ANALYTICAL CHEMISTRY
Walter J. Murphy, Editor

The Summer Symposium

THE Third Annual Summer Symposium, cosponsored by the Division of Analytical Chemistry and ANALYTICAL CHEMISTRY, will be held June 16 and 17 at Ohio State University, Columbus, Ohio. The complete program was published in the March issue of ANALYTICAL CHEMISTRY and also appears in the May 15 issue of *Chemical and Engineering News*.

The keynote address will be given by M. G. Mellon of Purdue University. His subject, "The Role of Separations in Analytical Chemistry," will set the stage for a series of eleven papers arranged for by H. A. Laitinen of the University of Illinois, general chairman of the symposium. Ralph M. Evans, superintendent of the Color Control Department, Eastman Kodak Company, will be the dinner speaker, and Charles W. Foulk, professor emeritus of the department of chemistry of Ohio State, will be the toastmaster.

We have heard the view expressed that too many special meetings of analysts are held. We do not subscribe to this viewpoint, for each of the meetings held so far this year has been well attended and the reactions of those attending have been most favorable.

Special symposia such as those sponsored by Louisiana State University, the Pittsburgh Section of the AMERICAN CHEMICAL SOCIETY, and the Division of Analytical Chemistry and ANALYTICAL CHEMISTRY provide opportunities for programs usually unsuited for national meetings of the AMERICAN CHEMICAL SOCIETY. Developments in both the fundamental and applied aspects of analytical chemistry are coming forth in such an accelerated volume, that suitable outlet for the dissemination of such information requires more than that which is possible at national meetings of the A.C.S.

As long as the programs provided at special symposia are of the caliber noted this year, we see little likelihood of any diminution of interest. Attendance at these symposia is important, to keep abreast of the fast-moving developments in analytical chemistry.

Reagent Chemicals

WE were especially pleased at the action of the Council of the AMERICAN CHEMICAL SOCIETY taken in Philadelphia last month in declaring the latest edition of "Reagent Chemicals" as official A.C.S. specifications.

Edward Wichers, chairman of the Committee on Analytical Reagents, and his associates have labored long and arduously on the revision. We are happy to report that material is about to go to the printer.

We have prevailed on W. D. Collins, secretary of the committee, to prepare a brief history of the committee and its work which will appear in *Chemical and Engineering News* when the book is ready for sale. Analysts the world over owe a debt of gratitude to the Committee on Analytical Reagents for its fine work in digesting data, critically reviewing them, and assembling them in useful form. Such labor reflects great credit on the AMERICAN CHEMICAL SOCIETY.

Graduate Fellowships

JOHN T. Byrne of the Massachusetts Institute of Technology has been selected as the 1950–51 recipient of the Merck graduate fellowship in analytical chemistry, sponsored by Merck & Co., Inc., and administered by the AMERICAN CHEMICAL SOCIETY. This is the second very promising young analyst to benefit from the generous and forward-looking action of Merck & Co.

"One swallow does not make a summer." Other companies must recognize the desirability of supporting graduate study in the field of analytical chemistry if industry is to have available capable leaders in the future.

Review Reprints Available

REPRINTS of the Second Annual Review of Analytical Chemistry (January and February issues) are now available at $1.50 per copy. A limited number of reprints of the first review (published in 1949) are still available at $1.50. In order to assist those who would like to have both reviews, a special combination rate of $2.50 has been set for the 1949 and 1950 reprints. Orders with remittances should be sent to the Special Publications Department, AMERICAN CHEMICAL SOCIETY, 1155 Sixteenth St., N.W., Washington 6, D. C.

Theory, Scope, and Methods of Recrystallization

R. STUART TIPSON
Mellon Institute, Pittsburgh 13, Pa.

Properly applied to appropriate mixtures, recrystallization is often the best method for the separation and purification of organic compounds. The conditions under which a single compound may be isolated, or two compounds may be "completely" separated, are discussed; attention is drawn to mixtures inseparable by recrystallization and to those which yield only one pure component. The applicability of simple batchwise and of continuous countercurrent recrystallization is considered. Schemes for batchwise, countercurrent fractional recrystallization are outlined, and the distribution of the components in the phases after each recrystallization cycle is investigated.

IN BIOCHEMISTRY and synthetic organic chemistry there is probably no greater thrill than that derived when a substance which has stubbornly defied crystallization is obtained in crystalline form. Why is the chemist so pleased to get crystals? Only incidentally does he delight in their beauty. Primarily, he is gratified because a way is opened up, first, for purification of the material by recrystallization, and secondly, for recognition of its purity and chemical identity by virtue of the special physical properties exhibited by crystals—e.g., melting point, crystallographic characteristics, and x-ray diffraction.

The process of recrystallization is one of the oldest methods known for the separation and purification of organic compounds. When properly applied to appropriate mixtures it is often the best method for sorting out the molecules. However, at one time it fell into disrepute, when certain mixtures not amenable to this treatment were encountered. Thus, the fact that a product has been repeatedly recrystallized and has a sharp melting point, unchanged by further recrystallization, does not prove that it is pure; it may be a eutectic composition or a special type of solid solution.

GENERAL PRINCIPLES OF RECRYSTALLIZATION

In order that a mixture may be recrystallized, it is obviously essential that the desired components shall first have been obtained crystalline. It will be assumed that crystallization has been effected, yielding a batch of crystals of compound A contaminated with compound B, substances which do not form a compound or compounds with each other. The problem is either (1) to separate A from B, possibly obtaining both A and B in pure condition, or (2) to purify either A or B. The discussion is limited to a consideration of the behavior of supersaturated solutions, including supercooled melts.

Let us first consider the simplest case, in which the mixture can be melted without decomposition of A or B. When the melt is cooled, in the presence of nuclei of A and B, solid solutions of A and B may or may not be deposited.

Solid Solutions Not Formed. If solid solutions are not formed we have a simple eutectogenic system as shown in Figure 1. If A is present in excess (relative to the eutectic composition, *C*) it will crystallize first and continue to crystallize with slow cooling until the eutectic temperature *D* is reached, when the eutectic composition of A and B will crystallize. On the other hand, if B is present in excess of composition *C*, compound B will crystallize until the eutectic temperature is reached. In the absence of secondary effects due to adsorption and inclusion, these crystals of A or B will be practically pure. If they are filtered off just before the eutectic temperature *D* is reached and recrystallized a few times in the same way, they are usually obtained very pure. Exceptions to the above are encountered if nucleating crystals of one component are unobtainable or deliberately withheld, or if one component crystallizes much more slowly than the other, when it may be possible to pass the eutectic temperature without deposition of the mixture having the eutectic composition.

It is therefore evident that, if A and B give a simple eutectic, it is possible to obtain the component present in excess in pure condition. It is not possible to obtain both A and B pure from the recrystallization of a given mixture under equilibrium conditions In order to obtain the other component pure, it is necessary to apply some other technique which will separate the eutectic composition. If the starting material already had the eutectic composition *C*, no separation by recrystallization is possible, except by overshooting the eutectic point. Thus, this kind of system gives an all-or-none effect as regards purification of one component. In a sense, it resembles distillation of two immiscible liquids.

In recrystallization from the melt, a series of fractions of crystals is obtained by stepwise cooling. If of comparable purity, the fractions are combined and recrystallized; if not, each fraction is remelted and again fractionated by stepwise cooling. Thus, the steps in one recrystallization cycle are: (1) melt the mixture, (2) supercool the melt, (3) nucleate, if necessary, (4) encourage growth of the crystals, and (5) separate the crystals from the melt. (Another method which is applicable involves stepwise partial melting of the crystals.) In certain cases such fractionations of an impure melt are highly effective, as in the purification of mobile, chemically stable liquids such as benzene, *p*-xylene, benzoic acid, and acetanilide. However, as a rule, they are not very practical because of such factors as high viscosity (which may retard crystallization), chemical instability, and difficulty of removal of crystals if the melt is viscous.

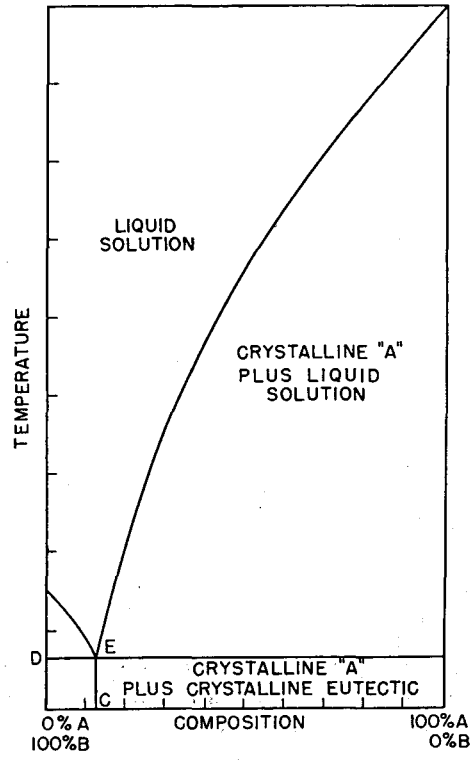


Figure 1. Relation between Composition and Freezing Temperature of Mixtures of Two Components Giving a Eutectic Only, Naphthalene-Benzene System (*13*)

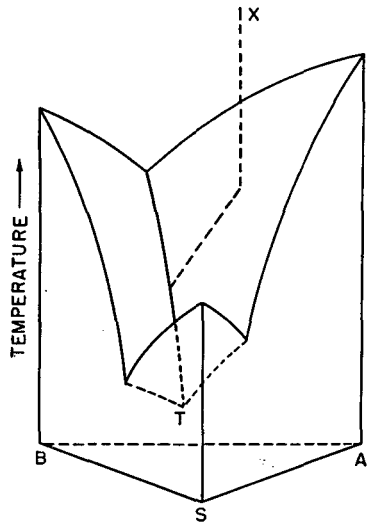


Figure 2. Relation between Composition and Freezing Temperature of Hypothetical Three-Component Solution Depositing No Solid Solutions

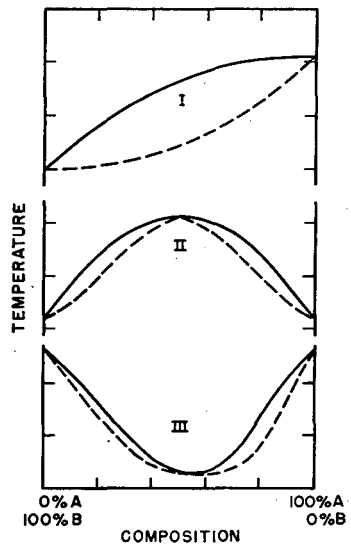


Figure 3. Freezing Points of Hypothetical Continuous Series of Solid Solutions

As a rule, it is preferable to recrystallize from a suitable solvent, *S*, which does not form solid solutions with the solutes (Figure 2). It must be borne in mind that, depending on the proportions of A, B, and *S* there is the possibility of deposition, on slow stepwise cooling—e.g., from *X*—of (1) any one component, if present in excess of its eutectic compositions; (2) any one binary eutectic composition, if present in excess of the ternary eutectic; and (3) the ternary eutectic, *T*. Apart from its polar effects, the solvent may be regarded as an inert medium which dilutes the system, lowering the viscosity so that crystallization may proceed at a reasonable rate, and providing a mother liquor in which the eutectic of A with B remains when the desired crystals are filtered off. One or two simple recrystallizations will usually suffice to give extremely pure crystals. In fact, in this event, the most complete purification possible by recrystallization then results.

The methods employed for the isolation of successive fractions of crystals are, of course, essentially those used for obtaining a supersaturated solution. Thus, by stepwise cooling to successively lower temperatures, a series of crops of crystals is obtained. It is sometimes possible to use differential crystallizability. Another method consists in stepwise introduction of a suitable third solute which may be a solid, a gas, or a poor solvent. Finally, controlled fractional evaporation may be employed. Combinations of these methods are often advisable.

Solid Solutions Formed. If A and B form solid solutions but no compound with each other, there are five possibilities. If A and B form a continuous series of solid solutions (see Figure 3) and the melting point of every possible composition lies between those of pure A and B (see curve I), the first crystals deposited are richer in one component than was the original melt. The melt naturally becomes correspondingly poorer in this component. Consequently, by repeated fractional recrystallization it is possible eventually to isolate pure A and pure B. This is the only two-component system which gives both components pure by fractional recrystallization of a given composition under equilibrium conditions. The diagram (I, Figure 3) resembles that for the fractional distillation of two liquids giving no azeotrope.

In other systems, there may be a composition of maximum (II) or minimum (III) melting point. In this event, purification proceeds only as far as pure A or pure B as one fraction, and the composition of maximum or minimum melting point as the other fraction. The result obtained thus depends on which side of this constant composition the original composition had been. If the starting material has the composition of maximum or minimum melting point, no separation is possible. The situation is precisely that encountered with azeotropes in distillation.

If there is a discontinuous series of solid solutions, there may be a eutectic of two types of solid solution, or there may be a system with a transition point. Similar principles apply to such systems.

Should a suitable solvent (which does not form solid solutions with the solutes) be added, in appropriate proportion, results similar to the above will be obtained. The possibility of deposition of the various eutectics with solvent must again be borne in mind—see, for example, Figure 4, which represents the only case in which both A and B can eventually be obtained pure from one starting composition, under equilibrium conditions.

SCHEMES FOR REPEATED RECRYSTALLIZATION

All fractionation procedures, whether involving distillation, extraction, diffusion, sublimation, or recrystallization, are schematically much the same, but the terms normally employed to describe the processes are different, as shown in Table I.

Subsequent discussion is confined to consideration of recrystallization from an added solvent, as a means of separation or purification. Such recrystallization is a repetitive process. After a suitable solvent has been chosen, the steps in one recrystallization cycle are as follows: (1) dissolution of the crude substance in the solvent (and removal of any insoluble impurity); (2) preparation of a supersaturated solution; (3) nucleating, if necessary; (4) growth of the crystals—i.e., usually, equilibration; (5) separation of these crystals from the mother liquor; (6) washing, if necessary; and (7) drying the crystals. When a supersaturated solution of compound A is caused to crystallize and is kept until equilibrated—i.e., until crystallization is complete at the chosen temperature—there is a distribution of A

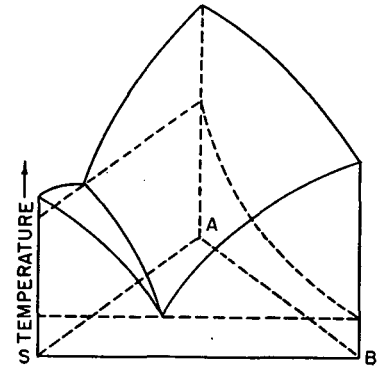


Figure 4. Relation between Freezing Temperature and Composition of Hypothetical Three-Component Solution

Continuous series of solid solutions of A and B with no maximum or minimum freezing point

Table I. Comparable Terms Employed in Three Fractionation Procedures

Recrystallization	Distillation	Liquid-Liquid Extraction
Crystallizand	Distilland	Extrahend
One recrystallization cycle	One simple distillation	One equilibration
Crystals	Distillate	Extract
Mother liquor	Still residue	Raffinate
Distribution constant = weight of A as crystals / weight of A in mother liquor		Partition coefficient = weight of A in extract / weight of A in raffinate

between crystals and mother liquor which is usually expressed as per cent yield (ratio of the weight of crystals to the original weight of A) but which can be expressed as a distribution constant relative to the two phases as shown in Table I. For pure A and a given solvent, this ratio will be a constant, provided that the proportion of solvent to solute is constant, the upper and lower temperature limits are accurately controlled, and equilibrium is reached in the recrystallization.

There are three general ways of contacting phases in a separation process: (1) simple batchwise, (2) cascade or batchwise countercurrent, and (3) continuous or differential countercurrent treatment.

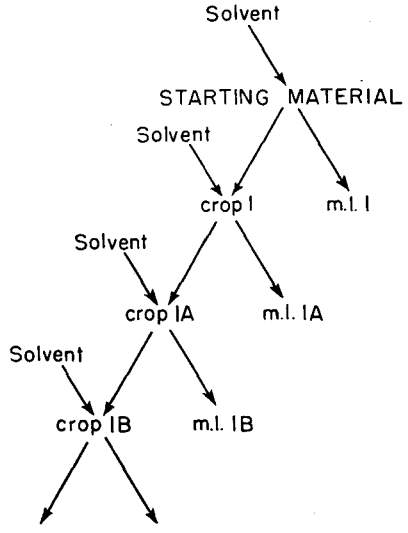


Figure 5. Simple Repeated Recrystallization

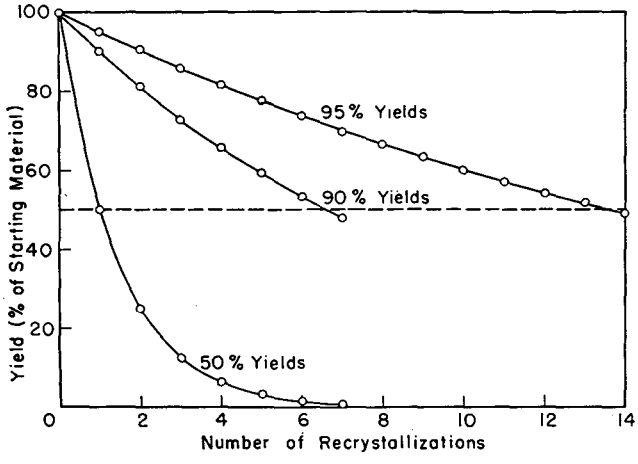


Figure 6. Typical Successive Yields for Repeated Simple Recrystallizations

Simple Batchwise Repeated Recrystallization. For purification involving removal of a small proportion of impurity, a number of simple recrystallizations may be performed as shown in Figure 5. After each recrystallization, an estimate of the degree of purification achieved is made. The mother liquors (m.l.) are discarded, reworked, or, if A and B are eutectogenic, they are pooled and their components are separated by some other method. Simple repeated recrystallization may be regarded as a multiple or successive contact process using fresh solvent at each stage. It is analogous to a one-stage batchwise extraction, which Hunter and Nash (*12*) call "multiple-contact extraction." In each recrystallization there is equilibration corresponding to that achieved by thorough shaking in multiple fractional extraction.

Two important points are the yield of crystals and the purification effected in each recrystallization cycle. Figure 6 compares the successive yields on repeatedly recrystallizing material, at yields of 95, 90, and 50% per cycle. Thirteen recrystallizations at 95% give about the same final yield (50%) as six at 90% yield per cycle. In this graph, and those which follow, the lines joining the points are drawn in as a visual aid; the process is actually discontinuous.

Table II. Ways of Expressing Enrichment

(1)[a]	$\frac{\text{Weight of A in crystals separated}}{\text{weight of A in mother liquor}}$
(2)[a,b]	$\frac{\text{Concentration of A in crystals separated}}{\text{concentration of A in original crystals}}$
(3)[a]	$\frac{\text{Concentration of A in crystals separated}}{\text{concentration of A in crystals next separated (from mother liquor)}}$

[a] (*23*). [b] (*24*).

If A and B are eutectogenic there will not be a great deal of B as impurity in A (or vice versa) after the first recrystallization, and therefore we may be able so to choose the solvent, its amount, and the upper and lower temperature limits as to obtain a good yield—e.g., 95%—at each recrystallization cycle after the first. Because only a few recrystallizations will usually be necessary, the final yield may still be high and the product very pure.

However, if A and B give solid solutions—e.g., of the type shown in Figure 4, to which the ensuing discussion is mainly confined—the situation is different. In the first place, the procedure may entail great loss of material because many recrystallizations may be necessary. Secondly, if the proportion of impurity removed at each stage is constant, this series will constitute the first term of a binomial expansion. Thus, although it is usually fairly easy to raise the purity from 80 to 90%, it is more tedious to raise it from 90 to 99%, still more tedious to raise it to 99.9%, and usually extremely tedious to raise it to 99.99%. That is because 100% purity is approached asymptotically.

There are various ways of expressing enrichment (*23*), as shown in Table II. In the following discussion, the first is employed as being the ratio most intelligible to and convenient for the organic chemist.

Let us consider a simple, hypothetical, numerical example. Suppose that we start with 200 grams of a 50–50 mixture of A and B. The solvent, its amount, and the higher and lower operating temperatures are so chosen that, for B, half goes into the crystals and half remains in the mother liquor at each recrystallization stage. Hence, the distribution constant for

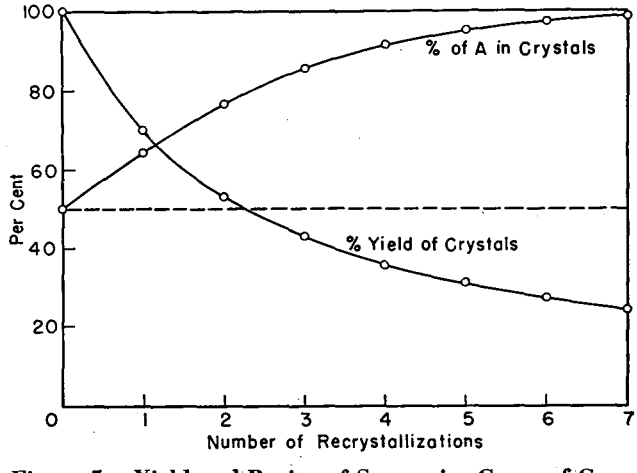


Figure 7. Yield and Purity of Successive Crops of Crystals in a Repeated Simple Recrystallization

In terms of % A
$K_A = 9$; $K_B = 1$

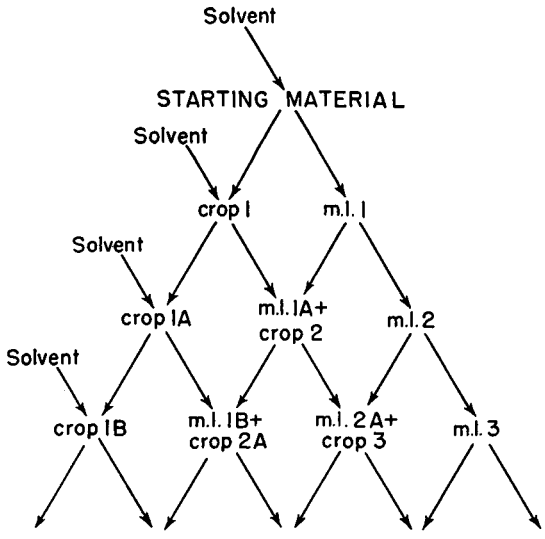


Figure 8. Triangular Fractional Recrystallization

$B = K_B = \frac{\text{weight of B in crystals}}{\text{weight of B in mother liquor}} = 1$. We shall suppose that, under these conditions, 90% of A crystallizes out in each step, and 10% stays in the mother liquor—i.e., $K_A = 9$. [It is assumed that conditions can be achieved such that K_A and K_B really are constants. If the solid solutions and liquid solution which are in equilibrium are not too concentrated, this can be accomplished with such systems as thiophene-benzene in bromoform (*2*), phenol-benzene or piperidine-benzene in benzil (*4*) and *p*-dichlorobenzene–*p*-dibromobenzene in alcohol (*17*). If K_A and K_B are not constants, the schematic principles involved will be similar, but the mathematics will be more complicated (*10, 15, 21*). An important factor is the speed of crystallization (*21*).]

The results of several simple recrystallizations under these conditions are shown in Figure 7, from which it is seen that, after seven recrystallizations, crystals containing 98.4% of A are obtained, but in only a 24.3% yield. In this example, very favorable distribution constants of 9 and 1 were chosen. But if the constants were 1.5 and 1, many more recrystallizations would be necessary to give the same degree of purity, and the loss of product would be much greater.

Cascade or Batchwise Countercurrent Recrystallization. In order to avoid the losses entailed in discarding the mother liquors, the principle of cascade fractional recrystallization was introduced. The schematic principles involved resemble those of cascade fractionation by extraction (*6*), precipitation (*9*), or distillation in a bubblecap still.

Cascade recrystallization differs from simple repeated recrystallization in that both the crystals and the mother liquor (m.l.) are repeatedly fractionated. In separation of A from B, to obtain both A and B, use of this process (or of continuous recrystallization) is essential. The procedure is normally performed batchwise and is an example of countercurrent distribution—that is, if one solute moves to the right, the other moves to the left. Such a scheme, often referred to as "triangular" fractional recrystallization, is shown in Figure 8. As a rule it is, like Craig's countercurrent liquid-liquid distribution (*7*), a discontinuous process operating at complete equilibrium in each cycle.

Separations depend upon the difference in the partition (between crystals and mother liquor) for A and B, respectively (*7, 16, 26*). It is assumed that, at equilibrium, the distribution ratio of one solute between the two phases is independent both of the absolute value of the concentrations and of the presence of other solutes; this is not necessarily true at high concentrations. The degree of separation achieved also depends on the number of recrystallizations applied; compounds A and(or) B may be isolated in any desired degree of purity by repeating the recrystallizations enough times (*25*).

Consider the case where unit mass of a solute is dissolved and the solution so treated that fraction x crystallizes out and $(1 - x)$ is the weight in the mother liquor. This crop of weight x is now recrystallized in the same manner, so that the same proportion again crystallizes, giving a crop of weight x^2. This is repeated many times. We can then draw up the table, showing the weight of material in each fraction, depicted in Figure 9. The numbers in front of each term in these expressions are none other than those in Omar Khayyám's mystical triangle, which led to his discovery of the binomial theorem. We see that the quantity in each row is a term in the binomial expansion

$$[x + (1 - x)]^n = 1$$

where n is the number of the row. [The same principle applies in systematic liquid-liquid extraction (*5*).]

Thus, when applied to the separation of two solutes, the over-all process can be set up to follow the binomial theorem. It is most efficient when a constant numerical fraction of the preceding weight is present as the crystals collected in each recrystallization step. The optimum value of this fraction will depend on the shape of the temperature-composition diagram of the original mixture.

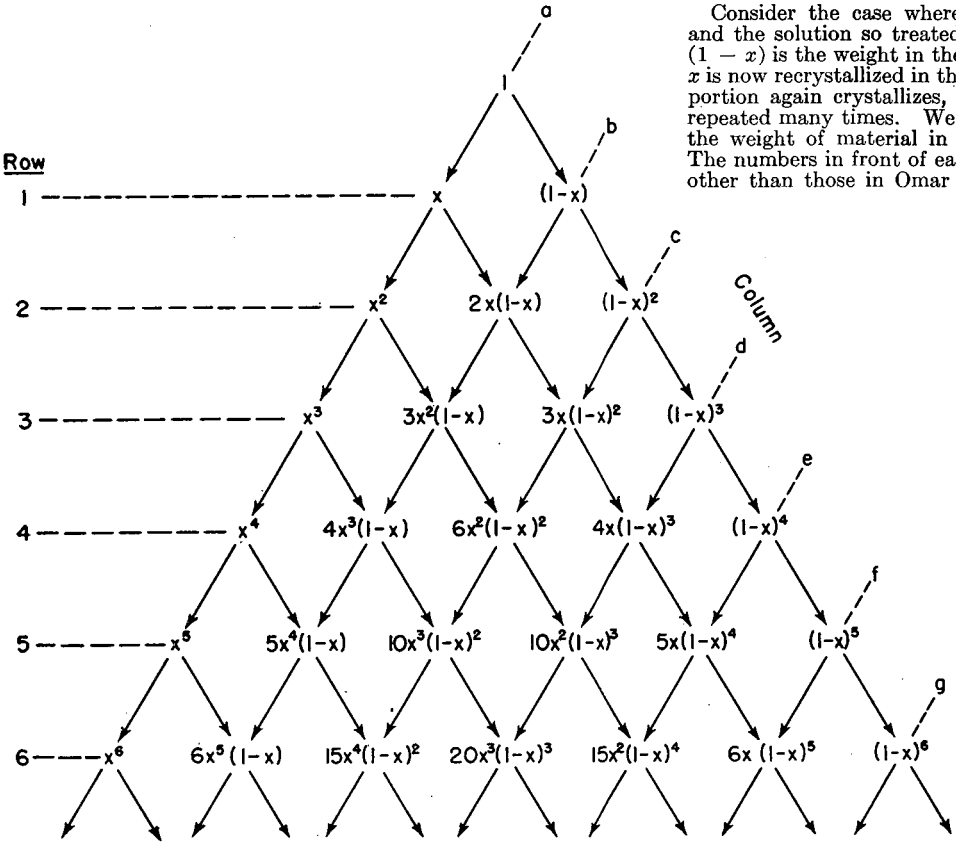


Figure 9. Weights of Fractions in Systematic Triangular Fractional Recrystallization

In 1886, Crookes (*9*) came to the conclusion that, for an approximately 1 to 1 binary mixture (presumably having a fairly symmetrical temperature-composition diagram), the best separation (in terms of labor, and yield of both components) requires that x and $(1 - x)$ be equal—i.e., that half the material is crystallized out and half remains in the mother liquor—in each recrystallization cycle (*11*, *14*). The same principle has been discussed in relation to the separation of gases by diffusion (*18*, *19*). [Of course, if we wished to obtain very pure A in small amount in a few steps, it would pay to crystallize out a small percentage of A in each step. Similarly, the most rapid purification of B may result when the crystal crops are large. Consequently, it is sometimes desirable to produce a variable crop size n the recrystallization scheme —e.g., by slight additional evaporation during each fractionation—thus giving more rapid purification of small amounts of both A and B.]

First of all, let us examine the yields of each fraction in a "triangular" recrystallization of 100 grams of starting material, with a 50% yield per recrystallization. The weights of each fraction are shown in Figure 10. If we now plot the column letter against the weight of the fraction—e.g., at the sixth and tenth rows —symmetrical distribution curves are obtained for a single solute crystallizing from a solution at $K = 1$. It is found that the weights of the different fractions gradually decrease from the middle out toward the two ends, following the bell-shaped distribution curve or normal curve of error and becoming identical with it when n is infinite. In Craig's countercurrent extraction apparatus, this distribution occurs in a set of tubes arranged consecutively (*7*). Secondly, we find that the maximum of this distribution curve migrates along the row according to the row number. Thirdly, if we now plot the distribution curves when one third of the solute crystallizes out in each step—i.e., when $K = 0.5$—it will be seen that the rate of migration of the peak is related to the distribution constant. Furthermore, it is noted that, in this instance, there is a skew distribution.

If we have two solutes, A and B, of different distribution constants, which migrate independently of each other, the resultant curve may be directly additive—i.e., it may be a summation of two such distribution curves, with regard to content of A and B, and may therefore be skew, as shown in Figure 11. This is

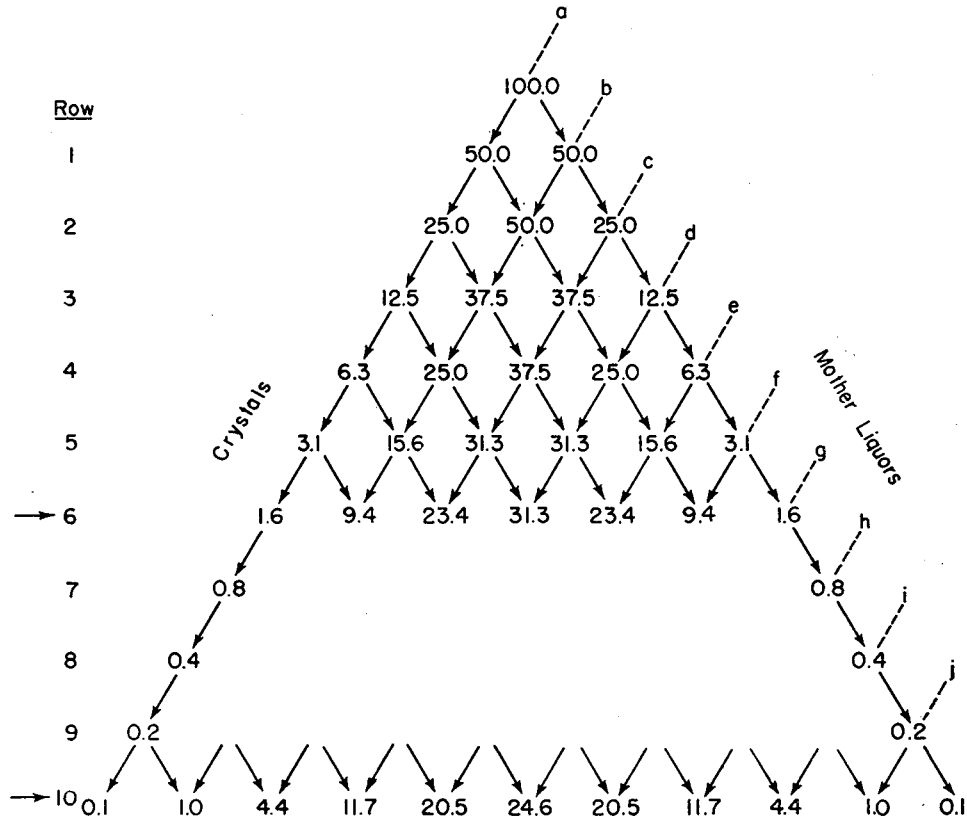


Figure 10. Actual Yields in Triangular Fractional Recrystallization

To nearest 0.1 gram
100 grams of material at 50% yield per recrystallization

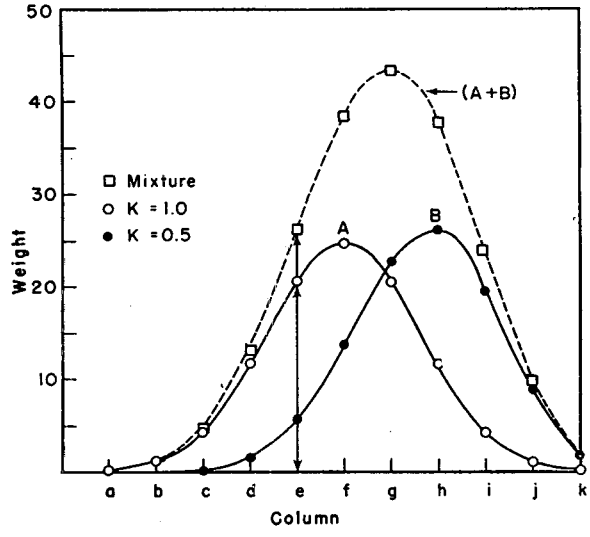


Figure 11. Tenth Row of Hypothetical Triangular Fractional Recrystallization

50–50 mixture of A and B
$K_A = 1.0$; $K_B = 0.5$

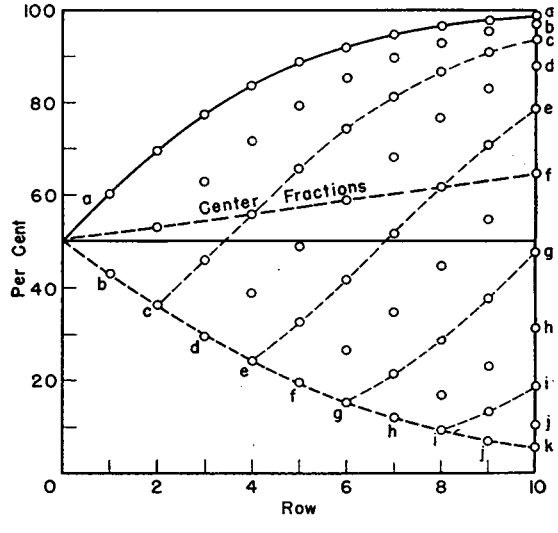


Figure 12. Purity of Every Fraction in Hypothetical Triangular Fractional Crystallization

In terms of % A
50–50 mixture of A and B
$K_A = 1.0$; $K_B = 0.5$

precisely analogous to what happens in Craig's countercurrent scheme of liquid-liquid extraction (*6*, *7*, *27*). If the ratio of the distribution constants of A and B is large, separation is not too difficult. If K_A/K_B is greater than 2, many fewer recrystallizations are needed than if K_A/K_B is less than 2. The nearer the ratio approaches unity, the greater the number of recrystallizations necessary; and when it equals unity A and B are impossible to separate. It is therefore advisable to search for a solvent which gives a large ratio, or else to operate with derivatives of A and B with a more desirable ratio in the chosen solvent.

Let us now consider the purity of each fraction, with respect to A or B. After a few points have been established, we can obviously read off the per cent purity of any fraction from the curves (Figure 11), without extensive calculation. It is only necessary to divide the ordinate for A or B by the ordinate on the summation curve at any particular column. We then obtain the purity curves in terms of % A and % B—that is, a combination of the terms of two binomial series expresses the per cent purity of each fraction. Thus, if the weight of A equals 1 and the weight of B equals 1 in the original mixture, and if in row 1, the weight of A in the crystals equals x and the weight of B in the crystals equals p, then the weight of A in the mother liquor equals $(1 - x)$ and the weight of B in the mother liquor equals $(1 - p)$. Therefore, at equilibrium, $K_A = x/(1 - x)$ or $x = K_A/(1 + K_A)$ and $K_B = p/(1 - p)$ or $p = K_B/(1 + K_B)$. The per cent of A in every fraction (every row and every column) may then be expressed as shown in Table III, where $(1 - x) = y$ and $(1 - p) = q$.

By means of Table III, let us now draw the curves expressing the per cent purity of every fraction for the hypothetical case when $K_A = 1$ and $K_B = 0.5$—i.e., $K_A/K_B = 2$. Two ways of doing this are shown in Figures 12 and 13. It is seen that the center fractions show gradual enrichment with regard to A and

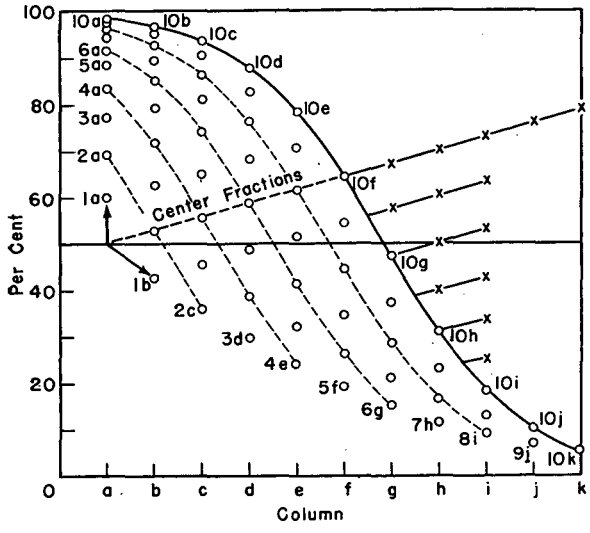


Figure 13. Purity of Every Fraction in a Hypothetical Triangular Fractional Recrystallization

In terms of % A
50–50 mixture of A and B
K_A = 1.0; K_B = 0.5

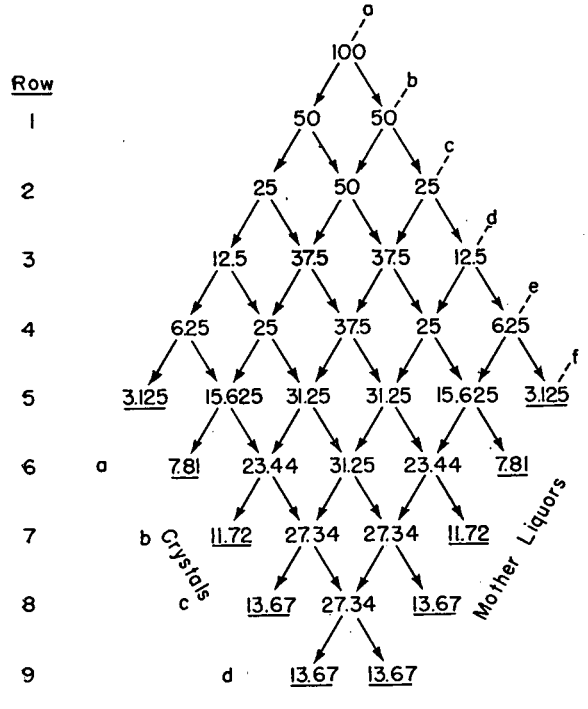


Figure 14. Weights of Fractions in Diamond Fractional Recrystallization

100 grams of material at 50% yield per recrystallization

Table III. "Purity" of Every Fraction (% of A) in a "Triangular" Recrystallization

Column Row	*a*	*b*	*c*	*d*	*e*	*f*
1	$\frac{x}{x+p}$	$\frac{y}{y+q}$				
2	$\frac{x^2}{x^2+p^2}$	$\frac{xy}{xy+pq}$	$\frac{y^2}{y^2+q^2}$			
3	$\frac{x^3}{x^3+p^3}$	$\frac{x^2y}{x^2y+p^2q}$	$\frac{xy^2}{xy^2+pq^2}$	$\frac{y^3}{y^3+q^3}$		
4	$\frac{x^4}{x^4+p^4}$	$\frac{x^3y}{x^3y+p^3q}$	$\frac{x^2y^2}{x^2y^2+p^2q^2}$	$\frac{xy^3}{xy^3+pq^3}$	$\frac{y^4}{y^4+q^4}$	
5	$\frac{x^5}{x^5+p^5}$	$\frac{x^4y}{x^4y+p^4q}$	$\frac{x^3y^2}{x^3y^2+p^3q^2}$	$\frac{x^2y^3}{x^2y^3+p^2q^3}$	$\frac{xy^4}{xy^4+pq^4}$	$\frac{y^5}{y^5+q}$
n	$\frac{x^n}{x^n+p^n}$	etc.				

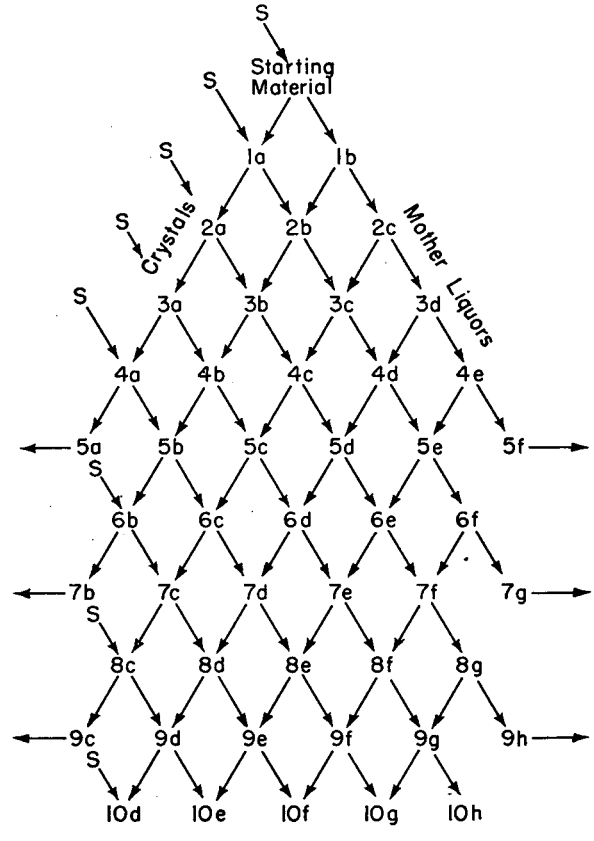


Figure 15. Double Withdrawal Scheme of Fractional Recrystallization

the "outer" fractions are asymptotic to 100% A and 100% B. The purity of fractions still to be obtained can then be read off, as at points X in Figure 13. It is seen that the crystal fractions are progressively enriched in compound A, while the mother liquor fractions are progressively impoverished in A as the number of recrystallizations increases. We see that the enrichment tends toward infinity, but that the purer the fraction the less its weight. Hence, unless the object is to prepare a small sample of very pure material, the simple triangular scheme is not of much use.

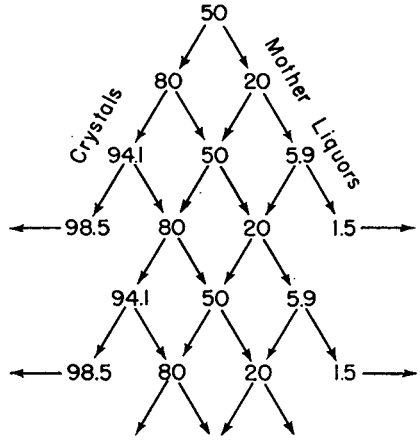


Figure 16. Purity of Fractions in Hypothetical Double Withdrawal Fractional Recrystallization

In terms of % A
50–50 mixture of A and B
K_A = 4.00; K_B = 0.25

Consequently, in order to improve the yield, the "diamond" scheme of fractionation was introduced. When the outermost fractions have reached the desired degree of purity, at the nth row, they are set aside and fractionation is continued as shown in Figure 14. A further row of recrystallizations is made, and the outer fractions are again removed. This is repeated until all the material is obtained as crystals or final mother liquor. It is useful to combine enriched fractions and again fractionally recrystallize (*20*). Except in special cases, this system is not very good because fractions of different purities are recombined. In those instances where K_A is the reciprocal of K_B, each fraction has the same composition as the fraction "vertically" above it in the triangular scheme; and in even-numbered rows, the largest part, corresponding to the middle term, has the original composition.

A better method is therefore the double-withdrawal scheme depicted in Figure 15. For example, fractions 5a and 5f are withdrawn. The recrystallization is continued to row 7, and then fractions 7b and 7g are withdrawn. After many such withdrawals all the crystal fractions 5a, 7b, etc., are united and recrystallized. The mother liquor fractions 5f, 7g, etc., are also united, crystallized, and recrystallized. This same scheme is used in liquid-liquid countercurrent distribution. Hunter and Nash (*12*) call it "fractional distribution by multiple contact." The system is profitably employed when the ratio of the distribution constants is large, so that an effective separation can be obtained in a few rows. It is most satisfactory when K_A is the reciprocal of K_B. Thus if, in a hypothetical case, K_A = 4.00 and K_B = 0.25, and we start with 50 grams of A plus 50 grams of B, multiple fractional distribution gives fractions having the purities shown in Figure 16, in yields shown in Figure 17.

An alternative method is fractional recrystallization of aliquots, shown in Figure 18. It is useful when the main component is fairly soluble in the hot but only sparingly soluble in the cold solvent and when it is desired to use as little solvent as possible. The same portion of solvent is, up to a point, repeatedly used. The starting material is divided into several equal parts, 1, 2, 3, etc. Part 1 is recrystallized from the minimum volume of hot solvent, giving crop 1A and mother liquor 1A. Part 2 is then recrystallized from mother liquor 1A, yielding crop 2A and mother liquor 2A, which is, in turn, used for the recrystallization of part 3, to give crop 3A. Crop 1A is now recrystallized from pure solvent, giving crop 1B and mother liquor 1B. The latter is used for the recrystallization of crop 2A, and so on. The procedure is repeated until crop 1 (A or B or C, etc.) is pure. Then attention is concentrated on the purification of crop 2 (A, B, C, etc.).

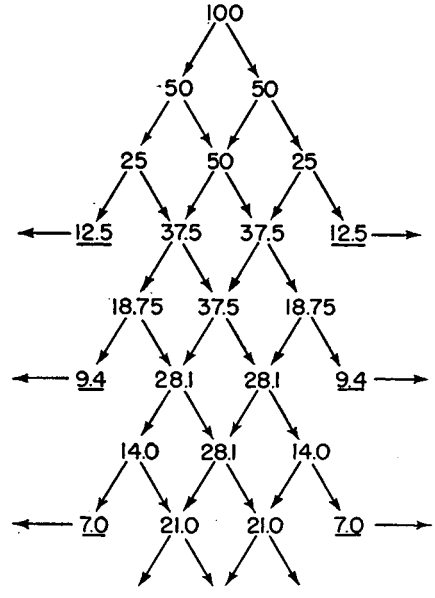


Figure 17. Weights of Fractions in Double Withdrawal Fractional Recrystallization

100 grams of material at 50% yield per recrystallization

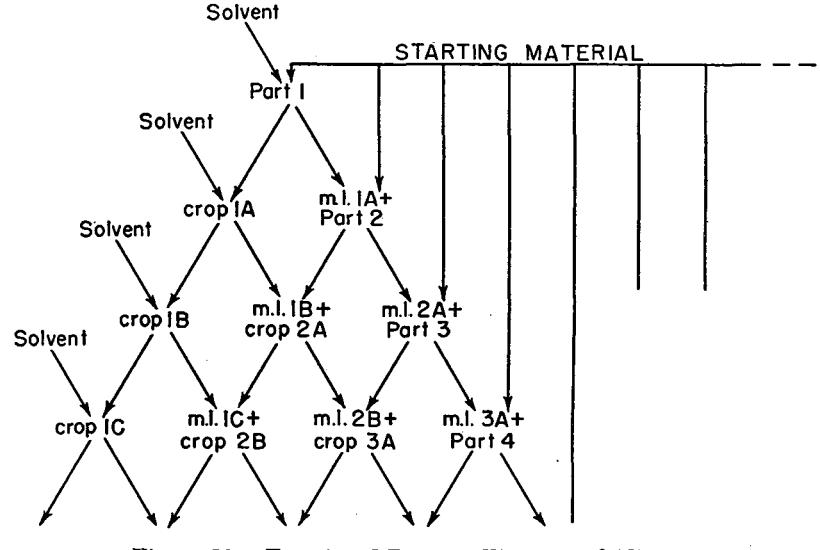


Figure 18. Fractional Recrystallization of Aliquots

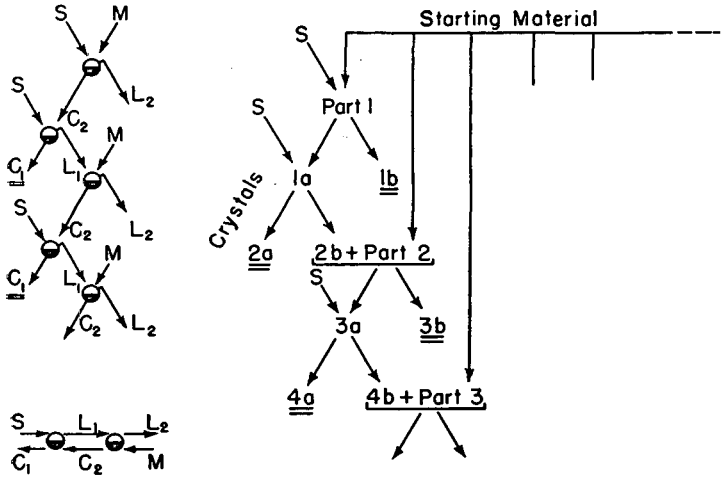


Figure 19. Two-Stage Batch Countercurrent Recrystallization

The same system is used in liquid-liquid extraction. Hunter and Nash (*12*) call it "pseudo-countercurrent extraction." Where the ratio between the distribution constants is very large, and extreme purity is not desired, the procedure may be shortened by using a two-stage or three-stage scheme, similar to those used in liquid-liquid extraction (*22*), as shown in Figures 19 and 20. If both A and B are desired, the system of crystallizing must be complete—that is, each fraction in a "positive" direction must conform with a fraction in the "negative" direction—but this does not mean that the crystals from each recrystallization must necessarily be transferred to the next dish positively, or that the mother liquor from every recrystallization must go into the next dish negatively. The crystals may be transferred to the second, third, or fourth dish positively, and the mother liquor may go into the second, third, or fourth dish negatively, depending upon the value of the enrichment factor (*24*). Of course, if only one component is desired, the foregoing schemes can often be greatly simplified by discarding highly impure fractions (*1*).

Continuous Recrystallization. Continuous recrystallization is a differential countercurrent method which consists in treatment in a vertical column or horizontal trough so arranged that the crystals continuously move in one direction and the liquid in the opposite direction. The crystals are brought in contact with mother liquor from a previous stage, and the crystals leaving the crystallizer meet fresh solvent. The applicability of the process is governed by the conditions previously outlined for systems which do or do not form solid solutions. If set up in a vertical tower, the crystals fall and the solvent rises, so that the phases flow continuously and countercurrently. In one form, crude material is introduced near the middle, purified crystals leave at the bottom where fresh solvent enters, and mother liquor leaves at the top. The column, in which equilibrium is not established at any point, may be visualized as a number of layers, each of which is equivalent to a plate. The efficiency can be increased by returning redissolved purified material to the apparatus, and the effect thus obtained is comparable theoretically to that of reflux in distillation. A similar principle is employed in countercurrent extraction with "extract reflux." The process is used industrially but has apparently not been employed extensively in the laboratory.

In a device patented (*3*) in 1947, a vertical column packed with a filter bed—e.g., of sand—is employed to give a system greatly resembling a chromatogram. The column is initially jacketed with a coolant. The material to be fractionated is placed at the top of the column and solvent is added there. The coolant is then very slowly drained away, the top of the column is warmed, and a temperature-gradient is established. Material dissolves at the top and then crystallizes out again lower down the column. As solvent moves downward, these crystals redissolve and again crystallize out lower down, so that there is multiple redissolution and recrystallization. Although solvent, solution, and crystals move in the same direction, the effect is countercurrent if the rates of movement are different. The apparatus may be used for recrystallization of quantities of 1 kg. or more, and for recrystallization from a melt, without added solvent.

SOME ADVANTAGES AND DISADVANTAGES OF RECRYSTALLIZATION

The advantages of recrystallization as a method for separation and purification are as follows. It yields the material in a form susceptible to special tests characteristic of the crystalline state. Oftentimes it may be accomplished without elaborate apparatus. It may readily be carried out on a macro or semimicro scale, but special apparatus and techniques are necessary for microrecrystallization. It may often be applied to materials not readily separated by other means—e.g., an azeotropic mixture. It may be applied to materials which decompose on attempted distillation. Recrystallization from a solvent is particularly indicated for substances sensitive to heat—e.g., those which decompose at their melting points—for it can often be accomplished either without heating at all, or by heating of very short duration. It is also applied to substances whose supercooled melts are very viscous. Recrystallization is superior to Craig's countercurrent extraction method (*8*) in its present state of development, when applied to separations on a large scale, but as a microtechnique for estimation of purity, it is probably not so useful as the latter. However, a pure substance or a constant-composition mixture will, under equilibrium conditions, give a symmetrical distribution curve at the rows of a systematic triangular recrystallization, and this might be employed as a form of solubility analysis.

On the other hand, mixtures inseparable by recrystallization may often be separated by other methods. Furthermore, recrystallization procedures cannot be applied until crystal nuclei have been obtained, and this may require long waiting. Batchwise, countercurrent recrystallization may be slow and tedious. Only a few stages are possible without great expenditure of labor, whereas liquid-liquid distribution lends itself admirably to mechanical

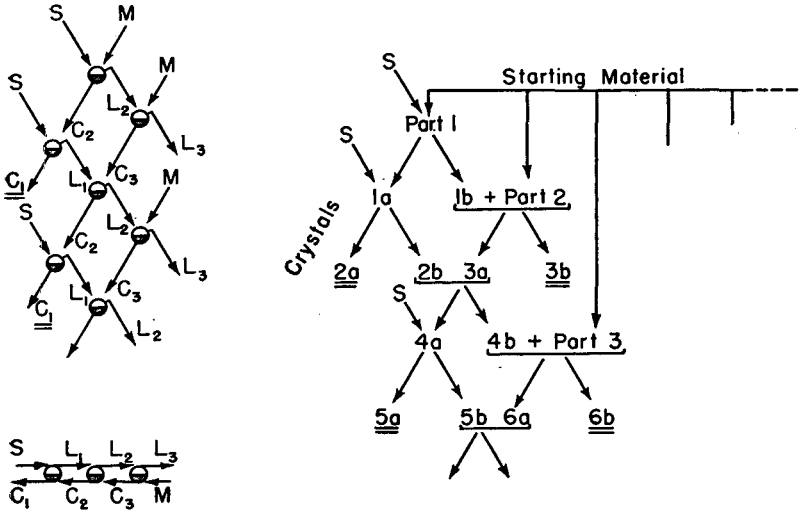


Figure 20. Three-Stage Batch Countercurrent Recrystallization

transfers and a large number of stages are readily achieved. Thus, with Craig's present apparatus, 3000 to 4000 quantitative extractions per hour can be performed.

ACKNOWLEDGMENT

The author gratefully acknowledges certain suggestions made by John R. Anderson of Mellon Institute and Lyman C. Craig of the Rockefeller Institute for Medical Research.

LITERATURE CITED

(1) Baxter, G. P., and Behrens, E. E., *J. Am. Chem. Soc.*, **54**, 591 (1932).
(2) Beckmann, E., *Z. physik. Chem.*, **22**, 609 (1897).
(3) Bowman, J. R. (to Gulf Oil and Development Co.), U. S. Patent 2,427,042 (Sept. 9, 1947).
(4) Bruni, G., *Gazz. chim. ital.*, **28**, 249, 259 (1898).
(5) Bush, M. T., and Densen, P. M., ANAL. CHEM., **20**, 121 (1948).
(6) Craig, L. C., *Federation Proc.*, 7, No. 3, 469 (1948).
(7) Craig, L. C., *J. Biol. Chem.*, **155**, 519 (1944).
(8) Craig, L. C., Mighton, H., Titus, E., and Golumbic, C., ANAL. CHEM., **20**, 134 (1948).
(9) Crookes, William, *Chem. News*, **54**, 131, 155 (1886).
(10) Doerner, H. A., and Hoskins, W. M., *J. Am. Chem. Soc.*, **47**, 662 (1925).
(11) Harkins, W. D., and Mortimer, B., *Phil. Mag.* [7], **6**, 601 (1928).
(12) Hunter, T. G., and Nash, A. W., *Ind. Eng. Chem.*, **27**, 836 (1935).
(13) International Critical Tables, Vol. IV, p. 177, New York, McGraw-Hill Book Co., 1928.
(14) James, C., *J. Am. Chem. Soc.*, **30**, 182 (1908).
(15) Käding, H., Mumbrauer, R., and Riehl, N., *Z. physik. Chem.*, **A 161**, 362 (1932).
(16) Martin, A. J. P., and Synge, R. L. M., *Biochem. J.*, **35**, 1358 (1941).
(17) Meyer, G., *Rec. trav. chim.*, **42**, 301 (1923).
(18) Mulliken, R. S., and Harkins, W. D., *J. Am. Chem. Soc.*, **44**, 37 (1922).
(19) Rayleigh, Lord, *Phil. Mag.* [5], **42**, 493 (1896).
(20) Richards, T. W., and Hall, N. F., *J. Am. Chem. Soc.*, **39**, 531 (1917).
(21) Riehl, N., *Z. physik. Chem.*, **A177**, 224 (1936).
(22) Rushton, J. H., *Ind. Eng. Chem.*, **29**, 309 (1937).
(23) Schlundt, H., *U. S. Bur. Mines, Tech. Paper* **265**, 31 (1922).
(24) Scholl, C. E., *J. Am. Chem. Soc.*, **42**, 889 (1920).
(25) Sunier, A. A., *J. Phys. Chem.*, **33**, 577 (1929).
(26) Varteressian, K. A., and Fenske, M. R., *Ind. Eng. Chem.*, **28**, 1353 (1936); **29**, 270 (1937).
(27) Williamson, B., and Craig, L. C., *J. Biol. Chem.*, **168**, 687 (1947).

RECEIVED July 1, 1949. Based on a paper presented before the Division of Physical and Inorganic Chemistry, Symposium on Separation and Purification of Organic Compounds, at the 115th Meeting of the AMERICAN CHEMICAL SOCIETY, San Francisco, Calif.

Purification by Fractional Melting

J. G. ASTON AND S. V. R. MASTRANGELO
Pennsylvania State College, State College, Pa.

The use of the solid-liquid equilibrium for purification by the method of fractional melting is shown to be highly efficient. This method makes it possible to observe the progress of purification inasmuch as the liquid fraction is always separated from the solid fraction under equilibrium conditions. Examples of the progress of purification of several materials are given.

ALTHOUGH crystallization from a solvent is, of course, one of the oldest and most widely used methods of separation, the possibility of using solid-liquid equilibria in a manner analogous to vapor-liquid equilibria for efficient separation has not been widely recognized. The separation of a single pure solid from a liquid of two components is similar to the removal of the vapor of a pure substance from a liquid mixture with a nonvolatile substance.

Whenever the solid-liquid equilibrium has been used for separations similar to those that are possible in the various types of liquid-vapor equilibria, the process used has been essentially crystallization (*3*). Such separations can be carried out by fractional melting with much greater convenience and ease of control. The present paper describes the work on the single-step separation of a purer substance as a solid from a liquid mixture in equilibrium with it, usually where no solid solution occurred, by fractional melting, and points out its advantages over fractional crystallization.

In theory, the substance may be obtained 100% pure and the maximum possible yield of pure substance by such a process is easily calculated by a material balance from the initial composition, if the eutectic diagram is known or can be computed using Raoult's law. In practice, if this process is carried out by cooling and removing the crystals which separate until the eutectic is reached, such yields of pure material are frequently not obtained.

The reason for this is illustrated best by an attempt to free *cis*-2-butene from the accompanying *trans*-2-butene by freezing. Starting with a sample of *cis*-2-butene with 3.1 mole % impurity, of which 2.9 mole % was the trans variety, about 10% of the liquid was frozen with vigorous stirring, using a bath no colder than necessary to get a convenient rate of cooling. In spite of the fact that the impurities (including the *trans*-2-butene) were both shown not to form solid solutions with the *cis*-2-butene, the solid that separated contained 3.4 mole % impurity. This was shown to be due to the fact that the eutectic mixture separated at the walls and while the liquid was poured off some crystals of the pure *cis*-2-butene melted by conduction down the stirring mechanism and were poured off as liquid, and the impurity remained behind as eutectic on the supercooled walls. Efforts to eliminate the temperature gradients responsible, by customary methods, such as an imposition of an air bath between the sample tube and refrigerant, produced little improvement.

The role of the solvent in fractional crystallization is to make it easier to get equilibrium by stirring, and this offsets the disadvantage of introducing a third component. The present paper shows how these difficulties can be removed by fractional melting of the solidified sample, under equilibrium conditions throughout.

APPARATUS

Four batch-type purifiers have been built and used at one time or another. All consist of a vessel with a large conducting surface inside to produce thermal equilibrium, and a heater. In this vessel the system is first entirely frozen and then fractionally melted. A predetermined amount of heat can be added electrically to this vessel; adiabatic conditions are maintained by an electrically heated shield which surrounds it to eliminate radiation and conduction. The radiation shield is surrounded by a copper sheath to equalize temperature and the whole is immersed in a refrigeration bath. Adiabatic conditions are maintained by keeping the shield at the same temperature as the melting vessel with the aid of copper-constantan difference thermocouples.

Apparatus 1. This has a capacity of 100 ml. and has conduction vanes in the form of a spiral stirrer similar to that used

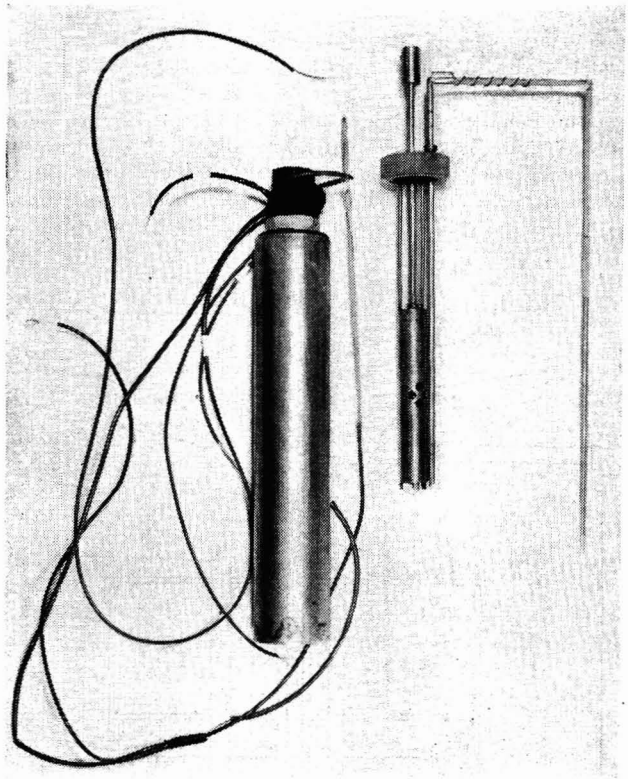


Figure 1. Fractional Melting Apparatus 1
Spiral stirrer and housing removed

in some meat grinders. Figure 1 is a photograph of this apparatus with spiral stirrer and housing removed (right of photograph).

Apparatus 2. This also has a capacity of 100 ml., but heat conduction is provided by a roll of copper gauze.

Apparatus 3. The capacity of the melting vessel is 500 ml. It has horizontal vanes of thin perforated copper separated by springs. The system of vanes can be compressed from above. This apparatus is described in detail below.

Apparatus 4. This is similar to apparatus 3, except that the compressible vanes are replaced by radial vanes. Figure 2 is a photograph of the apparatus with the bracket for mounting it in the large Dewar.

Description of Apparatus 3. Figure 3 is a drawing of apparatus 3 with explanatory legend. The melting chamber, 13, of 500-ml. capacity is equipped with the system of vanes, 14, 15, and 16 which can be compressed by the rod, 3, operating through the stuffing box, 2. The guide rods, 15, form the core of the springs which keep the vanes separated when not under compression. The heater, consisting of 25 ohms of B. and S. No. 30, D.S.C. copper wire, is wrapped on the outside wall which had previously been coated with a layer of Bakelite lacquer and baked at 120° C. for 3 hours. The heater was coated with Bakelite and similarly baked after wrapping.

The transfer tube, 5, is used to remove the melted material. It is fitted with a glass wool filter, 19, held in place by a constriction above and a drilled plug, 20, below. In order to avoid liquid's freezing in it, heat is supplied to the upper section by a No. 18 B. and S. bare copper wire, 4, passing down the center to the constriction. This wire passes out of the tube at the elbow and is wrapped round and soldered to the outside of the tube in the warm region.

The cylindrical radiation and conduction shield, 12, is closed at the top and bottom with disks of copper $^1/_8$ inch thick. The cylinder has a wall $^1/_{16}$ inch thick, which is wrapped with a heater of 180 ohms of B. and S. No. 30 D.S.C. manganin wire fastened with Bakelite in the same way as is the heater on the melting chamber.

The temperature equalizer, 11, consists of a copper cylinder $^1/_{16}$ inch thick. The cover is soldered to the top and is provided with four openings which are large enough to allow the Monel tubes, 6, 7, 8, and 9, to pass through without making thermal contact there. This prevents the formation of cold spots which might produce plugs consisting of solidified material. The bottom of the temperature equalizer is closed by means of a copper disk $^1/_{16}$ inch thick.

The outer envelope, 10, is made of Monel metal $^1/_{16}$ inch thick, and is provided with a Monel tube for evacuation. This reduces the heat leak by gaseous conduction to and from the melting chamber. Cork sheet insulation, 17 and 18, between the melting chamber and the shield, and between the shield and and temperature equalizer, is also provided for this purpose, so that evacuation may be dispensed with without seriously affecting the efficiency of the purification.

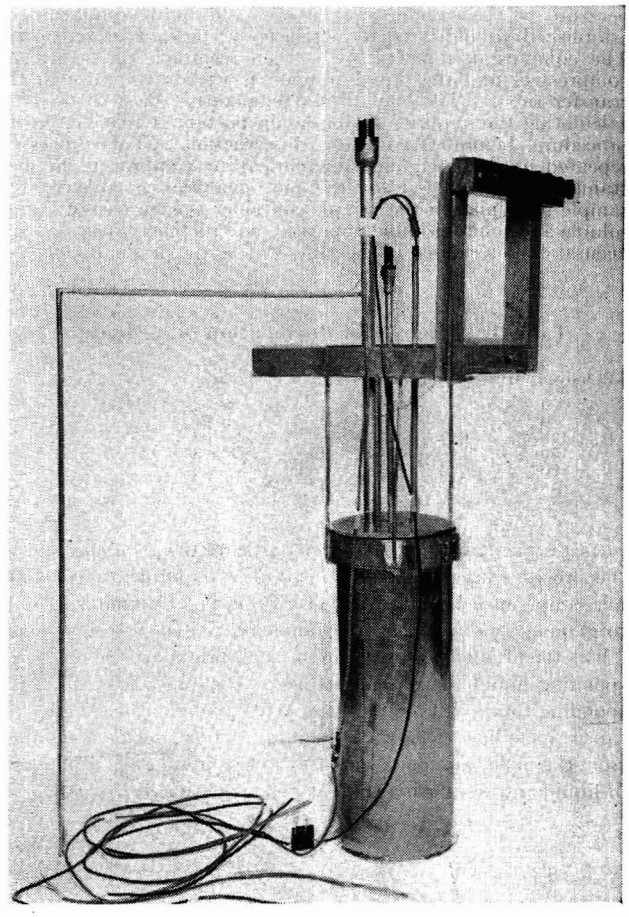


Figure 2. Fractional Melting Apparatus 4
With bracket for mounting on large Dewar

A calibrated copper-constantan thermocouple which is imbedded in paraffin contained in a long, thin-walled glass tube is used for observing the temperature of the sample in the melting chamber. The glass tube containing the thermocouple passes into the apparatus through tube 9. A copper-constantan difference thermocouple registers the difference in temperature between the melting chamber and the shield. The two junctions of this difference thermocouple are soldered to the walls under the respective heaters. These thermocouples will register temperature differences of about 0.0015°.

The entire apparatus is suspended inside a glass Dewar, which contains the refrigerating bath.

EXPERIMENTAL PROCEDURE

The unpurified material is first dried by a suitable procedure and then transferred to the sample container, excluding air. During this process and also during the cooling that follows, in which the sample is crystallized and finally brought to a temperature of about 20° below the melting point, the cryostat envelope is supplied with helium gas under reduced pressure. The helium gas is then pumped out and the shield is heated to the temperature of the melting chamber. Adiabatic conditions are then maintained throughout the rest of the procedure.

The original purity of the sample may now be obtained by the usual calorimetric method (*1*, *4*). The rises in temperature after adding increments of energy are observed and the re-

ciprocals of the fraction of sample melted are plotted against the corresponding equilibrium temperatures. The value of the quantity ($T_{100\%}$–$T_{50\%}$) and the heat of fusion data obtained are used for calculating the mole per cent impurity from the melting point equation and the melting point of the pure material equal to ($2T_{100\%}$–$T_{50\%}$). This part of the procedure may be omitted if a small test run is made in another apparatus.

The sample is cooled down again and recrystallized. A simple calculation from the heat of fusion data obtained during the purity determination will then decide the time required to melt any given fraction of the sample. For impurities of about 3 or 4 mole %, a 10% fraction is usually melted. The temperature of the sample is then observed until equilibrium is established, as indicated by a relatively constant or zero drift. The equilibrium temperature is then recorded, the vanes are compressed, and the liquid fraction is withdrawn through the transfer tube. (The equilibrium temperature may be used for calculating the impurity in the liquid portion at this step in the procedure, before withdrawing this fraction.) This process is repeated until the equilibrium temperatures approach the constant value calculated for the pure material as above. The sample remaining in the sample container is then free of liquid-soluble and solid-insoluble impurities and its purity may be more accurately determined in the same way as the original purity.

Table I. Progress of Purification of *n*-Heptane

Removed, Ml.	Equilibrium Temperature, ° C.	Purity, %	Yield, %
0	182.108	99.5	100
50	182.498	99.91	90
100	182.514	99.92	80
150	182.577	99.996	70
200			60

As a typical example which demonstrates the great efficiency of this process with apparatus 3, the progress of purification of a 500-ml. sample of *n*-heptane is given (Table I). Column 1 lists the total quantity of liquid sample removed after each step. Column 2 lists the absolute temperature of equilibrium before the corresponding liquid fraction is removed. Column 3 lists the corresponding total purity of the sample remaining in the sample container, including the solid and liquid fractions. This is obtained from the mole fraction of impurity in the liquid and the amounts of liquid and solid using the following equations:

$$P\% = 100\, Lf\,(T_o - T_n)\, Y_n/RT_o^2$$

$$Yn = \left(Qn - \int C_{pn}dT_n\right) \Big/ \left(Q_{\text{total}} - \sum_{i=1}^{n-1}\left[Q_i - \int C_{pi}dT_i\right]\right)$$

where

$p\%$ is the total mole % impurity

Lf is the heat of fusion in calories per mole of the major component

T_o is the melting point of the pure major component in degrees Kelvin

T_n is the absolute temperature of equilibrium at the nth step

Y_n is the corresponding fraction of material melted based on the total amount of material in the melting chamber at this step

R is the ideal gas constant, 1.987 calories per mole

Q_n is the amount of energy used for heating and melting the material in the melting chamber for the nth step

$\int C_{pn}dT$ is the amount of energy which produces the rise in temperature during the energy input (C_{pn} is the average heat capacity of the nth fraction based on interpolation of the solid and liquid heat capacity curves in the region of melting, and dT is the rise in temperature during the energy input)

Q_{total} is the total energy input required to melt the original quantity of material placed in the melting chamber

In Table I, column 4 lists the yield corresponding to the purity listed in column 3 based on the original weight of material.

Reference to Table I shows that the original purity of the sample is 99.5 mole % (row 1, with all the material present). After one 10% fraction was melted, the liquid fraction withdrawn and a second 10% fraction of the original sample melted, the purity rose to 99.91 mole %. It was possible to obtain a 90% yield of material with this purity. However, the second liquid fraction was withdrawn and a new fraction melted. The equilibrium temperature indicates that this sample was 99.92 mole % pure and an 80% yield was obtainable. After only 30% of the sample was removed the purity rose to 99.996 mole %, and after the last fraction was removed a purity determination made in the usual calori-

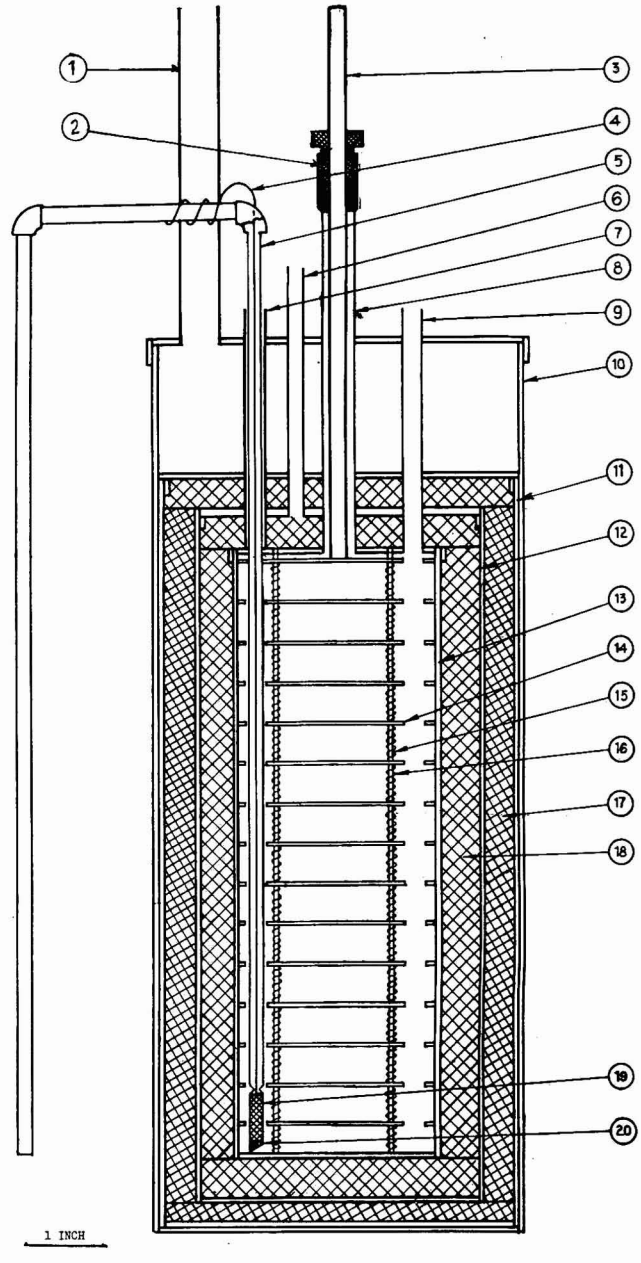


Figure 3. Fractional Melting Apparatus 3

1. Monel tube for evacuation
2. Stuffing box
3. Activator rod for vane system
4. No. 18 copper wire conduction heater
5. Transfer tube
6. Monel tube for electrical supply wires
7. Monel tube for transfer tube exit
8. Monel tube for activator rod exit
9. Monel filling tube and thermocouple exit
10. Outer envelope (cryostat)
11. Temperature equalizer
12. Shield
13. Melting chamber
14. Vane
15. Guide rod
16. Spring
17, 18. Cork sheet insulation
19. Glass wool filter
20. Drilled plug

Table II. Yields Obtained for *cis*-2-Butene

Yield, %	70	60	50
Purity, %	99.83	99.94	99.998

Table III. Progress of Purification of Other Samples

Compound	Original Purity, %	Purity, %	Yield, %
Cyclohexane	85.0	98.95	53.4
		99.14	45.5
		99.22	37.8
Cyclohexane	99.22	99.75	33.0
2,2,4-Trimethylpentane	99.6	99.99	78.0
n-Heptane	99.4	100	78.0
trans-2-Pentene	95.7	99.96	70.0
		99.99	60.0
2,2,4-Trimethylpentane	99.6	99.98	80.0
		99.99	70.0

metric way indicated that the purified sample had a purity of 100% within the accuracy of the measurement (±0.005 mole %).

A sample of *cis*-2-butene [melting point 134.260° K., ΔH_f = 1746.8 calories per mole (*5*)] containing an impurity of 3.9 mole % [mostly *trans*-2-butene melting point 167.61° K., ΔH_f = 2331.9 calories per mole (*2*)] was purified in apparatus 1, which employs a spiral stirring mechanism in place of the compressible vanes. The yields obtainable for the corresponding purities, based on the total quantity of material, are listed in Table II. Such yields would be very difficult to obtain by distillation.

It is interesting to compare with the theoretical yield the possible yields obtainable for *cis*-2-butene by stopping the process at any step in the procedure with the theoretical yield. A calculation made by solving the melting point equations for *cis*-2-butene and *trans*-2-butene at the eutectic temperature, using the above values for the melting point, shows that the eutectic temperature is 131.17° K. and the eutectic mixture consists of 1.43 *trans*-2-butene and 85.7% *cis*-2-butene. Hence, the per cent of eutectic mixture in a sample of *cis*-2-butene containing a 3.9 mole % impurity of *trans*-2-butene is 27.3 mole % and the theoretical yield of pure *cis*-2-butene is 72.7%. From Table I it is apparent that, although a 60% yield of the 99.94 mole % material based on the original weight of material is obtainable, this is 82.5% of the theoretically possible yield for this type of process.

The results of purifying other samples in various types of fractional melting apparatus are given in Table III. Column 1 lists the compound being purified; column 2 lists the original purity; and columns 3 and 4 list the corresponding purities and yields obtained, the latter based on the total quantity of material used. The materials corresponding to the yields in column 4 were not removed each time but were instead used for further melting to give the next corresponding purity and yield.

The results for cyclohexane are of doubtful validity, because the sample is reputed to contain about 1 mole % of methylcyclopentane, which forms solid solutions with cyclohexane (*6*).

Combined with the rough fractional distillation of the eutectic mixture and of early liquid fractions withdrawn, this method should, in most cases, prove superior to precise fractionation when only the major component is to be separated in the pure state. The truth of this statement can be seen in the separation of the geometric isomers of 2-butene and of 2-pentene.

ACKNOWLEDGMENT

The authors wish to thank the Phillips Petroleum Company for the financial help which made this work possible.

LITERATURE CITED

(1) Aston, J. G., Fink, H. L., Tooke, J. W., and Cines, M. R., ANAL. CHEM., **19**, 218 (1947).
(2) Guttman, L., and Pitzer, K. S., *J. Am. Chem. Soc.*, **67**, 324 (1945).
(3) Hicks, M. M., *Bur. Standards J. Research*, **2**, 483 (1929).
(4) Johnston, H. L., and Giauque, W. F., *J. Am. Chem. Soc.*, **51**, 3194 (1929).
(5) Scott, R. B., Ferguson, W. J., and Brickwedde, F. G., *J. Research Natl. Bur. Standards*, **33**, 1 (1944).
(6) Tooke, J. W., and Aston, J. G., *J. Am. Chem. Soc.*, **67**, 2275 (1945).

RECEIVED August 6, 1949.

Criteria of Analytical Methods for Clinical Chemistry

R. M. ARCHIBALD

The Hospital of the Rockefeller Institute for Medical Research, New York 21, N. Y.

Analytical reports from most clinical laboratories are unreliable, sometimes because of errors in the collection and labeling of specimens, but more often because of inadequate procedures within the laboratory. Unreliability results largely from inadequate supervision of analysts who may lack proper technical training or personality qualifications. Some improvement should result from selection of simple methods capable of giving accurate results in the hands of the personnel available. Explicit typed or printed directions free of ambiguity should be in the hands of each analyst. An aliquot from a large stock pool should be carried through all steps, including calculations, with each set of determinations. Calculations should be checked by some responsible individual in addition to the analyst. The laboratory director must be provided with adequate motivation devote the necessary time to supervise the analytical staff properly. Attributes of suitable analytical methods are considered.

IN NOVEMBER 1947, Belk and Sunderman (*4*) reported on a survey of the accuracy of measurements of calcium, urea, sodium chloride, hemoglobin, glucose, and serum protein as carried out in 40 to 50 hospitals in Pennsylvania, New Jersey, and Delaware. Aliquots of solutions of known strength for each substance to be measured were sent to the laboratories included in the survey; reports were made anonymously. The survey showed that almost two thirds of the laboratories reported results that were unsatisfactory from the point of view of accuracy.

In the case of calcium, the concentrations reported ranged from less than one third to more than twice the correct value; with urea, from one tenth of the correct value up to 35% too high. Similar unsatisfactory results were reported for sodium chloride, hemoglobin, and serum protein. For glucose there was more than a 14-fold difference between the highest and lowest values reported. Shuey and Cebel (*9*) found similar unsatisfactory results reported from U. S. Army and Air Force hospital laboratories not only for chemical analysis of blood, urine, and milk but also for identification of parasites or cultures of bacteria and fungi.

Little wonder, therefore, that clinicians frequently find it difficult to correlate laboratory findings with the clinical condition of patients, that the clinical staffs of hospitals distrust the results from clinical laboratories, and that some clinicians, when giving case reports, make a point of distinguishing between data originating in their own divisional laboratory and data coming from a central laboratory. This unsatisfactory state of affairs should be corrected.

It is commonly believed that the unreliability of results from clinical laboratories is due largely to inadequate training of the technical staff. This indeed is probably the chief cause of the trouble, and consideration of the proper qualifications, selection, and training of technicians for clinical chemical laboratories might well be the subject of a profitable symposium. However, other factors also are involved; the problem is more complex than it at first appears, and a careful analysis of the factors involved is desirable.

SELECTION OF METHODS

Conditions for maximum accuracy and usefulness of results exist in a clinical laboratory when the staff is properly selected and trained, adequate methods are employed, and adequate facilities (apparatus, chemicals, and working conditions) are available. Although the most important of these factors appears to be adequate training and qualification of the analyst, even a good analyst is at a disadvantage in attempting to provide an adequate number of accurate analyses with inadequate methods. And analysts who have only a moderate amount of training can obtain adequate results with good methods, but are likely to obtain erroneous values with inadequate methods.

The present task is to list and evaluate those criteria which make a method suitable for use in a clinical laboratory, taking into consideration the caliber of personality and training commonly found in the staff of a hospital or public health laboratory. Such consideration may help the director of clinical laboratories in selecting methods that will give satisfactory results and are best suited for the conditions at hand.

Proper selection of method can decrease the time required for each determination, thereby decreasing the effective load of work per technician, and also increase the precision of each determination. An ideal method measures accurately the substances or material to be determined (this implies that the method is specific); is rapid and permits early reporting of results and economy of labor; is simple (it provides less chance of error and requires less special training); and is economical—that is, it requires a minimum number of hours of work, employs relatively inexpensive reagents, and requires the simplest, most foolproof apparatus.

Another important consideration is the selection of methods that do not involve exposure to dangerous or obnoxious fumes, such as cyanide or benzene. This factor is more concerned with welfare of staff than accuracy of results.

Although no method is ideal, we can try to approach the ideal by wise selection of the methods now available and by the development of new and better methods suited to the conditions at hand.

