety trap and when the tube is evacuated, seal off at the capillary. Shake the tube so as to break the inner capillary containing the liquid sample, and place in a furnace preheated to 400° C., allowing it to remain for 15 minutes. No precautions are necessary in handling these tubes and they can safely be removed from the hot furnace. When the tube is cool, wash the outside and open it by cutting just below the shoulder; add 1 ml. of ethanol to destroy excess potassium and wash the sides down with hot water. Form a lip on the tube by heating the open end and pressing with forceps.

Procedure for Argentimetric Titration. Transfer the solution through the sintered-glass funnel into a 125-ml. Erlenmeyer flask containing 5 ml. of 0.01 *N* potassium chloride. Wash the reaction tube four times with hot water and add a drop of 1 to 3 nitric acid to each washing. Add several alundum boiling stones to the solution in the Erlenmeyer and bring to a boil on an electric hot plate at low heat. Add about 5 mg. of barium carbonate to the hot solution, and acidify if it does not prove acid. Continue boiling for about 10 minutes. This removes the hydrocyanic acid formed in the presence of nitrogen. In the presence of sulfur, boil at least 2 hours with replacement of evaporated water to assure quantitative removal of the hydrogen sulfide, then carefully add excess barium carbonate until an undissolved residue of about 50 mg. remains. Add 1 ml. of dichlorofluorescein indicator and 10 ml. of acetone to the cooled solution and titrate with 0.01 *N* silver nitrate, using side lighting and dimming overhead lights to increase sharpness of the end point (*3*).

Procedure for Silver Iodate Method and Iodometric Titration. Transfer the solution in the reaction tube to a 50-ml. beaker containing a drop of alizarin red indicator and a small stirring rod. Wash the reaction tube four times with hot water, adding a drop of phosphoric acid solution to each washing unless the indicator in the beaker turns yellow, in which case use only one more drop. Neutralize the solution in the beaker to a yellow color with phosphoric acid and add one drop in excess. Concentrate on a hot plate to the proper volume (1 mg. of chlorine or 2.4 mg. of bromine should be in about 10 ml. of solution). Add about 50 mg. of solid silver iodate and allow the hot solution to remain on the hot plate for 2 minutes, with occasional stirring. Allow to cool and filter through a sintered-glass funnel into a 125-ml. glass-stoppered Erlenmeyer. Wash the beaker five times with small portions of 75% ethanol. Dilute the solution in the Erlenmeyer with distilled water to about 75 ml. Add 0.5 gram of solid potassium iodide and 5 ml. of 6 *N* sulfuric acid. Allow to stand for 2 minutes and titrate with 0.04 *N* sodium thiosulfate to a starch end point.

Table I. Recovery of Halide by Silver Iodate Procedure

Halide	Taken *Mg.*	Recovered *Mg.*	Deviation *Parts/1000*
Chloride	0.8865	0.8910	+5
	1.064	1.070	+6
	1.241	1.243	+2
	1.418	1.408	−7
	1.596	1.584	−8
	1.773	1.763	−6
	1.950	1.941	−5
Bromide	1.998	2.016	+9
	2.398	2.408	+4
	2.797	2.794	−1
	3.197	3.207	+3
	3.596	3.593	−1
	3.996	3.990	−2

Table II. Precision and Accuracy of Microdetermination of Bromo Compounds by Iodate Method

Compound	No. of Detns.	Theory %	Found, Av. %	Precision %
o-Bromobenzoic acid	4	39.76	39.72	±0.16
Benzalacetophenone dibromide	4	43.42	43.39	±0.18

RESULTS

Table I shows the results obtained by the iodate procedure on solutions of potassium chloride and potassium bromide of known concentration. When the same procedure was tried with decomposition products, the results for chlorine were not satisfactory but bromo compounds gave good results on samples not containing nitrogen (Table II). No sulfur-containing compounds were tried.

Table III. Precision and Accuracy of Argentimetric Microdetermination of Chloro Compounds

Compound	No. of Detns.	Theory %	Found, Av. %	Precision %
Chlorobenzene	4	31.50	31.44	±0.06
p-Chlorophenoxyacetic acid	7	19.00	19.00	±0.12
p-Chlorothymol	4	19.20	19.25	±0.13
2,4-Dinitrochlorobenzene	5	17.50	17.52	±0.09
Benzyl isothiourea hydrochloride	3	17.49	17.55	±0.11

Table IV. Precision and Accuracy of Argentimetric Semimicrodetermination of Bromo Compounds

Compound	No. of Detns.	Theory %	Found, Av. %	Precision %
o-Bromobenzoic acid	4	39.76	39.81	±0.10
Benzalacetophenone dibromide	4	43.42	43.53	±0.13
p-Nitrobromobenzene	3	39.56	39.47	±0.09
Bromobenzene	3	50.90	50.74	±0.16

The argentimetric titration worked well on a micro scale with compounds containing chlorine alone or in combination with nitrogen and sulfur (Table III), but it did not work with bromo compounds. With these compounds, however, good results were obtained on a semimicro scale (10- to 20-mg. samples) and using 0.015 *N* silver nitrate (Table IV).

Calculations. ARGENTIMETRIC

$$\% \text{ halide} = \frac{\text{ml.} \times \text{normality} \times \text{equivalent weight} \times 100}{\text{weight of sample (mg.)}}$$

IODATE

$$\% \text{ bromide} = \frac{\text{ml.} \times \text{normality} \times 79.9 \times 100}{6 \times \text{weight of sample (mg.)}}$$

Equivalent weight for chlorine = 35.5

Equivalent weight for bromine = 79.9

CONCLUSION

Decomposition of halo-organic compounds with potassium in a sealed, evacuated tube on a micro scale was found practical.

The chloride formed could be determined, after proper treatment, by titrating with silver nitrate using dichlorofluorescein indicator.

The bromide formed could be determined on a semimicro scale by a similar argentimetric titration or, if no other acid-forming elements were present, on a micro scale by treatment to convert the potassium halide to potassium iodate and determining as usual.

These methods have been used in the laboratory of the Ortho Research Foundation for the past few months and have proved convenient and reliable.

ACKNOWLEDGMENT

The author wishes to thank John Cozzolino for his work and suggestions in the preparation of this paper.

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Determination of Small Amounts of Water

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A method, employing azeotropic distillation and subsequent determination of the water in the distillate by titration with Karl Fischer reagent, has been applied to samples of oils, greases, deposits, additive concentrates, etc., containing as little as 0.0002% of water. The determination is made in a special distillation system protected from atmospheric moisture, after the system has been dried by partial distillation of the azeotrope former used in the analysis itself. The method is accurate to 0.3 mg. of water.

THE reported chemical methods employing the Karl Fischer reagent for determining very small amounts of water in petroleum fractions or other liquids involve a direct determination of the water in the sample with the Fischer reagent.

Aepli and McCarter (*1*) determined water in liquid petroleum fractions by titrating the sample directly in a large flask protected from atmospheric moisture. The method has disadvantages in that a large amount of sample is required, which makes direct titrations unwieldy, and the mixture in the titrating flask exists in two phases, necessitating the extraction of the water with Fischer reagent. Snyder and Clark (*10*) also applied the direct titration procedure to petroleum fractions, using a solvent to effect a single phase in the titration vessel. This method also involves titration of large volumes of liquid, and it is necessary to prepare and keep quantities of dry solvent.

Weaver and Riley (*12*) have developed a method for determining water in gases by measuring the change in electrical conductance of a hygroscopic film. They have done a small amount of work on liquid samples; however, the amounts of water reported are not very low. Evans and Davenport (*5*) have described a manometric procedure for small amounts of water in insulating oils. Their method is limited to oils, and would not be applicable to greases or semisolid materials such as engine deposits. Benning, Ebert, and Irwin (*4*) applied infrared spectroscopy for water in Freons, using the strong absorption band of water at 2.67 microns, but state that compounds containing hydrogen will also absorb at this wave length. This rules out the application of the method to a wide range of organic liquids. Obviously, the A.S.T.M. distillation method (*3*) is not suitable for very small amounts of water, for direct visual volume measurement of the water is required. If water contents are very low, it is possible that the entire amount contained in the sample will remain dissolved in the distillate.

During the course of this investigation, a paper by Suter (*11*) described a method for water in inorganic alkaline materials. Water was separated from the sample by distillation with xylene and determined in the distillate with Karl Fischer reagent. This procedure, although suitable for high water contents, does not allow the precision required for very small amounts of water; the blank determinations are greater than the total water often determined by the present method. It was found in the present investigation that ground-glass joints, as found in Suter's apparatus, could not be tolerated where small amounts of water were being determined. Opening the system to atmospheric moisture, as is done in the reported method, cannot be tolerated where amounts of water are low.

The present method employs azeotropic distillation and determination of the water in the distillate with Karl Fischer reagent. The necessity for keeping a dry solvent, or correcting for the blank on the solvent, is eliminated. The determination is made in a system completely protected from atmospheric water. The use of large samples is necessary; however, the final titration is more convenient because the water is concentrated into a comparatively small volume of liquid. Even in the case of dark samples the distillate is water-white; it is therefore possible to titrate the water without the aid of potentiometric devices for determining the end point.

Snyder and Clark (*10*) have reported a number of substances that interfere with the Karl Fischer reagent. These substances will interfere in the present method only if they are volatile under the conditions of the test.

This method was developed primarily for new and used petroleum products including greases, although by the choice of a proper azeotrope former, it may be applied to other substances. The method has been applied over the past year to other organic liquids—for example, additive concentrates, and trichloroethylene—and to engine deposits and sludges.

REAGENTS

Solvent or Azeotrope Former. In the present work, benzene was used for all samples except greases. Pyridine was used for greases.

Karl Fischer Reagent. The reagent may be prepared (*9, 13*) or purchased. It should be equivalent to 1.5 to 2 mg. of water per milliliter of reagent.

Water-in-Methanol Solution. This solution should contain 1.5 to 2 mg. of water per milliliter. Because commercial anhydrous methanol sometimes contains as much as 1 mg. of water per milliliter, its water content should be estimated and adjusted to the desired concentration.

APPARATUS

The apparatus, shown assembled in Figure 1, consists of a distilling flask, a receiver and titration flask, and two 25-ml. automatic burets, the tips of which pass through 18/9 ball joints which fit on the titration flask. One automatic buret is for Karl Fischer reagent, the other for water-in-methanol.

A magnetic stirrer (Arthur H. Thomas Co.), a heating mantle, and a Variac are also required, as well as an instrument for determining the end point of the titration. The dead-stop method described by Foulk and Bawden (*6*) is satisfactory, and the Fischer Titrimeter or any pH meter may be used. This part of the apparatus is not absolutely necessary, as the end point may be determined visually.

PROCEDURE

Standardization of Reagents. The apparatus, except the distilling flask, is assembled as shown in Figure 1 and the reagents are standardized according to the procedure of Almy, Griffin, and Wilcox (*2*). It is more convenient to use this method than a distillation. Table I shows that factors obtained by both procedures are essentially the same.

Table I. Standardization of Reagent

	Direct Procedure	Distillation
Factor, mg. of H_2O/ml. of reagent	3.02 2.98	3.06 3.00

Determination of Water. The apparatus is completely assembled, and 500 to 600 ml. of the solvent to be used as the azeotrope former are introduced into the distilling flask and distilled until 50 to 75 ml. of distillate have been collected. The heat is reduced so that the solvent does not distill, and a small excess of Karl Fischer reagent is added to the distillate in the receiver and back-titrated with water-in-methanol. The distillation and titration are repeated and continued until a 50- to 75-ml. portion of distillate contains no water. At this point the apparatus is anhydrous and ready for the sample to be introduced into the distilling flask.

If the sample is liquid, it may be conveniently added, without opening the system, from a separatory funnel with a long stem passing through a glass stopper fitted with a rubber sleeve at the top of the distilling flask. The funnel is placed in position with the tip of the stem above the top condenser prior to the initial dehydrating step. To add sample, the funnel is lowered until the tip of the stem passes through the upper condenser and the desired amount of sample is allowed to run in. Sample weight is obtained by weighing the funnel before and after sampling. For greases, a piston and cylinder arrangement as shown in Figure 2 is used. As above, this apparatus is placed in position before the initial dehydration, and is lowered through the top condenser to add the sample. Sample weight is obtained by weighing the grease sampler before and after adding sample.

The distillation is allowed to continue for 30 minutes, during which time 50 to 75 ml. of distillate are collected. The distillation is stopped, and a small measured excess of Karl Fischer reagent is added to the distillate in the receiver and back-titrated with water-in-methanol. The distillation is repeated and continued until a 50- to 75-ml. portion of distillate contains no water. The first distillate usually contains all of the water; however, if the water content is comparatively high (0.1%), additional distillation may be necessary. Six or seven distillations may be made from a 500 to 600-ml. charge of azeotrope former.

Calculations. The per cent by weight of water is calculated from the equation:

$$\% \text{ water} = \frac{(A\text{-}BR)\,F}{10C}$$

where A = ml. of Karl Fischer reagent
B = ml. of water-in-methanol
R = ratio of Karl Fischer reagent to water-in-methanol
F = factor, mg of H_2O per ml. of Fischer reagent
C = weight of sample, grams

DISCUSSION

A suitable solvent or azeotrope former for use in this method should be a good solvent for the sample. Its azeotrope with water should be rich enough to bring about complete removal of water from the sample in a minimum number of distillations. It should hold the removed water in solution in the distillate.

The benzene-water azeotrope boils at 69.2° C. and contains 8.8% water (*7*). The solubility of water in benzene is 0.057% at 20° C. (*8*). Thus, benzene, being an excellent solvent for many substances, is suitable as an azeotrope former when very small amounts of water are determined.

No special attempt was made in this work to fractionate the vapors, the excess of benzene acting as a solvent for any water that might separate when the vapors are cooled in the bottom condenser. In addition, the inner surfaces of the distilling flask were carefully cleaned initially, so that condensing vapors drained completely, thus further ensuring the complete collection of the water in the receiver. If the water content of the sample is so large that water separates from the condensed vapors, a smaller sample should be taken or a method more suitable for large concentrations should be applied. Ground-glass joints or

Table II. Analysis of Samples of Known Water Content

Sample	Solvent	Sample	H_2O Added	H_2O Recovered
		Grams.	%	%
Lithium grease	Pyridine	10	0.0261	0. 240
Sodium grease	Pyridine	20	0.0670	0.0699
Calcium grease	Pyridine	20	0.159	0.161
Sodium-calcium grease	Pyridine	20	0.173	0.171
Oil 1	Benzene	600	0.00022	0.00021–0.00019
2	Benzene	415	0.00038	0.00034–0.00038
3	Benzene	300	0.00377	0.00374
4	Benzene	300	0.00755	0.00748
5	Benzene	300	0.0219	0.0220
Trichloroethylene[a]	Benzene	29.32	...	0.004 0.004
Trichloroethylene[a]	Benzene	29.32	...	0.000 0.000

[a] Last two samples are different batches.

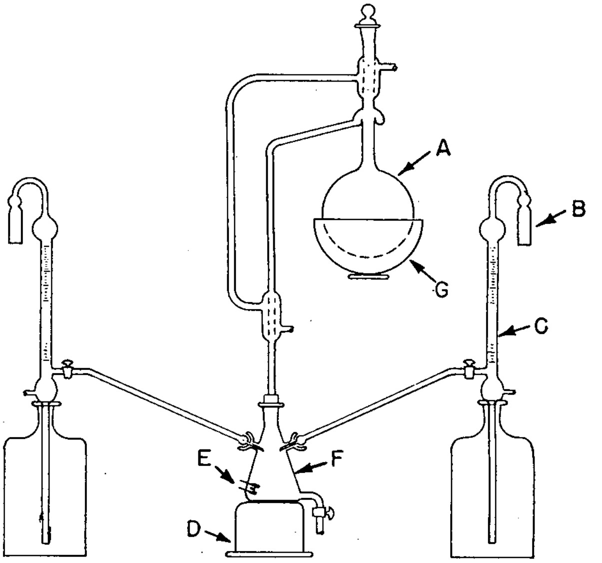

Figure 1. Apparatus for Determination of Water

A. Distilling flask
B. Drying tube
C. 25-ml. automatic burets
D. Magnetic stirrer
E. Electrodes
F. Receiver and titration flask
G. Heating mantle

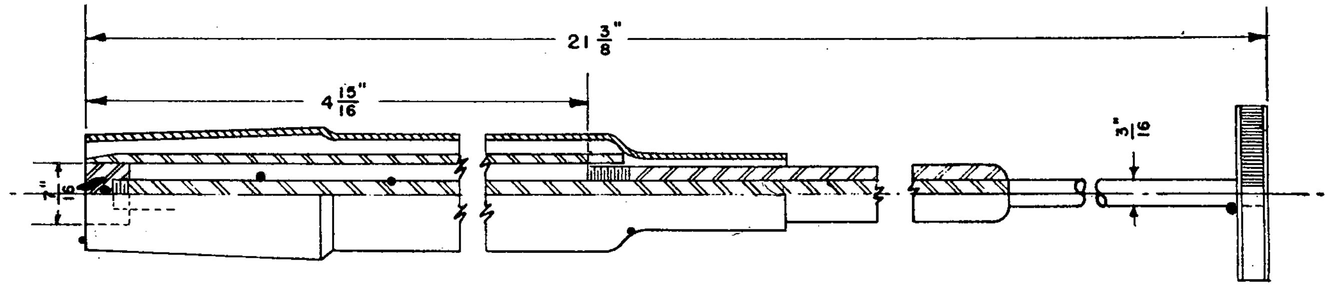

Figure 2. Grease-Sampling Apparatus

Table III. Results Obtained by Present and A.S.T.M. Distillation Methods

Sample	% H_2O, A.S.T.M.	% H_2O, Karl Fischer
Calcium grease	0.8 1.0	0.91 0.92
Sodium grease	None	0.038 0.040
Sodium grease	Less than 0.1	0.098 0.100
Lithium grease	Less than 0.1	0.099 0.103

stopcocks should not be used in the direct path of the distilling vapors, as they will hold, by capillary action, part of the first water-rich distillate, and complete recovery of water will not be accomplished.

Although benzene was found to be a suitable solvent for most substances analyzed, it was difficult to disperse some greases in it, even on prolonged boiling. Greases were much easier to disperse in pyridine and formed a clear solution with the boiling solvent. The pyridine-water azeotrope boils at 96.7° C., and contains 43% water, and because water is miscible with pyridine, even large amounts will remain dissolved in the distillate. In the analysis of greases difficulty with foaming was overcome by the addition of a few milligrams of an antifoam agent to the pyridine prior to the initial dehydration. The commercially available DC Antifoam A was found to be excellent for this purpose.

Table II shows the results of a series of samples of known water content. These samples were prepared by first dehydrating the solvent and sample as described in the procedure, cooling the mixture, and adding a weighed amount of water, either from a weighing pipet, or by adding more solvent of known water content. Table III shows a comparison of results obtained on different greases by the present method and by the A.S.T.M. distillation method.

ACCURACY

The method is accurate to 0.3 mg. of water, as shown by the data in Table II, which are typical results obtained from a large number of determinations.

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Report on Recommended Specifications for Microchemical Apparatus

Carbon-Hydrogen, Dumas Nitrogen, Sulfur, and Halogen

Committee for the Standardization of Microchemical Apparatus, Division of Analytical Chemistry, AMERICAN CHEMICAL SOCIETY

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RECOMMENDED specifications for microchemical apparatus to be used in connection with the carbon-hydrogen, Dumas nitrogen, halogen, and sulfur determinations have been published by the Committee for the Standardization of Microchemical Apparatus, which was first appointed in 1937 (*5–7*). At the 112th Meeting of the American Chemical Society held in New York in September 1947, a new committee was appointed by P. J. Elving, then chairman of the Division of Analytical and Micro Chemistry, to continue this work. This committee has declared its intentions to be as follows (*1*):

1. Where needed, to revise apparatus recommended by the former Committee for the Standardization of Microchemical Apparatus.
2. To recommend specifications for other items of quantitative micro-, semimicro-, and ultramicroapparatus. After this, attention will be given to the qualitative field.

All recommendations will be made with the understanding that the specifications represent the best thought at the present time. Additional revisions will be made when necessary. Primary consideration will be given to glass apparatus.

The committee has held twelve meetings and has reviewed all of the previously recommended specifications. Changes are being presented for many items to increase efficiency, add strength, and simplify the manufacture. Much experimental work was done in the members' laboratories before some of these changes were adopted. This report includes both the unchanged and the revised recommended specifications, so that all may be together for easy reference. Participation of chemists interested in microanalysis is invited by the committee. For this purpose questionnaires have been and will be circulated.

RECOMMENDATIONS

Carbon-Hydrogen (*7*). No changes have been made in the following:

Preheater (Figure 1)
Constant-Temperature Chamber (Figure 2)
Glass Parts for Pressure Regulator (Figure 5)
Mariotte Bottle (Figure 6)
Guard Tube (Figure 8)
Platinum Boat (Figure 9)

The following changes are recommended in the specifications for the absorption tubes (Figure 3), combustion tube with side arm (Figure 4), and bubble counter–U-tube (Figure 7).

ABSORPTION TUBES (FIGURE 3). The opening in the bottom of the stopper and that in the baffle have been increased from 0.25 ± 0.05 mm. to 0.4 ± 0.1 mm. to minimize the condensation of water at these points. An additional tube of greater length is also recommended:

a. Length of absorption chamber 80 mm. (over-all length 170 mm.)
b (new). Length of absorption chamber 100 mm. (over-all length 190 mm.)

No markings or etching should appear on the tube.

COMBUSTION TUBE WITH SIDE ARM (FIGURE 4). The specifications remain unchanged except that all three ends are to be smooth and both the angle between the main part of the combustion tube and the side arm and the angle of bend of the side arm are now 90° ± 5°.

BUBBLE COUNTER–U-TUBE (FIGURE 7). The diameter of the inner tube of the bubble counter has been increased from 9 to 10 mm. to 12 to 14 mm. in order to diminish backing up of the liquid in the bubble counter due to changes of pressure in the system and to minimize the effect of foaming of the liquid.

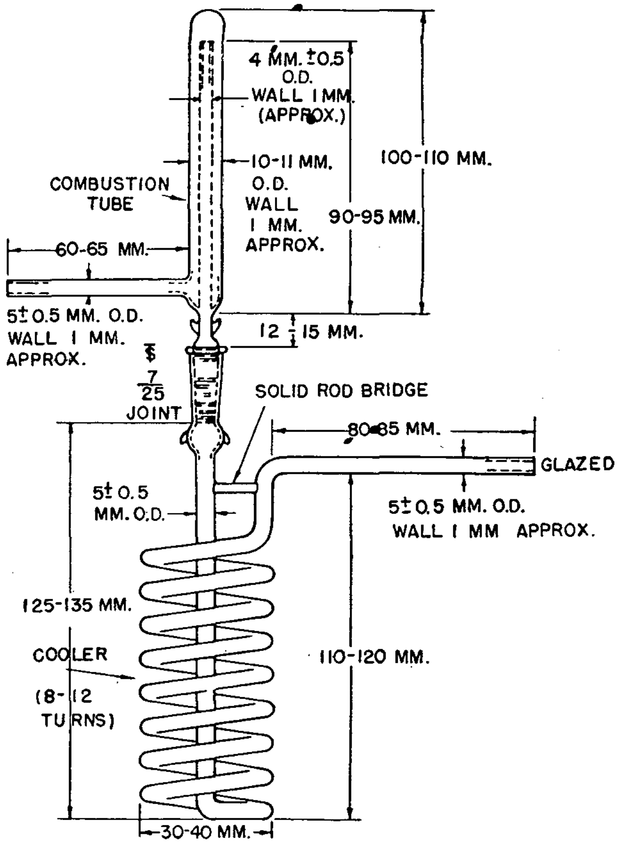

Figure 1. Preheater

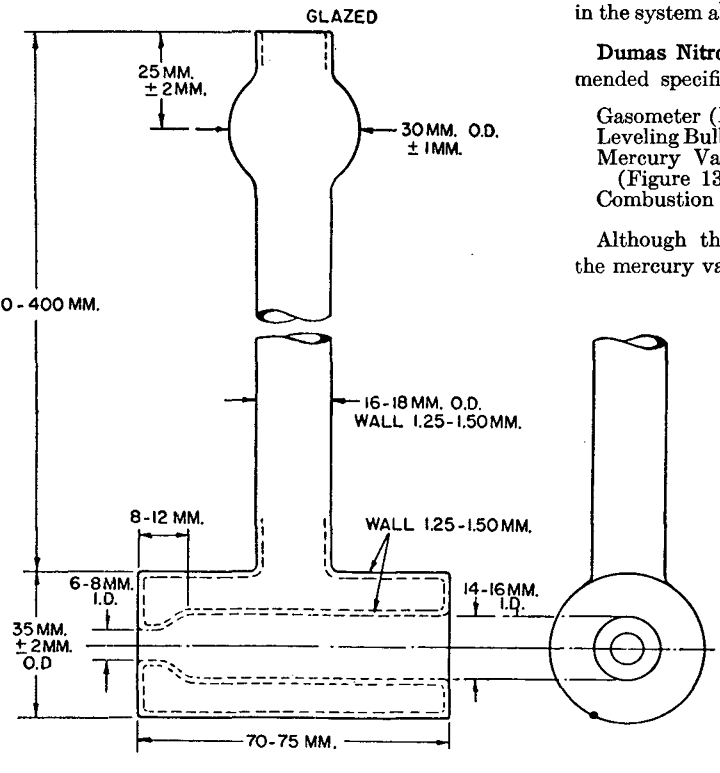

Figure 2. Constant-Temperature Chamber

Dumas Nitrogen (*6, 7*). No changes have been made in the recommended specifications of the following:

Gasometer (Figure 10)
Leveling Bulb for Use with Gasometer (Figure 11)
Mercury Valve for Carbon Dioxide Generator for Use with Dry Ice (Figure 13)
Combustion Tube with Tip (Figure 16)

Although there are no changes in the recommended specifications for the mercury valve for the carbon dioxide generator for use with dry ice, the wording in parentheses describing the thread to take the 18-mm. cap has been changed from "Glass Container Association specifications" to "Glass Container Manufacturers' Institute specifications."

