ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

Things to Come

DECEMBER is the traditional month to report things to come, but we have reserved the last month of the year to discuss the services to analytical chemistry of those who have served as members of the Advisory Board of ANALYTICAL CHEMISTRY and to introduce those who are about to assume this responsibility.

The January and February issues will again repeat the reviews of progress in fundamental and applied research in analytical chemistry. The reviews in 1950 will cover the developments of 1949 exclusively (except in a few instances where subjects are reviewed for the first time). The initial reviews undertook to bring analysts up to date on the advances made during the war years and the period between 1946 and 1947. This annual feature is designed to meet the needs of an era of specialization in the field of analytical chemistry.

Again we wish to express our indebtedness to the authors who have contributed to the 1950 review. What greater manifestation of the spirit of professional responsibility to their field of scientific endeavor can be shown by chemical analysts? Recognized as specialists in their respective fields, these men without thought of monetary reward have willingly placed their services at the call of their associates.

Crystallographic Data

EACH month for nearly two years we have published crystallographic data under the byline "Contributed by Armour Research Foundation of Illinois Institute of Technology."

We take full responsibility for what appears to be a misunderstanding on the part of our readers. This valuable month-by-month contribution to the advancement of knowledge in an important and highly specialized field is not intended to be the sole responsibility of W. C. McCrone and his associates of Armour.

Contributions from other organizations are desired. Indeed, readers cannot expect Dr. McCrone to carry on this work without assistance from other laboratories interested in the field. To emphasize this point in the future, when contributions are made by other laboratories the individuals and their laboratory connection will be identified in the published fundamental data, so that recognition will be forthcoming not only in ANALYTICAL CHEMISTRY but in the abstract journals as well.

Dr. McCrone joins us in soliciting data from laboratories throughout the world. To facilitate handling, we request that data be cleared through Dr. McCrone.

All the type presenting data to date has been held at the printing plant in the expectation that this information might be desired in reprint form by a large number of analysts. An expression from our readers on this point would be helpful.

A Father Power Memorial Award

ANALYTICAL chemists will be delighted to learn that Fordham University has announced an annual award to be known as The Father Power Memorial Award. Analytical chemistry, and particularly the field of microchemistry, owes much to Father Power, and the Division of Analytical Chemistry suffered an irreparable loss in Father Power's untimely death.

Father Power frequently referred to the definition of the alchemist, "The chymists are a strange class of mortals impelled by an almost insane impulse to seek their pleasure among smoke and vapor, soot and flame, poisons and poverty, yet among all these evils I seem to live so sweetly that may I die if I would change places with the Persian King." These prophetic words form the basis of a plaque that honors Father Power's memory in the Chemistry Building at Fordham University.

Several years ago we joined a few chemists visiting a well known chemist about to die from a disease that perhaps some day chemistry will eradicate. As we left the hospital in mid-town Manhattan in order to leave two men of science together for a few moments of privacy, a man of no special faith remarked, "I hope when my hour has come that I may be comforted by holding close the hand of Father Power."

Father Power voluntarily lived a life of poverty, and earned the distinction of a renowned scientist among his fellow scientists solely as a result of his noteworthy contributions to chemistry which he loved and served second only to his love of man and his spiritual duties. On that evening when his friend faced the Great Experiment, on countless evenings while pursuing his priestly duties and scientific experiments, the Father Power we knew and revered would have refused without the slightest hesitancy to change places with the Persian King. Less than a year after this related incident, Father Power himself completed the Great Experiment.

Organic Reagents

A Group of Twelve Papers

Presented at the Second Annual Summer Symposium sponsored by the Division of Analytical and Micro Chemistry and *Analytical Chemistry*, Wesleyan University, Middletown, Conn., June 24 and 25, 1949

The Role of Organic Reagents

In the Chemistry of Specific, Selective, and Sensitive Reactions

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Translated by RALPH E. OESPER, *University of Cincinnati, Cincinnati, Ohio*

The chemistry of specific, selective, and sensitive reactions has the following two tasks as its main objectives: (1) the gathering and classification of, and commenting on, experimental findings which are connected, either directly or indirectly, with the specificity, selectivity, and sensitivity of analytical methods. This material is to be found both in analytical papers and in papers on other branches of chemistry; (2) the selection from analytical papers of experimental findings that are of interest to other fields of chemistry; these are primarily to be sought in original papers and not in abstracts. This leads to a better understanding of the effectiveness of organic reagents; it points to problems still to be examined, especially to the discovery of new organic reagents, and the improvement of analytical methods based on the use of organic reagents; and finally, it shows the necessity of correcting erroneous generalizations. To demonstrate the significance of the chemistry of specific, selective, and sensitive reactions there are discussed here instances taken from the action of atomic groupings in organic reagents, new complex compounds formed by organic reagents, real and false equilibria by formation of metallo-organic compounds, masking of reactions, and inner complexes and adsorption compounds and lakes.

ORGANIC reagents play an important role in modern methods of analysis, which are based on chemical reactions of the materials that are to be detected or determined. This is evidenced by numerous comprehensive publications and periodical surveys in the journals of practically all countries (*26*), and by the space which the text- and handbooks of analytical chemistry devote to discussion of the employment of organic reagents. The first essay in English on the use of organic reagents in inorganic analysis was published by Feigl (*26*). The acquired knowledge in this field has now become so great that three excellent special works on organic analytical reagents have been published recently (*50, 98, 108*).

Some authors include under this heading all organic compounds that are used in the course of analytical operations. This, however, does not conform to the requirement that a compound is designated as a reagent only if it reacts with a material which is to be detected or determined. Among organic compounds that function as solvents, extraction media, flotation agents, wash materials, protective colloids, etc., it is a question not of reacting materials, but of analytical aids, which admittedly are sometimes of the highest importance in carrying out analytical processes. Such auxiliary materials also include organic materials that are employed as indicators of all varieties or as primary standards in titrimetry. If the requisite limitations are enforced regarding organic reagents, another generalization must also be taken into consideration—that the employment of organic reagents in chemical analysis is as old as analysis itself. If this were true, the tremendous development of preparative organic chemistry during the second half of the 19th century would certainly have stimulated an intensive use of organic reagents. This was not the case; in fact, many organic compounds, whose convenient preparation or even reactivity with inorganic substances has been well known for decades, have been recognized as reagents only within a comparatively few years.

An inspection of the literature reveals that up to about 1905 organic reagents were recommended and employed in inorganic analysis to a relatively limited extent. Grateful tribute should be paid to Cazeneuve, Denigès, Griess, Illinsky, Knorre, Pozzi-Escot, and Raschig, pioneers in the utilization of organic reagents. Hence the stimulus to make extensive use of organic reagents did not come from organic chemistry, which had long possessed an arsenal of analytically usable organic compounds. For a very long time analytical chemistry felt no urge to pay attention to this storehouse. The merit of having done this belongs to the few workers who first appreciated the prime analytical importance of the Werner complex chemistry to the formation of metallo-organic compounds. The introduction of dimethylglyoxime and dicyandiamidine as nickel reagents (Tschugaeff, 1905; Grossmann, 1907), of nitrosophenylhydroxylamine as a copper and iron reagent (Baudisch, 1911), and of alizarin as an aluminum reagent (Atack, 1915) ushered in, beginning around 1920, a new tendency in analytical chemistry. This movement is characterized by a far-reaching consideration of coordination chemistry, and above all by the employment of organic reagents for the production of soluble and insoluble metallo-organic complex compounds (*21*).

Organic reagents can be regarded from two viewpoints. The first is purely practical, in that the sole consideration is whether and how an organic reagent makes it possible to accomplish an analytical task. In this case, important factors are sensitivity, certainty, speed, and accuracy of tests or determinations. In the foreground is the prescribed succession of operations—the "procedure" of an analytical method. In this regard, an analytical method may be characterized by an organic reagent, and both the method and the reagents will retain their interesting features as long as they are not displaced by something better.

The second viewpoint takes account not only of the practical solution of an analytical problem, but all the details of an analytical method and their scientific bases. In general, this is taken to mean the application of physical-chemical laws to analytical procedures. Especial emphasis is placed here on a numerical conception of the conditions under which chemical changes can be carried out to reach analytical objectives, taking due account of the stoichiometry, equilibria, and kinetics of the reactions involved. This method of scientifically treating analytical procedures was originated by Wilhelm Ostwald, continued by W. Böttger, and has become a fundamental of modern analytical chemistry, particularly through the work of I. M. Kolthoff. Its essence is that the qualitative phenomena of chemical changes are, as a rule, taken as a priori facts, and attention is directed primarily to their quantitative aspects. However, of no less importance to a scientific continuation of the classical analytical method of working, which rests on the utilization of chemical reactions, is the scientific treatment of the following questions, through which the qualitative phenomena are brought to the front: How is it possible to arrive at new analytically useful reactions and reagents? How can known reactions be used in analysis with the highest efficiency? How can improvements of analytical procedures be discovered?

The present state of chemical knowledge will not provide precise answers to all parts of these questions, especially if nothing beyond physical-chemical laws is taken into consideration. Nevertheless, pure empiricism is not the only way out of this difficulty; points of reference and guiding principles are available for handling these problems. In the first place, it is necessary to consider in detail the chemism of known unequivocal and sensitive reactions. Efforts must be made to secure a clear understanding of the relations between the composition and constitution of reactants and reaction products, as well as of the relation of the particular type of reaction to the phenomena which are the bases of an analytical employment: solubility, volatility, color, fluorescence, etc. Further requirements are an extensive acquaintance with and intimate consideration of all the factors that may influence the certainty and sensitivity of reactions in a positive or negative direction. In such studies, especial attention must be given to many provinces of chemistry, particularly when the study includes phenomena which—though not of direct analytical use—often are instances of events that may take place in analytical processes.

There is at hand a great fund of factual knowledge which can be drawn on for solving these problems. The assembling, arrangment, and appraisal of this material are among the most important tasks of the chemistry of specific, selective, and sensitive reactions (*21*). A further task is to retain, from analytical studies, observations that are of significance with respect to other fields of chemistry. Accordingly, within the framework of the chemistry of specific, selective, and sensitive reactions, organic reagents are not treated exclusively because of their analytical usefulness. Their behavior can contribute greatly to extending the knowledge of specificity, selectivity, and sensitivity, and their characteristics can aid in securing insight into other provinces of chemistry.

GROUP ACTION IN ORGANIC REAGENTS

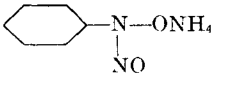
The reaction ability of organic compounds is always due to the activity of certain groups, which can be termed reactive or affine (binding) groups. These may involve (1) acidic groups, whose hydrogen atoms are replaceable by metals; (2) atoms or groups that can be bonded to metal atoms through auxiliary valences; (3) the simultaneous action of principal and auxiliary valences localized on different parts of the organic molecule; and (4) condensation reactions, usually those that proceed through elimination of water. The products of chemical changes 1 to 4 are not necessarily stoichiometrically defined and isolable compounds. The same types of reaction and combining forms can also result from surface reactions of solids with no production of stoichiometrically definable compounds. Such products are known as adsorption compounds, and interesting insights into their formation have recently been given by organic reagents.

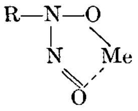
The principle of group actions in organic reactions, now generally accepted, contributes greatly to an understanding of the effect of organic reagents on the composition and constitution of the reaction product. It provides an initial orientation as to the classes of organic compounds that are likely to offer success in the search for new reagents. The presence of certain groups is a necessity for the reactivity of an organic compound, but by itself does not ensure the occurrence of an analytically usable reaction. The latter requires that the reaction products possess certain characteristics. Among these are insolubility in water, solubility in organic liquids, and color in the solid or dissolved state under normal or ultraviolet light. In addition, the reaction products should be produced rapidly and by even small amounts of the material being sought or determined. The reaction should be confined to a particular material or to a highly restricted number of substances (specific and selective action).

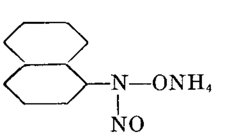
At present no definite predictions can be made as to which conditions satisfy the requirements. At first glance it would appear hopeless to seek new organic reagents by any means except pure empiricism. Fortunately, this is not the case. Previous experiences concerning the activity of organic reagents, whose number is steadily increasing, point to definite regularities whose observance is very helpful. Thus, there is no absolute independence of group action. The production of metallo-organic compounds is dependent on pH, and this relationship is often so pronounced that it constitutes an important factor for the attainment of selective and sometimes even specific actions of an organic reagent. Furthermore, it is sometimes possible by the addition of masking agents to lower the concentration of some ionic species to the point at which certain precipitations or color reactions fail. The action of such masking agents, which frequently are organic compounds, may be remarkably selective. Finally, the action of organic reagents may be annulled by the presence of reducing or oxidizing agents. Hence, the analytically useful reactivity of a certain group in organic compounds should never be considered apart from the entire reaction theater.

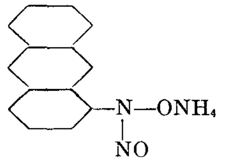
The external effect of the reaction milieu on the reactivity of a given group in organic compounds is accompanied by the important influence that may be exerted by the rest of the molecule of which the group is a part. Inasmuch as it is often possible to modify this residue very materially by introducing other atoms or groups, it becomes experimentally possible to test the activity of the reactive groups with respect to their reliability or variability. Such studies furnish information of practical as well as theoretical interest.

The earliest studies of group actions were made by Baudisch (*3*, *4*). After demonstrating that the ammonium salt of nitrosophenylhydroxylamine (cupferron I) precipitates copper and iron from acid solutions, he tested the behavior of derivatives of cupferron and found that the introduction of groups into the phenyl ring of cupferron does not impair the precipitating action of the active NO and NOH groups. The salts of cupferron, to which coordination formula Ia can be ascribed, and its derivatives are inner complexes. Neocupferron, II, which was recommended by Baudisch and Holmes (*5*), has a greater precipitating sensitivity than the parent compound. This reveals the so-called size or weighting effect exerted by the part of the molecule attached to the affine groups. It is probable that the increased size which would result from the attachment of a third ring, as shown in III, could lead to a still more sensitive precipitating reagent.


I

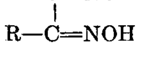

Ia

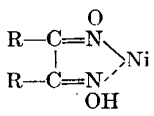

II

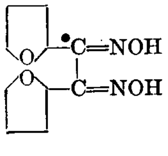

III

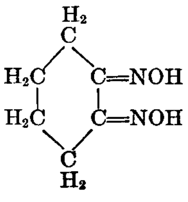
Practically all water-insoluble metal salts of the nitrosoaryl hydroxylamines are soluble in organic liquids, especially chloroform. This is in conformity with their character as inner complexes. The color of the solid copper and iron salts is not identical with that of the molecularly dissolved materials. The water-insoluble salts show a striking instability toward hydroxyl ions, and hence are converted by alkalies into the corresponding metal hydroxides and the soluble alkali salts of the organic compound. The metal cupferronate is readily decomposed by heat, which indicates that in general the decomposability of the organic components is retained in the salts. Thus, noteworthy information concerning the properties of metallo-organic compounds can be secured from studies of group actions of organic reagents.

Interesting examples of the tracing out of group actions are provided by the 1,2-dioximes (*16*). Tschugaeff's (*92*) discovery that nickel can be quantitatively precipitated by dimethylglyoxime and benzildioxime was subsequently substantiated with numerous 1,2-dioximes (*78*). The nickel inner complexes of 1,2-dioximes, IV, in which the two NOH groups are in the anti position, are assigned the coordination structure, IVa. Soule (*87*) recommended furildioxime as a nickel reagent, even though it is only slightly soluble in water. Wallach (*95*) found that cyclohexanedionedioxime, VI, which he prepared and which is readily water-soluble, precipitates nickel quantitatively, and in fact has a higher precipitating sensitivity than dimethylglyoxime. This remarkable finding demonstrates that the precipitating action due to a given grouping may be independent of the solubility or insolubility in water of the organic reagent. Diehl and associates (*94*) have recently described a convenient method for preparing cyclohexanedionedioxime and have pointed out the advantages of this water-soluble reagent for the detection and quantitative determination of nickel and palladium.

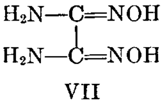

$$\begin{array}{l}\mathrm{R{-}C{=}NOH}\\ \quad|\\ \mathrm{R{-}C{=}NOH}\end{array}$$
IV

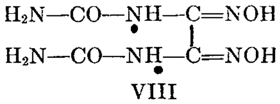

IVa

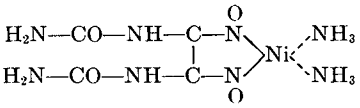

V


VI

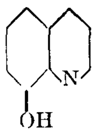
From the standpoint of group action, special interest is attached to dioximes in which the active groups are bound to nitrogen rather than to carbon atoms. Such compounds are represented by oxalenediamide oxime (*12*) and oxalenediuramide oxime (*33*). The former, VII, forms an inner complex nickel salt of the same constitution as the nickel salts of other 1,2-dioximes. In contrast, VIII behaves as a dibasic acid and, in ammoniacal solution, produces an insoluble diammine, whose coordination formula is shown by VIIIa. Hence, it must be assumed that the —CONH$_2$ group exerts a distinct acidifying action. This effect obviously is related to the configuration of the two hydroxy groups in the amphi position (*57*).

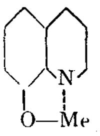

$$\begin{array}{l}\mathrm{H_2N{-}C{=}NOH}\\ \qquad|\\ \mathrm{H_2N{-}C{=}NOH}\end{array}$$
VII

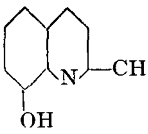

$$\begin{array}{l}\mathrm{H_2N{-}CO{-}NH{-}C{=}NOH}\\ \qquad\qquad\qquad|\\ \mathrm{H_2N{-}CO{-}NH{-}C{=}NOH}\end{array}$$
VIII


VIIIa

That group actions in organic precipitants are not always so independent of the rest of the organic molecule as in the examples cited is revealed by a comparison of 8-hydroxyquinoline (8-quinolinol) IX, and 8-hydroxyquinaldine, X. The former has found extensive use as a precipitant (*7, 9*) for numerous metals in acetate-buffered solutions or in ammoniacal solutions masked with tartrate. Inner complexes with the general coordination formula are formed.

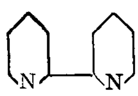

IX

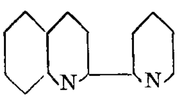

IXa

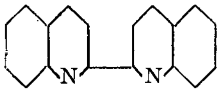

X

It would be natural to assume that compounds IX and X would exhibit analogous precipitating action, as they differ merely by one methyl group. This is the case, with the notable exception of their behavior toward aluminum. Merritt and Walker (*72*) found that aluminum is not precipitated by X, and made analytical use of their observation. They assume that the increase in volume due to the methyl group prevents a stable binding with the aluminum atom.

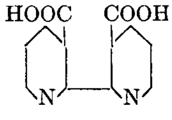
The fact that the slight increment in volume due to the introduction of a methyl group into an organic molecule can have a marked effect may be seen from studies of bipyridine derivatives by Smirnoff (*85*) and Willink and Wibaut (*106*). As early as 1895, Blau (*10*) found that ferrous salts combine with three molecules of α,α'-bipyridine, XI, to produce soluble red salts, which contain the complex cation $[\mathrm{Fe}(\alpha,\alpha'\text{-dip})_3]^{++}$. The formation of this colored cation in acidic solution was recommended 30 years later for the detection and colorimetric determination of iron (*36, 58*). The authors mentioned found that derivatives XII to XVI of bipyridine do not give a color reaction with ferrous salts. Perhaps these compounds are not capable of forming red hexammine ions of iron because of their excessive spatial requirements.


XI (α,α'-dip)

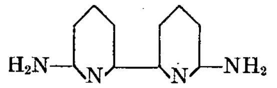

XII



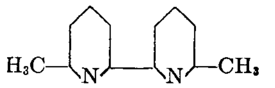
XIII



XIV



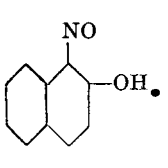
XV



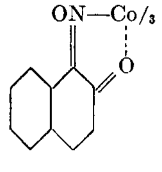
XVI

Tartarini (*91*) was the earliest to observe that cuprous salts are likewise capable of coordinating with α,α'-bipyridine to produce colored compounds. It is interesting that α,α'-diquinolyl, XIII, forms a purple-red complex ion, $[Cu(\alpha,\alpha\text{-quin})_2]^+$, with cuprous ion (*11*). Hoste (*61*) recently described a very sensitive, strictly specific test for copper and also a colorimetric method of determining this metal, which is based on the production of this tetrammine ion. It is probable that not only diquinolyl but also all other bipyridine derivatives, which do not form hexammine ions with ferrous ion, will be found to react with cuprous ions, for this latter reaction occurs with the less space-demanding tetrammine ions. If so, it will have been demonstrated that the selective action of a given group is raised to specificity by the volume increase derived from the attached organic molecular remainder. In any case, it may be concluded that steric factors can play significant parts in determining the reactivity of certain groups in the organic molecule.

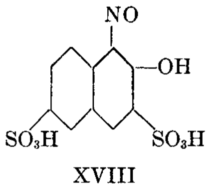
Because water-solubility is a very desirable characteristic in an organic reagent, and organic compounds can be made water-soluble by the introduction of sulfonic groups, the behavior of sulfonated derivatives of organic precipitating agents raises interesting questions. This matter was experimentally explored for the first time in 1928, when van Klooster (*65*) studied 1-nitroso-2-hydroxy-3,6-naphthalenedisulfonic acid, XVIII, commonly known as nitroso R salt. This compound is a derivative of 1-nitroso-2-naphthol, XVII, whose action as precipitant for cobalt was discovered as early as 1885 by Illinsky and Knorre (*62*). The cobalt inner complex, XVIIa, is produced in this instance. van Klooster found that a red coloration develops in acetate-buffered solution, is stable against subsequent addition of mineral acid, and makes possible a sensitive colorimetric determination of cobalt. The color reaction probably is due to the production of the water-soluble cobalt compound, XVIIIa.



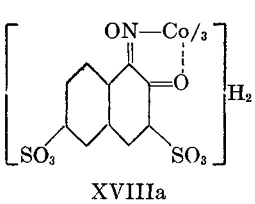
XVII



XVIIa



XVIII

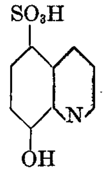


XVIIIa

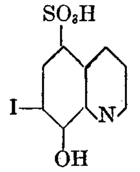
Comparison of coordination formulas XVIIa and XVIIIa shows that the inner complex–forming group has not lost its reactivity through the introduction of sulfonic groups. However, a water-insoluble precipitant has been converted to a water-soluble color reagent, which leads to a water-soluble compound with a colored inner complex anion. In addition, nitroso R salt exhibits a greater selectivity than the parent compound.

1-Nitroso-2-naphthol precipitates not only cobaltic, but also cupric, nickel, and ferric salts; of these only the cobalt compound is acid-stable. In the light of present experiences with group action in organic compounds, it seems to be a matter of course that the isomer of 1-nitroso-2-naphthol—i.e., 2-nitroso-1-naphthol—must also be a precipitant for cobalt. Because, however, the recognition of the significance of group actions is rather recent, the pertinent trials were not made until 1919. Bellucci (*6*) found that the anticipated precipitating action is about eight times as sensitive as that of the isomeric 1-nitroso compound. The fact that 2-nitroso-1-naphthol-4-sulfonic acid gives a color reaction with cobalt salts was observed as early as 1885 by Hoffmann (*59*), but it was not until 1938 that Sarver (*81*) made a thorough study of this reaction and applied it to the colorimetric determination of cobalt.

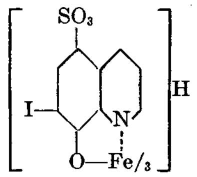
It is not a mere coincidence that effects of sulfonic groups, similar to those just discussed, are again observed with 8-hydroxyquinoline, IX, which reacts with numerous metal ions to produce insoluble inner complexes of the coordination structure IXa. The sulfonated derivatives, 8-hydroxyquinoline-5-sulfonic acid, XIX, and 7-iodo-8-hydroxyquinoline-5-sulfonic acid, XX, are not precipitants under the conditions prevailing in the oxine precipitations from dilute solutions. However, this does not signify that the inner complex–forming groups in the two sulfonic acids have lost their activity. This is shown clearly by their action toward solutions of ferric salts. Oxine gives a brown-green precipitate, whereas XIX and XX yield brown and brown-red solutions, respectively. Yoe (*107*) found XX to be a sensitive and extremely selective iron reagent, which makes possible a colorimetric determination of iron. He named this reagent ferron. Inasmuch as the intensity maximum of the color reaction is at the ratio 1 iron to 3 ferron, there can be little doubt that coordination formula XXa can be assigned to the soluble colored ferric product, even though the compound has not yet been isolated.



XIX



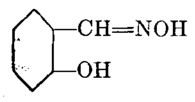
XX



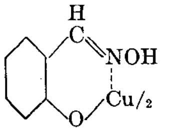
XXa

Here again, a precipitant has been modified into a color reagent by the introduction of sulfonic groups. Compounds analogous to XXa have not yet been detected in the case of other metals that can be precipitated as oxinates. However, an inner complex anionic binding of the metal is very probable because when the metal salt solutions are treated with an excess of XIX or XX, no precipitation follows if the system is then made basic with ammonia or caustic alkali (*22*). This signifies that a masking agent has been formed by the inclusion of a sulfonic group in the oxine molecule. It appears thus that the introduction of sulfonic groups into organic precipitants does not destroy the activity of inner complex–forming groups, but produces soluble electrolytes with inner complex anions instead of insoluble inner complexes. This indicates an interesting way by which new color reagents and masking agents may be obtained.

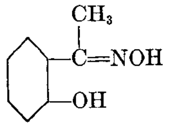
Because hydroxy and carboxy groups are known to confer water-solubility on organic compounds, it seems logical to inquire whether the introduction of these groups will affect organic inner complex formers in the same ways as the inclusion of sulfonic groups. Ephraim found that salicylaldoxime, XXI, is a selective precipitant for copper, with which it forms a yellow-green inner complex, XXIa. A gravimetric method has been based on this finding (*19*). His investigations of the behavior of derivatives of salicylaldoxime showed (*20*) that compounds XXII to XXV have the same precipitating action as XXI.



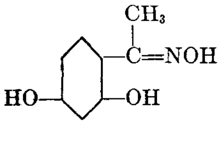
XXI



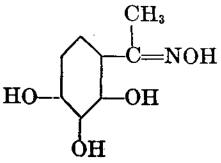
XXIa



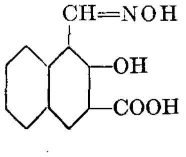
XXII



XXIII



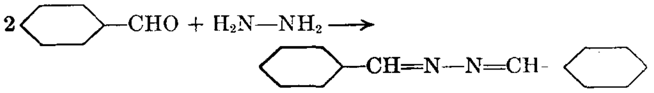
XXIV



XXV

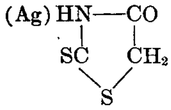
It appears, therefore, that the introduction of solubilizing hydroxy groups into the molecule of an organic precipitant does not annul the precipitating action of its characteristic groups. This finding can be utilized in the analytical development of group actions.

Fichter and Goldach (46) described a test for hydrazine based on the formation of a water-insoluble aldazine, resulting from the condensation of hydrazine with benzaldehyde:

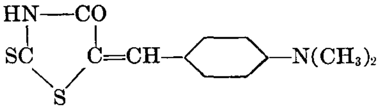


Because alcoholic solutions of benzaldehyde must be used, the aldehyde may precipitate in the aqueous solution. Reasoning from the knowledge that the precipitation action of the aldehyde group is preserved even after the introduction of hydroxy groups into the benzaldehyde molecule, Feigl and Schwarz (43) tested the action of water-soluble salicylaldehyde and found that it likewise produces an insoluble aldazine. This reagent permits a more reliable test for hydrazine, and its use provides a suitable weighing form in the gravimetric determination of hydrazine.

Interesting applications of group actions are based on the fact that organic reagents, which produce colorless precipitates with metal ions, can often be easily modified, by preparative procedures, to produce derivatives that give colored precipitates. Thus, a study of the reactivity of rhodanine, XXVI, showed that the ability to produce insoluble silver salts (through action of the imino group) is retained in all rhodanine derivatives with an intact imino group. Such derivatives are easily obtained by means of condensation reactions of the carbonyl or methylene group of rhodanine. Thus it was possible to obtain dimethylaminobenzylidene rhodanine, XXVII, the first organic reagent that forms a deep colored (red) silver salt (29).



XXVI

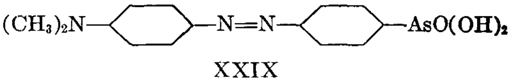


XXVII

The arsonic acids, XXVIII, demonstrate a similar color effect following the introduction of new groups. Their precipitating action is analogous to that of arsenic acid. In particular, the precipitation of zirconium as the white insoluble salt of alkyl arsonic acids has been recommended for the gravimetric determination of this metal (52). The precipitating action of the $AsO(OH_2)$ group is preserved in *p*-dimethylaminoazophenylarsonic acid, XXIX. However, the action is improved because of the intense red color of the zirconium salt. The use of XXIX is the basis of a sensitive test and a colorimetric procedure for determining zirconium (39, 55).

$R—AsO(OH)_2$

XXVIII



XXIX

The establishment of the fact that particular *group actions are* retained after certain other groups are put into organic compounds is of great importance in improving organic reagents or discovering new ones. However, the fact that group actions of reagents can also be nullified by introducing other groups is of no less importance, for sometimes the selectivity and sensitivity of organic reagents can be raised through such effects. Modern studies of the analytical application of organic reagents are giving ever-increasing consideration to group actions. Experimental studies which broaden and deepen knowledge of group reactions are of significance to the chemistry of specific, selective, and sensitive reactions, even when they do not lead to results of immediate analytical utility.

DISCOVERY OF NEW COMPLEX COMPOUNDS THROUGH USE OF ORGANIC REAGENTS

The activity of certain atoms and groups in organic compounds is not merely of analytical interest with respect to the specificity, selectivity, and sensitivity of a reagent. Often the composition, constitution, and properties of metallo-organic compounds are of great importance to complex chemistry. Sometimes during analytical studies, new metallo-organic compounds have been isolated which can be fitted into certain classes of complex compounds, and thus the systematics of complex compounds have been enriched; members of totally new classes of complex compounds have been discovered in the course of analytical research.

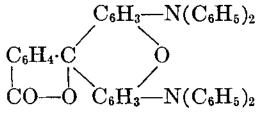
Willard and Smith (86, 105) found that tetraphenylarsonium chloride, XXX, is an excellent precipitant for large-volume and complex anions. Here is shown clearly the influence of the ionic volume on the occurrence of precipitation reactions, an effect that is met in numerous precipitations of alkaloid bases by means of complex acids. The findings of Willard and Smith have stimulated researches on the behavior of other cations of large volume. It is now known that the ferrous bipyridyl sulfate, XXXI (40), and the cation of the dye rhodamine, XXXII (68), are selective precipitants for complex ions and can be used analytically.

$(C_6H_5)_4As^+Cl^-$

XXX

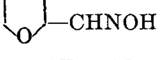
$[Fe(\alpha,\alpha'\text{-dip})_3]^{++}SO_4^{--}$

XXXI

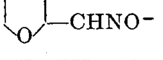


XXXII

Hayes and Chandlee (54), while working out a new specific method for the gravimetric determination of palladium, discovered interesting complex compounds of *β*-furfuraldoxime, XXXIII. Because of the replaceability of the hydrogen atom of its NOH group, this compound is an acid possessing the radical XXXIIIa. As it is known from the coordination formula of palladium dimethylglyoximate that one oxime group can be coordinated on palladium through its nitrogen atom, the question arises as to how the NOH group functions in furfuraldoxime. Hayes and Chandlee found that the yellow compound, A, is precipitated from acidic palladium solutions that contain chloride ions. This compound corresponds to the diammine type that is so frequently encountered among complex palladium salts (38). This type of compound might be expected to be produced with all soluble palladium salts, no matter what the corresponding anion, but such is not the case. The insoluble intercalation compound, B, was obtained from solutions of palladium sulfate, while solutions of palladium nitrate produced compound C.



XXXIII (F)



XXXIIIa (f)

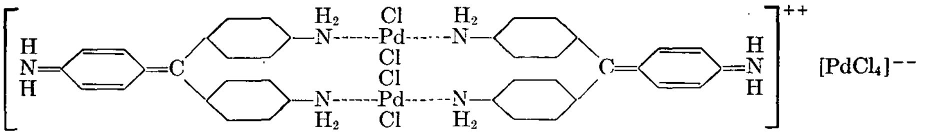
$PdCl_2.2F$ $PdSO_4.4F$ $Pdf_2.2F$
A B C

Accordingly, furfuraldoxime presents an unusual case in which the composition of the precipitate is affected by the anion which was originally combined with the cation. Among the palladium compounds of furfuraldoxime, A has the lowest solubility, so that its production is always assured by the addition of chloride ions.

The interesting observations by Hayes and Chandlee on the divergent behavior of furfuraldoxime toward palladium sulfate and nitrate are not cited in the abstracts of their paper because these results are not of direct analytical significance. This omission demonstrates plainly the danger that findings of importance to other fields may not receive proper attention if they are made in the course of analytical investigations. The chemistry of specific, selective, and sensitive reactions performs an important task in that it lays hold of such findings and indicates their significance.

Other instances in which organic reagents led to new complex compounds were offered through the behavior of masked ammoniacal thallic salt solutions toward 8-hydroxyquinoline, thionalide, and other organic precipitants. Precipitates of formula-pure, stable inner complexes of trivalent thallium, hitherto unknown, were produced in this manner (*27*). Thallic oxinate and dibromoxinate are very suitable for the selective detection and gravimetric determination of thallium (*32*). It is probable that other thallic inner complexes, which invariably contain a low percentage of thallium, will also be of analytical use. In view of the fact that thallium salts have pharmacological action, the inner complexes of this metal, which are soluble in organic liquids, may possess an interest of their own.

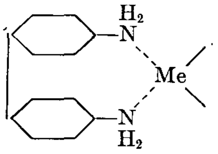
The elucidation of analytically valuable effects, or of those that are observed as by-products of analytical researches, is a fundamental objective of the chemistry of specific, selective, and sensitive reactions. West and Amis (*104*) discovered a sensitive reaction of palladium with fuchsin hydrochloride (Fs), which depends on the production of the brown compound $3PdCl_2.2Fs$. From his studies West (*103*) has concluded this palladium compound can be represented by the coordination formula XXXIV:



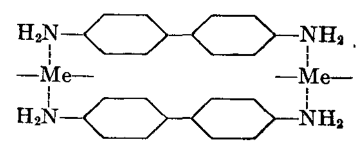
XXXIV ($3PdCl_2.2Fs$)

No complex compounds of this type had been observed previously.

These conclusions from West's studies make it necessary to re-examine the complex compounds of benzidine (Bzd). It has been recommended that $Cu(CNS)_2.Bzd$ be employed in quantitative analysis for the precipitation of copper (*89*) and $Hg(CN)_2.Bzd$ as a weighing form in the determination of mercury (*71*). These materials belong to a class of complex compounds of the general formula $MeX_2.Bzd$, that were studied by Herzog (*56*), Spacu (*88*), and Barceló (*2*). Spacu views these complexes as interpenetration compounds, $[MeBzd]X_2$. This formulation has been accepted in the analytical literature (*99*) but cogent arguments can be brought against it. The occupation of two coordination positions on one metal atom by one molecule of benzidine, as shown in XXXV, is impossible on steric grounds because of the too great distance between the two NH_2 groups. The formulation as a bimolar chelate compound with the coordination formula XXXVI is much more probable. This is analogous to the complex palladium fuchsin compound.



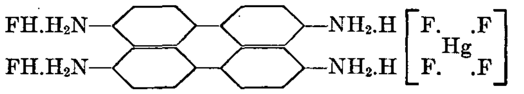
XXXV



XXXVI

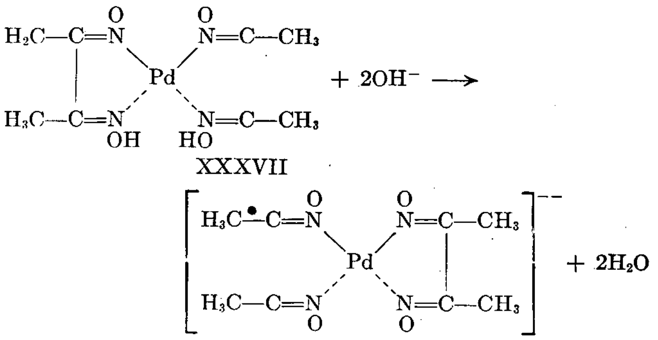
These examples make it plain that metallo-organic compounds, discovered in the course of analytical studies, may be of considerable importance to complex chemistry. The compounds isolated by West and Spacu lead to the expectation that polynuclear stable chelate compounds with considerably greater numbers of rings are much more possible than was hitherto assumed. Reaction possibilities, which had previously passed unnoticed, were thus indicated.

Not only may reactions involving organic reagents result in the production of new metallo-organic complex compounds of surprising structure, but surprising reaction paths may be traversed. Pertusi (*75*) proved that a white crystalline compound, $2Bzd.4HF.HgF_2$, precipitates from fluoride solutions on the addition of an acetic acid solution of benzidine and mercuric succinimide. The product is so stable that it provides a sensitive and specific test for fluoride and is the basis of a gravimetric determination (*73*). No coordination formula for this remarkable mercury compound has been given. It has been suggested that it is an addition compound of benzidine hydrofluoride with mercuric fluoride:



The complex compound discovered by Pertusi is unique with respect to both its composition and the unsymmetrical binding of mercuric fluoride. There is the further singularity that the use of mercuric succinimide is essential to its production. Accordingly, this presents another instance in which the composition of the precipitate produced by the action of an organic reagent with a cation is influenced by the anion originally associated with the cation. Such effects should be taken fully into account in the production of molecular compounds.

Feigl and Suter (*44*) made some interesting observations in studies designed to elucidate Cooper's discovery (*14*) that palladium dimethylglyoxime, XXXVII, is soluble in alkali hydroxide. This solubility results from the fact that this inner complex has the character of an acid because of the two unsalified and merely coordinatively bound NOH groups. These oxime groups are salified by strong alkali to produce water-soluble alkali salts possessing inner complex palladium-bearing anions, XXXVIII.



XXXVII

XXXVIII

or $$Pd(DH)_2 + 2KOH \longrightarrow K_2[PdD_2] + 2H_2O$$

Accordingly, palladium dimethylglyoxime should be regarded as an amphoteric compound, which functions as an inner complex in neutral or acid media and as an acid in alkaline media. The justification for speaking of a dimethylglyoxime palladium acid was supplied by the isolation of its potassium, barium, manganese, palladium, and lead salts. The lead salt is particularly interesting because of its scarlet color and the brown palladium salt for being a dimer of the hitherto unknown yellow palladium dimethylglyoxime. The findings relative to palladium dimethylglyoxime make it very likely that other inner complexes of organic compounds, which contain two acid groups, one salified, the other coordinated, may dissolve in caustic alkalies to form alkali salts of acids with inner complex metal-bearing anions. (Salicylaldoxime, isatin-β-oxime, phenylglyoxilic acid oxime, etc., are cases in point.) The production of such water-soluble alkali salts has a certain similarity to the formation of alkali salts of sulfonated inner complex formers. In both cases, the production of inner complex metal-bearing anions indicates masking possibilities.

The palladium in the alkaline solution of palladium dimethylglyoxime is masked toward practically all reactions. However, the dimethylglyoxime is also masked against the familiar action of nickel ions. Hence, organic compounds can be masked as a consequence of their inner complex binding, and this opens interesting prospects. The behavior of alkaline solutions of palladium dimethylglyoxime toward cyanide ions is remarkable (*35*). The reaction is:

$$[PdD_2]^{--} + 4CN^- \longrightarrow [Pd(CN)_4]^{--} + 2D^{--}$$

This corresponds to a demasking of the dimethylglyoxime, which in this way can be brought to reactivity with nickel to produce the familiar red compound. A specific and selective test for cyanide in alkaline solution was developed from this behavior, and presumably a quantitative procedure can be worked out. All the previous methods of detecting cyanide involved the liberation of volatile hydrocyanic acid and its subsequent identification. The new test is thus a distinct step forward. It can be used to reveal cyanide in illuminating gas.

The literature of analytical chemistry contains numerous other statements concerning metallo-organic compounds, which have analytical usefulness, but their method of formation and constitution have not been satisfactorily explained or have been incorrectly interpreted. Certain reactions have been described, even though the empirical formulas of the reaction products are not known. Certainly, a laboratory study of such statements would lead to many new compounds and reaction possibilities.

TRUE AND FALSE EQUILIBRIA IN REACTIONS WITH ORGANIC REAGENTS

Every chemical reaction leads to an equilibrium, characterized by the equilibrium constant, which represents the ratio of some power of the concentrations of the starting and final materials. Special analytical interest attaches to the establishment of equilibrium in reactions in which the hydrogen ion concentration plays a role, as in all reactions of organic reagents that are acidic in character. Independence of the acidity is unusual in such instances. Usually, the establishment of certain pH values is necessary to secure practically quantitative reactions. Careful studies have demonstrated that sometimes the selectivity of organic precipitants can be substantially increased by establishing certain pH values and maintaining certain pH ranges.

Particularly noteworthy are the effects of an extraction in the case of pH-dependent precipitation reactions which lead to metallo-organic compounds that are soluble in organic liquids. It is then possible at certain pH values to secure complete reaction, a result that could not have been expected from the equilibrium of the precipitation reaction. This was proved for the first time by Sandell (*79*) in the reaction of gallium salts with 8-hydroxyquinoline (oxine). The pH range for the quantitative precipitation of gallium oxinate lies between 6 and 8. Consequently, no visible formation of this salt occurs at pH 2.6 to 3. Sandell found that all the gallium passes, as oxinate, into the organic solvent if a solution of oxine and the metal salt is shaken with chloroform at this pH. Gallium can be detected and determined by this procedure even though other oxinate-forming metals are present.

Even though no perceptible precipitation of gallium oxinate occurs at pH ~3, total absence of reaction at this acidity is not necessarily indicated. Every precipitation reaction, and therefore also the reaction

$$Ga^{+++} + 3HOx \rightleftharpoons Ga(Ox)_3 + 3H^+$$

begins and proceeds to a slight extent even prior to the visible separation of a precipitate—i.e., before its solubility product is exceeded. In the case of the gallium-oxine reaction at pH ~3, the solution contains a slight quantity of molecularly dissolved gallium oxinate in equilibrium with its dissociation products. However, gallium oxinate is readily soluble in chloroform, and when the solution is shaken with the solvent, the oxinate distributes itself between the two liquids. Because the partition equilibrium greatly favors the chloroform, the gallium oxinate is extracted from the water solution, and is then replenished by the renewed formation of the salt. If the extraction with chloroform is repeated, this process continues until the gallium is entirely converted into oxinate and collected in the chloroform.

This extraction of gallium at pH values below those at which it is precipitated from water solutions is the only known instance of the analytical utilization of an equilibrium shift of this kind. However, an analogous behavior may be anticipated with such inner complexes as dissociate to only a slight extent in aqueous solution and are soluble in organic liquids. In fact (*28*), acid solutions of alkali vanadate, which exhibit no change when oxine is added, form a green layer when they are shaken with chloroform; this indicates that chloroform-soluble vanadium oxinate has formed. It is obvious that a selective extraction of inner complexes combined with the establishment of definite pH values can be of great significance in enhancing the selectivity of organic reagents.

Generally, organic reagents react directly with the substances to be detected or determined. But in certain cases a substance reacts only by shifting the equilibrium of another reaction in which an organic reagent is a partner. This equilibrium reaction may depend on pH or a redox reaction. Feigl and da Silva (*34*) showed that the filtrate obtained from a solution of a nickel salt that had been treated with an excess of dimethylglyoxime is a very sensitive reagent for basic materials of all kinds. As a result of the incomplete precipitation of the nickel-dimethylglyoxime from a neutral nickel solution, there is established the equilibrium:

$$Ni^{++} + 2DH_2 \rightleftharpoons Ni(DH)_2 + 2H^+$$

The solution is therefore saturated with nickel dimethylglyoxime and contains all the reactants for producing this inner complex, and also free hydrogen ions at a concentration just sufficient to prevent formation of the precipitate. Every material that withdraws hydrogen ions from the equilibrium will consequently occasion the precipitation of red nickel-dimethylglyoxime in an amount that is equivalent to the hydrogen ions consumed. Basic materials which dissolve so slightly in water that they have no effect on acid-base indicators—e.g., calcium carbonate, magnesium ammonium phosphate, calcium arsenate—react on their surfaces with hydrogen ions of the equilibrium solution, and thus cause deposition of red nickel dimethylglyoxime. Many practical applications can be made. For instance, it is easy to distinguish quickly between "fusible and infusible white precipitate" ($HgCl_2.2NH_3$ and $HgNH_2Cl$), a problem that ordinarily requires a quantitative determination of the mercury-nitrogen ratio. Calcite and dolomite can be readily distinguished by this means, as well as alumina either anhydrous or hydrated. It is clear that equilibrium solutions can be prepared from other acid-soluble inner complexes. The nickel-thionalide equilibrium solution has proved very suitable.

A counterpart of the acid equilibrium solution of nickel dimethylglyoxime is found in a basic equilibrium solution of silver chromate. This is prepared by shaking an excess of silver chromate with a solution of ethylenediamine (en) and then filtering (*30*). The bright yellow solution houses the equilibrium:

$$[\text{Ag en}]_2\text{CrO}_4 \rightleftharpoons \text{Ag}_2\text{CrO}_4 + 2\text{en}$$

When solids or dissolved materials which bind ethylenediamine react with this equilibrium solution, red-brown silver chromate precipitates as a consequence of the disturbance of the equilibrium. Water-insoluble materials with acid characteristics can be thus revealed topochemically in a manner that is completely analogous to the detection of basic materials by means of the acid equilibrium solution. Positive results with acid and basic equilibrium solutions are given also by solid substances which withdraw hydrogen ions or ethylenediamine molecules from equilibrium solutions through adsorption. Instances are alumina and hydrated silica.

An example of the application of an equilibrium disturbance was given by Velluz and Pesez (*93*). Their method of detecting ammonia and volatile organic bases is based on the fact that a solution containing zinc and oxinate ions will produce fluorescing zinc oxinate on contact with bases. The reaction is claimed to be superior to the litmus reaction for ammonia.

Equilibrium shifts, which can be put to analytical use, are also possible with redox reactions that involve organic compounds.

The earliest known example is the sensitive test for zinc described by Cone and Cady (*13*). It is based on the finding that in the presence of zinc ions diethylaniline is oxidized to colored quinoidal compounds by potassium ferricyanide in strongly acidified solution. This action is due to the formation of acid-insoluble zinc ferrocyanide, which thus removes ferrocyanide ions derived from the redox equilibrium. Later, Szebelledy and Tanay (*90*) recommended *p*-phenetidine as the reducing compound. Lapin's (*69*) test for ferrocyanide by means of tetrabase (*p*-tetramethyldiaminebiphenylmethane) depends on the removal of ferrocyanide ions by zinc or mercury ions. Other pertinent examples are the sensitive tests for copper and cyanide ions (*66, 76, 84*) in the system: copper–benzidine(*o*-tolidine)–cyanide-(thiocyanate), and the Eegriwe (*18*) test for magnesium in the system: *p*-aminophenol–ammonia–magnesium, which is based on the production of a colored magnesium lake.

In all these cases, the tests are dependent on the formation of colored oxidation products of organic reagents, which do not form these products in the absence of the materials to be detected. The term "induced oxidation" is sometimes incorrectly applied in such instances. According to the physicochemical nomenclature an induced reaction requires the simultaneous occurrence of an inducing reaction with a common reaction partner, the "actor." The examples just cited do not involve this type of inducing reaction or the action of an actor; rather they involve exclusively the shifting of redox equilibria through the removal of a reaction product.

The examples stressed concern true equilibria; there exist also "false equilibria" in precipitation reactions of inorganic ions by organic reagents. The term false equilibrium is applied when a precipitate is acid-resistant within a pH range where it is not formed. This phenomenon is outwardly analogous to the familiar behavior of cobalt and nickel sulfides, which cannot be brought down from acid solutions, but once precipitated from alkaline media are resistant to dilute acids. Strictly speaking, it is not possible to state what the true equilibrium is in the case of a false equilibrium; the question remains undecided as to whether the pH value, which limits the precipitability, determines the true equilibrium, or whether this is governed by the much lower pH value, which makes possible a solution of a precipitate. If the first condition is accepted, the dissolution is delayed, whereas if the second is held to be valid, the precipitation is delayed.

The abnormal resistance of precipitates, which are considered from the standpoint of a false equilibrium, must be demonstrated immediately following the production of the compound. Consequently, it has nothing to do with "aging" of precipitates. The latter is a process that can be followed, with respect to the elapsed time, especially in the case of precipitation of hydrated oxides. The precipitate, in such cases, exhibits progressive resistance to attack by acids or bases.

The following instances of false equilibria in the action of organic precipitants possessing acid character are taken from the literature (*98*).

Cobaltic nitrosonaphthol can be precipitated from acetic acid solution, but once formed it is stable against mineral acids. Rubeanic acid precipitates copper from acetic acid, and cobalt or nickel from ammoniacal solution. The metal rubeanates are resistant to dilute mineral or acetic acid. Cobaltic thioglycollic acid anilide has to be brought down from ammoniacal solution, but it then is stable against acetic acid. Palladium can be precipitated by 6-nitroquinoline from neutral solution; the precipitate is, however, resistant to strong hydrochloric acid. Only the sodium salt of dipicrylamine is able to precipitate potassium, cesium, and rubidium, whereas dipicrylamine itself is ineffective. On the other hand, the precipitated alkali salts are resistant to dilute acids.

All the preceding organic compounds are inner complexes, and it is probable that a study of the behavior of other inner complexes would reveal further instances of acid resistance, which should not exist according to the precipitation conditions. Nevertheless, such anomalies should not be viewed as a characteristic of inner complexes. The examples of acidic equilibrium solutions of nickel dimethylglyoxime and nickel thionalide showed that doubtless there are typical inner complexes, whose formation and behavior toward hydrogen ions conform exactly to a true equilibrium. It is remarkable that all the organic precipitants referred to previously have one property in common: Their solutions (in water or organic liquids) present an equilibrium between the tautomeric forms of inactive pseudo-aci form and an active aci form. When such equilibria exist, the concentration of the aci form is invariably increased by a rise in pH. This provides a plausible explanation of the fact that these reagents are to be used at pH values that guarantee a sufficiently high concentration of precipitating organic anions, but it does not explain why the formed precipitates are resistant to far lower pH values. The equilibria between pseudo-aci and aci forms of a precipitant immediately set themselves up, a fact that is readily discerned from the dissolution of the particular organic compound in alkali and its reprecipitation when this solution is made acid.

The question arises as to whether it is possible to have precipitation or solution anomalies of metallo-organic compounds without the participation of hydrogen ions. This has thus far received no attention, although when organic reagents are used the influence of pH on the formation and stability of metallo-organic compounds is of particular practical importance. However, two instances show that false equilibria can occur even with reactions that are independent of pH.

Hammett and Sottery (*53*) proved that aluminum can be precipitated by aurintricarboxylic acid only from acetic acid–acetate buffered solution, and that an ammoniacal solution of the reagent brings down nothing but hydrated alumina. Consequently, it should be possible to convert this precipitated inner complex into hydrated alumina by means of ammonia. This change does not take place. In contrast, the chromium salt of aurintricarboxylic acid, which can be precipitated under the same conditions as the aluminum salt, is decomposed by ammonia. Recently it was found (*35*) that palladium solutions containing cyanide ions, or more correctly complex Pd(CN)_4^{--} ions, produce no precipitate on treatment with oxine. The establishment of an equilibrium would therefore demand that precipitated palladium oxinate be soluble in alkali cyanide. However, palladium oxinate remains unaltered even when warmed with alkali cyanide. Other palladium inner complexes with 1,2-dioximes and salicylaldoxime show a normal behavior in that they are not precipitable from solutions of complex palladium cyanide, and once they are brought down, they dissolve immediately in alkali cyanide solutions.

There is, as yet, no completely plausible and experimentally confirmed explanation as to why false equilibria arise in the precipitation and solution of metallo-organic compounds (*25*). It is not known whether an abnormally delayed precipitation or dissolution is involved. The writer believes the latter to be more probable, and that this anomaly corresponds to a more or less persistent passivity. Hence the term "passive precipitates" seems appropriate for characterizing this anomaly. Such passivity may be the result of an abnormal low tendency by the particles of the precipitate to add and retain water on their surface (hydration). In other words, hydrophobia is exhibited, and this stands in the way of a direct contact with hydrogen ions, and also impedes a surface dissociation of the precipitate particles. Possibly the reason for the hydrophobia of inner complexes is the fact that in their molecules the metal atoms may be enclosed on all sides by organic radicals that of themselves have no hydrophilic groups and hence possess no centers for hydration. There is a relation to the fact that so many inner complexes are not readily wetted by water, and also to their frequent solubility in organic liquids.

The assumption that hydration is connected with the normal behavior of inner complexes, and that the lack of hydration is related to a passivity, is supported by observations of metal oxinates toward dilute hydrochloric acid (*37*).

As no metal oxinate can be precipitated unless the pH of the solution exceeds a certain value (with a few exceptions the lower limit is pH 4.5), all freshly precipitated metal oxinates should dissolve immediately in 3 *N* hydrochloric acid. This is true of the majority of the oxinates, including those that are known to carry water of crystallization; their anhydrous products obtained after drying at 120° C. display no passivity. In contrast, the freshly precipitated oxinates of aluminum, iron, chromium, and gallium dissolve just as sluggishly or just as little in this acid as the dried products. Especially impressive is the comparison of aluminum oxinate and thallium oxinate trihydrate, which are precipitable in the same pH range. The thallium salt cannot be completely dehydrated even by prolonged drying at 120° C. (*32*). It dissolves immediately in acid. In contrast, freshly precipitated or dried aluminum oxinate in 3 *N* hydrochloric acid remains unaltered at first and noticeable solution occurs only gradually.

The fact that metal oxinates which carry water of crystallization show no passivity, even after they are dehydrated, is probably due to their being immediately rehydrated on contact with dilute hydrochloric acid, whereas the passive metal oxinates dissolve only to the extent corresponding to their lesser hydration capability.

Experimental studies will be needed to determine the soundness of this tentative assumption of a passivity of metal oxinates due to hydrophobia, and to determine whether it must be taken into account in all instances of an abnormally sluggish dissolution of inner complex salts. Passivity can arise from other causes, and can occur with other organo-metallic compounds. The assembling of experimental material for researches on false equilibria and for testing their possible analytical employment is one duty of the chemistry of specific, selective, and sensitive reactions.

MASKING OF REACTIONS AND BEHAVIOR OF INNER COMPLEXES

When assembling and arranging material on chemistry of specific, selective, and sensitive reactions, it was found that the literature of analytical chemistry contains well-entrenched erroneous generalizations concerning organic reagents. Misstatements with respect to the terms "organic reagent" and "induced oxidation" have been pointed out above. There are similar unjustified generalizations concerning the formation of metallo-organic compounds.

In the first place, "masking" is often regarded as identical with the production of complexes. Masking is the appropriate term for the prevention of characteristic reactions that are normally shown by dissolved materials. In many cases, this hindering is due to the formation, through the aid of suitable masking agents, of soluble complex compounds to such an extent that the concentration of the ionic or molecular species originally present falls below the concentration necessary for the occurrence of a given reaction. However, this signifies that the stability of the resulting complex compound, and not the mere formation of the complex (*24*, *42*), is a factor of prime importance.

The masking of reactions of materials in true solution is one of the most effective means of improving the selectivity of precipitation and color reactions. Hence the search for organic compounds that can function as masking agents should be just as vigorous as attempts to find organic compounds that enter into precipitation or color reactions. Organic compounds that can accomplish maskings could be called "organic masking agents" in order to stress their special function. Up to the present, little attention has been directed to the fact that certain groups are doubtless involved when organic masking agents are active. It may be expected that due regard for group actions will facilitate the search for new selective masking agents, an endeavor that has thus far aroused little interest. The possibility of converting selective precipitants into masking reagents by introducing sulfonic groups has been discussed above. The solubility of certain inner complexes in alkali indicates that alkaline solutions of inner complex formers may be capable of serving as masking agents.

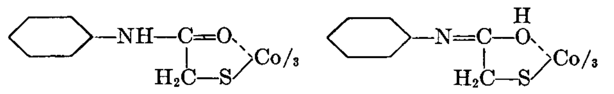
CHARACTERIZATION OF INNER COMPLEXES

Erroneous generalizations are encountered in the characterization of inner complexes—i.e., metallo-organic compounds in which a metal atom is linked with certain atoms of the organic molecule through principal and auxiliary valences to form a ring. It is frequently stated that inner complexes are distinguished by exceptional insolubility in water, stability against reagents, color, participation of functional groups responsible for ring formation in all reactions which result in opening the ring, inability to coordinate with further molecular species, and solubility in organic liquids. There are inner complexes to which this characterization applies. Now, however, when the number of inner complexes has increased so greatly, it is imperative to set matters right.

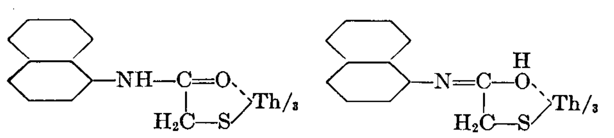
A great number of normal salts possess considerable water-insolubility. Insoluble sulfides, sulfates, chromates, oxyhydrates, etc., are examples. Other complex compounds are no more soluble in water than inner complexes. Probably the notion that inner complexes have an outstanding insolubility originated from the knowledge that the precipitation sensitivity of colored inner complexes as a rule is very high. However, the precipitation sensitivity, which is determined by the ease of perceiving small amounts of precipitate, is by no means a function solely of the insolubility of a precipitate. It depends also on the rapid overcoming of supersaturation, and the form species, color, and diffractive ability of the particles that make up the precipitate. The influence of such factors becomes readily apparent on comparing the precipitation of nickel by hydroxide and dimethylglyoxime. The production of red crystalline nickel dimethylglyoxime is perceptible in solutions which are so dilute that the light green amorphous nickel hydroxide precipitate can no longer be seen. Nevertheless, if the filtrate from a nickel hydroxide precipitation, or water saturated with nickel hydroxide, is tested with dimethylglyoxime, no trace of a nickel reaction can be observed.

Normal, insoluble salts, such as sulfides and sulfates, frequently prove much more resistant to attack by acids and bases than inner complexes. The latter, for the most part, are resistant to either acids or bases, but seldom to both, as is frequently true of insoluble normal compounds. In fact, there are known at present only two inner complexes whose resistance actually is abnormally great—cobaltic nitrosonaphthol and copper phthalocyanine (*15*)—which as yet has found no use in analytical work. These two, which are resistant to concentrated hydrochloric acid, dissolve in concentrated sulfuric acid, from which they are reprecipitated unaltered on the addition of water. The copper compound is not attacked by fused caustic, and can be sublimed in vacuo. However, no other inner complexes are even remotely so resistant as the naturally occurring silicates, sulfides, and certain ignited metal oxides.

Inner complexes do not possess abnormal stability, although in some cases components of inner complexes exhibit a stability that can legitimately be regarded as abnormal. Thus, trivalent cobalt and trivalent thallium form no known soluble or insoluble normal salts with organic or inorganic acids. Inorganic cobaltic or thallic compounds invariably contain the metal as a constituent of complex anions or complex cations, such as $[TlCl_4]^-$, $[Co(NH_3)_6]^{+++}$, and $[Co(NO_2)_3]^{---}$, or they are nonelectrolytic complex compounds. All these compounds are made up of three components. On the other hand, cobaltic and thallic inner complexes consist always of two components (*23*). This represents the simultaneous principal and auxiliary valence binding of the metal. Cobaltic nitrosonaphthol is a familiar instance of an inner complex trivalent cobalt salt. Further examples are the stable, water-insoluble compounds cobaltic thioglycollic acid anilide, XXXIX (*9*), and thallic thionalide, XL (*27*).



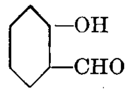
XXXIX



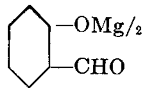
XL

In these inner complexes, there is stabilization not only of the higher valence of the metal, but also of the organic portion of the compound. Thioglycollic acid anilide and thionalide are mercaptans (thiols) and therefore, when not in combination, are rapidly oxidized even by air to the corresponding disulfides. As constituents of an inner complex, they are stable, despite their being combined with trivalent cobalt and thallium, whose reductions by a mercaptan would be a logical expectation.

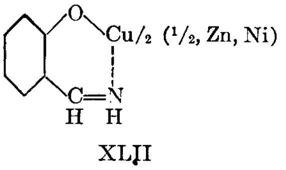
Pfeiffer and his associates (*77*) discovered a very impressive stabilization of an organic compound resulting from its incorporation into an inner complex. They found that compounds of very different kinds are produced by the reaction of metal salts with ammoniacal solutions of salicylaldehyde, XLI. Calcium and magnesium form fairly soluble nitrogen-free compounds, corresponding to XLIa. Zinc, copper, and nickel—i.e., ammine-producing metals—precipitate in the form of nitrogenous compounds, to which can be assigned the constitution of inner complexes of an imide of salicylaldehyde, XLII. The completely surprising thing about this divergent behavior is the fact that an imide of salicylaldehyde cannot be isolated in the free state. Therefore, its production in considerable amounts is occasioned solely through the formation of inner complexes. Accordingly, the zinc, nickel, and copper salts of salicylaldehyde, which have the structure XLIa, can be converted into inner complexes of salicylimide by treatment with ammonia.



XLI



XLIa



XLII

Other *o*-hydroxy aldehydes and *o*-hydroxy ketones behave similarly to salicylaldehyde and provide excellent instances of group actions. Duke (*17*) showed that accurate gravimetric methods of determining copper and nickel can be based on precipitation by means of ammoniacal solutions of salicylaldehyde and weighing of the resulting inner complexes. Duke also investigated the reactions of solutions of salicylaldehyde in methylamine and several derivatives of this primary amine, and obtained findings that are of great interest to the study of group actions.

Current generalizations concerning the color qualities of inner complexes need to be corrected. It is true that the first inner complexes used in analytical practice were distinguished by particularly intense color qualities. However, numerous inner complexes are only slightly colored, and others, such as zirconium mandelate (*67*) and thallic thionalide (*27*) are colorless. As yet, only a few cases have been found in which colorless components produce colored inner complexes. (An example is bismuth thionalide.) In the great majority of instances, inner complexes are colored when the metal ion, the inner complex–forming organic reagent, or both are colored. This rule also applies to normal salts. A peculiarity of the color quality of inner complexes is the fact that frequently with colored starting materials there is not only a deepening of color in the product, but frequently a characteristic color change. The color of inner complexes doubtless is connected with coordinative binding within the molecule, but coordinative binding is also found in other types of complex compounds. Accordingly, color and color intensification are also observed in addition and intercalation compounds. The color conferred by coordination of α,α'-bipyridine on ferrous and copper ions is a pertinent example.

The assertion that both functional groups attached to the metal atom react concurrently when inner complexes enter into chemical reactions is based on an extremely interesting study by Karrer and collaborators (*64*). They found that inner complexes of silver and *o*-amino and *o*-hydroxy acids react with alkyl halides in such manner that, in addition to silver halide, there is produced not only an ester of the carboxylic acid, but, at the same time, also *N*-alkyl and *O*-esters of the *o*-situated imino and hydroxy groups. However, this reaction course is not realized with all inner complex silver salts of this type nor with all alkyl halides; an analogous mode of reaction has, as yet, never been observed with other inner complexes. Hence, a simultaneous reaction of functional groups in inner complexes may not be taken as characteristic of this class of compounds.

When examining the question as to whether inner complexes are coördinatively saturated compounds, it is necessary to take account of their physical state. The great majority of solid inner complexes appear to be coordinatively saturated compounds, but there are notable exceptions. Inner complex metal oxinates with definite mole numbers of water of crystallization are known, in which the binding of water molecules cannot be brought into conformity with the maximum coordination numbers of the metal atoms contained in the inner complex. There is, for instance, a trihydrate of thallic oxinate, which can be dehydrated at 120° C. to a stable monohydrate (*32*). The inner complex thorium and uranyl oxinates hold an additional molecule of oxine with great tenacity (*8*). Cobaltic nitrosonaphthol, in which the cobalt has the coordination number 6, contains two molecules of water of crystallization. At present it is not possible to make precise statements concerning the localization—i.e., the points at which the coordinated water or oxine molecules are attached to the inner complexes.

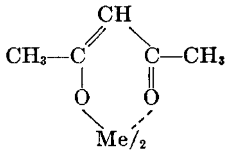
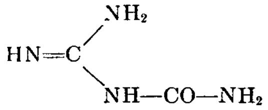
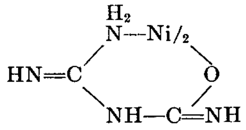
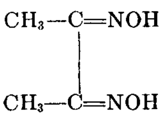
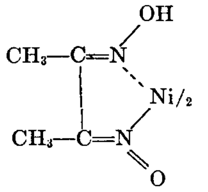
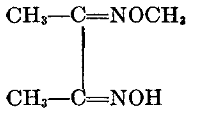
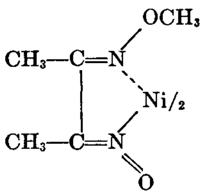
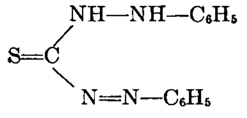
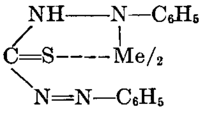
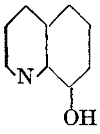
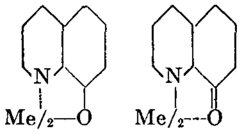
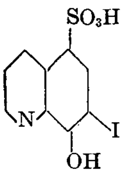
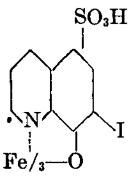
The solubility of inner complexes in organic solvents also can presumably be connected with their coordinative saturation. If it is assumed that dissolution represents a chemical process which leads to the production of a solvate, then solutions of inner complexes in organic liquids must be assumed to contain solvates of inner complexes. These solvates can be regarded as coordination compounds of molecules of the inner complex with molecules of the solvent.

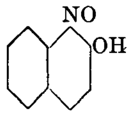
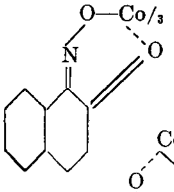
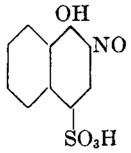
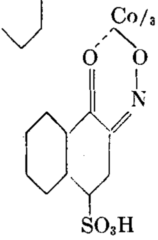
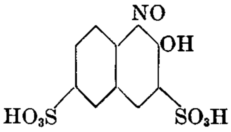
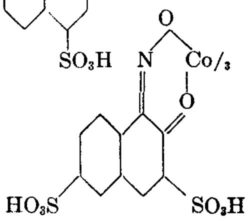
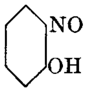
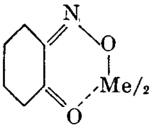
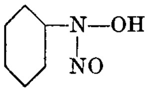
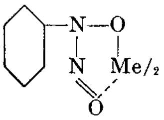
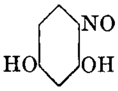
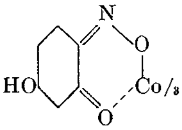
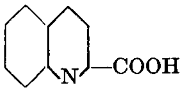
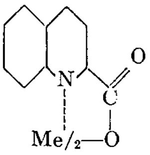
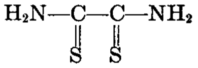
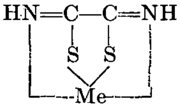
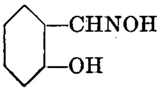
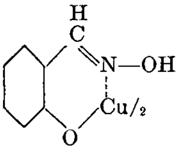
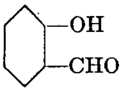
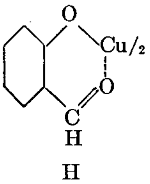
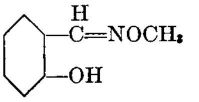
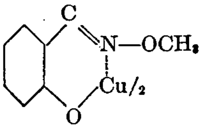
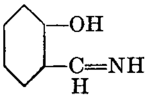
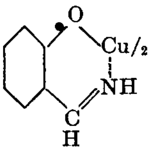
The solubility of inner complexes in organic liquids has an eminently practical analytical importance. The fact that they dissolve in liquids that are immiscible with water provides the possibility that inner complexes formed in water and suspended in it can be transported into the organic solvent by extraction. It, therefore, would be extremely advantageous if solubility in organic solvents actually were a characteristic of all inner complexes, but unfortunately this property does not prevail in such generalized form. Solubility in organic liquids to notable proportions is not general, nor have organic solvents been found to possess equal solution capabilities for inner complexes. Furthermore, different metal salts of the same inner complex former may show a thoroughly divergent behavior toward a particular solvent. The quantitative data concerning the solubility of inner complexes in organic liquids are still scanty, and there have been no studies of the action of mixtures of solvents. Consequently, there are no values available for comparing the solubilities of the salts with those of organic inner complex formers. Such data would have great theoretical and practical value in deepening the knowledge of inner complexes and solution processes.

Among organic solvents, a preferred position is occupied by chloroform; its solvent capability for inner complexes seems to be exceptionally great. Table I, which gives orientation relative to the chloroform solubility of various inner complexes, shows that there is a definite connection with group actions.

Studies of the solubility of inner complexes in organic liquids should take due account of dioxane (diethylene oxide). This cyclic ether, which is miscible with water and many organic liquids in all proportions, apparently is an excellent solvent for certain inner complexes. This property may have analytical importance, for dioxane can also serve as solvent for organic reagents that are insoluble in water. Feigl and Heisig (*38*) described the preparation of a fluorescing hydrosol of aluminum oxinate by adding a dioxane solution of this inner complex drop by drop to water. The literature of colloid chemistry contains no record of the preparation and study of hydrosols of inner complexes. It may be assumed that hydrosols of these salts and other metallo-organic compounds, and also of pure organic compounds, can be prepared with the aid of dioxane as solvent. Should this prove true, it will again demonstrate that findings derived from the chemistry of specific, selective, and sensitive

Table I. Solubility of Inner Complexes in Chloroform

No.	Inner Complex Forming Compound: Name	Inner Complex Forming Compound: Formula	Coordination Formula of Inner Complex	Solubility in $CHCl_3$
1	Acetylacetone	$CH_3.CO.CH_2.CO.CH_3$		Very sol.
2	Dicyandiamidine	 $HN{=}C\begin{array}{l}\diagup NH_2 \\ \diagdown NH-CO-NH_2\end{array}$		Insoluble
3	Dimethylglyoxime	 $\begin{array}{l}CH_3-C{=}NOH \\ \quad\;\, | \\ CH_3-C{=}NOH\end{array}$		Slightly sol.
4	Dimethylglyoxime mono-methyl ether	 $\begin{array}{l}CH_3-C{=}NOCH_3 \\ \quad\;\, | \\ CH_3-C{=}NOH\end{array}$		Very sol.
5	Diphenylthiocarbazone	 $S{=}C\begin{array}{l}\diagup NH-NH-C_6H_5 \\ \diagdown N{=}N-C_6H_5\end{array}$		Very sol.
6	1,8-Hydroxyquinoline			Very sol.
7	7-Iodo-8-hydroxyquinoline-5-sulfonic acid			Insoluble

8	Nitrosonaphthol, 1,2- and 2,1			Very sol.
9	Nitrosonaphthol-4-sulfonic acid			Insoluble
10	Nitrosonaphthol-3,6-disulfonic acid			Insoluble
11	Nitrosophenol			Very sol.
12	Nitrosophenylhydroxylamine			Very sol.
13	4-Nitrosoresorcinol			Insoluble
14	Quinaldinic acid			Insoluble
15	Rubeanic acid	 $\mathrm{H_2N{-}\underset{\underset{S}{\Vert}}{C}{-}\underset{\underset{S}{\Vert}}{C}{-}NH_2}$		Insoluble
16	Salicylaldoxime			Slightly sol.
17	Salicylaldehyde			Very sol.
18	Salicylaldoxime methyl ether			Very sol.
19	Salicylaldoximine			Insoluble

reactions can be very useful in fields with which analytical chemistry has no direct connection.

Inner complexes are soluble without decomposition in melts of the particular inner complex former (*41*). No tests have yet been made to determine whether solubility in melts is a property exhibited solely by inner complexes or whether normal salts of low-melting organic acids likewise show this behavior. It has been established that metal hydroxides and oxides and metal salts of inorganic and organic acids react with fused 8-hydroxyquinoline to form metal oxinates (*31*). The formation of the dark colored oxinates of iron and vanadium in this way makes possible rapid, sensitive, and selective tests for ferric oxide and vanadium pentoxide even in presence of greater quantities of other basic and acid metal oxides.