Specificity. The specificity of any analytical method depends not only on the specificity of the reaction involved in the measurement, be that development of color in a colorimetric method or formation of a precipitate in a gravimetric method, but more particularly on the preliminary fractionation of substances capable of reacting similarly by production of color or precipitate—for example, in Sperry's cholesterol method there is a preliminary fraction of α- and β-steroids (and other lipides) preceding application of the moderately-specific Liebermann-Burchard reaction. Methods that are sufficiently specific for analysis of plant material may not be adequately specific for work on blood or urine (and vice versa), because large amounts of interfering substances often present on the one hand may be almost never present on the other hand. Furthermore, methods which have been proved adequately specific for determination of substances in blood or urine of normal individuals or patients prior to therapy may be inadequately specific for determination of the same substances after therapy—for example, in the colorimetric determination of uric acid the amount of nonuric acid chromogen is small in the case of individuals not receiving medication, but after ingestion of aspirin or sodium salicylate at least one chromogenic degradation product (probably gentisate) is present (*5*, *6*). The clinical laboratory staff must be on the alert to detect, in previously adequate methods, inadequate specificity due to the presence of new therapeutic agents or their metabolic degradation products.

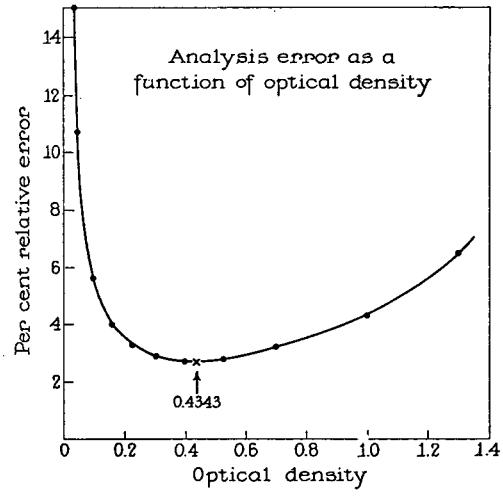


Figure 1. Relative Analysis Error as a Function of Optical Density

Relative analysis errors given on basis of 1% photometric error. Curve plotted from data recorded in Table I by Ayres (*2*). Ringbom (*6*) published similar curves with absorption as abscissa.

Accuracy and Precision. In considering the inherent accuracy or precision of a method apart from its specificity, particular attention will be paid to photometric methods of analysis because of the obvious advantages of photometry in a clinical chemical laboratory. An excellent summary of the literature concerned with accuracy in photometric analysis has been published recently by Ayres (*3*).

As has been shown by Twyman and Lothian (*12*), minimum error occurs when the transmittancy is 36.8% or the optical density 0.4343. As can be seen from Figure 1, the magnitude of the error involved in the photometric measurement increases enormously at optical densities below 0.2 or above 0.7. This corresponds to a range of transmittancy from 20 to 60%. Conditions of analysis should be so arranged that the optical density measurements fall between these two values. This usually can be achieved by adjustment of concentration of the solution, use of an absorption cell of a different thickness, adjustment of specific absorption coefficient—that is, by selection of wave length—or measurement against solutions of known concentration, rather than against a reagent blank.

The range of optical density or transmittancy corresponding to reasonable accuracy for any given system can be determined best by plotting absorptancy against the logarithm of concentration according to the method of Ringbom (*8*), as has been done in Figure 2. The point of greatest accuracy corresponds to that at which the curve has its steepest slope. Here, as for any other point on the curve, the relative analysis error for 1% photometric error is 230/slope. The slope is per cent change in absorption or transmittancy per tenfold change in concentration. Where Beer's law applies, this relative analysis error at the point of inflection is 2.72% (*3*) for a 1% photometric error. Accuracy is reasonably good over that steep portion of the curve which is

almost straight. The range of adequate accuracy is readily determined by inspection of the graph. This simple method of ascertaining the range of good accuracy for any given method is applicable whether or not the system follows Beer's law. Different systems which conform to Beer's law will have the same general form of curve with an inflection occurring at 36.8% transmittance. If the system does not conform to Beer's law, the inflection occurs at a different point. Neither system represented in Figure 2 conforms to Beer's law.

It is surprising how few authors of manuscripts on photometric methods have taken the trouble to ascertain the range over which their methods can be used with fair accuracy. Many, in apparent ignorance, recommend zero concentration as the lower limit of measurable concentration by their method; thus, they include a region in which the relative error is enormous. It is, of course, erroneous to assume that the full range of concentration over which Beer's law is followed is suitable for analysis. Adequate accuracy of a photometric method is not dependent upon whether or not Beer's law is followed. It is, of course, convenient to employ systems which conform to Beer's law, so that calculations can be made on the assumption that the optical densities of unknowns and standards are proportional to their concentrations. However, equally accurate analysis can be obtained using systems which do not conform to Beer's law, provided there are also determinations on standards slightly above and below the concentration of the unknown.

Consideration should be given to one other point which affects the accuracy of results, particularly in the regions of low optical density. This factor has nothing to do with the effect of the photometric error.

Most cuvettes used in photometry are calibrated on the basis of the transmittancy of two walls of the cuvette plus a thickness of solution of such strength as will give an over-all transmittancy between the ranges of 20 and 60%. In other words, two factors affect the over-all reading: the thickness of the colored solution, and the translucency, etc., of the two cuvette walls. Even though cuvettes giving an over-all transmittancy falling within a narrow range are selected, some cuvettes may fall within this range by virtue of having a shorter optical path compensated by a relative opacity or refractive factor in the wall of the cuvette. Furthermore, under the conditions ordinarily employed in calibration of cuvettes used for clinical work, the big factor influencing selection of proper cuvettes is the cell thickness. The variation in the transmittancy of the cuvette wall is a relatively small factor under these circumstances. However, if one uses these cuvettes with solutions having very low optical densities, a slight difference in the translucency of the cuvette walls becomes a relatively large factor. Where (as is seldom the case) one is obliged in photometry to use high transmittancies, the cuvettes should be matched with the aid of a solution of similarly high transmittancy.

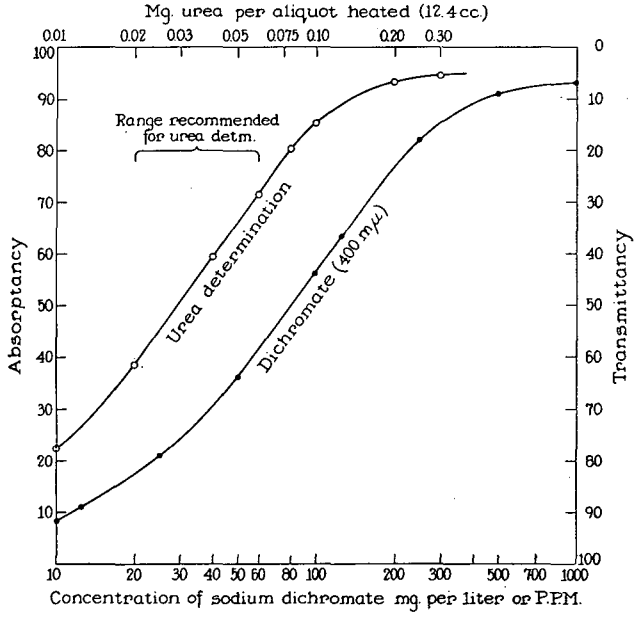


Figure 2. Standard Curves for Sodium Dichromate, 400 mμ, and for Red Color Produced in Colorimetric Determination of Urea (*1*)

Neither system conforms to Beer's law. Measurements were made in 18-mm. cuvettes in a Coleman 6A spectrophotometer.

SOURCES OF ERROR

Although it is important to select methods that are capable of giving accurate results, an inspection of the results of the survey reported by Belk and Sunderman (*4*) quickly shows that the widest discrepancies of results reported are probably due to factors other than faulty methods. It would appear profitable, therefore, to assess possible sources of error other than inadequate methods.

Collection of Sample. The initial step in the determination of the concentration of many substances in either blood or urine is the collection of the sample. As this takes place in the ward or the clinic, the laboratory technicians have nothing to do with it. However, mistakes can be made in the collection of samples sent to laboratories for analysis. Occasionally a sample becomes labeled with the name of the wrong patient. If blood for nonprotein nitrogen or calcium determination is collected in a tube containing ammonium oxalate, the concentrations reported by the laboratory are bound to differ from those circulating in the patient. A gross excess of anticoagulant has sometimes made it difficult to obtain clear filtrates. It is desirable that the ward and clinic staff recognize the importance of the correct amount of kind of anticoagulant for each determination, that clean receivers be used for collection of specimens of plasma or serum, and that specimens reach the laboratory promptly after collection. This is especially true in the case of samples sent for determination of glucose and is desirable even after the addition of fluoride.

Within the laboratory, mistakes result from the use of reagents of the wrong strength, poor operative technique, dirty apparatus, omitted portions of procedures, or other errors.

Reports of Results. Other very important causes of error are mistakes in the calculation of results or in the transcription of records. A misplaced decimal or omission of a dilution factor can easily spoil an otherwise adequate determination, and may well be the cause of a large number of the errors found in the survey reported by Belk and Sunderman. A fairly simple way of detecting errors due to use of reagents of wrong concentration, faulty operation of instruments, omission of part of a procedure, or errors in calculation is to run with every set of determinations a complete procedure including all the preliminary steps of preparation, using an aliquot from a large pool of an unknown. This pool should be reserved solely for use as a control and stored in a deep-freeze or other suitable location. If this control is run through all the steps involved in the preparation of the unknown samples and through the calculations simultaneously with the calculations of the unknowns, an error, if present, should be detected when the results are inspected. It would seem highly desirable to make routine practice the running of such a control with every set of determinations. The need for this control is probably greatest in laboratories that feel they can least afford the time for it; however, adoption of this suggestion would probably pay high dividends. Not only would errors be detected readily, but after several months the director of the laboratory would have available from collected reports an assessment of the reliability of his laboratory staff and of the methods with respect to each procedure so controlled.

What constitutes adequate accuracy of a procedure for use in a clinical chemical laboratory? The criteria of accuracy should depend not only on immediate use of the results, but also on what use someone may elect to make of them in the future. If results are to be used only as diagnostic aids or as guides in the

control of therapy, any result may be adequately accurate, provided it deviates from the true figure by not more than half of whatever deviation would be clinically significant for the determination in question. Most published methods are capable of providing this degree of accuracy. What constitutes a clinically significant difference for any one constituent is a question that may be by-passed for the moment, but the permissible percentage error will vary considerably from one determination to another. This standard is probably adequate for many occasional analyses for blood sugar, nonprotein nitrogen, blood urea, urea clearance, uric acid, etc., but not if the results are to be used to study the effect of some form of treatment on the blood or urine level of the constituent measured. In such an instance a much higher degree of accuracy would be required; for most research purposes higher criteria of accuracy must be set up. Even within the field of research the degree of accuracy necessary will vary with the problem and will depend on what use is to be made of the results.

No attempt is made here to specify which of several methods is best suited for the determination of any one constituent of biological material, for the methods best suited for one laboratory are not necessarily those best suited for another. Selection of the best method for each type of determination should depend upon the apparatus available, the work load per member of staff, training and personality, and to a very considerable extent on the use to which the data are to be put, not only in the immediate future but also in time to come.

Explicit Directions. Mention should be made of the need for careful preparation of explicit directions for the laboratory staff. Each step, from the receipt of the sample to the delivery of the calculated result, should be described in terms devoid of any ambiguity. Preparation of such directions usually requires considerable experience in the management of laboratory personnel. If any ambiguity remains in the final directions some technician is almost sure to find it and to proceed according to the unintended meaning.

Sperry (*10*) has pointed out the need for an authoritative manual which would serve for clinical chemists the same purpose served by the official manual for agricultural chemists (*2*). A technical manual (*13*) to some extent fills this need for the Army. "Quantitative Clinical Chemistry" (*7*) has long been an authoritative and indispensable guide for many methods. There is, nevertheless, a need for a manual which will undergo periodic and more frequent revision by a competent board.

For the sake of the patient who pays for the analyses done in the clinical laboratory, methods should be selected with an eye to economy, even though it is important to emphasize accuracy of results rather than minimum cost. Inasmuch as the technicians' time is a cost factor about nine times as great as that of either material or equipment, the cost of materials is a relatively small item. Therefore, emphasis should be placed on the selection of methods that can be completed quickly. Furthermore, if purchase of new apparatus facilitates more rapid work, a large initial outlay for equipment will soon be balanced by a saving in technicians' time (*11*).

At present, the patient (unaware) often gets poor value for his money. Even if it were to cost twice as much to obtain a reliable report, the patient would be getting a better value.

TRAINING OF TECHNICAL STAFF

The results from any procedure can be no better than the operator who conducts it. Therefore adequate training and motivation of the technical staff are prerequisites to good results. However important the chemical aspect of this training may be, even more important is that nontechnical training which starts at birth and determines the presence or absence of those essential personal traits that characterize the operator who is careful, systematic, conscientious, neat, thoughtful, and industrious.

Analytical results from industrial laboratories are, in general, more reliable than those from clinical laboratories because the directors of industrial laboratories, strongly motivated by financial considerations, exert greater control on the activities of the technicians than is often the case in clinical laboratories. The training of the director of an industrial laboratory is frequently chiefly chemical, whereas that of the chief in the clinical laboratory is often primarily medical. The director of the clinical laboratory must often split his time between laboratory duties and more lucrative clinical practice. In general, the salaries paid to hospital technicians are less than those paid to their industrial counterparts. Better salary would permit selection of the more desirable analysts.

SUMMARY AND RECOMMENDATIONS

The results of chemical analysis being reported by most clinical laboratories are very inadequate. Gross errors are partly due to mistakes made in the collection of the specimens in the wards or clinics, but to a very large extent are made within the confines of the laboratory itself.

Although much of the inadequacy can be attributed to personnel unqualified either because of personality characteristics or by lack of training, much can be done to improve the situation with present personnel.

The director of the laboratories should select methods capable of giving accurate results. Whenever possible, photometric measurements should be made only within the range of optical densities consistent with good accuracy. In most instances this is between 0.2 and 0.7.

The technical staff should be provided with explicit typewritten or printed directions, free of ambiguity.

Each set of determinations should include an aliquot from a large stock pool. This aliquot should be run through all the steps of the procedure and calculated simultaneously with the unknowns, so that if any reagents or instruments are not as they should be, if any step in the procedure is omitted, or if any error is made in the calculations this will become apparent.

Some responsible individual other than the technician should check the calculations before the results are reported.

From time to time, the director of the laboratory should provide the technicians with solutions, the concentration of which is known only to the director. Results from analysis of these will provide not only an index of the reliability of the members of the technical staff, but also an indication of the adequacy of the methods being employed.

The director of the clinical laboratory, whether an M.D. or not, should have the technical approach and the philosophy of the quantitative chemist. There should be adequate motivation for this director to devote the necessary time to the control of the technical staff.

LITERATURE CITED

(1) Archibald R. M., *J. Biol. Chem.*, **157**, 507 (1945).
(2) Assoc. Offic. Agr. Chemists, "Official and Tentative Methods of Analysis," 6th ed., 1945.
(3) Ayres, G. H., ANAL. CHEM., **21**, 652 (1949).
(4) Belk, W. P., and Sunderman, F. W., *Am. J. Clin. Path.*, **17**, 853 (1947).
(5) Kapp, E. M., and Coburn, A. F., *J. Biol. Chem.*, **145**, 549 (1942).
(6) Lutwak-Mann, C., *Biochem. J.*, **37**, 246 (1943).
(7) Peters, J. P., and Van Slyke, D. D., "Quantitative Clinical Chemistry," Vol. II, Baltimore, Williams & Wilkins Co., 1932.
(8) Ringbom, A., *Z. anal. Chem.*, **115**, 332 (1939).
(9) Shuey, H. E., and Cebel, J., *Bull. U. S. Army Med. Dept.*, **9**, 799 (1949).
(10) Sperry, W., *Chem. Eng. News*, **28**, 159 (1950).
(11) Stowe, W. P., *Am. J. Clin. Path.*, **9**, 239 (1939).
(12) Twyman, F., and Lothian, G. F., *Proc. Phys. Soc. (London)*, **45**, 643 (1933).
(13) U. S. Army, *Technical Manual* **8-227** (1946) (Restricted).

RECEIVED November 18, 1949. Presented before the Division of Biological Chemistry, Symposium on Clinical Chemistry, at the 116th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Conversion of Refractive Dispersions to Other Wave-Length Intervals

ALBERT SANKIN, C. C. MARTIN, AND M. R. LIPKIN
Sun Oil Company, Norwood, Pa.

A general relationship of the form $(n_x - n_y) \times 10^4 = K(n_u - n_v) \times 10^4 + K'$, where n_x, n_y, n_u, and n_v are refractive indexes of a single liquid at four different wave lengths, has been found to apply to liquids having F–C dispersions less than 250. This linear equation may be used for calculating one dispersion from another for both hydrocarbon and nonhydrocarbon liquids with an average deviation of two units of dispersion. The parameters, K and K', in the above equation are calculated from: $K = (\nu_x^3 - \nu_y^3)/(\nu_u^3 - \nu_v^3)$ and $K' = C_2 \times 10^4[\nu_x^2 - \nu_y^2 - K(\nu_u^2 - \nu_v^2)]$ in which ν is defined as the reciprocal of wave length. Satisfactory agreement with most data is obtained by considering C_2 to be the same for all liquids having F–C dispersions less than 250. The above expressions have been derived from the refractive index–wave-length equation: $n = C_1 + C_2\nu^2 + C_3\nu^3$, in which the appearance of ν^3 has been empirically verified, although it seems to disagree with generally accepted dispersion theory.

BOTH refractive index and refractive dispersion have been widely used in the field of hydrocarbon analysis (*13*, *19*, *24*, *26*, and references therein). Work in this field has been hampered by lack of uniformity in choice of wave lengths of light with which these properties have been obtained.

The *D* line of sodium is now widely used for refractive indexes and most refractometers utilizing white light are calibrated to give readings in terms of this line. There is no such general agreement in regard to the wave lengths to be used in determining refractive dispersion. Although correlations have generally used the dispersion for the *F* and *C* lines of hydrogen, $(n_F - n_C) \times 10^4$, Dixmier (*4*), Thorne, Murphy, and Ball (*33*), and Ward and Fulweiler (*35*) have worked with other combinations of wave lengths. The use of the *g* line of mercury and the *D* line of sodium has been advocated (*33*) because these two lines are more easily obtained experimentally than are the *F* and *C* lines. Furthermore, the *g–D* dispersion is larger than the *F–C* dispersion, which minimizes the effect of experimental error.

A simple method for the calculation of a desired refractive index or dispersion from data at other wave lengths would enhance the value of existing correlations by enabling an analyst to use them even when his experimental data are not for the same wave lengths. Such a method would also enable a correlator to use more of the available literature data than has heretofore been possible.

Various equations have been proposed for the relationship between refractive index and wave length. This subject has been reviewed from the physicists' viewpoint by Korff and Breit (*17*) and from the petroleum chemists' viewpoint by Thorne, Murphy, and Ball (*33*) and by Ward, Kurtz, and Fulweiler (*37*).

In the following review λ is the wave length at which the refractive index, n, is measured and $\nu = 1/\lambda$.

The Sellmeier-Drude equation (*5*, *31*):

$$n^2 - 1 = \sum \frac{A_i}{\nu_i^2 - \nu^2} \qquad (1)$$

can be derived theoretically both by classical and quantum mechanical methods (*17*). In this equation each ν_i is a characteristic molecular constant, equal to the reciprocal of wave length at an absorption band, and there is a term for every absorption band.

There is some theoretical basis for writing Equation 1 with the Lorentz-Lorenz (*27*, *28*) expression substituted for $n^2 - 1$. However, a set of data agreeing with one form of this equation can be made to agree as well with the other form, as long as the temperature is held constant, so that the density does not vary. Because there is no way of distinguishing between the two forms of Equation 1 on the basis of experimental data, the Lorentz-Lorenz form will not be further considered.

Inasmuch as the number of terms which might be anticipated for Equation 1 would make it extremely difficult to apply the equation directly, the assumption is frequently made that only one term is significant. The assumption of the one-term Sellmeier-Drude equation:

$$n^2 - 1 = \frac{A}{\nu_o^2 - \nu^2} \qquad (2)$$

is reasonable because, at a given wave length, one of the terms in Equation 1 may have a much smaller denominator than any other. Because the most important characteristic frequencies affecting dispersion in the visible range are those for the electronic transitions in the ultraviolet range (wave length about 0.1 micron or 1000 A. for hydrocarbons), it is reasonable to expect that the parameters in Equation 2 would be related to electronic configuration. This is borne out by the work of Kurtz and Ward (*20*) and Kurtz and Lipkin (*18*), which shows that these parameters can be calculated from consideration of the valence electrons in saturated hydrocarbons.

The Cauchy equation:

$$n = A + \frac{B}{\lambda^2} + \frac{C}{\lambda^4} + \ldots = A + B\nu^2 + C\nu^4 + \ldots \qquad (3)$$

although it was originally deduced independently (*17*), may be derived directly from Equation 1 or 2 (*16*), providing ν is smaller than ν_i, which is generally the case when ν is in or near the visible range.

The simplest equation which has been used to represent the relationship between refractive index and wave length is the simplified Cauchy equation:

$$n = A + B/\lambda^2 = A + B\nu^2 \qquad (4)$$

If n_x, n_y, n_u, and n_v are the refractive indexes when ν has the values ν_x, ν_y, ν_u, and ν_v, respectively, then from Equation 4:

$$n_x - n_y = \frac{\nu_x^2 - \nu_y^2}{\nu_u^2 - \nu_v^2}(n_u - n_v) \qquad (5)$$

This form of the simplified Cauchy equation involves no empirical constants and therefore is useful for petroleum fractions (*36*). It fails, however, to represent adequately the properties of samples having relatively high dispersions, such as aromatic hydrocarbons (*33*).

The Hartmann equation (*14*):

$$n = n_o + \frac{C}{(\lambda - \lambda_o)^a} \qquad (6)$$

and the simplified Hartmann equation (*14*):

$$n = n_o + \frac{C}{\lambda - \lambda_o} \quad (7)$$

are strictly empirical. It has recently been shown that Equation 6 may be applied to a large number of hydrocarbons if a is kept equal to 1.6 (*8*):

$$n = n_o + \frac{C}{(\lambda - \lambda_o)^{1.6}} \quad (8)$$

Some of the parameters in these equations have been found to be constant for certain groups of liquids (*8, 18, 20*). However, the calculation of one dispersion from another, using any but the simplified Cauchy (Equation 5), necessitates empirical evaluation of parameters for individual liquids, or some knowledge of compositions of the liquids being considered. The usefulness of the equation is, therefore, limited.

This paper presents an empirical relationship between refractive index and wave length which can be used for the calculation of one dispersion from any other. The method involves no empirical evaluation of parameters, and may be applied to both hydrocarbon and nonhydrocarbon liquids having F–C dispersions less than 250. The results obtained are within experimental error for most samples.

DEVELOPMENT OF CORRELATION

In 1946 Lipkin and Martin (*25*) published a method for the calculation of F–C specific dispersion, $\frac{(n_F - n_C) \times 10^4}{d}$, of hydrocarbon liquids from density, molecular weight, and the sodium D refractive index. This method was based upon the linear relationship between F–C specific dispersion and a function of molecular weight, density, and refractive index. It was reasoned that there was nothing unique about F–C specific dispersion, and therefore a linear relationship should exist between any specific dispersion and the previously mentioned function. This, in turn,

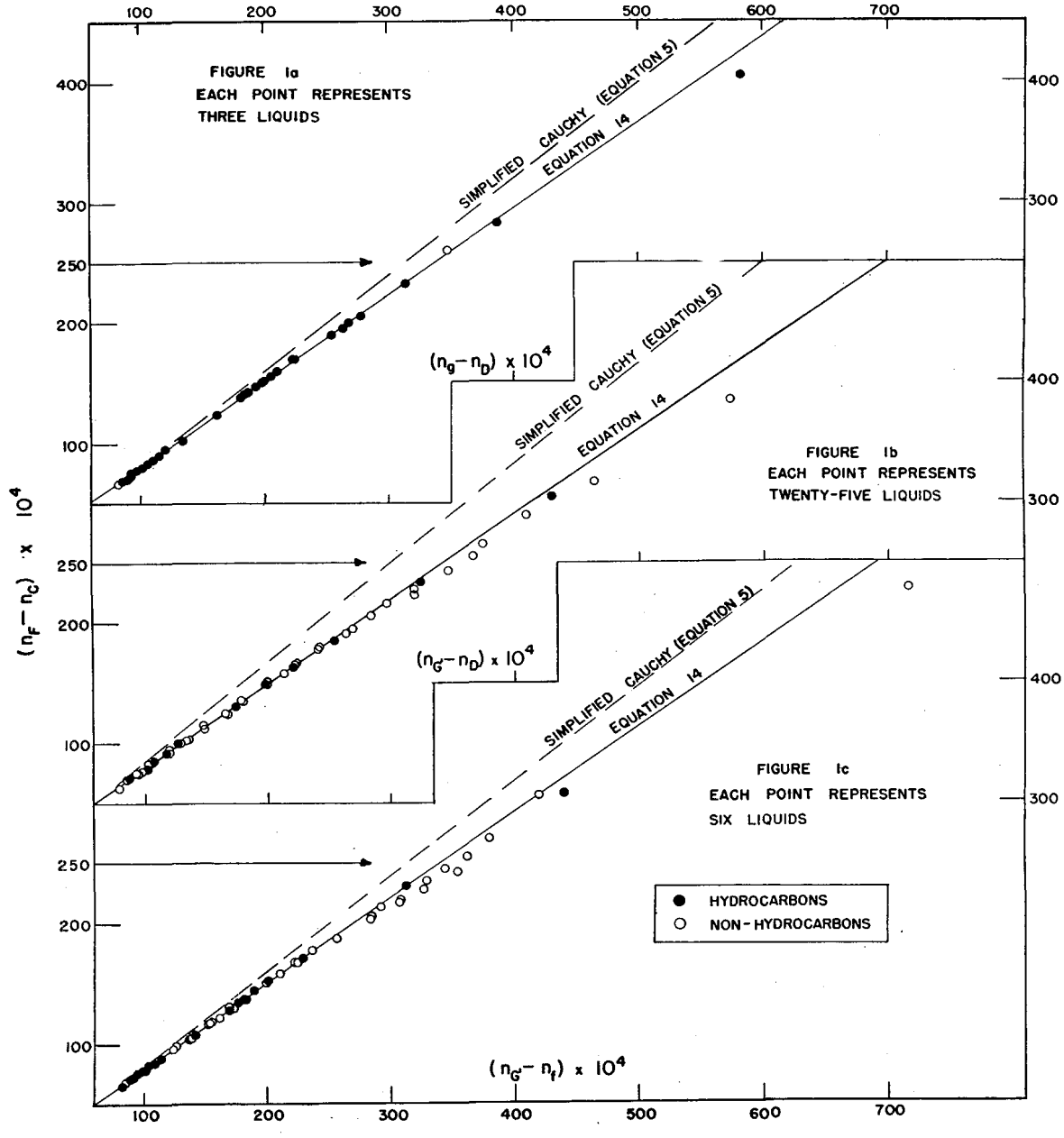


Figure 1. Linear Relationships between Dispersions Involving Commonly Used Wave Lengths of Light

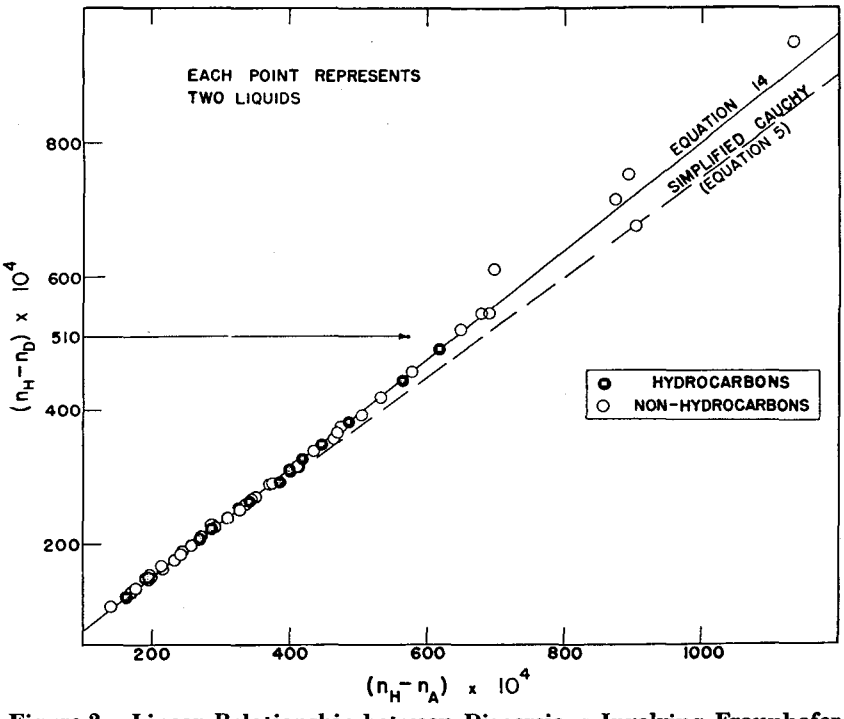


Figure 2. Linear Relationship between Dispersions Involving Fraunhofer Lines at Extremes of Visible Spectrum

implied a linear relationship between any two specific dispersions of a liquid, and probably between any two dispersions. Empirically, the linear relationship between dispersions was found to be a slightly better representation of the data than was the one involving specific dispersions. Although these relations were predicted on the basis of a correlation which is applicable only to hydrocarbons, they are satisfactory for most nonhydrocarbons.

Figures 1 and 2 are plots of one dispersion against another for various combinations of wave lengths. These graphs include vastly different types of liquids, both hydrocarbon and nonhydrocarbon. The data appearing in these graphs and all the data used in the subsequent development and testing of this method are from the following sources: (*1–3, 6–12, 15, 21–23, 29, 30, 32–35, 38*).

Because the various graphs in this paper contain the data for a total of more than 2000 liquids, some reduction in the number of points actually plotted was necessary. It was desirable to preserve as closely as possible with reduced numbers of points the trends shown by all the liquids. Therefore, the average points were calculated from liquids which were close to one another on the graphs, while the distinction between hydrocarbons and nonhydrocarbons was the only one that was made as to type. The ratios between numbers of liquids and numbers of points shown in the figures are approximate; strict adherence to these ratios would have resulted in some cases in the distortion of trends.

The simplified Cauchy equation (Equation 5) can be seen to represent only the very low dispersion liquids. The data, however, follow straight lines from the lower limit of liquid dispersions up to 250 *F–C* dispersion or, in the case of Figure 2, 510 *H–D* dispersion. These lines have neither the slopes nor the zero intercepts predicted by the simplified Cauchy.

DERIVATION OF EQUATION

Because none of the previously discussed equations would predict the linear relationships which were found empirically, the following generalized power series relating refractive index and wave length for a single liquid was considered:

$$n = C_1 + C_2\nu^s + C_3\nu^t \qquad (9)$$

where s and t are undetermined exponents. If n_x, n_y, n_u, and n_v are once again the refractive indexes when $\nu = \nu_x$, ν_y, ν_u, and ν_v, respectively, then:

$$n_x = C_1 + C_2\nu_x^s + C_3\nu_x^t$$

$$n_y = C_1 + C_2\nu_y^s + C_3\nu_y^t$$

$$n_x - n_y = C_2(\nu_x^s - \nu_y^s) + C_3(\nu_x^t - \nu_y^t)$$

$$C_3 = \frac{n_x - n_y}{\nu_x^t - \nu_y^t} - C_2\left[\frac{\nu_x^s - \nu_y^s}{\nu_x^t - \nu_y^t}\right]$$

Similarly:

$$C_3 = \frac{n_u - n_v}{\nu_u^t - \nu_v^t} - C_2\left[\frac{\nu_u^s - \nu_v^s}{\nu_u^t - \nu_v^t}\right]$$

Equating the two values of C_3 and solving for x–y dispersion:

$$n_x - n_y = \frac{\nu_x^t - \nu_y^t}{\nu_u^t - \nu_v^t}(n_u - n_v) + C_2\left\{\nu_x^s - \nu_y^s - \left(\frac{\nu_x^t - \nu_y^t}{\nu_u^t - \nu_v^t}\right)(\nu_u^s - \nu_v^s)\right\} \qquad (10)$$

If t, C_2, and s were the same for all liquids, Equation 10 would be linear and would not pass through the origin. This is the type of equation needed to represent the dispersion data. In order to determine if t could be constant, the slopes of the lines in Figures 1 and 2 were calculated by the method of least squares from the data for the hydrocarbon liquids having *F–C* dispersions less than 250 or *H–D* dispersions less than 510. Each of these "least squares slopes" was equated to the expression for the slope in Equation 10 and t was evaluated therefrom.

Table I. Evalation of Exponent *t*

Figure	Least Squares Slope	*t* from Least Squares Slope	Calculated Slope if $t = 3.00$
1a	0.721	2.97	0.718
1b	0.684	3.23	0.704
1c	0.730	2.72	0.708
2	0.800	2.83	0.809
Av.	...	2.94	...

Nonhydrocarbons were not included in this evaluation because some of them show evidence of systematic deviation and, although the correlation is satisfactory for most nonhydrocarbons, it is primarily intended for use in hydrocarbon analysis.

As a further check on the value of t, it was desirable to calculate this parameter from the data for a large number of wave lengths. Unfortunately, there are few reliable data in the literature for wave lengths other than those already used for this purpose. It was therefore decided to use only the excellent data available for distilled water (*34*) and toluene (*29*). These compounds differ sufficiently in dispersion to allow high precision in the evaluation of t. This evaluation was performed for various combinations of seven wave lengths in the visible spectrum. The average value of t thus obtained was 2.88 with almost negligible deviations from this value.

Although the agreement between the values of t from the data in Table I and from water and toluene was not perfect, it was close enough to indicate its probable constancy. Because the average calculated value of t was very close to an integer, it was convenient to assume $t = 3.00$ in the further development of this method. As can be seen from the last column of Table I, the

values of the slope calculated from $t = 3.00$ are not far from the empirical values.

Constant values of s and C_2 cannot be established as definitely as could the value of t, for a change in one can generally be compensated by a change in the value of the other. It was possible, however, to judge whether s should be greater or smaller than t. There were several choices for a value of s which, when considered together with a value of t equal to 3.0, gave systematic power series. Some of them were $s = 1.0$, 1.5, 2.0, 4.0, 5.0, and 6.0. Inasmuch as the first three were empirically indistinguishable from each other, 2.0 was chosen for comparison with 4.0 and 6.0. This comparison was made using combinations of refractive indexes for visible and ultraviolet radiation. The visible range is too small to be used alone without a serious loss of precision.

Refractive indexes in the ultraviolet range recently were measured on 21 hydrocarbons by Lauer (*22*, *23*). Of these, 14 were American Petroleum Institute–National Bureau of Standards hydrocarbons on which indexes in the visible range had been reported by Garrett (*9*) or Forziati (*8*). Visible and ultraviolet data for these compounds were substituted in the following equation in which the wave lengths are expressed in microns. (The micron, 10,000 A., although not generally used in the visible and ultraviolet ranges, is the most convenient unit of wave length when reciprocals are to be calculated.)

$$(n_x - n_{0.3500}) \times 10^4 = K\,(n_{0.2500} - n_{0.4861}) \times 10^4 + K' \quad (11)$$

where $$K = \left[\left(\frac{1}{\lambda_x}\right)^3 - \left(\frac{1}{0.3500}\right)^3\right] \div \left[\left(\frac{1}{0.2500}\right)^3 - \left(\frac{1}{0.4861}\right)^3\right] \quad (11a)$$

and $$K' = C_2 \left\{\left(\frac{1}{\lambda_x}\right)^s - \left(\frac{1}{0.3500}\right)^s - K\left[\left(\frac{1}{0.2500}\right)^s - \left(\frac{1}{0.4861}\right)^s\right]\right\} \times 10^4 \quad (11b)$$

Table II. Evaluation of Exponent s

s Assumed Equal to	λ_x[a]	K	Agreement of Calcd. and Exptl. $(n_x - n_{3500}) \times 10^4$: Av. deviation	Deviation of av.
2.0	0.2947	0.285	1	0
4.0	0.2817	0.387	4	+4
6.0	0.2726	0.471	8	+8

[a] Because refractive indexes at these wave lengths were not experimentally determined, they were obtained by linear interpolation between the two closest experimental values. This was considered sufficiently accurate because indexes were reported at intervals of 0.01 micron or 100 A.

Table III. Wave Lengths of Some Visible Spectral Lines

(Values of ν^2 and ν^3 listed may be substituted directly in Equation 14)

Color	Source	Designation	Wave Length, Micron	ν^2, Microns^{-2}	ν^3, Microns^{-3}
Violet	Ca	H	0.39685	6.3496	16.0002
Violet	H	h or $H\delta$	0.41018	5.9438	14.4908
Blue-violet	H	G' or $H\gamma$	0.43405	5.3080	12.2290
Blue-violet	Hg	g	0.43583	5.2645	12.0792
Blue-violet	He	i	0.44715	5.0015	11.1853
Blue	He	c	0.47131	4.5017	9.5514
Blue	H	F or $H\beta$	0.48613	4.2315	8.7043
Blue-green	He	v	0.50157	3.9750	7.9252
Green	Hg	e	0.54607	3.3535	6.1411
Yellow-orange	He	f, E or d	0.58756	2.8966	4.9299
Yellow-orange	Na	D_1D_2 or D	0.58926[a]	2.8800	4.8874
Red	H	C or $H\alpha$	0.65628	2.3218	3.5377
Red	He		0.66782	2.2423	3.3576
Red	O	A	0.7608[a]	1.7277	2.2709

[a] Average value of doublet lines.

Three values of λ_x were chosen so that K' would equal zero if s equals 2.0, 4.0, and 6.0, respectively. Because this eliminated C_2, deviations from the equation could then be attributed to an incorrect assumption of the value of s. The assumption that $s = 2.0$ yielded the best results, as shown in Table II.

Equation 9 therefore became

$$n = C_1 + C_2\nu^2 + C_3\nu^3 \quad (12)$$

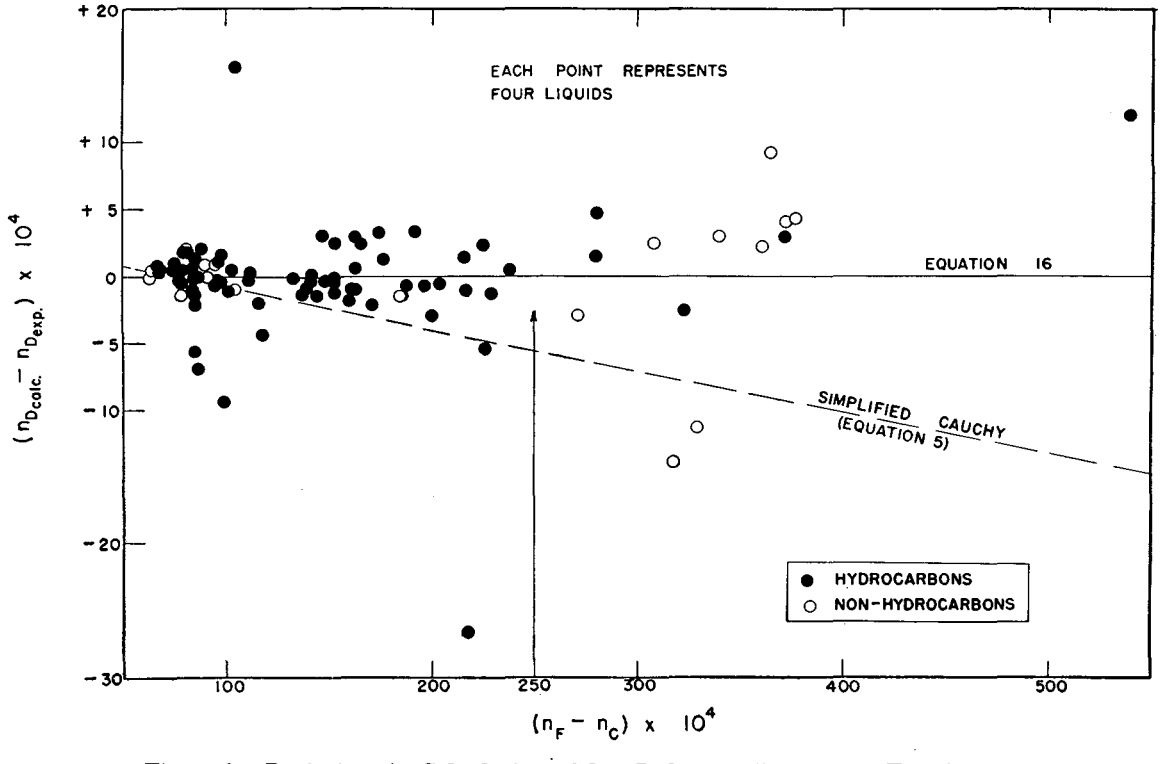


Figure 3. Deviations in Calculation of One Refractive Index from Two Others

Table IV. Agreement of Data with Equation 14 for Various Combinations of Wave Lengths

[Calculated minus experimental $(n_x - n_y) \times 10^4$ tabulated for liquids having F–C dispersions less than 250]

Wave length, Micron[a]						Plotted in	All Data			Concordant Data			Discordant Data		
λ_x	λ_y	λ_u	λ_v	K	K'	Figure	No.	Av. dev.	Dev. av.	No.	Av. dev.	Dev. av.	No.	Av. dev.	Dev. av.
0.4861	0.6563	0.4358	0.5893	0.718	+7.5	1,*a*	152	1.2	+0.1	152	1.2	+0.1	0	...	...
0.4861	0.6563	0.4340	0.5893	0.704	+7.6	1,*b*	1199	3.2	+1.4	1102	2.0	+0.7	97	16.8	+9.9
0.4861	0.6563	0.4340	0.5876	0.708	+7.7	1,*c*	285	3.1	+2.4	254	1.7	+0.9	31	14.8	+14.3
0.3968	0.5893	0.3968	0.7608	0.809	−10	2	111	3	−2[b]	111	3	−2[b]	0	...	...
0.5893	0.6563	0.4861	0.6563	0.261[c]	+2.3	3	410	1.4	−0.3	396	1.0	−0.1	14	12.9	−6.3
0.4861	0.6563	0.3000	0.4000	0.241	+28	...	22	8	+8	22	8	+8	0	...	...
0.2947	0.3500	0.2500	0.4861	0.285	0	...	21	2	+1	21	2	+1	0	...	...
0.2800	0.3500	0.2500	0.5893	0.376	−13	...	21	3	+2[b]	21	3	+2[b]	0	...	...
						Total	2221	2.7	+1.1	2079	1.8	+0.5	142	16.0	+9.2

[a] Sources of visible wave lengths used here may be found in Table III.
[b] Deviations of average are reported with reversed signs when K' is negative. (If a sample deviates positively from Equation 14 when K' is positive the same sample deviates negatively for a combination of wave lengths for which K' is negative.)
[c] Equation 16 used here. In this case calculated minus experimental $n_D \times 10^4$ is tabulated.

Table V. Agreement of Data with Equation 14 for Various Types of Liquids

[Calculated minus experimental $(n_x - n_y) \times 10^4$ tabulated for liquids having F–C dispersions less than 250]

Type of Sample[a]	All Data			Concordant Data			Discordant Data		
	No.	Av. dev.	Dev. av.	No.	Av. dev.	Dev. av.	No.	Av. dev.	Dev. av.
Saturated compounds	297	1.6	+1.3	293	1.5	+1.3	4	12.3	+3.8
Benzenes	149	1.8	−0.3	149	1.8	−0.3	0	...	...
Naphthalenes	2	1.1	+0.5	2	1.1	+0.5	0	...	...
Olefins	246	2.4	+0.1	231	1.7	−0.2	15	12.6	+5.3
Atomic bridged compounds	16	9.2	+8.9	11	1.6	+1.2	5	25.9	+25.9
Conjugated aromatic olefins	58	6.0	−0.7	48	1.9	−0.3	10	25.8	−2.6
Acetylenes	20	1.7	−0.9	20	1.7	−0.9	0	...	...
Nonhydrocarbons	1302	3.1	+1.5	1194	2.0	+0.7	108	15.2	+10.3
Mixtures[b]	113	1.0	−0.3	113	1.0	−0.3	0	...	...
Glasses	18	1.7	−1.7	18	1.7	−1.7	0	...	...
Total	2221	2.7	+1.1	2079	1.8	+0.5	142	16.0	+9.2

[a] If a liquid could logically be placed in more than one of these groups, it has been placed in the one with the lowest listing. Thus a mixture of acetylenes would be placed under "mixtures" and an olefinic acetylene would be placed under "acetylenes."
[b] This group consists mainly of petroleum fractions.

Table VI. Distribution of Deviations from Equation 14

[Calculated minus experimental $(n_x - n_y) \times 10^4$ tabulated for liquids having F–C dispersions less than 250]

Deviations	Number	% of Total
< −20	10	< 1
−11 to −20	9	< 1
−8, −9, −10	17	1
−6, −7	36	2
−5	20	1
−4	34	2
−3	77	3
−2	149	7
−1	250	11
0	506	23
+1	356	16
+2	311	14
+3	143	6
+4	81	4
+5	52	2
+6, +7	49	2
+8, +9, +10	41	2
+11 to +20	46	2
> +20	34	2
Total	2221	100

The exponent of the second term has been proved to be between 0 and 3.0 and is probably between 1.0 and 2.0.

Although the C_1 term does not appear in Equation 10 and therefore is not verified by any calculations with which this paper is directly concerned, such a term must be included to make Equation 12 valid. In fact, C_1 will always be almost as large as n. For example, the data in the visible range for toluene (*29*) may be represented within experimental error by:

$$n^{20} = 1.47657 + 0.00477\nu^2 + 0.00135\nu^3 \qquad (13)$$

with ν expressed in reciprocal microns.

The best average value for C_2 in the 50 to 250 F–C dispersion range was found and Equation 10 became:

$$(n_x - n_y) \times 10^4 = K\,(n_u - n_v) \times 10^4 + K' \qquad (14)$$

where

$$K = \frac{\nu_x^3 - \nu_y^3}{\nu_u^3 - \nu_v^3} \qquad (14a)$$

and $K' = C_2 \times 10^4\,[\nu_x^2 - \nu_y^2 - K(\nu_u^2 - \nu_v^2)] =$

$$38.0\,[\nu_x^2 - \nu_y^2 - K\,(\nu_u^2 - \nu_v^2)] \qquad (14b)$$

Equation 14 may be used for calculating any desired dispersion of a liquid from any given one, providing the F–C dispersion is less than 250.

Table III lists the wave lengths in microns of the most frequently used visible spectral lines, and also values of ν^2 and ν^3 which may be substituted in Equation 14a and 14b. Thus, for the g–D to F–C conversion:

$$K = \frac{(8.7043 - 3.5377)}{(12.0792 - 4.8874)} = 0.718$$

$$K' = 38.0\,[4.2315 - 2.3218 - 0.718\,(5.2645 - 2.8800)] = 7.5$$

$$(n_F - n_C) \times 10^4 = 0.718\,(n_g - n_D) \times 10^4 + 7.5 \qquad (15)$$

With this equation it is possible to use the existing methods of hydrocarbon analysis based upon F–C dispersion while experimentally determining the more convenient g–D. If a given conversion is to be used frequently, it may be desirable to make a plot of the type shown in Figures 1 and 2, calculated from Equation 14.

TESTING OF CORRELATION

Figures 1 and 2 show the agreement of the data with Equation 14. Figure 3, on the other hand, illustrates another application of this correlation. If v is allowed to equal y, Equation 14 becomes:

$$n_x = (1 - K)\,n_y + Kn_u + 10^{-4}K' \qquad (16)$$

Using Equation 16 it is possible to calculate a desired refractive index for a liquid from two other refractive indexes, providing the F–C dispersion of the liquid is less than 250. Figure 3 is a plot of deviations from Equation 16 against F–C dispersion for the calculation of the sodium D refractive index from those of the F and C lines of hydrogen.

Table VII. Conversion of Dispersions for API–NBS

Type	No. of Compounds	Calculated Minus Experimental $(n_F - n_C) \times 10^4$ Av. deviation	Deviation of av.	Max. deviation
Paraffins	18	1.7	+1.7	+2.2
Cycloparaffins	21	0.9	+0.9	+1.1
Alkylbenzenes	21	1.3	−1.3	−2.3
	60	1.3	+0.4	−2.3

The agreement of the data for Figures 1 to 3 as well as some calculations for the ultraviolet range are summarized in Tables IV to VI. The tables include data at many different temperatures with no distinction made among them. Most of these data are in the range 10° to 40° C. and within this range temperature has no appreciable effect. The indications are that Equation 14 with the average value of C_2 will satisfactorily represent data up to 100° C., providing all measurements on the sample are made at the same temperature. However, this has not been completely checked.

The data are separated into groups of concordant and discordant data. The discordant data are for liquids which, for a particular conversion, show deviations greater than 3.33 times the average deviation for all liquids.

Table IV shows that the average deviations are generally largest for those calculations involving the largest magnitudes of K'. This indicates that the principal source of error in the correlation is the assumption of a constant value for C_2 in Equation 14. The average deviation from a particular calculation is in general less than 30% of the magnitude of K'. For most calculations in the visible range this is less than 2.0, which approaches the experimental accuracy of most of the literature data. The data for the *g–D* to *F–C* conversion show the most consistent agreement with the correlation. These dispersions are relatively easily measured and have been determined most recently.

As an illustration of the application of the *g–D* to *F–C* conversion to a group of accurate hydrocarbon data recently obtained in a single laboratory, we may consider the dispersions of 60 A.P.I.–N.B.S. hydrocarbons reported by Forziati of the National Bureau of Standards (*8*). Table VII summarizes these data.

Table V shows that only 4% of the hydrocarbons are listed as discordant and that few of the discordant data are for the types of hydrocarbons that are most likely to be found in petroleum. Furthermore, the petroleum fractions, which constitute more than 80% of the "mixtures," show excellent agreement with the correlation. A possible explanation of the large deviations of some olefins and conjugated aromatic olefins is the experimental difficulty caused by the tendency of these compounds to undergo polymerization and atmospheric oxidation. In the 180 to 250 *F–C* dispersion range some nonhydrocarbons have been found for which the *F–C* dispersions calculated from the *G'D* and *G'f* dispersions are larger than the experimental values. This is the only definite evidence of serious systematic deviation that has as yet been found.

Even with the inclusion of the discordant data, Table VI shows that half of the liquids have deviations of 1 or less and more than 70% of them have deviations of no more than 2.

Most naphthalenes have *F–C* dispersions of almost 300 and are, therefore, beyond the range of this correlation, as are anthracenes and phenanthrenes. Most of the very few data in the high dispersion range are for nonhydrocarbons. In fact, with the exception of the previously mentioned polynuclear aromatics, none of the liquids having *F–C* dispersions beyond the range of the correlation are likely to be found in petroleum.

This correlation should be satisfactory for liquid mixtures having *F–C* dispersions less than 250 even if they have some high dispersion components.

DISCUSSION

A study of the calculations and of the figures indicates that Equation 12 can be extended, as follows, so that it applies to liquids which have *F–C* dispersions higher than 250:

$$n = C_1 + C_2\nu^2 + C_3\nu^3 + C_4\nu^4 + \ldots \qquad (17)$$

It would be extremely difficult to evaluate empirically both the exponents and coefficients for all the significant terms in this equation. However, consideration of the data shows that all the coefficients in this series are probably positive.

For samples of very low dispersion, such as gases and saturated liquid hydrocarbons, it is known that the simplified Cauchy equation (Equation 4) adequately represents the data with C_2 increasing proportionally to the dispersion. At about the lower limit of liquid dispersions C_3 becomes appreciable and C_2 increases less rapidly than before, perhaps approaching a maximum. An increase in C_4 compensates for a continued increase in C_2, and makes it possible to use an average value for C_2 in Equation 14.

This average value of C_2 is too large for saturated hydrocarbons, causing them to have an appreciable deviation of average (see Table V). Fortunately, these compounds usually deviate less than two units of dispersion from the correlation. The calculated values for the exponent t of 2.7 and 2.8 shown in Table I are low, probably because saturated hydrocarbon data predominate in the two groups involved. The fact that water also has a very low dispersion caused the low value of t calculated from the data for water and toluene.

At relatively high dispersions C_4 is so large that its effect is no longer minimized by the opposing effect of C_2 and Equation 14 is no longer valid. The high dispersion data will always deviate from Equation 14 in a direction away from the simplified Cauchy equation. This is to be expected, because data agreeing with Equation 17 (four or more terms) should be more nearly represented by Equation 14 (three terms) than by the simplified Cauchy (two terms).

If refractive indexes are available at three wave lengths and the index at a fourth is desired to ±0.0001 or better, it is recommended that the three available indexes be substituted in Equation 16 and K' be calculated. After C_2 has been calculated from K' at the three available wave lengths, this value may be used in Equation 16 for the calculation of the refractive index at the fourth wave length.

For liquids of very low dispersion, the simplified Cauchy equation (Equation 5) may give results as reliable as Equation 14 using the average value of C_2. The procedure outlined in the previous paragraph, however, will give better results than the simplified Cauchy in any case. This procedure can also be used for liquids of *F–C* dispersion slightly larger than 250 or for samples at temperatures over 100° C. However, for liquids of *F–C* dispersion over 320 it would be better to have four experimental indexes and use Equation 17. It is not known as yet what the maximum dispersion is at which a four-term equation is valid.

On the basis of the work described, the following conclusions have been reached:

Refractive index may be represented within experimental error in the visible and near ultraviolet ranges at any temperature by a power series with positive coefficients, the first term of which involves ν^0, and the third, ν^3.

Because of the ν^3 term this series cannot be simply derived from Equation 1 and therefore seems to disagree with present theory.

LITERATURE CITED

(1) Auwers, K. V., and Eisenlohr, F., *Ber.*, 43, 806 (1910).
(2) Auwers, K. V., and Westermann, H., *Ibid.*, 54, 2993 (1921).
(3) Campbell, K. N., and Eby, L. T., *J. Am. Chem. Soc.*, 63, 2683 (1941).
(4) Dixmier, G., *Chimie & Industrie*, Special No., 338 (1926).
(5) Drude, P., *Ann. Physik*, 14, 677 (1904).
(6) Egloff, G., "Physical Properties of Hydrocarbons," New York, Reinhold Publishing Corp., Vol. I (1939), II (1940), III (1946), IV (1947).
(7) Farmer, E. H., and Warren, F. L., *J. Chem. Soc.*, 134, 3221 (1931).
(8) Forziati, A. F., to be published in *J. Research Natl. Bur. Standards.*
(9) Garrett, A. B., Am. Petroleum Inst., Research Project 45 at Ohio State University, Serial No. 43, "Refractive Indices and Densities of some A.P.I. Hydrocarbons," 1944.
(10) Geldof, H., and Wibaut, J. P., *Rec. trav. chim.*, 67, 105 (1948).
(11) Gladstone, J. H., *J. Chem. Soc.*, 45, 241 (1884).
(12) *Ibid.*, 59, 290 (1891).
(13) Gooding, R. M., Adams, N. G., and Rall, H. T., IND. ENG. CHEM., ANAL. ED., 18, 2 (1946).

(14) Hardy, A. C., and Perrin, F. H., "Principles of Optics," New York, McGraw-Hill Book Co., 1932.
(15) "International Critical Tables," New York, McGraw-Hill Book Co., pp. 104–5, Vol. II, 1927; pp. 34–62, Vol. VII, 1930.
(16) Jenkins, F. A., and White, H. E., "Fundamentals of Physical Optics," New York, McGraw-Hill Book Co., 1937.
(17) Korff, S. A., and Breit, G., *Rev. Modern Phys.*, 4, 471 (1932).
(18) Kurtz, S. S., Jr., and Lipkin, M. R., *J. Am. Chem. Soc.*, 63, 2158 (1941).
(19) Kurtz, S. S., Jr., Mills, I. W., Martin, C. C., Harvey, W. T., and Lipkin, M. R., ANAL. CHEM., 19, 175 (1947).
(20) Kurtz, S. S., Jr., and Ward, A. L., *J. Franklin Inst.*, 224, 583, 697 (1937).
(21) Lantz, V., Shell Development Co., Emeryville, Calif., data contributed to Am. Petroleum Inst., Research Project 42, private communication.
(22) Lauer, J. L., *J. Chem. Phys.*, 16, 612 (1948).
(23) Lauer, J. L., "Refractive Index of Several Hydrocarbons in the Near Ultraviolet Wave-Length Region," doctorate thesis, University of Pennsylvania, 1947.
(24) Lipkin, M. R., and Martin, C. C., IND. ENG. CHEM., ANAL. ED., 18, 380 (1946).
(25) *Ibid.*, p. 433.
(26) Lipkin, M. R., Sankin, A., and Martin, C. C., ANAL. CHEM., 20, 598 (1948).
(27) Lorentz, H. A., *Wied. Ann.*, 9, 641 (1880).
(28) Lorenz, L. V., *Ibid.*, 11, 70 (1880).
(29) National Bureau of Standards, Certificate for Standard Samples 211a, 217, and 218.
(30) Perkin, W. H., *J. Chem. Soc.*, 69, 1025 (1896).
(31) Sellmeier, W., *Pogg. Ann.*, 143, 272 (1871); 145, 399, 520 (1872); 147, 386, 525 (1872).
(32) Sun Oil Co., unpublished data.
(33) Thorne, H. M., Murphy, W., and Ball, J. S., IND. ENG. CHEM., ANAL. ED., 17, 481 (1945).
(34) Tilton, L. W., and Taylor, J. K., *J. Research Natl. Bur. Standards*, 20, 419 (1938).
(35) Ward, A. L., and Fulweiler, W. H., IND. ENG. CHEM., ANAL. ED., 6, 396 (1934).
(36) Ward, A. L., and Kurtz, S. S., Jr., *Ibid.*, 10, 559 (1938).
(37) Ward, A. L., Kurtz, S. S., Jr., and Fulweiler, W. H., "Science of Petroleum," ed. by Dunstan, A. E., Nash, A. W., Brooks, B. T., and Tizard, H., Vol. II, p. 1137, London, Oxford University Press, 1938.
(38) Zeiss, Carl, Inc., data supplied with Pulfrich refractometer.

RECEIVED July 15, 1949. Presented before the Division of Physical and Inorganic Chemistry at the 116th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Polarographic Estimation of Chloramphenicol (Chloromycetin)

GLENN B. HESS, *Chas. Pfizer and Co., Inc., Brooklyn, N. Y.*

A polarographic method for the estimation of chloramphenicol (Chloromycetin) is presented. The reduction of chloramphenicol and of its hydrolysis product, 1-*p*-nitrophenyl-2-amino-1,3-propanediol, at the dropping mercury electrode has been studied at various pH values and the analysis of the polarographic waves is given. The reaction is irreversible and involves four electrons in the first wave, indicating preliminary reduction of the nitro group to the hydroxylamine.

THE development of fermentation and recovery processes for the production of chloramphenicol (Chloromycetin) for use as an antibiotic made necessary the concomitant development of rapid analytical methods for its determination in fermentation broths, concentrates, and solids. Before publication of the structure, it was known (*4*) that chloramphenicol contained covalently bound chlorine and a group giving an aryl amine on treatment with hydrochloric acid and zinc. This reaction, together with diazotization and coupling, has been used as a method for the colorimetric estimation of chloramphenicol (*4*). Because the compound could evidently be reduced and preliminary polarographic tests, without hydrolysis, showed well defined diffusion currents, a study was made of the behavior of chloramphenicol at the dropping mercury electrode, to determine the optimal conditions and to develop an assay method.

After publication of the structure, as *d*-threo-1-*p*-nitrophenyl-2-dichloroacetamido-1,3-propanediol (*5*), it was realized that the nitro group was probably the reactive portion of the molecule, inasmuch as nitro compounds are, in general, reducible at the dropping mercury electrode (*1*, *2*, *6*). For comparative purposes, the biologically inactive free amine, 1-*p*-nitrophenyl-2-amino-1,3-propanediol, obtained by hydrolysis of chloramphenicol with the splitting off of dichloroacetic acid, was prepared and studied under similar conditions to determine the effect of the dichloroaceto group on the polarographic reduction.

APPARATUS

A Sargent Model XXI recording polarograph was used for all the reported work. Two types of cells have been used, an H-type cell containing a saturated calomel electrode in one leg for investigative work, and a flask-type cell with a quiet mercury pool anode for routine use. Separate capillaries have been used with each cell; that for the H-type cell delivered at the rate of 2.588 mg. per second with an $m^{2/3}t^{1/6}$ value of 2.281, whereas that for the flask-type cells delivered at the rate of 2.321 mg. per second with an $m^{2/3}t^{1/6}$ value of 2.184. All determinations were made in a constant temperature room which was controlled at 24° ± 0.5° C.

EXPERIMENTAL

1-*p*-Nitrophenyl-2-amino-1,3-propanediol was prepared by hydrolyzing purified chloramphenicol in a slight excess of 0.5 *N* hydrochloric acid for 0.5 hour at reflux temperature. The solution was cooled, washed with ether and with chloroform to remove the dichloroacetic acid together with any unhydrolyzed chloroamphenicol, and evaporated to dryness under vacuum. The resultant amine hydrochloride was washed with a small amount of alcohol, then with ether, and dried.

For the investigation of the effect of pH on the reduction, master aqueous solutions, approximately 3 millimolar, of chloramphenicol and, later, of the 1-*p*-nitrophenyl-2-amino-1,3-propanediol hydrochloride were prepared. Bioassay potency was taken as the measure of purity of the chloramphenicol. Clark and Lubs buffers, hydrochloric acid–potassium chloride for pH 2, sodium hydroxide–potassium acid phthalate for pH 4 and 6, and sodium hydroxide–potassium chloride–boric acid for pH 8 and 10 were prepared in double strength solution. Just before use, equal volumes of master solution and double strength buffer solution were mixed, and the pH was checked by Beckman pH meter. Dissolved oxygen was removed by bubbling nitrogen through the solution. A trace of thymol was added to the chloramphenicol solutions as a maximum suppressor, because preliminary tests showed thymol to have less depressive effect on the diffusion current than gelatin. Methyl red was used as maximum suppressor for alkaline solutions of the amine hydrochloride and thymol in the acidic solutions.

Because chloramphenicol, buffered at pH 4, was found to give the best-defined and most reproducible first wave, and hydrolysis of the compound could be expected to be minimal at this pH, this buffer value was chosen for the routine determination of chloramphenicol. Potassium acid phthalate, monopotassium phosphate, and pyridine–pyridine hydrochloride buffers have been used with equally good results. The buffers have been prepared as concentrates so that upon dilution with chloramphenicol solutions, the proper pH is achieved. When the chloramphenicol concentrations were corrected for the bioassay potency, it was found that concentrations ranging from 100 to 1000 micrograms per milliliter gave values of the current-concentration ratio in excellent agreement, and that concentrations as low as 30 micrograms per milliliter could be assayed with only slight error.

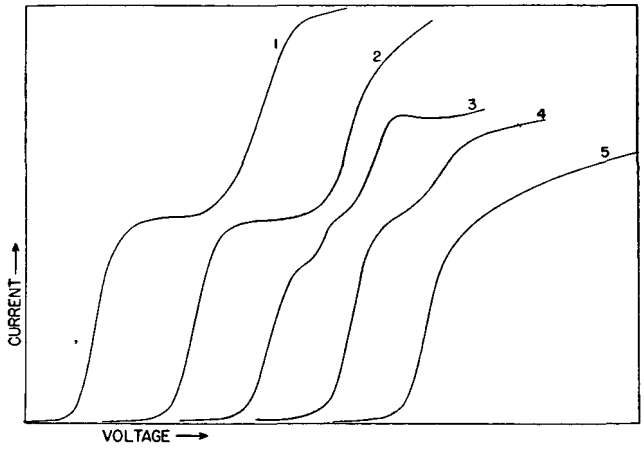


Figure 1. Polarograms of Chloramphenicol

1. In Clark and Lubs buffer at pH 2.2
2. At pH 4.2
3. At pH 6.4
4. At pH 8.1
5. At pH 10.1

Curves have been offset for clarity. For numerical values of $E_{1/2}$, see Table I

In routine assay of chloramphenicol, as solid material or aqueous solution, a solution is prepared 0.2 molar with respect to buffer and containing 300 to 500 micrograms of chloramphenicol when possible, but if necessary as low as 30 micrograms per milliliter. A portion of this solution is placed in the cell, a few crystals of thymol are added, and the solution is deaerated by bubbling for several minutes with wet oxygen-free nitrogen, and then electrolyzed between −0.4 and −1.1 volts versus the quiet mercury pool electrode. The diffusion current is determined by the method of midlines and the concentration of active material calculated from the current-concentration ratio, previously determined from the polarographic waves obtained with several concentrations of three biologically standardized lots.

DISCUSSION

Chloramphenicol and its hydrolysis product, 1-*p*-nitrophenyl-2-amino-1,3-propanediol, gave well defined primary waves which corresponded closely, both in half-wave potential and in diffusion current, in all buffers above pH 2.2. Because of this similarity, it would be impossible to differentiate between chloramphenicol and its hydrolysis product under the conditions used. However, it appears that the hydrolysis product is not a natural decomposition product or by-product, for in the polarographic assay of fermentation broths, concentrates, impure solids, and waste liquors, only a few samples of waste liquor have shown significant differences from the bioassay results. In the assay of 36 consecutive lots of finished material, assayed by polarograph, by the chemical method previously mentioned, and by biological methods, the polarographic assays showed a standard deviation of 3% from the biological assay and of 1.7% from the chemical assay. Polarographic assays were on the average slightly higher than bioassay results and slightly lower than chemical assay results.

The diffusion coefficient of chloramphenicol was calculated from the form of the Stokes-Einstein equation, $D = 3.32 \times 10^{-5}/V^{1/3}$ (*3*), where V is the molar volume. Determination of the density of crystalline chloramphenicol, calculation of the molar volume, and application of this relationship gave a value of 5.59×10^{-6} cm. sec.$^{-1}$ as the diffusion coefficient. Using the diffusion current values obtained at pH 4 in standardizing the routine method, application of the Ilkovič equation gave a value for N of 4.0 electrons. This value indicates that the nitro group of the compound is first reduced to the corresponding hydroxylamine, as has been found the case for other nitro compounds (*1*, *2*). A composite plot of $\log i/(i_d - i)$ versus E gave a straight line with a slope of 0.091, while the Heyrovský-Ilkovič equation requires a slope of 0.015 for a reaction involving 4 electrons. Consequently, the reduction of chloramphenicol is in all probability irreversible.

Representative polarographic curves obtained with chloramphenicol at the various pH values are shown in Figure 1 and the results obtained with both chloramphenicol and 1-*p*-nitrophenyl-2-amino-1,3-propanediol hydrochloride are combined in Table I. The values of the half-wave potentials versus the saturated calomel electrode have been corrected for the IR drop due to the resistance included in the circuit by the cell and the damping resistance. Diffusion currents are given in terms of the diffusion current constant, $I = i/cm^{2/3}t^{1/6}$, which is relatively independent of the characteristics of the dropping mercury electrode being used.

A perusal of the table or chart will show that the diffusion current of the first wave of chloramphenicol is practically constant at all pH values, while the half-wave potential shifts to more negative values at regular intervals with increasing pH. On the other hand, the half-wave potential of the final wave, which occurs in all buffers up to pH 10, is practically constant, while the diffusion current of this wave decreases markedly with increasing pH values. Because of this variation in half-wave potentials, the two waves interfere somewhat at pH values of 6 and 8. This interference, and lack of a well-defined diffusion current after the second wave, make these pH values unsuitable for analytical purposes.

The hydrolysis product of chloramphenicol, 1-*p*-nitrophenyl-2-amino-1,3-propanediol, shows a well-defined first wave, which corresponds closely, both in half-wave potential and in diffusion current, to the first wave of chloramphenicol at all pH values except 2.2, where this compound behaves anomalously. Poorly defined second waves, not included in the data of Table I, appear in the polarograms of this material, occurring at half-wave potential of about −1.0 volt at pH 2.2 and increasing in negative value with increasing alkalinity of the buffer even more rapidly than do the potentials of the first wave.

The occurrence of the break in the first wave of chloramphenicol at pH 6.4 has not as yet been explained. The wave in this buffer

Table I. Half-Wave Reduction Potentials (Volts *vs.* S.C.E.) and Diffusion Current Constants

pH	Chloramphenicol $E_{1/2}$	Chloramphenicol I	1-*p*-Nitrophenyl-2-amino-1,3-propanediol $E_{1/2}$	1-*p*-Nitrophenyl-2-amino-1,3-propanediol I
2.2	−0.35	5.84	−0.53	7.08
	−1.09	5.26	...	...
4.2	−0.48	5.75	−0.45	5.79
	−1.14	3.84	...	...
6.4	−0.60	4.42	−0.53	5.98
	−0.85	1.45	...	...
	−1.07	2.42	...	...
8.1	−0.69	5.85	−0.64	5.59
	−1.10	1.96	...	...
10.1	−0.79	5.90	−0.71	5.85

$E_{1/2}$ is corrected for IR drop.

I is $\frac{i}{Cm^{2/3}t^{1/6}}$.

has not been completely reproducible, as the maximum is difficult to suppress, and in some instances a double maximum has been observed. The behavior of 1-*p*-nitrophenyl-2-amino-1,3-propanediol in buffer of pH 2.2 with increased diffusion current and reversal of the pH-potential curve is thought to be due to the characteristics of the ionic amine hydrochloride, which would be formed in the hydrochloric acid–potassium chloride buffer.

SUMMARY

The behavior of chloramphenicol and of its hydrolysis product, 1-*p*-nitrophenyl-2-amino-1,3-propanediol, at the dropping mercury electrode has been studied, and the polarographic waves have been analyzed. The reaction is irreversible and involves 4 electrons, with preliminary reduction of the nitro group to the hydroxylamine. A method for the routine analysis of broths, concentrates, and solids in buffer of pH 4 has been shown to be accurate within the limits of the polarographic technique.

LITERATURE CITED

(1) Cropper, W. P., and Astle, M. J., *J. Am. Chem. Soc.*, **65**, 2395 (1943).
(2) Dennis, S. F., Powell, A. S., and Astle, M. J., *Ibid.*, **71**, 1484 (1949).
(3) Kolthoff and Lingane, "Polarography," p. 48, New York, Interscience Publishers, 1941.
(4) Parke, Davis and Co., private communication.
(5) Rebstock, M. C., Crook, H. M., Controulis, J., and Bartz, O. R., paper presented before Division of Medicinal Chemistry at 115th Meeting of AM. CHEM. SOC., San Francisco, Calif.
(6) Shikata, M., *Trans. Faraday Soc.*, **21**, 42 (1945).

RECEIVED December 2, 1949. Presented before the Division of Analytical and Micro Chemistry at the 116th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Qualitative Scheme of Analysis for the Common Sugars

T. H. WHITEHEAD AND W. C. BRADBURY[1], *University of Georgia, Athens, Ga.*

A systematic scheme of qualitative analysis is given for mixtures of sucrose, glucose, fructose, maltose, and lactose in solid or liquid samples. The presence of starch and dextrin does not interfere. This scheme is applicable to mixtures of sugars and most food products. The procedures call for no reagents that are difficult to prepare or store, no specialized laboratory equipment, and no unusual techniques. The scheme of analysis is based upon removing fructose from the solid sample with 90% ethyl alcohol, in which it is soluble but other sugars and starches are not. Fructose is confirmed by a specific test. Lactose is next removed from the residue by dissolving it in 50% ethyl alcohol and its presence is confirmed with the formation of its osazone. Sucrose is detected by its color reaction with cobalt nitrate or by the Raybin test with diazouracil. Glucose and maltose are separated by forming the osazones and taking advantage of their difference in solubility at 87° C. The methods are capable of detecting 5 mg. of sucrose and fructose or 200 mg. of the other sugars in a sample.

CHEMISTS who are frequently required to make qualitative and quantitative analyses of natural and manufactured food products recognize the need for a simple, rapid, and reliable scheme for determining sucrose, glucose, maltose, lactose, and fructose in the presence of starch and dextrins. There is no lack of tests for individual sugars or groups of sugars, but mixtures containing three or more sugars are difficult to analyze. Most quantitative methods for the accurate analysis of sugar mixtures require a knowledge of the qualitative composition of the mixture before the analysis is begun. The practice of reporting reducing sugars "in terms of glucose" gives no indication of the true composition of the mixture. Unless attention is given to the presence of starch or dextrins, they may be converted to glucose during the analysis and introduce a large error.

The scheme of analysis given here is satisfactory for the sugars mentioned above in the presence of starch and dextrins. Because the high cost and low sweetening value of other sugars preclude their use in most food products in competition with the common sugars, this scheme is suitable for such products.

REAGENTS

1-Naphthol Solution. Dissolve 15 grams of 1-naphthol, melting point 95–96° C., in 100 ml. of chloroform.

Fehling's Solution. A. Dissolve 34.6 grams of cupric sulfate pentahydrate in 400 ml. of water, add 0.5 ml. of 36 *N* sulfuric acid, and make up to 500 ml. with water.

B. Dissolve 172 grams of Rochelle salt, U.S.P., in 300 ml. of water, add 62.5 ml. of saturated sodium hydroxide solution, and make up to 500 ml. with water.

Ethyl Alcohol, 90%. Dilute 95% ethyl alcohol, U.S.P., with water until the specific gravity of the mixture at 20° C. is 0.8305.

Ethyl Alcohol, 50%. Dilute 95% ethyl alcohol, U.S.P., with water until the specific gravity of the mixture at 20° C. is 0.9316.

Phenylhydrazine base. Redistill Eastman Kodak Company No. 329 under reduced pressure at frequent intervals. It becomes unreactive after long exposure to sunlight and air.

Dinitrosalicylic Acid Reagent. Dissolve 2.0 grams of 3,5-dinitrosalicylic acid (Eastman Kodak No. 1802) in 70 ml. of water at 80° to 90° C., and add 10 ml. of sodium carbonate solution containing 20 grams of sodium carbonate per 100 ml. of water. When this mixture is cool, dilute it to 100 ml. with water.

Sodium Hydroxide Solution. Dissolve 1.5 grams of C.P. sodium hydroxide in enough water to make 100 ml. of solution.

Cobaltous Nitrate Solution. Dissolve 5.0 grams of cobaltous nitrate hexahydrate in 50 ml. of water, add 1 drop of 15 *N* nitric acid, and make up to 100 ml. with water.

Acetic Acid Solution. Dissolve 50 ml. of glacial acetic acid in 50 ml. of water.

Potassium Hydroxide Solution, 50%. Dissolve 50 grams of C.P. potassium hydroxide in 50 ml. of water.

PROCEDURES

Preliminary Examination. If the sample is a liquid, use 3 ml. If the sample is a solid, shake 50 mg. with 3 ml. of water and filter if all of the sample does not dissolve. Wash the residue on the filter paper with 0.5-ml. portions of water until the filtrate has a volume of 3 ml. (All filtrations in this scheme which call for filter paper require Whatman No. 40 paper or its equivalent.)

Using 3 ml. of sample solution, perform the Molisch test (*2*) to determine if any carbohydrate is present. This is done by adding 3 drops of the 1-naphthol solution to the 3-ml. sample. Shake

[1] Present address, Upjohn Laboratories, Kalamazoo, Mich.

well and then underlay the solution with 1 ml. of 36 *N* sulfuric acid. Carbohydrates produce a violet color at the interface of the two liquid layers. This color often changes to black and on dilution with 5 ml. of water yields a dull violet precipitate. If this test is negative, no sugar is present in the sample.

If the Molisch test is positive, dissolve 0.5 gram of the solid in 50 ml. of water, filter if necessary, and wash the residue with small portions of water to make 50 ml. of filtrate. If the sample is a liquid, dilute 10 ml. to 50 ml. with water. Mix 25 ml. each of Fehling's solutions A and B and to this add the 50 ml. of sample solution. Place the beaker containing this 100-ml. mixture in a larger beaker containing boiling water. At the end of 5 minutes, remove the smaller beaker and observe the result. If there is no precipitate of red cuprous oxide, or only a trace, the proper size of sample is 4 grams of solid. If there is a precipitate of cuprous oxide amounting to about 150 mg., the proper size of sample is 2 grams. If the Fehling's solution is decolorized, or nearly so, and the precipitate is large, the proper size of sample is 1 gram.

If the sample is a solid, weigh 1, 2, or 4 grams as determined above. If the sample is a liquid, evaporate 10 ml. to dryness on a water bath, then weigh 1, 2, or 4 grams of the solid residue.

Decolorizing Sample. If the sample is highly colored, decolorize by the usual methods with alumina cream or lead acetate (*1*).

Testing for Fructose. Weigh 0.2 gram of solid, or take 1 ml. of liquid, and make up to 3 ml. with water. Add to this solution 1 ml. of dinitrosalicylic acid reagent and 1 ml. of sodium hydroxide solution and allow it to stand for 2 hours at room temperature (20° to 25° C.). A red-orange color shows more than 5 mg. of fructose. Other sugars give a yellow color, which is a negative test (*4*).

Separation and Identification of Fructose. Weigh 1, 2, or 4 grams of solid sample as determined previously and place it in a 150-ml. beaker. Cover this with 50 ml. of 90% ethyl alcohol, stir well with a glass rod, and carefully break up any lumps. Place a watch glass over the beaker and allow it to stand for 45 minutes with occasional stirring.

Filter the mixture through Whatman No. 40 paper and catch the filtrate in a clean 125-ml. Erlenmeyer flask. Pour the filtrate over the residue three times to ensure solution of fructose from the residue. The filtrate contains fructose while the residue contains the other sugars, starch, and dextrins.

If desired, the filtrate may be tested again for fructose by evaporating the filtrate to 10-ml. volume, taking 3 ml. of this, and performing the test previously described for fructose (*4*).

Separation and Identification of Lactose. The residue from the above treatment with 90% ethyl alcohol contains the other sugars with starch and dextrins. Wash the residue with 10 ml. of 90% ethyl alcohol solution to remove the last traces of fructose, and discard the wash solution. Place the filter paper with residue on it in a Büchner funnel connected through a suction flask to the vacuum line and apply the vacuum until no liquid comes through the funnel.

Transfer the residue to a 150-ml. beaker and cover it with 20 ml. of 50% ethyl alcohol solution. Stir well with a glass rod and break up any lumps present (lumping usually indicates the presence of maltose). Filter the mixture through Whatman paper. The filtrate contains sucrose, glucose, and maltose. The residue contains lactose, starch, dextrins, and traces of other sugars.

Wash the residue with five 10-ml. portions of 50% ethyl alcohol solution, and discard the washings. Transfer the residue, if any, to a small beaker and dissolve it in 5 ml. of water. Any undissolved matter is starch or dextrin. Filter the mixture, if necessary, and to the filtrate add 0.5 ml. of acetic acid solution and 0.5 ml. of phenylhydrazine base. Shake this mixture well, pour it into a test tube, and immerse the tube in a water bath at 87° C. Prepare a Büchner funnel with Whatman paper and connect to the vacuum line. At the end of 45 minutes pour the mixture from the test tube quickly into the Büchner funnel, add 50 ml. of water to the filtrate, and allow it to cool to 20° C. A yellow precipitate is the osazone of lactose. For additional confirmation the crystals may be examined under a microscope and compared with known crystals of lactosazone.

Identification of Sucrose. The filtrate obtained from treating the sample with 50% ethyl alcohol contains sucrose, glucose, and maltose. This has a total volume of 20 ml. Boil this solution gently with a Bunsen flame until the volume is reduced to 10 ml., add 10 ml. of water to make a total volume of 20 ml., and divide it into two portions of 5 and 15 ml.

To the 5-ml. portion add 4 drops of cobalt nitrate solution and 2 drops of the potassium hydroxide solution. Shake the mixture and let it stand for 1 minute. An amethyst-blue color in the solution confirms sucrose (a dirty green precipitate suggests glucose or a blue precipitate suggests maltose).

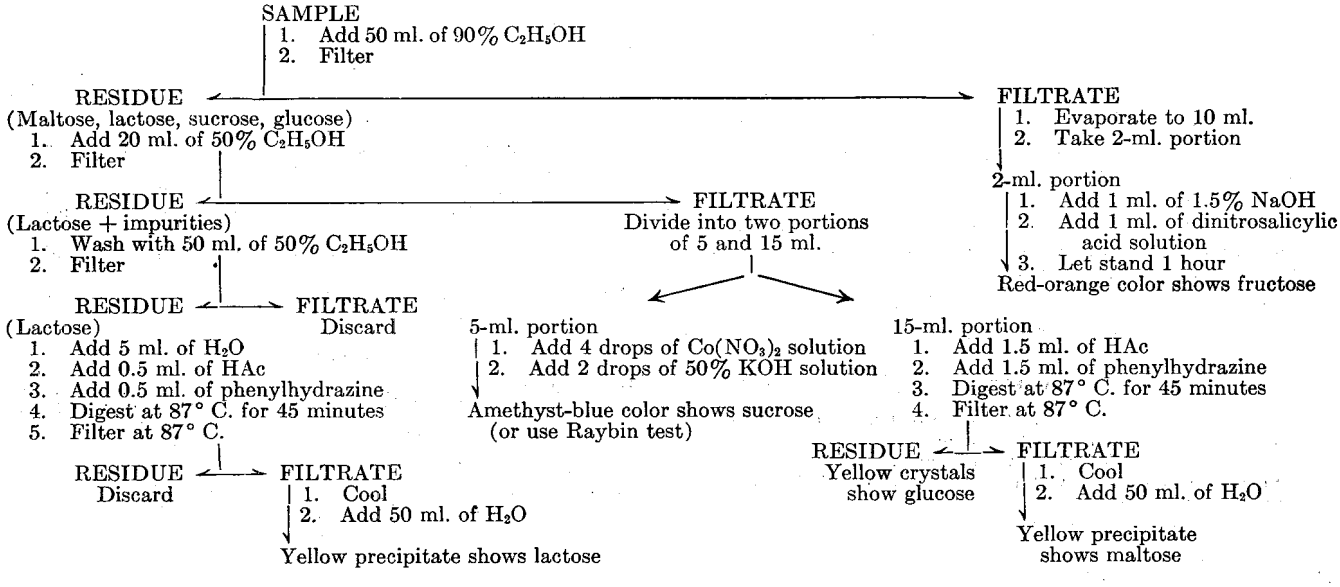
The Raybin test (*3*) for sucrose may be used instead of the cobalt nitrate test given above. To the 5-ml. solution, add 10 mg. of sodium hydroxide, and cool the mixture to 10° C. in an ice bath. Add 7 to 10 mg. of diazouracil and shake well. A blue-green color confirms sucrose. Other colors such as yellow or brown are negative.

Separation of Maltose and Glucose. To the 15-ml. portion described above, add 1.5 ml. of acetic acid solution and 1.5 ml. of phenylhydrazine base. Shake well, transfer to a test tube, and immerse the tube in a water bath at 87° C. Allow the tube to remain in the water bath at 87° C. for 45 minutes. Scratch the side of the tube occasionally with a glass rod to hasten the crystallization. At the end of 45 minutes, filter the mixture quickly through a Büchner funnel as described for lactose. A residue of yellow crystals on the filter paper is the osazone of glucose and the filtrate contains the osazone of maltose.

Confirmation of Maltose and Glucose. Remove the residue and examine the crystals under a microscope. Compare them with known crystals of glucosazone. Add 50 ml. of water to the filtrate, cool to 20° C., and note the formation of a precipitate. A yellow crystalline precipitate is the osazone of maltose. Examine the crystals under a microscope for additional confirmation.

SCHEMATIC DIAGRAM

To summarize the procedures given above and to show their relationship, a flow sheet is given.



DISCUSSION

If fructose is absent from the sample, the initial treatment of the sample with 90% ethyl alcohol may be omitted, as well as the evaporation of a liquid sample to dryness. The analysis may be started by shaking a solid sample of proper weight with 20 ml. of 50% ethyl alcohol or by adding 10 ml. of absolute ethyl alcohol to 10 ml. of a liquid sample, and then proceeding as directed for the separation and identification of lactose.

If fructose is present, it is essential to remove it first. Therefore a sample size must be chosen to permit the removal of fructose with 50 ml. of 90% ethyl alcohol solution. At 20° C., 50 ml. of 90% ethyl alcohol dissolve 1.7 grams of anhydrous fructose. However, if water is present in a liquid sample considerable amounts of other sugars will be removed by the 90% ethyl alcohol treatment and may result in negative tests later in the analysis. If fructose is not completely removed, it will give an erroneous test for glucose, because their osazones are identical.

It is necessary to wash the residue containing lactose with additional portions of 50% ethyl alcohol to remove any maltose that may remain. Maltose behaves like lactose in the formation of an osazone, but their osazones are not identical and can be differentiated under the microscope.