PRECISION NITROMETER AND LEVELING BULBS (FIGURE 12). The top above the stopcock has been changed from a funnel shape to that of a cylinder of approximately 9.5-ml. capacity to prevent spillage. A minimum inside diameter of 2 mm. is specified for: (*a*) the capillary between the cylindrical top and the stopcock; (*b*) the holes of the barrel of the stopcock; and (*c*) the top of the graduated tube. The markings on the graduated portion are recommended to be placed on the left-hand side as shown in Figure 12 to make it possible to read the nitrometer when the combustion train is set up either left to right (nitrometer on the right), or right to left (nitrometer on the left). Another modification is the addition of the socket of a spherical joint to the capillary side arm for connection to the stopcock (or needle valve). This joint has been added to eliminate the use of rubber tubing, thus

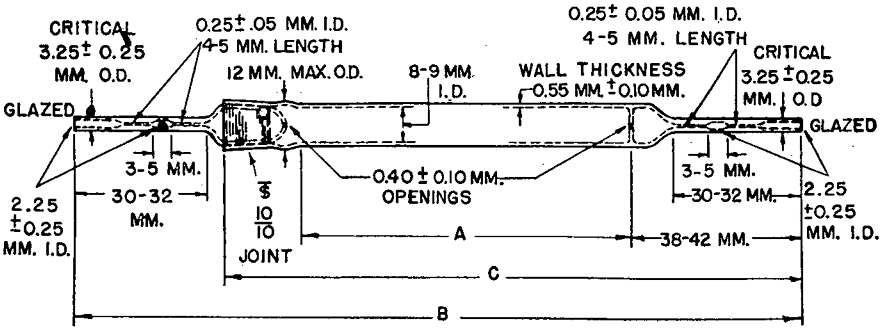

Figure 3. Absorption Tube

No markings or etchings on outside

Size	Approx. Length for Absorbent, Mm.	Over-all Length with Stopper, Mm.	Length of Body, Mm.
	A	B	C
A	80	170 ± 5	135 ± 5
B	100	190 ± 5	155 ± 5

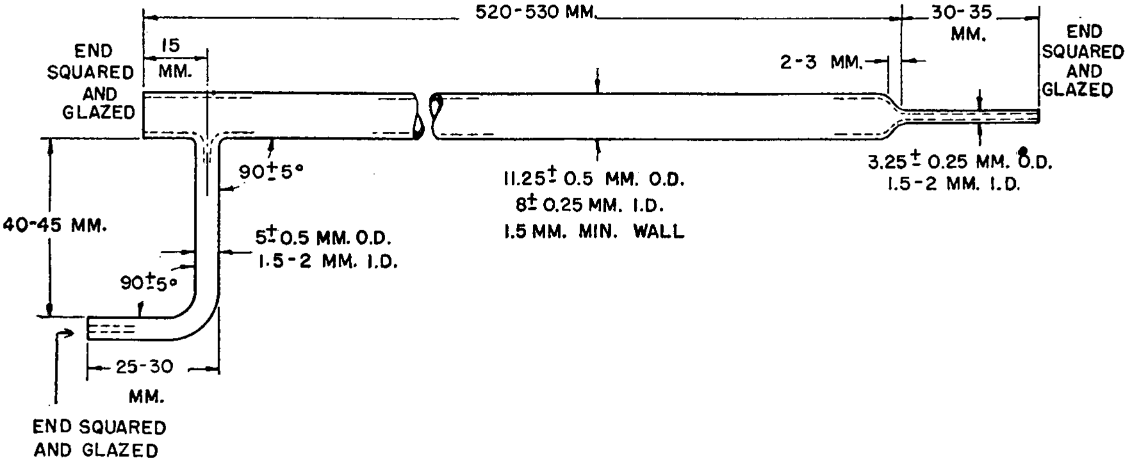

Figure 4. Combustion Tube with Side Arm

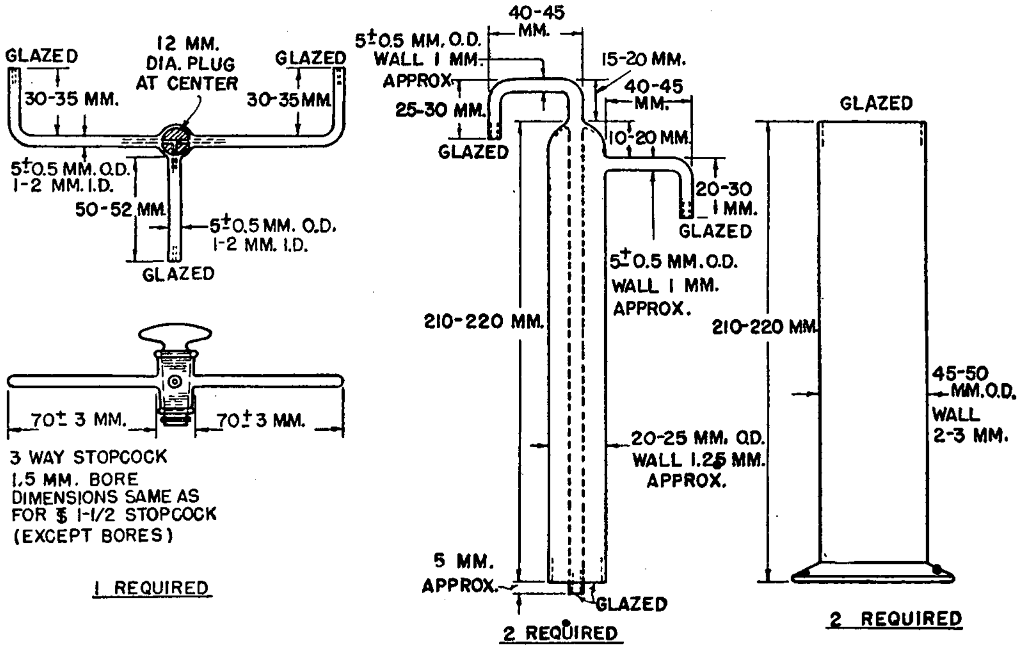

Figure 5. Glass Parts for Pressure Regulator

reducing the danger of breaking the side arm of the nitrometer. A corresponding change has been made in the Dumas stopcock (Figure 15). (The two parts of the spherical joint should be greased and securely held together by one of the mechanical clamps available for this purpose.) By virtue of the universal action of the spherical joint, alignment of these parts is unnecessary. Other minor changes have been made:

Length of calibrated portion
Total length
Dimensions of short side arm
Note regarding lower part of reservoir
Note regarding inside diameter of capillary at joint with reservoir
A leveling bulb of the conventional type has been added

The committee has no specifications for calibrating these micronitrometers, but suggests the following procedure in use by the National Bureau of Standards (*2*):

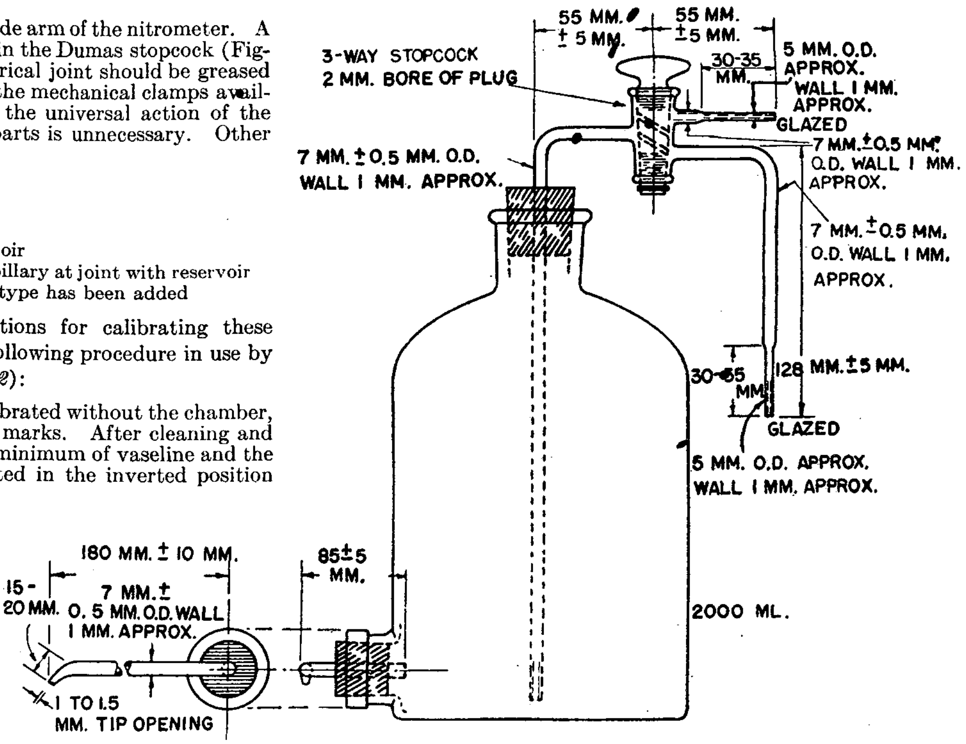

Figure 6. Mariotte Bottle

> The azotometer [nitrometer] is calibrated without the chamber, at the 0.1, 0.3, 0.5, 0.8, 1.1, and 1.5 marks. After cleaning and drying, the cock is lubricated with a minimum of vaseline and the instrument is tared and then mounted in the inverted position with the cock closed. The theromometer is suspended beside it and a transparent screen is placed around the assembly. The mercury is put in a small glass reservoir provided with a stopcock and having a long slender delivery tube which is inserted into the tube of the azotometer [nitrometer] down to the cock, for the initial filling at the 0.1 mark.
>
> Carefully run in the mercury, raising the delivery stem as the filling progresses until the orifice is just above the surface. Fill nearly to the test mark, remove the stem, and by means of a long slender steel rod, probe around the filled portion of the azotometer [nitrometer] to work out air pockets. Insert the delivery stem nearly to the surface of the mercury and fill to slightly below the mark, agitate the tube to round the meniscus, and, if necessary, add or remove mercury to complete the setting on the line; or if preferred, simply observe the point at which the meniscus stands, and correct accordingly. Round the meniscus for every reading. After observing the temperature, the azotometer [nitrometer] is allowed to stand for about 3 minutes, when the setting and temperature are checked, and, if satisfactory, the azotometer [nitrometer] is weighed. The azotometer [nitrometer] is again clamped in position and after standing for 5 min-

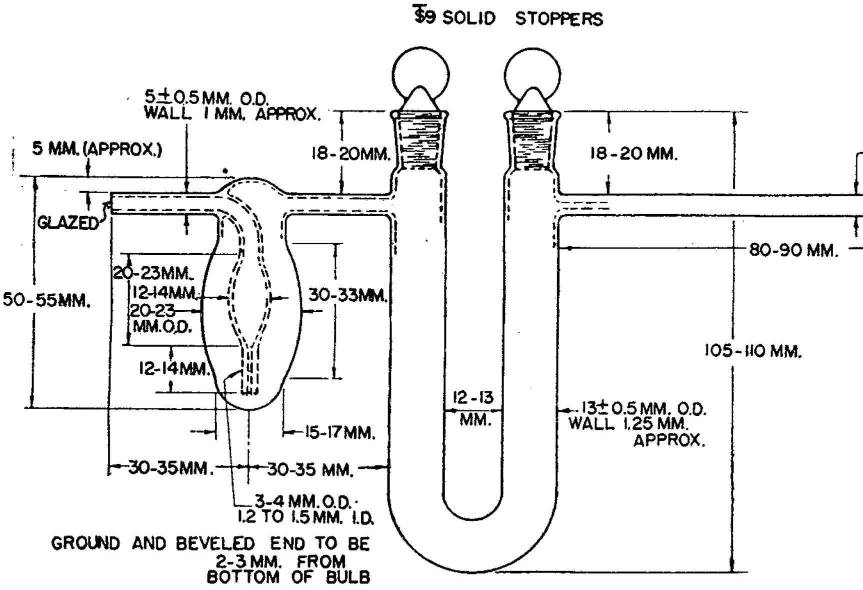

Figure 7. Bubble Counter–U-Tube

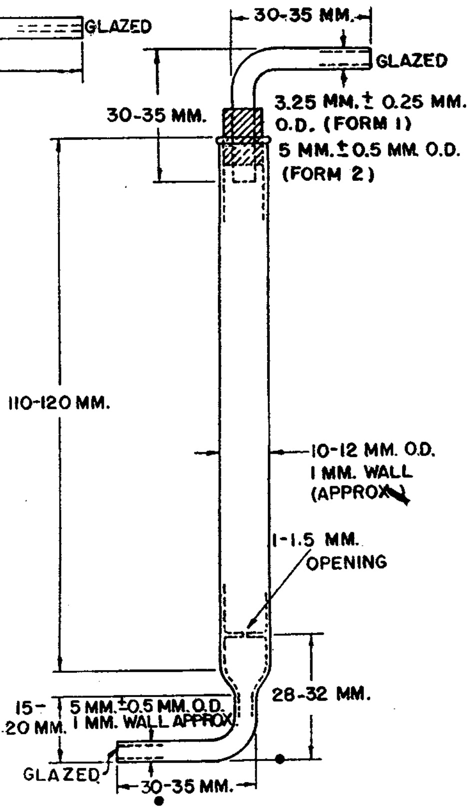

Figure 8. Guard Tube

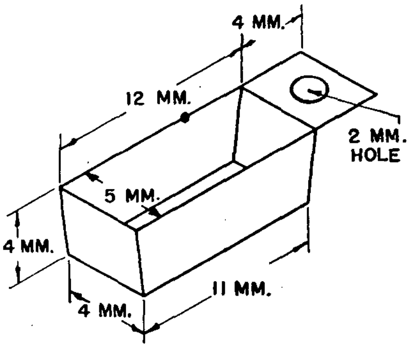

Figure 9. Platinum Boat

Weight 0.7 to 1.0 gram
3.5% rhodium alloy

utes, the delivery stem is inserted, the filling is made to the next test mark, and the instrument is weighed as before. The same procedure is followed for the other points. Air pockets at the cock seem to be the main source of trouble.

In general practice, the actual volume above 50% KOH solution in contact with glass walls is accepted as being 0.001 ml. less than that found by the mercury calibration, based on a determination by the Physikalisch-Technischen Reichsanstalt.

Standard Kipp Generator, 2000 Ml. (Figure 14). The specifications for this apparatus have undergone several changes upon the recommendation of the sole American manufacturer.

The inside collar has a 50/12 joint, making possible the deletion of the descriptive phrase "ground to fit" in the old specifications and elimination of the diameter dimensions at the lower end of the collar. The radius of curvature at the intersection of the bottom and side wall of the body is now specified to be about 20 mm. to prevent sharp angles. There is a slight change in the over-all height of the body from 341 ± 10 mm. to 356 ± 10 mm. and also in the height of the constriction from 109 ± .5 mm. to 124 ± 5 mm. The outside surface of the side tubulature of the body is now glazed instead of ground. The diameters of the lower end of the stem of the funnel have been changed: the outside diameter from 14 ± 2 to 18 ± 3, the inside diameter from 11 ± 1 to 7.5 mm. minimum. The distance from the lower end of the stem to the top of the ground zone has been changed from 310 ± 5 mm. to 335 ± 5 mm.

Dumas Stopcock (Figure 15). After the publication of the first specifications of the article (*7*), an error was detected in the drawing for the stopcock bore. Correction of this was made in a subsequent publication (*6*). Further modifications have been made to obtain greater strength as well as to include the ball member of a spherical joint for connection to the precision nitrometer (Figure 12).

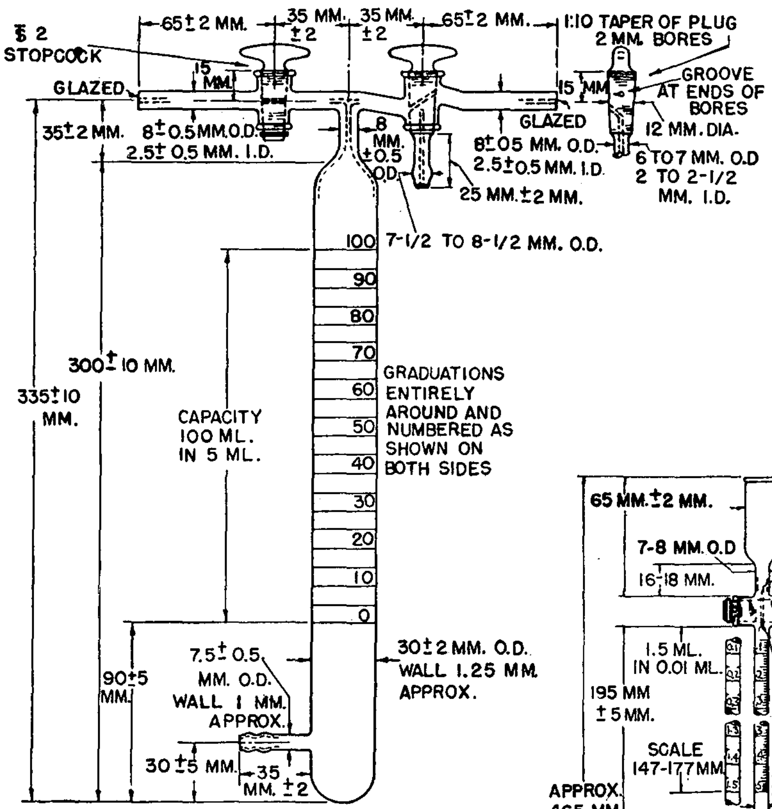

Figure 10. Gasometer

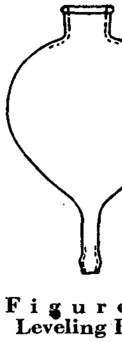

Figure 11. Leveling Bulb

250 ml., for use with gasometer

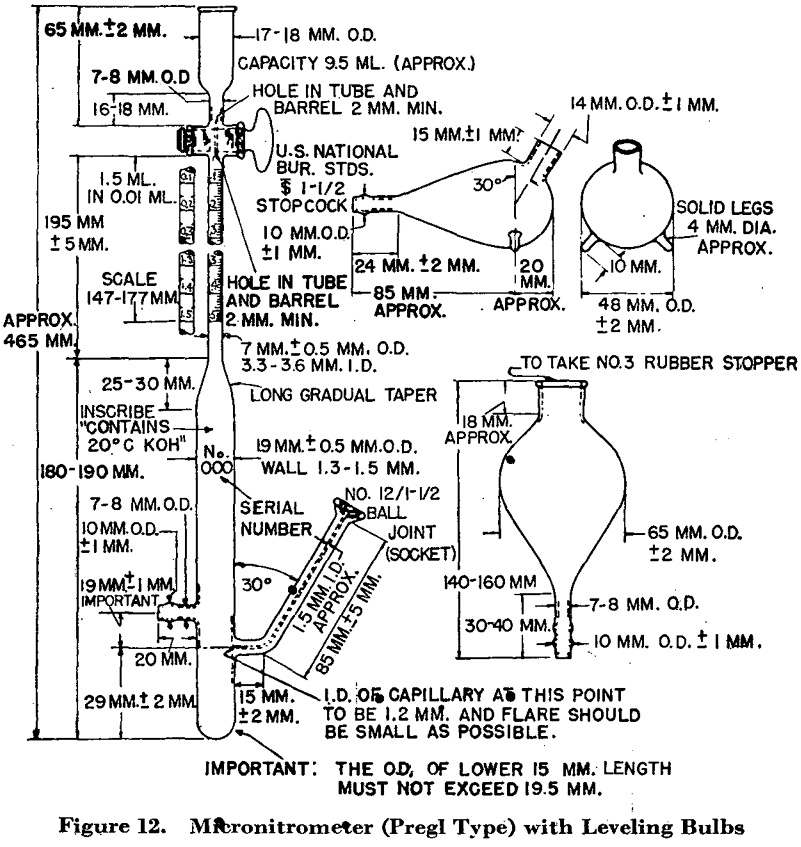

Figure 12. Micronitrometer (Pregl Type) with Leveling Bulbs

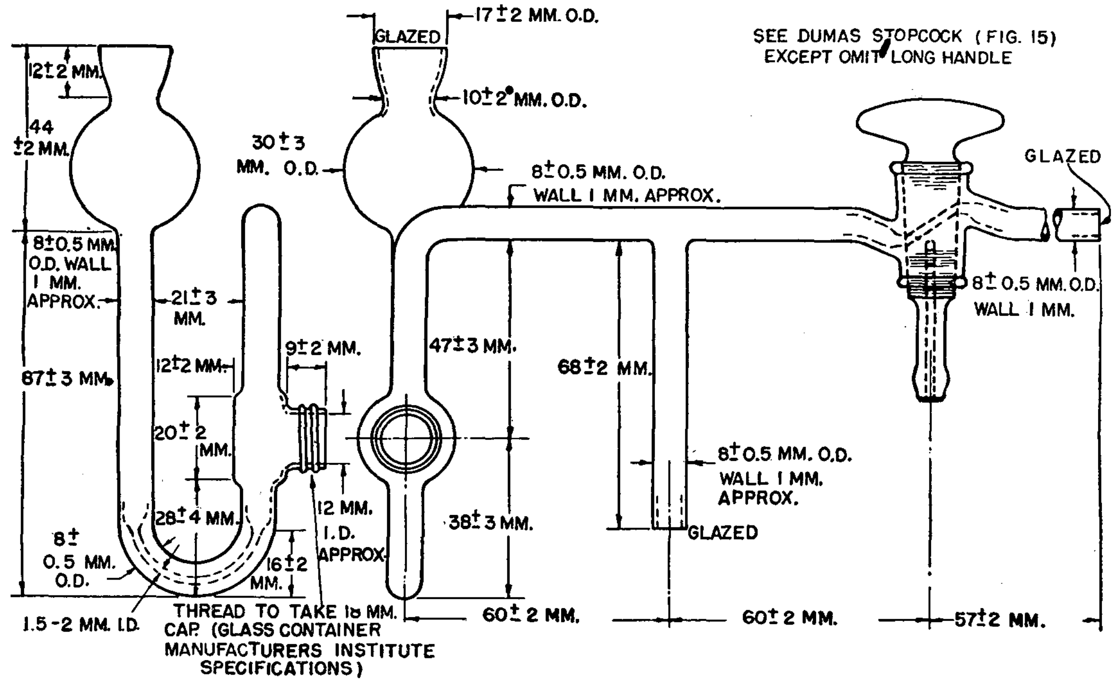

Figure 13. Mercury Valve for Carbon Dioxide Generator for Use with Dry Ice

All-Metal Needle Valve (Figure 17). The committee has included the all-metal needle valve of the Hershberg-Southworth type (*8*), a new item, because of its general acceptance. Specifications are given in Figure 17. The side arm, although specified at 140 mm., can be shortened, as desired, to fit a particular assembly. Connection of the side arms to both the combustion tube and the nitrometer may be made by means of either heavy-walled rubber tubing or the proper ground joints. The ground joints may be cemented to the metal tube or connected by heavy-walled rubber tubing.

Sulfur and Halogen (*5*). No changes have been made in the following:

Porcelain Crucible (Figure 19)
Glass Crucible Holder for Water Bath (Figure 20)
Porcelain Filter Crucible and Ignition Dish (Figure 21)
Crucible Filter Assembly (Figure 22)
Siphon, Receiver, and Inner Container for Barium Sulfate Filtration (Figure 23)
Air Filter (Figure 27)
Snipe Feather (Figure 30)
Large-Size Glass Test Tube for Metal Microbomb (Figure 36)

Changes have been made in the following:

Porcelain Filter Stick (Figure 18). This piece has been slightly changed with respect to the attachment of the filter plate. The length has been increased to about 55 mm. and the thickness of the filter plate has been specified as 2 mm. Tolerances have been established.

Glass Dome, Metal Crucible Desiccator, and Metal Block (Figure 24). Specifications are given for a new metal block to be used in connection with the glass dome and metal crucible desiccator. The block has the advantage of providing usable top and bottom surfaces, with and without rim. It has been designed so that there will be a minimum of play when transporting it in the metal crucible desiccator. With it, supported items may be carried with greater safety.

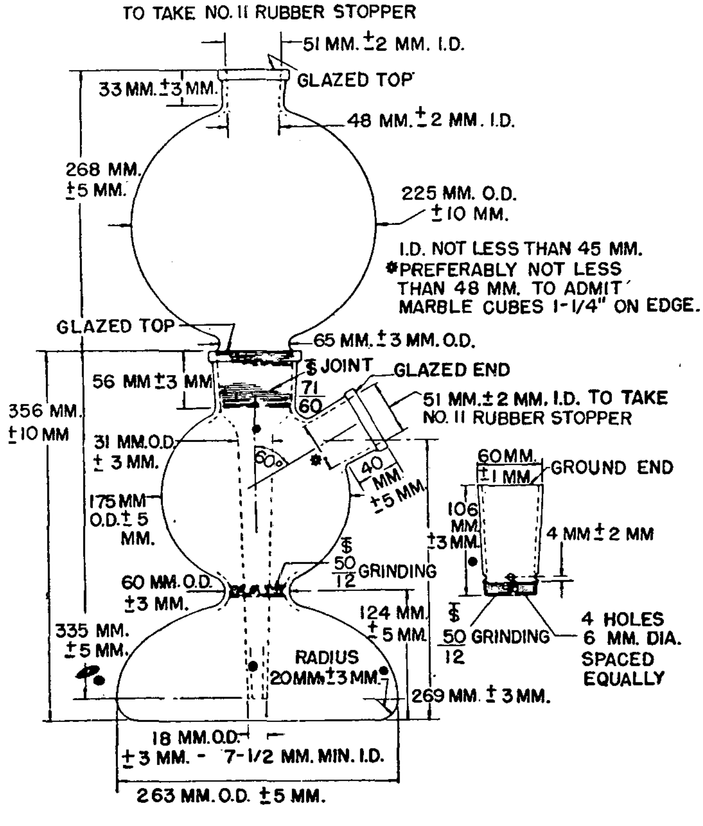

Figure 14. Standard Kipp Generator (2000 Ml.)

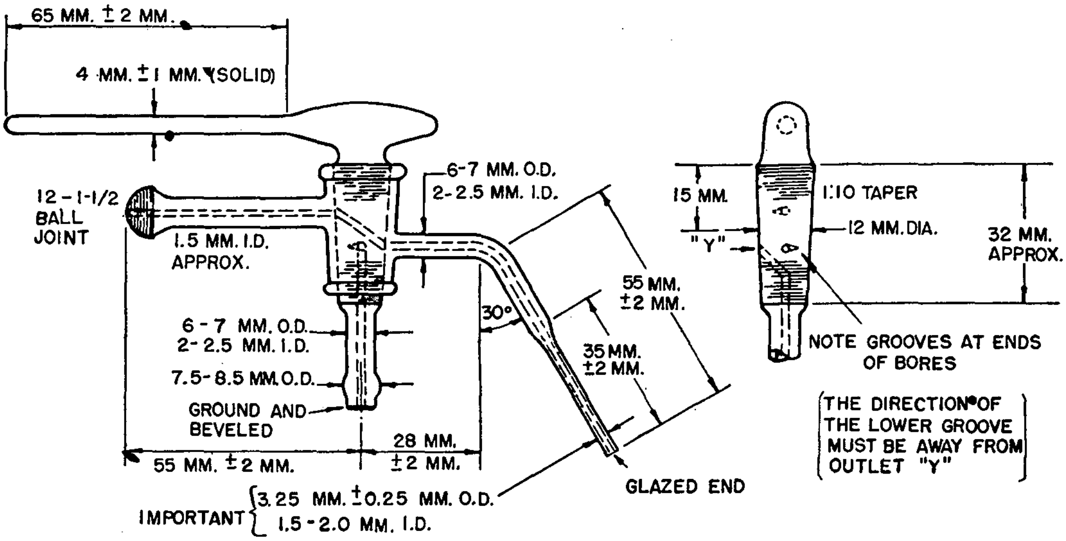

Figure 15. Dumas Stopcock

Micro Evaporating Dishes for Sulfur Determination (Figure 25). These items have been made more sturdy by specifying a wall thickness of 1.5 to 2.5 mm.

Platinum Microware for Halogen and Sulfur Determinations (Figure 26). Tolerances are now included in the specifications of the crucible, Munroe crucible, and filter stick.

The contact star has been revised completely (*4*). The item made according to the old specifications was fragile and needed constant reshaping. The new item consists of a perforated outside cylinder into which has been spot-welded a heavier star to form a sturdy and serviceable platinum contact.