The experiences accumulated thus far indicate that inner complexes cannot be characterized with certainty by means of special chemical properties or peculiarities of solubility and color. All such generalizations have proved to be in error. However, this error doubtless stimulated the search for organic reagents which because of their constitution are capable of forming inner complexes. In numerous cases, reactions which lead to inner complexes have proved to be selective, sometimes even specific, when conducted under appropriate conditions. Practically all such reactions possess high sensitivity and have therefore found application in qualitative and quantitative microanalysis. If, in addition, consideration is given to the significance of inner complexes for complex chemistry, for questions of color, solubility, and passivity, etc., it is understandable that the treatment of these salts within the bounds of the chemistry of specific, selective, and sensitive reactions is of the greatest importance.

ADSORPTION COMPOUNDS AND LAKES

A rather considerable number of organic reagents, at suitable pH values, react with metal salt solutions to precipitate products whose composition is not constant, but depends on the reaction conditions. As a rule, the molecular ratio between precipitated material and reagent is much in favor of the former. Similar combinations of materials, whose composition shows still more extreme departures from stoichiometrically definable compounds, are known in colloid chemistry as the result of adsorptions. Reasoning correctly that such precipitations had some sort of connection with adsorption, the term "adsorption compound" was a logical choice. Flagg (*51*) has introduced the appropriate term "adsorption precipitant" for organic reagents that bring about the precipitation of adsorption compounds. There is as yet no clear understanding of the details of the action of such adsorption precipitants or reagents, and Flagg is justified in stating "that the term is largely a disguise for ignorance of basic facts." Tannin precipitations and the formation of metal lakes which are of analytical importance fall into this class. An approach from the standpoint of the chemistry of specific, selective, and sensitive reactions offers new and interesting aspects.

TANNIN PRECIPITATES

The classic studies of Schoeller and his school have demonstrated that tannin functions as a precipitant for numerous metals, and that this action can be used as the basis of a series of excellent precipitation and separation procedures (*82*). Schoeller's explanation (*83*) of tannin precipitations is very enlightening, at first glance, and has been incorporated in the analytical literature.

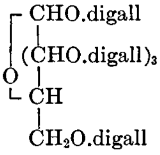
He points out that the solutions of salts of iron, aluminum, chromium, etc., which are precipitable by tannin, are without exception solutions of hydrolyzable metal salts and consequently contain hydrolysis products (metal oxyhydrates) in hydrosol form. Such sol particles are positively charged. Aqueous tannin solutions are colloidal dispersions in which the tannin particles carry negative charges. Therefore, the colloidal particles can undergo mutual discharge and flocculation with continuous disturbance of the hydrolysis equilibrium until the metal has been quantitatively precipitated. This explanation cannot be accepted as applying to all precipitations brought about by tannin.

Metals can be quantitatively precipitated by tannin from ammoniacal or slightly acid solutions which are masked with tartrate. Such solutions contain practically no oxyhydrate particles produced by hydrolysis, but complex metal-tartrate ions. Furthermore, weakly acid solutions of alkali salts of tungstic and molybdic acids, and acid solutions of complex niobium and tantalum compounds, when treated with tannin yield quantitative precipitations, in the form of adsorption compounds of the respective metal acids. Acid solutions of molybdates and tungstates contain negatively charged particles of the acid anhydrides, which cannot be flocculated by negatively charged tannin particles. Finally, most metal precipitates produced by the action of tannin are highly colored. This contradicts the experience that no considerable color changes accompany the mutual flocculation of oppositely charged hydrosols. Thus tannin precipitations cannot be due exclusively to mutual coagulation of colloid particles, as has been assumed, and consequently other explanations must be sought.

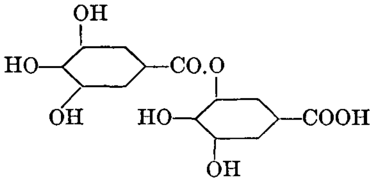
A clearer idea of the action of tannin is obtained if consideration is given to (1) surface reactions of colloidal particles suspended in water, and (2) the reaction capability of tannin due to the groups it contains.

Surface reactions of colloid particles should be regarded from the general standpoint of the reaction of solids with dissolved reactants. In this case, a reaction need not proceed solely via dissolved portions of the solid; ions or atoms of the surface of the solid can also act directly. The products of such surface reactions may form an independent new soluble or insoluble phase, but reaction products may also remain in the phase association of the solid. The latter condition is possible only when reaction-capable ions or atoms of the lattice surface do not leave the original phase association even after the reaction has taken place. This corresponds to an incomplete, nonuniversal reaction of surface atoms or ions, which is restricted to the surface layers of the solid and leaves its interior unaltered. This type of reaction leads to an accumulation of an initially dissolved reactant in the surface of a solid, and corresponds to the concept of an adsorption (*45*). A model example of such a surface reaction is provided when pure alumina is treated with dilute acid or alkali in preparation for chromatographic studies. No separate phase made up of the aluminum salt of the particular acid or of alkali aluminate is formed; rather acid radicals or alkali are held on the surface of the alumina—i.e., basic aluminum salt or aluminate is produced there. Consequently, in both cases, the surface reacts and fixes the reaction product. The mode of reaction of alumina powder holds also for alumina particles in sol form, and analogous surface reactions can be readily conceived with colloidal particles of other basic and acid metal oxides.

In order to understand the reactivity of tannin and to bring it into accord with the adsorption model on alumina just cited, it is necessary to consider the composition and constitution of tannin. The latter is a collective name given to water-soluble mixtures found in many plants and consisting of esterlike compounds of various sugars with phenolcarboxylic acids. Pentagalloylglucose (*48*, *63*) is surprisingly similar to Chinese tannin, and perhaps is its chief constituent. This compound, which has been prepared synthetically, has the structure:



Pentogalloylglucose



m-Digallic acid

Their polyphenol components are characteristic of all tannins. Despite their high molecular weights, tannins are water-soluble because they contain so many phenolic hydroxy groups. Their water solutions contain both true and colloidally dissolved tannin. There is little doubt that the content of phenol groups confers reactivity on colloidal particles of tannin as well as on dissolved tannin molecules. Therefore, solutions of hydrolyzable metal salts can react in different ways with colloidally dispersed and genuinely dissolved tannin. Surface reactions of hydrolysis products may occur with either dissolved or colloidal tannin, surface reactions of colloidal tannin particles with metal ions are possible, and finally there can be reactions between metal ions and tannin in true solution. All these possibilities lead to metal-tannin bindings, and the particular conditions will determine which reaction will prevail. However, under no circumstances will the resulting precipitate be homogeneous nor will it conform to a stoichiometric formula. It is also apparent that complex metal ions in ammoniacal solution will react with colloidal or truly dissolved tannin in so far as the products are not soluble in water.

The assumption that the reactivity of tannin is due to its phenol groups provides a good explanation as to why metal acids are precipitable by tannin. Phenols can react with metal acids to form phenol esters, as shown by the precipitation of formula-pure esters of tungstic, molybdic, and vanadic acids by 8-hydroxyquinoline (*97*). An esterlike binding between tannin and metal acid can obviously occur as well on the surface of colloidal acid anhydride particles as on the surface of colloidal tannin particles. The intense color of metal-bearing tannin adsorption compounds is likewise in harmony with the idea that the tannin is reacting chemically. As shown by its formula, tannin contains many ortho- situated hydroxy groups. When a metal salifies a group of this kind it is possible for the metal to be coordinated with the oxygen of a neighboring hydroxy group; inner complex bindings result, with the color effects that so frequently accompany them. In fact, the precipitation products of the noncolloidal gallic and digallic acid have approximately the same color as tannin precipitates.

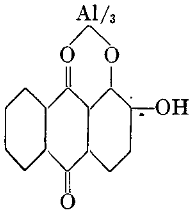
Hence tannin precipitates can be regarded as products of surface reactions of colloid particles, in which phenolic hydroxy groups of the tannin participate. However, the products of such surface reactions are not metal tannates or tannin esters of metal acids in an independent phase, but the tannin binding takes place on the surface of colloidal particles, whose interior remains unaffected. The designation "adsorption compound" thus acquires real meaning because tannin precipitations involve effects that can be viewed from the standpoint of adsorption as well as chemical binding.

FORMATION OF METAL LAKES

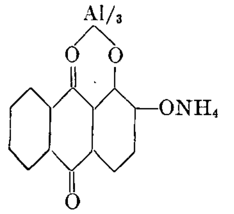
The term "lake" (color lake or metal color lake) comes from the chemical technology of textile dyeing. It signifies the fixing of dyes by inorganic metal compounds, usually oxides or oxyhydrates. There is no uniform concept as to the nature of the fixing. In a fundamental study, Werner (*102*) showed that lake-forming dyes are inner complex formers, and that colorless inner complex–producing organic compounds, which form colored metal salts, are irreversibly bound by the corresponding oxides in a way analogous to that by which dyes are held by metal mordants. On the other hand, Weiser (*96*), on the basis of careful experimental investigations, champions the view that the taking up of dyestuffs by the so-called mordants is an adsorption process which does not lead to chemical compounds. Both concepts are discussed in connection with metal color lakes in the literature of organic, colloid, and analytical chemistry (*47, 49, 60, 80, 100*).

Reactions that lead to lakes play an important role in analytical chemistry. After Atack (*1*) showed that the aluminum-alizarin reaction could be utilized for the sensitive detection and colorimetric determination of aluminum, many lake-producing reactions were described for the detection and determination of various metals (*101*). Most of the lake-forming dyes are acidic compounds, whose constitution is such that they are fundamentally capable of producing inner complexes. Although this fact seems to support Werner's assumption that lakes are inner complexes, such inner complexes have been actually isolated in surprisingly few instances, and then only under conditions that do not correspond to those prevailing during the production of lakes in analytical procedures.

Aluminum-alizarin lake is often cited as a model of lake production. Moehlau (*74*) prepared a formula-pure inner complex aluminum alizarinate, XLIII, and described its properties. This salt, which was obtained by the reaction of monopotassium alizarinate with aluminum chloride, is insoluble in 1 *N* hydrochloric acid and dissolves in ammonium hydroxide. Moehlau correctly assumed that an ammonium salt of an aluminum alizarinic acid, XLIV, is produced and he substantiated this idea by the isolation of the corresponding alkali earth salts.



XLIII



XLIV

Moehlau's findings appear to have been completely forgotten, or their significance with respect to the aluminum-alizarin lake was not appreciated. They demonstrate that the red aluminum-alizarin lake cannot be either an inner complex aluminum alizarinate or a mixture of this salt with unchanged alumina. The red lake, produced by adding ammoniacal alizarin solution to aluminum solutions and then acidifying with acetic acid, has distinctly different properties from the compound isolated by Moehlau. The lake is resistant to ammonia and is decomposed by hydrochloric acid much more dilute than 1 *N*.

Discussions of the formation, composition, and constitution of color lakes must take due account of the rule that lake reactions employed in analysis occur in the pH range of the respective metal hydroxides (oxyhydrates) which are then in the sol or gel form. It is therefore probably the metal hydroxide, and not the metal ions, which reacts in the aqueous solution with the dyes. This provides a tie in with the metal tannin precipitations discussed above, which were viewed as surface reaction products of sol particles. It is plausible that alizarin reacts with alumina:

$$[\mathrm{Al(OH)_3}]_x + \mathrm{HAliz} \longrightarrow [\mathrm{Al(OH)_3}]_{x-1}.\mathrm{(OH)_2.AlAliz} + \mathrm{H_2O}$$

This schematic representation indicates that the aluminum atoms of the alumina surface do not all react with these alizarin molecules, which would result in the production of aluminum alizarinate as an independent phase. The reaction proceeds only partially with principal and auxiliary valence binding of the alizarin on the surface of the alumina without forming a new phase. This corresponds to a chemical adsorption of alizarin. In the purely formal sense, it signifies a formation of basic aluminum alizarinate in the surface of the alumina. It might also be stated that an "alizarinization" of the alumina surface occurs. The reaction picture presented for the aluminum-alizarin lake can be appropriately applied to metal lakes of dyes, which possess inner complex–forming groups. Therefore, such lakes are to be regarded as adsorption compounds, in which dyestuffs are held by principal and auxiliary valences to metal atoms on the surface of gel or sol particles of the respective hydroxides (oxyhydrates). Because of this binding, color lakes have a similarity to inner complexes. This is shown by the fact that lakes frequently have a more intense or different color from that of the free dye. This divergence is the basis of the analytical usefulness of many lake reactions.

In the color lakes there is realized an inner complex binding which cannot be accomplished by a reaction of metal and dyestuff ions with production of inner complexes. As shown by Moehlau, it is not permissible to rule out the possibility of a production of inner complex metal salts of acid dyes under special circumstances. In isolated cases, including the analytical formation of lakes, products may closely approach the composition of inner complexes. This has been recently shown by Liebhafsky and Winslow (*70*) within the system hafnium-alizarin. However, all experience indicates that the production of adsorption compounds is the preferred mode of reaction.

Feigl and Zocher (*45*) discovered interesting examples of the behavior of organic inner complex formers in surface reactions with metal oxides.

If alumina is shaken with a concentrated chloroform solution of 8-hydroxyquinoline, the filtrate exhibits a yellow fluorescence. This shows that a chloroform-soluble fluorescent compound has been formed. However, the alumina, which appears to be unchanged, likewise fluoresces and this fluorescence persists despite prolonged washing with chloroform. Inasmuch as the fluorescence of metal oxinates is connected with the inner complex binding of the metal, it must be supposed that the surface of the alumina is "oxinated," analogous to the "alizarination" of the surface of the alumina in the aluminum-alizarin lakes. If the experiment is repeated with increasingly diluted oxine solutions, a region is reached in which no aluminum oxinate is formed as a separate phase, but there is only a holding of the oxine on the surface. This latter attachment is clearly revealed by the fluorescence of the alumina. It appears, therefore, that the inner complex binding of the oxine on the surface of the alumina—i.e., the chemical adsorption of oxine—is a preliminary stage in the formation of aluminum oxinate.

The sulfonic acids of oxine give no precipitate with water solutions of aluminum salts, and as yet no aluminum compounds of this type have been isolated in solid form. If, however, alumina is treated with aqueous solutions of these sulfonic acids, fluorescing products are obtained. This indicates that inner complex bonds have been formed through surface reactions, even though this binding cannot be accomplished in the form of solid inner complexes. The behavior of oxine sulfonic acids toward alumina is further evidence that the reactivity of inner complex–forming groups in organic compounds is not annulled by the introduction of sulfonic groups. The chemical reaction of oxine and its sulfonic acids with alumina, which can be followed through the formation of fluorescing products, demonstrates the reaction possibilities of dyes, whose constitution makes them potential inner complex formers. The inner complex binding of oxine on the surface of alumina corresponds to the inner complex binding of dyes on the surface of alumina. In one case, fluorescence results; in the other, the color characteristic of lakes appears.

When considering these color lakes from the standpoint of complex chemistry, it is essential to keep in mind that the deepening of a color or a color change is not unique with inner complex binding; such color phenomena can also arise through purely coordinative binding. Accordingly, it may be expected that "addition lakes" must exist as well as "inner complex lakes." The former would be colored adsorption compounds in which the pertinent surface reaction consists of an addition reaction—i.e., coordination of dyestuff molecules. The production of such lakes has now been indubitably demonstrated (*70*) in studies dealing with the action of organic bases on palladium cyanide. Strong diacid bases (B), such as ethylenediamine and α,α'-bipyridine, form addition compounds of the type $Pd(CN)_2.B$. These compounds correspond to the long-known diammine $Pd(CN)_2.2NH_3$, which is obtained by treating palladium cyanide with ammonia. Weak organic bases are not capable of producing diammines as a separate phase. However, their coordination capability is revealed by an attachment on the palladium atoms of the surface of palladium cyanide. Adsorption compounds of palladium cyanide with organic bases are obtained. Colored adsorption compounds—color lakes—are obtained when colored weak organic bases are used. In the majority of cases, the color of these addition lakes is more intense, and often different from the color of the corresponding basic dyes.

The behavior of acid-base indicators, which contain NH_2 or alkylated NH_2 groups, is especially interesting. They form lakes with palladium cyanide, and the color of the product is essentially that of the particular indicator in acid solution. This effect probably presents a contribution to the resonance theory of acid-base indicators. The addition lakes of palladium cyanide are examples of not only a new class of color lakes but also a new class of metallo-organic adsorption compounds. Hitherto, the known examples of this class of adsorption compounds invariably had basic or acid metal oxides as their inorganic components.

CONCLUSIONS

The foregoing considerations and experiments, which constitute an attempt to elucidate the formation and constitution of lakes, lead also to results that are interesting from the standpoint of new analytical methods.

The adsorption of oxine on the surface of alumina, forming a fluorescent product, can be used as a highly sensitive test for the detection of oxine (*45*). When 0.5 liter of oxine dissolved in water or organic liquid is shaken with alumina and filtered, a fluorescent product is obtained, even at a dilution of 1 to 10,000,000,000. This is a good example of the sensitivity of a test based on detection of traces.

Small quantities of calcium, magnesium, or aluminum in paper can be detected by spotting the paper with a solution of oxine or sulfonated oxine, or by exposing the paper to the vapors of oxine at room temperature. Fluorescent flecks are obtained through the contact of oxinate-forming materials (*37*).

Palladium cyanide can be specifically identified by spotting with an appropriate indicator solution; colored addition lakes are formed (*32*).

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Polysubstituted 1,10-Phenanthrolines and Bipyridines as Multiple Range Redox Indicators

Further Applications as Specific Organic Analytical Reagents

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THE "ferroin" reaction involves chelate ring formations having as their basis the specific group configuration $(=N-\overset{\|}{C}-\overset{\|}{C}-N=)$. This specific group occurs in the heterocyclic ring nitrogen compounds, of which the following are the most familiar examples: 2,2′-bipyridine; 1,10-phenanthroline; and 2,2′,2″-terpyridine.

This type of compound is capable of being altered over a wide range of modifications through the substitution of various type replacement groups, such as methyl, phenyl, carboxy, nitro, and halogen, for one or more of the hydrogens of the parent substance. These derivatives of the parent substances can be shown to affect the properties of the resulting reagents under the following categories:

Formation of the complex divalent ferrous cations with bipyridine, 1,10-phenanthroline, and terpyridine for use as redox indicators.

Use of the above formulations in the photometric determination of iron, copper, molybdenum, and cobalt, and the detection of other metals.

As anion precipitants for separations and determinations such as perchlorate, periodate, persulfate, and thiocyanate from chlorate, iodate, sulfate, and cyanide, respectively.

As masking reagents (in the quantitative separation of traces of aluminum in the presence of large amounts of iron, as a typical example).

HISTORICAL

General. The preparation, properties, and analytical applications in the use of the organic reagents having the ferroin specific grouping thus far investigated are described by Smith and Richter (*38*), Walden and Edmonds (*42*), and Oesper (*31*). The most recently published source of reference and the most complete

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bibliography covering the subject of bipyridine and related compounds is that of Welcher (*45*), which cites more than 100 literature references and includes details of preparative procedures and analytical applications.

The classification of organic chemical reagents generally follows the scheme of defining the specific functional group to which they owe their principal analytical applicability. The ferroin reaction group $(=N-\overset{\|}{C}-\overset{\|}{C}-N=)$ requires that the nitrogen originate in a cylic structure. An example of this grouping which does not result in the ferroin reaction is found in the compounds ethylenediamine and 8-aminoquinoline, as the nitrogen atoms are not both a portion of a ring structure. The unusual value of this type of compound lies in this prerequisite of heterocyclic ring nitrogen structure, for it multiplies the number of modified compounds that may be obtained. Associated with this advantage is the fact that each substituted 1,10-phenanthroline compound assures a modification in chemical and physical properties. These gradations in property are in a great many cases additive in a systematic manner. This property should make it possible to predict desirably modified compounds and eliminate the need for operations of synthesis which afford no predictable advantage.

1,10-Phenanthroline is a monoacid base (*27*). The instability constants for the ferroin ion, $FePh_3^{++}$, and its oxidized form, the ferriin ion, $FePh_3^{+++}$, were determined by Lee, Kolthoff, and Leussing (*27*). The reactions involved are:

$$PhH^{++} \rightleftarrows Ph + H^{+} \qquad K_A = 1.1 \times 10^{-5}$$

$$FePh_3^{++} \rightleftarrows Fe^{++} + 3Ph \qquad K_{\text{diss. ferroin}} = 5 \times 10^{-22}$$

$$FePh_3^{+++} \rightleftarrows Fe^{+++} + 3Ph \qquad K_{\text{diss. ferriin}} = 8 \times 10^{-15}$$

The dissociation constants for the ferroin and ferriin were determined in 0.05 M sulfuric acid and all reaction constants refer to 25° C. Similar studies were previously made by Dwyer and his associates on 1,10-phenanthroline (*11*) and bipyridine (*12*). 1,10-Phenanthroline as well as some of its substituted derivatives form monohydrates which are definite chemical compounds with sharp and reproducible melting points. Infrared data indicate the the water molecule is held through two hydrogen bonds to the ring nitrogens (*38*). The hydration of 1,10-phenanthroline and its dissociation when heated are reversible. The reaction involved is:

$$C_{12}H_8N_2.H_2O\ (s) \rightleftarrows C_{12}H_8N_2\ (s) + H_2O\ (g)$$

The temperature-vapor pressure data for this reaction have been determined, and from this measurement the hydrogen bond strength can be readily calculated. The 5-bromo- and 5-methyl-1,10-phenanthroline monohydrates were similarly investigated. The thermodynamic quantity, ΔH, for this dissociation reaction represents the amount of energy required to remove one mole of water and break two hydrogen bonds. Thus $\Delta H/_2$ represents the strength of a single hydrogen bond. This value was found by Fritz, Cagle, and Smith (*14*) to be 7.25 kg. cal. per mole for the unsubstituted compound. A similar determination of the deuterium bond has been provisionally found to give the value 7.30 kg. cal. per mole and is therefore somewhat stronger, as would be expected.

The ferroin-type complex cations are remarkably stable and permanent under a variety of conditions of formation and chemical environment. They are permanently stable in water or buffered solutions over the pH range of 2.5 to 9.0. Their instability constants are favorably high and a large number of diverse cations are without interference effects. Even under the conditions of lower pH from strong mineral acids, the various complex ferroin ions are remarkably stable and may be employed as redox indicators. Large excesses of strong oxidizing agents may be applied without effecting the decomposition of the organic base. Strong mineral acids lower the value of the instability constant and dis ociate the complex, but the reaction is reversed when the pH of the solution is increased to 2.5 to 3.0. A pH greater than 10 is required to affect the stability of the complex and cause precipitation of ferrous hydroxide.

Redox Indicators. The use of internal redox indicators had its first widespread analytical application with the introduction by Knop (*24*) of diphenylamine for use in the determination of iron by dichromate titration in 1924. The redox potential of diphenylamine is 0.76 volt. This potential is inconveniently low and was raised to 0.84 volt by the introduction of barium diphenylamine sulfonate (*26*). Diphenylbenzidine (*25*) had already been employed as an improvement over diphenylamine, and by the use of its sulfonic acid derivative the oxidation potential is raised to 0.87 volt. For cerium(IV) and vanadium(V) oxidations, N-phenylanthranilic acid has been shown (*41*) to be an improved redox indicator subsequent to the introduction of the ferroin type redox indicators. The latter has an oxidation potential of 1.06 volts. Additional nonreversible redox indicators have been described (*15, 33*).

The ferroin reaction had been known since 1889 as described by Blau (*2*). It was first suggested for use as a reversible redox indicator reaction by Walden, Hammett, and Chapman (*43*) in 1931, and was shown to be admirably adapted to cerium(IV) oxidimetry by Walden, Hammett, and Chapman (*44*) in 1933. The determination of the oxidation potential of the 1,10-phenanthroline ferrous ion was originally given as 1.14 volts. This value was first found by Getz (*16*) to require correction to 1.06 volts, but was first published as a correction by Hume and Kolthoff (*23*).

The first preparation of a substituted 1,10-phenanthroline, the 5-nitro-1,10-phenanthroline, was described by Hammett, Walden, and Edmonds (*20*) and the method of synthesis later improved as described by Smith and Cagle (*36*). The 5-nitroferroin has redox potential of 1.25 volts (*20*). The use of the tri-bipyridine ruthenium(II) ion as a redox indicator was described by Steigman, Birnbaum, and Edmonds (*40*) and its oxidation potential given the value of 1.33 volts This value has been redetermined and corrected in the work of the present paper.

The use of 5-nitroferroin as indicator in the microvolumetric determination of calcium in biological materials has been described by Salomon, Gabrio, and Smith (*32*). The extension of this basic procedure to the microdetermination of arsenic and of iron was investigated by Smith and Fritz (*37*). These reactions depend for their precision on the high cerium(IV) oxidation potential attained through the use of perchloratoceric acid in the presence of perchloric acid. Only 5-nitro-1,10-phenanthroline ferrous sulfate is suitable for these reactions.

The selection of a ferroin-type redox indicator best suited to the titration of ferrous solution employing dichromate as oxidant has led to the use of 5,6-dimethylferroin (*34*). This ferroin reactant provides a ferrous complex cation with an oxidation potential of 0.97 volt in formal acid solutions and provides a better indicator than any formerly employed for this routine analytical procedure. The end-point color change is sharp in either hydrochloric or sulfuric acid solutions.

The effect of symmetrical substitution of methyl groups on the properties of dimethyl-2,2′-bipyridines in complexing the ferrous ion (*5*) and the use of the tri-2,2′-bipyridine ferrous complex as an indicator in the determination of iron (*4*) have been described by Cagle and Smith. This work corrects the original erroneous assumption that the tri-2,2′-bipyridine ferrous ion is unsuited for this titration because of instability in acid solution.

Substituted terpyridine derivatives have not as yet been prepared. The preparation of 2,2′,2″-terpyridine for commercial distribution has but recently been developed. The oxidation potential of the tri-terpyridine ferrous ion has not been determined accurately because of its instability in solutions of low pH. Work by the present authors has disclosed that this value is somewhat lower than the corresponding bipyridine complex under similar conditions.

Photometric Reagents. The ferroin reaction in quantitative

The organic compounds of the bipyridine, 1,10-phenanthroline, and terpyridine type, having the specific functional group, $=N-\underset{\|}{C}-\underset{\|}{C}-N=$, represent reagents which provide for an almost limitless group of modifications through the substitution of a variety of elements and radicals in the place of their normal pyridine ring hydrogens and hydrogens of the benzine ring bridge of 1,10-phenanthroline. Common substitutions are $-CH_3$, $-Cl$, $-Br$, $-NO_2$, $-OH$, $-OCH_3$, $-COOH$, and other radicals, often as mono-, di-, tri-, and tetra-substitutions either symmetrically or unsymmetrically arranged. Modified form organic reagents, previously described, have been shown to be materially altered in their specific functional group activity by each such substitution. Applications of these compounds are four in number: (1) as "color detectives" in the spectrophotometric determination of iron and copper; and in the form of their ferrous complex ions as (2) oxidation-reduction indicators, (3) anion precipitants, and (4) masking reagents. A general review of this class of compounds of record is given in this paper. In addition, 22 new methyl substituted 1,10-phenanthrolines have been studied to determine individually the physical constants related to the above-mentioned four specific applications: the spectrophotometric evaluation of the wave length of maximum absorption and the molecular extinction coefficients of the ferrous and ferric complex ions. The determination of the oxidation potential of the ferric-ferrous systems has been experimentally evaluated and the determination of the oxidation potential of 1,10-phenanthroline as the ruthenous chloride complex has been reinvestigated with resultant corrections to published values and extension in the range of acidities studied. The compounds here described have been shown to exhibit properties which are predictable from reagent to reagent (based upon the type of molecular alteration) with high accuracy. Oxidation-reduction indicators are described and their transition potentials determined covering the range 0.84 to 1.30 volts and intermediate values in units of 0.01 to 0.03 volt. Preferred reagents for use in the most sensitive colorimetric determination of iron are indicated.

spectrophotometric determinations is characterized by extreme sensitivity. The ferroins provide molecular extinction coefficients which make possible the detection and determination of extremely small amounts of certain metal ions. Iron, for example, in quantities as low as 1 mg. in a cubic meter of water may be determined. This fact is not unique in the realm of spectrophotometric analysis, but requires a molecular extinction coefficient of definitely greater value than 10,000. Dithizone is capable of greater sensitivity in the determination of copper, attaining a molecular extinction coefficient of 21,800 (*1*), but requires the use of an immiscible organic solvent and an extraction process. As an illustration of the delicacy of the ferroin reaction in quantitative analysis, it is to be noted that permanganate ion has a molecular extinction coefficient of 2340 (*29*). The colors of ferroin cations fall almost entirely in the spectral region between 475 and 525 mμ, a desirable feature. Ferroin compounds outside this range have been described (*19*). Their properties fulfill all the desired aims for such procedures as outlined by Mellon (*29*).

The influence of diverse ions upon the ferroin reaction applied to spectrophotometric analyses has been thoroughly investigated by Mellon and his co-workers (*38*), and in hydrogen fluoride medium by Gillis (*17*).

Anion Precipitants. The substituted 1,10-phenanthrolines fall under the classification of organic anion precipitants. Like other anion precipitants, such as benzidine, nitron, and tetraphenylarsonium chloride, they are complex organic compounds of high molecular weight. Wide differences in the solubility of the various anion precipitates result from the alterations produced by the introduction of substituent groups for the replaceable hydrogens in the 3,8 positions. There are two types of anion precipitation: salt formation through linkage with nitrogen, the organic precipitant being in this case monobasic; and precipitation by the ferroin ion, in which case the cation is dibasic.

The precipitates formed are crystalline and of definite composition. In the case in which the first type compound is formed, the determination may be carried out photometrically, in addition to gravimetrically, by subsequent formation of the red ferroin ion after solution of the original precipitate under modified conditions. The second type of precipitation, employing the ferroin ion as precipitant, results in the formation of precipitates having a much higher molecular weight. This principle makes possible quantitative determinations based upon centrifugation and volume measurement of the capillary-confined precipitate (*10*). Inasmuch as complex ions of metals other than ferrous iron—namely, Cd^{++}, Cu^{++}, Zn^{++}, Co^{++}, Ni^{++}, Cr^{+++}, and Ru^{++}—with 1,10-phenanthroline are known, these may also be employed in anion precipitation, and have been studied to a limited extent under the procedures of microscopy (*28*).

The anions which form the most insoluble precipitates with ferroins are periodate, perchlorate, persulfate, thiocyanate, tungstate, fluotantalate, and others. Separations are possible of fluotantalate from fluocolumbate (*18*), periodate from iodate, perchlorate from chlorate, peroxydisulfate from sulfate, thiocyanate from cyanide, and other appropriate types of differentiation. Having in mind the extensive number of permutations possible through the use of substituted 1,10-phenanthrolines and the formation of the large group of substituted ferroins possible, all of which materially affect variations in property of the resulting precipitate, a very great number of new procedures are seen to be possible.

Masking Reagents. The use of 2,2′-bipyridine as a masking reagent in the separation and determination of small amounts of aluminum in the presence of large amounts of iron, such as is necessary in the analysis of iron ore, has been described by Smith and Cagle (*35*). In this work methods for the practical recovery of the spent reagent are the basis for the practicality of the procedure.

PREPARATION OF MATERIALS

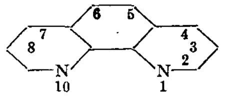
The preparation of various bipyridine and terpyridine is described by Morgan and Burstall (*30*). Snyder and Freier (*39*) describe the preparation of various 1,10-phenanthrolines by means other than a Skroup synthesis. The preparation of 23 symmetrical dimethyl-1,10-phenanthrolines by Case (*6–9*) has been noted. Other recently described materials were reported by Smith and Richter (*38*).

The cost of materials in the group of 1,10-phenanthrolines and related ferroin-type reagents is not a serious factor in all known applications, for the reason that either such small amounts are required or the recovery of spent reagents is readily accomplished.

Most of the ferroin reacting organic molecules under discussion have low solubility in water. For the preparation of the ferrous complexes this limitation is not troublesome because of their high

Table I. Mono-, Di-, Tri-, and Tetramethyl Derivatives of 1,10-Phenanthroline

(2,9 positions omitted)



Monomethyl Derivatives	Dimethyl Derivatives	Trimethyl Derivatives	Tetramethyl Derivatives
$\underline{(3)}$ $\underline{(4)}$ $\underline{(5)}$	$\underline{(3, 4)}$ $\underline{(3, 5)}$ $\underline{(3, 6)}$	(3, 4, 5) $\underline{(3, 4, 6)}$ $\underline{(3, 4, 7)}$	(3, 4, 5, 6) (3, 4, 5, 7) (3, 4, 5, 8)
	$\underline{(3, 7)}$ $\underline{(3, 8)}$ $\underline{(4, 5)}$	$\underline{(3, 4, 8)}$ $\underline{(3, 5, 6)}$ $\underline{(3, 5, 7)}$	$\underline{(3, 4, 6, 7)}$ $\underline{(3, 4, 6, 8)}$ $\underline{(3, 4, 7, 8)}$
	$\underline{(4, 6)}$ $\underline{(4, 7)}$ $\underline{(5, 6)}$	$\underline{(3, 5, 8)}$ $\underline{(3, 6, 7)}$ (3, 6, 8)	(3, 5, 6, 7) $\underline{(3, 5, 6, 7)}$ (4, 5, 6, 7)
		(3, 7, 8) (4, 5, 6) $\underline{(4, 5, 7)}$	

Table II. Bromo-1,10-phenanthrolines

Monobromo Derivatives	Dibromo Derivatives	Tribromo Derivatives	Tetrabromo Derivatives
(5)	(3, 5) (3, 6) (5, 6)	(3, 4, 5) (3, 5, 6)	(3, 5, 6, 8)

dissociation constants of the order of 1×10^{-15} to 1×10^{-22} (*11, 12, 27*).

The newly prepared reagents, the determination of the physical constants of which is reported in this paper, are listed in Table I.

A number of bromo-1,10-phenanthrolines have been prepared as listed in Table II. The 5-bromo-1,10-phenanthroline may be prepared by direct bromination of the parent substance (*38*). The remaining bromo derivatives have been prepared by F. H. Case, and a description of their synthesis is being prepared for publication. Those underscored in Table I are herein described.

DETERMINATION OF OXIDATION POTENTIAL

The procedure involved has been fully described by Smith and Richter (*38*), Section 4. The process involves the differential potentiometric titration of a mixture of a reference solution and one of the ferrous complex cation in question, by the use of a suitable oxidant, employing an appropriate acid concentration. The necessary data are obtained upon observing the proper formal oxidation potential for the ferrous-ferric system, followed by the determination of the ferroin-ferriin system, and continuing the titration to the determination of the oxidation potential of the oxidant at the point equal to twice the requisite amount of oxidant for the other two systems. It is assumed that the formality of acid employed is not allowed to alter throughout the entire process. If the first and last single electrode potentials are found to be correctly duplicated in such manner, the unknown single electrode potential of the ferroin-ferriin system may be considered to be established to an equal degree of accuracy. Such determinations are frequently studied employing 0.1 or 1.0 *F* hydrogen ion concentrations in sulfuric acid solution with sulfatoceric acid as oxidant.

As an illustrative procedure, the reinvestigation of the determination of the following reaction, first investigated by Steigman, Birnbaum, and Edmonds (*40*), was carried out.

$$(C_{10}H_8N_2)_3Ru^{+++} + e^- \rightleftarrows (C_{10}H_8N_2)_3Ru^{++}$$

The ruthenious bipyridine complex in the form of the chloride hexahydrate was prepared as described by Burstall (*3*). Solutions of this material in various strengths of sulfuric acid from 2 to 16 *F* hydrogen ion concentration were prepared. Ferrous sulfate solutions in identical media were added and the two reducing agents were then oxidized by titrations employing sulfatoceric acid in the same strength sulfuric acid. The typical potentiometric titration graph shown in Figure 1 for the condition of 6 *F* sulfuric acid solution was obtained. The same scheme was followed using nitric acid solutions in 0.1 *F* and 1.0 *F* hydrogen ion concentrations in order to have a sufficiently large end-point break. The values shown in Table III were thus determined.

An examination of the data of Figure 1 and Table III indicates that at 1 *F* hydrogen ion concentration the value for the oxidation potential of 1.25 volts was obtained. This value is considerably lower than the 1.33 volts found by Steigman, Birnbaum, and Edmonds. In the determination at 12 *F* hydrogen ion concentration (Figure 1), the oxidation potential for the ferrous-ferric system was found to be 0.70 volt, the correct formal oxidation potential. The oxidation potential for the ruthenous complex–ruthenic complex system may be read from the titration graph to be 1.07 with an accuracy of ±0.01 volt. The value for the oxidation potential of the cerium(IV)–cerium(III)

Table III. Oxidation Potential of Ruthenous-Ruthenic Bipyridine Complex Cation in 0.1 *F* to 16 *F* Hydrogen Ion Concentrations

Hydrogen ion formality	0.1	1.0	4.0	8.0	12.0	16.0
Oxidation potential, volts	1.27	1.25	1.21	1.16	1.07	0.98

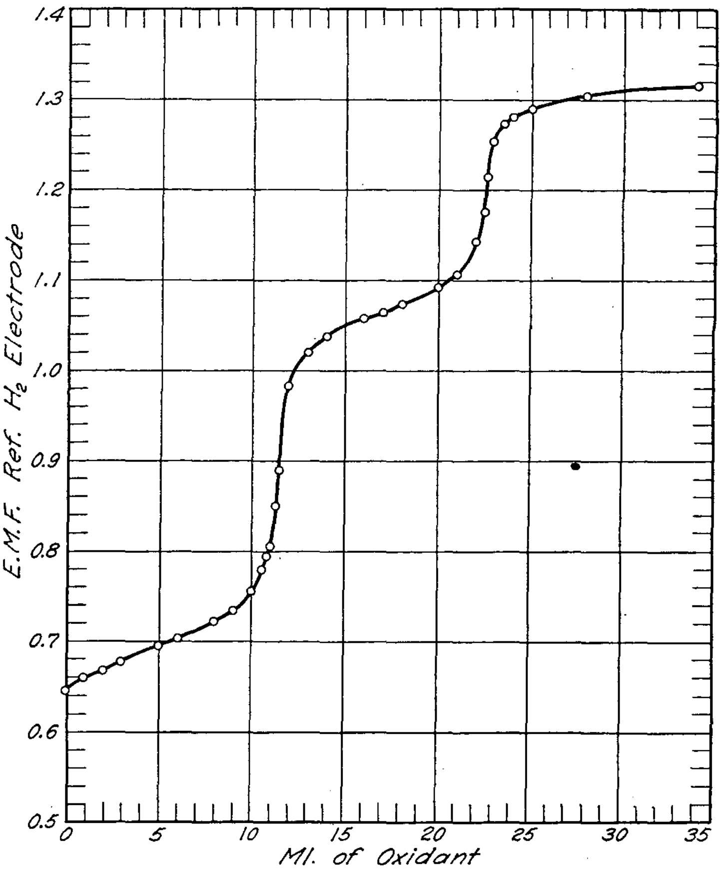


Figure 1. Potentiometric Titration of Ferrous and Ruthenium 2,2′-Bipyridine by Cerium(IV)

system was found to be somewhat low because of the presence of chloride ions from the ruthenium complex, which are known to lower this value from its true magnitude—namely, 1.42 volts.

This type of determination involves unit electron exchange for all three oxidation-reduction systems involved. The reaction is reversible, and under the conditions employed all products are suitably stable during the period required for the titration.

DETERMINATION OF SPECTROPHOTOMETRIC CONSTANTS

The spectrophotometric examination of aqueous solutions of the compounds investigated was carried out in 1-cm. cells on the General Electric recording spectrophotometer (*29*) over a range of 400 to 700 mμ. The ferroin solutions were prepared by weighing appropriate amounts of a standard iron solution from weight burets. This solution was adjusted to the transition point of Congo red paper, after reduction with sodium bisulfite, and a suitable amount of the phenanthroline added before the solution is made up to volume. Concentrations used varied from 1 to 8 p.p.m. of iron.

The spectrophotometric examination of tri-bipyridine ruthenious chloride hexahydrate, $(C_{12}H_8N_2)_3RuCl_2.6H_2O$, has not previously been described. Solutions of this compound of concentrations $2 \times 10^{-6} M$ to $7.5 \times 10^{-5} M$ were prepared by dissolving the required amount of reagent in 2 liters of conductivity water, and examined as described above. The solutions were re-examined after 12 months' storage, and their colors were found to be stable within 2% over this period of time. The spectrophotometric results are shown in Figure 2. The wave length of maximum absorption is seen to be 453 mμ, and the molecular extinction coefficient is calculated to be 14,500. Beer's law was found to hold over the concentration range.

ANION PRECIPITATION

For this application, the 1,10-phenanthrolines serve in two capacities. The ferrous complex ion of the selected phenanthroline acts as the precipitant, or the phenanthroline itself may act as precipitant. A procedure employing the first type of reaction has been described (*38*) for the determination of the anions perchlorate, periodate, persulfate, and molybdate. By reversing the process, iron may be determined by carrying out the ferroin reaction in the presence of excess perchlorate ion. These reactions may be employed in the presence of ferric iron, fluorides, sulfates, phosphates, iodates, and other common anions. The separation of molybdenum from tungsten was not successful if the unsubstituted 1,10-phenanthroline ferrous ion was used as precipitant.

The use of 3,5,6,8-tetramethyl-1,10-phenanthroline to precipitate ferrous iron as the perchlorate is quantitative, as shown by the fact that following the precipitation the filtrate is colorless. The same tetramethylphenanthroline may be employed as a monoacid base for the determination of perchlorate ion in the presence of chlorate, nitrate, bromate, and iodate (*13*). The major interferences are few, and include silver, mercury, tin, and cadmium. The precipitant was added in the form of 0.1% solution of the substituted phenanthroline in 0.1 *N* hydrochloric acid. Tests for qualitative sensitivity indicate that the limit of identification is approximately 0.01 mg. of perchlorate in 100 ml. of solution. The method is particularly well adapted to microdeterminations. A double precipitation is required with intermediate solution of the precipitated perchlorate in hot water if preponderant amounts of chlorate are present. Nitrate and sulfate show no interference when present.

Quantitative determinations of the perchlorate anion on a micro scale were carried out by precipitation of the perchlorate ion with a hydrochloric acid solution of the tetramethyl-1,10-phenanthroline. Microprecipitation and filtration beakers of 9-ml. capacity were employed. The precipitate was filtered and washed using a few 0.5-ml. portions of cold water containing a trace of precipitant, dried at 110° C., and weighed. The precipitate has the theoretical composition which calls for 8.32% nitrogen (found, 8.40 and 8.23% nitrogen; average, 8.31% microanalyses by Clark Microanalytical Laboratories, Urbana, Ill.). Results from a series of test reactions showed the method to be accurate to within 2% for amounts as low as 1 mg. or less.

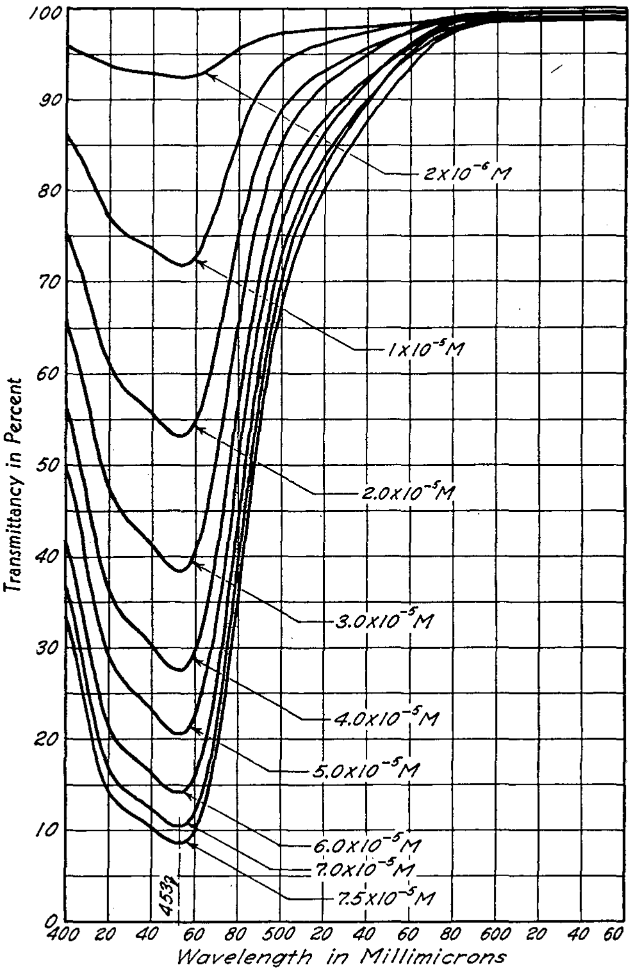


Figure 2. Spectrophotometric Analysis of Ruthenious-2,2′-Bipyridine Chloride Solutions

The high molecular weights involved in the case of precipitates formed by either of the methods available make the above described processes ideal for estimations by centrifugal separation and capillary tube calibration to determine the volume of the precipitate formed. Employing the many available alterations in property found in the substituted 1,10-phenanthrolines, a complete survey of these reagents following the schemes just described should lead to many new selective precipitation procedures of analytical significance.

MASKING REAGENTS

The separation of small amounts of aluminum from large amounts of iron—as for example in the analysis of iron ore—has been successfully accomplished as described by Smith and Cagle (*35*).

The method is based upon the preparation of the solution of the ore sample for the separation by exactly the same procedure as that employed in the phenylhydrazine precipitation of aluminum.

The reduced iron is then complexed by the addition of a 10% excess of 2,2′-bipyridine dissolved in alcohol. The solution is maintained at a pH of 3 to 4 and the ferroin reaction results in the formation of a bipyridine complex ferrous cation. The solution is now made basic with excess ammonium carbonate, and the precipitate formed is aluminum hydroxide together with any titanium and phosphate present, exactly as with the phenylhydrazine precipitation procedure. The great advantage from this procedure is that the precipitate need not be dissolved and reprecipitated as ordinarily required, and the precipitated aluminum hydroxide does not require the fine adjustment of the pH required by the phenylhydrazine process.

The spent bipyridine in the filtrate may be easily recovered. The filtrate from the aluminum precipitation is made strongly alkaline with sodium hydroxide and is steam-distilled to recover the bipyridine in 95% yield. The filtrates from a large number of determinations may be combined and steam-distilled at one time. In case 1,10-phenanthroline is used in place of the bipyridine, the same process applies but the recovered product must be extracted with benzene instead of being steam-distilled.

Attempts to use the colorless metal complexes of 1,10-phenanthroline such as those of zinc, nickel, cadmium, etc., as masking reagents have not as yet been developed.

DISCUSSION OF RESULTS

Effect of Methyl Group Substitutions on Physical Constants of Ferrous Complexes of 1,10-Phenanthrolines. The data obtained from the spectrophotometric examination of maximum absorption give the values for calculating the molecular extinction coefficient, and a test for the conformance of Beer's law. By storing the solution thus studied and repeating the spectrophotometric examination after several months, the permanency of the ferroins could be established. The resulting data confirmed the fact that Beer's law was valid in each case, and that the solutions were permanent for 3 to 4 months at least, and probably for longer periods of time. The molecular extinction coefficient is a very important physical constant. The higher this value becomes, the more sensitive the color reaction with ferrous ions. The values for the oxidation potential of the various methylferroins are also included with the various physical constants shown in Table IV.

From examination of the data of Table IV, it is found that the wave length of maximum absorption varies from 496 mμ in the case of the ferrous complex of 3,8-dimethyl-1,10-phenanthroline, to 520 mμ for the 5,6-dimethylferroin in steps of 1 to 3 mμ. From the data for ferroin and its 3-, 4-, and 5-methyl derivatives, the basis of the predicted or calculated values shown in column three is obtained. The 4,7 positions raise the wave length of maximum absorption 1 mμ, the 5,6 positions raise the value 5 mμ, and the 3,8 positions lower it 8 mμ. The values calculated in column three duplicate the experimentally determined values tabulated in column two exactly in ten cases, and the variation is in no case greater than 2 mμ, except for two cases of 4 mμ with two of the tetra-substituted products.

Table IV. Effect of Methyl Group Substitution on Physical Constants of Ferrous Complexes of 1,10-Phenanthroline Derivatives

Methyl Substituted Derivative	Wave Length Maximum Absorption, mμ		Molecular Extinction Coefficient	Redox Potentials, Volts		
				In 0.1 *F* Acid		In 1.0 *F* acid, found
	Found	Calcd.		Found	Calcd.	
1,10-Phenanthroline	510	...	11,100	1.10	...	1.06
3	502	...	11,500	1.07	...	1.03
4	511	...	13,500	...	...	...
5	515	...	12,200	1.06	...	...
3,4	505	504	13,700	0.97	0.96	0.93
3,5	506	507	12,500	...	1.03	...
3,6	506	507	12,200	...	1.03	...
3,7	502	504	12,600	...	0.96	...
3,8	496	496	11,500	1.03	1.04	...
4,5	516	516	12,700	0.95	0.95	...
4,6	516	516	12,700	0.95	0.95	...
4,7	512	512	14,000	0.88	0.88	0.87
5,6	520	520	12,600	1.00	1.02	0.97
3,4,6	510	508	12,400	0.92	0.92	...
3,4,7	504	504	14,000	0.88	0.85	...
3,4,8	497	495	13,000	...	0.93	...
3,5,6	512	512	11,800	...	0.99	...
3,5,7	507	509	12,500	0.93	0.92	0.89
3,5,8	500	499	11,900	0.99	0.99	...
3,6,7	510	508	13,600	...	0.92	...
4,5,7	517	517	14,500	...	0.84	...
3,4,6,7	510	510	14,000	0.84	0.81	...
3,4,6,8	504	501	11,600	0.89	0.89	...
3,4,7,8	500	496	13,800	0.85	0.82	...
3,4,6,8	504	504	11,600	0.93	0.96	...

No such conformity can be claimed for the influence of methyl substituted ferroins on the molecular extinction coefficient, except that in all cases the value is increased. The increase is least pronounced for substitutions in the 3,8 positions and is greatest for the 4,7 positions. The use of the 4,5,7-trimethylferroin gives the highest molecular extinction coefficient of all those thus far prepared. Its value is 14,500 compared to 11,100 for the unsubstituted ferroin. A greater value would be predicted for the 4,5,6,7-tetramethyl ferroin, which has not as yet been prepared.

As a color-producing reagent for use in the quantitative determination of iron, the first popularly applied ferroin reaction was that of bipyridine. Here the molecular extinction coefficient is 8650 and the wave length of maximum absorption is 522 mμ. It was proposed to use 2,2′,2″-terpyridine, therefore, as a preferred reagent, because in the form of its ferrous complex, its molecular extinction coefficient is 12,500 and the wave length of maximum absorption is 552 mμ. Because 2,2′,2″-terpyridine was not commercially available, 1,10-phenanthroline soon supplanted 2,2′-bipyridine in the spectrophotometric determination of iron with the favorable value of 11,100 for its molecular extinction coefficient and 510 mμ as its wave length of maximum absorption. The available sensitivity of the ferroin reaction is thus increased further by the introduction of methyl substitutions to a molecular extinction coefficient of 14,500 for the 4,5,7-trimethylferroin.

The redox potentials of the ferroins vary over a wide range (0.84 to 1.30 volts in 0.1 *F* acid solution) by proper selection of the organic base. The redox potential may be lowered by increasing the hydrogen ion concentration. The lowering of the redox potential by this means brings about a diminution of approximately 0.1 volt from 0.1 *M* to 8 *M* acid strength, and approximately 0.18 volt between 8 *M* and 16 *M*. The stability of the ferroins toward heat is limited to temperatures below 50° C.

The redox potentials of the ferroins follow a predictable pattern of unusual exactitude, as seen in Table IV, columns 4 and 5. Methyl groups in the 3 or 8 position lower the value 0.03 volt, the 5 and 6 positions lower it 0.04 volt, and the 4 and 7 positions lower it by 0.11 volt. The redox potential can be varied in steps of 0.01 to 0.03 volt over the range covered (0.84 to 1.10 volts). The 4,5,7-trimethylferroin has not only the highest molecular extinction coefficient, but also one of the lowest potentials, thus indicating the possibility of use as indicator for iron titrations with dichromate. The oxidation potential of the 4,5,6,7-tetramethylferroin would be predicted to have a potential of 0.81 volt in 0.1 *F* acid solutions. In a similar solution, the as yet untested 5,6-dibromoferroin would be predicted to have a potential of 1.22 volts.

The variation in properties of the methyl substituted ferroins indicates that substitutions for the 4,7 hydrogens effect the greatest change in the oxidation potential, whereas the 5,6 and 3,8 positions effect a much smaller change. The effect upon the molecular extinction coefficient is in the same order, but the trend is reversed when considering the effect upon the wave length of maximum absorption. Assuming that the same order of influence is exerted by substituents other than methyl, if a choice of new syntheses were to be made, the substitutions in the 4,7 positions would be preferred.

The variations in properties of the mono-, di-, tri-, and tetramethyl-1,10-phenanthrolines have thus been shown to be spe-

Table V. Values of Wave Length of Maximum Absorption and Molecular Extinction Coefficient of Substituted 1,10-Phenanthroline Ferrous Complex Ions

Methyl Substituted 1,10-Phenanthroline	Wave Length Maximum Absorption, mμ		Molecular Extinction Coefficient
	Calcd.	Found	
3	...	640	990
4	...	575	...
5	...	595	...
3, 4	625	...	...
3, 5	650	650	800
3, 6	645	655	930
3, 7	625	627	1070
3, 8	690	690	880
4, 5	580	575	950
4, 6	580	580	1100
4, 7	560	560	1500
5, 6	600	...	...
3, 4, 6	630	645	1040
3, 4, 7	610	625	1160
3, 4, 8	675	676	...
3, 5, 6	650	650	780
3, 5, 7	630	630	960
3, 5, 8	695	685	850
3, 6, 7	630	...	...
4, 5, 7	565	560	1080
3, 4, 6, 7	615	617	975
3, 4, 6, 8	680	674	...
3, 4, 7, 8	660	655	1150
3, 5, 6, 8	700	690	...

cific and additive. These compounds represent the first type of organic analytical reagents thus far known, whose analytical application and functional group activities may be predicted on the basis of alterations in structure not involving changes in the functional group itself.

Spectrophotometric Constants of Complex Ions in Oxidized or Ferriin Form. The study giving physical constants, such as those shown in Table IV as applied to the same colored ferrous complex ions after oxidation to their ferriin complex ions, has not previously been recorded. The figures given in Table V show these values.

From an examination of these values it is found that the wave length of maximum absorption of the ferriin complex ions varies from 560 mμ for the 4,7-dimethyl- and 4,5,7-trimethyl-1,10-phenanthroline ferric complex ions to 690 mμ for the 3,8-dimethyl- and 3,5,6,8-tetramethyl-1,10-phenathroline ferric complexes. Here again the values found and calculated are very close in agreement as noted for their comparable reduced ferroin form.

The calculation of the molecular extinction coefficient are shown in column 4 of Table V. The highest value found, 1500, is that for the 4,7-dimethyl-1,10-phenanthroline ferriin complex ion and the lowest, 780, with the 3,5,6-trimethyl complex. In general, the values for the molecular extinction coefficient of the ferriin complex ions are approximately 10% of the corresponding ferroin complex ions. This fact accounts for a general required addition of approximately 0.06 volt to the visual transition potential of these substituted methyl-1,10-phenanthroline complex ferroin indicators as compared to their potentiometrically observed values.

NEW DEVELOPMENTS IN FERROIN TYPE COMPOUNDS

The past five years have resulted in the following advances, which were formerly (*38*) outlined as desired research projects:

The use of the double Skraup synthesis starting with *o*-phenylenediamine in the synthesis of 1,10-phenanthroline has been described. This process was first commercially developed by Hieber and Muhlbauer (*22*).

The preparation of 8-nitroquinoline by direct nitration of quinoline has been shown to be readily accomplished, but it is no longer necessary to avoid a double Skraup reaction in the preparation of 1,10-phenanthroline.

The desired development of substituted phenanthrolines to give lower redox potentials in the range 0.8 to 1.0 volt has been accomplished.

The recovery of spent reagent following the use of phenanthroline and bipyridine as masking reagents has been found practicable.

The commercial preparation of 2,2′-bipyridine by the method of Hein and Schwedler (*21*) has been put into practice.

The molecular extinction coefficient value of substituted ferroins has been increased from 11,100 for the unsubstituted ferroin to 14,500 in 4,5,7-trimethyl-1,10-phenanthroline. This development makes available a much more sensitive reagent for use in the colorimetric determination of iron. A description of the many practical routine determinations of iron in food products, fruit juices, beer, wine, and biological materials has been included in the publication of Smith and Richter (*38*).

The commercial preparation of 2,2′,2″-terpyridine has been provided.

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Water-Soluble 1,2-Dioximes as Analytical Reagents

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The water-soluble 1,2-dioximes possess an advantage over reagents for nickel and palladium, such as dimethylglyoxime, which must be made up in organic solvents, where the danger of contamination of the nickel precipitate with excess reagent is always imminent. Discussed in this paper are the 1,2-dioximes reported as being water-soluble—diaminoglyoxime, α-furildioxime, 1,2-cyclopentanedionedioxime, 1,2-cyclohexanedionedioxime, and 1,2-cycloheptanedionedioxime. Only the latter two compounds are sufficiently soluble in cool water to allow them to be made up and used in aqueous solution at room temperature. 1,2-Cycloheptanedionedioxime was found to be superior in many ways to the previously reported water-soluble 1,2-dioximes. This reagent reacts with nickel ions to form a yellow precipitate that can be used for the gravimetric determination of this metal. Quantitative precipitation is obtained at pH 2.7 and greater. Nickel may be determined in solutions containing acetate, tartrate, chloride, citrate, perchlorate, sulfate, sulfosalicylate, nitrate, and thiocyanate anions. Nickel is satisfactorily determined in the presence of aluminum, chromium, manganese, vanadium, lead, magnesium, zinc, cadmium, arsenic, antimony, beryllium, iron, titanium, molybdenum, cobalt, bismuth, and copper, and in several alloys. A rapid, direct method for determining nickel in steels containing both cobalt and copper is described.

As OPPOSED to such reagents as dimethylglyoxime, the use of water-soluble 1,2-dioximes minimizes the danger of contaminating the nickel precipitate with excess reagent and avoids the solvent action of alcohol or acetone on this precipitate. It is more convenient and economical to use a stable aqueous solution of a water-soluble reagent.

Several attempts have been made to solubilize dimethylglyoxime and thus eliminate some of its disadvantages.

Kasey (*8*) recommended the use of a 2% solution, prepared by slowly adding a solution containing sodium hydroxide to hot water containing solid dimethylglyoxime. Semon and Damerell (*17*) describe the preparation of sodium dimethylglyoximate octahydrate by dissolving solid dimethylglyoxime in aqueous sodium hydroxide solution and then adding ethanol to precipitate the octahydrate. This preparation, of course, does not eliminate the use of an organic solvent. Hillebrand and Lundell (*6*) and Lundell, Hoffman, and Bright (*11*) suggest the use of ammonium hydroxide for solution of dimethylglyoxime when ethanol is not readily available. Such solutions are not stable for long periods of time (*1*). Raithel (*13*) recommended preparing the dimethylglyoxime reagent by thoroughly mixing equal weights of sodium peroxide and solid dimethylglyoxime and dissolving this mixture in distilled water followed by dilution. Diehl, Henn, and Goodwine (*3*) pointed out the explosive hazard of preparing the reagent solution as Raithel recommended and showed that decomposition of such a solution was even more rapid than that of a solution of dimethylglyoxime in 1% sodium hydroxide.

The need for water-soluble 1,2-dioximes is apparent, for none of the proposed schemes to solubilize dimethylglyoxime has eliminated the use of an organic solvent and at the same time resulted in a stable aqueous solution of dimethylglyoxime.

DIAMINOGLYOXIME

Diaminoglyoxime, called niccolox, has been proposed by Kuras (*9, 10*) as a reagent for the micro- and macrogravimetric determination of nickel. This reagent is easily prepared by reaction of cyanogen with hydroxylamine according to the procedure of Fischer (*5*). Diaminoglyoxime, although soluble in hot water, is difficultly soluble in cold water and alcohol. The orange-yellow nickel compound, which contains 20.05% nickel, is soluble in acid but insoluble in slightly ammoniacal solutions. It does not tend to creep and is stable toward drying. Nickel can be determined in the presence of sulfate, chloride, nitrate, manganese, chromium, aluminum, zinc, and the alkali metals. However, the use of this reagent is definitely limited by the fact that iron and cobalt seriously interfere.

ALPHA-FURILDIOXIME

Alpha-furildioxime was first reported by Tschugaeff (*19*) in 1905 as one of twelve 1,2-dioximes found to form colored complexes with nickel. It was not until 1925, however, that Soule (*18*) described it as a water-soluble compound, suitable for analytical work, and recommended it as a specific reagent for the detection and determination of nickel.

A satisfactory method for preparing α-furildioxime from furfural has been worked out by Reed, Banks, and Diehl (*16*). The solubility of this reagent in water at room temperature was found to be 0.79 gram (0.0033 mole) per liter (*15*), which is very nearly the same as the solubility of dimethylglyoxime—i.e., 0.40 gram (0.0034 mole) per liter. However, a 2% solution can be prepared (*18*) in hot water and used while hot. Other workers (*15*) have used a 2% solution of the reagent in 30% ethanol, which is stable at room temperature for long periods of time.

Reed and Banks (*15*) have shown that α-furildioxime can be used to separate nickel but they do not recommend its use for determining this metal because the nickel-reagent ratio in the nickel precipitate has been found to vary from 1:2 to 1:1 with increasing pH.

The yellow palladium compound, which contains 19.57% palladium, is quantitatively precipitated over the pH range 0.2 to 6 and it filters satisfactorily. It is stable toward drying and as much as 150% excess reagent has no noticeable effect on the results. Palladium can be satisfactorily determined in the presence of various anions and cations and can be effectively separated from platinum by a single precipitation.

Alpha-furildioxime, although a satisfactory reagent for the separation and determination of palladium, leaves much to be desired as to solubility in water and as an analytical reagent for nickel.

1,2-CYCLOPENTANEDIONEDIOXIME

1,2-Cyclopentanedionedioxime, although soluble in water to the extent of 1.3 grams (0.01 mole) per liter, has very little promise as an analytical reagent because of the very narrow pH range over which the nickel compound is insoluble.

1,2-CYCLOHEXANEDIONEDIOXIME

In 1924 Wallach (*23*) reported that 1,2-cyclohexanedionedioxime, now called nioxime, yielded a scarlet precipitate with nickel and was a very sensitive qualitative test for nickel. Seven years later Feigl (*4*) pointed out that this compound should be an ideal reagent for nickel, inasmuch as its solubility in water would constitute a significant advantage over dimethylglyoxime. It was not until 1945, however, that Rauh, Smith, Banks, and Diehl (*14*) succeeded in obtaining a sufficient amount of 1,2-cyclohexanedionedioxime to make a detailed study of its properties and uses as an analytical reagent. The solubility of nioxime in water at 21.5° C. is 8.2 grams (0.058 mole) per liter or 17 times greater than the corresponding molar solubility of dimethylglyoxime.

Wenger, Monnier, and Rusconi (*24*) have used nioxime to determine nickel by a semiquantitative method based on the limit of detection of the reaction.

Voter, Banks, and Diehl (*21*) have shown that nioxime can satisfactorily be used for the gravimetric determination of nickel in the presence of many of the common anions and cations, provided an empirical factor is used to correct for the amount of excess reagent that is carried down. Even though nioxime offers several advantages over dimethylglyoxime, such as water-solubility, increased sensitivity, quantitative precipitation down to pH 3, and a lower factor for nickel, its use as an analytical reagent for nickel is nevertheless limited because no single-precipitation method has been reported which makes it possible to determine nickel in the presence of iron. Nickel nioxime precipitates are difficult to filter and the steps necessary to alleviate this trouble are time-consuming.

Nioxime quantitatively precipitates palladium from solutions, the pH of which are as low as 1, and has been shown to be satisfactory as an analytical reagent for this metal (*22*). In addition to the advantages of water-solubility and increased sensitivity, the use of nioxime also makes possible a considerable saving of time, because palladium nioxime can be filtered from a hot solution after a brief digestion period.

Johnson and Simmons (*7*) in 1946 showed that the color of nickel nioxime, stabilized with gum arabic solution, can be used as the basis for a spectrophotometric method of determining nickel in steels.

1,2-CYCLOHEPTANEDIONEDIOXIME

Because nioxime was found to possess some distinct advantages over dimethylglyoxime as an analytical reagent for nickel and palladium, it was thought that an investigation of the properties of some of the higher homologs of 1,2-cyclohexanedionedioxime might be worth while.

In this connection, Vander Haar, Voter, and Banks (*20*) devised a method of preparing 1,2-cycloheptanedionedioxime (heptoxime) from cycloheptanone. It was soon noted that this compound possessed characteristics which would make it a valuable reagent for nickel, in that the previously stated disadvantages exhibited by nioxime were not experienced with heptoxime. Heptoxime, which forms a yellow precipitate with nickel ions, possesses almost all the good characteristics of both dimethylglyoxime and nioxime without their disadvantages.

Presumably heptoxime reacts with nickel ions, as do other 1,2-dioximes, to give molecules of the resonance-stabilized square coplanar configuration.

Physical Properties. Heptoxime, a white, crystalline solid with a molecular weight of 156.18, melts at 179–180° C. when recrystallized from water.

By precipitating a measured volume of a saturated heptoxime solution with an excess of nickel, the solubility of heptoxime in water was found to be 4.8 grams (0.031 mole) per liter at 19.5° C. Although this is somewhat less than the solubility of nioxime, it is still more than 9 times greater than the corresponding molar solubility of dimethylglyoxime.

Reagents. A saturated aqueous solution of heptoxime was used.

A standard nickel chloride solution was prepared from Mond nickel obtained from the International Nickel Company (*21*). This solution was analyzed electrolytically and found to contain 0.001991 gram of nickel per gram of solution. All samples from this solution were measured by use of weight burets.

The following solutions were made up by dissolving the reagent-grade chemicals in water: Citric acid solution, 1 gram per 3 ml. of solution. Sodium sulfite solution, 1 gram per 10 ml. of solution. Ammonium thiocyanate solution, 1 gram per 2 ml. of solution. Ammonium acetate solution, 1 gram per 5 ml. of solution.

Gravimetric Determination of Nickel. Procedures for determining nickel with dimethylglyoxime call for precipitation from neutral or mildly ammoniacal solutions (*2*). However, nickel heptoxime is best precipitated from a slightly acid medium. This constitutes a distinct advantage, for nickel may be separated from certain cations without the use of complexing agents which would be needed under the pH conditions required for the quantitative precipitation of nickel with dimethylglyoxime. Precipitation of nickel ions with heptoxime was found to be quantitative at pH values of 2.7 or greater.

The nickel heptoxime precipitate is of such a character that quantitative operations are easily performed. It does not tend to creep as does nickel dimethylglyoxime. Nickel heptoxime was dried for as long as 5 hours at 120° C. without apparent decomposition or loss of weight.

A series of determinations in which the amounts of nickel taken were varied indicated that samples of nickel from 6 to 61 mg. could be determined successfully (Table I). The solution volume was 200 ml. for each sample except determination 5, where a larger volume was required because of the voluminous character of the precipitate.

The results of several determinations in which the per cent excess of heptoxime was varied are shown in Table II. It is apparent that excess reagent does not noticeably affect the results. Evidence obtained indicates that the presence of ammonium acetate helps prevent adsorption of excess reagent by the nickel heptoxime.

The effect of various anions on the determination of nickel was studied. These anions, added as their ammonium salts or as the respective acids, were found not to interfere in the determination of nickel. Typical results are shown in Table III.

Table I. Determination of Nickel

Detn.	Nickel Taken	Weight of Precipitate	Nickel Found	Error
	Gram	*Gram*	*Gram*	*Mg.*
1	0.0063	0.0400	0.0064	+0.1
2	0.0139	0.0878	0.0140	+0.1
3	0.0225	0.1421	0.0226	+0.1
4	0.0345	0.2168	0.0345	0.0
5[a]	0.0614	0.3866	0.0615	+0.1

[a] Solution containing sample diluted to 600 ml.

Table II. Effect of Excess Heptoxime on Determination of Nickel

Detn.	Excess Heptoxime Added[a]	Nickel Taken	Weight of Precipitate	Nickel Found	Error
	%	*Gram*	*Gram*	*Gram*	*Mg.*
1	30	0.0235	0.1486	0.0236	+0.1
2	50	0.0241	0.1521	0.0242	+0.1
3	80	0.0242	0.1525	0.0243	+0.1
4	100	0.0261	0.1648	0.0262	+0.1
5	150	0.0232	0.1468	0.0233	+0.1

[a] 2 grams of ammonium acetate also present.

Table III. Effect of Various Anions upon Determination of Nickel

Anion Present	Anion	Nickel Taken	Weight of Precipitate	Nickel Found	Error
	Grams	*Gram*	*Gram*	*Gram*	*Mg.*
Acetate	1.6	0.0224	0.1408	0.0224	0.0
Tartrate	9.0	0.0222	0.1397	0.0222	0.0
Chloride	1.4	0.0236	0.1495	0.0238	+0.2
Citrate	2.0	0.0235	0.1483	0.0236	+0.1
Perchlorate	1.8	0.0243	0.1525	0.0243	0.0
Sulfate	1.5	0.0234	0.1484	0.0236	+0.2
Sulfosalicylate	2.0	0.0259	0.1625	0.0258	−0.1
Nitrate	1.7	0.0224	0.1406	0.0224	0.0
Thiocyanate	1.5	0.0218	0.1375	0.0219	+0.1

Nickel was determined in the presence of aluminum, chromic, manganous, vanadate, plumbous, magnesium, zinc, cadmium, antimonite, arsenite, beryllium, ferric, titanous, cuprous, cobaltous, molybdate, and bismuth ions. Aluminum, chromic, antimonite, arsenite, ferric, titanic, and bismuth ions, when present, must be complexed with either tartrate or citrate to prevent their coprecipitation as hydroxides. Cobaltous ions react with heptoxime to form a brown complex compound which remains in solutions if the cobalt concentration is not too high. However, if an appreciable amount of cobaltous ion is present, a reprecipitation may be necessary. Enough heptoxime must be added not only to precipitate the nickel but also to complex the cobaltous ions. Copper ions react with heptoxime, yielding an insoluble brown precipitate. However, this interference is eliminated by taking advantage of the fact that cuprous thiocyanate is soluble in excess thiocyanate (*25*). The soluble complex formed effectively masks the cuprous ions and prevents their reaction with heptoxime. Acetate is added to prevent the precipitation of plumbous chloride when lead is present. These data are shown in Table IV.

Table IV. Effect of Various Metal Ions on Determination of Nickel

Ion Present	Ion	Nickel Taken	Weight of Precipitate	Nickel Found	Error
	Gram	*Gram*	*Gram*	*Gram*	*Mg.*
Aluminum[a]	0.2	0.0241	0.1517	0.0241	0.0
Chromic[a]	0.2	0.0251	0.1580	0.0251	0.0
Manganous	0.3	0.0242	0.1542	0.0244	+0.2
Vanadate	0.3	0.0240	0.1516	0.0241	+0.1
Plumbous[b]	0.05	0.0233	0.1474	0.0234	+0.1
Magnesium Zinc Cadmium	0.2 each	0.0224	0.1416	0.0225	+0.1
Antimonite[a] Arsenite[a]	0.3 each	0.0224	0.1408	0.0224	0.0
Beryllium	0.2	0.0219	0.1395	0.0222	+0.3
Ferric[a]	1.0	0.0231	0.1461	0.0232	+0.1
Titanic[c]	0.1	0.0215	0.1365	0.0217	+0.2
Cuprous[d]	0.01	0.0202	0.1273	0.0202	0.0
Cobaltous	0.007	0.0213	0.1356	0.0216	+0.3
Molybdate	0.2	0.0244	0.1546	0.0246	+0.2
Bismuth[a]	0.1	0.0230	0.1459	0.0232	+0.2

[a] Complexed with tartrate.
[b] Acetate present.
[c] Complexed with citrate.
[d] Complexed with thiocyanate.

Recommended Procedure. The following procedure is for the determination of nickel in iron and steel containing both cobalt and copper.