The residue remaining after lactose is dissolved by water contains starch and dextrins. This may be dissolved in boiling water and tested for starch or dextrin with a solution of iodine in potassium iodide. If any starch or dextrin dissolves with the lactose it is converted to glucose by the acetic acid, but this does not interfere with the test for lactose, because the glucosazone is insoluble at 87° C. and will be filtered out before the osazone of lactose precipitates.

The presence of maltose in a sample is often indicated by its characteristic odor and its marked tendency to absorb water and cake. When a mixture of maltose and glucose is treated with acetic acid and phenylhydrazine base, the mixture becomes cloudy at once if maltose is present. It remains clear for several minutes if glucose alone is present.

It is essential to evaporate the solution of sucrose, glucose, and maltose in 50% ethyl alcohol solution to half its volume in order to remove excess alcohol. The presence of ethyl alcohol delays the formation of the osazones beyond 45 minutes. The mixture cannot be held at 87° C. longer than 45 minutes because sucrose may hydrolyze and give a false test for glucose. It does not interfere under the conditions of this scheme.

The tests given in this scheme detect 5 mg. of fructose or sucrose and 200 mg. of glucose, lactose, and maltose. The Raybin test for sucrose is stated by Raybin (*3*) to detect 50 mg. of sucrose in 5 ml. of solution.

The scheme is not a complete scheme for all sugars, because no provision is made for the less common pentose sugars or for mannose and raffinose. However, these are not usually found in food products.

LITERATURE CITED

(1) Assoc. Offic. Agr. Chemists, "Official and Tentative Methods of Analysis," 6th ed., p. 562, 1945.
(2) Molisch, H., *Monatsh.*, 7, 198–209 (1886).
(3) Raybin, H. W., *J. Am. Chem. Soc.*, 55, 2603 (1933).
(4) Whitehead, T. H., and Bradbury, W. C., ANAL. CHEM., 21, 1430 (1949).

RECEIVED December 5, 1949. Presented in part before the Meeting-in-Miniature, Georgia Section, AMERICAN CHEMICAL SOCIETY, Atlanta, Ga., November 18, 1949.

Conductometric Titrations with Organic Reagents

Determination of Magnesium with Calcium Saccharate in Presence of Calcium

J. F. CORWIN, A. P. DRESEL[1], AND G. E. OSUCH, *Antioch College, Yellow Springs, Ohio*

The use of calcium saccharate for the volumetric determination of magnesium has been found suitable for titrating magnesium either alone or in the presence of limited amounts of calcium. The conductometric method of analysis was used and the end point determined by calculation.

CALCIUM saccharate has been found satisfactory for the direct conductometric titration of magnesium. This reagent and saccharic acid have been used in the gravimetric determination of magnesium in limestone (*2, 3*) and in the detection of magnesium in the presence of barium, calcium, and strontium (*4*). When a standard solution of calcium saccharate is added to a solution containing magnesium, the insoluble precipitate of trimagnesium saccharate is formed and calcium ions replace the magnesium ions in solution. The replacement of the magnesium ions in solution by the faster-moving calcium ions causes a slight decrease in resistance before the equivalence point is reached. After the end point, further addition of concentrated calcium saccharate solution causes a sharp decrease in resistance. Typical titration curves are shown in Figure 1. Magnesium can be titrated either alone or in the presence of calcium, provided that the amount of calcium does not exceed the amount of magnesium present. Other metals of the alkaline earth group and those that appear in small quantities in the usual limestone analysis do not interfere with the reaction, but as in most conductometric procedures, high concentration of conducting ions must be avoided.

[1] Present address, Eastman Kodak Company, Rochester, N. Y.

APPARATUS

The 60-cycle, Leeds & Northrup, industrial conductivity equipment was used. The dip cell for high conductivity solutions was modified by removing the glass shield. A 300-ml. beaker served as a reaction vessel. The selection of concentrations that allowed at least 200-ml. volume of solution in the reaction vessel to not over 10 ml. of titrant added eliminated the necessity of volume corrections. The reaction vessel was kept at constant temperature.

SOLUTIONS

The magnesium chloride was prepared by adding approximately 17 grams of magnesium chloride hexahydrate to 2 liters of water and was standardized gravimetrically by the precipitation of the magnesium as magnesium ammonium phosphate.

The calcium saccharate solution was prepared by adding 54 grams of pure calcium saccharate, obtained from the Fisher Scientific Company, to 500 ml. of boiled water. Because calcium saccharate reacts with the carbon dioxide in the air, the solution was stored in a glass bottle with an Ascarite absorption tube on the inlet and a pinch clamp on the outlet. Nevertheless, owing to the slow decomposition of the salt, the solution was standardized before each series of titrations or, at least, every other day.

PROCEDURE

The standardization procedure consisted of pipetting 25 ml. of the magnesium chloride solution into a 300-ml. electrolytic

Table I. Calcium Saccharate *vs.* a Synthetic Mixture of Magnesium Chloride and Calcium Nitrate

Mg Taken	Calcium Saccharate Used	Mg Recovered	Difference	Ca Added
Gram	*Ml.*	*Gram*	*Gram*	*Gram*
0.0261	7.60	0.0260	0.0001	0.0200
0.0232	6.80	0.0232	0.0000	0.0200
0.0232	6.86	0.0234	0.0002	0.0200
0.0237	6.96	0.0238	0.0001	0.0200
0.0237	6.96	0.0238	0.0001	0.0200

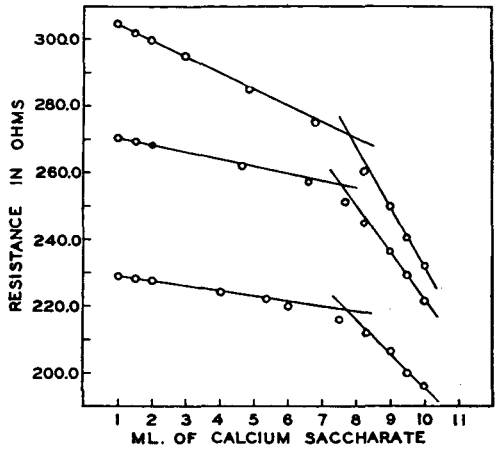


Figure 1. Calcium Saccharate *vs.* Magnesium Solution Containing Calcium Ions

beaker and diluting with water to make approximately 200 ml. of solution. The beaker was placed in a constant temperature bath and the calcium saccharate was added in about 0.5-ml. portions from a microburet. Because the magnesium saccharate complex is somewhat soluble, 1 ml. of titrant was added and the solution was stirred constantly for 10 minutes before the first resistance reading was taken. All subsequent readings were made when the resistance became constant or at 10-minute intervals after each addition.

In order to see if this procedure would be effective in the presence of calcium, calcium nitrate was added to the magnesium chloride solution. As long as the amount of calcium was less than the amount of magnesium, it had no apparent effect on the reaction.

Typical titration data are listed in Table I for a series of determinations which contained the maximum amount of calcium allowable. The results in this table were obtained by calculation rather than graphically. By selecting the linear values of resistance and titrant before and after the equivalence point, respectively, and substituting them in the equation for a straight line, a pair of equations may be obtained that when solved simultaneously will give the equivalence point. A complete description of the calculation has been given in a previous publication (*1*).

DISCUSSION

Less than 1% error is obtained by this method of determining magnesium and it is not necessary to separate calcium from the magnesium if the amount of calcium is less than the amount of magnesium. The titration can be made in 1 hour and the calculations can be completed in 10 minutes.

CONCLUSION

A method for the volumetric determination of magnesium is described, the accuracy of which varies from 0.4 to 0.8 mg. of magnesium per 100 mg. of magnesium taken. The method is fairly rapid and can be carried out in the presence of limited amounts of calcium.

LITERATURE CITED

(1) Corwin and Moyer, IND. ENG. CHEM., ANAL. ED., **18**, 302 (1946).
(2) Shead and Heinrich, *Ibid.*, **2**, 388 (1930).
(3) Shead and Valla, *Ibid.*, **4**, 246 (1932).
(4) Tananaev and Lovi, *J. Applied Chem. (U.S.S.R.)*, **10**, 1112 (1937).

RECEIVED November 28, 1949. Work done under Grant-in-Aid from the Frederick Gardner Cottrell Fund, Research Corporation, New York, N. Y.

Application of the Lead Reductor to Determination of Uranium

WILLIAM D. COOKE[1], FRED HAZEL, AND WALLACE M. McNABB
University of Pennsylvania, Philadelphia, Pa.

A NUMBER of metallic reducing agents have been proposed for the determination of uranium. Reduction of uranium (VI) in a Jones reductor gives a mixture of uranium(IV) and uranium(III). The solution is aerated to oxidize uranium(III) to uranium(IV). Birnbaum and Edmonds (*1*) used a silver reductor for reduction to the quadrivalent state. This procedure necessitated a high temperature, 60° to 90° C., and controlled acidity, 4 *N* hydrochloric acid. However, at least in small amounts, the extent of reduction depends upon the temperature and the rate of passage through the column (*6*). Someya (*2*) found that uniform reduction of uranium could be carried out in strong acid solutions by means of liquid lead and bismuth amalgams. Koblic (*4*) reduced uranium quantitatively to the quadrivalent state with lead. However, his procedure is inconvenient because the solutions have to be boiled in an inert atmosphere of carbon dioxide in the presence of hydrochloric acid for at least 0.5 hour.

[1] Present address, Frick Chemical Laboratory, Princeton University, Princeton, N. J.

Treadwell (*11*) used a lead reductor instead of a Jones reductor for various metals but did not include uranium. The use of the lead reductor probably has been limited because of the difficulty of applying it to sulfuric acid solutions.

When sulfuric acid solutions are reduced with lead, an adherent film of lead sulfate is formed which soon decreases the efficiency of the reducing agent. The formation of the lead sulfate film can be prevented by the presence of hydrochloric acid. If the concentration of hydrochloric acid in the solution is greater than 2.5 *N*, no lead sulfate is formed even after continued use. The successful application of the lead reductor to the determination of uranium is described below.

Solutions of uranium(VI) were reduced to uranium(IV) in both hydrochloric and sulfuric acid solutions. The acidity was not critical and could be varied within wide limits. However, when sulfate ions were present, it was found necessary to add sufficient hydrochloric acid to prevent the formation of lead sulfate. The reduced uranium was caught in a solution of ferric sulfate.

Uranium can be reduced quantitatively to the quadrivalent state with lead. High concentrations of sulfuric acid can be tolerated, provided a sufficient concentration of hydrochloric acid is present. Reduction is rapid and amalgamation of the metal is unnecessary. The uranium(IV) is determined indirectly by the addition of an excess of iron(III) and subsequent titration of iron(II) with standard dichromate solution.

The iron(II) formed during the oxidation of the uranium to uranium(VI) by iron(III) was titrated with dichromate according to the method of Kolthoff and Sandell (*5*).

Any iron present in the uranium solution will be reduced and interfere with the procedure described. Nessle (*7*) has proposed a method for determining uranium(IV) in the presence of iron(II). This consists of titrating the solution potentiometrically with standard ferric sulfate solution at an elevated temperature in an inert atmosphere.

PREPARATION AND STORAGE OF REDUCTOR

The reductor consisted of a column of reagent grade granulated lead 25 cm. long and 2 cm. in diameter.

When the reductor was stored overnight it was covered with a solution of 10% hydrochloric acid containing about 0.1% ferric ion. Unless this small amount of ferric ion was added during storage, the first determination was a few tenths per cent too low. Before use the column was washed with six 25-ml. portions of 1 to 15 hydrochloric acid.

PROCEDURE

About 50 ml. of solution containing 40 to 200 mg. of uranium and 0 to 9 *N* in sulfuric acid concentration were made 3 *N* in respect to hydrochloric acid. The solution was poured through the reductor and caught in 10 ml. of 5% ferric sulfate solution. The reductor was washed with five or six 25-ml. portions of 1 to 15 hydrochloric acid, 10 ml. of 85% phosphoric acid were added, and the titration was carried out with 0.05 *N* potassium dichromate. Diphenylamine sulfonate indicator (0.5 ml. of 0.3% solution) was added a few milliliters before the end point. A correction of 0.10 ml. of 0.05 *N* potassium dichloromate was subtracted as the indicator blank. [Since the completion of this work, a new indicator, 5,6-dimethyl-1,10-phenanthroline, has been proposed for the ferrous-dichromate titration (*9*). The small correction and the resistance to oxidation of this indicator would be of value in this titration.]

RATE OF REDUCTION

The rate of reduction of uranium(VI) to uranium(IV) was found to be high. Solutions containing 0.1 gram of uranium were completely reduced when passed through the reductor at the rate of 175 ml. per minute. Higher rates of flow were not attempted because they could not be measured conveniently.

To find whether prolonged use would decrease the rate of reduction, 50 samples (approximately 0.1 gram of uranium each) were determined and the above procedure was repeated. Reduction was complete at flow rates as high as 175 ml. per minute.

ACCURACY

A solution of uranyl sulfate was standardized gravimetrically according to the method of Hillebrand and Lundell (*3*) as modified by Someya (*10*). The uranium was precipitated from carbonate-free solutions as ammonium uranate, ignited to the oxide, and weighed. Ten-milliliter samples of the solution yielded 0.2408, 0.2410, and 0.2408 gram of U_3O_8.

Aliquot portions of this solution were analyzed by the proposed method. Limits were set at 0.04 to 0.2 gram. The higher limit was chosen arbitrarily at 0.2 gram, assuming that this would be as high a concentration as would be encountered ordinarily. The lower limit, 0.04 gram, was chosen because it corresponds, roughly to the beginning of the semimicro range. The results are shown in Table I.

DISCUSSION

The use of metallic lead as a reducing agent compares favorably with amalgamated zinc. Test lead can be obtained with a high degree of purity and a blank is not necessary. Because lead does not evolve hydrogen from acids, amalgamation is unnecessary, and the reduction proceeds with theoretical efficiency. Complications caused by the evolution of hydrogen are not encountered (*8*).

In using a Jones reductor, air must be excluded from the column to prevent low results. With the lead reductor such precautions are not necessary and solutions can be run through an air-filled column without introducing an error.

Ammonium ion, which must be removed prior to treatment with amalgamated reductors, does not interfere. Solutions containing acetate ions can be reduced with lead, so that the indirect titration of sodium can be accomplished. Nickel does not interfere but, as in the case of the Jones reductor, nitrate ion must be removed. The breadth of the method is indicated by the results given in Table II, where values obtained under different conditions are compared with the calculated value.

Table I. Determination of Uranium

Uranium Taken	Vol. Calculated	Vol. Found	Error	
Gram	*Ml.*	*Ml.*	*Ml.*	*%*
0.2128	33.52	33.45	−0.07	0.2
		33.46	−0.06	0.2
		33.49	−0.03	0.1
		33.48	−0.04	0.1
		33.53	+0.01	0.0
0.1425	23.48	23.54	+0.06	0.2
		23.46	−0.02	0.1
		23.43	−0.05	0.2
		23.48	0.00	0.0
		23.48	0.00	0.0
0.1017	16.76	16.75	−0.01	0.1
		16.79	+0.03	0.2
		16.73	−0.03	0.2
		16.73	−0.03	0.2
		16.73	−0.03	0.2
0.04072	6.71	6.74	+0.03	0.5
		6.71	0.00	0.0
		6.72	+0.01	0.1
		6.74	+0.03	0.5
		6.74	+0.03	0.5

Table II. Effect of Conditions

(Calculated value, 16.76 ml.)

Solution	Ml. Required
3 *N* HCl	16.75
3 *N* HCl + 3 *N* H_2SO_4	16.79
3 *N* HCl + 9 *N* H_2SO_4	16.82
Air-filled reductor	16.75
5% ammonium ion	16.75
5% acetate ion	16.75

LITERATURE CITED

(1) Birnbaum, Nathan, and Edmonds, S. M., IND. ENG. CHEM., ANAL. ED., **12**, 155 (1940).
(2) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," p. 103, New York, John Wiley & Sons, 1929.
(3) *Ibid.*, p. 368.
(4) Koblic, O., *Chem. Listy.*, **19**, 1 (1925).
(5) Kolthoff, I. M., and Sandell, E. B., "Textbook of Quantitative Inorganic Analysis," p. 609, New York, John Wiley & Sons, 1943.
(6) MacInnes, D. A., and Longsworth, L. G., U. S. Atomic Energy Commission, *Document* **MDDC-910** (1942).
(7) Nessle, G. J., *et al.*, U. S. Atomic Energy Commission, *Document* **MDDC-1123** (1947).
(8) Nilakantan, P., and Jayaraman, N., IND. ENG. CHEM., ANAL. ED., **11**, 339 (1939).
(9) Smith, G. F., and Brandt, W. W., ANAL. CHEM., **21**, 948 (1949).
(10) Someya, Kinichi, *Z. anorg. Chem.*, **152**, 378 (1926).
(11) Treadwell, W. D., *Helv. Chim. Acta.*, 5, 806 (1922).

RECEIVED November 21, 1949.

Cellulose Ester Viscosities by the Ball-Drop Method

CARL J. MALM, LEO B. GENUNG, AND GEORGE B. LAPHAM
Eastman Kodak Company, Rochester, N. Y.

The ball-drop method is useful for measuring viscosities of concentrated solutions of cellulose esters. Several ball-drop viscometers are described, which are readily constructed from simple apparatus, are useful over a considerable range of viscosity, and are easily calibrated. Viscosity results expressed in seconds are acceptable for control purposes, but they should be converted to absolute or kinematic units for engineering purposes and for a more thorough understanding of the properties of the solutions. Procedures for the measurement of viscosities and determination of conversion factors are presented. The Faxen equation is particularly useful for the latter purpose. If viscosity data are to have practical significance, it is necessary to use solvents, concentration, and temperature closely duplicating use conditions. Pronounced viscosity effects may be caused by variations in concentration; temperature; solubility; density, viscosity, and purity of solvent; and presence of certain ions. It is not possible to predict viscosities of concentrated solutions accurately from data obtained on dilute solutions.

VISCOSITIES of solutions of cellulose esters are measured under many different conditions. The viscosities of dilute solutions, including intrinsic and inherent viscosities (*4*), are of considerable value as a measure of an average molecular weight or degree of polymerization. They show the extent of degradation, which influences the general level of physical properties. Viscosity determinations in dilute solutions are, however, of only secondary usefulness as a measure of the viscosity which a given cellulose ester will have in the more highly concentrated solutions encountered in commercial practice. Other factors including temperature, solubility of the ester in the solvent, and the presence of certain metallic ions may produce increases in viscosity to the point of gel formation. The measurement of viscosities of solutions of cellulose esters under practical conditions is, therefore, necessary and of considerable importance for production control.

The ball-drop method for the measurement of viscosity is widely used because of the simplicity of apparatus required and the versatility attainable. By varying the ball-drop distance and the diameter and density of the ball, viscosities may be measured conveniently over the range 10 to 10,000 poises. Older ball-drop viscometers tended to use comparatively large balls in tall, narrow tubes and long distances of fall. In some of the newer apparatus small balls are used in large containers to decrease the wall effect and minimize errors due to improper centering of the ball. An exception is the Hoeppler rolling-ball viscometer, a very precise reference instrument in which comparatively large balls roll through a tube of uniform dimensions mounted at an angle from vertical.

Viscosity results may be expressed in absolute units (poises or centipoises), in kinematic units (stokes or centistokes), or more commonly in commercial practice, in seconds. The latter unit is practical for production control purposes because it does not involve a calculation. The other units are preferable, in general, because they do not require a complete description of the apparatus in order to define a viscosity, and viscosities determined with various apparatus are directly comparable.

The conversion of ball-drop seconds to absolute or kinematic units is simple and convenient because equations, particularly that of Faxen (*5*), can be used to calculate conversion factors. The use of this equation was thoroughly investigated by Bacon (*3*), whose conclusions as to its general usefulness have been confirmed in this laboratory.

The Faxen equation is actually the expression of Stokes (*9*) applied to the falling-ball viscosity method with a correction for wall effect. The Stokes equation and viscometry based on it involve the following assumptions (*3*):

a. The motion of the sphere relative to the fluid is slow.
b. The fluid is infinite in extent.
c. The fluid is homogeneous.
d. The sphere is rigid.
e. The motion is steady and free from accelerations.
f. There is no slip between fluid and sphere—i.e., the fluid film in contact with the sphere moves with the same velocity and direction.

The Faxen equation provides a correction for assumption *b*. The other requirements are more often fulfilled, as discussed by Bacon (*3*).

Stokes Equation.

$$\eta = \frac{2gr^2(b - s)t}{9L} \tag{1}$$

where
η = absolute viscosity, poises
g = acceleration due to gravity, cm. per sq. sec.
r = radius of ball, cm.
b = density of ball, grams per cc.
s = density of fluid, grams per cc.
t = time of fall, seconds
L = ball-drop distance, cm.

Faxen Equation.

$$\eta = \frac{2gr^2(b - s)t}{9L}\left[1 - 2.104(d/D) + 2.09\,(d/D)^3 - 0.95\,(d/D)^5\right] \tag{2}$$

where
d = diameter of ball, cm.
D = diameter of tube, cm.

Equation 2 simplifies to the form customarily used for the calculation of ball-drop viscosities:

$$\eta = K(b - s)t \tag{3}$$

where K, the apparatus constant, may be determined experimentally with fluids of known viscosity and density, such as oils from the National Bureau of Standards. This constant may also be calculated, for from Equations 2 and 3 it can be seen that

$$K = \frac{2\,gr^2}{9L}\left[1 - 2.104\,(d/D) + 2.09\,(d/D)^3 - 0.95\,(d/D)^5\right] \tag{4}$$

Equation 4 was used for the calculation of apparatus constants reported in some of the following sections. The calculated values usually agree closely with values obtained by calibration.

Kinematic viscosities in stokes, ν, can be calculated from absolute viscosities in poises and the solution density by the following relationship:

$$\nu = \frac{\eta}{s}$$

APPARATUS

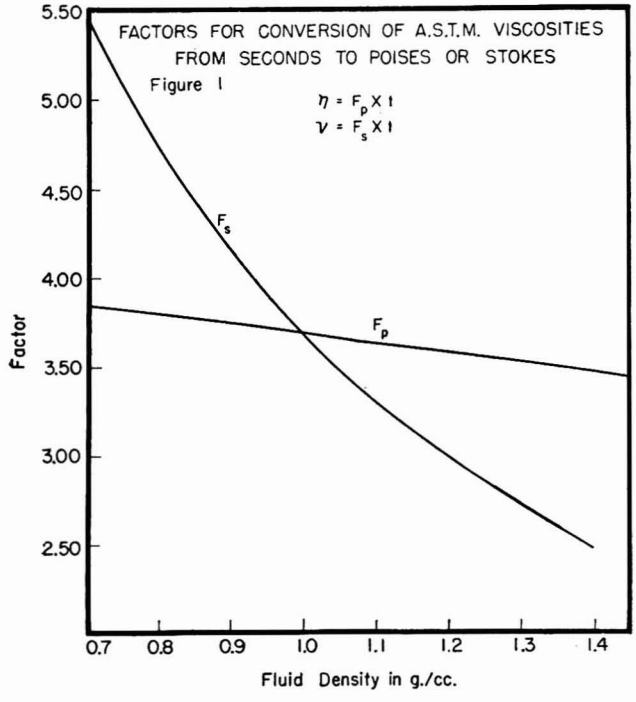
Many different ball-drop viscometers of varying dimensions are now in common use. Some of the confusion thus created can be

avoided by converting the viscosities in seconds to poises or stokes.

A.S.T.M. Viscometer. The A.S.T.M. specifications for cellulose acetate (*2*) and for cellulose nitrate (*1*) described a ball-drop viscometer consisting of $^5/_{16}$-inch steel balls which are timed through a 10-inch (25 4-cm.) drop in a cylinder 14 inches long with an inside diameter of 1 inch. A temperature of 25.0° ± 0.1° C. is specified.

The tolerances permitted and calculation of the apparatus constant, K, by means of the Faxes equation are as follows:

r = radius of ball = 0.1563 ± 0.00025 inch or 0.397 ± 0.0005 cm.



d = diameter of ball = 0.3125 ± 0.0005 inch or 0.794 ± 0.001 cm.

Weight of ball = 2.035 ± 0.010 grams

b = ball density = $\dfrac{2.035}{1.333 \times 3.1416 \times 0.397^3}$ = 7.76 grams per cc.

D = diameter of cylinder = 1.0 ± 0.02 inches or 2.54 ± 0.05 cm.

L = ball-drop distance = 10.0 ± 0.10 inches or 25.4 ± 0.25 cm.

g = acceleration of gravity = 980.4 at Rochester, N. Y.

Substituting the above data into Equation 4 gives:

$$K = 0.545$$

Viscosities measured in this apparatus may be calculated as follows:

$$\eta = 0.545(7.76 - s)t \text{ for viscosity in poises} \quad (6)$$

$$\nu = \frac{0.545(7.76 - s)t}{s} \text{ for viscosity in stokes} \quad (7)$$

Factors F_p and F_s for converting viscosities in seconds to poises or stokes vary for a specified viscometer with the density of the fluid. These factors for the A.S.T.M. viscometer can be read from Figure 1 when the fluid density, s, is known; the calculation is then made as follows:

$$\eta = F_p \times t \quad (8)$$

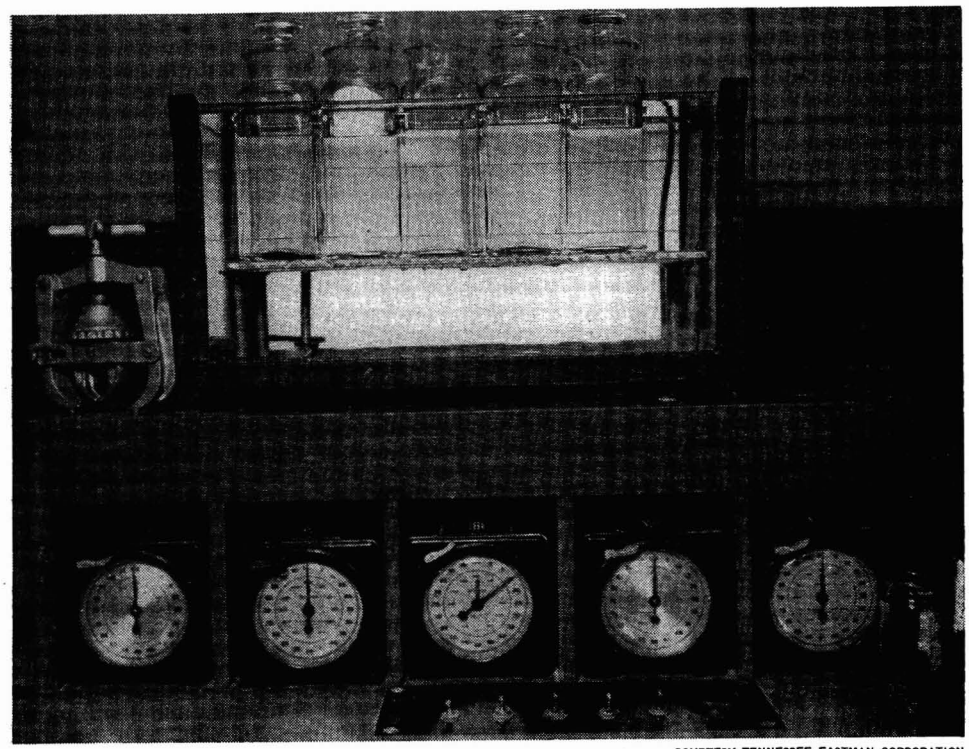
$$\nu = F_s \times t \quad (9)$$

This apparatus has a number of disadvantages: too high ratio of ball to cylinder diameters; difficulty of centering balls, which may lead to considerable wall-effect errors; too short distance between the surface of the fluid and the top timing mark to assure establishment of constant ball velocity in low viscosity solutions; and possibility of solvent loss during transfer of solutions.

Bottle Viscometer. Cellulose ester viscosities were determined for many years in these laboratories in an apparatus similar to the A.S.T.M. viscometer.

Steel balls of $^5/_{16}$-inch diameter were used in cylinders $1^9/_{16}$ inches in inside diameter, 14 inches high, and with a 10-inch ball-drop distance at a temperature of 20° C. Because this method has the same disadvantages as the A.S.T.M. method, a bottle viscometer was developed in the laboratories of the Tennessee Eastman Corporation. The dimensions were selected so that the viscosities in seconds are practically identical with those given by the tube viscometer.

The bottles used are 2.5 inches square, 8 inches high, glass-stoppered, with a capacity of 500 ml. Usually $^1/_8$-inch steel balls weighing 0.1295 to 0.1310 gram are used, but $^1/_8$-inch aluminum or $^1/_{16}$-inch steel or aluminum balls may also be used to extend the working range to lower viscosities. The ball-drop distance is 2.25 inches, with the lower mark 1 inch above the bottom (outside) of the bottle. The bottles may be marked individually on three sides, or more conveniently, marks may be provided on the front and back of the glass water bath in which the viscosity measurement is made (see Figure 2).



COURTESY TENNESSEE EASTMAN CORPORATION

Figure 2. Bottle Viscometer

The square-bottle apparatus has the following advantages: a small ball is used in a container of comparatively large

Table I. Calibration Data for Bottle Viscometer

Bottle
L, 2.25 inches or 5.715 cm.
D, side to side, 6.0 cm.
D, corner to corner, 7.6 cm.

Ball	1/8-Inch Steel	1/8-Inch Aluminum	1/16-Inch Steel
Av. diameter, inch	0.1247	0.1250	0.0625
Av. radius, cm.	0.1584	0.1588	0.0794
Av. weight, gram	0.1302	0.04736	0.01629
Av. density, grams/cc.	7.84	2.82	7.76
Ball constants			
Using Bur. Standards oil			
493.0 poises at 30° C.	0.875	0.873	0.236
208.9 poises at 40° C.	0.847	0.860	0.235
Using oil standardized by Hoeppler viscometer, 107.5 poises at 25° C.	0.846	...	0.229
Calcd. by Equation 4			
Side to side diameter	0.850	0.855	0.227
Corner to corner diameter	0.872	0.877	0.229

cross section to avoid errors due to wall effect; the ball passes through a column of solution sufficient, ordinarily, to bring the ball to constant velocity; and the viscosity is measured in the container in which the solution is prepared, thus avoiding solvent loss errors during transfer. Square bottles were chosen in preference to round bottles in order that the same solution and container can be used for observations of color and haze. Its disadvantages are the difficulty of removing the glass stopper (a mechanical stopper puller is often required) and occasional trouble in avoiding solvent loss at the stopper. These difficulties are sometimes avoided by using a metal foil–covered rubber stopper instead of the glass stopper. When the viscosity is to be measured, the rubber stopper is removed without disturbing the foil. Then a small hole is made in the center of the foil through which the balls are dropped.

Table I presents calibration data and dimensions for 1/8-inch steel and aluminum balls and for 1/16-inch steel balls. Apparatus constants calculated by Equation 4 are compared with calibration data obtained using a Bureau of Standards oil and a Hoeppler viscometer. Because the cross section of the bottles is not circular, calculations have been made assuming the container diameter to be the minimum (side to side) and the maximum (corner to corner). The true value would be expected to lie between these limits. Bureau of Standards calibration data on standard viscosity sample P-7 were:

Temperature, ° C.	Density	Poises	Stokes
30	0.8885	493.0	554.9
40	0.8831	208.9	236.6

The Hoeppler viscometer was calibrated against water, and the ball constants obtained were further checked against various Bureau of Standards oils. A blend of castor oil and dibutyl phthalate (density 1.024) was found by means of the Hoeppler to have a viscosity of 107.5 poises at 25° C. This oil was then used for calibration of the square-bottle apparatus.

The following apparatus constants, based on the data in Table I, are recommended for use with this viscometer: $K = 0.86$ for 1/8-inch balls. $K = 0.23$ for 1/16-inch balls.

Equation 4 shows that these ball constants vary with diameter but not with the density of the ball. It also shows that the change in ball constant with temperature should be very slight, for this depends principally on the coefficients of expansion of glass and the ball metal.

Equations for the calculation of absolute and kinematic viscosities using various balls are:

1/8-Inch Steel Balls

$$\eta = 0.86(7.8 - s)t \quad (10) \qquad \nu = \frac{0.86(7.8 - s)t}{s} \quad (11)$$

1/8-Inch Aluminum Balls

$$\eta = 0.86(2.8 - s)t \quad (12) \qquad \nu = \frac{0.86(2.8 - s)t}{s} \quad (13)$$

1/16-Inch Steel Balls

$$\eta = 0.23(7.8 - s)t \quad (14) \qquad \nu = \frac{0.23(7.8 - s)t}{s} \quad (15)$$

1/16-Inch Aluminum Balls

$$\eta = 0.23(2.8 - s)t \quad (16) \qquad \nu = \frac{0.23(2.8 - s)t}{s} \quad (17)$$

Graphs, such as those shown in Figure 1 for obtaining conversion factors, can be prepared readily using these relationships.

This apparatus can be used over a wide range of temperature without significant errors in the apparatus constant or ball density.

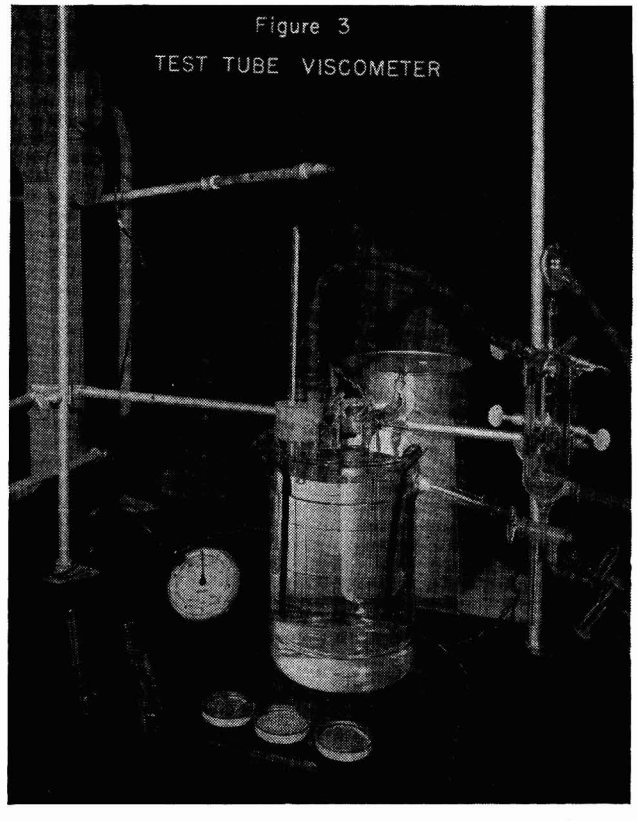
Test Tube Viscometers. Practical viscometers for special applications can be set up simply by making use of test tubes. Malm, Salo, and Vivian (*7*) describe the use of 38 × 150 mm. test tubes and 1/16-inch steel balls with a 1.25-inch fall distance.

For general laboratory purposes, 32 × 200 mm. test tubes are used with 1/16-inch and 1/8-inch steel and aluminum balls

Table II. Calibration Data for 32 × 200 Mm. Test Tube Viscometer

Tube
D (internal), 2.9 ± 0.1 cm.
L, 2 inches or 5.08 cm.

Balls	1/8-Inch Steel	1/8-Inch Aluminum	1/16-Inch Steel	1/16-Inch Aluminum
d, inch	0.1250 ± 0.0003		0.0625 ± 0.0002	
Weight, gram	0.1296–0.1308	0.0466–0.0472	0.0162–0.0164	0.0059
Density, grams/cc.	7.76	2.80	7.78	2.82
Ball constants				
Calcd. by Equation 4	0.836	0.836	0.239	0.239
By calibration with Bur. Standards oil				
8.835 poises at 25° C.	...	...	...	0.235
439 poises at 30° C.	0.830	...	0.241	...
208.9 poises at 40° C.	0.831	...	0.241	...
Useful range, poises	100–1500	40–100	30–100	10–40



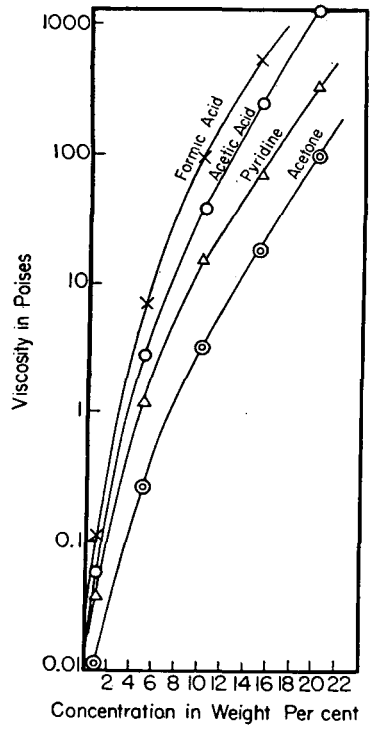


Figure 4. Viscosity-Concentration Curves for Cellulose Acetate Butyrate (13% Acetyl and 37% Butyryl) in Four Solvents

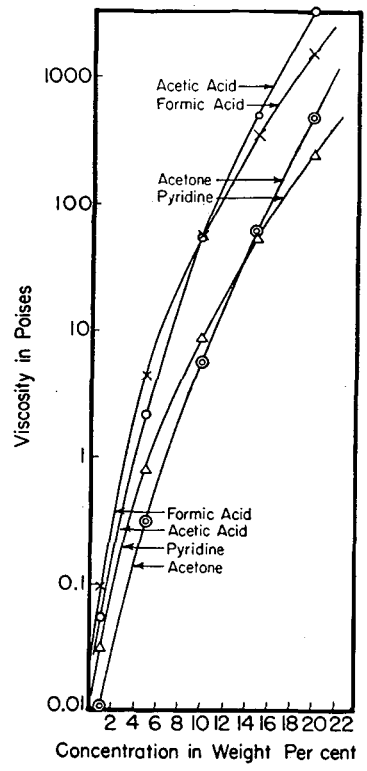


Figure 5. Viscosity-Concentration Curves for Cellulose Acetate Propionate (24% Acetyl and 15% Propionyl) in Four Solvents

with a ball-drop distance of 2 inches (see Figure 3). Viscosities from 10 to 1500 poises can be measured readily with this simple apparatus. The data are shown in Table II, and use of the following constants is recommended for this apparatus: $K = 0.83$ for $^1/_8$-inch balls. $K = 0.24$ for $^1/_{16}$-inch balls.

The equations for the calculation of absolute and kinematic viscosities are like Equations 10 to 17, except for the substitution of these K values.

Although melts can be prepared in test tubes and their viscosities measured directly, it is usually necessary to prepare solutions of cellulose esters in bottles and transfer to tubes, taking precautions to avoid solvent loss. These solutions cannot be prepared in tubes satisfactorily because the bulk of the cellulose ester is too great to provide a sufficient volume of solution, and mixing is difficult in such an elongated container.

PROCEDURE

The sample is dried at a temperature well below its softening point. Two hours at 100° to 110° C. are usually satisfactory for commercial cellulose esters. The required amount is then weighed quickly to avoid error due to moisture absorption and the specified weight of solvent is added.

An accurate procedure is as follows: A little more than the required weight of cellulose ester is dried in the bottle which is to be used, the bottle is stoppered and cooled, and then enough sample is removed to leave the exact weight required. The solvent can often be added volumetrically from an automatic pipet. In this case the size of the sample is adjusted to conform to the weight of solvent dispensed. The weight of bottle plus solution should be recorded and checked just before viscosity determination to prove that no solvent has been lost.

The mixture is allowed to stand stoppered until the cellulose ester is partly dissolved, and the bottle is then tumbled end over end, usually overnight or until a smooth, uniform solution is obtained. In certain cases it may be necessary to warm the sample and tumble again before a good solution is obtained.

The solution is then transferred to the viscometer (unless the container itself serves as the viscometer), taking precautions to avoid solvent loss. When the solution is free from air bubbles and is known to be at the required temperature, clean balls are dropped through the center of the column of liquid, and the time of fall through the marked distance is measured with a stop clock. Ordinarily, the average time for three balls is taken as the viscosity in seconds.

Certain additional precautions should be taken. The size and density of ball should be selected to give a slow enough rate of fall to ensure constant velocity through the distance timed, to give a time great enough to be measured with sufficient accuracy, and to avoid turbulence. If the viscosity is less than about 10 poises or if the time of fall is less than about 20 seconds, some other method of viscosity measurement, such as the use of a pipet, should be chosen. Good temperature control is important, as indicated by the viscosity-temperature curves in Figure 7. When the measurement is made at a temperature greatly different from that of the room, it is advisable to bring the balls to temperature before use.

CHOICE OF VISCOSITY UNITS

Absolute viscosities are probably the best measure of the resistance to flow of fluids through pipes. Kinematic viscosities, on the other hand, best express the resistance to flow by gravity.

Practical viscosities in seconds may not give accurate comparisons between two samples, particularly when the fluid densities vary appreciably. The fact that the effect of density is in opposite directions in the cases of ball-drop seconds and kinematic viscosities leads to difficulties in interpreting ball-drop values. The kinematic viscosity decreases sharply with increasing density, whereas the ball-drop time increases because the driving force depends on the difference in densities of ball and solution.

The following examples illustrate some variations in viscosities which are encountered in plant practice.

Two solutions, one of density 0.85 and the other of 1.20, have viscosities of 80 seconds as measured in the A.S.T.M. ball-drop viscometer. Using factors obtained from Figure 1, their absolute and kinematic viscosities are found to be as follows:

Density	Ball-Drop, Seconds	Absolute Viscosity, Poises	Kinematic Viscosity, Stokes
0.85	80	300	354
1.20	80	286	238

If these solutions have the same absolute viscosity (300 poises), the relationships are found to be:

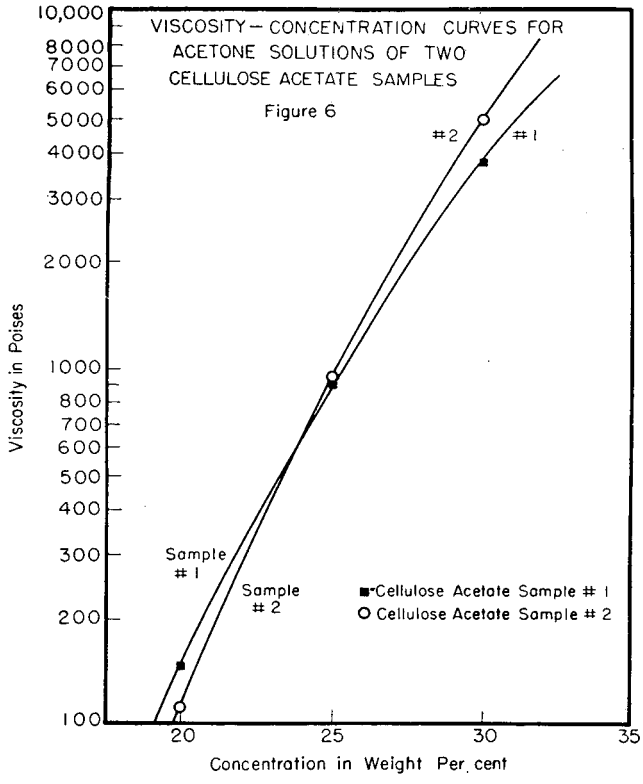
Density	Ball-Drop, Seconds	Absolute Viscosity, Poises	Kinematic Viscosity, Stokes
0.85	80	300	354
1.20	84	300	250

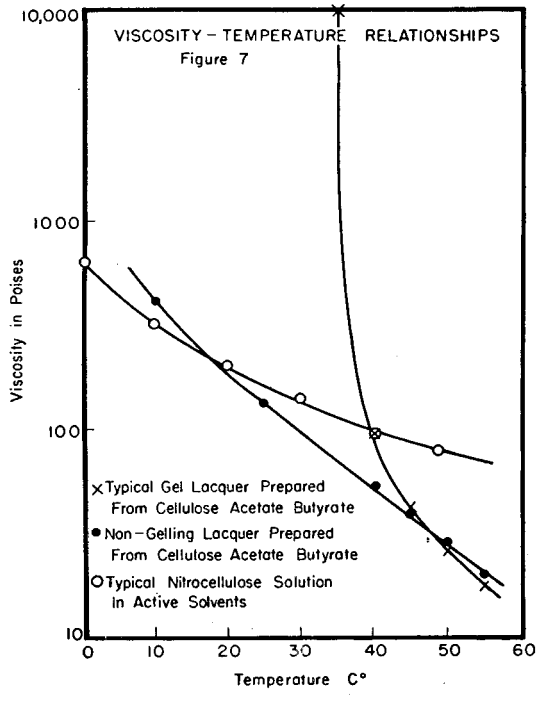
In the third case, where the two samples have the same kinematic viscosity (354 stokes), the relationships are:

Density	Ball-Drop, Seconds	Absolute Viscosity, Poises	Kinematic Viscosity, Stokes
0.85	80	300	354
1.20	119	425	354

VISCOSITIES OF CELLULOSE ESTER SOLUTIONS

The concentration of cellulose ester in a solution is only one of several variables affecting the viscosity. Temperature, solubility, density of the solvent, purity of the solvent, and the presence of certain ions may have pronounced effects. It is, therefore, necessary to measure viscosities of these materials with the composition, concentration, and temperature as close as prac-

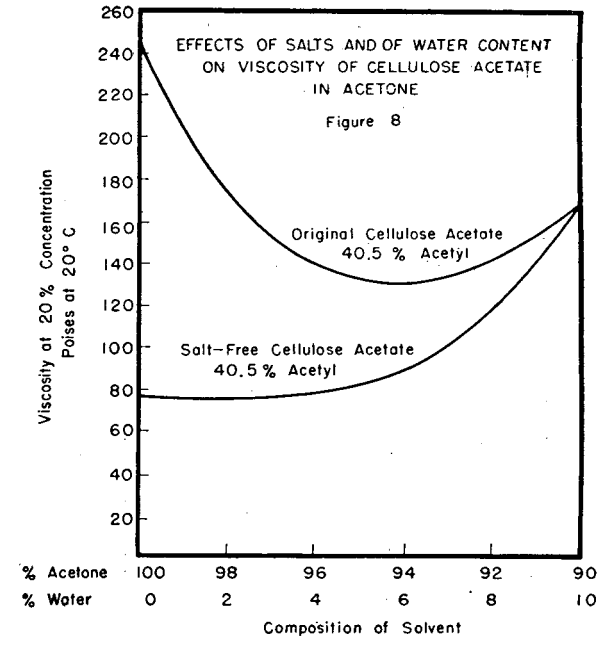




ticable to use conditions, if the results are to be of practical significance.

Effects of Solubility and Density. Figure 4 shows viscosity-concentration curves for a cellulose acetate butyrate (13% acetyl and 37% butyryl) in acetone, pyridine, acetic acid, and formic acid, all of which are good solvents for this ester. The viscosities increase in the same order as the viscosities and densities of these solvents.

A different set of solubility relationships is shown in Figure 5,



which presents viscosity-concentration curves for solutions of a cellulose acetate propionate sample (24% acetyl and 15% propionyl) in the same four solvents. At lower concentrations, less than 8%, the same relationship holds as in Figure 4. However, when the concentration is raised, some of the curves cross. Pyridine and formic acid are good solvents for this ester, but acetone and acetic acid are more nearly borderline solvents, and this limitation in solubility is shown by the steeper viscosity-concentration curves.

Solubility differences depending on the cellulose used for esterification and the method used for manufacturing the ester may also be encountered. Figure 6 shows viscosity-concentration relationships for acetone solutions of two different samples of cellulose acetate. The curves show that sample 2 has a lower viscosity at lower concentrations and a higher viscosity at high concentrations than sample 1.

Effect of Temperature. Viscosity variations with temperature may follow several different relationships, again depending on solubility, as described by Malm and Smith (*8*). Figure 7 contrasts the viscosity variation with temperature of a nitrocellulose solution in active solvents, a nongelling lacquer solution prepared using cellulose acetate butyrate, and a cellulose acetate butyrate gel lacquer.

Effects of Salt Content and of Water in Acetone. Figure 8 shows the variation in viscosity with water content of an acetone solution of cellulose acetate (40.5% acetyl) and also for the same cellulose acetate after treatment to remove salts (*6*). These salts may produce a considerable increase in viscosity (threefold in this case for the acetone solution). The presence of increasing amounts of water first appreciably lowers, then increases the viscosity of the salt-containing sample. The salt-free sample has better solubility in acetone; its viscosity is lower and the presence of increasing amounts of water has only a small effect until more than 5% has been added.

ACKNOWLEDGMENT

The authors gratefully acknowledge the assistance of William Kuchmy and John V. Fleckenstein.

LITERATURE CITED

(1) Am. Soc. Testing Materials, A.S.T.M. Designation D 301–33 for Soluble Nitrocellulose, Section 11.

(2) Am. Soc. Testing Materials, A.S.T.M. Designation D 871–46T for Testing Cellulose Acetate.
(3) Bacon, L. R., *J. Franklin Inst.*, **221**, 251–73 (1936).
(4) Cragg, L. H., *J. Colloid Sci.*, **1**, 261–9 (1946).
(5) Faxen, H., dissertation, Uppsala, 1921; *Ann. Physik*, 68, 89 (1922); *Arkiv Mat. Astron. Fysik*, **17**, No. 27 (1922).
(6) Malm, C. J., U. S. Patent 2,126,489 (Aug. 9, 1938).
(7) Malm, C. J., Salo, M., and Vivian, H. F., *Ind. Eng. Chem.*, **39**, 168–9 (1947).
(8) Malm, C. J., and Smith, H. L., Jr., *Ibid.*, **38**, 941 (1946).
(9) Stokes, G., *Trans. Cambridge Phil. Soc.*, **8** (1845); **9** (1851); Collected Papers, Vol. 1.

RECEIVED October 4, 1949. Presented before the Division of Cellulose Chemistry at the 116th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Determination of Small Amounts of Triethylene Glycol in Air

SAUL KAYE WITH THE TECHNICAL ASSISTANCE OF ANNE C. ADAMS
University of Chicago, Chicago 37, Ill.

A method is described for the quantitative determination of triethylene glycol vapor in air. By sampling 30 liters of air, concentrations of the order of 2 micrograms per liter (0.002 p.p.m.) may be determined simply and rapidly. This concentration is in the range usually required in processes of air sterilization. The air is passed through concentrated sulfuric acid, which absorbs all the triethylene glycol present; the acid solution is heated on a steam bath for 30 minutes and cooled to 30° C., and then a freshly prepared solution of 1-naphthol in sulfuric acid is added. The intensity of the yellow color formed is measured spectrophotometrically, and is proportional to the quantity of triethylene glycol present. The presence of water affects the development of color; different calibration curves must therefore be used for different relative humidities. A formula relates the optical density of the treated sample to both the water and the glycol content, so triethylene glycol concentrations may be calculated directly from spectrophotometer readings. When conditions of analytical treatment are carefully controlled, the method gives deviations of about ±5% of the glycol concentrations.

THE vapors of triethylene glycol and of propylene glycol have been found to possess marked bactericidal activity when dispersed in small quantities in the air (*3*, *6*, *7*). The former compound, having much the lower vapor pressure (0.0013 mm. of mercury at 25° C., *4*), is the more efficient, and is currently receiving widespread application as an aerial disinfectant for occupied premises.

The bactericidal effectiveness of triethylene glycol vapor depends upon the extent to which the atmosphere is saturated with this vapor. The saturation concentration of triethylene glycol vapor in the air in the absence of water vapor has been reported by Wise and Puck (*11*) to be of the order of 11 micrograms of glycol per liter of air at 25° C., and the variation of this saturation concentration with relative humidity and temperature of the air has been determined (*10*).

Losses of unknown magnitude, caused by condensation and by ventilation of the room, make it desirable to have an analytical method of determining the concentration of triethylene glycol in the air. This method must be sufficiently sensitive to determine quantities of glycol in the saturation range, between 1 and 10 micrograms per liter of air, at normal temperatures and relative humidities.

Wise, Puck, and Stral (*12*) bubbled the air through water and determined colorimetrically the extent to which an aliquot of the aqueous glycol solution reduced a standard dichromate–sulfuric acid mixture. Because of the small quantities of triethylene glycol which must be analyzed, large volumes of air (ca. 300 liters) and long sampling periods must be used. Another method of determining triethylene glycol (*2*, *5*) depends upon the condensation of the vapor upon a cooled surface, and subsequent measurement of the amount of light scattering caused by this condensed film. Instruments embodying this principle (glycometers) have been built and successfully used in these laboratories for several years. They are, however, complicated and expensive, and are not yet commercially available.

This paper describes a simple, rapid, and reasonably precise method for the analysis of triethylene glycol in air. Concentrated sulfuric acid is used as the collecting medium for the glycol, and the analysis is performed spectrophotometrically by determining the intensity of the color produced upon the addition of 1-naphthol to the heated acid solution of glycol.

APPARATUS AND MATERIALS

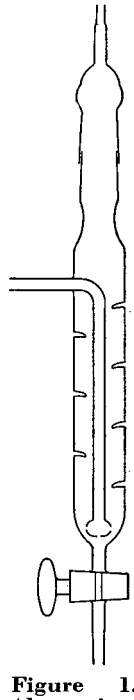


Figure 1. Absorption Tube

Sampler. The sampler used is a glass absorption tube (Figure 1). Air is drawn through the tube by means of a vacuum pump, which is protected from corrosion by a soda-lime tube placed in the line. The flow of air is controlled by a suitable critical flow orifice installed between the sampler and the pump. It is desirable to have as little absorbing fluid as possible left in the tube after draining, and to prevent the liquid from splashing over into the vacuum system during operation. The Vigreux-type indentations have been found helpful in the second regard. When 10 ml. of sulfuric acid are used in the tube illustrated, whose over-all height is 26 cm., the acid level is about 3 cm. above the outlet. When air is being drawn through the acid at a rate of 15 liters per minute, the acid level rises to about 6 cm. above the base. The arrangement of the ground-glass joint (24/40) at the cap is convenient for refilling the bubbler. The sulfuric acid acts as stopcock lubricant; both the regular and the silicone-base types of stopcock grease were found to interfere with the test. The rubber retaining washer must be removed from the stopcock.

Other Equipment. A spectrophotometer (Coleman Universal, Model 14, was used in this laboratory), a steam bath, and a water bath capable of maintaining a temperature of 30° C. are required. A vacuum pump and appropriate flowmeters should be available for taking the samples.

Reagents. Triethylene glycol (air sterilization grade, Carbide and Carbon Chemicals

Corporation) distilled between 104° and 105° C. at 0.4 mm. of mercury, was employed as the standard. Concentrated sulfuric acid, reagent grade, and 1-naphthol, melting point 95–96° (Eastman Kodak), are the other reagents.

SAMPLING PROCEDURE

Ten-milliliter portions of concentrated sulfuric acid are dispensed, by means of an acid buret, into heavy-walled borosilicate glass ignition tubes, 25 × 200 mm. These tubes are placed in a rack and covered to protect them from dust. When a sample is required, the contents of one of the tubes are poured into the absorbing tube and the ignition tube is clamped below to receive the liquid after air has been drawn through. With the bubbler illustrated, 9.8 of the original 10.0 ml. drain from the apparatus in about 15 seconds. Thus, if successive samples are to be taken from atmospheres of changing glycol content, it is well to rinse the absorption tube with sulfuric acid between samples. For tests on more static atmospheres, however, the carry-over of glycol from one sample to the next introduces negligible error if only short intervals intervene.

The method is most accurate when the sample contains between 0.05 and 0.25 mg. of triethylene glycol. A maximum of 18 liters of air per minute may be drawn through 10 ml. of acid in the sampler illustrated without observable loss by splashing, while air flow rates between 18 and 12 liters per minute have not produced observable differences in collection efficiency. In order to simplify the procedure, the authors have installed standard orifices in vacuum lines, so that a constant rate of 15.0 liters of air per minute is maintained. All samples are taken for 2 minutes when normal "glycolized" atmospheres are being analyzed; the 30 liters of air thus sampled provide the amount of glycol required to perform the spectrophotometric determination in the most accurate range. The time of sampling may be varied for particularly low or high glycol concentrations.

ANALYTICAL PROCEDURE

After a series of samples has been taken, the ignition tubes are placed in a boiling water bath for 30 minutes, then removed and placed on a bath at 30° C. (A rack that can be transferred from one bath to the other is a convenience here.) The contents of the tubes reach bath temperature in about 5 minutes, and this interval is used for the preparation of the 1-naphthol solution, which must be freshly made immediately before each test. The reagent is dissolved in concentrated sulfuric acid; 0.100 gram is added for each 21.0 ml. of sulfuric acid used. When the ignition tubes have cooled to 30° C., 1 ml. of the 1-naphthol solution is added to each, and the mixtures are shaken. The tubes and contents are allowed to remain at 30° C. for an additional 15 minutes to allow full color development, and are then transferred to individually calibrated cuvettes. A tube containing only 10 ml. of unaerated sulfuric acid is boiled and cooled along with the samples, is treated with naphthol solution, and then serves as the blank for the entire series. The intensity of yellow color produced in the test samples is compared with the blank by measuring the relative transmittance of light of wave length 410 mμ, where maximum absorption occurs, as was determined by a wave length–transmittance experiment.

CALIBRATION CURVE

A calibration curve must be prepared to relate the measured per cent transmittance to the glycol content.

A stock solution is prepared by slowly adding cold sulfuric acid to 1.000 gram of triethylene glycol until the volume is nearly 100 ml. The final dilution to 100 ml. is made at room temperature. Appropriate volumes of this concentrated solution are diluted with sulfuric acid, so that a series of solutions containing from 0.01 to 0.40 mg. of triethylene glycol per milliliter is obtained. Such standard solutions have been kept in a refrigerator for months without a significant change in response. One milliliter of each of these solutions is added to 9 ml. of concentrated sulfuric acid contained in an ignition tube, and these standards are then treated as described above.

Line *A* on Figure 2 indicates the relation of optical density, $D = (2 - \log_{10} \%\ \text{transmittance})$ to the amount of triethylene glycol in each sample. The relationship is a straight line up to at least 0.25 mg. of glycol in the sample, and is reproducible within the limits of accuracy of the instrument (approximately 3% of the measured glycol concentration for transmittances between 20 and 70% of the control, or optical densities from 0.1 to 0.7).

EFFECT OF WATER

Samples of triethylene glycol and acid to which more water has been added develop less color with the naphthol reagent than they would were no water added. If this is not taken into consideration high humidities can introduce serious error into the determination of the triethylene glycol content of the air. In Figure 2, *A* represents the transmittance *vs.* glycol relationship in the absence of water, while *C* indicates the relation in the presence of 0.50 ml. of water in each sample. From a series of tests with different amounts of added water, one obtains a family of straight lines of differing slope. By relating the slopes of these lines to the water content, we obtain, for our test conditions, the relation

$$\text{Milligrams of TEG} = \frac{D}{2.6 - 3(\text{grams of } H_2O)}$$

where the weights of triethylene glycol and of water are those added to the sample, and D is the optical density. Knowing the volume, the temperature, and the relative humidity of the air being sampled, we may convert optical densities to glycol concentrations by this expression.

Calculation is considerably simplified when the air being sampled is of constant temperature, and when a constant volume is taken. Thus, for 30-liter samples, at about 23° C., substitution in the above formula gives

$$\text{Micrograms of TEG/liter} = \frac{1780D}{145 - RH}$$

where RH is the relative humidity.

A psychrometric determination is required with each sample. Although this dependance upon the humidity of the air introduces an additional variable, it does not in practice operate as a serious drawback to the use of the method, because the relative humidity of the air is of primary importance in determining the effectiveness of glycol as an aerial disinfectant, and must be regularly determined in the course of any program utilizing glycol. The fact that the analytical procedure for glycol requires a knowledge of the humidity, then, introduces no new complications.

COLLECTION EFFICIENCY

Wise, Puck, and Stral (*12*) have reported that the first of two absorption tubes containing water succeeded in removing only about 70% of the glycol from the air; when two such tubes were used in series, the total recovery was approximately 90%. With 10 ml. of sulfuric acid as the collection medium, a single absorber appears to be completely efficient, as indicated by the close agreement of concentrations determined analytically in saturated atmospheres with saturation concentrations determined from vapor pressure measurements. Furthermore, when two acid absorbers were used in series, there was at no time any measurable amount of glycol recovered in the second sampler.

ACCURACY AND PRECISION OF METHOD

The accuracy of the results obtained from air containing triethylene glycol is not readily determined, because the normal methods of volatilizing this material into the room, by the use of a heated surface, lead to losses of not inconsiderable size. Thus, in this laboratory (*9*) it has been estimated that only about 50% of the quantity of material lost from a vaporizing device appears in the air of even a sealed room. British workers (*1*) have confirmed and extended these findings. In order to obtain atmospheres containing triethylene glycol vapor in concentrations which could be estimated by reasonably certain independent methods, a

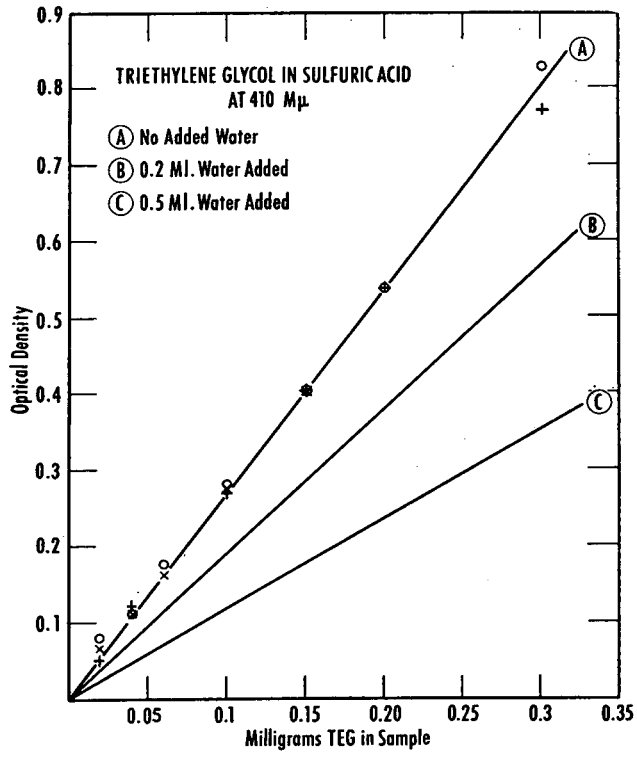


Figure 2. Calibration Curve

Table I. Triethylene Glycol Concentration of Saturated Air

	Test I	Test II
Temperature, ° C.	23.9	20.0
Relative humidity, %	58	54
Analytical concn., micrograms/liter	2.91, 2.91, 2.70, 2.61, 3.06, 2.96, 2.67	1.95, 1.72, 1.72, 2.11, 1.85, 1.95, 1.95, 1.95
Mean analytical concn.	2.83 ± 0.15	1.90 ± 0.10
Saturation concn. (Wise and Puck, *11*)	2.75	1.95

tightly sealed experimental room (*8*) was used. Several large bed sheets were soaked with liquid glycol, and hung in the chamber for 2 days with a large fan playing directly upon them. It is reasonably certain that at the end of this time the air was saturated with triethylene glycol vapor. The results of two such tests are presented in Table I.

The average deviation from the mean of the analytical values is seen to be ±5.3% in both tests, and the saturation concentrations, determined in a different fashion, fall within this range.

In another test, two 30-liter air samples were taken simultaneously from an experimental chamber into which triethylene glycol was being heat-volatilized continuously. This method of evaporation usually produces mist as well as vapor, and thus the absolute concentration at any time is not known. The results obtained from several such pairs of samples analyzed by the above method are presented in Table II. The average of the deviations of each pair from its mean is 2.5%.

INTERFERING SUBSTANCES

In a previous method for the analysis of triethylene glycol (*12*) ordinary room air often gave readings which formed a significant proportion of those obtained from air containing glycol. A series of tests in the authors' laboratory and in several other "normal" atmospheres indicated that ordinary room air gives a negative or negligible response when tested by this method. A red-orange color developed, however, when air containing a large amount of tobacco smoke was sampled. Gross amounts of dust or traces of rubber or soap in the ignition tubes imparted a deep red color to the samples when naphthol was added, whereas traces of copper and iron caused a green coloration. The effect of various organic materials has thus far been investigated only in a preliminary way. The test is not specific for triethylene glycol, for propylene glycol and diethylene glycol give qualitatively similar results.

An important source of error will be introduced into any of the methods used for determination of triethylene glycol if the temperature of the surface from which it is heat-volatilized is high enough to cause thermal decomposition. Tentatively, this temperature may be taken as 140° C.

Table II. Triethylene Glycol Concentrations Determined by Pairs of Samplers

Pair No.	Analytical Concentration, γ/Liter Sampler 1	Sampler 2	Mean
1	1.98	1.75	1.87
2	2.09	2.06	2.08
3	2.44	2.22	2.33
4	2.44	2.21	2.33
5	2.40	2.45	2.43
6	2.92	2.89	2.91
7	3.05	3.06	3.06

DISCUSSION

The 1-naphthol solution must be prepared immediately before use; the authors have not yet found a solvent in which the naphthol is stable, which does not interfere in some way with the test. If the naphthol solution is permitted to stand for more than 15 minutes, color formation is greatly diminished. This diminution in color also occurs if the 1-naphthol solution is added to the hot acid solutions of glycol.

At least 30 minutes in the steam bath are necessary; increasing this time to 1 hour appears to produce no greater color, while heating for more than 1 hour appears to decrease color formation. The color develops to a maximum within 15 minutes after addition of the naphthol reagent, and fades but slowly thereafter.

The authors are at present attempting to determine the mechanism of this reaction, whose details have been determined in an empirical fashion. The method has, however, provided a useful tool for performing routine analyses of the air in experimental rooms.

ACKNOWLEDGMENT

The authors are indebted to E. W. Dunklin for supplying redistilled triethylene glycol.

LITERATURE CITED

(1) Bourdillon, R. B., Lidwell, O. M., and Lovelock, J. E., "Studies in Air Hygiene," p. 122, London, Medical Research Council, 1948.
(2) Puck, T. T., *Rev. Sci. Instruments*, **19**, 16 (1948).
(3) Puck, T. T., Robertson, O. H., Hamburger, M., and Hurst, V., *J. Infectious Diseases*, **76**, 216 (1945).
(4) Puck, T. T., and Wise, H., *J. Phys. Chem.*, **50**, 329 (1946).
(5) Puck, T. T., Wise, H., and Robertson, O. H., *J. Exptl. Med.*, **80**, 377 (1944).
(6) Robertson, O. H., Bigg, E., Puck, T. T., and Miller, B. F., *Ibid.*, **75**, 593 (1942).
(7) Robertson, O. H., Puck, T. T., Lemon, H. M., and Loosli, C. G., *Science*, **97**, 142 (1943).
(8) Robertson, O. H., Puck, T. T., and Wise, H., *J. Exptl. Med.*, **84**, 559 (1946).
(9) Wise, H., *Ind. Eng. Chem.*, **41**, 633 (1949).
(10) Wise, H., Ph.D. thesis, University of Chicago, 1947.
(11) Wise, H., and Puck, T. T., *Science*, **105**, 556 (1947).
(12) Wise, H., Puck, T. T., and Stral, H. M., *J. Biol. Chem.*, **150**, 61 (1943).

RECEIVED September 30, 1949. Presented before the Division of Analytical and Micro Chemistry at the 116th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J. Work done under a grant from the Biological Department, Chemical Corps, Camp Detrick, Frederick, Md.

Simultaneous Spectrophotometric Determination of Cobalt, Copper, and Iron

R. E. KITSON, *Polychemicals Department, E. I. du Pont de Nemours & Company, Inc., Wilmington, Del.*

An examination of the thiocyanate-acetone system led to the development of a colorimetric method for the simultaneous determination of cobalt, copper, and iron. The method involves development of color in a single solution followed by spectrophotometric measurements at three wave lengths: 380, 480, and 625 mμ. The procedure is simple, rapid, and free of serious interferences, but it is not applicable to amounts of copper smaller than 50 micrograms. A modified procedure was developed for the specific determination of cobalt.

THE direct colorimetric determination of cobalt or copper is relatively simple in the presence of each other or in solutions containing iron. Iron is most frequently determined by the *o*-phenanthroline and the thiocyanate methods, but these procedures are subject to interference from both cobalt and copper (*9*). In the thiocyanate method, this interference takes the form of complexes of colors distinctly different from that of the ferric thiocyanate. Cobaltous thiocyanate in 50% acetone is an intense blue, whereas the cupric thiocyanate complex in the same medium possesses a dull, orange-brown color. It seemed likely that a suitable set of conditions might be found under which the three colored complexes would form in the same solution. Then measurements at three different wave lengths on a spectrophotometer should permit the determination of all three metallic ions in the same solution. A careful study of the thiocyanate colors actually led to a rapid, simple method for the simultaneous determination of cobalt, copper, and iron.

The thiocyanate system, particularly as applied to the determination of iron, has been the subject of extensive studies (*2*). The reader is referred to the thorough reports of Woods and Mellon (*15*) and Peters and French (*6*). Because of the similarity of color development conditions to those of Woods and Mellon, little experimental work was done on the iron thiocyanate system in the present research.

The thiocyanate method for cobalt has not been studied as extensively as the corresponding procedure for iron. Although the basic color reaction was discovered by Vogel and others around 1870, Tomula (*12*) appears to have been the first to use the reaction on a quantitative basis. Modifications of the original method, including the reduction of the ferric thiocyanate with chlorostannous acid or other reductants and the solvent extraction of the cobalt complex, have been published by Putsché and Malooly (*8*), Young and Hall (*16*), Vorontzov (*13*), and Kolthoff (*4*).

So far as the author knows, the copper thiocyanate complex has never been proposed as a basis for the determination of copper.

APPARATUS AND SOLUTIONS

Transmittancy measurements were made with a Beckman Model DU spectrophotometer. The blue-sensitive phototube was used for all measurements except where transmittancy curves over the region 620 to 1000 mμ were being measured. The tungsten source and Corex absorption cells were used throughout. Transmittancy measurements in all cases were made against a blank containing the reagents.

Standard solutions of cobalt, copper, and iron were prepared by dissolving weighed amounts of the pure metals in nitric acid, removing most of the excess acid by evaporation, and diluting the resulting solutions to known volumes with water. Stock solutions contained from 1 to 1.5 mg. of metal per ml., and were suitably diluted to provide working standards of the desired concentration. Enough acid was added to the working standards to prevent hydrolysis of the metallic ions.

Ammonium thiocyanate (C.P., 250 grams) was dissolved in distilled water, and the resulting solution was diluted to 500 ml.

The chlorostannous acid reagent was prepared by dissolving 20 grams of C.P. stannous chloride in 40 ml. of concentrated hydrochloric acid, and diluting to 100 ml. with distilled water. Metallic tin was added to stabilize the solution.

Saturated ammonium peroxydisulfate solution was obtained by mixing an excess of the C.P. salt with water and allowing the mixture to stand for 24 hours with occasional shaking.

The concentrated nitric, sulfuric, and hydrochloric acids were C.P. chemicals, as was the acetone.

COLOR REACTION

Much has been written about the intense red color of the ferric thiocyanate system, which apparently is due to a complex ion such as $Fe(SCN)^{++}$ or $Fe(SCN)_6^{---}$ (*1, 5, 10, 11*). The intensity of the color is somewhat greater in solutions containing high concentrations of an organic solvent of low dielectric constant, such as acetone, ethyl alcohol, or methyl Cellosolve (*14*) than in an aqueous system. The color fades very slowly upon standing, owing to the autoreduction of iron to the ferrous state. This reduction may be delayed by the addition of suitable holding oxidants (*7, 15*).

Unlike the ferric thiocyanate color, the bright blue cobaltous thiocyanate color, which may be due to the complex ion $Co(SCN)_4^{--}$ (*8, 9*), does not appear in aqueous solution. In solutions containing more than about 40% ethyl alcohol, acetone, or similar materials, however, the color develops immediately. It is stable, and cannot be destroyed by reductants.

The cupric thiocyanate complex, like the cobalt complex, develops only in solutions containing organic solvents such as acetone. It is probably due to a complex ion and is readily reduced, being so unstable that a holding oxidant must be used to make accurate color measurements.

The spectral transmittancy curves for the three colors are given in Figure 1. The wave lengths of maximum absorption of the three colored complexes, together with their relative strengths, are given in Table I.

EFFECT OF VARIABLES ON COLOR DEVELOPMENT

Acetone Concentration. As reported by Woods and Mellon (*15*), increasing concentrations of acetone increase the intensity of the iron color. A strictly similar effect is not observed with the cobalt color, because this color does not exist in aqueous systems.

Table I. Intensity of Thiocyanate Complexes at Wave Lengths of Maximum Absorption[a]

Metal Ion	Wave Length, mμ 380	480	625
Co^{++}	0	0	29
Cu^{++}	28	19	3
Fe^{+++}	60	300	17

[a] Expressed as specific extinction, k, where

$$k = \frac{\text{absorbancy}}{(\text{concentration, mg./ml.})(\text{cell length, cm.})}$$

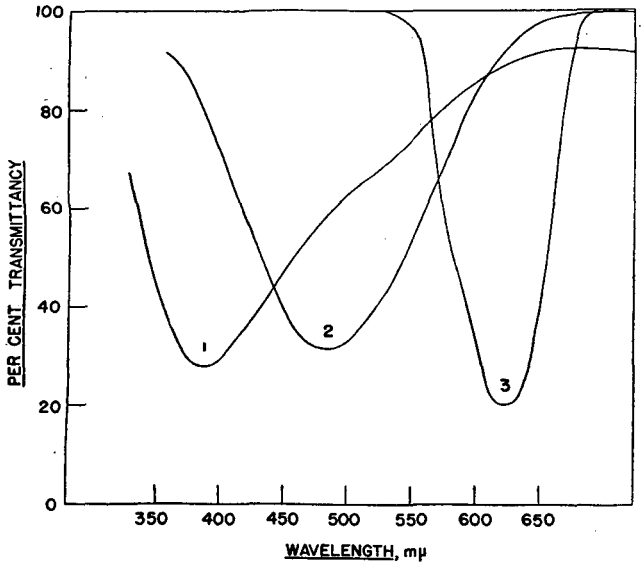


Figure 1. Spectral Transmittancy Curves of Copper, Iron, and Cobalt Thiocyanate Complexes

1. Ca. 20γ Cu^{++}/ml.
2. Ca. 2γ Fe^{+++}/ml.
3. Ca. 15γ Co^{++}/ml.

Table II. Range of Obedience to Beer's Law for Cobaltous, Cupric, and Ferric Thiocyanate Systems

[Concentrations expressed as γ/ml. (p.p.m.) of final colored solution]

Metal Ion	Wave Length, mμ		
	380	480	625
Co^{++}	...	...	0–60
Cu^{++}	0–30	0–60	0–90
Fe^{+++}	0–6	0–6	0–6

At acetone concentrations up to 40%, the color intensity increases very rapidly with increases in acetone concentration (*12*), but above 40% acetone, the rate of color increase is much slower. A similar behavior is noted for the copper color. High acetone concentrations apparently have no effect on the colors except to increase their intensity slightly. The amount of acetone used is limited by the low solubility of many inorganic salts in an acetone-water medium and by the need to keep the total volume of reagents (including acetone) small to permit the use of large aliquots of sample solution. The best compromise appears to be 50% acetone in the final volume. At concentrations much below this figure, small changes in acetone concentration have a very important effect on the intensities of the cobalt and copper colors. Fifty per cent acetone, in a 50-ml. final volume, permits the use of 15 ml. of sample, which is generally adequate.

Acid Concentration. The thiocyanate color systems require an acid environment. Many investigators have used either nitric or hydrochloric acids, and the author found either to be satisfactory in all three of the color reactions. An amount corresponding to 2 ml. of the concentrated acid per 50-ml. final volume is satisfactory in each case. Sulfuric acid in the copper color reaction tends to increase the intensity of the absorption at 480 mμ. Although this effect is not serious, it led to the rejection of sulfuric acid as the acidifying agent.

Thiocyanate Concentration. If excess thiocyanate is present, thiocyanate concentration has no effect on the development of any of the colors; 2.5 ml. of the 50% solution are used.

Copper, Cobalt, and Iron Concentrations. Each of the three color systems obeys Beer's law over limited ranges. Their behaviors at the various wave lengths of interest are summarized in Table II.

Holding Oxidants. Much has been written about holding oxidants in the iron thiocyanate system (*7*, *15*). In modern photometric practice, where the intensity of the iron thiocyanate color can be measured within a few minutes after development, no holding oxidant is needed. This is not true of the cupric thiocyanate system. This color fades rapidly and must be held by some means if maximum precision is sought. Potassium periodate is too strong an oxidant. Under conditions of the work reported here it produces a yellow coloration with the other reagents which causes very high values for copper. Small amounts of potassium peroxydisulfate, however, were found to provide the proper action. The amount must be carefully controlled. An excess causes a marked yellow coloration which results in high copper values; 0.5 ml. of a saturated aqueous solution, added before color development, is satisfactory. Hydrogen peroxide, frequently used in the ferric thiocyanate system, does not prevent autoreduction of the cupric thiocyanate.

Order of Addition of Reagents. In the cobalt and iron reactions, the order of addition of reagents apparently is not significant. This is not true of the copper thiocyanate system, however. Addition of acetone to a system containing copper, acid, oxidant, and thiocyanate, or of thiocyanate to a system containing the other reagents, failed to produce consistent colors. Apparently both the thiocyanate and acetone are important to the color reaction, and small local concentrations of these reagents become magnified in effect at the instant of color development. Solutions of ammonium thiocyanate in acetone developed yellow colors upon standing. A freshly prepared solution of thiocyanate in acetone proved to be satisfactory, and its use is recommended.

Diverse Ions. In the study of interfering ions, the following color development procedure was used (the contents of the flasks were mixed thoroughly after each addition).

To a 50-ml. volumetric flask containing a solution of a known amount of the metal being examined and a known amount of the diverse ion were added 2 ml. of concentrated hydrochloric acid. Next, 0.5 ml. of a saturated solution of ammonium peroxydisulfate was added. Into a 50-ml. Erlenmeyer flask were pipetted 25 ml. of acetone and 2.5 ml. of 50% (w/v) aqueous ammonium thiocyanate solution. This solution was added to the contents of the volumetric flask. The contents of the latter flask were then diluted to volume with water. The color intensity was measured at the appropriate wave lengths in 1-cm. cells against a blank containing the reagents and the diverse ion. Approximately 50 diverse ions were tested in the cobalt system and in the copper system. In each case, 0.5 mg. of either cobalt or copper was used with 100 times this concentration of the diverse ion. If interference was observed at this concentration of diverse ion, successively smaller concentrations of the ion were tried until a concentration was found giving less than 5% interference. Woods and Mellon (*15*) have made a similar study on the iron thiocyanate system, and this work was not repeated.

Of the ions tested, the following did not interfere in the cobalt or copper procedures when present in concentrations 100 times that of the measured ion: acetate, aluminum, ammonium, antimony, arsenate, arsenite, beryllium, cadmium, carbonate, chloride, formate, lithium, magnesium, manganese, nickel, nitrate, oxalate, phosphate, potassium, silicate, sodium, sulfate, thorium, zinc, and zirconium.

The following ions interfered in the cobalt and copper procedures and must be absent from the sample to be analyzed, or must be removed by suitable techniques before color development: barium, calcium, lead, mercurous, silver, strontium, thiosulfate, and tungstate, which gave precipitates with one or another of the reagents; ceric, chromic, and dichromate ions, which interfered by their own color; and bismuthyl, molybdate, and titanium, which reacted with the reagents to give intensely colored compounds.

The effect of the remaining diverse ions is summarized in Table III.

Direct Procedure for Cobalt Determination. Both the cupric and ferric thiocyanate complexes can be reduced to colorless cuprous and ferrous complexes. If the determination of cobalt

Table III. Effect of Certain Ions on Color Development

	Ion Sought					
	Cobalt		Copper		Cobalt (with Stannous Chloride)	
Ion	Effect	Max. permissible amount[a], mg.	Effect	Max. permissible amount[a], mg.	Effect	Max. permissible amount[a], mg.
Bromide	None	>50	Reduces color intensity	5	None	>50
Chlorate	Yellow color with reagents	>50[b]	Yellow color with reagents	5	None	>50
Iodide	Yellow color with reagents	>50[b]	Yellow color with reagents	0.6	None	>50
Mercuric	None	>50	Reduces color intensity	10	None	>50
Nitrite	Reduces color intensity	5	Destroys color	None	Reduces color intensity	10
Sulfite	Yellow color with reagents	>50[b]	Yellow color with reagents	None	Intense yellow color with reagents	None
Uranyl	Intense yellow color with reagents	5	Intense yellow color with reagents	None	Intense yellow color with reagents	5
Vanadate	Yellow color with reagents	5	Yellow color with reagents	None	Intense red color with reagents	None

[a] In presence of 0.59 mg. of cobalt or copper.
[b] Yellow colors seldom interfere with spectrophotometric measurement of cobalt alone at 625 mμ.

Table IV. Analyses of Known Cobalt-Copper-Iron Solutions[a]

	Cobalt		Copper		Iron	
Solution	Added	Found	Added	Found	Added	Found
	γ	γ	γ	γ	γ	γ
1	298	306	298	263	..	..
2	745	755	298	271	..	..
3	745	755	595	610	..	..
4	298	296	..	..	102	101
5	745	755	..	5	61	63
6	119	122	..	..	102	103
7	..	..	119	109	102	101
8	..	..	298	291	102	104
9	119	125	119	98	41	43
10	745	750	1490	1440	102	91

[a] Over-all precision of method does not generally permit number of significant figures given here for illustrative purposes.

alone is desired, the deliberate reduction of copper and iron speeds up the cobalt determination.

Of the various substances available, chlorostannous acid was chosen as a stable, readily available reductant. The chlorostannous acid is added as a 20% solution in dilute hydrochloric acid. The strength of the acid is so chosen that 5 ml. of the reductant contain the equivalent of 2 ml. of concentrated hydrochloric acid. The amount of reductant added is unimportant, provided an excess is present. The effects of other variables, except diverse ions, on the color development are essentially the same as in the absence of chlorostannous acid.

In the study of the effects of diverse ions, the same order of addition was used as in the unmodified procedure. However, 5 ml. of chlorostannous reagent were used in place of the hydrochloric acid, and the holding oxidant was omitted. The premixing of the acetone and thiocyanate was also omitted in most cases, because it was unimportant in any but the copper thiocyanate color reaction.

Of the ions tested, the following did not interfere when present at concentrations 100 times that of the cobalt: acetate, aluminum, ammonium, antimony, arsenate, arsenite, beryllium, bismuth, borate, bromide, cadmium, carbonate, chlorate, chloride, formate, iodide, lead, lithium, magnesium, manganese, nickel, nitrate, oxalate, phosphate, potassium, silicate, sodium, sulfate, thiosulfate, thorium, titanium, zinc, and zirconium.

The following ions interfered in the cobalt determination, and must be absent from the sample, or removed by suitable techniques before the color development: barium, calcium, mercurous, silver, strontium, and tungstate ions, which gave precipitates with one or another of the reagents; chromic and dichromate ions, which interfered by their own colors; and ceric, molybdate, and vanadate ions, which reacted with the reagents to give intense colors.

The effect of the remaining diverse ions is summarized in Table III.

Analysis of Known Mixtures. In order to test the method, a number of known mixtures of cobalt, copper, and iron were prepared and analyzed using the procedure detailed above under diverse ions. Typical results are given in Table IV.

Calculation of Results. The theory of photometric analysis of mixtures of two or more colors has been discussed (*3*). In the present case, three equations in three unknowns are required. These equations take the form

$$A_{380} = k_{380}^{Cu}C_{Cu} + k_{380}^{Fe}C_{Fe} + k_{380}^{Co}C_{Co} \quad (1)$$

$$A_{480} = k_{480}^{Cu}C_{Cu} + k_{480}^{Fe}C_{Fe} + k_{480}^{Co}C_{Co} \quad (2)$$

$$A_{625} = k_{625}^{Cu}C_{Cu} + k_{625}^{Fe}C_{Fe} + k_{625}^{Co}C_{Co} \quad (3)$$

Fortunately for the simplicity of the method, k_{380}^{Co}, and k_{480}^{Co} are essentially zero. This permits the solution of Equations 1 and 2 as equations in two unknowns. The concentration of cobalt can then be calculated from Equation 3 after the concentrations of copper and iron have been calculated. Thus,

$$C_{Cu} = \frac{A_{380} \times k_{480}^{Fe} - A_{480}k_{380}^{Fe}}{k_{380}^{Cu}k_{480}^{Fe} - k_{380}^{Fe}k_{480}^{Cu}} \quad (4)$$

$$C_{Fe} = \frac{A_{480} - C_{Cu}k_{480}^{Cu}}{k_{480}^{Fe}} \quad (5)$$

$$C_{Co} = \frac{A_{625} - C_{Cu}k_{625}^{Cu} - C_{Fe}k_{625}^{Fe}}{k_{625}^{Co}} \quad (6)$$

RECOMMENDED PROCEDURE

Weigh or otherwise measure a portion of the sample containing 50 to 500 micrograms of cobalt and/or copper and 5 to 50 micrograms of iron into a 50-ml. volumetric flask. The cobalt, copper, and iron must be in the ionic form. Organic material must previously have been destroyed, usually by ashing. Known interferences must be removed by suitable chemical treatment. To the volumetric flask, add 2 ml. of concentrated hydrochloric acid, 0.5 ml. of saturated ammonium peroxydisulfate, and a freshly prepared mixture of 25 ml. of acetone and 2.5 ml. of 50% (w/v) aqueous ammonium thiocyanate. Mix thoroughly during each addition. Dilute the solution to volume with distilled water. Measure the absorbancy of the solution against a blank of the reagents within 10 minutes of color development at 380, 480, and 625 mμ. Calculate the cobalt, copper, and iron concentrations using Equations 4, 5, and 6.