Weighing Tube (Figure 28). For the sake of sturdiness the diameter of the rod has been increased to 2.5 to 3 mm. and the inside diameter of the sample receptacle has been increased to 5 to 5.5 mm.

Weighing Tube with Cap (Figure 29). The rod is specified to have a uniform diameter of 2.5 to 3 mm. for added strength.

Combustion Tube with Inner Spiral (Figure 31). The tip is now specified to be flat with a slight bevel, ground, and polished. The solid spiral rod and center rod have been increased in size to 2.0 ± 0.25 mm. diameter.

Filter Tube (Figure 32). The top of the filter tube has been

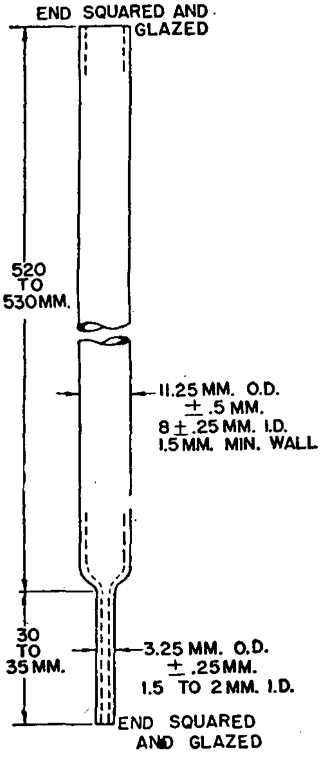

Figure 16. Combustion Tube with Tip

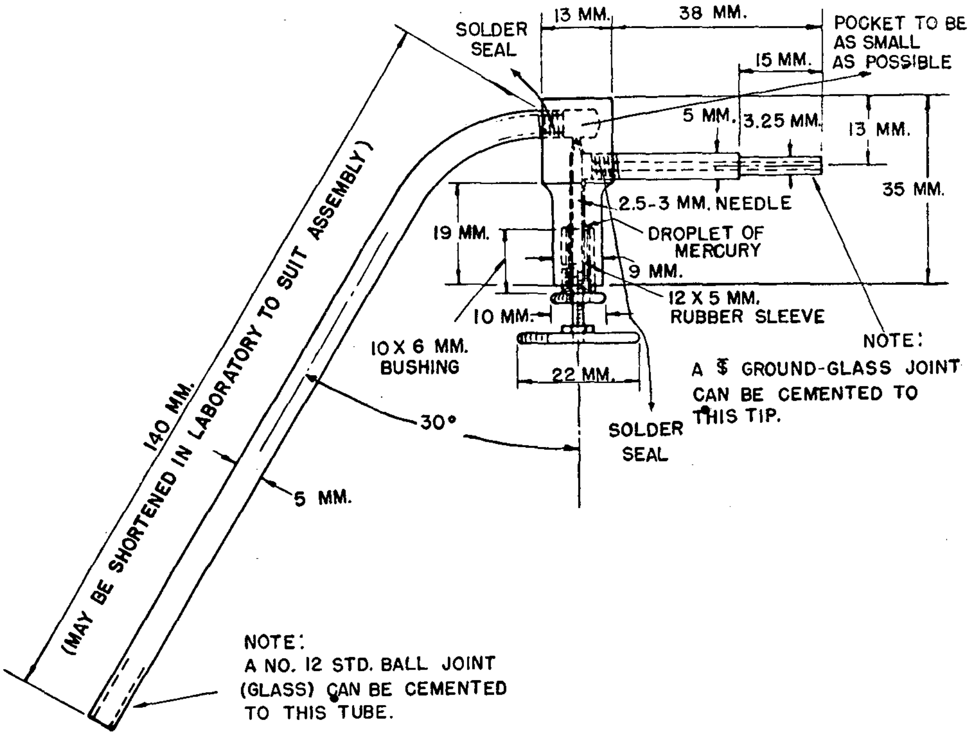

Figure 17. All-Metal Needle Valve (Hershberg-Southworth Type)

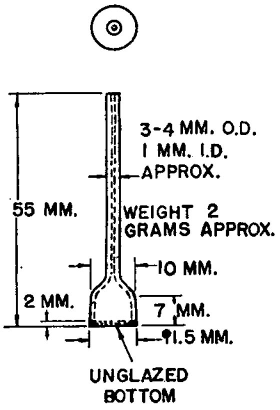

Figure 18. Porcelain Filter Stick

Dimensions approximate

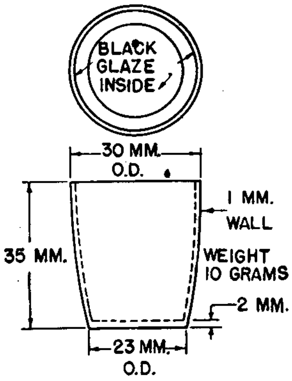

Figure 19. Porcelain Crucible

Dimensions approximate

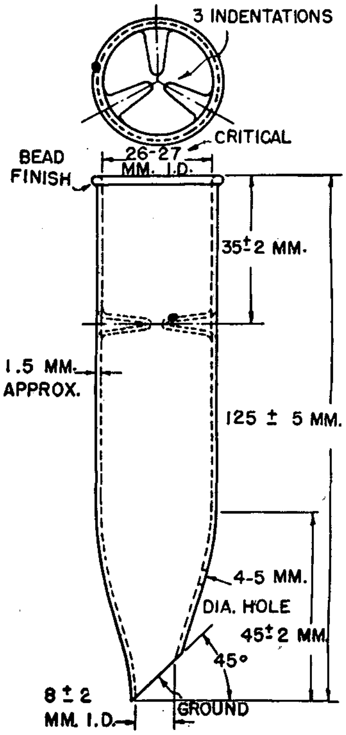

Figure 20. Glass Crucible Holder for Water Bath

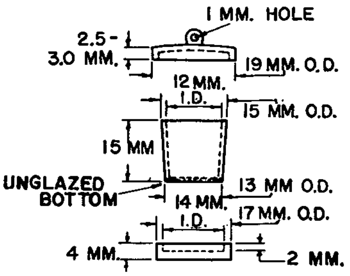

Figure 21. Filter Crucible and Ignition Dish (Porcelain)

Dimensions approximate

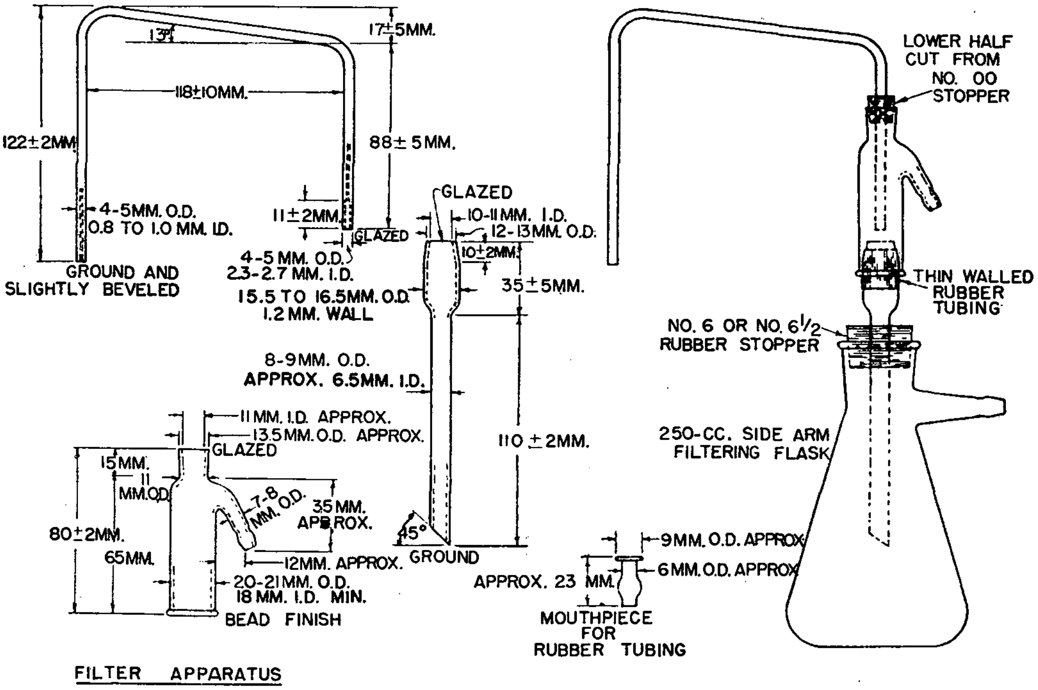

Figure 22. Crucible Filter Assembly

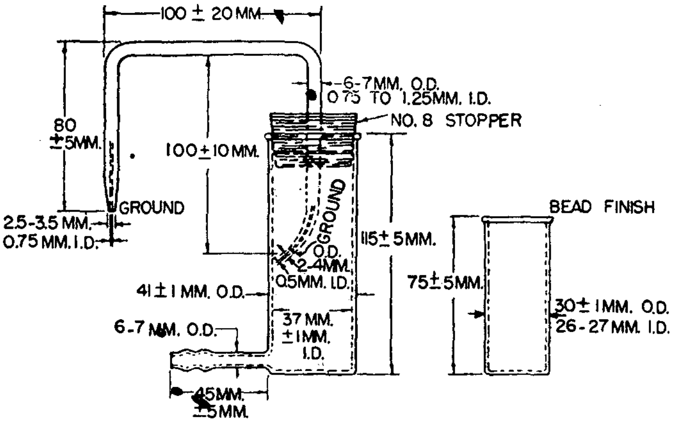

Figure 23. Siphon, Receiver, and Inner Container for Barium Sulfate Filtration

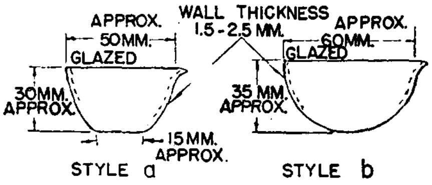

Figure 25. Micro Evaporating Dishes for Sulfur Determination

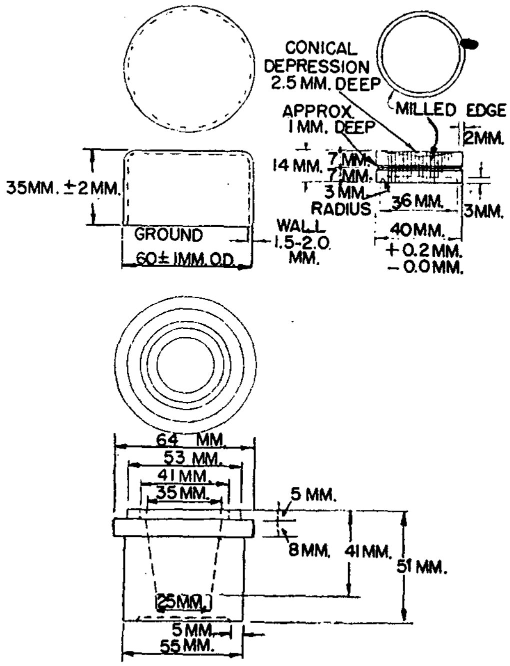

Figure 24. Metal Crucible Desiccator with Glass Dome and Metal Block

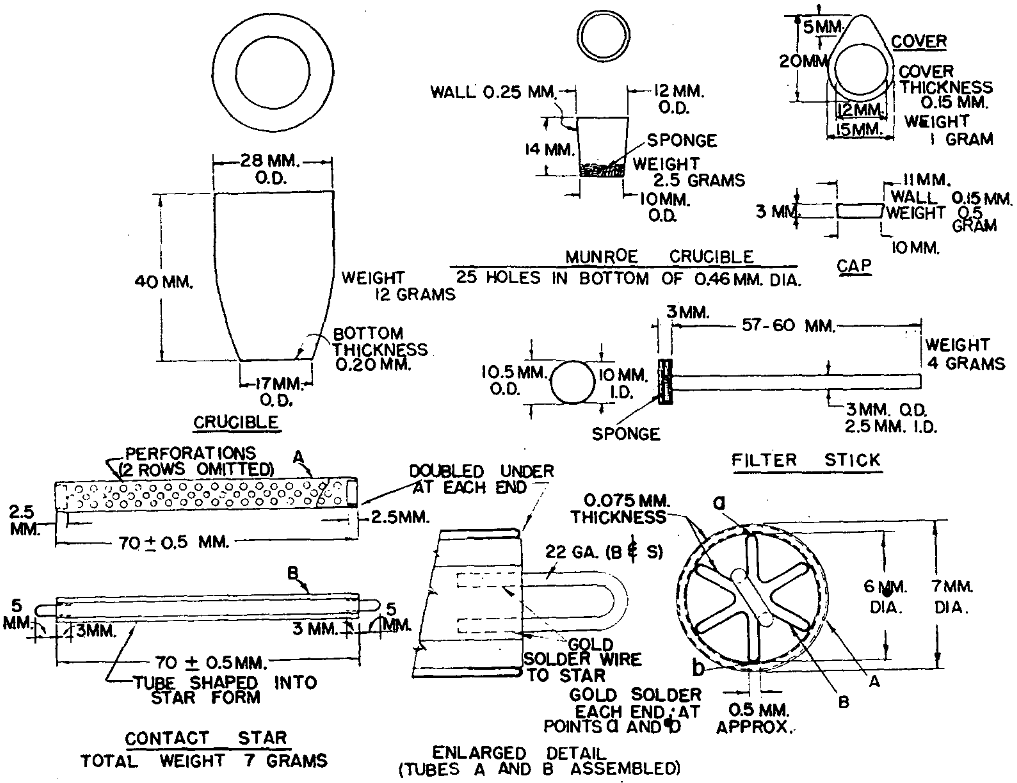

Figure 26. Platinum Microware for Halogen and Sulfur Determinations

Tolerances on Platinum (Unless Otherwise Shown)	
Height, mm.	±0.5
Outside-inside diameter, mm.	±0.5
Thickness, mm.	±0.01
Weight, gram	±0.5

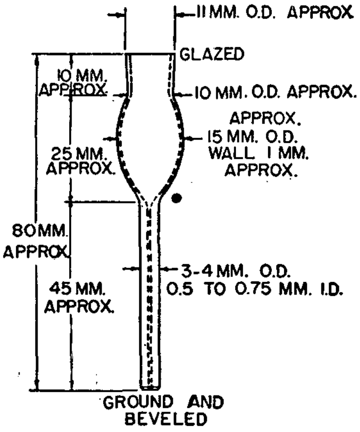

Figure 27. Air Filter

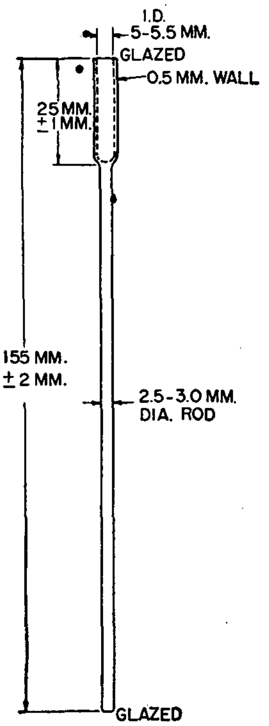

Figure 28. Weighing Tube

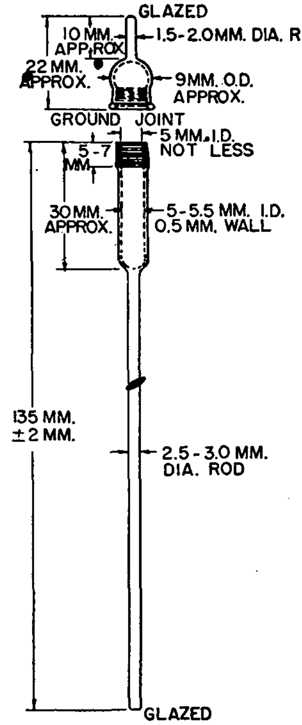

Figure 29. Weighing Tube with Cap

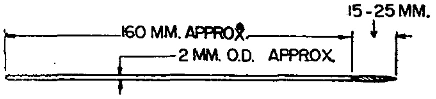

Figure 30. Snipe Feather

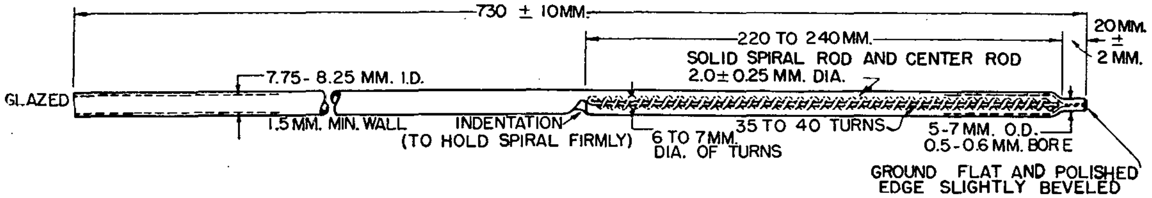

Figure 31. Combustion Tube with Inner Spiral

changed to call for a taper of 1 in 10. Collaborative tests have been made by committee members to determine the most desirable porosity of the filter plate. As a result the committee recommends that the filter plate have an average pore diameter of 15 to 25 microns.

Filtration Assembly (Figure 33). The few changes here are corrections in the old drawing. This showed the outlet tube protruding too far beneath the stopper into the filter tube and also indicated a base for the test tube. The present committee does not recommend specifications for a base and has deleted it.

Graduated Wash Bottle (Figure 34). The dimension for the base has been increased to 50 to 60 mm.

Sintered-Glass Funnel with Test Tube (Figure 35). The assembly shown replaces the sintered-glass funnel previously recommended (*5*). It has the advantage of a ₸ ground joint in place of the former rubber stopper. The funnel may be used interchangeably in evaporation and filtration apparatus (*3*).

ACKNOWLEDGMENT

The committee wishes to express its thanks to the officers of the Division of Analytical Chemistry for their cooperation; to the many persons who have furnished information in answer to its questionnaires; and, in particular, to the various companies

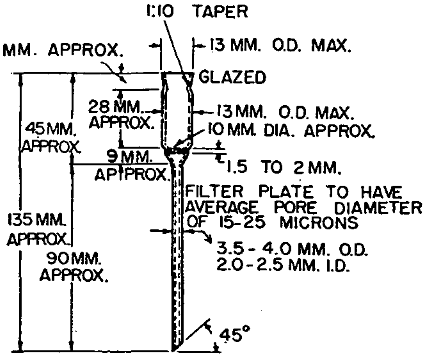

Figure 32. Filter Tube

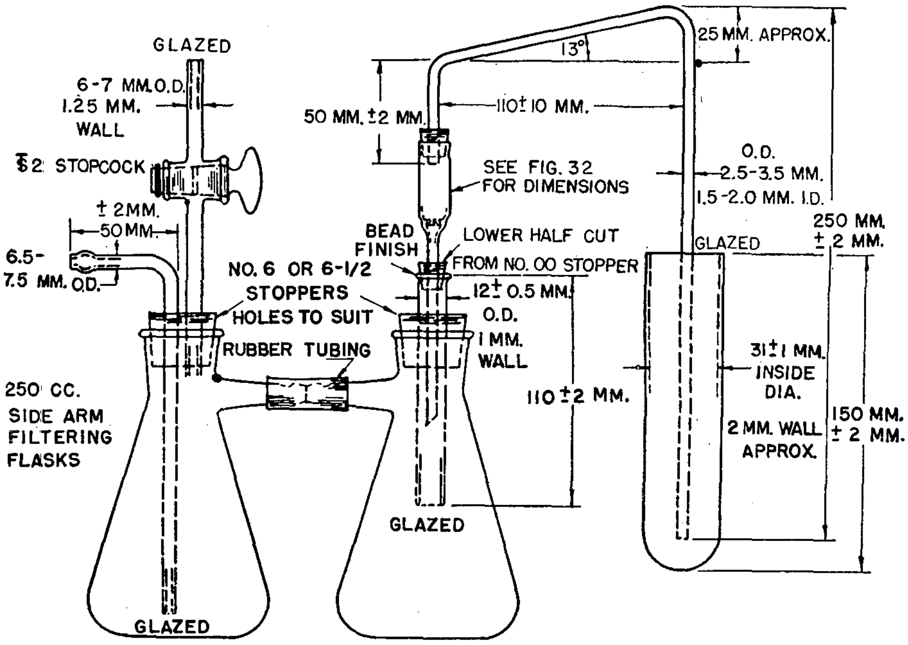

Figure 33. Filtration Assembly

and institutions of the committee members for the time and facilities necessary for the accomplishment of the work reported.

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(2) Mulligan, G. C., National Bureau of Standards, Washington, D. C., private communication to Al Steyermark.
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(6) *Ibid.*, p. 476.
(7) Royer, G. L., Alber, H. K., Hallett, L. T., Spikes, W. F., and Kuck, J. A., *Ibid.*, 13, 574 (1941).
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Received August 4, 1949. Presented before the Division of Analytical and Micro Chemistry at the 115th Meeting of the American Chemical Society, San Francisco, Calif. That part pertaining to the micro Dumas determination was presented as a report to the Division of Analytical and Micro Chemistry at the 114th Meeting of the American Chemical Society, St. Louis, Mo.

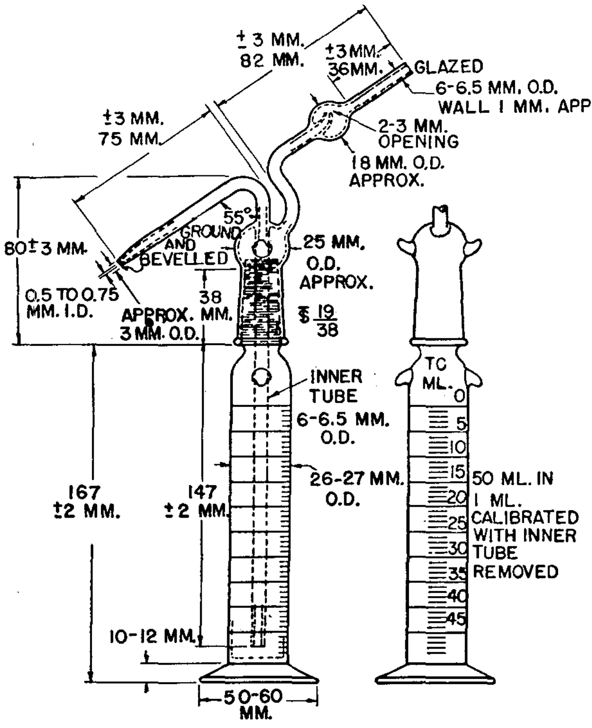

Figure 34. Graduated Wash Bottle

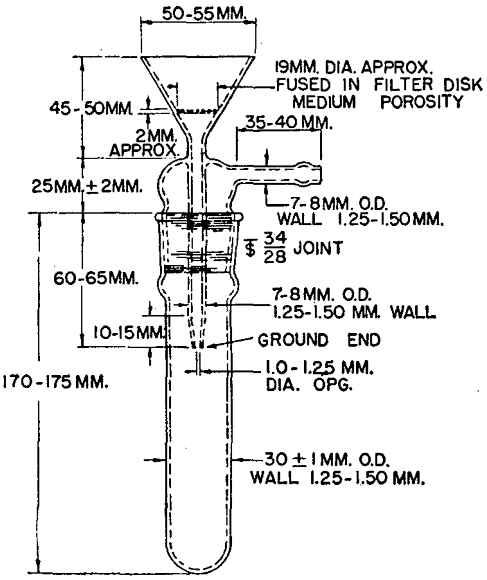

Figure 35. Sintered-Glass Funnel with Test Tube

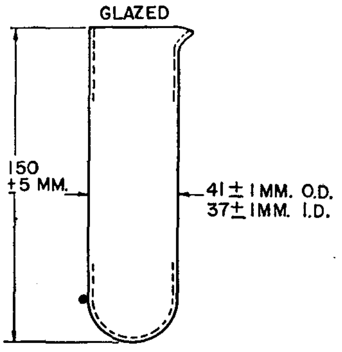

Figure 36. Large-Size Glass Test Tube for Metal Microbomb

NOTES ON ANALYTICAL PROCEDURES . . .

Determination of Unsaturation in Heavy Hydrocarbon Gases by Catalytic Hydrogenation

Boiling Water-Jacketed Catalyst

JESSE H. SHIVELY, FLOYD PHILGREEN, AND HARRY LEVIN
The Texas Company, Beacon, N. Y.

THE catalytic hydrogenation method described by McMillan, Cole, and Ritchie (*1*) has been widely used for the determination of unsaturates in normally gaseous hydrocarbons. Refinements of this method, involving corrections for the nonideality of gases, have been described by Robey and Morrell (*3*), who also extended the method to include C_5 hydrocarbons which they rendered gaseous by diluting with hydrogen 1 to 2.

Other methods commonly used for determining gaseous unsaturates are based on bromine number determination or absorption of the unsaturates in aqueous solutions of various reagents. These methods supplement catalytic hydrogenation, which is the only rapid method giving a reliable indication of the total number of unsaturated linkages per average molecule of gas—commonly expressed as "per cent unsaturation," as though the molecule contained one double bond.

The value obtained in the preliminary hydrogenation run is discarded, its purpose being only to establish adsorption equilibrium between the catalyst and the residual gases—that is, to saturate the catalyst. Occasionally one preliminary run is insufficient and it is necessary to discard the results of two runs on a sample.

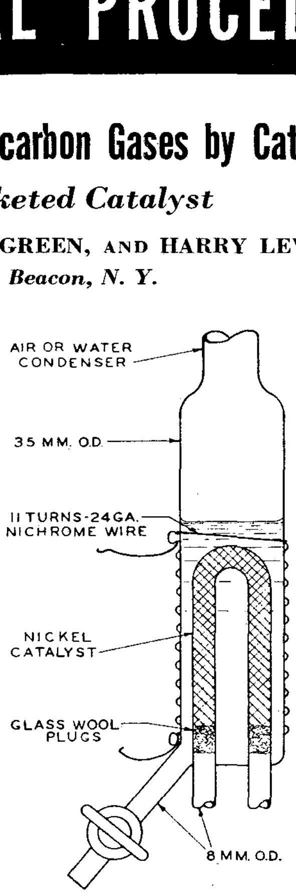

Figure 1. Water-Jacketed Hydrogenation Catalyst

Table I. Analyses of Simple Mixtures of C_5 Hydrocarbons

	Unsaturation, Per Cent			
		Found		
	Present	Preliminary run	Run 1	Run 2
Using Unheated Catalyst				
Sample 1 99.9% mixed pentanes 0.1% pentenes	0.1[a]	5.0	1.2[b]	0.3
Sample 2 20.8% 2-methylbutane 54.0% *n*-pentane 25.2% 1-pentene	25.2[c]	29	25	24
Using Boiling Water–Jacketed Catalyst				
Sample 3 2-Pentenes	C.P.	99	98	98
Sample 4 77.6% *n*-pentane 22.4% 2-pentenes	22.4[c]	23	22	22
Sample 5 87.9% *n*-pentane 12.1% 2-pentenes	12.1[c]	13	13	13

[a] Determined by bromine titration.
[b] Catalyst apparently not adequately presaturated. This value should be discarded.
[c] Blended.

Table II. Per Cent Unsaturation of Complex C_5 Low Temperature Fractional Distillation Fractions[a]

(Boiling Range 18° to 50° C.)