Weigh a sample that contains approximately 20 mg. of nickel into a 500-ml. Erlenmeyer flask, and dissolve the sample in an appropriate acid or acid mixture. Decompose any carbides with 10 ml. of nitric acid. Add 10 ml. of 60% perchloric acid per gram of sample taken. After fuming begins, boil the solution for 15 minutes. Add 4 volumes of water and, after dissolving any salts, filter off the silica. Wash with 1% hydrochloric acid and then with water. To the filtrate add 18 ml. of the citric acid solution for each gram of sample taken plus 3 ml. in excess. Filter all solutions being added to the sample solution. If lead is known to be present add 5 ml. of ammonium acetate solution. Add 10 ml. of sodium sulfite solution. After diluting the solution to 200 ml. adjust the pH to about 3.5 with ammonium hydroxide. Warm the solution to about 50° C. and add 20 ml. of ammonium thiocyanate solution. Stir until any precipitate of cuprous thiocyanate dissolves. For samples high in copper content, an additional 10 ml. of ammonium thiocyanate solution may be needed to dissolve this precipitate. As soon as the solution is clear, slowly add with constant stirring 15 ml. of saturated heptoxime solution for each 10 mg. of nickel present plus 5 ml. in excess. Digest at about 80° C. for 10 minutes. Allow the beaker to stand in cool tap water for 30 minutes. Filter the solution through a weighed filter crucible of medium porosity, keeping the crucible filled with liquid. Wash with cold water and dry at 110° to 120° C. for at least 1 hour. The factor for nickel is 0.1590.

It is important that the pH be adjusted to about 3.5 before addition of the ammonium thiocyanate in order to prevent the formation of insoluble perthiocyanic acid (*12*).

This procedure can readily be adapted for determining nickel in the presence of any of the metallic ions shown in Table IV. The metallic ions present determine the complexing agents that must be used. When tartrate or citrate is not used, 5 ml. of the ammonium acetate solution should be added to buffer the solution. In most cases, a solution volume of about 200 ml. and a pH of about 4.0 are preferable.

Table V. Determination of Nickel in Steel and an Aluminum Alloy

Material	Nickel Present	Nickel Found	Av. Found
	%	%	%
N.B.S. cast iron No. 115	15.89	15.82	
		15.93	
		15.94	
		15.84	15.88
N.B.S. 18 chromium-9 nickel steel No. 101c	9.27	9.29	
		9.26	9.28
		9.23[a]	
		9.28[a]	9.26
N.B.S. nickel steel No. 33c	3.28	3.25	
		3.26	
		3.27	3.26
N.B.S. nickel-molybdenum steel No. 111a	1.75	1.75	
		1.75	
		1.74[b]	
		1.75[b]	1.75
N.B.S. chromium-nickel-molybdenum steel No. 139	0.563	0.558	
		0.556	0.557
N.B.S. aluminum-base alloy No. 85a	0.41	0.42	
		0.42	0.42

[a] Analysis run by analyst unfamiliar with this procedure.
[b] To these samples were added 5 mg. of Cu^{++} and 5 mg. of Co^{++}. Samples contained 22.5 and 24.5 mg. of nickel, respectively.

The recommended procedure for determining nickel in steels containing both cobalt and copper was tested on five National Bureau of Standards steels representing a wide range of nickel content. An aluminum-base alloy was also analyzed for nickel. The results shown in Table V attest the accuracy of this simple and direct procedure.

Adaptation of this procedure, so that dimethylglyoxime could be used as the precipitating agent, was unsuccessful, as nickel dimethylglyoxime is somewhat soluble in the presence of thiocyanate ions.

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Precipitation of Thorium from Homogeneous Solution

Separation from Rare Earths of Monazite Sand with Tetrachlorophthalic Acid

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The reaction between thorium salts and certain dicarboxylic acids such as succinic, phthalic, and tetrachlorophthalic, proceeds extremely slowly in aqueous solution at room temperature. A visible precipitate does not form for many hours, whereas a gelatinous precipitate is obtained if the solutions are at or near boiling. When a clear solution of thorium and a dicarboxylic acid are warmed to 70° to 85° C., with continuous stirring, a thorium salt slowly precipitates in a dense, crystalline, and readily filterable form. Tetrachlorophthalic acid is the only one studied which quantitatively precipitates thorium. Upon double precipitation at pH 1.0 to 1.2, this acid effects quantitative separation of thorium from the large amounts of rare earths normally found in monazite sand. This precipitation is accomplished by a relatively simple procedure. The precipitate is ignited to the oxide.

PRECIPITATES obtained by the method of precipitation from homogeneous solution possess more desirable characteristics for analytical separations than do those obtained by the conventional procedure. The method has been successfully employed for the determination of aluminum (*8*), gallium (*4*), thorium (*6*), zirconium (*7*), and magnesium (*1*), and for the separation of zirconium and hafnium (*5*).

For effecting the separation of thorium from the rare earths, two dicarboxylic acids have been reported in the literature as successful. The fumaric acid method (*2*) separates thorium from the rare earths in 40% ethanol as a white flocculent precipitate. Sebacic acid (*3*) is described as effecting this separation by precipitating thorium in a granular and voluminous form, but it is the experience of the authors that the product is too gelatinous. In neither method was less than 50 mg. of thorium oxide quantitatively precipitated.

The precipitation of thorium from homogeneous solution employing a dicarboxylic acid appeared to offer several advantages—formation of a dense granular precipitate, separation from the rare earths, separation of small quantities of thorium, and weighing of the precipitate as the thorium salt without recourse to ignition to thorium oxide which practically every gravimetric method requires. Only the first three of these objectives were attained.

Tetrachlorophthalic acid and the other dicarboxylic acids that were studied seem to precipitate thorium in the method which is described here by a mechanism quite different from the case where either urea (*4, 6, 8*) or an ester of oxalic acid (*1, 6*) is used. The hydrolysis of urea produces ammonia, which in turn results in an increase in hydroxyl ion necessary for precipitation. The hydrolysis of an ester of oxalic acid directly results in the production of the necessary precipitant.

In the method described in this paper, a solution of tetrachlorophthalic acid is added directly to the solution of thorium and rare earths. At room temperature, a visible precipitate does not form for several hours. When the solution is gently warmed to 70° to 85° C., with mechanical stirring, the thorium salt slowly precipitates in a dense, crystalline, and readily filterable form. The exact mechanism of this precipitation from homogeneous solution has not yet been determined. It is probably due to the increased dissociation, with rise in temperature, of a complex of thorium with hydroxyl ion with concurrent precipitation of a thorium salt. Various treatments that are commonly used to induce precipitation from supersaturated states were of no influence in effecting precipitation of the thorium salt from cool solutions.

PRELIMINARY INVESTIGATIONS WITH DICARBOXYLIC ACIDS

Materials Used. Pure thorium and rare earth perchlorates were prepared as for the urea method (*6*). All dicarboxylic acids were C.P. products.

Succinic Acid. The addition of a hot succinic acid solution to a solution containing thorium resulted in a gelatinous precipitate which was converted on long digestion to a crystalline precipitate. The addition of a cool succinic acid solution to a cool solution containing thorium resulted in the slow production of a dense crystalline precipitate upon slow heating and rapid stirring.

Approximately 80% of the thorium present can be precipitated from a solution of pH 3.3 containing 10 grams of succinic acid and 0.1 gram of thorium oxide by this latter method.

Other Dicarboxylic Acids. Of the various acids used, the following behaved in a manner similar to succinic: dibromosuccinic, phthalic, and 3-nitrophthalic acids. Precipitation was not complete with any of these.

Adipic, 1,2-naphthalic, and 1,8-naphthalic acids produced gelatinous precipitates.

Hexahydrophthalic acid did not precipitate thorium.

PRECIPITATION OF THORIUM WITH TETRACHLOROPHTHALIC ACID

Preliminary Investigation. Of the various dicarboxylic acids tried, only tetrachlorophthalic acid quantitatively precipitated thorium in a dense crystalline form. In Figure 1 can be seen a comparison of the precipitates obtained by the reaction of thorium and tetrachlorophthalic acid under two sets of conditions.

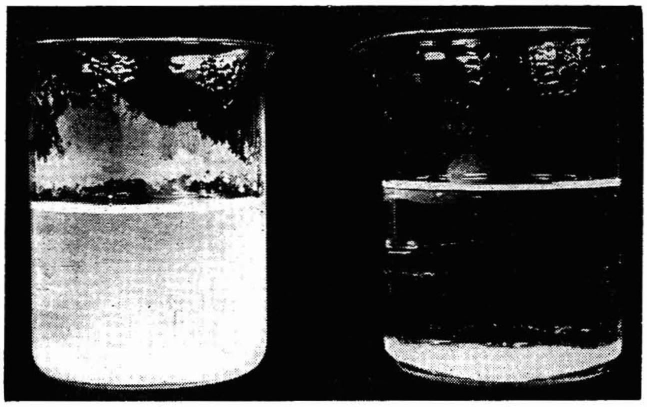

Figure 1. Comparison of Precipitates Obtained by Addition of Tetrachlorophthalic Acid to Thorium under Varying Conditions

A. By addition of hot solution of acid to hot thorium solution. Precipitate settles and becomes less gelatinous upon digestion
B. By precipitation from homogeneous solution

Composition of Precipitate. Ignition tests on the precipitate obtained from homogeneous solution at pH 1.0 indicate that for each thorium ion there is the equivalent of two tetrachlorophthalate ions and approximately three water molecules. Tetrachlorophthalic acid itself crystallizes from aqueous solution as the hemihydrate. On the assumption that thorium replaces only one hydrogen ion of tetrachlorophthalic acid at pH 1.0 and that the hemihydrate still exists, this would leave two hydroxyl ions. Thus, the precipitate is probably a basic salt. At any rate, the slight variation in composition of the precipitate does not permit direct weighing of the thorium salt but requires ignition to thorium oxide.

Effect of pH. The separation of thorium from the rare earths is accomplished through control of pH. At pH 2.0, trivalent cerium is also precipitated by tetrachlorophthalic acid. Below this pH value only thorium is precipitated from a mixture containing lanthanum, trivalent cerium, praseodymium, neodymium, and yttrium. The effect of pH on the solubility of the thorium salt is shown in Table I.

Table I. Effect of pH

pH	ThO_2 Taken[a] *Gram*	ThO_2 Found *Gram*	Difference *Mg.*
0.50	0.1072	0.0816	−25.6
0.75	0.1072	0.1065	−0.7
0.90	0.1072	0.1068	−0.4
1.00[b]	0.1072	0.1072	0.0
1.20	0.1072	0.1072	0.0

[a] Amount of acid used represents 2.5 times theoretical required for 0.1 gram of ThO_2.
[b] Quantitative precipitation at this pH confirmed by several experiments.

Table II. Single Precipitation of Thorium

(Rare earths taken = 0.388 gram La_2O_3, 0.407 gram CeO_2, 0.088 gram Pr_6O_{11}, 0.238 gram Nd_2O_3, 0.017 gram Y_2O_3)

No.	ThO_2 Taken *Gram*	ThO_2 Found *Gram*	Difference *Mg.*
1[a]	0.1072	0.1181	+10.9
2[a]	0.0536	0.0595	+5.9
3[b]	0.0011	0.0014	+0.3

[a] pH 1.2.
[b] pH 1.0.

Table III. Double Precipitation of Thorium[a]

(Rare earths taken = 0.388 gram La_2O_3, 0.407 gram CeO_2, 0.088 gram Pr_6O_{11}, 0.238 gram Nd_2O_3, 0.017 gram Y_2O_3)

No.	ThO_2 Taken *Gram*	ThO_2 Found *Gram*	Difference *Mg.*
1	0.1072	0.1071	−0.1
2	0.1072	0.1072	0.0
3	0.1072	0.1075	+0.3
4	0.0536	0.0536	0.0
5	0.0536	0.0538	+0.2
6	0.0107	0.0107	0.0
7	0.0107	0.0107	0.0

[a] All precipitations carried out at pH 1.0 to 1.1.

Effect of Ignition. Subjecting the precipitate to a high temperature results in a volatilization loss of some thorium, which can be prevented by a low temperature destruction of the organic anion. This is accomplished by ignition of the previously dried precipitate at 375° C. for approximately 1 hour, with subsequent ignition at 850° C. to thorium oxide.

SEPARATION OF THORIUM FROM RARE EARTHS

Single Precipitation. The results obtained with a single precipitation are shown in Table II. The volume of precipitate obtained with even as little as 1.1 mg. of thorium oxide is sufficient to be conveniently handled. The results in Table II indicate the necessity for reprecipitation. If the precipitation is carried out at pH 1.0, the amount of impurity will be reduced considerably—for example, in the case of a separation with amounts comparable to sample 1 of Table II, only about 3 or 4 mg. of rare earth oxides will be found with the thorium.

In this set of experiments as well as with the double precipitation, the amount of tetrachlorophthalic acid used was two and one half times the theoretical required for 0.1 gram of thorium oxide.

Double Precipitation. In Table III are given the results of the separation of thorium from the rare earths by double precipitation.

DETERMINATION OF THORIUM IN MONAZITE SAND

Modification of Urea Method. For the decomposition of monazite sand with perchloric acid, the urea method (*6*) prescribes the use of a 50-gram sample and the subsequent use of an aliquot portion. This portion of the procedure is now modified to employ individual 1-gram samples when finely ground monazite sand is available.

Another change prescribes the adjustment of pH of the solution containing thorium and rare earth oxalates obtained from the initial methyl oxalate precipitation. In the original procedure, a few milligrams of the rare earths remain in solution and sometimes postprecipitate. Although no thorium could be found with this small quantity of precipitate, it appeared advisable to adjust the pH to ensure complete precipitation of the rare earths also. This occurs at pH 0.8. This latter value is also obtained without any pH adjustment at the conclusion of the second methyl oxalate precipitation.

Decomposition of Monazite Sand with Perchloric Acid. Weigh 1 gram of finely ground monazite sand into a 250-ml.

Erlenmeyer flask. Add 5 ml. of concentrated nitric acid and 30 ml. of 70% perchloric acid, bring to gentle boiling, and continue for 1 to 1.5 hours after initial evolution of dense white fumes of perchloric acid. Do not cover the flask during this decomposition.

Cool, and add 15 ml. of water, then 5 ml. of 3% hydrogen peroxide. Warm gently for several minutes to clarify the solution. If the bulk of the gelatinous mass of phosphates obtained upon addition of water does not redissolve, add 5 to 10 ml. of concentrated hydrochloric acid. These phosphates may not readily dissolve if an excessive amount of perchloric acid is evaporated during the decomposition period instead of being returned by reflux action. However, a solid residue of zircon, quartz, rutile, ilmenite, and dehydrated silica may be present. Cool the solution, then filter to remove this residue, and wash thoroughly with cool 1 to 3 nitric acid.

Precipitation of Thorium and Rare Earth Oxalates with Methyl Oxalate. Evaporate the filtrate obtained from the perchloric acid decomposition to dense white fumes in a 600-ml. beaker. Cool, and add 50 ml. of water, followed by 5 ml. of 3% hydrogen peroxide. Warm gently for several minutes until the solution becomes clear, then add 100 ml. of water. Cool this solution, then add concentrated ammonium hydroxide dropwise to the appearance of a small amount of gelatinous precipitate which does not redissolve. Quickly add 10 ml. of concentrated hydrochloric acid and allow the solution to stand for 5 minutes.

Add 6 grams of methyl oxalate. Employ mechanical stirring and warm gently, 70° to 85° C., and continue for 30 minutes after the initial precipitate appears. Then add a hot solution of 8 grams of oxalic acid in 200 ml. of water. Stir and keep warm for an additional 30 minutes.

Cool this solution to room temperature, then adjust the pH to 0.8 to 0.9 by the dropwise addition of dilute ammonium hydroxide. Filter through an ashless filter paper and wash ten times with a cool 2% oxalic acid solution adjusted to pH 1.0 with hydrochloric acid. Do not attempt to transfer the bulk of the precipitate but wash by decantation as much as possible.

Transfer the filter and precipitate back to the 600-ml. beaker containing the oxalates, then proceed as directed in the urea method (*6*) for the reprecipitation of thorium and rare earths with methyl oxalate.

Separation of Thorium from Rare Earths with Tetrachlorophthalic Acid. Cool the solution of thorium and rare earth perchlorates obtained from the second methyl oxalate precipitation. Add 200 ml. of water and 1 gram of sodium iodide, then adjust the pH to 1.5 to 1.6. Add 200 ml. of a solution of tetrachlorophthalic acid containing 3 grams per liter. This amount of acid represents 2.5 times the theoretical required to precipitate 0.1 gram of thorium oxide. Adjust the pH to 1.0 to 1.1. Place the beaker on a temperature-controlled hot plate designed to reach 70° to 80° C. Employ rapid mechanical stirring. The precipitate will begin to form in approximately 20 minutes for amounts of thorium oxide in the range of 50 to 100 mg. Complete precipitation will be effected in 1.5 hours after initial formation. Where the amount of thorium is considerably less than 50 mg., the initial precipitate may not appear for 1 to 2 hours or longer.

Filter the hot solution, then wash with a cool 0.1% tetrachlorophthalic acid solution adjusted to pH 1.5 with hydrochloric acid. Do not attempt to remove every trace of precipitate from the beaker walls. Next convert the thorium salt to insoluble hydrous thorium oxide by treating the precipitate with hot 2% sodium hydroxide. Wash the stirring rod, beaker walls, and filter paper at least ten times with this solution to remove the sodium tetrachlorophthalate.

Redissolve the hydrous thorium oxide with hot 2 *N* hydrochloric acid, catching the filtrate in the original precipitation beaker. Wash the stirring rod and beaker walls with the hot acid.

Reprecipitate the thorium with tetrachlorophthalic acid as in the previous case. Transfer the precipitate quantitatively to a previously ignited and weighed No. 3010 Selas crucible. Wash thoroughly with a cool 0.1% tetrachlorophthalic acid solution adjusted to pH 1.5.

Dry the crucible at 110° C., then place in a cold muffle furnace and slowly raise the temperature to 350° C. and hold for 45 minutes. Next slowly raise the temperature to 850° C. and hold for 1 hour. Weigh the precipitate as ThO_2.

Table IV. Analysis of Monazite Sand

Sample	Urea		Tetrachlorophthalic Acid	
	Sample *Grams*	ThO_2 %	Sample *Grams*	ThO_2 %
1	1.2325	7.84	1.7350	7.76
2	1.1413	7.79	1.1215	7.76
Av.		7.81		7.76

ANALYSIS OF A MONAZITE SAND

The sand taken for analysis was of Indian origin. It was ground to a 100-mesh size, then analyzed by both the urea and the tetrachlorophthalic acid methods. Data obtained are given in Table IV.

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Extraction of Cupferrates

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THE solubility of ferric cupferrate in ether was indicated in 1896 by Bamberger and Ekecrantz (*6*), who reported that the precipitate formed when ferric chloride and nitrosophenylhydroxylamine were mixed, though similar in appearance to ferric hydroxide, was soluble in ether with the production of a red-brown color. Baudisch (*9*) and Auger and associates (*3*) reported that various cupferrates were soluble in organic solvents. Baudisch and King (*10*) suggested that ferric cupferrate be freed from certain coprecipitated impurities by dissolving it on the filter paper with chloroform, ether, or acetone.

One of the earliest to use extraction of their cupferrates as a means of separating metallic ions was Meunier (*35*), who found that all iron(III), titanium, and copper could be removed from a (1 + 9) hydrochloric acid solution by extraction with chloroform using excess cupferron. The clear aqueous layer gave no

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The theory of the extraction of cupferrates is presented, together with certain simplified theoretical relationships. The properties of various cupferrates are reviewed briefly. Some experimental data are given on the extraction of various cupferrates as a function of acidity and excess of cupferron. Some advantages of the extraction procedure as contrasted with the precipitation techniques in cupferron separations are pointed out.

reaction for iron with thiocyanate or with ferrocyanide. After these metals were removed and the pH was adjusted to 2.5 to 4.5, chromium, gallium, and aluminum could be precipitated by cupferron.

Bertrand (*14*) stated that extraction with cupferron plus chloroform removed molybdenum quantitatively at a concentration of 5 micrograms per liter if the aqueous layer had a pH greater than 1. Paulais (*37*) found that the removal of microgram quantities of iron was possible by extraction with chloroform plus cupferron and that the iron could be estimated by the color of the chloroform extract.

The authors have found extraction to be far superior to precipitation for the removal of microgram to milligram quantities of elements that form chloroform- or ether-soluble cupferrates. The removal by extraction is quantitative, whereas the removal of iron by precipitation from concentrated solutions of uranium or other salts is often far from complete and even tenths of milligrams of iron fail to precipitate (*17*).

Extraction has the advantage that a number of elements of the acid hydrogen sulfide group—e.g., tin, molybdenum, etc.—are almost completely removed, and for this reason it is often desirable to precede the hydrogen sulfide separation by a cupferron extraction. The excess of cupferron may, to a great extent, be removed from the aqueous layer by extraction. The recovery of the extracted elements from the solvent layer is practicable on either a micro or a macro scale.

THEORY OF EXTRACTION OF CUPFERRATES

It is assumed that the metal exists in the aqueous phase either as hydrated ions, here represented as M^{+n} for simplicity, or as the undissociated cupferrate of the metal, $MR_{n_{aq.}}$; the subscript *aq.* is used for substances in the aqueous phase and *org.* for substances in the organic solvent. In certain instances the metal may exist as oxygenated cations or anions, $MO_y^{(n-2y)}$, or as other complexes such as citrates, tartrates, halides, etc., and in these cases appropriate mass law expressions will need to be used. In the following treatment, which is general for a reagent of type HR, the symbol HR is used for the free acid—e.g., hydrogen cupferrate—R^- for the anion of the acid, and $R_{tot.}$ for the total amount of reagent introduced, or in this case for total cupferron added to the system. The following five relationships may be used in deriving a general expression for the distribution, D, provided the cupferrates of the metals are not associated in the organic phases, which seems to be the case as far as present evidence is concerned. By definition, D is the ratio of metal in the organic solvent to metal in the aqueous phase.

$$K_1 = \frac{\gamma_{HR_{org.}} \; [HR]_{org.}}{\gamma_{HR_{aq.}} \; [HR]_{aq.}} \tag{1}$$

$$K_2 = \frac{\gamma_{H^+}[H^+]\gamma_{R^-}[R^-]}{\gamma_{HR_{aq.}} \; [HR]_{aq.}} \tag{2}$$

$$K_3 = \frac{\gamma_{MR_{n_{aq.}}} \; [MR_n]_{aq.}}{\gamma_{M^{+n}} \; [M^{+n}]\gamma^n_{R^-}[R^-]^n} \tag{3}$$

$$K_4 = \frac{\gamma_{MR_{n_{org.}}} \; [MR_n]_{org.}}{\gamma_{MR_{n_{aq.}}} \; [MR_n]_{aq.}} \tag{4}$$

$$R_{tot.} = [HR]_{org.}V_{org.} + [HR]_{aq.}V_{aq.} + [R^-]V_{aq.} + n[MR_n]_{org.} \; V_{org.} + n[MR_n]_{aq.} \; V_{aq.} \tag{5}$$

By substituting values from Equations 1 to 4 in 5 and simplifying, the following expression for the distribution results:

$$\frac{1}{D} = \frac{V_{aq.} \; \gamma_{MR_{n_{org.}}}}{V_{org.} \; \gamma_{MR_{n_{aq.}}} \; K_4} + \frac{V_{aq.} \; V^{n-1}_{org.}\gamma_{MR_{n_{org.}}}}{K_3K_4\gamma_{M^{+n}}\gamma^n_{R^-}} \times \left\{ \frac{\left(\frac{\gamma_{R^-}\gamma_{H^+}K_1}{\gamma_{HR_{org.}} \; K_2} + \frac{\gamma_{R^-}\gamma_{H^+}V_{aq.}}{\gamma_{HR_{aq.}} \, K_2V_{org.}}\right)[H^+] + \frac{V_{aq.}}{V_{org.}}}{R_{tot.} - n[MR_n]_{org.}\left(1 + \frac{\gamma_{MR_{n_{org.}}} \; V_{aq.}}{\gamma_{MR_{n_{aq.}}} \; V_{org.}K_4}\right)} \right\}^n \tag{6}$$

Here $(MR_n)_{org.}$ is total moles of MR_n in the organic phase. Little is known about the specific constants for the various cupferrates. The value of K_1 at room temperature is 2×10^2 for chloroform-water. Hantzsch (*28*) found for K_2 the value $10^{-5.24}$. In general, the cupferrates precipitate from aqueous solution and dissolve readily in organic solvents. K_4 may therefore be safely assumed to be large in most cases. Making allowance for the values of K_1, K_2, and K_4, Equation 6 to good approximation becomes:

$$\frac{1}{D} = \frac{V_{aq.}V^{n-1}_{org.}\gamma_{MR_{n_{org.}}}}{K_3K_4\gamma_{M^{+n}}\gamma^n_{R^-}} \left\{ \frac{\frac{\gamma_{R^-}\gamma_{H^+}K_1}{\gamma_{HR_{org.}}K_2}[\mathrm{H}^+]}{R_{tot.} - n[MR_n]_{org.}} \right\}^n \tag{7}$$

From these considerations it is apparent that the extraction of a cupferrate to an organic solvent is favored by low acidity; by an appreciable excess of the reagent over the stoichiometric amount; by a small volume of organic liquid when n is 2 or greater; and by a small volume of the aqueous phase. Insufficient data are available for a rigorous test of the foregoing expressions. Such data as have been obtained are in reasonable agreement with the general outlines of the theory.

A further approximation of Equation 7 may be made by taking the activity coefficients as unity, and assuming all the excess cupferron to be in the organic layer. It is thus found that:

$$D^* = \frac{K_2^nK_3K_4[HR]^n_{org.}}{K_1^n[\mathrm{H}^+]^n} \tag{8}$$

In this expression D^* is the distribution coefficient—i.e., the ratio of concentration of metal in the organic solvent to that in the aqueous phase. The data that will be presented are in terms of the approximate Equation 8.

PROPERTIES OF VARIOUS CUPFERRATES

The following brief review presents the chief analytical properties of the cupferrates with special reference to extraction. A general summary of the earlier literature on cupferron and neoferron has been published (*46*), including data on precipitation.

PROPERTIES OF CUPFERRATES

Aluminum. Forms at pH 2 to 5 (*35*); completely precipitated from neutral solution, not at all from acid solution (*43*); quantitatively precipitated from 0.005 *N* acid (*39*), not at all from 0.5 *N* acid (*42*); readily soluble in ether (*9*). Small amounts of aluminum are completely extracted at pH 2 to 5 by cupferron plus chloroform (*35*).

Ammonium. White; easily soluble in water and hot alcohol; difficultly soluble in cold alcohol (*5*).

Antimony. QUINQUIVALENT. Not precipitated under any conditions (*3, 43*); not extracted from (1 + 9) sulfuric acid by cupferron plus chloroform according to authors' observations.

TRIVALENT. Sb(Cup)$_3$; white; precipitates almost completely from weakly acidic solution with excess of cupferron (*3, 43*); very soluble in neutral organic solvents except alcohol (*3*); quantitatively extracted from (1 + 9) sulfuric acid by cupferron plus chloroform according to local observations.

Arsenic (III and V). Neither form of arsenic is precipitated by cupferron under any conditions (*43, 44*).

Barium. Ba(Cup)$_2$ (*4*); Ba(Cup)$_2$.2H$_2$O (*3*); white (*3*); forms a true salt from neutral solutions (*3, 9, 33*).

Beryllium. Not precipitated from sulfuric acid solution containing tartrate (*31*).

Bismuth. Bi(Cup)$_3$ forms in acid solution (*3*) and is quantitatively precipitated from hydrochloric and sulfuric acid solutions by excess cupferron (*41, 43*); soluble in toluene or methyl ethyl ketone (*3*).

Cadmium. Cd(Cup)$_2$ (*3*); white (*1, 33*); precipitated from neutral solution, or slightly acidic but not from strongly acidic solution—e. g., 0.5 *N* (*9, 42, 43*). Quantitatively precipitated from neutral solution (*29*); insoluble in organic solvents (*9*) except boiling alcohol or ether (*3*); soluble in sodium carbonate and ammonium hydroxide (*29*).

Calcium. Ca(Cup)$_2$.2H$_2$O; white (*3*); slightly soluble in boiling water (*3*); forms a true salt (*9, 33*).

Cerium. QUADRIVALENT. Ce(Cup)$_4$, rust colored; forms in acidic solution and easily soluble in organic solvents (*3*).

TRIVALENT. Ce(Cup)$_3$, yellow, formed in neutral solution (*3*); not as completely precipitated from acidic solution as Ce(IV) (*32*); insoluble in organic solvents (*3*), and not extracted from (1 + 9) hydrochloric acid by cupferron plus ethyl acetate (*24*).

Chromium(III). Partially precipitated from neutral or slightly acidic solutions (*43*), but not from 0.5 *N* acid (*42*); not extracted from (1 + 9) hydrochloric acid by cupferron plus ethyl acetate. Bivalent chromium is not precipitated from (1 + 13) hydrochloric acid (*24*).

Cobalt. Co(Cup)$_2$, flesh colored or rose, (*3, 9*); formed in neutral (*3, 7, 43*) or dilute acetic acid solution (*43*); not precipitated from strong mineral acid (*9, 43*) nor from 0.5 *N* acid (*42*); soluble in ethyl acetate or ether with the formation of a deep red color (*9*); soluble in alcohol (*3*).

Columbium. White, according to local observation; precipitated quantitatively from rather concentrated sulfuric acid (1 + 9) containing tartaric and oxalic acids (*38*); precipitated quantitatively in preference to ferrous iron from (1 + 4) hydrochloric acid. The precipitate is insoluble in chloroform, but if chloroform plus the hydrogen cupferrate is shaken with an aqueous solution of columbium, appreciable amounts of columbium can be transferred to the chloroform layer.

Copper. Cu(Cup)$_2$, light bluish green, gray green (*9, 29*), or greenish white (*8*); incompletely precipitated from strong mineral acids (*15, 43*) but quantitatively precipitated from acetic acid or very dilute hydrochloric acid by excess cupferron (*15*); quantitatively precipitated with iron from (1 + 4) hydrochloric acid (*7*) and the copper cupferrate may be completely extracted from the Fe(Cup)$_3$ by 6 *N* ammonia, leaving not even a spectrographic trace (*7*). Soluble in chloroform (*9, 14*) and toluene plus nitrobenzene (*8*); difficultly soluble in ether (*8*); extracted completely by cupferron plus chloroform from (1 + 9) hydrochloric acid (*35*), but not completely according to local experiments from (1 + 99) to (1 + 7) sulfuric acid.

Gallium. Precipitated completely from 0.3 *M*, very incompletely from 0.5 *M*, and not at all from 0.7 *M* sulfuric acid (*13*); completely precipitated from (1 + 12) sulfuric acid by excess cupferron (*45*).

Indium. In(Cup)$_3$, white, formed in acid solution (*3*); not precipitated from (1 + 5) hydrochloric and only partially from (1 + 8) hydrochloric acid (*3, 34*); very soluble in alcohol, benzene, and chloroform (*3*).

Iron. FERRIC. Fe(Cup)$_3$ (*9, 15*), reddish brown (*5*); quantitatively precipitated from (1 + 4) hydrochloric acid (*15*) leaving not even a spectrographic trace (*7*); completely precipitated from (1 + 4) sulfuric acid (*32*); stable toward ammonia but decomposed to ferric hydroxide by sodium hydroxide (*7*); soluble in ether and chloroform (*9*). Extracted quantitatively from (1 + 9) hydrochloric acid by cupferron and chloroform (*35, 37*), or by ether or ethyl acetate (*24*), leaving not even a spectrographic trace (*7*); quantitatively extracted from (1 + 9) sulfuric acid by cupferron plus ether (*17*).

FERROUS. Practically completely precipitated from weak mineral acid solution (*43*).

Lead. Pb(Cup)$_2$, white (*3*); partially precipitated from neutral or weak acetic acid (*3, 43*); not precipitated from 0.5 *N* acid (*42*); insoluble in organic solvents (*9*); slightly soluble in alcohol (*3*).

Manganese (II). Partially precipitated from neutral solution but not from dilute acetic acid (*43*); not precipitated from 0.5 *N* acid (*42*); soluble in ether (*9*).

Mercury. MERCURIC. Hg(Cup)$_2$, white; precipitated from faintly acidic solution (*3*); partially precipitated from neutral solution, not from weak acetic acid (*43*), and incompletely from 0.5 *N* acid (*42*); soluble in alcohol, benzene, and chloroform (*3*).

MERCUROUS. Hg$_2$(Cup)$_2$ (*3*); precipitated from acidic solution (*3, 43*); quantitatively precipitated from 0.5 *N* acid (*42*); insoluble in neutral organic solvents except hot alcohol (*3*).

Molybdenum (VI). CupMoO$_3$ and (Cup)$_2$MoO$_3$. The former is soluble in alcohol and insoluble in benzene, whereas (Cup)$_2$MoO$_3$ is very soluble in benzene and chloroform. Both substances form in acid solution (*3*). If pH is greater than 1, 5 micrograms of molybdenum per liter are quantitatively extracted by cupferron plus chloroform (*14*). According to local studies less than 4 micrograms remain in the aqueous phase of 100 ml. of (1 + 49) to (2 + 9) sulfuric acid after extraction by cupferron plus chloroform. Molybdenum is completely extracted from (2 + 9) hydrochloric acid by cupferron plus ethyl acetate (*24*).

Neodymium. Reddish white; insoluble in organic solvents (*9*).

Nickel. Ni(Cup)$_2$ (*3*); green; formed only in neutral solution (*3, 9*); partially precipitated from neutral or acetic acid solution and not from stronger acid (*43*); not precipitated from 0.5 *N* acid (*42*); soluble in organic solvents (*9*).

Platinum (IV). Not precipitated from (1 + 19) sulfuric acid (*47*).

Potassium. KCup, white; readily soluble in water, difficultly in methyl or ethyl alcohol; explodes on heating (*4*).

Scandium. Not precipitated from sulfuric acid solution containing tartrate (*31*).

Silver. AgCup (*3, 4*); white (*4*); formed only in neutral solution (*3*); partially precipitated from neutral solution, not from dilute acetic acid (*43*); not precipitated from 0.5 *N* acid (*42*); almost completely insoluble in neutral organic solvents (*3*).

Sodium. NaCup; white; readily soluble in water, difficultly soluble in methyl or ethyl alcohol; explodes on heating (*4*).

Strontium. Sr(Cup)$_2$.2H$_2$O, white (*3*); forms in neutral solution (*33*); somewhat soluble in boiling water (*3*).

Tantalum. Quantitatively precipitated from strong sulfuric acid solution containing tartaric and oxalic acids (*38*); quantitatively precipitated in preference to ferrous iron in (1 + 4) hydrochloric acid (*20*).

Thorium. Incompletely precipitated from dilute mineral acid solution but completely precipitated from acetic acid (*32, 48*). The precipitate readily passes through filter paper under light suction (*48*). Small amounts of thorium are almost completely precipitated from buffered ammonium carbonate solution (*21*). Freely soluble in ether, difficultly soluble in chloroform (*25*); almost completely extracted from (1 + 9) hydrochloric acid by cupferron plus ethyl acetate (*24*).

Tin. STANNIC. Sn(Cup)$_4$, white (*3*); completely precipitated from dilute mineral acid (*43*); quantitatively precipitated from 1.5 *N* acid by excess cupferron (*40*), from mildly acidic solutions containing hydrofluoric acid and boric acid (*26*); very soluble in chloroform (*3*); completely extracted from (1 + 9) hydrochloric acid solution by cupferron plus ethyl acetate (*24*).

STANNOUS. Sn(Cup)$_2$, white (*3*); practically completely precipitated from dilute mineral acid solution and quantitatively from 1.5 *N* acid by excess cupferron (*40*); soluble in benzene and chloroform (*3*).

Titanium. Ti(Cup)$_2$, canary yellow (*11, 12*); quantitatively precipitated from acid solution (*10, 11*), also from (1 + 49) to (1 + 19) sulfuric acid containing tartrate (*47*); quantitatively precipitated from (2 + 3) sulfuric acid (*7, 32*); precipitates in preference to iron(II) (*19*). Soluble in ethyl alcohol (*11*) and more soluble in chloroform than in ether according to authors' observations; completely extracted from (1 + 9) hydrochloric acid by cupferron plus chloroform (*35*), and also by cupferron plus ether or ethyl acetate (*24*).

Tungsten. Partially precipitated from (1 + 19) to (1 + 4) sulfuric acid (*32*); stated to be quantitatively precipitated from (2 + 3) sulfuric acid (*7*). Extraction with cupferron plus ethyl acetate only partially removes tungsten from (1 + 9) hydrochloric acid containing phosphoric acid, whether iron is present or absent, by cupferron plus ethyl acetate (*24*).

Uranium. SEXIVALENT. $UO_2NH_4(Cup)_3$ (*27*); yellow (*9*, *33*, *36*); forms on addition of cupferron to uranyl nitrate solution (*9*); precipitates from neutral solutions (*33*). Uranium is precipitated from very dilute (1 to 30,000) neutral solution with characteristic form (*36*). Insoluble in organic solvents (*9*, *27*). There appear to be two forms, one soluble and the other insoluble in ethyl ether; $UO_2NH_4(Cup)_3$ is the insoluble variety (*27*). From very concentrated solutions in (1 + 9) sulfuric acid milligram quantities of uranium(VI) are extracted to an equal volume of chloroform, if excess of cupferron is placed in the system.

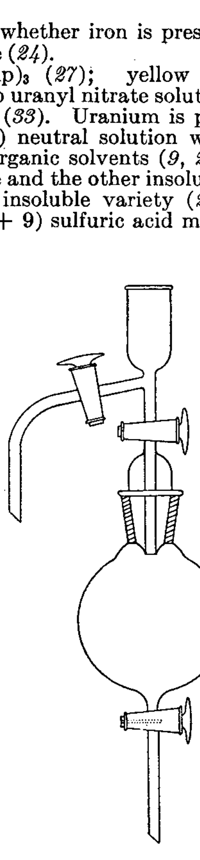

Figure 1. Apparatus for Extractions with Ether Plus Cupferron

After equilibrium is established, ethereal and aqueous layers are displaced upward by introducing mercury through stopcock at bottom of bulb

QUADRIVALENT. $U(Cup)_4$, brown; completely precipitated from acidic solution (*2*); quantitatively precipitated from (1 + 24) to (1 + 12) sulfuric acid (*30*). The authors found that the precipitate decomposes on washing unless the solution contains hydroxylamine hydrochloride in addition to cupferron. Soluble in chloroform and other neutral organic solvents (*2*). Milligram amounts of uranium(IV) are incompletely extracted from aqueous acid by hydrogen cupferrate in chloroform but almost completely by ethereal hydrogen cupferrate (*25*). According to authors' experiments macroamounts of uranium(IV) are quantitatively extracted from (1 + 19) sulfuric acid containing hydroxylamine hydrochloride by ethereal hydrogen cupferrate. In the presence of saturated mercury-zinc amalgam, submilligram quantities of uranium (IV) are quantitatively extracted from (1 + 19) sulfuric acid by ethereal hydrogen hydrogen cupferrate, leaving not even a spectrographic trace of uranium in the aqueous layer according to authors' experience.

Vanadium. The quinquevalent compound is mahogany red and both vanadium(IV and V) are completely precipitated from (1 + 99) hydrochloric or sulfuric acid; the precipitation from neutral solution is not quantitative (*50*). Vanadium(V) is completely precipitated in presence of tungsten without addition of hydrofluoric acid by careful acidity adjustment (*18*). Vanadium(V) is completely extracted from (1 + 9) hydrochloric acid by cupferron and ethyl acetate (*24*) and even microgram amounts are completely extracted from (1 + 9) sulfuric acid by cupferron plus ether (*23*). Trivalent vanadium forms a brownish black precipitate that is difficultly soluble in water (*16*).

Zinc. $Zn(Cup)_2$ is white and forms in neutral solution (*3*, *9*). It is not precipitated from buffered acetic acid (*43*). Even from neutral solution the precipitation is not quantitative with excess cupferron (*29*). No precipitation occurs in 0.5 *N* acid (*42*). Dissolves readily in ether (*9*); somewhat soluble in water (*29*).

Zirconium. $Zr(Cup)_4$ (*22*) is white (*22*, *32*) and is precipitated quantitatively from acidic solutions (*7*, *10*, *22*). The precipitation is quantitative from (1 + 19) sulfuric acid (*1*, *49*) or from (2 + 3) sulfuric acid (*32*). The precipitate is moderately soluble in ethyl acetate (*24*).

From the foregoing summary and from experience of others as well as the authors it is known that copper, iron(III), tin(IV), titanium, uranium(IV), and vanadium(V) are completely extracted from (1 + 9) hydrochloric acid and molybdenum(VI) is substantially completely removed. From (1 + 9) sulfuric acid copper is much less completely extracted, but the other elements are completely removed, except molybdenum where detectable microgram amounts remain in the aqueous layer after three extractions.

Antimony(III), bismuth, cerium(IV), gallium, indium, and thorium are completely precipitable under certain conditions of acidity, as shown in the preceding summary. All these precipitates are soluble in one or more of the neutral organic solvents. Little detailed information is available regarding their extraction. The authors' observations are that antimony(III) is completely extracted along with iron, titanium, vanadium, etc., from (1 + 9) sulfuric acid.

The following elements are completely precipitated from rather strongly acidic solutions: columbium, tantalum, tungsten, mercury(I), and zirconium. When the precipitates have formed in quantity they do not dissolve readily in organic solvents. Observations on columbium indicate that by extracting with chloroform containing hydrogen cupferrate it is possible to transfer appreciable quantities of columbium to chloroform. Detailed experiments have not been made to determine whether this procedure is generally applicable to the other difficultly soluble cupferrates.

EXPERIMENTAL

Extraction experiments were performed either with separatory funnels of conventional types when chloroform was the solvent, or with the special inverted funnel device shown in Figure 1 when ether was solvent. In the latter case the aqueous solution was forced up by mercury with the aid of a leveling bulb, not shown in Figure 1, and the ethereal layer was drawn off at the top. In microextractions the ethereal layers were drawn off with a pipet.

The determinations of the substances in the two layers were made by conventional volumetric and gravimetric procedures for macroamounts, or photometrically with the aid of a Beckman Model DU quartz prism spectrophotometer for microquantities.

Cupferron from both Eastman Kodak Company and the G. Frederick Smith Chemical Company was used. Samples of copper, molybdenum, uranium, and other materials were of analytical reagent purity. The metals and salts were tested for significant impurities by sensitive procedures in connection with other critical work.

Distribution of Hydrogen Cupferrate between Acidified Aqueous Solutions and Chloroform. These experiments were made by W. E. Bunce of this laboratory. Weighed amounts of cupferron were treated with known amounts of sulfuric acid in excess of that necessary to form the hydrogen cupferrate. After the aqueous layer was shaken to equilibrium with an equal volume of chloroform at room temperature, the phases were separated and the aqueous layer was filtered. The hydrogen cupferrate in the clear filtrate was estimated by the color produced by adding an excess of ferric solution (reverse of the procedure of Paulais, *37*). It was found experimentally that there was a linear relation between the extinction and the concentration of cupferron over the range listed in these experiments. Fresh white cupferron was selected and the same sample was used in the distribution experiments as in the photometric calibrations. The results are presented in Table I.

The average value for D^* is $2.1_1 \times 10^2$, and it appears to be relatively independent of the excess of free sulfuric acid.

Distribution of Copper Cupferrate to Chloroform. The aqueous and the chloroform layers were each of 100-ml. volume. The copper remaining in the aqueous layer after equilibrium was established was estimated colorimetrically. These experiments were made by W. H. Davenport, Jr. See Table II.

In all but three of these experiments the distribution is so small that slight experimental errors will have a marked effect on the observed value of D^*. In the three cases where the observed distribution is 0.19, 0.35, and 0.25 the approximate Equation 8

Table I. Distribution of Hydrogen Cupferrate between Chloroform and Aqueous Solution

Aqueous Layer	Chloroform Layer	Free[a] H_2SO_4	Cupferron Used	Cupferron in Aqueous Phase	D^* $C_{chlor.}/C_{aq.}$
Ml.	*Ml.*	*Moles* × *10*^{2}	*Grams*	*Mg.*	
50	50	0.9	2.500	11.6	214
50	50	1.0	2.500	12.0	207
50	50	3.7	2.500	11.9	209
50	50	8.2	2.500	11.3	220
100	100	8.4	2.000	8.8	226
100	100	5.8	10.000	51.2	194

[a] Sulfuric acid in excess of that necessary to convert cupferron into hydrogen cupferrate.

Table II. Distribution of Copper between Aqueous Solutions and Chloroform

(In each case 50.0 mg. of copper were present in system)

Free H_2SO_4 Sp. Gr. 1.84 *Ml./100 ml.*	Total Cupferron *Gram*	Copper in Aqueous Phase at Equilibrium *Mg.*	D^* $C_{chlor.}/C_{aq.}$
2.0	0.25	42	0.19
4.0	0.25	47	0.064
6.0	0.25	47	0.064
6.0	1.0	37	0.35
8.0	0.25	48	0.042
8.0	1.0	40	0.25
10.0	0.25	49	0.02
12.0	0.25	About 50	About 0.0

used in the form $K\bullet = D^* \frac{[H^+]^2}{[HCup]^2}$ yields values of K of 5.2, 4.2, and 5.3 × 10^2, respectively. The free cupferron acid is taken as the difference between the total cupferron and the cupferron combined with the copper in the chloroform layer. In the above expression $K = \frac{K_2^2 K_3 K_4}{K_1^2}$.

Table III. Extraction of Molybdenum(VI) by Cupferron plus Chloroform

A. At Fixed Acidity, 10 Ml. of H_2SO_4, Sp. Gr. 1.84, per 100 Ml.

Mo taken, mg.	10	20	30	40	50
Mo in aqueous layer, mg.	0.001	0.004	0.003	0.003	0.003

B. At Fixed Concentration, 20 Mg. of Mo per 100 Ml.

H_2SO_4, sp. gr. 1.84, ml. per 100 ml.	2.0	6.0	10.0	14.0	18.0
Mo in aqueous layer, mg.	0.003	0.004	0.001	0.001	0.001

A graphic representation of expected relationships and experimental data is indicated in Figure 2. In these calculations K is taken as 4.9 × 10^2.

Molybdenum. Bertrand found that at pH greater than 1 even as little as 5 micrograms of molybdenum(VI) per liter could be extracted quantitatively (*14*). He stated that extraction was incomplete at lower pH and it became of interest to find out the degree of incompleteness of the extraction. These experiments were made by D. R. Norton. In each case three successive extractions of the 100-ml. aqueous phase were made with 50.0-ml. portions of chloroform. The results are indicated in Table III.

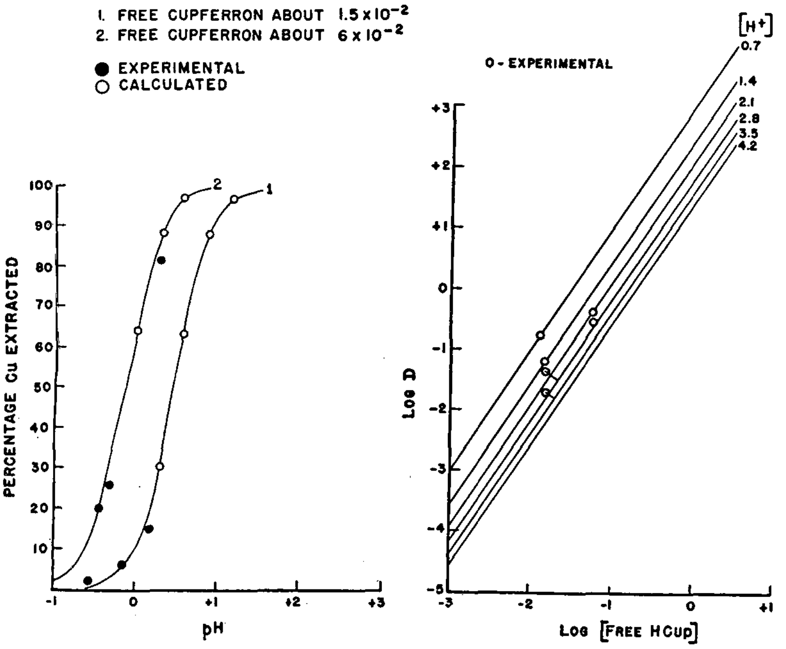

Figure 2. Extraction of Copper Cupferrate

Left. Effect of pH upon per cent extracted. *Right.* Effect of concentration of free hydrogen cupferrate

Table IV. Effects of Excess Cupferron and Variable Acidity on Extraction of Uranium(IV) to Ether

A. At Constant Acidity. Aqueous Layer, 300 Ml., 1.47 *N* in Sulfuric Acid. Ether Layer 100 Ml.

Hydrogen Cupferrate Used *Moles × 10⁴*	U(IV) Taken *Moles × 10⁴*	U in Ether at Equilibrium *Moles × 10⁴*	D^* C_{ether}/C_{water}
29.5	7.95	2.91	1.84
40.2	7.95	4.51	3.95
53.6	7.95	5.85	8.35
80.4	7.95	7.69	88.4

B. At Variable Acidity with Constant Excess of Cupferron. Aqueous Layer, 300 Ml. Ether 100 Ml. 40.2 × 10^{-4} Mole of Hydrogen Cupferrate, 7.95 × 10^{-4} Mole of U(IV)

Sulfuric Acid Normality	U in Aqueous Layer	U in Ether	
0.98	1.77	6.18	10.5
1.47	3.29	4.66	4.3
2.06	3.99	3.96	3.0
2.65	4.93	3.02	1.8

This evidence and certain observations made in the course of volumetric work indicate that the recovery of molybdenum by extraction is adequate for most purposes, although a few micrograms remain in the aqueous layer even after three extractions with additional cupferron and chloroform.

Uranium(IV). The extraction of the uranium(IV) cupferrate was performed as follows:

The uranium in (1 + 19) sulfuric acid was passed through a Jones reductor and the reductor was washed with sufficient acid to bring the volume to 300 ml. The reduced solution had added to it 5 grams of hydroxylamine hydrochloride. The solution in the inverted extraction apparatus (Figure 1) was cooled in ice water and the appropriate weight of cupferron was dissolved in cooled water, acidified, and extracted with 100 ml. of ether. The ethereal extract was then added to the cold uranium solution and the mixture was vigorously agitated. After equilibrium was established, the ether phase was removed at the top of the apparatus by displacing the aqueous layer upward by mercury. After removal of the ether and destruction of the cupferron the uranium was estimated by titration with ceric sulfate. The cupferron was decomposed by nitric and sulfuric acids, followed by perchloric acid treatment. The uranium was reduced by liquid zinc amalgam. The values obtained are shown in Table IV.

If a constant is calculated in the same manner as was done for copper, $K = D^* \frac{[H^+]^4}{[HCup]^4}$ from Equation 8, the experiments under A, Table IV, give values of K of 0.75, 0.76, 0.48, and 0.68 × 10^8. The values for D^* under A are proportional to the fourth power of the hydrogen cupferrate concentration. The values under B for K are 1.7, 0.92, 1.52, and 1.42 × 10^8.

Although the agreement between theory and observation in these studies leaves much to be desired, only a rough approximation treatment is possible in the absence of activity data, lack of temperature control, and rough approximation of concentrations of cupferron and acid.

Recovery of Substances from Organic Extracts. In studies not detailed here the authors have had numerous occasions to use the extraction of milligram to microgram amounts of iron, molybdenum, titanium, vanadium, etc., from concentrated aqueous solutions of uranium or other elements.

In such procedures the total amount of cupferron used is from 1 to 4 ml. of 5% solution per 50 ml. of aqueous phase. Two 10-ml. portions of ether are used and after being washed twice with water the elements are removed from the ether by two treatments with 10-ml. portions of 6 *N* nitric acid, shaking thoroughly until the ether layer is definitely brown. The aqueous nitric acid extracts are then evaporated with sulfuric acid and evaporation with nitric and sulfuric acid is repeated until the organic matter is destroyed.

Recovery of Macroamounts of Elements from Chloroform Extracts. The following procedure has been utilized frequently by Pekola with good success:

To the 600-ml. beaker containing the chloroform extracts are added 100 ml. of nitric acid (1 to 1). A stirring rod is inserted and the chloroform evaporated on a steam or hot water bath. When no more chloroform appears to go off, the beaker is removed and 50 ml. of 1 to 1 sulfuric acid are added. The beaker is heated on a hot plate at full heat; the beaker and solution are kept in motion by rotating with beaker tongs until the solution boils. Unless the last globules of chloroform are removed in this manner mechanical loss may occur. The solution is evaporated at medium heat until light fumes of sulfur trioxide are given off. After cooling, 50 ml. of concentrated nitric acid are added and evaporated to heavy fumes of sulfur trioxide. To the fuming solution perchloric acid is added not more than 2 drops at a time, waiting for the reaction to cease each time before making further additions to a total of 1 ml. The solution is removed from the hot plate, the sides and cover are washed down, 10 ml. of concentrated nitric acid and 5 ml. of perchloric acid are added, and the solution is evaporated to fumes. The heat is increased sufficiently to boil off the perchloric acid. The solution is cooled, and the cover and sides of the beaker are washed down and taken to fuming again. If necessary, the perchloric acid treatment is repeated.

The amount of 1 to 1 sulfuric acid and the other acids may be reduced if small amounts of cupferrates and excess cupferron are present. The procedure given is designed to handle a 1.0-gram sample containing as high as 70 to 80% of extractable elements—e.g., iron, titanium, zirconium, etc.

CONCLUSIONS

The extraction of cupferrates to chloroform has been found to be a very effective procedure for removing iron, titanium, molybdenum, vanadium, etc., prior to the estimation of other substances. The procedure is effective in the collection of microgram to milligram quantities of iron, titanium, vanadium, etc., prior to their estimation by colorimetric methods. A fuller account of experience here and in other laboratories is to be published in the National Nuclear Energy Series. The method sometimes makes unnecessary the use of a hydrogen sulfide separation; this is frequently true in connection with the volumetric estimation of uranium, where by proper choice of initial acid attack, use of a suitable reductant, and the use of standard ceric sulfate it is possible to avoid the interfering action of antimony, arsenic, titanium, vanadium, molybdenum, and iron. Following such removal of other elements it is possible to make use of oxidation or reduction treatments and pH control to prepare the solution for further extractions or precipitations involving cupferron.

Considerable flexibility is possible by control of excess cupferron and hydrogen ion concentration. The possibilities can be roughly estimated from published data on the solubilities of the cupferrates and from the theoretical treatment that has been outlined. There is much need for more critical experiments and the accumulation of further quantitative data.

ACKNOWLEDGMENT

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Determination of End Unsaturation in Organic Compounds

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A chemical method for detecting and determining terminal or end unsaturation in mixtures of unsaturated organic compounds has been developed. The unsaturated compounds are treated with a slight excess of potassium permanganate to form glycols which are split by means of periodic acid. Because formaldehyde is a product of this reaction when terminal double bonds are present, determination of formaldehyde affords a specific method for determining end unsaturation. Formaldehyde is separated from the reaction mixture by distillation and is determined spectrophotometrically by means of chromotropic acid.

A VARIETY of methods have been published for the determination of olefinic unsaturation in organic compounds. The chemical methods for this determination break down into two general categories—addition reactions and hydrogenation. The physicochemical methods are rather diversified and include infrared absorption, ultraviolet absorption, mass spectrometry, Raman spectrometry, and polarography.

The chemical methods for the determination of olefinic unsaturation are used chiefly for the determination of total unsaturation, whereas the physicochemical methods are used principally for the determination of the position of the unsaturation or even the exact chemical compound. At least two chemical methods have been suggested, however, for determining the position of the unsaturated group or groups in organic compounds. Dienometry, which was proposed almost simultaneously by two groups of investigators (*2*, *6*) utilizes the Diels-Alder reaction for determining the amount of conjugated unsaturation. Kolthoff and Lee (*7*) and later Saffer and Johnson (*9*) suggested methods for determining internal and external unsaturation in polymers based on the difference in the rate of reaction of perbenzoic acid with these two types of bonds.

Because of the lack of a simple chemical method to determine end or terminal unsaturation in organic compounds, it was decided to investigate the possibilities of a method which would be specific for this type of olefinic linkage. It is well known that olefins are converted into glycols with potassium permanganate. Because terminal unsaturated groups are transformed to 1,2-glycols which are split with periodic acid to yield formaldehyde and a higher aldehyde, the determination of formaldehyde would then afford a specific indication of this type of unsaturation.

The idea of using the Malaprade reaction together with a distillation to remove the formaldehyde for the determination of 1,2-glycols has been suggested (*3–5*, *8*, *10*). However, in none of these investigations has a specific reagent for formaldehyde been used. A sensitive and specific spectrophotometric method for determining formaldehyde with chromotropic acid has been described by Bricker and Johnson (*1*). Therefore, a combination of these methods should provide a comparatively simple chemical procedure for determining end unsaturation.

REAGENTS AND APPARATUS

Ethanol, 95%

Potassium permanganate solution, 1% aqueous solution

Periodic acid solution, 200 mg. per ml., obtained from the G. Frederick Smith Chemical Company

Chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid), obtained from the Paragon Division, Matheson Chemical Company

Sulfuric acid, concentrated

The distillation apparatus is illustrated in Figure 1.

PROCEDURE

Dissolve a weighed quantity of the unsaturated compound in ethanol and then dilute it to a definite volume with this solvent. Pipet a suitable sized aliquot (not more than 3 ml.) of this solution which will yield not over 2 mg. of formaldehyde into a glass-stoppered test tube. Add sufficient ethanol so that a total of 3 ml. of alcohol are present and then add 1 ml. of the periodic acid solution. Now add the potassium permanganate solution in 0.2-ml. portions until a pink color persists in the supernatant liquid for 1 minute. Alternatively, if the amount of total unsaturation is known, 0.5 ml. more than the calculated amount of the potassium permanganate solution can be added in 0.2-ml. portions without observing so closely the color of the supernatant liquid.

Place the test tube in the distillation apparatus (Figure 1) and continue to distill the reaction mixture until 10 or 15 ml. of distillate are collected. Read the volume of the distillate collected and then transfer 1.00 ml. of this solution to a test tube containing 50 mg. of chromotropic acid. Add 5 ml. of concentrated sulfuric acid to the test tube and then heat the resulting colored solution in a boiling water bath for 30 minutes. Cool the test tube and finally dilute the solution to 50 ml. in a volumetric flask. Measure the optical density of this solution against a reagent blank at 570 mμ.

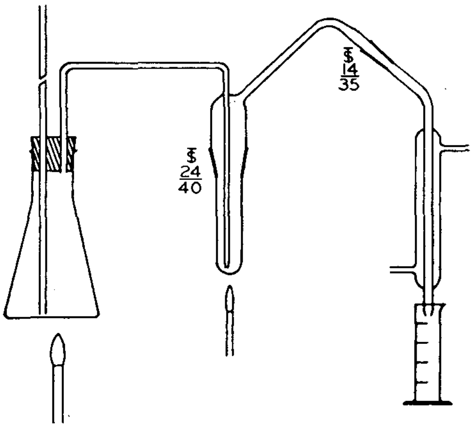
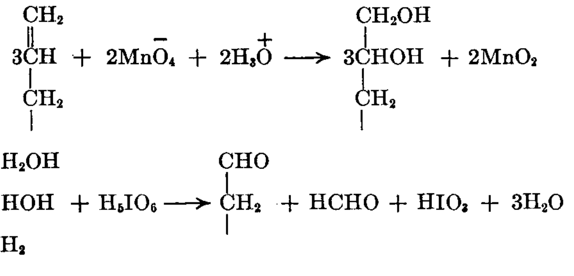


Figure 1. Distillation Apparatus

From a calibration curve obtained from known amounts of formaldehyde and by multiplying by the number of milliliters in the distillate, the amount of formaldehyde in the entire distillate is obtained. The formaldehyde found by making a blank determination on all reagents is now subtracted to give the amount of formaldehyde actually obtained from the end unsaturation in the sample.

DEVELOPMENT OF METHOD

The chemical reactions which are used in this procedure to determine end unsaturation can be shown by the following equations:



$$\begin{array}{c}CH_2\\ \|\\ 3CH\\ |\\ CH_2\\ |\end{array} + 2Mn\overset{-}{O_4} + 2H_3\overset{+}{O} \longrightarrow \begin{array}{c}CH_2OH\\ |\\ 3CHOH\\ |\\ CH_2\\ |\end{array} + 2MnO_2$$

$$\begin{array}{c}CH_2OH\\ |\\ CHOH\\ |\\ CH_2\\ |\end{array} + H_5IO_6 \longrightarrow \begin{array}{c}CHO\\ |\\ CH_2\\ |\end{array} + HCHO + HIO_3 + 3H_2O$$

In order to investigate the analytical possibilities of these reactions, it was necessary to determine how well small amounts of formaldehyde could be distilled from solution. Although the papers dealing with the determination of glycols by periodic acid oxidation followed by distillation do not imply any difficulty in distilling formaldehyde quantitatively, it is known that this compound is strongly hydrated in solution and, therefore, is not distilled as readily as one would predict from its physical properties. About 1.3 mg. of formaldehyde in 5 ml. of water were placed in a test tube and then distilled in the apparatus shown in Figure 1. Two 10.0-ml. distillates were collected and analyzed for formaldehyde. Inasmuch as the first distillate contained over 98% of the formaldehyde, it was decided that 10 to 15 ml. of distillate would be adequate to recover the formaldehyde quantitatively.

It is important to distill the solution in the test tube almost to dryness at least once before the distillation is stopped. This can be done easily by regulating the two burners shown in Figure 1 so that the distillation from the test tube proceeds much faster than that in the steam-generating flask. The continuous stream of gas bubbles from the steam-generating flask prevents the reaction mixture in the test tube from bumping during the distillation and also aids in sweeping the formaldehyde into the distillate.

Because the reaction mixture contained strong oxidizing agents, it was important to determine if the formaldehyde could be recovered from solutions containing potassium permanganate and periodic acid.

Identical amounts of formaldehyde (about 0.5 mg.) in 2.0 ml. of water were added to 3.0 ml. of 95% ethyl alcohol and then treated with 0.00, 0.50, and 1.00 ml. of potassium permanganate solution, respectively. These solutions were distilled and the distillates were analyzed according to the recommended procedure. The recoveries of formaldehyde were 99, 28, and 21%. Another series of 2.0-ml. aliquots of the formaldehyde solution was taken and 1.0 ml. of periodic acid and 3.0 ml. of water were added to each solution. No ethyl alcohol was added to any of these solutions but 0.00, 0.20, and 0.50 ml. of potassium permanganate solution were added, respectively. The recovery of formaldehyde was 99, 23, and less than 2% from these solutions. To each of three additional 2.0-ml. aliquots of the formaldehyde solution, 1.0 ml. of periodic acid and 3.0 ml. of ethyl alcohol were added. Then 0.00, 0.50, and 1.0 ml. of potassium permanganate solution were added, respectively. The recovery of formaldehyde was 100, 98, and 94%.

These experiments indicate the importance of the ethyl alcohol in the recommended procedure. Formaldehyde cannot be recovered from an aqueous solution of potassium permanganate and periodic acid but it can be distilled well from an alcoholic solution. This is believed to be due to the reaction of formaldehyde with ethanol in dilute acid solution to form ethylal. This acetal is stable and undergoes oxidation rather slowly. The possibility that the formaldehyde may all be present in the distillate as ethylal causes no interference in its determination with chromotropic acid. This reagent has been shown to be especially useful for determining combined formaldehyde in linear or cyclic formals.

In the recommended procedure, the periodic acid is added before the potassium permanganate. It was shown in a series of experiments that when potassium permanganate was added prior to the periodic acid, about 30% less formaldehyde was obtained than when the recommended procedure was followed. These data can be explained if it is assumed that periodic acid must be present to react immediately with the glycol as it is formed, and thus prevent the attack by excess permanganate on the glycol to yield products other than formaldehyde. This explanation implies that the rate of attack by periodic acid on a glycol is considerably faster than that of permanganate.

The ethyl alcohol used in the recommended procedure also serves to destroy the excess potassium permanganate before it can appreciably oxidize the formaldehyde or any compounds which should yield formaldehyde in the procedure. This was shown in a series of experiments in which constant amounts (10.4 mg.) of undecylenic acid dissolved in dilute sodium hydroxide were treated with sulfuric acid to a pH of about 7. Then 1 ml. of periodic acid solution and varying amounts of potassium permanganate solution were added to each sample. The results of these experiments showed that the amount of formaldehyde obtained increased to a maximum value and then decreased rapidly as the amount of permanganate in excess of the theoretical amount to form the glycol was added. However, when alcohol was used in a similar series of experiments, the recovery of formaldehyde was considerably higher than the maximum value obtained when no alcohol was present. Furthermore, the recovery of formaldehyde was practically constant in these experiments as long as sufficient permanganate was present to form the glycol, but not more than 6 to 8 mg. in excess.

Some experiments were run to determine how long the reaction mixture should stand prior to the distillation. The recovery of formaldehyde was essentially constant when the reaction mixture was allowed to stand 0, 1, 2, and 5 hours between the addition of the potassium permanganate and the actual distillation. Therefore, it was concluded that the time of standing of the mixture prior to distillation was not a critical factor.

RESULTS AND APPLICATIONS

A qualitative study on several compounds was carried out in order to test the conclusion that only compounds that contain a terminal olefinic group would yield a positive test for formaldehyde by the recommended procedure. The following compounds gave a very strong test for formaldehyde and, therefore, indicated terminal unsaturation: allyl alcohol, allyl chloride, allyl bromide, allyl isothiocyanate, allyl thiourea, styrene, isoprene, pinene, citral, citronellol, itaconic acid, and α-methylenebutyrolactone. The following compounds produced little or no more formaldehyde than the blank and consequently indicated that few, if any, terminal olefinic linkages were present: oleic acid, cinnamic acid, indene, coumarin, and indole. Citraconic and aconitic acids gave inconclusive results because small samples showed a considerable amount of formaldehyde whereas large samples gave very little formaldehyde. The results from these compounds clearly indicate that the recommended procedure provides a convenient qualitative method for detecting end unsaturation in a variety of organic compounds.

In order to determine the quantitative possibilities of the procedure, several standard solutions from a sample of commercially available 10,11-undecylenic acid were prepared by dissolving weighed quantities of the material in ethyl alcohol and diluting to 25.0 ml. in volumetric flasks. Various sized aliquots of these solutions were analyzed by the recommended procedure. The precision of multiple analyses on the same sized aliquot was within 5% or better in all cases. A plot of the total formaldehyde recovered in the distillates versus the number of milligrams of

Table I. Determination of Undecylenic Acid

Azelaic Acid Taken *Mg.*	Undecylenic Acid Taken *Mg.*	Undecylenic Acid Found *Mg.*	Deviation *Mg.*
100	2.95	2.95	±0.00
100	7.86	8.05	+0.19
Oleic Acid Taken			
18.8	2.02	1.96	−0.06
19.0	2.02	1.89	−0.13
19.5	2.02	1.97	−0.05
85.7	3.00	2.85	−0.15
160	2.02	1.83	−0.19
19.0	5.05	4.87	−0.18
58.0	5.05	4.85	−0.20
75.0	5.05	4.92	−0.13
105.5	5.05	4.78	−0.27
87.5	8.01	8.10	+0.09
Ricinoleic Acid Taken			
10.4	2.02	2.17	+0.15
10.4	2.02	1.98	−0.04
20.8	2.02	2.17	+0.15
20.8	2.02	1.96	−0.06
31.2	2.02	1.84	−0.18
31.2	2.02	1.86	−0.16
41.6	2.02	1.86	−0.16
41.6	2.02	1.82	−0.20
11.0	5.05	4.92	−0.13
11.0	5.05	5.04	−0.01
21.9	5.05	4.87	−0.18
21.9	5.05	4.76	−0.29
32.9	5.05	4.79	−0.26
32.9	5.05	4.84	−0.21
43.0	5.05	4.87	−0.18
43.0	5.05	4.79	−0.26

undecylenic acid taken is shown in Figure 2. These results, which were obtained by three different chemists, indicate that the recovery of formaldehyde is linear with the smaller samples of unsaturated acid taken. With the larger samples, the recovery of formaldehyde appears to deviate slightly from linearity.

After this calibration curve was obtained, a study was made to see whether or not undecylenic acid could be determined in the presence of azelaic acid, oleic acid, or ricinoleic acid. In each case small amounts of undecylenic acid were added to weighed portions of the other acid. The results of these experiments, listed in Table I, indicate that undecylenic acid can be determined fairly accurately in the presence of these acids.

Additional quantitative studies were made with styrene and butyl acrylate. Linear calibration lines were obtained with both of these compounds, as is shown in Figure 2. These two compounds were selected in order to determine whether or not the general procedure could be used to detect terminal double bonds in styrene and acrylate polymers of low molecular weight. The calibration curves indicate that the method may be applicable and work involving these polymers is now in progress.

When various sized aliquots of a standard solution of itaconic acid were analyzed, a linear calibration line was again obtained. However, this line intersected the ordinate at a value higher than the blank. This showed that the smaller samples of this acid gave abnormally high amounts of formaldehyde and that the percentage of formaldehyde obtained decreased as the weight of sample increased. The multiplicity of functional groups in itaconic acid probably caused this discrepancy. Further work will have to be done in order to determine if the procedure can be modified to overcome this anomalous behavior.

The formaldehyde obtained from compounds containing end unsaturation is not stoichiometric and, therefore, the amount of formaldehyde found cannot be used to calculate directly the percentage of end unsaturation. The percentage of formaldehyde produced by a certain end-unsaturated compound appears to be constant over a considerable concentration range but varies somewhat with each compound. Therefore, to apply this procedure quantitatively, it is necessary to prepare a calibration curve with a pure sample of the compound to be determined and then use this curve in all subsequent determinations of this substance.

The validity of the use of such a procedure is indicated by the agreement of multiple determinations on the same compound by various chemists.

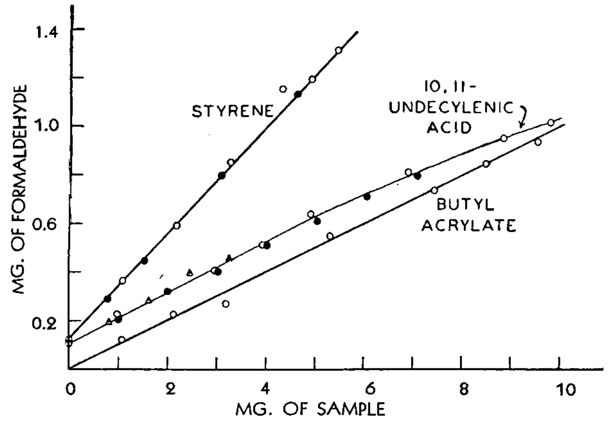


Figure 2. Calibration Curves for Various Compounds

Open circles, closed circles, and triangles show results of various chemists. All values for butyl acrylate were lowered so that lines would not intersect

INTERFERENCES

The limitations so far encountered in this procedure fall into two categories: the formation of formaldehyde from the chemical attack on compounds which do not contain terminal olefinic groups, and the interference caused by various volatile compounds on the development of the formaldehyde color with chromotropic acid.

Any organic substances other than terminal unsaturated compounds which would yield formaldehyde on treatment with either potassium permanganate or periodic acid would interfere with this method—for example, methyl alcohol or any 1,2-glycols that may be present with the original sample would cause high results.

A more serious limitation was observed when dealing with organic compounds in which rearrangements can take place. Citraconic and aconitic acids should not show any terminal unsaturation. However, small samples, less than 3 mg. of these compounds, gave abnormally high yields of formaldehyde but when larger samples were taken the amount of formaldehyde decreased as the size of the sample increased. Furthermore, the formaldehyde recovered from these acids was not too reproducible on duplicate runs. Both acids form α- and β-hydroxy acids during the glycol formation and it is possible that these structures change so that a terminal olefinic group is actually present. As such rearrangements can cause a serious interference, it would appear that this method must be confined to the determination of end-unsaturated groups which are isolated from other functional groups.

Another limitation of the method was encountered when trying to determine safrol in the presence of isosafrol. Both compounds are apparently completely oxidized by potassium permanganate and thus large amounts of this reagent must be added. This causes some difficulty in the subsequent distillation. Furthermore, the cyclic formal which is present in both of these compounds is not completely destroyed and consequently formaldehyde is found in the distillate from both of these samples. Although isosafrol gives much less formaldehyde than safrol, the difference does not appear to be sufficiently reproducible to permit a quantitative determination or separation of these compounds.

In attempting to determine end-unsaturated compounds in the presence of large amounts of benzene, phenyl ethyl alcohol, benzaldehyde, and acetone, it has been found that these compounds are distilled with the formaldehyde and cause a serious inhibition of the development of the color with chromotropic acid. Work is now in progress to eliminate this type of interference.

ACKNOWLEDGMENT

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Preparation and Colorimetric Properties of Aluminon

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Commercial preparations of the dye called Aluminon vary in quality; some are worthless for analytical purposes. Methylenedisalicylic acid as prepared here, which with salicylic acid is used to make Aluminon, was found to contain a low amount of monomer probably because phenolic resins are formed during its preparation. It is not expedient to remove all the resins but rather to keep the amount of polymer low. The molecular weight of methylenedisalicylic acid is 288. By using a product with an average value of 310 or less, a satisfactory reagent can be made and directions are given for its preparation. It is more soluble in water than the acid form of commercial Aluminon and therefore an ammonium salt is not required. Spectrophotometric measurements of different lots indicate that a reagent can be satisfactorily reproduced. Studies were made in the ultraviolet and visible portions of the spectrum of aqueous solutions of commercial Aluminons and of others made by the method described. Changes in spectral characteristics with changes in hydrogen ion concentrations were also studied. Errors which may be introduced by the presence of the colorless, carbinol "faded" form of the dye are indicated. Changes in absorbancy which accompany metal complex formation are given and typical calibration curves at selected wave lengths that may be employed with colorimeters are shown.