If cobalt alone is sought, add 5 ml. of the chlorostannous acid reagent in place of the hydrochloric acid and peroxydisulfate reagents in the above procedure. Measure the blue-colored solution at 625 mμ, and calculate the concentration of cobalt from the equation

$$C_{Co} = \frac{A_{625}}{k_{625}^{Co}} \quad (7)$$

or from a suitable plot of absorbancy *vs.* concentration.

DISCUSSION OF RESULTS

The proposed general procedure for the simultaneous determination of cobalt, copper, and iron is generally applicable to a wide variety of samples containing more than about 50 micrograms of cobalt and/or copper, and more than 5 micrograms of iron. It is not suited to the direct determination of amounts of copper much under 50 micrograms, because the difficulties due to fading are magnified in this range. Neither is it suitable for the determination of traces of any of the three metals in the presence of large amounts of the others (such as the determination of traces of cobalt in iron) without prior separation. Despite these limitations, the method is a valuable tool. It permits the simultaneous determination of the three metals in a fraction of the time that

would be required for the individual determinations by other procedures. Within the limitations given, the method is accurate and precise to ±10%. The calculations, although more involved than those of ordinary colorimetric determinations, are simple and readily handled by nontechnical personnel.

Of the interferences encountered, none appears to be serious. Many of the interfering anions can be readily destroyed by proper chemical treatment—for instance, sulfite and nitrite are destroyed by heating the sample with acid. Others can be readily removed by either a dry or wet ashing procedure.

Some workers (*9*) have reported that nickel interferes in the cobalt and iron determinations, but the author was unable to substantiate this claim for nickel-iron, nickel-copper, or nickel-cobalt ratios of 200 to 1. Above this point, slight bleachings of the iron, copper, and cobalt colors were observed.

The method of calculation used may be subject to some criticism. The extinction coefficients of cobaltous thiocyanate at 480 and 380 mμ are not exactly zero. Measurements with concentrated cobalt solutions and 10-cm. cells indicated the k values (see Table I) to be about 0.008 and 0.006. Because these values were very small in comparison to the values for iron and copper at these wave lengths, they were neglected. This permits the use of a simpler set of calculations.

The most likely source of serious error arises from an unsuspected yellow color in the final solution. Yellows generally have absorption maxima in the region of 350 mμ, and yellows too faint to be observed visually can show appreciable absorptions at 380 mμ, thus causing serious errors in the copper analyses. Prime sources of such yellows in the recommended method are old reagents and unsuspected oxidations during development of the colors.

ACKNOWLEDGMENT

The author wishes to acknowledge with thanks the assistance of John Mitchell, Jr., and Donald Milton Smith in the development of the methods and in the preparation of the manuscript. Thanks are also due to H. D. Deveraux, W. J. Joyce, and R. G. Wooleyhan, who did much of the experimental work.

LITERATURE CITED

(1) Bent, H. E., and French, C. L., *J. Am. Chem. Soc.*, **63**, 568 (1941).
(2) Brown, E. A., IND. ENG. CHEM., ANAL. ED., **17**, 228 (1945).
(3) Gibb, R. P., Jr., "Optical Methods of Chemical Analysis," pp. 113 ff., New York, McGraw-Hill Book Co., 1942.
(4) Kolthoff, I. M., *Mikrochemie* (N.S.), **2**, 176 (1930).
(5) Møller, M., *Kem. Maanedsblad*, **18**, 138 (1937).
(6) Peters, C. A., and French, C. L., IND. ENG. CHEM., ANAL. ED., **13**, 604 (1941).
(7) Peters, C. A., McMasters, M. M., and French, C. L., *Ibid.*, **11**, 502 (1939).
(8) Putsché, H. M., and Malooly, W. F., ANAL. CHEM., **19**, 236 (1947).
(9) Sandell, E. B., "Colorimetric Determinations of Traces of Metals," pp. 202–5, 263–71, New York, Interscience Publishers, 1944.
(10) Schlesinger, H. I., *J. Am. Chem. Soc.*, 63, 1765 (1941).
(11) Schlesinger, H. I., and Van Valkenburgh, H. B., *Ibid.*, **53**, 1212 (1931).
(12) Tomula, E. S., *Z. anal. Chem.*, 83, 6 (1931).
(13) Vorontzov, R. V., *J. Applied Chem. (U.S.S.R.)*, **8**, 555 (1935).
(14) Winsor, H. W., IND. ENG. CHEM., ANAL. ED., **9**, 453 (1937).
(15) Woods, J. T., and Mellon, M. G., *Ibid.*, **13**, 551 (1941).
(16) Young, R. S., and Hall, A. J., ANAL. CHEM., **18**, 264 (1946).

RECEIVED November 30, 1949. Presented at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, February 15 to 17, 1950.

Application of Flame Spectrophotometry to Water Analysis

Determination of Sodium, Potassium, and Calcium

PHILIP W. WEST, PATRICIA FOLSE, AND DEAN MONTGOMERY
Louisiana State University, Baton Rouge, La.

A method is presented for the rapid determination of sodium, potassium, and calcium in waters by means of flame spectrophotometry. Small samples may be routinely analyzed with an accuracy comparable to that obtained using conventional methods. Interfering effects of diverse ions have been obviated by the addition of "radiation buffers."

CONVENTIONAL methods for the mineral analysis of water are tedious and time-consuming procedures requiring the best efforts of a trained analyst. Spectroscopic determination, which would eliminate extensive chemical treatment or separation, has for many years attracted the attention of analysts. The high cost of instruments together with the complexity of ordinary spectroscopic techniques has, however, restricted development along these lines. The introduction of the flame unit for use with the Beckman DU spectrophotometer has now provided a tool of great potential value in this field and the present investigation was undertaken to ascertain the value of such instruments in water analysis.

Introduction of metal ions such as sodium, potassium, and calcium into flames of relatively high temperature produces intense radiations, and the intensities of these radiations are proportional to the concentrations of the salts introduced into the flame. To measure small variations in concentration by means of this phenomenon it is necessary to measure the radiant energy intensity with a photometer or spectrophotometer (*6*).

Barnes, Richardson, Berry, and Hood (*1*) proposed a simple flame photometer employing filters for isolating the desired spectral regions and photocells for use in measuring radiation intensities. With such an instrument they were able to determine sodium and potassium within an error range of ±3%. Berry, Chappell, and Barnes (*2*) modified the instrument to incorporate the use of internal standards and so reduced the expected range of error to ±1.2%. The combination of a prism-type spectrophotometer and a suitable flame unit has the apparent advantage of greater monochromacity and much greater flexibility. Because the spectrophotometer has become a basic instrument in most laboratories, such a combination provides a logical extension of its use.

Table I. Interferences Due to Anions

Anion	P.P.M.	Sodium, P.P.M. 80	50	20	Potassium, P.P.M. 80	50	20	Calcium, P.P.M. 80	50	20
SO_4^{--}	50	79	47	20	76	46	20	83	51	21
	100	79	47	20	77	48	21	83	51	21
	500	79	48	20	77	48	21	80	51	22
	1000	79	48	20	76	49	20	79	51	21
HCO_3^-	50	85	53	20	78	48	20	79	50	21
	100	83	53	21	73	49	19	79	50	20
	500	85	53	21	77	48	17	12	7	6
	1000	85	52	21	77	44	18	3	3	2
Cl^-	50	78	50	21	78	50	20	79	..	20
	100	80	49	19	78	50	19	78	49	20
	500	80	50	20	78	50	20	80	50	20
	1000	80	48	21	79	49	20	79	51	20

APPARATUS

The instrument employed in these investigations was a Beckman flame spectrophotometer consisting of a Model DU spectrophotometer and a Beckman flame unit. The latter provides all necessary housing, meters, and connections for fuel, air, oxygen, and cooling water, as well as the sample atomizer and vaporizing chamber. A hot flame is produced by a mixture of gas and oxygen, both of which may be adjusted independently; injected at the base of this flame is the vaporized aqueous sample. The emitted radiation resulting from the flame excitation of the cations present in the sample is reflected into the spectrophotometer, which functions in its normal manner. Because no appropriate designation of radiation units is provided on the spectrophotometer, the intensities of emissions are customarily recorded in terms of percentage transmittance.

The high temperature flame required for excitation was obtained by use of the natural gas supplied to the local laboratory together with U.S.P. tank oxygen. The air line in the laboratory was found to be contaminated with water and subject to wide variations in pressure; this difficulty was overcome by use of a rotary pressure pump equipped with suitable scrubbers.

SOLUTIONS

Standards. The sodium and potassium standards used in this work were prepared by diluting to volume appropriate weights of reagent grade sodium and potassium chlorides. Reagent grade calcium carbonate was carefully dissolved using hydrochloric acid and diluted to volume to obtain standard calcium chloride. Each standard solution was made up to contain 0.5 mg. of the desired metal for each milliliter of solution and was stored in borosilicate glassware.

Radiation Buffer for Sodium. Distilled water was successively saturated with reagent quality chlorides of calcium, potassium, and magnesium. The solutions were filtered after each saturation.

Radiation Buffer for Potassium. Distilled water was successively saturated and filtered with reagent quality chlorides of sodium, calcium, and magnesium.

Radiation Buffer for Calcium. Distilled water was successively saturated and filtered with reagent quality chloride of sodium, potassium, and magnesium.

EXPERIMENTAL

A preliminary study was made using pure solutions of individual cations to determine whether or not sufficient sensitivity and precision were obtainable for the concentration ranges of minerals in water. The sensitivity was adequate in the case of sodium, potassium, and calcium for distinguishing differences in concentration of only 1 or 2 p.p.m. A linear response to concentration was not found, nor was there similarity between the respective curves of response *vs.* concentration for the different elements. Checks made over a period of several months indicated the instrument gave constant performance.

Studies of Interferences. Because chloride, sulfate, and bicarbonate are the anions most likely to be present in significant amounts in water samples, a study was made of possible interfering effects of these substances upon the emission strengths of the ions to be determined. A series of test solutions was prepared containing these anions in a wide range of concentrations together with sodium, potassium, and calcium ions in the normal concentration range of these cations in water. These solutions were subjected to analysis by means of the flame spectrophotometer and the results obtained are shown in Table I. Bicarbonate ions greatly suppress the emissions of calcium ions and this difficulty was first obviated by acidifying and heating the solution to remove the bicarbonate. This operation was later made unnecessary by a modification in procedure which provided for a general elimination of interfering effects.

Besides the effect of anions, the radiant energy emissions of metal ions are affected by the presence of other cations, a phenomenon already familiar in spectroscopy (*3*). The error produced may be positive or negative and the amount of the error is dependent upon the concentration and identity of the metals involved. Table II illustrates the errors produced as a result of the presence of an extraneous cation. These findings are in agreement with the results reported by Ivanov (*5*), who states that for flame excitation, the emission intensity of an alkali is increased if another alkali is present. Because of the extent and variable nature of these interferences an investigation was undertaken to develop "radiation buffers" for use in providing uniform radiation characteristics for the metals sodium, potassium, and calcium. It was thought that if high consistent concentrations of diverse cations were added to each sample, small concentration variations within the samples themselves would be without effect upon the emission strength of the metal ion to be determined spectrophotometrically. Such a procedure is similar to that proposed by Duffendack, Wiley, and Owens (*4*) for emission spectrography.

To prevent serious dilutions of the samples it was necessary to obtain high concentrations of the diverse cations by addition of small volumes of very concentrated solutions of the radiation buffers. An arbitrary concentration of the radiation buffers in the samples was obtained by adding one volume of the appropriate buffer to 25 volumes of sample. To test the buffers, solutions containing extraneous cations and the buffers, as well as the ions to be determined, were prepared and analyzed with the flame spectrophotometer. The results obtained are shown in Table III and predict the failure of diverse cation concentrations as high as 500 p.p.m. to alter the emission intensity of the ion sought. Furthermore, if bicarbonate ions are present in concentrations of 1000 p.p.m. or less the buffer prohibits any decrease in intensity of calcium emissions. Therefore it may be stated that for almost any water sample likely to be encountered the addition of radiation buffer solutions will prevent erroneous results caused by diverse interfering ions.

Table II. Interference of Cations

Extraneous Cation, P.P.M.		Sodium, P.P.M. 10	50	100	Potassium, P.P.M. 10	50	100	Calcium, P.P.M. 10	50	100
Na^+	0	..	..	..	13	51	100	12	50	100
	50	..	..	..	15	57	105	14	54	103
	100	..	..	..	17	59	107	14	56	106
	500	..	..	..	23	66	115	20	59	110
K^+	0	11	51	100	..	..	..	12	50	90
	50	11	52	105	..	..	..	14	52	106
	100	13	57	113	..	..	..	14	52	107
	500	15	64	123	..	..	..	14	56	107
Ca^{++}	0	11	51	100	13	52	100	..	..	..
	50	12	52	105	13	52	101	..	..	..
	100	12	51	105	13	53	100	..	..	..
	500	14	51	104	14	56	100	..	..	..

Table III. Interference Effects with Radiation Buffer Present

Extraneous Cation, P.P.M.		Sodium, P.P.M. 90	50	10	Potassium, P.P.M. 90	50	10	Calcium, P.P.M. 90	50	10
Na	90	..	..	..	90	52	12	93	52	11
	500	..	..	..	90	51	11	94	51	11
K	90	84	48	10	..	..	..	92	54	12
	500	84	47	11	..	..	..	91	54	10
Ca	90	88	48	9	90	48	12	..	..	..
	500	89	48	9	87	49	9	..	..	..

PROCEDURE

Standard Curves. Working curves of concentration *vs.* percentage transmittance should be prepared for sodium, potassium, and calcium from standards containing the proper amount of the appropriate radiation buffer; the wave lengths to be used for the emission measurements are 589, 767, and 556 mμ, respectively. The latter value is chosen in preference to the 626 mμ line because a smaller spectrophotometer slit width is permitted and this is more important than the small decrease in flame background obtainable with the longer wave length. It was also more satisfactory than 423 mμ, which was also investigated.

The most concentrated standard of a series of standards made so as to encompass the entire range of expected concentrations is introduced into the flame and the proper spectrophotometer sensitivity is selected. The response of the instrument to each remaining member of standard samples and a blank containing the buffer is then determined. Each point on the calibration curve should be corrected for background luminosity. Working curves should be checked each month and each time new radiation buffers are prepared.

Analytical Procedure. The following procedure refers to sodium determinations, but is also typical of the method used in the determination of potassium and calcium.

One milliliter of sodium radiation buffer is added to a 25-ml. portion of the sample and the solution is thoroughly mixed. The spectrophotometer is adjusted with a standard sample to provide the same response as that obtained in preparing the standardization curve. The response of the instrument to the unknown sample is determined and corrected for luminosity background; an average of three readings is made to eliminate random error. The sodium concentration in the sample is then obtained by comparison with the calibration curve. For very accurate work a standard sample having a concentration approximately equal to that of the sample may be used as a check point on the calibration curve.

RESULTS

Precision and Sensitivity. Standard deviations, determined from the data used in making the standardization curves, are given in Table IV. The sensitivity of the instrument is not the same for the three cations. Concentration differences of 1 or 2 p.p.m. can be easily detected for sodium and potassium, whereas changes of 3 or 4 p.p.m. can be ascertained for calcium. Reference to the standardization curves (Figure 1) graphically illustrates this sensitivity variation.

Table IV. Deviation of Standard Determinations

Ion Concn., P.P.M.	Sodium σ_{20} % transmittance	Sodium σ_{20} P.P.M.	Potassium σ_{20} % transmittance	Potassium σ_{20} P.P.M.	Calcium σ_{19} % transmittance	Calcium σ_{19} P.P.M.
90	1.19	1.80	1.25	1.50	2.03	4.06
50	1.09	1.45	1.60	1.60	1.41	2.82
10	0.99	1.00	0.55	0.55	1.14	2.28
Av.	1.05	1.40	1.20	1.20	1.53	3.06

Table V. Comparison of Flame Spectrophotometer Analyses with Chemical Analyses of Waters

Sample	Sodium	Potassium	Calcium
Verdigris River	28 (33)	3 (2.5)	57 (55)
	49 (48)	3 (5)	56 (60)
Neosho River	17 (19)	3 (2.4)	52 (57)
	11 (9.9)	3 (3.5)	27 (31)
Spring River	6 (9.7)	2 (4.0)	35 (34)
Arkansas River	234 (223)	6 (—)	92 (88)
Kiamichi River	6 (6.0)	2(2.8)	0 (3.1)
Rush Creek	113	3	88
	106 (118)	3 (8.0)	90 (96)
	98	3	114
Canadian River	196	5	67
	196 (195)	5 (18)	67 (70)
	196	5	67

Values indicated in parentheses reported by U. S. Geological Survey Laboratory, Stillwater, Okla.
Concentrations expressed in terms of parts per million.

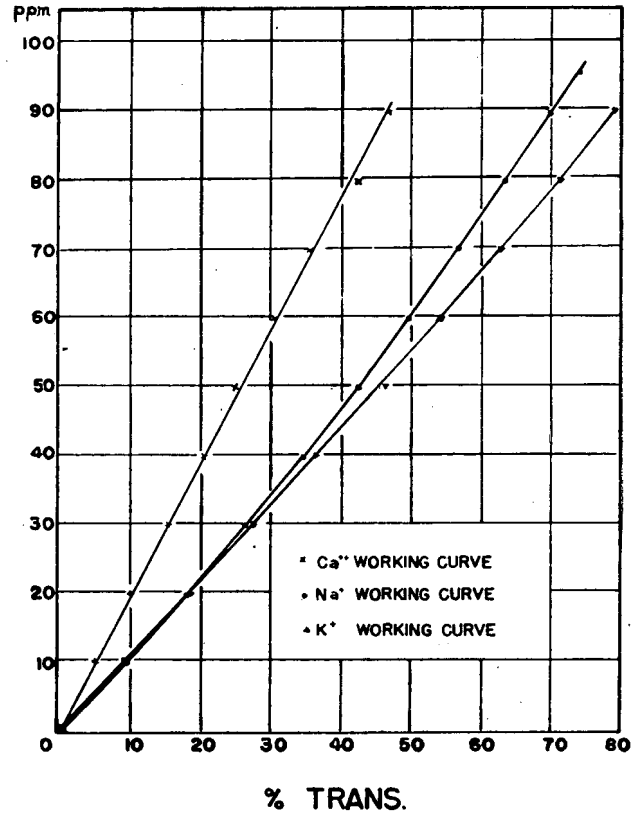


Figure 1. Working Curves for Calcium, Sodium, and Potassium

Table VI. Water Analysis Project Report

Analyzed by U. S. Geological Survey Laboratory, Stillwater, Okla.
Unpublished records, subject to revision, in cooperation with Engineering Experiment Station, Oklahoma Planning and Resources Board, and others

	Silica (SiO_2)	Iron (Fe)	Calcium (Ca)	Magnesium (Mg)	Sodium (Na)	Potassium (K)	Bicarbonate (HCO_3)	Sulfate (SO_4)	Chloride (Cl)	Fluoride (F)	Nitrate (NO_3)	Dissolved Solids	Hardness as $CaCO_3$ Total	Hardness as $CaCO_3$ Noncarb.	pH
	Parts Per Million														
1	14	0.00	88	20	223		184	130	340	0.2	5.5	944	302	150	8.4
2	14	0.08	3.1	2.2	6.0	2.8	16	5.0	4.2	0	0.5	56	17	4	7.3
3	14	1.0	31	5.4	9.9	3.5	80	33	8.0	0.1	8.0	160	100	34	7.7
4	8.0	0.10	34	5.8	9.7	4.0	73	61	6.0	0.3	10	187	109	49	7.5
5	13	0.10	57	11	19	2.4	151	72	17	0	7.0	281	187	64	7.8
6	12	0	96	62	118	8.0	271	201	212	0	3.0	920	494	272	8.3
7	13	0.02	70	28	195	18	179	30	380	0.1	5.0	852	290	143	7.6
8	11	0.16	60	11	48	5.0	163	40	84	0.2	4.0	361	194	61	7.8
9	8.0	0.16	55	9.5	33	2.5	171	33	54	0.1	3.0	303	176	36	7.7

1. Arkansas River at Webber Falls, Okla.
2. Kiamichi River near Belzoni, Okla.
3. Neosho River near Wagoner, Okla.
4. Spring River near Quapaw, Okla.
5. Neosho River near Commerce, Okla.
6. Rush Creek at Purdy, Okla.
7. Deep Fork of Canadian at Dewar, Okla.
8. Verdigris River near Inola, Okla.
9. Verdigris River near Claremore, Okla.

Table VII. Analytical Recoveries of Added Ions

Sample (Rivers)	Sodium, P.P.M.			Potassium, P.P.M.			Calcium, P.P.M.		
	Original	Added	Found	Original	Added	Found	Original	Added	Found
1	28 (33)	10	41	3 (2.5)	10	13	57 (55)	10	69
2	49 (48)	10	62	3 (5)	10	13	56 (60)	10	67
3	6 (9.7)	30	38	2 (4)	30	32	35 (34)	30	68
4	...	50	57	...	50	54	...	50	90
5	11 (9.9)	30	41	3 (3.5)	30	35	27 (31)	30	62
6	...	50	61	...	50	55	...	50	83

1. Verdigris River
2. Verdigris River
3. Spring River
4. Spring River
5. Neosho River
6. Neosho River

Values in parentheses, chemical analysis by U. S. Geological Laboratory in Stillwater, Okla.

Analytical Determinations. Table V compares analytical determinations made with the flame spectrophotometer to those obtained with classical methods of chemical analysis. It can be seen that the results obtained are in general agreement. In practically every instance the deviations between reported values and the experimental findings are within the limits common to internal checks in duplicate analyses of waters by chemical procedures. Where deviations exist, final interpretation should be tempered by the thought that chemical analyses may not be absolute and repeated checks by both methods might reconcile apparent differences due to method.

Recovery Studies. Four samples of the waters listed in Table VI were selected for recovery studies; Table VII tabulates the results of these determinations. The additional concentrations of minerals in the samples were obtained by adding the necessary amount of appropriate standard to the original solution. (The calculations were based on the original concentration as determined with the flame spectrophotometer.)

SUMMARY

The small quantities of sodium, potassium, and calcium normally found in water can be easily and quickly determined with a flame spectrophotometer. Reliable results can be obtained for sodium and potassium, while calcium can be determined with sufficient accuracy to warrant application of the method for most routine work. Radiation buffers have been developed which serve to minimize interfering effects of diverse ions. The concentrations of the ions sought are determined by reference to semipermanent calibration curves. Small quantities of sample are sufficient and no chemical treatment of the samples is necessary. The calibrations as well as the determinations can be done by an operator without extensive chemical or instrumental experience. The development of a higher flame temperature might permit the application of this method to magnesium determinations as well.

ACKNOWLEDGMENT

The authors wish to express their appreciation to the Division of Research Grants and Fellowships of the U. S. Public Health Service for financial support of this investigation.

LITERATURE CITED

(1) Barnes, R. B., Richardson, D., Berry, J. W., and Hood, R. L., IND. ENG. CHEM., ANAL. ED., 17, 605 (1945).
(2) Berry, J. W., Chappell, D. G., and Barnes, R. B., *Ibid.*, 18, 19 (1946).
(3) Brode, W. R., and Silverthorn, R. W., "Proceedings of Sixth Summer Conference on Spectroscopy and Its Applications," pp. 60–5, New York, John Wiley & Sons, 1939.
(4) Duffendack, O. S., Wiley, F. H., and Owens, J. S., IND. ENG. CHEM., ANAL. ED., 7, 410 (1935).
(5) Ivanov, D. N., *Zavodskaya Lab.*, 10, 401 (1941).
(6) Parks, T. D., Johnson, H. O., and Lykken, Louis, ANAL. CHEM., 20, 822 (1948).

RECEIVED November 3, 1949. Presented before the Division of Water, Sewage, and Sanitation Chemistry at the 117th Meeting of the AMERICAN CHEMICAL SOCIETY, Detroit, Mich.

Colorimetric Determination of o- and m-Dihydroxyphenols

HOBART H. WILLARD AND A. L. WOOTEN[1]
University of Michigan, Ann Arbor, Mich.

IN PREVIOUS work (*1*) on the volumetric determination of resorcinol it was found that on iodination in the presence of catechol a dark insoluble precipitate formed. It has been found that this reaction is very selective for *m*- and *o*-dihydroxyphenols. After the excess iodine is destroyed the precipitate is dissolved by the addition of acetone and the resulting grape-blue color is measured. Beer's law is obeyed over the range 0 to 75 p.p.m.

REAGENTS

Iodine Solution, 0.1 *N*. Six and one-half grams of iodine and 10 grams of potassium iodide are dissolved in a little water and diluted to 1 liter. It is not necessary to standardize this reagent.

Sodium Thiosulfate Solution, 0.1 *N*. Twenty-five grams of sodium thiosulfate are dissolved in 1 liter of freshly boiled water containing 0.1 gram of sodium carbonate. It is unnecessary to standardize this reagent.

Starch Solution. A 1% starch solution containing 2% potassium iodide.

Buffer. The buffer is acetic acid–sodium acetate, molar in acetate ion. For the determination of resorcinol or catechol pH should be 5.7; for the determination of phloroglucinol, 6.0.

[1] Present address, Reichhold Chemicals, Inc., Ferndale, Mich.

Reagent grade acetone, 0.05% resorcinol solution, and 0.05% catechol solution are used.

PROCEDURE

Determination of Resorcinol. Take a neutral sample of no more than 15 ml. containing no more than 0.75 mg. of resorcinol, and add 10 ml. of buffer, 10 ml. of 0.05% catechol solution, and 15 ml. of 0.1 *N* iodine solution. After 1 minute titrate the excess iodine with sodium thiosulfate and starch. Transfer the sample to a 100-ml. volumetric flask, add 50 ml. of acetone to dissolve the precipitate, and dilute to 100.0 ml. with distilled water. Measure the intensity of the color at 725 mμ. Refer to a standardization curve for the resorcinol content.

Determination of Catechol. The same procedure is followed, except that 0.05% resorcinol is added rather than catechol. Although the product is the same in both cases, separate standardization curves are necessary.

Determination of Phloroglucinol. The resorcinol procedure is followed, except that a buffer of pH 6.0 is necessary. A new standardization curve is required.

If a *m*-dihydroxyphenol is iodinated in the presence of an *o*-dihydroxyphenol, a dark colored addition compound is formed. This precipitate dissolves on the addition of an equal volume of acetone to give a grape-blue color. The intensity of this color is proportional to either the *m*- or *o*-dihydroxyphenol, depending on which is present in the lesser amount. The optimum concentration range is 0 to 50 p.p.m. This could be made considerably smaller by use of more concentrated reagents. Of 25 phenols tested, only the *o*- and *m*-dihydroxyphenols gave this reaction.

EXPERIMENTAL

Table I shows that the color body is formed from equal amounts of resorcinol and catechol. The color produced by resorcinol in the absence of catechol is due to aristol formation. A blank in the determination of catechol corrects for this. Resorcinol alone gives a red or pink color; in the presence of catechol the color is blue-violet. This blank may be eliminated by the use of a spectrophotometer.

In the experiments reported in Tables II to VIII the variables in the determination of resorcinol were tested one by one. The procedure given herewith was followed using 0.750 mg. of resorcinol. Very similar results were obtained in the determination of catechol and phloroglucinol.

Table I. Formation of Color Body

Resorcinol, mg.	0.00	0.60	1.20	1.50	1.80	2.40	3.00
Catechol, mg.	3.00	2.40	1.80	1.50	1.20	0.60	0.00
Optical density	0.5	362	740	890	780	520	185

Table II. Effect of Catechol-Resorcinol Ratio

Resorcinol, Mg.	Catechol, Mg.	Molar Ratio, Catechol/ Resorcinol	Optical Density
0.750	0.750	1.0	452
0.750	1.500	2.0	520
0.750	2.250	3.0	522
0.750	3.750	5.0	522
0.750	6.000	8.0	518

Table III. Effect of Amount of Buffer

Buffer Added, Ml.	Optical Density
2.0	480
4.0	503
6.0	512
8.0	526
10.0	525

Table IV. Effect of pH on Rate of Reaction

pH of Buffer	Time of Reaction, Minutes	Optical Density
5.3	0.25	385
5.3	0.50	435
5.3	1.00	440
5.3	2.00	452
5.3	5.00	470
5.3	10.00	504
5.3	20.00	520
5.3	30.00	525
5.7	1.00	530
5.7	2.00	525
5.7	3.00	527

Table V. Effect of Amount of Iodine

Iodine, Ml.	Optical Density
1.5	455
3.0	460
6.0	477
9.0	509
12.0	525
15.0	525

Table VI. Effect of Excess Sodium Thiosulfate

Excess Sodium Thiosulfate, Ml.	Density After 0.5 min.	After 1.0 min.	After 5.0 min.	After 10.0 min.
0.0	525	525	525	525
1.0	526	526	525	519
5.0	520	520	495	467

Table VII. Effect of Acetone-Water Ratio

Acetone, % by Volume	Optical Density
35	420
50	520
60	516

Table VIII. Standardization Curve

Resorcinol, Mg.	Optical Density
0.000	17.7
0.150	114.0
0.300	212.3
0.450	308
0.600	420
0.750	500

DISCUSSION

As Table II indicates, at least twice the theoretical amount of catechol must be present if the reaction is to be complete in 1 minute. In the procedures above, the amount specified is about five times the theoretical. A blank should, of course, be run on the catechol alone. One batch that contained 0.75% resorcinol was analyzed by this method.

The amount of buffer specified is about 25% more than the minimum necessary (Table III).

The pH of the buffer is critical (Table IV). The buffer should be rechecked frequently.

It is surprising that such a large excess of iodine is necessary to complete the reaction (Table V). Approximately an eightfold excess is necessary. The procedure specifies a tenfold excess.

The bleaching of the color in the presence of excess thiosulfate is not surprising. The mere acidification of the sample is sufficient to bleach the color with the liberation of free iodine.

Resorcinol has been determined by this method in the presence of 50 times as much of each of the following phenols with an error of less than 1%: *o*-cresol, *m*-cresol, *p*-cresol, phenol, *o*-phenylphenol, *p*-phenylphenol, *o-tert*-butylphenol, *p-tert*-butylphenol, *p-tert*-amylphenol, salicylaldehyde, *p*-hydroxybenzaldehyde, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, salicylic acid, *p*-aminophenol, *m*-aminophenol, *o*-aminophenol, *o*-nitrophenol, and *m*-nitrophenol. Hydroquinone interferes in this large excess, but not when it is present in the same order of magnitude as the resorcinol.

ACKNOWLEDGMENT

One of the authors wishes to express thanks to Reichhold Chemicals, Inc., for the financial aid that made this work possible.

LITERATURE CITED

(1) Willard, H. H., and Wooten, A. L., ANAL. CHEM., 22, 585 (1950).

RECEIVED August 31, 1949. From a dissertation submitted by A. L. Wooten to the Graduate School of the University of Michigan in partial fulfillment of the requirements for the degree of doctor of philosophy in chemistry.

Estimation of Beta-Activities from Bremsstrahlung Measurements in an Ionization Chamber

P. C. TOMPKINS[1], LEON WISH, AND W. T. BURNETT, JR.
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

The radioactivity of a beta-emitting nuclide can be determined by using an ion chamber to measure the *Bremsstrahlung* radiation originating in the source and its container. The method is particularly applicable to the measurement of high intensity irradiation sources. The advantages of the method are: (1) Ionization chambers have a much wider range, stability, and linearity of response than conventional Geiger-Müller instruments; (2) the activity may be quantitated in solutions or solids, particularly those which are highly hydrated, without the necessity of chemical processing; and (3) the comparison with beta-activities as measured in the usual way provides an additional criterion of radioactive purity. The major limitations are: (1) an extreme sensitivity to the presence of a gamma-emitting impurity; (2) the relatively high activities required; and (3) variations arising from an inhomogeneous composition of the source and its immediate environs.

THE use of beta-emitters at activity levels greater than 1 mc. has made it desirable to develop procedures that can be used to quantitate such activities directly on the original material. A procedure for the purpose has been successfully developed by the authors utilizing a high-pressure ionization chamber which is normally used for gamma-radiation measurements. Preliminary studies (*17*) have demonstrated that this instrument could be satisfactorily adapted to the quantitative determination of the activity of a pure beta-emitting isotope (^{32}P) by measuring the ion current produced by secondary electromagnetic radiation originating in the source and its container.

The procedure depends on the fact that, when energetic beta-particles are absorbed by matter, a part of the energy is lost through the production of *Bremsstrahlung* (secondary electromagnetic radiation). Because the chamber and container walls are thick enough to absorb all of the beta-particles, the ion current produced in the chamber by the *Bremsstrahlung* is quantitatively related to the activity of the sample. The chamber is constructed in such a way that essentially 4π geometry is achieved in the collection of photons. Furthermore, the *Bremsstrahlung* measurements are affected very little by self-absorption and by variations arising from an inhomogeneous distribution of the radioelement within the source.

This paper describes studies which have been made to identify the important variables which must be controlled for quantitative *Bremsstrahlung* measurements.

APPARATUS, MATERIAL, AND EXPERIMENTAL METHODS

The gamma-ray ionization chamber was essentially the same as that described by Stephenson (*2*) and Jones and Overman (*10*). In Figure 1 are shown the main features of the instrument.

The amplifying and measuring system consisted of a Model 30 vibrating reed electrometer (Allied Physics Corporation, Pasadena, Calif.), and a Brown recording potentiometer, the amplified current being fed through a resistor of 10^{11}, 10^{10}, 10^{9}, or 10^{8} ohms and the voltage drop recorded. The current was calculated using Ohm's law.

Isotopes. The radioisotopes used for *Bremsstrahlung* measurements (^{32}P, ^{90}Sr, ^{90}Y, and ^{35}S) were obtained from the Operations Division of the Oak Ridge National Laboratory, Oak Ridge, Tenn. (*1*). They were essentially carrier-free—i.e., of very high specific activity. Up to the present time, no gamma-radiation has been shown to exist in the decay of these elements. All samples were subjected to radiochemical analysis and found to be free from radioactive impurities within the limits of the analysis. In addition, *Bremsstrahlung* decay curves, usually over a period of 6 to 10 half-lives, and absorption curves with a standard beta-counter, served as a further check on the purity of the materials.

[1] Present address, Naval Radiological Defense Laboratory, San Francisco, Calif.

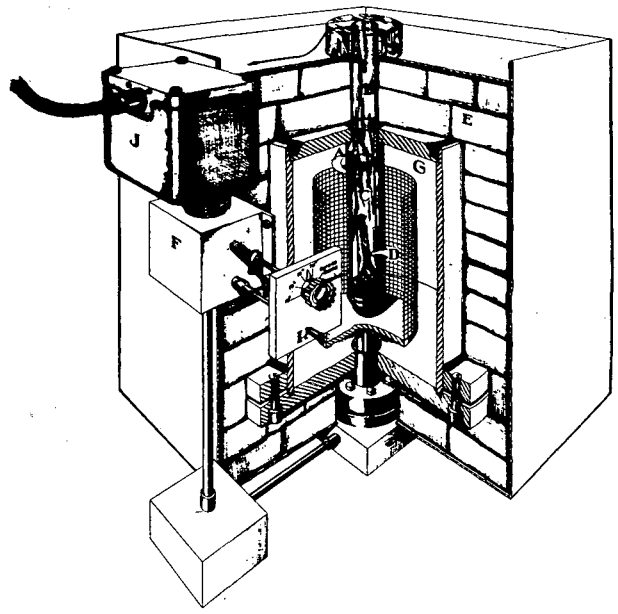


Figure 1. Cutaway View of Gamma-Ray Ionization Chamber

A. Steel thimble
B. Lucite thimble (to protect steel thimble from contamination)
C. Lusteroid container
D. Volumetric flask containing sample
E. Lead shielding
F. Resistor box
G. Gas chamber filled with 20 atm. of argon
H. Toggle switch
I. Leads to main box of vibrating reed electrometer
J. Vibrating reed head

RESULTS AND DISCUSSION

Calibration of Ion Chamber. The absolute disintegration rate of the various beta-emitters was determined by the method developed by Zumwalt in 1948 (*18*). To calibrate the ion chamber, the ion currents produced by dilute acid solutions of each radionuclide contained in 10-ml. volumetric flasks were measured. Then, the number of microcuries required to produce a micromicroampere (10^{-12}) of ion current was calculated for each nuclide.

The activities in terms of microcuries per micromicroampere are shown in Figure 2 as a function of the maximum energy of the beta-particles. It is evident that the *Bremsstrahlung* method will be most useful with beta-emitters whose maximum energy is greater than 1 m.e.v. For example, the activity range of

Table I. Calibration of Ion Chamber for ^{32}P

μc. of ^{32}P/10 Ml. of Solution[a]	Ion Current[b], $\mu\mu$amp.	μc. of $^{32}P/\mu\mu$amp.
24,000	493	48.7
12,000	246	48.9
6,000	125	48.0
3,000	61.9	48.5
1,500	31.1	48.3
750	15.6	48.1
375	7.79	48.2
188	3.89	48.3
94	1.94	48.5
47.0	0.980	48.0
23.5	0.483	48.7
11.8	0.244	48.3
5.90	0.122	48.3
		Av. = 48.4[c]

[a] Determined with Geiger-Müller counter, limiting accuracy ±3%.
[b] Corrected for background current and linearity.
[c] Standard deviation = 0.27 μc./$\mu\mu$amp.

$^{90}Sr(E_{max.} = 0.641$ m.e.v.) is only 5.3% of that of $^{90}Y(E_{max.} = 2.2$ m.e.v.). Therefore, it would take approximately 20 times as much ^{90}Sr to give the same instrument reading as ^{90}Y. Weak beta-emitters such as ^{14}C and ^{35}S require about 250 μc. for detection, and 4.5 mc. for the production of an ion current equal to the background current.

The data for ^{32}P are summarized in Table I. The current produced by the ^{32}P *Bremsstrahlung* was found to be directly proportional to the activity within the limits of experimental error after correcting for background and linearity of response of the instrument (*10*). Background for the chamber was constant at 0.180 ± 0.005 $\mu\mu$amp. This represented the limit of precision in determining the average displacement of the pen on the Brown recording potentiometer. Thus, a sample yielding an ion current equal to background, which corresponded to an activity of 8.7 μc. ^{32}P, could be measured with a precision of about 4% assuming that the standard error of difference is $\sqrt{(0.005)^2 + (0.005)^2} = \pm 0.007$. However, the precision increased as the ion current increased, so that the background current could be ignored when the ion current was greater than 20 to 30 $\mu\mu$amp. On the other hand, factors such as dilution or sampling errors became important at the higher activity levels.

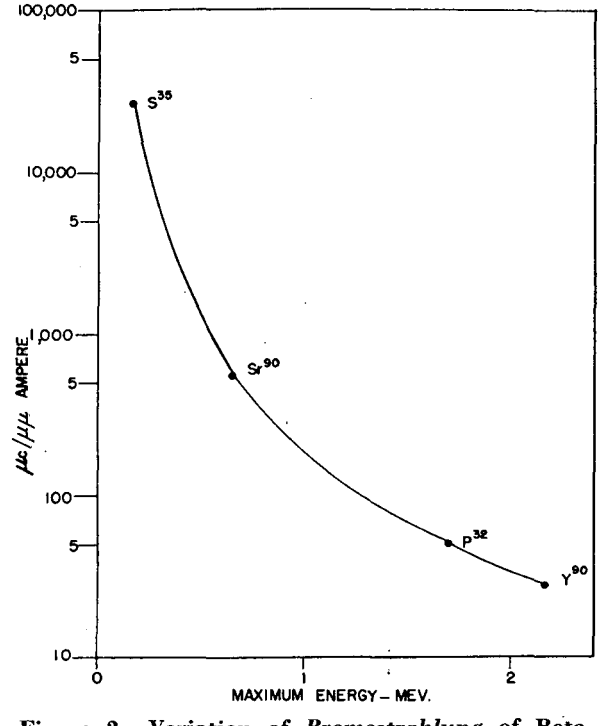


Figure 2. Variation of *Bremsstrahlung* of Beta-Emitters with Energy

The lowest ^{32}P activity that can be detected by the instrument is 1 μc., which gives an ion current of approximately 0.02 $\mu\mu$amp. The upper limit of the instrument for ^{32}P is about 500 mc.

Purity. Because the ion chamber used for these measurements was designed to measure gamma-photons, the measurements are extremely sensitive to the presence of gamma-emitting impurities in the sample. This is somewhat of a disadvantage, in that the beta-emitters must be correspondingly free from such impurities before the ion current can be interpreted quantitatively in terms of the disintegration rate of the beta-emitter. On the other hand, this characteristic can be turned to practical use, for it provides a new basis for determining the purity of a given sample.

As previously indicated, the isotopes under investigation were found to be radioactively pure by following their decay on the ion chamber. The high stability of the amplifying system together with its wide linear range made it possible to follow even short-lived isotopes over 5 to 10 half-lives. Decay curves of ^{32}P and ^{90}Y are shown in Figure 3. The samples decayed in a strictly exponential manner over a time span greater than 5 half-lives. Values of 14.3 ± 0.1 days and 64 ± 1 hours were obtained for the half-lives of ^{32}P and ^{90}Y, respectively. These are in good agreement with published data (*3, 6–8, 11–14, 16*).

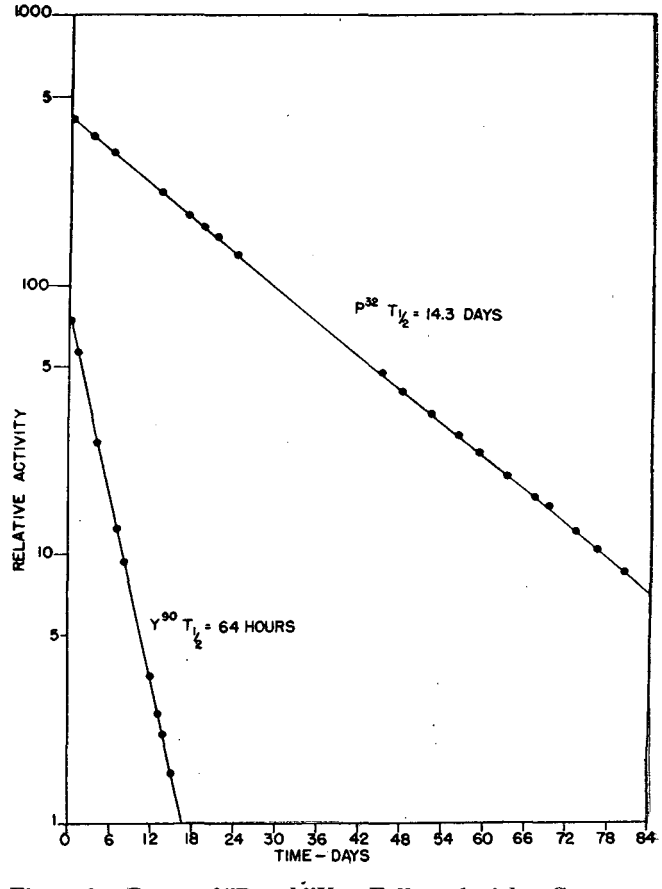


Figure 3. Decay of ^{32}P and ^{90}Y as Followed with a Gamma-Ray Chamber

Description of Variables. The ion current per unit of activity has been found to differ from that produced under standard conditions (10 ml. of solution contained in a 10-ml. volumetric flask) when the experimental conditions are altered in one of the following ways: (1) surface to volume ratio of the sample, (2) composition of the sample container, (3) concentration and atomic number of solutes, and (4) physical state of the sample—i.e., wet and dry precipitates and solids. However, the deviations are reproducible and their direction is predictable from the Bethe-

Heitler equation (*9*, *15*). This equation is often used in its simplified form:

$$-\left(\frac{dE}{dx}\right)_{rad} = NEZ^2\phi(E) \qquad (1)$$

where $(dE/dx)_{rad}$ is the rate at which energy is dissipated per unit thickness of absorber by the production of *Bremsstrahlung*, N is the number of atoms of atomic number, Z, per unit thickness of absorber, and E is the energy of the beta-particles. The term $\phi(E)$, a slowly varying linear function of the logarithm of E (*9*), may be disregarded for the present purpose.

Self-Absorption. If all the beta-particles are absorbed in a medium wherein the average N and Z are homogeneously distributed—e.g., a solution—the number of induced photons per millicurie of the beta-activity should be independent of the radionuclide distribution in the medium except for variations arising from self-absorption of the induced photons. However, when part of the beta-particle energy is expended in one medium and part in another, the Bethe-Heitler equation applies independently to each. Thus, beta-particles absorbed in a solution produce photons with an "efficiency" characteristic of the solution, whereas those absorbed by the glass walls of the container produce photons with a higher "efficiency" (therefore, more photons per millicurie) depending upon the density and average atomic number of the glass. The same applies to any particles that penetrate the glass and are absorbed in the Lucite thimble of the chamber.

The *Bremsstrahlung* photons are produced in a continuous spectrum by electrons of a given energy, E. The intensity distribution is roughly uniform over the entire frequency range (*15*) within a factor of 2. The maximum energy cannot be greater than E. If the energy distribution be integrated—e.g., with E as a variable, from zero to the initial energy, E_0, and then over the beta-ray spectrum of the nuclide in question—the final distribution of photon energies will include only a small fraction of hard radiation near $E_{max.}$. Unless the preponderant soft radiation is removed, the ion current may be so sensitive to self-absorption in the sample that the procedure becomes worthless as an analytical tool. Absorption curves of ^{32}P *Bremsstrahlung* which were obtained in connection with another problem (*4*) indicate that only about 10% of the photons will pass through the $^3/_{16}$-inch steel liner of the pressure chamber. Moreover, the experimental results reported in the following sections indicate that self-absorption does not interfere significantly with the *Bremsstrahlung* measurements.

Effect of Sample Size and Container Composition. Because a fraction of the beta-particle energy will be expended in the sample container, the surface to volume ratio of the sample and the shape and composition of the sample container become interrelated variables. These were evaluated by measuring the ion current produced by the same quantity of activity in different volumes of dilute nitric acid (pH 1) contained in 10-ml. volumetric flasks, 50- and 100-ml. glass test tubes, and 50- and 100-ml. Lusteroid test tubes. The measurements were made using carrier-free ^{32}P, ^{90}Y, and $^{90}Sr - {}^{90}Y$ in radioactive equilibrium. The results are illustrated in Figure 4, where the ratio of the ionization current produced at different volumes to that produced from a 10-ml. volume has been plotted against the volume of solution. The volume effect is the same for all of the radionuclides. The volume of the container does not appear to be a significant factor.

In the Lusteroid test tubes, the ionization relative to that produced from a 10-ml. volume is unity between volumes of 8 and 17 ml. Outside this range (4 to 8 and 17 to 21), the deviations are 1% or less. With volumes between 21 and 25 ml. there is a de-

Table II. Relationship between Production of *Bremsstrahlung* from ^{32}P Beta-Particles and Z of Absorbing Medium

Cation Absorber[a]	Z of Cation Absorber	Observed Increase in Ion Current over Control[b], %	Calculated[c] Increase in Ion Current, %
Ca^{++}	20	2.0	1.9
Co^{++}	27	3.5	3.5
Sr^{++}	38	7.0	6.9
Ba^{++}	56	15	15
Hg^{++}	80	31	31

[a] 0.42 *M* in 0.1 *M* HNO_3.
[b] ^{32}P in 0.1 *M* HNO_3 as control.
[c] $0.0048Z^2$.

Table III. Ion Currents Produced by *Bremsstrahlung* from ^{32}P Beta-Particles in Solutions Containing Various Concentrations of Calcium Nitrate

Concentration of Calcium Nitrate *Moles/liter*	Increase in Ion Current Calculated[a] %	Observed Ion Current *μμamp.*[b]	Corrected[a] Ion Current *μμamp.*	Deviation from Control[c] %
Control	..	6.09	6.09	..
0.2	0.6	6.13	6.09	0.0
0.5	1.5	6.24	6.13	+0.6
1.5	4.5	6.35	6.06	−0.5
2.5	7.5	6.62	6.12	+0.5
4.5	13.5	6.87	5.94	−2.5

[a] Calculated on basis that % increase = 3 *M*, where *M* is moles of calcium nitrate/liter.
[b] Samples contained in 10-ml. volumetric flasks.
[c] ^{32}P in 0.1 *M* HNO_3.

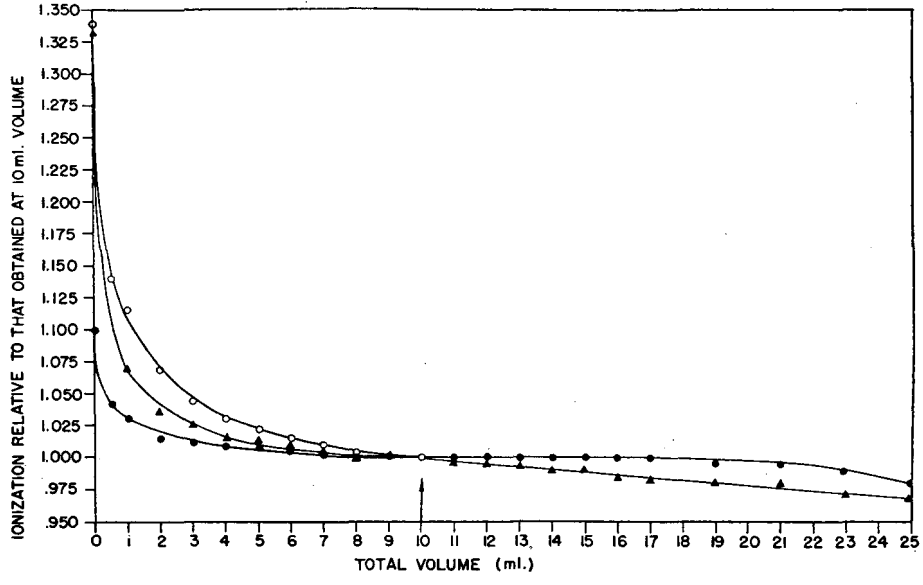


Figure 4. Effect of Volume and Container on Ionization Produced by *Bremsstrahlung*

○ 10-ml. volumetric flask
▲ 50- and 100-ml. borosilicate glass test tubes
● 50- and 100-ml. Lusteroid test tubes

crease of the order of 3% which is attributed to the fact that some of the induced photons fall outside the effective volume of the ionization chamber. The increase in the slope of the curve beginning at about 7 ml. and rising sharply below a solution volume of 1 ml. can be attributed to the fact that more electromagnetic radiation is induced per beta-particle in the Lusteroid than in water.

In glass test tubes, the gradual decrease in the ionization current as the volume of solution increased between 7 and 25 ml. is considered to indicate that a significant fraction of the total *Bremsstrahlung* originates in the glass even at these volumes.

The increase in the ion current resulting from an increasing surface-to-volume ratio was marked when each essentially weightless radionuclide was evaporated to dryness in the container. For glass, the increase was of the order of 30% as compared to 10% for Lusteroid.

Effects of Solutes. According to Equation 1, the ion current should increase by a factor roughly proportional to the square of the atomic number of the absorber. This was investigated by comparing both ^{32}P and ^{90}Y in 0.42 *M* solutions of calcium, cobalt, strontium, barium, and mercury (as their nitrates) in 0.1 *M* nitric acid with a solution containing the same quantity of activity in 0.1 *M* nitric acid as a standard. Thus, only the *Z* of the cation was variable. It was found that the various cations—calcium, cobalt, strontium, barium, and mercury—increased the ion current by 2, 3, 7, 15, and 31%, respectively. The data for ^{32}P are given in Table II. These results confirm the theoretical prediction. Furthermore, the equation, % increase = $0.0048Z^2$, represents the data for both ^{32}P and ^{90}Y, the correction being good to ±10%.

The effect of increasing calcium nitrate concentrations upon the production of ion current, as well as the effect of independently increasing the nitric acid concentration, was studied using both ^{32}P and ^{90}Y in the former and ^{32}P in the latter case.

The ion current per unit activity should increase linearly with the solute concentration at low concentrations—i.e., *N* in Equation 1—but would be expected to deviate from this relation as the solute concentration is increased enough to influence significantly the average atomic number of the medium in which the radioelement is dissolved. It was found that the increase in the ion current produced by either ^{32}P or ^{90}Y was linear up to a concentration of 3 *M* calcium nitrate, a 3% increase in ionization per mole calcium nitrate per liter being observed. At higher concentrations, there was a saturation effect which was more marked with ^{32}P than with ^{90}Y. Thus, for ^{32}P, the increase at 4.5 *M* calcium nitrate was 10% and at 10 *M* calcium nitrate only 15%. For ^{90}Y, the increase at 4.5 *M* calcium nitrate was 13%. The effect at the 10 *M* concentration of the solute was not investigated. The results obtained with ^{90}Y are shown in Table III.

The increase in the ion current produced by ^{32}P *Bremsstrahlung* was also proportional to the concentration of nitric acid, being <0.5% for concentrations less than 1 *M* and 1, 3, and 4% for 5 *M* nitric acid, concentrated nitric acid, and fuming nitric acid, respectively. The influence of nitric acid is less than that of calcium nitrate and can usually be disregarded unless a precision of the order of ±1% is required. The equation, % increase = 0.22 *M*, represents the data for all concentrations of nitric acid, the correction being valid to ±10%.

Although the results indicate that the production of ion current depends upon the solute concentration, the required control of the experimental conditions should usually not be too critical for successful application of the method. The influence of varying the concentration of salts of heavy metals was not investigated.

Effect of Precipitates. The effect of precipitates on the *Bremsstrahlung* measurements was investigated by adding phosphoric acid to ^{32}P solutions contained in two test tubes, and precipitating bismuth phosphate in one tube and calcium phosphate in another. The ion current produced by each was continuously recorded as the precipitate settled. The precipitates were centrifuged, the supernatant solutions removed, and the precipitates dried. The solutions and precipitates were then measured separately. A similar experiment was carried out with ^{90}Y, the yttrium carrier (10 mg. per 10 ml.) being precipitated as the hydroxide and as the oxalate.

The results, shown in Table IV, indicate that uncontrolled measurements on precipitates and suspensions must be ruled out for accurate analytical work. Moreover, the marked difference between the dense crystalline precipitates, bismuth phosphate and yttrium oxalate, and the gelatinous yttrium hydroxide, shows that the water concentration of the absorbing medium is one of the most important variables in this type of measurement. Moreover, with crystalline precipitates, the ion current per unit activity varies sharply with the degree of packing. Also, the influence of the container composition is effectively illustrated by the difference observed with yttrium oxalate in glass and Lusteroid containers. These results are consistent with Equation 1 which predicts that reproducible results for ionization per unit activity can be obtained on solid materials only when the composition of the solid is homogeneous—i.e., average *Z* is constant. If gelatinous precipitates are involved and plastic containers can be used, it should be possible to set up reproducible conditions, for an inhomogeneous distribution of the radioelement in the suspension would not be critical. The results of another investigation (*4*) are in agreement with the above conclusions.

Table IV. Effect of Precipitation of Added Absorber upon Production of Ion Currents by *Bremsstrahlung* from ^{32}P and ^{90}Y Beta-Particles

Added Absorber[a]	Calcium Phosphate	Bismuth Phosphate	Yttrium Hydroxide	Yttrium Oxalate	
	Per Cent Increase in Ion Current over Control[b]				
	Glass	Glass	Glass	Glass	Lusteroid
5 seconds after precipitation	0	0	0	0	0
5 minutes after precipitation	2.5	4.0	0	4.5	4.2
After centrifuging precipitate	..	24.5	3.3	10.0	4.0
After drying precipitate	33.1	43.1	..	33.2	21.0

[a] 10 mg. per 10 ml. of 0.1 *M* HNO_3.
[b] ^{32}P or ^{90}Y in 0.1 *M* HNO_3.

CONCLUSIONS

The radioactivity of a pure beta-emitting nuclide can be accurately determined by measuring the ionization produced by the *Bremsstrahlung* radiation originating in the source and its container. This ionization can be interpreted directly in terms of the absolute disintegration rate of the nuclide under investigation by proper calibration of a high-pressure ionization chamber.

The major limitations of the *Bremsstrahlung* method of measuring radioactivities are (1) an extreme sensitivity to the presence of a gamma-emitting impurity; (2) the requirements of relatively high activities; and (3) variations arising from an inhomogeneous composition of the medium and its immediate environs.

On the other hand, the wide range, stability, and linearity of response of the instrument make possible the accurate determination of the half-life of many radionuclides. One may determine directly beta-activities in many solutions and solids, particularly those which are highly hydrated, which could not otherwise be measured without extensive processing. Thus, it has been applied to the determination of ^{32}P in the excreta and tissues of animals which had received therapeutic doses of this isotope (*5*). A trace of a gamma-emitting impurity, not detectable on the Geiger-Müller beta-counter, can be picked up with the ion chamber, so that it should be possible to develop rapid analytical procedures for the determination of radiation purity.

The method does not appear suitable for extensive application to chemical processing problems involving high salt concentrations, precipitates and suspensions, tracer studies at low activity

levels, or the accurate measurement of weak beta-emitters such as ^{14}C and ^{35}S below the 100-mc. level.

LITERATURE CITED

(1) Atomic Energy Commission, Isotopes Division, "Radioisotopes Catalogue," September 1947.
(2) Atomic Energy Commission, Isotopes Division, "Simplified Ionization Chamber," Isotopes Division, *Circ.* A-7 (1949).
(3) Bothe, W., *Z. Naturforsch.*, 1, 179 (1946).
(4) Burnett, W. T., Jr., Tompkins, P. C., and Wish, L., Oak Ridge National Laboratory, *Rept.* **ORNL-263** (1949).
(5) Burnett, W. T., Jr., Tompkins, P. C., Wish, L., Beyl, G. E., and Huddleston, M., Oak Ridge National Laboratory, *Rept.* **ORNL-266** (1949).
(6) Glendenin, L. E., and Coryell, C. D., *Plutonium Project Record*, **9B**, 7.11.2 (1946).
(7) Goldschmidt, B. L., and Morgan, F., Atomic Energy Commission, **MC-11** (August 1943).
(8) Grummitt, W. E., and Wilkinson, G., *Nature*, 158, 163 (1946).
(9) Heitler, W., "Quantum Theory of Radiation," London, Oxford University Press, 1936.
(10) Jones, J. W., and Overman, R. T., "Use and Calibration of a 100% Geometry Ion Chamber," Oak Ridge National Laboratory, **Mon C-399** (1948).
(11) Nottorf, R. W., *Plutonium Project Record*, **9B**, 7.11.1 (1946).
(12) Nuclear Data Committee, Clinton National Laboratory, *Nucleonics*, 2, Part 2, 111, 133 (May 1948).
(13) Overman, R. T., Abstracts of 111th Meeting, *Am. Chem. Soc.*, p. 44P, 1947.
(14) Plutonium Project, *J. Am. Chem. Soc.*, 68, 2411 (1946).
(15) Rasetti, F., "Elements of Nuclear Physics," Philadelphia, Pa., Prentice-Hall, 1936.
(16) Stewart, D. W., Lawson, J. L., and Cork, J. M., *Phys. Rev.*, 52, 901 (1937).
(17) Tompkins, P. C., and Wish, L., Oak Ridge National Laboratory, *Rept.* **ORNL-314** (1949).
(18) Zumwalt, L. R., personal communication.

RECEIVED August 12, 1949. Work done under Contract W-7405-Eng-26 for the Atomic Energy Commission, Oak Ridge, Tenn.

Qualitative Determination of Carboxylic Esters

Scope and Limitations of the Hydroxamic Acid Test

ROBERT E. BUCKLES AND CHARLES J. THELEN
State University of Iowa, Iowa City, Iowa

A study of the scope and limitations of the hydroxamic acid test for carboxylic esters has led to the development of three modified test procedures. These procedures make it possible to distinguish between acid derivatives that react with hydroxylamine to form hydroxamic acids and compounds that do not. They are used further to distinguish esters from the more reactive acid chlorides and acid anhydrides when limited to compounds which contain no nitrogen. These modified procedures have been especially successful when applied to polymers, plasticizers, fats, and oils. The test can also be used to detect esters in mixtures.

A NUMBER of the more popular, recent textbooks (*6*, *8*, *9*) of qualitative organic analysis give the saponification of an ester to the alcohol and the salt of the acid as the only reliable, specific classification test for esters. Dependence on this test alone in the qualitative analysis for esters has been unsatisfactory both in research and in courses in qualitative organic analysis. This article discusses the scope and limitations of the hydroxamic acid test as a specific class reaction for esters.

The preparation of a hydroxamic acid by the reaction of hydroxylamine with a suitable derivative of a carboxylic acid has been a well-known reaction for a long time (*14*).

$$RCOOR' + NH_2OH \longrightarrow RCONHOH + R'OH$$

$$RCOCl + NH_2OH \longrightarrow RCONHOH + HCl$$

$$(RCO)_2O + NH_2OH \longrightarrow RCONHOH + RCOOH$$

Hydroxamic acids can be detected easily and fairly specifically by the addition of ferric chloride solution under conditions acidic enough to keep ferric hydroxide from precipitating. A deep magenta color is observed as a result of this reaction. The reaction that takes place is formulated by Sidgwick (*10*) as follows:

$$3RCONHOH + FeCl_3 \longrightarrow \left[R{-}C(=O){-}NH{-}O\right]_3 Fe + 3H^+ + 3Cl^-$$

Various methods of synthesis of a hydroxamic acid followed by its detection with ferric chloride solution have been used by Davidson (*2*) as the bases for classification tests for several types of organic compounds including esters.

During the past year this test as applied to esters has been tried out on a considerable number of compounds in order to evaluate it. It has been found necessary to devise modifications. Three useful test procedures have been developed.

PROCEDURES

The compounds used in testing these procedures were either samples of commercial products or the more highly purified compounds sold by chemical supply houses. No attempt was made to purify any of these samples further.

A. Dissolve a drop or a few crystals of the compound to be tested in 1 ml. of 95% alcohol and add 1 ml. of 1 *N* hydrochloric acid. Note the color produced when 1 drop of 10% ferric chloride is added to the solution.

B. Mix 1 drop or several crystals of the compound with 1 ml. of 0.5 *N* hydroxylamine hydrochloride in 95% ethyl alcohol. Add 0.2 ml. of 6 *N* aqueous sodium hydroxide, heat the mixture to boiling, and after the solution has cooled slightly add 2 ml. of 1 *N* hydrochloric acid. If the solution is cloudy add 2 ml. of 95% ethyl alcohol. Observe the color produced when 1 drop of 10% ferric chloride solution is added. If the color caused by the drop of ferric chloride solution does not remain when it is mixed into the test solution, keep adding the reagent dropwise until the observed color pervades the entire test solution. Usually only 1 drop of the ferric chloride solution is necessary. Compare the color with that produced by test A. A positive test will be a distinct burgundy or magenta color as compared with the yellow observed when the original compound is tested with ferric chloride in the presence of acid. [The color observed in this test is the one listed as magenta (*12*) in the illustrations under the word "color."] It is best to observe the color of the test solution within 5 minutes after adding the ferric chloride solution. In

many cases, however, the colors of test solutions remain unchanged for hours.

C. If test B is positive, repeat it without adding the sodium hydroxide and with only 1 ml. of the hydrochloric acid for acidification.

RESULTS

Carboxylic Esters. The following esters gave solutions of deep magenta color when used in procedure B:

Methyl formate
Ethyl formate
Isoamyl formate
n-Amyl formate
Methyl acetate
Isobutyl acetate
Benzyl acetate
Phenyl acetate
Methyl butyrate
Ethyl crotonate
Ethyl α,β-dibromobutyrate
Isoamyl salicylate
Phenyl salicylate
Ethyl *p*-hydroxybenzoate
Ethyl acetoacetate
Ethyl malonate
n-Butyl sebacate
Ethyl phthalate
n-Butyl phthalate
Phenyl phthalate
Methyl 2,2′,6,6′-tetranitro-4,4′-diphenyldicarboxylate
n-Butyl tartrate
n-Butyl citrate
n-Butyl stearate
Methyl benzoate
Phenyl benzoate
Methyl cinnamate
Methyl *m*-nitrocinnamate
Ethyl α,β-dibromo-β-phenylpropionate
Ethyl lactate
n-Butyl lactate
Methylsalicylate
n-Butyl acetylricinoleate
Ethyl glycinate hydrochloride
Triacetin
Tripropionin
Cottonseed oil
Linseed oil
Coumarin
α-Gluconolactone
Polylactic acid
Polyvinyl acetate
Methyl oxalate
Ethyl oxalate
Ethyl oxalpropionate

The following esters gave medium magenta colors when used in test B:

Hydrogenated cottonseed oil
Glyptal resin
Polymethyl methacrylate

Isobutyl acid phthalate and *sec*-octyl acid phthalate usually gave weak tests when used in test B. It was necessary to add the hydroxylamine to the ester before the base was added. If the reverse order was followed the test was negative (yellow solution).

Oxalates and any ester which cleaved to give oxalic acid (ethyl oxalpropionate) required more ferric chloride than the average ester in order to give a persistent test. The same behavior was observed with methyl acetate containing oxalic acid.

All the esters gave yellow solutions when used in test A. Only phenyl acetate gave a medium magenta solution when used in test C; all the others gave yellow solutions.

Esters of Other Acids. The following esters gave negative tests to procedure B:

Ethyl carbonate
Ethyl carbamate
Ethyl chloroformate
Methyl *p*-toluenesulfonate
Methyl sulfate
Ethyl sulfate
Ethyl nitrate
Butyl phosphate

Trihalomethyl Compounds. Solutions of deep magenta color were obtained when benzotrichloride and chloral hydrate were used in test B. The following compounds gave solutions of medium magenta color:

Chloroform
Bromoform
Carbon tetrabromide
Bromotrichloromethane
Trichloroacetic acid

Negative tests (yellow solutions) were obtained with iodoform, carbon tetrachloride, and 1,1,2-trichloro-1,2-dibromoethane.

Acid Anhydrides. The following acid anhydrides gave solutions of deep magenta color when used in either test B or C:

Acetic anhydride
Succinic anhydride
Maleic anhydride
Phthalic anhydride
3-Nitrophthalic anhydride

Acid Chlorides. Benzoyl chloride and *p*-phenylbenzoyl chloride gave deep magenta solutions with tests B and C. Benzenesulfonyl chloride and thionyl chloride gave yellow solutions to both tests.

Carboxylic Acids. Formic acid and phthalic acid gave solutions of medium magenta color with both tests B and C. Lactic acid (85%) gave a deep magenta color by test B only. Acetic, benzoic, *dl*-malic, succinic, glutaric, and citric acids all gave yellow solutions by test B.

Phenols. Phenols gave yellow solutions by both tests A and B.

Aldehydes. Weakly positive tests were often observed with the following aldehydes:

Formaldehyde
Benzaldehyde
p-Hydroxybenzaldehyde
m-Nitrobenzaldehyde
Vanillin
Anisaldehyde

Other aldehydes—e.g., isobutyraldehyde, *n*-butyraldehyde, and *n*-heptaldehyde—always gave negative tests.

Amides. Formamide gave a deep magenta solution by test B. The following amides gave medium magenta tests:

Acetamide
Formanilide
Benzamide
Diacetylhydrazine
Phthalhydrazide

Negative tests were given by urea, butyramide, acetanilide, *N*-benzoyl-*p*-bromoaniline, *N*-benzoyl-2-naphthylamine, and *N*-acetyl-1-naphthylamine.

Imides. The following imides gave deep magenta colors by test B:

Diacetamide
Succinimide
Phthalimide
4-Nitrophthalimide

Medium to light magenta tests were given by the following imides:

N-*n*-Amyl-3-nitrophthalimide
Biuret
Benzoylurea
N-Methyl-*N*′-acetylurea

A negative test was given by diethylbarbituric acid.

Nitriles. Acetonitrile and propionitrile gave light magenta tests by procedure B. Valeronitrile, benzonitrile, and phenylacetonitrile gave yellow tests.

Isocyanates. Phenyl, *p*-tolyl, and α-naphthyl isocyanates gave deep magenta tests by procedure C but only very weak or negative tests by procedure B. *p*-Nitrophenyl isocyanate gave a light magenta solution with test C; it was negative with test B. Phenyl isothiocyanate was negative to both tests B and C.

Nitro Compounds. The following nitro compounds gave deep red solutions when used in test B whether hydroxylamine hydrochloride was added or not:

Nitromethane
2-Nitropropane
2-Nitro-1-butanol
2-Nitro-1-chlorobutane

Test A gave yellow solutions in these cases. Nitrobenzene and *p*-nitrotoluene gave yellow solutions when used in test B. Nitroaromatic esters and anhydrides gave normal results (see above).

Esters in Mixtures. Methyl benzoate when mixed with an equal amount of each of the following compounds gave solutions of deep magenta color when used in test B:

Propionic acid
n-Octyl alcohol
Propylene glycol
Benzaldehyde
n-Butyraldehyde
Formamide
Diethylaniline
n-Butylamine
Ethylaniline

With a large excess of *n*-butyraldehyde in the mixture better results were obtained when the test mixture was allowed to stand 5 or 10 minutes before addition of ferric chloride solution. Isoamyl acetate mixed with acetone gave a negative test by procedure B unless two or three times as much hydroxylamine hydrochloride as usual was used. Then a positive test was observed.

Methyl acetate mixed with an equal amount of each of the following compounds gave positive results varying from weak to medium by procedure B:

Formaldehyde
Isobutyraldehyde
n-Heptaldehyde
Aldol

Mixed with the following compounds methyl acetate gave solutions of deep magenta color when used in test B:

Acetaldehyde	Diethyl ketone
Acetal	Methyl *n*-hexyl ketone
Methyl ethyl ketone	Acetophenone

DISCUSSION

Generally speaking, esters will give hydroxamic acids only when heated with hydroxylamine in the presence of base (test B). This hydroxamic acid test is fairly satisfactory as a specific classification test for carboxylic esters if it is limited to compounds which do not contain nitrogen. The weakly positive tests often obtained with aldehydes which have no hydrogen on α-carbon may be due to ester formation in an accompanying Canizzaro reaction. Generally these tests are very weak and should not be mistaken for the strong ester tests. Trihalomethyl compounds often simulate esters and give positive tests, presumably by way of orthoacid derivatives as summed up in the following reaction:

$$RCX_3 + 3OH^- + NH_2OH \longrightarrow RCONHOH + 3X^- + 2H_2O$$

Most of these trihalomethyl compounds that would be encountered, however, would be insoluble in cold, concentrated sulfuric acid and would give a negative ferrox test (*1*). Exceptions would be such compounds as chloral hydrate and trichloroacetic acid.

The hydroxamic acid test does not give positive results with carbonates, urethans, chloroformates, sulfonates, and esters of inorganic acids. This, of course, is a limitation on its use as a general ester test.

The test is strongly positive with both simple and complex carboxylic esters. Even esters of high molecular weight such as glycerides of fatty acids and polymeric esters give good results. Monoesters of dicarboxylic acids tend to saponify so fast that usually only weak tests are observed. Oxalates or any ester which yields oxalic acid on saponification requires more ferric chloride than the usual ester to give a satisfactory positive test. Presumably a complex forms between ferric chloride and oxalic acid. Lactic acid acts as an ester in this test. This behavior is simply further proof of the generally accepted view that lactic acid contains a large percentage of lactyllactic acid as well as higher polymeric esters (*11*).

A phenolic group either in the original compound or formed as a result of the hydroxylaminolysis of the ester group does not hinder the test with ferric chloride solution. The presence of acid in the solution would depress the formation of the colored ion expected from the reaction of ferric chloride with a phenol (*13*):

$$6ArOH + FeCl_3 \rightleftharpoons Fe(OAr)_6^{---} + 6H^+ + 3Cl^-$$

Such a dependence on acid concentration has not been observed in the color reaction between ferric chloride and a hydroxamic acid.

The use of the hydroxamic acid test for esters as a preliminary test on both water-soluble mixtures and water-insoluble mixtures is feasible. Most types of compounds accompanying esters in mixtures do not interfere with the test. Aldehydes and ketones may compete successfully for the hydroxylamine and make the test weak or negative, but the addition of more hydroxylamine hydrochloride solution than called for in the procedure will correct this deficiency.

The formation of hydroxamic acids from anhydrides and chlorides of carboxylic acids when they react with hydroxylamine hydrochloride even in the absence of base serves as a test to distinguish these compounds from the less reactive esters as well as from the nitrogen-containing acid derivatives which give positive tests according to procedure B only. Isocyanates give positive results by test C (*4*), but they give negative tests in the presence of base. Presumably the hydroxamic acid derived from the substituted carbamic acid is saponified. It has also been reported (*5*) that ketenes react with hydroxylamine hydrochloride to form hydroxamic acids. Both formic and phthalic acids act like anhydrides in the hydroxamic acid test. This is not unusual, since formic acid in much of its chemical behavior is more like an anhydride than other carboxylic acids are. It is a good formylating agent both for amines and alcohols. The commercial phthalic acid used may well be contaminated by phthalic anhydride and thus give the test as an anhydride.

Many of the nitrogen-containing derivatives of carboxylic acids also give positive tests. Imides, which are the nitrogen analogs of anhydrides, give the most consistent results. Some amides and nitriles also give positive tests, but the results are not consistent enough to make the test useful for these types of compounds.

Nitro compounds with enolizable hydrogen react with sodium hydroxide to yield an ion. The action of acid on this ion yields the aci form of the nitro compound immediately. This aci form slowly reverts to the normal form on standing (*7*):

$$RCH_2NO_2 + OH^- \rightleftharpoons (RCHNO_2)^- + H_2O$$

$$(RCHNO_2)^- + H_3O^+ \overset{\text{rapid}}{\rightleftharpoons} RCH{=}N\begin{matrix}\nearrow O\\ \searrow OH\end{matrix} + H_2O$$

$$(RCHNO_2)^- + H_3O^+ \overset{\text{slow}}{\longrightarrow} RCH_2NO_2 + H_2O$$

If the solution is tested with ferric chloride while an appreciable amount of the aci form is still present, a deep red color will be observed (*3*). Hence these nitro compounds give color reactions when test B is applied to them even when no hydroxylamine hydrochloride is used.

ACKNOWLEDGMENT

The authors would like to express their appreciation to Ralph L. Shriner for the many suggestions which aided this work.

SUMMARY

The scope and limitations of the hydroxamic acid test for carboxylic esters have been investigated.

Three procedures have been developed to enable the hydroxamic acid test to be used as a specific class reaction for carboxylic esters when applied to compounds that do not contain nitrogen. The hydroxamic acid test was found to give satisfactory results not only with simple carboxylic esters, but also with polymeric esters and with glycerides of fatty acids. It was also satisfactory for testing esters in mixtures.

Positive tests (misleading from the standpoint of the application of the test to esters) with nitrogen-containing compounds, trihalomethyl compounds, and aldehydes have also been investigated and discussed.

LITERATURE CITED

(1) Davidson, D., IND. ENG. CHEM., ANAL. ED., **12**, 40 (1940).
(2) Davidson, D., *J. Chem. Education*, **17**, 81 (1940).
(3) Hantzsch, A., and Shultze, O. W., *Ber.*, **29**, 699 (1896).
(4) Hurd, C. D., *J. Am. Chem. Soc.*, **45**, 1472 (1923).
(5) Jones, L. W., and Hurd, C. D., *Ibid.*, **43**, 2422 (1921).
(6) McElvain, S. M., "Characterization of Organic Compounds," New York, Macmillan Co., 1946.
(7) Maron, S. H., and La Mer, V. K., *Ibid.*, **61**, 692 (1939).
(8) Schneider, F., "Qualitative Organic Microanalysis," New York, John Wiley & Sons, 1946.
(9) Shriner, R. L., and Fuson, R. C., "Systematic Identification of Organic Compounds," 3rd ed., New York, John Wiley & Sons, 1948.
(10) Sidgwick, N. V., "Organic Chemistry of Nitrogen," p. 198, New York, Oxford University Press, 1937.
(11) Watson, P. D., *Ind. Eng. Chem.*, **32**, 399 (1940).
(12) Webster, "New International Dictionary," 2nd ed., unabridged.
(13) Wesp, E. F., and Brode, W. R., *J. Am. Chem. Soc.*, **56**, 1037 (1934).
(14) Yale, H. L., *Chem. Rev.*, **33**, 209 (1943).

RECEIVED October 23, 1949.

Spectrophotometric Determination of Cinchona Alkaloids

HELEN S. GRANT AND J. H. JONES[1]

Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

The composition of a mixture of quinine (or quinidine) and cinchonine (or cinchonidine) can be accurately calculated from the optical density at 316 and 348 mμ of a solution of the alkaloids in 0.1 *N* hydrochloric acid by the use of simultaneous equations. Mixtures of cinchona alkaloids, such as the alkaloids from cinchona bark, behave spectrophotometrically as a simple two-component system and the amount of quinine-type and cinchonine-type alkaloids in such mixtures can be determined by the same procedure. A simple, rapid method for analysis of small samples of cinchona bark based on this fact has been developed. The method may be used to determine the total alkaloid content and the amount of quinine-type alkaloids. The latter value is a reasonably reliable estimate of the quinine content.

A KNOWLEDGE of the alkaloid content of the bark of nursery stock of cinchona trees is of value in the selection of seedlings which will produce high-quinine–yielding trees. To permit an intelligent selection of the planting stock with the least loss, analyses of a considerable number of very small samples of bark are needed. Although a complete analysis of the component alkaloids of the young bark would be preferable, a knowledge of the quinine and total alkaloid content is sufficient to permit intelligent selection of seedlings. The work reported here was undertaken with these objectives in mind.

Gravimetric methods for the analysis of cinchona bark are reasonably accurate, but are lengthy and tedious, and require large samples of bark; consequently, they are not suitable for the purposes being considered. A review of previous work on the spectrophotometry of cinchona alkaloids suggested that a spectrophotometric method of analysis would meet the objectives of this work.

The ultraviolet absorption spectra of the cinchona alkaloids have been determined by a number of workers. In dilute acid solution, quinine and quinidine have absorption peaks of about 250, 318, and 348 mμ. The peaks for cinchonine and cinchonidine are at about 235 and 315 mμ. Carol (*1*) has shown that quinine dissolved in 0.1 *N* hydrochloric acid can be determined spectrophotometrically at 250.5, 318, and 347.5 mμ if interfering substances are absent. Recently, Loustalot and Pagan (*3*) have shown that the quinine plus quinidine content of cinchona bark can be determined from the absorption of an acidified alcoholic extract of the bark at 380 mμ (using a 30 mμ slit width). They determined the total alkaloid content of the bark by a volumetric procedure. Stimson and Renter (*4*) have suggested that the quinine content of totaquine may be determined from the absorption at 331 mμ in neutral alcohol. By using the total weight of the alkaloids and the ultraviolet absorption of the mixture, Fuchs and Kampitsch (*2*) have determined the quinine and cinchonine contents of mixtures containing these two alkaloids.

This paper reports the results of a study of the spectrophotometric analysis of mixtures of cinchona alkaloids.

[1] Present address, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.

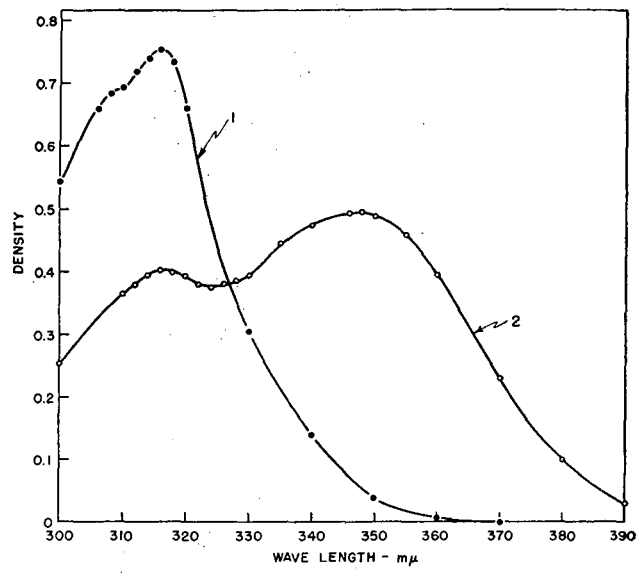


Figure 1. Absorption Spectra of Principal Cinchona Alkaloids

1. Cinchonine and cinchonidine
2. Quinine and quinidine

Concn., 30 mg. per liter
Solvent, 0.1 *N* HCl
Cells, 1 cm.

Table I. Analyses of Known Mixtures of Cinchona Alkaloids

Mixture No.	Composition of Mixture[a]				Q Alkaloids		C Alkaloids		Total Alkaloids	
	Q	Qd	C	Cd	Added	Found	Added	Found	Added	Found
1	1.50	..	..	1.50	1.50	1.48	1.50	1.53	3.00	3.01
2	..	0.75	0.75	..	0.75	0.74	0.75	0.73	1.50	1.48
3	..	0.60	0.60	..	0.60	0.59	0.60	0.60	1.20	1.19
4	2.25	..	..	0.75	2.25	2.26	0.75	0.74	3.00	3.00
5	0.75	..	..	2.25	0.75	0.75	2.25	2.30	3.00	3.05
6	3.00	..	0.60	..	3.00	3.00	0.60	0.59	3.60	3.59
7	3.00	..	0.30	..	3.00	2.98	0.30	0.26	3.30	3.25
8	0.30	..	3.00	..	0.30	0.31	3.00	2.99	3.30	3.30
9	3.00	..	0.15	..	3.00	2.99	0.15	0.14	3.15	3.12
10	0.15	..	3.00	..	0.15	0.15	3.00	2.97	3.15	3.12
11	0.90	0.30	1.50	0.90	1.20	1.18	2.40	2.43	3.60	3.62
12	0.90	..	1.50	0.90	0.90	0.90	2.40	2.43	3.30	3.32
13	0.60	..	3.00	0.60	0.60	0.61	3.60	3.60	4.20	4.21

[a] In mg. per 100 ml. of solution taken for spectrophotometric analysis. Q, quinine. Qd, quinidine. C, cinchonine. Cd, cinchonidine.

EXPERIMENTAL

The spectrophotometric data given in this report were obtained with a Beckman Model DU spectrophotometer. A slit width of approximately 2 mμ was used in all measurements. Matched 1-cm. Corex cells were used.

The alkaloids used as standards were recrystallized several times from alcohol or benzene and dried to constant weight at 100° C. The melting points of the purified materials agreed with the literature values.

The spectral absorption curves of equal weights of

quinine, quinidine, cinchonine, and cinchonidine in 0.1 N hydrochloric acid, measured against a control of 0.1 N hydrochloric acid, are shown in Figure 1. The curves for quinine and quinidine appear to be identical and are shown as a simple curve. Each compound has absorption maxima at 318 and 348 mμ. The curves for cinchonine and cinchonidine also appear to be identical and are shown in Figure 1 as a single curve. In the region selected for study, these compounds have a single absorption peak at 316 mμ. The curves in Figure 1 are in good agreement with the results of other workers.

Concentration *vs.* optical density data show that quinine, quinidine, cinchonine, and cinchonidine dissolved in 0.1 N hydrochloric acid obey Beer's law to within ±1.0% at the absorption peaks for concentrations between 10 and 60 mg. per liter. Each of the alkaloids may be determined spectrophotometrically, therefore, if interfering substances are absent.