	Unheated Catalyst					Boiling Water–Jacketed Catalyst			
Cut	Preliminary run	Run 1	Run 2	Run 3	Run 4	Preliminary run	Run 1	Run 2	Run 3
1	117	104	108	1[illegible]1	117	105	103	103	103
2	129	116	114	116	...	107	107	107	...
3	114	109	106	115	103	108	102	104	104
4	110	108	109	...	...	106	105	...	...
5	117	110	109	...	...	106	105	...	...
6	113	102	94	100	...	96	95	...	...

[a] Chiefly pentenes and pentadienes.

Initial attempts to utilize the conventional room temperature hydrogenation method as a step in the complete analysis of certain complex C_5 cuts from analytical low temperature fractional distillations met with little success (Table II), though the unsaturation of simple mixtures prepared from pure C_5 hydrocarbons was determined moderately well (Table I).

The apparatus of McMillan, Cole, and Ritchie (*1*) was used after modifying it so the catalyst was equipped with a boiling water jacket (Figure 1). When reducing the catalyst boiling triethylene glycol (280° to 290° C.) was used instead of water. All stopcocks were lubricated with hydrocarbon-insoluble lubricant (*2*), which is essential in C_5 analysis.

The better precision obtainable with the heated catalyst is apparent from Table II. The high values for unsaturation found in preliminary runs with unheated catalyst, in contrast to much lower values found in succeeding runs on the same sample, indicate that adsorption on the catalyst was excessive. When the temperature of the catalyst was maintained at 100° C. throughout the analysis, the C_5 hydrocarbons exhibited low adsorption characteristics similar to lighter hydrocarbons at room temperature, and though the exact compositions of these samples are unknown the results with the heated catalyst are probably more reliable for this reason. Even the saturating run generally agrees very well with subsequent runs under these conditions.

In the analysis of simple C_5 mixtures acceptable results were obtained by either method (Table I), but those from the preliminary runs were usable only with the hot catalyst. The time required with the latter was generally about half that necessary with the conventional catalyst tube.

The original method called for presweeping the catalyst with hydrogen before every determination. It subsequently proved more practical to eliminate this step between duplicate determinations on the same sample, inasmuch as after the first test the catalyst is in equilibrium with residual reaction gases, and

Table III. Effect of Hydrogen Presweeping of Catalyst

	Composition	Total Unsaturation, Per Cent			
			Found		
		Present	Preliminary run	Run 1	Run 2
1.	(Swept catalyst with hydrogen)				
2.	2-Methylpropene	99.5[a]	100.7	99.7	99.6
3.	1,3-Butadiene	196.4[b]	197.7	197.5	197.5
4.	1-Butene	99.9[a]	100.0	99.8	...
5.	(Swept catalyst with hydrogen)				
6.	*n*-Butane 1-Butene	10.1[c]	11.2	10.3	10.4
7.	*n*-Butane 1,3-Butadiene	49.1[c]	49.5	49.5	49.3
8.	*n*-Butane	0.0[a]	0.0	0.3	0.3
9.	*n*-Butane 1-Butene 1,3-Butadiene	128.8[c]	128.6	128.6	128.5
10.	(Swept catalyst with hydrogen)				
11.	Ethene, commercial	Unknown	95.2	95.6	95.5
12.	*n*-Butane 1-Butene 1,3-Butadiene	128.8[c]	129.2	128.6	...
13.	(Swept catalyst with hydrogen)				
14.	20.8% 2-methylbutane 25.2% 1-pentene 54.0% *n*-pentane	25.2[c]	24.8	24.6	25.0
15.	99.9% mixed pentanes 0.1% pentenes	0.1[a]	0.0	0.3	−0.3

[a] Determined by bromine titration.

[b] Minimum unsaturation; determined with maleic anhydride.

[c] Blended.

the latter technique was employed in the present work except where otherwise stated. The data in Table III were obtained with the hot catalyst in the order in which they appear. The catalyst was not swept with hydrogen except as indicated, and then only to ascertain the effect. When the catalyst had not been swept with hydrogen as a prior step, the results of preliminary runs were never significantly different from succeeding runs, even though the composition of successive samples varied widely—for example, C_2 preceded C_4 hydrocarbons and 0% unsaturation preceded 128%. Even with prior hydrogen sweeping of the catalyst, preliminary runs gave values which differed little from subsequent runs, in contrast to large differences when C_5 hydrocarbons were run at room temperature with conventional presweeping (Table II).

Catalyst life has been so good that the authors suspect it may undergo a certain amount of autoregeneration. Unlike hydrogenation at room temperature, the present method has been used successfully on undried gases with aqueous solutions or moist mercury in the burets of the hydrogenation apparatus. This proved particularly convenient in hydrogenating moist gases left from Orsat analyses. However, it is considered good practice to avoid exposing the catalyst to moisture unnecessarily when cold and not in use and this is accomplished by closing the stopcocks at its base, thereby isolating the wet burets.

The hot water–jacketed catalyst has also been applied to the analysis of gaseous mixtures containing cyclopentene, cyclopentadiene, ethylacetylene, and vinylacetylene. The reaction with cyclopentene proceeds normally. That cleavage of the ring did not occur at the temperature of boiling water was established by mass spectrometric examination of the reaction products. Slightly exaggerated analytical results are obtained, however, when essentially pure cyclopentadiene or ethylacetylene is subjected to catalytic hydrogenation over the heated catalyst. The high values probably result from dimerization followed by absorption of gaseous hydrocarbon in the condensed dimer. Liquid product has actually been observed on the confining mercury following a determination on pure ethylacetylene. Mixtures containing less than 10% ethyl- or vinylacetylene have been analyzed with satisfactory results.

ACKNOWLEDGMENT

The authors express their appreciation to W. L. Slater for suggestions on the design of the boiling water–jacketed catalyst.

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Purification of O,O-Diethyl O-*p*-Nitrophenyl Thiophosphate (Parathion) for Use as a Primary Standard

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THE increased use of the new insecticide, parathion, has created an urgent need for a simple method of obtaining the pure compound from technical grade material for use as an analytical standard. The only published criterion for evaluating the purity (*1*) of parathion has as its basis a nitrogen analysis. The presence of any contaminant containing nitrogen would make this procedure questionable. The following method has been used in this laboratory to prepare parathion in a pure state for use as a standard.

PROCEDURE

A 30-gram sample of technical parathion (a dark brown oil with a strong garliclike odor) was washed by decantation with small portions of petroleum ether (Skellysolve A), using a total of 200 ml. The parathion was then taken up in 50 ml. of ether and shaken with 25-ml. portions of 10% sodium carbonate until the aqueous solution was no longer colored. This required about 200 ml. of the wash solution. The ether solution was then dried over anhydrous sodium sulfate and was finally passed through a plug of dried cotton. The dried ether solution was filtered, using mild suction, through a column (8 cm. long and 3 cm. in diameter) containing a mixture (*1*) of 2 parts of Attapulgus clay (an attapulgite clay) and 1 part of Hyflo Super-cel (a diatomaceous earth), previously made wet with ether. The ether was removed on the steam bath and the last traces were removed by a stream of dried nitrogen, leaving a yellow oily residue. To test the purity at this point, a portion of this residue was chilled in dry ice for one hour. Scratching soon induced crystallization. The melting point of the pale amber crystalline mass was about 1° C. The main body of the oil was dissolved in 25 ml. of ether, and Skellysolve A was added at room temperature to the point of incipient turbidity. This solution was placed overnight in the freezing compartment of a refrigerator at a temperature of −15° C. In the morning there was a mass of white needles, which were freed from the amber mother liquor by decantation and washed twice with 10-ml. portions of a precooled mixed solvent consisting of equal parts of Skellysolve A and ether. The crystals were taken up in about 50 ml. of ether, dried over anhydrous sodium sulfate, and passed through dried cotton; the ether was removed on the steam bath, and the last traces of solvent were removed by a current of dried nitrogen. The parathion thus obtained (about 5 grams) was a very pale yellow liquid without odor, which crystallized into almost colorless long needles melting sharply at 6° C.

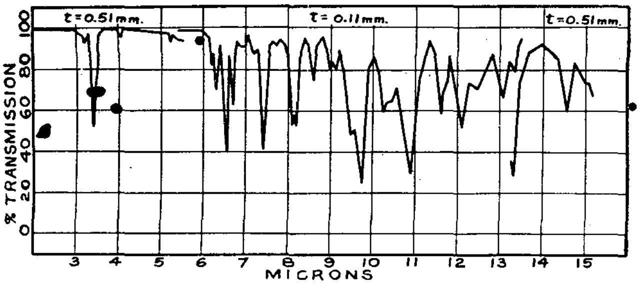

Figure 1. Infrared Spectrogram of Parathion

Temperature, 25° C.
State, 23 grams per liter of carbon tetrachloride (2.0 to 7.5 μ); 22 grams per liter of carbon disulfide (7.5 to 15.0 μ)

Absorption Maxima, Microns

3.24
3.36
4.08
5.22
6.19
6.29
6.54
6.69
7.18
7.43
7.75
8.11
8.17
8.61
8.99
9.12
9.49
9.74
10.27
10.47^{S}
10.82
11.64
12.13
12.53
13.09
13.35
14.57
15.0^{S}

The purified parathion was stored in the refrigerator. It was found highly toxic to houseflies. The infrared spectrogram is shown in Figure 1.

Analysis. Calculated for $C_{10}H_{14}NO_5PS$: C, 41.23; H, 4.85; N, 4.81; P, 10.64; S, 11.01. Found: C, 41.42; H, 4.76; N, 4.94; P, 10.75; S, 11.35.

Physical Constants. n_D^{25}, 1.5370; d_4^{25}, 1.2655.

Substituting these values in the Lorenz-Lorentz formula gives a molecular refraction of 71.88 compared to a value of 70.40 calculated from the atomic refractivity values of Jones *et al.* (*2*).

Since this paper was submitted for publication a modification in procedure from this point has been successfully used.

The oil is dissolved in about twice its volume of absolute ethanol, instead of in a mixed solvent. Crystallization is induced by cooling in dry ice. As in the original procedure, a mass of white needles is obtained by holding the solution at about −15° C. overnight. The crystals are separated from the amber mother liquor by decantation and washed sparingly with precooled ethanol. The yellow oil obtained when the crystals melt is then transferred to a beaker, and placed in a vacuum desiccator over phosphorus pentoxide. Vacuum is applied cautiously at first until most of the ethanol has been drawn off. The final purified product is transferred to a bottle and stored in the refrigerator. Additional product of slightly lower purity may be recovered by working up the mother liquor in the same way.

ACKNOWLEDGMENT

The authors wish to thank H. L. Cupples of this bureau for the infrared spectrogram included in this paper.

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Determining Densities and Adsorption of Gases by Solids

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AN APPARATUS for determining the surface area or specific surface of powdered materials from measurements of the permeability to air of a column of the materials is described in the patent literature (*2*). It has been modified to permit use in determining the densities of materials and the adsorption of gases by powdered materials, and this paper describes the modifications and the method of use. Data are given for the densities of a number of materials determined by this method, as compared to their densities determined by the conventional liquid displacement method.

PRINCIPLE OF METHOD

In determining the volume of a material for density calculation, use is made of the principle that the time, t, required for a quantity of gas to escape from a system through an orifice from pressure, p_1, to a pressure, p_2, at constant temperature is proportional to the volume of the system. Any change, V_s, in the volume of the system incurred by the introduction of the material is proportional then to the change in time required for the pressure to drop through the same pressure range or:

$$V_s = \frac{V_1(t_1 - t_2)}{t_1} \qquad (1)$$

where $V_s = V_1 - V_2$ = volume of sample
V_1 = volume of system without sample
V_2 = volume of system with sample
t_1 = time of pressure drop without sample
t_2 = time of pressure drop with sample

APPARATUS

The apparatus is shown schematically in Figure 1. That part of the apparatus used in determining the specific surface of powders consists of a mercury manometer carrying two enamel-covered Nichrome wire electrodes of different lengths which operate an electric timer, an air chamber with an inlet valve, and a sample cup holder. In determining densities, the sample cup holder supports an adapter which comprises a sample container of approximately 1-liter capacity and an air tank equipped with cover, rubber gasket, cover clamp, and a small orifice through which gas can escape to the atmosphere.

METHOD OF OPERATION

The sample container with and again without the sample is placed in the air tank and air under pressure is admitted to the system through the inlet valve until the mercury column is above the end of the shorter electrode. During the time required for the pressure to be built up, air is escaping through the orifice, so that the orifice attains the temperature of the air within the system. Experience has shown that the orifice attains this temperature if the mercury column is maintained above the end of the shorter electrode for approximately one minute before the inlet valve is closed. When the mercury column breaks contact with the end of the shorter electrode, it actuates the electric timer, which operates until the mercury column breaks contact with the end of the longer electrode. In this manner, the time required for the pressure to be reduced, by escape of air through the orifice, from p_1 to p_2 is measured automatically. This is recorded as t_1 for V_1, the volume of the system with the empty sample container in place in the air tank.

This operation is repeated after the sample is placed in the sample container and the time required for the air to escape from this system is recorded as t_2. The volume of the system, V_1, is previously determined in the same manner, using a steel plug of known volume in place of the sample.

The density of the material is then calculated by the equation

$$d = \frac{W}{V} \qquad (2)$$

where d = density
W = weight of sample in grams
V_s = volume of sample in cc.

Table I. Results of Tests for Densities of Granular Materials by Liquid and Air Displacement Methods

	Density at 24.0° + 0.5° C.	
Material	Liquid displacement[a]	Air displacement
Cement A	3.18	3.16
Cement B	3.20	3.19
Cement C	3.07	3.07
Cement D	2.86	2.86
Cement E	3.08	3.08
Gypsum	2.42	2.43
Blast furnace slag	2.72	2.72
Pulverized limestone	2.70	2.69
Silica sand	2.64	2.64
Masons' sand	2.67	2.69
Pea gravel	2.79	2.77
Olivine	3.21	3.21
Topaz	3.27	3.29
Chrome ore	4.37	4.35
Iron oxide	4.46	4.45
Barytes	4.38	4.37
Fused alumina	3.92	3.91
Dead burned magnesite	3.11	3.13
Crushed firebrick	2.70	2.69
Crushed chert	2.62	2.63
Coal	1.32	1.34
Charcoal	1.43	1.45[b]
White pine sawdust	...	1.55

[a] Standard method for test for specific gravity of hydraulic cement (*1*). Results corrected to density at 24.0° C.

[b] Result determined on paste of material with water.

PREPARATION OF SAMPLE

The present design of the apparatus permits the use of large samples which may be weighed on laboratory scales other than chemical balances. However, the principle may be readily applied to test smaller samples, although a correspondingly greater accuracy of weighing would be necessary. Bulky or fluffy materials may be compacted with the use of a sample container having a filter cloth bottom and a tamper having a filter cloth cover, each connected to an air aspirator. In use, the sample is pressed into place in the container with the tamper as the vacuum is applied to both the bottom and top surfaces.

TESTS OF MATERIALS THAT ADSORB AIR STRONGLY

Certain powders having high specific surface adsorb large quantities of gas under relatively low pressures. However, because the method just described may be used with liquids as well as solids, the density of these highly adsorbent powders may be determined by covering them with a liquid. The procedure followed is to determine the volume of a paste consisting of a known volume of water and a known weight of the powder. The determined volume of the paste is the combined volume of the water and powder. Some of these powders, such as charcoal, are difficult to wet with water but this can be overcome by using a small quantity of a surface-active agent in the water. The density of a sample of charcoal determined in this way was found to be 1.45 at 24.0° C. as compared to a density of 1.43 determined by immersion in kerosene.

The volume of air adsorbed by such materials over the range of pressure of p_1 to p_2 may be determined by determining the density as described above and then determining t_2 for the dry powder. Then, from the weight of the dry powder and density, the volume of the dry sample, V_s, may be calculated from Equation 2 for use in Equation 3.

$$V_A = V_1\left(\frac{t_2}{t_1} - 1\right) + V_s \qquad (3)$$

where V_A = volume of gas adsorbed
V_1 = volume of system with empty sample container
V_s = W/d = absolute volume of solid sample
t_1 = time for pressure to drop from p_1 to p_2 with empty sample container
t_2 = time for pressure to drop from p_1 to p_2 with adsorbing sample in sample container

The volume of gas adsorbed per gram of sample, V_a, may be calculated by dividing the total volume adsorbed by the weight of the sample used:

$$V_a = \frac{V_A}{W} \qquad (4)$$

A test with powdered charcoal in the pressure range of p_1 = 948 mm. and p_2 = 566 mm. of mercury, at a barometric pressure of 743 mm. and a temperature of 24° C., gave the following results:

Density by paste method, d = 1.45
Weight of sample, W = 481.0 grams
Volume of dry solid, V_s $= \dfrac{481.0}{1.45} = 331.6$
Volume of apparatus, V_1 = 1752.0 cc.

t_1 = 89.9 seconds
t_2 = 97.7 seconds

$$V_A = 1752\left(\frac{97.7}{89.9} - 1\right) + 331.6$$

= 483.6 cc. of air adsorbed

or

$$V_a = \frac{483.6}{481.0} = 1.005 \text{ cc. per gram of charcoal}$$

DISCUSSION OF RESULTS

The method described was designed primarily to obtain density data for use in calculating the specific surface of a powdered material from data on the permeability to air of a column of the material. As may be seen from Table I, the densities determined by this method agree closely with the densities determined by the liquid displacement method.

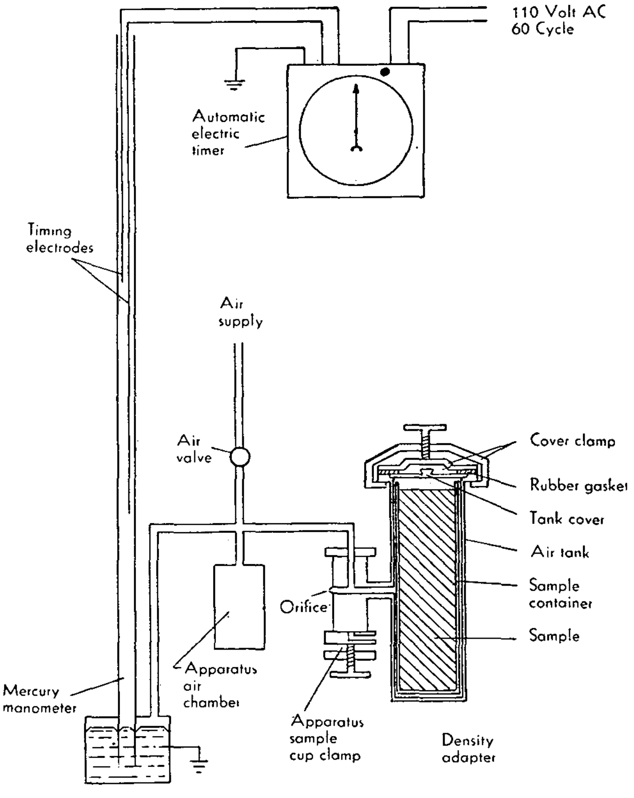

Figure 1. Density Adapter and Air Permeability Specific Surface Apparatus

This method eliminates the need of selecting a suitable liquid for the density determination and the difficult operation of eliminating air from the powder, which are so essential in the liquid displacement method. Although the method was developed primarily for use with powdered materials, it may be used with solids of various shapes and sizes and with liquids.

Data are given in Table I for a wide range of materials ranging in particle size from peak gravel to powdered charcoal. The specific gravities by the liquid displacement method were determined in kerosene and were converted to densities at 24.0° C., at which temperature the densities by the air displacement method were determined. The values obtained by the two methods were identical for a number of the materials and did not vary by more than 0.02 for the others.

This method provides a simple means of determining the amount of gas adsorbed by powders. As used, the equipment operates over only one range of pressures, but it can be adjusted to cover a number of ranges. As the authors have no particular interest in adsorption, this phase of the study was limited to one measurement with powdered charcoal.

In the operation of this apparatus, there are two steps in which the temperature of the sample tends to be reduced below that of the room temperature, 24.0° ± 0.5° C., at which the tests were made. The first is that of increasing the pressure in the apparatus from atmospheric pressure to above the pressure, p_1, in which compressed air at approximately 90 pounds per square inch is admitted into the apparatus and allowed to expand to about 26 pounds per square inch. The second is that of reducing the pressure of the air in the system during the test from above p_1 to p_2 when air is allowed to escape to the atmosphere. However, the quantity of air undergoing these expansions amounts to somewhat less than 2 liters and the expanded air is in contact with the sample for less than 3 minutes. Hence, because this quantity of air is small and its heat capacity extremely small in relation to that of the apparatus, it is believed that the temperature of the sample is not reduced significantly below the temperature of the room during the period of the test.

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Use of Liquid Aniline for Determining Sulfuric Acid in the Presence of Other Acids

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IF A small volume of dilute sulfuric acid is added to dry aniline the water is readily dissolved and the aniline sulfate formed is so insoluble in the aniline that it is precipitated almost quantitatively. Hydrochloric, nitric, and sulfonic acids give no precipitates. Furthermore, the presence of the latter acids in very large excess fails to influence the quantitative precipitation of aniline sulfate.

If aniline sulfate so precipitated is filtered and washed free of the soluble acids with more liquid aniline, the precipitate can be dissolved in water and readily titrated as sulfuric acid without interference from the aniline.

On the basis of these observations a rapid and accurate method was developed for determining sulfuric acid in the presence of sulfonic acids. This application has limited usefulness, but it suggests an analytical usefulness of salt-forming organic liquids in a manner not generally exploited.

Table I. Recovery of Sulfuric Acid in 0.4-Ml. Samples of Varying Concentration by Aniline Method

Known *Gram*	Found (Experimental) *Gram*	Difference *Gram*	Recovery (Corrected)[a] *Gram*	Recovery %
0.00207	0.00192	0.00015	0.00207	100.0
0.00408	0.00398	0.00010	0.00413	101.2
0.00600	0.00589	0.00011	0.00604	100.6
0.00816	0.00792	0.00024	0.00807	98.9
0.01010	0.00994	0.00016	0.01009	99.9
0.01226	0.01212	0.00014	0.01227	100.1
0.01423	0.01403	0.00020	0.01418	99.7
0.01616	0.01602	0.00014	0.01617	100.1
0.01792	0.01773	0.00019	0.01788	99.8
0.01955	0.01945	0.00010	0.01960	100.3

[a] Experimentally found value corrected for solubility of 0.00015 gram of H_2SO_4 in 25 ml. of aniline.

Table II. Effect of Volume of Water on Recovery of Sulfuric Acid by Aniline Method

Water in 25 Ml. Aniline *Ml.*	Recovery of 0.004 g. H_2SO_4 (Uncorrected) %
0.0625	97.5
0.125	98.7
0.187	99.5
0.250	99.7
0.312	99.0
0.375	99.3
0.437	98.6
0.500	96.9
0.562	96.9
0.625	91.5
0.750	87.2
1.000	70.7

Exactly 0.40 ml. of the aqueous solution is added to 25 ml. of dry aniline in a test tube or small Erlenmeyer flask. The mixture is shaken, allowed to stand for 5 minutes for complete precipitation of the aniline sulfate, and then filtered by vacuum through a Whatman No. 5 filter paper, preferably on a small Hirsch funnel. The precipitation vessel is rinsed twice with 1-ml. portions of aniline, and these are used to wash the precipitate on the filter to remove the small amount of aniline carrying the soluble acids.

The filter paper and precipitate are carefully transferred to a 50-ml. beaker and all the aniline and aniline sulfate remaining in the funnel are washed into the beaker with distilled water. The volume of water is brought to about 25 ml. The aniline sulfate must be entirely dissolved, but it is not necessary to remove the filter paper. The aniline sulfate is titrated with 0.01 *M* sodium hydroxide using phenolphthalein.

Table I shows known and found sulfuric acid in a series of experiments where sulfuric acid alone was present. The average of the differences should represent the solubility of sulfuric acid in aniline under these conditions. With a correction made for this solubility, the figures in the last column are obtained. In these determinations the weight of sulfuric acid varied from 0.002 to 0.02 gram, corresponding to 0.4 ml. of 0.05 to 0.5 *M* sulfuric acid. Maximum error of single determinations made at ten different concentrations in this range was 1.2%; average error was 0.4%. These figures are obtained from the last column of Table I, where correction for solubility of aniline sulfate in aniline has been made.

Variables studied other than concentration of sulfuric acid were: amount of water added, presence of hydrochloric and nitric acids, presence of sulfonic acids, and presence of the cations, sodium, potassium, and ammonium.

Table II shows the per cent recovery of 0.0040-gram samples of sulfuric acid when different small volumes of water are added with the acid to 25 ml. of aniline. Recovery is substantially constant and complete up to 0.40 ml. of water. These figures are not corrected for the solubility of aniline sulfate in the various aniline-water mixtures.

Addition of 0.40 ml. of 5.0 molar hydrochloric acid or 5.0 molar nitric acid alone produced no precipitate. A solution containing 0.00390 gram of sulfuric acid in 0.40 ml. gave a corrected recovery of 0.00392 gram in the absence of added acids. In the presence of 1.0 molar hydrochloric and 1.0 molar nitric acid the corrected recovery for two experiments was 0.00387 and 0.00369 gram.

m-Cresolsulfonic acid (hydroxy-*m*-toluenesulfonic acid) failed to produce a precipitate with aniline, as did Negatan (Negatol, Lilly), a colloidal product containing a high percentage of sulfonic acid groups. In the presence of this sulfonated colloid comprising 50% by weight of the 0.40-ml. aqueous sample, 0.004 gram of sulfuric acid was precipitated quantitatively.

The following acids at the concentration shown have also been found to form precipitates of their aniline salts when 0.40 ml. of their aqueous solution is added to 25 ml. of aniline: sulfurous (saturated), phosphoric (1 *M*), oxalic (0.5 *M*), and tartaric (0.5 *M*). The residual solubilities of these acids in aniline have not been studied. The following acids gave no trace of precipitate when added to aniline in this same proportion: hypochlorous (saturated), hydrochloric (0.5 to 5 *M*), perchloric (0.5 *M*), hydrobromic (0.5 *M*), hydrosulfuric (saturated), nitric (0.5 to 5 *M*), boric (saturated), arsenious (saturated), acetic (0.5 *M*), trichloroacetic (0.5 *M*), benzoic (saturated), malonic (0.5 *M*), fumaric (saturated), succinic (0.5 *M*), citric (0.5 *M*), *o*-phthalic (saturated), lactic (0.5 *M*), salicylic (saturated), and aspartic (saturated). Monobasic amino acids such as glutamic (saturated), alanine (0.5 *M*), methionine (saturated), and glycine (0.5 *M*) are precipitated, probably as the zwitter-ions rather than as the aniline salts.

When inorganic cations (as sodium, potassium, and ammonium) are present along with sulfate ions they are quantitatively precipitated from aniline as the inorganic sulfates. It is not unlikely that the inorganic sulfates could be separated on the filter from aniline sulfate, as by ether washing, and separately accounted for.

Satisfactory aniline can be prepared by distilling wet technical grade aniline. The water is first carried over in a milky emulsion, after which the clear dry aniline distills over. In this way it is very easy repeatedly to recover satisfactory aniline for re-use.

RECEIVED December 1, 1948.

Modification of an Adiabatic Specific Heat Calorimeter

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IN A recent paper (*3*) an adiabatic calorimeter for determination of the specific heat of solids and liquids was described. A number of changes in the construction and method of operation have since been made, which have markedly increased the efficiency of the instrument.