THE reagent Aluminon is described as the ammonium salt of aurintricarboxylic acid. Preparations of it, offered for the colorimetric determination of small amounts of aluminum, vary in quality, frequently lack sensitivity, and have caused considerable trouble in laboratories of the steel industry and elsewhere.

In the experimental work reported here, a method is described for the preparation of a reagent with properties that may be reproduced well in successive lots. Spectrophotometric measurements have been made of dyes prepared by this method and of two commercial preparations.

PREPARATION OF A REAGENT

Two procedures (*1, 5*) originally proposed for the preparation of Aluminon yield unsatisfactory products, and modifications (*6, 7, 12*) of them appear to produce little improvement.

In the method of preparation (*5*) devised by K. Sandmeyer, sodium nitrite is added to concentrated sulfuric acid and to this mixture salicylic acid is added. Formaldehyde is then slowly introduced. In the procedure of Caro (*1*), sodium nitrite is added to concentrated sulfuric acid and an intimate mixture of equivalent amounts of methylenedisalicylic acid and salicylic acid is added. The second method seems preferable, but the dye formed lacks sensitivity. Holaday (*7*) improved this procedure by adding a second portion of nitrite and sulfuric acid and in this manner improved the dye.

The amounts of monomeric aurintricarboxylic acid must be low in products made by Sandmeyer's and Caro's procedures for the following reasons. In the published discussions of the preparations of methylenedisalicylic acid it appears to be assumed that only the monomer is present. However, when a reaction mixture containing salicylic acid, formaldehyde, and a mineral acid is heated for several hours, resinous products result, doubtless of the phenol-formaldehyde type. Clemmensen and Heitman (*2*), who used a 50% aqueous solution of sulfuric acid as a condensing agent, obtained a product which after washing with hot water melted at 238° C. In the course of this work, methylenedisalicylic acid was prepared by their procedure, but only a small amount of pure monomer could be isolated from it. Kahl (*8*), who used hydrochloric acid as a condensing agent, purified the crude product of the reaction by dissolving it in a 50% hot aqueous solution of acetic acid, filtering, and precipitating the soluble material by pouring the solution into an excess of distilled water. In this way, he obtained an acid that melted at 242° C.

A rapid method for the preparation of methylenedisalicylic acid is described below. One product of this procedure was found by titration to have an average molecular weight of 300. Its melting point was 247° C. The molecular weight of the monomer is 288. Methylenedisalicylic acid with an average molecular weight of 310 or less was found to be suitable for the preparation of Aluminon with satisfactory properties. It is not necessary to use a pure monomer because of uncertainties in the subsequent reaction when Aluminon is prepared.

When monomeric methylenedisalicylic acid and salicylic acid are converted to Aluminon, two products can be formed. One is aurintricarboxylic acid and the second is formaurindicarboxylic acid which is produced by reaction with methylenedisalicylic acid alone. Kahl (*8*), who first prepared formaurindicarboxylic acid, observed that it forms colored lakes with aluminum, barium, and calcium.

From several unsatisfactory commercial preparations of Aluminon, it was possible to separate colored impurities in the following manner:

An aqueous solution of the ammonium salt was acidified, and the precipitate separated by filtration. The precipitate was dissolved in acetone, precipitated with benzene, and filtered. After three or four such treatments, a black powder separated from the acetone, probably formed by slow coagulation. When the powder was dissolved in a colorless solution of Aluminon buffered with ammonium acetate, a red color was produced.

Such colored impurities may be resinous products formed when highly polymerized methylenedisalicylic acid is used to make Aluminon. They are not present if the acid has an average molecular weight of 300 or less. The following procedure for the preparation of a reagent is based on this fact. A product of this method is more soluble in water than the acid form of commercial Aluminon. It is therefore not necessary to prepare the ammonium salt. Formaurindicarboxylic acid also was made according to this method.

Alizarinsulfonic acid is another reagent for the colorimetric estimation of aluminum and its use suggests that a sulfonated Aluminon might be advantageous because it would not require the addition of a stabilizing protective colloid. A mixture of equivalent amounts of methylenedisalicylic acid and 5-sulfosalicylic acid was treated by the following procedure for the preparation of Aluminon. The product contained 0.99% sulfur instead of the theoretical 6.37%, if only a monosulfonated aurintricarboxylic acid had been formed. Presumably some condensation had occurred. The remainder was assumed to be formaurindicarboxylic acid.

Aluminon was also directly sulfonated with fuming sulfuric acid. The percentage of sulfur found in different lots varied, but an aqueous solution of each preparation produced a color with at least 1 microgram of aluminum. During sulfonation and subsequent isolation of the sulfonated products, great care was required to prevent contamination by traces of aluminum and of other elements which form colored complexes. No further work was done on this subject because the degree of sulfonation in different products varied too much to permit the preparation of a reagent of uniform quality.

PREPARATION OF METHYLENEDISALICYLIC ACID

Reagents used for the preparation of methylene disalicylic acid (3,3′-dicarboxy-4,4′-dihydroxydiphenylmethane) are: 80 grams of salicylic acid, 7.0 grams of trioxane, 100 ml. of glacial acetic acid, and 1 ml. of concentrated sulfuric acid dissolved in 5 ml. of glacial acetic acid.

Add the salicylic acid and trioxane to the glacial acetic acid and heat the mixture to 95° C. Remove the source of heat and add to the mixture the solution of sulfuric acid in glacial acetic acid. The temperature will rise 5° or more. Probably traces of acetyl sulfate are formed which act as a catalyst for the reaction. If the solution of sulfuric acid in acetic acid is heated before it is added, a violent reaction may result.

After 5 minutes, pour the mixture into 4 liters of distilled water and allow to remain, or stir, until a clear supernatant liquid is formed, and filter. Add the filtered material to 400 ml. of a mixture of equal volumes of glacial acetic acid and distilled water and again filter. Pour the filtrate into distilled water, separate the precipitate, and wash it with successive portions of hot distilled water until all acetic acid has been removed. Dry the precipitate in air and afterward in a vacuum with a desiccant, at room temperature. The yield is 20 grams or more.

The molecular weights found by titration of several lots prepared by the procedure above varied between 298 and 304. With one of them a value of 301 was found; and with this lot, by ebulliometry, the Physical Chemistry Section of this bureau obtained a value of 300.

PREPARATION OF ALUMINON

In the following procedure, a mixture of methylenedisalicylic acid and salicylic acid is treated twice as recommended by Holaday (*7*).

Reagent A is made up of 20 grams of methylenedisalicylic acid prepared by the above procedure, and 10 grams of salicylic acid. The acids are finely powdered and intimately mixed.

Reagent B. Two portions are made: 60 ml. of concentrated sulfuric acid and 10 grams of sodium nitrite.

Cool the sulfuric acid to the temperature of crushed ice. Powder the nitrite in an agate mortar and mix quickly small portions with the acid to avoid the formation of red fumes.

Surround with ice one portion of Reagent B contained in a 400-ml. beaker. Stir small quantities of A into it with a thermometer at a rate that permits the temperature to be held not higher than 5° C. After all has been added, allow the mixture to remain in ice for about 1 hour. Then add and mix the second portion of B which has been chilled to 5° C. or below. After another hour, remove the ice and allow the mixture to remain at room temperature for about 12 hours. Pour the mixture, in small amounts while stirring, into 4 liters of distilled water previously cooled to about 2° C. and filter at once with suction. Add the filtered material to 2 liters of distilled water, stir well, and again filter. Wash the filtered material once, and allow it to dry first in the air and then in a vacuum over a desiccant at room temperature. The yield is 22 grams or more.

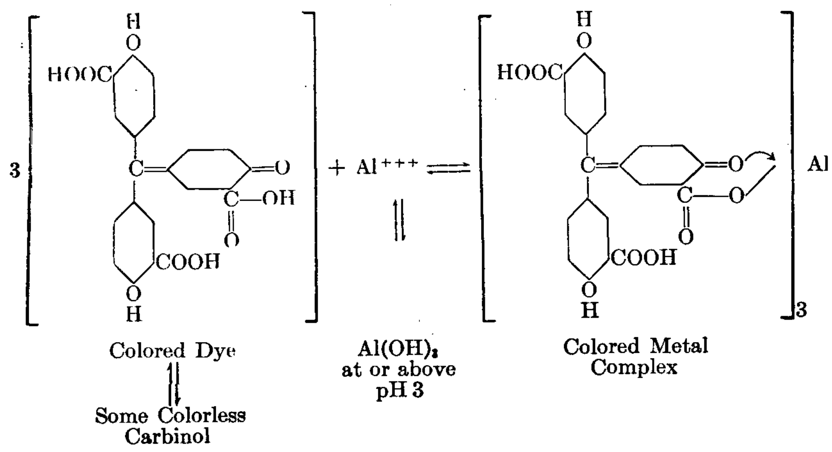
COLORED COMPLEX OF ALUMINUM FORMED BY ALUMINON

Most dyes do not exist as "pure" organic compounds. Extensive studies have recently been made on the rate of fading of many sulfonephthalein indicators in strongly alkaline solutions (*11*). The indicators were of the highest purity obtainable, but the spectrophotometric data indicated that in the purified crystalline indicator some of the dye existed as a colorless carbinol form. The same may be true in the case of Aluminon. Changes in color of aqueous solutions may indicate instability. Not only may some colorless carbinol form of the dye be present but in this case some dicarboxylic acid may be mixed with tricarboxylic acid. The amount of colorless carbinol present in the dye may depend upon the methods and conditions under which the dye is synthesized. For instance, a freshly prepared solution of a dye containing some carbinol may deepen in color for a few days if the pH of the solution is favorable.

For compounds of this type some knowledge of the various equilibrium relationships or the dissociation constants of the carboxyl groups and of the hydroxyl groups is essential for optimum utilization. The dissociation constants of a simple molecule such as salicylic acid are easy to evaluate.

Expressed as pK, the negative of the logarithm of the dissociation constant, pK_1 for the carboxyl group is about 4 and pK_2 for the hydroxyl group is about 10. In the case of aurintricarboxylic acid, however, the dissociation of the groups may not occur simultaneously and may overlap. Thus, the constants can only be approximated.

In metal complex formation, the following reactions may occur:



One would not expect the formation of chelated compounds to require much time. However, suppression of such complex formation may occur if the pH is substantially lower than that of the carboxyl dissociation. The possibility also exists that some of the dye itself may be in its colorless carbinol (faded) form, and this would apparently retard the complete reaction. At pH 3 and higher, insoluble aluminum hydroxide is formed. At much higher pH, the dye itself develops a more intense color, but the rate of fading is also high. Therefore, maximum color development probably cannot be achieved and a compromise must be made. Using the reagent Aluminon as free acid buffered at pH 4.2, the complex formation with aluminum is practically complete in 20 minutes at room temperature (25° C.). Under these conditions it is not necessary to use heat or stabilizers such as gelatin or gum arabic.

The introduction of a sulfonate group ortho to the central carbon atom in the phthalein indicators greatly increased the solubility and stability (*3*). Thus we have the familiar series of stable indicators, the sulfonephthaleins. In an analogous case perhaps an orthosulfonic acid group in the aurintricarboxylic acid would make the acid stronger and more soluble. Assuming that a sulfonate group would also form chelated compounds, the reaction could take place at a lower pH and a metal complex probably would be more stable. However, the synthesis of such a compound as an Aluminon reagent is a problem in itself.

SPECTROPHOTOMETRIC MEASUREMENTS

Several workers have reported on the instability of the reagent Aluminon under various conditions. All used the ammonium salt of the reagent.

Winter, Thrun, and Bird (*13*), using a Duboscq colorimeter, reported maximum color formation in 10 minutes at pH 4.5 to 5 after heating to 80° C. They noted that the dye itself is decolorized at pH 7 and also that the lake begins to fade at pH 7.3. Craft and Makepeace (*4*) using a Coleman spectrophotometer also made an extensive study of the visible portion of the spectrum. Using ammonium acetate buffers, they studied the effect of pH upon the development of color which accompanies the formation of a metal complex. Roller (*10*) noted that the yellow color of the dye in alkaline solution and its red color in acid solution practically neutralize each other at pH 6.3 and suggested the use of this pH in which the blank of buffer and dye is practically colorless. The importance of a precisely determined calibration curve is also emphasized by Olsen, Gee, and McLendon (*9*).

Preliminary measurements with a Beckman spectrophotometer at this bureau confirmed these results and showed stock solutions of the ammonium salt of aurintricarboxylic acid to be unstable. The absorbancies of the aluminum complexes changed over a period of time even when buffered at different pH values. Some changes were noted in the ultraviolet as well as in the visible portion of the spectrum. Hence, spectrophotometric measurements were made at frequent wave-length settings over a spectral range of 200 to 400 mμ. The solutions were prepared and kept at a constant temperature of 25° C. Hydrogen-ion concentrations were controlled by use of acetic acid–sodium hydroxide buffers or of various concentrations of hydrochloric acid or of sodium hydroxide. pH measurements were made of all solutions by use of a commercial glass-electrode assembly.

The curves in Figure 1 illustrate the differences in samples of Aluminon obtained from two different sources and in three different lots of dye (free acid) prepared at this bureau.

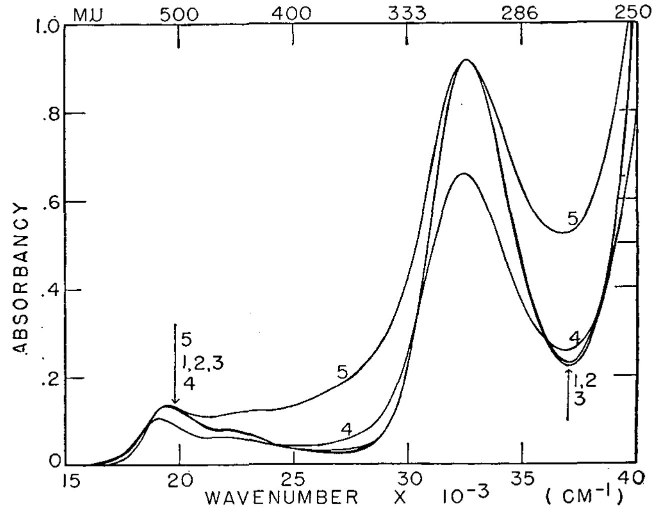


Figure 1. Comparison of Absorbancies of Samples of Aluminon from Different Sources

All the dyes were in 5 × 10^{-5} molar aqueous solutions and were measured relative to water in 2-cm. absorption cells. Transmittancy readings were made on the Beckman instrument at 2 to 5 mμ intervals. In Figure 1 absorbancy values (absorbancy = $-\log_{10}$ transmittancy) are plotted as a function of wave number ($1/\lambda$) with the corresponding wave lengths shown at the top of the graph. Curves 1, 2, and 3 in Figure 1 represent the dyes synthesized at the bureau, and it is readily seen that they are reproducible. Curves 4 and 5 represent the two commercial dyes and show differences throughout most of the spectral range. Curve 5 shows a shift in the wave length of maximum absorption in the visible, which also denotes a change in hue of this dye. Because metal complex formation increases the absorbancy values of the bands in the visible, it is easily seen that in the determination of the same amount of aluminum the two commercial dyes would give different results.

The curves in Figure 2 show the absorbancies of formaurindicarboxylic acid (curve 1) and aurintricarboxylic acid (curve 2).

Changes in color of the reproducible free acid dyes with change in hydrogen ion concentration were then studied. Transmittancy measurements of 5 × 10^{-5} *M* dye in buffers were made relative to the respective buffers in 2-cm. absorption cells at 25° C. The two sets of curves in Figure 3 represent the various

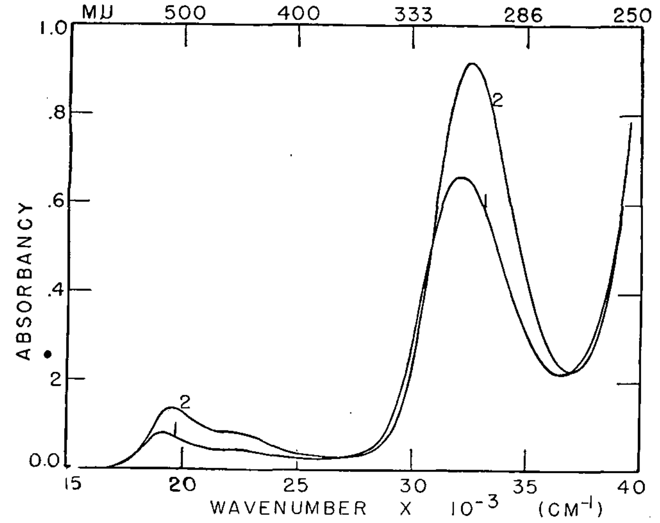


Figure 2. Comparison of Absorbancies of Formaurindicarboxylic Acid (1) and Aurintricarboxylic Acid (2)

stages in the overlapping dissociation of the carboxyl groups in aurintricarboxylic acid.

There is first a slight increase in color with decrease in hydrogen ion concentration (increase in pH), and then a decrease in color. The first group of curves, 1 through 5, inclusive, shows the dye at pH values 3.4, 3.9, 4.2, 4.4, and 4.6, respectively. The second group of curves, 5 through 12, represents the dye at pH values 4.6, 4.8, 5.0, 5.2, 5.4, 5.5, and 5.8, respectively. At pH 5.8 the solution is almost colorless, as shown by the higher transmittancy values at about 525 mμ. The changes in the main band in the ultraviolet are slight. Above pH 7, the dye changes to a deep pink color. The pink color at about pH 12.0, however, is fugitive. The fading reaction may be somewhat similar to that reported for the stable sulfonephthalein indicators in great excess of alkali (*11*), or of phenolphthalein (*3*).

In the case of Aluminon, only the practical aspects of the problem were considered, and the results of the fading reaction are shown in Figure 4.

Transmittancy measurements of 10^{-4} *M* dye with enough sodium hydroxide added to give a pH of 11.9 were made in 1-cm. absorption cells. The spectrophotometric measurements were made as soon as possible after addition of the alkali, and at stated intervals thereafter. Curves 1 through 7 were made at the end of 0.5, 1, 2, 3, 4, 5, and 24 hours, respectively. The initial deep pink color in the visible gradually decreases until a slightly colored equilibrium mixture is obtained at the end of 24 hours. After this there is little change in the visible, but significant changes in the molecule are indicated by the ultraviolet data, particularly at about 380 and 275 mμ. Curves 8 through 12 represent the faded dye after 2, 5, 7, 9, and 15 days, respectively. The use of stronger alkali would accelerate the rate of initial fading and perhaps show greater changes in the ultraviolet.

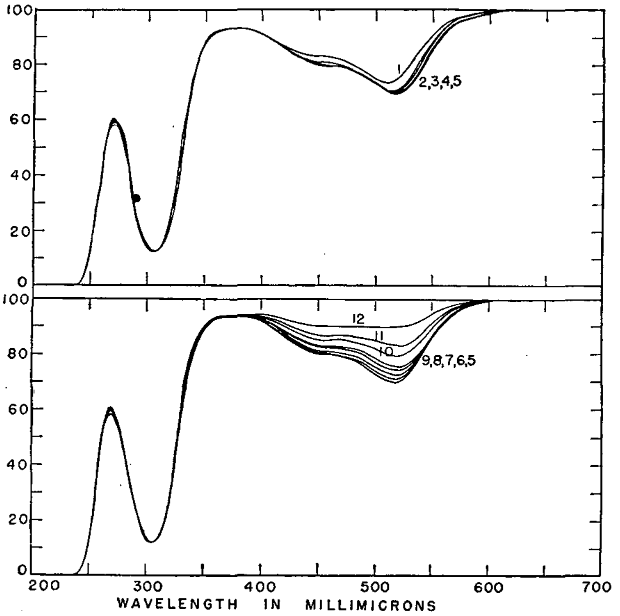


Figure 3. Effect of pH on Spectral Characteristics of Aluminon

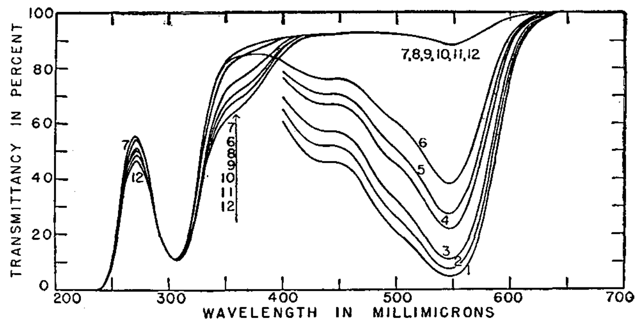


Figure 4. Alkaline Fading of Aluminon at pH 11.9

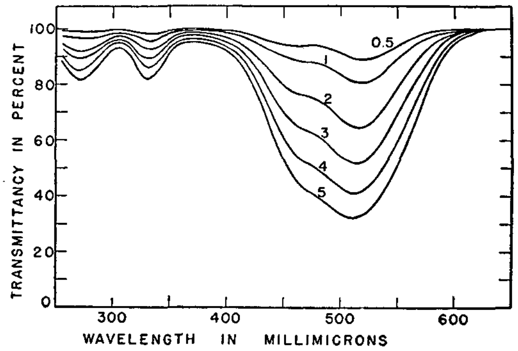


Figure 5. Transmittancy of Aluminum Complex of Aluminon Formed at pH 4.2 Relative to Dye Alone at pH 4.2

The data demonstrate the seriousness of errors which can be introduced if the dye is subjected to treatment with alkali, or, if in making up stock solutions, alkali is added to dissolve the dye, then allowed to stand before adjusting the pH downward with acid. In the case of the sulfonephthalein indicators, the reaction was for the most part reversible, and color could be regenerated by bringing the solutions again to the favorable lower pH. Aluminon, however, seems to be permanently changed after prolonged reaction with sufficient alkali. The technique formerly used in the determination of aluminum, which consisted of roughly adjusting the pH by means of ammonium hydroxide and hydrochloric acid, may be responsible for some variation in the results. Not only should the stock solution of Aluminon reagent be promptly adjusted to a pH of about 4.2, but the test solution should also be brought to this same pH before formation of the complex for the most accurate results.

In studying the changes in spectral characteristics which accompany aluminum complex formation with Aluminon, several series of observations were made throughout the ultraviolet and the visible, using different buffers and various amounts of dye and of aluminum. The curves shown in Figure 5 are representative of such a series made at pH 4.2. The transmittancy measurements of the aluminum complex were made relative to the initial amount of the dye itself, in buffer. It is recognized that some of the dye is taken up by the complex and that the amount must vary with the amount of aluminum. Water has been used in the solvent cell, or buffer, but the most precise calibration curves were obtained by using the buffered dye in the solvent cell. By following such a procedure, the transmittancy values are as shown in the figure. The ultraviolet data are of little use but were obtained in order to get an over-all picture of the complex formation. The curves represent the addition of 0.5, 1, 2, 3, 4, and 5 ml. of 2×10^{-4} *M* aluminum chloride in 100 ml. of buffered dye.

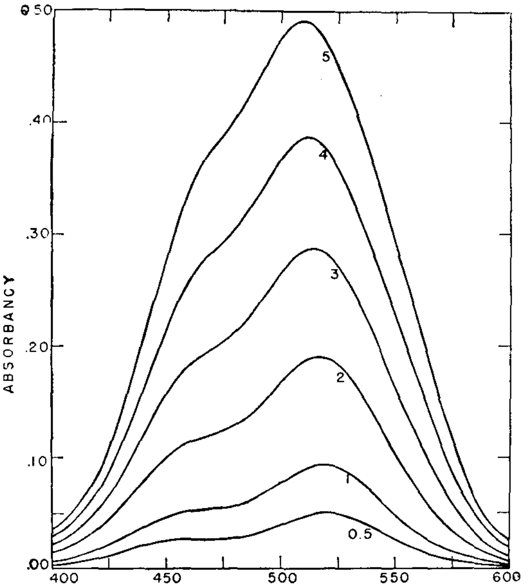


Figure 6. Absorbancy Values of Aluminum Complex of Aluminon Relative to Dye Alone

On the absorbancy basis, the same data are given in Figure 6 for the visible range. The numbers on the curves are the same as those of Figure 5 and represent the same amounts of aluminum. It is clearly seen that the metal complex makes an appreciable increase in absorbancy throughout the bands in the visible. Using the same data for calibration curves, the absorbancy values at stated wave lengths are plotted as a function of the amounts of aluminum in 100 ml. of solution as shown in Figure 7. Any of the selected or other wave lengths may be used. However, near the wave lengths of maximum absorption, straight lines show conformity to Beer's law, while some curvature may be expected over the steeper portions of the bands. For use with a colorimeter using a 525 mμ filter, for example, one would expect to obtain straight-line calibration curves if the photocell response of the particular colorimeter used is linear. It is not necessary to use a spectrophotometer for accurate results in routine analysis, but it is desirable to make a precise calibration curve for each lot of dye regardless of the instrument used.

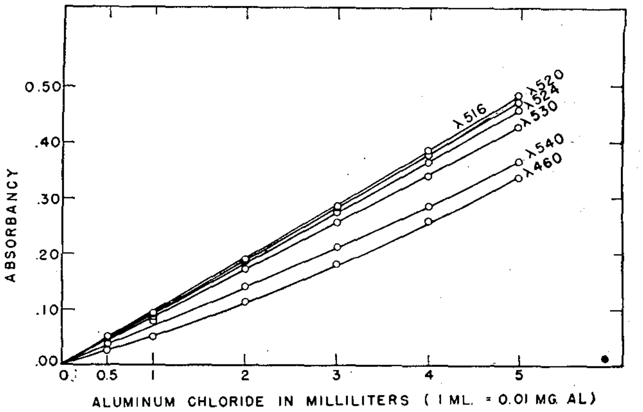


Figure 7. Calibration Curves

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Spectrophotometric Study of Spot Tests

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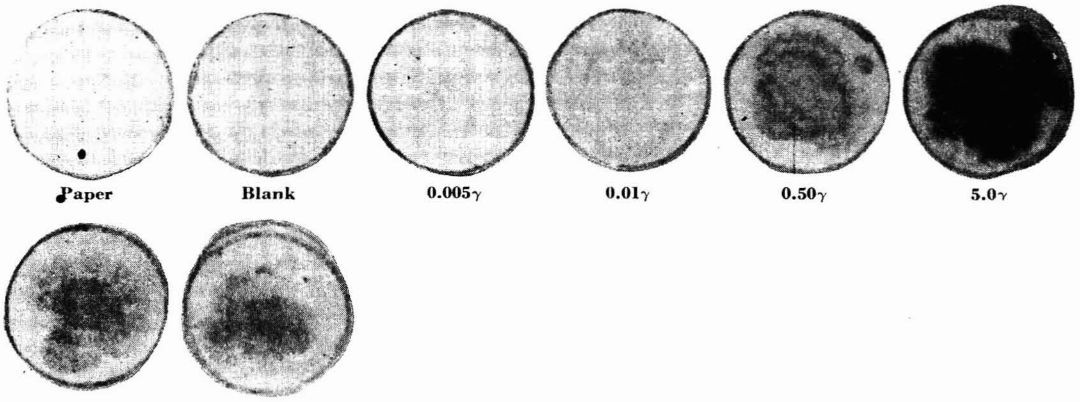
ABOUT ten years ago, an attempt was made in this laboratory to answer certain fundamental questions about spot tests by measuring the spots on a General Electric recording spectrophotometer (*4*). In particular, the authors hoped to discover whether the results of reflectance and transmittance measurements ever obey Beer's law, and whether one type of measurement is preferable to the other. Two tests, radically different in their chemistry, were chosen for investigation—namely, the detection of cupric ion with α-benzoin oxime, and the identification of silver by the deposition of that element through the action of a developer containing silver ion on exposed silver bromide derived from the silver in the sample (*2*, *3*). The results of this work are intrinsically valuable, and they have a bearing on recent efforts to make filter-paper chromatography quantitative (*1*, *5*).

If a series of spots is to be evaluated on the spectrophotometer, it is desirable to have the beam incident upon a constant fraction of the sample. If this fraction is constant, it need not be known. If it varies, its values will have to be known if the consequent deviations from Beer's law are to be calculated. The simplest way of satisfying the requirement of constancy is to distribute the spot uniformly over a constant area, which overlaps the incident beam. The copper spots were confined by using Yagoda test papers with an inner circle 1.2 cm. in diameter. The silver

The α-benzoin oxime test for copper and the identification of silver by the action of a photographic developer were studied spectrophotometrically in transmittance and in reflectance. The results indicate the former type of measurement to be preferable for the quantitative evaluation of spot tests, and show that such tests can give transmittance data closely in accord with Beer's law.



Paper Blank 0.005γ 0.01γ 0.50γ 5.0γ

0.10γ 0.05γ

Figure 1. Silver Spot Tests Obtained by Action of Photographic Developer

Diameter of circles is 1.6 cm.; that of mask 1.1 cm. Upper row. 1 Metol treatment. Dried at 105° C. Lower row. Dried at 28° C. *Left.* 1 Metol treatment. *Right.* 2 Metol treatments

was deposited on filter paper, as uniformly as possible, within a circle 1.6 cm. in diameter. (The black silver deposits, Figure 1, were much less uniform than those of the green copper compound.)

Masking was employed in order to make certain that the measured changes in reflectance or transmittance were due entirely to the sample. To accomplish this, a twofold mask of white paper was used in the former type of measurement, and a single black mask in the transmittance work. Both kinds of masks had circular holes 1.1 cm. in diameter; the beam was appreciably larger.

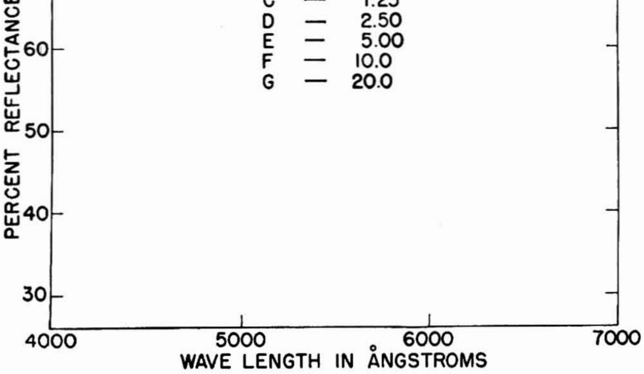


Figure 2. Reflectance Curves for α-Benzoin Oxime Spot Test for Copper

For the systems under investigation, Beer's law may be written in a form

$$\text{Log } I_B/I = k(m) \qquad (1)$$

identical with the equation used to evaluate measurements of x-ray absorption. In Equation 1, I_B and I represent the radiant energies of a given wave length emergent, respectively, from the "blank" and from the sample, k is a proportionality constant, and m is the mass in micrograms of the sample. The experimental results are evaluated by plotting $\log I_B/I$ against m.

COPPER TEST

Reflectance Measurements. For both reflectance and transmittance tests, the center areas of the Yagoda test papers were impregnated with α-benzoin oxime. A 0.02-ml. drop of a solution containing 4% by weight of the oxime in 95% ethyl alcohol was spread uniformly over the area. After 5 minutes, evaporation of the solvent was complete at room temperature and the paper was ready for use.

The desired quantity of copper was then added (as sulfate) to the impregnated center area in a drop of 0.02-ml. volume. A 2-minute exposure of the paper to the fumes of concentrated ammonium hydroxide followed immediately. Reflectance measurements (Figure 2) were then made on the green spots that appeared in the center areas.

The reflectance data for 4400 Å. were used as a basis for the curve in Figure 3, the value of I_B being taken from curve *A*, Figure 2.

According to Figure 3, Equation 1 seems to apply to these data only as a limiting law for amounts of added copper smaller than, say, 1 microgram. This state of affairs is entirely reasonable. Inasmuch as α-benzoin oxime was added in large excess (800 micrograms), lack of this reagent could scarcely be responsible for the curvature in Figure 3. It seems likely, however, that the proportion of added copper fixed at or near the surface by the α-benzoin oxime decreases as the amount of added copper increases. The cupric ion escaping into the paper has little or no effect on reflectance. The law of diminishing returns is consequently responsible for the shape of the curve in Figure 3. The fact that Equation 1 applies to the results of transmittance measurements (see below) strengthens the foregoing argument, for such measurements should indicate all the copper in the paper.

It is obvious from Figure 3 that the limit of identification for these reflectance measurements is comparable with 0.05 to 0.1 microgram as given by Feigl (*3*).

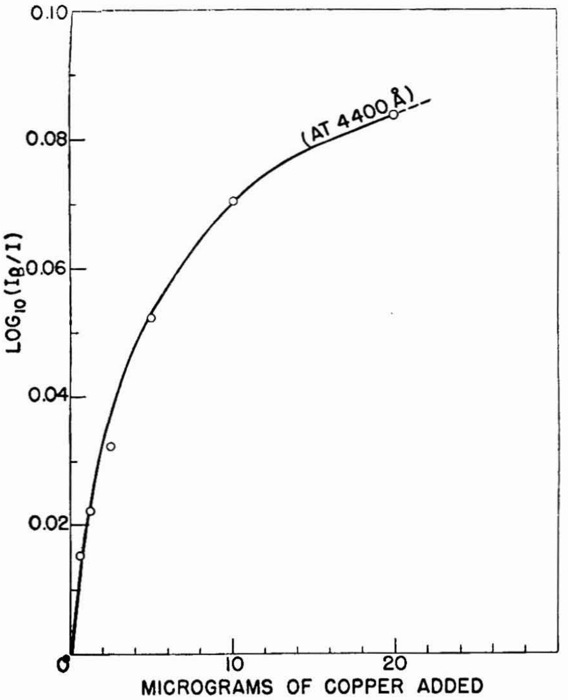


Figure 3. Beer's Law Plot for Data of Figure 2

Transmittance Measurements. Even had the reflectance data been in accord with Equation 1, transmittance measurements on similar spots would have been welcome as permitting a comparison of the two techniques. Owing to the absorption of radiant energy by the relatively thick paper, the transmitted beams were of low intensity, and a special cam that magnified the readings fivefold was used in the spectrophotometer. Furthermore, the papers (Yagoda No. 598) varied enough in thickness to make advisable an individual determination of I_B for each paper.

Except for the following slight modifications, the spots for transmittance measurements were prepared as described above.

After the alcohol had evaporated in the impregnation with α-benzoin oxime, a 0.01-ml. drop of distilled water was allowed to fall on the center area, which was then exposed for 2 minutes to the fumes of concentrated ammonium hydroxide. After about 5 minutes, when the paper had dried, the transmittance of the center area was measured to establish I_B. The desired amount of cupric ion was then added in a drop of 0.01 ml. After the color had been developed by a second 2-minute exposure to the ammoniacal fumes, the transmittance was again measured to establish I. The results are given in Figures 4 and 5.

The most striking feature of the transmittance data is their excellent agreement with Beer's law. The small intercept on the ordinate axis of Figure 5 probably results from a slight decrease in transmittance, not attributable to added cupric ion, that occurs between the transmittance measurements that establish I_B and I—for example, the impregnated paper may have darkened a little. (The intercept in Figure 5 corresponds to a decrease in transmittance from only 15 to 14%.) Although the distribution of absorbing centers is probably uniform in the plane of the paper, this distribution necessarily undergoes a sharp negative gradient in the direction of the beam. Figure 5 is thus experimental proof that Beer's law can be valid (or nearly so) even when the concentration of absorbing material is not constant.

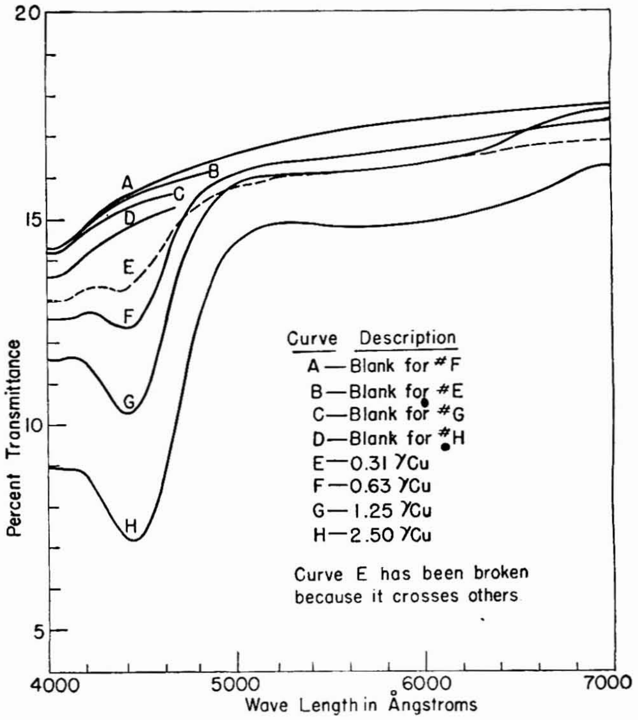


Figure 4. Transmittance Curves for α-Benzoin Oxime Spot Test for Copper

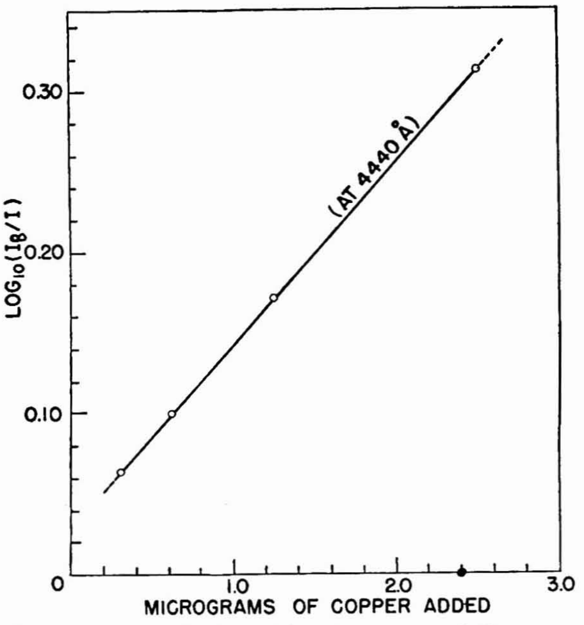


Figure 5. Beer's Law Plot for Data of Figure 4

A comparison of the reflectance and transmittance data leads to these conclusions: The reflectance and transmittance curves for the green compound formed by α-benzoin oxime and cupric ion resemble each other. Only the latter curves give data in agreement with Equation 1 over a wide range of log I_B/I values. The maximum value (near 4440 Å.) of this quantity per microgram of added cupric ion is 0.02 in reflectance and 0.11 in transmittance, to the considerable advantage of the latter technique. Consequently, the limit of identification is considerably the lower in transmittance.

The foregoing work was successful in that it yielded fundamental information about spot tests. When an attempt was made several years later to apply the method in a practical problem, some difficulties appeared. The Yagoda papers then available were so thick that the spectrophotometric results suffered from too great a reduction in the intensity of the beam. The transmittance data were less satisfactory when the sample contained only a trace of copper because repressing the interference of metallic cations present in large excess greatly reduced the sensitivity of the test (*3*).

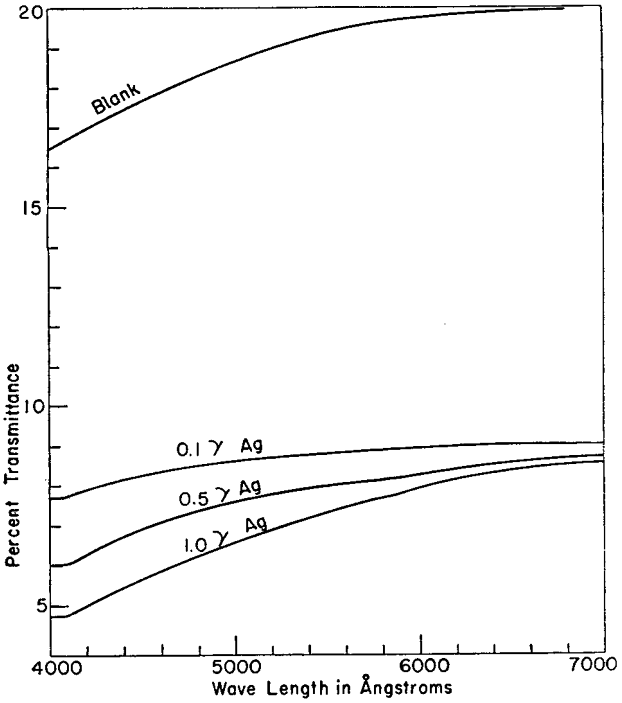


Figure 6. Transmittance Curves for Silver Spot Tests (Single Development)

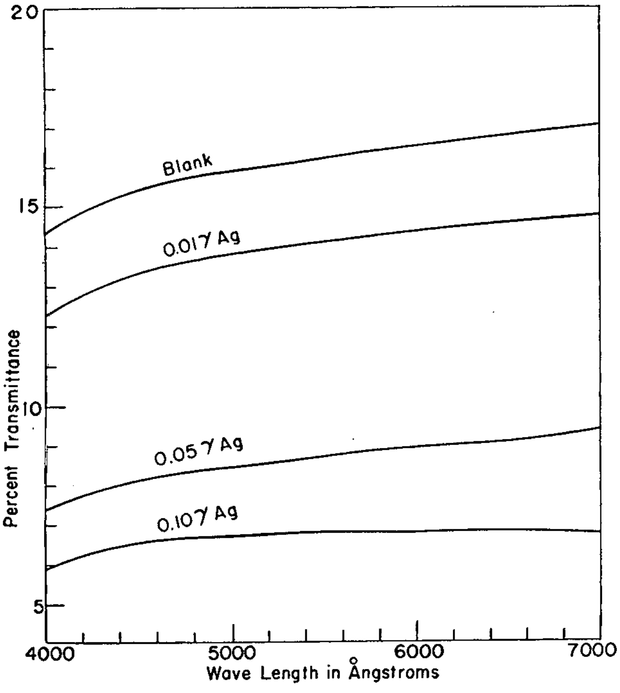


Figure 7. Transmittance Curves Obtained for Spot Tests (Double Development)

SILVER TEST

It is well known that silver can be precipitated by the action of a photographic developer containing silver ion upon a paper in which the silver ion to be identified has been fixed as the bromide (*2*). Transmittances were measured for two series of silver spots prepared as follows:

Single Development. The silver ion to be added, contained in a 0.01-ml. drop, was placed at the center of a circle about 1.6 cm. in diameter drawn in pencil on a piece of filter paper (Schleicher and Schuell, No. 589). The paper was then soaked for 0.5 minute in 0.02 *M* potassium bromide solution in order to fix the silver. Excess potassium bromide was then removed by soaking the paper in eight changes of distilled water within the next 5 minutes. The developer solution was then prepared according to the directions of Feigl by adding silver nitrate to a Metol (*p*-methylaminophenol sulfate) solution containing citric acid; 0.07% of gum arabic was added as stabilizer. The paper on which the sample had been fixed was submerged in the developer solution for 12 minutes in the dark. It was then removed, rinsed successively in distilled water and in acetone, and dried at room temperature. Transmittance measurements were then made with the results given in Figure 6. The special magnifying cam was used in the spectrophotometer for all transmittance measurements on silver samples.

Double Development. To increase the sensitivity of the test, a series of silver samples was put through two complete developments; the developer solution was renewed but the technique was otherwise unchanged. The transmittance curves for this series are given in Figure 7.

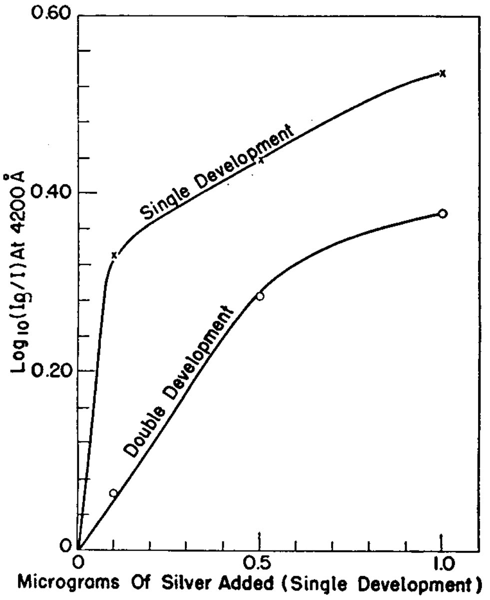


Figure 8. Beer's Law Plots for Data of Figures 6 and 7

Figure 8 is a plot based upon Equation 1, for both series of silver tests. Clearly, the data are not concordant with Equation 1. This is no doubt due in part to the irregular way in which the silver was deposited (see Figure 1), but the more complex chemistry of the test must also be held responsible. Figure 8 shows (1) that the test is extremely sensitive, the lower limit of identification (at least with double development) being near the 0.005 microgram given by Feigl (*2*); (2) that the second development increases the sensitivity; and (3) that, as the silver bromide initially fixed increases in amount, it soon becomes less effective in promoting the deposition of silver.

The foregoing method for estimating silver has been successfully applied in practical problems. Because the silver in the sample is separated from the other constituents when it is fixed as the bromide, the method has a decided advantage over the α-benzoin oxime test for copper, in which repressing the interference of other ions reduces the sensitivity of the test. The precision with which silver can be determined by the transmittance measurements described above is difficult to estimate, but it probably approaches ±10% under the best conditions.

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2nd Annual Summer Symposium — Organic Reagents

Organic Reagents in Inorganic Analysis

Sources of Error and Interferences

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Such problems as reagent isomerism and tautomerism, isomerism of reaction products, and variations in coordination numbers may be mentioned as peculiar but important aspects in the use of organic reagents for inorganic analysis. A general survey of these and other problems connected with the use of organic compounds for qualitative and quantitative work is presented.

THE general applicability of organic reagents for inorganic analysis seems to compare most favorably with that of inorganic reagents from almost every standpoint. On the basis of reliability, which is the sole consideration covered in the present discussion, they can be assumed to possess a number of distinct advantages. This discussion is therefore introduced not as a deterrent to the use of such reagents but rather in the hope that by directing attention to the modes of interfering reactions and the sources of general errors, more rapid development and utilization of organic reagents for analytical purposes can be promoted.

Brief mention is justified of some of the factors contributing favorably to the use of organic reagents when considered on the basis of reliability. A very significant characteristic of many such reagents is a high degree of selectivity, or even specificity, when applied under appropriate conditions. Specificity is one of the most important attributes in analytical chemistry, and although progress of real significance along such lines is already marked (*1–3*), continued development can be anticipated through the invaluable investigations of such men as Feigl, Baudisch, Yoe, Kolthoff, and other brilliant pioneers in this phase of scientific development.

Factors other than specificity add to the reliability of organic reagents. Inorganic-organic compounds encountered in gravimetric analyses usually have such low solubilities that solubility effects are of minor significance. Such substances, particularly of the chelate type, are usually nonhygroscopic in character, which is a most important attribute for gravimetry. Of prime importance, however, are the very favorable equivalent weights of practically all inorganic-organic precipitates, such that very small chemical factors occur in gravimetry; in titrimetric procedures involving indirect methods such as the bromate technique (iodometric), equivalent weights of metallic ions may be as little as a tenth or even $^1/_{24}$th of the ionic weight.

PURITY OF REAGENTS

One would be naïve indeed to assume that analytical chemists fail to appreciate the importance of purity of reagents. Organic reagents are of somewhat special character as compared to inorganic compounds. Someone once said that organic chemistry is the chemistry of reactions and side reactions. Organic reagents are sometimes contaminated with impurities—these may be derived from side reactions in the process of manufacture or they may result from general instability of the reagents or decomposition of their solutions. An interesting but extreme example of the importance of impurities in organic reagents has been noted by West and Hamilton (*9*), who have obtained evidence that resorcinol, which is often used as a reagent for the colorimetric detection and determination of zinc, is not itself active in this instance. Instead, an impurity present only as minute traces in resorcinol serves to produce the test color. Fales (*1*) has called attention to an important instance where impurities present in a reagent may lead to unexpected error. He has noted that 1-nitroso-2-naphthol may contain an impurity which reacts with nickel to give a precipitate. This impurity was found in a number of commercial samples of the reagent as well as in material freshly prepared in the laboratory. The indication in this case is that some side reaction occurs in the normal preparative procedure to produce the active impurity. That the 1-nitroso-2-naphthol is itself stable is evidenced by the observation of Fales that solutions of the reagent that have stood for a few days can be used with confidence, because the deleterious impurity settles out as a brown precipitate.

ERRORS IN QUALITATIVE ANALYSIS

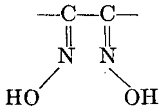
Errors resulting from the use of organic reagents for qualitative inorganic analysis usually take the form of simultaneous competitive reactions. In fact, a very important form of interference, the so-called "negative" or "masking" interference, is due very often to concurrent reactions which involve the reagent and reduce its effective concentration so low that the characterizing test reaction is inhibited. The more generally understood type of interference is the one involving the formation of reaction products similar to those of the true test reactions. Such false tests are most often due to the presence of ions which are very similar to the ion sought, so that analogous reactions occur which may be indistinguishable from the test reaction itself. Detailed consideration of the topic of interferences encountered in qualitative analysis has been included in a paper by West (*8*).

ERRORS IN QUANTITATIVE ANALYSIS

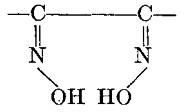
A wide variety of sources of error is possible in the application of organic reagents to quantitative work. Fortunately, such possibilities seldom materialize, and by careful attention to the details of procedure most potential hazards can be avoided.

Gravimetry. Probably the most general source of error in gravimetric work results from the low solubility of many organic precipitants in water. Excess reagents, when added to aqueous solutions, may result in the precipitation of the reagent and consequent contamination of the precipitate. Such contamination obviously introduces serious error where the reaction product is to be dried and weighed as such. True, it is often expedient to convert such precipitates to the form of the metal oxides before weighing and thus obviate errors due to contamination by the reagent, but such procedures invalidate one of the significant advantages of organic precipitants—the possibility of very favorable equivalent weights. It seems preferable, therefore, to recognize the source of such error and then circumvent it by carefully avoiding harmful excesses of reagent; most precipitates involved are so insoluble that the addition of excess reagent for mass action effects is unnecessary.

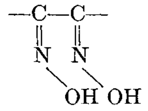
Isomerism is somewhat overlooked in analytical chemistry but cannot be avoided as an important source of error. Isomeric forms may be encountered in the reagent itself and in the reaction product; in either case wide variation in analytical chemical behavior may result. The well known case of the reactivities of the dioximes may be cited in this regard, inasmuch as such wide differences exist between the α-, β-, and γ-dioximes in connection with their reactions with metallic ions. The configuration of these isomers is depicted as:



α

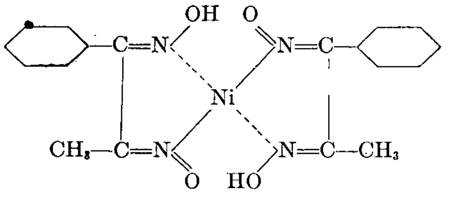


β

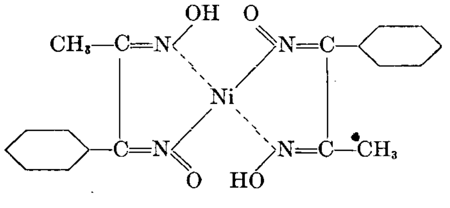


γ

The α form is highly selective in its reactions, the β isomer gives no reaction with metals, and the γ form reacts with many metals but is of no value because of its almost total lack of selectivity. A second type of isomerism involves the reaction product itself and was considered as early as 1905 by Tschugaeff (*7*). Where unsymmetrical molecules are employed, cis-trans isomers may be formed which vary considerably in their physical characteristics. Sugden has shown (*6*) that methylbenzylglyoxime can form such isomeric compounds in its reactions with nickel, as shown by formulas I and II:



I



II

He was able to produce these products, identical in molecular weight, but differing in both solubility and melting point. Obviously, such situations are of importance to the analyst, although equilibrium conditions ordinarily exist so that under controlled conditions of analytical experiment, products of reproducible properties are obtained. In the case cited above, for example, the α form was found to melt at 168° C. while the β form melted at 75–77° C. The β isomer was thermolabile and was rapidly transformed at 120° C. to produce an equilibrium mixture melting at 152° C. It can be assumed that the solubility of the mixture would vary at different temperatures and in different solvents and that change of solvent would affect the equilibrium position; such a precipitate would require careful study before reliable analytical procedures could be devised.

Coprecipitation and postprecipitation phenomena may also be sources of error in the gravimetric application of organic reagents, but little positive evidence is available to show the extent. A survey of accepted procedures employing organic precipitants fails to disclose references to such difficulties and it is interesting to speculate concerning the effect of coordination on the tendency toward coprecipitation. In the case of chelate formation where coordination spheres of the central atom are filled, little coprecipitation should occur through adsorption. In such cases, precipitation itself should be a more complex process than where normal salt formation is involved; as a consequence, there should be a reduced tendency for occlusion and mixed crystal formation because not only the ionic radii of the coprecipitating ions would need to be of the same order of magnitude as the ion being determined but, in addition, the coprecipitating ions should have corresponding tendencies toward coordination. Because of the relatively large size of organic precipitants, few inorganic ions can replace them in a crystal lattice without undue strain—another condition minimizing the likelihood of occlusion.

Low results sometimes are caused by competitive reactions which reduce the effective concentration of the reagent. For example, in the determination of nickel by precipitation with dimethylglyoxime in the presence of zinc, cobalt, and copper, extra amounts of reagent are required because of the formation of soluble complexes of these metals.

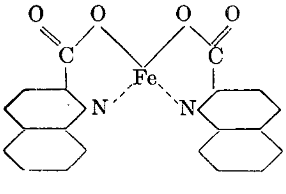
The volatility, together with the decomposition, of precipitates constitutes an important source of error. Especially in the case of chelate compounds, volatility of precipitates is appreciable and low temperature drying will usually be required for accurate work. In cases where precipitates must be ignited, great care should be taken and it is well to add oxalic acid or ammonium nitrate to the precipitate prior to the ignition as a means of obviating excessive volatilization.

Titrimetry. Few errors in titrimetric analysis involving organic reagents can be attributed solely to the use of organic compounds. Purity of the reagents can be a factor, especially where oxidimetric methods are used, and, similarly, stability of the reagents and of the reaction products should be considered. Where the bromate procedure is employed, volatilization of bromine or of the iodine liberated can constitute a serious source of error.

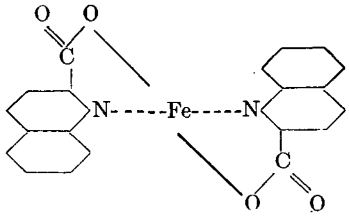
One of the most serious criticisms of many titrimetric procedures is the utilization of empirical reactions in indirect technique. However, such methods are giving good results and although some of the reactions employed may be complicated, they are often stoichiometric in nature.

Colorimetry. Three main types of errors in colorimetric work are generally peculiar to organic reagents: isomerism of reagents and reaction products, tautomeric shifts, and competitive reactions. In the case of isomerism the most important types would seem to involve reaction products. Such possibilities can be shown by reference to the investigation of Ray and Bose (*4*) of the cis-trans isomerism of ferrous quinaldate. See structural formulas III and IV, next page.

Two forms, differing in color, were demonstrated. In the case



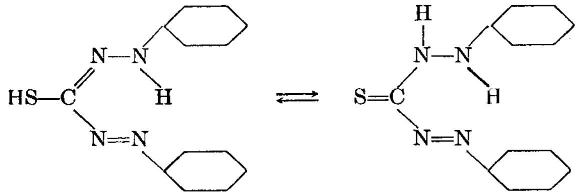
III



IV

cited, a red compound representing one of the isomers proved to be unstable and was transformed into a stable violet form. Although this illustration is mainly of academic interest, potential dangers of this type of phenomenon are being encountered in practice.

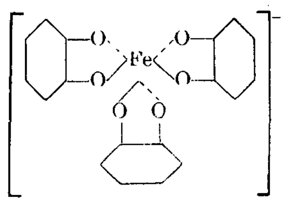
Tautomeric equilibria are of real importance in colorimetric work: the well known example of diphenylthiocarbazone serves to illustrate this. Diphenylthiocarbazone exists in either the keto or enol forms, depending on the pH of the solution.



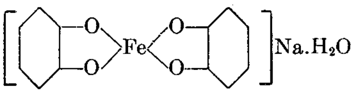
It is well known that the form of the reagent affects its reactions and thus becomes a factor in quantitative analysis. In most cases involving tautomerism, careful control of the hydrogen ion concentration serves to establish reproducible conditions.

The significance of competitive reactions in colorimetry is of special interest because so many of the color-forming reagents lend themselves readily to complexation. Where soluble but colorless complexes are formed by diverse ions, low results may be caused because of the lowered concentration of the reagent. The practical importance of this phenomenon is attested to by the fact that even the reliable 1,1′-bipyridine method for the determination of iron suffers from interferences when large amounts of zinc are present: the deleterious effect of zinc is undoubtedly due to the utilization of the reagent in the formation of ammine-type complexes.

A fourth type of interference may be best described as a coordination number phenomenon. In certain cases the coordinating capacity of metals may vary, depending on the solvent, temperature, pH, and concentration of complexing agent. Where there is a variation in the amount of addenda coordinated, a variation in color can be anticipated. Yoe and Jones (*10*) in their studies of sodium 1,2-dihydroxybenzene-3,5-disulfonate found that three complexes were formed with ferric iron, depending on the pH. At low pH values a blue complex was found to exist. As the pH was raised a sudden change to a violet colored compound occurred at pH 5.7, which was then gradually converted to a red form at pH 9.0 to 10.0. They ascribed this phenomenon to a variation in coordination number of the iron from 4 to 6. This theory is consistent with the findings of Reihlen (*5*), that a red iron-pyrocatechol complex exists of the type



while a blue salt having a formula



could be isolated. By careful attention to the details of procedure such variations can be eliminated. The possibility of interferences arising from such shifts should be kept in mind, however, because many colorimetric tests depend on the chemical behavior of coordinated compounds.

SUMMARY

A survey of the sources of error and interferences occurring in the use of organic reagents indicates many possible hazards. Actual experience, on the other hand, shows that organic reagents compare very favorably with inorganic reagents. There is considerable evidence, in fact, to show that organic compounds can be used with greater confidence, and with the development of more compounds that are specific in their reactions it seems highly probable that the analytical chemist will depend more and more on organic reagents.

Users of organic reagents should keep in mind the special properties of organic compounds and of organic-inorganic compounds, as well as such factors as solubility of reagents, stability, and purity of organic compounds. It is extremely important also that the analyst be familiar with the chemistry of coordinated compounds, for much of the work involving organic reagents actually entails the use and behavior of complex ions and molecules.

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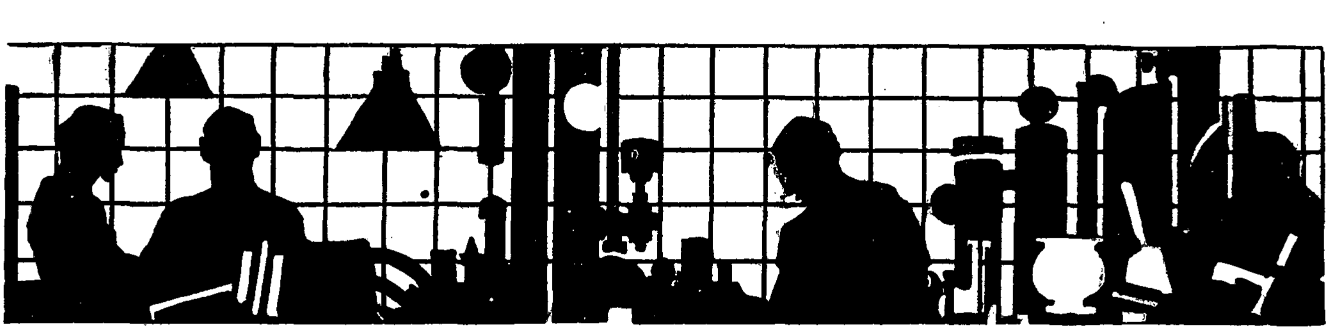

2nd Annual Summer Symposium — Organic Reagents

Colorimetric Determination of Boron Using 1,1′-Dianthrimide

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Sixty organic compounds were tested qualitatively in concentrated sulfuric acid for their suitability as either colorimetric or fluorometric reagents for boron. Twenty of these gave color and seventeen fluorescence changes. Further testing in a quantitative way led to a choice of 1-amino-4-hydroxyanthraquinone as a fluorescent and 1,1′-dianthrimide as a colorimetric reagent. The more satisfactory colorimetric method is described in detail and compared with other methods. In the usual procedures for determining boron in plant material, losses of boron during ashing of the sample may occur. A method for wet ashing such samples, by which losses of boron can be minimized, is also given.

THE importance of traces of boron in such diverse fields as agriculture and the steel industry makes of interest any new reagent for its determination. The one dealt with here is not ideal, but seems to have some points of superiority over quinalizarin. Of a large number of compounds dissolved in concentrated sulfuric acid and tested qualitatively, 20 gave a color change with boron and were tested further. The final choice was 1,1′-dianthrimide (1,1′-dianthraquinoylamine) and a colorimetric method using this reagent is described.

The two most widely used colorimetric methods employ quinalizarin (*2*) or curcumin (*4*) as reagents. Quinalizarin seems to be the more widely used because it is less subject to interference by other elements and because determinations may be made easily and quickly. The reagent, however, is not particularly suited for use with a filter photometer or spectrophotometer, for there is a considerable overlapping of the absorption bands of the reacted and unreacted reagent (*7*). Consequently, there is a wide divergence from Beer's law and a colorimeter can be used over only a limited range of boron concentration. The authors have had difficulty in finding concentrated sulfuric acid low enough in boron to permit utilization of this limited range. The color change (from greenish yellow to blue) in the present method is suitable for use with a filter photometer and less difficulty has been experienced in finding sulfuric acid sufficiently low in boron.

EXPERIMENTAL

The reagents were tested qualitatively as follows: Sufficient reagent was dissolved in C.P. concentrated sulfuric acid to give a moderately deep color. [It was necessary to obtain sulfuric acid from several sources and test each bottle to find lots sufficiently low in boron for most of this work. A good test reagent is quinalizarin (1,2,5,8-tetrahydroxyanthroquinone) which gives a pink solution in concentrated sulfuric acid low in boron and becomes increasingly blue as the boron concentration is increased.] This was divided into six portions of approximately 6 ml. each in low-boron glass test tubes (Corning's alkali-resistant). To three of these tubes was added approximately 1 mg. of C.P. boric acid. One pair of tubes was kept in a desiccator at room temperature (approximately 20° C.). Another pair was heated for 5 hours at 80° C., and the remaining pair was heated for 5 hours at 105° C. in an electric oven. Any color or fluorescent changes were noted before and again after heating. Fluorescent observations were made in the Beckman spectrophotometer (Model DU) by observing the solution in 1-cm. Corex tubes from above, while the wavelength scale was slowly turned from 250 to 700 mμ. The slits were opened wide and the room was kept dark to facilitate observation. In some instances it was difficult to distinguish between light scattering by the solution and true fluorescence. Only if the wave length of the observed light was different from that of the exciting light was the phenomenon termed fluorescence. If there was some doubt as to whether a compound gave a change with boric acid, the test was repeated using varying concentrations of the compound in sulfuric acid.

Many of the compounds are listed below according to their color or fluorescent change with boric acid. In several instances these changes have been noted by others.

Compounds which give a color change are hydroxyanthraquinones: 1,5-, 1,2,5,8-; 1,2,4,5,6,8-, and carminic acid; 1,10-phenanthraquinones: 1,2-di- and 1,2,4-tri-; the 1-hydroxy-2,4-*p*-aminophenyl sulfonic acid derivative of anthraquinone: anthraquinone dyes (designated to save space by the color index of Rowe, *6*): 1085, 1078 (a 1,4-diamino derivative), 1053, and 1054 (basic structure 1,5-dihydroxy-4,8-diaminoanthraquinone); 1-amino-4-hydroxyanthraquinone and C.I. 1073, which is a derivative of this compound. Several 1,1′-dianthrimides: 8-amino-4,4′-diamino- and 4,4′-diamino-8-nitro-. 1,4′4′,1″-trianthrimide, pentanthrimide, the chromotrop dyes C.I. 53 and 56, and morpholquinone also give a color change.

The anthraquinones which give a change in fluorescence are: anthraquinone itself; 1,2-dihydroxy-; 1-amino-4-hydroxy-; 1-amino-; 1-amino-2,4-dibromo-; 1-chloro-5-amino-; 1-amino-8-chloro-; 1-chloro-5-nitro-; α-malein amino-; 2,6-disulfo-; 1-chloro-5-benzamido-; *N*-methylamino-; and C.I. 1078. Quinizarin, leucoquinizarin, curcumin, and 2-hydroxy-3-naphthoic acid give a change in fluorescence.

The following compounds give no change in color or fluorescence: the chromotrop dyes of C.I. 90, 302, and 508, and three 1,1′-dianthrimides: 8-nitro-4,4′-dinitro-; 4,4′- and 4,5′-dibenzamino-. Judging from the method of preparation, these samples were probably contaminated with boron and might have proved suitable reagents if purified. These compounds also give no change: 1,3,4-trihydroxy-9,10-phenanthraquinone; 1,2,4-, 1,4,5-; 1,4,5,8-, and 1,2,4,5,8-hydroxyanthraquinones; 2-, 2,6-, and 1,4,5,8-aminoanthraquinones; 1,8- and 1,4,5,8-chloroanthraquinones; benzanthronylaminoanthraquinone; benzoin; *p*- and *o*-naphthazarin; anthrone; and phthiocol.

Following the qualitative tests, absorption spectra of the compounds showing a color change were determined using a Beckman spectrophotometer. Where the curves with and without boric acid indicated that the compound might be useful as a quantitative reagent, a measured amount was added in concentrated sulfuric acid to a series of boron standards prepared in porcelain crucibles by evaporating aliquots of a standard boric acid solution to dryness on a steam plate in the presence of 2 or 3 ml. of a 0.1 *N* suspension of calcium hydroxide. After holding for various lengths of time at several temperatures, the standards were read in a photoelectric colorimeter fitted with a combination of Corning glass color filters chosen on the basis of the spectrophotometric data previously obtained. On the basis of these tests, dyes of color index 1085, 1053, 1054, and 56, 8-nitro-1,1′-dianthrimide, 1,1′-dianthrimide, and carminic acid appeared promising. Dianthrimide was chosen as the best of these because of adherence to

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Beer's law, sensitivity, ease of obtaining it pure or of purifying it, and reproducibility of results.

Eckert and Steiner (*3*) describe a method for the synthesis of 1,1′-dianthrimide and review the early patent literature in which the color change of various dianthrimides with boric acid may be found.

Quinizarin, leucoquinizarin, and 1-amino-4-hydroxyanthraquinone were investigated as to their adaptability to quantitative fluorescent analysis. Radley (*5*) has recommended the latter compound as a qualitative reagent and it proved to be the most suitable for quantitative work. The reaction is carried out much as it is with dianthrimide, except that no heating is required. When the intensity of fluorescence is determined in a photoelectric fluorometer with a 1° filter transmitting the 546 mμ mercury line (Corning filters 3486, 5120, and 4308) and with Corning filter 3480 as the 2° filter, a linear response to boron concentration is found. Results agreeing fairly well with those obtained by the colorimetric method were obtained on a variety of plant samples. Somewhat more consistent results are obtained with the dianthrimide method and it is preferred by the authors.

DESCRIPTION OF METHOD

Reagents. Stock solution of 1,1′-dianthrimide. (Until this compound is obtainable through supply houses, a sample will be furnished upon request.) Make a solution of 400 mg. per 100 ml. of concentrated sulfuric acid low in boron (test using quinalizarin). This solution will keep for several months in the refrigerator.

Working solution of reagent. Dilute the stock solution 1 to 20 with concentrated sulfuric acid. Use a freshly diluted solution each day.

Sulfuric acid, 0.07 *N*.

Calcium hydroxide, c.p. Most lots are sufficiently low in boron. Make a 0.1 *N* suspension in water.

Hydrogen peroxide, 90% (Becco Sales Corp., Buffalo 7, N. Y).

Boron standard, 100 micrograms per ml. Dissolve 0.5715 gram of c.p. orthoboric acid in 100 ml. of water. Dilute 1 to 100 for the working standard.

Keep all solutions in low-boron glass. Corning's alkali-resistant glass or Kimble's glassware is satisfactory.

In cleaning glassware, avoid using chromic-sulfuric acid solution or concentrated nitric acid. If necessary to clean with acid, use hot 1 to 1 hydrochloric.

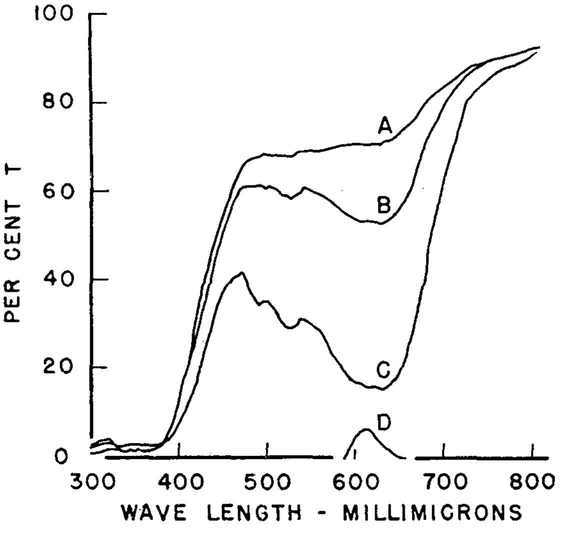

Figure 1. Absorption Spectrum of 1,1′-Dianthrimide with and without Added Boron

Curves obtained with a Model DU Beckman spectrophotometer using a 1-cm. cell. Concentration of reagent, 8 mg. per 100 ml. of concentrated sulfuric acid. *A*, no added boron; *B*, 0.1 microgram of boron; *C*, 0.6 microgram of boron per ml. of reagent. All tubes heated for 5 hours at 80° C. *D* is transmittance curve for filter combination (Corning 9780 one half standard thickness and 2403 standard thickness) used in colorimeter

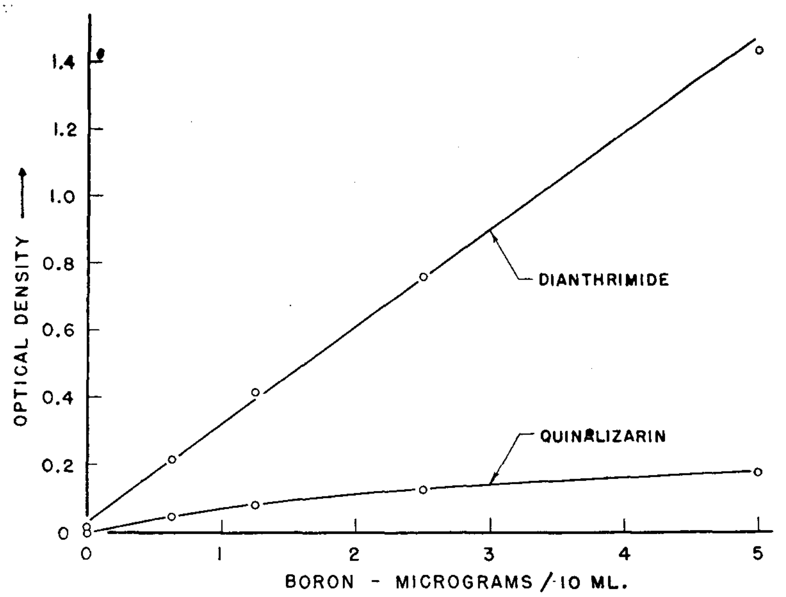

Figure 2. Calibration Curves for Boron Using 1,1′-Dianthrimide and Quinalizarin

Ashing Procedure. Two methods were used for ashing plant material—a dry-ashing procedure, and a wet digestion. The dry-ashing procedure is given because it was used in obtaining results by which the dianthrimide method could be compared with two other methods, and in work leading to the choice of 1,1′-dianthrimide. Data presented below indicate that during dry ashing a loss of boron occurs with many plant samples. The wet-ashing procedure is, therefore, recommended.

WET DIGESTION. To a 25- to 50-mg. sample of finely ground plant material in a 20 × 100 mm. Corning alkali-resistant glass test tube add 2 ml. of concentrated sulfuric acid and approximately 10 mg. of solid calcium hydroxide. Heat gently over a low Bunsen flame until charring occurs. Cool and add 1 drop of 90% hydrogen peroxide. Heat gently at first and then more vigorously until fumes of sulfur trioxide appear. Cool, and repeat the peroxide and fuming treatment. With most plant samples 4 drops of the peroxide are sufficient to give a colorless solution. Prepare standard tubes containing 0.5, 1.5, and 3.0 micrograms of boron by adding 1 ml. of 0.1 *N* calcium hydroxide suspension to suitable aliquots of the standard solution and taking to dryness in an oven at 125° C. To these standards and a blank tube add 2 ml. of sulfuric acid and 10 mg. of calcium hydroxide and treat exactly as the samples. (The loss of boron during the digestion of the standards is negligible and this step could probably be omitted.)