Examination of Figure 1 shows that in 0.1 N hydrochloric acid the optical density per unit weight of quinine (and quinidine) is about one half that of cinchonine (and cinchonidine) at 316 mμ but is nearly seven times as great at 348 mμ. Because this is true, it should be possible to calculate accurately the composition of a mixture of quinine (or quinidine) and cinchonine (or cinchonidine) dissolved in 0.1 N hydrochloric acid from the optical density of the solution at 316 and 348 mμ by the use of simultaneous equations. A mixture of all four of the alkaloids should behave as a simple two-component system and the spectrophotometric analysis should give the quinine plus quinidine and the cinchonine plus cinchonidine content of the mixture. Obviously, the data cannot be used to resolve mixtures of quinine and quinidine, or cinchonine and cinchonidine.

Results of the spectrophotometric analysis of mixtures of known amounts of the four principal cinchona alkaloids are shown in Table I. (In these analyses the amounts of quinine-type and cinchonine-type alkaloids were calculated from the densities of the standards and the mixtures at 316 and 348 mμ by the method of simultaneous equations.) The average error in the determination of the total alkaloids is less than 1% and the largest error is 2%. In most cases equally accurate results are obtained for the amount of each type of alkaloid.

The mixtures shown in Table I contain only the four principal cinchona alkaloids. The alkaloids from cinchona bark contain a considerable proportion (10 to 50%) of the "so-called" amorphous alkaloids. Because most of the alkaloids in the bark are closely related chemically and the available data indicate that the absorption spectra of the closely related cinchona alkaloids are very similar, it appears probable that the amorphous alkaloids have absorption spectra very similar to that of either quinine or cinchonine. If this is true, the presence of the amorphous alkaloids should not introduce an appreciable error in the spectrophotometric determination of total alkaloids.

The results of the analysis of several samples of alkaloids that contained amorphous alkaloids are shown in Table II. It is apparent that the spectrophotometric method gives a good estimate of the total alkaloid content of such mixtures. It also appears reasonable to assume that the results for quinine-type and cinchonine-type alkaloids are valid indications of the composition of the mixture.

The spectrophotometric method was applied to the determination of the total alkaloids in cinchona bark as follows:

A small sample of the powdered bark, usually 1 gram, was mixed with a few milliliters of 10% sodium hydroxide solution in a 200-ml. flask, 100 ml. of benzene were added, and the flask and contents were weighed. The flask was connected to a reflux condenser and the benzene was boiled gently until the alkaloids were completely extracted (3 to 6 hours). After cooling to room temperature, any benzene lost was replaced. A 50-ml. aliquot of the benzene was transferred to a separatory funnel and the alkaloids were extracted with several small portions of 0.1 N hydrochloric acid. The combined acid extracts were boiled 2 to 3 minutes to expel any suspended benzene, cooled, and diluted to exactly 1000 ml.

The optical density of the acid solution of the alkaloids was determined at 316 and 348 mμ. From these data and density of the standard solutions of quinine and cinchonine (30 mg. per liter in 0.1 N hydrochloric acid), the alkaloid content of the solution was calculated from the simultaneous equations,

$$D_{316} = XD_q^{316} + YD_c^{316}$$

$$D_{348} = XD_q^{348} + YD_c^{348}$$

where X and Y are the amounts of quinine-type and cinchonine-type alkaloids; D_{316} and D_{348} are the optical densities of the sample solution at 316 and 348 mμ; and D_q^{316}, D_q^{348}, D_c^{316}, and D_c^{348} are the optical densities per unit weight of the two standards at the respective wave lengths.

Table II. Analyses of Mixtures Containing Amorphous Alkaloids

Sample	Sample Weight	Analysis: Q alkaloids	Analysis: C alkaloids	Total Alkaloids
	Gram	*Gram*	*Gram*	*Gram*
Totaquine alkaloids	0.300	0.039	0.255	0.294
Totaquine alkaloids	0.300	0.040	0.253	0.293
Bark (1) alkaloids	0.300	0.096	0.207	0.303
Bark (2) alkaloids	0.300	0.099	0.206	0.305
Amorphous alkaloids[a]	0.600	0.193	0.398	0.591

[a] Bark alkaloids after removal of cinchonine, quinine, and cinchonidine by U.S.P. XII method.

Typical results obtained by this procedure are shown in Table III, together with the results of gravimetric analysis of the same samples. In most cases the results by the spectrophotometric and gravimetric methods do not differ by more than 2% of the alkaloid content of the sample. The average deviation between replicates for the bark samples shown in Table III was approximately 2%.

DISCUSSION

The proposed spectrophotometric method separates the cinchona alkaloids into two groups—the quinine type and the cinchonine type. The amount of quinine-type alkaloids gives directly the maximum amount of quinine that could be present. Loustalot and Pagan (*3*) have shown that the absorption in dilute acid at 380 mμ gives an accurate estimate of the quinine plus quinidine content of many cinchona barks. The quinine-type alkaloids shown by the proposed method should give an equally accurate estimate of the quinine plus quinidine content.

The quinine content of several samples analyzed in this work has varied from 65 to 100% of the quinine-type alkaloids shown by spectrophotometric analysis. Even if the spectrophotometric method does not give a very accurate estimate of the quinine content in all cases, it should still be valuable as a rapid preliminary test.

The extraction procedure described has proved satisfactory. It would appear, however, that spectrophotometric determination could be used with other extraction procedures that quantitatively extract the alkaloids and eliminate interfering substances.

The presence of excessive coloring matter is recognized as a possible source of error, although no interference from this source could be detected in samples examined in the course of this work. Benzene extracts much less of the coloring matter fron cinchona bark than does alcohol.

Although relatively few bark samples were analyzed, those examined were said to represent young, mature, and very old bark from several commercial species of cinchona.

The U.S.P. XII method for the analysis of totaquine consists of the following steps:

Cinchonine is precipitated as the alkaloid and weighed.

Quinine and cinchonidine are precipitated together as the tartrates and the amount of each alkaloid is calculated from the

weight of the precipitate and the optical rotation of a solution of the precipitate.

Quinidine is precipitated as the iodide and the quinidine content is calculated from the weight of the precipitate.

Each of the above precipitates can be analyzed conveniently for its alkaloid content spectrophotometrically. The precipitates are dissolved in a measured volume of 0.1 *N* hydrochloric acid and the optical density of the solution at the appropriate wave length (or wave lengths) is determined.

The spectrophotometric determination is particularly advantageous in the analysis of the tartrate precipitate, inasmuch as the accuracy of the results obtained by the U.S.P. method, when applied to natural mixtures, is questionable. Tests of several tartrate precipitates indicate that the spectrophotometric method gives results that agree with those calculated from the methoxyl and nitrogen content of the precipitate. These data together with the results on known mixtures indicate that the spectrophotometric method gives accurate results for the quinine and cinchonidine content of the tartrate precipitate.

Table III. Analyses of Cinchona Bark and Totaquine

Sample[a]	Spectrophotometric			Gravimetric
	Cinchonine alkaloids	Quinine alkaloids	Total alkaloids	Total Alkaloids
	%	%	%	%
Totaquine	62.1	10.8	72.9	72.8
Bark 1	6.5	2.9	9.5	9.4
Bark 2	6.4	2.9	9.3	9.2
Bark 3	5.2	3.0	8.2	8.7
Bark 4a	2.4	1.6	3.9	..
Bark 4b	2.0	2.8	4.7	..
Bark 4c	2.7	2.0	4.7	..
Bark 4d	2.2	2.1	4.3	..
Bark 4e	2.2	3.2	5.4	..
Bark 4 (av.)	..	..	4.6	4.7

[a] All results are the average of two or more determinations except for bark 4. Barks 4a to 4e are samples from individual young trees. Gravimetric value for bark 4 was obtained on a composite sample of equal amounts of barks 4a to 4e.

SUMMARY

The composition of a mixture of quinine (or quinidine) and cinchonine (or cinchonidine) can be determined accurately from the optical density at 316 and 348 mμ of a solution of the mixture in 0.1 *N* hydrochloric acid. A mixture of all four alkaloids behaves spectrophotometrically as a simple two-component mixture and the quinine plus quinidine and the cinchonine plus cinchonidine content of the mixture can be determined in the same way.

The spectrophotometric method is also applicable to the analysis of mixtures containing the so-called amorphous cinchona alkaloids. A simple, rapid method for the determination of the total alkaloids in cinchona bark, based on this fact, may be used to determine the total alkaloid content and the amount of quinine-type alkaloids in the bark. The latter value should be a reasonably reliable estimate of the quinine content.

Each of the precipitates obtained in the U.S.P. XII method of analysis of totaquine can be dissolved in 0.1 *N* hydrochloric acid and the alkaloid content determined spectrophotometrically. The spectrophotometric method is particularly advantageous for the analysis of the quinine plus cinchonidine tartrate precipitate.

Typical results obtained by the various spectrophotometric procedures are given.

LITERATURE CITED

(1) Carol, J., *J. Assoc. Offic. Agr. Chemists*, 26, 238 (1943).
(2) Fuchs, V. L., and Kampitsch, A., *Scientia Pharm.*, 6, 125 (1935).
(3) Loustalot, A. J., and Pagan, C., *J. Assoc. Offic. Agr. Chemists*, 30, 154 (1947).
(4) Stimson, M. M., and Renter, M. A., *J. Am. Chem. Soc.*, 68, 1192 (1946).

RECEIVED October 27, 1949. Presented at the All-Day Technical Conference, Chicago Section, AMERICAN CHEMICAL SOCIETY, Chicago, Ill., December 26, 1947. Extract from laboratory studies of Mexican resources as part of the Mexican Industrial Development program conducted by the Armour Research Foundation of Illinois Institute of Technology under the sponsorship of the Bank of Mexico.

Determination of Potential Yeast Fermentables in Cereal Grains

R. T. WISTHOFF AND R. J. ALLGEIER
U. S. Industrial Chemicals, Inc., Baltimore, Md.

Total fermentable carbohydrate is determined in cereal grains used in fermentation processes by a method which selects the fermentable portion more precisely than those used hitherto. When this method is employed and the grain ferments under optimum conditions, the fermentation efficiencies are very close to 100%, which means that the analytical results correspond closely to the actual fermentables of the grain. The procedure consists in washing a finely ground sample of the grain to remove sugar, then dissolving out the starch with hydrochloric acid of suitable strength. The latter solution is heated to hydrolyze the starch to dextrose, and sugar is determined on both the washings and the hydrolyzed starch by the Lane and Eynon method. The fermentation and analytical results show the superiority of the proposed method over the acid hydrolysis procedure of the Association of Official Agricultural Chemists, when the purpose is to determine fermentable material in the grain.

ALTHOUGH procedures for the direct determination of starch in complex substances have been studied for over a century, there has been a need for a method of accurately determining total fermentable carbohydrates in grains. In 1846 Krocker (*4*) under Liebig's direction developed an ingenious fermentation procedure for the determination of the true starch content of all the commonly known cereal grains and legume seeds. With a few exceptions surprisingly good agreement was obtained as compared with current knowledge of the starch content of these materials.

Methods for the determination of starch have been based upon a number of its properties, and certain of its characteristics have been used analytically for a number of years; an adequate review of the literature was covered by Clendenning (*3*). Recently Rask (*6*) reported improvements in the A.O.A.C. tentative method (*1*) and the authors have applied his technique in part to cereal grains. Fermentations were made with Dent corn, wheat, rye, and milo maize in such a manner as to obtain the maximum possible fermentation efficiency from the type of grain under test. Sugar was determined by the method herein described and by the

Table I. Available Dextrose and Fermentation Data on Milo Maize

Detn. No.	Dextrose: By dispersion, %	Dextrose: In washings, %	Dextrose: Total available, %	Dextrose: By acid hydrolysis, %	Fermentation Efficiency: Acid hydrolysis, %	Fermentation Efficiency: Dispersion method, %
			Milo Maize 1			
1	71.08		73.45			
	71.02	2.37	73.39	76.44	97.18	101.60
2	71.27		73.63			
	71.37		73.73			
	71.45	2.36	73.81	...	96.97	101.38
3	71.37		73.62		95.37	99.78
	71.13		73.38		95.52	99.87
	71.41	2.25	73.66	76.88	95.76	100.12
Av.	71.26	2.33	73.59	76.66	96.16	100.55
Greatest deviation from average	0.34		0.30			
			Milo Maize 2			
1	72.09		74.97		97.01	100.46
	71.79	2.88	74.67	76.95	97.31	100.77
					96.56	100.00
2	71.51		74.45			
	71.79		74.73			
	71.59	2.94	74.53	76.84	96.68	100.12
3	71.80		74.79		97.31	100.77
	71.70		74.69		96.65	100.09
	72.00	2.99	74.99	77.37		
Av.	71.78	2.94	74.73	77.05	96.92	100.37
Greatest deviation from average	0.43		0.37			

conventional hydrochloric acid hydrolysis procedure (*2*). Comparisons of the fermentation results are given, using these two sugar determinations as representing the total fermentables.

REAGENTS

Ethyl alcohol, 190-proof. Ethyl ether, anhydrous. Hydrochloric acid, 20.5 to 21.0 grams per 100 ml. Sodium hydroxide, 25% solution. Sodium hydroxide, 2 *N*.

Lead acetate, neutral, saturated solution. Potassium oxalate, dried powder. Ammonium bifluoride, $NH_4F.HF$, 1% solution.

Fehling's Solution A, 69.28 grams of cupric sulfate per liter. Fehling's Solution B, 346 grams of Rochelle salts and 100 grams of sodium hydroxide per liter.

Methylene blue indicator, 1.0% solution.

PROCEDURE

Preparation of Sample. Two grams of grain to be tested are ground to pass a U. S. Sieve No. 40 screen and accurately weighed. The grain is placed in a funnel, which has been prepared by carefully fitting a 9-cm. No. 2 Whatman filter paper into a 6.5-cm. long-stemmed funnel, washing with distilled water and then with 190-proof alcohol, and drying at room temperature for several minutes.

The sample is then introduced, washed three times with ethyl ether, three times with 190-proof alcohol, six times with water, three times with 190-proof alcohol, and three times with ethyl ether, and allowed to drain after each washing. Ten milliliters of liquid are employed in each case. The sample is allowed to dry overnight at room temperature or 30 minutes at 50° C. to evaporate the ether. The filtrate of the composite washings is retained.

Determination of Dextrose in Composite Washings. The washings obtained in the treatment are evaporated on a steam bath to about 25-ml. volume and are transferred into a 100-ml. volumetric flask with a total of 25 ml. of water in three washings, using a policeman. Hydrolysis is effected with 10 ml. of 1 to 1 hydrochloric acid in a boiling water bath for 1 hour with subsequent cooling to room temperature. The solution is neutralized with 25% sodium hydroxide to the phenolphthalein end point and 10 ml. of saturated neutral lead acetate solution are added. The mixture is diluted to volume with water at 20° C. and after standing for a few minutes is filtered through a dry No. 12 Whatman folded filter paper; the first 25 ml. are returned to the filter. Ten grams of potassium oxalate are added to the filtrate and after stirring well the precipitate is removed by filtration through No. 12 Whatman folded filter paper; the first 25 ml. are returned to the filter. Total sugar as dextrose is determined on the filtrate by the method of Lane and Eynon (*5*).

Determination of Dextrose in Washed Grain by Dispersion and Lane and Eynon Technique. The method of dispersing starch in hydrochloric acid as described by Rask (*6*) was applied to the washed and dried material obtained in the treatment of the 2-gram sample. The procedure described by Rask (*6*) is followed in detail to the point where the dispersed starch solution has been completely filtered through the sintered-glass crucible.

Table II. Available Dextrose and Fermentation Data on Dent Corn

Detn. No.	Dextrose: By dispersion, %	Dextrose: In washings, %	Dextrose: Total available, %	Dextrose: By acid hydrolysis, %	Fermentation Efficiency: Acid hydrolysis, %	Fermentation Efficiency: Dispersion method, %
			Dent Corn, Grade 1			
1	67.92		71.55			
	67.84	3.63	71.47	80.47	88.71	99.65
2	68.07		71.66		89.12	100.10
	68.00		71.59		88.60	99.52
	68.18	3.59	71.77	80.32		
3	67.85		71.39		88.86	99.81
	67.75		71.29		89.00	99.97
	67.93	3.54	71.47	80.47	88.74	99.68
Av.	67.94	3.59	71.52	80.42	88.84	99.79
Greatest deviation from average	0.35		0.35			
			Dent Corn, Grade 2			
1	66.71		70.62			
	67.19	3.91	71.10	79.36	89.37	100.75
2	66.67		70.65			
	66.47		70.45		89.69	101.11
	66.75	3.98	70.73	80.35	88.82	100.13
3	67.20		71.03		89.23	100.58
	67.12		70.95		88.94	100.26
	67.04	3.83	70.87	80.10	89.51	100.91
Av.	66.89	3.91	70.80	79.94	89.26	100.62
Greatest deviation from average	0.63		0.49			

Table III. Available Dextrose and Fermentation Data on Whole Wheat

Detn. No.	Dextrose: By dispersion, %	Dextrose: In washings, %	Dextrose: Total available, %	Dextrose: By acid hydrolysis, %	Fermentation Efficiency: Acid hydrolysis, %	Fermentation Efficiency: Dispersion method, %
			Soft Winter Wheat			
1	65.76		69.52			
	66.00	3.76	69.76	76.00	90.00	98.45
2	65.64		69.42			
	66.08		69.86		90.09	98.55
	66.00	3.78	69.78	...	90.09	98.55
3	66.36		69.94		89.67	98.08
	66.16		69.74		89.64	98.05
	66.12	3.58	69.70	76.44	89.79	98.22
Av.	66.02	3.70	69.72	76.22	89.88	98.32
Greatest deviation from average	0.58		0.43			
			Hard Winter Wheat			
1	59.01		63.57			
	58.85	4.56	63.41	70.89	91.05	101.80
2	58.67		63.21			
	58.53		63.07		90.08	100.72
	58.39	4.54	62.93	70.89	91.05	101.80
3	59.01		63.59		89.56	100.14
	58.69		63.27		89.01	99.53
	59.01	4.58	63.59	70.89	88.89	99.39
Av.	58.77	4.56	63.33	70.89	89.94	100.56
Greatest deviation from average	0.65		0.63			

The starch solution is then treated as follows, instead of precipitating the pure starch with alcohol.

Three 25-ml. aliquots are pipetted into 250-ml. volumetric flasks, and 50 ml. of 2 *N* sodium hydroxide solution are added to neutralize about two thirds of the dispersing acid. Hydrolysis is effected in a boiling water bath for 1 hour with subsequent cooling to room temperature. The resulting solution is neutralized with 2 *N* sodium hydroxide to the phenolphthalein end point and 10 ml. of saturated neutral lead acetate were added. Distilled water is added to volume at 20° C. and shaken. After standing for a few minutes it is filtered and clarified as in the determination of dextrose in the washings. The total sugar as dextrose is obtained by the Lane and Eynon method (*5*). The combined total of dextrose in the washings and in the washed grain is the total available dextrose shown in the tables. These values are on the "grain as received" basis.

Fermentation of Cereal Grains. Preliminary experiments demonstrated that the best fermentation conditions for milo maize, corn, and wheat are: premalting with 1% malt, based on the weight of grain at 63° to 66° C. for 30 minutes, increasing the temperature slowly to 90° C., holding at 90° to 93° C. for 30 minutes, and autoclaving at 20 pounds steam pressure for 1 hour. The mash is then cooled to 63° C., malted with 7% malt, based on grain weight, for 30 minutes at 63° to 66° C., cooled to 32° C., and seeded with 4% by volume of a grain yeast in malt extract. To prevent infection 0.01% ammonium bifluoride on the mash volume is added to the fermentations. The incubation time is 120 hours at 30° C., after which alcohol is removed by distillation.

The rye samples require different treatment to obtain maximum efficiency. Ten per cent malt and 5% clarase (a commercial product containing diastatic enzymes produced by Takamine Laboratories, Clifton, N. J.) are employed on the grain weight basis and the temperature is slowly raised to 54° C., held for 60 minutes, and then slowly raised to 63° C. and held for 60 minutes at 63° to 66° C. The mash is then cooled to 32° C., and adjusted to a pH of 4.9 with sulfuric acid, and 0.5 ml. of antifoam and 0.01% ammonium bifluoride on the mash volume basis are added. The percentage of inoculum, period of fermentation, and incubation temperature are the same as those employed above. The authors allowed 120 hours for incubation in order to obtain as nearly complete fermentation as possible for comparative purposes.

Calculations of Fermentation Efficiency

$$\% \text{ efficiency} = \frac{\text{total grams of alcohol produced} \times 100}{\text{grams of grain} \times \% \text{ dextrose} \times 0.4829}$$

Suitable corrections are made for the alcohol introduced with the seed yeast. The factor 0.4829 represents grams of alcohol to be expected per gram of dextrose on the basis of the chemical transformation of dextrose to alcohol, corrected for the small loss (about 5%) due to production of yeast cell substance and fermentation by-products. This is the so-called Pasteur factor.

Table IV. Available Dextrose and Fermentation Data on Rye

Detn. No.	Dextrose: By dispersion, %	Dextrose: In washings, %	Dextrose: Total available, %	Dextrose: By acid hydrolysis, %	Fermentation Efficiency: Acid hydrolysis, %	Fermentation Efficiency: Dispersion method, %
			Rye Sample 1			
1	59.86		65.58			
	60.14	5.72	65.86	70.89	90.20	98.99
2	59.38		65.12		90.57	99.38
	59.84	5.74	65.58	72.10	90.80	99.64
	59.64		65.38			
3	59.20		65.06		91.03	99.89
	59.46	5.86	65.32	72.10	91.06	99.83
	59.32		65.18		90.90	99.75
Av.	59.61	5.77	65.38	71.70	90.76	99.60
Greatest deviation from average	0.89		0.73			
			Rye Sample 2			
1	57.99		66.31	71.70	91.36	99.25
	57.87	8.32	66.19		91.79	99.71
2	57.74		66.40	71.51	92.28	100.25
	57.54	8.66	66.20		92.15	100.11
	57.48		66.14		92.15	100.11
3	57.51		65.95			
	57.32	8.44	65.76	71.89	...	...
	57.44		65.88			
Av.	57.61	8.47	66.10	71.70	91.95	99.89
Greatest deviation from average	0.66		0.51			

Table V. Summary of Available Dextrose and Fermentation Data on Cereal Grains

Grain	Dextrose: By dispersion, %	Dextrose: In washings, %	Dextrose: Total available, %	Dextrose: By acid hydrolysis, %	Fermentation Efficiency: Acid hydrolysis, %	Fermentation Efficiency: Dispersion method, %
Dent corn 1 grade	67.94	3.59	71.52	80.42	88.84	99.79
Dent corn 2 grade	66.89	3.91	70.80	79.94	89.26	100.62
Soft winter wheat	66.02	3.70	69.72	76.22	89.88	98.32
Hard winter wheat	58.77	4.56	63.33	70.89	89.94	100.56
Rye sample 1	59.61	5.77	65.38	71.70	90.76	99.60
Rye sample 2	57.61	8.47	66.10	71.70	91.95	99.89
Milo maize sample 1	71.26	2.33	73.59	76.66	96.16	100.55
Milo maize sample 2	71.78	2.94	74.72	77.05	96.92	100.37
Av.						99.96
Barley malt	47.86	13.47	61.33	67.78		

EXPERIMENTAL RESULTS

Table I shows comparative results obtained on two samples of milo maize from different sources. In sample 1 the average total available dextrose is 73.59% and that obtained by acid hydrolysis is 76.66%, a difference of 3.07%. In sample 2 the parallel figures are 74.73 and 77.05%, a difference of 2.32%. Calculations of fermentation efficiency based on the alcohol values obtained (average of 5 fermentations) gave 96.16% based on acid hydrolysis and 100.55% based on the dispersion method in the first sample, and 96.92% as compared with 100.37% in the second sample. In all the samples studied, higher apparent starch content was found with the acid hydrolysis procedure.

In the case of Dent corn two grades were analyzed, Nos. 1 and 2 (Table II). The difference between the dextrose content by the two methods is more pronounced than in milo maize, being 8.90% in the No. 1 grade and 9.14% in the No. 2 grade. The efficiencies by the dispersion method gave 99.79 and 100.62%, respectively, as compared to 88.84 and 89.26% by the acid hydrolysis method.

Two varieties of whole wheat, soft winter and hard winter, were analyzed and the results are shown in Table III. The average differences in dextrose content in the two methods are 6.50% in the soft grade and 7.56% in the hard grade. The average fermentation efficiencies for the soft and hard winter varieties by the acid hydrolysis method were 89.88 and 89.94%, whereas the proposed method gave 98.32 and 100.56%, respectively.

Two samples of rye from different sources, shown as Nos. 1 and 2 (Table IV), were analyzed by the two procedures. Differences of 6.32 and 5.60% dextrose between the two methods were obtained. Again the method discussed gave lower starch content. The average fermentation efficiencies for samples 1 and 2 by the acid hydrolysis method were 90.76 and 91.95%, while the proposed method gave 99.60 and 99.89%, respectively.

Table V summarizes the data on all the grains studied. The method applied to two samples each of Dent corn, wheat, rye, and milo maize gave fermentation efficiencies of the order of 100%. The fermentation efficiencies calculated on the basis of the acid hydrolysis procedure varied considerably: Dent corn 88 to 89%, wheat 89 to 90%, rye 90 to 92%, and milo maize 96 to 97%. Milo maize fermentation efficiencies were the highest of the series

by the acid hydrolysis method. The results indicate that the described method determines only potential yeast fermentable substances.

Critical techniques in the procedure are the draining of each solvent before the addition of the succeeding one in the washing of the grain, the correct concentration of the dispersion hydrochloric acid, and following other details as outlined. The accuracy and reproducibility of the analyses depend on these factors. Under the conditions mentioned, the water-soluble carbohydrates are determined separately from the pure starch which is hydrolyzed mildly in order to exclude dextrins and hydrolyzable materials other than starch. When the method is carefully followed according to described procedure, fermentation efficiencies very close to 100% are obtained. In the analyses of cereal grains these results are superior to those obtained by the acid hydrolysis procedure.

ACKNOWLEDGMENT

The authors wish to express their appreciation to F. M. Hildebrandt for his helpful suggestions during the course of this work.

LITERATURE CITED

(1) Assoc. Offic. Agr. Chemists, "Official and Tentative Methods of Analysis," 6th ed., p. 249, 1945.
(2) *Ibid.*, p. 410.
(3) Clendenning, K. A., *Can. J. Research*, **23B**, 239–59 (1945).
(4) Krocker, *Ann.*, 58, 212–27 (1846).
(5) Lane, J. H., and Eynon, Lewis, "Determination of Reducing Sugars by Fehling's Solution with Methylene Blue Indicator," London, England, Norman Rodger, 1934.
(6) Rask, O. S., *J. Assoc. Offic. Agr. Chemists*, 30, 376–8 (1947).

RECEIVED September 28, 1949. Presented before the Division of Agricultural and Food Chemistry at the 116th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Analysis of Data on Plastics Life Tests

W. L. GORE, *E. I. du Pont de Nemours & Company, Inc., Arlington, N. J.*

Analytical methods of treating data are outlined, which are generally applicable where individual tests may be divided into two categories (good and bad) and where multiple tests may be run, so that the classifications can be expressed as fractions. Analysis of data on life of plastics is illustrated.

PLASTICS generally lose strength and toughness when they are exposed outdoors to sunlight or are heated for any considerable period of time in the presence of oxygen. The variations in resistance to heat and sunlight are large, and modifications in composition or the addition of stabilizers will often result in important changes in the resistance of a given plastic.

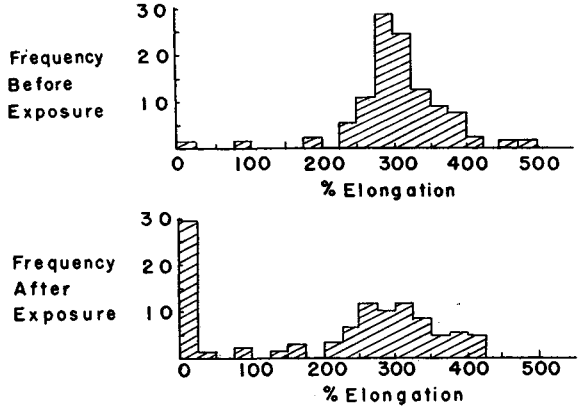


Figure 1. Distribution of Elongation of Nylon
Before and after 3 months' outdoor weathering

Because outdoor and high temperature uses for plastics are important, reliable tests of useful life under various conditions of exposure are required, but a number of difficulties are encountered in making these life tests. Measurements of properties such as tensile strength and elongation are variable and unreliable. Figure 1 shows the frequency distribution of elongation for an extruded nylon wire sample before exposure and after exposure outdoors for 3 months. Evidently the embrittlement occurred in a spotty fashion; in some of the tests results were equivalent to the original average value for the material, whereas others were very low. The samples tested can be divided into two classifications—those that are tough and those that contain a brittle spot somewhere in the test section. It is apparent that this method of classifying the data is the most reasonable one, because the average elongation is likely to be a figure that does not represent any of the actual material and this average is extremely subject to chance fluctuations, depending on whether tough or brittle test lengths are chosen.

A survey of heat embrittlement and outdoor weathering embrittlement data for a number of plastics has shown that the exposure tends to generate random points of embrittlement rather than a continuous and homogeneous decrease in strength and toughness. With this concept of the phenomena of heat and weather degradation of plastics, one is faced with confusion in defining the "failure" point. Should the failure point be defined as the time of exposure that gives a single brittle test? an intermediate fraction of brittle tests? all tests brittle? From a functional point of view, the criterion of failure is the time when the first incipient embrittlement occurs. This exposure time is obviously impractical of measurement—for example, a specification of 1% defective as the failure point requires about 230 tests without a failure to be 90% sure that the material is less

Table I. Mandrel Tests

(50 wraps for each exposure)

Exposure, Min.	f, Fraction Breaking	Δf, Change in Fraction Breaking	d	Δfd	Δfd^2
240	0.00	0.10	−3	−0.30	+0.90
270	0.10	0.22	−2	−0.44	+0.88
300	0.32	0.20	−1	−0.20	+0.20
330	0.52	0.08	0	0	0
360	0.60	0.28	+1	+0.28	+0.28
390	0.88	0.12	+2	+0.24	+0.48
420	1.00			$\Sigma\Delta fd$ −0.42	$\Sigma\Delta fd^2$ 2.74

$\bar{X}$ (average) $= X_o + C(\Sigma\Delta fd) = 345 + 30(-0.42) = 332$ minutes

(Standard deviation) $= C\sqrt{\Sigma\Delta fd^2 - (\Sigma\Delta fd)^2} = 30\sqrt{2.74 - (-0.42)^2} = 48$ minutes

C = time interval between samples (30 minutes)
X_o = working origin (for purposes of calculation)
d = time intervals deviation of exposure time from X_o

than 1% defective, and at least one failure must be found in 53 tests in order to be 90% sure that the true per cent defective is greater than 1%. The same difficulty is encountered if the criterion of failure is chosen as the exposure time that causes 100% (or 99%) failure in test lengths. Therefore, from the standpoint of the practical requirements of measurement, the observations must be made at intermediate percentages of failure and, if necessary, the results extrapolated to very small percentages. According to statistical theory, the most reproducible point for defining failure is the time of exposure to give 50% failures in the test lengths.

TESTS METHODS AND ANALYSIS OF DATA

One method of testing flexible plastics for embrittlement from heat or ultraviolet exposure consists in wrapping a strip of extruded wire around a steel mandrel of approximately the same diameter as the plastic wire. The unexposed sample of wire can be wrapped around the mandrel without breaking, but after continued exposure to degrading conditions, brittle spots begin to appear until the material is so embrittled that no portion of the exposed wire can be bent around the mandrel without breaking.

A sample of a nylon in the form of extruded wire 0.045 inch in diameter was exposed in a 200° C. air oven and tested at 30-minute intervals for embrittlement by wrapping it around a 0.060-inch steel mandrel. The results of this testing are shown in Table I and Figure 2.

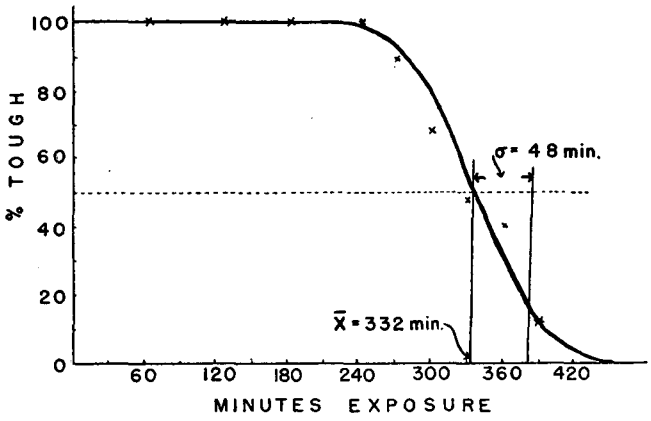


Figure 2. Results of Mandrel Test of Nylon

A temperature of 200° C. is extremely high for any organic material to withstand in the presence of oxygen and only polytetrafluoroethylene is practical for use at such a high temperature. Most thermoplastics lose their useful strength properties at 100° C. or only a little above. Accelerated heat aging tests of nylons have shown little correlation with their performance at lower temperatures, and therefore it appears that new degradation mechanisms may be initiated at the higher temperatures.

The calculations outlined in Table I show that the time of exposure giving 50% embrittlement, as measured by the mandrel wrap test, is 332 minutes, and that the variation in fraction of wraps failing the test gives a calculated standard deviation of 48 minutes. Alternative methods of treating such data have also been suggested (*2, 4–6*). A number of questions present themselves in regard to this test:

1. What is the estimated time of exposure of nylon at 200° C. in air to give incipient embrittlement? How long must the exposure be continued to give complete embrittlement?
2. How reliable are the estimates of exposure time to give 50% embrittlement and of the variation in fraction brittle with exposure time?

The answers to the first question require an assumption regarding the form of the distribution for degree of embrittlement with time of exposure. The author's data with nylon heat and light degradation do not discredit the hypothesis that these distributions are in accordance with the law of random errors (the Gaussian or normal distribution), although no theoretical reasoning is known which supports this hypothesis. However, a good enough representation of observed data is obtained to permit practical problems to be solved, and it is then possible to calculate the times of exposure that give incipient and complete embrittlement. Tables of the integrals of the normal distribution curve (*3*) give the fraction of the distribution relative to the deviation of the measured variable (time of exposure) in standard deviation units. Thus, from such a table, approximately 2.2% embrittlement is expected at 2 standard deviation units less exposure than that which gives 50% embrittlement. Two standard deviations ($2 \times 48 = 96$ minutes) less than 332 minutes are equal to 236 minutes' exposure and this might be given as an estimate of the safe exposure time of nylon at 200° C. in air before embrittlement to bending will occur.

The questions as to the reliability of the average time, $\bar{X}$, and the standard deviation, σ, are answered for practical purposes by the application of standard methods of estimating errors for statistics calculated from random samples of a normally distributed continuous variate. [Epstein and Churchman (*1*) give the precise errors involved. The treatment outlined here leads to slightly greater estimates of the errors than is correct.] The 95% assurance limits for the calculated average are given as

$$\bar{X} = 332 \pm \frac{2\sigma}{\sqrt{N}} = 332 \pm \frac{2 \times 48}{\sqrt{50}} = 332 \pm 13.6 \text{ minutes}$$

and for the calculated standard deviation as

$$\bar{X} = 48 \pm \frac{2\sigma}{\sqrt{2N}} = 48 \pm \frac{2 \times 48}{\sqrt{100}} = 48 \pm 9.6 \text{ minutes}$$

where N is the number of independent tests made after each exposure period.

When a sufficient amount of testing has been done with a given type of plastic so that the value of the standard deviation is known with some confidence, it is possible to simplify the test procedure by making only one test at such an exposure time that an intermediate fraction defective is obtained, preferably in the range between 0.20 and 0.80. This may be accomplished by making tests at exposure intervals of about every two standard deviations. With the test of nylon recorded in Table I, the exposure periods could be increased from 30 minutes to 90 or 100 minutes. (About 3 tests would have been required at 100-minute intervals as compared to the 14 which were made at 30-minute test intervals.)

The use of this simplified test procedure again involves the assumption of normality of distribution and the use of a table of integrals of the normal error function. If f_o symbolizes the fraction breaking after a time of exposure X_o, and the standard deviation may be assumed to be equal to a given value, σ, the value t_o corresponding to f_o may be determined from the normal integral table where t_o is the number of standard deviation units by which X_o, the exposure time, differs from $\bar{X}$, the exposure time giving 50% of the tests breaking. $\bar{X}$ is then found from

$$\bar{X} = X_o - \sigma t_o$$

The error of $\bar{X}$ can be estimated by calculating the 95% assurance limits by the formula

$$\bar{X} \pm 2\left(\frac{1.25\sigma}{\sqrt{N}}\right)$$

This estimation is correct only when the test is made at the 50% exposure period and becomes somewhat larger at other

exposure periods. It is believed to be practical for this use, however.

If the test is made at an exposure period which gives close to 50% defective tests, the t_0 term is very small and therefore the value used for σ, the standard deviation, is of little importance in estimating $\bar{X}$.

APPLICATION OF ANALYSIS

The analytical methods of treating data outlined here are generally applicable where the degradation can be measured by a classification of individual tests into two categories (good and bad) and where it is possible to run multiple tests so that the classifications can be expressed as a fraction. Fewer than 20 or 25 trials to an evaluation usually give results too unreliable to be of much use, and 50 to 100 trials are desirable.

Tensile strength, elongation, and bending brittleness data have been analyzed by the foregoing schemes. Exposures have been made outdoors and under ultraviolet from carbon arc lamps, at elevated temperatures in air and under oxygen pressure, and at very high temperatures in the absence of oxygen where pyrolysis was the cause of degradation. The advantages of the use of an intermediate degree of degradation as the criterion to be measured are the greater precision with which these points can be measured as compared to the points of incipient or complete embrittlement and the methods required for the measurement of intermediate degradation also permit an estimate of the reliability of results. Such an estimate is to a large degree lacking in data collected to measure very small or very large fractions of degradation.

LITERATURE CITED

(1) Epstein, Benjamin, and Churchman, C. W., *Ann. Math. Stat.*, **15**, No.1, 90–6 (March 1944).
(2) Finney, D. J., "Probit Analysis, A Statistical Treatment of the Sigmoid Response Curve," London and New York, Cambridge University Press, 1947; Berkson, Joseph, *J. Am. Statistical Assoc.*, **45**, 148–53 (1948).
(3) Hodgman, C. D., ed., "Handbook of Chemistry and Physics," Akron, Ohio, Chemical Rubber Publishing Co.
(4) Knudsen, L. F., and Curtis, J. M., *J. Am. Statistical Assoc.*, **42**, 282–96 (1947).
(5) Litchfield, J. T., and Wilcoxon, F., *J. Pharmacol. Exptl. Therap.*, **96**, 99–113 (1949).
(6) Miller, L. C., *Ann. N. Y. Acad. Sci.*, in press.

RECEIVED August 6, 1949. Presented before the Division of Paint, Varnish, and Plastics Chemistry at the 116th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Analytical Applications of 8-Hydroxyquinoline Derivatives of Gallium and Thallium

THERALD MOELLER AND ALVIN J. COHEN[1], *University of Illinois, Urbana, Ill.*

Precipitation of gallium and thallium(III) from aqueous solutions with 8-hydroxyquinoline is quantitative at pH values above 3.1 and 3.8, respectively, and provides bases for procedures for the gravimetric determination of these cations. Precipitation of thallium(I) with this reagent is incomplete even under optimum conditions. The 8-hydroxyquinoline derivatives of all three ions dissolve readily in chloroform to yellow solutions which undergo photochemical decomposition, at a rate that increases in the order gallium–thallium(III)–thallium(I). Spectrophotometric studies on chloroform solutions over the range 300 to 1000 mμ indicate each compound to be characterized by broad absorption bands in the ranges 320 to 328, 335 to 338, and 392.5 to 402 mμ. Absorption bands in the last range obey Beer's law and are free from interference by 8-hydroxyquinoline. Both gallium and thallium(III) may be determined spectrophotometrically as 8-hydroxyquinoline derivatives, but the photochemical sensitivities of thallium(I) solutions preclude use for quantitative work. Gallium can be extracted completely from acetate-buffered (pH 3.0 to 6.2) salt solutions by shaking with chloroform solutions of 8-hydroxyquinoline, but extraction of thallium(III) is never complete. Combination of extraction and spectrophotometric techniques permits determination of gallium in microgram quantities.

THE suitability of 8-hydroxyquinoline (oxine) as a reagent for the gravimetric and colorimetric determination of aluminum (*5*, *10*, *12*) and indium (*4*, *9*, *14*) has been established. The reagent has also been adapted to the gravimetric determination of gallium (*4*), and in a paper which appeared shortly after very similar work had been completed by the authors (*11*), Feigl and Baumfeld (*3*) discussed its use for the gravimetric determination of thallium(III).

The yellow gallium, thallium(III), and thallium(I) derivatives of 8-hydroxyquinoline—i.e., oxinates—dissolve readily in solvents such as chloroform. Sandell (*15*, *16*) has shown that chloroform solutions of the gallium compound give a detectable yellow fluorescence when as little as 0.1 microgram of the metal is present in a 10-ml. volume. A fluorometric method for gallium based upon examination of solutions prepared by extracting the oxinate into chloroform has resulted. Lacroix (*7*) has extended Sandell's method to the determination of 1.4 to 4.28 microgram quantities of gallium in bauxites. Feigl and Baumfeld (*3*) employed the yellow color of thallium(III) oxinate in chloroform to indicate the presence of thallium.

The colorimetric characteristics of nonaqueous solutions of the 8-hydroxyquinoline derivatives of gallium and thallium have escaped consideration. The success which has attended use of the oxinates of aluminum (*5*, *10*, *12*) and indium (*9*) for the colorimetric determination of these elements suggests the development of similar procedures for gallium and thallium.

APPARATUS AND MATERIALS

All absorption spectra measurements reported were made with a Beckman Model DU quartz spectrophotometer, using 5-cm. cells with quartz windows. A Beckman Model H pH meter, the glass electrode of which was calibrated frequently against potassium hydrogen tartrate buffers, was used for all pH measurements.

Gallium metal (obtained from The Eagle-Picher Company,

[1] Present address, Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, Calif.

Joplin, Mo.), containing only spectroscopic traces of other elements, was converted to the sulfate by digestion with nitric and sulfuric acids, followed by crystallization. Solutions prepared from the crystalline sulfate and standardized by the gravimetric 8-hydroxyquinoline procedure (*4*) were used as sources of gallium ion. Solutions containing thallium(III) were obtained by dissolving weighed quantities of dried (150° C., 1.25 hours), chemically pure thallium(III) oxide in sulfuric acid. Ordinary chemically pure thallium(I) sulfate was used without further purification as a source of thallium(I) ion. Chloroform used was of analytical reagent quality and contained 0.75% ethyl alcohol by volume as a preservative. The 8-hydroxyquinoline employed was an Eastman product. All other chemicals were of analytical reagent quality. To avoid interferences by trace amounts of metal ions, aqueous solutions used in all colorimetric work were prepared with conductivity water, and correcting blanks were run on the reagents.

PRECIPITATION OF 8-HYDROXYQUINOLINE DERIVATIVES

Measured volumes of standard gallium sulfate–sulfuric acid solutions (pH 1.81) were precipitated at 75° C. by addition of slight excesses of 5% solutions of 8-hydroxyquinoline in either ethyl alcohol or acetic acid, followed by dropwise addition of 5 *N* aqueous ammonia. The first permanent precipitate was noted at an average pH of 2.82. Although precipitation was shown analytically to be complete at an average pH of 3.10, the suspensions were adjusted to pH 7.0 to 7.5 to provide an ample factor of safety in the reaction. These suspensions were digested for 30 minutes on the steam bath, cooled, and filtered through weighed sintered-glass crucibles. The precipitates were weighed after being washed with 20 ml. of hot water, dried for 2 hours at 120°, and cooled. Negative deviations of 1.4 to 4.4 mg. in 167 to 196 mg. of the oxinate demonstrated the quantitative nature of the precipitation of gallium by this modification of the Geilmann and Wrigge procedure (*4*). A sample of the compound prepared by this procedure for spectrophotometric studies was a yellow, microcrystalline solid containing 13.76% gallium [calculated for $Ga(C_9H_6NO)_3$, 13.88%].

Thallium(III) oxinate was prepared by essentially the same procedure (*2*), except that both precipitation and digestion were carried out at room temperature. Best results were obtained with solutions containing about 10 ml. of water to each milligram of thallium to be precipitated. Upon addition of ammonia, the first permanent precipitate appeared at average pH 3.0, and precipitation was analytically complete at average pH 3.7 to 3.8. Final adjustment of pH to 4 to 8, followed by digesting for 2 hours at 20° to 30° C., washing with hot water, drying at 120° C. for 1.5 to 2 hours, and weighing, showed precipitation to be quantitative.

Representative samples of the yellow, crystalline product darkened at 167° C. and melted with decomposition over the range 167–169° (uncorrected). The comparatively low melting point (corresponding anhydrous oxinates of gallium and indium melt above 287° C.) suggests the presence of water in the dried product. On the basis of thallium contents determined by the iodide procedure, Feigl and Baumfeld (*3*) reported that drying at 100° C. yielded a trihydrate and that at 110° to 120° C. not all combined water could be removed without decomposition of the compound. The authors have observed no decomposition of the compound at 120° C. Their own average analyses of thallium 31.20 [by method of Spacu and Pop (*17*)], carbon 50.29, nitrogen 6.57, and hydrogen 3.14, when compared with thallium 32.09, carbon 50.92, nitrogen 6.60, and hydrogen 2.85 as calculated for anhydrous $Tl(C_9H_6NO)_3$, and thallium 31.21, carbon 49.52, nitrogen 6.42, and hydrogen 3.08 as calculated for a 1-hydrate, suggest the presence of some hydrate water. However, because thallium determinations carried out on pure salt solutions by the same procedure (*17*) were always low, the measured thallium content is a poor criterion of true composition. The other values are interpreted, therefore, as indicating the presence of only slight amounts of water after drying at 120° C., and the composition of the dried product is closer to that of the anhydrous compound than to that of a monohydrate as suggested by Feigl and Baumfeld.

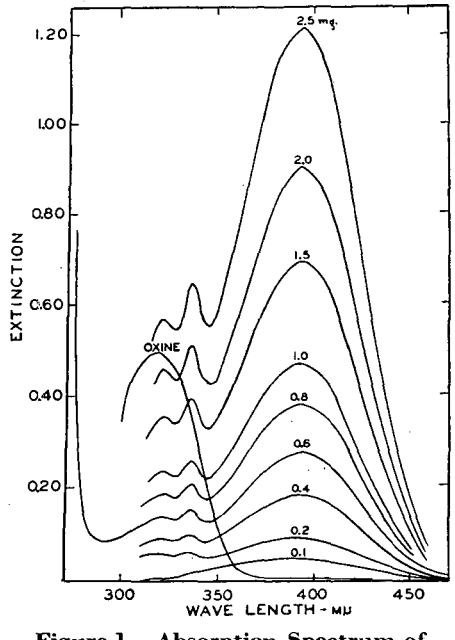


Figure 1. Absorption Spectrum of Gallium Oxinate in Chloroform

Use of 8-hydroxyquinoline under the conditions outlined was recommended independently (*11*) for the gravimetric determination of thallium(III). Excellent precision with an average error of about −0.8% was obtained over a range of 1 to 200 mg. of thallium. The precipitate is characterized by a favorable factor, and the method possesses greater inherent accuracy than the chromate procedure recommended from a comparative study by Chrétien and Longi (*1*).

Thallium(I) oxinate was prepared by a modification of the method of Rey (*13*). Optimum conditions involved adding a boiling solution containing 1 gram of thallium [as thallium(I) sulfate] and 2 ml. of 15 *N* aqueous ammonia in 48 ml. of water to 100 ml. of a boiling 3 *N* aqueous ammonia solution containing 0.711 gram of 8-hydroxyquinoline. The finely divided, yellow crystalline precipitate, which formed immediately, was removed (after cooling) by filtering through sintered glass, washed with hot water, and dried at 120° C. for 2.5 hours. The product darkened at 219° C. and melted with decomposition at 220° C. (uncorrected). The experimentally determined composition (*17*) of thallium 56.42, carbon 32.38, nitrogen 4.15, and hydrogen 1.87 is to be compared with thallium 58.64, carbon 31.01, nitrogen 4.02, and hydrogen 1.74 calculated for $Tl(C_9H_6NO)$. Based upon this composition, the yield was 87%. Because modification of the procedure failed to improve the yield or precipitate all the thallium(I) taken, this compound is not suited to the gravimetric determination of thallium.

SPECTROPHOTOMETRIC EXAMINATION OF CHLOROFORM SOLUTIONS OF GALLIUM AND THALLIUM DERIVATIVES OF 8-HYDROXYQUINOLINE

A stock chloroform solution of gallium oxinate, containing the equivalent of 1000 mg. of gallium per liter, was prepared from a weighed quantity of the compound. A series of chloroform solutions of varying gallium contents prepared by appropriate dilution of this stock solution was then examined spectrophotometrically over the range 310 to 600 mμ.

The spectra of certain of these solutions were measured to 1000 mμ, while the spectrum of the solution containing the equivalent of 0.6 mg. of gallium per liter was extended down to 252 mμ. A very intense absorption band was found in the region 254 to 268 mμ. However, this band is too close to the intense 251 mμ band of 8-hydroxyquinoline to permit its use for analytical purposes. At wave lengths greater than 460 mμ, no characteristic absorption was noted. As a consequence, the major portion of the representative data summarized in Figure 1 embraces only the 310 to 460 mμ region. Included in this figure are comparative data for a chloroform solution containing 6.203 mg. of 8-hydroxyquinoline (equivalent to 1 mg. of gallium) per liter. The intensity of the absorption at 392.5 mμ limited meas-

urements to solutions containing the equivalent of not more than 2.5 mg. of gallium per liter.

Characteristic absorption bands are apparent at 392.5, 335, and 320 mμ, displacements of these bands with concentration changes being negligible. The 392.5 mμ band corresponds closely to the 395 mμ bands reported previously for aluminum (*10*) and indium (*9*) oxinates. Although the 320 mμ band corresponds almost exactly to the 318 mμ band of 8-hydroxyquinoline, it is characterized by an average equivalent extinction coefficient of 1.16×10^3 as compared with a value of 2.34×10^3 for 8-hydroxyquinoline and cannot be due to the reagent as such. Inasmuch as the broad oxine band centering at 318 mμ would markedly affect both the 320 and 335 mμ bands if even a small amount of 8-hydroxyquinoline were present in solution with the gallium compound, only the 392.5 mμ band is of potential analytical significance. As shown in Figure 1, this band is free from interference by oxine.

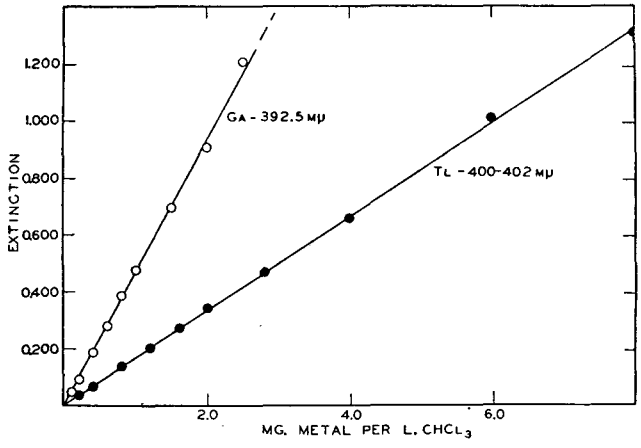


Figure 2. Beer's Law Relationships for Oxinates of Gallium and Thallium(III)

Data characterizing the concentration dependence of this absorption band are summarized in Table I. Values of the absorption coefficient—i.e., the k of the Beer-Lambert relation $\log I_0/I = kcl$—average to 92.8 when the concentration, c, is expressed in grams of gallium per liter and the light path, l, is 5 cm. Calculated molecular extinction coefficients average to 6.47×10^3, from which the average equivalent extinction coefficient is 2.16×10^3. This adherence of the absorption data to Beer's law is shown graphically in Figure 2. Greatest accuracy can be expected with the Beckman instrument at this wave length in the range 0.4 to 1.8 mg. of gallium per liter of chloroform. The lower limit of detection of gallium by this means is of the order of 5×10^{-6} gram.

Chloroform solutions of gallium oxinate undergo slow photochemical decomposition, especially when exposed to bright sunlight. However, they yield reproducible absorption spectra over periods of several hours in the subdued light of the laboratory.

Table I. Absorption Spectra Data for Gallium Oxinate in Chloroform at 392.5 Mμ

Concentration, Mg. Ga/L. $CHCl_3$	Extinction	Absorption Coefficient[a]	Molecular Extinction Coefficient ($\times 10^{-3}$)
0.10	0.046	92.0	6.41
0.20	0.090	90.0	6.28
0.25	0.118	94.4	6.58
0.30	0.131	87.3	6.09
0.40	0.184	92.0	6.41
0.50	0.215	86.0	6.00
0.60	0.277	92.3	6.44
0.70	0.343	98.0	6.83
0.80	0.382	95.5	6.66
0.90	0.438	97.3	6.78
1.00	0.471	94.2	6.57
1.50	0.695	92.7	6.46
2.00	0.905	90.5	6.31
2.50	1.21	96.8	6.75
		Av. 92.8	6.47

[a] Calculated for gallium concentrations in grams per liter and for 5-cm. cells.

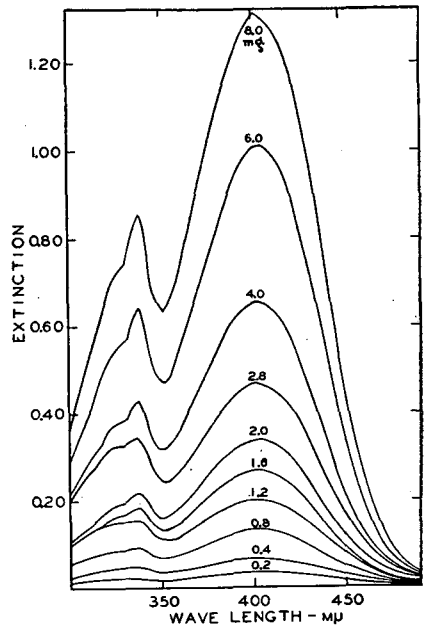


Figure 3. Absorption Spectrum of Thallium(III) Oxinate in Chloroform

Similar spectrophotometric studies were made upon chloroform solutions of thallium(III) oxinate prepared by appropriate dilution of a stock solution containing the equivalent of 200 mg. of thallium per liter. As indicated in Figure 3, the spectra of such solutions resemble those of gallium oxinate solutions very closely. Characteristic absorption bands center at 328, 338, and 400 mμ, and no other bands were found in the region 300 to 1000 mμ. Because the wave length of maximum absorption at the third band changes slightly with concentration, this band is best described by the wave-length range 400 to 402 mμ. Experimentally, the wave length should be adjusted to give maximum absorption in this region. As with gallium, this is the only band potentially useful for analytical purposes.

Numerical data characterizing absorption at 400 to 402 mμ are summarized in Table II, the average molecular and specific extinction coefficients being 6.79×10^3 and 2.26×10^3, respectively. This adherence to Beer's law is shown graphically in Figure 2. Optimum thallium concentrations for best results with the Beckman instrument would embrace the range 1.2 to 5.0 mg. per liter of chloroform, the minimum quantity of thallium detectable being of the order of 1×10^{-6} gram. Feigl and Baumfeld (*3*) report the visible detection of 5 micrograms of thallium as oxinate in chloroform solution.

Chloroform solutions of thallium(III) oxinate undergo more rapid photochemical decomposition than those of the gallium compound. However, reproducible absorption spectra were obtained when solutions were prepared under red light, protected from the light of the laboratory, and examined within 4 hours after preparation.

Spectrophotometric studies on chloroform solutions of thallium (I) oxinate were limited by the extreme thermal and photochemical sensitivities of these solutions. These solutions are char-

Table II. Absorption Spectra Data for Thallium(III) Oxinate in Chloroform at 400 to 402 Mμ

Concentration, Mg. Tl/L. $CHCl_3$	Extinction	Absorption Coefficient[a]	Molecular Extinction Coefficient ($\times 10^{-3}$)
0.20	0.033	33.0	6.75
0.40	0.064	32.0	6.55
0.80	0.134	33.5	6.85
1.20	0.199	33.2	6.79
1.60	0.269	33.6	6.87
2.00	0.339	33.9	6.93
2.80	0.467	33.3	6.81
4.00	0.655	32.8	6.70
6.00	1.01	33.7	6.89
8.00	1.31	32.5	6.64
		Av. 33.2	6.79

[a] Calculated for thallium concentrations in grams per liter and for 5-cm. cells.

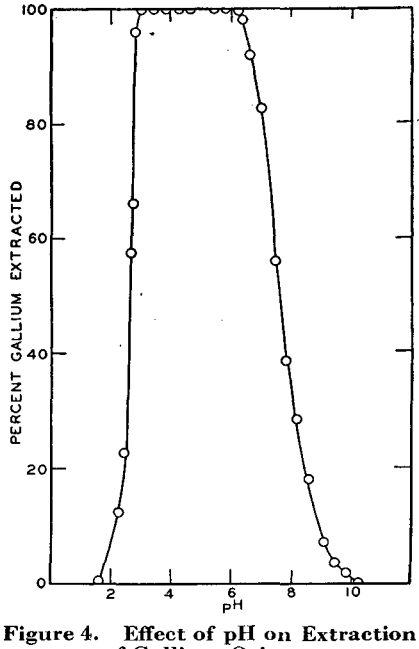


Figure 4. Effect of pH on Extraction of Gallium Oxinate

acterized by four absorption bands centering at 310, 325, 338, and 395 mμ, respectively, over the spectral region 300 to 1000 mμ. The band at 310 mμ may be due to partial decomposition of the compound. The others are comparable with those for gallium and thallium(III) oxinates. Although absorption at 395 mμ follows Beer's law, strict adherence to this relation is difficult to achieve because of decomposition. Reproducible results were obtained only when solutions were prepared under a red photographic safe-light by dissolving the oxinate in chloroform at 0° C. and examined within 5 minutes after they had warmed to room temperature. The absorption characteristics of such solutions are of no analytical importance.

EXTRACTION OF GALLIUM AND THALLIUM(III) INTO CHLOROFORM AS OXINATES

Sandell (*15*, *16*) reported complete extraction of gallium into chloroform as oxinate in the pH range 2.6 to 3.0, but gave no supporting data. Lacroix (*6–8*) calculated extraction to be complete above pH 2 and suggested graphically that complete removal of gallium from the aqueous layer might be expected at any pH above 2. However, this calculated curve was verified by only three experimental points, all at low pH values. No extraction data are available for thallium(III) oxinate.

A comprehensive study of the extraction characteristics of gallium and thallium(III) as oxinates was carried out. The procedure following amounted to adjusting the pH of a 25-ml. aqueous sulfate solution (4 to 5 mg. of the metal per liter) to an appropriate value, shaking with four times the equivalent of 8-hydroxyquinoline as four successive 5- to 10-ml. portions of a chloroform solution, diluting the combined extracts to 25 to 50 ml. with chloroform, and determining the gallium or thallium content of the extract spectrophotometrically, using the 392.5 mμ and 400 to 402 mμ bands and the working curves in Figure 2. Blanks were run at each pH to correct for extraction of traces of impurities from the reagents, and the thallium(III) extracts were protected from light. Values of pH up to 4.6 were obtained by adding powdered sodium acetate. Higher pH values were obtained by buffering to pH 4.6 with sodium acetate and adding 3 to 5 *N* sodium hydroxide. Entry of oxine into the aqueous layer raised its pH after extraction 0.2 to 0.4 unit.

As shown in Figure 4, extraction of the gallium compound begins at slightly below pH 1.8 but is complete only at pH values of 3.0 or above. Complete extraction is obtained over the pH range 3.0 to 6.2, the lack of completeness at higher pH values characterizing the oxinates of other metals (*9*, *10*) being apparent. Corresponding extraction pH values for aluminum and indium are 4.2 and 3.2, respectively (*9*, *10*).

Appreciable extraction of thallium(III) was obtained only above pH 6.0, with maximum extraction being limited to the rather narrow pH interval 6.5 to 7.0. Even in this range, only incomplete removal of thallium from the aqueous layer was effected by the four equivalents of 8-hydroxyquinoline found adequate for aluminum (*10*), gallium, and indium (*9*). Only 86 to 89% of the thallium present was extracted when 15 to 50 times the calculated quantity of 8-hydroxyquinoline was used. It must be concluded that acetate-buffered solutions are not suited to the recovery of thallium(III) by this means. Inasmuch as the precipitation of hydrous thallium(III) oxide is also markedly inhibited by acetate ion, the presence of a comparatively stable acetothallium complex is suggested. Since uncomplexed thallium(III) can exist only in solutions of such low pH that oxine itself would be removed into the aqueous layer, the presence of some complex of intermediate stability would appear essential to the quantitative extraction of the material by this procedure. Such a complex has not been found.

SPECTROPHOTOMETRIC DETERMINATION OF GALLIUM AND THALLIUM(III)

Like aluminum (*10*) and indium (*9*), gallium can be determined colorimetrically by extracting its oxinate into chloroform at controlled pH and examining the extract spectrophotometrically. Although this procedure offers excellent promise for the determination of gallium in trace quantities, it is nonspecific to the extent that many other cations are also extracted and give oxinates with very similar absorption spectra. Ions such as aluminum, indium, thallium(III), tin(II), copper(II), bismuth, iron(II or III), nickel, and cobalt extract to greater or lesser extents at pH values of 3.0 or above (*10*). Determination of gallium in the presence of any of these would be possible only by careful pH control and then only if the amount extracted at pH 3 is very small. On the other hand, ions such as magnesium, calcium, strontium, barium, zinc, cadmium, mercury(II), tin(IV), lead, manganese(II), chromium(III), and silver(I) are not extracted appreciably at pH values below 3.5 (*10*); so a sufficient margin of safety exists. The situation with gallium closely parallels that with indium (*9*). In the absence of interferences, gallium may be determined with an absolute error of less than 1% in quantities ranging from 0.4 to 1.8 mg. per liter of chloroform.

Spectrophotometric determination of thallium(III) as oxinate can be effected by precipitating the compound, dissolving in chloroform, and examining the chloroform solution at 400 to 402 mμ. Quantitative results may be expected with somewhat less than milligram quantities of thallium per liter of chloroform.

However, any cation forming an insoluble oxinate at pH values of 4 or above will interfere, and the procedure is less clean-cut than that for gallium.

ACKNOWLEDGMENT

The authors wish to express sincere appreciation to the Office of Naval Research for the support which rendered this investigation possible.

LITERATURE CITED

(1) Chrétien, A., and Longi, Y., *Bull. soc. chim.*, **11**, 245 (1944).
(2) Feigl, F., *Nature*, **161**, 436 (1948).
(3) Feigl, F., and Baumfeld, L., *Anal. Chim. Acta*, **3**, 83 (1949).
(4) Geilmann, W., and Wrigge, F. W., *Z. anorg. allgem. Chem.*, **209**, 129 (1932).
(5) Gentry, C. H. R., and Sherrington, L. G., *Analyst*, **71**, 432 (1946).
(6) Lacroix, S., *Anal. Chim. Acta*, **1**, 260 (1947).
(7) *Ibid.*, **2**, 167 (1948).
(8) Lacroix, S., dissertation, University of Paris, 1948.
(9) Moeller, T., IND. ENG. CHEM., ANAL. ED., **15**, 270 (1943).
(10) *Ibid.*, p. 346.
(11) Moeller, T., and Cohen, A. J., *Bimonthly Report* **6**, **NR 052060, N6ori-71, T. O. XVII**, University of Illinois, April 1 to June 1, 1948.
(12) Parks, T. D., and Lykken, L., ANAL. CHEM., **20**, 1102 (1948).
(13) Rey, A. B., *Ion*, **7**, 389 (1947).
(14) Royer, G. L., IND. ENG. CHEM., ANAL. ED., **12**, 439 (1940).
(15) Sandell, E. B., ANAL. CHEM., **19**, 63 (1947).
(16) Sandell, E. B., IND. ENG. CHEM., ANAL. ED., **13**, 844 (1941).
(17) Spacu, G., and Pop, A., *Z. anal. Chem.*, **120**, 322 (1940).

RECEIVED April 12, 1949. Abstracted from papers presented at the 114th Meeting of the AMERICAN CHEMICAL SOCIETY, Portland, Ore., and at the 115th Meeting of the AMERICAN CHEMICAL SOCIETY, San Francisco, Calif.

Infrared Spectrometric Determination of Deuterium Oxide in Water

VERNON THORNTON AND F. E. CONDON
Phillips Petroleum Company, Bartlesville, Okla.

The deuterium oxide content of deuterium oxide–water mixtures containing 3 to 100% deuterium oxide is determined with a precision of 1 part in 100 from the absorbance of the 3.98-micron deuterium oxide band in the infrared absorption spectrum of a sample diluted with water so that the deuterium oxide concentration is in a measurable range. The per cent deuterium oxide is read from a calibration curve. The time required for each determination is approximately 10 minutes.

IN A continuation of a study of spectra and structure (*2*), acetone-d_6 was prepared from acetone by repeated deuterium-hydrogen exchange with 99.8% deuterium oxide in the presence of potassium carbonate as catalyst (*1*). Progress of the exchange reaction in its intermediate stages was followed by determining the deuterium oxide content of the water remaining after removal of the acetone-d_x by fractional distillation. This determination was accomplished by measuring the intensity of the 3.98-micron deuterium oxide band in the infrared absorption spectrum of a sample diluted with water so that the deuterium oxide content was about 3%. The deuterium oxide content of the diluted sample was read from a calibration curve prepared by determining the absorbances of synthetic blends of water and 99.8% deuterium oxide. The deuterium oxide content of the sample before dilution was then simply calculated from the weights of the sample before and after dilution and the measured deuterium oxide content of the diluted sample.

Samples for calibration and for analysis were prepared in a 10-ml. glass-stoppered graduated cylinder or mixing bottle. About 0.1 to 0.5 gram of 99.8% deuterium oxide or of unknown was transferred to the tared cylinder by means of a medicine dropper, care being taken to avoid prolonged exposure of the material to atmospheric moisture, and weighed to the nearest 0.1 mg. Enough distilled water was added to bring the deuterium oxide content of the mixture to 0 to 5% (preferably about 3% for unknowns) and its weight was obtained. The contents of the cylinder were then mixed, first by rotating the cylinder in an inclined position so as to avoid contact with the ground-glass surfaces, and finally by inversion of the cylinder a number of times. About 0.5 gram of diluted sample is required for use in filling the infrared absorption cell.

The absorbance of the diluted sample in a calcium fluoride cell, 0.043 mm. thick, was determined with a Perkin-Elmer Model 12B infrared spectrometer with a sodium chloride prism, GM amplifier, and Speedomax G recorder. With the wave-length drive set at 3.98 microns and the recorder in motion, a record was made by the "shutter in–shutter out" technique, from which the absorbance was computed in the customary manner. The absorbance of water in the same cell was redetermined at the time of each analysis as a check of the calibration, and, if necessary, a correction was applied to the absorbance of the unknown. The time required for each determination was approximately 10 minutes.

Figure 1 shows the calibration curve obtained in this work. From these data, it was estimated that 0 to 3% deuterium oxide

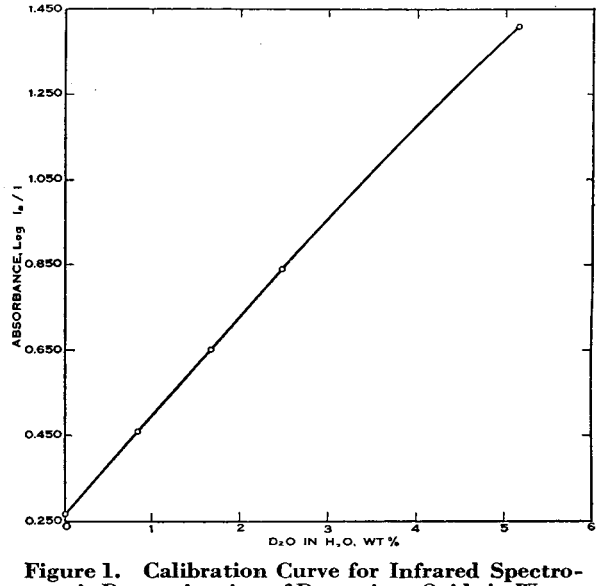


Figure 1. Calibration Curve for Infrared Spectrometric Determination of Deuterium Oxide in Water

in water can be determined in this way with a precision of 0.03 percentage unit, and that larger concentrations can be determined with a precision of 0.03 to 1 percentage unit, depending on the dilution necessary to bring the deuterium oxide content to within the measurable range (about 3% for greatest precision of measurement of the absorbance in a 0.043-mm. cell).

LITERATURE CITED

(1) Halford, J. O., Anderson, L. C., Bates, J. R., and Swisher, R. D., *J. Am. Chem. Soc.*, 57, 1663 (1935).
(2) McMurry, H. L., Thornton, V., and Condon, F. E., *J. Chem. Phys.*, 17, 918 (1949).

RECEIVED November 18, 1949. Report 774-49R, Phillips Petroleum Company.

Ferrate Oxidimetry

Oxidation of Arsenite with Potassium Ferrate(VI)

J. M. SCHREYER, G. W. THOMPSON[1], AND L. T. OCKERMAN
University of Kentucky, Lexington, Ky.

Methods of analyzing potassium ferrate(VI) are described. The methods are based upon the oxidizing property of the ferrate(VI) ion and the determination of the total iron present in the compound.

THE availability of potassium ferrate(VI) in a state of high purity (*4*) and its potentiality as a new and powerful oxidizing agent indicated the necessity of developing methods of analysis for the compound.

The impurities usually present in samples of potassium ferrate (VI) are potassium chloride and hydrous ferric oxide. For the analysis of impure as well as highly purified samples, use of the oxidizing property of the ferrate(VI) ion seemed the most logical approach for the development of suitable methods.

De Mollins (*3*) reported the quantitative reduction of the ferrate(VI) ion by means of the iodide ion in acid solution.

$$2FeO_4^{--} + 8I^- + 16H^+ \longrightarrow 2Fe^{++} + 8H_2O + 4I_2$$

In view of the expected reduction of any ferric ions present as an impurity and the instability of the ferrate(VI) ion in acid solution, it appeared impossible that this reaction would be a quantitative measure of the ferrate(VI) ion present in a sample.

The method developed in this laboratory makes use of the increased stability of the ferrate(VI) ion in strongly alkaline solution and is based upon the reduction of the ferrate(VI) ion to ferric ion in alkaline arsenite solution. A weighed sample of potassium ferrate(VI) is added to a standard alkaline arsenite solution containing a quantity of arsenite in excess of that required for the reduction of the ferrate(VI) ion. The excess arsenite is back-titrated with standard bromate or standard cerate solution. The following equation represents the chemical reaction upon which the method is based:

$$2FeO_4^{--} + 3AsO_3^{---} + 11H_2O \longrightarrow 2Fe(OH)_3(H_2O)_3 + 3AsO_4^{---} + 4OH^-$$

For confirmation by an independent method, the samples under investigation are analyzed to determine the total iron present in the compound. The hydrous ferric oxide impurity is removed by solution of the sample in sodium hydroxide solution and subsequent filtration. After the filtrate is acidified, the ferric ions are reduced to the ferrous state and titrated with a standard cerate solution.

DEVELOPMENT OF METHODS

Preliminary studies showed increased stability of the ferrate (VI) ion in alkaline solutions. Investigations were undertaken to determine the optimum alkalinities of arsenite solutions used in the determination of potassium ferrate(VI) in a sample. The alkalinities of the solutions were varied between approximately neutral and 10 molar in sodium hydroxide. As shown in Figure 1, higher percentages of potassium ferrate(VI) were found in those determinations conducted in the more alkaline arsenite solutions.

One of the difficulties encountered in the use of potassium bromate for the standardization of highly alkaline arsenite solutions was the fact that the concentration of hydrochloric acid at the end point was found to be critical. Reproducible results in standardization of alkaline arsenite solutions were obtained only if the concentration of hydrochloric acid was 1.5 to 2.0 *N*.

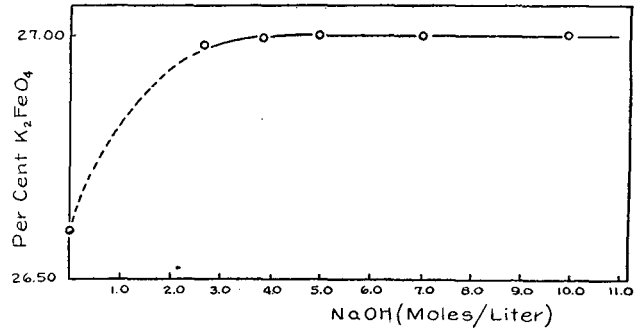


Figure 1. Average Per Cent Potassium Ferrate *vs.* Molarity of Sodium Hydroxide

Smith (*5*) found that Gyory's method (*1*) for the determination of arsenite with bromate need not be conducted in heated solutions. He reported that if methyl orange was used as an indicator in the titration conducted at room temperature, the response to the first excess drop of oxidant required at least 30 seconds. Gyory's original method seemed preferable because the solutions were already hot as a result of the neutralization of the strongly alkaline arsenite solutions that were employed.

No difficulty was encountered in standardizing strongly alkaline arsenite solutions with standard cerate solutions.

In the investigation of the method based on the determination of total iron present in a sample, results were obtained which were not in agreement with those obtained by the arsenite methods. The error was found to be caused by the presence of certain reducible substances in the caustic solution used to dissolve the potassium ferrate(VI). Heinemann and Rohn (*2*) have reported

[1] Present address, 2632 Dominguez St., Long Beach, Calif.

small quantities of reduced and reducible sulfur compounds, sodium sulfide and sodium thiosulfate, in commercial caustic soda. Blanks carried through the entire procedure were found to be significant, and satisfactory agreement with the results of the arsenite methods was obtained only when blanks were employed.

SOLUTIONS REQUIRED

Alkaline Arsenite Solution, approximately 0.375 *N* AsO_3 Weigh out 9.27 grams of pure dry arsenious oxide and dissolve in 200 ml. of 1 *N* sodium hydroxide. Mix with approximately 240 ml. of saturated sodium hydroxide solution. When using the alkaline arsenite solution in the arsenite-bromate method (see procedure), standardize the arsenite solution as follows: Pipet 10 ml. of the alkaline arsenite solution into a 500-ml. Erlenmeyer flask and add 225 ml. of distilled water and 65 ml. of concentrated hydrochloric acid. Heat to 70° to 80° C., add 2 drops of methyl orange, and titrate immediately with standard bromate solution using a 75-ml. buret. An additional drop of methyl orange may be required before the end point is reached because of fading of the color.

Standard Cerate Solutions, approximately 0.075 *N* and 0.02 *N*. Standardize against pure arsenious oxide.

Osmium Tetroxide Solution, 0.01 *M*. Dissolve 0.25 gram of osmate in 100 ml. of 0.1 *N* sulfuric acid.

o-Phenanthroline Ferrous Complex Solution, 0.025 *M*.

Stannous Chloride Solution. Dissolve 150 grams of iron-free stannous chloride dihydrate in 1 liter of 1 to 2 hydrochloric acid.

Mercuric Chloride Solution, 5% solution in water.

Sulfuric Acid Solution, 1 to 5.

Methyl Orange Solution, 0.5 to 1 gram per liter of water.

Sodium Hydroxide Solution, saturated.

Sodium Hydroxide Solution, 8 *M*.

Hydrochloric Acid, C.P.

ANALYTICAL PROCEDURES

Procedure for Arsenite-Bromate Method. Weigh a sample, containing approximately 0.1 gram of potassium ferrate, into a 500-ml. flask containing 10 ml. of the alkaline arsenite solution. Standardize the alkaline arsenite solution against the standard bromate solution immediately prior to use. Add the weighed potassium ferrate sample to the alkaline arsenite solution carefully, and particularly if the flask is wet with water, do not allow the sample to strike the side of the flask. Add 2 drops of methyl orange solution, 225 ml. of distilled water, and 65 ml. of concentrated hydrochloric acid. Heat the solution to 70° to 80° C., and titrate immediately with standard bromate solution. Introduce another drop of methyl orange near the end point, and approach the end point cautiously while vigorously swirling the solution. The end point is marked by a sudden change from golden yellow to greenish yellow. From the known titer of the arsenite solution and the volume of the standard bromate solution used, calculate the per cent of potassium ferrate as follows:

$$\text{Per cent } K_2FeO_4 = \frac{(10 \times N\ AsO_3^{---} - \text{ml. of } BrO_3^- \times N\ BrO_3^-) \times K_2FeO_4}{3000 \times \text{weight of sample}} \times 100$$

Procedure for Arsenite-Cerate Method. Weigh a sample, containing approximately 0.1 gram of potassium ferrate, into a 500-ml. flask containing 10 ml. of the alkaline arsenite solution. Standardize the alkaline arsenite solution against the standard cerate solution immediately prior to use. Observe the same precautions as in the arsenite-bromate method in adding the sample to the arsenite solution. Add 100 ml. of water and acidify with 100 ml. of 1 to 5 sulfuric acid. Cool to 20° to 25° C. and add 2 drops of osmate solution. Add 1 drop of *o*-phenanthroline ferrous complex indicator and titrate with the standard cerate solution. From the known titer of the alkaline arsenite solution and the volume of standard cerate solution used, calculate the per cent potassium ferrate as follows:

$$\text{Per cent } K_2FeO_4 = \frac{(10 \times N\ AsO_3^{---} - \text{ml. of } Ce^{+4} \times N\ Ce^{+4}) \times K_2FeO_4}{3000 \times \text{weight of sample}} \times 100$$

Procedure for Total Iron Method. Weigh a sample, containing approximately 0.1 gram of potassium ferrate, into a clean, oven-dried fritted-glass filter of 50-ml. capacity and fine porosity. Pipet 15 ml. of 8 *M* sodium hydroxide solution into a 25-ml. graduate. Add the sodium hydroxide solution in three or four portions to the potassium ferrate(VI) sample, stir with a glass rod, and filter continuously at the full capacity of a vacuum pump. Wash the graduate three times with a few milliliters of distilled water and pour these washings onto the filter. Release the suction tube and remove the filter. Hold the filter over the mouth of the flask and wash the bottom of the filter with a few milliliters of distilled water. Acidify the solution with 15 ml. of concentrated hydrochloric acid and heat nearly to boiling. Reduce the ferric ions with stannous chloride solution. Add only 1 or 2 drops excess of stannous chloride solution. Cool immediately to 20° to 25° C. and add 15 ml. of mercuric chloride reagent. Add 250 ml. of distilled water and 2 drops of *o*-phenanthroline ferrous complex indicator, and titrate immediately with the standard cerate solution.

Carry a blank through the entire process. Use 2 drops of stannous chloride solution for the reduction of this blank. Correct the volume of cerate solution used in the analysis by subtracting the volume of cerate solution used in the blank.

Calculate the per cent potassium ferrate as follows:

$$\text{Per cent } K_2FeO_4 = \frac{(N\ Ce^{+4} \times \text{ml. of } Ce^{+4}) \times K_2FeO_4}{1000 \times \text{weight of sample}} \times 100$$

Table I. Comparison of Methods for Analysis of Potassium Ferrate

Sample No.	Potassium Ferrate, %		
	Total iron	Arsenite-bromate method	Arsenite-cerate method
1	26.28	26.30	26.32
	26.35	26.29	26.31
	26.32	26.35	26.30
	26.30	26.32	26.33
	26.34	...	26.29
2	63.37	63.40	63.43
	63.35	63.35	63.42
	63.34	63.43	63.37
	63.45	63.39	63.40
3	94.56	...	94.41
	94.25	...	94.29
	...	...	94.55

DISCUSSION OF RESULTS

The results of analysis by both the arsenite and total iron methods, which are totally independent of each other, are observed to be in satisfactory agreement. The arsenite-bromate and arsenite-cerate methods gave equally satisfactory results; however, back-titration using the cerate solution is preferable to back-titration with the bromate solution. The bromate titration is carried out while the solution is hot; considerable experience is needed to obtain reproducible results, and the acidity in hydrochloric acid must be carefully controlled. The arsenite-cerate and total-iron methods are highly recommended for the routine analysis of potassium ferrate(VI) samples. The arsenite-cerate method is not recommended for the analysis of solutions of potassium ferrate(VI) which are highly decomposed and contain large quantities of hydrous ferric oxide, because the *o*-phenanthroline end point is obscured by the color of excess ferric ions.

ACKNOWLEDGMENT

The authors gratefully acknowledge the contribution to this work of W. E. Caldwell, Department of Chemistry, Oregon State College, who originally suggested an investigation of the oxidation of the arsenite ion by the ferrate(VI) ion.

LITERATURE CITED

(1) Gyory, Stefan, *Z. anal. Chem.*, 32, 415–21 (1893).
(2) Heinemann and Rohn, IND. ENG. CHEM., ANAL. ED., 9, 458 (1937).
(3) Mollins, J. de, *Bull. soc. chim.*, 16 (2), 246 (1871)
(4) Schreyer, J. M., thesis, "Higher Valence Compounds of Iron," Oregon State College, Corvallis, Ore., 1948; summary and preparation presented for publication.
(5) Smith, G. F., *J. Am. Ceram. Soc.*, 29, 143–5 (1946).

RECEIVED November 18, 1949.

Siloxene as a Chemiluminescent Indicator in Titration

FREDERIC KENNY AND R. B. KURTZ, *Hunter College, New York, N. Y.*

The indicator described emits light in the presence of an oxidant. When a minute excess of oxidant is added beyond the stoichiometric point, the entire liquid emits light. This is taken as the end point. Highly colored components such as cobaltous ion do not interfere in the titration of ferrous ion with ceric sulfate solution.

WÖHLER in 1863 treated calcium silicide with concentrated hydrochloric acid and obtained a yellow, insoluble product which he called "silicone" (*13*). The silicone consisted of bright, orange-yellow leaflets, insoluble in all inert solvents, and pseudomorphic with the calcium silicide. On heating in air it burst into flame. On heating, while enclosed, it evolved hydrogen and silicon hydrides. If prepared with more dilute hydrochloric acid, it was lighter in color. In sunlight it bleached. Under water, in sunlight, it evolved a steady stream of bubbles of hydrogen and was oxidized to a white product which Wöhler called "leucone." Since Wöhler, several investigators have attempted to elucidate the structure of the various silicon derivatives involved (*12*), but most of our knowledge of their structures is due to Kautsky (*3*, *4*).

NOMENCLATURE

The name "silicone," unfortunately, is the same as that now universally applied to the technically important, polymeric $R_2Si{=}O$ compounds (R = organic radical or hydrogen) as given by Kipping (*11*).

The silicones of Wöhler and Kautsky, which are the ones discussed in this paper, contain covalent silicon-silicon bonds in rings of six silicon atoms, also silicon-oxygen, silicon-chlorine, and silicon-hydrogen bonds, and no organic constituents, whereas the industrial silicones contain no silicon-silicon bonds, but do contain silicon-carbon bonds as well as silicon-oxygen bonds. Wöhler's silicone, which is used in this research as an indicator is referred to here as siloxene indicator.

SILOXENE INDICATOR

Structure and Reactions. According to Kautsky (*3*, *4*) a solid, yellow "silicone" consists of thin sheets of atoms, one molecule thick. These sheets are stacked one upon the other to form a solid pseudocrystal pseudomorphic with the original calcium silicide crystal. Actually, there is sufficient space between the sheets to allow a reagent solution to penetrate almost instantly from their edges toward their centers. The looseness, or interlamellar distance of the sheets, varies with methods of preparation, a propyl alcohol preparation giving considerable distance, and the method described herein making them rather tightly bound (*6*). Throughout most reactions of substitution, hydrolysis, and oxidation the lamellar structure remains the same, the dimensions and shape of the crystal altering little. The silicon atoms are arranged in hexagons which are all connected in one network plane, forming one lamella. Each hexagon is bridged to an adjacent one by oxygen atoms (*4*, *7*). The plane is probably folded periodically along its surface to accommodate oxygen atoms. The reactive groups—e.g., hydroxyl, chlorine, or hydrogen—are on both sides of this plane (*1*, *4*). Wöhler's silicone is not a compound having a definite chemical composition, but is a variable mixture of siloxene, polyhydroxysiloxenes, polychlorosiloxenes, and various oxidized siloxenes in which oxygen atoms have been inserted between silicon atoms of silicon 6-rings previously joined with silicon-silicon bonds. Figure 1 shows siloxene, chlorosiloxene, hydroxysiloxene, and an oxidized siloxene.