The primary aims in modifying the calorimeter were to decrease the time required for each determination, to facilitate the process of cleaning and assembling the apparatus, and to reduce the lost time resulting from gasket leaks, short circuits, and other mechanical failures.

The modified calorimeter is shown in cross section in Figure 1. The external apparatus was changed from that shown in the previous publication only by the addition of a galvanometer circuit.

The calorimeter is now mounted rigidly in the adiabatic shield by three Pyrex tubes, *A*, threaded for small machine screws. The shield is, in turn, connected to the lid of the main brass can by a Transite post, *B*. The bottom of the shield, *C*, was also made removable permitting access to the calorimeter bomb without removing the entire shield or disturbing any electrical connections. The whole apparatus may be inverted at will to facilitate cleaning and loading.

The calorimeter bomb is closed by a lid consisting of a silver plate, *D*, backed by a steel ring, and bolted against a flat, silver-faced, steel flange on the calorimeter with 12 machine screws. The gasket is a ring of annealed 20-gage copper wire. This method of closure has been uniformly effective, even when considerable pressures developed in the calorimeter.

The heating wire for the adiabatic shield has been extended to the lid of shield. The six thermocouples controlling the temperature of the shield are made in a continuous series, the three for the shield being located in clips on the inside of the shield lid, *E*. Only the copper leads for this thermel are connected to the main binding post, *F*. These changes have eliminated temperature differences between the body and the lid of the adiabatic shield, and made adjustment of the shield-calorimeter temperature difference unnecessary.

A new series of six thermocouples, *G*, was installed—three in clips in the body of the adiabatic shield opposing three in the lid of the outside can. These are connected to a Rubicon Type 3400-H indicating galvanometer. This makes it possible to keep the temperature difference between the bath and the adiabatic shield constant, even during the heating periods.

The operating efficiency has been increased by use of a revised procedure. A heating range of approximately 15° C. is now used (formerly 8° C.). Determinations are made in a continuous series with the final equilibrium period for one determination serving as the initial period for the next. In this manner, four or five determinations are made per day. A second operator is needed during the heating periods to adjust the oil-bath temperature.

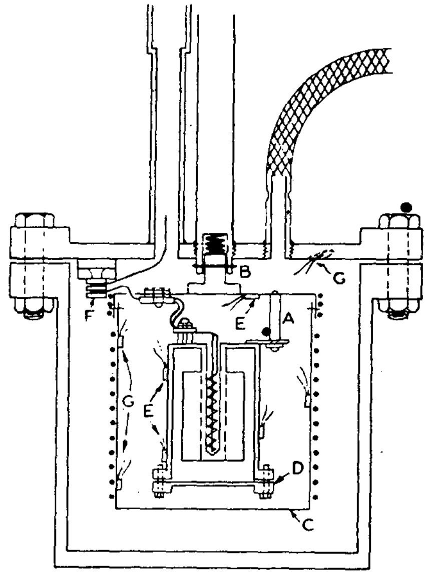

Figure 1. Modified Calorimeter

Although it was not necessary to recalibrate the resistance heater thermometer, the modifications made necessitated redetermination of the heat capacity of the calorimeter. During recalibration, it was desired to determine the influence not only of temperature but also of power, time of heating, and temperature rise on the apparent heat capacity of the calorimeter.

For the calibration, the calorimeter was loaded with 120 gram of crushed vitreous silica, whose specific heat is known (*2*). Th heat capacity of the empty calorimeter was determined by dif ference. The details of the method and calculation of results ar given in the earlier paper (*3*). Measurements were made ove four temperature ranges (50° to 75°, 125° to 145°, 200° to 225 and 275° to 295° C.), covering each range on two consecutiv working days. On each day, three to five runs were made usin combinations of two powers (approximately 1.5 and 3.0 watts and two heating times (10 and 20 minutes), giving temperatur rises of approximately 6° and 12° C.

The most satisfactory method of analyzing a mass of unbal anced data such as these is multiple correlation. The procedur is described by Brownlee (*1*) for systems of one dependent and three independent variables. The process is essentially that of determining, by the method of least squares, the partial correlation coefficients of each variable and the regression equation. The latter is an equation for the dependent variable, in this case apparent heat capacity of the calorimeter, in terms of the three independent variables, average temperature, power, and temperature rise. The regression coefficients are tested for significance by comparing the ratio of the coefficients to their standard error with statistical tables for that ratio.

The regression equation thus found is:

$$\text{Heat capacity (cal./° C.)} = 40.25 + 0.01501t\,(\text{av., ° C.}) - 0.1180w\,(\text{watts}) + 0.01846\Delta t\,(\text{° C.})$$

The coefficients for power and temperature rise were found to be not statistically significant, even at the 0.10 level. This does not prove that they have no importance, but rather indicates that the effect caused by variation of these quantities is small compared to other errors in the method. In order to minimize variations due to changes in operating conditions, a standard procedure (heating rate of 3 watts and temperature rise of 10° to 15° C.) is used whenever practicable. When other conditions are used, the regression coefficients give the correction to be applied to the apparent heat capacity of the calorimeter.

The equation used for calculating the heat capacity at any temperature was developed from experimental data taken, under the standard conditions, at about *68°, 141°, and 214°* C. The constants were adjusted slightly to fit the data at 288° C.

$$\text{Heat capacity (cal./° C.)} = 39.62 + 0.0248t - 3.15 \times 10^{-5}t^2$$

where t = average temperature, ° C. This equation, which constitutes the calibration equation of the instrument, fits the experimental data to within ±0.2%.

Since the procedure and mechanical changes described above were made, the calorimeter has been in regular service for an average of four determinations per day without loss of time for any mechanical or electrical failure. The time required to clean and load the calorimeter was considerably shortened, for it is no longer necessary to remove the shield or the calorimeter or to disturb any electrical connection. The precision equals or excels that of the unmodified instrument—that is, within ±0.5%.

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Liquid Sampling for Analysis by Mass Spectrometer

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ANALYSES of liquid mixtures by mass spectrometer require introduction of minute amounts of calibrating liquids (on the order of 0.001 ml.) and measurement of these amounts to about 1%. Sample introduction usually consists of expanding the liquid directly into the high vacuum system of the spectrometer by touching a pipet to a sintered-glass disk under mercury (*3*). Two methods for quantitative measurement of small amounts of iquid have been published: The volume of liquid is measured by a micropipet constructed of thermometer tubing (*3*), or the pressure of the expanded vapor is measured by a specially designed micromanometer which operates in the pressure range of to 100 microns of mercury (*4*).

The method herein described is a volume-measuring modification which is simple, time-saving, and sufficiently accurate, and nvolves no reading errors.

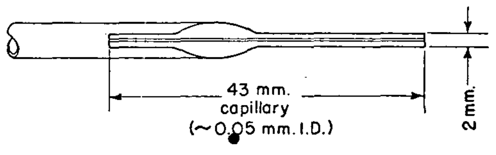

Figure 1. Self-Filling 0.001-Ml. Micropipet

Bottom of tip ground on emery cloth if necessary to make good contact with sintered disk

The instrument used is the Consolidated mass spectrometer. n addition to the conventional gas-handling system of this instrument, the method requires only the usual mercury–sintered isk valve and a self-filling 0.001-ml. micropipet (*1, 2*) (Figure 1; vailable commercially in sizes 0.001 to 0.010 ml.).

When dipped in a liquid, the small section of capillary tubing ecomes filled only to the top by capillary action; then the liquid s delivered completely to the vacuum system by touching the ipet tip to the sintered disk under mercury. Air is allowed to ow through the capillary following the sample until all visible roplets of liquid are swept into the instrument. As the amount of air thus introduced is immaterial to analysis, the mass peaks for air need not be measured. Quantitative results for hydrocarbons appear to be best if the pressure of sample plus air in the 4-liter vacuum system is kept near a Pirani-gage reading of 0.20 ± 0.03 mm. Under these conditions, the amount of air represents 20 to 30% of the total; no detrimental effects on the mass spectrometer filament have been noted. An alternative method, sealing with mercury, consists of filling the capillary with the sample and then pouring enough mercury into the pipet to cover the upper tip. Thus mercury, instead of air, flows through the capillary following the liquid sample.

Table I. Reproducibility of Liquid Introduction into Mass Spectrometer by 0.001-Ml. Self-Filling Micropipet

Component	Mass	Av. Peak Height: Air flow	Av. Peak Height: Mercury seal	Maximum Spread, %: Air flow	Maximum Spread, %: Mercury seal	Av. % Deviation from Average: Air flow	Av. % Deviation from Average: Mercury seal
n-Heptane	100	550 (5)[a]	500 (5)	1.8	4.6	0.6	1.4
n-Octane	114	281 (5)	271 (5)	2.8	8.5	0.7	2.0
n-Nonane	128	225 (6)	225 (7)	4.5	7.3	1.6	2.5
Xylenes	106	213 (6)	212 (6)	3.8	8.6	1.2	2.1

[a] Figures in parenthesis represent number of runs.

The methods permit sufficiently good quantitative analysis, as shown by the reproducibility tests in Table I for *n*-heptane, *n*-octane, *n*-nonane, and a xylene mixture, both with air flowing through the capillary and with mercury sealing. Precision of both methods apparently decreases with the vapor pressure. The air-flow method is somewhat more precise in all four cases; accuracy of this method is demonstrated by analyses of a typical 6-component synthetic blend, mainly octanes (Table II). Some inconsistency is expected for the mercury-seal method, inasmuch as droplets of liquid occasionally are trapped inside the capillary by mercury. Use of this method is essential when air must be excluded.

Table II. Mass Spectrometer Analyses of Synthetic Blend Using 0.001-Ml. Self-Filling Micropipet

(Air-flow method used for calibrations and samples)

Component	Synthetic Composition	Analysis 1	Analysis 2
	Volume %		
n-Nonane	3.2	3.5	3.9
n-Octane	74.8	73.2	75.3
2-Methylheptane	7.0	6.9	7.0
3-Methylheptane	6.9	7.2	6.4
4-Methylheptane	2.9	4.3	2.9
n-Heptane	5.2	4.9	4.5

Calculating time is appreciably decreased by the use of this pipet. It is not necessary to calculate percentage patterns from the spectra of pure calibration compounds because the amount of sample, and, therefore, the spectral peak height, are always the same, within experimental error. For the same reason, sensitivity coefficients (peak heights ÷ sample pressure or volume) are not needed, for the peaks themselves serve this purpose. Because the partial volumes of the constituents add up to near 1.0, approximate percentages are obtained immediately, although normalization to 1.0, or 100%, is usually necessary.

Application with increased accuracy to C_4 alcohols, C_3 acids, and other oxygenated compounds has been obtained by a modification of the mercury seal method, which consists of forcibly squirting sample and mercury through the pipet and onto the sintered disk.

ACKNOWLEDGMENT

The authors wish to thank Sidney Katz, Institute of Gas Technology, Chicago, Ill., for describing the use of the self-filling pipet in microchemistry.

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2-Nitro-1,3-indandione

Promising Reagent for Identification of Organic Bases

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In 1936 Wanag (*4*) described a new acidic reagent, 2-nitro-1,3-indandione, which formed salts with both inorganic cations and organic bases such as the aliphatic and aromatic amines and the nitrogen heterocycles. These derivatives were reported (*4–6*) to be crystalline, nonhygroscopic, water-soluble compounds which, with few exceptions, gave sharp melting points. Rosenthaler (*3*) applied the reagent to the study of alkaloids but other than reporting crystal formation gave no analytical or melting point data. Later Müller (*1*) extended the work to a number of miscellaneous compounds such as histamine, tyramine, and arginine.

2-Nitro-1,3-indandione is a strong acid; hence many of its salts hydrolyze in an aqueous medium to yield acidic solutions. The extent of the hydrolysis depends on the ionization constant of the base used in the preparation, which in most cases is so small as to permit the titration of the free acid, and thus make it possible to determine the neutral equivalent of the salt. This property, unrecognized by Wanag, and not possessed by the usual amine derivatives, gives unusual promise to 2-nitro-1,3-indandione as a reagent for the identification of organic bases. For these reasons the work of Wanag was continued in this laboratory, with particular attention to the acid properties of the salts.

Because only a limited number of heterocyclic derivatives were described by Wanag and others, most of the additional preparative work was devoted to the heterocyclic compounds. The salts were originally prepared by the addition of an aqueous solution of the acid to a dilute hydrochloric acid solution of the amine. As 2-nitro-1,3-indandione is very insoluble in dilute hydrochloric acid, impure salts were often obtained, which required several recrystallizations. Experiments with other solvents indicated that acetone was a more suitable reaction medium, inasmuch as both the organic bases and the free acid were usually soluble in this medium, whereas the salts either precipitated immediately or slowly crystallized. In a few instances the salts precipitated as oils which failed to crystallize.

Except for compounds in which the nitrogen was not functionally basic, such as uracil, xanthine, or acetanilide, all others gave derivatives with this reagent. The alkylamines form crystalline salts which do not hydrolyze sufficiently to give neutralization equivalent measurements. The bulk of the nitrogenous bases, however, gave derivatives which with few exceptions (due to color interference) could be titrated.

In certain cases, such as the titration of the 4-quinazolone derivative, abnormal amounts of alkali were required, owing to the partial neutralization of the enol form of the quinazolone. In almost every instance in which the ionization constants of the bases were known to be less than 10^{-6} the indandione derivative gave a titration value within 2% of theory (with the exceptions cited above).

Although melting point data pertaining to the indandionates are to be found in the literature, earlier workers failed to point out that these values were for the most part decomposition points which are difficult to reproduce, and hence variations of several degrees often were noted among individual observers. However, the derivatives of 2-nitro-1,3-indandione are very easily prepared, and this makes this reagent extremely useful for isolation as well as characterization purposes.

2-Nitro-1,3-indandione was first prepared according to the directions of Wanag (*4, 5*) by the nitration of 1,3-indandione with cold fuming nitric acid in glacial acetic acid under conditions which gave rather erratic yields. In this laboratory it was discovered that more reproducible results were obtained with ordinary concentrated nitric acid containing oxides of nitrogen. These oxides have a marked influence on the course of the reactions, favoring the formation of nitration rather than oxidation products.

EXPERIMENTAL

2-Nitro-1,3-indandione. Dissolve 20 grams of 1,3-indandione in 200 ml. of glacial acetic acid and raise the temperature of solution to 48° C. Add 20 ml. of 50 to 60% nitric acid visibly colored with the oxides of nitrogen. Shake or stir 5 to 10 seconds and place the flask immediately under cold running tap water while continuing the agitation for several additional minutes. After cooling for 30 to 60 minutes, remove as much of the mother liquid as possible by filtration. Dissolve the crude 2-nitro-1,3-indandione in 500 ml. of cold water, decolorize with charcoal at 10° to

Table I. Titration Data

Salts of 2-Nitro-1,3-indandione	M.P. (Dec.) of Salt, ° C.	Neutral Equivalent		Solvent Used	% Carbon		% Hydrogen		% Nitrogen	
		Theory	Found		Theory	Found	Theory	Found	Theory	Found
Acetamidine	254–7[a]		U.T.T.[b]	Water	...	...	...	...	...	...
Acridine	183	370	373	Acetone	...	...	...	...	...	...
Adenine	...		U.T.T.		51.5	51.3	3.65	3.45	...	...
dl-Alanine	197–8[c]	140	266	Acetone	51.4	51.4	4.32	4.38	...	...
p-Aminoacetanilide	214	341	340	Acetone	...	...	...	...	...	...
p-Aminoacetophenone	191–4	326	332	Acetone	...	...	...	...	...	...
o-Aminoacetophenone	164–6	326	320	Acetone	62.6	62.1	4.32	4.20	...	...
p-Aminoazobenzene	...		U.T.T.	Acetone	...	...	...	...	...	...
m-Aminobenzoic acid	204–5	164	163	Acetone	...	...	...	...	...	...
p-Aminobenzoic acid	203–5	164	164	Acetone	...	...	...	...	...	...
p-Aminobiphenyl	196	360	369	Acetone	...	...	...	...	...	...
o-Aminophenol	...	300	296	Water	...	...	...	...	...	...
p-Aminophenol	...	300	298	Acetone	...	...	...	...	...	...
m-Aminophenol	201–3	300	306	Alcohol	...	...	...	...	...	...
α-Aminopyridine	189	285	284	Acetone	...	...	...	...	...	...
5-Aminouracil.H_2O	...	336	315	Water	46.4	46.8	3.60	3.45	16.7	16.5
Ammonia	222–4	...	U.T.T.	Water	...	...	...	...	...	...
Aniline	185–7	284	284	Acetone	...	...	...	...	...	...
o-Anisidine	176–7	314	312	Acetone	...	...	...	...	...	...
p-Anisidine	191–2	314	313	Acetone	...	...	...	...	...	...
Anthranilic acid.$^3/_2H_2O$	174–5	177	175	Acetone	54.1	54.2	4.26	4.15	7.88	8.11
Benzamidine	189–90	...	U.T.T.	Water	...	...	...	...	...	...
Benzylamine	178–9	...	U.T.T.	Acetone	...	...	...	...	...	...
Bornylamine	224–5	344	347	Alcohol	...	...	...	...	...	...
n-Butylamine	143–4	...	U.T.T.	Acetone	...	...	...	...	...	...
5-Bromo-8-nitroquinoline	138	444	453	Acetone	48.7	48.9	2.27	2.38	...	...
Caffeine	177	385	379	Acetone	53.0	53.2	3.92	3.93	...	...
m-Chloroaniline	179–80	318	317	Acetone	...	...	...	...	...	...
o-Chloroaniline	168–9	318	315	Acetone	...	...	...	...	...	...
p-Chloroaniline	187–9	318	314	Acetone	...	...	...	...	...	...
7-Chloro-4-quinazolone	192	372	314	Acetone	54.9	54.9	2.71	2.73	11.31	11.27
Cinchonine.1H_2O (di salt)	167–8	347	329	Water	64.0	64.2	4.91	5.12	8.06	7.98
Creatine	...	322	329	Water	48.5	48.4	4.38	4.72	...	...
Creatinine	...	304	305	Water	51.3	50.9	3.98	4.06	...	...
Cyclohexylamine	221–2	...	U.T.T.	Acetone	...	...	...	...	...	...
o-Dianisidine	216–17	313	308	Acetone	...	...	...	...	...	...
Diazoaminobenzene (di salt)	176–8	290	287	Acetone	62.2	62.1	3.65	4.31	...	...
Dibenzylamine	202–4	388	381	Acetone	...	...	...	...	...	...
4,7-Dichloroquinoline	156–7	389	386	Acetone	55.6	55.1	2.59	2.54	...	...
Dicyclohexylamine	222	...	U.T.T.	Water	67.7	68.0	7.58	7.75	...	...
Di-*n*-decylamine	70–1	...	U.T.T.	Acetone	...	...	...	...	5.73	5.76
Diethylamine	183	...	U.T.T.	Water	...	...	...	...	...	...
p-Diethylaminobenzaldehyde	113–14	368	375	Acetone	65.2	65.0	5.47	5.72	...	...
Diisoamylamine	189–91	...	U.T.T.	Acetone	...	...	...	...	...	...
Di-2-ethylhexylamine	147	...	U.T.T.	Acetone	69.4	69.1	9.32	9.30	...	...
Di-*n*-hexylamine	113–14	...	U.T.T.	Acetone	67.0	67.2	8.57	8.80	...	...
Dimethylamine	210	...	U.T.T.	Water	...	...	...	...	...	...
N,*N*-Dimethylaniline	133	312	316	Water	...	...	...	...	...	...
Di-*n*-nonylamine	92	...	U.T.T.	Acetone	...	...	...	...	6.08	5.88
Ethylamine	205–6	...	U.T.T.	Water	...	...	...	...	...	...
N-Ethylaniline	174–5	312	305	Acetone	...	...	...	...	...	...
Ethylenediamine	208	...	U.T.T.	Acetone	...	...	...	...	...	...
Ethyl nicotinate	127	342	339	Acetone	59.7	59.4	4.12	4.10	...	...
N-Ethyl-*o*-toluidine	179–82	326	329	Acetone	...	...	...	...	...	...
Glycine	...	133	253	Water	...	...	...	...	...	...
Guanidine	258	...	U.T.T.	Water	...	...	...	...	...	...
Guanine	...	342	337	Water	49.1	48.8	2.95	3.03	...	...
Histidine	...	269	263	Water	...	...	...	...	...	...
Hydrazine	...	223	227	Water	48.4	48.7	4.07	4.10	...	...
1-Hydroxyproline	...	322	311	Water	52.2	51.8	4.38	4.77	...	...
Isoquinoline	177	320	312	Acetone	...	...	...	...	...	...
2-Methoxy-6,9-dichloroacridine	138	469	460	Acetone	58.9	58.9–	3.01	3.20	...	...
5-Methoxy-8-nitroquinoline	158	396	400	Acetone	57.7	57.3	3.31	3.47	...	...
Methylamine	194–9	...	U.T.T.	Water	...	...	...	...	...	...
N-Methylaniline	174–6	298	290	Acetone	...	...	...	...	...	...
Methyl anthranilate	150–1	342	337	Acetone	59.7	59.8	4.14	4.24	...	...
5-Methyl-8-nitroquinoline	159	379	371	Acetone	60.2	59.9	3.45	3.56	...	...
6-Methylquinoline	156–8	334	332	Acetone	68.3	68.1	4.22	4.22	...	...
7-Methylquinoline	171	334	330	Acetone	...	...	...	...	8.38	8.74
α-Naphthylamine	207–10	334	335	Acetone	...	...	...	...	...	...
β-Naphthylamine	186	334	333	Acetone	...	...	...	...	...	...
Nicotinic acid	169	157	161	Acetone	57.4	57.2	3.21	3.66	...	...
m-Nitroaniline	182	...	U.T.T.	Acetone	...	...	...	...	...	...
o-Nitroaniline	143–5	329	328	Acetone	54.7	54.6	3.37	3.36	...	...
p-Nitroaniline	172	329	321	Acetone	...	...	...	...	...	...
5-Nitroquinoline	153–4	365	369	Acetone	59.2	58.9	3.04	3.21	...	...
8-Nitroquinoline	152–3	365	356	Acetone	59.2	58.9	3.04	3.16	...	...
m-Phenylenediamine (mono salt)	196–7	299	301	Water	60.2	59.9	4.38	4.54	...	...
p-Phenylenediamine (di salt)	246–8	245	246	Acetone	...	...	...	...	...	...
p-Phenetidine	191–3	328	331	Acetone	...	...	...	...	...	...
Phenylhydrazine	...	299	301	Alcohol	...	...	...	...	...	...
α-Picoline	147–8	284	278	Acetone	...	...	...	...	...	...
β-Picoline	141	284	281	Acetone	63.4	63.2	4.25	4.28	...	...
γ-Picoline	160–2	284	278	Acetone	63.4	62.9	4.25	4.23	...	...
Pipecolinic acid.1H_2O	133–4	338	334	Water	53.3	53.3	5.37	5.50	8.28	8.36
Piperidine	...	...	U.T.T.	Water	...	...	...	...	...	...
Pyridine	166–8	270	272	Acetone	...	...	...	...	...	...
4-Quinazolone	195	337	317	Acetone	60.5	60.3	3.29	3.43	12.45	12.43
Quinine.1H_2O (di salt)	210	...	...	...	63.0	62.5	5.01	5.06	7.72	7.79
Quinoline	148–9	320	325	Acetone	...	...	...	...	...	...
Semicarbazide	186–9	266	264	Acetone	45.1	45.0	3.79	4.01	...	...
Strychnine	226	525	521	Acetone	...	...	...	...	...	...
Sulfanilamide	203–5	363	364	Acetone	...	...	...	...	...	...
Theobromine	...	371	351	Water	51.8	52.1	3.53	3.87	...	...
o-Tolidine (di salt)	217–20	297	298	Acetone	...	...	...	...	...	...
m-Toluidine	184–6	298	296	Acetone	...	...	...	...	...	...
o-Toluidine	185–6	298	296	Acetone	...	...	...	...	...	...
p-Toluidine	183–4	298	294	Acetone	...	...	...	...	...	...
Urea.1H_2O	160	269	270	Water	44.6	44.6	4.12	4.26	15.60	15.85
2,4-Xylidine	184–7	312	312	Acetone	...	...	...	...	...	...

[a] All melting points in this column were taken with a melting point block and are corrected.
[b] U.T.T. unable to titrate.
[c] Compounds with no M.P. data gave indeterminate melting points.

A number of salts formed oils rather than crystalline derivatives: indandionates of 6-aminohexanol, 2,4-dimethylquinazoline, novocaine, and 1-tyrosine.

15°, add 300 ml. of concentrated hydrochloric acid, and allow to stand for several hours; yield, 73%. (2-Nitro-1,3-indandione is commercially available from Krishell Laboratories, Portland, Ore.)

Preparation of Salts of 2-Nitro-1,3-indandione. Prepare a stock solution of the acid by dissolving 5 grams of 2-nitro-1,3-indandione in 150 ml. of acetone. Dissolve approximately 150 mg. of the test compound in a minimum amount of acetone, alcohol, or water and then add it to a slight excess of the stock solution. Place salts that do not crystallize immediately in a refrigerator for several hours, then filter, wash, and recrystallize from ethanol, water, or aqueous alcohol. The salts thus prepared have fairly sharp melting points (usually with decomposition).

The neutralization equivalents were determined according to the directions of Niederl and Niederl (*2*), using a phenolphthalein indicator. The data obtained from the titration of some of the compounds described by Wanag, together with those synthesized in this laboratory, are listed in Table I.

SUMMARY

The salt formation of 2-nitro-1,3-indandione as described by Wanag has been confirmed; over 40 new salts have been prepared and the titration characteristics of approximately 100 2-nitro-1,3-indandionates have been studied. 2-Nitro-1,3-indandione formed well defined salts with a wide variety of compounds, including the simple and substituted amines, alkaloids, nitrogen heterocycles, amino acids, amides, and halo, azo, and nitroamines. Most of the salts were colored; a few contained water of crystallization. Fairly accurate *neutralization equivalents* may be determined for the salts of weak and moderately weak bases such as quinoline or aniline but not for the stronger bases such as mono-, di-, and trialkyl amines, morpholine, and piperidine.

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Molecular Still for Small Volumes

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THE semimicro molecular still described by Breger (*1*) is similar in principle to one with which the author is familiar. The still is simple to make and was designed to accommodate materials either too viscous or too small in volume to be distilled in any of the moving-film type stills now commercially available.

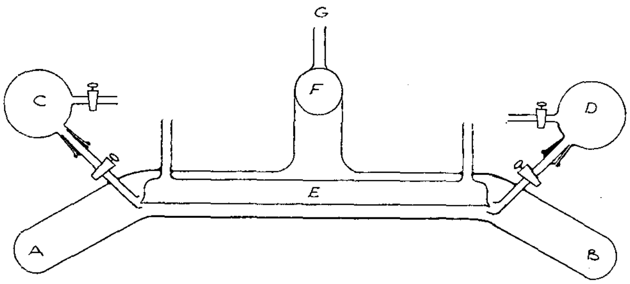

Figure 1. Molecular Still

Whereas the smallest sample distilled to date was a 14.5-gram mixture of lubricant oils which was divided into seven 2-gram cuts, smaller samples could be handled, and, if necessary, a model of smaller dimensions could be built.