Place all tubes in an oven to dry any moisture condensed on the sides of the tubes, and add 5 ml. of the dianthrimide solution. Heat in an oven at 90° C. for 3 hours, cool in a desiccator, and dilute the contents of each tube with 5 ml. of concentrated sulfuric acid. The colored solutions are stable for several hours. Read in Evelyn colorimeter tubes with the blank set at 100. A filter transmitting in the region at 620 mμ is used. A combination of Corning filters 9780, one half standard thickness, and 2403 standard thickness was used in this work. A set of standards and a blank should be run each time. For samples high in boron the digest can be made to a volume of 5 or 10 ml. with concentrated sulfuric acid and an aliquot taken for analysis.

DRY ASHING. Ash a 0.5-gram sample in a covered porcelain crucible overnight at 600° C. Dissolve the ash with four or five portions of 0.07 *N* sulfuric acid, warming to hasten solution. Transfer the successive solutions and any insoluble matter to a volumetric flask and make to a volume of 25 ml. After the insoluble material has settled, transfer an aliquot containing 0.3 to 3 micrograms of boron to a 20 × 100 mm. test tube, add sufficient 0.1 *N* calcium hydroxide suspension to make alkaline, and evaporate to dryness in an oven at 125° C. Add 5 ml. of the reagent to these tubes and to suitable standards and blanks taken to dryness with calcium hydroxide and proceed with the determination as described above.

Table I. Boron Content of Alfalfa Samples

Sample No.	Curcumin[a]	Quinalizarin[b]		Dianthrimide	
	Micrograms of boron per gram				
			Av.		Av.
1	15	9, 11	10	13, 12	12.5
2	15	9, 8	8.5	9, 10	9.5
3	60	58, 58	58	63, 64	63.5
4	70	74, 74	74	76, 76	76
5	30	17, 18	17.5	23, 25	24
6	25	25, 28	26.5	22, 23	22.5
7	50	49, 47	48	56, 60	58
8	60	51, 49	50	60, 60	60

[a] Naftel method (*4*). The authors are indebted to J. E. Dawson, Cornell University, who made these determinations.

[b] Method of Berger and Truog (*2*). Results by quinalizarin and dianthrimide methods were obtained on aliquots of dilute acid extract of ash of a single sample in each case.

Table II. Recovery of Boron Added to Plant Samples

Sample	B in 25-Mg. Sample	B in Sample +0.625 B	B Recovered	Recovery
	γ	γ	γ	%
Carrot tops				
1	0.93	1.57	0.64	102
2	1.08	1.78	0.70	112
3	1.93	2.52	0.59	94
Turnip tops				
1	1.06	1.63	0.57	91
2	1.26	1.86	0.60	96
Alfalfa				
1	0.62	1.20	0.58	93
2	1.51	2.18	0.67	107
				Av. 101

RESULTS AND DISCUSSION

The absorption spectrum of 1,1′-dianthrimide with and without added boric acid is shown in Figure 1. Added boron gives a broad absorption band centered near 620 mμ and the reagent alone does not absorb light excessively at this wave length. This is in contrast to quinalizarin and most of the other reagents tested, where reagent and reagent plus boron curves overlap to such an extent that the Lambert-Beer law is not followed (*7*). Figure 2 shows a standard curve for both 1,1′-dianthrimide and quinalizarin. The fact that 1,1′-dianthrimide gives a colored complex which follows the Lambert-Beer law is advantageous for work with a photoelectric colorimeter, in that fewer points are required to define the standard curve and a greater change in optical density for a given change in boron concentration is obtained. A further advantage lies in the fact that a greater contamination of concentrated sulfuric acid with boron can be tolerated.

A few tests for interferences were made. Sulfate, chloride, tungstate, arsenate, and arsenite were found not to interfere. Fluoride in concentrations found in plant samples does not interfere but gives low results at higher concentrations. Nitrate and nitrite interfere, but are usually eliminated during ashing or can be removed from solution by adding a little Devarda's alloy and evaporating to dryness with a calcium hydroxide solution. Oxidizing agents such as chromate, periodate, and perchlorate interfere. The following cations were tested and found not to interfere: sodium, potassium, calcium, zinc, cupric, manganese, aluminum, and beryllium.

The boron content of several alfalfa samples was found to be essentially alike when determined by the present method and by two others (Table I). The validity of the method was tested further and found satisfactory by determining the recovery of added boron using the wet-digestion procedure (Table II).

Winsor (*8*) by the analysis of fumes given off during the dry ashing of plant materials has shown that losses of boron occur during this operation. Indirect evidence presented in Table III indicates that plant samples vary considerably in this respect. The four samples of alfalfa give the same values when either wet or dry ashed. Both turnip and carrot tops, however, give considerably higher values when wet digested. Presumably the dry-ashed samples are low because of losses of boron during ashing.

Austin and McHargue (*1*) recommend the addition of a saturated solution of barium hydroxide to the sample in a platinum crucible prior to ashing at 450° C. to prevent losses of boron. Their procedure was used in obtaining the values shown in Table IV. These values are essentially the same as those obtained by ashing in porcelain crucibles without the addition of a fixative, whereas the wet-ashing values are considerably higher. The latter values are more in line with those expected from a consideration of the boron content of the nutrient solutions in which these plants were grown. Individual values in this table give some idea as to the reproducibility of results.

REACTION BETWEEN REAGENT AND BORON

A few observations may throw some light on the nature of the reaction between boric acid and 1,1′-dianthrimide in concentrated sulfuric acid.

The reaction producing the blue color is dependent upon the nature of the vessel in which the reaction occurs, the temperature and duration of heating, and the concentration of reagent and of boric acid. The reaction occurs about equally readily in porcelain and in Corning alkali-resistant glassware, while only a partial color development occurs in Evelyn colorimeter tubes. In platinum ware, the reagent is unstable and darkens with no blue color formation.

Table III. Dry *vs.* Wet Ashing in Preparation of Plant Samples for Boron Determination

Sample	Dry Ashing	Wet Ashing
	γ/*g.*	γ/*g.*
Alfalfa		
A	12.5[a]	13.3
B	10.0	10.3
C	63.0	62.5
D	76.0	73.3
Turnip tops		
A	25.0	32.4
B	33.0	41.2
Carrot tops		
A	24.0	48.0
B	208	285
C	181	278
D	106	188

[a] Each value is average of two or more determinations.

Table IV. Comparison of Two Dry-Ashing Procedures with Wet-Digestion Procedure

	Method of Ashing					
	Dry with $Ba(OH)_2$[b]		Dry without $Ba(OH)_2$[c]		Wet[d]	
Sample		Av.		Av.		Av.
	γ/*g.*	γ/*g.*	γ/*g.*	γ/*g.*	γ/*g.*	γ/*g.*
1	61.3 77.3	69.3	73.0 62.3	67.7	77.2 80.6	78.9
2	60.0 75.6	67.8	61.2 58.8	60.0	85.3 85.3	85.3
3	59.5 63.8	61.7	... 78.3	78.3	160 163	162
4	70.0 70.0	70.0	71.5 71.3	71.4	164 183	174
5	128 131	130	110 129	120	303 290	297
6	148 113	131	129 110	120	328 305	317

[a] Carrot tops. Boron content of nutrient solution, 0.5 p.p.m. for 1 and 2, 2 p.p.m. for 3 and 4, and 5 p.p.m. for 5 and 6.

[b] Ashed according to procedure of Austin and McHargue (*1*) in platinum crucibles.

[c] Ashed as in [a] but without fixative and in porcelain crucibles.

[d] In samples 1 and 2, wet digested, values are for single determination. In all other cases values are average obtained on duplicate aliquots taken from single ashing.

At a temperature of 90° C. about 3 hours are required for maximum color development, while at 80° C. about 5 hours are required. At room temperature the reaction is very slow.

If a solution of the reagent is diluted with a little less than an equal volume of either absolute ethanol or methanol, the color changes from a yellowish green to colorless. This solution

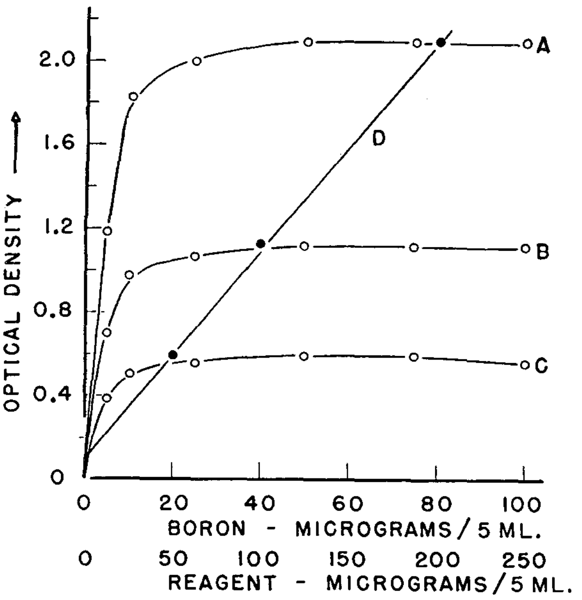

Figure 3. Relationship between Color Developed and Concentrations of Reagent and Boron

will give a blue color with boric acid after several hours at room temperature. To obtain the same intensity of color, however, 5 to 10 times as much boron is required as in the regular procedure.

In Figure 3 a family of three curves is shown.

Curve *A* was obtained by running a series of reactions using a reagent concentration of 200 micrograms per 5 ml. of acid and with boron (as boric acid) concentrations as indicated. After heating at 80° C. for 5 hours the optical density was determined using a 620 $m\mu$ filter and Evelyn colorimeter tubes. Curves *B* and *C* were obtained similarly, except that the reagent concentration was 100 micrograms per 5 ml. in *B* and 50 micrograms per 5 ml. in *C*. In each case the maximum color was attained at a boron concentration of approximately 40 micrograms per 5 ml. Calculation shows that at this point in the case of *A*, the ratio of atoms of boron per molecule of dianthrimide is about 8, whereas in *B* the ratio is 16, and in *C* is 32. Using data found in a discussion of the quinalizarin method published by Weinberg, Proctor, and Milner (*7*) the comparable ratio for this reagent at the concentration studied was calculated to be approximately 125. Curve *D* shows the relationship between the maximum color developed and reagent concentration and it can be seen that the Lambert-Beer law holds in this case.

Finally, the instability of the colored boron complex should be noted. If an excess of boric acid is added to a solution of the reagent and heated, a very deep blue color results. If the solution is then poured into water, the reagent precipitates out as fine red crystals, leaving a clear colorless dilute acid phase. The reagent may then be filtered, washed a few times with water, dried, and redissolved in concentrated sulfuric acid and found to have the same absorption spectrum (300 to 1000 $m\mu$) as the original reagent.

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Color Reaction of Beryllium with Alkannin and Naphthazarin

Spectrophotometric Studies

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MOST of the methods that have been proposed for the determination of beryllium have proved unsuitable for dealing with microgram quantities. The quinalizarin method of Fischer (*6*) can readily detect 0.5 microgram of beryllium, but the reagent is too unstable in the alkaline medium employed by Fischer to permit convenient quantitative work; furthermore, the color change from violet to blue is not a desirable one. The quinizarin-2-sulfonic acid method proposed by Fairhall *et al.* (*8*) has been found extremely sensitive to salt concentration, and in general yields reliable results only when conditions are rigorously controlled (*3*). Aurintricarboxylic acid (aluminon) has been tried in this laboratory as a colorimetric reagent for beryllium, but the color reaction lacks sensitivity and is undesirable for several other reasons (*9*). The various fluorometric methods (*7, 8, 10, 11, 14*) have been found inaccurate in the authors' laboratory. In the quinizarin method, for example, the optimal pH is about 11.5, and very small variations from this value lead to erratic results; it is difficult to buffer in this region (*13*). Spectrographic methods (*1, 2*) are reported to be very good from the standpoint of sensitivity and specificity, but are somewhat lacking in precision. In view of the increasing interest in beryllium, and certain deficiencies in existing methods, it appears desirable to pub-

A rapid, sensitive, and accurate spectrophotometric method is described for the determination of microgram quantities of beryllium, using either alkannin or naphthazarin as the reagent. From 1 to 20 micrograms of beryllium in 20 ml. can be determined with a probable error of less than 3% (standard deviation, 4.5%); as little as 0.1 microgram can be determined with somewhat less accuracy. Optimal conditions for the method have been found by studying the various factors that influence the color of the reagent with beryllium, such as pH, dye concentration, time of standing, and various extraneous ions. This method is subject to considerable interference from other elements, so that isolation of beryllium will be necessary in the analysis of biological samples, minerals, alloys, etc., but the method has proved extremely useful where the analysis of fairly pure beryllium solutions is desired; the accuracy, sensitivity, and ease of carrying out the procedure compare favorably with other methods.

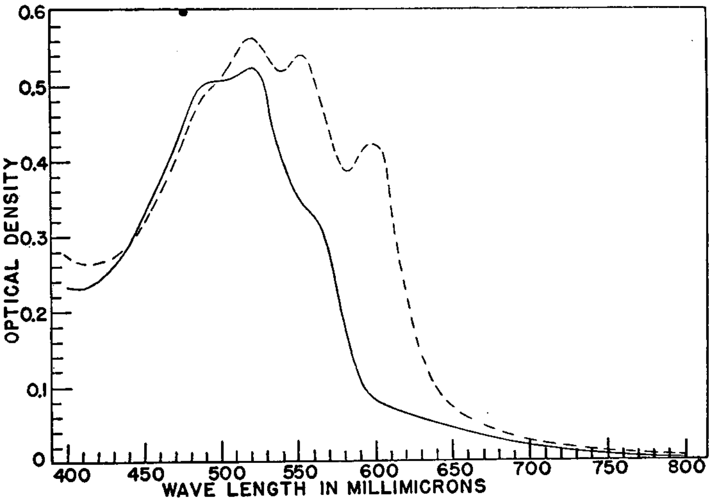

Figure 1. Absorption Spectra of Blank and Beryllium Solutions

4.65 γ of Be, 5 ml. of buffer, 0.2 ml. of gum arabic solution, 1 mg. of alkannin
Total volume 20 ml. pH 6.5. Read *vs.* distilled water
Solid line, blank
Dashed line, beryllium solution

lish a new spectrophotometric method which has proved rapid, sensitive, and accurate.

Alkannin and naphthazarin were used in a qualitative test for beryllium by Dubsky *et al.* (*4, 5*); these workers carried out the test in the presence of ethylenediamine, which, they believed, reacted with alkannin or naphthazarin to form a diimine which then formed a chelate complex with beryllium. In any case, alkannin or naphthazarin gives a blue color with ethylenediamine which goes over to a purple in the presence of beryllium. This approach has the disadvantage that the reagents are unstable in the alkaline medium, and the colors fade rapidly. On the other hand, the authors have found that ethylenediamine is not essential, and that a suitable color difference can be developed at a lower pH where the dye is stable, the red color going toward the blue in the presence of beryllium.

APPARATUS AND REAGENTS

All absorption spectra and spectrophotometric readings were made with a Beckman Model DU quartz photoelectric spectrophotometer. pH measurements were made with a Beckman laboratory model pH meter.

Alkannin. It was found possible to set up a quantitative method for beryllium using an alcoholic extract of the roots of *Alkanna tinctoria*, but variations among various extracts made it desirable to isolate the pure compound alkannin (*12*). In the studies that follow, solutions of the pure material, 0.4 mg. per ml. in redistilled 1,4-dioxane, were employed.

Naphthazarin. Certain difficulties in obtaining pure alkannin from various batches of roots led to synthesis of the analogous compound, naphthazarin (*12*). A solution of 0.4 mg. per ml. in redistilled 1,4-dioxane was employed.

Standard Beryllium Solutions. These were prepared by dissolving about 1 gram of the metal in dilute hydrochloric acid, removing a slight insoluble residue, and diluting to 1 liter with distilled water. The residue contained beryllium and silicon, as shown by the spectrograph, but the solution was essentially pure with respect to beryllium. Samples of this solution were evaporated to dryness after addition of a little sulfuric acid, and ignited to beryllium oxide for accurate analysis. This fairly acid stock solution could be kept indefinitely; dilutions in the microgram range were freshly prepared frequently to eliminate the possibility of changes due to adsorption, base exchange with the glass container, etc.

Borate-Mannitol Buffer. Various buffers were tried. Phosphate buffers interfered with the color reaction, as did bisulfite. A borate-mannitol buffer was found satisfactory. A solution of 0.5 *M* boric acid was made up in 10% mannitol and adjusted to pH 5.0 with sodium hydroxide. A trace of thymol was added to inhibit microorganisms, and the solution was stored in the refrigerator when not in use. Under these conditions, it can be kept indefinitely. This buffer changes pH on dilution, so that solutions prepared for spectrophotometric analysis as described below had a final pH of 6.5.

Gum Arabic. The beryllium-dye color lake would not remain in clear solution, but tended to flocculate on standing. This effect was easily countered by the addition of gum arabic as a stabilizer. A 0.5% solution was prepared for this purpose.

EXPERIMENTAL

Absorption Spectra. Absorption spectra were studied to determine a suitable wave length for further studies. It can be seen from Figure 1 that at pH 6.5 there is a large difference between blank and beryllium samples at 600 mμ and that the blank is fairly low at this wave length. A similar picture is seen with naphthazarin. Other curves were run at several pH values, and 600 mμ was optimal in all cases, so that working at this wave length in a further study of the effect of pH on the color reaction was justified—that is, although the actual spectra are shifted by changing pH, the net values obtained by reading beryllium samples against blanks will be maximal at 600 mμ.

Effect of pH. Figure 2 shows that with alkannin, the maximal color is developed above a pH of about 5, beyond which pH changes are not significant over a range of at least 3 pH units. A similar curve is obtained with naphthazarin. The reagents are unstable above a pH of about 8; the colors fade rapidly, and routine quantitative work would be difficult. These compounds are pH indicators; they change from red through a series of purples to blue in the region of pH 8, and because of their instability the alkaline region has not been investigated as regards a suitable wave length at which to work. A pH of about 6.5 has been found to be entirely satisfactory; the borate-mannitol buffer is efficient in this region, the colors are stable, and small variations in pH do not introduce errors. A system of 1 part of dioxane and 3 parts of water is involved, where the question of pH becomes somewhat complicated; directly measured, uncorrected pH values have been correlated with optical density, a procedure satisfactory for the purposes.

Effect of Dye Concentration. It can be seen from Figure 3 that alkannin alone does not conform to the Beer-Lambert law; the blank curve tends to flatten, so that it is possible to work at a level of 2 mg. of dye without incurring too high a blank. This quantity of dye has proved satisfactory with both alkannin and naphthazarin, in that it is a sufficient excess to give fairly linear standard curves over the range of beryllium concentrations in question.

Effect of Time. Figure 4 shows that, after a small initial increase, the readings on a naphthazarin-beryllium solution remained essentially constant for some time. No significant changes occur over the time normally required to prepare and read a series of samples. Fading of the colors is observed in solutions which have stood for longer periods—e.g., overnight. Alkannin behaves similarly.

Effect of Heating. Heating up to 80° C. was found to have very little effect on color development where 2 mg. of alkannin were used. During some preliminary studies where 1 mg. of alkannin was employed, a slower color development than that described above was observed, and it was found that heating the solutions hastened the development of maximal intensity. Boiling the solutions resulted in almost immediate fading of the colors.

Recommended Procedure for Analysis of Pure Beryllium Solutions. On the basis of the data described above, a method has been set up which has served very well in the analysis of pure beryllium solutions such as were obtained in certain physicochemical studies of solubilities of beryllium compounds, etc.

To the beryllium sample, containing from 1 to 30 micrograms of beryllium in 9.5 ml. or less, 5 ml. of the buffer, 0.5 ml. of the gum arabic solution, and 5 ml. of alkannin or naphthazarin solution are added, and the volume is adjusted to 20 ml. with distilled water. This sample is read against a similarly prepared blank on the spectrophotometer at 600 mμ.

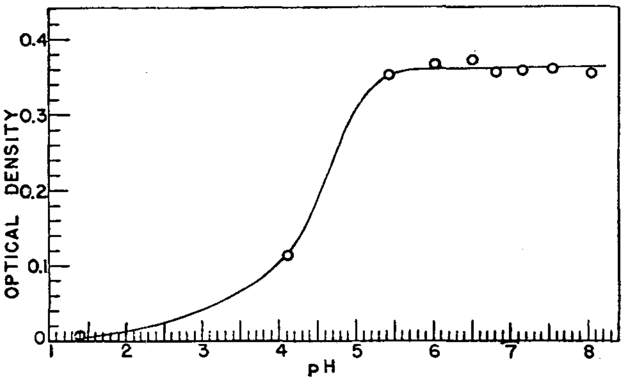

Figure 2. Effect of pH

4.65 γ of Be, 5 ml. of buffer, 0.5 ml. of gum arabic solution, 2 mg. of alkannin, adjusted to various pH values with hydrochloric acid or sodium hydroxide. Total volume 20 ml. Read *vs.* blanks adjusted to same pH values

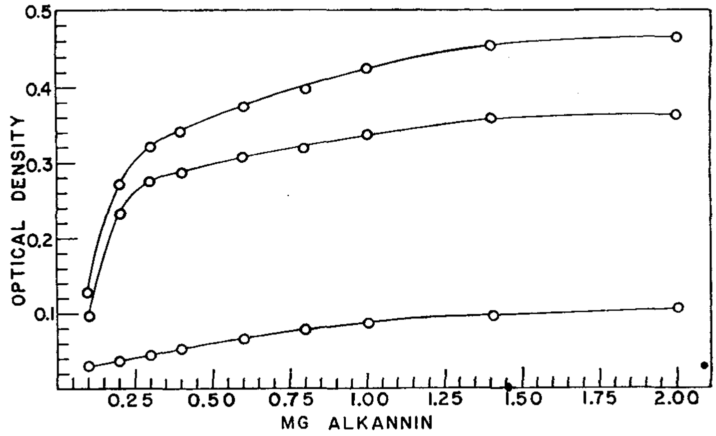

Figure 3. Effect of Dye Concentration

4.65 γ of Be, 5 ml. of buffer, 0.5 ml. of gum arabic solution, varying amounts of alkannin. Total volume 20 ml. pH 6.5. Read *vs.* distilled water
Upper. Be solutions
Middle. Net values
Lower. Blanks

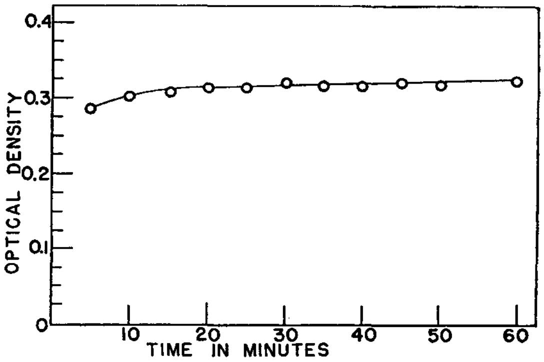

Figure 4. Effect of Time

4.65 γ of Be, 5 ml. of buffer, 0.5 ml. of gum arabic solution, 2 mg. of naphthazarin. Total volume 20 ml. pH 6.5. Read *vs.* blank at timed intervals after mixing

Figure 5 shows a typical standard curve. Beyond about 10 micrograms the solutions become too dense for the most accurate spectrophotometric readings; the upper limit of the method is set by the instrument. Figure 6 shows a standard curve over that part of the range which may be considered as the most precise from the standpoint of the instrument. It is possible to go down to as little as 0.1 microgram of beryllium by reducing the final volume to 10 ml.; half-volumes of the buffer, dye, and gum arabic are employed. Figure 7 shows a standard curve for the region below 1 microgram.

Results. Three groups of "unknowns" were made up by a disinterested worker and analyzed by the above procedure (Tables I, II, and III). By comparing these tables, it can be seen that the absolute error increases with increasing beryllium content, but that the increase is not proportional, so that per cent error decreases with larger amounts of beryllium.

Interferences. Several ions that might be encountered later were tested for interference with the determination of beryllium

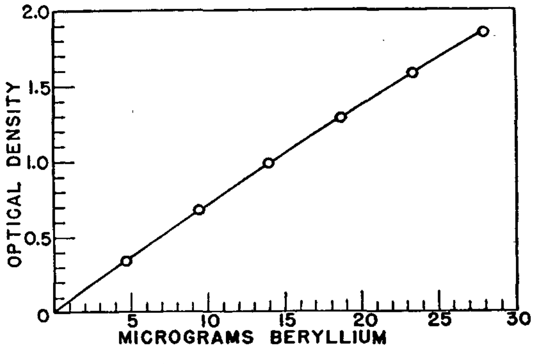

Figure 5. Standard Curve with Alkannin

Table I. Determination of Beryllium in Synthetic Unknowns

(Range 0 to 1 γ in 10 ml.)

Be Added, γ	Be Found, γ
0.93	0.93
0.28	0.33
0.39	0.44
0.19	0.19
0.61	0.65
0.79	0.82
0.49	0.55

Av. absolute error, γ	0.033	Probable error, %	6.6
Av. % error	7.6	Standard deviation, %	9.9

by this method. Table IV summarizes the effects of these ions in several concentrations. It was found that alkannin and naphthazarin had essentially the same susceptibility to interference by these extraneous elements.

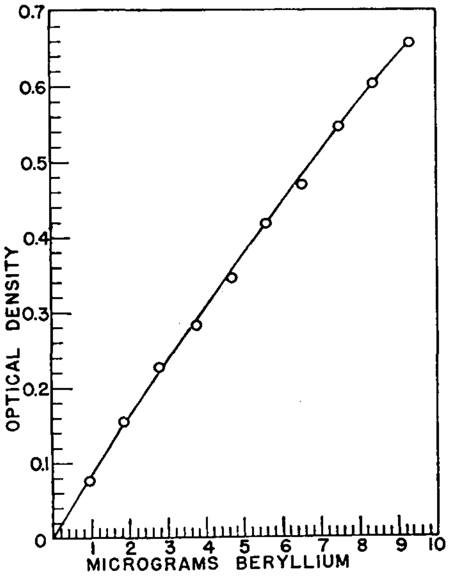

Figure 6. Standard Curve with Alkannin

Table II. Determination of Beryllium in Synthetic Unknowns

(Range 1 to 10 γ in 20 ml.)

Be Added, γ	Be Found, γ
1.39	1.40
1.86	1.85
2.79	2.75
1.21	1.15
1.58	1.40
3.35	3.30
4.08	3.95
7.90	8.15
5.58	5.70
2.32	2.20
8.36	8.60
6.60	6.60
4.65	4.60
7.25	7.45
5.77	6.30
5.12	5.05
3.72	3.50
8.18	8.40
6.98	7.25

Av. absolute error, γ	0.145	Probable error, %	2.8
Av. % error	3.4	Standard deviation, %	4.2

Table III. Determination of Beryllium in Synthetic Unknowns

(Range 10 to 20 γ in 20 ml.)

Be Added, γ	Be Found, γ
20.9	20.5
12.8	13.1
11.2	11.6
14.4	14.7
19.5	19.2
15.6	15.7
16.7	16.4
18.1	18.0

Av. absolute error γ	0.275	Probable error, %	1.4
Av. % error	1.8	Standard deviation, %	2.1

Table IV. Per Cent Error Introduced by Addition of Various Ions to 4.65 Micrograms of Beryllium

Ion Added, γ	1	10	100	1000
Ca^{++}	0	0	0	0
Mg^{++}	0	0	+10	+45
Zn^{++}	0	+5	+27	+150
Cu^{++}	0	+7	[a]	[a]
Fe^{+++}	0	+8	+35	[a]
Al^{++}	0	+27	+77	[a]
F^{-}	0	−8	−56	−95
PO_4^{---}	0	0	−30	−70

[a] Visible precipitate made colorimetric measurement impossible.

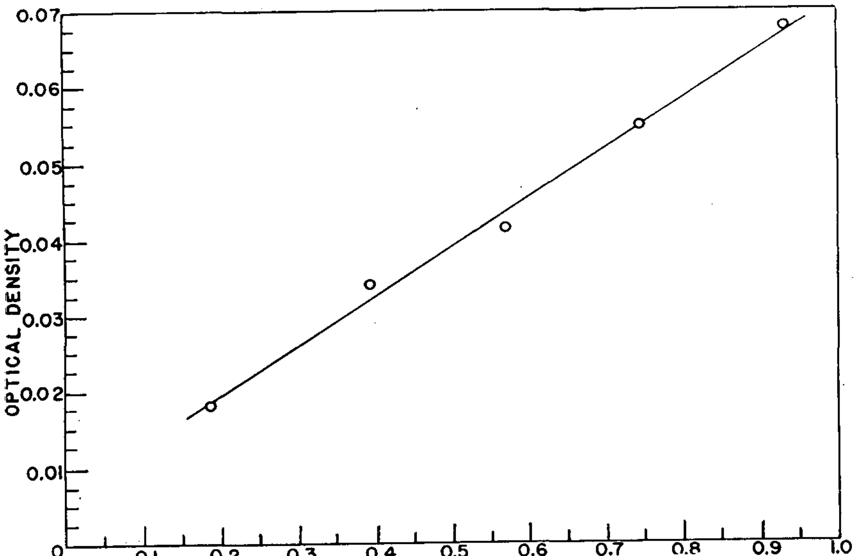

Figure 7. Standard Curve in Range below 1 γ with Naphthazarin

The effect of total salt concentration was studied, using sodium chloride. This salt does not alter optical densities so long as the colored materials remain in solution, but exerts its effect by causing flocculation of the latter. This effect depends on the amount of gum arabic added to stabilize the system. With no gum arabic present, the buffer alone is sufficient to cause precipitation. With the amount of gum arabic used in these studies, salt concentrations as high as 0.2 *M* (calculated for the final solution taken for spectrophotometry and exclusive of the buffer) can be tolerated. The amount of gum arabic used can be varied to meet the conditions encountered. The use of dioxane as solvent for the dyes might be mentioned. Not only are the free dyes more soluble in dioxane than in most common organic solvents miscible with water, but there is less tendency for the beryllium-dye lake to come out of solution when dioxane is present.

DISCUSSION

In the analysis of fairly pure beryllium samples, alkannin and naphthazarin yield results that compare favorably with other methods which have been proposed. Advantages of the method include the fact that color development is spontaneous and rapid, extremely rigorous pH control is not necessary, and the colored solutions are stable for a sufficient time to permit convenient quantitative work. The sensitivity is as great as that of other methods except the spectrographic, and the accuracy seems adequate. The two reagents serve equally well, conditions are essentially the same for employing either one, and it becomes a matter of convenience as to which is to be used; naphthazarin is somewhat more easily obtained than alkannin, so that it may be the reagent of choice. Like most of the beryllium methods previously employed, alkannin and naphthazarin are subject to considerable interference from extraneous elements, and it appears that at least a partial isolation of beryllium will be necessary before application of the method to the analysis of such things as biological samples, minerals, alloys, etc.

In analyzing such small amounts of material, special precautions must be observed to avoid contamination. These precautions have been well pointed out by Cholak and Hubbard (*2*).

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2nd Annual Summer Symposium — Organic Reagents

Preparation of Alkannin and Naphthazarin

For Use as Reagents for Beryllium

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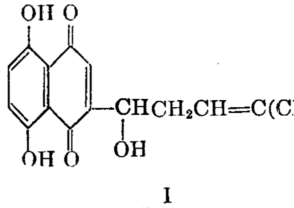
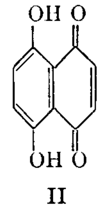
Akannin and naphthazarin were shown by Underwood and Neuman to be equivalent reagents for the microdetermination of beryllium. Alkannin (a substituted naphthazarin), which forms only a small fraction of the colored matter extractable from alkanet root (*Alkanna tinctoria*), was shown to be the only constituent active with beryllium. The bulk of the colored material appears to be a polymer of alkannin, and evidence indicates a dimer. The isolation procedure for alkannin was thoroughly studied. The difficulties in obtaining large amounts of alkannin made it desirable to find a reagent that could be more readily procured. Naphthazarin was synthesized according to the method of Zahn and Ochwat, but the purification procedure was modified. The synthetic method was found to be much simpler than extraction from the root. Absorption spectra of the compounds alone in carbon tetrachloride and at several pH's in aqueous medium, with and without beryllium, were determined.

IN SEEKING a more satisfactory method for the microdetermination of beryllium than any previously reported, Underwood and Neuman (*8*) developed first a method using alkannin and then an equivalent procedure using naphthazarin. Because alkannin is a substituted naphthazarin, the two compounds would be expected to behave similarly.



I
Alkannin



II
Naphthazarin

Formanek (*6*) first referred to the color reaction of beryllium with an alcoholic extract of the root *Alkanna tinctoria*. Dubsky and Krametz (*4*) used both alkannin and naphthazarin in the microdetection of beryllium in a solution containing ethylenediamine. The alcoholic extract of *Alkanna tinctoria* (roots were obtained from the S. B. Penick Company) was tried, and a method for the microdetermination of beryllium was developed using such an extract. The variation from batch to batch of root led to the isolation of the active ingredient, which was alkannin. Alkannin itself forms only a few per cent of the highly colored material extractable from the root, and it was shown that the nonalkannin material gave no color reaction with beryllium.

Brockmann (*2*) made a very complete study of alkannin and its related compounds, and gave directions for its extraction and isolation. He extracted the root with petroleum ether and then extracted the red material from the solvent with sodium hydroxide solution. After the deep blue alkali solution was washed several times with benzene and petroleum ether, the addition of acetic acid caused the precipitation of a red material. This was recrystallized repeatedly from benzene to give pure alkannin which melted at 148°C.

Underwood and Neuman (*9*) attempted to follow Brockmann's directions, but found that alkannin was too soluble in benzene to permit recrystallization. Evaporation of the solvent left a tarry, resinous mass. A different procedure was employed for the purification. After precipitation with acetic acid, the solid was

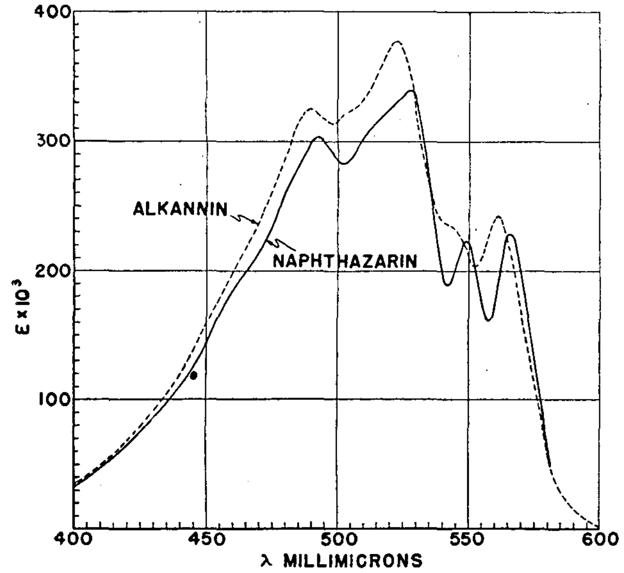


Figure 1. Absorption Spectra of Alkannin and Naphthazarin in Carbon Tetrachloride, 5×10^{-5} *M*

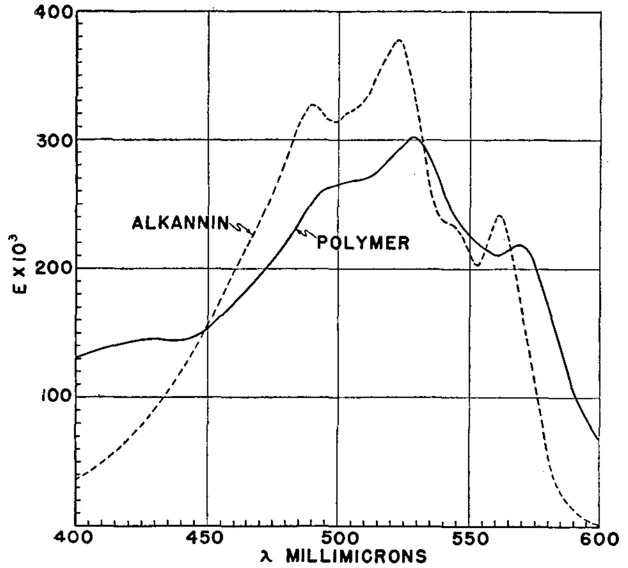


Figure 2. Absorption Spectra of Alkannin and Alkannin Polymer in Carbon Tetrachloride

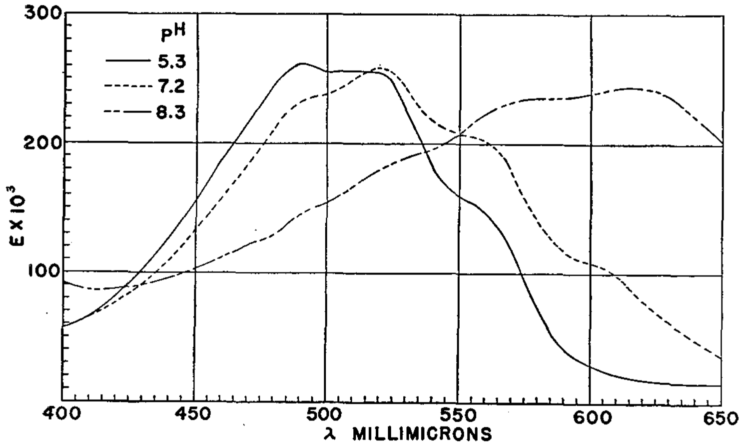


Figure 3. Absorption Spectra of Naphthazarin in Aqueous Medium at Different pH's

taken up with sodium hydroxide, then reprecipitated with acetic acid. This cycle was repeated five or six times. The final precipitation with acetic acid produced bright red flocs which were filtered and washed thoroughly with water. The solid was dissolved in alcohol and precipitated by dilution with water. The dried product melted at 143–4° C. Other workers attempting to follow this procedure on a larger scale obtained a considerable quantity of crude material from which no pure alkannin could be derived.

A thorough study of the isolation procedure yielded a number of interesting observations. The bulk of the solid precipitated upon the addition of acetic acid was not alkannin but what appeared to be a polymer of it. The nature of the precipitate was such that it clogged the filter and made washing extremely difficult, and the early workers left unwashed cakes of this material for considerable periods of time before processing them further. No alkannin could be isolated from such cakes. Evidence indicates that polymerization of the alkannin occurs through the side chain, and the reaction was favored by the prolonged contact with acetic acid and sodium acetate in the unwashed cake. The bleaching of alkaline solutions upon standing in air indicates that oxidation occurs and destroys the chromophoric group. When contacts with acid and alkali were kept to a minimum and the temperature was kept low, good yields of pure alkannin were obtained. Despite all precautions, the large bulk of the material extracted from the root was the polymer. The material probably exists as such in the root, and the alkannin content may be a seasonal function, as has been found to be the case for other natural products. The polymer is useless as a reagent, as it gives no noticeable color with beryllium under the conditions tried.

Because of the difficulty encountered in the preparation of large quantities of alkannin, attention was turned to a synthetic material. Naphthazarin was formerly available commercially as alizarin black, but attempts to obtain a sample were unsuccessful. Several syntheses reported in the literature were tried. The first was that of a patent by Ellis (*5*), which consisted of a condensation of succinic anhydride with hydroquinone in the presence of boric and sulfuric acids to yield a hydroxy-substituted β-benzoylpropionic acid, which was subjected to simultaneous ring closure and oxidation without isolation. From 100 grams of succinic anhydride and 40 grams of hydroquinone it was possible to isolate only 400 mg. of pure naphthazarin.

A synthesis according to Zahn and Ochwat (*10*) proved much more satisfactory. In this reaction maleic anhydride and hydroquinone are added to a melt of aluminum chloride and sodium chloride. The method of purification was modified, and pure naphthazarin was obtained in good yield.

ISOLATION OF ALKANNIN

A continuous extraction apparatus was constructed by using a 4-liter filter flask to contain the ground root. The side neck was connected so that the overflow from the flask fed into a heated flask, from which the solvent was distilled and flowed into a tube that extended to the bottom of the filter flask. In this way the solvent flowed up through the ground root and was concentrated in a flask. The filter flask contained 1 kg. of the root, and the colored matter was concentrated to 500 ml. in a 1-liter flask. One batch was extracted in one day. Inasmuch as the procedure of Brockmann in which the petroleum ether extract was treated with sodium hydroxide resulted in emulsions, the solvent was blown off with a stream of air before treatment with alkali.

Heating on a steam bath may be employed to hasten the process. The residue is cooled, and 250 ml. of 1 *N* sodium hydroxide are added and stirred vigorously for 5 minutes. Then 150 ml. of water are added, stirred for a few minutes, and the mixture is centrifuged to settle the solid matter. The supernatant liquid is saved, and the solid is stirred for 5 minutes with 100 ml. of 1 *N* sodium

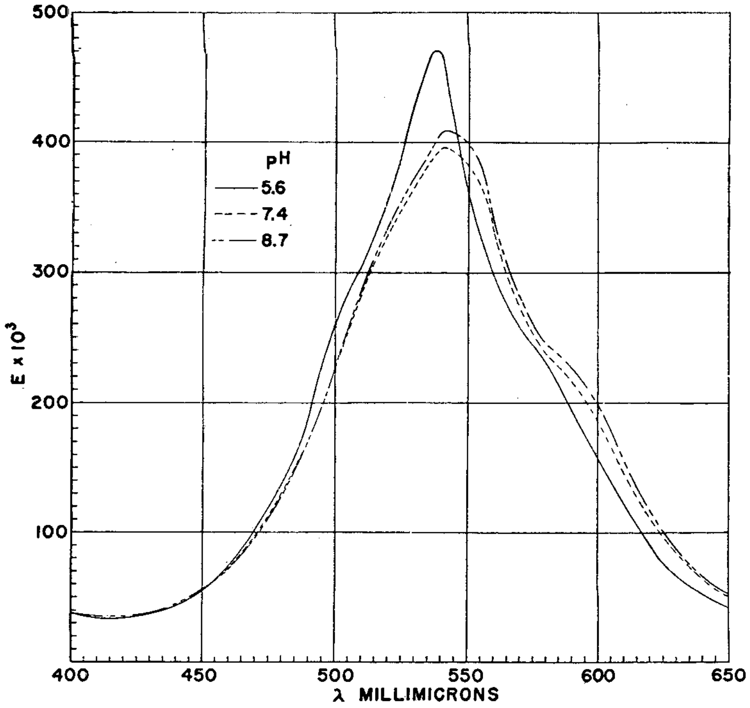


Figure 4. Absorption Spectra of Naphthazarin in Aqueous Medium with Excess Beryllium at Different pH's

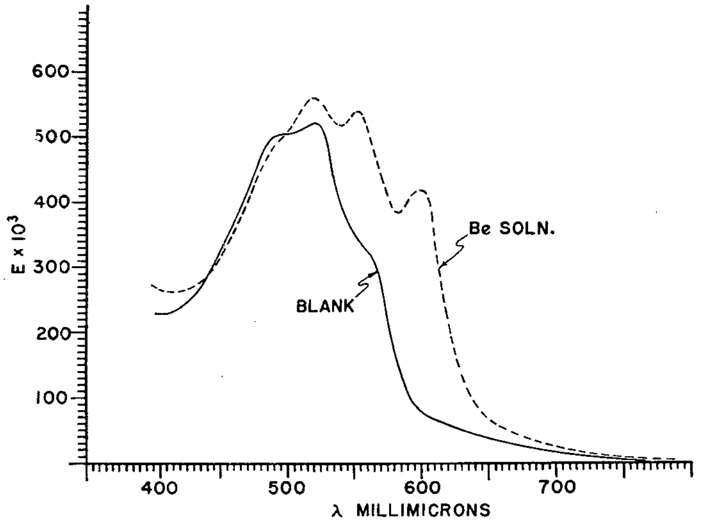


Figure 5. Absorption Spectra of Alkannin with and without Beryllium in Aqueous Medium at pH 6.5

hydroxide and 150 ml. of water. The mixture is centrifuged again and the supernatant liquid is combined with the previous liquid. The solid matter remaining from the centrifuging operation is discarded. This solid is insoluble in both sodium hydroxide and acetic acid solutions. Centrifugation is employed rather than filtration because the filters are clogged by the mixture. The sodium hydroxide solution is acidified by the addition of acetic acid (1 to 1) until the solution turns red. The solid material is settled by centrifuging, and the supernatant liquid is discarded. This solid is dissolved in 200 ml. of 1 *N* sodium hydroxide solution, and any insoluble material is separated by centrifuging. The alkaline solution is again acidified with acetic acid, and the solid is settled by centrifuging.

The solid is washed with water and centrifuged again. It is freed from any solution by use of suction, or by centrifuging if cups for drying solids are available. If the solid is gummy, it is chilled with dry ice and powdered. The solid is dissolved by dropping the powder into 250 ml. of alcohol during stirring. After stirring for about 15 minutes, the alcohol solution is filtered. Most of the solid is nonalkannin material and will not dissolve in alcohol. The alkannin is precipitated by diluting the alcohol solution with four or five times its volume of water. The suspension is centrifuged to settle as much of the solid as possible. The supernatant liquid is poured through a Büchner funnel, using a Whatman No. 41 filter paper. Finally, the solid is transferred to the filter and washed with a little water. As much of the liquid as possible is removed by suction and the solid allowed to dry. The solid is dissolved in as little alcohol as possible and the procedure of precipitation by dilution with water is repeated. A third precipitation from an alcohol solution gives a very pure product, melting at 143–4° C.

The solid left after the first alcohol extraction is stirred with alcohol and filtered again. Heating is not recommended because the solid will become gummy. This extraction with alcohol is continued until dilution with water followed by centrifuging yields no appreciable amount of solid. Some additional alkannin may be recovered from the colloidal supernatant liquid of the first few alcohol extractions by precipitating the solid with the addition of sodium chloride. The colloidal particles appear to have a negative charge, as sodium chloride is much more effective in coagulation than sodium sulfate. This solid should be filtered off and washed with water. After drying, the solid is dissolved in alcohol, and the solution is filtered and diluted with water. Running through the cycle several times will give about 10% more of the pure material.

Contact with sodium hydroxide and acetic acid should be kept as brief as possible, and temperatures should be kept below 20° C. If it is necessary to interrupt the procedure after treatment with either of the above reagents, the solutions should be kept in a refrigerator. The crude alkannin precipitated by acetic acid should not be allowed to stand for longer than 1 hour after separation from the liquid. It should be redissolved in sodium hydroxide or covered with distilled water and kept refrigerated.

The yields were low and variable, ranging from 700 mg. to 4 grams per kg. of root. Inasmuch as the dye is contained only in the bark of the root, it would be much more efficient to extract the bark alone if there were an easy way to separate it from the bulk of the root. Part of the variation in yield may be attributed to a difference in the amount of bark present in the different batches extracted.

PREPARATION OF NAPHTHAZARIN

A synthesis according to Zahn and Ochwat (*10*) gave the best yield.

A mixture of 10 grams of maleic anhydride and 11 grams of hydroquinone is added to a melt of 100 grams of anhydrous aluminum chloride and 20 grams of sodium chloride at 180° and heated to 200–220° C. The melt turns blue-red with foaming and solidifies after about an hour. It is allowed to cool, the solid mass is pulverized and boiled with water, and concentrated hydrochloric acid is added until the purple color of the aluminum-naphthazarin complex turns to a brown color due to precipitated naphthazarin. The crude naphthazarin is dried and extracted with benzene. Zahn and Ochwat evaporate the benzene and sublime the residue. Sublimation was not satisfactory for the final purification because impurities sublimed along with naphthazarin.

The benzene solution is extracted with 2 *N* sodium hydroxide, and the benzene layer is discarded. The aqueous layer is acidified with hydrochloric acid until the blue color disappears, and the naphthazarin comes down as a red-brown precipitate. The precipitate is filtered off, washed with water, dried in a vacuum desiccator, and recrystallized from "practical heptane," a high-boiling petroleum ether fraction. A yield of about 2 grams is obtained. Because naphthazarin slowly decomposed on heating in the open air, no melting point is reported and it was necessary to prepare the diacetate to characterize the material obtained by following the above procedure. A golden yellow material was obtained melting sharply at 192° C., in good agreement with reported values for the diacetate.

SUBLIMATION

Alkannin sublimes readily at a temperature of 130° C. and a pressure of 10^{-2} mm. Even when pure alkannin was sublimed using temperatures as low as 120°, a large amount remained in the heated portion of the tube as a dark, tarry mass. This residue was not appreciably soluble in alcohol but was readily soluble in benzene.

The "alkannin polymer" obtained as the main bulk of the colored material in the root gave no sublimate at temperatures up to 180° C. and pressures from 10^{-2} to 10^{-3} mm.

Naphthazarin sublimes readily under the conditions employed for alkannin. The material in the heated portion of the sublimer remains a bright red, in contrast to alkannin, which decomposes.

ABSORPTION SPECTRA

The absorption spectra of alkannin, alkannin polymer, and naphthazarin were determined in carbon tetrachloride solutions in which the chromophore concentrations were equal.

It can be seen from Figure 1 that the spectra of alkannin and naphthazarin are similar. The curve for alkannin checks very closely with that obtained by Brockmann (*2*). Figure 2 shows the spectra of alkannin and alkannin polymer and here again the curves are similar, indicating that the same chromophoric groups are involved.

In aqueous media, the spectra depend upon the pH. Figure 3 shows the spectra of an aqueous solution of the same concentration as for the carbon tetrachloride solution at several pH's. The solution goes from a red at 5.3 to a blue at 8.3.

Figure 4 shows the effect of adding a large excess of beryllium (ratio of 7 to 1 or more). The spectra undergo a great change due to the formation of the beryllium-dye complex, but pH has little effect on the spectra of the complex. When beryllium is added to an excess of the dye at constant pH, a combination of the curves shown in Figures 3 and 4 is obtained, and Figure 5 shows the difference between such a curve and a dye curve. Curves such as these were used by Underwood and Neuman to select the optimum wave length and pH for analytical purposes.

When an excess of beryllium is added to a solution containing the alkannin polymer the curves in Figure 6 are obtained. Beryllium does not affect the spectrum of the polymer.

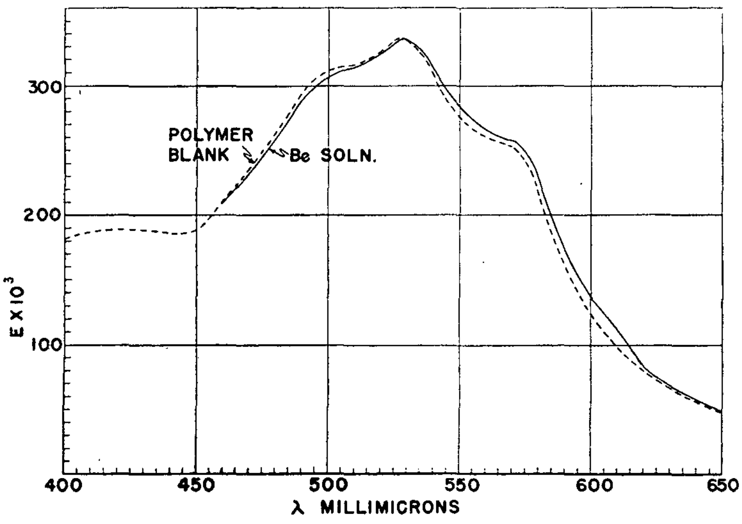


Figure 6. Absorption Spectra of Alkannin Polymer with and without Beryllium in Aqueous Medium at pH 6.5

OPTICAL ROTATION

Alkannin has one asymmetric carbon atom, and it would be expected to be optically active. Brockmann (*2*) measured its optical activity using the cadmium red line at 643.85 mμ because of too much absorption in the conventional yellow sodium region.

A special spectrum cadmium-mercury H4 type lamp was used with a Corning 2404 filter to cut out the mercury lines. A Franz Schmidt and Haensch No. 9123 polarimeter with a vernier reading to 0.01° was used. The solutions were necessarily dilute in order to pass enough light, and the angle measured was very small. For this reason twenty readings were taken on each blank and each sample, and the results were treated statistically. In chloroform the following results were obtained:

$$(\alpha)^{27}_{cd} = (-0.223 \times 100)/(1 \times 0.0901) = -248° \pm 9°$$
$$(\alpha)^{27}_{cd} = (-0.261 \times 100)/(1 \times 0.1028) = -254° \pm 7°$$

Brockmann reported −225° and −227° C. on two samples, and his values are not too far from those reported here.

The alkannin polymer showed no optical rotation.

DISCUSSION

The empirical formula and structure of alkannin were disputed until the work of Brockmann. Raudnitz, Redlich, and Fiedler (*7*) gave the formula $C_{16}H_{14}O_4$, whereas Dieterle, Salomon, and Nosseck (*3*) gave $C_{15}H_{14}O_4$, but neither group postulated a structure with oxygen in the side chain. Betrabet and Chakravarti (*1*) gave the formula $C_{30}H_{26}O_8$ to alkannin, and their description of the compound fits that of the polymer reported in this work. Brockmann found the formula to be $C_{16}H_{16}O_5$ with the structure as shown above. He attributed the discrepancies to the fact that none of the previous workers had obtained pure alkannin. The results of the present work would indicate that those before Brockmann worked with either the polymer or an impure compound. A rough determination of the molecular weight of the polymer by the melting point depression of camphor gave a value of 520 to 580. This would indicate that the polymer was dimer of alkannin which has a molecular weight of 288. The polymer shows the same chromophoric group as alkannin by absorption spectra studies, but it shows no optical rotation. The latter fact shows that the OH in the side chain reacts, inasmuch as the asymmetry of the molecule is destroyed. This would indicate that the polymerization occurred in the side chain. Other factors which are consistent with the higher molecular weight of the polymer are its inability to undergo sublimation and the increase in the melting point. The polymer is also less soluble in alcohol than alkannin, although it remains extremely soluble in benzene.

The reactivity of the side chain of alkannin may be seen in its behavior during sublimation. At temperatures as low as 120° at a pressure of 10^{-2} mm. a large amount of pure alkannin turns to the nonvolatile, benzene-soluble mass. This is in contrast to naphthazarin, which has the same structure without the side chain, is completely volatile, and undergoes no change under similar conditions.

The specific rotation of alkannin in chloroform solution is not too far from that reported by Brockmann. However, if the calculations in the conventional formula are carried out using his data, a value one tenth that which he reports would be obtained. Because the magnitude of the angle measured is almost the same as that found in this work, it is believed that his concentration is expressed in grams per liter instead of the grams per 100 cc. used in the formula for specific rotation. If the value he gives were grams per 100 cc., the solution would be so highly colored that it would be impossible to pass enough light to operate a polarimeter. If his concentrations are interpreted to be grams per liter, they would be approximately the same as for the solutions in this work.

No difficulties were encountered in following the synthesis of naphthazarin reported by Zahn and Ochwat, although it was necessary to modify the method of purification. Practical heptane, a high-boiling petroleum ether, was found to be an excellent solvent for recrystallizing naphthazarin. Naphthazarin may be synthesized in the necessary quantities much more readily than an equivalent amount of alkannin may be extracted from the root. Inasmuch as Underwood and Neuman (*8*) have shown the two reagents to be equivalent, it is recommended that naphthazarin be used in the microdetermination of beryllium.

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[END OF SYMPOSIUM]

Chloroform Extraction of Ferric Cupferrate

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The distribution of ferric iron between chloroform and an aqueous phase containing much chloride in the presence of nitrosophenylhydroxylamine is governed by the relation

$$\frac{[\mathrm{FeCf_3}]_{\mathrm{CHCl_3}}}{\Sigma[\mathrm{Fe}]_{\mathrm{H_2O}}} = K' \frac{[\mathrm{HCf}]^3{}_{\mathrm{CHCl_3}}}{[\mathrm{H^+}]^3{}_{\mathrm{H_2O}}}$$

The value of *K′* depends upon the chloride concentration and also upon the indifferent electrolyte concentration. Values of *K′* for various chloride concentrations are reported.

THERE are a considerable number of scattered references in the analytical literature on the extraction of metal cupferrates from aqueous medium by various immiscible organic solvents such as chloroform, carbon tetrachloride, and ether. The metals extracted are those forming cupferrates slightly soluble in mineral acid medium—e.g., ferric iron, vanadium, and molybdenum. Particularly, the extraction separation of ferric iron from such metals as aluminum and beryllium has been applied by a number of workers.

It seemed worth while to determine the value of the extraction coefficient of ferric cupferrate as an aid to the evaluation of the separation. The instability of cupferron in acid solutions prevents accurate quantitative data from being obtained, but even approximate results should have some analytical utility.

DISTRIBUTION OF A METAL BETWEEN AQUEOUS AND IMMISCIBLE SOLVENT PHASES IN PRESENCE OF CUPFERRON

The following equilibria exist in this system:

$$\mathrm{HCf} \rightleftharpoons \mathrm{H^+} + \mathrm{Cf^-} \text{ (aqueous phase). (HCf = nitrosophenylhydroxylamine)} \quad (1)$$

$$\underset{\text{Aqueous phase}}{(\mathrm{HCf})_w} \rightleftharpoons \underset{\text{Organic solvent}}{(\mathrm{HCf})_o} \quad (2)$$

$$\mathrm{MCf}_m \rightleftharpoons \mathrm{M}^{+m} + m\,\mathrm{Cf^-} \text{ (aqueous phase). } (\mathrm{M}^{+m} = \text{metal ion}) \quad (3)$$

$$\underset{\text{Aqueous phase}}{(\mathrm{MCf}_m)_w} \rightleftharpoons \underset{\text{Organic solvent}}{(\mathrm{MCf}_m)_o} \quad (4)$$

The respective equilibrium constants are:

$$\frac{[\mathrm{H^+}][\mathrm{Cf^-}]}{[\mathrm{HCf}]_w} = k_{\mathrm{Cf}}$$

$$\frac{[\mathrm{HCf}]_o}{[\mathrm{HCf}]_w} = p_{\mathrm{Cf}}$$ (partition coefficient of nitrosophenylhydroxylamine between organic solvent and water)

$$\frac{[\mathrm{M}^{+m}][\mathrm{Cf^-}]^m}{[\mathrm{MCf}_m]_w} = k_{\mathrm{M}}$$

$$\frac{[\mathrm{MCf}_m]_o}{[\mathrm{MCf}_m]_w} = p_{\mathrm{M}}$$

The combination of these expressions gives:

$$\frac{[\mathrm{MCf}_m]_o}{[\mathrm{M}^{+m}]} = \frac{p_{\mathrm{M}}}{k_{\mathrm{M}}}\left(\frac{k_{\mathrm{Cf}}}{p_{\mathrm{Cf}}}\right)^m \frac{[\mathrm{HCf}]_o^m}{[\mathrm{H^+}]^m} = K\frac{[\mathrm{HCf}]}{[\mathrm{H^+}]^m}$$

The value of the equilibrium constant, *K*, may be found by direct experiment. It may also be obtained by calculation if certain data are known:

$$\frac{p_{\mathrm{M}}}{k_{\mathrm{M}}} = \frac{[\mathrm{MCf}_m]_o}{[\mathrm{M}^{+m}][\mathrm{Cf^-}]^m}$$

$$\frac{k_{\mathrm{Cf}}}{p_{\mathrm{Cf}}} = \frac{[\mathrm{H^+}][\mathrm{Cf^-}]}{[\mathrm{HCf}]_o}$$

The value of $p_{\mathrm{M}}/k_{\mathrm{M}}$ is obtained by dividing the molar solubility of the metal cupferrate in the organic solvent by the solubility product in water saturated with the organic solvent; the value of $k_{\mathrm{Cf}}/p_{\mathrm{Cf}}$ is found similarly. The values of the solubility products of the cupferrates of ferric iron, copper(II), aluminum, bismuth, and tin(IV) have been reported(*1*). The solubility of the cupferrates in organic solvents does not appear to have been determined. The solubility of nitrosophenylhydroxylamine in organic solvents and in water has not been reported. The dissociation constant, k_{Cf}, of nitrosophenylhydroxylamine is approximately 5×10^{-5} (*1*). The value of the partition coefficient, p_{Cf}, for nitrosophenylhydroxylamine between chloroform and water has not been determined exactly, but it is known to be greater than 100. This means that the amount of nitrosophenylhydroxylamine remaining in the aqueous phase, acidified with a mineral acid, after shaking with chloroform, is usually negligibly small. The amount of undissociated metal cupferrate in water solution is extremely small and need not be considered.

Table II. Extraction of Ferric Cupferrate by Chloroform from Hydrochloric Acid Solutions

Concn. of HCl, M	Original $\Sigma[Fe]_{H_2O}$, $M \times 10^3$	Original $[HCf]_{H_2O}$, $M \times 10^3$	Final $\Sigma[Fe]_{H_2O}$, $M \times 10^3$	Final $[FeCf_3]_{CHCl_3}$, $M \times 10^3$	Final $[HCf]_{CHCl_3}$, $M \times 10^3$	K'
1.00	....	....	....	....	....	6×10^{8}[a]
2.00	1.80	7.2	0.67	1.13	3.8	2.4×10^{8}
2.00	1.80	7.2	0.73	1.07	3.9	2.1×10^{8}
2.00	1.80	14.4	0.095	1.70	9.3	1.8×10^{8}
						Av. 2.1×10^{8}
3.00	1.80	7.1	1.25	0.55	5.4	7.4×10^{7}
3.00	1.80	10.6	0.80	1.00	7.6	7.6×10^{7}
						Av. 7.5×10^{7}
4.00	1.80	10.8	1.29	0.51	9.3	3.2×10^{7}
4.00	1.80	14.4	0.97	0.83	11.9	3.2×10^{7}
						Av. 3.2×10^{7}

[a] Average of 15 values ranging from 1.4×10^7 to 15×10^8. Variability probably due to experimentally unfavorable distribution ratio (most of iron goes into chloroform phase).

The analyst is interested in the distribution of a metal in all its forms between the two phases. This means that account must be taken of metal species other than the simple ion—i.e., complex ions and slightly dissociated molecules. If the equilibrium constants of all the reactions involved and the concentration of the complex-forming agents are known, $[M^{+m}]$ in the equation can be replaced by an expression involving these and the total metal concentration in the aqueous phase. If the concentration of the complex-forming substance is large compared to that of the metal, it can be taken to remain approximately constant as the total concentration of metal varies, and a simple expression is obtained for the ratio of the metal forms in the two phases under specified conditions, provided the complexing substance is univalent.

As an example, suppose that M^{+m} forms a series of complexes with chloride ion. The aqueous solution will contain the species M^{+m}, MCl^{+m-1}, MCl_2^{+m-2} ..... MCl_n^{m-n}. At a sufficiently high acidity, the hydrolysis of M^{+m} can be neglected. The sum of concentrations of the metal in all its forms in the aqueous phase is given by:

$$\Sigma[M]_w = [M^{+m}]_w \left\{ 1 + \frac{[Cl^-]}{K_1} + \frac{[Cl^-]^2}{K_1K_2} + \cdots \right\}$$

Therefore, for a constant concentration (actually activity) of Cl^-, we have the relation:

$$[M^{+m}]_w = \Sigma[M]_w \times \text{constant}$$

$$\therefore \frac{[MCf_m]_o}{\Sigma[M]_w} = K' \frac{[HCf]_o^m}{[H^+]^m}$$

K' is a constant for a specified concentration of Cl^- at constant ionic strength and in the absence of other complex-forming substances.

As long as there is no real or virtual association of the metal in either phase—i.e., if the species in the aqueous phase contain only one atom of M—the fraction of total M in the organic phase will be independent of the total amount of metal present.

K′ FOR FERRIC CUPFERRATE

The preceding expression was tested for ferric iron in a mixture of hydrochloric acid and sodium chloride in which the chloride concentration was maintained constant at 4 *M*. The values of constant K' are shown in Table I. In these and other experiments the procedure was as follows:

The ferric chloride solution, containing the requisite amount of hydrochloric acid and sodium chloride, was treated with a freshly prepared cupferron solution (prepared from a 99.8% pure product) in a separatory funnel. The volume of the solution after the addition of cupferron was 10.0 ml. After mixing, 10.0 ml. of chloroform were added immediately and the mixture was shaken for 2 minutes. Special experiments showed that this period of shaking sufficed for attainment of distribution equilibrium. The chloroform was allowed to settle and an aliquot portion of the clear aqueous phase was evaporated almost to dryness with nitric acid and a little sulfuric acid to destroy any organic matter present. The residue was taken up in water and iron was determined colorimetrically by the *o*-phenanthroline method, with hydroquinone as reducing agent. A blank was always run on the acids. The distribution experiments were carried out at 23 ± 1° C.

Table I. Extraction of Ferric Cupferrate by Chloroform from Aqueous Solution of Constant Chloride Ion Concentration (4 *M*)

Concn. of HCl, M	Concn. of NaCl, M	Original $\Sigma[Fe]_{H_2O}$, $M \times 10^3$	Original $[HCf]_{H_2O}$, $M \times 10^3$	Final $\Sigma[Fe]_{H_2O}$, $M \times 10^3$	Final $[FeCf_3]_{CHCl_3}$, $M \times 10^3$	Final $[HCf]_{CHCl_3}$, $M \times 10^3$	$K' \times 10^{-7}$
4.00	0	1.80	10.8	1.29	0.51	9.3	3.2[a]
4.00	0	1.80	14.4	0.97	0.83	11.9	3.2
							Av. 3.2
3.00	1.00	1.80	7.2	1.37	0.43	5.9	4.2
3.00	1.00	1.80	10.7	1.22	0.67	8.7	2.5
3.00	1.00	1.80	10.6	1.14	0.66	8.6	2.5
3.00	1.00	1.80	14.2	0.81	0.99	11.2	2.3
3.00	1.00	3.60	21.4	1.00	2.60	13.5	2.9
3.00	1.00	3.60	28.4	0.44	3.16	18.9	2.9
							Av. 2.9
2.00	2.00	1.80	7.2	1.17	0.63	7.2	2.9
2.00	2.00	1.80	7.2	1.07	0.73	5.0	4.4
2.00	2.00	1.80	10.8	0.72	1.08	7.5	2.9[b]
2.00	2.00	1.80	14.4	0.38	1.42	10.1	2.9
2.00	2.00	2.70	14.4	0.75	1.95	8.5	3.5
2.00	2.00	2.70	21.5	0.19	2.51	13.9	4.2
							Av. 3.5
1.00	3.00	1.80	7.2	0.65	1.15	3.7	3.4
1.00	3.00	1.80	7.2	0.65	1.15	3.7	3.5
1.00	3.00	1.80	14.4	0.07	1.73	9.1	3.3
							Av. 3.4

[a] When ferric cupferrate precipitate was allowed to stand 5 minutes before extraction with chloroform under conditions similar to above, apparent value of K' was found to be 1.5×10^7, thus showing effect of decomposition of nitrosophenylhydroxylamine.

[b] When ferric cupferrate was allowed to stand 5 minutes before extraction, apparent value of K' was 1.6×10^7.

Values of K' for various hydrochloric acid concentrations (no other chloride present) are given in Table II.

At constant chloride concentration, K' in the equation

$$\frac{[FeCf_3]_o}{\Sigma[Fe]_w} = K' \frac{[HCf]_o^3}{[H^+]^3}$$

is seen to remain constant, within the limits of experimental error in this unstable system, as $[FeCf_3]_o/\Sigma[Fe]_w$ is varied from about 0.3 to 25 and $[H^+]$ is varied from 1 to 4. With increasing chloride concentration the value of K' decreases in accordance with the greater transformation of Fe^{+++} into $FeCl^{++}$, $FeCl_2^+$, $FeCl_3$, and possibly other species (*2*). A plot of log K' versus hydrochloric acid concentration appears to give a straight line in the range 1 to 4 M (Figure 1). No great accuracy is claimed for the values of K' reported, and the close conformity to linearity may be fortuitous.

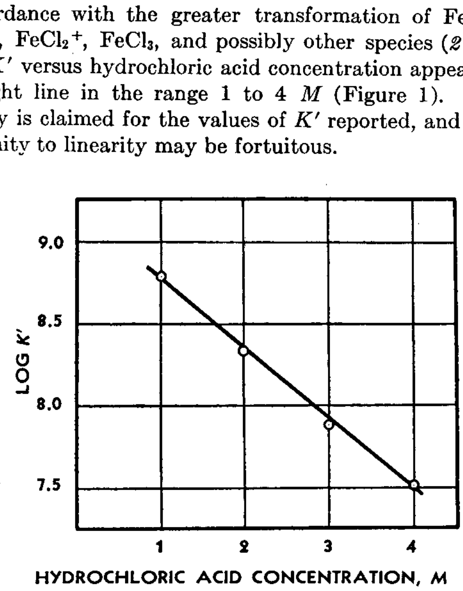

Figure 1. Variation of K' for Ferric Cupferrate in Chloroform with Hydrochloric Acid Concentration

A few determinations of K' in 1 M perchloric acid were made. The value 7×10^9 was obtained (average of 5 values ranging from 3.6×10^9 to 1×10^{10}), but this figure must be regarded as very tentative because of the unfavorable experimental conditions.

When the concentration of free nitrosophenylhydroxylamine in the chloroform phase is 0.1 M the ratio $[FeCf_3]_o/\Sigma[Fe]_w$ for 1 M hydrochloric acid medium is 6×10^5, on the basis of the above value of K'. *A double extraction under these conditions (final* concentration of excess reagent 0.1 M in the chloroform) should therefore reduce the ferric iron concentration of the aqueous solution to a very small value. The extent of extraction of such metals as aluminum, which do not readily give a cupferrate precipitate in mineral acid medium, cannot be stated at present, although a rough estimate of K' for aluminum can be based on the reported solubility products of ferric and aluminum cupferrates, which are, respectively, 1×10^{-25} and 2×10^{-19} according to Pyatnitskiĭ. If the solubilities of ferric and aluminum cupferrate in chloroform are roughly the same, the value of K' for aluminum cupferrate should be of the order 3×10^2 in 1 M hydrochloric acid.

The magnitude of this value indicates that the hydrogen ion and cupferron concentration may have to be controlled with some care for a satisfactory separation when much iron is present. As a matter of fact, aluminum cupferrate tends to be extracted from sulfuric acid solutions less acid than 4 N (*3*). If the extraction equilibrium is reversible (the reversibility does not seem to have been tested), a better separation can be achieved by applying the principle of retrograde extraction—i.e., the chloroform solution of ferric cupferrate containing a little aluminum cupferrate and excess cupferron is shaken with a hydrochloric acid solution of suitable concentration containing added cupferron. By proper choice of conditions, the major part of the aluminum in the chloroform phase can be removed without transfer of any significant amount of iron.

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Quantitative Study of Reaction between Beryllium and Quinizarin-2-sulfonic Acid

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FAIRHALL (*1*) has reported a colorimetric method for the determination of beryllium based on its reaction with an anthraquinone dye, quinizarin-2-sulfonic acid. An attempt to duplicate Fairhall's results led to a critical study of the factors involved in the reaction. The data obtained served as the basis for a revised procedure which permits the analysis of beryllium in quantities from 1 to 20 micrograms with a probable error of 3.1%.

EXPERIMENTAL

The procedure described by Fairhall (*1*) served as a point of departure for these studies.

In investigating various anthraquinone derivatives as color reagents for beryllium, 1,4-dihydroxyanthraquinone-2-sulfonic acid (quinizarin-2-sulfonic acid), buffered at pH 7.0 with ammonium acetate, was found to give a red color which is proportional to the amount of beryllium. The color develops rapidly reaches a maximum in 5 minutes, and does not fade for several hours. The most satisfactory range for colorimetric comparison in a visual colorimeter is 1 to 20 micrograms of beryllium.

No color developed when beryllium and the dye were mixed under the conditions given by Fairhall (*1*). Heat (100° C. for 10 minutes) was necessary to develop the color. Because ammonium acetate is not an effective buffer in the region of pH 7.0, a search for a more suitable buffer was instituted. Histidine monohydrochloride was found to be a fairly effective buffer at pH 6.5 and it did not interfere with the color reaction as did phosphate, bisulfite, borate, and maleate. It was also observed that the colored lake formed between beryllium and the dye could be easily salted out, but that this separation was prevented by the addition of gum arabic as a color stabilizer. With these preliminary changes in the Fairhall procedure, the reaction was studied in more detail.

Instruments. Measurements of absorption spectra were made with a Model DU Beckman quartz spectrophotometer, using the

A critical study has been made of factors affecting the reaction between beryllium and quinizarin-2-sulfonic acid, an anthraquinone dye suggested by Fairhall as a color agent for beryllium. Under optimal conditions of pH, temperature, etc., quinizarin-2-sulfonic acid may be used satisfactorily for the determination of microquantities of beryllium (1 to 20 micrograms) with a standard deviation of 4.65%. However, the conditions of operation must be rigorously controlled and numerous substances interfere markedly with color formation.