Siloxene is the first product formed by the interaction of calcium silicide and hydrochloric acid (*4*, *5*). The reaction for a single lamella (*4*) is:

$$6n\,H^+ + 3n\,CaSi_2 + 3n\,H_2O \longrightarrow \underset{\text{(Siloxene)}}{(Si_6H_6O_3)_n} + 3n\,H_2 + 3n\,Ca^{++}$$

Kautsky states that unless the acid is diluted with much alcohol, and the reaction is carried out over a period of many hours, at a low temperature, and in darkness, the siloxene forms chlorosiloxenes, partially, and, by hydrolysis of the latter, hydroxysiloxenes (*4*). Higher temperatures, light, and aqueous acid result in oxidized forms. The layers of silicon atoms appear to be preserved intact throughout the above transformations. Pure siloxene consists of white leaflets. The siloxene lamella (*4*, *7*) consists of hexagonal molecular units of the composition $Si_6H_6O_3$, containing silicon 6-rings completely surrounded by oxygen atoms and connected together edgewise, through the oxygens, so that the formula of the lamella may be written $(Si_6H_6O_3)_n$, and the entire crystal $m(Si_6H_6O_3)_n$, where m equals the number of similar lamellae in

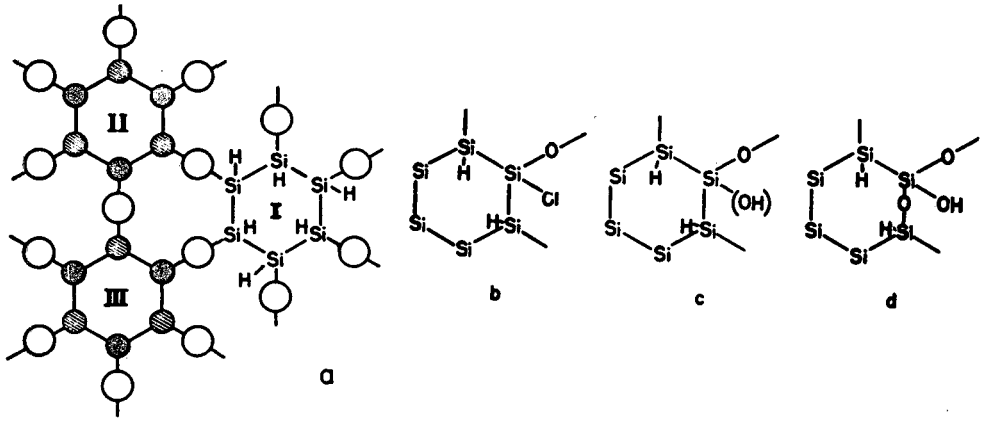


Figure 1. Siloxene, Chlorosiloxene, and Oxidized Siloxene

a. Siloxene (colorless, weakly fluorescent). Three identical six-membered rings are drawn in two different ways. The open circles indicate oxygen atoms (*4*, *7*). The small cross-hatched circles in rings II and III indicate silicon atoms (*4*, *7*), which are in a plane probably at least 1A. above the plane of the silicon atoms represented by the small black circles (*4*, *7*). The hydrogen atoms are omitted in rings II and III. In ring I three of the hydrogens are above and three are below the silicon atoms (*4*).
b, *c*, *d*. A single silicon atom diagramed with its four neighboring atoms.
b. Chlorosiloxene [colored, fluorescent (*8*), chemiluminescent]
c. Hydroxysiloxene [(colored, fluorescent, chemiluminescent) (*8*)]. Several additional hydroxy groups increase the color and the fluorescence.
d. Oxidized hydroxysiloxene (colorless, nonfluorescent). After complete oxidation there is no Si—Si bond. Silicic acid is the final product (*4*, *10*).

the solid crystal. Kautsky has estimated (*6*) that 100 mg. of siloxene have a reactive surface of about 2736 square feet. The hydrogens in it are replaceable analogously to the hydrogens in benzene.

The silicon 6-ring is a chromophore and a luminophore (*4*). The colors of the silicones depend upon the number and extent of substitution of the hydroxysiloxene rings present. The pure hydroxysiloxenes have the following colors: $Si_6H_6O_3$ (colorless), $Si_6H_5O_3(OH)$ (yellow), $Si_6H_4O_3(OH)_2$ (brown-red), $Si_6H_3O_3(OH)_3$ (red), $Si_6H_2O_3(OH)_4$ (brown-violet), and $Si_6O_3(OH)_6$ (black). Kautsky states (*4, 9, 10*) that the colors, fluorescences, and chemiluminescences of the silicones depend on the number and extent of substitution of the hydroxysiloxene rings present, and that these properties are more pronounced for silicone than for a pure specimen of an individual hydroxysiloxene. He states further that silicones possibly contain small areas of hydroxysiloxenes surrounded by oxidized siloxenes and that these hydroxysiloxene areas function as chemiluminescence centers when energy reaches them as a result of oxidation of another part of the silicone network. The energy for the activation of the chemiluminescence centers can be supplied by treating a suspension of silicone in dilute acid with strong oxidizing agents such as chromic acid, potassium permanganate, nitric acid, ceric solutions, and per compounds.

The "oxidation-potential" required for light emission of silicone has not heretofore been determined, but should be rather high, as strong oxidizing agents are required. The oxidation reaction appears to be strictly nonreversible; once oxidized, the reduced forms cannot be regenerated.

Preparation. "Siloxene indicator" is the name applied in this research to Wöhler's silicone. Its preparation is extremely simple.

Calcium silicide (Eimer & Amend, technical grade) was well powdered with a mortar and pestle. Five grams of this powder were placed in an 800-ml. beaker in a hood. The room was not darkened. Concentrated hydrochloric acid (50 ml.) was added and the mixture was agitated and stirred for several minutes with a long stirring rod. A vigorous reaction gradually developed, with spontaneous heating, foaming, and evolution of much hydrogen chloride vapor. After it subsided, 25 ml. more of concentrated hydrochloric acid were added, and the mixture was boiled gently and stirred for 5 minutes. Then 150 ml. of water were added, and it was boiled 5 minutes longer. The suspension of yellow silicone was decanted from any gray, unreacted calcium silicide onto a Büchner filter funnel. It was washed on the filter with water, then with 95% ethyl alcohol, and finally with ether. The yellow product was spread out on a porous plate to dry, and stored in a brown bottle.

It is stable for only a few days when dry, but for a longer time if kept under dilute hydrochloric acid. If prepared by this method, the material obtained is neither spontaneously flammable nor explosive. The time required for preparation of 5 grams of indicator was 20 minutes.

Use and Advantages. There are many situations in the field of analytical chemistry in which a titration cannot be made because the color of the solution makes it impossible to observe the color change of any indicator which might be selected. The authors have demonstrated that in such situations chemiluminescence can be used to locate the end point. Several types of titration are possible.

One situation in which the chemiluminescent siloxene indicator can be used to advantage is the oxidimetric titration of solutions, the color of which would interfere with the detection of the end point of any of the known redox indicators or with the end point of potassium permanganate.

When a very slight excess of oxidant is present in the solution being titrated, light is emitted by the indicator. If the excess is merely local, a momentary bright spot is produced in the solution. A minute excess of oxidant beyond the stoichiometric point will cause the entire solution to emit light. When the titrations are carried out in the dark, the end point is taken as the point at which the outline of the entire liquid is visible.

For the research in hand the amount of siloxene indicator used in each titration was approximately 100 mg. Titrations employing different amounts of indicator showed that variations of several milligrams had little or no effect upon the end point. The effect on the end point of extremely small or extremely large amounts of indicator is being investigated and will be reported later.

A desirable feature of siloxene indicator is its prompt reaction to excess of oxidant. Consequently, no indicator catalyst is required.

EXPERIMENTAL

Use of Siloxene Indicator in the Titration of Ferrous Ion with Ceric Sulfate Solution. Approximately 0.1 *M* solutions of ferrous ammonium sulfate and ceric sulfate were prepared and were compared by titrating 25.00-ml. portions of the ferrous solution with the ceric sulfate. A vacuum tube potentiometer of the type described by Garman and Droz (*2*) was used to locate the end point. The meter readings could be made to within 2 mv. on a scale which had been carefully calibrated against a series of potentials measured with a Leeds & Northrup 7660-A vacuum tube potentiometer. A saturated calomel reference electrode and a gold indicator electrode were used. The cell temperature was not controlled thermostatically. The maximum variation of the laboratory temperature, however, was about 2°, which corresponds to a variation of about 2 mv.

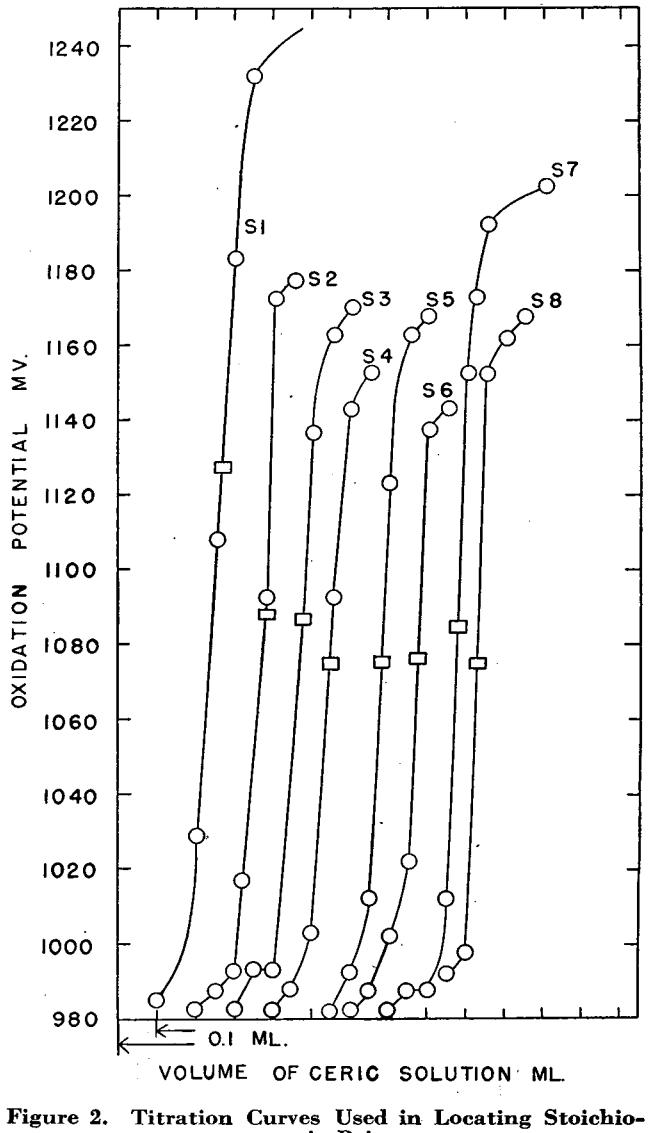


Figure 2. Titration Curves Used in Locating Stoichiometric Point

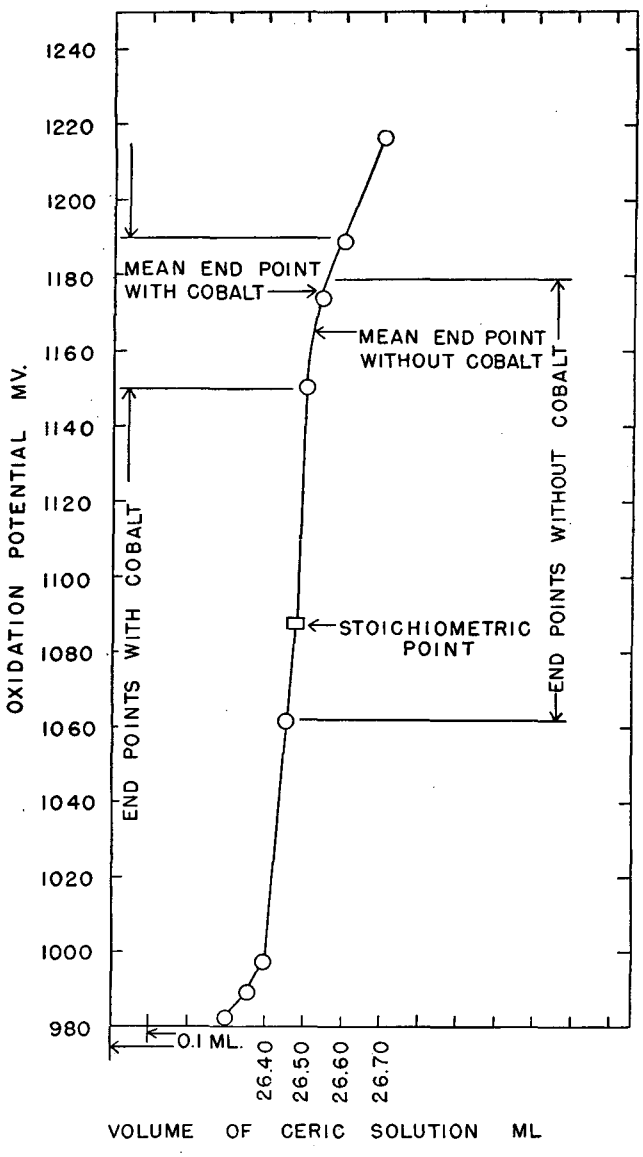


Figure 3. Average Curve Showing Range of End Points without Cobalt and with Cobalt Using Siloxene

in the voltage of the cell. The darkroom in which the titration was carried out was equipped with a convenient light which could be turned on or off as the occasion demanded.

The results of eight titrations of the ferrous solution with the ceric solution are given in Figure 2.

Because the curve for the reaction

$$Fe^{++} + Ce^{++++} \rightarrow Fe^{+++} + Ce^{+++}$$

is symmetrical with respect to the stoichiometric point, the midpoint of the nearly vertical portion of each curve was taken as the end point. The results of this graphical method are given in Table I, together with the corresponding potentiometer readings and oxidation potentials. The latter are referred to the normal hydrogen electrode.

With respect to the buret readings, the average deviation of a single observation was thus 0.01 ml., which for the case in hand yields a precision of 0.50 part per 1000.

The mean end point potential of 1087 mv. agrees fairly well with theoretically calculated values and with the results of numerous experimenters.

Because the jump in potential near the stoichiometric point is

Table I. Volume of Ceric Solution Equivalent to 25.00 Ml. of Ferrous Solution

(At 30° ± 2° C. and corresponding potentials)

Titration	Ceric Solution	Deviation from Mean	Potential Difference	Oxidation Potential	Deviation from Mean
	Ml.	*Ml.*	*Mv.*	*Mv.*	*Mv.*
S1	26.47	0.01	886	1128	41
S2	26.48	0.00	845	1087	0
S3	26.48	0.00	845	1087	0
S4	26.45	0.03	833	1075	12
S5	26.48	0.00	832	1074	13
S6	26.48	0.00	834	1076	11
S7	26.48	0.00	834	1076	11
S8	26.43	0.05	851	1093	6
Mean	26.48	0.01	845	1087	12

Table II. Volume of Ceric Solution Equivalent to 25.00 Ml. of Ferrous Solution

(Siloxene indicator at 30° ± 2° C. and corresponding potentials)

Titration	Ceric Solution	Deviation from Mean	Potentiometer Reading	Oxidation Potential	Deviation from Mean
	Ml.	*Ml.*	*Mv.*	*Mv.*	*Mv.*
1	26.50	0.02	980	1222	41
2	26.50	0.02	850	1092	71
3	26.55	0.03	920	1162	1
4	26.55	0.03	925	1167	4
5	26.50	0.02	970	1212	49
6	26.50	0.02	955	1197	34
7	26.45	0.07	920	1162	1
8	26.56	0.04	920	1162	1
9	26.55	0.03	950	1192	27
Mean	26.52	0.03	932	1174	25

about 30 mv. per 0.02 ml. of ceric solution, the precision of the potential measurements in this region is not high. This, of course, can be improved by control of temperature and by the use of a microburet.

From the results shown in Table I, the average curve shown in Figure 3 was plotted. In order to test the performance of siloxene indicator in the titration of ferrous ion with ceric ion, eighteen 25.00-ml. portions of ferrous solution were titrated. In each case the end point was determined in the dark, the indicator being added in a lighted room about 2 ml. prior to the end point.

In order to test the effectiveness of the indicator in the presence of a highly colored solution, half of the titrations were of the ferrous solution alone and half were of the same ferrous solution to which 5 ml. of 0.5 *M* cobaltous nitrate solution had been added. These solutions were approximately 0.10 *M* with respect to cobaltous ion and at the end point they were approximately 0.05 *M*. The depth of color due to cobalt at the end point was decidedly more pronounced than the usual permanganate end point and sufficiently deep to prevent the use of permanganate or the use of the common redox indicators. The concentration of cobaltous ion employed was purely arbitrary and is not to be construed as the upper limit of concentration permissible. Work is in progress to determine this upper limit, if any, and also to determine the application of chemiluminescent indicators to other titrations involving the presence of various colored components.

The results of the titrations of the ferrous solutions containing no cobalt are shown in Table II and those of the solution containing cobalt are shown in Table III. The potentiometer readings and corresponding oxidation potentials are given for each titration. The intensity of the light emitted at the end point did not seem to be materially affected by the presence of the cobalt. To test a more extreme case of interference with light emission, a titration was carried out on a ferrous solution containing 10 ml. of India ink. A satisfactory end point was obtained in this solution.

The limits between which the end points lie for ferrous solutions that contain cobalt and for those that do not contain cobalt are shown in Figure 3.

With respect to the buret readings of Table II, the average deviation of a single observation was thus 0.03 ml., which for the case in hand yields a precision of 1.1 parts per 1000. The indicator

Table III. Volume of Ceric Solution Equivalent to 25.00 Ml. of Ferrous Solution

(5 ml. of 0.5 *M* cobaltous nitrate added, using siloxene indicator at 30° ± 2° C. and corresponding potentials)

Titration	Ceric Solution	Deviation from Mean	Potentiometer Reading	Oxidation Potential	Deviation from Mean
	Ml.	*Ml.*	*Mv.*	*Mv.*	*Mv.*
10	26.60	0.05	895	1137	36
11	26.55	0.00	940	1182	9
12	26.56	0.01	970	1212	39
13	26.55	0.00	935	1177	4
14	26.60	0.05	965	1207	34
15	26.54	0.01	960	1202	29
16	26.50	0.05	910	1152	21
17	26.55	0.00	940	1182	3
18	26.55	0.00	860	1102	71
Mean	26.55	0.02	931	1173	27

error—namely, 26.52 − 26.48 or 0.04 ml.—corresponded to less than 1 drop from the buret, and for the case in hand amounts to 1.5 parts per 1000.

The potential at which the indicator emits enough light to give the end point appears to be about 87 mv. higher than the stoichiometric potential.

In the titrations of Table II and in all subsequent titrations the volume of solution at the end point was approximately 55 to 60 ml.

With respect to the buret readings of Table III, the average deviation of a single observation was thus 0.02 ml., which for the case in hand yields a precision of 0.75 part per 1000. The indicator error is 26.55 − 26.48 or 0.07 ml., 0.03 ml. more than in the absence of cobalt. The end-point potential appears to be about 86 mv. above the stoichiometric potential.

Table IV. Volume of Ceric Solution Equivalent to 25.00 Ml. of Ferrous Solution

(Siloxene indicator added prior to titration and at 2 ml. before end point)

	Prior Addition of Indicator			Indicator Added 2 Ml. before End Point		
Titration	Time of exposure	Ceric solution	Dev. from mean	Time of exposure	Ceric solution	Dev. from mean
	Min.	*Ml.*	*Ml.*	*Min.*	*Ml.*	*Ml.*
1	4	27.05	0.03	3	27.00	0.00
2	6	27.10	0.08	3	27.05	0.05
3	5	27.05	0.03	4	27.02	0.02
4	6	26.98	0.04	3	27.02	0.02
5	5	26.94	0.08	2	27.00	0.00
6	8	27.03	0.01	3	26.98	0.02
7	5	27.01	0.01	2	26.97	0.03
8	8	27.00	0.02	3	26.98	0.02
9	5	26.95	0.07	7	26.96	0.04
10	6	27.06	0.04	2	27.00	0.00
Mean	6	27.02	0.04	3	27.00	0.02

If the results of Table III and Table IV are combined, the eighteen samples yield an average deviation of 0.02 ml., an end point of 26.54 ml. of ceric solution, an indicator error of 0.05 ml., and an accuracy of 1.9 parts per 1000. With samples requiring 40.00 ml. of ceric solution instead of 26.54 ml. the accuracy would be 1.3 parts per 1000. If, of course, the indicator error was applied as a correction, a decidedly higher degree of accuracy would be obtainable. In many instances it would be possible to use siloxene indicator in the standardization of the ceric solution and thereby eliminate most or all of the indicator error. The potential required to give the light at the end point is approximately 86 mv. above the stoichiometric potential. The oxidation potential at the end point appears to be about 1173 mv.

In order to determine what effect, if any, is produced on the end point by adding the indicator at the beginning of the titration instead of at a point 2 ml. prior to the end point, ten titrations of each sort were carried out on 25.00-ml. portions of a ferrous solution. The time of exposure of the indicator to the solution was noted in each case (Table IV).

In Table IV it is seen that exposure of the indicator to the solution throughout the entire titration increased the time of exposure by about 3 minutes but had little or no effect on the end point.

A determination was made of the pH of the iron solution at the end point of the indicator, as well as the pH of a suspension of the indicator in 50 ml. of distilled water. These measurements, made with a glass electrode and a Leeds & Northrup 7660-A vacuum tube potentiometer, yielded 0.23 for the pH of the former and 3.59 for the pH of the latter. The pH at the end point of the iron solution would, of course, vary with the acidity of the ferrous and ceric solutions and the volumes of each used. Attempts by the authors to obtain satisfactory light emission of siloxene indicator in neutral or basic solutions have failed. Experiments to determine the minimum acidity necessary are in progress and will be reported on later. However, preliminary results indicate that the value is in the neighborhood of pH 2.

The decidedly acid pH of the suspension of siloxene indicator in distilled water probably results from the presence of residual hydrochloric acid in the indicator as well as hydrolysis of the chlorosiloxenes present.

A direct determination of the indicator correction was carried out by preparing a solution of sulfuric acid having the same pH as that of the end point solution—namely, 0.23—by diluting 5.8 ml. of 18 *M* sulfuric acid to 250 ml. One hundred milligrams of indicator were added to 50-ml. portions of the solution and titrated with ceric solution from a microburet, the readings of which could be estimated to 0.001 ml. Half of the solutions tested were also 0.05 *M* with respect to cobaltous ion, whereas the other half contained no cobalt. The results are shown in Table V.

In Table II the indicator correction was shown to be 0.04 ml. for solutions containing no cobalt, which is substantially the same as the directly measured value of 0.035 ml. given in Table V.

In Table III the indicator correction was shown to be 0.03 ml. more in a solution which is 0.05 *M* with respect to cobaltous ion than in a solution containing no cobalt. Thus, the total correction is seen to be 0.07 ml. This is substantially the same as the directly measured value of 0.068 ml. shown in Table V.

SUMMARY

This paper describes a new method of titration which employs a chemiluminescent material as indicator. When siloxene indicator is used in titrating ferrous ion, a precision of 0.02 ml. is obtained which corresponds to 0.75 part per 1000.

An indicator error of 0.04 ml. was determined for solutions containing no cobalt. Without the use of this correction, iron was titrated with an accuracy of 1.5 parts per 1000. This would correspond with an accuracy of 1.0 part per 1000 if sufficient ferrous solution were taken to require 40.00 ml. of ceric solution. By applying the indicator correction or by using siloxene indicator in standardizing the ceric solution, a more accurate result could be obtained.

The presence at the end point of 0.05 *M* cobaltous solution increased the end point by only 0.03 ml. and had little effect on the end point potential.

Table V. Direct Determination of Indicator Correction with Ceric Solution

(On 100-mg. portions of indicator in sulfuric acid solutions having end point pH, with and without 0.05 *M* cobaltous ion)

	Ceric Solution Used	
Titration	Solutions containing no cobalt	Solutions containing 0.05 *M* cobaltous ion
	Ml.	*Ml.*
1	0.035	0.071
2	0.035	0.075
3	0.038	0.067
4	0.039	0.066
5	0.030	0.060
Mean	0.035	0.068

No indicator catalyst is required

Potential measurements in the vicinity of the stoichiometric point yielded an oxidation potential at the end point of 1173 ± 26 mv., referred to the normal hydrogen electrode.

The siloxene indicator, to function properly, must be used in acid solution.

Methods described may be applicable to many determinations involving the presence of a highly colored or opaque component.

LITERATURE CITED

(1) Bohm, J., and Hassel, O., *Z. anorg. Chem.*, **160**, 161 (1927).
(2) Garman, R. L., and Droz, M. E., IND. ENG. CHEM. ANAL. ED., **11**, 398 (1939).
(3) Hückel, W., "Anorganische Strukturchemie," p. 926, Stuttgart, F. Enke Verlag, 1948.
(4) Kautsky, H., *Kolloid Z.*, **102**, 1 (1943).
(5) Kautsky, H., *Z. anorg. Chem.*, **117**, 215 (1921).
(6) *Ibid.*, **236**, 127 (1938).
(7) Kautsky, H., and Gaubatz, E., *Ibid.*, **191**, 384 (1930).
(8) Kautsky, H., and Hertzberg, G., *Ibid.*, **139**, 157 (1924).
(9) Kautsky, H., and Neitzke, O., *Z. Physik*, **31**, 61 (1925).
(10) Kautsky, H., and Zocher, H., *Ibid.*, **9**, 269 (1922).
(11) Kipping, F. S., *J. Chem. Soc.*, **101**, 2106 (1912).
(12) Mellor, J. W., "Comprehensive Treatise on Inorganic Chemistry," Vol. 6, pp. 231, 236, London, Longmans, Green & Co., 1925.
(13) Wöhler, F., *Ann.*, **127**, 263 (1863).

RECEIVED October 5, 1949.

Determination of Boron in Magnesite and Fused Magnesia

WALLACE M. HAZEL AND GERALDINE H. OGILVIE
Norton Company Research Laboratory, Chippawa, Ontario, Canada

A method for the determination of boron in magnesite and fused magnesia is described. The method is direct, requires very little time, and is subject to no interference from the usual impurities found in magnesite. Data are presented showing the accuracy and precision obtainable. The method entails the solution of the sample in hydrochloric acid under a reflux condenser, and precipitation of the iron, titanium, calcium, and magnesium with sodium carbonate and sodium hydroxide. The solution containing the precipitate is made up to a definite volume and filtered, and an aliquot is taken for the boron titration. This is made with 0.1 *N* sodium hydroxide after the addition of mannitol.

MAGNESITE from some foreign deposits, especially India, contains only a few parts per million of boron. Magnesite from some domestic deposits contains boron, and magnesites produced from the extraction and calcination of magnesium hydroxide from sea water contain very appreciable amounts of boron.

The increased use of high grade periclase for the embedding medium of resistor elements in hot plates, immersion heaters, stove units, etc., has resulted in an investigation of the various factors affecting the conductivity of this embedding medium. It has been found that the presence of even small amounts of boron is detrimental to periclase used for this purpose.

The presence of boron in the periclase gives a marked increase in the slope of the curve of resistance versus temperature at the higher temperatures of operation. At low temperatures the effect is not so great. The change of the slope of this curve means that as the amount of boron increases, the conductivity of the periclase becomes greater. It actually becomes dangerous and undesirable as an insulator material, especially when used in household appliances. When boron is present in this material it also has a tendency to glaze over the individual grains, causing sintering of the insulating medium. During sintering the resistor itself is attacked, resulting in early failure of the element.

Because many of the available magnesites contain appreciable boron, it has been necessary to develop an accurate and reliable method for determining boron in all concentrations in incoming magnesite, and in all the resultant fusions of this material.

In the authors' laboratory, it has been the practice to determine the boron content of these products by spectrographic methods when the concentrations are low, and by wet chemical methods when the boron is high. Boron determinations up to 1000 p.p.m. (0.10% boron) are carried out spectrographically by a method developed in the laboratory, which is intended for publication at a later date.

An accurate determination of boron is not a simple test for the analyst. Many methods have been proposed (*1, 2, 6, 8*), but most are subject to long tedious separations in which boron is lost, to high blanks from the reagents used, and to volatility of the boric acid in aqueous and acid solutions. The classical method of Chapin (*2*) is perhaps the most widely used procedure, but the high unreliable blanks obtained by this method make the determination of small amounts of boron exceedingly difficult and unreliable. These high blanks are occasioned, no doubt, by the fact that the calcium chloride, which is used as a dehydrating agent before the distillation of the boric acid as methyl borate, contains appreciable amounts of boron. The large amount of the dehydrating agent necessary makes this method rather inaccurate, especially for smaller amounts of boron.

Many thousands of boron analyses have been made in this laboratory on all types of materials including elemental boron. The authors have consistently tried to develop fast, accurate, and dependable methods for boron. From their experience in this type of work, the method here described has been worked out. They feel that it satisfies all the above requirements for the determination of boron in magnesite and fused magnesia products.

EXPERIMENTAL

A sample of very high grade India magnesite was selected as the starting material. This sample contained less than 0.5 p.p.m. of boron by spectrographic analysis, and was a part of the magnesite from which spectrographic standards are prepared. Besides the boron, the material contained as impurities about 1.50% silica, 0.06% ferric oxide, 1.70% calcium oxide, and traces of minor constituents such as alumina, titania, manganese oxide, sodium oxide, and sulfate. This material, because of its negligible boron content and the noninterference of the other constituents, made an excellent starting material for preliminary study.

A standard solution of boric acid was made from recrystallized J. T. Baker's reagent grade boric acid. Chemical analysis

Table I. Accuracy of Volumetric Determination of Boron in Magnesia

H_3BO_3 Addition to 5 G. MgO (1 Ml. = 2.5 Mg. B)	NaOH Titration (1 Ml. = 0.001321 G. boron)	Boron Added	Boron Recovered after Blank Subtraction	Deviation
Ml.	*Ml.*	*Mg.*	*Mg.*	*Mg.*
0.00	0.35			
	0.35			
0.60	0.70	0.3	0.5	+0.2
	0.65	0.3	0.4	+0.1
	0.70	0.3	0.5	+0.2
1.00	0.83	0.5	0.6	+0.1
	0.80	0.5	0.6	+0.1
	0.80	0.5	0.6	+0.1
2.00	1.25	1.0	1.2	+0.2
	1.20	1.0	1.1	+0.1
	1.15	1.0	1.1	+0.1
4.00	2.00	2.0	2.2	+0.2
	1.95	2.0	2.1	+0.1
	1.90	2.0	2.0	0.0
6.00	2.60	3.0	3.0	0.0
	2.55	3.0	2.9	−0.1
	2.65	3.0	3.0	0.0
8.00	3.30	4.0	3.9	−0.1
	3.30	4.0	3.9	−0.1
	3.30	4.0	3.9	−0.1
10.00	4.00	5.0	4.8	−0.2
	4.00	5.0	4.8	−0.2
	4.00	5.0	4.8	−0.2
12.00	4.75	6.0	5.8	−0.2
	4.80	6.0	5.9	−0.1
	4.80	6.0	5.9	−0.1
14.00	5.48	7.0	6.8	−0.2
	5.55	7.0	6.9	−0.1
	5.55	7.0	6.9	−0.1
15.00	5.88	7.5	7.3	−0.2
	5.88	7.5	7.3	−0.2
16.00	6.28	8.0	7.8	−0.2
	6.40	8.0	8.0	0.0
	6.43	8.0	8.0	0.0
18.00	7.10	9.0	8.9	−0.1
	7.05	9.0	8.9	−0.1
	7.10	9.0	8.9	−0.1
20.00	7.85	10.0	9.9	−0.1
	7.95	10.0	10.0	0.0
	7.90	10.0	10.0	0.0
30.00	11.50	15.0	14.7	−0.3
	11.40	15.0	14.6	−0.4
	11.40	15.0	14.6	−0.4
40.00	15.30	20.0	19.7	−0.3
	15.35	20.0	19.8	−0.2
	15.30	20.0	19.7	−0.3

showed this material to be 99.99% H_3BO_3. A solution of 14.289 grams of the salt in 1 liter contained 2.5 mg. of boron per ml.

$$\frac{14.289 \times 0.17495}{1000} = 0.0025 \text{ gram of boron per ml.}$$

The standard boric acid solution was added to 5-gram samples of the India magnesite, so that the percentage of boron in the samples to be titrated ranged from 0.03 to 2.00%. Triplicate analyses were made throughout the whole range.

Five-gram samples of the India magnesite were weighed into round-bottomed digestion flasks, and after the additions of various amounts of the standard boric acid solution, the samples were dissolved under reflux with 1 to 1 hydrochloric acid. The flask was cooled, and the solution was nearly neutralized with sodium hydroxide and poured into a solution of sodium hydroxide and sodium carbonate contained in a 500-ml. volumetric flask to precipitate the magnesium as hydroxide. The carbonate was included to ensure the complete precipitation of calcium (*5*), which if not removed might cause low results because of the formation of relatively insoluble calcium borate. The boron remained in the solution, which was diluted to 500 ml. and mixed well before the heavy precipitate was filtered off through a dry paper. A 100-ml. aliquot was taken for the boron titration. This aliquot, containing one fifth of the original boron addition, now represented the boron percentage on the basis of a 1-gram sample.

The solution was adjusted to a pH of 7, using *p*-nitrophenol indicator, and digested to precipitate any alumina or trace amounts of iron, titanium, etc., which might have remained in solution. After filtering and washing, the solution was freed of carbon dioxide by acidifying and boiling under reduced pressure. The presence of carbon dioxide interferes in a sodium hydroxide titration. When cool, the sample was adjusted to a pH of 7 and titrated with 0.1 *N* sodium hydroxide to a phenolphthalein end point. Mannitol was added during the titration to convert the boron to a form in which it could be titrated (*3*, *4*).

Table I shows the various amounts of the standard boric acid solution which were added to the magnesite samples, together with the sodium hydroxide titration for each determination.

REAGENTS REQUIRED

Hydrochloric acid, 1 to 1. Concentrated hydrochloric acid (specific gravity 1.19) diluted with an equal volume of water.

Sodium hydroxide, 10%. Solution of C.P. pellets in distilled water.

Sodium carbonate. Anhydrous C.P. powder.

p-Nitrophenol indicator. One gram dissolved in 75 ml. of neutral ethyl alcohol and diluted to 100 ml. with water.

Concentrated sodium hydroxide, C.P. sodium hydroxide pellets, (75 grams) dissolved in 75 ml. of carbon dioxide–free distilled water. Carbonate allowed to settle or filtered off.

Stock sodium hydroxide. One volume of concentrated sodium hydroxide mixed with 1 volume of carbon dioxide–free distilled water.

Sodium hydroxide, 0.1 *N*. Concentrated sodium hydroxide solution, 25 ml. in 4 liters carbon dioxide–free distilled water. Standardized against potassium acid phthalate or recrystallized boric acid.

Hydrochloric acid, 0.1 *N*. Approximately 8.45 ml. of concentrated hydrochloric acid (specific gravity 1.19) per liter of distilled water.

Phenolphthalein. One gram dissolved in 100 ml. of neutral ethyl alcohol, and made up to 200 ml. with water.

Mannitol. Reagent grade, from Atlas Powder Co., Wilmington, Del.

PROCEDURE

Weigh 5 grams of the magnesium oxide sample into a 250- or 300-ml. round-bottomed flask with a ground-glass standard taper neck. Add 35 ml. of 1 to 1 hydrochloric acid and connect the flask to a water condenser having a ground-glass joint. Heat the flask gently with a medium hot plate until the sample is completely in solution.

Remove from heat and cool the sample. Rinse down the condenser and disconnect the flask. Add 10% sodium hydroxide dropwise until the solution is nearly neutral. No indicator is used for this adjustment. When the solution is approaching the neutral point, vigorous stirring will just redissolve the precipitate formed by the drop of sodium hydroxide (*7*).

Pour the nearly neutralized solution slowly and with constant swirling into a 500-ml. volumetric flask containing 15 grams of sodium hydroxide and 2 grams of sodium carbonate in 50 ml. of water. Cool, dilute to the mark, and mix well.

Filter through a dry No. 41 Whatman filter paper, until sufficient solution is obtained for a 100-ml. aliquot. Pipet this amount into a 250-ml. borosilicate glass beaker.

Adjust the solution to a pH of 7 as follows: Add 4 drops of *p*-nitrophenol indicator. This gives a brilliant yellow color in the alkaline solution. Add 1 to 1 hydrochloric acid dropwise with stirring until the color is just discharged, then make dropwise additions of 0.1 *N* sodium hydroxide until the first definite yellow stage is reached. If necessary, approach the end point a second time by discharging the yellow color with 0.1 *N* hydrochloric acid, and again neutralize slowly with 0.1 *N* sodium hydroxide. At the neutral point, 1 drop of 0.1 *N* hydrochloric acid should discharge the yellow color, leaving a water-white solution, then 1 drop of the 0.1 *N* sodium hydroxide should give the definite yellow color, which can be taken as the neutral point.

Digest at about 65° C. for 1 hour. Filter into a 1000-ml. pressure flask (preferably with a side arm) through a 9-cm. No. 40 Whatman filter paper. Wash well with hot water. Acidify the filtrate with 1 to 1 hydrochloric acid, still making use of the *p*-nitrophenol indicator, and add about 3 drops of acid in excess. Carefully warm the flask until the solution is about 70° C. Transfer to a suction pump and boil under reduced pressure until all the gas is expelled from the solution (*9*). A No. 8 rubber stopper can be used conveniently in the neck of the flask and the suction applied on the side arm. Care must be taken to avoid

loss through splashing; the suction can be controlled to prevent too violent agitation during the boiling. A shield should be placed around the flask to guard against injury from broken glass, in case the flask should be defective and collapse under vacuum. In years of experience the authors have never had a flask collapse in this laboratory.

Cool, and wash down the stopper and the inside of the flask with water which has been boiled to rid it of carbon dioxide, and then cooled. Nearly neutralize with the stock sodium hydroxide solution, and finally adjust to pH 7 with dilute hydrochloric acid and sodium hydroxide as described above.

Add 1 ml. of phenolphthalein indicator and 1 gram of mannitol. Titrate with approximately 0.1 *N* sodium hydroxide solution to a pink color; discharge the color with another gram of mannitol and again titrate to a pink color. Continue the alternate addition of mannitol and sodium hydroxide until the mannitol no longer fades the phenolphthalein end point. This titration has been described in detail (*9*).

It is very essential that a blank determination should be carried through all the steps of the procedure (*1*).

Calculate the per cent boron present. One milliliter of 0.1000 *N* sodium hydroxide is equivalent to 0.001082 gram of boron.

$$\text{(Titration} - \text{blank) ml. of 0.1 } N \text{ NaOH} \times 0.001082 \times 100 = \% \text{ boron}$$

DISCUSSION OF RESULTS

The preliminary investigations were carried out to assure sufficient accuracy over the range of values that might be expected for this material.

To someone not familiar with this type of titration, the blanks may appear to be too high for accurate work. The blank is not an inherent fault of the determination due to impurities, etc., in the reagents used. It merely represents the number of milliliters of sodium hydroxide necessary to change the solution being titrated from a pH of 7.0 (the starting point) to a pH of approximately 11.0, which is the neutralization point in the boric acid titration (*3*, *4*). The blanks are very constant and absolutely reliable.

Boron was added throughout the range from 0.03 to 2.00%. These amounts cover the entire range of boron content expected for material of this type. There is no reason to believe that the method would not be just as accurate for the higher ranges. A similar type of titration is already in use in this laboratory for very high-boron materials, and no discrepancies have been noted.

Table I clearly shows that no boron is precipitated as an insoluble compound during the sodium hydroxide precipitation.

Table II. Determination of Boron in Magnesite Samples

Sample	B_2O_3 Content, Duplicate Analyses %	Difference *Mg. B_2O_3*	Sample	B_2O_3 Content, Duplicate Analyses %	Difference *Mg. B_2O_3*
1[a]	0.04 0.04	0.0	12[a]	0.27 0.38	0.1
2	0.08 0.08	0.0	13	0.30 0.32	0.2
3[a]	0.08 0.09	0.1	14	0.34 0.34	0.0
4	0.06 0.06	0.0	15[a]	0.35 0.35	0.0
5	0.11 0.12	0.1	16[a]	0.37 0.38	0.1
6	0.17 0.17	0.0	17	0.39 0.39	0.0
7[a]	0.17 0.17	0.0	18	0.42 0.44	0.2
8[a]	0.17 0.19	0.2	19[a]	0.43 0.43	0.0
9	0.11 0.11	0.0	20[a]	0.44 0.46	0.2
10	0.19 0.21	0.2	21[a]	0.32 0.35	0.3
11	0.18 0.20	0.2	22	0.29 0.33	0.4

[a] Analyses by different operators.

This is not true of all other basic hydroxides—e.g., the use of ammonium hydroxide would cause precipitation of the calcium as insoluble calcium borate, giving low results for boron. Ammonium hydroxide would also cause interference in the titration and is to be avoided in any work of this type.

In Table II a number of typical results are shown for magnesite and fused magnesia samples. The values represent raw materials, as well as fusions of mixtures of both high and low boron-bearing magnesites. Each of the analyses shown was made in duplicate, and in some cases the duplicate determinations were performed by different operators.

Table III. Boric Acid in Magnesite Samples after Boric Acid Additions

B_2O_3 in Magnesite *Mg.*	B_2O_3 Added as H_3BO_3 *Mg.*	B_2O_3 Total *Mg.*	B_2O_3 Recovered *Mg.*	Deviation *Mg.*
3.1	2.8	5.9	5.8	−0.1
3.2	5.6	8.8	8.8	0.0
3.6	2.8	6.4	6.4	0.0
3.6	5.6	9.2	9.3	+0.1
4.2	2.8	7.0	7.1	+0.1
4.2	5.6	9.8	9.8	0.0
4.6	2.8	7.4	7.3	−0.1
4.6	5.6	10.2	10.4	+0.2

The greatest variation in the results shown in Table II represents a difference of only approximately 0.1 ml. of 0.1 *N* sodium hydroxide. In most of the analyses shown the differences represent 0.05 ml. or less of the sodium hydroxide solution. These analyses were not specially selected, but represent regular determinations taken at random from the files.

After the boron content of several regular magnesite samples had been established, as shown in Table II, further work was carried out by adding 25 and 50 mg. of the recrystallized boric acid to 5-gram portions of these previously analyzed samples. The total boron present was then determined as described above.

The excellent recovery in each case showed that the original values represented all of the boron present in the sample, and that no losses occur as the concentration of boron is increased. These results are shown in Table III.

ACKNOWLEDGMENT

The authors wish to express their appreciation to the directors of the Norton Company, Worcester, Mass., for permission to publish this material.

They also wish to express their thanks to M. F. Beecher, director of research and development, and to A. H. Ballard, associate director of research, for their encouragement and interest in the development of new and practical methods of analysis in this laboratory.

LITERATURE CITED

(1) Allen, E. T., and Zies, E. G., *J. Am. Ceram. Soc.*, 1, 739 (1918).
(2) Chapin, W. H., *J. Am. Chem. Soc.*, 30, 1691 (1908).
(3) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," p. 611, New York, John Wiley & Sons, 1929.
(4) Krantz, J. C., Jr., Carr, C. J., and Beck, F. F., *J. Phys. Chem.*, 40, 927 (1936).
(5) Lundell, G. E. F., and Hoffman, J. I., "Outlines of Methods of Chemical Analysis," p. 83, New York, John Wiley & Sons, 1938.
(6) Lundell, G. E. F., Hoffman, J. I., and Bright, H. A., "Chemical Analysis of Iron and Steel," p. 396, New York, John Wiley & Sons, 1931.
(7) Scott, W. W., "Standard Methods of Chemical Analysis," 5th ed., p. 33, New York, D. Van Nostrand Co., 1939.
(8) *Ibid.*, p. 162.
(9) *Ibid.*, p. 183.

RECEIVED November 25, 1949.

Rapid Fat Determination in Plant Control of Cacao Products

Centrifuge Method

FRANCIS X. KOBE, *Rockwood & Co., 88 Washington Ave., Brooklyn 5, N. Y.*

A method for the centrifugal fat extraction of cacao products is described. Data indicate the relative rate of extraction and comparison is made with the official method of the Association of Official Agricultural Chemists.

FATS and oils of vegetable origin usually occur naturally in a partially free state. They may lie at or near the surface of the plant product, in capillary spaces formed by the rupture of cellular structure, or encased in unruptured cells. Their removal may be effected quantitatively by solution in various solvents.

Studies of analytical procedures based on the extraction technique are complicated, however, by several factors. These include the fineness or degree of grind, the quantity and composition of the extractable material, choice of solvent, temperature, and moisture content.

Perhaps the most commonly employed method for total fat determination by the extraction process is that described by Lewkowitsch (*25*), which consists essentially of extracting a prepared sample with a suitable solvent in a Soxhlet apparatus for several hours. Through the years, many modifications of this method have been introduced, some for the purpose of increasing its accuracy, others with a view toward increasing its practicality.

Hanus (*11*), Kreutz (*21*), and Heller (*14*) were among the first to suggest improvements. Devices such as Johnson (*35*), Underwriter's (*20*), and Butt (*31*) extractors have found wide application.

Lepper and Waterman (*24*) devised the present official method of the Association of Official Agricultural Chemists, involving a series of extractions in a Knorr tube.

The dairy industry has for many years used the Roese-Gottlieb method, performing the extraction in a Röhrig tube or Mojonnier flask (*2*). Hillig (*17*) suggested the use of acid hydrolysis in the manner of Hertwig (*15*) and Fellenberg (*9*). Offut (*28*) and Jansen (*19*) also adopted this technique. Other variations include the methods of Bailey and Walker (*3*), Macara and Hinton (*26*), Francis (*10*), Sabine (*30*), Stone (*33*), and Micaelli and Desnuelle (*27*).

Herty (*16*) and Harris (*12*) deviated from the usual procedures by basing their analyses on the specific gravity of the fat-solvent extracts.

Richter (*29*) developed a method based on the refractive index of the fat extract. This avenue of approach has been followed by Wesson (*34*), Coleman and Fellows (*6*), Demikovskiĭ (*7*), Ermakov (*8*), Hasse and Bake (*13*), Leithe (*22*), Leithe and Heinz (*23*), Bielefeldt (*4*), Stanley (*32*), and Brüchner (*5*).

The centrifugal method of fat extraction as outlined by Hughes (*18*) consisted of successively extracting the fat-bearing substance with ether and centrifuging the ether layer after each extraction.

In the corresponding centrifugal extractive procedure which this paper describes, petroleum ether (benzine) is used, principally because it is inexpensive and requires no special preparation other than distillation below 60° C. It will not dissolve theobromine, the cacao alkaloid, to the same extent as does ethyl ether, nor is it affected by traces of moisture which would dissolve sugar and other nonfatty material that may be present.

The progressive extraction principles upon which this determination is based may be demonstrated in a simple manner.

If we assume that a 2-gram sample of chocolate containing 50% cocoa butter is progressively washed with 25-gram portions of petroleum ether, we have approximated the conditions for a fat determination. The solid matter (cocoa solids-not-fat, carbohydrates, etc.) is wet by the same solution of fat and approximately 2 grams or less of such solution are always left in contact with the solids remaining in the centrifuge tube. These 2 grams of solution carry a certain amount of the original fat which will be removed by subsequent extractions.

The effect of three such extractions is shown in Table I.

The accurate estimation of fat in any shredded or ground food product depends on the distance through which the solvent must pass to reach the fat and the distance through which it must return. Diffusion or dialysis and ease of solubility are of the utmost importance. Time is also a critical factor.

One very important assumption is that a complete state of equilibrium exists between all of the solvent and all of the fat present in the system at the time of centrifugal separation. This is usually the case with most cocoas and chocolates when 3 to 5 minutes are allowed to elapse between the time the dry matter is wetted by the solvent and the time the solids are separated in the centrifuge. The time for equilibrium is only a matter of seconds in extremely finely divided matter. Thus it is an advantage to work with the most finely ground material possible.

Table I. Progressive Centrifugal Extraction of Cacao[a]

Original sample 2 grams
1.0 gram of cocoa matter
1.0 gram of cocoa butter

Separation	Residue, Grams: Cocoa matter	Cocoa butter	Residual petroleum ether	Extract, Grams	Fat Extracted: Gram	Fat Extracted: %	Cumulative % Fat Extracted
1	1.0000	0.0760	1.9240	24.00	0.9240	46.20	46.20
2	1.0000	0.0060	1.9940	25.00	0.0700	3.50	49.70
3	1.0000	0.0010	1.9990	25.00	0.0050	0.25	49.95

(Fat in solvent-free residue, 0.10%)

[a] 25.00 grams of petroleum ether used in each separation.

CENTRIFUGE METHOD FOR RAPID FAT DETERMINATION

Apparatus. Heavy-walled borosilicate glass centrifuge tubes, ungraduated, with lip, round-bottomed, annealed. Length, 120 ± 2 mm., outside diameter, 28 ± 1 mm. Capacity, 50 ml.

Petroleum ether, distilled below 60° C.

Aluminum beaker, 250 ml.

International centrifuge, size 2, 0.75 hp., 110 volts, 6 amperes.

Preparation of Sample. In determining fat by this method, the need for a well-prepared, representative sample cannot be overemphasized, particularly in the analysis of cacao beans, cacao

Table II. Routine Plant Control of Cacao Products

Sample	Average % Fat	Weight of Sample, Grams	Method	No. of Extractions
Raw cacao beans	48–51	2	Direct	4 (sugar added)
Chocolate liquor	54	2	Direct	4 (sugar added)
Ice cream coatings	50–60	2	Direct	4
All other coatings	27–45	2	Indirect	3
Ice cream flakes	25–35	2	Direct	4
Cocoas	6–25	2	Direct	3
Low fat cocoa	0–6	10–30	Direct	4
Cocoa press-cake[a]	6–25	2	Indirect	3
Expeller feed	25–35	2	Direct	4
Expeller cake	4–10	2	Direct	3
Cacao shell	2–5	10–30	Direct	3

[a] Subsequently analyzed in form of cocoa.

nibs, expeller feed, and cacao shell. They are prepared by grinding once in a Wiley type mill with an appropriate screen or in a Quaker mill followed by passage through a Wiley mill or a micropulverizer. Press cake samples are obtained by scraping the cake and sieving the scrapings through a screen of approximately 60-mesh.

Procedure. Place a thoroughly clean 250-ml. aluminum beaker in a drying oven at 100° C. for approximately 5 minutes, transfer to a desiccator, cool, and weigh.

Weigh out approximately 2 grams of the material to be analyzed into a previously weighed 50-ml. lipped centrifuge tube, add 30 ml. of petroleum ether to the material in the tube, and stir with an aluminum rod. If the sample is sweet chocolate or chocolate liquor (baking chocolate), it should be in a fluid condition and free from lumps. After stirring, raise the rod slowly and rinse off with a small quantity of petroleum ether from a wash bottle. Provide the tube with a cork stopper and balance in a centrifuge cup. Centrifuge until the supernatant liquid is clear.

Remove the tube from the centrifuge and pour the extract carefully into the weighed aluminum beaker. Wash the lip of the tube and the stopper with additional petroleum ether, adding the washings to the extract. While the solvent containing the extracted fat is being evaporated over a hot plate, add 30 ml. or more of fresh petroleum ether to the extraction tube and stir carefully in order to disperse the centrifuged solids. Complete the extraction using the same precautions as in the first extraction. Three extractions are usually sufficient, but for samples such as chocolate liquors, ice cream coatings, and ice cream flakes, containing considerable fat, four extractions are recommended.

Allow the solvent in the beaker to evaporate almost to dryness and then pass a current of air over it, using a rubber bulb aspirator. After the odor of solvent has completely disappeared, cool the beaker in a desiccator and weigh. Report the gain in weight as total fat; it includes not only the saponifiable glyceryl esters but also all other extractable matter.

DISCUSSION OF PROCEDURE

Various materials settle at different rates. Light-sweet and milk coatings settle rapidly, baking chocolate more slowly. The rate of settling for most chocolates appears to be a function of the cocoa matter present. Where difficulties are experienced in obtaining a clear, supernatant liquid, a small portion of finely powdered sugar (1 to 2 grams) is sometimes added to the sample. The fat spreads on the sugar crystals and the increased bulk gives a more rapid-settling material. Sugar is normally added to ground cacao beans and chocolate liquors, directly after weighing the sample. In nearly every instance, clarification requires 5 to 10 minutes, but in some cases only 2 minutes are required. The tubes are usually revolved at a speed of 2500 to 2800 r.p.m., at relative centrifugal forces of 1379 and 1730, respectively.

In evaporating the solvent, the beaker containing the extracted fat is placed on an electric hot plate, maintained at 90° to 100° C. At times extracts boil up so suddenly, because of superheating, that the solution foams over the sides of the beaker, thereby ruining the analysis. To prevent this, the extract may either be brought slowly up to temperature on a cooler segment of the plate, or a strip of asbestos or wire screen may be interposed between the beaker and the hot plate.

Because of the tendency for the fat to creep up the sides, to the top of the beaker, small containers should not be used. The 250-ml. aluminum beaker has been found to give best results. If petroleum ether, in pure or extract form, is added to a hot beaker, the solution will crackle and spatter as it contacts the hot metal. To obviate the possibility of fat loss from this source, the beaker should be removed from the hot plate and cooled slightly before

Table III. Comparison of Total Fat by Centrifuge and A.O.A.C. Methods

Sample	Centrifuging Time at 2500 R.P.M.[a], Min.	Progressive Extraction, Direct Method, % 1st	2nd	3rd	4th	Total	Indirect, %	A.O.A.C., %[b]
Light sweet	2	27.44	0.71	..	..	28.15	28.20	28.20
		27.45	0.69	..	..	28.14	28.17	
		27.39	0.75	0.01	..	28.15	28.30	28.29
		27.41	0.69	0.13	..	28.23	28.20	
	Av.	27.42	0.71	0.03	..	28.16	28.22	28.25
Light sweet	2	27.45	0.70	0.03	..	28.18	28.20	28.23
		27.48	0.68	0.06	..	28.22	28.24	
		27.46	0.67	0.01	..	28.14	28.21	28.31
		27.49	0.71	..	..	28.20	28.32	
	Av.	27.47	0.69	0.03	..	28.19	28.24	28.27
Dark sweet	4–5	32.69	0.97	0.11	..	33.77	33.90	33.85
		32.50	1.33	..	..	33.83	33.82	
		32.70	1.08	..	..	33.78	33.85	33.83
		32.66	1.14	0.02	..	33.82	33.84	
	Av.	32.64	1.13	0.03	..	33.80	33.85	33.84
Dark sweet	4–5	32.22	0.92	0.11	..	33.25	33.31	33.28
		32.28	0.87	0.05	..	33.20	33.36	
		32.26	0.95	0.02	..	33.23	33.28	33.37
		32.30	0.88	0.06	..	33.24	33.34	
	Av.	32.26	0.91	0.06	..	33.23	33.32	33.33
Semi-sweet	8–9	29.43	0.93	0.24	..	30.61	30.74	30.77
		29.43	1.07	0.16	..	30.66	30.88	
		29.92	0.78	0.02	..	30.72	30.81	30.71
		29.82	0.82	0.03	..	30.67	30.79	
	Av.	29.65	0.90	0.11	..	30.66	30.81	30.74
Semi-sweet	8–9	29.65	0.78	0.12	..	30.55	30.64	30.59
		29.72	0.81	0.02	..	30.55	30.75	
		29.75	0.69	0.06	..	30.50	30.76	30.63
		29.68	0.85	0.01	..	30.54	30.61	
	Av.	29.70	0.78	0.05	..	30.53	30.69	30.61
Bitter-sweet	9–10	36.55	0.92	0.10	..	37.57	37.69	37.64
		36.00	1.57	0.06	..	37.63	37.70	
		36.64	0.99	0.04	..	37.67	37.80	37.53
		36.85	0.68	0.05	..	37.58	37.68	
	Av.	36.51	1.04	0.06	..	37.61	37.72	37.59
Bitter-sweet	9–10	36.55	0.88	0.03	..	37.46	37.52	37.43
		36.45	0.97	0.09	..	37.51	37.60	
		36.23	1.22	0.02	..	37.47	37.58	37.49
		36.32	1.04	0.05	..	37.41	37.49	
	Av.	36.38	1.03	0.05	..	37.46	37.55	37.46
Liquor	10–11	47.73	5.53	1.00	0.06	54.32	...	54.52
		47.65	6.06	0.72	..	54.43	...	
		46.44	6.78	0.90	0.14	54.26	...	54.41
		48.62	4.95	0.75	0.15	54.47	...	
	Av.	47.61	5.83	0.84	0.09	54.37	...	54.47
Liquor	10–11	46.66	6.01	1.21	0.25	54.13	...	54.12
		47.04	5.98	0.86	0.13	54.01	...	
		47.91	4.99	0.90	0.18	53.98	...	54.01
		47.51	5.70	0.67	0.06	53.94	...	
	Av.	47.28	5.67	0.91	0.16	54.02	...	54.07
Milk chocolate	3	29.12	0.62	..	..	29.74	29.78	29.89
		29.13	0.74	0.01	..	29.88	29.95	
		28.97	0.79	0.05	..	29.81	29.99	29.80
		29.11	0.68	0.01	..	29.80	29.82	
	Av.	29.08	0.71	0.02	..	29.81	29.89	29.85
Milk chocolate	3	28.33	0.76	0.06	..	29.15	29.18	29.07
		28.46	0.59	..	..	29.05	29.14	
		28.38	0.68	0.04	..	29.10	29.24	29.10
		28.22	0.77	0.03	..	29.02	29.13	
	Av.	28.35	0.70	0.03	..	29.08	29.17	29.09
Breakfast cocoa	6	22.75	0.96	0.13	..	23.84	23.99	23.84
		22.46	1.00	0.24	0.02	23.72	23.86	
		22.28	1.26	0.26	..	23.80	23.97	23.90
		22.06	1.12	0.45	0.11	23.74	23.85	
	Av.	22.39	1.09	0.27	0.03	23.78	23.92	23.87
Ice cream coating	6–7	51.89	2.27	0.23	..	54.39	54.49	54.47
		52.31	1.87	0.08	..	54.26	54.46	
		51.83	2.35	0.13	..	54.31	54.55	54.40
		53.18	1.18	0.02	..	54.38	54.42	
	Av.	52.30	1.92	0.12	..	54.34	54.48	54.44

[a] Does not include: 1 minute or less required for stirring sample with petroleum ether in extraction tube; 1 to 1.25 minutes required for centrifuge to come to rest without braking action.
[b] (*1*), Method 1.

any additions are made. This precautionary measure results in only slight loss of time but the danger of spoiling the analysis is avoided.

In transferring extracts to the beaker, it is wise to pour carefully to prevent agitating the centrifuged solids. The tube should be inverted over the beaker and the lip washed with care.

The weight of the sample ordinarily taken for analysis is 2 grams. In the case of low-fat or solvent-extracted cocoas, samples of 10 to 30 grams are advisable. This necessitates the use of a larger extraction tube or cell. A 250-ml. centrifuge bottle has been found practical. The procedure is the same as for the smaller samples, except that 70 to 80 ml. of petroleum ether must be used per extraction. The speed of the centrifuge under these conditions is approximately 2000 r.p.m., or a relative centrifugal force of 1060.

For very rapid determinations it is possible to use what may be termed an "indirect" determination, wherein the tube is weighed and the loss in weight after extraction is accounted for as fat. This procedure cannot be considered as accurate as the "direct" method, but it suffices in many cases. The tube may be dried in a warm atmosphere at 60° to 65° C. Special care must be taken not to overheat the tube, dehydrate the defatted sample, or cause the addition of moisture. Usually the higher the percentage of cocoa matter present, the greater is the inaccuracy of this indirect method.

Table II lists cocoa products usually examined for fat, together with routine plant procedures for their respective analyses.

Table III represents a comparison between the official A.O.A.C. method (*1*) using a Knorr extraction tube and the method outlined above.

SUMMARY

In most cases the centrifuge method compares favorably with the official method. The speed of the centrifuge was set at 2500 r.p.m., but it could easily be increased, depending on the type and condition of the equipment. In progressing through the range of chocolates, from a light-sweet, dark-sweet, semisweet, bittersweet to a chocolate liquor, the time required to obtain a clear extract increases. Time is gained in this method, however, by evaporating the extracts while subsequent extractions are being made. Although the results given by the indirect method are good, unusual care was exercised in these comparative analyses. In normal plant control, the results are not as close, particularly in the case of cocoas, where the high cocoa matter content is sometimes associated with relatively high moistures.

The centrifuge method has been used in the laboratories of Rockwood & Co. for at least 20 years and has proved satisfactory (*36*).

LITERATURE CITED

(1) Assoc. Offic. Agr. Chemists., "Official and Tentative Methods of Analysis," 6th ed., p. 229, 1945.
(2) *Ibid.*, p. 309; *Z. Nahr. Genussm.*, 9, 531 (1905).
(3) Bailey, L. H., and Walker, P. H., *J. Ind. Eng. Chem.*, 6, 497 (1914).
(4) Bielefeldt, J., *Kem. Maanedsblad*, 21, 8–11 (1940); *Chem. Zentr.*, I, 2874 (1940).
(5) Brüchner, R., *Chem. Ztg.*, 66, 229–30 (1942).
(6) Coleman, D. A., and Fellows, H. C., U. S. Dept. Agr., *Dept. Bull.* 1471 (1927); *Tech. Bull.* 71 (1928).
(7) Demikovskiĭ, *Maslobolno Zhirovoe Delo*, 11, 171 (1935).
(8) Ermakov, *Ibid.*, 11, 282 (1935).
(9) Fellenberg, *Mitt. Lebensm. Hyg.*, 25, 316 (1934).
(10) Francis, A. C., *Bull. offic. office intern. cacao chocolat*, 7, Sond.-Nr., 80–1 (1937); *Chem. Zentr.*, II, 4258 (1937).
(11) Hanus, *Z. Nahr. Genussm.*, 2738 (1906).
(12) Harris, C. P., IND. ENG. CHEM., ANAL. ED., 2, 410 (1930).
(13) Hasse and Bake, *Chem. Ztg.*, 47, 557 (1923).
(14) Heller, *Apoth. Ztg.*, 31, 330 (1916).
(15) Hertwig, *J. Assoc. Offic. Agr. Chemists*, 6, 508 (1923).
(16) Herty, *J. Ind. Eng. Chem.*, 1, 76 (1909).
(17) Hillig, F., *J. Assoc. Offic. Agr. Chemists*, 18, 454 (1935).
(18) Hughes, *Chem. News*, 119, 1041 (1919).
(19) Jansen, G. R., *Bull. offic. office intern. cacao chocolat*, 6, 135–45 (1936); *Chem. Zentr.*, II, 206 (1936).
(20) Joint Rubber Insulation Committee, *J. Ind. Eng. Chem.*, 6, 78 (1914).
(21) Kreutz, *Z. Nahr. Genussm.*, 15, 680 (1908); 16, 584 (1908).
(22) Leithe, W., *Angew. Chem.*, 47, 734–6 (1934).
(23) Leithe, W., and Heinz, H., *Z. Untersuch. Lebensm.*, 72, 414–18 (1936).
(24) Lepper and Waterman, *J. Assoc. Offic. Agr. Chemists*, 9, 46, 461 (1926).
(25) Lewkowitsch, "Chemical Technology of Oils, Fats, and Waxes," 6th ed., Vol. 1, p. 288, New York, Macmillan Co., 1921.
(26) Macara, T., and Hinton, C. L., *Bull. offic. office intern. cacao chocolat*, 7, Sond.-Nr., 86 (1937); *Chem. Zentr.*, II, 4258 (1937).
(27) Micaelli, O., and Desnuelle, P., *Bull. mens. ITERG (Inst. tech. études et recherches corps gras)*, No. 7, 31–3 (1948).
(28) Offut, M. L., *J. Assoc. Offic. Agr. Chemists*, 28, 482–3 (1945).
(29) Richter, *Z. Nahr. Genussm.*, 24, 312 (1912).
(30) Sabine, D. B., *Chemist-Analyst*, 30, 79 (1941).
(31) Soybean Analysis Committee, *Oil and Soap*, 21, 305 (1944).
(32) Stanley, J., IND. ENG. CHEM., ANAL. ED., 9, 132–5 (1937).
(33) Stone, C., *J. Assoc. Offic. Agr. Chemists*, 31, 188 (1948).
(34) Wesson, *Cotton Oil Press*, 4, No. 3, 70 (1920).
(35) Winton and Leach, "Food Inspection and Analysis," 4th ed., p. 54, New York, John Wiley & Sons 1920.
(36) Zenlea, B. J., unpublished papers.

RECEIVED November 3, 1949.

Colorimetric Method for Estimating Small Amounts of Aldrin (Compound 118)

A. A. DANISH AND REX E. LIDOV, *Julius Hyman & Company, Denver, Colo.*

THE synthesis of an alkali-stable highly potent insect toxicant, called aldrin (Compound 118) has been announced recently (*2*). Structurally aldrin is 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene (I). The chemistry and applications of this material have been discussed by Lidov, Bluestone, Soloway, and Kearns (*3*).

The availability of aldrin for wide scale experimentation in the field of agricultural, household, and public health uses makes necessary a method for determining minute amounts such as would be present in spray or dust residues on plants and in biological fluids and tissues.

An analytical procedure based on the bicycloheptene structure of aldrin offers a more or less specific method, and is the subject of this paper.

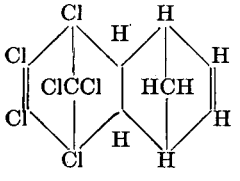
Phenyl azide reacts at the double bond of the halogen-free bicycloheptene ring of aldrin to form a phenyldihydrotriazole derivative (aldrin-phenyldihydrotriazole, II). This type of reaction has been discussed by Alder and Stein (*1*).

The triazole derivative, in turn, produces an intensely colored substance, III, with a diazonium compound under the experimental conditions devised for this analysis. The reactions leading to the formation of the colored substance have not yet been fully elucidated, but it appears that opening of the triazole ring as

A colorimetric procedure is described for the determination of small amounts of aldrin (Compound 118, 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene). Reaction with phenyl azide to form a dihydrotriazole derivative and subsequent treatment with diazotized dinitroaniline in strongly acid medium produce an intense red color. Amounts of the insect toxicant of 10 to 40 micrograms in the final 10-ml. aliquot are readily determined with a spectrophotometer. Commonly used insect toxicants do not interfere.

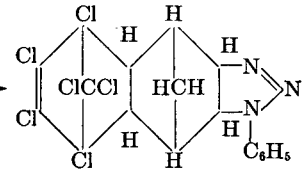
well as coupling is involved. The chemistry of the color formation will be discussed in another publication.

Aldrin
I



+ $C_6H_5N_3$ →

Aldrin-phenyldihydrotriazole
II



II + dinitrophenyl diazonium salt → colored compound (III)

The aforementioned series of reactions provides a basis for a colorimetric analytical method for aldrin in which the commonly used agricultural chemicals do not interfere. The procedure described herein permits the estimation of as little as 10 micrograms of aldrin, and has been successfully applied to the analysis of this insect toxicant in insecticidal dusts, in film residues on glass and paper, in human and animal urine, and in mixture with other insecticides. Application of this procedure to the determination of aldrin in milk and in spray and dust residues on plants appears promising.

METHOD

The analytical procedure for aldrin consists of the following basic steps:

1. Quantitative formation of aldrin-phenyldihydrotriazole (II) by heating aldrin with an excess of phenyl azide liquid, in absence of solvent, and then removing unreacted azide with a mechanical vacuum pump. (Because of the small amounts of aldrin and phenyl azide reacting, the presence of conveniently measured amounts of solvent makes the rate of dihydrotriazole formation too slow to be practicable.)
2. Treating the triazole residue in alcoholic hydrochloric acid with an excess of diazotized 2,4-dinitroaniline, and then acidifying strongly to produce an intensely red colored compound (III). (The presence of hydrochloric acid is essential. Other strong acids, in the absence of hydrochloric acid, do not lead to the desired color formation.)
3. Evaluation of the developed red color, using a spectrophotometer and a standard transmittance-concentration curve from data on known amounts of purified aldrin-phenyldihydrotriazole.

Purified aldrin-phenyldihydrotriazole, prepared from aldrin and phenyl azide, was used for plotting a standard optical transmittance curve.

A stock solution of the triazole in hexane was made up and diluted to various strengths, and 1.0-ml. aliquots of the diluted solutions were carried through the procedure described below. The transmittance of the colored solutions obtained from 10 to 50 micrograms of the aldrin-phenyldihydrotriazole was plotted against concentration to make a standard curve as shown in Figure 2. In subsequent analyses, the amount of aldrin is readily calculated from the amount of dihydrotriazole formed.

Absorption curves of the colored solutions were run on a Beckman spectrophotometer using 1.00-cm. quartz cells. A typical curve, shown in Figure 1, has an absorption maximum at about 515 millimicrons. The Coleman Junior spectrophotometer was used for routine determinations of aldrin throughout this study.

PREPARATION OF STANDARD ALDRIN-PHENYLDIHYDROTRIAZOLE CURVE

Reagents. Phenyl Azide (freshly distilled), boiling point 49–50° C., at 5 mm. The phenyl azide was prepared according to the method of Lindsay and Allen (*4*).

Aldrin. A material recrystallized from methanol and melting at 100–102° is satisfactory. A sample of crystalline aldrin may be obtained from Julius Hyman & Company, Rocky Mountain Arsenal, Denver, Colo.

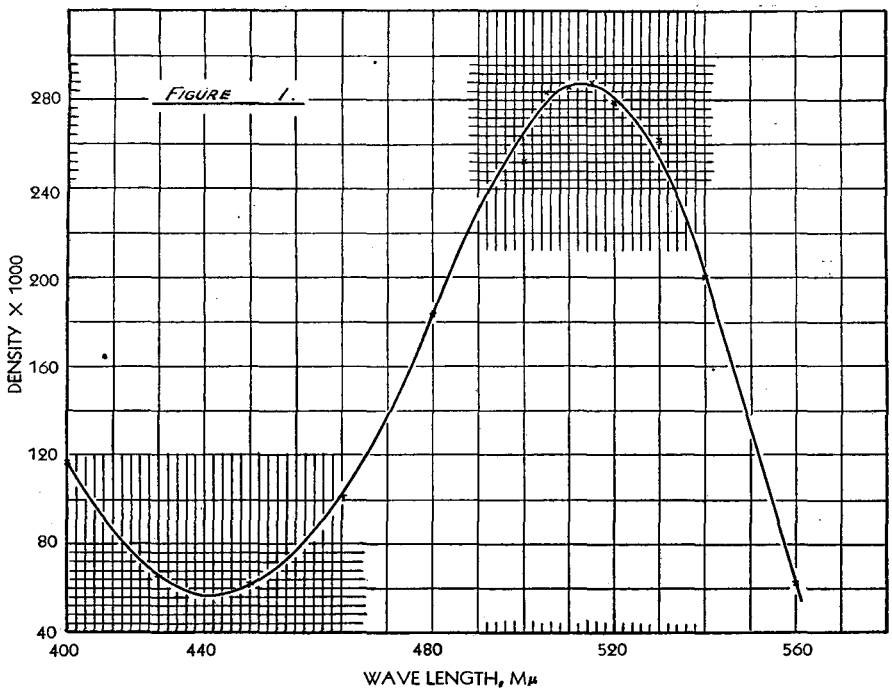
Crystalline Aldrin-phenyldihydrotriazole. The dihydrotriazole is prepared by refluxing a solution containing 10.0 grams of aldrin and 4.0 grams of phenyl azide in 30 ml. of hexane for 40 minutes. The dihydrotriazole begins to precipitate out during the refluxing period. The white crystalline solid is collected by suction filtration, recrystallized once from 1 to 5 benzene–ethyl alcohol, and dried in a vacuum desiccator. Melting point is 174° C. (decomposes). (The melting point is determined by plunging the capillary tube containing the triazole in the melting point bath at 164° and heating at the rate causing a 2° rise in temperature per minute.) Purity may be determined by total nitrogen analysis (theory is 8.67%).

Other reagents used are: 2,4-dinitroaniline, Eastman; sodium nitrite, finely powdered, reagent grade; concentrated hydrochloric acid; acetone, C.P.; phosphoric acid, 85%, C.P.; absolute ethyl alcohol; concentrated sulfuric acid, C.P.; hexane, redistilled (Skellysolve B, b.p. 60° to 70°); and sulfuric acid, 2 to 1, 2 volumes concentrated sulfuric acid with 1 volume distilled water.

Color Diluting Solution. This is prepared by mixing 5 volumes of absolute ethyl alcohol, 1 volume of concentrated hydrochloric acid, and 4 volumes of 2 to 1 sulfuric acid. It is made up just before use. (In the equation for calculation of results, this solution is referred to as Solution B.)

Diazotized 2,4-Dinitroaniline. A slight modification of Schoutissen's procedure is employed (*5*). A solution of 1.5 grams of 2,4-dinitroaniline in 30.0 ml. of concentrated sulfuric acid is prepared by heating to 90°. The solution is cooled to 0° and then 0.7 gram of finely powdered sodium nitrite is sifted onto the sulfuric acid solution at 0°. After standing for 1 to 2 hours, at a temperature below 15°, the sodium nitrite dissolves. To the resultant solution, 40.0 ml. of 85% phosphoric acid are added with stirring. The temperature should not exceed 40° during the phosphoric acid addition. After standing at room temperature for an additional 2 hours, the preparation is ready for use. The final solution is pale yellow and is stable at room temperature for at least a week. On prolonged standing the reagent solution darkens progressively to a deep orange, and should be discarded.

Stock Standard Solution of Aldrin-phenyldihydrotriazole. Exactly 0.1000 gram of aldrin-phenyldihydrotriazole is dissolved in 5 ml. of acetone and enough hexane is added to make 1000 ml. One milliliter contains 100 micrograms.



STOCK STANDARD SOLUTION OF ALDRIN. Exactly 0.1000 gram of aldrin is dissolved in hexane to make 1000 ml. of solution. Each milliliter contains 100 micrograms of aldrin.

Apparatus. A Coleman Junior Model 6A spectrophotometer, with tubular cells 9 mm. in diameter is used (Catalog No. 6-302).

Beakers; 10 to 1000-ml. volumetric flasks; 1-ml. volumetric pipet, serological pipet; steam bath; vacuum desiccator; vacuum pump; and water aspirator are also required.

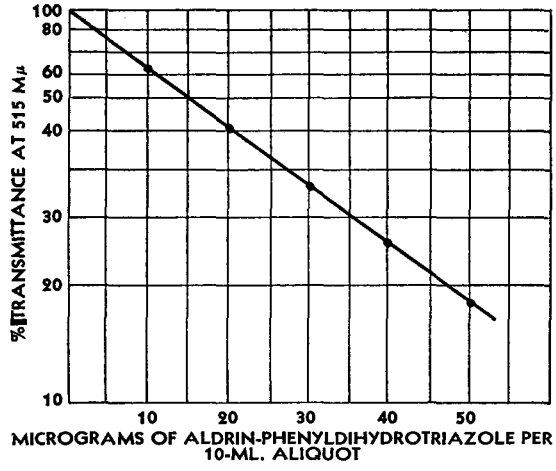


Figure 2

Procedure A. Five aliquots of the triazole stock solution of 5, 10, 15, 20, and 25 ml. are introduced into 50-ml. volumetric flasks. Each aliquot is diluted to 50 ml. with hexane, making working standards equivalent to 10, 20, 30, 40, and 50 micrograms of triazole per ml., respectively. Using a volumetric pipet, 1 ml. of each working standard is quantitatively transferred to a cylindrical spectrophotometer cell and 1.0 ml. of hexane, which serves as a blank, is placed in another cell. Two drops of phenyl azide (30 to 40 mg.) are added to each cell, care being taken that none of the azide liquid strikes the glass walls.

The hexane is evaporated completely at room temperature by placing the cells in a vacuum desiccator attached to a water aspirator. The evaporation requires 10 to 20 minutes. A drop of oily residue remains. This evaporation is handled conveniently by placing the cells in a beaker containing a 2.5-cm. level of mineral oil at room temperature and transferring the whole to the desiccator. The oil bath prevents excessive cooling of the hexane solution during the evaporation.

After complete removal of solvent, the vacuum is broken gently and the colorimeter cells are then heated in an oven at 75° to 80° C. for exactly 30 minutes. It is during this heating period that, in an actual analysis, any aldrin is quantitatively converted to the triazole.

The excess phenyl azide is then removed in vacuo at 1 to 2 mm. by almost completely immersing each cell in a beaker of water at 45° to 50° and attaching each cell directly to the vacuum pump for 3 minutes. The nearly colorless film residue at the bottom of the cell is now ready for coupling with diazotized dinitroaniline.

The film residue is dissolved in 5.0 ml. of absolute ethyl alcohol and then 1.0 ml. of concentrated hydrochloric acid and 0.3 ml. of diazotized dinitroaniline solution are added. A serological 1.0-ml. pipet, graduated in tenths, is used to measure out the diazotized dinitroaniline solution. The solutions are mixed well and allowed to stand for 20 minutes, during which an orange color develops. Finally, 3.7 ml. of 2 to 1 sulfuric acid are added slowly to each solution to make a final volume of 10 ml. The solutions are mixed well and allowed to stand for at least 3 minutes. An intense red color is produced at this point. In the equations for calculation of results, this solution is called Solution A. The blank solution is used to set the galvanometer index line at 100% transmittance at 515 millimicrons, and the transmittance of each red solution is measured. The standard curve is prepared by plotting per cent transmittance against concentration expressed as micrograms of aldrin-phenyldihydrotriazole per 10 ml.

DETERMINATION OF ALDRIN IN HEXANE SOLUTIONS

Reagents and apparatus are the same as those used in the preparation of the standard curve.

Procedure B. A hexane solution of aldrin is diluted or concentrated so as to bring the aldrin content within a range of 15 to 150 micrograms per ml. In cases where the hexane solution requires concentration, the evaporation is carried out in a beaker on a steam bath with a gentle stream of air passing over the surface. The concentrated or diluted solution of aldrin is washed with hexane into a volumetric flask made up to volume with the hexane washings. One milliliter of the adjusted aldrin solution is precisely measured into a spectrophotometer cell, 2 drops of phenyl azide are added, and the dihydrotriazole is quantitatively formed and then treated with diazotized dinitroaniline to produce the red color as in the preparation of the standard curve. A blank, starting with 1.0 ml. of hexane and 2 drops of azide, is run at the same time.

The final blank solution is set at 100% transmittance and the transmittance of the test solution is then measured. Reading from the standard curve, one obtains the number of micrograms of triazole.

CALCULATION OF RESULTS

The percentage of aldrin present in the samples analyzed can be obtained by means of the following equations.

$$\% \text{ aldrin} = \frac{\text{micrograms of dihydrotriazole read from standard curve} \times \left(\frac{\text{volume of Solution A} + \text{volume of Solution B}}{\text{volume of Solution A}}\right) \times \text{aliquot ratio} \times 0.754}{\text{grams of test sample} \times 10{,}000}$$

The 0.754 factor represents the ratio of the molecular weight of aldrin to that of the aldrin-phenyldihydrotriazole. Thus:

$$\text{Micrograms of aldrin} = \text{Micrograms of triazole} \times 0.754$$

Using this conversion factor, the standard aldrin-phenyldihydrotriazole curve may be replotted, if desired, to read directly in micrograms of aldrin. In that case the calculation becomes:

$$\% \text{ aldrin} = \frac{\text{micrograms of aldrin read from standard curve} \times \left(\frac{\text{volume of Solution A} + \text{volume of Solution B}}{\text{volume of Solution A}}\right) \times \text{aliquot ratio}}{\text{grams of test sample} \times 10{,}000}$$

The factor in parenthesis is omitted when Solution A is not diluted with Solution B.

The aliquot ratio is the ratio of the total volume of the solution containing aldrin to the volume of that solution taken for analysis.

The conversion of micrograms to grams requires a factor of 10^{-6}. The conversion of the analytical result to a percental basis requires multiplication by 10^2. The figure 10,000 in the denominators of the above equations accounts for these two factors.

NOTES ON PROCEDURE

On heating, phenyl azide decomposes partially to give nonvolatile products which form colors with diazonium compounds. To minimize the presence of these impurities, the quantity of phenyl azide is limited to 2 drops in the triazole formation step.

If the color intensity of the final solution corresponds to more than 50 micrograms of triazole (15% transmittance), dilution of the sample and blank with color diluting solution will give a transmittance value within a more desirable range for color intensity measurement. In equations for calculation of results the color diluting solution is called Solution B. Should it be found that more than 250 micrograms of dihydrotriazole had been present before dilution, the entire analysis should be repeated, after readjusting the concentration of aldrin test solution to 20 to 40 micrograms per ml., using hexane as the diluent. This step is necessary because the amount of phenyl azide in the first determination may have been insufficient for quantitative conversion of all the aldrin to the dihydrotriazole. On the other hand, should the transmittance be equivalent to less than 20 micrograms of dihydrotriazole, the analysis should also be repeated, starting with 2 or 3 ml. of the hexane solution of aldrin and 2 drops of phenyl azide, evaporating the solvent in a vacuum desiccator, and continuing the determinination as in the preparation of the standard curve.

The transmittance readings should be taken within 5 to 10 minutes after red color development. The developed red color is stable on standing up to at least 2 hours when working with crystalline dihydrotriazole only; however, when phenyl azide is used, as it must be in an actual analysis for aldrin, some of its nonvolatile thermal decomposition products develop colors on standing.

It appears that the decomposition of phenyl azide is significantly accelerated by direct exposure to light. Consequently, during all steps of the procedure in which phenyl azide is present, including that of its removal in vacuo, exposure of the reaction mixtures to direct light must be avoided.

The phenyl azide reagent should be stored in the cold and distilled on the day it is used in order to minimize extraneous color formation.

Complete removal of excess phenyl azide after triazole formation is necessary to ensure reproducible results.

From the manipulative standpoint, the critical step lies in the vacuum evaporation of the solvent from the solution of aldrin and the two drops of phenyl azide. Care must be observed that no foaming or undue chilling occurs during the evaporation; undue chilling may cause some aldrin to crystallize out of contact with the phenyl azide and prevent quantitative formation of the dihydrotriazole.

The application of vacuum and its release must at all times be gradual, so that none of the crystalline aldrin or its dihydrotriazole is swept out by a surging air stream. A three-way stopcock may be conveniently used for this purpose.

The aliquots of aldrin solution should be pipetted at the same temperature as that at which it was made up to volume.

DISCUSSION

Hexane was found to be a convenient extracting solvent for insecticidal dusts containing aldrin.

To determine whether any loss of aldrin occurs on evaporation, a stock solution of aldrin in hexane was made up, and aliquots containing 20 to 250 micrograms of aldrin were measured into tall-form beakers and diluted to 250 ml. with hexane. The solutions were evaporated to 10 ml. on a steam bath with a jet of air passing gently over the liquid surface. Following the same procedure, solutions of aldrin of varying concentrations were reduced to convenient final volumes. After concentration, in the manner described, the aldrin content of the final concentrates was determined.

The data of Table I show a 2 to 6% loss of aldrin, depending on the volume of hexane evaporated. The loss is roughly 2 to 3% for each 250 ml. of hexane evaporated. Where a 94 to 98% recovery is not sufficiently accurate for the objectives sought, a correction can be applied to compensate for evaporative loss.

The complete procedure was tested by analyzing hexane solutions containing known amounts of aldrin. The recoveries, shown in Table II, average about 96.5%.

To test further the accuracy of the method, synthetic mixtures of aldrin with its insecticidally active epoxide derivative, Compound 497, were prepared (*2*). The results, shown in Table III, indicate that aldrin can be determined accurately in the presence of gross amounts of structurally related material.

This colorimetric procedure is applicable to the estimation of aldrin in cow's urine.