CONSTRUCTION

The still is constructed of 26-mm. outside diameter Pyrex tubing having an over-all length of 380 mm. The internal water or compressed air-cooled condenser, *E*, is 200 × 14 mm. in outside diameter. The still is heated by means of a Nichrome wire heating coil wound on the outside of the still between the receiver tubes, and the current is regulated by a variable-voltage transformer. Data for a temperature-voltage calibration curve were taken before the still was set up for operation. The still is evacuated through a 29/42 standard-taper ground-glass joint which is in the line perpendicular to the plane of the drawing at *F*, allowing the still to rotate about *F* in the plane of the drawing. The vacuum is measured by a McLeod gage connected to the 8-mm. tube, *G*, by new rubber pressure tubing.

It has been observed that a stream of oil flowing over a hot surface has a strong tendency to occupy as small an area as possible in spite of efforts to spread it out. Therefore, as satisfactory results were obtained with the simple form of the still shown, various ideas for altering the evaporation surface to cause spreading of the distilland into a thinner film have been rejected.

OPERATION

The material to be distilled is poured through one of the receiver tubes into either end, *A* or *B*, of the still. Vacuum is then applied to the still to effect a preliminary degassing, after which the still is tipped to cause the distilland to flow slowly along the surface below the condenser, *E*, to the opposite end. Next, the still is tipped to cause the distilland to flow slowly back to its original position. The rocking of the still is continued while the distilland is thoroughly degassed as indicated by the reduction of the pressure to about 1 micron. Most molecular distillations can be carried out at an absolute pressure of from 1 to 5 microns. If any trouble is experienced in obtaining this vacuum with a reasonably high pumping capacity, all rubber connections in the system must be made with new rubber, the outer surface of which is treated with a good high-vacuum grease such as the silicone grease marketed by Dow-Corning. Then, while rocking is continued, the temperature is raised; this causes distillation to begin, which washes the spattered distilland from the condenser. The receiver-tube stopcocks are kept closed, after the initial evacuation of receivers *C* and *D*, during both the degassing and cleansing operations. During the actual distillation operation, the rocking of the still is necessarily intermittent, because time is required to allow the distillate to drain from the condenser through the receiver tubes into the receivers. The receiver-tube stopcocks are used to keep the distillate from flowing back from the receivers into the still when it is rocked.

By use of an auxiliary vacuum source, it is possible to take cuts without releasing the vacuum on the still. The receiver-tube stopcock is closed and the vacuum released on the receiver, the distillate is poured out, vacuum is again applied to the distillate receiver from the auxiliary source, and finally the receiver-tube stopcock is reopened.

ACKNOWLEDGMENT

The assistance of Kenneth W. Reeser is gratefully acknowledged.

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RECEIVED January 22, 1949.

Constant-Flow Buret Based on Principle of Mariotte Flask

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IN MANY experimental procedures it is necessary or desirable to use a device for introducing or metering a liquid at a constant rate of flow. Of the arrangements that may be used for this purpose, one based upon the principle of the Mariotte flask is advantageous because a model capable of high precision can be constructed by even a relatively unskilled technician. Furthermore, a wide variety of rates of flow can be obtained by simple adjustment of the effective working head and/or the rate-regulating tip.

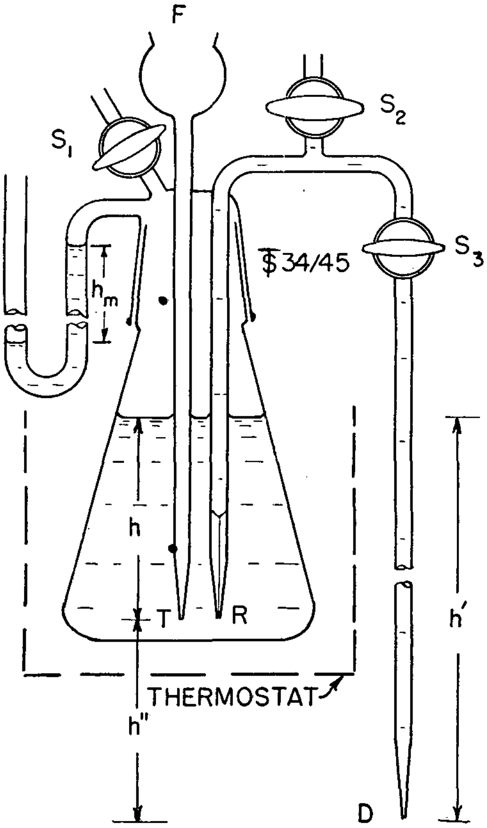

Figure 1. Buret

Apparatus of this type has been reported by Zentner (*2*). In the present paper, the factors affecting the precision of operation of such devices are discussed and experimentally verified and an improved design capable of high precision is described.

EXPERIMENTAL

A diagram of the apparatus is shown in Figure 1. A 34/45 ₮ male joint is sealed to a 500-ml. Erlenmeyer flask. The delivery tube and solution-air inlet tube are ring-sealed into the cap, which also has sealed to it a stopcock to facilitate the partial exhaustion of the flask. (An open-end manometer, filled with the same liquid as the flask, was sealed to the apparatus used for study of the behavior of the buret.) In operation, the flask is filled with the liquid and, with stopcock S_3 closed, suction is applied through stopcock S_2 until the liquid rises into the horizontal portion of the tube. The delivery tube, *D*, is then inserted into a beaker containing the liquid, and suction is applied again at S_2 until the tube is filled completely. With S_2 and S_3 closed, suction is applied to S_1 until air bubbles issue freely from the tip, *T*. The flask is now ready to deliver liquid at a constant rate of flow, by opening stopcock S_3.

Table I. Rates of Flow Using Air Inlet Tube with Radius of 3 Mm.

h	Manometer Reading, h_m	Bubble Pressure, h_b	Rate of Flow
Cm.	*Cm.*	*Cm.*	*G./min.*
4.97	5.50	0.53	0.2637
4.38	4.87	0.49	0.2649
3.83	4.40	0.57	0.2634
2.62	3.30	0.68	0.2630
2.00	2.48	0.48	0.2688
1.67	2.20	0.52	0.2625
1.28	1.75	0.47	0.2608
0.86	1.41	0.55	0.2599

The rate of flow is determined by the effective pressure head, *H*, the dimensions of the rate-restricting tip, *R*, and the viscosity of the liquid. Inasmuch as the viscosity of aqueous solutions has a temperature coefficient of about 1 or 2% per degree, it is necessary to place the flask in a bath, the temperature of which is maintained constant to about 0.1° C. to ensure precise results. The rest of the delivery tube need not be thermostated.

From an inspection of Figure 1, it is evident that the effective hydrostatic head, *H*, is given by

$$H = h' - h_m$$

where h_m is the reading of the manometer.

Because

$$h_m = h + h_b$$

where h_b is the excess pressure required to form a bubble, and

$$h' = h + h''$$

then

$$H = h'' - h_b$$

The bubble pressure, h_b, is at a maximum when the bubble is approximately hemispherical. Any further diminution of the pressure above the liquid causes the bubble to become unstable and it is detached from the tip. The introduction of this air causes the pressure, h_m, to decrease and the liquid may rise into *T*. As more liquid leaves the flask the pressure in the vessel is reduced and a new bubble of air forms at *T*.

All this theory was verified by an experimental study in which tips of various diameters were installed at *T* and several values of h'' were employed. The flow rates were measured by weighing the effluent from *D* while measurements of h and h_m were made with a cathetometer.

Results found when using a tip, *T*, of radius 3 mm., and a head, h'', of 14 cm. are given in Table I. The first column gives the height of liquid above the air inlet tip, and the second gives the maximum value for the manometer reading. When a bubble entered the air space above the liquid the manometer reading decreased by several millimeters and then rose again to a maximum value at the time the next bubble was detached. The difference of columns 1 and 2 is the maximum bubble pressure, h_b, tabulated in column 3. Column 4 gives the rate of flow observed for each value of h. The average deviation of these values from the mean is ±0.6%.

Another series of experiments with a tip of radius 3 mm., but with a head, h'', of 85 cm. gave values for the rate of flow that showed an average deviation from the mean of ±0.3%.

The rates of flow given in Table I are not instantaneous values but averages obtained by collecting drops over a period of several minutes. The rate at any moment varies, owing to the fluctua-

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Table II. Rates of Flow with Fine Capillary Air Inlet Tube

h	Manometer Reading, *hm*	Bubble Pressure, *hb*	Rate of Flow
Cm.	*Cm.*	*Cm.*	*G./min.*
4.01	21.95	17.94	0.9822
3.35	21.28	17.93	0.9816
2.03	19.97	17.94	0.9822
0.90	18.86	17.96	0.9816

tion of the pressure above the liquid. In the experiment where the head was 85 cm., the rate of bubbling at tip *T*, and hence the fluctuations in h_m, varied from 2 to 20 seconds per bubble for values of *h* between 5 and 0.6 cm., respectively. In the experiment where the head was 14 cm., the rate of bubbling was approximately four times these values. Consequently, the rates of flow for short periods of time probably differed significantly from the tabulated values.

The most satisfactory design makes use of a tube drawn out to a very fine capillary at *T* and has a relatively large value of h''. The results found with such an apparatus, given in Table II, show that the rate of flow is constant within the experimental error of measurement. The fluctuations of the manometer reading were not greater than 0.1 mm.; for $h = 5.5$ cm. the rate of bubble formation was 0.70 second per bubble, whereas for $h = 0.36$ cm. the rate was 0.97 second per bubble. Because of the rapid rate of bubbling, the small variation in this rate, and the small fluctuation of h_m, it is believed that the constancy of the rate of flow over short periods of time did not differ significantly from the values measured over the longer interval.

The precision of apparatus built according to the above description is attested by its use in automatic titrations in which the reagent is added at a constant rate and the amount of reaction is determined by the elapsed time from the start to the end point (*1*). The results found in such service are comparable with those obtained using the best volumetric analytical technique.

The disadvantage of the requirement that the apparatus be thermostated is offset by the advantages of flexibility of rate of delivery and simplicity of construction. For metering large volumes of liquids at precise rates, modifications similar to those of Zentner (*2*) may be used, provided the restrictions discussed in the present paper are followed.

SUMMARY

A constant-flow buret of high precision is based on the principle of the Mariotte flask. By placing the rate-restricting tip in the liquid to be delivered, thermostating the flask, and using a fine capillary tip for the air inlet, rates of flow constant to better than 0.1% over both short and long periods of time have been obtained.

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Modified Thyratron Thermoregulator Circuit

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THE simple on and off thyratron thermoregulator circuit such as that of Garrett (*1*) is very reliable because the thyratron will carry enough current to operate a 250-watt heater directly and thus eliminate the usual relay. However, the circuit described incorporates two small batteries which have a limited life of service, especially if they become overheated by close proximity to the thyratron or otherwise. These batteries may be eliminated by the exceedingly simple modification of this circuit shown in Figure 1. Here the circuit is entirely alternating current-operated. The current through the thermoregulator cannot be more than 3 or 4 microamperes, which is low enough to prevent appreciable corrosion of the mercury contact.

The circuit is self-explanatory except for one or two details. The two connections marked *B* and *C* must be made so that the grid and anode are opposite in phase with respect to the anode return. If, on first trial, the tube refuses to show control when the thermoregulator leads are shorted together, leads *B* and *C* must be interchanged.

On first glance it appears that current is always flowing through both the heater and the fan. This is true, but it must be remembered that the impedance of a small fan motor is much larger than that of a heater. When the tube is not firing, the fan runs by means of current through the heater. This current is too small to dissipate appreciable heat in the heater. When the tube fires, the fan is shorted out by the tube and so stops running, while at the same time the heater is activated. The fan is directed at the water surface of the thermostat and serves to cool it. By this means the thermostat may be operated several degrees below room temperature (*1*).

This circuit has given satisfactory service for more than a year for the control of two large water thermostats (approximately 40-gallon capacity). Schwenk (*2*) has recently proposed a somewhat similar circuit, but has used a much higher voltage and lower resistance in the thermoregulator circuit and the thermoregulator is connected directly across the power line. The latter is undesirable for safety's sake and it is desirable to use as low a potential and as high a resistance as possible in this part of the circuit to minimize corrosion at the mercury contact. This circuit is being used with thermoregulators which are open to the air. Undoubtedly this consideration is much less important if closed thermoregulators are used, out of contact with the air. The present circuit uses one thousand times less current through the thermoregulator than does Schwenk's circuit.

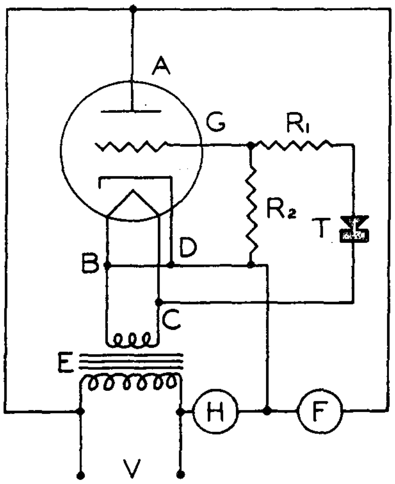

Figure 1. Modified Circuit

Tube FG57
A. **Anode (cap)**
B, C. **Connections**
D. **Grid and anode return**
E. **5-volt transformer**
F. **Fan**
G. **Grid**
H. **Thermostat heater**
R_1. **300,000 ohms**
R_2. **1 megohm**
T. **Thermoregulator**
V. **120-volt power line**

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Received February 14, 1949.

Stabilized Magic Eye Indicator for Karl Fischer Titrations

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A SIMPLE three-tube titration instrument is described which is unaffected by ordinary line voltage fluctuations. The shadow angle of the magic eye changes by about 5° for a 10% line voltage change. Component tolerances are 10% or greater. The instrument is usable with any platinum-platinum titration cell, the effective resistance of which, at the end point, is between 100 ohms and 1 megohm.

DISCUSSION

The use of the electrometric "dead-stop" end-point technique (*1*) is generally accepted as the most satisfactory method of carrying out Karl Fischer moisture titrations. A number of instruments have been applied to the determination of this end point. For the sake of simplicity, the straightforward galvanometer circuit is preferred by some (*7*). This circuit and its modifications perform satisfactorily. However, as has been shown by Potts (*4*), optimum sensitivity requires the use of a special low-resistance titration cell, matching the internal resistance of the galvanometer. A cheaper and more rugged cell, of high resistance, may be used, if an electronic amplifier is substituted for the galvanometer. An electronic amplifier, in itself, may also be somewhat more durable than a galvanometer.

Several electronic "magic eye" circuits suitable for the present purpose have been described (*2*, *3*, *5*, *6*), and that described by the writer has been in use in this laboratory for several years. All these circuits, however, are somewhat unstable to line voltage fluctuations, and several, including the writer's, are sensitive as to certain component tolerances. In particular, difficulty has been encountered in duplicates of the writer's model, because of the wide variations in target current between individual magic eye tubes. In order to eliminate the nuisance of line voltage drift and adjustments necessitated by aging of components, the circuit described here was developed. In addition to improved stability and noncritical components, it is very flexible as to the resistance of the cell with which it is used, and is very simple in construction and operation.

The circuit diagram of the instrument is given in Figure 1. As opposed to the galvanometer circuit, in which a constant voltage is applied to the cell, it is convenient with an electronic amplifier to pass a constant current through the cell, and to measure the change in potential across the electrodes as the titration proceeds. A satisfactory approximation may be obtained by connecting the cell, through a relatively high resistance, to a fixed voltage source. Inasmuch as the effective resistance change of the cell at the end point of a Karl Fischer titration is of the order of tenfold, extreme sensitivity in the amplifier circuit is not required.

Using a high-mu triode driving a 6E5 (magic eye) tube, full shadow deflection is obtained from a 0.1-volt change in electrode potential, a value that is easily obtained by supplying the cell and series resistor with about 0.5 volt. In the present circuit, this series resistor is made variable, so as to permit adjustment of the cell current in accordance with the end-point resistance of the particular cell used. One side of the input is grounded, and a shielded cable is used to connect the electrodes, to minimize stray pickup.

One section of a 6SL7 twin triode is used as the amplifier in this circuit, direct-coupled to a conventionally operating 6E5 magic eye tube. In order to minimize grid current, the tube is operated at a very low plate current, and gain is maintained by use of a high value of plate load. Any alternating current pickup is by-passed by the grid-to-cathode capacitor at the 6E5 tube. This part of the circuit is conventional. The effects of line voltage fluctuations are neutralized by use of the second triode of the 6SL7 as a cathode follower, which is cathode-coupled to the amplifier triode. The low effective plate resistance of the cathode follower minimizes degeneration of amplifier gain by the high value of cathode resistor. By means of this circuit, any change in supply voltage affecting the grid voltage of the amplifier affects its cathode voltage to almost the same extent, thereby maintaining a substantially constant bias. Drift may also be introduced by changes in amplifier heater voltage. In this circuit such an effect is neutralized by the equal effect on the cathode of the cathode follower, and is minimized by the reduction of heater voltage to 5.7 volts, by means of a series resistor. The canceling of heater voltage variations by means of the cathode follower depends, for its effectiveness, upon close matching between the cathodes of both triodes. Although twin triodes will generally match fairly closely in this respect, an occasional tube will be found unsatisfactory. Examples of variations between 6SL7 tubes are shown in Table I.

Where the additional expense is justified, almost perfect performance may be obtained by use of the Type 6SU7 tube, which is directly interchangeable with the 6SL7. Using this more care-

Table I. Effect of Line Voltage on Magic Eye Shadow Angle

6SL7 Make	RCA	RCA	RCA	Tung-Sol	Lafayette	Unmarked
	Shadow Angle, Degrees					
Line voltage						
120	0	0	0	0	0	0
110	5	..	5	5	..	..
100	10	..	15	10	20	45
90	45	10	30	15	..	..
80	..	..	..	20	30	90

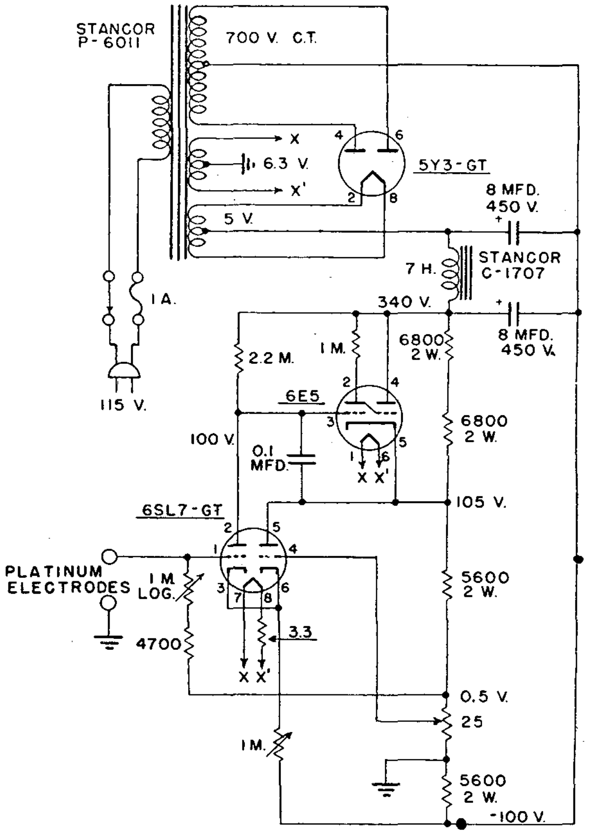

Figure 1. Schematic Diagram

All resistors 0.5 watt except where noted. M. Megohm fully matched tube, line voltage variations as high as 40% have negligible effect on the magic eye shadow angle.

The cathode follower triode serves the additional function of a zero adjustment, for setting the eye shadow angle at the start of a titration. This is accomplished by the potentiometer in its grid circuit, by means of which the grid is returned to the same potential as that of the amplifier grid, as indicated by the shadow angle. This is desirable, in any case, in order to achieve the best cancellation of line voltage changes.

Because of the differences between individual 6SL7 tubes, it is necessary to provide a compensating control: the potentiometer in the 6SL7 cathode circuit. It ordinarily need be adjusted only on changing tubes. To make this adjustment, the electrodes are disconnected from the amplifier, the zero-adjusting potentiometer is rotated to the stop, in the direction which opens the eye, and the compensating potentiometer is then adjusted until the eye just closes. (When the back-titration procedure is followed, the shadow angle is adjusted to just under the maximum.)

With the exception of the 6SL7 tube, none of the component values in the circuit is critical, and 10% tolerances are adequate. In most cases, the choice of the 6SL7 may also be considered unimportant, so long as it is made by a reliable manufacturer. Only where extreme line voltage fluctuations are the rule will it be necessary to select 6SL7's or to use the more carefully matched tube type mentioned.

Operating procedure is the same as with circuits previously described, except that a polarizing current control replaces the sensitivity control. For any given cell, a position of maximum sensitivity will be found for this control. Generally, something less than maximum sensitivity will be adequate, and this control normally need not be changed after initial adjustment to the cell used.

In actual performance tests, the instrument has been entirely satisfactory. When compared with the unit formerly used, the lack of flutter and drift in the eye position is immediately apparent. In addition, the instrument is ready for use only 30 seconds after being turned on, because warm-up drift is effectively cancelled. Titrations were performed with cells of various effective resistances, with equal success. Satisfactory results were obtained with an electrode system consisting of a pair of 18-gage platinum wires, sealed through glass tubes, and then ground off flush with the glass. A spacing of about 1 cm. between electrodes was used. This cell has the obvious advantage of eliminating the problem frequently encountered in other cells, in which the wires break off close to the glass seal. Cleaning of the electrodes is also facilitated.

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Determination of Small Amounts of Zirconium with Mandelic Acid

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MANDELIC acid was recently found to be a specific reagent for the precipitation of zirconium. Kumins (*2*) investigated this reagent and recommended it for the determination of 0.020 to 0.3 gram of zirconium oxide in the presence of titanium, iron, vanadium, aluminum, chromium, thorium, cerium, tin, barium, calcium, copper, bismuth, antimony, and cadmium.

Because analyses must be made frequently of samples containing only a few milligrams of zirconium oxide, this investigation was undertaken to find if mandelic acid might be used in this range. For comparison, the same size of sample was run by the phosphate method, which is perhaps the most widely used method for the determination of small amounts of zirconium in ores and alloys (*1*, *3*).

EXPERIMENTAL

A solution of zirconyl chloride was prepared and standardized by precipitation with both ammonium hydroxide and cupferron. Aliquot portions of this standard solution were used for analyses.

The zirconium was determined with mandelic acid, using the procedure of Kumins (*2*) and by precipitation with ammonium phosphate from a 3.6 N sulfuric acid solution, using the procedure of Hillebrand and Lundell (*1*). (Kumins did not specify exactly the concentration of hydrochloric acid to be used. This is not critical, for quantitative precipitations were obtained in samples ranging from 0.1 to 8 M in hydrochloric acid. All determinations were made from 2 M hydrochloric acid solutions in this investigation.) The results given in Table I are averages of several closely agreeing determinations.

Table I. Determination of Zirconium in Pure Solutions

Mandelic Acid Method			Phosphate Method		
ZrO_2 taken, g.	ZrO_2 found, g.	Error, g.	ZrO_2 taken, g.	ZrO_2 found, g.	Error, g.
0.015[illegible]	0.0154	+0.0001	0.0153	0.0155	+0.0002
0.0077	0.0077	0.0	0.0077	0.0076	−0.0001
0.0031	0.0030	−0.0001	0.0031	0.0031	0.0
0.0015	0.0016	+0.0001	0.0015	0.0016	+0.0001
0.0008	0.0007	−0.0001	0.0008	0.0009	+0.0001
0.00015	0.0002	+0.00005	0.00015	0.00015	0.0

Table II. Determination of Zirconium in Presence of Aluminum, Iron, and Titanium[a]

Mandelic Acid Method			Phosphate Method[b]		
ZrO_2 taken, g.	ZrO_2 found, g.	Error, g.	ZrO_2 taken, g.	ZrO_2 found, g.	Error, g.
0.0153	0.0156	+0.0003	0.0153	0.0162	+0.0000
0.0077	0.0078	+0.0001	0.0077	0.0083	+0.0006
0.0031	0.0030	−0.0001	0.0031	0.0032[c]	+0.0001
0.0015	0.0015	0.0	0.0015	No precipitate obtained	
0.0008	0.0008	0.0	0.0008	No precipitate obtained	
0.00015	0.0002	+0.00005	0.00015	No precipitate obtained	

[a] 0.100 gram Al^{+++} + 0.100 gram Fe^{+++} + 0.025 gram Ti^{++++} added to each sample as chloride.
[b] 10 ml. of 3% H_2O_2 added to these samples to form pertitanic acid.
[c] Precipitation complete only when sample was allowed to stand 3 days after addition of ammonium phosphate.

These data show that the mandelic acid method is as accurate as the phosphate method in determining small amounts of zirconium. A complete determination of zirconium may be made within an hour, using mandelic acid, whereas a much longer period is needed for the phosphate method.

The two methods were also compared by determining zirconium in solutions containing aluminum, ferric, and titanium ions. These ions were used as impurities because they are the ones commonly associated with zirconium.

Table II shows that accurate determinations can be made with mandelic acid in the presence of large amounts of aluminum, iron, and titanium.

The phosphate method, however, gave rather erratic results in the presence of these impurities. When less than 3 mg. of zirconium oxide was present, no precipitate was obtained even upon the addition of 20 grams of ammonium phosphate. Obviously, the mandelic method is the superior method.

Kumins gave no data concerning separations from cobalt, magnesium, manganese, mercury, nickel, uranium, and zinc. Separations from these elements were studied and the results are shown in Table III.

These data show that successful separations can be made from cobalt, magnesium, manganese, mercury, nickel, uranium, and zinc. The precipitates formed in the presence of cobalt and manganese were discolored slightly, showing some contamination.

Solutions of hafnium chloride, when treated with mandelic acid, gave a precipitate of the same nature as those of zirconium mandelate. These precipitates were ignited and weighed, and compared with those obtained by precipitation with ammonium hydroxide:

Weight of HfO_2 by precipitation with ammonium hydroxide (average), 0.0410 gram

Weight of HfO_2 by precipitation with mandelic acid (average), 0.0407 gram

Table III. Effect of Impurities

Impurity (0.250 G.)	ZrO_2 Taken, G.	ZrO_2 Found, G.	Error, G.
Co^{++} as $CoCl_2$	0.0766	0.0771	+0.0005
	0.0153	0.0156	+0.0003
Mg^{++} as $MgCl_2$	0.0766	0.0768	+0.0002
	0.0153	0.0156	+0.0003
Mn^{++} as $MnCl_2$	0.0766	0.0772	+0.0006
	0.0153	0.0157	+0.0004
Hg^{++} as $HgCl_2$	0.0766	0.0768	+0.0002
	0.0153	0.0154	+0.0001
Ni^{++} as $NiCl_2$	0.0766	0.0768	+0.0002
	0.0153	0.0155	+0.0002
UO_2^{++} as $UO_2(NO_3)_2$	0.0766	0.0769	+0.0003
	0.0153	0.0156	+0.0003
Zn^{++} as $ZnCl_2$	0.0766	0.0768	+0.0002
	0.0153	0.0156	+0.0003

Mandelic acid, like all other reagents that precipitate zirconium, also precipitates hafnium quantitatively. Hence, in using mandelic acid for the determination of zirconium, one actually determines zirconium plus hafnium.

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Received February 23, 1949.