"blue-sensitive" phototube and 1-cm. Corex absorption cells. The colorimetric readings were made with a Klett-Summerson photoelectric colorimeter using the appropriate filter (interference type, Farrand Optical Company, New York, N. Y.). A Beckman pH meter, laboratory model G, was used for all pH determinations.

Reagents. Technical grade 1,4-dihydroxyanthraquinone-2-sulfonic acid obtained from the Eastman Kodak Company, Rochester, N. Y., was purified by dissolving the sodium salt in water, acidifying with hydrochloric acid, pouring into 95% ethyl alcohol, and filtering the precipitate which formed. The reagent was prepared as a 0.16% aqueous solution.

Histidine monohydrochloride monohydrate was obtained from Nutritional Research Biochemicals, Cleveland, Ohio. The buffer was prepared as 0.1 *M* solution containing 1% gum arabic as a colloid stabilizer and adjusted to the proper pH by additions of 2.0 *N* sodium hydroxide. A crystal of thymol was added to retard bacterial decomposition of the reagent.

The standard beryllium solution was prepared by dissolving 1.000 gram of spectrographically pure beryllium in dilute hydrochloric acid. The solution was transferred to a 1-liter volumetric flask and made up to volume with distilled water. The final acid concentration was approximately 0.1 *N*. Portions of this stock solution were evaporated to dryness, and the residues were ignited to beryllium oxide and weighed; the average value of 1.00 mg. of beryllium per ml. was found. For analysis, appropriate dilutions of this stock solution were made.

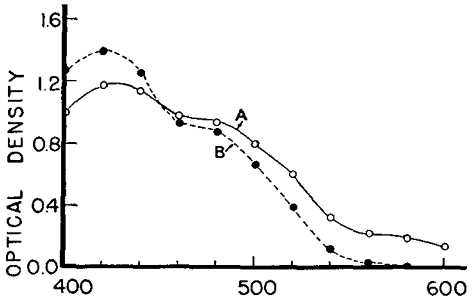

Figure 1. Absorption Spectra of Beryllium-Dye Complex *vs.* Reagent Blank

A. Beryllium-dye complex
B. Reagent blank

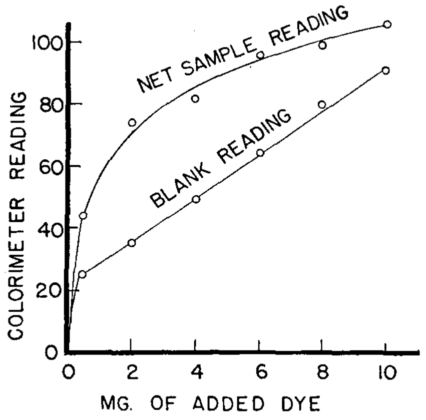

Figure 2. Colorimetric Response of Beryllium-Dye Complex *vs.* Reagent Blank

Measured against function of dye concentration

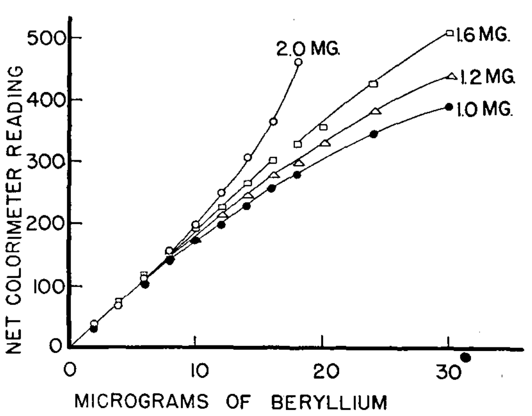

Figure 3. Colorimetric Response of Beryllium-Dye Complex as Function of Dye Concentration

Effect of pH on Absorption Spectra. The color of the beryllium-dye complex was markedly affected by variations in pH. Accordingly, absorption spectra of the blank and the beryllium-dye complex were studied over the pH range from 4.0 to 9.0. The curves were obtained with solutions containing 6.0 micrograms of beryllium and 0.4 ml. of 0.5% aqueous 1,4-dihydroxyanthraquinone-2-sulfonic acid, buffered with 2 ml. of 0.1 *M* histidine monohydrochloride in 1% gum arabic solution, all in a total volume of 20 ml. Blanks identical in every way, but lacking beryllium, were also prepared. The solutions were heated at 100° C. for 10 minutes and cooled to room temperature under tap water prior to reading.

It was observed that, at acid pH, beryllium affected the absorption of the dye only slightly. At alkaline pH (8.8), beryllium increased absorption of the dye at wave lengths from 500 to 600 mμ, but the blank itself absorbed strongly. Between pH 6 and 7 the beryllium-dye complex showed some absorption from 550 to 600 mμ where the absorption of the blank was negligible. It was evident that, for color contrast (beryllium complex *vs.* blank), a wave length of 575 mμ was optimal and the reaction should be buffered at pH 6.5 (cf. Figure 1).

Effect of Dye Concentration. A series of solutions was prepared containing 4.0 micrograms of beryllium, 2 ml. of buffer solution (pH 6.5), varying amounts of dye solution, and distilled water to give a total volume of 20 ml. Blanks (no beryllium) were prepared for each dye level. The plot of colorimetric response *vs.* dye concentration, shown in Figure 2, indicates that a dye concentration of about 2 mg. in a total volume of 20 ml. is optimal—i.e., there was a large net color difference with a low blank.

Because previous studies (*2*) have shown that, under certain circumstances, the optimal quantity of dye varies as a function of the amount of beryllium being analyzed, a series of standard curves was obtained employing varying quantities of dye. In these experiments, the volume was 25 ml., pH was 6.5, buffer concentration was 0.01 *N*, and the beryllium content varied from 1.0 to 30 micrograms. The results are presented in Figure 3.

It is apparent that the color-beryllium relation does not conform to Beer's law. A series of curves was obtained, the curvature of which varied with the quantity of dye added. An almost linear curve was observed with 1.6 mg. of dye. This quantity was considered optimal and was employed in most subsequent studies.

Effect of Buffer Concentration. A series of standard curves was obtained with varying concentrations of histidine buffer. In this series, the following conditions were employed: pH 6.5, volume 25 ml., amount of dye 2 mg., beryllium content 2 to 16 micrograms. Three concentrations of buffer were studied: 0.1, 0.01, and 0.005 *M*. The results are presented in Figure 4.

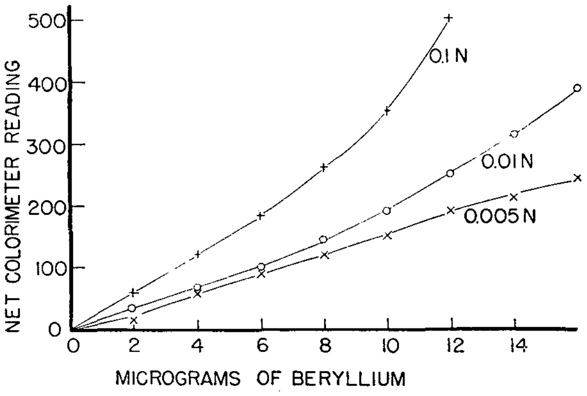

Figure 4. Colorimetric Response of Beryllium-Dye Complex as Function of Salt Concentration

Increasing the concentration of buffer increased the color intensity. Not only did beryllium samples show greater color intensity; the blank was also increased. For this reason, a compromise was chosen as optimal: 0.01 *M* buffer was employed in subsequent work.

Similar effects were noted when varying quantities of sodium chloride were added to the solutions being analyzed. It appears that the color reaction is sensitive to variations in the ionic strength of the solution and not to a specific interaction with the buffer.

Effect of Time on Stability. A series of beryllium standards in the concentration range 1 to 20 micrograms per 20 ml. of solution was prepared together with blanks, heated at 100° C. for 10 minutes, cooled to room temperature under tap water, and read in a Klett-Summerson photoelectric colorimeter equipped with a Farrand interference filter at 575 mμ.

Readings taken over a 2-hour time interval to determine whether there was any appreciable drift with respect to time showed that the solutions were relatively stable for at least 2 hours. In fact, there was a slight increase (3 to 4%) in color intensity after standing.

The time of heating will vary somewhat with the diameter of the tubes employed by the analyst. Wide-diameter tubes may require as long as 30 minutes to reach full color development. Because overheating tends to reduce the color density, optimal conditions cannot be stated categorically but must be arrived at by experimentation.

PROCEDURE

Based on the conditions outlined above, the following procedure was adopted. Blanks and standards are analyzed with each group of unknowns.

Table I. Recovery of Known Quantities of Beryllium

Added γ	Found γ	Added γ	Found γ
0.50	0.55	8.0	7.9, 8.4
1.0	0.95, 1.0, 1.0	9.0	8.7, 9.5, 9.4
2.0	2.2, 2.0	10.0	10.0, 10.3
3.0	3.2, 3.1, 3.2	11.0	11.3
4.0	4.3, 4.2	13.0	13.6
5.0	5.1, 5.2, 5.3	15.0	15.4
6.0	6.2, 6.1	17.0	17.4
7.0	6.8, 7.3, 7.4	19.0	18.6

Table II. Effect of Interfering Substances

Foreign Ion	Amount Required to Give Interference Equivalent to 0.5 γ of Be	Interference[a] Effect
	Mg.	
Zn^{++}	2.5	H
Ca^{++}	1.2	H
NO_3^-	1.2	H
Cu^{++}	0.5	H + CC
PO_4^{---}	0.3	H
Mg^{++}	0.3	H
Al^{+++}	0.05	H + Ppt
F^-	0.005	L

[a] L reading lower and H reading higher than solutions containing beryllium standard. CC, color change; Ppt, precipitation.

One to 15 ml. of the solution to be analyzed were taken, the pH was adjusted to approximately 6, and the solution was transferred to a tube graduated at 20 ml. Two milliliters of histidine buffer and 1.0 ml. of a 0.16% aqueous solution of quinizarin-2-sulfonic acid were added and the mixture was diluted to a volume of 20 ml. The tube was immersed in a boiling water bath for 10 minutes and cooled in tap water and the optical density was determined by means of a Beckman spectrophotometer (at 575 mμ) or a Klett-Summerson colorimeter equipped with a filter (interference type, 575 mμ, Farrand Optical Company).

Optical measurements were converted to micrograms of beryllium by means of a standard calibration curve which is essentially a straight line.

Accuracy. A series of unknown solutions of pure beryllium chloride was prepared by a disinterested person and analyzed by the procedure given above. The results are presented in Table I. In view of the small amounts of beryllium analyzed, the accuracy was considered satisfactory.

Interfering Substances. The influence of foreign ions was investigated. The experiments were made on solutions containing 10 micrograms of beryllium in 20-ml. volume. The values on Table II indicate the quantities of interfering ions which cause an error equivalent to 0.5 microgram of beryllium.

The common anions which might be used in the preparation of samples (sulfate and chloride) fortunately do not materially affect the color reaction, if the total molarity does not exceed 0.005. Many common cations, however, and fluoride and phosphate interfere markedly and thereby limit the applicability of the method.

Discussion. Quinizarin-2-sulfonic acid, suggested by Fairhall (*1*) as a colorimetric agent for beryllium, can be used successfully for the accurate analysis of beryllium only if the conditions of color development are rigorously controlled. Necessary precautions must be taken to exclude interfering substances, in particular, phosphate, fluoride, and aluminum. Because the method requires the use of pure solutions, its applicability is at present restricted to analyses of beryllium dusts as used in inhalation studies and analyses of air samples around beryllium plants. The method may be applied to other substances if the beryllium is first isolated.

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Spectroscopic Analysis of Isomeric Xylene Mixtures

Method for Analysis of Mixtures with Overlapping Absorption Bands

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An ultraviolet spectroscopic method is described for the analysis of mixtures with overlapping absorption bands, and is applied to isomeric xylene mixtures. This method involves the preparation of a working chart from the difference in measured optical densities at selected wave lengths and the known composition of the samples. From ultraviolet absorption measurements at four wave lengths, the composition of isomeric xylene mixtures can be read from the working chart. The mean deviation between the known composition of synthetic isomeric xylene samples and their composition as obtained from the working chart is less than 1%. Application of the method is rapid and does not require measurement of extinction coefficients or involve solvent and cell corrections, and eliminates extraneous errors due to "fingerprints," etc. Departure from Beer's law does not affect accuracy of results.

THE usual method for the spectroscopic analysis of multicomponent mixtures with overlapping absorption bands requires the determination of the extinction coefficient of each component at wave lengths where absorption is to be measured. For a mixture of n components, absorption measurements at n wave lengths, followed by the solution of the n simultaneous equations, gives the concentration of the various components. Variations of this method have been used by preparing working curves for the binary mixtures and making corrections for the component having the least absorption (*2*, *5*). Robertson, Ginsburg, and Matsen (*6*) were able to analyze a mixture of isomeric cresols with an overlapping absorption band by making corrections for the absorption of two of the components, the concentration of which had been determined from absorption measurements using independent bands.

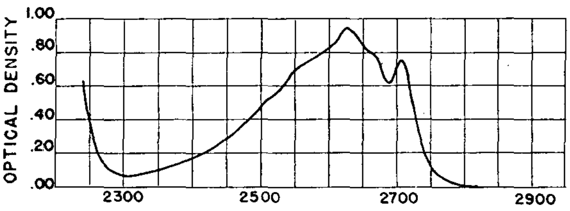

Figure 1. Ultraviolet Absorption Spectrogram of 1,2-Dimethylbenzene (*o*-Xylene)

Inspection of the spectrograms of the isomeric xylenes as shown in Figures 1, 2, and 3 shows that no relatively independent bands exist and that the extinction coefficients of the isomers at the wave lengths practicable for analysis are of the same order of magnitude. Because the accuracy obtained by the solution of n simultaneous equations is determined by the accuracy of determination of the extinction coefficients and their order of magnitude, as well as that of the absorption measurements at n wave lengths, any error in the determination of extinction coefficients or the measurement of total absorption may affect the results considerably. The preparation of a working chart from the difference in measured optical densities of synthetic mixtures, at selected wave lenths, tends to reduce the precautions required for the securing of accurate values, inasmuch as extinction coefficients are not required and any extraneous absorption, which is constant at the two wave lengths used, cancels. Corrections for any deviation from Beer's law are eliminated, because the composition of the synthetic mixtures covers the entire composition range of the analytical samples and any deviation from Beer's law will appear in the working chart.

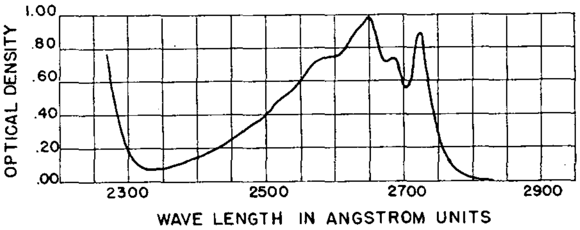

Figure 2. Ultraviolet Absorption Spectrogram of 1,3-Dimethylbenzene (*m*-Xylene)

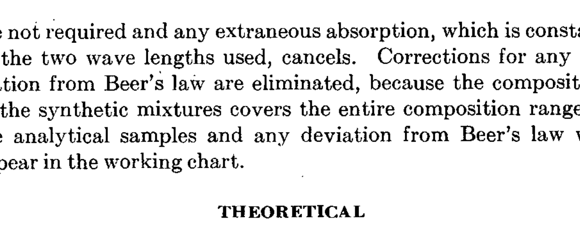

Figure 3. Ultraviolet Absorption Spectrogram of 1,4-Dimethylbenzene (*p*-Xylene)

THEORETICAL

For a single component, the analytical procedure is reduced to the preparation of a working curve by plotting $\log I_0/I$, the optical density, against concentration. If the absorbing substance obeys Beer's law, the working curve is a straight line according to the relationship

$$\log I_0/I = \epsilon c d$$

where ϵ is the extinction coefficient, c is the concentration, and d is the thickness of the absorbing material. A mixture of independently absorbing substances absorbs light according to the equation

$$\log I_0/I = (\epsilon_1 c_1 + \epsilon_2 c_2 + \ldots)d$$

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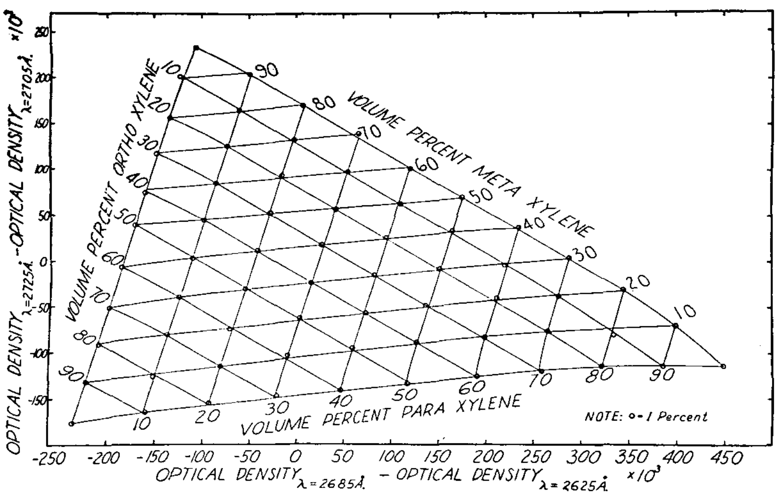

Figure 4. Working Chart for Analysis of Xylene Mixtures

where ϵ_1, ϵ_2 . . . are the extinction coefficients of noninteracting substances having concentrations c_1, c_2 . . ., respectively. The difference in density between bands from two different compounds in a single mixture is a more sensitive function of concentration than the density of either band alone, and those regions of maximum and minimum absorption give the greatest sensitivity. By the method of differences, the optical densities at two wave lengths are required for a single measurement. With constant total concentration, two distinct measurements are sufficient to determine the composition of a three-component system, thus requiring optical density measurements at three wave lengths. Measurements at four wave lengths may provide a greater sensitivity, as the wave lengths selected for the measurement of one component may not be a sensitive function of concentration for the second.

Absorption measurements for a three-component mixture are:

$$E^{tot}_{\lambda_1} = \epsilon'_{\lambda_1}c_1 + \epsilon''_{\lambda_1}c_2 + \epsilon'''_{\lambda_1}c_3 \qquad (1)$$

$$E^{to}_{\lambda_2} = \epsilon'_{\lambda_2}c_1 + \epsilon''_{\lambda_2}c_2 + \epsilon'''_{\lambda_2}c_3 \qquad (2)$$

$$E^{tot}_{\lambda_1} - E^{tot}_{\lambda_2} = (\epsilon'_{\lambda_1} - \epsilon'_{\lambda_2})\,c_1 + (\epsilon''_{\lambda_1} - \epsilon''_{\lambda_2})\,c_2 + (\epsilon'''_{\lambda_1} - \epsilon'''_{\lambda_2})c_3 \qquad (3)$$

where E^{tot} is the optical density of the mixture. A similar relationship can be written for the difference in optical densities at wave lengths λ_3 and λ_4. By fixing the concentration of one component with the total concentration constant, and varying the concentration of the two remaining, a straight-line relationship is expected between the difference in optical densities at two wave lengths, λ_1 and λ_2, and the composition of the mixture. All lines representing fixed quantities of one component are parallel. Similarly, lines representing constant concentrations of the second component will be parallel and have a slope different from that of the first if their extinction coefficients at the two wave lengths differ. A difference of optical densities at wave lengths λ_3 and λ_4 fixes the points of intersection of these two groups of parallel lines, thus giving the composition of the mixture. The above relationships are shown graphically in Figure 4 for the ternary system *o*-, *m*-, and *p*-xylene, which served as a working chart for the analysis of isomeric xylene mixtures.

Any extraneous absorption due to solvent, cells, fingerprints, etc., appearing in Equations 1 and 2 is eliminated in Equation 3, if constant for the wave lengths selected.

The curvature of the constant percentage lines in those regions of high concentration of the para component, as shown in Figure 4, indicates a deviation from Beer's law.

ANALYSIS OF SPECTROGRAMS

For maximum sensitivity from a working chart as described above, regions of maximum absorption of one component must coincide with regions of minimum absorption of the other components, and the wave lengths selected should provide a maximum difference in absorption for a single component. From inspection of the spectrograms of *o*-, *m*-, and *p*-xylene shown in Figures 1, 2, and 3, it is noted that *p*-xylene has maximum absorption bands at 2685 and 2745 Å. with relatively weak absorption at wave lengths 2620 and 2720 Å. Strong bands for the meta isomer are found at 2650 and 2725 Å. with minimum absorption at 2675 and 2705 Å. The ortho component has maxima at 2630 and 2710 Å. with a minimum at 2690 Å.

Applying the criteria stated above, wave lengths 2685 and 2625 Å. were selected for measurement of the para content. A maximum at 2725 Å. and minimum at 2705 Å. gave a measurement of the meta content equal in sensitivity to about two thirds of that of the para isomer. The positions, 2705 and 2625 Å., corresponding to minimum absorption for the meta and para isomers, respectively, coincide with positions of maximum absorption for the ortho component. The ortho isomer has a minimum at 2685 Å. which coincides with a maximum of the para; thus the wave lengths selected serve equally well for the measurement of the ortho content.

As Figure 4 shows, the difference in optical density, $E_{2685\text{ Å.}} - E_{2625\text{ Å.}}$, at wave lengths 2685 and 2625 Å. was plotted against the difference in optical density, $E_{2725\text{ Å.}} - E_{2705\text{ Å.}}$, at wave lengths 2725 and 2705 Å.

APPARATUS AND REAGENTS

The instrument used in this work was a Beckman quartz spectrophotometer (*1*), model DU, equipped for ultraviolet spectroscopy. The absorption cells were quartz, 1 cm. in length. The solvent, 2,2,4-trimethylpentane (iso-octane) was purified by selective adsorption on silica gel (*3*, *4*). Pure grade iso-octane, 99 mole % purity, obtained from the Phillips Petroleum Company, was passed through the silica gel column until its transmittancy equaled or excelled that of a standard sample obtained from the National Bureau of Standards. The solvent from the analyzed samples was reclaimed by passage through the silica gel column. The activated silica gel, No. 22-08-08-226, was obtained from the Davison Chemical Corporation.

PREPARATION OF THE WORKING CHART

Synthetic samples were prepared from "standard samples" of hydrocarbons obtained from the National Bureau of Standards. The purity of the standard samples was as follows: *m*-xylene 99.94 ± 0.04%, *p*-xylene 99.93 ± 0.03%, *o*-xylene 99.99 ± 0.007%.

In preparing the synthetic samples, 0.65 ml. of the standard sample, measured with a pipet, was introduced into 25 ml. of solvent (pipet) which had been weighed previously on an analytical balance. From the weight of the resulting solution, the weight of the isomeric xylene was obtained. One milliliter of this solution (pipet) was added to 75 ml. of solvent measured from calibrated pipets. Samples of each isomer were prepared in an analogous manner. The ratio of the weights of 0.65 ml. of the standard samples checked the ratio of the densities of the isomers to within 0.04%. All measurements were made at 25° ± 0.1° C. from a constant-temperature bath. By means of 1-ml. and 5-ml. pipets calibrated and found to deliver volumes in the ratio of 1 to 5 to within 0.13%, 10-ml. samples were prepared, varying in composition by 10%.

The absorption of the samples was measured at the four wave lengths designated above and plotted as shown in Figure 4. This graph, covering the entire composition range on the ternary composition axes, served as a working chart for the analysis of experimental samples. The same pipets and quantities were used in preparing the experimental samples for analysis.

ACCURACY OF THE METHOD

The triangular coordinates of Figure 4, constructed from the measured absorption of the synthetic samples, indicate the

Table I. Analyses of Isomeric Xylene Mixtures

Sample	Actual Composition, Volume %			Observed Composition, Volume %		
	Ortho	Meta	Para	Ortho	Meta	Para
I	26.9	25.0	48.1	27.4	24.9	47.8
II	28.8	41.5	29.7	28.9	41.8	29.3
III	56.3	18.0	25.7	55.0	19.1	25.6
III	56.3	18.0	25.7	55.5	18.7	25.6
IV	14.3	71.4	14.3	14.2	71.4	14.4
V	33.3	33.3	33.3	33.7	33.8	32.7

accuracy of the method. The variation in distance between lines of constant percentage does not exceed ±0.5% except in the regions of high concentration of one component; also, the experimental points do not vary by more than ±0.5% from straight constant percentage lines except in those regions near the vertexes and a few points along the 0% para coordinate. The intersections of the constant percentage lines at the experimentally determined points, also, indicate the accuracy of construction of the absorption diagram. As the preparation of the 10-ml. samples used for calibration purposes is omitted in the analysis of isomeric xylene mixtures, the analysis of a mixture would be expected to be more accurate than the location of any point used in the preparation of the working chart.

To test further the accuracy of the method, synthetic samples, prepared from synthetic mixtures of the pure hydrocarbons, were analyzed (Table I).

The two analyses of sample III indicate a possible error in the preparation of the synthetic hydrocarbon mixture. A decrease in concentration of total xylenes would probably eliminate the curvature of the constant percentage lines in regions of high concentration of one component, thus giving a triangular coordinate system.

ACKNOWLEDGMENT

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Determination of Sugar in Forage Plants

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The time required to make sugar determinations on forage plants has been considerably shortened. Extracting either green or dried material with an alcoholic solution in the Waring Blendor was as complete as with the longer A.O.A.C. method employing a Soxhlet extraction. Carotene and sugar were extracted simultaneously from green material by the "foaming mixture" in the blender. Clarification of the extract with lead did not alter the amount of reducing substances found in the plants studied.

THE soluble carbohydrate fraction of forage crops may account for as much as 20% of the feeding value of the crop. During the harvesting procedures a large proportion of these labile constituents may be lost, especially under adverse harvesting conditions (*1*, *8*). Recently there has been an increase in the use of artificial methods of drying forages, and many studies are being conducted to determine the effect of the various drying procedures on the amount of nutrients preserved (*1*, *8*). It is also desirable to study the relative loss of sugars when the crop is cured by various processes, but such a study is handicapped by the lengthy procedure used to perform sugar analyses on the forage.

The object of the present study was to determine whether the usual procedure for sugar analysis could be shortened by using a more rapid method of extraction and by eliminating the lead clarification of the extracts. Because the carotene content is also of interest when the forages are preserved by different methods, an attempt was made to determine both the sugar and the carotene content by one extraction of the same sample of green material.

EXPERIMENTAL

Two methods of extracting dry forage plants were compared. The dry forage material was ground in a Wiley mill, using the screen with 1.0-mm. holes, and 2.0-gram samples were used for all extractions. In one method of extraction, the procedure described for plant tissues by the Association of Official Agricultural Chemists (*2*) was followed, except that a 50 to 60% solution of ethyl alcohol as used for feeds was used in place of 80% alcohol. Others (*3*, *5*) also have found this substitution satisfactory. In this procedure, preliminary heating of the forage material in alcohol on a steam bath was found to be necessary in order to extract all the sugar from the tissue.

The second or experimental extraction procedure used was to extract the dry material with 200 ml. of 75% alcohol in a Waring Blendor for 5 to 7 minutes. The alcohol solution was then filtered by using a Büchner funnel or fritted filter with vacuum and the residue was washed five to six times with 50% alcohol. During the investigations it was found that the filtration process was hastened by layering Hyflo Super-cel suspended in 70% alcohol over the filter paper on the Büchner or over the fritted filter.

Green forage material was extracted by three procedures. In each case 5.0 grams of material were used. Procedure 1 was the method described by the Association of Official Agricultural Chemists (*2*), as modified for use with the dry material. Procedure 2 was the method using the Waring Blendor, with 75% alcohol, for extraction purposes. The third procedure combined the extraction of carotene and sugar from the same sample by employing the "foaming mixture" (*6*). This consisted of using an extraction mixture of 100 ml. of absolute alcohol and 70 ml. of Skellysolve B. The water content of the green material was usually sufficient to give the necessary proportions of alcohol to Skellysolve to produce a desirable foaming mixture. For use in the Waring Blendor the sample was cut as fine as possible (1.25-cm. lengths or less).

Table I. Effect of Method of Extracting Dried Plant Material on Amount of Sugar Found (Dry Basis)

Plant	Sample No.	Date Dried	A.O.A.C., 50–60% Ethanol %	Waring Blendor, 75% Ethanol %
Blue grass	28	10/31/46	11.35	11.50
	28[a]	10/31/46	11.23	11.92
Orchard grass	29	10/31/46	5.38	5.29
Oats	31	10/31/46	13.53	13.57
	31[a]	10/31/46	13.69	13.58
Barley	30	10/31/46	12.95	12.46
	30	10/31/46	12.82	12.33
	30	10/31/46		12.40
	30[a]	10/31/46	12.90	12.48
Ladino clover	27	10/31/46	6.84	6.88
	27[a]	10/31/46	6.91	6.90
Alfalfa	26	10/31/46	6.45	6.45
	26[a]	10/31/46	6.66	6.60
	75	8/45	4.33	4.28
	76	8/45	4.55	4.60
	77	8/45	4.57	4.75
	78	8/45	5.21	5.30
	79	8/45	6.01	5.88
	80	8/45	5.80	6.05
	81	8/45	5.16	5.35
	82	8/45	5.97	6.05
	83	8/45	5.74	6.05
	84	8/45	4.96	5.49
	85	8/45	5.60	5.98
Av. 23 samples			7.77	7.81
Standard error			±0.71	±0.69

[a] Clarified sample.

with a paper cutter, mixed well, and then weighed. The sample was extracted in the blender for 5 to 7 minutes, filtered by vacuum, and washed 5 to 6 times with 50% alcohol. The combined extracts were put in a separatory funnel, where 10% aqueous sodium chloride was added to reduce the alcohol content to approximately 80%. Then 50 ml. of Skellysolve F were added. Two definite layers form after gentle shaking. The epiphase was used for carotene determinations and the hypophase was used for sugar determinations.

The filtered extract from all the different methods of extraction was evaporated on a steam bath to a volume of 10 to 20 ml., then diluted to a given volume. At this point some of the extracts were divided into two portions, for determination of the sugar content with and without clarification of the extract. One aliquot was taken directly for inversion. Another aliquot was clarified by using neutral lead acetate, followed by sodium oxalate, before inversion. Inversion was performed by adding 10 ml. of 20.45% hydrochloric acid per 50 ml. of extract, letting the solution stand for 18 hours, neutralizing it with sodium hydroxide, and diluting further if necessary.

The method of determining reduced copper after boiling the diluted extract with the copper sulfate–alkaline tartrate solutions (*2*) was the dichromate–ferric sulfate–diphenylamine procedure as described by Leonard *et al.* (*5*). Sufficient time, a few minutes with stirring, must be allowed for the cuprous oxide to dissolve completely in the ferric sulfate (*4*). The method was found very satisfactory and, after a little practice, duplicate determinations on a forage extract agreed within 1% of each other.

Each figure presented in the tables is an average of two or three determinations on that particular sample.

RESULTS

The amount of sugar found in dried forage plants by the two methods of extraction is given in Table I. It is evident that the same amount of sugar was extracted in 5 to 7 minutes by using the Waring Blendor as by following the much longer procedure outlined by the Association of Official Agricultural Chemists (*2*).

The results of extracting green forage plants by the three procedures are given in Table II. The amount of sugar extracted by each of the three methods was the same.

With the Waring Blendor procedure the samples that contained a high amount of sugar, over 18% (dry basis), showed a small amount of sugar in the residue when it was re-extracted with 75% alcohol. The amount of sugar found by re-extracting the residue was negligible in all cases where less than 18% sugar was found in the plant tissue.

In order to determine whether the refluxing process as used in the Association of Official Agricultural Chemists procedure had any effect on the sugar in the refluxing liquid, an alcoholic extract of plant tissue was refluxed overnight in the Soxhlet apparatus. It contained less sugar after the overnight refluxing, but the loss was small (2%). This would indicate that prolonged or continued refluxing of a sample could result in a low value for the sugar content of the sample.

The results when sugar was determined on the extracts before and after clarification are shown in Table III. In this comparison only the extract from the green oat plant shows a decrease in reducing substances when clarified with lead. Extracts from the other green and dried forage plants examined show no change in amount of reducing substances when clarified with neutral lead acetate.

Extracts obtained by all three extraction procedures, after evaporation and dilution, were used in determining the effect of clearing with lead. All give the same result.

Table II. Effect of Method of Extracting Green Plant Material on Amount of Sugar Found (Dry Basis)

Plant	Sample No.	Date Sample Taken	A.O.A.C., 50–60% Ethanol %	Waring Blendor: 75% ethanol %	Waring Blendor: Ethanol Skellysolve B %
Bluegrass	19	11/13/46	16.0	16.2	15.7
	20	11/14/46	...	10.8	10.7
Alfalfa	21	11/21/46	13.7	13.3	13.3
	21[a]	11/21/46	13.7	13.6	13.3
Oats	22	12/ 3/46	24.9	24.7	24.4
	22[a]	12/ 3/46	23.3	23.9	23.8
Orchard grass	23	12/ 9/46	18.3	18.3	19.3
	23[a]	12/ 9/46	18.1	18.0	18.9
Ladino clover	24	12/12/46	8.87	8.95	8.89
	24[a]	12/12/46	8.96	...	8.87
Dallis grass (greenhouse)	32	1/29/47	3.63	3.98	3.84
	32[a]	1/29/47	...	4.06	3.93
Bermuda grass (greenhouse)	33	1/29/47	2.16	2.10	2.17
	33[a]	1/29/47	...	...	2.17
Av. 10 samples			14.27	14.30	14.36
Standard error			±2.39	±2.40	±2.42
Av. 12 samples				13.16	13.19
Standard error				±2.16	±2.19

Clarified sample.

DISCUSSION

The results of these experiments indicate that rapid extraction with the Waring Blendor could be used in place of the longer Soxhlet extraction procedure for determining sugar in forage materials. This would eliminate entirely the time-consuming operation of the preliminary heating of the sample in alcohol on a steam bath before initiating the Soxhlet extraction. One example to show that this process was necessary is given in Table III, samples 76 and 76a. Sample 76 averaged 4.55% sugar but sample 76a, which was not given the preliminary heating in alcohol, averaged 4.25% sugar.

The size of the standard error of the mean in these comparisons is not a measure of the accuracy of the method but indicates that the sugar content of the samples analyzed shows the same variation with each method of extraction.

The comparison of methods of extraction reported here for forage plants is in agreement with the comparisons reported by Leonard *et al.* for apple tissue (*5*). They found that the Waring Blendor procedure with 70% alcohol gave the same result as the extracting procedure described by the Association of Official Agricultural Chemists with either 80 or 50% alcohol. Moyer and Holgate (*7*) have used the Waring Blendor with 85% ethyl alcohol to extract sugar from frozen vegetables, with satisfactory results. Recently Waldron *et al.* (*10*) have shown that extracting green vegetables and young bromegrass with 80% ethanol in the Waring Blendor gave comparable results to the Soxhlet extraction procedure as outlined by the Association of Official Agricultural Chemists.

The results of these three separate investigations and the results in this study indicate that the Waring Blendor could be used to replace the longer Soxhlet extraction procedure to extract sugar from many other materials.

When one wishes to determine both the carotene content and the sugar content of the same sample of green forage, considerable time can be saved by using the single extraction procedure for both constituents as carried out in this study.

The comparison between clarification and no clarification indicates very little need for the clarification process in determining sugars in these forage plants. Leonard *et al.* (*5*) found little difference between the results obtained by clarifying or not clarifying the extracts from apple tissue, although one variety of apple showed a decrease in reducing substances when lead clarification was performed. The young green oat plant, 15 to 25 cm. (6 to 10 inches) high, used in this study also showed a decrease in reducing substances when the extract was clarified. It is possible that the stage of maturity of the plant or other factors may alter the results obtained in the clarification comparison. However, comparisons were made on alfalfa from the very young stage up to the hay stage with no differences due to lead clarification of the extract.

Certainly any one performing a large number of sugar analyses on plant products would be benefited by determining what effect, if any, the lead clarification process has on the reducing substances in the extracts under consideration.

In this study clarification with mercuric nitrate which has been recommended for green apple leaves (*9*) was not attempted, nor was the sodium hydroxide–zinc sulfate clarification process (*7*) used.

Table III. Effect of Clearing Alcoholic Extracts of Grasses and Legumes on Amount of Invert Sugar Found in Extract (Dry Basis)

		Fresh Material			Dried Material	
Plant	Sample No.	Not clarified, %	Clarified, %	Sample No.	Not clarified, %	Clarified, %
Blue grass	25	10.90	11.10	28	11.35	11.23
	25	11.03	11.47	28	11.50	11.92
				28–1	11.63	11.75
				28–1	11.63	11.72
Orchard grass	23	18.3	18.1	29	5.38	5.4
	23	18.3	18.0	..	...	...
	23	19.3	18.9	..	...	...
Dallis grass	32	3.84	3.93	..	...	...
	32	3.98	4.06	..	...	...
Bermuda grass	33	2.17	2.17	..	...	...
Barley	..	...	...	30	12.95	12.90
				30	12.46	12.48
Oats	22	24.9	23.3	31	13.53	13.69
	22	24.7	23.9	31	13.57	13.58
	22	24.4	23.8			
		(24.7)	(23.7)			
Ladino clover	24	8.87	8.96	27	6.84	6.91
	24	8.89	8.87	27	6.88	6.90
Alfalfa	21	13.7	13.7	26	6.45	6.66
	21	13.3	13.6	26	6.45	6.60
	21	13.3	13.3	85	5.61	5.58
				84	5.05	4.86
Av. 13 samples				83	5.74	5.74
(excluding oats)		11.22	11.24	82	6.01	5.93
Standard error		±1.56	±1.53	81	5.18	5.13
				80	5.94	5.66
				79	6.15	5.87
				78	5.23	5.19
				77	4.55	4.59
				75	4.30	4.36
				76	4.52	4.57
				76[a]	4.22	4.27
				Av. 25 samples	7.72	7.78
				Standard error	±0.67	±0.70

[a] No preliminary heating on steam bath.

PROCEDURE

As a result of experiments and the reports of others (*5, 7, 10*) the following procedures are recommended for trial in the determination of sugars in forage plants.

Extraction of Material. A. Dried Material. Extract approximately 2.0 grams (sample size should vary inversely to the sugar content) of a finely ground representative sample of the dried forage with 200 ml. of 75% ethyl alcohol in the Waring Blendor for 5 to 7 minutes. Filter the mixture, using a Büchner funnel and vacuum with a layer of Hyflo Super-cel over the filter paper. Wash 5 to 6 times with either 50 or 70% alcohol.

B. Green Material for Sugar. Use 3 to 10 grams of a representative sample of material, cut as finely as possible without forcing large quantities of juice from the stems and leaves. Extract 5 to 7 minutes in the Waring Blendor with 200 to 250 ml. of alcohol so the final concentration of alcohol is over 70%. Filter and wash as described for dry material.

C. Green Material for Sugar and Carotene. Use 3 to 20 grams prepared as described in B. Extract for 5 to 7 minutes in the Waring Blendor with 100 ml. of absolute alcohol and 70 ml. of Skellysolve B. It occasionally may be necessary to add more alcohol (or less frequently Skellysolve) to produce the desired foaming mixture (*6*), depending on the water content of the sample. Filter and wash the mixture. This can be accomplished by means of a fritted-glass filter with an adaptor, so that vacuum can be applied when it is placed over a large separatory funnel. Separate the phases by adding aqueous sodium chloride and 50 ml. of Skellysolve F, and shake gently. Draw off the hypophase and wash the epiphase once with water. For carotene determinations the hypophase may be re-extracted with Skellysolve F.

The total alcoholic extract may be evaporated or an aliquot used for this purpose. Dilute and invert according to A.O.A.C. specifications. When hydrochloric acid is to be used for inversion it should be neutralized with sodium hydroxide to short of neutrality and neutralization completed by careful addition of sodium bicarbonate. Use aliquots of the inverted solution for sugar determination after making the desired dilution. The method for sugar determination used in this report is satisfactory (*5*). Other investigators have used different methods with equal satisfaction (*1, 2, 7, 9, 10*).

It appears that sugar in forage plants as well as apples (*5*) and frozen or green vegetables (*7, 10*) can be extracted as completely in 5 to 7 minutes in the Waring Blendor as by using the much longer Soxhlet extraction procedure. Carotene and sugar can be extracted simultaneously from green forage material by using a mixture of ethanol and Skellysolve and the blender. When aqueous sodium chloride is added to the extract, carotene remains in the epiphase while the hypophase may be used for sugar analysis. Clarification of the extract with lead was found to be unnecessary for the common forage plants used in this study. By employing the shorter but adequate procedures outlined in this study the time required for a sugar analysis on plant materials can be reduced to less than half the time required by the usual A.O.A.C. procedures.

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Effect of Constituent Materials upon Spectrographic Measurement of Seven Impurity Elements

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Inorganic powder samples weighing 350 micrograms were burned to completion in a direct current arc and the effects of constituent elements upon the spectrographic measurement of the seven impurity elements, tin, vanadium, beryllium, cadmium, cobalt, antimony, and manganese were studied.

THE work of Goldschmidt and Peters (*2*) brought into sharp focus the fact of differential volatilization, that elements present in a carbon arc do not emit their spectra simultaneously. They completely volatilized in a carbon arc a cupeled lead bead containing other metals. During the 170 seconds required for complete volatilization, lead appeared in the spectra within the first 20 seconds and disappeared in 50 seconds. Gold did not appear until after 20 seconds, rhodium until after 75 seconds of arcing.

If one volatilizes a sample in a carbon arc, the initial time of emission of a particular element will vary with the volatility of other elements present. Slavin (*4*) has pointed out the failure of intensity methods when a constant exposure time is used to record photographically the emission spectrum of the same element in materials of different composition. He proposed, as a basis for quantitative analysis, the total energy of the emission until all the specimen has been burned in a carbon arc, eliminating the time factor.

Consideration of the foregoing indicates that the use of the carbon arc as a source in quantitative work calls for complete volatilization of the sample. It is desirable, then, to use a sample weight small enough so that it can be burned to completion in a reasonable time. That a very small sample size need not limit quantitative measurements has been shown by Fitz and Murray (*1*). Concerned with the rapid quantitative measurement of minute samples of inorganic powders, they developed a method by which they determined tin, silicon, aluminum, iron, magnesium, copper, manganese, nickel, calcium, and titanium in various silicate materials with an accuracy of 10%. In general, the sample weight was 1.0 mg. but the method encompasses weights ranging from 0.1 to 5.0 mg. The sample was mixed with a large excess of pure powder mixture which serves as buffer and internal standard. Weighed proportions of the mixture were pressed into a pellet and burned completely in a direct current arc. The line intensity ratios between selected lines of sample constituent and internal standard were determined photometrically and converted to weight by reference to calibration curves which were prepared from synthetic standards containing oxides of the ten metals in varying proportions.

These factors point to a broader approach to analytical problems, which is further emphasized by the semiquantitative method of Harvey (*3*). He uses for analysis a 10-mg. sample which is completely volatilized in a direct current carbon arc. Ratios are densitometrically established between selected lines and background and are multiplied by sensitivity factors that have been previously determined for particular spectrum lines in a particular matrix. Thus, prepared standards are eliminated and only a plate calibration curve is needed. The limit of error is 30 to 50%.

In this work, attention was directed to the following points as a basis for a method of semiquantitative analysis of inorganic powders.

Weight of Sample. It was desired to use a minimum weight which would completely volatilize in a reasonable period. At the same time the weight must be large enough to give an accurate intercomparison of samples having different matrices and containing varying numbers of impurity elements. Both 50- and 100-microgram samples were tried but failed to give reproducible results; 350 micrograms were finally used. The region between 100 and 350 micrograms has not been examined.

Presence of Impurity Elements in Sample, excited in the direct current arc. Of the 37 elements used as impurities, seven elements of common occurrence in the laboratory were studied. The choice of seven simply brought the initial investigation within a reasonable scope.

Use of Base Materials (sodium chloride, lead oxide, iron powder, magnesium oxide, and carbon). Because of the limitations of the work these base materials were arbitrarily chosen to cover a wide range of volatility and are not intended to be completely representative. Sodium chloride volatilizes far more readily than many of the impurity elements added to it. Carbon, on the other hand, represents the opposite condition, and lead oxide, iron powder, and magnesium oxide fall between the two extremes of sodium chloride and carbon.

Preparation of Standard Curves for each of the seven elements in each of the five matrices. This was followed by an examination of these 35 curves to determine the effect of the impurities and matrices upon the spectrographic measurement of the seven impurity elements studied.

PREPARATION OF STANDARDS

Two grams of each standard were prepared by weighing out the requisite amounts of base material and impurity elements

Table I. Composition of Standards

(Eleven series of standards: three having carbon matrix, three in sodium chloride matrix, three in magnesium oxide matrix, one in lead oxide matrix, and one in powdered iron matrix)

Series	Base Material	Range of Impurity Elements, P.P.M.	Impurity Elements Present
A B C	Carbon Sodium chloride Magnesium oxide	10,000–100,000	Sb, Au, Pb, Sn, Cd, Mn, Co (7 impurity elements)
D E F	Carbon Sodium chloride Magnesium oxide	5000–50,000	Zn, Be, Sb, Au, Pb, Sn, Cd, Mn, Co, V, As, Ag (12 impurity elements)
G H I	Carbon Sodium chloride Magnesium oxide	50–10,000	B, Ca, Cd, Fe, Si, Be, Zn, Li, K, Cr, Mn, As, Ag, Sn, In, Sb, Au, Pb, Tl, Ni, Co, Pd, V, Rh, Hg, Al, Cu, Ge, Se, Rb, Sr, Ru, Te, Cs, Ba, W, Ir (37 impurity elements)
J	Lead oxide	100–50,000	Sb, Au, Sn, Cd, Mn, Co, V, As, Ag, Be, Na, Zn (12 impurity elements)
K	Iron powder	100–50,000	Sb, Au, Sn, Cd, Mn, Co, V, As, Ag, Zn, Be, Na (12 impurity elements)

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as the metal, oxide, or chloride. These were mixed and hand-ground in an agate mortar for 15 minutes, then placed on a mixing mill for 1 hour, and finally hand-ground for an additional 15 minutes.

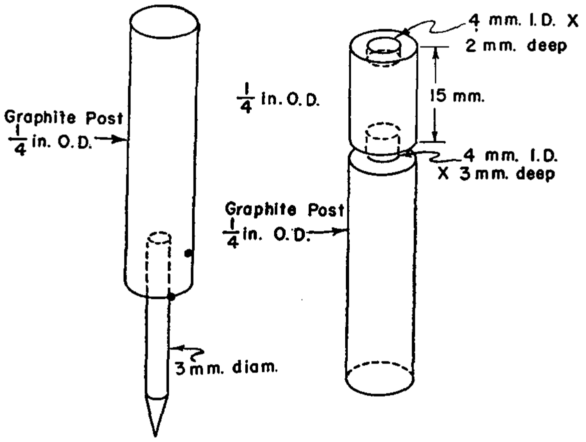

Figure 1. Dimensions of Graphite Electrode

Eleven series of standards in five base materials were made up (Table I). Three series were of carbon base containing, respectively, 7, 12, and 37 impurity elements. Three each were of sodium chloride and magnesium oxide base prepared in the same manner as the carbon series. The lead oxide base contained twelve impurities; the iron powder, twelve. Consideration of Table I will make clear the fact that for impurity element concentration ranges of 20,000 to 50,000 p.p.m., the combined amounts of elements added as impurities are of the same order of magnitude as the base material.

Intensity ratio values derived from series A, B, and C were not used in the final calculations because the three standard series so closely resembled series D, E, and F in number and range of impurities.

For every set of standards, moving plates were run to determine maximum time necessary for the complete burning of the sample. The sample was made up of the mixture of the base material and impurity elements as standard, and the graphite containing the internal standard elements, molybdenum and bismuth. The time was set at 2 minutes.

DETAILS OF ANALYTICAL PROCEDURE

The Wadsworth fully automatic stigmatic grating spectrograph, 21-foot grating 15,000 lines per inch (2.5 cm.) (Jarrell-Ash Company) was used. The optical system consisted of a 350-mm. quartz lens focused beyond the slit, and a step sector. The slit height was 14 mm.; the slit width, 25 microns.

Electrodes were prepared from special spectrographic graphite (National Carbon Company). Figure 1 illustrates the electrode setup. The lower electrode (positive) consisted of a 15-mm. length of $^1/_4$-inch carbon rod, in the lower end of which a 3-mm. crater with a 4-mm. inside diameter was drilled, for mounting on a carbon post. In the upper end a 2-mm. crater, with a 4-mm. inside diameter, was drilled. In this the charge was placed. The upper electrode, made from $^1/_8$-inch carbon rod, was cut to a sharp point and inserted in the upper carbon electrode holder. Both upper and lower electrodes were preburned for 35 seconds.

All weighings were made on an assay balance. A thin platinum spatula, about 6 cm. in length, was used to handle the powders. The material to be weighed was placed on a watch glass 3 cm. in diameter, in which an off-center hole (2 to 3 mm. in diameter) had been blown toward the underside of the glass. This provided a slight lip which fitted easily into the mouth of the electrode crater, and the material was transferred to the crater by brushing it carefully through the hole with a fine camel's-hair brush; 350 micrograms of standard and 250 micrograms of internal standard (20% molybdenum and 10% bismuth in a powdered carbon base) were weighed separately, transferred to the crater, and distributed evenly over the crater floor by tapping gently. To avoid loss of charge with initial arcing, a drop of collodion (ether and collodion, 1 to 1 by volume) was added.

Charges were burned for 2 minutes at 11.2 amperes and 250 volts input, with a 4-mm. electrode separation, kept constant by manual operation. The upper electrode was changed for each sample.

Eastman SA-1 plates were used and developed in D-19 at 18° ± 1° C. for 3 minutes.

Lines Used

Be 3130.416/Mo 3132.594	Mn 2576.104/Bi 2897.975
Sb 2877.915/Bi 2897.975	Sn 3175.019/Mo 3170.347
V 3183.982/Mo 3170.347	Co 3044.005/Mo 3170.347
Cd 3261.057/Mo 3170.347	

PREPARATION AND USE OF CURVES

Plate calibration curves were run and intensity ratios calculated from transmittance values obtained by the use of the ARL-Dietert microphotometer.

With a 14-mm. slit height, the step sector gave greater latitude in reading the spectra of elements of lesser sensitivity and those of greater sensitivity on the same step spectrum. By application of the step sector ratio (1.5) the microphotometer measurements for lines in different steps were converted to actual relative intensities.

Every sample was run in quadruplicate. The internal standard line (bismuth or molybdenum), used for each impurity element, was assigned on the basis of proximity to the line of the element under examination. For the four intensity ratios thus found, any value the deviation of which exceeded four times the average deviation of the remaining intensity ratios was dis-

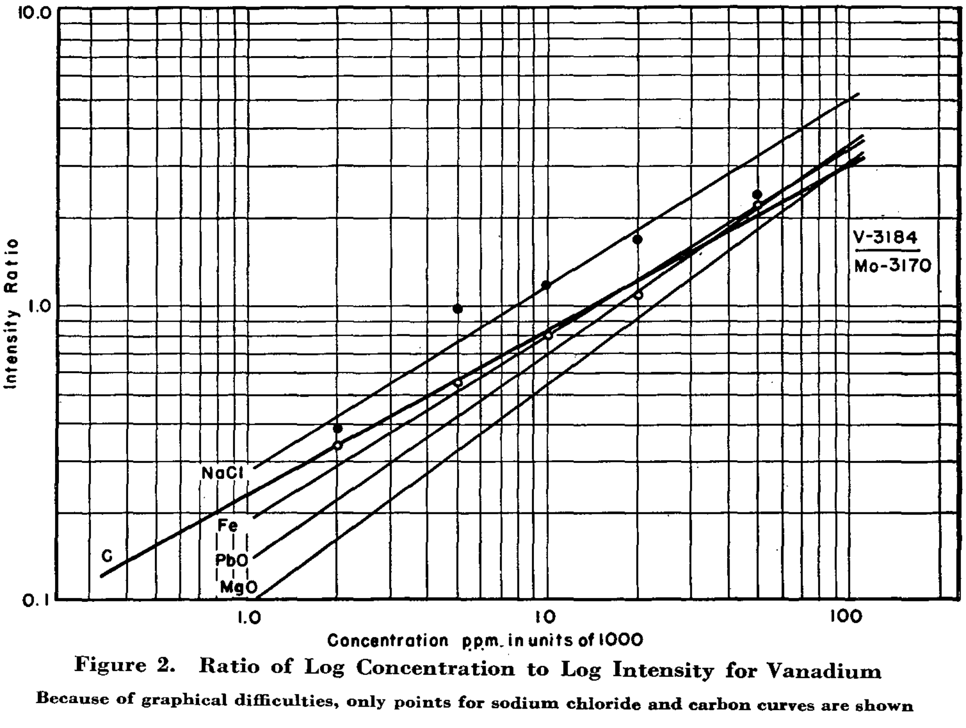

Figure 2. Ratio of Log Concentration to Log Intensity for Vanadium

Because of graphical difficulties, only points for sodium chloride and carbon curves are shown carded (20 out of 700 were rejected). The mean intensity ratio was used to plot log of concentration against log of intensity ratio for a given element in a given matrix and the best possible curve was drawn through these points. Figure 2 shows such curves for one element.

Curves were prepared for the seven impurity elements, tin, cadmium, antimony, cobalt, manganese, beryllium, and vanadium. Table II shows the series of standards from which the mean intensity ratio was chosen for each concentration of each element.

For example, in plotting the curves for vanadium (Figure 2) the range of the curves covers 2000 to 50,000 p.p.m. For the three curves of vanadium in carbon, sodium chloride, and magnesium oxide, the points for 2000 and 5000 p.p.m. were drawn from the data of series G, H, and I. The points 10,000, 20,000, and 50,000 p.p.m. were taken from the data of series D, E, and F. For the curves of vanadium in lead oxide and iron, series J and K were used. Thus, a standard curve for an impurity in a given matrix is made up of points representing the intensity ratios for a given concentration chosen at random, with no deliberate regard for anything but the base material.

This procedure gave 35 standard curves (seven impurity elements in each of the five matrices). Then the curve for each element in a given matrix was made the reference curve. All other intensity ratios for that element, in all other matrices, were referred to this curve. The amount of the element present as represented by its intensity ratio was read from the reference curve. This was repeated for each element in each matrix until every intensity ratio value of each impurity element in each matrix had been referred to the reference curve.

Table II. Series of Standards Used for Curves

Impurity Elements	Range on Standard Curve, P.P.M.	Combinations of Standards Series Used for Curves							
		D	E	F	G	H	I	J	K
		(12)[a]			(37)			(12)	(12)
Vanadium	2000–50,000	*	*	*	*	*	*	*	*
Cadmium	5000–50,000	*	*	*				*	*
Beryllium	100–1000				*	*	*	*	*
Tin	[illegible]000–50,000	*	*	*	*	*	*	*	*
Cobalt	5000–50,000	*	*	*				*	*
Antimony	5000–50,000	*	*	*				*	*
Manganese	200–10,000				*	*	*	*	*
Impurity range for given standard series, p.p.m.		5,000–50,000			50–10,000			100–50,000	100–50,000

[a] Numbers in parentheses indicate number of impurity elements present in given matrix. Specific elements are listed in Table I.

Table III. Measurement of Impurity Elements

(Standard deviation from known amount expressed as factor)

Element	Matrix	Deviation Factors (Quotients)								
Tin	MgO	1.5	1.6	1.4	2.0	1.3	1.6	...	...	...
	C	1.6	1.5	1.5	1.7	4.6	1.4	...	...	...
	NaCl	1.6	1.6	1.9	1.4	1.3	1.3	...	...	...
	PbO	2.5	2.6	1.7	2.6	1.4	1.4	...	...	...
	Fe	3.9	4.6	3.1	4.8	2.5	2.6	...	...	...
Cobalt	MgO	1.7	1.7	1.6	3.0	...	...	...	...	...
	C	1.6	1.3	1.3	2.2	...	...	...	...	...
	NaCl	1.4	1.6	1.7	1.6	...	...	...	...	...
	PbO	3.4	3.0	1.8	3.1	...	...	...	...	...
	Fe	2.1	2.6	1.9	3.3	...	...	...	...	...
Manganese	MgO	1.1	1.3	1.4	1.9	2.6	2.3	3.5	3.7	...
	C	1.7	1.7	1.6	1.5	1.4	1.5	1.7	1.7	...
	NaCl	1.3	1.3	1.6	2.0	2.7	2.3	3.4	3.4	...
	PbO	1.3	1.3	1.6	2.1	2.4	3.5	2.9	2.8	...
	Fe	1.4	1.4	1.5	1.6	1.8	1.6	1.8	1.9	...
Vanadium	MgO	1.3	2.0	2.3	3.3	2.7	...	...	...	...
	C	1.3	1.6	1.8	2.0	1.5	...	...	...	...
	NaCl	1.7	1.6	1.7	1.5	1.7	...	...	...	...
	PbO	1.2	1.4	1.7	2.0	1.5	...	...	...	...
	Fe	1.2	1.5	1.8	2.4	1.6	...	...	...	...
Cadmium	MgO	1.1	1.2	1.2	1.7	...	...	...	...	...
	C	1.4	1.2	1.5	1.5	...	...	...	...	...
	NaCl	1.4	1.2	1.4	1.4	...	...	...	...	...
	PbO	3.1	1.4	1.7	1.6	...	...	...	...	...
	Fe	1.5	1.6	1.8	2.5	...	...	...	...	...
Beryllium	MgO	...	...	...	...	...	2.1	2.2	2.3	2.1
	C	...	...	...	...	...	1.6	1.7	1.7	1.8
	NaCl	...	...	...	...	...	1.5	1.5	1.7	1.9
	PbO	...	...	...	...	...	1.6	1.6	1.6	1.7
	Fe	...	...	...	...	...	1.6	1.9	2.2	2.7
Antimony	MgO	1.1	1.2	1.4	1.6	...	...	...	...	...
	C	1.1	1.2	1.3	1.4	...	...	...	...	...
	NaCl	1.2	1.2	1.4	1.5	...	...	...	...	...
	PbO	1.2	1.4	1.6	1.7	...	...	...	...	...
	Fe	1.2	1.4	1.4	1.6	...	...	...	...	...
Concentration range, p.p.m.		50,000	20,000	10,000	5,000	2,000	1,000	500	200	100

For example, the curve of beryllium in magnesium oxide was made the reference curve. All intensity ratios for beryllium in sodium chloride, carbon, lead oxide, and iron were then converted to concentration of beryllium present by reading them from the magnesium oxide curve. The curve of beryllium in sodium chloride was made the next reference curve and all intensity ratios for beryllium in magnesium oxide, carbon, lead oxide, and iron were converted to concentration of beryllium. Tabulation of the values so read presented an evaluation of the measurement of a known amount of an impurity element, regardless of the matrix in which found or the number of additional impurity elements present in the matrix.

From this tabulation the standard deviation for the measured values of a known amount of any one of the seven impurities considered, was calculated. Table III summarizes the standard deviations, expressed as factors, for the measured values of known amounts of each impurity element as referred to its curve in one matrix. It is noted that measurement of 50,000 p.p.m. of tin in the four matrices, carbon, sodium chloride, lead oxide, and iron, when read from the curve of tin in magnesium oxide, may be expected to fall within a range of 33,000 to 75,000 p.p.m. Its measurement in the matrices magnesium oxide, carbon, sodium chloride, and iron, when read from the curve of tin in lead oxide, falls in the range of 20,000 to 125,000.

On the basis of the preceding discussion, Table IV summarizes the choice of matrices which may best be used for preparing one standard curve for an impurity element. The amount of the impurity in all matrices referred to the curve of the element in any one of these matrices shows a factor of deviation which does not exceed 2. The standards of manganese ranging from 200 to 50,000 p.p.m. may be prepared in either carbon or iron powder. Assuming the choice of carbon, analysis may then be run for manganese occurring in materials whose major constituent is magnesium oxide, carbon, sodium chloride, lead oxide, iron, or a combination of these. The intensity ratio of Mn 2576/Bi 2898 for the sample analyzed is referred to the standard curve of manganese in carbon. The amount of manganese in the sample, as read from the curve, will have a lower and upper limit, respectively, of 50 and 200%.

CONCLUSIONS

If one burns to completion a 350-microgram sample of any one of the five matrices, magnesium oxide, carbon, sodium chloride, lead oxide, and iron, containing the impurity elements, tin, cadmium, cobalt, vanadium, beryllium, antimony, and manganese, the following conclusions may be drawn:

Under the conditions described in this paper, the spectrographic determination of an element in one matrix may be made with reference to an analytical curve prepared for the element in another matrix.

The error in the spectrographic measurement of any one of these seven elements may be limited,

Table IV. Best Matrix Material for Standard Curve

(Maximum deviation factor of impurity element from true amount does not exceed 2)

Element	Range, P.P.M.	MgO	C	NaCl	PbO	Fe
Be	100–1000		*	*	*	
Sb	5000–50,000	*	*	*	*	*
V	2000–50,000		*	*	*	
Cd	5000–50,000	*	*	*		
Mn	200–50,000		*			*
Sn	1000–50,000	*		*		
Co	5000–50,000			*		

by the proper choice of matrix for the standard curve, to a high factor of 2 and a low factor of 0.5.

The presence of a large number of impurities or a large quantity of any one element does not affect these limiting factors.

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Photometric Determination of Sulfur in Metals and Alloys

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A photometric method for the determination of sulfur in metals and alloys is described. The method consists of solution of the sample in a mixture of hydrochloric and nitric acids, destruction of the nitric acid, reduction of the sulfate to sulfide with the aid of hydriodic acid, distillation of the hydrogen sulfide into a solution of ammonium hydroxide, and finally precipitation as colloidal lead sulfide and measurement of the transmittancy of the sulfide sol at 370 mμ.

A METHOD published by the author a few years ago (*2–4*) has been used extensively in these laboratories for the determination of sulfur in metals and alloys. It consists of solution of the alloy in a mixture of nitric and hydrochloric acids, destruction of the nitric acid, reduction of the sulfate to sulfide with the aid of hydriodic acid and distillation, and iodometric titration of the resultant hydrogen sulfide. In order to increase the sensitivity of this method it has been modified to provide for a photometric determination of the sulfur as lead sulfide—a procedure suggested by the work of Field and Oldach (*1*). Suitable techniques have been developed to make it applicable to the determination of 1 to 50 micrograms of sulfur in 0.1 to 1 gram of most of the common metals and alloys.

APPARATUS

Distillation Apparatus. The distillation apparatus diagramed in Figure 1 was used.

Photoelectric Photometer. An Evelyn photometer was used in the present investigation and the procedure was written with this instrument in mind, but any instrument of comparative quality is suitable.

REAGENTS

Standard Potassium Sulfate Solution (1 ml. = 5 micrograms of sulfur). Transfer 27.2 mg. of pure dry potassium sulfate to a 1-liter volumetric flask, dissolve, and dilute to the mark with distilled water.

Redistilled Hydrochloric Acid. Transfer 500 ml. of hydrochloric acid (specific gravity 1.19) and 450 ml. of distilled water to a 1500-ml. standard-taper round-bottomed Pyrex flask and add a few grains of 12-mesh silicon carbide. Connect to an all-glass water-cooled distillation apparatus and heat to boiling. When enough of the acid has been distilled over to wash out the condenser system and to ensure that the acid coming over is constant boiling—e.g., 50 ml.—replace the receiver with a clean Pyrex bottle. Continue the distillation until the volume in the flask has been reduced to 100 ml. Stopper and reserve the distilled acid.

Redistilled Nitric Acid. Distill undiluted nitric acid (specific gravity 1.42) in the manner described above into a clean Pyrex bottle, discarding the first 25-ml. portion of acid that comes over. Stopper and reserve.

Redistilled Formic Acid. Oxidize the sulfite in commercial formic acid by titration with 0.1 *N* potassium permanganate until the solution acquires a pink tint which persists for a few seconds. Distill as directed above. Stopper and reserve.

Hydriodic Acid–Hypophosphorous Acid Mixture. Transfer 100 ml. of hydriodic acid (specific gravity 1.70) and 25 ml. of hypophosphorous acid (50%) to a clean 250-ml. Vycor Erlenmeyer flask. Add a few grains of clean 12-mesh silicon carbide, heat to vigorous boiling without a cover on a hot electric plate, and boil for exactly 3 minutes. Cover and cool to room temperature. Ignore any iodine that may appear in the solution on standing.

Lead Citrate Solution. Dissolve 5 grams of lead nitrate in 50 ml. of distilled water and dilute to 100 ml. Add 20 grams of citric acid and warm to dissolve. If a precipitate appears on standing, use only the supernatant solution or allow to stand overnight and then decant from the precipitate.

PROCEDURE

Preparation of Calibration Curve. Add from a buret 0, 2, 4, 6, 8, and 10 ml. of the standard potassium sulfate solution (1 ml. = 5 micrograms of sulfur) to clean 50-ml. standard-taper Pyrex Erlenmeyer flasks (Figure 1). (Take care to prevent contamination of the samples with sulfate from unclean glassware or impure chemicals. Whenever an acid or acid mixture is called for, use the redistilled acids described under reagents.) Carry each sample separately through the steps indicated below.

Evaporate nearly to dryness on a hot plate, in an oven or even over a flame, providing the flaming is done in a hood and the flask is held in such a position that any sulfur acids from the flame do not enter the flask. Cool to room temperature and add 5 ml. of hydrochloric acid. Add 15 ml. of hydriodic-hypophosphorous acid mixture, making sure that a drop or two falls on the ground-glass joint, so that the latter will be wet in the subsequent distillation. Stopper at once with the distillation head and immediately dip the arm into 15 ml. of ammonium hydroxide (1 to 2) in a clean 25-ml. volumetric flask. [It is imperative that the volumetric flask and the side arm of the distillation head be freed of any lead salts (that may be present from a previous analysis) by washing in nitric acid (1 to 1) and then distilled water.]

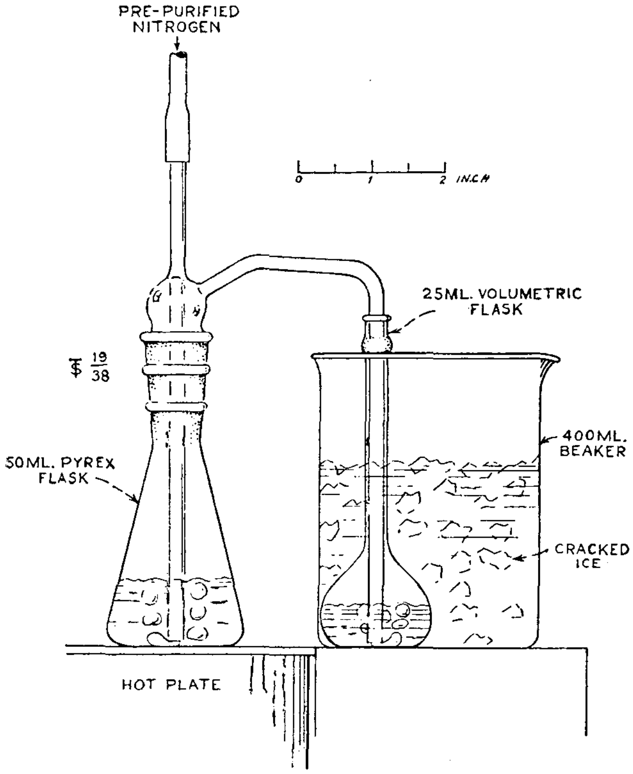

Figure 1. Distillation Apparatus

Place the Erlenmeyer flask on a definite spot on a hot plate, so that the center of the flask is 5 cm. in from the edge of the plate. Arrange to surround the volumetric flask with a crushed ice bath as indicated in Figure 1. The surface temperature of the hot plate should be previously adjusted with a Variac, so that a thermometer will read 185° ± 3° C. when its bulb is suspended near the bottom of a 30-ml. beaker (containing 15 ml. of Dow Corning 550 silicone oil) placed next to the spot on the plate reserved for the 50-ml. Erlenmeyer flask. (The temperature chosen for the distillation is not critical, providing that it is somewhere in the region of 185° C. and is maintained constant.)

Connect the distillation head immediately to a tank of pure nitrogen (previously adjusted to a definite rate of flow), using a rubber tubing that is not likely to give off any sulfur or sulfur compounds to the nitrogen that passes through it. (To prevent oxidation of the sulfide it is essential that the sample be at room temperature and that the nitrogen line be connected immediately, in order that removal of air from the flasks will be completed before distillation of the sulfide begins.) The rate of flow of nitrogen must be rather closely controlled if reproducible results are to be obtained. To do this it is recommended that the flow be regulated by means of the usual pressure gage until the rate is such that 100 ml. of water are displaced in 30 ± 1 seconds from an inverted water-filled 100-ml. graduate when the end of the rubber hose is held under the graduate as the latter is suspended to a depth of 2.5 cm. in a water bath.

Distill for exactly 5 minutes after the first appearance of white fumes escaping from the volumetric flask, keeping the latter in intimate contact with crushed iced by removal of excess water and replacement with ice. (Sulfide will be lost if the ammonium hydroxide solution is not kept cold. Even with this precaution the amount of sulfide that can be safely held is limited.) Finally remove and separate the flasks. Immediately pipet 1 ml. of the lead citrate solution to the volumetric flask, swirl, dilute to the mark, mix well, and transfer to a dry absorption cell. Read the per cent transmittancy at 370 mμ with the instrument set at 100% transmittancy with distilled water. (It is essential that all operations from the finish of the distillation to the reading of the per cent transmittancy be done as quickly as is convenient, in order to prevent oxidation of the ammonium sulfide and to obtain the photometric reading before appreciable agglomeration of the colloidal lead sulfide takes place.)

Prepare a standard curve by plotting, on semilog paper, micrograms of sulfur against per cent transmittancy values that have been corrected for the blank. The latter should correspond to not more than about 0.5 microgram of sulfur. The corrected per cent transmittancy values are obtained as follows:

$$\frac{\%\ T \text{ of sample } vs. \text{ H}_2\text{O}}{\%\ T \text{ of blank } vs. \text{ H}_2\text{O}} \times 100 = \text{corrected } \%\ T$$

Analysis of Sample. Transfer 0.1 to 1 gram of the sample, depending on the sulfur content, to a clean 50-ml. standard-taper Pyrex Erlenmeyer flask (Figure 1). (For samples that dissolve with unusual violence—tin, aluminum, and magnesium alloys—the initial solution is best accomplished in a clean covered 150-ml. beaker.) For a 1-gram sample add 10 ml. of a freshly mixed solution of 80 ml. of hydrochloric acid and 20 ml. of nitric acid. (If a sample smaller than 1 gram was taken in order to keep the amount of sulfur to be determined below 50 micrograms, it will be necessary to reduce proportionately the amount of solvent acids used, or boil off the excess after solution of the sample, in order to avoid using too much formic acid.) Cover and heat gently to start dissolution of the sample. Cool in an ice bath if the initial reaction becomes too violent. Continue to heat gently until solution of the sample is complete. (If hydrolysis occurs at any time up to the point where complete destruction of the nitrates has been accomplished, add 5 ml. of hydrochloric acid.)

Place the flask on a hot plate with surface temperature of about 130° C. and add from a medicine dropper about 0.5 ml. of formic acid. Cool the flask in an ice bath if the reaction becomes too violent. Continue the addition of small portions of formic acid until gas ceases to be evolved from the solution and it is evident that nitrogen acids have been completely destroyed. In general, not more than 1 or 2 ml. of formic acid will be required. Boil down as far as possible—to 1- or 2-ml. volume—on a flame to expel formic acid. Add 10 ml. of hydrochloric acid and boil down to 5 ml. on a flame. Cool to room temperature.

Add 15 ml. of hydriodic-hypophosphorous acid mixture, cap with the distillation head, and dip the arm into 15 ml. of ammonium hydroxide (1 to 2) in a clean 25-ml. volumetric flask as directed above. (For copper and copper alloys it is best to use 20 ml. of the hydriodic-hypophosphorous acid mixture to ensure complete solution of the cuprous iodide and thus to prevent bumping.) If heat is generated on adding the acid mixture or if iodine is liberated in the solution in the distilling flask, cool to room temperature, allow to stand, and shake occasionally until the iodine has been reduced. Finally place the flask on the hot plate, connected to the nitrogen, and proceed with the distillation and photometric determination as directed above. Run a blank through the entire procedure, using equivalent quantities of acids and taking precautions not to lose sulfuric acid during the evaporations. The blank should correspond to not more than about 1 microgram of sulfur. (The destruction of the nitric acid with formic acid is more difficult to initiate when running a blank than it is for a sample. This suggests that the reduction reaction is catalyzed by certain metal ions.) Obtain the corrected per cent transmittancy for the sample and read from the standard curve the weight of sulfur present in the sample.

PRECAUTIONS AND MODIFICATIONS OF PROCEDURE REQUIRED IN ANALYSIS OF CERTAIN METALS

Arsenic, Antimony, Tin, and Germanium. Arsenic precipitates first as the iodide and then as metal during the sulfide distillation. For this reason it is best to limit the sample size to 0.5 gram to minimize bumping. If a larger sample is to be analyzed it may be best to resort to removal of the arsenic by distillation as bromide, using the method described below for selenium.