To 100-gram batches of 1-day-old samples of cow's urine were added 0.05, 0.1, and 0.5 mg. of aldrin in acetone to give 0.5, 1.0, and 5.0 p.p.m., respectively. The urines were then extracted with

Table I. Recovery of Aldrin after Evaporative Concentration of Hexane

Hexane Solution Concentrated, from	Aldrin, γ		Recovery, %
	Added	Recovered	
250 to 10 ml.	20	19	95
	50	49	98
	100	97	97
	125	120	96
	150	142	95
	200	188	94
	250	241	96
500 to 50 ml.	1000	920	92
500 to 25ml.	500	460	92
100 to 10 ml.	100	97.5	97.5

Table II. Analyses of Known Amounts of Aldrin

Taken for Analysis, γ	Found Present, γ	Recovery, %
10	8.8	88.0
15	14.5	96.5
20	19.3	96.5
25	24.2	96.0
30	28.8	96.0
35	35.4	101.0
40	40.0	100.0

Table III. Analyses of Known Amount of Aldrin in Compound 497

Aldrin Added to Compound 497, %	Aldrin Found, %
3.8	4.5
16.0	16.6
25.3	25.8

Table IV. Recovery of Aldrin Added to Cow's Urine

Aldrin Added, P.P.M.	Aldrin Found, P.P.M. Uncorrected for blank	Aldrin Found, P.P.M. Corrected for blank	Recovery Corrected for Blank, %
0	0.20[a]	...	..
0	0.20[a]	...	..
0.5	0.62	0.40	80
0.5	0.60	0.41	82
1.0	1.10	0.90	90
1.0	1.00	0.93	93
5.0	5.45	4.91	98
5.0	5.36	4.84	97

[a] Calculated as aldrin.

two 50-ml. batches of hexane. Occasional emulsions were broken by centrifuging. The hexane extracts were dried with anhydrous sodium sulfate, filtered, evaporatively concentrated, and analyzed for aldrin as described under Procedure B. The results of these analyses are shown in Table IV. Similar experiments with human urine gave slightly better recoveries.

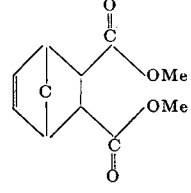
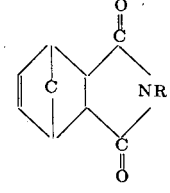
For analysis of aldrin commercial dusts or wettable powders, a preliminary extraction with hexane is necessary. A Soxhlet apparatus containing a sample large enough to be representative is satisfactory. A minimum of 30 minutes' extraction time has been used, and 1 hour is recommended.

INTERFERENCES

The commonly used organic insect toxicants do not interfere in the analysis of aldrin by this new procedure. Hexane solutions of chlordan, DDT, methoxychlor, hexachlorocyclohexane (BHC), and toxaphene treated according to the procedure for determining aldrin gave a pale yellow color similar to that of the blank.

Dimalone [bicyclo-(2.2.1)-5-heptene-2,3-dicarboxylic acid dimethyl ester] and Octacide 264 [the *N*-octyl imide of bicyclo-(2.2.1)-5-heptene-2,3-dicarboxylic acid] do produce a red color with an absorption maximum in the same region as that obtained in the analysis of aldrin. However, because Dimalone is an insect repellent and Octacide 264 is a pyrethrum synergist, neither of these products is likely to be encountered in commercial mixtures of aldrin. The response to the colorimetric test for aldrin of some chemicals commonly used for insect control is listed in Table V.

Table V. Response of Some Substances Used for Insect Control to Color Test for Aldrin

Interfering Substances		Noninterfering Substances
Repellent	Pyrethrum synergist	Chlordan
		DDT
Dimalone	Octacide 264	Methoxychlor
		Hexachlorocyclohexane (BHC)
		Toxaphene
		Compound 497

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Yuji A. Tajima of this laboratory in conducting the spectroscopic determinations and to Phebe Hines for aid in carrying out the analysis.

LITERATURE CITED

(1) Alder, K., and Stein, G., *Ann.*, **485**, 211 (1931).
(2) *Chem. Eng. News*, **26**, 3854 (1948).
(3) Lidov, R. E., Bluestone, H., Soloway, S. B., and Kearns, C. W., *Advances in Chemistry Series*, **1**, 175 (1950).
(4) Lindsay, R. O., and Allen, C. F. H., "Organic Syntheses," Vol. 22, p. 96, New York, John Wiley & Sons, 1942.
(5) Schoutissen, H. A. J., *J. Am. Chem. Soc.*, **55**, 4535 (1933).

RECEIVED April 12, 1949. Presented before the Division of Agricultural and Food Chemistry, Symposium on Economic Poisons, at the 115th Meeting of the AMERICAN CHEMICAL SOCIETY, San Francisco, Calif.

Polarographic Determination of O,O-Diethyl O-*p*-Nitrophenyl Thiophosphate (Parathion)

C. V. BOWEN AND FRED I. EDWARDS, JR.

Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Beltsville, Md.

Parathion may be determined quantitatively by means of the polarograph. The electrolysis is carried out in an acetone-water solution with 0.05 *N* potassium chloride as electrolyte, and 0.01% gelatin as suppressor at 25° ± 0.5° C. An accuracy of ±1% is obtained. Several commercial products were analyzed.

THE only reported method for the estimation of *O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate (parathion) is a colorimetric procedure (*1*) based upon the reduction of the nitro group to an amino group with subsequent diazotization and coupling with *N*-(1-naphthyl)-ethylenediamine to produce a color that may be measured. This procedure has had application in the determination of spray and dust residues where 90% recovery is satisfactory, but is not suitable for use in the assay of technical materials. Consequently, reliable and sensitive methods of analysis are greatly needed for this new and highly toxic material in insecticidal formulations. Because the reduction occurs so readily with zinc in the above-mentioned procedure and nitrobenzene (*6*) was the first organic compound to be investigated with the polarograph, it was considered probable that parathion would be easily reduced at the dropping mercury electrode and thus be determined by this means.

APPARATUS

A Sargent Model XXI polarograph was used in this investigation. The reduction was carried out in an H-type electrolysis cell with a saturated calomel reference cell in one arm (*5*). A thermostatically controlled water bath maintained the cell at 25° ± 0.5° C. During the recording of the polarogram the air stirrer was stopped in order to eliminate vibration and the heating system was disconnected at the bench outlet to remove the possibility of stray current effect (*3*). It was observed that other operating

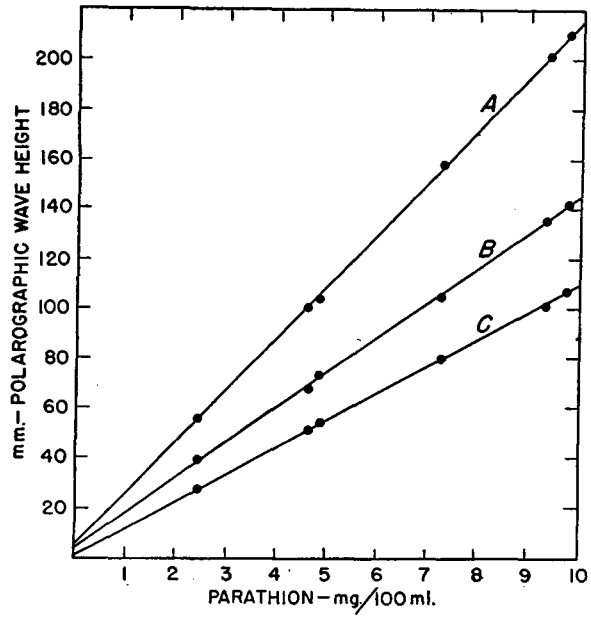


Figure 1. Standard Curves for Parathion Determination

Sensitivity, microampere per millimeter, *A*, 0.020; *B*, 0.030; *C*, 0.040

electrical appliances, such as hot plates on the same bench, had a stray current effect on the polarograph.

PREPARATION OF STANDARD CURVES

The *O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate used in the preparation of the standard curves was obtained by isolation from a high-purity technical parathion according to the method devised by Edwards and Hall (*2*). It was a crystalline material that melted sharply at 6° C. The physical constants were in agreement with those published by Fletcher *et al.* (*4*).

A sample of 0.4863 gram of this purified *O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate was dissolved in acetone to make 1 liter of standard solution. A 20-ml. aliquot, containing 9.73 mg., was placed in a 100-ml. volumetric flask and 30 ml. of acetone were added. Then 0.35 gram of potassium chloride and 0.6 gram of acetic acid were dissolved in about 25 ml. of water and added to the acetone solution; 0.01 gram of gelatin was dissolved in a few milliliters of water by warming, cooled, and added to the above, and the solution was brought to the mark with water. (This solution is 0.05 *N* with respect to potassium chloride, and 0.1 *N* with respect to acetic acid, and contains 0.01% of gelatin and 50% of acetone in water.) The acetic acid was added to prevent any hydrolysis of the ester during the electrolysis.

The sample side of the H cell was emptied and rinsed by means of suction without being removed from the thermostat bath. The used mercury was retained in the suction flask. The cell was rinsed well with acetone and then with a portion of the sample solution before being filled with the sample solution. Prior to the electrolysis oil-pumped nitrogen was bubbled through the sample solution for 10 minutes to remove dissolved oxygen. The nitrogen was passed through a 1 to 1 acetone-water solution before it reached the sample solution. For electrolysis the dropping mercury electrode was placed firmly in the cell and the polarograph set to record the wave at 0 to −1.5 volts at a sensitivity of 0.020 microampere with maximum damping. Waves were recorded in duplicate for 0.020-, 0.030-, and 0.040-microampere sensitivity to allow for considerable leeway in the size of the sample.

The sensitivities of the polarograph refer to the microamperes corresponding to 1-mm. deflection of the recorder. Polarographic waves were obtained in the same manner for 7.29-, 4.86-, and 2.43-mg. samples of parathion. Figure 1 shows the average wave height for from 2 to 10 determinations at each concentration plotted against the concentration to give the standard curves.

After some of the standardization polarograms had been obtained, it was decided that considerable time could be saved by using an aqueous stock solution of twice the normality of potassium chloride and acetic acid desired in the sample to be analyzed instead of weighing these materials for each determination. The gelatin was weighed fresh each day.

Table I. Analysis of Commercial Samples of Technical Parathion

Sample No.	Parathion Found, %	Sample No.	Parathion Found, %
1	84.7	4	93.1
	83.9		92.0
	85.3		93.2
	Av. 84.6		Av. 92.8
2	92.3	5	94.4
	91.5		93.5
	92.0		95.3
	Av. 91.9		Av. 94.4
3	98.1		
	97.5		
	97.7		
	Av. 97.8		

Table II. Analysis of Parathion Formulations

Material	Parathion Present, %	Parathion Found: Soxhlet extraction, %	Parathion Found: Flask extraction, %
Commercial dusts			
Sample 1 (25%)	...	...	24.0
			24.0
			24.1
			Av. 24.0
Sample 2 (25%)	...	24.4	24.0
		24.1	24.1
		24.4	24.0
		Av. 24.3	Av. 24.0
Sample 3 (1%)	...	...	0.97
			0.92
			0.94
			Av. 0.94
Wettable powders			
Sample 1 (25%)	...	...	24.0
			23.7
			23.9
			Av. 23.9
Sample 2 (25%)	...	23.3	23.9
		23.3	23.7
		23.6	23.8
		Av. 23.4	Av. 23.8
Synthetic dusts			
Sample 1	10.0	...	9.7
			9.7
			9.8
			Av. 9.7
Sample 2	12.2	12.2	
		12.0	
		12.1	
		Av. 12.1	
Sample 3	29.1	29.2	
		29.3	
		29.3	
		Av. 29.3	

ANALYSIS OF COMMERCIAL PRODUCTS

Technical parathion samples were analyzed in the same manner as the standard samples (Table I). For dust formulations at least 1 gram was taken for a sample and made to volume with acetone to obtain an extract containing approximately 1 mg. per ml. of parathion based on the manufacturer's claims. The sample was shaken intermittently for 1 hour, and allowed to stand for 10 minutes, and a portion was centrifuged in a glass-stoppered tube until clear. An aliquot of this clear solution to contain approximately 10 mg. was taken and the procedure described was followed. The recovery of parathion from dusts by this procedure was checked by extracting two of these commercial dusts in a Soxhlet apparatus. The results were found to be within the limits of accuracy of the method, as shown in Table II. Dusts of known parathion content prepared in this laboratory were

analyzed after Soxhlet extraction, and the recovery as shown in Table II was found to give results also within the limits of accuracy of the method.

DISCUSSION

Normal curves for the polarograms were obtained with the technical materials as well as with the purified sample. The decomposition potential of −0.30 volt and a half-wave potential of −0.39 volt were obtained against the saturated calomel electrode.

p-Nitrophenol, which is a major contaminant of the technical parathion, does not reduce at the dropping mercury electrode until after the parathion has been completely reduced, and consequently does not interfere with the curve obtained in the analysis. The decomposition and half-wave potentials for *p*-nitrophenol under the conditions for the determination of parathion were found to be −0.45 and −0.68 volt, respectively.

Diethyl *p*-nitrophenyl phosphate, the oxygen analog of parathion, was investigated to ascertain whether it would interfere, if present, in the determination of parathion. It was found, however, under the conditions used in this method to have a decomposition potential of −0.37 volt and a half-wave potential of −0.47 volt.

A mixture consisting of one third parathion and two thirds oxygen analog, instead of giving the anticipated broken wave beginning at the decomposition voltage for parathion, gave a normal curve with a decomposition potential of −0.34 volt. This indicates an interference if small amounts of the oxygen analog should be present, a situation not likely to occur with present methods of synthesis (*4*).

The polarographic method of analysis of parathion as described here has an accuracy of ±1%, and 2 mg. of *O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate per 100 ml. of solution are apparently a minimum concentration for the sensitivities investigated. However, the polarograph used is equipped with resistors, so that a sensitivity of 0.003 microampere per millimeter may be used. At this sensitivity it would be possible to obtain a sufficient wave height to determine parathion at a concentration of less than 1 p.p.m.

LITERATURE CITED

(1) Averell, P. R., and Norris, M. V., ANAL. CHEM., **20**, 753–6 (1948).
(2) Edwards, F. I., and Hall, S. A., *Ibid.*, **21**, 1567 (1949).
(3) English, F. L., *Ibid.*, **20**, 889–91 (1948).
(4) Fletcher, J. H., Hamilton, J. C., Hechenbleikner, I., Hoegberg, E. I., Sertl, B. J., and Cassaday, J. T., *J. Am. Chem. Soc.*, **70**, 3943–4 (1948).
(5) Kolthoff, I. M., and Lingane, J. J., "Polarography," New York, Interscience Publishers, 1946.
(6) Shikata, M., *Trans. Faraday Soc.*, **21**, 42–52 (1925).

RECEIVED October 1, 1949. Presented before the Division of Agricultural and Food Chemistry, Symposium on Economic Poisons, at the 115th Meeting of the AMERICAN CHEMICAL SOCIETY, San Francisco, Calif.

Quantitative Determination of Certain Flavonol-3-glycosides

By Paper Partition Chromatography

THOMAS B. GAGE AND SIMON H. WENDER, *University of Oklahoma, Norman, Okla.*

Binary mixtures of rutin, quercitin, isoquercitin, robinin, and xanthorhamnin and ternary mixtures of rutin, quercitin, and isoquercitin have been separated quantitatively by paper partition chromatography. The mixtures, containing 10 to 40 micrograms of each pigment, were chromatographed on Whatman No. 1 filter paper with *n*-butanol–acetic acid–water (40-10-50 volume %). For absorption spectra determinations, blank filter paper strips were carried through the chromatographic procedure. The individual pigment zones (located under ultraviolet light) and corresponding areas of the blank strips were cut out and extracted by capillary leaching for 1 hour with 0.5% aqueous aluminum chloride solution. The optical density of the flavonoid–aluminum chloride complexes at their respective absorption maxima was determined with the Beckman Model DU spectrophotometer. Per cent recoveries, calculated on the ratio of optical density of each recovered pigment to the optical density of the aluminum chloride complex (containing the same initial amount of nonchromatographed flavonoid pigment), ranged between 92 and 95%. The flavonoid standards may each be carried through the chromatographic procedure on individual paper strips and the aluminum chloride complex formed by the leaching process. This procedure adjusts the standard for the losses occurring on the filter paper during chromatography. Recoveries of 98 to 101% have been calculated for mixtures after correcting in this manner for losses occurring during chromatography.

SEVERAL approaches to the quantitative determination of flavonoid pigments have been offered in recent years. Porter, Brice, Copley, and Couch (*4*) have developed a spectrophotometric assay method for mixtures of rutin and quercetin based upon their absorption maxima in the ultraviolet region. This method is excellent for samples known to contain only rutin and quercetin, but it has not yet been adapted for mixtures where other flavonoid pigments are also present. A colorimetric method of analysis for total content of quercetinlike substances has been described by Wilson *et al.* (*7*). This method was later adapted for fluorometric analysis by Glazko and co-workers (*3*). The latter two methods depend upon the color or fluorescence of certain flavonoid pigments in the presence of boric acid and are valuable in the determination of total content of quercetinlike substances. The amounts of individual flavonoid compounds present in mixtures, however, generally cannot be determined by the latter two procedures. Porter, Dickel, and Couch (*5*) have used the absorption spectrum of the rutin–aluminum chloride complex to determine rutin in urine. The authors noted that the presence of other flavonoid pigments interfered with the determination of rutin because of their absorption maxima in the vicinity of the rutin–aluminum chloride complex.

Wender and Gage (*6*) have recently adapted the method of paper partition chromatography (*1*), coupled with the use of chromogenic sprays, to the qualitative separation and identification of mixtures of flavonoid pigments. This method has now

Table I. Separation of Binary Mixtures of Flavonol Glycosides

Flavonoids	Amount Spotted, γ	Wave Length, Mμ	Optical Density Of standards	Optical Density Of recovered pigment	Recovered γ	Recovered %
Rutin	23.1	415	0.153	0.144	21.7	94.2
Quercitin	19.5	408	0.140	0.1325	18.4	94.6
Rutin	23.1	415	0.153	0.142	21.4	92.8
Isoquercitin	16.0	415	0.145	0.1365	15.1	94.1
Quercitin	19.5	408	0.140	0.132	18.4	94.2
Isoquercitin	16.0	415	0.145	0.1365	15.1	94.1
Xanthorhamnin	32.2	409	0.135	0.1275	30.4	94.5
Isoquercitin	16.0	415	0.145	0.1355	14.9	93.4
Robinin	24.0	342	0.112	0.1055	22.6	94.3
Quercitin	19.5	408	0.140	0.133	18.5	95.0
Robinin	24.0	342	0.112	0.105	22.5	93.8
Isoquercitin	16.0	415	0.145	0.129	14.2	88.9

been extended to the quantitative separation and estimation of the individual compounds present in a mixture. The procedure involves: (1) the separation of mixtures containing two or three of the flavonol-3-glycosides—rutin, quercitin, isoquercitin, xanthorhamnin, and robinin—by paper partition chromatography; (2) removal of the pigments from the filter paper by leaching with aqueous aluminum chloride solution; and (3) spectrophotometric assay of the individual aluminum chloride complexes of the flavonoids at their respective absorption maxima. Although the method is not necessarily limited to the five glycosides reported on in this paper, it did not work satisfactorily with the aglycones of these pigments.

MATERIALS AND REAGENTS

Aluminum chloride, 0.5% aqueous solution.

Butanol–acetic acid–water. Forty milliliters of *n*-butanol, 10 ml. of glacial acetic acid, and 50 ml. of distilled water were well mixed and the two layers separated.

Flavonoid stock solutions. Solutions of the five flavonol-3-glycosides included in this report were prepared in 95% ethyl alcohol with the following concentrations: rutin, 0.925 mg. per ml.; quercitin, 0.806 mg. per ml.; xanthorhamnin, 0.967 mg. per ml.; isoquercitin, 0.50 mg. per ml.; robinin, 0.50 mg. per ml.

PROCEDURE

Whatman No. 1 filter paper strips, 2.5 × 56 cm., were spotted 8 cm. from one end with 23.1 micrograms of rutin and 19.5 micrograms of quercitin in 95% ethly alcohol solution. The strips were allowed to dry and were then placed in the upper troughs of one-dimensional chromatogram chambers (*6*). Additional blank strips were inserted in the chambers to provide solvent blanks for the spectrophotometric assays. The lower trough of each chamber had been previously filled with the water-rich layer of the 3-component system, 40% *n*-butanol, 10% glacial acetic acid, and 50% distilled water. The upper trough was filled with the alcohol-rich layer of the above solvent system and the chamber was sealed air-tight by means of a securely weighted ground-glass plate. The strips were irrigated for 13 to 14 hours, during which time the solvent moved a total distance of 38 to 40 cm. beyond the line marking the initial location of the pigments. After this period, the strips were removed from the chambers and air-dried on racks.

When the strips had dried, the solvent front and pigment zones were located by their characteristic fluorescence in ultraviolet light. Each pigment zone was outlined lightly with pencil while under the ultraviolet lamp. The sections of filter paper containing the pigment zones were cut out of the paper strips, allowing a sufficient amount of paper at each end of the pigment zones for convenience in handling. Corresponding sections from the blank strips were also removed. The paper segments were shaped to a point at one end and suspended in the leaching chamber as shown in Figure 1. A watch glass (11-cm. diameter), fitted with a filter paper circle of the same size and filled with 0.5% aqueous aluminum chloride solution, served as a solvent trough. Two Petri dishes were used to support the watch glass at an appropriate height. The end of each paper segment was held in place against the edge of the filter paper circle by means of capillary attraction between the two wet paper surfaces. The pointed end of each paper strip was inserted in a 5-ml. beaker. The aluminum chloride solution moved by capillary action down the filter paper strips, carrying with it the flavonoid glycoside in the form of the aluminum chloride complex. The blank filter paper strips were also leached in this manner to provide solvent blanks for the Beckman Model DU spectrophotometer.

The leaching chamber was covered with a glass dome and the leaching process was allowed to continue for 30 to 45 minutes. The cover was then removed and the strips were carefully washed with a few microdrops of the leaching solvent applied with a micropipet. The volume of solution required to remove each pigment from the filter paper segments amounted to about 2 ml. The extracts were transferred to 5-ml. volumetric flasks by means of micropipets. The beakers were rinsed with the aluminum chloride solution and the washings added to the volumetric flasks. The flasks were made up to volume with 0.5% aluminum chloride solution and the optical density of each solution was determined at the wave length of its absorption maximum in the region, 340 to 420 mμ (Table I). The leachings from the blank filter paper strips were used for solvent blanks in the spectrophotometer.

For the purpose of calculating the per cent recovery of each flavonoid pigment, 5-ml. samples of the aluminum chloride complexes of rutin and quercitin were prepared, using the same initial amount of each pigment (23.1 micrograms of rutin and 19.5 micrograms of quercitin). The optical density of each of these samples was determined in the spectrophotometer at the wave

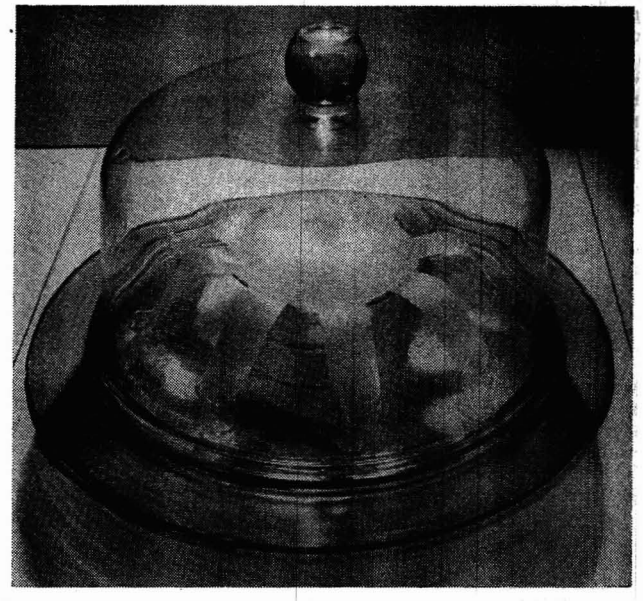


Figure 1. Photograph of Leaching Chamber

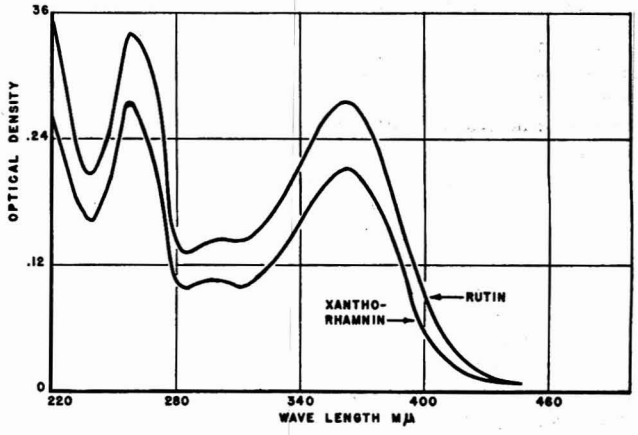


Figure 2. Absorption Spectra of Rutin (0.00825 Gram per Liter) and Xanthorhamnin (0.00967 Gram per Liter) in 95% Ethyl Alcohol Solution

1-cm. cells

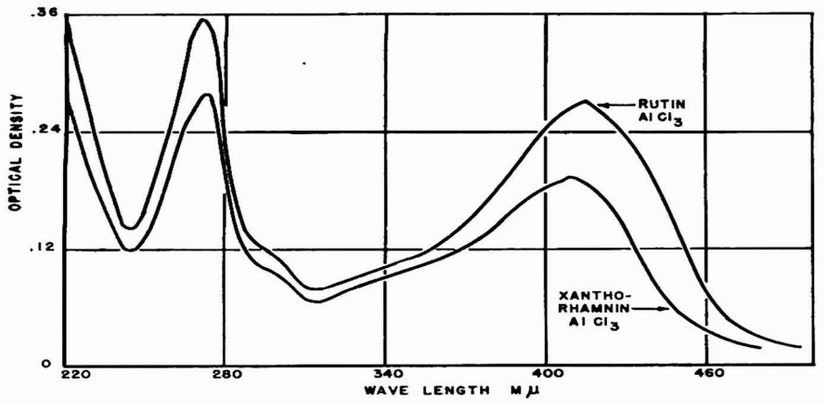


Figure 3. Absorption Spectra of Rutin and Xanthorhamnin in 0.5% Aqueous Aluminum Chloride Solution

1-cm. cells

length of its absorption maximum in the region, 340 to 420 mμ (Table I). The ratio of optical density of the chromatographed and separated flavonoid complex to the optical density of the nonchromatographed pigment provided a measure of per cent recovery of each pigment (Table I).

In the same manner, binary mixtures of rutin and isoquercitin, quercitin and isoquercitin, xanthorhamnin and isoquercitin, robinin and quercitin, and robinin and isoquercitin were separated by paper partition chromatography and the amount of each pigment recovered from the filter paper in the form of the aluminum chloride complex was determined (Table I).

Ternary mixtures of rutin, quercitin, and isoquercitin were also prepared and separated by this method (Table II).

Calculations. The extinction coefficient for each pigment was calculated from the optical density of the standard samples by the following equation:

$$K = \frac{D_o}{C \times L}$$

where K is the extinction coefficient at the wave length of the absorption maxima listed in Tables I and II; D_o is the optical density of the sample; C is the concentration in grams per liter; and L is the light path through the absorption cell in centimeters. The per cent recovery was calculated on the basis of

$$\% \text{ recovery} = \frac{D \times 100}{K \times L \times C} = \frac{D \times 100}{D_o}$$

where D is the optical density of the recovered sample, and D_o is the optical density of the standard sample.

RESULTS AND DISCUSSION

The results of the recovery experiment described in this paper have been collected in Tables I and II. Optical densities and recoveries are averages of duplicate determinations. The recoveries listed in the tables have not been corrected for losses occurring during the chromatographic and leaching procedures. Recovery estimates of 98 to 101% have been obtained when adjustment was made for such losses. To accomplish this, standards were based upon the amount of pigment recovered from individually chromatographed and recovered samples rather than upon the optical density of the nonchromatographed samples. This latter procedure would be appropriate for the estimation of the flavonoid content of plant extracts or other natural products.

The spectra of three of the five flavonol glycosides in ethyl alcohol and in 0.5% aqueous aluminum chloride solution are shown in Figures 2 to 4. The spectra of isoquercitin and quercitin are not shown, because these two pigments exhibit spectra similar to rutin and xanthorhamnin, respectively, in both solvent systems. Alcoholic aluminum chloride solution produces absorption spectra with the five flavonoids which are very similar in shape to the absorption spectra obtained in aqueous aluminum chloride solution. The aqueous medium was used because the pigments were more easily removed from the filter paper segments by the latter solvent.

Recovery of flavonoid pigments from the paper chromatogram strips by leaching with 95% ethyl alcohol has been studied (*2*), but the recoveries have not been as complete.

The absorption spectra of the aluminum chloride–flavonoid complexes are stable over a period of several hours. Because it is possible to leach the flavonoid pigment from the paper strips with aluminum chloride solution in less than 1 hour, no great error is involved in the use of this solvent due to instability of the complex formed.

The flavonol glycosides undergo some decomposition if allowed to remain on the completed chromatogram strips for prolonged periods of time; however, 6 to 12 hours' drying time does not entail any great loss in recovery.

Table II. Separation of Mixtures of Three Flavonol Glycosides

Flavonoids	Amount Spotted, γ	Wave Length, Mμ	Optical Density Of standards	Optical Density Of recovered pigment	Recovered γ	Recovered %
Rutin	23.1	415	0.153	0.142	21.4	92.8
Quercitin	19.5	408	0.140	0.131	18.2	93.6
Isoquercit in	16.0	415	0.145	0.130	14.4	89.7

Attempts to apply this method to the aglycones of rutin, xanthorhamnin, and robinin have not been successful because of difficulties encountered in leaching the complexes from the filter paper strips. The aglycones are much more strongly adsorbed on cellulose, in the presence of aluminum chloride, than the corresponding glycosides.

ACKNOWLEDGMENT

The samples of isoquercitin and robinin were kindly donated by the Pharmacology Laboratory, Bureau of Agricultural and In-

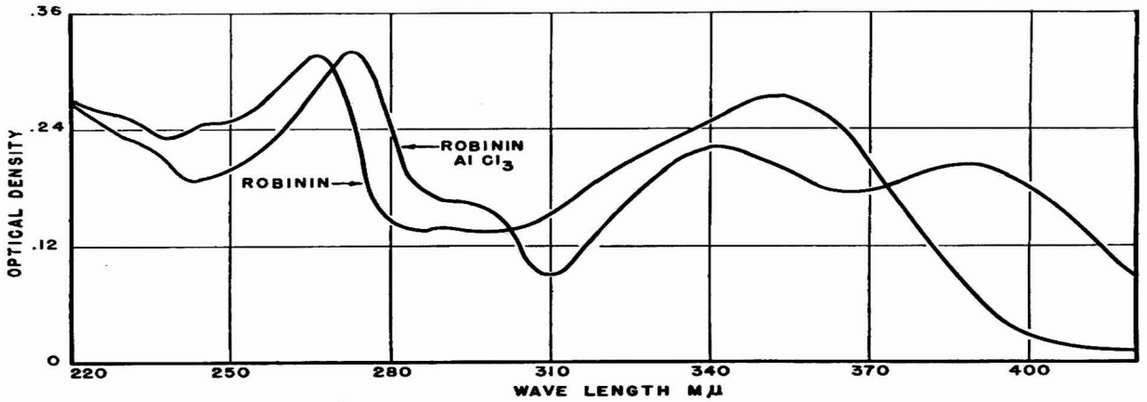


Figure 4. Absorption Spectrum of Robinin (0.0096 Gram per Liter) in 95% Ethyl Alcohol and 0.5% Aqueous Aluminum Chloride Solution

1-cm. cells

dustrial Chemistry, Albany, Calif.; the other pigments were purchased from the S. B. Penick Company, New York, N. Y.

LITERATURE CITED

(1) Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, 38, 224 (1948).
(2) Gage, T. B., and Wender, S. H., *Federation Proc.*, 8 (1), 293 (1949).
(3) Glazko, A. J., Adair, Foster, Papageorge, Evangeline, and Lewis, G. T., *Science*, 105, 48 (1947).
(4) Porter, W. L., Brice, B. A., Copley, M. J., and Couch, J. F., U. S. Dept. Agr., Bur. Agr. Ind. Chem., *Processed Circ.* AIC-159 (July 1947).
(5) Porter, W. L., Dickel, D. F., and Couch, J. F., *Arch. Biochem.*, 21, 273 (1949).
(6) Wender, S. H., and Gage, T. B., *Science*, 109, 287 (1949).
(7) Wilson, C. W., *J. Am. Chem. Soc.*, 61, 2303 (1939).

RECEIVED December 27, 1949. Taken from the dissertation submitted by Thomas B. Gage to the University of Oklahoma in partial fulfillment of the requirements for the Ph.D. degree, June 1950. This investigation was supported by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

Microdetermination of Carbon and Hydrogen in Organic Compounds

JOHN J. NAUGHTON AND MICHAEL M. FRODYMA[1]
University of Hawaii, Honolulu, T. H.

An apparatus and method for the determination of carbon and hydrogen in microsamples of organic compounds are described. The method consists of combustion of the sample in a modified Pregl apparatus, collection of the resulting water vapor and carbon dioxide in a dry ice trap and a liquid air trap, respectively, and, after pumping out the excess oxygen, determination of the water vapor and carbon dioxide manometrically in standard volumes. An analysis of a simple substance can be completed in 16 minutes, whereas a slight procedural modification necessary for nitrogen-containing compounds lengthens the determination to 23 minutes. The accuracy attainable for the determination of carbon is comparable, and of the hydrogen, superior, to the accuracy of the Pregl carbon-hydrogen method.

As a result of work on the determination of small amounts of carbon in steels by a low-pressure combustion method (*8*), it was thought feasible to extend a similar method to the analysis of organic compounds, and to include the determination of hydrogen. The method for carbon in steel was the outgrowth of the work of Yensen (*13*), with important modifications by Wooten and Guldner (*12*) and Murray and Ashley (*7*). It involves the combustion of the induction heated sample in oxygen in an all-glass high vacuum system, collection of the carbon dioxide by condensation in a low temperature trap (liquid air or liquid nitrogen), and after the excess oxygen is pumped out, determination of the carbon dioxide by a pressure measurement in a calibrated volume.

In organic analysis some attempts have previously been made to determine the gaseous products of a dry combustion either volumetrically or manometrically. Hackspill and his co-workers (*3, 4*) suggested heating the organic material with copper oxide in a vacuum and then treating the resultant gases in such a manner as to permit the volumetric determination of carbon, hydrogen, and nitrogen with a single sample. Schwarz (*11*) carried out some preliminary work which seemed to indicate that carbon-hydrogen analysis through the volumetric study of combustion gases was feasible. A novel approach to the problem was that of Berraz (*1*), who burned the sample in a vacuum with copper oxide, collected the products of combustion with a Toeppler pump, and then subjected these combustion gases successively to temperatures of −180° C. (liquid air), −80° C. (dry ice and acetone), and room temperature. By reading the pressure at each of the stages, he was able to determine nitrogen, carbon, and hydrogen with an accuracy comparable to other analytical micro methods. Unfortunately all these methods involve rather elaborate equipment, and are inherently more cumbersome than the gravimetric method.

[1] Present address, Department of Chemistry, George Washington University, Washington, D. C.

APPARATUS

The basic idea in this research was to develop an apparatus for the determination of carbon and hydrogen in organic micro samples, utilizing manometric and vacuum techniques, with as little modification of the familiar and well tried Pregl method as possible. The equipment is shown in Figure 1. The similarity to the conventional Pregl apparatus is readily evident, particularly in the oxygen purification system and the combustion equipment. The normal adsorption tubes are replaced by two freeze-out traps and a manometer. An appended copper–copper oxide furnace, *ml*, is used only for nitrogen-containing compounds. The whole is evacuated with a mechanical oil pump.

Purification of Oxygen. Oxygen from a tank is admitted through a pressure regulator, *a*, of the conventional type. From here it passes through an Ascarite-Anhydrone purifier, *b*, over a copper oxide–filled tube, *c* (50 cm. long × 1.5 cm. in diameter), maintained at about 500° C., through another Ascarite-Anhydrone tower, *d*, and finally through a U-type liquid air trap, *e* (18 cm. deep × 1.5 cm. in diameter at wide leg). The introduction of this liquid air trap is found to effect an improvement in the blank values. When liquid nitrogen is used as the coolant, large amounts of oxygen liquefy in this trap and are tied up uselessly. By keeping only sufficient liquid nitrogen in the Dewar flask around the trap, to maintain the level of the liquefied oxygen just above the crook of the trap, efficient purification is accomplished without excessive tie-up.

From this trap onward it is necessary to use an all-glass system. All rubber connectors were found to be the source of excessive blanks, especially when impregnated with wax, and less so when impregnated with low vapor pressure grease. Glass to glass seals of the ordinary Pyrex glass No. 774 of the system to the Pyrex glass No. 172 of the combustion tube are made without difficulty. All connecting tubing is of 5-mm. diameter.

Combustion System. The oxygen purification system is connected to the combustion tube through stopcock *f*. The combustion tube is of Pyrex glass No. 172, and is 60 cm. long and 1 cm. in diameter. To the mouth of this tube a ground-glass standard-taper joint, *g*, is sealed as shown in Figure 1. The female half of

the joint constitutes a cap. Stopcock *h* at the end of the cap is utilized in sweeping out the water vapor and carbon dioxide that are found to be present on the inside of the cap after it is opened to admit a sample. All stopcocks and ground-glass joints are lubricated with Apiezon N, which is a low vapor pressure grease distributed by the James G. Biddle Company, Philadelphia, Pa. The male section of the joint is sealed to the combustion tube to prevent chance contamination of the boat or sample being introduced, by contact with the lubricating grease.

A variety of catalytic fillings for the combustion tube were tried. The platinum filling of Friedrich (*2*) was sufficient for simple nonnitrogenous compounds. For the more complex type of compound and for larger samples the filling described by Niederl and Niederl (*9*, p. 107, "simple" filling) was found to give the best results. It consists of a roll of platinum wire, copper oxide, and a packing of electrodeposited silver crystals (*6*). The lead dioxide is omitted, for it was found to be totally inadequate for the removal of nitrogen oxides in determinations of nitrogen compounds by this method.

The combustion system is tilted, the axis of the combustion tube being at an angle of about 30° with the horizontal. It is thus possible to slide seven samples successively into the tube past heater *i* maintained at temperature.

The furnaces are kept at a temperature of about 650° to 700° C. Pyrex glass No. 172 successfully stands this temperature under vacuum without collapse or leakage. At the end of the combustion tube is trap *k* for use in the determination of nitrogen-containing substances.

The heating unit consists of an electric sample heater, *i*, and a Von Czoernig–Alber combustion micro furnace, *j*. This unit is distributed by the A. H. Thomas Company, Philadelphia, Pa.

Nitrogen Oxide Reduction System. The combustion system is connected to the gas measuring system and to the nitrogen oxide reduction system by means of the two-way stopcock, *l*. This latter system consists of a piece of Pyrex glass No. 172 (8 cm. × 1.5 cm. in diameter) filled with a 2-cm. section of copper oxide wire, a roll of freshly reduced copper gauze 4 cm. long, and another 2-cm. section of copper oxide. It is heated by means of a Bunsen burner equipped with a wing top. No oxidation of the reduced copper is noticeable after ten runs. If oxidation does occur accidentally, reduction can be accomplished by admission of a drop of pure methanol through the cold combustion tube, and passage of the vapor over the heated copper in the nitrogen oxide tube. Connection to the measuring system is made through stopcocks *m* and *n*.

Gas Measurement System. This system consists of two U-traps, *o* and *p*, in series (13 cm. deep, 1.5 cm. in diameter at wide leg), appropriate volumes *q* and *r*, and a T-type stopcock, *s*, which connects the traps to each other and to the two-liquid manometer, *t* (*5*). This manometer consists of two base tubes, shown at *t* (Figure 1), connected at their base to form a U. These tubes are 10 cm. long by 2.2 cm. in diameter. On the end of one tube is mounted a capillary, *x*, 40 cm. long by 1 mm. in inside diameter, which is connected to the distilling flask, *u*, and through a length of 5-mm. tubing and stopcock *v* to the pressure leg of the manometer. The manometer is prepared for use by being filled with pure mercury to about half of its volume. Amoil-S, a low vapor pressure and low density oil manufactured by Distillation Products, Inc., Rochester, N. Y., is then distilled under vacuum from the storage flask, *u*, into one leg of the manometer. During distillation of the manometer liquid, stopcock *v* is kept open, but when in use for pressure measurements this stopcock is closed, and the space above the Amoil-S is kept under vacuum.

The high sensitivity of this type of manometer stems from the evident fact that a small pressure-induced depression of the mercury level in the large-diameter tube at *w* will cause a great increase in the height of the oil in the 1-mm. capillary, *x*. The pressure on the mercury at *w* in normal operation never exceeds 40 mm. of mercury. Excessive pressure in the pressure leg of the manometer would blow through the mercury unless the precaution of opening stopcock *v* is taken. Assuming that a 0.2-mm. change in level of the oil is discernible, the manometer in use in this research has a sensitivity of 0.015 mm. of mercury.

It was found impossible to use a manometer with a single liquid of low density because of the solubility of the gases being measured (carbon dioxide and water vapor) in the liquids that might be used. Such troubles are not experienced with mercury exposed to the gases, as in the two-liquid manometer, and the high sensitivity is retained.

The size of bulbs *q* and *r* is governed by the size of the samples to be analyzed. For 2- to 5-mg. samples, the bulbs are 7 cm. in diameter in this apparatus. The size of the volume to be used in measuring water vapor, *q*, should be so regulated that the pressure will not exceed the water vapor pressure for the prevalent room temperature; otherwise, condensation to liquid water will take place.

The volumes of the carbon dioxide and water measuring systems are calibrated empirically by running a series of benzoic acid samples of high purity (Bureau of Standards Sample No. 140) and calculating what each millimeter rise in the level of the oil in the manometer represents in milligrams of carbon or hydrogen, as the case may be.

The gas measurement system is connected to the exhaust system through stopcock *y*.

Exhaust System. The pumping system consists of the U-trap, *z*, and a mechanical oil pump. The trap is necessary, for it is found that vapors diffuse at low pressures from the pump to the gas measuring system. This trap when submerged in liquid air catches this diffusate.

The connections to the mechanical pump are made through glass tubing and rubber vacuum tubing. The vacuum pump is the Duo-Seal model manufactured by the W. M. Welch Scientific Company, Chicago, Ill.

PROCEDURE

Essentially the analysis involves the combustion of the organic sample in an atmosphere of purified oxygen with the aid of a combustion catalyst.

The water vapor and carbon dioxide produced by the oxidation of the carbon and the hydrogen present in the sample are frozen out by passing the gases through traps surrounded by acetone–dry ice slush for the water vapor, and by liquid air for the carbon dioxide. Remaining traces of gases are removed by exhausting the combustion tube with a vacuum pump. The parts of the system containing the water vapor and carbon dioxide traps are

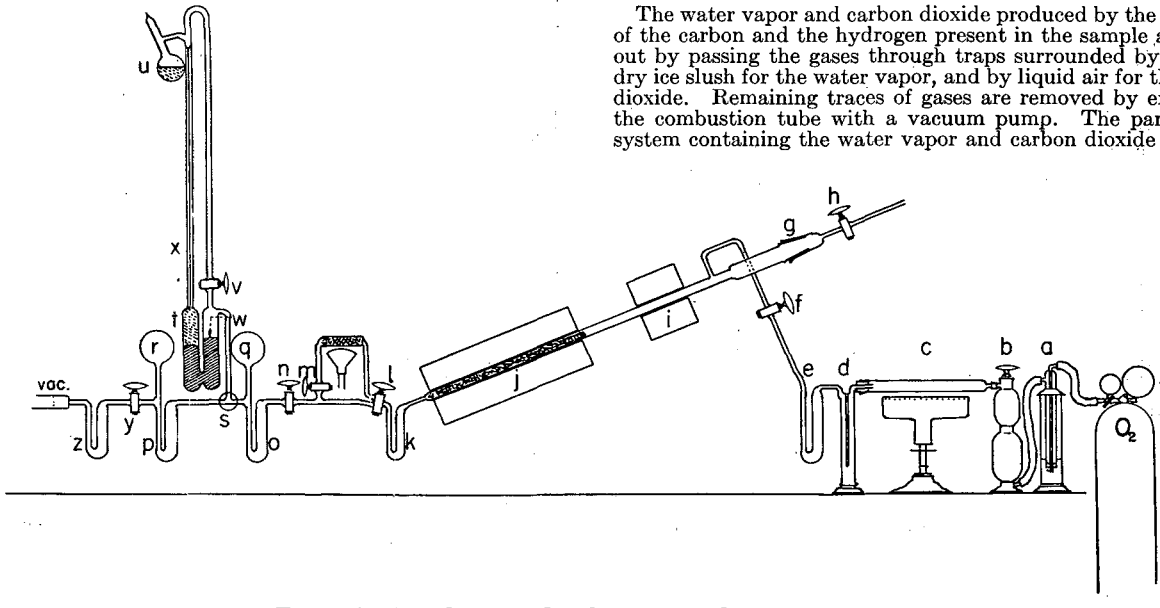


Figure 1. Purification, Combustion, and Analysis Train

isolated by means of stopcocks and the frozen gases are vaporized by replacing the freeze-out baths with beakers containing water at room temperature. The pressures exerted by the water vapor and the carbon dioxide in their respective compartments of calibrated volume are measured by means of the mercury-oil manometer, and, from these data, the quantities of carbon and hydrogen in the original sample are calculated.

Conditioning the System. Prior to the first determination of a series the system is conditioned by passing oxygen through it for approximately 15 minutes. To accomplish this the vacuum pump is first started. Stopcocks *n*, *v*, and *y* (Figure 1) are closed, and the T-cock at *s* is turned to connect traps *o* and *p* and the manometer, *t*. The valve system on the tank of oxygen serving as the oxygen source is set to provide a slow stream of gas which is admitted to the combustion tube by opening stopcock *f*. All heating units are turned on, and traps *e* and *z* are immersed in liquid air. Stopcock *y* is opened, two-way stopcock *l* is turned so that the nitrogen oxide reduction system is by-passed, and stopcock *n* is carefully opened until the oxygen pumping through the system registers a pressure of 30 mm. on the two-liquid manometer. The pressure is kept at this point at all times when a flow of oxygen is called for in the procedure. When so used the manometer acts as a flowmeter. A 30-mm. register on this flowmeter indicates that the oxygen is flowing at a rate of approximately 5 ml. per minute. After the furnaces have come to temperature, the flow of oxygen is continued for 15 minutes to complete the conditioning of the system. The system is then ready for use in analyses.

Analytical Procedure (nonnitrogenous compounds). With the oxygen flow at 30 mm. as in the conditioning run, trap *o* is immersed in a dry ice–acetone slush, and trap *p* is immersed in liquid air. Although the vapor pressure of water at the temperature of the dry ice–acetone mixture is about 10^{-3} mm., negligible error is introduced by loss of water vapor from this trap in the course of a normal determination, where procedures are not unduly prolonged.

The ground-glass cap at the upper end of the combustion tube is then removed and a platinum boat containing the sample is inserted and allowed to slide down the tube to the filling. The ground cap is then replaced and stopcock *h* is opened for 0.5 minute in order to sweep out the atmospheric gases which have entered the cap when it was removed from the combustion tube. The sample burner is lowered over the sample during the next half minute and, after it is in that position for 2 minutes, stopcock *f* is closed. The combustion tube is then evacuated, and stopcock *n* is gradually opened in such a manner that the pressure on the manometer-flowmeter does not exceed 30 mm. No effort is made to evacuate the system completely at this point.

Stopcock *f* is now carefully opened to a position where the oxygen is again flowing through the combustion tube at a pressure of 30 mm., thus sweeping out the residual gases at a low pressure. This sweep-out is maintained for 2 minutes, and then *f* is closed and the system is evacuated as completely as is possible. On completion of the evacuation, stopcocks *n* and *y* are closed and the system is ready for the measurement of the pressures of the collected gases.

The carbon dioxide trap, *p*, is first isolated from the water trap by turning the T-stopcock, *s*, to connect the trap to the manometer. The frozen carbon dioxide in the trap is evaporated by replacing the liquid air by a beaker of room temperature water. When the gas has reached room temperature the manometer is read and stopcock *y* is opened. As soon as all the carbon dioxide has been pumped out of the system, the manometer is read again. The difference between the two pressure readings is a measure of the amount of carbon dioxide evolved on combustion of the sample. The amount of water resulting from the combustion is measured by manipulating stopcock *s* so as to connect the water trap to the manometer and then repeating the procedure utilized in the measurement of the carbon dioxide.

An absolute calibration of the system could be made by independent measurements of the volumes of the carbon dioxide and water measuring sections. Through the use of the gas laws the weight of carbon dioxide and water represented by each pressure reading could be calculated. This would necessitate calculation of the conversion factor for the two-liquid manometer, through exact knowledge of the relative diameters of the capillary, *x*, and the large tube, *t* (Figure 1), and the relative densities of the mercury and the Amoil-S.

In this research a shorter scheme was followed, in that the manometer was empirically calibrated in terms of milligrams of carbon or hydrogen per millimeter of carbon dioxide or water vapor, respectively, by utilizing the results of a series of determinations on benzoic acid. Multiplying the appropriate pressure by the factor gives the results in milligrams of carbon or hydrogen, as the case may be. No temperature or blank corrections are found to be necessary to give results that fall within the acceptable range, as defined below.

Table I. Time Schedule

	Operation	Nonnitrogenous, Min.	Nitrogenous, Min.
1.	Setting of oxygen flow, introduction of sample, cap sweep-out	1	1
2.	Lowering heater, first combustion	2.5	2.5
3.	First evacuation of combustion tube	3	3
4.	Low pressure sweep-out of combustion tube	2	2
5.	Second evacuation of combustion tube	3	3
6.	Passing gases through nitrogen oxide reduction tube, third evacuation	Not done	7
7.	Determination of amounts of carbon dioxide and water vapor	4.5	4.5
	Total	16	23

After the determination is completed, stopcock *f* is opened to admit oxygen to the combustion tube and stopcock *n* is carefully opened until the manometer-flowmeter indicates a pressure of 30 mm. The system is now ready for the next determination. It is customary to run as many as seven determinations consecutively before removing any of the previously used boats from the combustion tube.

Analytical Procedure (nitrogenous compounds). For nitrogen-containing substances the procedure followed is the same as for nonnitrogenous compounds, up to the point when the evacuation of the system is completed after the low pressure sweep-out. However, trap *k* is cooled with liquid air during these steps, and the nitrogen oxide reduction tube is heated to about 650°.

After complete evacuation two-way stopcock *l* is turned to connect the combustion tube to the nitrogen oxide reduction tube. The liquid air around trap *k* is replaced by a beaker of room temperature water. After 1 minute stopcock *m* is carefully opened and the gases are allowed to flow through the system at a pressure of 5 mm. on the manometer-flowmeter until the system is again completely evacuated. The frozen-out gases are measured as in the case of the nonnitrogenous type of sample.

Time Schedule for Determinations. In Table I is detailed the time consumed in the various operations of the determinations of nonnitrogenous and nitrogenous organic substances. The time consumed in the weighing of the sample is not included.

EXPERIMENTAL RESULTS AND DISCUSSION

In the early stages of the research an effort was made to build the manometric apparatus around the standard Pregl equipment. Many experiments were performed on a number of variations of this equipment to show the inadvisability of such features as rubber connections in the evacuated portions of the system, and the advisability of using the steps in technique that are described in the procedure. The magnitude of the blank was used as a preliminary indication of the success of each innovation. Over two hundred determinations were necessary to achieve the final form of the apparatus. The average blank of the form of the apparatus as described amounts to 0.0003 mg. of hydrogen and 0.003 mg. of carbon.

As a basis of judgment of the comparative accuracy of the results obtained in these analyses, the conclusions of Niederl and Niederl (*9*, p. 132) as to the accuracy of the Pregl method are accepted as standard. They conclude that the tolerance for hydrogen is about 30 parts per thousand, and for carbon about 5 parts per thousand (or ±0.3% general average for both). Hereafter these values are referred to as the accepted tolerances for the determination of hydrogen and carbon.

Original successful determinations were made on samples weighing between 1 and 2 mg. These results are summarized in Table II. Attempts to run samples of less than 1 mg. were unsuccessful, in that the average accuracy achieved was outside

the tolerance. Results lead the authors to the conclusion that for such small samples the error in weighing on the type of microbalance used (Kuhlmann) was a large fraction of the allowable error for the determination, and in large measure accounts for the inaccuracy.

In Table II the high accuracy of the hydrogen determinations is noteworthy. The accuracy is well within the 30 parts per thousand allowable, the average deviation being about half of this. This may be explained by recalling that although the weight of a mole of water vapor is less than half that of a mole of carbon dioxide, the pressure exerted by a mole of each is the same. It is thus theoretically possible to determine hydrogen with the same degree of accuracy as carbon by the use of the procedure here outlined. Other factors mitigate against the complete achievement of this, however, notably the great adsorptive capacity of solid surfaces for water vapor.

For the apparatus used for the determinations shown in Table II, the factors for the conversion of pressure readings to milligrams of hydrogen and carbon were 0.000711 mg. of hydrogen per mm. of water vapor pressure, and 0.00448 mg. of carbon per mm. of carbon dioxide pressure.

An alteration in the apparatus was made in order to analyze samples weighing between 2 and 5 mg., in the hope (largely unfulfilled) of further increasing the accuracy of the determination. This change consisted of a replacement of bulbs *q* and *r* (Figure 1) by bulbs of larger capacity. The factors for conversion of pressure readings to milligrams of carbon and hydrogen were 0.01197 and 0.002023, respectively. Samples containing nitrogen, sulfur, and chlorine were also analyzed with this equipment (Table III). Corrections for blanks or for temperature changes have not been made. The benzoic acid results illustrate the precision of the calibration measurement.

A statistical analysis of the accuracy and precision of these results, of the type that Power (*10*) has so ably made for the Pregl method, is neither possible nor justifiable for the number of results here presented. This paper is intended as a preliminary report on an apparatus and method which have just been brought to the stage of yielding acceptable results. Further work will doubtless result in improvements which will be reflected in higher accuracy of individual results.

It will be noted from Tables II and III that the accuracy and precision vary considerably from the determinations on one substance to those on another. This results from the practice of running analyses in batches. All the analyses of one group seem to give results of comparable accuracy, dependent on the condition of the system at the time of the analyses. Much further work is necessary, however, to check these trends, and to account for the conditions which cause them.

These results were obtained under conditions which are far from ideal from the microanalyst's viewpoint. The determinations were carried out by relatively inexperienced operators in a climate noted for its high humidity. No provisions were made for temperature or humidity control in either the balance room or the room in which the analyses were carried out. In spite of these adverse conditions, however, the average accuracy of the results falls within the acceptable range, as previously defined.

It is felt that the major advantages offered by the manometric method here described, over the gravimetric method in widespread use, are decrease in the time necessary for an analysis, reduction of the number of micro weighings (usually five) to the one weighing of the sample, and elimination of the handling of the absorption tubes, with the attendant exacting procedures.

Table II. Determination of Carbon and Hydrogen in 1- to 2-Mg. Samples

Sample	Sample No.	% Carbon Experimental	Deviation from Theoretical	% Hydrogen Experimental	Deviation from Theoretical
Benzoic acid	1	69.01	+0.17	4.96	+0.01
Theoretical	2	68.33	−0.51	4.99	+0.04
% C = 68.84	3	68.74	−0.10	5.02	+0.07
% H = 4.95	4	68.94	+0.10	4.96	+0.01
	5	69.03	+0.19	4.93	−0.02
Sucrose	1	42.47	+0.37	6.54	+0.07
Theoretical	2	41.76	−0.34	6.61	+0.14
% C = 42.10	3	42.77	+0.67	6.61	+0.14
% H = 6.47	4	42.49	+0.39	6.51	+0.04
	5	39.95[a]	−2.15	6.29	−0.18
	6	42.31	+0.21	6.64	+0.17
	7	42.13	+0.03	6.60	+0.13
	Average deviation		±0.28		±0.09
	Average deviation, parts/thousand		±5		±16

[a] Value rejected, not used in calculating average deviation.

Table III. Determination of Carbon and Hydrogen in Samples of 2 to 5 Mg. Weight

(Samples containing nitrogen, sulfur, and chlorine)

Sample	Sample No.	% Carbon Experimental	Deviation from Theoretical	% Hydrogen Experimental	Deviation from Theoretical
Benzoic acid	1	68.68	−0.16	5.01	+0.06
Theoretical	2	68.02	−0.82	4.89	−0.06
% C = 68.84	3	68.98	+0.14	4.91	−0.04
% H = 4.95	4	68.96	+0.12	4.96	+0.01
	5	69.72	+0.88	5.02	+0.07
Sucrose	1	42.14	+0.04	6.50	+0.03
Theoretical	2	42.12	+0.02	6.47	0
% C = 42.10	3	42.19	+0.09	6.52	+0.05
% H = 6.47	4	41.92	−0.18	6.49	+0.02
Acetanilide	1	70.35	−0.74	6.81	+0.10
Theoretical	2	70.67	−0.42	6.62	−0.09
% C = 71.09	3	71.77	+0.68	6.86	+0.15
% H = 6.71					
Cystine	1	29.94	−0.05	5.02	+0.01
Theoretical	2	30.07	+0.08	5.12	+0.09
% C = 29.99	3	30.15	+0.16	4.99	−0.04
% H = 5.03	4	30.00	+0.01	4.94	−0.09
o-Chlorobenzoic acid	1	53.13	−0.56	3.22	0
Theoretical	2	53.81	+0.81	3.23	+0.01
% C = 53.69					
% H = 3.22					
	Average deviation		±0.29		±0.05
	Average deviation, parts/thousand		±5.5		±10

ACKNOWLEDGMENT

Thanks are due to all the members of the Chemistry Department of the University of Hawaii for their cooperation and suggestions, and particularly to Leonora N. Bilger for her interest and encouragement. The authors also wish to thank Hawaiian Gas Products, Ltd., for the donation of the liquid air used.

LITERATURE CITED

(1) Berraz, G., *Anales inst. invest. cienty. tecnol. (Argentina)*, 7, 70–80 (1937).
(2) Friedrich, A., "Die Praxis der quantitativen organischen Mikroanalyse," Leipzig and Vienna, F. Deuticke, 1933.
(3) Hackspill, L., and D'Huart, G., *Bull. soc. chim.*, **35**, 800–3 (1924).
(4) Hackspill, L., and Heeckeren, G., *Compt. rend.*, **177**, 59–60 (1923).
(5) Jaeger, F. M., "Optical Activity and High Temperature Measurements," pp. 265–8, New York, McGraw-Hill Book Co., 1930.
(6) MacNevin, W., and Clark, H. S., IND. ENG. CHEM., ANAL. ED., **10**, 338 (1938).
(7) Murray, W. M., Jr., and Ashley, S. E. Q., *Ibid.*, **16**, 242–8 (1944).
(8) Naughton, J. J., and Uhlig, H. H., *Ibid.*, **20**, 477–80 (1948).
(9) Niederl, J. B., and Niederl, V., "Organic Quantitative Microanalysis," New York, John Wiley & Sons, 1942.
(10) Power, F. W., IND. ENG. CHEM., ANAL. ED., **11**, 660–73 (1939).
(11) Schwarz, K., *Mikrochemie*, **14**, 271–9 (1934).
(12) Wooten, L. A., and Guldner, W. G., IND. ENG. CHEM., ANAL. ED., **14**, 835–8 (1942).
(13) Yensen, T. D., *Trans. Am. Electrochem. Soc.*, **37**, 227 (1920).

RECEIVED August 6, 1949. Abstracted from a dissertation presented by Michael M. Frodyma to the Graduate Division of the University of Hawaii in partial fulfillment of the requirements for the degree of master of science, June 1949.

Flame Photometric Determination of Calcium in Brucite and Magnesite

ROBERT E. MOSHER, EDWARD J. BIRD, AND A. J. BOYLE, *Wayne University, Detroit 1, Mich.*

In a rapid and accurate solution method for the determination of calcium oxide in magnesite and brucite the Beckman flame photometer is used. Analytical results compare favorably with the methanol or Caley and Elving procedure. Interferences in the determination of calcium oxide caused by the presence of metals common to magnesite and brucite are described. The time of analysis is greatly reduced, because filtration, precipitation, and titration operations are not required.

THE determination of calcium oxide in high-grade magnesite and brucite ores is of great importance to the refractories industry. As commonly done, this requires the separation of small amounts of calcium (2 to 5%) from large amounts of magnesium and for this purpose the Caley and Elving method (*5*) is widely used. This communication is concerned with the use of the Model DU Beckman spectrophotometer and Beckman flame photometer attachment for the rapid determination of calcium in magnesite and brucite.

REAGENTS

Standard Calcium Solution. Weigh 4.995 grams of dried, analytical reagent grade calcium carbonate into a 500-ml. beaker and add 200 ml. of distilled water and 20 ml. of concentrated hydrochloric acid in small portions. Transfer quantitatively to a 1-liter volumetric flask, dilute to the mark, and mix thoroughly. This solution contains 2.00 mg. of calcium per milliliter.

Solution A. Weigh 9.46 grams of reagent grade aluminum nitrate, $Al(NO_3)_3.9H_2O$, and 739.0 grams of recrystallized or calcium-free magnesium nitrate, $Mg(NO_3)_2.6H_2O$, into a 1-liter beaker containing 5 ml. of concentrated hydrochloric acid. Add 500 ml. of distilled water, heat gently, and stir until dissolved. In a separate 250-ml. beaker dissolve 1.72 grams of electrolytic iron in 40 ml. of 1 to 1 hydrochloric acid. Warm to hasten solution. Cool to room temperature, transfer both solutions to a 1-liter volumetric flask, dilute to the mark, and mix thoroughly. Should distilled magnesium be used instead of magnesium nitrate, it is advisable to dissolve it in hydrochloric acid.

PREPARATION OF SAMPLE

To a 1.0-gram sample of magnesite or brucite in a 250-ml. beaker, add 10 ml. of distilled water and 10 ml. of concentrated hydrochloric acid. Evaporate the solution to dryness and bake on the hot plate for 10 minutes. Cool and wash down the sides of the beaker with 5 ml. of 1 to 1 hydrochloric acid. Warm the solution to dissolve hydrolyzed salts, add 20 ml. of distilled water, and boil gently until the soluble salts are in solution. Cool and transfer the sample to a 250-ml. volumetric flask. Dilute to the mark with distilled water and mix thoroughly. Permit the solution to stand until the insolubles settle sufficiently to leave a clear supernatant solution. Approximately 5 ml. of the solution are required for analysis. The insolubles resulting from the acid solution of brucite or magnesite are very similar to those obtained from limestone and dolomite and consist almost entirely of silica. For this reason it is not customary to analyze these insolubles for calcium oxide.

PREPARATION OF STANDARD SOLUTIONS

Measure accurately 4, 6, and 8 ml. of the standard calcium solution into 250-ml. beakers. To each beaker add 5 ml. of solution A and 5 ml. of concentrated hydrochloric acid. Cover with watch glasses and proceed as for sample preparation.

FLAME PHOTOMETRIC PROCEDURE

General Principles. One end of a right-angled microcapillary aspirating tube is immersed in a portion of the prepared sample which is held in a 5-ml. sample cup and the other end is inserted into a heated glass chamber. At its entrance into this chamber, the capillary tube is encased in a glass sleeve carrying air at controlled pressures in the vicinity of 20 pounds per square inch. Aspiration and atomization of the sample are produced by expansion of the compressed air and proceed at a uniform rate as long as the pressure of the entering air is kept constant and the capillary tube is kept open. The resultant aerosol is conducted to the burner and mixed with oxygen and either natural gas or propane, which are likewise introduced under individually controlled pressures. Combustion occurs just after mixture and produces a spectrum characteristic of the elements activated and proportional in intensity to the concentration of these elements in the sample.

The light from the flame is transmitted to the standard Beckman spectrophotometer assembly, where it is separated into its component wave lengths by means of a quartz prism. A narrow region of the spectrum characteristic of the element to be measured is focused through a slit and projected onto a phototube, whereby a direct reading of the intensity is obtained. This reading is a measure of the total intensity of the light emitted by the burning gas—i.e., flame background—and the light emitted by the element or elements whose spectra fall within the region covered by the slit. To derive the latter, the flame background is measured using distilled water in the sample cup. The transmittance obtained is subtracted from the total intensity reading. Spectrophotometric readings of the intensity of the element under study are translated into measurements of its concentration by interpolation with readings of the flame intensity of lower and higher standard solutions, carried out consecutively under identical conditions.

Instrument Characteristics. Each flame photometer has certain individual characteristics with which the operator must become familiar, in order to obtain satisfactory results—for example, the bore of the capillary tube is subject to variation in different aspirators. Hence, air pressure must be adjusted to the optimum for each individual aspirator.

Differences are also observed in burners. The original burner in the authors' apparatus required a 3-cm. pressure of propane, which produced too intense a flame background for good analytical work, whereas a newer replacement gave an adequate flame without an objectionable background on a 1-cm. pressure of propane.

The optimal pressures of oxygen, as well as of air and hydrocarbon gas, vary with the burner and thus must be determined for the individual instrument. This is accomplished by measuring transmittance at difference pressures of oxygen while air and gas pressures are kept constant. With progressive increase in oxygen pressure, transmittance values rise to a peak and subsequently decline in a smooth curve, as exemplified by Figure 1. In the vicinity of the peak, relatively large variations in oxygen pressure cause relatively small changes in transmittance readings (*7*). It is in this less sensitive zone of oxygen, air, and gas pressures that transmittance readings are most accurate.

Adjustment of Apparatus for Analytical Procedures. In the operation of the instrument, it is necessary to control a number of interdependent variables. These are considered in the order followed in carrying out an analysis. The atomizing chamber should be preheated in order to avoid condensation of aerosol and consequent irregularity in delivery to the burner.

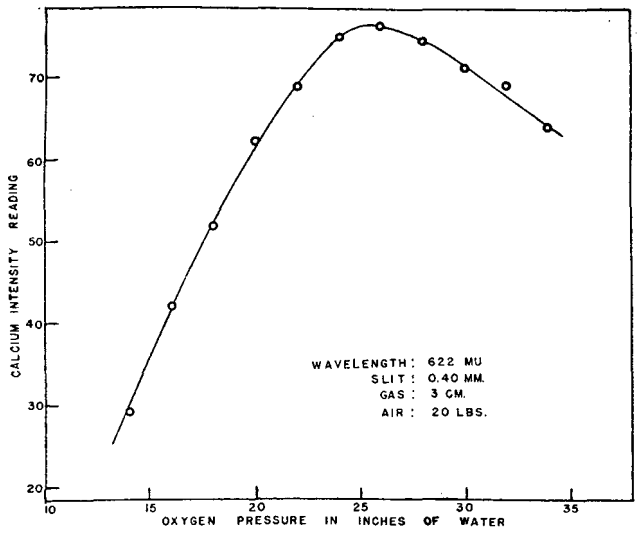


Figure 1. Effect of Oxygen Pressure

Initial Dark Current Check. The fixed switch is set at 0.1, the shutter is closed, and the spectrophotometer circuit is balanced to the zero point with the dark current rheostat. The fixed switch is then moved to the "check" position and, if the circuit is balanced, the needle should remain at the zero position.

Sensitivity Adjustment. The fixed switch is then turned to the 0.1 position. The variable rheostat is set at a point that provides optimal transmittance spread with minimal galvanometric fluctuation and is fixed at this position throughout the succeeding determinations.

Wave-length setting is made at 622 millimicrons for calcium.

Gas pressure is then adjusted to the optimal level for both the type of gas employed and the instrument. Natural gas is used at a pressure of 3 cm. of water for calcium determinations. If there are considerable fluctuations in line pressure, it is advisable to substitute bottled propane for natural gas. With the newer type of burner, propane may be used at a pressure of 1 cm. of water.

Air pressure is then set at the optimal level for the aspirator. This ranged from 15 to 20 pounds per square inch for the authors' instrument.

Oxygen pressure setting is made during aspiration of the middle standard. The oxygen pressure is adjusted to achieve peak transmittance (Figure 1) and is maintained at the same setting for the entire series of determinations.

Dark current recheck is made at this point, by closing the shutter and manipulating the dark current rheostat, if necessary, to bring the needle to the zero point. Repetition of the dark current check is made after each transmittance reading. The shutter is in the closed position for all dark current adjustments and is opened for all transmittance readings.

Slit width is then adjusted during aspiration of the middle standard, so that the transmittance setting is approximately 50. A notation is made of the exact reading. Slit width must be maintained constant for all the subsequent determinations. The usual slit width for calcium is in the vicinity of 0.2 mm.

Flame background is then measured during aspiration of distilled water. This transmittance reading is subtracted from that recorded during aspiration of the middle standard to obtain the intensity value for the element tested in the standard. The flame background is extremely low—i.e., about 1 to 2 divisions—at 622 millimicrons when either natural gas at 2 to 3 cm. or propane at 1 cm. is used as the flame source. Although the flame background tends to remain constant as long as other settings are unchanged, it should be rechecked after each sample analysis.

Sample Analysis. All instrumental settings employed for measurement of the middle standard are kept constant, except for the transmission dial. Pressure gages must be kept under surveillance to make certain of uniformity. A series of clean beakers is filled to the same level with the samples to be analyzed and with each of the standards. The first sample is then aspirated and the transmission dial is manipulated until the needle returns to the zero point and remains stable in this position. The reading on the dial, when the needle is in equilibrium at the zero point, is the transmittance value for the element under study plus the flame background. The latter is then remeasured and deducted from the total intensity reading.

The uniform aspiration of the sample, essential to stability of the needle, takes place within a few seconds. A diminishing reading suggests clogging of the aspirating tube. The plug is dislodged by disconnecting the atomizer and holding the finger over the air outlet so as to force air backward through the capillary until free bubbling occurs in a sample cup containing distilled water.

If the transmittance value of the sample is below that of the middle standard, the low standard is then measured; if above, the high standard is determined instead. To make certain of reproducibility, the transmittance value of the middle standard is redetermined and should fall within ±0.2 division of the original reading. If these conditions are met, the concentration of the element in the sample is determined by interpolation, because the relationship between transmittance values and concentration over the range covered by the standards is linear.

DISCUSSION

Flame excitation temperatures produce for the most part molecular band spectra of calcium oxide with calcium salts rather than atomic line spectra (*4*). Figure 2 shows various emission maxima obtained in the range from 400 to 700 mμ with a calcium chloride solution containing 1000 p.p.m. of calcium. Peaks *A* and *B* were determined at greater sensitivity settings than *C*. The same slit width of 0.15 mm. was used for the entire wavelength range demonstrated. The sodium maximum resulted from the inclusion of a few parts per million of sodium chloride in the calcium chloride solution used for these measurements. This shows the possibility of sodium interference (*1*, *2*) if exceptionally wide slit widths—i.e., 1 mm.—are used to measure small amounts of calcium at 622 mμ, which is the wave length found most suitable for its measurement.

A combination of a sufficiently narrow slit with a didymium filter may be used to increase selectivity of calcium measurement in the presence of sodium. Slit width of 0.15 to 0.35 mm. may be used for the determination of calcium in magnesite and brucite, inasmuch as elements common to these minerals do not produce spectral interference.

The standards used to establish concentration for the element to be measured must approximate as closely as possible the physical and chemical characteristics of the unknown (*3*). Titanium, manganese, and chromium in concentrations of five to ten times that ordinarily encountered in magnesite and brucite

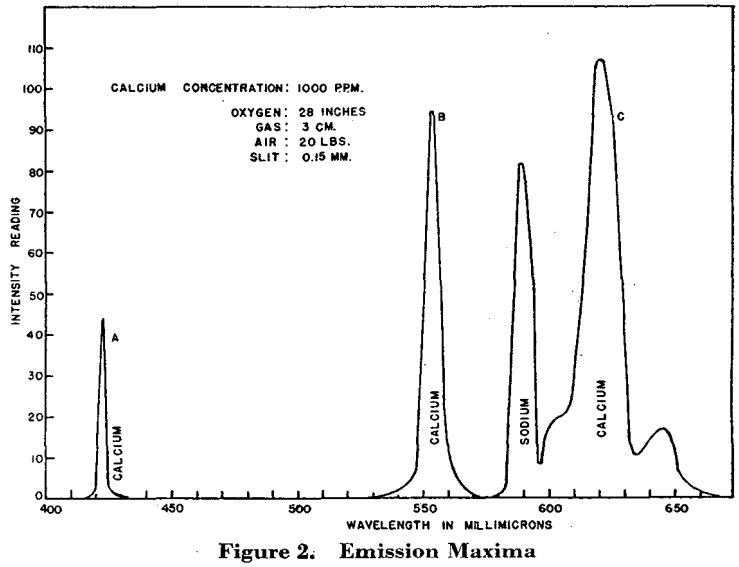


Figure 2. Emission Maxima

do not influence the emission intensity of calcium at 622 mμ. Aluminum, magnesium, and iron depress the intensity of calcium emission in the order given. The curves in Figure 3 were prepared by adding increasing amounts of aluminum, magnesium, and iron, respectively, to standard calcium (as chloride) solutions containing 60 p.p.m. of calcium. The depression caused by iron is insignificant as compared to aluminum or magnesium. The iron is added to the standard, however, to approximate the chemical composition of the sample.

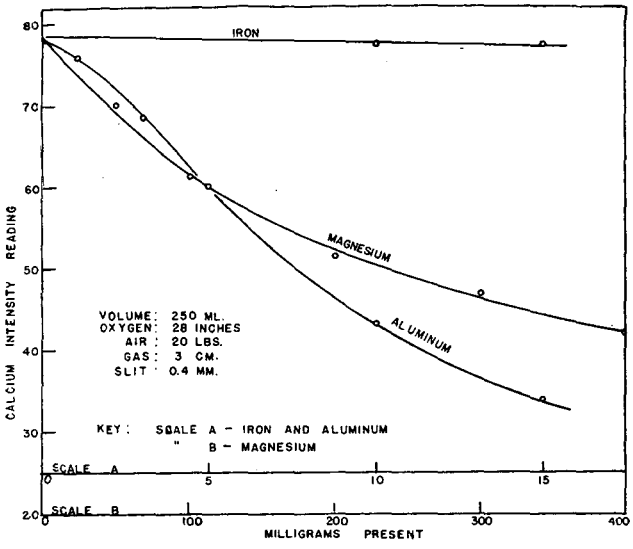


Figure 3. Effect of Aluminum, Magnesium, and Iron Additions

This marked depression exhibited by the introduction of relatively small amounts of aluminum in a solution containing only calcium is most striking. Mitchell and Robertson (*6*) suggest that it is due to absorption of calcium emission energy by aluminum and state that this effect is more pronounced in the high temperature zone of the flame. This phenomenon has been used as an indirect quantitative method for the determination of aluminum.

The following compounds were tried as additions to the calcium-aluminum chloride solution mixture to minimize the depressing effect of aluminum: malonic acid, gelatin, dextrin, citric acid, tartaric acid, 1-propanol, ammonium acetate, ammonium phosphate, ammonium nitrate, potassium chloride, and magnesium chloride. Several of the compounds listed will be recognized as those known to complex aluminum. Magnesium chloride proved to be the salt most effective in smoothing out the depression tendency of aluminum on calcium measurements.

Table I shows the combined effect of aluminum and iron on calcium emission intensity when a fixed amount of magnesium is present. The per cent depression is due almost entirely to the aluminum concentration. Table I is not to be confused with Figure 3, which shows the depressing effect on calcium emission intensity produced by aluminum, magnesium, and iron independently—i.e., 5 mg. of aluminum produce a little more depression than 100 mg. of magnesium. Their combined effect is not additive, however, as will be seen by examining Table I. Sample 7 of Table I contains 15 mg. of calcium and 175 mg. of magnesium. If the magnesium were not present, the initial calcium intensity reading would be approximately 33% greater (Figure 2). However, because all calcium measurements are to be made in the presence of about the same amount of magnesium, calcium-magnesium mixtures are arbitrarily considered to have zero depression. Sample 9 of Table I has 15 mg. of calcium, 175 mg. of magnesium plus 3.4 mg. of aluminum, and 8.6 mg. of iron. The per cent depression is 5.5, which is due almost entirely to the 3.4 mg. of aluminum, the iron having only a slight effect. Now 3.4 mg. of aluminum (Figure 3) will reduce the calcium emission intensity approximately 20% when measured with calcium alone. In the presence of 175 mg. of magnesium, however, the depression is only 5.5%. This illustrates the smoothing out effect of magnesium and demonstrates that the combined effects of magnesium and aluminum are not strictly additive and why it is essential to have an average, fixed amount of magnesium and aluminum in the standards with which samples are compared.

Table I. Combined Effect of Aluminum and Iron on Calcium Intensity

Sample	Ca *Mg.*	Mg *Mg.*	Al *Mg.*	Fe *Mg.*	Depression of Original Intensity Reading %	Remarks
1	6	350	...	...	0.0	Corresponds to 1.0-gram sample of brucite or magnesite containing 0.84% CaO
2	6	350	3.4	8.6	0.5	
3	6	350	6.8	17.2	0.7	
4	15	350	...	...	0.0	Corresponds to 1.0-gram sample of brucite or magnesite containing 2.10% CaO
5	15	350	3.4	8.6	1.7	
6	15	350	6.8	17.2	3.5	
7	15	175	...	...	0.0	Corresponds to 0.5-gram sample of brucite or magnesite containing 4.20% CaO
8	15	175	1.7	4.3	2.7	
9	15	175	3.4	8.6	5.5	

Each sample was made up to 250 ml. in a volumetric flask.

Table II. Additions of Known Amounts of Calcium to Analyzed Magnesite Sample

CaO Present, %	CaO Found (Flame Photometer), %	Error, %
1.50[a]	1.49	−0.66
1.60	1.58	−1.25
1.72	1.68	−2.32
1.88	1.86	−1.06
2.00	1.98	−1.00
2.18	2.20	+0.92

[a] Average of four samples by the method of Caley and Elving.

Table III. Comparison of Caley-Elving and Flame Photometric Methods for Calcium Oxide

Sample	Per Cent Calcium Oxide: Caley-Elving Method	Per Cent Calcium Oxide: Flame Photometer	Error, %
Magnesite	1.50	1.49	−0.66
	1.91	1.93	+1.04
	2.65	2.72	+2.64
Brucite	2.18	2.17	−0.46
	2.31	2.25	−2.59

To investigate recoveries, a sample of magnesite was analyzed for calcium oxide by the Caley-Elving method. Five samples of this magnesite were then prepared for flame photometric analysis and to each, calcium was added as chloride. The results are recorded in Table II. Table III shows the analysis of magnesite and brucite samples for CaO by the Caley-Elving and flame photometric methods.

LITERATURE CITED

(1) Barnes, R. B., Richardson, D., Berry, J. W., and Hood, R. L., IND. ENG. CHEM., ANAL. ED., **17**, 605–11 (1945).
(2) Beckman, A. O., National Technical Laboratories, South Pasadena, Calif., *Bull.* **193B**, June 1948.
(3) Berry, J. W., Chappell, D. G., and Barnes, R. B., IND. ENG. CHEM., ANAL. ED., **18**, 19–24 (1946).
(4) Brode, W. R., "Chemical Spectroscopy," 2nd ed., p. 53, New York, John Wiley & Sons, 1943.
(5) Caley, E. R., and Elving, P. J., IND. ENG. CHEM., ANAL. ED., **10**, 264–9 (1938).
(6) Mitchell, R. L., and Robertson, I. M., *J. Soc. Chem. Ind.*, **55**, 269–72T (1936).
(7) Mosher, R. E., *et al.*, *Am. J. Clin. Path.*, **19**, 461 (1949).

RECEIVED April 7, 1949.

Microdetermination of Unsaturated Fatty Acids by Alkali Isomerization

LEAH COHADAS BERK[1], NORMAN KRETCHMER[2], RALPH T. HOLMAN[3], AND GEORGE O. BURR[4]
University of Minnesota, Minneapolis, Minn.

Alkali isomerization of the polyene fatty acids produces characteristic ultraviolet absorption bands which may be used for quantitative estimations. When ethylene glycol–potassium hydroxide is used as reagent for this purpose, the background absorption produced during the heat treatment becomes a large portion of the total absorption and is variable, prohibiting use of 100-microgram quantities of fatty acids. This difficulty is avoided by the use of aqueous alkali at high temperature and pressure. Analytical data are presented for linoleic, linolenic, and arachidonic acids.

SINCE its introduction as the medium for conjugation of fatty acids (*6*), ethylene glycol–potassium hydroxide has been widely used in the spectrophotometric analysis of lipides (*1, 4, 8, 9*). Brice and Swain (*3*) have strongly criticized the use of glycol as the solvent for fats containing very small amounts of polyenoic acids, because of the strong and variable ultraviolet absorption developed on heating it in the presence of air. However, this high and variable background can be reduced by protection of the reagent by an atmosphere of nitrogen (*10*). In preliminary experiments with glycol–potassium hydroxide under air using the desired small sample size (100 micrograms), the blank absorption was variable and so high in comparison to that of the isomerized fatty acids that accurate measurement of the unsaturated fatty acids was prohibited. Substitution of glycerol for glycol did not materially improve the results. Another serious disadvantage common to these two reagents is that the low dilutions required for spectral examination in the measurement of 100-microgram quantities of fatty acids require the measurement of light absorption in very concentrated viscous solutions.

In view of the fact that conjugation of fatty acids takes place readily in aqueous potassium hydroxide at high temperatures (*2*), this was finally adopted as the solvent for the spectrophotometric determination of 100-microgram quantities of fatty acids. The absorption of potassium hydroxide in water rises rapidly at wave lengths shorter than 2300 A. At 2350 A., the position of the maximum for conjugated diene, the $E^{1\%}_{1\ cm.}$ is approximately 0.05, depending upon the concentration at which reading is made. This value is highly reproducible. Therefore, if the final reading is made in an alkali concentration of less than 1%, the background density is virtually eliminated as a source of error. Absorption due to potassium hydroxide approaches a zero value at 3000 A.

APPARATUS

The equipment consists of a source of high pressure steam, an upright, electrically heated boiler which feeds steam through a needle valve into the horizontal autoclave or bomb shown in Figure 1. The bomb, 10 cm. in diameter and 63 cm. deep, has a metal thermometer, a stainless steel lining, and a stainless steel sliding tray with eight holes of the right diameter to hold snugly the 30-ml. nickel crucibles that act as reaction vessels. The bomb is provided with an inlet copper pipe running the length of the bomb, which feeds steam through small lateral holes spaced throughout the length of the chamber. An outlet drain with a large valve is so placed to permit rapid release of pressure and drainage of condensate. The front lid is provided with a soft metal gasket and is easily opened and closed to admit samples. Nickel crucibles stand up well under prolonged use. The temperature of the bomb can be easily held within 1° C. by controlling the flow of steam with the needle valve. The bomb is mounted in a sheet iron tray with a water drain, and a spraying device is mounted above the bomb, permitting rapid cooling of the apparatus at the end of the heating period.

PROCEDURES

Preliminary work with linseed oil indicated that maximum conjugation of diene and triene was reached in 10 to 15 minutes at 180° C., using 6.0% potassium hydroxide as reagent. At least 20 minutes were required at 170° to attain the same degree of conjugation. At 160° conjugation did not reach a maximum in 40 minutes.

The effect of potassium hydroxide concentration in the reagent upon the degree of conjugation at the end of 15 minutes at 180° is shown in Figure 2. Linolenic acid was used, and it was found that 4 to 6% of potassium hydroxide was required to produce maximum conjugation, of both diene and triene. The 6% level of alkali was chosen for use in all subsequent studies.

Using 6% potassium hydroxide, a time study of the development of chromophores from arachidonic acid was made. Figure 3 shows these results together with the preliminary results on linseed oil. Triene conjugation from linseed oil remains relatively constant and high after 10 minutes' treatment. Diene conjugation, however, reaches a maximum at 10 minutes and decreases progressively thereafter. With arachidonic acid the characteristic tetraene band increases progressively, but has nearly attained its maximum at 15 minutes. Triene absorption from arachidonate also reaches its maximum by 15 minutes. A 15-minute reaction time was selected as the best compromise.

During the preliminary studies there was considerable variability, which was thought to be due to oxidation of the samples as the carrying solvent was evaporated from the nickel crucibles. A trace of hydroquinone in alcohol, amounting to 0.1% of the fatty acids, was added to the solution. This reduced the variability among replicates, presumably by its antioxidant effect. Tests showed that this material has no effect on the absorption spectra after the alkali "cook."

On the basis of the above preliminary work, the following final procedure was adopted for the study of purified lipides.