2-Anthraquinone Sulfonate Derivatives of Morphine and Codeine

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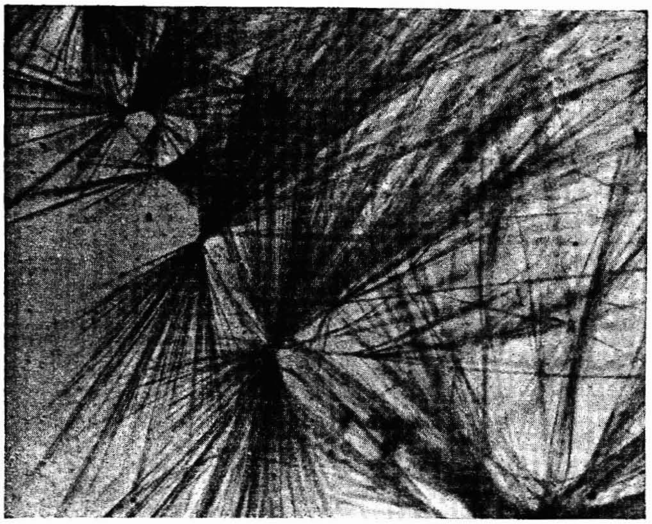

Figure 1. Morphine 2-Anthraquinone Sulfonate (×130)

IN TOXICOLOGICAL analyses of blood or tissue specimens it is often necessary to confirm the presence of small amounts of morphine or codeine. The alkaloids are usually isolated through one of the modifications of the Stass-Otto process (*6*) and the purified residue obtained after evaporation of the ether or chloroform extract is examined for the presence of basic substances.

Among the many color reactions which will detect the presence of morphine and codeine are Marquis' (*4*), Mecke's (*5*), and Froehde's (*1*) reagents. However, these color reactions do not differentiate between morphine or codeine as isolated from tissue specimens. There are also several crystal reagents, such as Kraut's (*2*), Wagner's (*7*). and Marme's (*3*), which form characteristic crystals with many of the organic bases, but actual distinction between morphine and codeine is difficult.

The authors find that a solution of sodium 2-anthraquinonesulfonate forms characteristic crystals with morphine and codeine, and that the melting points of these derivatives are sufficiently far apart to afford a means of specific identification for either.

Morphine and codeine are the only alkaloids of 19 tested which give a crystalline precipitate under the conditions outlined. Cocaine, pontocaine, procaine, metycaine, atropine, homatropine, aconitine, scopolamine, strychnine, brucine, caffeine, narceine, dilaudid, quinine, ergamine, pseudopelletierine, and nicotine do

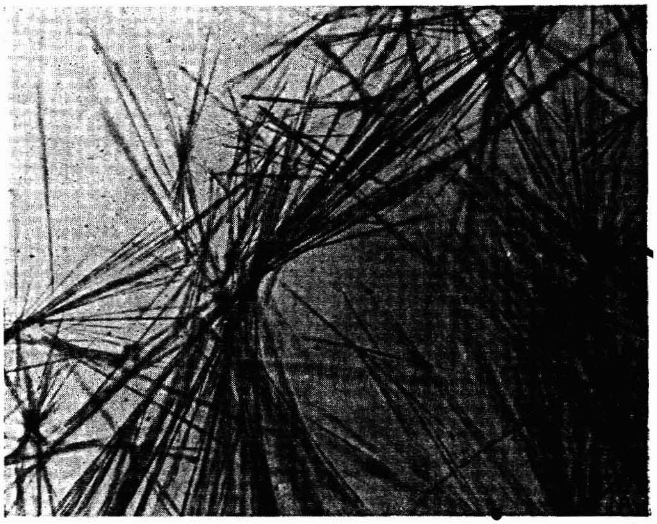

Figure 2. Codeine 2-Anthraquinone Sulfonate (×130)

not form insoluble 2-anthraquinone sulfonate salts in 6 *N* sulfuric acid at room temperature.

To make the reagent, 1 gram of sodium 2-anthraquinone sulfonate is added to 20 ml. of water containing 2 ml. of 3 *N* hydrochloric acid. The mixture is stirred for a few minutes, filtered, and stored in a brown bottle. Reagents are added to the preparations with a dropping pipet with the tip drawn out to a capillary 1 mm. in diameter. About 1 to 2 mg. of the dry purified residue obtained from the extract of tissue are placed on a slide and dissolved in one drop of 6 *N* sulfuric acid. One drop of the reagent is added directly to the drop on the slide, and the preparation is allowed to stand for 10 to 15 minutes for crystallization to occur. The crystals formed with morphine and codeine isolated from tissue samples are shown in Figures 1 and 2.

Excess solution is decanted from the preparation by means of a capillary pipet of 0.5-mm. bore and about 6 cm. in length. The pipet is placed vertically in the drop, and then slowly inclined until it is almost horizontal. Liquid rises in the tube by capillary action. The tube is then carefully removed without disturbing the crystals and a drop of water is added to the residue. The decantation and washing are repeated once more. The slide is dried in an oven at 100° C. and the melting point determined under the microscope.

The morphine derivative melts at 198° to 199° C., and its solubility in water at 20° C. is 0.85 mg. per ml. The codeine derivative melts at 175° to 176° C. and its solubility in water at 20° C. is 0.87 mg. per ml. Both derivatives are deep yellow in color when prepared in large amounts but appear colorless in minute amounts on a slide.

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Apparatus for Fractional Crystallization in Vacuum

HAROLD A. SCHERAGA[1] AND MILTON MANES[2], *Duke University, Durham, N. C.*

SEVERAL different designs have been reported for accomplishing the purification of liquids by fractional crystallization (*1-4*). However, they did not contain all the features that were desired for the purification of benzalchloride which was to be used for kinetic studies (*5*). In particular, an apparatus was desired in which all operations could be carried out in vacuum with the liquid being stirred during cooling. In addition, it was desirable to be able to determine cooling curves and pour off the rejected and accepted fractions without breaking the vacuum. The apparatus described below accomplishes this purpose without contamination of the liquid by the lubricant on the joints, and may be used for liquids that freeze within a very wide range of temperatures.

Two aspects of the crystallization apparatus are shown in Figures 1 and 2.

Figure 1 is a profile of the entire apparatus; Figure 2 represents a cross section of part of the apparatus, cut by a plane which is perpendicular to the plane of Figure 1 and passes through the inlet tube, sealed to the vessel at *A*. *A* is a point common to both drawings. The tubular vessel, in which the liquid was frozen, was constructed from 45-mm. outside diameter tubing and had a capacity of approximately 130 ml. Graduations on this vessel served to indicate the volume of liquid after successive pourings of unfrozen material. The vessel was charged through an inlet tube, *A* (Figure 2), which was inclined about 20° upward. The inlet tube was then closed with a cap made from a ground-glass joint, *J*, to which a stopcock, *K*, was sealed. The system was then immediately evacuated, with stirring, through stopcock *B* (Figure 1) to outgas the liquid at a pressure of 1 mm. of mercury. The stirrer was made from glass rod and had a glass-enclosed iron rod sealed to its top for magnetic operation. If it were desired to open the system without atmospheric contamination—e.g., to change receivers—a positive pressure of nitrogen could be maintained by admitting the gas through *L* and releasing it through *C* or *D*.

Cooling curves could be determined by means of a thermocouple inserted in the thermocouple well which contained a small pool of mercury at the bottom for good thermal contact—for example, with a three-junction copper-constantan thermocouple, used at about −20° C. in conjunction with a Leeds & Northrup type K potentiometer and a type R galvanometer, temperatures could be read to 0.01°, using suitable cooling baths for the crystallization.

Pouring off of unfrozen material as a "rejected" fraction was accomplished by rotating the vessel on joint *E*, a sintered-glass disk serving to catch any crystals that might be carried through with the liquid. All rejected fractions were poured through outlet *D* on the cow to a receiver. The raised ring-sealed tube, *H*, prevented any drippings on the bottom of the cow from running into the "accepted" receiver. When the accepted fraction was ready to be poured to the accepted receiver, the crystals were melted and the cow was rotated on joint *G* to bring outlet *C* into place. In many cases, the sintered-glass disk may not be necessary, as the crystals tend to remain behind during the pouring operation. In such cases, the absence of a disk facilitates pouring. A bulge in the back of the vessel near the top of the thermocouple well, shown in Figure 2 and indicated by the dotted oval in Figure 1, altered the geometry of the apparatus in a manner which aided the pouring operation.

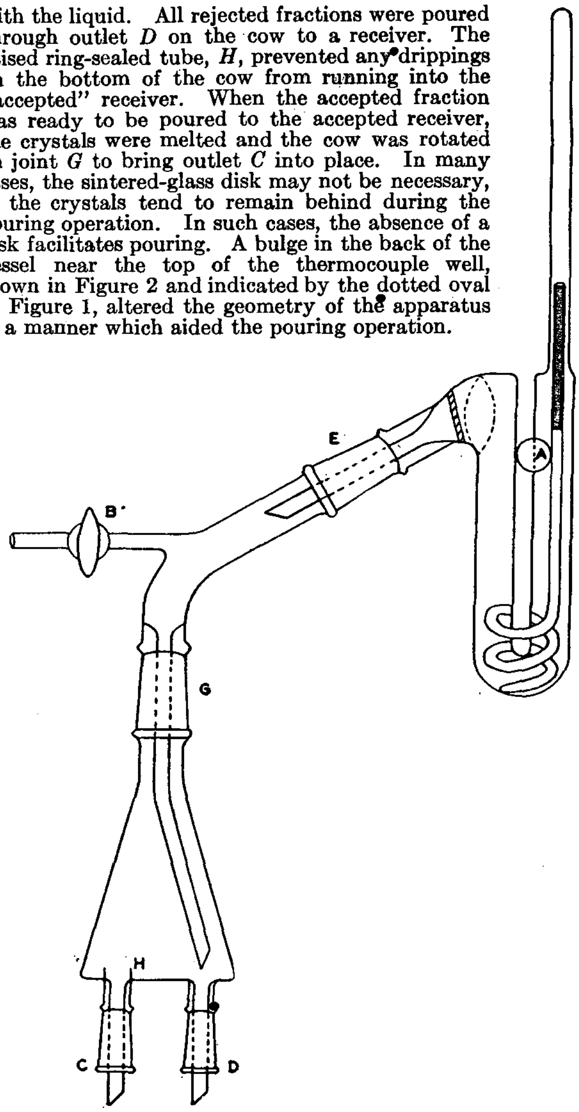

Figure 1. Crystallization Apparatus

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If it is desired to outgas the purified material at very low pressures in a system free of lubricant, the cow can be connected to an all-glass high-vacuum system at joint *C* and the liquid poured directly into a suitable container for subsequent distillation in a high vacuum. Before such distillation is performed, the liquid can be frozen in liquid air and the cow sealed off below *C* to eliminate the lubricant from the high vacuum system.

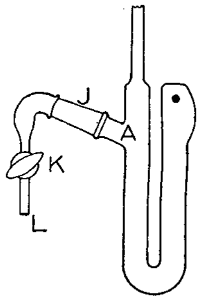

Figure 2. Tubular Vessel, Showing Inlet Tube

The application of this apparatus to the purification of benzalchloride has been discussed (*5*).

ACKNOWLEDGMENT

The authors would like to express their appreciation to D. E. Sampson, glassblower at the University of North Carolina, who made the apparatus, and to Marcus E. Hobbs and Douglas G. Hill for their advice and interest.

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Systematic Qualitative Tests for Certain Acidic Elements in Organic Compounds

Elimination of Interference by Cyanide

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A SYSTEM for the detection of nitrogen, chlorine, bromine, iodine, arsenic, sulfur, and phosphorus in a single 1-mg. sample of an organic compound (*1*) was based upon pyrolysis of the sample in the presence of zinc and calcium oxide (*3*), detection of the evolved ammonia in the event that nitrogen were present, followed by subsequent tests for the other elements using the pyrolysis residue. For the detection of halides a portion of the residue was extracted with water and the aqueous extract was tested for halide with silver nitrate, for bromide or iodide with fluorescein–chloramine-T, and for iodide with starch-nitrite.

During the past two years students have occasionally reported the presence of halide in nitrogenous compounds containing no halogen. This spurious test has its origin in the fact that some nitrogenous compounds when pyrolyzed with zinc and calcium oxide will occasionally form cyanide as well as ammonia and the student observing the formation of a precipitate of silver cyanide will report the presence of halogen. Although it is unlikely that an experienced observer would be misled, the fact that cyanide ion may also prevent the formation of eosin or tetraiodoeosin, as well as the starch-iodine color, thus offering the possibility that bromine and iodine may be reported absent when actually present, suggested the desirability of modifying the system to avoid all possible difficulties.

The interference by cyanide has been provided for in the modified tests described below by the addition to the systematic scheme of a test for cyanide (*2*) in case both nitrogen and halide have been *found present,* and a procedure for the *removal* of cyanide if present.

ADDENDUM TO SYSTEMATIC TESTS (*1*)

B-1. Tests for Halogen. In a 1- to 1.5-mm. thin-walled capillary a 4- to 5-mm. column of the aqueous extract of the pyrolysis residue is allowed to react with a 3- to 4-mm. column of a solution 0.5 *F* in silver nitrate and 3 *F* in nitric acid. The formation of a white or yellow precipitate within 30 seconds indicates the presence of cyanide, chloride, bromide, or iodide.

If nitrogen has been found to be absent (A), the test for bromide or iodide (B-2) and the test for iodide (B-3) are performed. If nitrogen has been found to be present the remainder of the aqueous extract is transferred with a capillary pipet to a 2-ml. beaker, 1 drop of a solution 0.1 *F* in sodium acetate and 0.1 *F* in acetic acid is added, and the beaker is covered with a circle of filter paper impregnated with 1 drop of a reagent freshly prepared by mixing equal volumes of 0.015 *F* aqueous cupric acetate and one half saturated aqueous benzidine acetate (*2*). The appearance of a blue spot on the paper within a few seconds indicates the presence of a cyanide. If cyanide is present, the filter paper is removed and the mixture is heated gently on a hot plate until the test for cyanide with a fresh circle of filter paper, impregnated with the cupric acetate–benzidine acetate reagent, is negative. Then the test is repeated for halide (B-1) and if positive (chlorine, bromine, or iodine present) tests B-2 (for bromide or iodide) and B-3 (for iodide) are performed.

RESULTS OBTAINED WITH MODIFIED SYSTEMATIC SCHEME

Cyanide can be detected without difficulty with the cupric acetate–benzidine acetate reagent when as little as 1 microgram of cyanide is present in the aqueous extract of the pyrolysis residue. A more sensitive test is not required; if less than 1 microgram of cyanide is present in the aqueous extract no significant interference by cyanide is observed. If cyanide is present in the aqueous extract of the pyrolysis residue, even in amounts as great as 100 to 200 micrograms, it can be removed by the recommended procedure to the point where no precipitate is obtained in the test for halide (B-1) if halogens are absent and where bromide and iodide in amounts as low as 5 to 10 micrograms can be detected without difficulty. To date there has been no indication that the amount of cyanide that may be formed during a pyrolysis can cause any difficulty in the tests for sulfur, arsenic, and phosphorus (*1*).

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Received December 27, 1948. Contribution 1260, Gates and Crellin Laboratories of Chemistry, California Institute of Technology.

Correspondence

Determination of Saponification Number

Sir: In the article on "Determination of Saponification Number" [Englis, D. T., and Reinschreiber, J. E., Anal. Chem., **21**, 602 (1949)], the curves for Figures 1 and 2 were transposed. The figure shown as 2 should appear over the title for Figure 1 and vice versa. This fact is readily evident from the descriptive matter on the graphs.

A statement in the sentence starting at the bottom of page 604 requires correction. It should read: "When the water content

Table I. Supplementary Determinations

Weight of Sample, Grams	Approximate % of Ethanol in Final Mixture	Saponification No.	Average
	Edible Oil A		
4.9146	19	188.4	188.6
4.9019	19	188.6	
5.4181	19	188.8	
5.3021	67	191.7	192.0
4.9110	65	192.6	
5.1188	67	191.7	
	Edible Oil B		
5.1381	19	190.4	191.1
5.2870	19	192.6	
5.0852	19	191.8	
4.7905	19	190.8	
5.1925	19	190.6	
5.1029	66	194.6	194.8
5.1663	67	194.8	
5.4418	68	194.8	
4.8367	66	194.6	
5.2580	69	195.2	

is high and an increased hydrolysis of the soap takes place, one would anticipate higher values for the back-titration of the excess alkali with correspondingly lower saponification numbers." Hence, the results for samples 22 and 23 are anomalous and appear to be in error. Supplementary determinations under similar solvent conditions have been made, in which all samples were saponified under reflux condensers. Some of the samples were then diluted with water to reduce the ethanol content to the indicated values before titration of the excess alkali. These solutions were consequently titrated at a higher dilution than in the previous experiments, in which additional heating had been employed to drive off the ethanol. As a result, in the earlier experiments the period of saponification had been prolonged beyond a 30-minute period in these cases. However, in none of the experiments was there evidence of incompletely saponified oil. The results of the supplementary determinations are given in Table I.

Under these conditions, with the final ethanol content about 19%, the saponification numbers have an average more than three units lower than when the ethanol content is about 65%—a value characteristic of usual correct operating conditions.

A further examination of the conditions which gave the high results for the original samples 22 and 23 will be made to establish whether they have resulted from other cause than random error.

The data reported in Table I of the original article were subjected to a statistical analysis in that a *t* test (Snedecor, G. W., "Statistical Methods," 4th ed., p. 75, Ames, Iowa, Iowa State College Press, 1948) was calculated for the two groups representing, respectively, 67 to 72% and 34 to 64% of ethanol in the final solution. The weights of oil were chosen at random in the two groups, so as to eliminate a variance from that source. The group method of calculation was used. The *t* value calculated was 0.73. The *t* values necessary for 1% and 5% levels of significance are 3.25 and 2.26, respectively. Thus, there is no significant difference between the two groups. This gives confirmation to the conclusion that the ethanol content can be varied from 34 to 72%.

University of Illinois
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D. T. Englis
James E. Reinschreiber
Louis A. Wollerman

CRYSTALLOGRAPHIC DATA

Contributed by Armour Research Foundation of Illinois Institute of Technology

25. 2,4,6-Trinitrotoluene (TNT)

TNT crystallizes from a variety of organic solvents to give well formed rods, tablets, and plates. The crystals from ethanol are elongated parallel to the *c* axis but other solvents—e.g., acetone, ether—often give crystals elongated parallel to the *b* axis.

There is no evidence of polymorphism for TNT.

Crystal Morphology (determined by W. C. McCrone).

Crystal System. Orthorhombic.

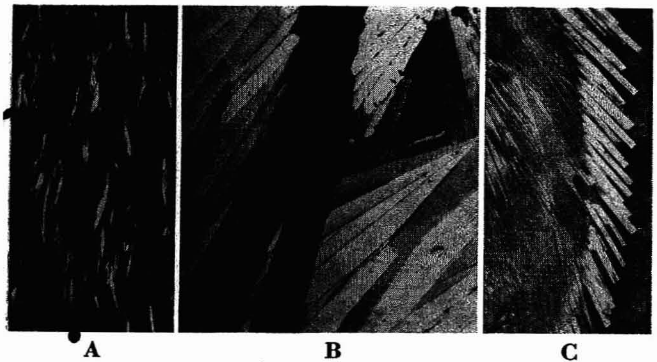

Figure 1. Trinitrotoluene Crystals

A = Grown from melt at room temperature; B = grown from melt at about 70° C.; C = grown from a thymol mixed fusion

Form and Habit. Usually elongated parallel to *a* or *c* depending on the solvent; acetone or ether (*b*), alcohol or melt (*c*). Shows the forms: brachy pinacoid {010}, prism {110}, and macrodome {061}.

Axial Ratio. $a:b:c$ = 0.375:1:0.153; 0.3793:1:0.1493 (*1*); 0.376:1:0.151 (*3*).

Interfacial Angles (Polar). $061 \wedge 0\bar{6}1 = 93°46$; $110 \wedge \bar{1}10 = 138°\,40$.

X-Ray Diffraction Data (determined by W. C. McCrone and A. Humphries).

Cell Dimensions. a = 14.99 Å., b = 40.0 Å., c = 6.10 Å.; a = 14.85 Å., b = 39.5 Å., c = 5.96 Å. (*3*).

Formula Weights per Cell. 16.

Formula Weights. 227.13.

Density. 1.654 (flotation).

Principal Lines

d	I/I_1	d	I/I_1
6.990	0.42	3.049	0.24
6.655	...	2.991	0.18
5.983	0.03	2.915	0.07
5.587	0.46	2.867	0.08
5.404	0.13	2.781	...
5.043	0.07	2.721	0.12
4.967	0.10	2.668	0.10
4.711	...	2.589	0.06
4.577	0.05	2.539	...
4.406	...	2.430	0.02
4.270	0.27	2.356	0.10
4.141	...	2.293	0.07
3.989	0.05	2.235	...
3.844	1.00	2.171	...
3.745	0.10	2.132	0.08
3.678		2.058	...
3.497	0.11	2.027	0.03
3.423		1.994	0.03
3.330	...	1.964	...
3.255	0.07	1.921	0.03
3.143	0.04	1.869	0.04

Optical Properties (determined by W. C. McCrone).

Refractive Indexes (5893 Å.; 25° C.).
$\alpha = 1.543 \pm 0.002$
$\beta = 1.674 \pm 0.002$
$\gamma = 1.717 \pm 0.004$.
Optic Axial Angles (5893 Å.; 25° C.).
$2V = 60°$.
$2E = 114°$.
Dispersion. $v > r$.
Optic Axial Plane. 001.
Sign of Double Refraction. Negative.
Acute Bisectrix. b.
Molecular Refraction (R) (5893 Å.; 25° C.).
$\sqrt[3]{\alpha\beta\gamma} = 1.641$.
R(calcd.) = 44.3.
R(obsd.) = 49.6.

Fusion Data (determined by W. C. McCrone).

Trinitrotoluene melts at 81° C. with neither sublimation nor decomposition. The melt supercools readily but usually crystallizes spontaneously within a few minutes. Crystal growth at room temperature is very rapid and gives fine curved rods and needles (Figure 1). At temperatures just below the melting point large broad rods are formed; these grow with a characteristic jagged crystal front. The direction of most rapid growth is parallel to c with b, and therefore BX_a, vertical. A mixed fusion with thymol (Figure 1) shows separate rods with 90° and 67° profile angles.

Trinitrotoluene crystallized from the melt and reheated to a temperature just below the melting point will show a characteristic secondary crystallization called "boundary migration" (*4*).

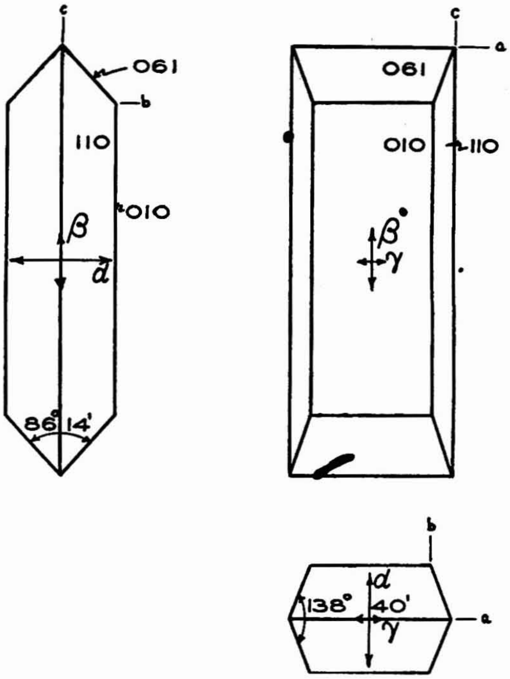

Figure 2. Orthographic Projection of Typical Crystal of Trinitrotoluene

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SCIENTIFIC COMMUNICATIONS

Determination of Vitamin A in the Unsaponifiable Fraction of Fish Liver Oils

At the American Chemical Society meeting held in Portland, Ore., September 13 to 17, 1948, we reported on a simplified method for obtaining the unsaponifiable fraction of fish liver oils for the estimation of vitamin A. The manuscript was not submitted for publication because experimental data were destroyed by explosion and fire in our plant just prior to the meeting, and subsequent limitations in time and facilities have prevented repetition of the original experimental work.

During recent months, there has been increasing interest in the use of the unsaponifiable fraction in the physicochemical estimation of vitamin A. In view of this, a number of workers who have investigated our method have urged that the procedure be published, even though supporting data are not available.

Weigh 0.1 to 0.25 gram of oil, depending on potency, into a 25 × 150-mm. low actinic test tube fitted with a 19/38 female standard-taper joint. Add 0.6 ml. of 50% potassium hydroxide solution and 6 ml. of alcohol. Place on a steam bath and reflux under an air condenser for 15 minutes or until the oil is completely dissolved. Then remove the condenser, and evaporate the alcohol under vacuum with nitrogen. As soon as this solvent is removed, add about 20 ml. of 1.5% barium chloride solution saturated with chloroform. Allow this mixture to cool, and pipet in exactly 20 ml. of water-washed chloroform. Shake this mixture thoroughly, and centrifuge it until the chloroform layer is clear or nearly so. The barium soaps will form a layer at the interface. Pipet 10 ml. of the chloroform solution into an amber or red volumetric flask. If vitamin A is to be determined by ultraviolet absorption, add 0.3 ml. of isopropyl alcohol and evaporate the mixture in the volumetric flask to dryness. The volumetric flask in which evaporation takes place can be held partly immersed at an angle in water at about 60° to 65° C. and vacuum applied, under a stream of nitrogen, slowly at first, with an aspirator. Take care not to overheat the residue, and as soon as the last of the solvent has been removed, fill the flask almost to its mark with isopropyl alcohol and mix it thoroughly. Allow the flask to come to room temperature, and then bring up to volume with isopropyl alcohol. If, after standing for an additional period of 1 to 2 minutes, the solution is cloudy, centrifuge it. The solution can then be used for further dilutions as may be required.

If the Carr-Price reagent is to be used, the chloroform aliquot may be dried by the addition of a few grains of sodium sulfate and then used directly.

It is generally believed that, when oils are saponified, the potassium soaps formed group themselves in micelles which have the property of absorbing vitamin A. The fact that it usually takes three or more washes with ethyl ether to extract the vitamin is evidence that vitamin A is difficult to remove from these micelles. In fact, even with numerous washings with ethyl ether vitamin A is often not removed quantitatively from the saponification mixture. Precipitating the soaps as barium salts breaks up the absorbing micelles and releases the vitamin A, thus eliminating the necessity for multiple extractions.

The barium salts formed dissolve slightly in the chloroform, and the solubility varies with the types of oils tested. These soaps are nearly insoluble in isopropyl alcohol, precipitate when the chloroform is evaporated, and for the most part do not redissolve in isopropyl alcohol. With some samples, the salts may form a cloud when the isopropyl alcohol is added, but usually the cloud can be settled by centrifugation. If an adequate centrifuge is not available, the solution can be filtered through a sintered-glass filter under a slight pressure of nitrogen.

In comparing the method with the currently used and mor laborious methods, slightly greater recovery and better precisio were obtained. The greater recovery does not appear to be du to failure to remove extraneous absorbing materials, as th absorption curves in the range 300 to 350 mμ were essentiall identical.

DIRK VERHAGE
ROBERT W. PAREN

Lyle Branchflower Co.
Seattle, Wash.