When analyzing antimony it is necessary to add an extra 5 ml. of hydrochloric acid with the 10 ml. of hydrochloric-nitric acid mixture used for solution of the sample. This precaution plus that of keeping the flask covered during solution of the sample will prevent hydrolysis of the antimony. The hydrolysis is harmful only in that it may prevent complete destruction of the nitrates in the solution.

Care must be taken in the solution of tin alloys to prevent loss of the sample due to the violent boiling that occurs as the divalent tin suddenly goes over to the oxidized state.

Germanium precipitates as the oxide on solution in the acid mixture. In order to reduce the bumping and to minimize the amount of germanium that accompanies the sulfide in the subsequent distillation, it is best to remove the bulk of the germanium by distillation as follows:

Dissolve 0.5 gram of the sample in 10 ml. of hydrochloric-nitric acid mixture in the usual manner. Remove the cover and boil down on a flame to about 0.5 ml. Repeat the distillation one or more times by adding 10 ml. of hydrochloric acid and boiling down to 0.5-ml. volume. Care must be taken to avoid loss of sulfuric acid. Finally add 10 ml. of hydrochloric acid, destroy the nitrates, and proceed with the distillation in the usual manner.

Some arsenic, tin, germanium, and probably antimony will accompany the sulfide in the distillation, but this usually causes no trouble. Evidence of the presence of these metals in the distillates comes from the fact that the absorbancy of the lead sulfide solutions increases rather than decreases on standing. It is necessary to have sufficient hypophosphorous acid present to ensure complete reduction of tin to the less volatile stannous state; otherwise marked contamination of the distillate will take place.

Tellurium and Selenium. Tellurium precipitates as the metal during the sulfide distillation. In order to minimize bumping it is best, therefore, to limit the sample size to 0.5 gram. Selenium metal cannot be analyzed as directed in the procedure, for although most of it precipitates as metallic selenium during the distillation, appreciable quantities accompany the sulfide and precipitate as lead selenide in the photometric analysis. In order to obtain correct results for sulfur it is necessary, therefore, to resort to removal of the selenium by distillation as bromide.

Dissolve 1 gram of selenium metal plus 0.25 gram of relatively sulfur-free zinc or zinc alloy—e.g., Bureau of Standards zinc base No. 94—in 10 ml. of the hydrochloric-nitric acid mixture in the usual manner. Boil off the excess acid on a flame until a precipitate begins to appear—i.e., reduce the solution to 1- or 2-ml. volume. Cool, add 10 ml. of redistilled hydrobromic acid (prepare the sulfur-free acid by distilling 1 liter of hydrobromic acid, specific gravity about 1.38, in an all-glass still discarding the first 50 ml. that comes over), and boil down on a flame to about 0.5-ml. volume. (There is danger of loss of sulfuric acid during the expulsion of the selenium if the sample is flamed to dryness and if zinc or some other metal is not present.) Transfer the flask to the hot plate used in the distillation of the sulfide and allow the rest of the hydrobromic acid to boil off, flaming the sides and top of the flask to expedite the expulsion of the acid. When all or nearly all of the excess hydrobromic acid has been expelled, cool the flask, add 2 ml. of hydrobromic acid, and repeat the distillation process. After the second removal of excess hydrobromic acid on the plate, add 2 ml. of hydrochloric-nitric acid mixture, boil down on a flame to 0.5-ml. volume, add 5 ml. of hydrochloric acid and 0.5 ml. of formic acid, boil down to 0.5 ml. on a flame, add 5 ml. of hydrochloric acid, cool, add 15 ml. of hydriodic-hypophosphorous acid mixture, and distill as usual. Run a reagent blank, adding the 0.25-gram portion of zinc or zinc alloy.

Lead Alloys. Because of the low solubility of lead chloride it is necessary, if solution in hydrochloric-nitric acid solution is desired, to obtain the sample in as great a state of subdivision as possible before solution of the alloy is attempted. Even so, it is not always possible nor indeed necessary to accomplish complete decomposition of lead alloys, for this will usually take place upon subsequent boiling with the distillation mixture. Decomposition is best accomplished by heating gently on an asbestos pad, keeping the flask covered to prevent loss of too much acid, and adding more hydrochloric acid if necessary. Because little or no oxidizing action occurs during solution of a lead alloy, more formic acid will be required than is the case in alloys of copper or tin.

Lead Metal. Metallic lead must be dissolved in nitric acid.

Dissolve 0.5 gram of the sample in a mixture of 4 ml. of distilled water and 1 ml. of nitric acid with gentle heating. When solution is complete, boil on a flame until the volume is reduced to 1 ml. or until salts begin to precipitate. Add 25 ml. of hydrochloric acid and heat to dissolve all the lead chloride. Treat with formic acid to destroy nitrates, and proceed in the recommended manner, adding 15 rather than 10 ml. of hydrochloric acid after the expulsion of the formic acid.

Silver and Silver Alloys. Silver alloys that can be decomposed in the recommended hydrochloric-nitric acid mixture can be analyzed as directed. If severe bumping is encountered it is best to remove the silver chloride before treatment with formic acid.

The following procedure is recommended for silver metal.

Dissolve 1 gram in a covered 100-ml. beaker in a mixture of 2 ml. of nitric acid and 6 ml. of water with gentle heating. Add 10 ml. of hydrochloric acid, stir, and warm gently to coagulate the precipitate. Allow to settle and tamp down the precipitate. Decant the solution into a 50-ml. standard-taper Erlenmeyer distilling flask and wash the precipitate twice by decantation, using 3-ml. portions of hydrochloric acid. Ignore traces of silver chloride that may precipitate on cooling. Boil the solution down to 15-ml. volume, add formic acid to destroy the nitric acid, and proceed with the analysis in the usual manner.

Mercury. In the analysis of metallic mercury dissolve a 1-gram portion in 5 ml. of nitric acid with gentle heating. Boil the solution down to 0.5 ml., add 10 ml. of hydrochloric acid followed by formic acid, and proceed in the usual manner.

Molybdenum. When molybdenum metal is analyzed by the unmodified method very low results are obtained. Thus, when a 1-gram sample is analyzed only about 15% of the sulfur is recovered. By reducing the sample size the percentage recovery can be greatly increased. When a 0.5-gram sample is taken the recovery is about 85% and for a 0.25-gram sample it is about 95%. The low results may be due to depletion of reducing power of hydriodic-hypophosphorous acid solution due to the large valence change that occurs in the reduction of the molybdenum during the distillation, but further work needs to be done to prove this. [Almost complete recovery of the sulfur can be obtained in the analysis of 1 gram of chromium (present as chromic chloride) or 1 gram of vanadium (present as ammonium vanadate)].

In view of the low results mentioned it is desirable, when analyzing samples containing over about 0.1 gram of molybdenum, to remove most of the latter by an ether extraction before performing the sulfur distillation.

Dissolve 0.5 gram of the sample in 5 ml. of hydrochloric-nitric acid solution in the usual manner. Boil down on a flame until the molybdenum just starts to precipitate. (Oxidizing acids must be present during the ether extraction to keep the molybdenum in the oxidized state; otherwise highly incomplete extraction will result.) Add 10 ml. of hydrochloric acid and heat to dissolve all material that has precipitated. Cool to room temperature. Pour the solution into a clean 150-ml. separatory funnel and wash the flask with 2 ml. of hydrochloric acid, added from a medicine dropper. Reserve the flask. Add 50 ml. of pure ethyl ether, stopper, and shake vigorously to extract the molybdenum—relieving the pressure by opening the stopcock or removing the top from time to time. After the excess pressure has been eliminated shake for a few seconds and then allow the layers to separate. Drain off the lower acid layer into the reserved flask. Add 5 ml. of hydrochloric acid to the separatory funnel and shake for a few seconds. Drain the acid into the flask. Place the flask on an electric hot plate and boil off the ether. Add about 1 ml. of formic acid and heat gently to destroy nitrates. Boil down on a flame to 5-ml. volume. Cool, add 15 ml. of hydriodic-hypophosphorous acid mixture, and continue as directed in the procedure.

Aluminum and Magnesium. Because of the large amount of heat generated during solution of aluminum and magnesium alloys, it is best to add small portions of the sample to the acid in the flask while the latter is resting in an ice bath. Even with these precautions enough acid may boil off to permit hydrolysis to take place. In view of this and because of the fact that some of these alloys contain considerable amounts of silicon, it is often more convenient to limit the sample size to 0.5 gram.

Nickel. To analyze metallic nickel, dissolve 1 gram of the sample in 10 ml. of nitric acid, boil off all the nitric acid, bake on a flame until most of the nitrates have been converted to oxides, cool, add 10 ml. of hydrochloric acid, heat to dissolve all the salts, destroy the nitrates, and proceed in the usual manner.

Chromium, Silicon, Tungsten, and Zirconium. Materials that are not soluble in nitric or hydrochloric-nitric acid mixtures cannot be analyzed by the recommended method. If the materials are alloyed sufficiently to permit solution or disintegration, an analysis can usually be performed. In some instances it may be possible to resort to a preliminary alkaline fusion.

Table I. Calibration Curve Data

No.	Sulfur, γ	% Transmittancy
1	0	98.00
2	10	64.00
3	20	42.75
4	30	25.50
5	40	16.25
6	50	9.50

DISCUSSION

During the development of the method, spectrophotometric investigation of the lead sulfide sol showed that the transmittancy decreases with decreasing wave length, which suggests that the effect measured may be, at least in part, light scattering rather than light absorption. At 350 mμ or thereabouts the blank, which consists of a mixture of lead nitrate, ammonium citrate, ammonium iodide, and ammonium chloride in dilute ammonium hydroxide solution, begins to exhibit absorption which becomes very marked at about 270 mμ (anions?). In order to avoid this interference, quantitative measurements are best made at a wave length in the region of 370 mμ. Beer's law is followed very well.

The quantitative reduction of sulfate to sulfide is difficult to achieve under the conditions employed in the present method. Several things influence the speed and completeness of the reduction. Among them are acid concentrations, time and temperature of distillation, rate of flow of nitrogen during distillation, etc. Because of the empirical nature of the method, therefore, it is necessary to standardize against sulfate instead of sulfide and to control all important variables.

Extreme precautions must be taken to ensure that none of the distilled sulfide is precipitated previous to the addition of the lead citrate solution; otherwise low results will be obtained due to agglomeration of the sol. Precipitation of the sulfide can usually be recognized visually, but it is safer to rely on the photometer for confirmation. When contamination of the distillate by metal ions is suspected it is best to transfer the distilled sample to a clean absorption cell and photometer as quickly as possible without dilution with water. If the reading is less than 100% *T*, contamination is indicated. A sample thus tested cannot be used for an analysis, because air oxidation of the sulfide is rapid.

During the development of the present method it was noted that serious contamination of the distillate with lead was encountered in the analysis of Bureau of Standards sample of lead base alloy No. 53b. The cause of this trouble was finally found to be associated with the excess formic acid present in the solution after the destruction of the nitric acid. Apparently some volatile organic compound of lead is formed under the conditions existing at the time of the sulfide distillation. Attempts to eliminate the lead contamination by the use of various types of condensers or liquid traps failed. The only way to prevent contamination was to boil off the formic acid previous to the sulfide distillation.

Further investigation on the influence of formic acid showed that the effect noted with lead could be detected in the analysis of many of the other metals under investigation. From Table III it will be seen that although the deleterious effect is not so pronounced as it is with lead, it cannot be ignored in accurate work. Oddly enough, very little lead appears to distill from solutions of lead-tin solders. When analyzing metals such as germanium, tin, arsenic, and antimony (whose sulfides are soluble in ammonium hydroxide), and several other metals such as molybdenum, removal of the formic acid will not be necessary. In such cases it will suffice to boil the solution down to 5 ml. after destruction of the nitric acid and before the addition of the hydriodic hypophosphorous acid mixture.

In the analysis of samples containing materials that precipitate during or after solution in nitric or nitric and hydrochloric acid solution, some of the nitrate may be occluded in the precipitate and thus escape destruction by the formic acid. The subsequent treatment with hydriodic-hypophosphorous acid mixture may free some or all of this nitrate and low results for sulfur will be obtained because some of the sulfide will be oxidized by the oxidizing acids that distill over. One common source of trouble is the hydrolysis of metal ions during solution of the sample. Hydrolysis of iron, aluminum, or tin can be prevented (or eliminated) by heating with additional hydrochloric acid. Materials such as lead, silicon, tungsten, and germanium precipitate during solution in the recommended hydrochloric-nitric acid mixture. Experience has shown that no difficulty is encountered with lead alloys as long as solution is made with hydrochloric-nitric acid mixture. On the other hand, when the lead alloy is dissolved in nitric acid, and hydrochloric acid is added subsequently, appreciable amounts of nitrate are occluded in the precipitated lead chloride. The only way to eliminate this condition is to add sufficient hydrochloric acid to prevent the precipitation of the lead chloride during the destruction of the nitric acid. Moderate quantities of silicon, tungsten, and germanium do not appear to cause trouble as long as decomposition of the alloy is made with the recommended hydrochloric-nitric acid mixture. If trouble is encountered with larger amounts of these metals, it may be

Table II. Determination of Sulfur in Various Metals and Alloys

No.		Sulfur Found %	Sulfur Recovered[a] γ
1	B. of S. ingot iron No. 55b (0.018% S)	0.017	..
2	B. of S. Mo-W steel No. 132 (0.004% S)	0.003	..
3	B. of S. 18 Cr-8 Ni steel No. 101b (0.023% S)	0.022	..
4	Vicalloy (50 Co, 40 Fe, 10 V)	0.011	..
5	Permalloy (55 Fe, 45 Ni)	0.0050	..
6	Mo Permalloy (80 Ni, 14 Fe, 5 Mo)	0.0015	..
7	Molybdenum metal	0.0004	25
8	A.S.T.M. electronic nickel No. H-1400	0.0014	24
9	Cobalt metal	0.0016	23
10	Manganese metal	0.0080	..
11	B. of S. zinc base No. 94	0.0003	25
12	Cadmium metal	0.0008	23
13	B. of S. aluminum base No. 85a	0.0000	25
14	Magnesium metal	0.0016	24
15	OFHC copper metal	0.0020	..
16[b]	Copper metal	0.0050	..
17	B. of S. manganese bronze No. 62a	0.0019	23
18	Bismuth metal	0.0011	25
19	Silver metal	0.0005	24
20	Mercury metal	0.0001	25
21	Lead metal	0.0002	23
22	B. of S. lead base No. 53b	0.0006	23
23	B. of S. solder No. 127	0.0005	..
24[b]	Solder sample	0.0014	..
25	B. of S. tin base No. 54c	0.0006	26
26	Germanium metal	0.0002	25
27[c]	Arsenic	0.0001	24
28	Antimony metal	0.0016	25
29	Tellurium metal	0.0004	27
30[d]	Selenium	0.0000	26

[a] Amount of sulfur recovered over that present in sample when 25 γ of sulfur were added.

[b] Values obtained by American Smelting and Refining Co., Barber, N. J., using an independent method were 0.0050% S for copper and 0.0015% S for solder.

[c] 1.3 grams of B. of S. No. 83a As_2O_3 analyzed.

[d] 1.8 grams of H_2SeO_3 analyzed.

necessary to remove the oxides by filtration before treatment with formic acid.

When the metal or alloy to be analyzed contains little or no reducible metal ions it is possible to use less hypophosphorous acid in the distillation mixture. On the other hand, in the analysis of metals such as molybdenum, vanadium, iron, copper, or tin the concentration recommended in the procedure must be used in order to assure rapid and complete reduction of the iodine formed. If the iodine is not reduced by the hypophosphorous acid before distillation takes place, some of it may be carried over into the ammonium hydroxide solution, resulting in oxidation of the sulfide when the latter is subsequently distilled over.

If necessary from an economy standpoint, the amount of distillation mixture or the concentration of hydriodic acid therein may be reduced in certain analyses. On the other hand, reduction is more rapid with the recommended distillation mixture and in addition, the latter has been adjusted to ensure solution of 1 gram of lead, tin, or copper during distillation.

EXPERIMENTAL

Typical calibration curve data, obtained as directed in the procedure, are recorded in Table I.

Various metals and alloys were analyzed as directed in the procedure, using the appropriate modifications whenever necessary. Formic acid was removed in all the analyses except that of molybdenum (Table II). In some cases the values given are an average of duplicate or triplicate analyses. In such instances the maximum spread in the analyses was of the order of 1 or 2 micrograms of sulfur. Because of the lack of suitable standards with which to "prove in" the method, it was necessary in many instances to resort to an indirect procedure for checking the method. This consisted of analyzing two samples of the metal or alloy, to one of which were added 25 micrograms of sulfur in the form of potassium sulfate. (The metal sample was added to a flask in which a 5-ml. aliquot of standard potassium sulfate had been evaporated nearly to dryness.) If the difference in the quantity of sulfur obtained in the two analyses was close to 25 micrograms, it was assumed that the method was satisfactory. The percentages of sulfur found from the first analysis are listed in the next to the last column of Table II. The differences in quantity of sulfur obtained in the first and second analysis are listed in the last column of Table II.

In order to investigate the effect of formic acid, several metal samples were dissolved and distilled as directed in the procedure. Upon completion of the distillation the solution in the volumetric flask was quickly transferred to a clean absorption cell without adding lead citrate or diluting to 25 ml. and photometered at 370 mμ. The process was repeated, with the exception that the excess formic acid was not boiled off. Instead, the solution was boiled down to 5 ml. after the destruction of the nitric acid. The results are recorded in Table III.

Table III. Effect of Formic Acid

No.	Sample	Formic Acid Present % T	Formic Acid Expelled % T
1	B. of S. ingot iron No. 55b	98	100
2	A.S.T.M. electronic nickel No. H-1400	98	100
3	B. of S. zinc base No. 94	98	100
4	OFHC copper metal	96	100
5	Bismuth metal	96	100
6	B. of S. solder No. 127	98	100
7	B. of S. lead base No. 53b	85	97
8	Lead (as $PbCl_2$)	89	98

ACKNOWLEDGMENT

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Determination of Zinc Oxide as a Residual of Zinc Powder

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A simple technique, which may be applicable in other cases, has been devised for determining the zinc oxide on powdered zinc. The powder was spread on a copper boat, covered, wrapped tightly in annealed copper foil, and heated in vacuum at 450° C. In this way, the zinc was completely removed and the zinc oxide could be brushed off and weighed on the microbalance.

IN 1946, this laboratory undertook to isolate, identify, and determine the zinc oxide present as a film on fine, reagent grade zinc powder. This objective was accomplished by obtaining the zinc oxide as a residual after the metal had been completely removed by a combination of physical processes carried out under conditions designed to minimize oxidation and loss of oxide by entrainment. The technique employed is new and may prove useful in other cases.

EXPERIMENTAL METHOD

The weighed sample of zinc powder (ca. 200 mg.) is spread evenly on the center portion of the "boat," which is made by splitting thin-walled (ca. 0.08 cm.) copper tubing (see Figure 1). After the boat has been covered with a similar section of tubing pressed into place, it is tightly wrapped in annealed copper foil about 0.003 cm. thick, the edges of which are folded over. Before being used, all copper is hydrogen-fired to remove oxide. Up to three wrapped boats are then stacked, and inserted in the Pyrex firing tube, and the apparatus (Figure 2) is assembled for a run. The tube is flushed for 10 minutes with line hydrogen, evacuated with a good oil vacuum pump for 10 minutes, flushed with line hydrogen for an hour (less would probably suffice), and then reconnected to the pump. The temperature of the furnace is next raised to 450° C. and held there for 30 minutes to complete the separation of zinc and zinc oxide. Furnace and tube are now allowed to cool, whereupon air is readmitted. After the boats have been removed and opened, the loosely adhering zinc oxide

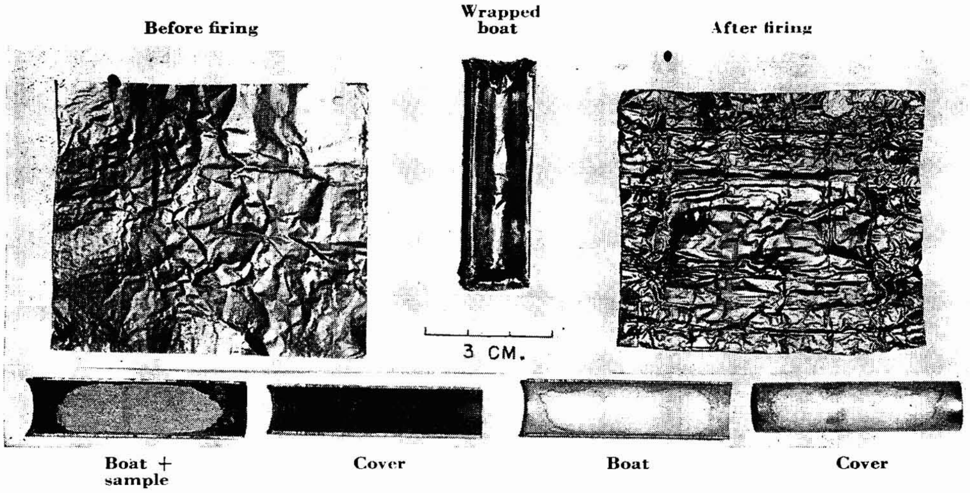

Figure 1. Boat at Various Stages in an Experiment

Note zinc-rich pattern after firing. Residual zinc oxide has been brushed out

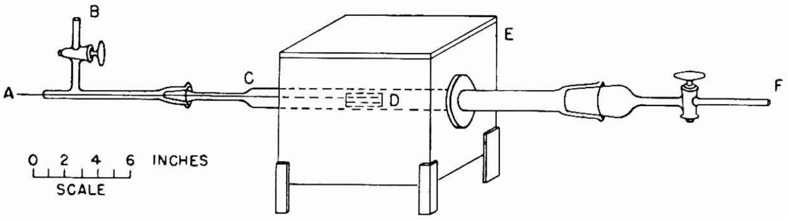

Figure 2. Apparatus with Thermocouple Added

A. Thermocouple—not required in usual analysis
B. Entry for hydrogen
C. Pyrex tube
D. Wrapped boats
E. Furnace
F. Exit connection for evacuation

powder is brushed onto a piece of platinum foil and weighed on a microbalance.

Data to show the reliability of the method are given in Table I. Results thus obtained are slightly high.

DISCUSSION

The foregoing technique should be applicable to the isolation and determination of other oxides, and to the analysis of alloys. The apparatus employed is available in most laboratories.

Shielding. During the preliminary work, in which the sample was shielded very little or not at all, rings of zinc formed on the tube just beyond the ends of the furnace and zinc oxide was found on the tube between the sample and the rings of zinc. Moreover, too much zinc oxide generally remained in the boat, and in variable amount.

Because zinc has a vapor pressure near 10^{-4} atmosphere at 400° C. (*2*), its evaporation under the experimental conditions is to be expected. Zinc oxide, however, is much less volatile. The residues from two experiments lost no weight upon being heated for 1 and then for 2 hours under the experimental conditions. Although the zinc oxide on the firing tube might possibly have been formed by the oxidation of evaporated metal, it is very much more likely to have been entrained by the vapor and then deposited on the tube. Effective shielding is therefore indicated. Such shielding would at the same time reduce the chance of stray oxidation during the run. Wrapping the covered boat in annealed, hydrogen-fired copper foil accomplished both objectives to a satisfactory extent, and prevented the formation of any visible deposit on the glass tube.

Stray Oxidation. Table I gives evidence of stray oxidation, inasmuch as oxygen contents above 1.17% were found when heating was prolonged. Determinations of zinc oxide were carried out in high vacuum (pressures usually not above 0.5 micron) at 400° C., other conditions remaining the same. For the powder of Table I, 1.12, 1.15, 1.12, and 1.13% oxygen was obtained in this way; zinc foil under the same conditions gave less than 0.01% oxygen. The conclusion therefore is that the results of Table I are high by about 0.05% owing to stray oxidation, which is not surprising when one remembers that no attempt was made to maintain high vacuum in the simple apparatus of Figure 2. Ordinarily, this error will not be great enough to warrant the use of the much more elaborate high-vacuum equipment.

Miscellaneous. The determination has been carried through with good results both above and below the melting point of zinc (419.4° C.), which proves that removal of the metal need not involve a liquid. (The copper-zinc diagram shows no phases melting below 419.4° C., *1*.) Evaporation of zinc and its diffusion into copper are instrumental in the process; even the outer wrapping of copper foil turns to brass.

The ease and reliability with which the residual oxide can be brushed off the boat are striking. On a 200-mg. sample, 0.01% of oxygen corresponds to about 0.1 mg. of zinc oxide.

Price (*3*) describes the determination of zinc in its alloys by evaporation into vacuum and gives references to earlier work. Vernon, Akeroyd, and Stroud (*4*) succeeded in isolating oxide films from zinc foil by the same method.

Table I. Oxygen Content of Reagent Grade Zinc Powder

Date	Minutes at 450° C. (Furnace)	% Oxygen
11/22/46	15	1.17, 1.18, 1.17
11/22/46	30	1.17, 1.18, 1.16
12/31/46	30	1.16, 1.17, 1.18
11/26/46	45	1.35, 1.28, 1.21
11/12/46	60	1.34, 1.33, 1.35
11/13/46	120	1.27, 1.27, 1.20

Zinc powder passed 325-mesh sieve.
Estimated average particle diameter (microscope), 30 or 35 microns.
Zinc content of powder obtained on small samples by weighing pyrophosphate, 98.80, 98.82%, with estimated accuracy of ±0.2%.
Maximum temperature of sample, 440° C.
Residual powder gave x-ray diffraction pattern of zinc oxide only and liberated no hydrogen from acid.

ACKNOWLEDGMENT

The authors are indebted to W. F. Gilliam for suggesting the problem; to J. S. Kasper for the x-ray diffraction work; to E. F. Fullam for establishing the particle size of the powder; and to A. C. Titus for determining its zinc content.

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Fluorometric Determination of Malic Acid and 2-Naphthol

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Quantitative fluorometric methods for the determination of malic acid and 2-naphthol are based upon the reaction of malic acid and 2-naphthol in sulfuric acid. The effect of variations in the operating conditions for each method is discussed. The method for malic acid is applicable to determinations of 1 to 30 micrograms of malic acid. Citric and succinic acids in large excess do not interfere with the malic acid determination, whereas tartaric acid in the ratio of 1 to 1 with malic acid causes a 3 to 5% error. The determination of malic acid in apple juice is described. The method for 2-naphthol is applicable to determinations of 1 to 14 micrograms of 2-naphthol. The presence of 1-naphthol in a 1 to 1 ratio with 2-naphthol causes an error of approximately 5%.

THE method of the Association of Official Agricultural Chemists (*1*) for the volumetric determination of total malic acid is long and tedious, and the results require an empirical correction. The fluorometric method of Barr (*2*), based upon the reaction of malic acid with resorcinol in concentrated sulfuric acid, cannot be used in the presence of citric acid. A fluorometric method which is relatively free of interferences, rapid, and simple in technique is described here. It is based upon the qualitative test for malic acid employed by Eegriwe (*4*) in which malic acid and 2-naphthol, when heated in concentrated sulfuric acid, produce a blue fluorescence. There is some evidence (*3, 5*) that 5,6-benzocoumarin is the fluorescing product which is formed by condensation of the intermediate, formyl acetic acid, with 2-naphthol.

APPARATUS

The fluorescence intensities are measured with a Lumetron fluorescence meter Model 402 EF with 25-ml. cells. The primary filter permits maximum transmittance in the spectral region of 365 mμ. The secondary filters consist of a combination of a yellow filter, furnished by the Photovolt Corporation for use in vitamin B_1 determinations, which does not transmit emission below 400 mμ, and a Corning lantern blue filter No. 5543. This combination of secondary filters permits maximum transmittance corresponding to the region of greatest fluorescence intensity of the final solution.

REAGENTS

Malic Acid. Technical grade *l*-malic acid was recrystallized from ethyl ether.

2-Naphthol. Technical grade 2-naphthol was purified by distillation.

Sulfuric Acid, 91.5 to 92.5%. One hundred milliliters of concentrated sulfuric acid are added to 7 ml. of water. The specific gravity (20°/4° C.) of the solution must be within the range of 1.822 and 1.826.

Lead Acetate Solution. Seventy-five grams of normal lead acetate are dissolved in water and 0.5 ml. of glacial acetic acid is added. The solution is diluted to 250 ml.

Sodium Salicylate Solution. Exactly 2.000 grams of reagent grade sodium salicylate are dissolved in water and diluted to 1000 ml. It may be preserved by the addition of several drops of toluene.

DETERMINATION OF MALIC ACID

Analytical Procedure for Solutions Free from Interferences. A sample solution containing 1 to 30 micrograms of malic acid, preferably 1 ml. or less, is accurately measured into a 10-ml. Erlenmeyer flask and evaporated to dryness in a 105° C. oven. One milliliter of reagent consisting of 12 mg. of 2-naphthol per 100 ml. of 92% sulfuric acid is added. The flask is tipped slightly to ensure complete wetting of the bottom surface and is then heated in an electric oven at 90° to 95° C. for 30 minutes. The flask is removed and cooled, after which the solution is transferred with water to a 100-ml. volumetric flask and brought to volume. The temperature of the solution is adjusted to 25° ± 1.5° C. and the fluorescence intensity reading is taken.

A straight-line calibration curve is prepared from instrument readings obtained from well-distributed points by carrying amounts of malic acid through the procedure described. The fluorescence meter is adjusted to give a reading of zero with a blank solution carried through the complete analytical procedure and a reading of 100 with the standard solution of sodium salicylate (2.000 grams per liter).

Table I. Reproducibility of Fluorometric Method for Determination of Malic Acid

Number of samples, N	31
Malic acid present in each sample	30.08 micrograms
Average fluorometric reading, $\bar{x}$	96.3 units
Average deviation of fluorometric readings	1.12 units
Standard deviation of fluorometric readings	1.28 units

Standard deviation = $\left[\frac{\Sigma(x - \bar{x})^2}{N - 1}\right]^{1/2}$

Discussion. The maximum fluorescence intensity is obtained if the sulfuric acid solution of 2-naphthol is prepared from 92% sulfuric acid. With 90 and 95% sulfuric acid the apparent fluorescence intensities are approximately two thirds of those obtained with the 92% acid. The concentration of 2-naphthol is limited to 120 micrograms per sample because larger concentrations produce excessive fluorescence in the blank. The solution of 2-naphthol in 92% sulfuric acid is stable for at least 2 weeks if stored in an amber bottle in a refrigerator. A calibration curve should be prepared for each batch of reagent.

The maximum fluorescence intensity is reached in less than 30 minutes at 90° C. and is not diminished by further heating. At 80° C. the maximum is reached in 28 minutes, whereas at 100° the maximum fluorescence is reached in 16 minutes. The maximum fluorescence readings obtained at 80° C. are comparable to those obtained at 90° C., whereas those obtained at 100° C. are 2 to 4% lower.

In the pH range of 0 to 5 and in any 3° temperature range between 20° and 30° C. the variation of fluorescence intensity of the final solution is within the experimental error. The fluorescence intensity is constant over long periods of standing.

Table I demonstrates the reproducibility of the fluorescence intensity readings for 31 samples, each of which consisted of 30.08 micrograms of malic acid.

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Table II. Separation of Pure Malic Acid

Malic Acid Present, Mg.	Malic Acid Found, Mg.	Error, %
50.0	49.9	
	49.9	
	49.2	
	49.9	
	Av. 49.7	0.6
40.0	41.1	
	41.2	
	41.6	
	41.3	
	Av. 41.3	3.3

Table III. Separation and Determination of Malic Acid in a Synthetic Mixture Containing Sucrose, Glucose, and Fructose

Malic Acid Present, Mg.	Malic Acid Found, Mg.	Error, %
40.0	38.9	
	38.9	
	39.7	
	38.1	
	Av. 38.9	2.8
40.0	39.5	
	38.9	
	41.2	
	39.7	
	Av. 39.8	0.5

The total malic acid is determined by this method, inasmuch as it was found that *l*-malic or *dl*-malic acid produces identical fluorescence intensities in this reaction.

As much as 0.5 mg. of either citric or succinic acids has no influence on the fluorescence readings. Tartaric acid if present in a ratio to malic acid of 1 to 1 will produce an error of 3 to 5%; for a ratio of 3 to 1 the error produced is 20 to 25%.

The procedure requires 1 hour, during which time a large number of samples may be run simultaneously.

Determination of Malic Acid in Apple Juice. The method of the Association of Official Agricultural Chemists (*1*) for the separation of malic acid from fruit juices has been modified to use smaller volumes. In general, the protein matter is coagulated and removed from the juices by filtration along with other insoluble material. The malic acid is precipitated as the lead salt, washed, and put back into solution as malic acid by precipitating the lead as its sulfide. The elapsed time required for a complete determination by the fluorometric method is 2.5 hours compared to nearly 24 hours for the standard A.O.A.C. method.

PROCEDURE FOR SEPARATION OF MALIC ACID FROM APPLE JUICE. A sample containing 20 to 75 mg. of malic acid is selected and the volume is brought to 15 ml. with water or by evaporation. The solution is transferred quantitatively to a 100-ml. volumetric flask and brought to volume with 95% ethyl alcohol. The solution is mixed, again brought to volume, and filtered through a folded filter paper covered with a watch glass. Seventy-five milliliters of the filtrate are pipeted into a 100-ml. centrifuge tube, and 10 mg. of citric acid and 1 ml. of lead acetate solution are added. The solution is thoroughly mixed, and centrifuged at 1000 r.p.m. for 15 minutes. The supernatant liquid is tested with lead acetate solution for complete precipitation. If additional precipitate appears, more lead acetate solution is added and the centrifuging is repeated. The liquid is carefully decanted and discarded, leaving the precipitate in the centrifuge tube. The precipitate is washed with 75 ml. of 80% ethyl alcohol added in small portions. A stirring rod is used to ensure a homogeneous mixture and is rinsed with the last portion of 80% alcohol. The solution is again centrifuged and decanted as before. The precipitate is suspended by adding 50 ml. of water in small portions while using the stirring rod for thorough mixing. The solution is saturated with hydrogen sulfide by a rapid stream of the gas. The mixture is transferred quantitatively to a 100-ml. volumetric flask and brought to volume with water. The contents are well mixed and filtered. Aliquot portions of the filtrate are taken for the determination of malic acid by the fluorometric method described.

DISCUSSION. The error incurred by passing known amounts of malic acid through the above separation and the fluorometric determination is illustrated in Table II. In each separation four aliquots were analyzed. These errors are within the same range as those found in the determination alone.

Malic acid can be separated from sugars normally found in apple juice with the same accuracy as from solutions free of these sugars. Thus in Table III the error for separations from synthetic solutions of 40 mg. of malic acid containing 100 mg. of sucrose, 150 mg. of *d*-glucose, and 250 mg. of *d*-fructose is illustrated. In each separation four aliquots were determined fluorometrically.

Results obtained by determining the amount of malic acid present in commercial apple juices before and after the addition of known amounts of malic acid are recorded in Table IV. Results are averages of four aliquots determined for each separation.

DETERMINATION OF 2-NAPHTHOL

Analytical Procedure. A solution containing 1 to 15 micrograms of 2-naphthol dissolved in benzene is measured volumetrically into a 10-ml. Erlenmeyer flask and evacuated to dryness by means of a vacuum desiccator and a water pump. One milliliter of reagent, consisting of 1 gram of malic acid dissolved in 100 ml. of 92% sulfuric acid and allowed to age at room temperature for 24 hours, is added. The flask is tipped slightly to ensure complete wetting of the sample, and is then placed in a 35° to 40° C. oven for 10 minutes. The contents are transferred quantitatively to a 100-ml. volumetric flask with water and brought to volume. The temperature should be 25° ± 1.5° C. when the fluorescence reading is taken.

A linear calibration curve is prepared from 2-naphthol samples which are treated as in the standardized procedure. Zero on the fluorescence meter may be adjusted with distilled water and the 100 setting obtained with the sodium salicylate solution (2.000 grams per liter).

DISCUSSION. Benzene was used as the solvent for the 2-naphthol in this investigation. The "aging" of the malic acid in the 92% sulfuric acid solution increases the fluorescence intensity approximately sixfold. This reagent is stable for 10 days if refrigerated. The same calibration curve may be used for successive batches of the solution of malic acid in 92% sulfuric acid if the 92% sulfuric acid is from the same source. The concentration of the malic acid is not critical.

The reaction goes to completion rapidly and after 3 or 4 minutes no change in the fluorescence intensity is noted. Above 25° C. the fluorescence intensities produced from the reaction are at a maximum. The fluorescence intensity is not increased at reaction temperatures above 40° C. but the possibility of interference due to decomposition of otherwise noninterfering substances is increased.

The fluorescence intensity of the blank is comparable to that

Table IV. Determination of Malic Acid in Commercial Apple Juice

Sample Taken *Ml.*	Malic Acid Added *Mg.*	Malic Acid Found *Mg.*	Malic Acid Found per 5 Ml. Juice *Mg.*	Malic Acid Found Less Malic Acid Added per 5 Ml. Juice *Mg.*
		Apple Juice A		
10	None	34.2	17.1	17.1
		34.4	17.2	17.2
		36.4	18.2	18.2
5	34.7	54.8	54.8	20.1
		51.7	51.7	17.0
5	57.8	73.6	73.6	15.8
		72.4	72.4	14.6
		Apple Juice B		
10	None	57.6	28.8	28.8
		57.8	28.9	28.9
		57.4	28.7	28.7
5	10.0	38.5	38.5	28.5
		39.2	39.2	29.2
		39.7	39.7	29.7
		40.8	40.8	30.8
5	20.0	47.4	47.4	27.4
		48.6	48.6	28.6
		49.2	49.2	29.2
		48.3	48.3	28.3

Table V. Reproducibility of Fluorometric Method for Determination of 2-Naphthol

Number of samples, N	48
2-Naphthol present in each sample	14.00 micrograms
Average fluorometric reading, $\bar{x}$	87.1 units
Average deviation of fluorometric readings	1.20 units
Standard deviation of fluorometric readings	1.35 units

produced by distilled water and therefore the latter may conveniently be substituted for the zero adjustment of the fluorescence meter.

Table V illustrates the reproducibility of the fluorescence intensity readings for 48 samples. Each sample consisted of 14.00 micrograms of 2-naphthol.

The fluorometric method may be used for the determination of 2-naphthol in the presence of 1-naphthol, if the concentration of 1-naphthol is not greater than that of 2-naphthol. If the amount of the 1-naphthol is equal to that of 2-naphthol, the error is approximately 5% positive. If the ratio of 1-naphthol to 2-naphthol is 5 to 1, the error is approximately 15% positive.

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HYDROCARBON TYPE ANALYSIS

Estimation of Six-Membered Ring Naphthenes

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A method of hydrocarbon type analysis of petroleum naphthas is described, based on examination of cuts of specified boiling ranges, prepared by fractionation of original and dearomatized samples. The cut points selected ensure the segregation of particular groups of hydrocarbons and contents of aromatics, paraffins, and total naphthenes are obtained in detail throughout the boiling range initial boiling point to 225° C. The "reactable" six-membered ring naphthene contents are determined by a dehydrogenation procedure and do not embrace geminal cyclohexane derivatives. The latter are included in the "unreactable" naphthene content, together with the cyclopentane derivatives.

FROM the commencement of the petroleum industry many investigations have been carried out with the object of correlating the physical properties and chemical constitution of naturally occurring hydrocarbon mixtures, but, until recent years, little progress had been made in the development of reliable analytical methods for the accurate assessment of hydrocarbon type composition. The analytical difficulties encountered in such work are extremely great and are due to the involved and complex chemical nature of the raw materials. Nevertheless, considerable effort has been directed toward the ultimate goal of analytical petroleum chemistry—i.e., the resolution of petroleum into individual hydrocarbons and other constituents—and much progress has been accomplished.

The hydrocarbons present in petroleum may be conventionally and broadly classified as paraffins, naphthenes, and aromatics, a classification that is sharp for the gasoline boiling range but becomes ambiguous for compounds of high molecular weight, which may contain the characteristic structure of all three types combined in the one molecule. For the purposes of the present paper, the simple classification is tenable, since the investigation has been restricted to materials boiling within the gasoline range. The paraffins represent the well-defined saturated open-chain structure hydrocarbons. The aromatics may be specified as cyclic hydrocarbons, as a predominant feature of their structure is the benzene ring with its characteristic resonating double bond unsaturation. The naphthenes have fully saturated carbon ring structures. In the absence of evidence to the contrary, straight-run petroleum fractions are considered to contain five- and six-membered ring naphthenes only and it is with the estimation of these two classes of compound that this investigation is concerned.

ESTIMATION OF HYDROCARBON TYPES

Fractional Distillation. The primary step in the estimation of hydrocarbon type is that of fractional distillation, and progress in recent years in this connection has been stupendous. Various forms of ultraefficient column packing have been designed and used with great success in the analytical laboratory, notably the glass and stainless steel helices developed by Fenske *et al.* (*3*, *4*, *10*, *11*), the spiral packings due to Podbielniak (*9*), and the spiral screen packing described by Lecky and Ewell (*7*). Using columns packed with such materials it has become normal practice to fractionate a gasoline sample into close boiling cuts, with subsequent analysis of these for aromatic, naphthene, and paraffin contents (*5*, *8*). By these means a hydrocarbon type analysis throughout the boiling range can be obtained. It has been realized that the removal of aromatic hydrocarbons, prior to the fractionation, facilitated estimation of naphthene and paraffin hydrocarbons. This fact is due partly to abnormal vapor pressure relationships existing between certain of these compounds and the aromatic hydrocarbons, and also to the reduction in the number of individual compounds present in the distillation charge.

The removal of aromatics, without affecting the other hydrocarbons present, is accomplished by the convenient process of selective adsorption. The sample is percolated through a column packed with silica gel adsorbent (each 100 grams of gel absorb 8 grams of aromatics) and the aromatic-free percolate of constant refractive index is segregated, equivalent to the naphthene-paraffin component, which can then be fractionated and analyzed. The accuracy and fullness of the resultant naphthene-paraffin analysis depend on the time expenditure permitted for the necessary fractionation. For ordinary routine analysis purposes, the distillation time may be as short as 40 hours, whereas for more exacting research programs greater precision in fractionation may be necessary. For the program of work comprising the subject matter of this paper, the fractionations have been those of the first or routine kind.

Two types of fractionating column have been employed, both equivalent to 40 theoretical plates in efficiency. The first comprised a 5-foot (150-cm.) length of 20-mm. inside diameter tubing packed with 0.125-inch Fenske glass helices, capable of handling distillation charges of 1 to 3 liters. The second consisted of an 18-inch (45-cm.) length of 16-mm. inside diameter tubing packed with $^1/_{16}$-inch stainless steel gauze cylinders, suitable for distilling smaller charges of 200 to 500 ml. The reflux ratio employed normally in such distillations was 40 to 1.

Estimation of Aromatics. The method of analysis for aromatic content aims at the determination, as far as possible, of individual aromatic hydrocarbons, and employs fractionation and ultraviolet spectroscopy. A sample of the original gasoline is fractionated (40 theoretical plates, 40 to 1 reflux ratio, not less than 20 to 1 charge–hold-up ratio) into cuts of specific boiling ranges:

1 I.B.P.- 60° C.
2 60–117° C. (benzene + toluene)
3 117–120° C.
4 120–126° C. (ethylbenzene)
5 126–140° C. (ethylbenzene + isomeric xylenes)
6 140–145° C. (xylenes, mainly *m*- and *o*-)
7 145–150° C.
8 150–175° C.
9 175–200° C.
10 200–225° C.

Each of the above cuts is examined by ultraviolet spectroscopic methods (*6*) for individual aromatic hydrocarbons likely to be present from a consideration of the boiling point—viz., cut 2, benzene and toluene, cut 5, ethylbenzene, *o*-, *m*-, and *p*-xylene, etc. Cut 3 is low in aromatic content and serves as a buffer cut, avoiding the estimation of ethylbenzene in high concentration of toluene. In the case of the higher boiling cuts, 150° to 175° C., 175° to 200° C., and 200° to 225° C., the number of isomers does not permit at the present juncture complete estimation of individuals and a total aromatic content only is obtained. Experience has shown that aromatics are absent from the cut of initial boiling point to 60° C. and its examination can be omitted. A knowledge of the weight per cent yields of the cuts, together with their determined aromatic contents, enables the total aromatic content of the sample to be calculated, including a detailed distribution throughout the whole boiling range.

Estimation of Naphthenes and Paraffins. Various methods for estimation of naphthene and paraffin contents are available, the majority based on relationships between physical properties. The particular method chosen here employs specific refraction measurements.

A quantity of the gasoline sample is dearomatized by percolation through silica gel and the aromatic-free percolate is distilled through a fractionating column (40 theoretical plates, 40 to 1 reflux ratio, not less than 20 to 1 charge–holdup ratio). Boiling point data on a weight basis are recorded and specific cuts are segregated, of boiling ranges:

1 I.B.P.- 15° C.
2 15- 32.5° C.
3 32.5- 45° C.
4 45- 66° C.
5 66- 75° C.
6 75- 85° C.
7 85- 95° C.
8 95–109° C.
9 109–120° C.
10 120–130° C.
11 130–150° C.
12 150–175° C.
13 175–200° C.
14 200–225° C.

These cut points were chosen with the deliberate intention of facilitating and simplifying the subsequent analysis for naphthene and paraffin hydrocarbons, and were based on experience gained by analyses carried out in greater detail. The advantage of the specific cut points may be best assessed by reference to Table I, wherein are listed the predominant hydrocarbons considered to be present.

Table I. Predominant Hydrocarbons in Specific Cuts

Boiling Range, ° C.	Paraffins	Naphthenes
I.B.P.–15	Butanes	. . .
15–32.5	Isopentane	. . .
32.5– 45	*n*-Pentane	Cyclopentane
45– 66	Isohexanes	Cyclopentane
66– 75	*n*-Hexane	Methylcyclopentane
75– 85	Dimethylpentanes	Cyclohexane
85– 95	Methylhexanes	Dimethylcyclopentanes
95–109	*n*-Heptane	Methylcyclohexane, ethylcyclopentane, and trimethylcyclopentanes
109–120	Iso-octanes	Trimethylcyclopentanes and dimethylcyclohexanes
120–130	*n*-Octane	C_8H_{16}, 5- and 6-membered ring
130–150	Iso- and *n*-nonanes	C_9H_{18}, 5- and 6-membered ring
150–175	Iso- and *n*-decanes	$C_{10}H_{20}$, 5- and 6-membered ring
175–200	Iso- and *n*-undecanes	$C_{11}H_{22}$, 5- and 6-membered ring
200–225	Iso- and *n*-dodecanes	$C_{12}H_{24}$, 5- and 6-membered ring

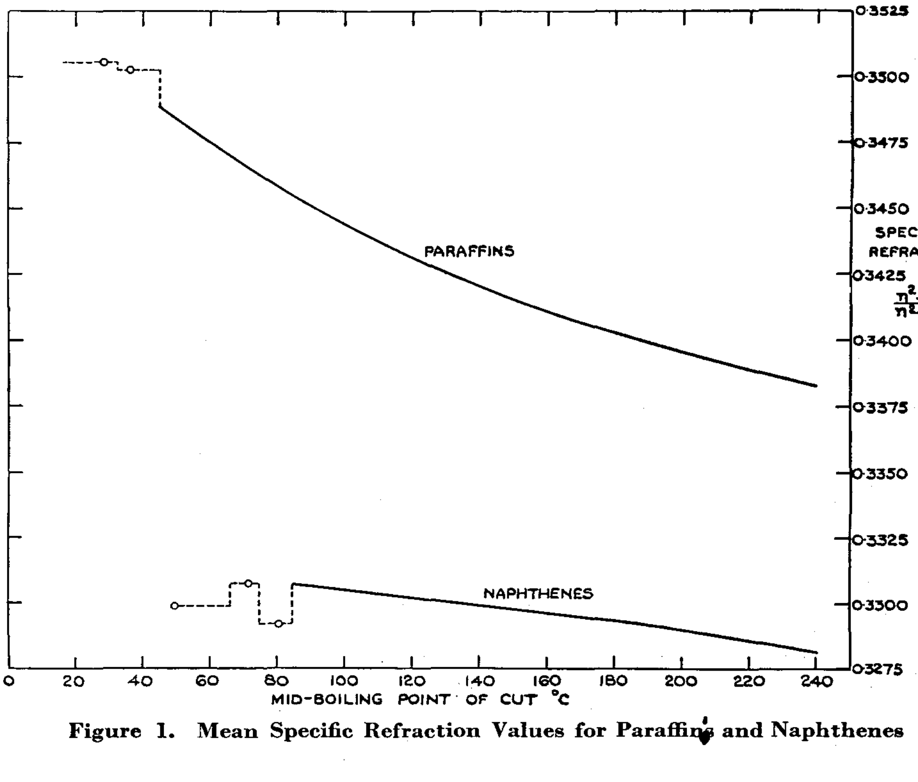

Figure 1. Mean Specific Refraction Values for Paraffins and Naphthenes

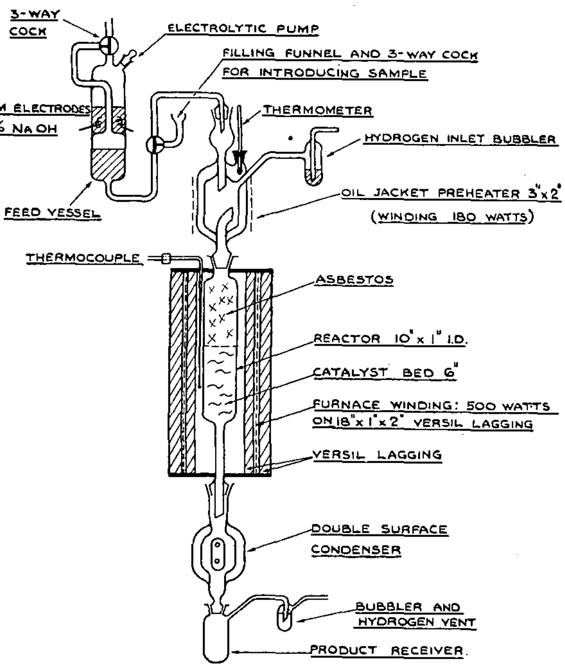

Figure 2. All-Glass Dehydrogenation Apparatus

The density at 20° C. in grams per milliliter (accuracy ±0.0001 gram per ml.) and the refractive index at 20° C., sodium D line (accuracy ±0.0001), of each cut are determined and specific refractions are calculated according to the formula:

$$\frac{n^2 - 1}{n^2 + 2} \times \frac{1}{d}$$

where d = density at 20° C., gram per ml. (by pycnometer, *2*) and n = refractive index at 20° C. (sodium D line) (by Abbe or Pulfrich instrument).

By linear interpolation, using mean values for the specific refractions of the naphthenes and paraffins assumed to be present, the naphthene and paraffin contents of each cut are determined and expressed as weight percentages of the sample.

A plot of the mean specific refraction values employed against mid-boiling point of cut is given in Figure 1, and has been constructed from the values listed in Table IX.

DIFFERENTIATION AND ESTIMATION OF NAPHTHENE TYPES

The scheme of analysis outlined above enables a hydrocarbon type composition of any straight-run gasoline to be calculated in terms of aromatics, paraffins, and naphthenes throughout the boiling range. It is of interest, for various reasons, to distinguish between, and, as far as possible, estimate the two main types of naphthene hydrocarbons—namely, the five-membered ring or cyclopentane derivatives and the six-membered ring or cyclohexane derivatives. For the boiling ranges below 95° C., this can be accomplished by reference to boiling point data and the total naphthene contents of the specified cuts, as the chosen cut points separate the following hydrocarbons: cyclopentane (boiling point 49.2° C.), methylcyclopentane (boiling point 71.9° C.), cyclohexane (boiling point 80.8° C.), and the dimethylcyclopentanes of boiling point 85° to 95° C. Above 95° C., however, substituted cyclopentane and cyclohexane derivatives occur together and recourse must be made to more elaborate methods. It was decided to investigate the reaction discovered by Zelinsky (*12*) and his co-workers (*13*)—namely, the dehydrogenation of cyclohexane derivatives to the corresponding benzene hydrocarbons in the presence of platinum or palladium catalysts:

$$C_6H_{11}R \leftrightarrow C_6H_5R + 3H_2$$

Subsequent estimation of the increase in aromaticity on dehydrogenation can furnish an analytical measure of the content of six-membered ring naphthenes. Zelinsky showed that, under certain conditions, the above reaction was quantitative and selective, and proceeded in one stage without formation of intermediate partially dehydrogenated cyclohexanes—i.e., cyclohexenes—and paraffins, and cyclopentane derivatives were unaffected.

Equipment. The apparatus used in the present investigation consisted of an all-glass reactor and preheater connected to a combined feed vessel and electrolytic pump, with receiver trap and condenser, the latter operated with methanol cooling at −30° C. For maintaining the slow feed rate of 6 ml. per hour, the electrolytic pump functioned in a most satisfactory manner. A diagrammatic sketch is given in Figure 2. The catalyst space measured 70 ml. and the dead space of 50 ml. at the top of the reactor was filled with Gooch asbestos.

Catalyst Preparation. The platinum catalyst was prepared from analytical grade platinum chloride, hydrochloroplatinic acid, $H_2PtCl_6.6H_2O$. A quantity of 30 grams of granular active charcoal was added to an aqueous solution containing 13.6 grams of platinum chloride, the whole was evaporated on a water bath, and the mixture was stirred until dry. The resulting solid was introduced into the catalyst space of the reactor and flow of hydrogen commenced. After flushing, generation of the active catalyst was achieved by raising the temperature at a rate to reach 300° C. at the end of 10 hours. The catalyst was then ready for use, and thereafter an atmosphere of hydrogen was maintained in the apparatus. The composition of the finished catalyst corresponded to 15% (weight) platinum, 85% (weight) charcoal.

Procedure. To render the apparatus free from leaks, heavy Audco grease No. 657 was used on all ground-glass joints save those of the feed vessel and pump, where the light grade, No. 356, proved satisfactory. Freedom from leaks was tested by checking the relative rates of entry and exit of hydrogen gas when no sample was passing. The feed was introduced through the filling funnel and three-way stopcock into the electrolytic pump and the preheater temperature was adjusted to 200° C.

Preliminary Experiments. As an initial test of the catalyst, methylcyclohexane was used as feed in a few preliminary experiments prior to the completion of the electrolytic pump, using gravity feed from a small buret into the preheater. The physical constants of the methylcyclohexane, density at 20° C., gram per ml., 0.7681, and refractive index $n^{20°\,C.}_D$, 1.4225, compared with literature values for the pure hydrocarbon of 0.7694 and 1.4231 and correspond to a purity of approximately 99%.

The refractive indexes of the first few drops of product approached that of toluene—namely, 1.4969—but rapidly declined for subsequent yields to that of the feed. The reason for this rapid loss of activity was not, at first, obvious and such variables as catalyst bed temperature and hydrogen rate were investigated without success. It was then realized that, because platinum-containing catalysts are notoriously sulfur-sensitive, the rubber connection between the temporary feed buret and the preheater might be responsible. Steps were taken, therefore, to ensure that all connections in the final design of apparatus were of glass.

Aromatic-free materials, produced by exhaustive extraction of aromatics by concentrated sulfuric acid, when dehydrogenation was attempted, exhibited the same phenomenom—i.e., rapid loss of catalyst activity. This was probably due to the presence of some sulfur-containing compounds formed during the acid treatment and not removable by the customary alkali wash. The technique of dearomatization by percolation through silica gel obviates this trouble, as the sulfur compounds present are selectively absorbed and one obtains a naphthene plus paraffin component, the sulfur content of which is <0.0005% (weight). As an additional safety precaution, all feed materials under examination were filtered through silica gel immediately prior to dehydrogenation, and no further recurrence of the above trouble was experienced.

A series of runs, with methylcyclohexane as feed, was then carried out under varying conditions in order to establish a standard procedure. In each case the first runnings were discarded until successive products of a constant refractive index (n^{20}_D ± 0.0003) were obtained. The results are listed in Table II. A continuous slight bleed of hydrogen gas (approximately 30 ml. per hour) was maintained through the apparatus during the

Table II. Dehydrogenation of Methylcyclohexane

Catalyst Bed Temp., ° C.	Feed Rate, Ml./Hour	Conversion to Toluene, %
300	12	60
300	6	84
325	12	85
325	6	96–97

above runs. It was essential to restrict this flow to a minimum, because the dehydrogenation reaction is of the reversible type, and lower conversions were obtained with higher rates of hydrogen throughput. The standard conditions of dehydrogenation adopted were:

Catalyst bed temperature	325° C.
Feed throughput	0.1 vol./vol./hour
Hydrogen throughput	5.0 vol./vol. liquid feed

These were considered satisfactory for the following reasons:

A 96 to 97% conversion (weight/weight) of naphthene to aromatic would ensure an accuracy of determination of 3 to 4% of the six-membered ring naphthene content.

Higher catalyst bed temperatures would be likely to cause breakdown of hydrocarbons by thermal cracking reactions.

Sufficient yield of dehydrogenated material for analysis is obtained over a running period of 3 to 4 hours.

Table III. 50/50 Volume Blend of Methylcyclohexane and 2,2,4-Trimethylpentane

	Refractive Index, n_D^{20}	Paraffin Content, Wt. %	Naphthene Content, Wt. %	Aromatic Content, Wt. %
Blend	1.4075	47.4	52.6	0.0
Dehydrogenated product	1.4393	48.6	1.6	49.8
Dearomatized dehydrogenated product	1.3929	96.8	3.2	0.0

For check purposes a blend of equal volumes of methylcyclohexane and 2,2,4-trimethylpentane was prepared. Theoretically, on dehydrogenation, the methylcyclohexane in such a blend is converted into toluene and removal of this aromatic hydrocarbon from the product of dehydrogenation would yield the unchanged isoparaffin. The data of Table III indicate that 98% of the six-membered ring naphthene was converted to toluene. The theoretical refractive index of the remaining aromatic-free product—unchanged iso-octane plus unconverted methylcyclohexane—calculates as 1.3928 (n_D^{20}), which is in close agreement with the experimental value of 1.3929 (n_D^{20}).

DEHYDROGENATION OF SYNTHETIC HYDROCARBONS

Before the method of estimating the cyclohexane derivatives by dehydrogenation outlined above could be applied generally to petroleum fractions, it was considered advisable to attempt demonstrations with regard to the behavior of individual hydrocarbons during the actual dehydrogenation procedure. The ideal analytical proof of validity, the testing of all possible hydrocarbons in all proportions, is unattainable.

In the case of naphthenes, if one restricts the examination to the cyclopentane and cyclohexane derivatives, we have one possible cyclopentane, one possible cyclohexane, and one possible methylcyclopentane—i.e., one isomer of formula C_5H_{10} and two isomers of formula C_6H_{12}. Of formula C_7H_{14}, however, five dimethylcyclopentanes are possible, as both the 1,2 and 1,3 substituted compounds can exist in cis and trans forms, also one ethylcyclopentane and one methylcyclohexane, a total of seven isomers, only one of which dehydrogenates to a benzene derivative. An increase to eight carbon atoms (C_8H_{16}) gives a possibility of eight trimethylcyclopentanes, five methylethyl cyclopentanes, two propyl cyclopentanes, seven dimethylcyclohexanes, and one ethylcyclohexane, making a total of 23 isomers. Of formula C_9H_{18}, there are 42 possible cyclopentane derivatives and 21 cyclohexane derivatives. Therefore, in the boiling range of approximately 150° C., it is possible, theoretically, to have a total of 96 five- and six-membered ring naphthenes. In addition, 70 paraffinic hydrocarbons can exist of formula $C_5H_{12} \longrightarrow C_9H_{20}$, also in the boiling range of approximately 150° C., making a grand total of 166 hydrocarbons.

Extension of the range to 175° C. covers the hydrocarbons containing 10 carbon atoms per molecule, the possibilities being 75 paraffins, 118 substituted cyclopentanes, and 87 substituted cyclohexanes—i.e., an additional 280 hydrocarbons. The situation is further complicated by the possible presence, above 150° C., of some 60 dicyclic hydrocarbons of varying types, some of which contain a cyclohexane ring in their structure and may conceivably dehydrogenate.

This enormous number of isomers, only a meager proportion of which have been isolated from petroleum or prepared synthetically precludes any thought of complete and comprehensive vindication.

A number of individual naphthene and paraffin hydrocarbons of purity closely approaching 100% were available and were dehydrogenated under the standard conditions—temperature 325° C., atmospheric pressure, and hydrogen flow at 30 ml. per hour. A quantity of approximately 20 ml. of each test sample hydrocarbon was processed and the refractive indexes (n_D^{20}) of the products were determined (Table IV). The observed differences, in the case of the paraffin hydrocarbons, correspond to a formation of less than 1% aromatic hydrocarbons—i.e., a negligible degree of reaction. The same conclusion holds good for the two monosubstituted cyclopentane derivatives, isopropyl- and *n*-propylcyclopentane. A slightly higher degree of reaction is noted for di- and trisubstituted cyclopentane derivatives, but ultraviolet spectroscopic analyses of the products indicated that the aromatic formation was of the order of 2 to 3% only (Table V). Bearing in mind the complexity of the fractions encountered in the application of the method in actual analysis, it is considered that such a value is of a satisfactorily low order and a general conclusion may be made that paraffins and five-membered ring naphthenes are affected to a negligible degree by the conditions of dehydrogenation employed.

Of the six-membered ring naphthenes in addition to methylcyclohexane, ethylcyclohexane and 1,3-dimethylcyclohexane (cis and trans) were both dehydrogenated to 99 to 100% aromatic materials. The slight extent of side reactions in the above two

Table IV. Dehydrogenation of Individual Hydrocarbons

Hydrocarbon	Purity, %	Refractive Index, n_D^{20}: Original	Refractive Index, n_D^{20}: After dehydrogenation
n-Heptane	99.9+	1.3878	1.3882
n-Octane	99+	1.3978	1.3980
2,2,4-Trimethylpentane	99.9+	1.3919	1.3921
n-Nonane	98.6	1.4055	1.4060
2,2,5-Trimethylhexane	99+	1.4000	1.4010
n-Decane	98.6	1.4119	1.4128
2,2,4,6,6-Pentamethylheptane	99+	1.4190	1.4190
1,3-Dimethylcyclopentane (trans)	99+	1.4090	1.4109
1,1,3-Trimethylcyclopentane	99+	1.4112	1.4128
Isopropylcyclopentane	99.0	1.4260	1.4264
n-Propylcyclopentane	99+	1.4263	1.4265
Methylcyclohexane	99+	1.4225	1.4930
Ethylcyclohexane	99.2	1.4332	1.4950
1,3-Dimethylcyclohexane (cis and trans)	96.5	1.4232	1.4962
1,1-Dimethylcyclohexane	98+	1.4290	1.4300

Table V. Ultraviolet Spectroscopic Analyses for Aromatics

(Dehydrogenation of individual hydrocarbons)

Hydrocarbon	Aromatic Content of Dehydrogenated Product, Wt. %: Ethylbenzene	*p*-Xylene	*m*-Xylene	*o*-Xylene	Total
1,3-Dimethylcyclopentane (trans)	0.7	0.3	0.3	0.6	1.9
1,1,3-Trimethylcyclopentane	0.2	0.6	1.9	0.7	3.4
Ethylcyclohexane	95.7	0.5	1.6	1.6	99.4
1,3-Dimethylcyclohexane (cis and trans)	...	3.2	92.8	4.0	100.0

cases can be readily appreciated by reference to ultraviolet spectroscopic analyses (Table V) of the products, each cyclohexane derivative giving rise to its corresponding benzene hydrocarbon. The remaining six-membered ring naphthene, 1,1-dimethylcyclohexane, proved to be little changed by the dehydrogenation conditions.

This confirms the conclusions of previous workers that geminal substituted cyclohexane derivatives—i.e., those with two substituent groups attached to the same carbon atom—cannot be considered true hexahydroaromatics, and therefore do not dehydrogenate to aromatic hydrocarbons. For this reason the results obtained by dehydrogenation methods in terms of five- and six-membered ring naphthenes may not represent the true composition. This is true particularly of the higher boiling range considered, as the chances of such disubstituted derivatives increase with rise in boiling point. The hydrocarbon 1,1-dimethylcyclohexane is the only geminal substituted compound among the 8 possible C_8H_{16} six-membered ring naphthenes. Of the 21 possible C_9H_{18} six-membered ring naphthenes, 4 have geminal carbon atoms, while the proportion increases to 27 out of 87 corresponding $C_{10}H_{20}$ hydrocarbons.

METHOD OF CALCULATION AND PRESENTATION OF RESULTS

The recommended method for the estimation of five- and six-membered ring naphthenes is based on the foregoing investigation.

Estimation of Aromatic Content of I.B.P.–225° C. material by fractionation into cuts of specified boiling range, followed by ultraviolet spectroscopic analysis for individual aromatic contents up to a boiling point of 150° C., and estimation of total aromatic content of cuts between 150° and 225° C.

Preparation of Aromatic-Free Material, I.B.P.–225° C., by dearomatization with silica gel (selective adsorption).

Fractionation of Aromatic-Free Material and estimation of total paraffin and naphthene contents of cuts of specified boiling range by linear interpolation of specific refraction data.

Estimation of Cyclopentane, Methylcyclopentane, Cyclohexane, and Dimethylcyclopentanes in the range I.B.P.–95° C. by reference to boiling point data and total paraffin and naphthene contents.

Dehydrogenation of Aromatic-Free Cuts of boiling ranges 95–109° C., 109–120° C., 120–130° C., 130–150° C., 150–175° C., 175–200° C., and 200–225° C. (obtained from fractionation of aromatic-free material) by processing over the platinum-charcoal catalyst under the standard conditions.

Estimation of Aromatic Contents of Dehydrogenated Cuts by ultraviolet spectroscopy or A.S.T.M. method (*1*).

Calculation of Results. If A = per cent weight of aromatics in dehydrogenated cut and C = corresponding weight of six-membered ring naphthenes in original cut, then

$$C = A \times \frac{\text{molecular weight of six-membered ring naphthenes}}{\text{molecular weight of aromatics}}$$

The weight of original cut corresponding to 100 parts of dehydrogenated product = $100 - A + C$.

$\therefore$ Weight per cent of six-membered ring naphthenes in original cut $= \frac{C}{100 - A + C} \times 100\%$.

The assumed values of molecular weights in the various cuts are:

Boiling Range of Cut, ° C.	Molecular Weight of Six-Membered Ring Naphthene	Molecular Weight of Corresponding Aromatic
95–109	98	92
109–120	112	106
120–130	112	106
130–150	126	120
150–175	140	134
175–200	154	148
200–225	168	162

The contents of six-membered ring naphthenes found will not include the geminal substituted compounds and are equivalent to the contents of "dehydrogenatable" or "reactable" six-membered ring naphthenes. The weight percentages of five-membered ring naphthenes obtained by difference will include the "unreactable" six-membered compounds:

(Total naphthene content) − (reactable six-membered ring naphthene content) = (five-membered ring naphthene content) + (unreactable six-membered ring naphthenes)

For simplicity, the final results are best expressed as "total," "reactable," and "unreactable" naphthene contents, provided always that the true meaning of the terms is understood, calculated on the basis of original sample equal to 100%, by reference to boiling point yield data and aromatic contents. A specimen analysis can be seen in Tables VI and VII.

USEFULNESS AND APPLICABILITY OF RESULTS

Though the techniques outlined have certain limitations regarding accuracy and interpretation, nevertheless such methods of distinguishing between reactable and unreactable naphthenes are of advantage, particularly in the case of the hydrocarbon type

Table VI. Paraffin and Naphthene Contents of 95° to 225° C. East Texas Naphtha

(Total aromatic content = 14.9 wt. %. Specimen analysis)

Boiling Range of Cut	Yield of Dearomatized Sample	Density at 20° C.	Refractive Index, $n^{20°C.}_D$	Specific Refraction, $\frac{n^2-1}{n^2+2} \times \frac{1}{d}$			Composition of Cut		Composition				Composition			
									Naphthenes		Paraffins		Naphthenes		Paraffins	
				Cut	Naphthene	Paraffin	Naphthene	Paraffins	Cut	Total	Cut	Total	Cut	Total	Cut	Total
° *C.*	*Wt. %*	*G./ml.*					*Wt. %*		*Wt. % of Dearomatized*				*Wt. % of Original*			
95–109	11.52	0.7365	1.4092	0.33582	0.33055	0.34445	62.1	37.9	7.15	7.15	4.37	4.37	6.08	6.08	3.72	3.72
109–120	7.60	0.7319	1.4082	0.33719	0.33030	0.34336	47.1	52.8	3.59	10.74	4.01	8.38	3.06	9.14	3.41	7.13
120–130	10.64	0.7379	1.4114	0.33678	0.33020	0.34299	48.6	51.4	5.17	15.91	5.47	13.85	4.40	13.54	4.65	11.78
130–150	21.86	0.7520	1.4189	0.33576	0.32995	0.34200	51.8	48.2	11.32	27.23	10.54	24.39	9.63	23.17	8.98	20.76
150–175	26.10	0.7645	1.4249	0.37440	0.32960	0.34097	57.8	42.2	15.09	42.32	11.01	35.40	12.84	36.01	9.37	30.13
175–200	13.96	0.7776	1.4324	0.33383	0.32925	0.34005	57.5	42.5	8.03	50.35	5.93	41.33	6.84	42.85	5.04	35.17
200–225	8.32	0.7886	1.4378	0.33274	0.32890	0.33945	61.7	38.3	5.13	55.48	3.19	44.52	4.36	47.21	2.72	37.89

Table VII. Reactable and Unreactable Naphthene Contents of 95° to 225° C. East Texas Naphtha

(Specimen analysis)

Boiling Range of Cut	Aromatic Content after Dehydrogenation	Weight % of Cut				Composition, Wt. %, of 95–225° C. Naphtha			
		Reactable naphthenes	Total naphthenes	Unreactable naphthenes	Total paraffins	Reactable naphthenes	Total naphthenes	Unreactable naphthenes	Total paraffins
° *C.*	*Wt. %*								
95–109	49.0	50.5	62.1	11.6	37.9	4.95	6.08	1.13	3.72
109–120	24.0	25.0	47.2	22.2	52.8	1.62	3.06	1.44	3.41
120–130	38.6	39.7	48.6	8.9	51.4	3.59	4.40	0.81	4.65
130–150	22.1	23.0	51.8	28.8	48.2	4.28	9.63	5.35	8.98
150–175	22.0	22.8	57.8	35.0	42.2	5.06	12.84	7.78	9.37
175–200	18.7	19.3	57.5	38.2	42.5	2.30	6.84	4.54	5.04
200–225	13.7	14.1	61.7	47.6	38.3	0.99	4.36	3.37	2.72

Table VIII. Detailed Hydrocarbon Type Composition of S/R Naphthas from Middle East Crudes

(Typical analyses)

Type	Boiling Range, ° C.	Predominant Hydrocarbons	Naphtha A			Naphtha B			Naphtha C			Naphtha D		
Paraffins	I.B.P.- 15	Butanes		...			...			...			...	
	15- 32.5	Isopentane		3.07			3.11			...			2.02	
	32.5- 45	*n*-Pentane		2.85			3.44			...			3.63	
	45- 66	Isohexanes		4.96			4.35			...			5.09	
	66- 75	*n*-Hexane		4.58			3.56			1.81			4.78	
	75- 85	Dimethylpentanes		0.96			0.79			0.36			0.92	
	85- 95	Methylhexanes		4.01			3.07			4.01			3.86	
	95-109	*n*-Heptane		5.40			4.21			5.88			5.83	
	109-120	Iso-octanes		3.47			3.41			4.53			3.39	
	120-130	*n*-Octane		4.92			4.11			5.48			5.68	
	130-150	Iso- and *n*-nonanes		6.86			6.20			8.22			7.78	
	150-175	Iso- and *n*-decanes		9.30			7.90			9.91			9.44	
	175-200	Iso- and *n*-undecanes		7.49			6.21			6.87			7.76	
	200-225	Iso- and *n*-dodecanes		5.91			4.58			2.31			7.06	
	Total			63.78			54.94			49.38			67.24	
Naphthenes	I.B.P.- 66	Cyclopentane	0.43		3.12	0.29		4.08	...		3.86	0.02		2.09
	66- 75	Methylcyclopentane	0.57			1.17			0.63			0.64		
	75- 85	Cyclohexane		0.47			0.95			1.02			0.56	
	85- 95	Dimethylcyclopentanes	1.65			1.67			2.21			0.87		
	95-109	Ethylcyclopentane and trimethylcyclopentanes	0.14		1.87	1.09		3.08	0.94		3.31	0.08		1.42
	95-109	Methylcyclohexane		1.73			1.99			2.37			1.34	
	109-120	Trimethylcyclopentanes and 1,1-dimethylcyclohexane	0.57		1.29	0.78		1.64	1.62		2.16	0.33		0.97
	109-120	Dimethylcyclohexanes		0.72			0.86			0.54			0.64	
	120-130	Unreactable C_8H_{16} naphthenes	0.01		1.35	0.69		1.98	1.61		2.71	0.17		1.26
	120-130	Reactable C_8H_{16} naphthenes		1.34			1.29			1.10			1.09	
	130-150	Unreactable C_9H_{18} naphthenes	1.62		3.07	2.05		3.72	3.73		4.96	1.01		2.52
	130-150	Reactable C_9H_{18} naphthenes		1.45			1.67			1.23			1.51	
	150-175	Unreactable $C_{10}H_{20}$ naphthenes	2.30		4.06	3.89		5.68	4.52		6.34	1.40		3.40
	150-175	Reactable $C_{10}H_{20}$ naphthenes		1.76			1.79			1.82			2.00	
	175-200	Unreactable $C_{11}H_{22}$ naphthenes	2.98		4.70	4.46		6.02	5.35		6.72	2.90		4.66
	175-200	Reactable $C_{11}H_{22}$ naphthenes		1.72			1.56			1.37			1.76	
	200-225	Unreactable $C_{12}H_{24}$ naphthenes	2.81		4.22	4.54		5.40	1.81		2.26	3.31		4.52
	200-225	Reactable $C_{12}H_{24}$ naphthenes		1.41			0.86			0.45			1.21	
	Total	Unreactable	13.08		23.68	20.63		31.60	22.42		32.32	10.73		20.84
		Reactable		10.60			10.97			9.90			10.11	
Aromatics	...	Benzene		0.05			0.63			0.48			0.21	
	...	Toluene		0.48			2.04			3.45			1.16	
	...	Ethylbenzene		0.17			0.57			1.36			0.40	
	...	*p*-Xylene		0.73			0.58			0.93			0.47	
	...	*m*-Xylene		1.04			1.48			2.46			1.03	
	...	*o*-Xylene		0.93			0.87			1.29			0.76	
	145-150	C_9H_{12} aromatics		0.06			0.07			...			0.07	
	150-175	C_9H_{12} aromatics		4.01			3.03			4.38			3.04	
	175-200	$C_{11}H_{14}$ aromatics		2.75			2.23			3.03			2.52	
	200-225	$C_{12}H_{16}$ aromatics		2.32			1.96			0.92			2.26	
	Total			12.54			13.46			18.30			11.92	

analysis of feed stocks and products, appertaining to processes wherein the essential reaction is thought to be aromatization by dehydrogenation of six-membered ring naphthenes—e.g., Hydroforming. Though the basis of this paper is the examination of a relatively small number of pure compounds, those hydrocarbons tested represent typical examples of the various classes and the inferences drawn are considered to be correct. As typical examples, the results obtained for the hydrocarbon type composition of a number of straight-run naphthas are given in Table VIII. The advantages to be gained by a knowledge of the make-up of petroleum fractions, in such detail, is self-evident.