A sample of fatty acid or oil is weighed, dissolved in Bloor's mixture of purified alcohol and ether (3 to 1), and made to a volume that contains approximately 100 micrograms of lipide in each milliliter and hydroquinone in an amount equal to 0.1% of the lipide. One-milliliter aliquot portions of lipide solutions are pipetted into the nickel crucibles and, without evaporation of the solvent, 1 ml. of 6% potassium hydroxide is added to each. Blanks are prepared containing 1 ml. of Bloor's mixture and 1 ml. of 6% potassium hydroxide. Two blanks and six samples

[1] Present address, 724 South Van Buren St., Green Bay, Wis.
[2] Present address, Long Island College of Medicine, Brooklyn, N. Y.
[3] Present address, Texas A. and M. College System, College Station, Tex.
[4] Present address, Experiment Station, Hawaiian Sugar Planters Association, Honolulu, T. H.

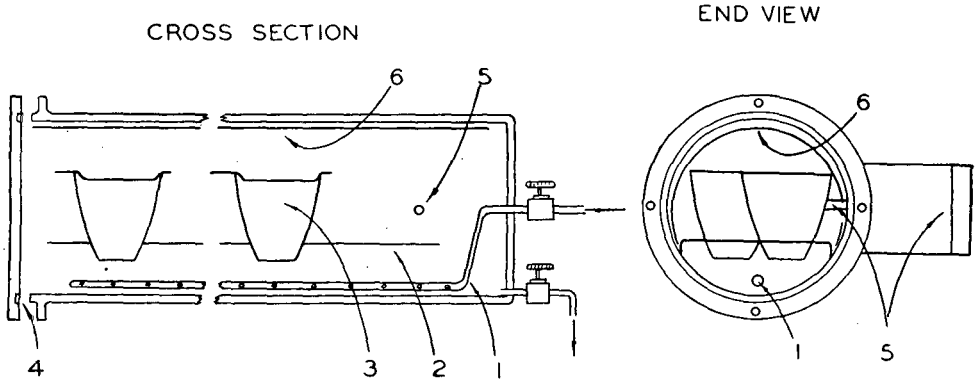


Figure 1. Bomb or Autoclave Used for Alkaline Isomerization

1. Steam inlet tube with lateral holes
2. Stainless steel tray for crucibles
3. Nickel crucibles with covers
4. Lid with lead gasket
5. Metal thermometer
6. Stainless steel shield

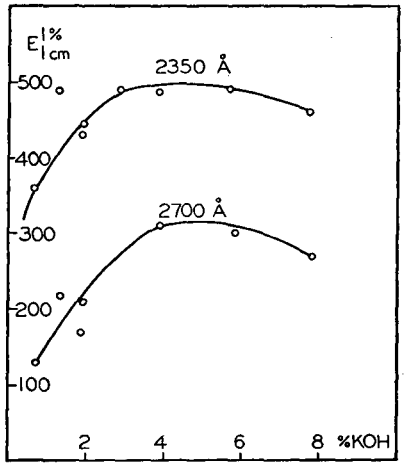


Figure 2. Effect of Potassium Hydroxide Concentration upon Degree of Isomerization of Linolenic Acid during 15 Minutes' Treatment at 180° C.

are run each time. The crucibles are covered with nickel covers. The bomb is closed and steam is allowed to enter slowly until the temperature reaches 100° C. with the outlet valve open for flushing out condensate, alcohol, ether, and air. The valve is closed and steam let in rapidly until the temperature reaches 180° C., when the timing is started. The temperature is held constant for 15 minutes, when the steam is cut off, the outlet valve opened wide, and the cold water spray turned on. As soon as the chamber is cool, it is opened and the contents of the crucibles are washed with water into 10-ml. volumetric flasks and diluted to volume. Spectral readings are taken immediately with a Beckman spectrophotometer, for the absorption was observed to decrease over the space of several hours.

RESULTS

Typical ultraviolet absorption curves were obtained by this method of alkali conjugation of linoleic, linolenic, and arachidonic acids. Working constants for the absorption at the maxima produced from these acids are given in Table I. Inasmuch as the practical limits of the method are determined by the densities of the band produced, it is clear that 0.8 mg. of arachidonic acid is required to give a density of 0.5 at 3025 A., using a 1-cm. cell. However, it is not necessary to dilute to 10 ml. for the readings at the longer wave lengths. By diluting only to 3.0 ml. and by making readings at densities of 0.1 to 0.2, it is possible to work with samples containing only 60 micrograms of arachidonic acid. In mixtures of linoleic and arachidonic acid (linolenic being absent) the 2700 A. absorption band of arachidonate may be used, thus increasing the sensitivity by a factor of 5. Similarly, with plant tissues containing fatty acids no more unsaturated than linolenic acid, it is possible to work accurately with samples containing as little as 50 micrograms of either linoleic or linolenic acids.

Mixtures of purified linoleic and arachidonic acids were analyzed using this micromethod, the 2700 Å. band being used as a measure of arachidonic acid. Results on three such mixtures (Table II), show method is applicable to the determination of these acids in mixtures.

The constants given in this paper are only indicative of the type of results possible with the method described. They should not be used as constants with other apparatus or by other investigators who may be working under different conditions. The degree of conjugation of these substances is dependent upon many factors, one of which certainly is the type of construction of the apparatus. During the development of this technique, this was very apparent; the constants fluctuated whenever a change of de-

Table I. $E^{1\%}_{1\ cm.}$ at Maxima of Absorption Bands Used for Analysis

(Calculated for free acid)

Acid	2350 A.	2700 A.	3025 A.
Linoleic	627	...	...
Linolenic	487	328	...
Arachidonic	425	258	63.6

Table II. Determination of Linoleic and Arachidonic Acids in Known Mixtures

Linoleic Acid			Arachidonic Acid		
Present, γ	Calcd., %	Obsd., %	Present, γ	Calcd., %	Obsd., %
31.6	23.6	29.5	102.6	76.4	70.0
62.4	47.6	26.7	68.6	52.4	47.1
93.6	73.2	75.7	34.3	26.8	28.0

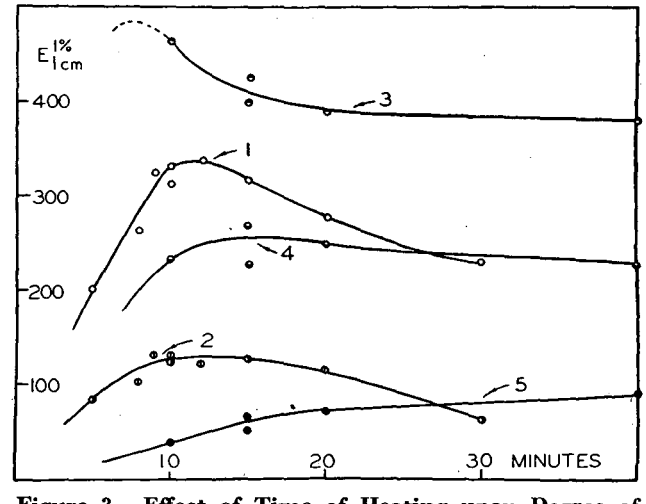


Figure 3. Effect of Time of Heating upon Degree of Isomerization with 6.0% Potassium Hydroxide at 180° C.

1. Conjugated diene from linseed oil
2. Conjugated triene from linseed oil
3. Conjugated diene from arachidonic acid
4. Conjugated triene from arachidonic acid
5. Conjugated tetraene from arachidonic acid

sign or repair was made on the bomb. It is recommended that investigators determine their own constants and check these periodically. The technique described was developed for application to the microanalysis of biological matter where only minute samples are available. Emphasis was laid on the determination of linoleic and arachidonic acids, which are important components of animal material. The method does not appear to be useful for the analysis of acids more unsaturated than arachidonic, where another technique is of far greater advantage (*5*). However, the method has been used successfully in the characterization of the lipides of liver particulate fractions (*7*).

LITERATURE CITED

(1) Beadle, B. W., and Kraybill, H. R., *J. Am. Chem. Soc.*, 66, 1232 (1944).
(2) Bradley, T. F., and Richardson, D., *Ind. Eng. Chem.*, 34, 237 (1942).
(3) Brice, B. A., and Swain, M. R., *J. Optical Soc. Am.*, 35, 532 (1945).
(4) Hilditch, T. P., Morton, R. A., and Riley, J. P., *Analyst*, 70, 62 (1945).
(5) Holman, R. T., and Burr, G. O., *Arch. Biochem.*, 19, 474 (1948).
(6) Kass, J. P., Miller, E. S., Hendrickson, J., and Burr, G. O., Abstracts 99th Meeting Am. Chem. Soc., p. 11, Cincinnati, 1940.
(7) Kretchmer, N., *Arch. Biochem.* (in press).
(8) Mitchell, J. H., Jr., Kraybill, H. R., and Zscheile, F. P., Ind. Eng. Chem., Anal. Ed., 15, 1 (1943).
(9) Mowry, D., Brode, W. R., and Brown, J. B., *J. Biol. Chem.*, 142, 671 (1942).
(10) O'Connor, R. T., Heinzelman, D. C., and Dollear, F. G., *Oil & Soap*, 22, 257 (1945).

Received August 8, 1949. Part of thesis presented by Leah Cohadas to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the M.S. degree, June 1945. The work was aided by grants from the National Dairy Council on behalf of the American Dairy Association and from the National Livestock and Meat Board.

Microdetermination of Formaldehyde with Chromotropic Acid

CLARK E. BRICKER AND W. AUBREY VAIL
Princeton University, Princeton, N. J.

The use of chromotropic acid as a specific reagent for determining formaldehyde has been extended so that it can be applied in the presence of large concentrations of various organic compounds. In the presence of chromotropic acid, these interfering organic compounds are removed by evaporation, whereas the formaldehyde is retained by the reagent. Subsequent addition of sulfuric acid develops the true purple color due to the formaldehyde present.

In several previous publications (*2*, *3*, *7*, *8*) chromotropic acid, 1,8-dihydroxynaphthalene-3,6-disulfonic acid, has been suggested as a suitable reagent for a spectrophotometric method to determine small amounts of formaldehyde. More recently, Speck (*10*) found that diacetyl yields formaldehyde when heated with sulfuric acid and applied chromotropic acid to determine this compound. Boos (*1*) developed a method for methanol in which the methanol is oxidized to formaldehyde, which is then determined by this same colorimetric reaction.

In endeavoring to determine terminal unsaturation by an indirect chemical method based on a formaldehyde determination, Bricker and Roberts (*4*) have pointed out that certain organic substances, such as benzene and acetone, inhibit the color formation of formaldehyde-chromotropic acid. In order to eliminate this interference, two color reactions for formaldehyde, Schiff's reagent and the potassium ferricyanide–phenylhydrazine method of Schryver (*9*) were studied extensively. These methods were proved to be no better and in most respects inferior to the chromotropic acid reaction. Furthermore, the volumetric method using the optimum conditions with hydroxylamine hydrochloride (*6*) was investigated and was found to lack the specificity and sensitivity of colorimetric methods. Consequently, a thorough study of the chromotropic acid reaction was undertaken to improve the specificity and reliability of this reagent for formaldehyde in the presence of organic compounds.

All previous methods using chromotropic acid as a formaldehyde reagent have developed the purple color by adding a definite volume of the solution to be analyzed to a solution of chromotropic acid. Sulfuric acid is then added and the resulting solution is heated for about 30 minutes in a boiling water bath. No positive interferences, except diacetyl (*10*) and possibly glycerylaldehyde, furfural, and some sugars (*5*), have ever been reported. However, if certain organic compounds are present with the formaldehyde, a less intense color is produced. This has been attributed to the fact that formaldehyde couples with many compounds in strong sulfuric acid and thereby prevents its color development with the reagent.

Because there is no apparent color change when chromotropic acid and a formaldehyde solution are heated, it has been assumed previously that there is no reaction between these two compounds unless sulfuric acid is present. In this investigation it has been found that chromotropic acid does react with or retain formaldehyde when a solution is evaporated to dryness. During this evaporation the volatile organic compounds which inhibit the purple dye formation are removed. When the sulfuric acid is added and the resulting solution is heated, the correct amount of the purple dye is produced.

RECOMMENDED PROCEDURE

Weigh 100 ± 10 mg. of chromotropic acid (obtained from Paragon Division of The Matheson Company, Inc.) into a 30-ml. beaker. Add a definite volume of the solution to be analyzed, no more than 1 ml. in volume or 100 micrograms in formaldehyde content. If less than 1 ml. of solution is taken, add sufficient water to make 1 ml. Evaporate the solution to dryness on a low temperature hot plate or in an oil bath whose temperature does not exceed 200° C. and heat the residue for at least 5 minutes after the last traces of liquid have disappeared from the sides of the beaker. Allow to cool and then add 5 ml. of concentrated sulfuric acid. Heat the resulting solution in boiling water for 30 minutes. Cool and dilute to 50 ml. with water in a volumetric flask. Allow the diluted solution to reach room temperature and then measure the optical density against a reagent blank at 570 mμ.

Several known quantities of formaldehyde are carried through this procedure in order to obtain a calibration curve. Because this curve is linear, the number of micrograms of formaldehyde

in an unknown sample is calculated by merely dividing the measured optical density by the density produced by 1 microgram of formaldehyde in the calibration curve.

EXPERIMENTAL

All spectrophotometric readings were made on a Beckman Model DU spectrophotometer using a slit width of 0.04 mm.

The first experiments using the recommended procedure with four 10-microgram quantities of formaldehyde per milliliter showed that the reproducibility of the method was extremely good. Therefore, it seemed likely that a reproducible amount of formaldehyde was retained by the reagent during the evaporation.

The temperature of the hot plate or oil bath during the evaporation should be such as not to cause spattering. Inasmuch as the temperatures of hot plates vary appreciably, the stability of the residues and the subsequent reproducibility of the purple color were studied by using an oil bath which was maintained at various temperatures. It was proved that the dried residues did not undergo any change if they were allowed to stand for 20 minutes at 170° C. Furthermore, the recovery of formaldehyde from mixtures of organic compounds was perfect when an oil bath maintained at a temperature as high as 200° C. was used for the evaporation step in the recommended procedure.

The amount of chromotropic acid necessary for a determination was determined in two ways.

In one series of experiments, a constant amount of formaldehyde in 1 ml. of solution was added to various weights of reagent. These data, shown in Table I, indicate that the optical density increased gradually as the weight of chromotropic acid was increased. There was, however, very little change in the optical density when a 100- or 150-mg. portion of reagent was used. Calibration curves with pure formaldehyde solutions were then run using 50, 100, and 150 mg., respectively, of chromotropic acid for each determination. All these calibration curves were linear to at least 100 micrograms of formaldehyde, whereas the older method (*3*) started to deviate from linearity above 60 micrograms. Although the calibration curves in which 100 or 150 mg. of reagent were used were practically superimposable, the curve obtained with 50 mg. had a slightly lower slope.

Table I. Variation of Color Produced with Weight of Reagent Used

Formaldehyde Taken	Chromotropic Acid Taken	Extinction, Duplicate Determinations	
Mg.	*Mg.*		
0.050	10.0	0.070	0.062
0.050	30.0	0.285	0.292
0.050	40.0	0.321	0.328
0.050	50.0	0.375	0.378
0.050	60.0	0.380	0.382
0.050	70.0	0.387	0.384
0.050	80.0	0.392	0.392
0.050	90.0	0.397	0.396
0.050	100.0	0.402	0.406
0.050	150.0	0.408	0.411

In view of these experiments, it was decided that better reproducibility and sensitivity could be expected if no less than 100 mg. of chromotropic acid were used for each determination.

It was found that the recovery of formaldehyde decreased if the amount of solution taken was much greater than 1 ml. Larger volumes than 1 ml. could be analyzed for formaldehyde by using proportionally larger quantities of reagent.

Other acids in addition to sulfuric acid were used to develop a color after the drying operation. When sirupy phosphoric acid was used, an orange-purple color was formed. Hydrochloric acid gave a pinkish purple color and acetic acid yielded a canary yellow. The optical densities at 570 mμ of these solutions were much lower in all cases than those obtained from an equal amount of formaldehyde when sulfuric acid was used. No further work was done with these acids because the sensitivity was so much lower.

Table II. Recovery of Formaldehyde

Ratio	Formaldehyde Added	Formaldehyde Found: Old procedure (*3*)	Formaldehyde Found: Recommended procedure
	Mg.	*Mg.*	*Mg.*
Acetaldehyde to formaldehyde			
10:1	0.050	0.048_6	0.049_1
40:1	0.050	0.046_1	0.048_1
100:1	0.050	0.044_0	0.044_8
100:1	0.010	0.004_2	0.009_4
200:1	0.010	0.003_6	0.008_4
Benzaldehyde to formaldehyde			
10:1	0.050	0.048_2	0.049_9
40:1	0.050	0.046_9	0.049_6
100:1	0.050	0.044_8	0.049_0
100:1	0.010	0.004_4	0.009_2
200:1	0.010	0.003_6	0.008_8
Benzene to formaldehyde			
1000:1	0.050	0.046_0	0.049_8
4000:1	0.050	0.038_1	0.049_1
10,000:1	0.050	0.028_9	0.048_9
10,000:1	0.010	0.003_2	0.009_8
20,000:1	0.010	0.002_0	0.009_9
Pyridine to formaldehyde			
1000:1	0.050	0.034_5	0.050_8
4000:1	0.050	0.024_3	0.050_8
10,000:1	0.050	0.015_5	0.050_6
10,000:1	0.010	0.004_8	0.009_6
20,000:1	0.010	0.003_1	0.009_9

APPLICATIONS AND INTERFERENCES

It has been shown (*3*) that *n*-propyl alcohol, *n*-amyl alcohol, methyl ethyl ketone, acetone, and other organic compounds inhibit the formaldehyde color development and thereby give low recoveries. This is also shown in Table II, where this same procedure was used to determine formaldehyde in the presence of acetaldehyde, benzene, pyridine, and benzaldehyde.

With the procedure recommended in this paper, formaldehyde recoveries have been investigated in the presence of 20 different organic compounds. The results of a few of these studies are shown in Table II.

The recommended procedure has proved suitable for determining as little as 1 part of formaldehyde in the presence of 20,000 parts of chloroform, carbon tetrachloride, methanol, ethyl alcohol, *n*-butyl alcohol, isobutyl alcohol, *sec*-butyl alcohol, *tert*-amyl alcohol, acetone, methyl ethyl ketone, pyridine, and benzene. The recovery of formaldehyde in the presence of acetic acid, propionic acid, and benzyl alcohol is probably reliable to only 1 part in 1000 or 2000 of these compounds. With acetaldehyde or benzaldehyde present, the recovery of formaldehyde is reliable to only about 1 part in 100. Benzoic acid shows the greatest interference, but by using small samples, the recovery of formaldehyde can be assumed to be quantitative in the presence of ten times as much of this compound. The fact that benzoic acid is not as volatile as the other compounds studied undoubtedly accounts for its marked interference.

When an attempt was made to determine formaldehyde in the presence of iodine, erratic results were obtained. There was no apparent correlation of formaldehyde recovered to the amount of iodine present. However, formaldehyde can be determined very accurately by the recommended procedure in the presence of at least 25 times as much iodine if the iodine is reduced with sodium sulfite before the sample is added to the chromotropic acid. The amount of sodium sulfite used is not critical; a 1000-fold excess did not appreciably effect the recovery.

The cyclic formals, such as are present in safrol and piperonal, yield formaldehyde on heating with acid. This formaldehyde of constitution can be determined by making use of the previously reported procedure (*3*). If the procedure reported in this paper is followed, these formals are removed by evaporation and only any free formaldehyde which was present originally is determined. Hence, a combination of the two chromotropic acid procedures

will serve to determine safrol or other similar compounds and formaldehyde in the presence of each other.

Speck (*10*) has pointed out that formaldehyde will interfere with the determination of diacetyl by his procedure. Samples of diacetyl when carried through the recommended procedure produce no color. Furthermore, diacetyl, at least in small concentrations, does not interfere with the determination of any free formaldehyde that may be present. Therefore, a combination of these procedures will serve to determine formaldehyde and diacetyl in the presence of each other.

Compounds which are not readily volatile at 170° C. may still interfere with the formaldehyde reaction. However, the usefulness of this reagent has been greatly extended with this modified procedure. This procedure gives a linear calibration line and thereby eliminates the necessity of using a calibration graph to determine the amount of formaldehyde corresponding to a given optical density.

ACKNOWLEDGMENT

The authors gratefully acknowledge the financial assistance of the grant made by E. I. du Pont de Nemours & Company to sponsor fundamental chemical research at Princeton University.

LITERATURE CITED

(1) Boos, R. N., ANAL. CHEM., **20**, 964 (1948).
(2) Boyd, M. J., and Logan, M. A., *J. Biol. Chem.*, **146**, 279 (1942).
(3) Bricker, C. E., and Johnson, H. R., IND. ENG. CHEM., ANAL. ED., **17**, 400 (1945).
(4) Bricker, C. E., and Roberts, K. H., ANAL. CHEM., **21**, 1331 (1949).
(5) Eegriwe, E., *Z. anal. Chem.*, **110**, 22 (1937).
(6) Huckabay, W. B., Newton, C. J., and Metler, A. V., ANAL. CHEM., **19**, 838 (1947).
(7) Kleinert, T., and Srepel, E., *Mikrochemie ver. Mikrochim. Acta*, **33**, 328 (1948).
(8) MacFayden, D. A., *J. Biol. Chem.*, **158**, 107 (1945).
(9) Schryver, S. B., *Proc. Roy. Soc. (London)*, **82B**, 226 (1909).
(10) Speck, J. C., Jr., ANAL. CHEM., **20**, 647 (1948).

RECEIVED November 29, 1949.

NOTES ON ANALYTICAL PROCEDURES . . .

Determination of Benzylpenicillin

DOROTHY J. HISCOX

Department of National Health and Welfare, Ottawa, Ontario, Canada

IN 1946, Page and Robinson (*6*) published a colorimetric method for the determination of benzylpenicillin, using *N*(1-naphthyl) ethylenediamine dihydrochloride as a color reagent. By this method the benzene ring of the penicillin was nitrated, and the nitro compound was reduced, then diazotized and coupled with the color reagent in the presence of ethyl alcohol. A violet color showing maximum absorption at a wave length of 560 mμ was produced.

This method did not give reproducible results when tested in this laboratory. During investigational work, two reasons for the failure of this method were found: An excess of sodium nitrite reacts with the color reagent, and under the conditions outlined by the authors, nitration was not complete. This is also true of other methods for the determination of benzylpenicillin which are based upon the nitration of the benzene ring (*1–3*). Under none of the conditions used in these methods could reproducible results be obtained when tested colorimetrically with *N*(1-naphthyl) ethylenediamine dihydrochloride. The error did not lie in the color development, as there was no significant variation in the color developed in numerous aliquots taken from single nitrations. Nitration was more complete with smaller samples of penicillin.

For routine work the simplest conditions for nitration would be to heat the sample with nitration mixture in a test tube in boiling water. It was decided to see if complete nitration could be effected under these conditions. Surprisingly, it was found that nitration was more complete when smaller aliquots of nitration mixtures were used. When the nitration mixture was broken down into its two components, it was shown that this result was due to the decrease in the amount of sulfuric acid present with the smaller aliquots.

Typical results are shown in Table I. With sample 1, as the amount of sulfuric acid increased, the color produced decreased. This could be due to the decrease in the concentration of potassium nitrate present in the mixture. However, samples 2 and 3 show that it was due to the amount of sulfuric acid present. With these samples, different amounts of the same nitration mixture were used, but the concentration of the potassium nitrate was unchanged. Nitration was more complete with the smaller amount of nitration mixture—that is, with less sulfuric acid present.

It was found that, when a sample of 0.5 mg. of penicillin or less is heated in boiling water for 2 hours with 0.5 ml. of a nitration mixture containing 60 grams of potassium nitrate in 100 ml. of concentrated sulfuric acid, nitration is complete. Under these conditions, reproducible results are obtained. The density of the color from an amount of aniline equivalent to 0.5 mg. of benzylpenicillin, as determined in a Beckman quartz spectrophotometer at 560 mμ, was 1.20 and 1.30. The density of the color from 0.5 mg. of benzylpenicillin was 1.19, 1.23, and 1.33. Although this cannot be taken as an absolute comparison, it is a good indication that nitration by the author's procedure is complete. When such a small amount of nitration mixture is used, it is necessary to add more sulfuric acid after nitration is completed before the color is developed to ensure its complete development.

Heat from 0.1 to 0.25 mg. of penicillin with 0.5 ml. of nitration mixture (60 grams of potassium nitrate in 100 ml. of concentrated sulfuric acid) in a 25 × 100 mm. test tube in boiling water for 2 hours. Add 5 ml. of water and 0.2 gram of granular zinc and heat an additional 15 minutes. Transfer to a 25-ml. volumetric flask, rinsing the tube with two 3-ml. portions of water and decanting the liquid from the zinc residue. Add 1 ml. of concen-

Table I. Effect of Sulfuric Acid on Nitration of Benzylpenicillin

Sample	KNO_3, G.	H_2SO_4, Ml.	% Absorption	
1	0.40	1.0	57.9	51.9
	0.40	1.5	17.4	16.9
	0.40	2.0	3.2	4.7
2	0.40	1.0	74.7	73.8
	0.80	2.0	27.3	24.2
3	0.25	0.5	89.9	90.2
	0.50	1.0	75.4	74.0

Table II. Determination of Benzylpenicillin by Three Methods

Sample	% Benzylpenicillin NEP[a]	UV[b]	NED[c]
41,369	89.4	89.0	89.7
41,518	90.5	87.1	96.8
42,522	93.1	94.8	96.8
43,678	94.3	98.7	97.1
46,385	89.4	90.0	95.2
46,574	88.6	91.0	87.2
46,975	89.5	92.0	91.2
48,623	88.8	91.0	91.1
48,743	88.5	89.1	86.8
48,774	94.6	92.0	96.2
48,949	93.8	91.0	92.0
50,233	93.4	92.9	94.0
50,345	92.5	95.8	97.0
50,346	93.3	93.9	95.2
49,523	92.4	95.8	94.8
49,038	88.3	92.9	95.1
Mean	91.3	92.3	93.5

[a] *N*-ethylpiperidine.
[b] Ultraviolet.
[c] *N*(1-naphthyl) ethylenediamine dihydrochloride.

trated sulfuric acid, cool, and add 1 ml. of 0.1% sodium nitrite and 1 ml. of 0.25% ammonium sulfamate, shaking well after the addition of each. Add 10 ml. of 95% alcohol. Add 1 ml. of 1.5% *N*(1-naphthyl) ethylenediamine dihydrochloride, prepared fresh daily. Make to volume. Let stand at least 1 hour and determine the density of the solution at 560 mμ in a Beckman quartz spectrophotometer.

Crystalline penicillin may be used, or the residue from a 1-ml. aliquot of a solution of penicillin in water or buffer which has been taken to dryness in boiling water. The procedure may be interrupted after nitration, but once reduction has taken place the color must be developed within 1 to 2 hours. With many samples of penicillin color development is complete within 15 minutes, but some samples require 1 hour for maximum color development. The color is stable for at least 3 hours after the maximum color is reached. Though different brands of *N*(1-naphthyl) ethylenediamine dihydrochloride possess different physical properties, under the above conditions the same results are obtained.

For reference the author established a regression line using the above procedure and amounts of sodium benzylpenicillin varying from 0.05 to 0.5 mg. The 67 points used were determined on 5 days during a period of 2 weeks. Two brands of *N*(1-naphthyl) ethylenediamine dihydrochloride and two standard benzylpenicillins were used. The equation of the line was $Y = 0.399032\,X - 0.0059$, where Y represents milligrams of benzylpenicillin and X is the density. The correlation coefficient was +0.995 and the standard error of prediction ±0.014 mg.

In Table II results obtained by this method on a number of commercial samples of penicillin are compared with results by the gravimetric *N*-ethylpiperidine (*5*) and the ultraviolet absorption (*4*) methods. Although these methods are based upon three different principles, they show good agreement. They vary greatly in the amount of time required, the size of sample used, and the precautions that must be observed. The choice of a method will depend upon the conditions existing in individual laboratories.

ACKNOWLEDGMENT

The author wishes to thank Merck & Co. and Ayerst, McKenna & Harrison, Ltd., both of Montreal, for gifts of crystalline benzylpenicillin.

LITERATURE CITED

(1) Boxer, G. E., and Everett, P. M., ANAL. CHEM., **21**, 670 (1949).
(2) Canback, T., *Farm. Rev.*, 46, 97 (1947).
(3) Del Vecchio, G., and Argenziano, R., *Bull. soc. ital. biol. spec.*, **22**, 1190 (1946).
(4) Levy, G. B., *et al.*, ANAL. CHEM., 20, 1159 (1948).
(5) Mader, W. J., and Buck, R. R., *Ibid.*, 20, 284 (1948).
(6) Page, J. E., and Robinson, F. A., *Nature*, 158, 910 (1946).

RECEIVED July 19, 1949.

Identification and Microdetermination of Nickel

In Presence of Iron by Means of 1,2-Cyclohexanedione Dioxime

H. I. FEINSTEIN

National Bureau of Standards, Washington, D. C.

ADVANTAGES and disadvantages in the use of 1,2-cyclohexanedione dioxime (called nioxime) as compared with dimethylglyoxime in the analytical chemistry of nickel and palladium have been reported (*2–4*). Voter, Banks, and Diehl (*3*) devised a procedure using this reagent for the gravimetric determination of nickel in the presence of a number of elements, but were not successful in preventing interference by iron. The cause of this difficulty was not explained, but it seems likely that the masking agents did not form sufficiently stable complexes at a pH of 4 to 5 to prevent oxidation of the reagent by ferric iron.

Accordingly, a change in the pH of the solution at the time of precipitation might eliminate the interference from iron. Therefore, experiments were performed using neutral or slightly ammoniacal solution at room temperature followed by digestion at 60° C. Furthermore, nioxime was added to the already neutralized solution, whereas Voter, Banks, and Diehl (*3*) adjusted the pH after the reagent was added. The use of acetate as a buffer was eliminated, because tartrate could function both as buffer and masking agent. It is believed that tartrate has no appreciable reducing action on ferric ion under the conditions of the procedure.

The procedure finally adopted was tested on three National Bureau of Standards standard samples of iron and steel. The results of nickel determinations employing single and double precipitation on these samples are given in Table I.

GRAVIMETRIC PROCEDURE

A weighed sample containing 10 to 25 mg. of nickel is transferred to a 400-ml. beaker and decomposed by accepted methods

Table I. Values Obtained for Nickel by Single and Double Precipitations

N.B.S. Standard Sample	Weight of Sample, G.	Nickel Present, %	Nickel Found, % Single precipitation	Nickel Found, % Double precipitation
33c[a]	0.5	3.28	3.32 3.32	3.27 3.26
82a[b]	1.0	1.07	1.12 1.12 1.12 1.11	1.10 1.10
101b[c]	0.1	8.99	9.17 9.13	
	0.2		9.18	8.90 8.99

[a] Nickel steel (SAE 2335). Mn 0.733; Cu 0.031; Cr 0.052; Mo 0.032; Al 0.032.
[b] Nickel-chromium cast iron. Mn 0.65; Si 2.06; Cu 0.08; Cr 0.33.
[c] 18 Chromium–9 nickel steel (SAE 30905). Mn 0.597; Cu 0.168; Cr 18.49; V 0.049; Mo 0.078; Co 0.078; Cb 0.062; Sn 0.012.

for nickel in iron and steel as when dimethylglyoxime is used (*1*). If an insoluble residue remains, it is filtered off and washed thoroughly with hot water. If this residue is suspected of containing nickel, the paper is burned, treated with hydrofluoric-sulfuric acid, evaporated to dryness, fumed off, fused with bisulfate, taken up with water and a little acid, and added to the main solution. This solution, having a volume of about 250 ml., is cooled to room temperature, tartaric acid solution (250 grams of tartaric acid and 10 ml. of nitric acid per liter, *1*) is added in the proportion of 20 ml. per gram of iron, and the solution is neutralized with ammonium hydroxide, using pHydrion paper. A slight excess of ammonium hydroxide does no harm. If necessary, it is cooled again to room temperature and 0.8% aqueous solution of nioxime is added (8 ml. per 10 mg. of nickel) dropwise, during constant stirring. The precipitate is digested at 60° C., with occasional stirring, for 40 minutes, filtered through a paper of medium porosity, and thoroughly washed with hot water.

The paper and precipitate are returned to the original beaker, and are decomposed in the usual manner with 10 ml. of concentrated sulfuric acid and the necessary quantity of concentrated nitric acid. The solution is diluted with water, heated, and filtered, and the residue is washed thoroughly with hot water. The precipitation in the filtrate is made exactly as before. The precipitate is filtered through a weighed glass or porcelain filter crucible of medium porosity, washed five times with hot water, and dried at 110° C. to constant weight. For routine analyses of many kinds, the second precipitation can be profitably omitted. The nickel content is calculated in the manner of Voter, Banks, and Diehl (*3*).

MICROSCOPICAL AND SPOT TESTS

A qualitative comparison was made of the microscopical tests for nickel on a droplet of nickel sulfate solution (containing 0.1 microgram of nickel), employing about 1 cu. mm. of 0.05% aqueous solution of nioxime and about 1 cu. mm. of saturated aqueous solution of dimethylglyoxime. It was observed that the nioxime reagent was more sensitive for nickel, and the precipitate appeared sooner and was visually deeper pink than the dimethylglyoxime compound. Under the microscope, the fine small fibrous masses of nickel nioxime (Figure 1) were bright pink and the larger dimethylglyoxime needles appeared almost black.

In another experiment, a droplet of a prepared solution of National Bureau of Standards sample 82a containing 0.35 microgram of nickel and a large excess of iron was placed on a slide and a few crystals of citric acid and a slight excess of ammonium hydroxide were added. The excess was allowed to evaporate, leaving a solution substantially neutral. On treating with a droplet of 0.8% aqueous nioxime solution, the red color of the nickel nioxime appeared immediately. Under the microscope, crystals were not distinguishable. The test was repeated using dimethylglyoxime. Under the microscope, it had the characteristic appearance of nickel dimethylglyoxime.

Two drops of a prepared solution of sample 82a containing about 0.07 microgram of nickel were placed on a spot plate in each of three depressions. Several crystals of citric acid were added to each and the solutions were made slightly alkaline with ammonium hydroxide. The excess ammonium hydroxide was allowed to evaporate, leaving a solution substantially neutral. To one solution was added a drop of 0.8% aqueous nioxime solution and to another a drop of saturated aqueous dimethylglyoxime solution, while the third was a control. The solution treated with nioxime was a pale but definite pink, whereas the one to which dimethylglyoxime was added was very faint pink or uncertain.

Figure 1. Nickel Compound of 1,2-Cyclohexanedione Dioxime Precipitated from Pure Nickel Sulfate Solution (×258)

When the above spot tests were repeated after making a separation of iron with ammonium hydroxide, nioxime gave a positive and dimethylglyoxime a negative result.

As a spot test for nickel, nioxime is very satisfactory. It is more sensitive than dimethylglyoxime. However, the particles of nickel nioxime are too small to be useful microscopically.

LITERATURE CITED

(1) Am. Soc. Testing Materials, "A.S.T.M. Methods of Chemical Analysis of Metals," p. 62–4, Philadelphia, American Society for Testing Materials, 1946.
(2) Johnson, W. C., and Simmons, M., *Analyst*, **71**, 554–6 (1946).
(3) Voter, R. C., Banks, C. V., and Diehl, H., ANAL. CHEM., **20**, 458–60 (1948).
(4) *Ibid.*, pp. 652–4.

RECEIVED April 15, 1949.

Iodometric Determination of Acid Value of Lac

N. R. KAMATH AND V. B. MAINKAR
Department of Chemical Technology, University of Bombay, Bombay, India

THE acid value of lac is generally determined by dissolving lac in a suitable solvent, usually alcohol, titrating the solution with a standard solution of alkali, either aqueous or alcoholic, and judging the end point by means of a suitable indicator. Thus, Weinberger and Gardner (*13*) recommend a solution of 5 grams of lac in 50 ml. of alcohol, titrated against 0.5 *N* alcoholic potassium hydroxide in as short a time as possible, using thymol blue as an indicator. This method has been adopted by the United States Shellac Importers' Association (*11*). On the other hand, the British Standards Institution (*3*) recommends the use of a 0.1 *N* alcoholic potassium hydroxide solution in conjunction with Alkali Blue 6B, as an external indicator; while in the case of bleached lac phenolphthalein is used as an indicator (*2*). The time taken for the titration has not been taken into consideration.

Gidvani and Dobbie (*5*) have made a thorough study of the methods recommended for the determination of the acid value of lac and have suggested that to get sufficiently reliable values it is necessary to use 0.1 *N* alkali, as higher concentration of alkali causes hydrolysis. Moreover, the time taken for the titration should be less than 2 minutes. That lac resin is easily saponified by alkali has been shown by Knott (*6*), who found that in an excess of even weak alkali (about 0.1 *N* potassium hydroxide)

Table I. Acid Values of Lac

Type of Lac	Iodometry	Alkalimetry: Universal indicator	Alkalimetry: Thymo blue
1[a] Dewaxed and decolorized shellac	69.05 68.95	68.11 67.87	74.23 73.16
2[a] Seed lac	54.46 56.40	54.91 54.01	67.74 69.60
3[a] Crown shellac	64.71 64.71	65.06 65.13	73.94 73.82
4[a] Dewaxed lemon shellac	65.89 66.03	65.72 67.11	73.13 73.62
5[a] Dewaxed garnet shellac	59.77 59.89	83.77	84.94 86.22
6 Washed seed lac	60.94 61.22	63.46 63.77	69.69 70.00
7 Dewaxed lac from (6)	61.44 61.37	65.45 65.75	70.84 71.12
8 Ether-soluble from (7)	70.49 73.70	80.42 79.50	91.28 88.14
9 Ether-insoluble from (7)	55.08 54.88	57.20 57.89	63.04 63.53

[a] Commercial samples

there is appreciable hydrolysis in a very short time. Thus, when a lac solution is titrated with alkali there is likely to be a certain amount of saponification and consequently higher results are obtained. The difficulties of obtaining a correct acid value are further enhanced by the use of indicators. In the titration of a weak acid with a strong base like potassium hydroxide, complete neutralization of the acid takes place only in the alkaline pH range. This results in further hydrolysis of lac, giving higher values depending on the indicator employed.

In order to overcome the difficulty of judging the end joint when indicators are used, Gardner and Whitmore (*4*) employed the potentiometric method, using a hydrogen electrode. They, however, found the preparation and maintenance of the hydrogen electrode rather elaborate, and in a later publication Murty, Weinberger, and Gardner (*9*) advocated the use of antimony or quinhydrone electrodes, which gave reproducible results. The potentiometric titrations require a much longer time, as one has to wait for constancy of voltage before taking a reading. In the light of the observations of Knott and others the potentiometric method would, therefore, give much higher results.

Thus none of the methods recommended for the determination of the acid value of lac is likely to give the correct values. Of the various methods that have been suggested for estimating the acid value of organic substances, without using an alkali, perhaps the most interesting method is the one based on iodometry, which is represented by the equation

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

Kolthoff (*7*) claims that acids of dissociation constant 10^{-6} may be determined by this method; the pH at the end of the titration is 7 to 7.3.

Lüdtke (*8*) has proposed and used the potassium iodide–potassium iodate method for the determination of the carboxyl content of oxycellulose. The Lüdtke method has been considerably modified and used by Nabar and Padmanabhan (*10*) in their work on oxycellulose. This method has also been used by Ruziczka (*12*) for oils, fats, lacquers, hydrolyzates, and organic acids, but the details of his method are not available.

In the iodometric method, no alkali is employed and the final pH is only 7 to 7.3; furthermore, the end point with starch as indicator should be easily discernible even with dark colored solutions. Such a method should, therefore, be eminently suitable for lac. However, as lac is insoluble in water, the method usually employed for aqueous solutions will have to be considerably modified.

In the original method proposed by Lüdtke (*8*) sodium chloride solution is added to a suspension of oxycellulose in water in order to bring out the carboxyl groups, followed by the addition of a solution of potassium iodide–potassium iodate; the iodine liberated is finally titrated with a standard solution of sodium thiosulfate using starch as indicator. When this procedure was employed in the case of lac, the value did not reach constancy even after 24 hours' contact.

Kolthoff has also stated that if the reaction with acid is carried out in the presence of sodium thiosulfate, equilibrium is established in a very short time, the excess of sodium thiosulfate being titrated with standard iodine solution. When this procedure was adapted in the case of lac, the end point was not very sharp. Starch as an indicator is not effective in the presence of large quantities of alcohol. In order to get a solvent mixture in which both the iodate and lac will remain in solution, various combinations were tried, including the replacement of the sodium chloride by other reagents. Excellent results could be obtained by employing excess of potassium iodide in 2 to 1 alcohol, adding the other reagents as aqueous solutions. Addition of small quantities of distilled water at an intermediate stage is also helpful in giving a homogeneous solution.

APPARATUS AND REAGENTS

Iodine value flasks of 250-ml. capacity are convenient.

Potassium Iodide Solution, 1 *M*. Dissolve 24.9 grams of C.P. potassium iodide in 100 ml. of 95% alcohol and 50 ml. of distilled water.

Potassium Iodate Solution, 0.1 *M*. Dissolve 3.21 grams of C.P. potassium iodate in 150 ml. of distilled water.

Sodium Thiosulfate Solution, 0.1 *N*. Dissolve 25 grams of sodium thiosulfate pentahydrate (reagent grade) in 1000 ml. of distilled water and standardize against potassium dichromate, after about 10 days.

Iodine Solution, 0.1 *N*. Dissolve 20 to 25 grams of potassium iodide in a minimum amount of water, add 12.7 grams of iodine (reagent grade), and dilute to 1000 ml. after all the iodine has been dissolved in the potassium iodide.

Starch Solution. Triturate 1 gram of soluble starch into a paste with a little cold water and slowly add to 200 ml. of boiling water. Boil 1 or 2 minutes to get a clear solution.

PROCEDURE

Weigh accurately about 0.4 gram of lac in a 250-ml. iodine value flask and dissolve it in 20 ml. of 95% alcohol, warming if necessary. When solution is complete, cool, and then add 10 ml. of 1 *M* potassium iodide solution, followed by 10 ml. of 0.1 *M* potassium iodate solution, 10 ml. of 0.1 *N* sodium thiosulfate, and then 10 ml. of distilled water. Shake thoroughly until a clear solution is obtained, then add 20 ml. of 0.1 *N* iodine solution. Mix well and immediately titrate the excess iodine with standard sodium thiosulfate, using starch as indicator.

Carry out a blank with all the reagents except lac.

The acid value is given by the expression

Acid value = (ml. of thiosulfate for lac − ml. of thiosulfate for blank) × (normality of thiosulfate) × 56.1/weight of lac

The end point with starch indicator is sharp.

In Table I the acid values of some commercial and authentic samples of lac are given. The values determined by the method recommended by Gidvani and Dobbie, using universal and thymol blue indicators, are included for comparison.

DISCUSSION

In Table I the values obtained with the iodometric method are, in general, lower than those obtained by the usual alkalimetric method, using either universal indicator or thymol blue. This is to be expected, as the hydrolysis that takes place in the alkalimetric method is entirely absent in the iodometric method. The higher values obtained in the case of garnet shellac by alkalimetry are evidently due to the difficulty of judging the end point, as garnet shellac gives a deep red solution. Of greater interest,

however, are the results obtained with ether-soluble lac. The values reported in the literature for this portion of lac are generally between 90 and 110, compared with the low value of 70 obtained by the present method. Possibly the ether-soluble portion of lac contains ester linkages highly susceptible to alkali; a structure involving a lactone linkage has been suggested by Bhowmik and Sen (*1*). The saponification value of the ether-soluble lac is about the same as that mentioned in the literature.

LITERATURE CITED

(1) Bhowmik, T., and Sen, H. K., *J. Indian Chem. Soc., Ind. Ed.*, 7, 160 (1944).
(2) British Standard Specification for Bleached Lac, No. 1284 (1946).
(3) British Standard Specification for Lac, No. 954 (1941).
(4) Gardner, W. H., and Whitmore, W. F., IND. ENG. CHEM., ANAL. ED., 1, 205 (1929).
(5) Gidvani, B. S., and Dobbie, J. M., London Shellac Research Bureau, *Bull.* 4 (1940).
(6) Knott, E. B., *J. Soc. Chem. Ind. (Trans.)*, 59, 252 (1940).
(7) Kolthoff, I. M., and Furman, N. H., "Volumetric Analysis," Vol. II, pp. 350–89, New York, John Wiley & Sons, 1929.
(8) Lüdtke, M., *Angew. Chem.*, 47, 520, 537 (1934).
(9) Murty, N. N., Weinberger, H., and Gardner, W. H., Indian Lac Research Institute, *Bull.* 25 (1936).
(10) Nabar, G. M., and Padmanabhan, C. V., private communication.
(11) Rangaswami, M., and Sen, H. K., "Handbook of Shellac Analysis," p. 65, Indian Lac Research Institute 1942.
(12) Ruziczka, W., *Farben-Chem.*, 13, 25 (1942); *Peintures, pigments, vernis*, 17, 610 (1942).
(13) Weinberger, H., and Gardner, W. H., IND. ENG. CHEM., ANAL. ED., 5, 267 (1933).

RECEIVED April 12, 1949.

Microdetermination of Iodine

An Improvement in Reflux Distillation Apparatus and Technique

J. W. THOMAS, L. A. SHINN, H. G. WISEMAN, AND L. A. MOORE

Bureau of Dairy Industry, United States Department of Agriculture, Washington, D. C.

THREE papers (*2, 3, 8*) describe the determination of iodine in small amounts of plasma by employing the catalytic effect of iodide on cerate reduction by arsenite. There are certain undesirable features of each proposed method. The Chaney micromethod (*3*) as modified by Taurog and Chaikoff (*8*) has given very low and variable recoveries (10 to 80%) in the authors' laboratory and in others (*2, 5*). Barker's modification (*2*) of the absorbing solution used by Talbot (*7*) is undesirable because it greatly lengthens an already long procedure.

The procedure outlined by Taurog and Chaikoff (*8*) and Chaney (*3*), consisting of wet-ashing the sample with chromic-sulfuric acids followed by distillation, has been adapted with two principal modifications, and used with good results. The addition of a small amount of arsenite to the 1.2 ml. of 1% sodium hydroxide in the absorption trap made possible satisfactory (94 to 100%) recoveries of added iodine. One or two drops of a sodium arsenite solution were used, made by dissolving 2.48 grams of arsenic trioxide in 15 ml. of water containing 1.7 grams of sodium hydroxide, diluting, neutralizing with sulfuric acid, adding 10 grams of sodium bicarbonate, and diluting to 500 ml. A solution of arsenic trioxide alone in sodium hydroxide would probably be adequate for the absorbing liquid. In other halide distillation techniques (*1, 6*) arsenite has been used in the trap in order to ensure quantitative recoveries. The reducing medium in the absorption trap probably prevents oxidation of the distilled iodide. Iodide is then determined in the distillate diluted to the desired volume, using the catalytic action of iodide on the reduction of cerate by arsenite.

A simplified reflux still (Figure 1) permits the operator to digest and distill from the same flask having but one standard-taper ground-glass joint. Actually a reflux still was found to be unnecessary for recovery of iodide, but the volume in the boiling flask must be maintained.

Add 25 ml. of redistilled water to the chromic acid–digested sample in a 500-ml. flask. Put 1.2 ml. of 1% sodium hydroxide and 1 drop of 0.1 *N* sodium arsenite solution into the absorption trap, which is well insulated by wrapping it with asbestos or cork. Then attach the flask to the reflux still and heat it. When distillation starts add 3.0 ml. of 50% phosphorous acid to the flask through the dropping funnel. It is not necessary to use pressure to force the phosphorous acid through the dropping funnel, as it is when using the Riggs modification of the Chaney still (*2, 5, 8*). Distill for 7 to 8 minutes, remove heat from flask, and draw off distillate into a 25-ml. volumetric flask. Wash the trap with small portions of warm redistilled water and collect these in the volumetric flask. It is much simpler and easier to wash out this absorption trap after each distillation than to wash the trap on the Riggs-Chaney still. The distillate volume is made up to 25 ml. when aliquots are taken for the colorimetric determination of iodide by its catalytic effect on reduction of cerate in the presence of excess arsenite.

The float valve is merely a thin-walled hollow glass sphere with a short tail. The capillary water return is 6 cm. long and is turned up 2 cm. Its inside diameter is 1 to 2 mm. The entire still was made by one of the authors (H.G.W.).

Details of other techniques and procedures have been adequately described (*2, 3, 8*).

With one exception all samples of C.P. chromic acid that were tried contained too much iodine for this procedure. However, a sample of Grasselli's technical grade was found to be sufficiently low in iodine (approximately 0.05 microgram per gram). Green-

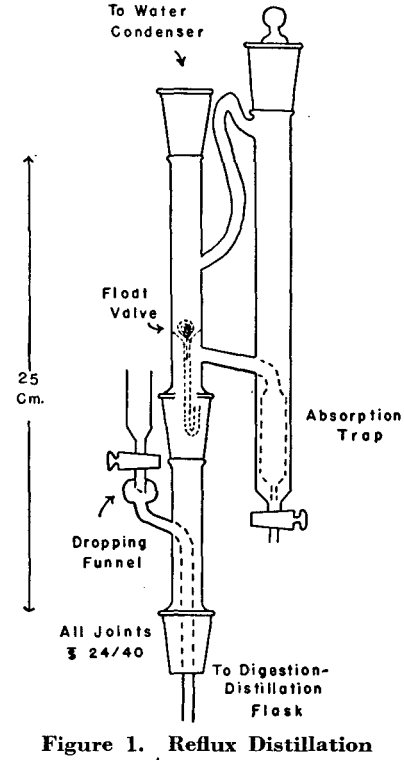


Figure 1. Reflux Distillation Apparatus

man (5) used a sample of Fisher's technical grade that contained nearly 0.03 microgram per gram. One sample of C.P. arsenic trioxide contained a substance that interfered with the iodide-catalyzed reduction of cerate by arsenite.

With the authors' still, boiling for 7 minutes after addition of the phosphorous acid gave recoveries of 94 to 100% when arsenite was used in the trap. Using arsenite in the distillation trap Greenman (5) reports recoveries averaging 95% for iodide in diiodotyrosine compared with low variable amounts without the use of arsenite. Since this manuscript was prepared another article describing a suitable still for this purpose has come to the attention of the authors (4).

LITERATURE CITED

(1) Alford, W. C., *J. Ind. Hyg. Toxicol.*, **29**, 396–9 (1947).
(2) Barker, S. B., *J. Biol. Chem.*, **173**, 715–24 (1948).
(3) Chaney, A. L., IND. ENG. CHEM., ANAL. ED., **12**, 179–81 (1940).
(4) Connor, A. C., *et al.*, *Surgery*, **25**, 510–17 (1949).
(5) Greenman, University of Pittsburgh, personal communication. 1948.
(6) Martinek, M. J., and Marti, W. C., IND. ENG. CHEM., ANAL. ED., **3**, 408 (1931).
(7) Talbot, N. B., Butler, A. M., Salzman, A. H., and Rodriguez, P. M., *J. Biol. Chem.*, **153**, 479–88 (1944).
(8) Taurog, A., and Chaikoff, I. L., *Ibid.*, **163**, 313–22 (1946).

RECEIVED June 8, 1949.

Polarographic Data on Zinc in Small Concentrations

Deviations from the Ilkovič Equation

LUDWIG S. CHAMPA AND ABRAHAM WALLACH

Division of Industrial Hygiene, Montana State Board of Health, Helena, Mont.

DURING the development of a method for the determination of small amounts of zinc in various concentrations of very dilute sulfuric acid by polarographic means, a lack of proportionality between wave height and concentration was noted. A closer study verified these initial findings and the results that were obtained under varying conditions of drop time and concentration are reported below.

APPARATUS

The determinations were made with a Sargent Model XII polarograph. Lithium chloride (0.1 *N*) was used as the supporting electrolyte, because maxima effects—reported with other alkali chlorides—were absent. The galvanometer calibration factor, according to the manufacturer, was 0.0051 microampere per millimeter.

The ratios of the sensitivity settings (2 to 5, 5 to 10, 10 to 20) were checked in triplicate, using various concentrations of cadmium (with zinc there was a noticeable decrease in diffusion current with an increase in applied e.m.f. after 1.0 volt), in order to make sure that conversion from each of these four sensitivities was permissible on the instrument. To ensure more accurate measurements of the wave heights, the concentration of cadmium in each case was such that the wave height for the less sensitive reading was from 46 to 60 mm. and that for the corresponding more sensitive reading was 112 to 120 mm. The maximum deviation during each set of runs varied from less than 0.5% for the 10, 20 comparison to less than 1% for the 5, 10 and 2, 5 comparisons. The ratio of average wave heights for the 10, 20 comparison was exactly 2.00; for the 5, 10 sensitivity comparison was 1.99 (instead of 2.00); and for the 2, 5 comparison was 2.53 (instead of 2.50).

EXPERIMENTAL

Kolthoff and Lingane (3) have stated that with a drop time between 3 and 6 seconds there is strict linear proportionality between the diffusion current and the concentration of metal ions analyzed. However, they mention that other investigators (1, 6) have claimed that at very small concentrations the diffusion current is greater than corresponds to strict linear proportionality with the concentration.

Because the authors were dealing with small amounts of zinc, a series of experiments was run with dropping times varying from 2.77 to 5.90 seconds to determine whether or not there was strict proportionality between current and concentration.

The first series of experiments was made with five different concentrations of zinc, varying from 0.10 to 0.005 mg. per ml. (1.53×10^{-3} to 0.77×10^{-4} *M*). A primary solution was prepared by dissolving zinc sulfate heptahydrate, and standardized by the potassium ferrocyanide method mentioned by Treadwell and Hall (7). From this solution a standard was prepared containing 0.1 mg. of zinc per ml. in 0.1 *N* lithium chloride. The other concentrations were obtained by taking aliquot portions (25 or 50 ml.) of the 0.1 *N* standard or weaker concentrations already made, and diluting them in proper volumetric flasks with 0.1 *N* lithium chloride.

The sensitivity used for 0.10 mg. per ml. was 20; for 0.05 mg. per ml., 10; for 0.02 mg. per ml., 5; and for 0.01 and 0.005 mg. per ml., 2. The temperature of the water bath was kept at 25° ± 0.2° C. In measuring the wave heights for zinc, it was found that reproducible results were best obtained by drawing the limiting current line parallel to the residual current whenever its angle with the horizontal was less than that formed by the residual current. (There is a decrease in diffusion current with an increase in e.m.f. above 1.0 volt.) Corrections for residual current were made, as indicated by Kolthoff and Lingane (2), in measuring diffusion current.

The polarograms obtained at the 3.0-second drop time were run in triplicate on each of two separate portions of solution. With the other drop times, determinations were mostly made in quadruplicate on the same portion of solution. Where the deviation on one of these four results was greater by four times the average deviation of the remaining results, this single result was discarded. The data are shown in Table I.

DISCUSSION

Ion Concentration. As can be seen from Table I, the ratio of diffusion current to concentration is not constant within the range of zinc concentrations tested, but increases progressively with decreasing concentrations at drop times less than 3.7 seconds, being more marked with lower drop times. The ratio of diffusion current to concentration also increases at higher drop times (4.18 and 4.90 seconds). Whether it is progressive or not was difficult to ascertain, because an experimental variation in measurement of the polarograms of even 0.5 mm. at these drop times can cause a relative difference of 1%. For example, if one were to use the 43.8 figure (drop time 4.18, concentration 0.005) the average wave height would be 43.5 and the per cent deviation 3.3 instead of 3.1.

Effective Pressure. In view of these observations, the data were analyzed to see whether (with all other factors constant) the diffusion current was proportional to the square root of the effective pressure on the dropping mercury. According to data indicated by Kolthoff and Lingane (4) the back pressure due to interfacial tension at the surface of the mercury drop varies, with usual capillaries used, from 2 to 3 cm. of mercury. In making this correction on the height of the mercury column, the figure 2.5 cm. was used. Any error due to a deviation of 0.5 cm. would make little difference in the square root of the effective heights used in the data in Table II.

The data in Table II indicate that although with a concentration of 0.1 mg. of zinc per ml., the ratio of $i_d/h^{1/2}$ does not vary to any greater degree with varying pressures of mercury columns than the data obtained by Maas (5), this is not so with lower concentrations of zinc. At a concentration of 0.005 mg. of zinc per ml., the deviation is about 14.2% with a difference in pressure of only 25%; and for a range of pressure that varies twofold, there is a deviation in the ratio of $i_d/h^{1/2}$ of about 17.9%.

Table I. Determination of Zinc

Dropping Time	Height of Hg Column	Zn Concn.	Wave Height	Height Corresponding to 20 Sensitivity	Ratio, Height/ Concn.	Deviation
Sec.	*Cm.*	*Mg./Ml.*	*Mm.*	*Mm.*		%
2.77	93.0	0.10	107.6			
			107.0			
			108.0			
			106.8			
		Av.	107.4	107.4	1074	..
		0.02	94.0			
			93.9			
			94.9			
			94.6			
		Av.	94.4	23.6	1180	9.9
		0.01	119.9			
			119.8			
			120.3			
		Av.	120.0	12.0	1200	11.8
		0.005	63.8			
			64.3			
			63.0			
			63.0			
		Av.	63.5	6.35	1270	18.3
3.00	87.0	0.10	103.4			
			104.2			
			103.6			
			102.3			
			102.4			
			103.5			
		Av.	103.2	103.2	1032	..
		0.05	104.7			
			103.2			
			103.4			
			104.6			
			103.6			
			102.8			
		Av.	103.7	51.8	1037	0.5
		0.02	85.3			
			85.2			
			85.7			
			85.0			
			84.5			
		Av.	85.1	21.3	1065	3.1
		0.01	117.7			
			116.4			
			116.9			
			118.0			
			116.3			
			117.3			
		Av.	117.1	11.7	1170	13.5
		0.005	61.2			
			61.3			
			61.4			
			61.3			
			61.7			
			61.0			
		Av.	61.3	6.13	1226	18.9
3.25	79.0	0.10	100.1			
			99.8			
			99.5			
		Av.	99.8	99.8	998	..
		0.05	102.0			
			101.3			
			100.6			
		Av.	101.3	50.7	1014	1.5
		0.02	82.7			
			82.2			
			81.9			
			82.3			
		Av.	82.3	20.6	1030	3.2
		0.01	106.4			
			105.0			
			106.0			
			105.5			
		Av.	105.7	10.6	1060	6.0
		0.005	54.8			
			55.2			
			54.8			
			54.9			
		Av.	54.9	5.49	1098	10.0
3.65	71.0	0.10	91.6			
			91.8			
			92.0			
			91.5			
		Av.	91.7	91.7	917	..
		0.05	92.1			
			92.0			
			93.4			
		Av.	92.5	46.3	926	1.0
		0.02	75.5			
			75.8			
			75.0			
			75.4			
		Av.	75.4	18.9	945	3.1
		0.01	96.4			
			96.0			
			95.9			
		Av.	96.1	9.61	961	4.8
		0.005	47.4			
			47.0			
			48.2			
			47.3			
		Av.	47.5	4.75	950	3.6
4.18	62.0	0.10	83.7			
			84.2			
			84.6			
		Av.	84.2	84.2	842	..
		0.02	69.9			
			69.3			
			69.4			
			70.0			
		Av.	69.6	17.4	870	3.3
		0.005	43.8 (?)			
			43.2			
			43.4			
			43.5			
		Av.	43.4	4.34	868	3.1
5.90	46.0	0.10	72.6			
			71.6			
			71.7			
		Av.	72.0	72.0	720	..
		0.02	59.5			
			60.0			
			60.0			
		Av.	59.8	15.0	750	4.1
		0.01	73.0			
			73.0			
			71.6			
		Av.	72.5	7.25	725	0.7
		0.005	36.3			
			36.1			
			36.4			
			36.2			
		Av.	36.2	3.62	724	0.6

In order to check any error by having assumed an incorrect back pressure due to interfacial tension, the applied height of mercury was plotted against the diffusion current squared.

If $i_d \propto \sqrt{h_{app.} - h_{s.t.}}$ where $h_{s.t.}$ is the back pressure due to interfacial tension, and $h_{app.}$ is the hydrostatic pressure on the mercury drops, then

$$i_d^2 \propto h_{app.} - h_{s.t.}$$

or

$$i_d^2 = Kh_{app.} - Kh_{s.t.}$$

Thus a straight line should be obtained if i_d^2 is plotted against $h_{app.}$ This was nearly so with the six points from the data on the concentration of 0.1 mg. of zinc per ml., but definitely not so with data obtained on the 0.02, 0.01, and 0.005 mg. of zinc per ml. runs.

Acid Concentration. Because the samples contained small amounts of sulfuric acid, a series of experiments was run to determine the effect on diffusion current by varying the concentration of acid. The authors did not have equipment for measuring the pH accurately and easily, so that the acid concentrations are expressed in normality. All runs were made with a dropping time of 3.0 seconds. Solutions containing no acid and 0.001, 0.002, 0.0025, and 0.005 N acids all having 0.10, 0.05, and 0.010 mg. of zinc per ml., respectively, were run in duplicate. The results showed that the diffusion current for each particular concentration of zinc, irrespective of acid concentration, did not vary more than 1% of the average of results obtained. Thus, in

Table II. Relation between Diffusion Current and Pressure on Dropping Mercury

h Applied	h Corrected	$h^{1/2}$ Corrected	Concentration of Zinc, Mg./Ml. 0.10		0.02		0.01		0.005	
			id	$id/h^{1/2}$	id	$id/h^{1/2}$	id	$id/h^{1/2}$	id	$id/h^{1/2}$
Cm.	*Cm.*	*Cm.*	*Mm.*		*Mm.*		*Mm.*		*Mm.*	
93.0	90.5	9.51	107.4	11.31	94.4	9.94	120.0	12.63	63.5	6.69
87.0	84.5	9.20	103.2	11.27	85.1	9.25	117.1	12.72	61.5	6.69
79.0	76.5	8.74	99.8	11.43	82.3	9.42	105.7	12.21	54.9	6.30
71.0	68.5	8.28	91.7	11.09	75.4	9.10	96.1	11.63	47.5	5.74
62.0	59.5	7.71	84.2	10.94	69.6	9.03	...	...	43.4	5.63
46.0	43.5	6.60	72.0	10.90	59.8	9.05	72.5	11.00	36.2	5.49

such weak concentrations of sulfuric acid, the ratio of diffusion current to the concentration of zinc—as in the experiments run in neutral solution—is also not constant but increases progressively.

SYNOPSIS

A series of experiments was run on polarographic analysis of zinc in concentrations from 0.005 to 0.10 mg. per ml. in various concentrations of sulfuric acid. At such low concentrations of zinc, the ratio of diffusion current to concentration increases progressively with decreasing concentration in either neutral or acid solutions up to 0.005 N, especially with smaller dropping times. Within this range of no acid to 0.005 N sulfuric acid, and especially at the lower concentrations of zinc, the ratio of the diffusion current to the square root of effective pressure on the mercury drop increases with a decrease in dropping time. Sulfuric acid concentration has no appreciable effect on diffusion current up to a strength of 0.005 N. The use of lithium chloride as a supporting electrolyte obviates the need for a suppressor of maxima usually obtained with other alkali chlorides when running zinc.

LITERATURE CITED

(1) Hamamoto, E., *Collection Czechoslov. Chem. Commun.*, **5**, 427 (1933).
(2) Kolthoff, I. M., and Lingane, J. J., "Polarography," p. 57, New York, Interscience Publishers, 1946, revised reprint.
(3) *Ibid.*, p. 60.
(4) *Ibid.*, pp. 67, 68.
(5) *Ibid.*, p. 71.
(6) Thanheiser, G., and Maassen, G., *Mitt. Kaiser-Wilhelm-Inst. Eisenforsch. Düsseldorf*, **19**, 27 (1937).
(7) Treadwell and Hall, "Analytical Chemistry," Vol. II, 5th Printing, p. 667, New York, John Wiley & Sons, 1947.

RECEIVED February 28, 1949.

Direct Determination of Tin in Pig Tin

SILVE KALLMANN
Ledoux & Co., 155 Sixth Ave., New York, N. Y.

THE evaluation of pig tin involves the determination of impurities, such as lead, copper, antimony, arsenic, bismuth, cadmium, zinc, iron, nickel, cobalt, and sulfur; tin is calculated "by difference." This indirect determination of tin provides an accurate tin figure and reveals the nature and percentage of contaminating elements. Unfortunately, it is very time-consuming and involved.

Frequently, the producer and buyer of pig tin are not so much interested in the impurities as in whether the material meets certain specifications based on its tin content. In such cases a rapid, accurate, and direct determination of tin is of great importance. Unfortunately, the usual iodometric determination of tin must be run on a small portion of the sample and is therefore scarcely more accurate than ±0.25%.

A method for the determination of tin in Bolivian tin concentrates (*1*) to a large extent obviates the difficulties of the usual iodometric determination of tin. Based on several years of experience with the method in its original form, and on the advice of several chemists, certain changes were worked out.

PROCEDURE

Weigh 5.0000-gram portions of pig tin in the form of sawings or drillings and also 5.0000-gram portions of standard C.P. tin into 750-ml. Erlenmeyer flasks. If an accuracy better than 0.05% is desired, use 10.0000-gram portions.

Add 100 ml. of concentrated hydrochloric acid and cover with a glass cover to avoid loss by spraying and to prevent oxidation of stannous chloride; 5 grams of tin usually dissolve in 2 to 3 hours in the cold acid. Gentle warming on a plate with a surface temperature not higher than 60° C. speeds up solution of the sample. Disregard any small residue consisting of undissolved copper, bismuth, or antimony. If the residual metallic sponge looks larger than a few milligrams and is suspected to hold back tin (particularly with lower grades of tin), add a few milligrams of potassium chlorate. Avoid any excess. The potassium chlorate will oxidize a small quantity of stannous chloride to stannic chloride, which in turn will dissolve any copper, antimony, or lead.

Add about 10 grams of sodium chloride and dilute to about 300 ml. with hot water. If potassium chlorate was used, precipitate any copper, antimony, or bismuth by warming gently with a few grams of iron wire or drillings low in carbon, keeping the flask covered continually with a small cover glass. Omit the treatment with iron if no potassium chlorate was used.

Introduce into the Erlenmeyer flasks two nickel strips or foils weighing at least 5 grams each. Close the flasks with rubber stoppers containing a bent-glass tube extending on the outside to the bottom of the flasks. Boil the solution gently for about 75 minutes, or until the volume has been reduced to about 200 ml., then seal the end of the glass tube with a hot solution of sodium bicarbonate in a 250-ml. beaker. Remove the flasks from the hot plate and cool in running water to below 15° C.

TITRATION

When dealing with 5.0000-gram portions of pig tin, weigh accurately 2.9400 grams of potassium iodate into small beakers, and add about 0.5 gram of sodium bicarbonate and about 100 ml. of water (60° to 70° C.) which has previously been boiled. Stir gently with a glass rod until the salts have dissolved but prevent any losses; 2.9400 grams of potassium iodate theoretically oxidize 4.8918 grams or 97.84% of the tin present in a 5-gram sample. Therefore less potassium iodate should be added for pig tins lower than 98%.

Prepare a dilute solution of potassium iodate by dissolving 3.0000 grams of potassium iodate in a 1000-ml. volumetric flask in warm water. Cool, fill to the mark with cold water, and mix.

Remove the rubber stopper from the Erlenmeyer flask containing the reduced tin solution and add immediately and

Table I. Accuracy of Potassium Iodate Method

Tin by Potassium Iodate Method[a], %	Tin Calculated by Difference, %
99.83	99.85
99.26	99.23
99.64	99.64
99.92	99.90
98.42	98.48
97.12	97.09

[a] Average of three.

Example

5 Grams of C.P. Standard Tin Taken		5 Grams of Pig Tin Taken	
KIO_3 added, grams	2.9400		2.9400
KIO_3 titrated, ml.	20.50		10.00
20.50 ml. KIO_3 ≡	0.0615 gram KIO_3	10.00 ml. KIO_3 ≡	0.0300 gram KIO_3
	2.9400		2.9400
Total	3.0015 grams KIO_3		2.9700 grams KIO_3
Factor.	$\frac{5.0000}{3.0015} = 1.6658$	Sn =	$\frac{2.9700 \times 1.6658 \times 100}{5}$
Theoretically.	$\frac{356.10}{214.02} = 1.6639$	Sn =	98.95%

quantitatively the solution of 2.9400 grams of potassium iodate, washing the beaker thoroughly with cold water. Agitate the Erlenmeyer flask at once in order to prevent any prolonged contact of the iodine that is formed upon the nickel strip and the precipitated antimony or copper. Add starch solution and titrate with the dilute potassium iodate solution (3 grams per liter) to the usual blue end point. If available, pass carbon dioxide gas into the Erlenmeyer flask from the moment the rubber stopper is removed.

Calculation. Theoretically, because 1 mole of potassium iodate ≡ 3 moles of tin, 214.02 grams of potassium iodate = 356.1 grams of tin.

ANALYTICAL RESULTS

The data in Table I show that results obtained in actual analysis by the potassium iodate method agree very well with those of the indirect method where tin is calculated by difference.

LITERATURE CITED

(1) Kallmann, S., IND. ENG. CHEM., ANAL. ED., 15, 166–74 (1943).

RECEIVED October 28, 1949.

CRYSTALLOGRAPHIC DATA

30. β-Alanine (β-Aminopropionic Acid)

Contributed by J. KRC, JR., AND W. C. MCCRONE, Armour Research Foundation, Illinois Institute of Technology, Chicago 16, Ill.

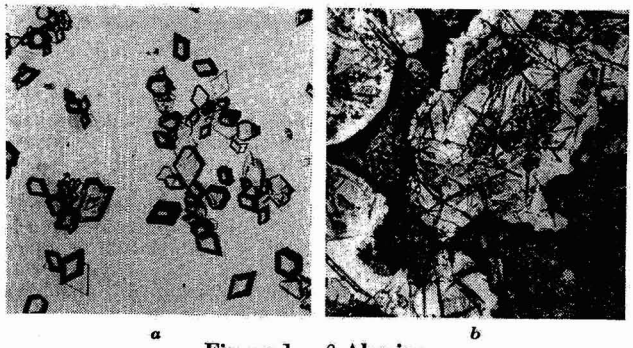
IN CONNECTION with the foundation's file of crystal data there are inquiries for crystallographic data on the following compounds: aureomycin, acetoacetanilide, diethyl carbamate, and heavy metal salts of fatty acids. It would be appreciated if anyone having unpublished and even fragmentary data on any of these compounds would send the information to W. C. McCrone, who will send it to the interested parties.

CRYSTALLOGRAPHIC DATA FOR β-ALANINE

$H_2N—CH_2—CH_2—COOH$

Structural formula of β-alanine

Excellent crystals of β-alanine are obtained from *n*-propyl alcohol and from water solutions (Figure 1). An orthographic



a *b*

Figure 1. β-Alanine

a. **Crystals from water**
b. **Fusion preparation. Dark areas are β-alanine and decomposition products. Lighter areas contain needlelike sublimate of β-alanine**

projection of a typical β-alanine crystal is shown in Figure 2. Pronounced 010 cleavage, together with the fact that α is parallel to *b*, indicates that the molecules lie in the 010 plane. No evidence of polymorphism was observed.

CRYSTAL MORPHOLOGY

Crystal System. Orthorhombic.

Form and Habit. Tablets lying on brachypinacoid {010} showing bipyramids {111} and macropinacoid {100}.

Axial Ratio. $a:b:c = 0.714:1:0.441$.

Interfacial Angles (Polar). 011 ∧ 01$\bar{1}$ = 132° 24′; 101 ∧ 10$\bar{1}$ = 116° 36′.

Cleavage. 010 strong; 101 slight.

X-RAY DIFFRACTION DATA

Cell Dimensions. a = 9.86 A.; b = 13.81 A.; c = 6.09 A.

Formula Weights per Cell. 8.

Formula Weight. 89.09.

Density. 1.412 (pycnometer); 1.418 (x-ray).

Principal Lines

d	I/I_1
6.86	0.25
4.83	1.00
4.54	0.21
4.14	0.45
3.44	0.83
3.35	0.51
3.03	0.50
2.665	0.20
2.569	0.30
2.413	0.16
2.344	Very weak
2.295	0.13
2.250	0.23
2.211	Very weak
2.167	0.28

OPTICAL PROPERTIES

Refractive Indexes (5893 A.; 25° C.). α = 1.519 ± 0.002; β = 1.591 ± 0.002; γ = 1.600 ± 0.002.

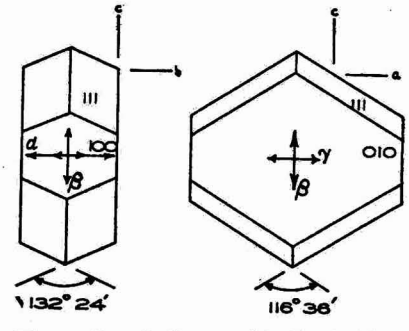


Figure 2. Orthographic Projection of Typical Crystal of β-Alanine

Optic Axial Angle (5893 A.; 25° C.). $2V = 48°$; $2E = 80°$.
Dispersion. $r > v$ moderately strong.
Optical Axial Plane. 001.
Sign of Double Refraction. Negative.
Acute Bisectrix. $b = \alpha$.
Extinction. Parallel and symmetrical.
Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.570$. R(calcd.) = 21.1. R(obsd.) = 20.7.

FUSION DATA

β-Alanine melts at 196° C. with decomposition. The melt supercools to a glass but can be made to crystallize by holding for several minutes at a temperature just below the melting point.

The authors are indebted to R. E. Score of the B. F. Goodrich Chemical Company for the sample of β-alanine used in this study.

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Section, Armour Research Foundation of the Illinois Institute of Technology, Chicago, Ill.

Book Reviews

A.S.T.M. Standards on Petroleum Products and Lubricants (with related information). Committee D–2. xvi + 735 pages. American Society for Testing Materials, 1916 Race St., Philadelphia 3, Pa., 1949. Price, $5.50 and $6.50.

The 1949 A.S.T.M. publication presents many changes over the 1948 edition.

The asphaltic material has been eliminated and the light hydrocarbon section expanded. Methods advanced to standards include D 873–49, Oxidation Stability (Aviation Gasoline); D 187–49, Burning Quality of Kerosene; D 664–49, Neutralization Value (Electrometric Titration); D 938–49, Congealing Point of Pharmaceutical Petrolatums; D 878–49, Chlorides and Sulfates (Insulating Oils); D 877–49, Dielectric Strength (Insulating Oils); D 268–49, Lacquer Solvents, Sampling and Testing; D 923–49 Sampling (Insulating Oils); D 924–49, Power Factor, Dielectric Constant (Insulating Oils); and E 1–49, Specifications, A.S.T.M. Thermometers. Method D 481, Acid Heat of Gasoline, has been discontinued, while D 663, Acid and Base Numbers by Color-Indicator Titration, D 270, Sampling, and D 894, Sulfur in Lubricating Oils Containing Additives, have been replaced by other procedures.

New tentative procedures include D 1017–49T, D 130–49T, D 117–47T, D 1012–49T, D 1018–49T, D 1078–49T, D 1015–49T, D 1016–49T, D 1019–49T, D 1026–49T, E 77–49T, and D 1020–49T to D 1025–49T, inclusive. A total of 25 methods is presented in twelve appendixes, which are followed by a list of the personnel of Committee D–2 and other information of interest.

E. L. BALDESCHWIELER

Normas de Pureza de los Reactivos para Análisis Químicos. *Comité de Reactivos para Análisis de la American Chemical Society.* Translated by *Casimiro Busquets*, Paseo de Gracia, 11, 4°, 4ª, Barcelona, Spain, 1948.

This Spanish translation includes all the specifications published by the Committee on Analytical Reagents, AMERICAN CHEMICAL SOCIETY, through March 1947. As indicated in the subtitle, the translator has incorporated in each specification all corrections and changes that have been published by the committee.

Work of the committee since 1947 has produced additional specifications and modifications of many tests which are being prepared for publication. Because the improvement in quality of reagent chemicals is in general a gradual process, it is probable that chemicals which conform to the specifications published through 1947 will satisfy most of the requirements and tests that may be published in the near future.

Spanish-speaking chemists may obtain information as to the availability of the book from the translator.

W. D. COLLINS

The Analyst's Calendar

Gordon Research Conferences

THE Gordon Research Conferences, sponsored by the American Association for the Advancement of Science and formerly known as the Gibson Island Research Conferences, are to be held from June 26 to September 1 at the Colby Junior College, New London, N. H., and the New Hampton School, New Hampton, N. H.

The program at the Colby Junior College will include sessions on catalysis, June 26 to July 2; petroleum, July 3 to 9; polymers, July 10 to 14; textiles, July 17 to 23; corrosion, July 24 to 28; instrumentation, July 31 to August 6; vitamins and metabolism, August 7 to 11; food and nutrition, August 14 to 20; medicinal chemistry, August 21 to 25; cancer, August 28 to September 1. The program at the New Hampton School includes: chemistry and physics of metals, July 3 to 7; current trends in analytical chemistry, July 10 to 14; organic coatings, July 17 to 21; ion exchange, July 24 to 28; microbiological deterioration, July 31 to August 4; physical methods in nucleic acid and protein research, August 28 to September 1. The full program was published in the March 27 issue of *Chemical and Engineering News*.

Current Trends in Analytical Chemistry
July 10 to 14, New Hampton, N. H.

Introductory Survey. N. H. FURMAN.
A Topic in Instrumental Analysis. R. H. MÜLLER.
Analysis by Chromatographic Adsorption. H. H. STRAIN.
Fluorescence Analysis. CHARLES E. WHITE.
Statistical Methods in Analysis. GRANT WERNIMONT.
Statistical Methods in Analysis. W. J. YOUDEN.
Electrical Methods in Analysis. J. J. LINGANE.
Electrical Methods in Analysis. W. E. CAMPBELL.
Organic Analysis. C. W. GOULD.

International Conference on Spectrography

The Groupement pour l'Avancement des Méthodes Spectrographiques, Paris, France, is organizing an International Conference on Spectrography, to be held in Strasbourg October 12 to 14, 1950, in which American delegates are invited to participate. Three meetings have already been held in Paris to discuss plans for the conference.

Society for Applied Spectroscopy. New York, N. Y., May 26 and 27
Symposium on Molecular Structure and Spectroscopy. Mendenhall Laboratory of Physics, Ohio State University, Columbus, Ohio, June 12 to 17
Third Annual Summer Symposium. Ohio State University, Columbus, Ohio, June 16 to 17
International Microchemical Congress. Graz, Austria, July 2 to 6
Instrument Conference and Exhibit. Instrument Society of America, Buffalo, N. Y, September 18 to 22

AIDS FOR THE ANALYST . . . . .

Use of Thorium Nitrate as a Radioactive Standard for Immersion-Type Geiger-Müller Tubes. Ray L. Shirley, Michigan State College, East Lansing, Mich. (Present address, University of Florida, Gainesville, Fla.)

BALE, Haven, and LeFevre [*Rev. Sci. Instruments*, **10**, 193 (1939)] designed an immersion-type Geiger-Müller counter tube (Distillation Products, Inc., Rochester, N. Y.) for measuring radioactivity of solutions. These tubes are made of glass, with a wall thickness of approximately 0.01 cm., and are not satisfactory for the assay of radioactive isotopes that have emissions less than 0.5 m.e.v. Bale and Bonner ("Physical Methods of Organic Chemistry," Vol. II, Chap. XXV, p. 1249, New York, Interscience Publishers, 1946) stated that a solution of 100 grams of potassium acetate per 100 ml. of water was a satisfactory standard for determining reproducibility of counts with tubes of this type.

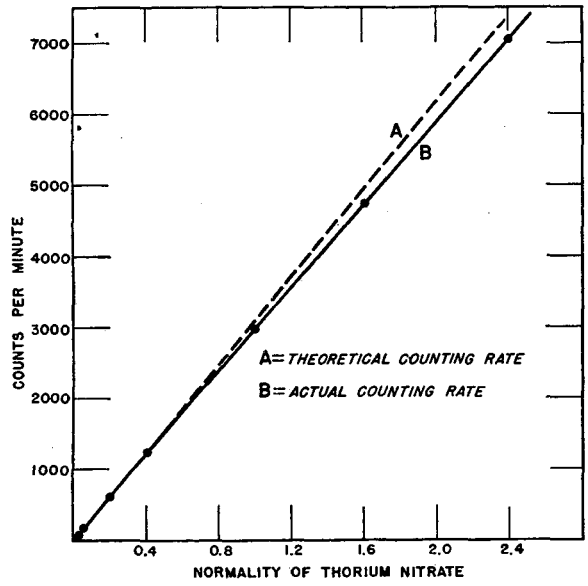


Figure 1. Effect of Increasing Concentrations of Thorium Nitrate on Counting Rate

The writer has found that thorium nitrate, compared to potassium salts, has advantages as a radioactive standard for immersion-type tubes, in that much greater β-particle radioactivity may be obtained per volume of solution. Five milliliters of a 0.02 N thorium nitrate solution used in this investigation were found to have the equivalent activity of the same volume of 5 N potassium acetate; 5 ml. of a 2.4 N solution of thorium nitrate had an activity of approximately 7000 counts per minute when assayed for radioactivity with the dipping-type tubes used in this laboratory. Thorium emits α-particles, has a half-life of approximately 1.3×10^{10} years, and is associated with its degradation products, some of which are β-particle emitters.

The concentrations of the various degradation products that are present in "pure" thorium nitrate, prepared at a known time, may be calculated by Bateman's method [*Proc. Cambridge Phil. Soc.*, **15**, 423 (1910)]. The mesothorium 2 content of thorium nitrate is largely responsible for the β-particles that penetrate the immersion-type counter tubes. Because this isotope is of short half-life, it rapidly comes to equilibrium with its parent, mesothorium 1. The latter element, having a half-life of 6.7 years, increases in concentration for many years before it comes to equilibrium with its parent, thorium. However, when relatively short periods are used for determining instrumental counting capacity, corrections for equilibrium changes should not be necessary.

Several different concentrations of thorium nitrate [$Th(NO_3)_4 \cdot 4H_2O$, Baker's C.P.] were prepared in aqueous solution and the activity was determined using an immersion-type Geiger-Müller tube in conjunction with a modified Neher-Harper quenching circuit and a Tracerlab, Inc., Autoscaler counter. Although the activity determined in these solutions was not a linear function of the concentration of thorium nitrate above a concentration of approximately 0.4 N, the reagent should have value as a radioactive standard to test for instrumental reproduction of counts at fairly high rates per unit of time. As shown graphically in Figure 1, where activity is plotted against concentration of thorium nitrate, the rate of counting begins to deviate from linearity after a counting rate of approximately 2000 counts per minute is obtained. The deviation from linearity increases with increased concentration until a variation of approximately 6% is observed at 7000 counts per minute or at a concentration of 2.4 N thorium nitrate.

The loss of activity with concentrations of thorium nitrate greater than 0.4 N may be due to the density of the solution (Bonner, J. F., Jr., and Bale, W. F., personal communication). It is probably not due to the β-emitting degradation products of thorium precipitating out of solution, as strong acidification of the standard solutions with nitric acid did not decrease the loss. Bale and Bonner ("Physical Methods of Organic Chemistry," Vol. II, Chap. XXV, p. 1249, New York, Interscience Publishers, 1946) reported a corresponding loss of radioactivity when tricalcium phosphate was added to solutions of radioactive phosphorus 32. In the present study a salt-free solution of phosphorus 32, obtained from Oak Ridge, Tenn., was found not to deviate from linearity of counts up to 7000 counts per minute with increased concentrations of the isotope.

Different preparations of thorium nitrate may be expected to vary in their radioactivity, depending on the time of separation of the thorium from its degradation products. The thorium nitrate used in this investigation had been commercially prepared at least 5 years before being used as a standard. Inasmuch as thorium nitrate is a common laboratory reagent, has a long half-life and a high radioactivity, and is very soluble in water, it appears to be a valuable radioactive standard for checking the reproducibility of counts of immersion-type Geiger-Müller tubes and corresponding counter circuits.

PUBLISHED with the permission of the Director of the Michigan Agricultural Experiment Station as journal article No. 1016 (n.s.).

New Standard Samples Issued by National Bureau of Standards

The National Bureau of Standards has added two new alloys to its list of analyzed standard samples.

Standard low-carbon silicon steel 131 is certified for carbon, 0.0028%. Price, $5.00 per 100-gram unit (prepaid)

Standard magnesium-base alloy is now available with a provisional certificate of analysis for the following constituents: aluminum 2.97%, zinc 1.05, manganese 0.45, silicon 0.012, copper 0.011, lead 0.003, iron 0.002, nickel 0.0009. Price, $4.00 per 100-gram unit (prepaid)