RECEIVED October 13, 1949.

Meeting of the Optical Society of America

THE following abstracts of papers given at the Optical Society of America's thirty-fourth annual meeting at Buffalo, N. Y., October 27 to 29, 1949, are of interest to analytical chemists.

Infrared Microspectroscopy. ELKAN R. BLOUT, GEORGE R. BIRD, AND DAVID S. GREY, Research Laboratory, Polaroid Corp.

The determination of the infrared spectra of much smaller amounts of materials than have been used hitherto has been made possible by the design and construction of microscope objectives achromatized over large portions of the spectrum. A simple infrared microspectrometer now in use in this laboratory makes use of spherical reflecting microscope objectives and a Perkin-Elmer infrared spectrometer. The objectives have a numerical aperture of 0.40 and, because they contain no refractive elements, they may be used to wave lengths well beyond those possible of attainment with prism spectrometers. The magnification to the entrance slit of the spectrometer is approximately 22 ×. With this instrument the authors have been able to determine the spectra of some fibers, certain crystals, and tissue areas. Data were presented which indicate the resolution obtainable, the minimum sample dimensions required, and other prerequisites for satisfactory results. Comparison data were presented on materials of biological interest, such as thymine (a single crystal *vs.* an evaporated film), phenyl alanine (single crystal *vs.* mineral oil mull), nucleohistone (bundle of fibers *vs.* cast film), and tissue sections. The spectra and some preliminary polarization measurements of some natural and synthetic fibrous materials, such as ramie, cellulose acetate, and polyvinyl alcohol, were also shown.

Color Phase Contrast. Requirements and Applications. CHARLES PROFFER SAYLOR, National Bureau of Standards, ARTHUR T. BRICE, Phase Films, Ross, Calif., AND F. ZERNIKE, University of Groningen, Netherlands.

In color phase contrast, which was first discussed by Zernike (National Academy of Sciences, spring meeting, 1948), several difficult requirements must be met for the attainment of maximum brilliance and usefulness. The system itself is one in which a controlled chromatism of the phase plate is sought instead of the customary attempt at equality of phase difference at all wave lengths. The thickness and dispersion of the phase ring in relation to the general aperture can be such that zero phase contrast will occur at one or more wave lengths of transition in color sensation. If the phase contrast is made high but opposite in spectral zones of relatively pure color sensation, striking and beautiful microscopical images can be formed with objects of low inherent contrast. The greatest advantage of this system lies not in the beauty of the images but in the clear distinction which is afforded between effects caused by scattering or general absorption and those caused by small differences of refractive index or thickness.

An Extension of Toraldo's Theory of Phase Contrast. L. IVAN EPSTEIN, Bausch & Lomb Optical Co.

G. Toraldo di Francia has calculated the light intensity distribution in the phase contrast image of a transparent, uniformly phase-shifting semiinfinite plane on a transparent background. He supposes Köhler illumination parallel to the axis of the microscope, so that the illuminating light comes to a focus in a point at the center of the back focal plane of the objective. The phase pattern is a circular spot in the middle of the aperture. The author has extended this theory to the case of an annular phase pattern filled with light. The theory of this form of image formation is merely a special case of a more general theory due to Duffieux and his students.

Fluorophotometric Determination of Uranium. GEORGE F PRICE, RENATO J. FERRETTI, AND SAMUEL SCHWARTZ, Argonn National Laboratory.

A fluorophotometric method for uranium determination ha been developed that covers a range from 10^{-10} to 10^{-4} gram, wit a standard error of about 2×10^{-11} gram at the lower limit, an which is so remarkably free from interferences that purificatio of unknowns is almost never required even when impurity concer trations are thousands or tens of thousands of times greater tha the uranium concentration. This method is a refinement of th well-known sodium fluoride bead test for uranium. The extrem sensitivity results principally from use of special photoelectr fluorophotometers described in the following paper. The abilit to tolerate enormous excesses of impurities depends on the fin ings that no substance investigated gives interfering fluorescenc in the procedure employed, and that per cent quenching of ura nium fluorescence is independent of uranium concentration (e cept at concentrations so high that uranium quenches itself which means that quenching can be reduced by reducing samp size and can be measured and corrected for by adding know amounts of uranium to portions of the unknowns.

Sensitive Fluorophotometers. GEORGE R. PRICE, RENATO FERRETTI, AND SAMUEL SCHWARTZ, Argonne National Labora tory.

A series of special fluorophotometers was developed for the ura nium determination method described in the preceding pape Most of these employed the H4 mercury arc and the 1P21 mult plier phototube plus a d.c. galvanometer. The best ones ga linear response within 3% over a hundred thousandfo range of light intensity, and were sufficiently sensitive to dete fluorescence in every liquid and solid tested except powdere graphite.

Fluorescence Studies of Plutonium and Neptunium. GEORG R. PRICE, Argonne National Laboratory.

A number of compounds of plutonium and neptunium we tested for fluorescence under a variety of optical conditions at di ferent temperatures and both in the pure state and in liquid an solid solution. Special search was made for resonance fluorescen of the plutonyl ion corresponding to a particularly sharp absor tion band in the infrared. No fluorescence was found that a peared to be characteristic of the metal ions themselves.

The Permanence of Glass Standards Issued to Check the Ph tometric Scale of Spectrophotometers. KASSON S. GIBSON AN MARION A. BELKNAP, National Bureau of Standards.

Shortly after the introduction of commercially available phot electric spectrophotometers, the National Bureau of Standar began issuing calibrated glass disks for assistance in checking th photometric scale of spectrophotometers. Although no comple account of this work has been published, reference has been ma to these standards in various publications. Certain of the glasses—a carbon yellow, a cobalt blue, and a copper green—we carefully measured and values of spectral transmittance adopt in 1933. Since then they have been mostly kept in the dark in f ing envelopes, with only occasional use. Remeasurement of the glasses after 16 years shows no certain change in their spectr transmittance. In addition, exposure tests have been made similar glasses over the past years by exposing them to south sk light (including sunlight) under a clear plate glass cover. Sm but definite changes of spectral transmittance have been fou or the carbon yellow and copper green glasses during this exposure, but the changes for the cobalt blue glass border on the uncertainties of measurement. These results indicate that, except with the most careless usage and exposure, the glass standards of spectral transmittance issued by the bureau should remain unchanged for a long time.

An Electrical Tristimulus Integrator. E. W. Peterson, H. H. Grossman, J. L. Saunderson, and V. J. Caldecourt, The Dow Chemical Co.

An automatic integrator has been attached to a General Electric recording spectrophotometer to give the tristimulus values X, Y, and Z of the spectrophotometric curve being recorded. The instrument uses the weighted ordinate method of integration, with the ordinates spaced every 10 mμ from 400 to 700 mμ. At each ordinate, a condenser for each of the three tristimulus funcions is given a charge which is proportional to the product of the ransmission or reflection reading times the values of the quantiies $(E_c\bar{x})$, $(E_c\bar{y})$, and $(E_c\bar{z})$ at that ordinate. After completion of he spectrophotometric curve, the 31 condensers of each function are paralleled and the sum of charges is determined by measurement of the three voltages on the three gangs of condensers. The voltage measurements are made automatically during the time the pectrophotometer is returning from 700 to 400 mμ, and the reults are read from calibrated clocks. Preliminary tests indicate hat an absolute accuracy of ±0.1% in the tristimulus values X, Y, and Z has been achieved.

An Improved Gloss Recorder. Richard S. Hunter, Henry A. Gardner Laboratory, Inc.

The design and improvement of a continuous gloss recorder were described. This type of recorder is used in paper mills to ndicate the uniformity of surface polish imparted to coated paper. Paper of the type used in the *Journal of the Optical Society of Amerca* until recently and many other magazines is coated on both ides with a clay-adhesive mixture and then polished to give suraces on which halftone prints will be clearly rendered. Experince with existing gloss recorders in paper mills has shown that ome of the paper dust which is always present tends to accumuate on optical elements of the exposure head and causes the glossmeter to change calibration with time, even though blasts of comressed air and other measures to prevent this dust accumulation re taken. In a new gloss exposure head, there are test and refernce beams which pass together through the only optical elements xposed to paper dust. Any accumulation of dust on these glass windows therefore diminishes the two beams equally but does not lter the ratio of one to the other, which is measured as gloss. Use of the stable measuring method developed by Brice and phoocells paired for temperature response produces a gloss recorder hat is stable in calibration over periods as long as a week.

Spectral Reflectance Measurements in the Ultraviolet. Nison A. Finkelstein, Massachusetts Institute of Technology.

The high speed automatic recorded spectrophotometer decribed by Harrison has been modified by addition of an integratng sphere and a hydrogen discharge source yielding a continuous ltraviolet and visible spectrum of high intensity. This source, developed with the assistance of Wyckoff from the design decribed by Allen, has twice the intensity and emits radiation hroughout 64 times as great a solid angle as do commercially vailable sources of this type. With this instrument, spectrophoometric reflectance curves are obtained from 2400 to 6000 Å. in minutes, using a slit width corresponding to a wave-length interval of 20 Å. This equipment has been used to measure the specral reflectance of living human skin.

The Analytical Equation of the Emulsion Calibration Curve of Photographic Photometry. Milton Green and T. Schreiber, Signal Corps Engineering Laboratories.

By a careful study of its preliminary curve, an accurate expresion for the emulsion calibration curve has been obtained. It nvolves five constants which are characteristic of the emulsion. A power series expansion of gamma-log intensity in terms of denity will, for three terms (this corresponds to five constants), give a very close fit also. Two constants of the above equations are [illegible]slational and only affect the location of the curve relative to he coordinate axes. A third constant can be used as a sealing actor which sets the units of measure of one coordinate axis relaive to the other. Thus, there are two constants remaining which determine the shape of the curve. If these two constants were dentical for all emulsions, a single appropriate transmittance cale would suffice to linearize all emulsion calibration curves. Experiment shows them to vary somewhat. Hence, a single transmittance scale that will linearize some emulsion curves fairly acurately will linearize others only approximately.

The Effect of Gaseous Atmospheres on Excitation in Emission Spectroscopy. B. F. Scribner, National Bureau of Standards. (No abstract available.)

Emission Spectra of Technetium. William F. Meggers and Bourdon F. Scribner, National Bureau of Standards.

Three milligrams of highly purified $^{43}Tc^{99}$ were loaned by the United States Atomic Energy Commission. Solutions containing 50 to 100 micrograms of Tc were dried on copper electrodes and excited with low and high energy discharges from an ARL multisource unit. A stigmatic concave grating was employed to photograph the spectra from 2100 to 9000 Å. within which limits more than 2000 lines characteristic of Tc were recorded. Wave lengths were measured relative to iron standards, relative intensities were estimated on a scale of 1 to 1000, and almost every line was definitely assigned either to neutral Tc atoms or to singly charged Tc^+ ions. The ground state of Tc atoms is established as $(4d^5\ 5s^2) \times {}^6S_{2^1/_2}$. The lowest level of the $(4d^6\ 5s)\ {}^6D$ term is at 2572.9 cm.$^{-1}$ whereas the associated ^4D term begins at 10516.5 cm.$^{-1}$ Almost all the stronger Tc I lines are explained as combinations of these even terms with odd terms identified as $(4d^5\ 5s\ 4p)\ {}^6P^0$ and $(4d^6\ 4p)\ {}^4(PDF)^0$ or $(4d^6\ 4p)\ {}^6(PDF)^0$. Tested by the combination principle the average error in measured wave lengths of Tc lines appears to be less than ±0.02 Å. Prominent lines of the Tc I spectrum have wave lengths 4297.05, 4262.27, 4238.19, and 3636.10 Å.; outstanding lines of the Tc II spectrum have wave lengths 2543.24, 2610.00, and 2647.02 Å.

Extensions to the Analysis of the Infrared Spectra of the Noble Gases. Curtis J. Humphreys, National Bureau of Standards.

Although the first spectra of neon, argon, krypton and xenon are among the most completely analyzed atomic spectra, there have long been a small number of missing levels particularly among those arising from the s^2p^5f configuration. The most intense combinations of such levels are in the infrared region making observations between microns 1 and 2 necessary to establish all of them. New observations have been made with the recently completed high resolution grating spectrometer. These observations cover the same range as those of Sittner and Peck, but include neon, and yield wave lengths of 13 krypton lines not previously reported. All new lines and all intense lines not classified by Sittner and Peck are accounted for as combinations of new or known levels. In accordance with the *jl*-coupling scheme discussed by Racah, the levels of the noble gases are arranged in pairs. These are extremely close for the *f*-levels, and in some instances have never been resolved. The location of all pairs has now been established or confirmed by infrared combinations. On the basis of the predictions of Edlén the separation of several of the close pairs has been undertaken, and the necessary conditions for resolution discussed.

Use of Briquetted samples in the Spectrochemical Analysis of Carbon and Alloy Steels and Other Metals. J. F. Woodruff, Armco Steel Corp.

At the Armco Steel Corporation, over a period of several years, a number of spectrochemical methods have been developed which utilize briquetted powders, drillings, millings, nibblings, and grindings. The paper described briquetted sample techniques for the routine analysis of steel, zinc, and zinc base alloys. Low and plain carbon steels are analyzed quantitatively for such elements as tin, lead, manganese, nickel, chromium, molybdenum, cobalt, titanium, aluminum, zinc, and boron. Zinc and zinc-base alloys are analyzed quantitatively for cadmium, iron, lead, tin, antimony, and aluminum. Operational details, such as sample holders, spectrum lines utilized, source units and source-unit constants employed, and precision and accuracy data over a 5-year period were discussed.

An Introductory Study of the Spectral Characteristics of Ferrous Alloys. J. Convey and J. K. Hurwitz, Canadian Bureau of Mines.

The wave lengths of the spectral lines of the low alloy steel spectrum have been measured for purposes of identification in the region 2327 to 4384 Å. The description of the observed lines included the following information: wave length, atomic origin, designation of the transition, excitation potential, visual intensity, and possible interferences. For the spectrochemistry of nickel, chromium, manganese, silicon, and tungsten in low alloy and stainless steels, homologous line pairs were selected primarily on the basis of similar excitation potentials and freedom from interference. To test the homologous nature of these line pairs, the spectra of low alloy and stainless steels were recorded photographically using a wide range of source conditions. An ARL multisource unit and a Baird Associates 3-meter grating spectrograph were used. Results indicate small lateral shifts in the working curves for large changes in the spark discharge. The high nickel and chromium content in stainless steel enhances the spectral lines of iron relative to those lines of the alloying elements with the exception of nickel. This phenomenon is being investigated more fully at the present time.

New Raman Spectrograph and Source. A. C. MENZIES, Hilger and Watts Ltd., London.

A two-prism spectrograph with novel features has been designed specially for the Raman effect, having two cameras, $F/5.7$ and $F/1.5$, mounted on the same arm, pivoted so that one takes the place of the other smoothly. Either rapid spectrograms or ones with greater dispersion are taken at will, and for research purposes the fast camera aids in selection of material or arrangement of illumination. Dispersions are 1 mm. for 18.8 and 63 Å., respectively, at 4358 Å. Alternatively, one position can be used for the camera chosen, and the other for a scanning device, so that change from photography to direct reading is simple. The source is a new arrangement in which no mirrors are incorporated, but consists of a light furnace depending upon the high reflectivity of materials such as magnesium oxide, so that lack of specular reflection is more than compensated by using some of the light more than once. With the combination, the complete Stokes spectrum of carbon tetrachloride can be seen with the naked eye, and all but the doublet in the anti-Stokes spectrum. Using 6 ml., the 991 cm.$^{-1}$ line of benzene is photographed in 0.5 second with the slow camera.

Infrared Spectrophotometer with Cathode-Ray Display. A. C. MENZIES, Hilger and Watts, Ltd., London.

An infrared spectrophotometer with cathode-ray presentation has been developed following the general principles of those made by Sutherland and by Thompson, but differing from theirs in details, particularly of electronic circuits. The detector is a thermocouple, having a time-constant of 15 milliseconds. The wavelength range covered is 1.5 to 25.7 μ, with 8 cams. Scanning time of the trace on the long-delay glow tube is variable, from 15 to 50 seconds in four steps. A small fast electrolytic recorder is incorporated, so that a note of the trace can be taken at will. Push-pull amplifiers are used: the preamplifier is compact and housed in the infrared spectrometer, and the valves used for it are of one type and not specially selected. The main amplifier is conventional, and output from it is d.c. restored to ground and the chopper component is filtered out. The remaining signal is d.c. amplified and applied to the vertical plates of the C.R.T. The instrument is suited for observations of transient phenomena, and for rapid analyses, selection, and control. Photographs of traces on the tube were shown, up to 23 μ.

The Carbon Arc as an Infrared Source. C. S. RUPERT AND JOHN STRONG, Johns Hopkins University.

The positive crater of the carbon arc is a highly satisfactory source for infrared spectroscopy. The brightness increase over the globar (approximately 7 × 6.5 μ and 5 × at 15 μ) permits a gain of between 2 and 3 in resolving power. Under correct operating conditions small changes in the arc current alter the size but not the temperature of the crater. The detector output is constant to 2% when the edges of the crater image are diaphragmed off at the entrance slit. Scattered light is intolerable in a single-prism monochromator, but negligible when a suitable double monochromator, such as a prism-grating combination, is used. The circular shape of the arc crater suggests the use of the image slicer designed to cut up a circular stellar image and put the light through a slit. The authors have constructed a three-division slicer. Second-hand low intensity motion picture projection arcs, generally available, are adaptable to spectroscopic use. The arc burns unattended for 15 to 30 minutes in an entirely satisfactory manner. This source should have particular value in increasing the signal to noise ratio for high resolution spectroscopy, infrared microscopy, and high speed scanning of the spectrum.

Spectroscopic Analysis of Segregates in Alloys. J. CONVEY, Canadian Bureau of Mines, Ottawa. (No abstract available.)

Comparative Results of Photographic and Direct Reading Methods of Analysis of High Alloy Steels. W. MAGRUN, M. WINDLE, AND J. H. JURMAIN, Baird Associates, Inc.

An evaluation was made of the three types of spectrographic equipment now commercially available—grating, prism, and direct reading—in their applicability to the analysis of a wide range of high alloy steels. The necessity and effectiveness of a method of compensation for the large variation of iron, the dilution method, were demonstrated. A comparison was made between results obtained both chemically and spectrographically.

A D.C. Arc Source and Automatic Controller. G. H. FETTERLEY AND W. M. HAZEL, Norton Company Research Laboratory.

An effective source of direct current having any desired output up to 45 amperes at 150 volts was described, being characterized by small size, light weight, low cost, and negligible maintenance. While it has superseded all other d.c. sources, regardless of cost, in the Norton Company laboratories, it is particularly recommended where cost is an important consideration. It is also a useful auxiliary unit where highly refractory materials are analyzed regularly, because these materials are sometimes difficult to volatilize completely with the familiar multipurpose type of source. An automatic current controller was also described as a separate unit, and can be used to hold the current within ±0.5 ampere at any selected value between 5 and 15 amperes.

New Spectrometer for Production Control Quantometer. M. F. HASLER, R. W. LINDHURST, J. W. KEMP, AND M. C. KRAUSHAAR, Applied Research Laboratories, Glendale, Calif.

A new vertical spectrometer has been developed which is particularly suited for routine direct-reading applications such as are encountered in production control work. Primary thermal and mechanical stability are achieved by using a heavy, aluminum-plate, box weldment as the basic mount for the various optical elements. All such elements, including the arc-spark stand, the fixed primary slit, the original diffraction grating, the receiver slits, the receiver condensing mirrors, and the multiplier phototubes are rigidly mounted to this weldment. This, in turn, is mounted on rubber shock absorbers inside a constant temperature sheet metal case. Constant temperature is achieved by running tap water through a system of tubing mounted in the walls of the case. Long-term variations in temperature are compensated by a monitor system which allows the operator very rapidly and accurately to position small 4-inch sections of the receiver slit system with respect to the spectrum. The net result is a very stable spectrometer which is independent of its surroundings both as to vibration and temperature. Details of design and construction were discussed.

Recent Advances in the Direct-Reading Analysis of High Percentage Alloys. M. F. HASLER, Applied Research Laboratories.

Three significant advances have been made in the past year in connection with the spectrochemical analysis of high percentage alloys. The first is the development of source conditions which, in combination with the latest direct-reading apparatus, allow a precision of analysis for high percentage constituents of from 0.3 to 0.4% of the quantity being measured, and this by means of a single determination. The second is the accomplishment of this high precision under excitation conditions which provide complete independence in the determination of one element from changes in the concentrations of the other elements. The third is the development of rigorous calculating methods which make possible the rapid analysis of all constituents, including those of high percentage, in any alloy system. The application of these advances to the analysis of stainless steels and tool steels was discussed in detail. The net result is the availability of high speed, direct-reading methods of analysis which can compete in accuracy, for all metallic constituents with routine chemical methods.

The Analyst's Calendar

Third Symposium on Analytical Chemistry. Louisiana State University, Baton Rouge, La., January 30 to February 2, 1950

Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. William Penn Hotel, Pittsburgh, Pa., February 15 to 17, 1950

Fifth Annual Microchemical Symposium. American Museum of Natural History, New York, N. Y., February 24 and 25, 1950

Chemical Institute of Canada, Analytical Division. Royal Connaught Hotel, Hamilton, Ontario, March 2 and 3, 1950. Second annual symposium

Third Annual Summer Symposium. Ohio State University, Columbus, Ohio, June 16 to 17, 1950

AIDS FOR THE ANALYST . . . . .

Portable Radioactivity Indicator for Low-Level Surveys. B. P. McKay and John L. Wood, University of Tennessee, Memphis, Tenn.

A SIMPLE monitor has been constructed for use of the chemist who is concerned with the qualitative detection of radioactivity. Useful in experiments carried out at the tracer level when it is known that no health hazard exists, the monitor emits loud clicks, and flashes a neon glow tube with impulses transmitted from a Geiger-Müller tube. When the tube is brought near a source of radiation, an increase in the rate of clicks is readily discernible at levels as low as twice the background. Contamination of beakers, spatulas, rubber gloves, etc., is instantly detected. The processes of decontamination may be monitored continuously until entirely effective.

The high voltage supply permits the use of commercially available Geiger-Müller tubes which operate between 800 and 2250 volts. Above 800 volts at the tube the alternating current meter reading is proportional to the high voltage direct current. Little lag due to thermal changes in the rectifier filament is noted above 800 volts at the tube. Figure 1 shows a 2 mg. per sq. cm. bell-type tube housed in a holder and attached to the instrument by a 4-foot cable.

The unit was constructed of components generally available at radio supply stores. The cost of the parts, less Geiger tube, amounted to approximately $40.

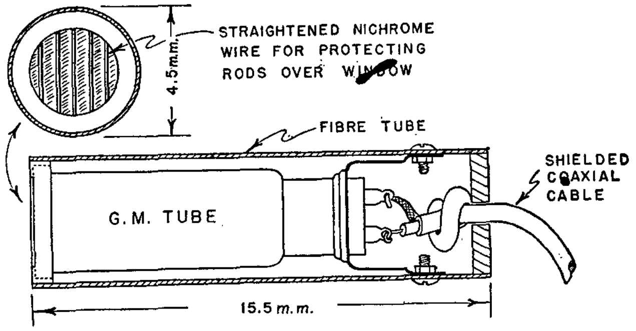

Figure 1. Bell-Type Tube

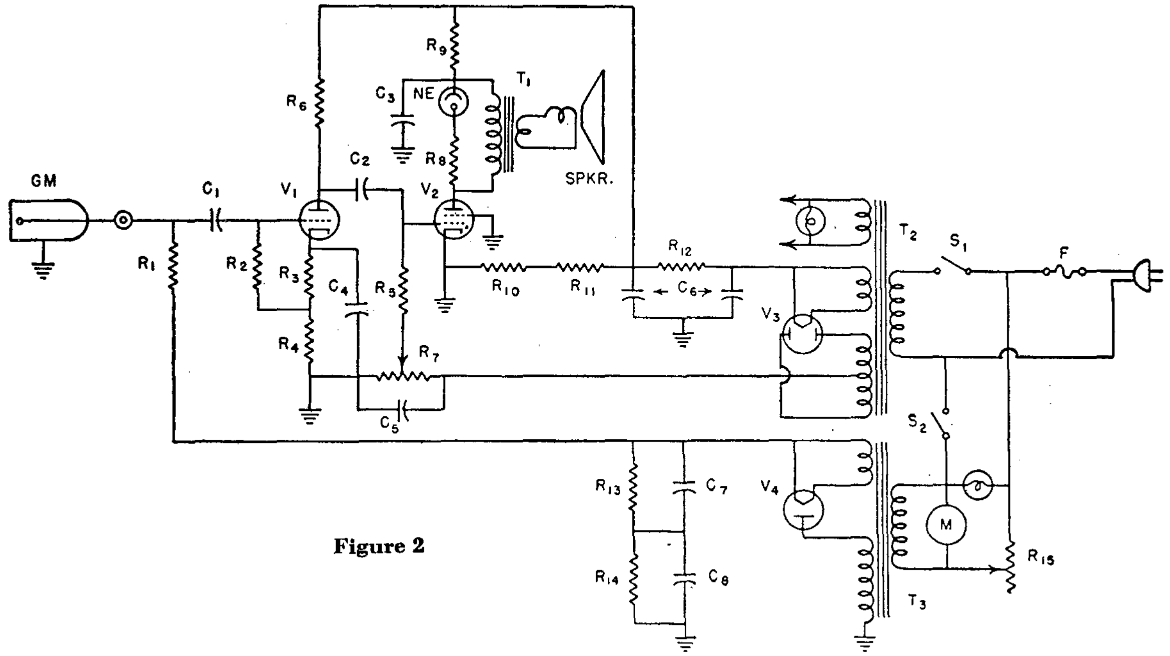

Figure 2

R_1.	20 megohms, 0.5 watt
R_2.	3.9 megohms, 0.5 watt
R_3.	220 ohms, 0.5 watt
R_4.	2,400 ohms, 0.5 watt
R_5.	220,000 ohms, 0.5 watt
R_6.	5000 ohms, 0.5 watt
R_7.	1000 ohms, wire wound
R_8.	1000 ohms, 1 watt
R_9.	180,000 ohms, 1 watt
R_{10}, R_{11}.	10,000 ohms, 10 watts
R_{12}.	2,000 ohms, 10 watts
R_{13}, R_{14}.	2 megohms, 2 watts
R_{15}.	1500 ohms, 25 watts (Ohmite No. 0159)
C_1.	50 mmfd., 2500 volts mica
C_2.	0.001 mfd., 600 volts
C_3.	0.05 mfd., 600 volts
C_4.	0.1 mfd., 400 volts
C_5.	25 mfd., 25 volts
C_6.	10 × 10 mfd., 450 volts
C_7, C_8.	2 mfd., 1500 volts
V_1.	1/2 of 6SN7 GT
V_2.	2050 Thyratron
V_3.	5Y3/GT
V_4.	2 × 2
NE.	NE 51 glow tube
T_1.	Thordarson No. T-14S82
T_2.	Thordarson No. T-22R00
T_3.	Thordarson No. T-22R44
S_1, S_2.	S.P.S.T. toggle
F.	1-amp. fuse
M.	0 to 150 volts a.c.
GM.	Any self-quenching Geiger-Müller tube having operating potential between 800 and 2250 volts d.c.

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