CONCLUSIONS

A method of hydrocarbon type analysis of straight-run naphthas is proposed, the main object of which is the estimation of the five- and six-membered ring naphthenes throughout the range of initial boiling point to 225° C. The procedure embodies estimation of aromatic content, estimation of paraffin and total naphthene contents, estimation of reactable naphthene content, and estimation of unreactable naphthene content.

The reactable naphthene content is obtained by a dehydrogenation procedure and does not include geminal cyclohexane derivatives. The latter are included in the unreactable naphthene content together with the total cyclopentane derivatives. The accuracy of determination in each case is:

Aromatic content	±1% of determination
Paraffin content	±2% of determination
Total naphthene content	±2% of determination
Reactable and unreactable naphthene content	±4% of determination

Table IX. Assumed Values for Specific Refraction of Naphthenes and Paraffins

Boiling Point, ° C.	Naphthenes	Paraffins
27.85 (Isopentane)	...	0.35057
36.07 (*n*-Pentane)	...	0.35020
49.26 (Cyclopentane)	0.32986	...
71.81 (Methylcyclopentane)	0.33074	...
80.74 (Cyclohexane)	0.32926	...
45	...	0.34890
65	...	0.34710
85	0.33075	0.34550
125	0.33020	0.34295
165	0.32960	0.34090
205	0.32890	0.33940
225	0.32850	0.33870

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Determination of Mannosidostreptomycin and Dihydromannosidostreptomycin

A Colorimetric Method

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Mannosidostreptomycin or dihydromannosidostreptomycin in samples of commercial streptomycin or dihydrostreptomycin solids may be determined colorimetrically by means of the anthrone reagent. This reagent (0.2% in 95% sulfuric acid) reacts with the mannose moiety, producing a characteristic color. The intensity of this color, compared with that produced by mannose standards, permits quantitative determination of mannosidostreptomycin and dihydromannosidostreptomycin. Streptomycin and dihydrostreptomycin at the low level of concentration used for this test (about 300 micrograms per ml.) do not interfere with the determinations.

THE procedure presented is based on the use of Dreywood's anthrone reagent (*1*) in sulfuric acid for the determination of carbohydrates. This method has been applied to the determination of the quantity of mannose derived from the hydrochloride or the sulfate salts of mannosidostreptomycin (streptomycin B) or dihydromannosidostreptomycin which is contained in the sample. [Subsequent to the authors' routine application of this reagent, a similar use of anthrone was announced, but no details of the procedure were made available (*2*).] This reagent is highly specific for carbohydrates, giving a characteristic blue-green color. No noncarbohydrate, except furfural, has been reported to give this color. It has been shown (*4*) that the anthrone reagent in sulfuric acid gives the same depth of color with a compound of a sugar as if the compound were first hydrolyzed and then the determination made.

Streptomycin and dihydrostreptomycin at the low level of concentration used for these determinations do not interfere with the test while similar absorption spectra (450 to 700 millimicrons) are obtained for mannose, mannosidostreptomycin, and material containing both streptomycin and mannosidostreptomycin (see Figure 1). D(+)-Mannose is used as a standard for the determination and over the range indicated reacts with anthrone following Beers' law (Figure 2).

APPARATUS

A Coleman Junior spectrophotometer was used for the measurements of color intensity, but any type of instrument is suitable if absorption measurements at a wave length of 620 millimicrons can be attained.

The size of the cuvette used in the instrument will affect the range of the test. For the results here reported, 25 × 105 mm. cuvettes were used. When cells of smaller diameter are used, the concentration of sample solution will need to be increased to obtain suitable intensity of color for accurate measurement.

Pyrex test tubes (inside diameter 22 mm.) are suitable reaction tubes. The uniformity of these tubes is important for reproducibility of results, as variation in size may effect the development of the heat of reaction. Because of the sensitivity of the test, chemical cleanliness of glassware is important. Freedom from traces of lint or fibers from cotton or filter paper is especially necessary in both glassware and water sources. Pipets or syringes are suitable for adding the reagent to the sample. The use of lubricants such as Cello-Seal with burets introduces errors.

REAGENTS AND SOLUTIONS

Anthrone Reagent. The reagent for the test is 0.2% anthrone prepared according to the method in "Organic Syntheses" (*3*) in 95% sulfuric acid (reagent grade). Fresh solutions are to be prepared daily.

Sulfuric Acid, 95%. The 95% sulfuric acid was prepared (*4*) by cautiously adding 1 liter of concentrated acid to 50 ml. of water and cooling.

D(+)-Mannose. C.P. reagent having a specific rotation of +14.25° was obtained from the Pfanstiehl Chemical Company.

PROCEDURE

It is necessary to standardize the reagent with a known concentration of mannose each time a series of determinations is made. With the size of cuvette here used, concentrations of mannose in water ranging from 5 to 50 micrograms per ml. gave color density sufficient for accurate determinations.

The concentration of streptomycin in solution used will be somewhat dependent upon the amount of mannosidostreptomycin or dihydromannosidostreptomycin contained, and the limits of detection desired when pure streptomycin or dihydrostreptomycin is to be authenticated. Usually a total of 300 micrograms per ml. is satisfactory for initial determinations. Inasmuch as the yield of mannose from pure mannosidostreptomycin approximates

20%, the concentration of the solution can be varied, depending upon the estimated content of the sample. For accurate determinations the sample should be of sufficient concentration so that the mannosidostreptomycin or dihydromannosidostreptomycin content will yield at least 5 micrograms per ml. of mannose.

To 5 ml. of the streptomycin solution or mannose standard, 10 ml. of the anthrone reagent are added and immediately mixed. When the heat of reaction has subsided to room temperature (the period of standing should be kept uniform for each series of analyses, including the standard and blank), the solutions are transferred to matched cuvettes or colorimeter tubes for measurement of the density of absorption at 620 millimicrons. Included with each series of analyses is a blank for zero adjustment of the measuring instrument. This blank consists of 5 ml. distilled water plus 10 ml. of the reagent and is prepared concurrently with the other samples.

CALCULATION OF RESULTS

For each series of determinations the conversion factor, *K*, is established using mannose standards (see Figure 2).

$$K = \frac{C \text{ (mannose concentration)}}{D \text{ (density of absorption)}}$$

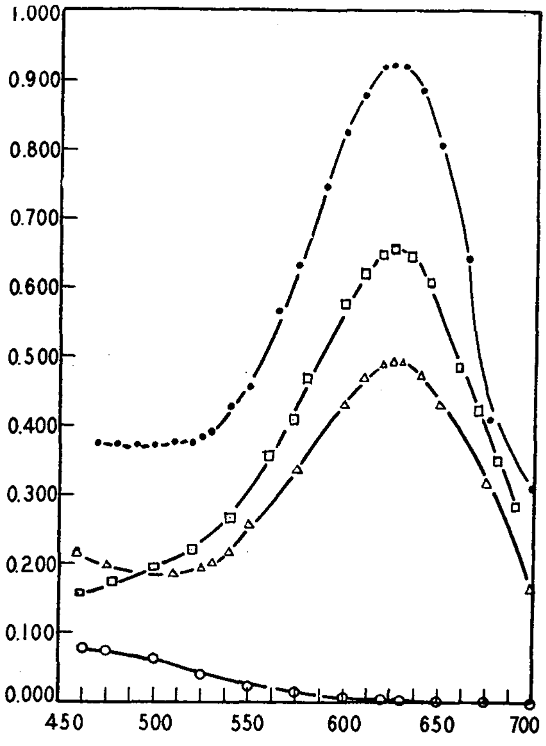

Figure 1. Absorption Curves of Anthrone Reaction Products

□ Mannose
○ Crystalline streptomycin hydrochloride (300 γ/ml.)
● Mannosidostreptomycin sulfate (300 γ/ml.)
△ Mixture of streptomycin and mannosidostreptomycin

Using this factor the mannose content of the streptomycin sample is determined. From the mannose content the mannosidostreptomycin or dihydromannosidostreptomycin content may then be calculated. In the results here given the mannose content of mannosidostreptomycin hydrochloride is taken as 21.14%, while for the corresponding sulfate 20.28% is assumed. When the total weight of mannosidostreptomycin in the sample is known, the content in the original material can be computed.

RESULTS

In Table I are shown the results of several analyses. The anthrone procedure compares favorably with the countercurrent distribution procedure (*5*) which has been authenticated for these types of samples.

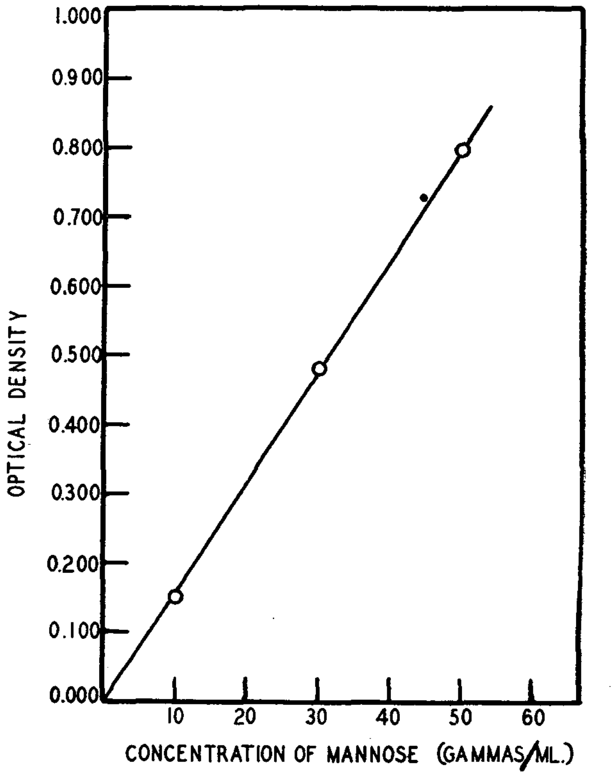

Figure 2. Relation of Color Density Due to Anthrone Reaction Using Varying Quantities of D(+)-Mannose

Table I. Analysis of Mannosidostreptomycin in Samples of Streptomycin

Streptomycin Sample	% Mannosidostreptomycin: Anthrone method (colorimetric)	% Mannosidostreptomycin: Countercurrent distribution method
A	0.5	0.0
B	2.3	2.0
C	6.5	6.0
D	25.0	24.0
E	46.0	45.0
F	52.0	50.0
G	57.0	56.0
H	58.0	56.0
I	97.0	98.0
J[a]	0.0	...
K[a]	57.0	...

[a] Samples of dihydrostreptomycin preparations and corresponding per cent of dihydromannosidostreptomycin.

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Colorimetric Determination of Nitrates and Nitric Acid Esters

Isomeric Xylenols as Reagents

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A study of the six isomeric xylenols (dimethyl phenols) as reagents for the colorimetric determination of the nitrate ion was undertaken. From nitration and absorption spectra studies it was shown that 3,4-xylenol (3,4-dimethyl phenol) was the best reagent. Methods for the colorimetric determination of inorganic nitrate and of nitric acid esters in the presence of stabilizers such as diphenylamine are described. They are based upon the fact that nitrates can be hydrolyzed by 80% sulfuric acid to yield nitric acid. The nitric acid liberated nitrates 3,4-xylenol, forming 6-nitro-3,4-xylenol which is steam-distilled from the reaction mixture and collected in an alkaline solution, forming the deeply colored sodium salt. This salt is capable of accurate colorimetric determination. The colored system, the nitration reaction, and the hydrolysis reactions are discussed. The recommended concentration range is from 0.10 to 0.35 mg. of nitrate nitrogen in 100 ml. of solution, using a cell depth of 1 cm.

THIS paper deals with a study of the isomeric xylenols as reagents for the colorimetric determination of nitrate, and the colorimetric determination of nitrates and nitric acid esters. The method is based upon the fact that nitrates yield nitric acid on treatment with sulfuric acid. The nitric acid liberated is used to nitrate a xylenol. The nitroxylenol is then steam-distilled from the reaction mixture, and the distillate is collected in an aqueous solution of sodium hydroxide, forming the deeply colored sodium salt of the nitroxylenol. This salt can be determined colorimetrically.

A literature survey indicated that only 2,4-xylenol had been proposed (*1, 2, 6, 9, 12, 14, 15, 18*) as a reagent for nitrate analysis.

Table I. Summary of Data on Nitro Derivatives of Isomeric Xylenols

Compound	M.P. (Micro.)	Wave Length at Maximum, Na Salt in H_2O	Molecular Extinction Coefficient (Maximum)	Wave Length at Minimum, Na Salt in H_2O	Molecular Extinction Coefficient (Minimum)	Ratio, $E^{max.}_{mol.}/E^{min.}_{mol.}$
	° *C.*	*mμ*		*mμ*		
6-Nitro-2,3-xylenol	48.5– 49.0	430	4741	362	1934	2.45
4,6-Dinitro-2,3-xylenol	83.5– 87.0	378	11024	326	5256	2.09
6-Nitro-2,4-xylenol	72.3– 73.0	446	4465	355–356	1021	4.37
2-Nitro-3,4-xylenol	71.8– 72.4	418–420	780.8	325–332	456.4	1.73
6-Nitro-3,4-xylenol	86.8– 87.5	432	5378	353	1346	4.03
2,6-Dinitro-3,4-xylenol	126.5–127.5	426	6049	336	997.0	6.06
2-Nitro-3,5-xylenol	66.0– 66.5	412	1603	327	860.3	1.86
2-Nitro-3,6-xylenol	34.0– 34.5	424	800.5	329	487.7	1.64
4-Nitro-3,6-xylenol	120.5–122.0	410	18412	291	709.2	25.96

APPARATUS AND REAGENTS

Apparatus. The optical density measurements were made with a Beckman Model DU spectrophotometer. Absorption cells with a 0.998-cm. solution thickness were used.

The all-glass steam distillation apparatus was made from 10-mm. Pyrex tubing and consisted of a water-cooled condenser (15-cm. jacket), and a 250-ml. round-bottomed flask. A standard-taper (24/40) joint connects the two.

Reagents. 3,4-Xylenol was obtained from the Eastman Kodak Company (Catalog No. 1155). 2,3-Xylenol was obtained through the courtesy of Lee I. Smith, University of Minnesota. 2,4-Xylenol, 3,5-xylenol, and 2,5-xylenol were obtained from the Reilly Tar and Chemical Corporation, Indianapolis, Ind.

3,4-Xylenol reagent, 2 and 8% acetone solutions.

Sulfuric acid (80 weight %). Mix 660 ml. of c.p. sulfuric acid (specific gravity 1.84) with 300 ml. of distilled water and cool before using.

Sodium hydroxide, 2% aqueous solution.

Standard potassium nitrate solution. Dissolve 0.3610 gram of potassium nitrate, which has been dried at 120° C., in distilled water to make 500 ml. of solution. One milliliter of the standard solution contains exactly 0.1 mg. of nitrogen.

STUDY OF ISOMERIC XYLENOLS

Preparation and Absorption Spectra of Nitroxylenols. In order to study the isomeric xylenols for use as reagents it was first necessary to prepare the nitro derivatives, and to study their absorption spectra. Below are given in brief the data concerning their preparation and absorption spectra. A detailed account of their preparation is given by Holler, Huggett, and Rathmann (*8*).

2,3-XYLENOL. When 2,3-xylenol is nitrated, it yields 6-nitro-2,3-xylenol, 4,6-dinitro-2,3-xylenol, and presumably some 4-nitro-2,3-xylenol, although none of the 4-nitro derivative was isolated from the nitration mixture. Only the 4,6-dinitro compound had been prepared previously (*11*). The structure was proved for the 6-nitro- and 4,6-dinitro-2,3-xylenols (*8*). Figure 1 gives the absorption spectra for the sodium salts of 6-nitro- and 4,6-dinitro-2,3-xylenols. The spectrophotometric properties are given in Table I.

2,4-XYLENOL. 6-Nitro-2,4-xylenol was obtained by the nitration of 2,4-xylenol (*13, 17*). Only one mononitro derivative is obtained on nitration. The absorption spectrum of the sodium salt is given in Figure 1 and the other data are given in Table I.

3,4-XYLENOL. 3,4-Xylenol is very easily nitrated (*5, 7*), yielding 6-nitro-3,4-xylenol and 2-nitro-3,4-xylenol in the ratio of approximately 6 parts of the 6-nitro derivative to 1 part of the 2-nitro compound. With excess nitric acid the 2,6-dinitro derivative is formed. 2-Nitro-3,4-xylenol had not been previously prepared. After this paper had been submitted for publication the preparation of this compound was described by Mueller and Pelton (*10*). It crystallizes from petroleum ether in orange needles melting at 71.8° to 72.4° C. The structure was proved for the 2-nitro, 6-nitro, and 2,6-dinitro derivatives (*8*). Figures 1 and 2 give the absorption spectra for the sodium salts of all three nitro derivatives and Table I gives the other data.

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3,5-Xylenol. 3,5-Xylenol, being a symmetrical molecule, yields on nitration only one *o*-nitrophenol (2-nitro-3,5-xylenol, *3*), but also gives a *p*-nitro derivative (4-nitro-3,5-xylenol, *16*). The absorption spectrum for 2-nitro-3,5-xylenol (sodium salt) is given in Figure 2 and the other data are in Table I.

The 4-nitro compound was not prepared.

3,6-Xylenol. A mixture of 2-nitro- and 4-nitro-3,6-xylenol is formed on the nitration of 3,6-xylenol (*4*). The two isomers can be separated by steam distillation, the 2-nitro compound steam-distilling and leaving the 4-nitro derivative in the residue. Figure 2 gives the absorption spectrum for the sodium salt of 2-nitro-3,6-xylenol and Table I the other data. The sodium salt of the 4-nitro derivative has the spectrum as shown in Figure 1.

2,6-Xylenol. No *o*-nitro derivatives can be formed from 2,6-xylenol; therefore this isomer was not studied.

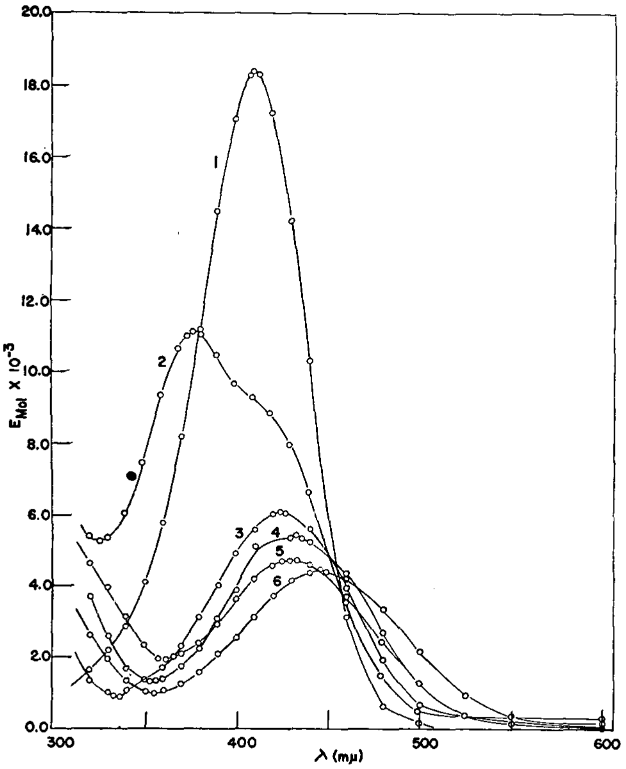

Figure 1. Molecular Extinction Curves for Sodium Salts

Water used as solvent

1. 4-Nitro-3,6-xylenol
2. 4,6-Dinitro-2,3-xylenol
3. 2,6-Dinitro-3,4-xylenol
4. 6-Nitro-3,4-xylenol
5. 6-Nitro-2,3-xylenol
6. 6-Nitro-2,4-xylenol

Discussion. If a certain isomeric xylenol is to be of value as a reagent in the determination of nitrate, it should possess the following properties:

On nitration the incoming nitro group should go ortho to the hydroxyl group. Only *o*-nitroxylenols steam-distill.

The incoming nitro group should not enter a position adjacent to a methyl group. Methyl groups force the nitro group to twist out of the plane of the ring (steric hindrance) and the loss of coplanarity causes a great reduction of the molecular extinction coefficient of the compound in question.

The xylenol should yield only one mononitro derivative on nitration.

The molecular extinction coefficient of the sodium salt of the *o*-nitroxylenol should be as large as possible, so that the method will give the required sensitivity.

The first step in the testing of the different xylenols consisted of preparing the *o*-nitroxylenol and measuring the absorption spectrum of the sodium salt. It was found that the *o*-nitro derivatives of 3,5-xylenol (2-nitro-3,5-xylenol) and 3,6-xylenol (2-nitro-3,6-xylenol), in which the methyl group was adjacent to the nitro group, had a much lower value for the molecular extinction coefficients than did the nitro derivatives of 2,3-xylenol (6-nitro-2,3-xylenol), 2,4-xylenol (6-nitro-2,4-xylenol), and 3,4-xylenol (6-nitro-3,4-xylenol) (Table I and Figure 1). Because of this fact, 3,5-xylenol and 3,6-xylenol were discarded for use as reagents; 2,6-xylenol was discarded because no *o*-nitro derivatives are possible.

The next step in the testing of the remaining xylenols was to run standard solutions of potassium nitrate in order to obtain a colorimetric factor that could be applied to the analysis of nitrates.

One- to 5-ml. aliquots of standard potassium nitrate solution (0.3610 gram per 500 ml.) were measured into a 250-ml. round-bottomed flask and the solution was evaporated to dryness on a steam bath. The flask was cooled and 1 ml. of a 2% acetone solution of the xylenol was added; 15 ml. of 80% (weight) sulfuric acid was run in, and the mixture was swirled for 10 minutes and then allowed to sit for 20 minutes more. The solution was then diluted with 100 ml. of water and steam-distilled. The distillate was collected in a 100-ml. volumetric flask containing 5 ml. of 2% sodium hydroxide solution. After the distillation was complete (80 ml. collected), the solution was cooled to 20° C., diluted to the mark, mixed, and filtered, and the maximum optical density was measured on a Beckman spectrophotometer.

As is noted in Table II, 2,4-xylenol does not give a constant value for the colorimetric factor; this indicates that in the nitration reaction the nitration of the phenol is not constant but probably gives unwanted side reactions. Owing to this fact, 2,4-xylenol was discarded for use as a reagent. 3,4-Xylenol, on the

Table II. Testing of Xylenols with Standard Solutions of Potassium Nitrate

Xylenol	Nitrate Nitrogen Added, Mg.	Factor, Concentration of N, Mg. / Max. Optical Density
2,4-Xylenol	0.099	0.399
	0.198	0.410
	0.199	0.406
	0.199	0.417
	0.297	0.427
3,4-Xylenol	0.100	0.412
	0.150	0.412
	0.200	0.412
	0.250	0.412
	0.300	0.412

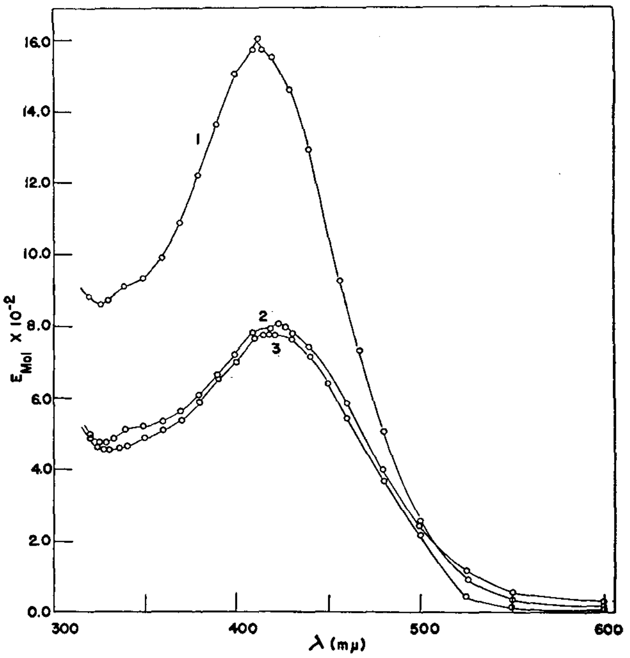

Figure 2. Molecular Extinction Curves for Sodium Salts

1. 2-Nitro-3,5-xylenol
2. 2-Nitro-3,6-xylenol
3. 2-Nitro-3,4-xylenol

Water used as solvent

Table III. Analysis of Nitrate Solutions

Nitrate Nitrogen Present	Nitrate Nitrogen Found	Deviation	Deviation
Mg.	*Mg.*	*Mg.*	%
0.100	0.099	−0.001	1.0
0.100	0.100	±0.000	0.0
0.150	0.149	−0.001	0.7
0.200	0.198	−0.002	1.0
0.200	0.199	−0.001	0.5
0.250	0.247	−0.003	1.2
0.300	0.300	±0.000	0.0
0.300	0.297	−0.003	1.0

Table IV. Effect of Diverse Ions

Foreign Ion Added		Potassium Nitrate Present	Potassium Nitrate Found	Nature of Interference
	Mg.	*Mg.*	*Mg.*	
None	None	1.04	1.04	
Cl^-	5	1.04	0.69	Hydrochloric acid evolved reacts with nitric acid liberated
	10	1.04	0.62	
Fe^{++}	100	1.04	1.05	
Fe^{+++}	100	1.04	1.06	
H_2O_2	10	1.04	0.47	Reacts with liberated nitric acid
Mn^{++}	100	1.04	1.06	
NH_4^+	100	1.04	1.04	
NO_2^-	1	1.04	1.11	Nitrous acid liberated reacts with reagent giving colored products which steam-distill
	5	1.04	1.10	
	10	1.04	1.14	
Pb^{++}	100	1.04	1.04	
PO_4^{---}	100	1.04	1.05	
SO_3^{--}	100	1.04	1.06	
Zn^{++}	100	1.04	1.06	

other hand, yields a constant value of the factor and was chosen over 2,3-xylenol because of the greater molecular extinction coefficient for the sodium salt of the 6-nitro derivative. On nitration 3,4-xylenol yields two mononitroxylenols (2-nitro- and 6-nitro-3,4-xylenol) that are steam-distillable, whereas 2,3-xylenol can presumably yield a nonsteam-distillable *p*-nitro derivative (4-nitro-2,3-xylenol) that would decrease the sensitivity of the method. Therefore, 3,4-xylenol is the best of the six isomeric xylenols as a reagent for the colorimetric determination of nitrates because:

The molecular extinction coefficient of the sodium salt of the 6-nitro-3,4-xylenol has a greater value than any of the other *o*-nitro derivatives of the other isomeric xylenols.

3,4-Xylenol yields on nitration two mononitro isomers which are both steam-distillable.

3,4-Xylenol can be obtained commercially (Eastman Kodak Company, Catalog No. 1155) in high purity.

COLORIMETRIC DETERMINATION OF NITRATES AND NITRIC ACID ESTERS

The following colorimetric methods were used for the determination of inorganic nitrate and nitric acid esters using 3,4-xylenol as the reagent.

Inorganic Nitrate. Preparation of Calibration Curve. Measure into 250-ml. round-bottomed flasks (Ŧ24/40) quantities of standard potassium nitrate solution (0.3610 gram per 500 ml.) ranging from 0.5 to 3.5 ml. Evaporate nearly to dryness on a steam bath, and cool to room temperature. Add 1 ml. of a 2% acetone solution of 3,4-xylenol and 15 ml. of sulfuric acid (80 weight %), and mix. Swirl gently for the first 10 minutes and then allow to stand for 20 minutes longer. Add 150 ml. of distilled water and a boiling chip, and attach the all-glass condenser. Steam-distill the mixture, collecting the distillate in a 100-ml. volumetric flask which contains 5 ml. of a 2% sodium hydroxide solution. After the distillation is complete (70 to 80 ml. distilled over), cool to 20° C., and dilute to the mark with water. Mix thoroughly, and filter the yellow solution through a wad of cotton placed in a No. 42 Whatman paper. Transfer a portion to an absorption cell and measure the optical density or percentage transmittancy of the yellow solution at a wave length of 432 mμ, using distilled water as a reference solution. The readings obtained yield a calibration curve covering the range of 0.0 to 0.3 mg. of nitrate nitrogen.

Procedure. Pipet out a quantity of material to contain between 0.10 and 0.35 mg. of nitrate nitrogen and transfer to a 250-ml. round-bottomed flask (Ŧ24/40). Evaporate nearly to dryness on a steam bath, and cool to room temperature. Add 1 ml. of a 2% acetone solution of 3,4-xylenol and 15 ml. of sulfuric acid (80 weight %), and mix. Swirl gently for the first 10 minutes and then allow to stand for 20 minutes longer. Add 150 ml. of distilled water and a boiling chip, and steam-distill the mixture. Collect the distillate in a 100-ml. volumetric flask which contains 5 ml. of 2% sodium hydroxide solution. After the distillation is complete (70 to 80 ml. distilled over), cool to 20° C., and dilute to the mark with water. Mix, filter, and measure the intensity of the color as in the preparation of the calibration curve.

The recommended concentration range is from 0.10 to 0.35 mg. of nitrate nitrogen in 100 ml. of solution, using a cell depth of 1 cm.

Table III gives the results of running a number of samples of potassium nitrate by this method.

Effect of Diverse Ions. The various cations and anions that are likely to be found in samples containing inorganic nitrate were tested for their interference in the colorimetric determination (Table IV). Known amounts of potassium nitrate and foreign ion were mixed and the nitrate analysis was carried out as above. The sulfate salts of the cations (lead as acetate), and the anions as the sodium salts were used to check the effect of diverse ions. With the ions studied, the only interferences were from chloride, nitrite, and hydrogen peroxide, which can be easily removed at the beginning of the analysis. The treatment of the cold solution with saturated silver sulfate solution, with the subsequent filtration of the precipitated silver chloride, removes the chlorides. Nitrites may easily be eliminated by treatment of the aliquot (slightly acidified with acetic acid) before evaporating down with 20% sulfamic acid solution. The excess sulfamic acid is hydrolyzed by hot water and is destroyed while the aliquot is evaporating on the steam bath. The hydrogen peroxide can be destroyed by treatment of the original solution with 0.5% potassium permanganate solution.

Nitric Acid Esters in Presence of Stabilizers. In the methods given below small amounts of nitric acid esters may be determined in the presence of stabilizers, such as diphenylamine, diethyldiphenylurea, and their derivatives, which are added to commercial explosives.

Procedures. The inorganic nitrate calibration curve was used for the following methods.

Decyl Nitrate. Weigh out an amount of material to contain between 0.07 and 01.25 gram of decyl nitrate, and transfer to a 250-ml. volumetric flask, which contains 5 ml. of 3,4-xylenol reagent (8% solution). Mix thoroughly, and add with swirling 150 ml. of sulfuric acid (80 weight %). Stopper the flask, wire on the stopper, place the flask and contents in a shaker, and shake (240 to 250 oscillations per minute) for 30 minutes. After shaking remove the stopper, cool to 20° C., and dilute to the mark with sulfuric acid (80 weight %). Foaming may be eliminated by adding 1 to 2 drops of petroleum ether before diluting. Mix thoroughly, pipet out a 10-ml. aliquot for analysis, and run the aliquot into a 250-ml. round-bottomed flask containing 150 ml. of distilled water. Wash out the pipet (calibrated "to contain") with water, then with acetone, and finally again with water. Add a porous chip to the flask, attach the condenser, and allow the mixture to steam-distill. Catch the distillate in a 100-ml. volumetric flask, which contains 5 ml. of aqueous 2% sodium hydroxide. After the distillation is complete (70 to 80 ml. distilled over), cool the distillate to 20° C., and dilute to the mark with water. Filter the yellow solution through a small wad of cotton placed in a No. 42 Whatman paper. Measure the intensity of the color as in the preparation of the calibration curve.

n-Amyl Nitrate. Weigh out an amount of material to contain between 0.07 and 0.10 gram of *n*-amyl nitrate, and transfer to 100-ml. volumetric flask. Dissolve by adding 25 to 50 ml. of petroleum ether (30° to 60° C.). Adjust to 20° C., and dilute to the mark with petroleum ether (30° to 60° C.). Take a 5-ml. aliquot for analysis, and run it into a 50-ml. glass-stoppered, Pyrex, Erlenmeyer flask. Add 1 ml. of 3,4-xylenol reagent (2%), mix, and add 15 ml. of sulfuric acid (80 weight %). Stopper the flask, wire on the stopper, place the flask and contents in a shaker, and shake for 30 minutes (240 to 250 oscillations per minute). Cool the reaction mixture in an ice bath and remove the stopper, then pour the reaction mixture into a 250-ml. round-bottomed flask that contains 80 ml. of water. Rinse the flask with water, 2 to 3 ml. of acetone, and again with water. Add a boiling chip, attach the condenser, and steam-distill the mixture from an oil bath. Collect the distillate, and treat it as in the analysis of decyl nitrate, diluting to

Table V. Analysis of Standard Solutions of *n*-Amyl Nitrate

n-Amyl Nitrate Present *Mg.*	*n*-Amyl Nitrate Found *Mg.*	Deviation *Mg.*	Deviation %
0.76	0.76	±0.00	0.0
0.76	0.76	±0.00	0.0
1.52	1.50	−0.02	1.3
1.52	1.50	−0.02	1.3
2.28	2.27	−0.01	0.5
2.28	2.25	−0.03	1.3
3.04	3.02	−0.02	1.0
3.80	3.80	±0.00	0.0
3.80	3.75	−0.05	1.3

Table VI. Analysis of Pure Samples of Decyl Nitrate

Decyl Nitrate Added *Mg.*	Decyl Nitrate Found *Mg.*	Deviation *Mg.*	Deviation %
110.5	110.6	+0.1	0.1
110.5	111.0	+0.5	0.5
101.1	100.2	−0.9	0.9
112.2	110.9	−1.3	1.2
107.6	108.3	+0.8	0.8
113.7	114.2	+0.5	0.4
117.0	116.3	−0.7	0.6

the mark with water, and pipetting off the petroleum ether before filtering the solution.

RESULTS

Standard solutions of *n*-amyl nitrate in petroleum ether were made up and run by the method given (Table V).

The results obtained by running a number of pure samples of decyl nitrate are given in Table VI. Good agreement was obtained.

Discussion. Decyl nitrate could not be determined in the presence of petroleum ether because of incomplete extraction by sulfuric acid. Thus, initial solution of the sample in petroleum ether was not made. Large amounts of *n*-amyl nitrate (0.1 gram) may be determined by the use of the decyl nitrate procedure.

Colored System. Figure 3, curve 1, gives the optical density–wave length curve obtained when the method is standardized with potassium nitrate (0.29 mg. of nitrate nitrogen). This colored system was found to have a maximum optical density at a wave length of 432 mμ and a minimum optical density at 353 mμ. This indicates that in the procedure the nitric acid formed by the hydrolysis nitrates the 3,4-xylenol to yield the 6-nitro compound. The ratio of the maximum optical density to the minimum optical density in this case was found to be only 2.65, indicating that in the nitration other products besides 6-nitro-3,4-xylenol are formed, and that these absorb rather strongly in the region of the minimum.

This was investigated by extracting an acidified portion of the steam distillate with petroleum ether, chromatographing the extract on a column of silicic acid–Celite (2 to 1), and developing the chromatogram with 5% diethyl ether in petroleum ether. The chromatogram obtained showed an upper band, light yellow in color, which was identified by its melting point and absorption spectrum as 2-nitro-3,4-xylenol, and a canary-yellow lower band, which was similarly identified as 6-nitro-3,4-xylenol.

Figure 3 also shows more clearly the relationships between the mononitroxylenols that are found in the steam distillate.

A sample of potassium nitrate (0.29 mg. of nitrate nitrogen) was run as in the preparation of calibration curve. The absorption spectrum of a portion of the accurately diluted steam distillate was taken (curve 1). After the measurement was made, this portion was quantitatively recombined with the remainder, and the whole acidified with sulfuric acid. This solution was then extracted with petroleum ether (30° to 60° C.), and the extract chromatographed on a column of silicic acid–Celite (2 to 1). The zones were cut, each was eluted with diethyl ether, and the ether was evaporated off. The individual residues were then each dissolved in 5 ml. of 2% sodium hydroxide and diluted accurately up to the original volume of 100 ml. The absorption spectra of the solutions were then measured.

Curve 2 gives the spectrum for the lower zone of the chromatogram, which was previously identified as 6-nitro-3,4-xylenol. The spectrum of the upper zone of the chromatogram, shown to be 2-nitro-3,4-xylenol, is given in curve 3. The absorption spectrum of the sodium salt of 3,4-xylenol is shown in curve 4. The concentration of this was equivalent to that used in the procedure. Curve 5 gives the results of adding together curves 2, 3, and 4. The difference between curves 1 and 5 is probably due to the slight loss of the nitro derivatives on extracting, chromatographing, and eluting.

No 2,6-dinitro-3,4-xylenol was found, as would be expected, when an excess of 3,4-xylenol is present. The colored system was stable for over 2 months.

Curve 4 gives the optical density–wave length curve for the sodium salt of 3,4-xylenol. The concentration of the salt is 0.0238 gram per 100 ml. of solution, which is equivalent to that used in the procedure. Note the very low absorption at 432 mμ.

The recommended concentration range is from 0.10 to 0.35 mg. of nitrate nitrogen in 100 ml. of solution, using a cell depth of 1 cm.

Nitration and Hydrolysis Reactions. As in all nitrations, the yields of the 2-nitro- and the 6-nitro-3,4-xylenols are not stoichiometric. A six- to sevenfold excess of 3,4-xylenol is used, and the conditions of the hydrolysis of the nitric acid ester and nitration of the phenol are controlled; thus, reproducible and accurate results can be obtained.

Stabilizers, such as diphenylamine, diethyldiphenylurea, and their derivatives, appear to have no effect on the nitration reaction. This is probably due to the fact that 80% sulfuric acid is used, and the nitric acid liberated nitrates the 3,4-xylenol before nitrating or oxidizing the diphenylamine, diethyldiphenylurea, or their derivatives.

It was found that 20 minutes' shaking was adequate for obtaining complete reaction. The shaker speed was between 240 and 250 oscillations per minute.

In the hydrolysis of the ester and the nitration of the 3,4-xylenol, 80% sulfuric acid resulted in the maximum recovery of the ester. A more dilute or a more concentrated acid gave much lower recoveries.

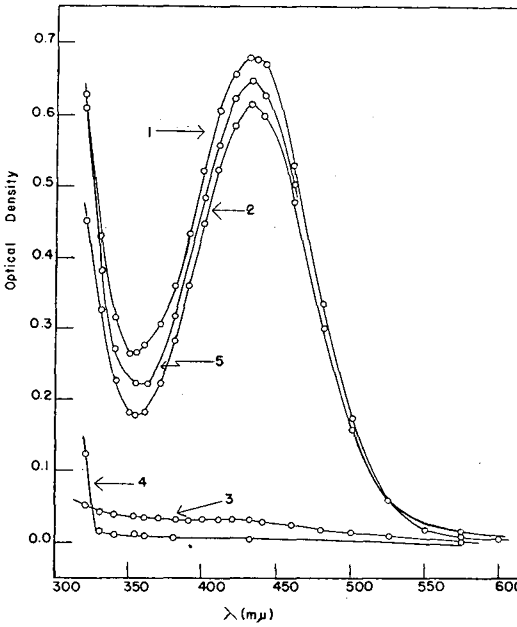

Figure 3. Constituents of Steam Distillate

1. Colored system obtained when method is standardized with 0.29 mg. of nitrate nitrogen
2. Lower zone of chromatogram (6-nitro-3,4-xylenol)
3. Upper zone of chromatogram (2-nitro-3,4-xylenol)
4. Sodium salt of 3,4-xylenol (0.0238 gram per 100 ml.)
5. Curve produced by adding curves 2, 3, and 4. All solutions diluted to 100 ml.

Table VII. Analysis of Synthetic Samples

Stabilizer Added	Decyl Nitrate Added Mg.	Decyl Nitrate Found Mg.	Deviation Mg.	Deviation %
Diphenylamine	117.0	116.3	−0.7	0.6
	110.0	109.5	−0.5	0.5
Diphenylamine derivatives (mixture of 2-nitrodiphenylamine, 2,4-dinitrodiphenylamine, p-nitrosodiphenylamine)	113.7	114.2	+0.5	0.4
	108.8	109.1	+0.4	0.5
Diethyldiphenylurea	105.5	104.2	−1.3	1.2
	121.3	120.2	−1.1	0.9

It is essential to have a shaking apparatus that tends to homogenize the reaction mixture as well as to shake it, and the shaking action should be vigorous. A shaker speed of 240 to 250 complete oscillations per minute was sufficient to effect the complete hydrolysis of the nitric acid esters.

A number of synthetic samples were made up containing decyl nitrate together with roughly equal weights of diphenylamine, or diphenylamine derivatives, or diethyldiphenylurea. These were then analyzed. Table VII gives the results of these runs. No interference was found from these compounds.

Interferences. Organic nitrites cause serious errors in the determination and should be absent from the materials to be analyzed. Nitrites when hydrolyzed in acid solution give nitrous acid, which reacts with the 3,4-xylenol to give a variety of steam-distillable colored products.

Other Nitric Acid Esters. The application of this method to nitrocellulose was attempted. Low results were obtained with nitrocellulose due to incomplete hydrolysis in 80% sulfuric acid. The hydrolysis was very slow (about 6 hours were required to reach a steady value) and only 87.5% of the nitrocellulose was recovered.

Nitroglycerin and diethylene glycol dinitrate are very easily hydrolyzed by dilute sulfuric acid. These esters were not run by the above method; however, the method should be readily applicable to their analysis and should yield good results.

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Spectrophotometric Determination of Uracil, Thiouracil, and Related Compounds

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A COLORIMETRIC method for assaying barbiturates, introduced by Dille and Koppanyi (*2*), is based on the color produced when a barbiturate reacts with a cobalt salt in an alkaline medium. Further investigations (*1, 3–6*) describe optimum conditions and application of this reaction to the barbiturates. During a study of this reaction in the determination of phenobarbital it was noted that compounds with —CONHCO— and —CONHCS— groups interfered in the reaction by giving similar color. This interference prompted investigation of the color reaction for possible use in assay of compounds containing these groups. Among the compounds tested were uracil, thiouracil, and propylthiouracil and it was found that by using conditions similar to those used for the barbiturates, reproducible quantitative results accurate to within about 1 or 2% could be obtained. These results are noted in Table I.

REAGENTS REQUIRED

Chloroform, C.P. grade. Dry over anhydrous sodium sulfate.

Methyl Alcohol Absolute, C.P. grade. Dry over anhydrous sodium sulfate.

Cobalt Acetate Reagent. Dry cobalt acetate for 2 hours at 100° C. Dissolve 0.25 gram of dried cobalt acetate in 200 ml. of dried absolute methyl alcohol, C.P. grade.

Isopropylamine Reagent A. Dilute 50 ml. of isopropylamine (Eastman) to 200 ml. with dried chloroform.

Isopropylamine Reagent B. Dilute 50 ml. of isopropylamine (Eastman) to 200 ml. with absolute methyl alcohol.

ANALYTICAL PROCEDURE

Because optimum conditions for the determination of the —CONHCO— and —CONHCS— groups vary, an example of each is presented.

Compounds with —CONHCS— Group. Accurately weigh a dry sample sufficient to give a concentration of approximately 1 mg. per ml. and transfer to a volumetric flask. Dissolve the sample in methanol and dilute to volume. Transfer exactly 5 ml. to a dry 25-ml. volumetric flask and add reagents in the following order: 5 ml. of isopropylamine reagent A, 5 ml. of cobalt acetate reagent, and dilute to volume with chloroform. Mix thoroughly and take readings at 530 mμ. Run a blank on reagents consisting of 5 ml. of cobalt acetate reagent, 5 ml. of isopropylamine reagent A, and 5 ml. of methanol, diluting to volume with chloroform. Subtract the blank reading from that obtained for the sample. Prepare standard graphs, using the dried standard compound dissolved in methanol in suitable concentrations.

Compounds with —CONHCO— Group. Accurately weigh a dry sample sufficient to give a concentration of 0.75 mg. per ml.

Uracils, thiouracils, and related compounds react with cobalt salts in anhydrous alkaline medium to form stable color complexes. The complexes formed obey Beer's law within limits and this reaction may be used for accurate quantitative measurements. Optimum conditions for color development and application of this reaction to analysis of some pharmaceuticals and biological compounds are described.

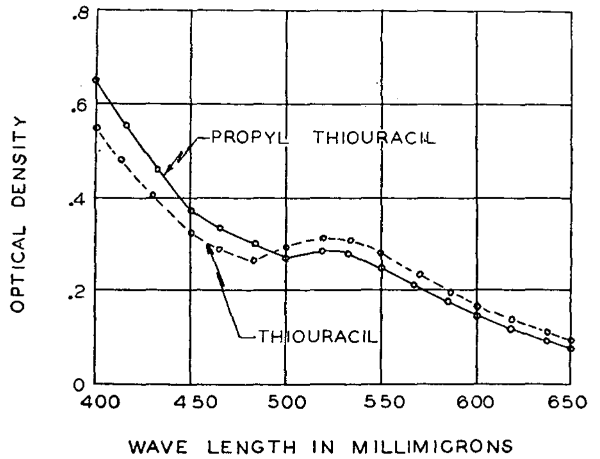


Figure 1. Absorption Spectrum of Thiouracil and Propylthiouracil

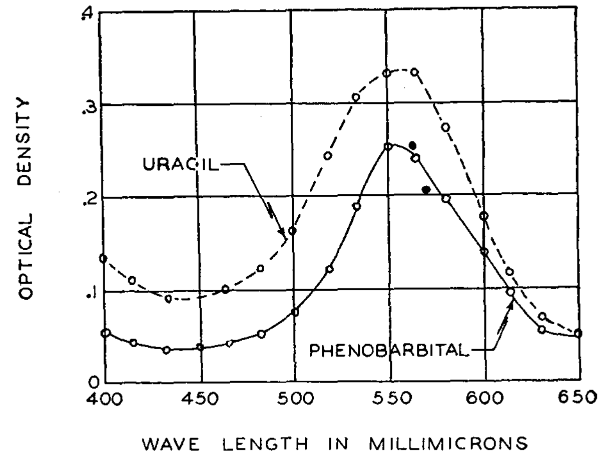


Figure 2. Absorption Spectrum of Uracil and Phenobarbital

and transfer to a volumetric flask. Dissolve the sample in dry chloroform and dilute to volume. Transfer exactly 5 ml. to a dry 25-ml. volumetric flask, add 5 ml. of cobalt acetate reagent, and 5 ml. of isopropylamine reagent B, and dilute to volume with chloroform. Mix thoroughly and take readings at 560 mμ. Run a blank on reagents and subtract the value obtained from the sample reading. Prepare standard graphs using the dried standard compound dissolved in chloroform in suitable concentrations.

EXPERIMENTAL

The data for the absorption curves were obtained using a Coleman Model 11 spectrophotometer and are represented in Figures 1 and 2.

Figure 1 includes curves for thiouracil and propylthiouracil. Both compounds contain the —CONHCS— group and show maximum absorption in the visible spectrum at 530 mμ and at some point in the ultraviolet spectrum.

Figure 2 shows curves for uracil and phenobarbital, which contain the —CONHCO— group and exhibit maximum absorption at 560 mμ.

The color forms almost immediately after addition of the reagents and is stable for several hours. For best results the medium used in this reaction should be a chloroform-methanol mixture in the ratio of 3 to 2. Either methanol or chloroform solvent may be used for dissolving the sample. The 3 to 2 chloroform-methanol ratio may be accomplished in either case by proper choice of diluent in preparing reagents. If difficulty is experienced in dissolving a sample in either of these solvents, a small amount of isopropylamine added to the solvent often aids solution.

Table I. Determination of Uracil, Thiouracil, Propylthiouracil, and Phenobarbital

Compound	Added	Found	Recovery
	Mg.	*Mg.*	%
Uracil	3.75	3.75	100.0
	3.75	3.76	100.3
	3.00	2.99	99.6
Thiouracil	5.00	4.91	98.2
	5.00	5.03	100.6
	4.00	3.95	98.8
Propylthiouracil	6.00	5.98	99.7
	7.50	7.48	99.7
	7.50	7.45	99.3
Phenobarbital	5.00	4.98	99.6
	5.00	4.98	99.6
	2.50	2.48	99.2

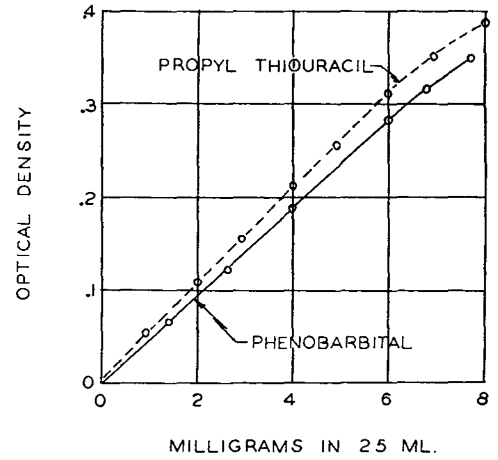


Figure 3. Calibration Curves for Phenobarbital and Propylthiouracil

It is important to carry out the reaction under anhydrous conditions, as the presence of moisture will cause a gradual fading of color. The sample, solvents, and reagents must be completely dry to ensure development of a maximum stable color.

Figure 3 shows calibration curves for phenobarbital and propylthiouracil, made using a Coleman Model 11 spectrophotometer. The curves obey Beer's law within certain limits of concentration and fall off at varying points of higher concentration, depending upon the compound tested.

DISCUSSION

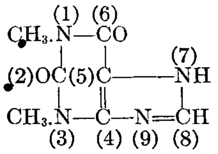
Numerous tests with various compounds indicate that the color reaction is rather specific for compounds possessing the —CON-

HCO— or —CONHCS— group, which may be found in aliphatic compounds or aromatic heterocyclic compounds.

Some examples of compounds having the —CONHCO— group and forming a blue-violet color complex with cobalt are: phenobarbital, barbital, pentobarbital, theobromine, phthalimide, alloxan, biuret, and uracil.

The red color complex formed with cobalt and the —CONHCS— group is evidently a composite color, for the solution has more than one absorption maximum. Propylthiouracil, and thiopental are examples of compounds that contain this group and form the red color complex.

Theophylline presents an interesting exception to the above groups. Theophylline



was found to give a blue-violet color complex similar to those compounds with a —CONHCO— group. It is supposed that the functional group is formed with atoms 2, 6, and 7 to make the same complex as is formed with uracil and similar compounds.

This reaction will find application in various fields of chemistry, particularly in biological and pharmaceutical chemistry.

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Determination of Calcium on Soil Extracts and Plant Ash by Chloranilic Acid

Compensating Errors Caused by Presence of Magnesium and Iron

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Colorimetric determinations of calcium in soil extracts and plant ash by the chloranilic acid procedure are subject to error due to the presence of iron and magnesium. These errors may be compensating, as iron increases and magnesium decreases the intensity of the chloranilic acid color. Magnesium and iron cause additional errors by inhibiting precipitation of calcium chloranilate. A corrective procedure for use when all samples of a group have nearly the same magnesium and iron content is suggested.

THE authors have noted that the chloranilic acid colorimetric procedure for determination of calcium as proposed by Tyner (*7*) frequently gives results that are at variance with standard oxalate procedures (*1*) by from 10 to 25% in analysis of soil extracts and plant ash. Investigation has shown that these errors were due to the presence of magnesium and/or iron and that the error may be positive or negative, large or small, depending on the relative concentration of these elements.

Additions of magnesium reduce the intensity of the chloranilic acid color (Figure 1), and prevent calcium precipitation (Figure 2). As reported by Tyner (*7*), additions of ferric iron to chloranilic acid increase the intensity of the color (Figure 1) and also prevent precipitation of calcium (Figure 2). It may also be noted in Figure 2 that the effects of iron and magnesium in depressing calcium precipitation are additive.

Data for Figure 2 were obtained by additions of magnesium sulfate, ferric chloride, and magnesium sulfate plus ferric chloride to solutions containing 0.786 mg. of calcium. The calcium chloranilate was then precipitated by Tyner's procedure (*7*). This precipitate was dissolved and the calcium determined by reprecipitation as oxalate, using a volumetric micro procedure (*1*). These points were also verified by washing the calcium chloranilate precipitates with dilute (25 p.p.m.) chloranilic acid, transferring the precipitate and filter paper to a beaker containing 50 ml. of 0.1 *N* hydrochloric acid, dissolving the precipitate and, after filtering, reading in the photometer. A straight line following Beer's law was obtained over the range from 0 to 1.5 mg. of calcium in solutions where iron and magnesium were omitted. The inhibiting effects of iron and magnesium shown in Figure 2 were also noted for other concentrations of calcium.

The effect of magnesium is contrary to the report of Tyner (*7*), who stated that magnesium is coprecipitated or occluded in the calcium chloranilate precipitate. This was proved incorrect by spectrographic analysis of a calcium chloranilate precipitate produced in the presence of 1.25 mg. of magnesium. Recovery of magnesium in the calcium chloranilate precipitate containing 0.629 mg. of calcium was of the magnitude of 0.005 mg. of magnesium. Even this small amount is probably the result of incomplete washing of the filter paper and precipitate. Undoubtedly the lighter color of chloranilic acid caused by the presence of magnesium led Tyner to the erroneous conclusion that magnesium was coprecipitated.

The possible errors which could be caused by small amounts of iron or magnesium and how they may compensate for each other are clearly shown in Figure 3. Variations shown here are within the range of concentrations that might be expected in using the chloranilic acid procedure for calcium determination in plant tissues (*2, 6*). Greater variations are found in soil extracts. Plant

Table I. Comparison of Calcium Determinations in Carpet Grass by Oxalate, Chloranilic Acid, and Chloranilic Acid Plus Magnesium Procedures

Sample No.	Calcium[a] by Oxalate	Calcium by Chloranilic Acid, 25 Mg. of Mg in Stds.	Calcium by Chloranilic Acid	Magnesium in Sample Aliquot
	Mg./g.	*Mg./g.*	*Mg./g.*	*Mg.*
1	3.19	3.08	3.36	0.17
2	3.40	3.40	3.68	0.19
3	3.29	3.28	3.56	0.20
4	4.04	4.08	4.36	0.22
5	2.18	2.06	2.36	0.23
6	3.60	3.68	3.96	0.24
7	3.83	3.92	4.16	0.28
8	1.68	1.76	2.04	0.28
9	2.18	2.20	2.52	0.31
10	4.66	4.72	4.96	0.35

[a] Volumetric micro procedure (*1*).

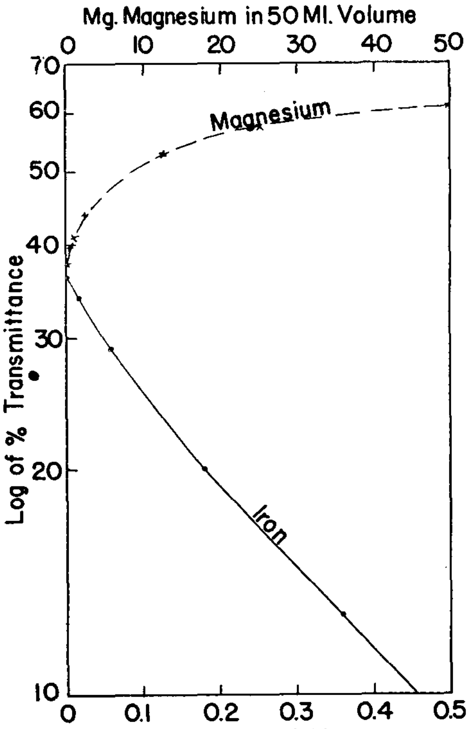

Figure 1. Effect of Magnesium and Iron on Transmittance of Chloranilic Acid at 525 mμ

Cenco filter 87309 B

tissues not specifically prepared for iron analysis are often ground in steel mills that introduce more iron into the sample and greater possible errors (*5*). In many cases the magnesium and iron content of plant tissues may nearly compensate for one another, but in others large errors may result.

The authors found that by rapid determination of the iron (*4*) and magnesium (*3*) content in aliquots of unknown solutions before analysis for calcium, and then by addition of equivalent amounts of iron and magnesium to the standard solutions, very good agreement with standard calcium oxalate precipitation procedures was obtained. An illustration of partial correction by addition of magnesium alone to the standards is given in Table I. This procedure is practical only when all samples of a group have nearly the same iron and magnesium contents.

Analysts should accept calcium analyses by the chloranilic acid procedure with caution when iron and/or magnesium may be present, unless these factors are considered in the determination.

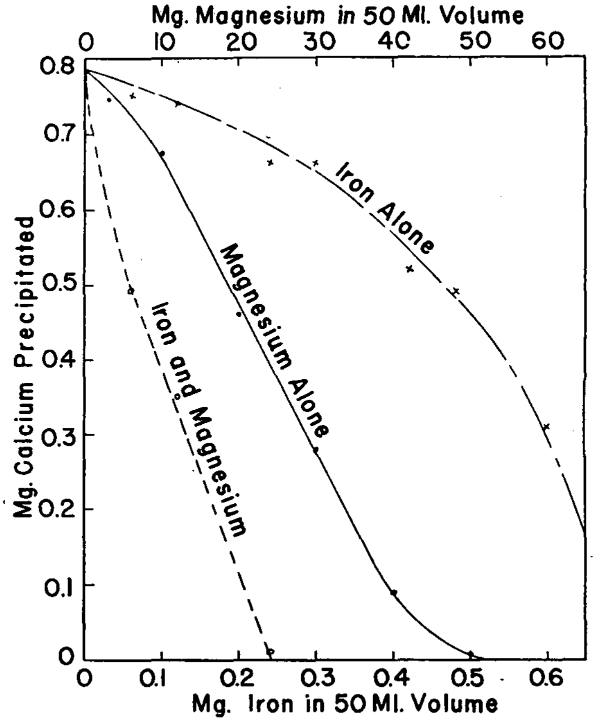

Figure 2. Effect of Magnesium and Iron on Precipitation of Calcium Chloranilate from a Solution Containing 0.876 Mg. of Calcium in Chloranilic Acid

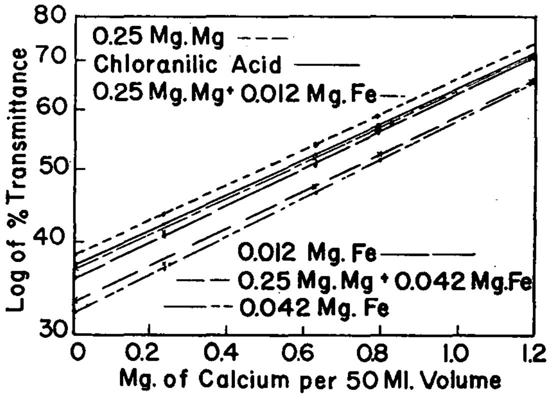

Figure 3. Effect of Magnesium and Iron on Calcium-Chloranilic Acid Transmittance Curve at 525 mμ

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Determination of Nordihydroguaiaretic Acid in the Leaf of *Larrea divaricata* (Creosote Bush)

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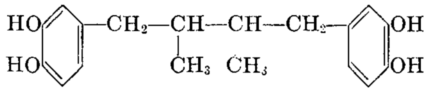
A simple method for the determination of nordihydroguaiaretic acid in the leaf of the creosote bush is based on the reaction of nordihydroguaiaretic acid with ammonium molybdate to yield an orange colored complex. Only small amounts of compounds that interfere with the reaction were found to be present and corrections may be applied. The method should be applicable to the determination of added nordihydroguaiaretic acid in fats and oils and various food products.

NORDIHYDROGUAIARETIC acid (N.D.G.A.) is a valuable antioxidant for fats and oils. It occurs in the desert plant *Larrea divaricata* Cav., familiarly known as the creosote bush. Its structure (*3*, *8*) is as follows:



Lundberg and Halvorson (*5*) developed methods for determining nordihydroguaiaretic acid in fats and oils, involving the reduction of gold chloride and adaptations of the Gibbs indophenol (*2*) and Emmerie and Engel (*1*) procedures. It was found in the authors' laboratories that these methods are not well suited for determining the compound in the creosote bush leaf. The gold chloride reagents react with various oxidizing agents. Light and oxidizing and reducing agents interfere with the indophenol reaction. The Emmerie and Engel reaction is dependent upon the time allowed for color development. When samples of creosote bush extract and pure nordihydroguaiaretic acid were compared using this method, the rates of change in color intensity differed, introducing a source of error.

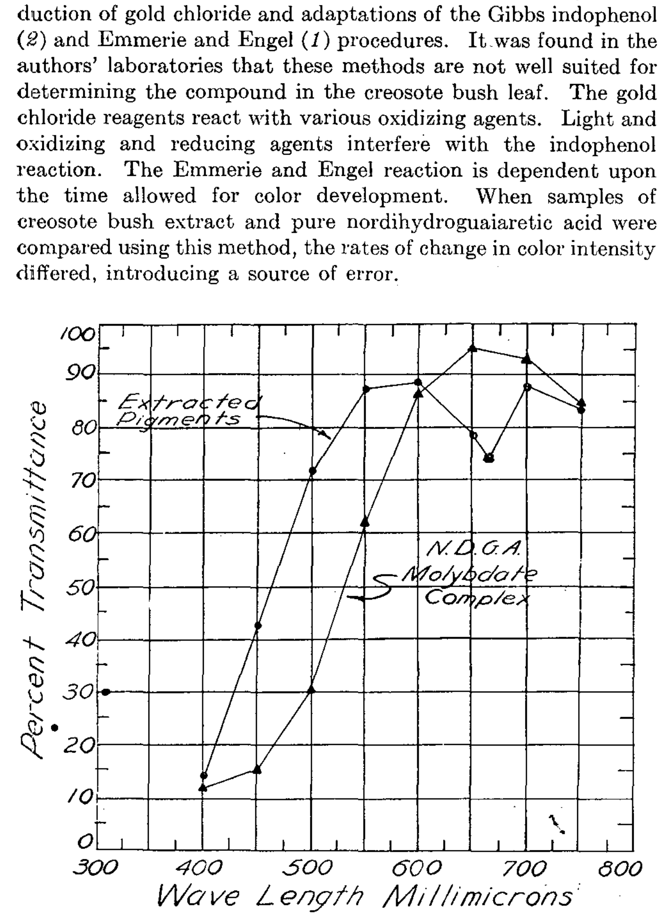


Figure 1. Comparison of Transmittance Curves of Extracted Pigments and of Nordihydroguaiaretic Acid–Molybdate Complex

Extracted pigments, 1 gram of leaf extracted with alcohol and diluted to 250 ml. nordihydroguaiaretic acid, 350 micrograms per ml. (equivalent to amount in above extract)

Because the creosote bush is the commercial source of the material, a dependable analytical method for its determination in the leaf is important.

DEVELOPMENT OF QUANTITATIVE COLORIMETRIC TEST

No reactions were found which were specific for nordihydroguaiaretic acid, but it did form colored solutions and precipitates with many phenolic group reagents, including ferric alum, ammonium vanadate, copper sulfate–ammonium hydroxide reagent, osmic acid, sodium hydroxide, ferric chloride, ammonium molybdate, neutral and basic lead acetate, and silver nitrate. It formed yellow and red diazo compounds with nitrous acid and aromatic amines such as benzidine and sulfanilic acid and a blood-red color with nitrous acid and urea.

Several of these reagents gave promise of utilization in quantitative tests. Of these, the ammonium molybdate reagent was rather arbitrarily chosen for more complete study and, because subsequent work proved it to be satisfactory, no further work was done with the other reagents.

Solutions of ammonium molybdate (1 to 3%) gave a stable, light-resistant, instantaneously developed orange color with alcoholic solutions of nordihydroguaiaretic acid. The intensity was proportional to the concentration of nordihydroguaiaretic acid and the reaction was sensitive to a concentration of 25 micrograms per ml. The chief disadvantage of ammonium molybdate was the fact that the reaction was also given by other compounds containing *o*-dihydroxybenzene groups such as tannic acid, gallic acid, pyrogallol, and catechol. The reaction is, in fact, the basis of Martini's colorimetric method (*6*) for the determination of molybdenum, using catechol as a reagent.

The colors developed with the above compounds seemed to be spectrophotometrically the same, a phenomenon apparently related to the presence of the common *o*-dihydroxybenzene groups. On an equal weight basis, the intensities varied as follows: Tannic and gallic acids gave about one half the color intensity of nordihydroguaiaretic acid, catechol three fourths, and pyrogallol about the same. This means that the error introduced by the presence of any of these compounds would be of corresponding magnitude.

A standard curve was prepared using alcoholic solutions of pure nordihydroguaiaretic acid containing from 50 to 500 micrograms per ml. (concentration plotted against log of per cent transmittance). The orange color was developed by adding 10 ml. of 1% ammonium molybdate to 5 ml. of the solution. The color developed instantaneously and was read at a wave length of 500 mμ, on the Coleman (Model 11) spectrophotometer.

Although lower wave lengths were more sensitive to nordihydroguaiaretic acid, 500 mμ was chosen because it represented a wave length of adequate sensitivity and minimum pigment interference (Figure 1). The amounts of pigments and nordihydroguaiaretic acid used in preparing Figure 1 were roughly equivalent to their relative proportions in the leaf. That interference is at a minimum at 500 mμ is indicated by the fact that the difference between the transmittance curves is at a maximum.

SELECTION OF SOLVENT FOR EXTRACTION

Creosote bush samples were extracted for 16 hours with the following solvents: benzene, isopropyl alcohol, dioxane, petroleum ether, acetone, *tert*-butyl alcohol, methyl alcohol, ethyl ether, formic acid, toluene, chloroform, carbon tetrachloride, carbon disulfide, ethyl alcohol, pyridine, and water. The extracts were freed of solvent, diluted with alcohol to the proper concentration range, and tested with molybdate. Blanks were prepared to correct for the pigmentation of the solutions. The maximum results were obtained in the cases of ethyl and methyl alcohols and no significant increases occurred on further extraction from 16 to 60 hours.

In the first 90 minutes 95% of the nordihydroguaiaretic acid extractable with alcohol was removed from the leaf and the extraction was essentially complete after 4 hours. Table I gives typical results from several tests.

Table I. Effect of Time of Extraction on Nordihydroguaiaretic Acid Extracted from Creosote Bush Leaf with Ethyl Alcohol

Time Extracted	N.D.G.A.[a]
Hours	%
1.5	7.98
3	0.01
4	0.10
5	0.00
7	0.01
23	0.09
Total	8.19

[a] Results would include any *o*-dihydroxybenzene compounds present as N.D.G.A.

It was tentatively decided to use ethyl alcohol as the solvent. Because the recorded values would include not only nordihydroguaiaretic acid, but also the effects of any other compounds present capable of reacting colorimetrically with the molybdate reagent, it was necessary to determine the extent of any possible interference by such compounds.

ESTIMATION OF POSSIBLE INTERFERING COMPOUNDS

Water-Soluble *o*-Dihydroxybenzene Compounds. *o*-Dihydroxybenzene compounds constitute a potential source of interference, as they react colorimetrically with molybdate reagent.

The common *o*-dihydroxybenzene compounds, gallic acid, pyrogallol, catechol, and tannic acid, are from 150 to 15,000 times as water-soluble as the slightly soluble nordihydroguaiaretic acid. This difference in solubility was made the basis for determining the amount of interference caused by these water-soluble compounds.

Different weight samples of solvent-free leaf extract were refluxed with 100 ml. of water. Light yellow, almost colloidal, precipitates of impure nordihydroguaiaretic acid formed on cooling, proving the solutions to be saturated. The solutions would also contain all or most of the more soluble *o*-dihydroxybenzene compounds, and the amounts of these compounds in solution would vary with the weight of samples extracted. The solutions were filtered at 25° C., the filtrates were tested with molybdate reagent, and the transmittance was determined on the spectrophotometer. Results were in grams of nordihydroguaiaretic acid, even though they included the effects of other *o*-dihydroxybenzene and possibly other water-soluble compounds.

The interference caused by these compounds was determined mathematically through the use of simultaneous equations.

Let X = concentration of N.D.G.A. in grams necessary to saturate 100 ml. of water in the presence of leaf extract.

Let Y = amount of interference (expressed as grams of N.D.G.A.) caused by water-soluble compounds other than N.D.G.A. when b grams of leaf extract are extracted with 100 ml. of water.

Then $2Y$ = the amount of interference caused by water-soluble compounds other than N.D.G.A. when $2b$ grams of leaf extract are extracted with 100 ml. of water.

Then $X + Y$ = apparent grams of N.D.G.A. found when b grams of leaf extract are extracted with 100 ml. of water.

$X + 2Y$ = apparent grams of N.D.G.A. found when $2b$ grams of leaf extract are extracted with 100 ml. of water.

Values for X and Y were obtained by solving the equations.

A number of experimental determinations were made using different amounts of leaf extract and equations were set up for each. The resulting combinations of equations, solved for X, yielded values which ranged from 0.04 to 0.05 gram and averaged 0.047 gram. This average value was used as the solubility of nordihydroguaiaretic acid in 100 ml. of water at 25° C. in the presence of an excess of leaf extract and was substituted in the equations to obtain values for the amount of interference caused by water-soluble compounds or Y.

The results are listed in Table II and calculations are included to show the amount of interference to be anticipated if the initial leaf sample were 5 grams and the amount of extract were 1.5 grams. In the method finally adopted, 5-gram samples were used and when extracted with ethyl alcohol yielded approximately 1.5 grams. Values on such samples would be about 0.4% too high, owing to the interference of water-soluble compounds.

The error is minor when the total values of 8 to 10% are considered and may be compensated for by introducing a correction.

Tannins. Traces of tannins were reported by Waller (*10*) and because some are water-soluble and some are water-insoluble, there was no assurance that they had been extracted as part of the water-soluble group. An attempt was therefore made to estimate them separately.

Only one method for the determination of tannic acid was found which could be modified for use in the presence of nordihydroguaiaretic acid. The reaction was described by Mitchell (*7, 9*) and involved the formation of a violet color on mixing a buffered solution of tannic acid and a ferrous sulfate–sodium potassium tartrate reagent. It was sensitive to concentrations as low as 20 micrograms per ml. but above 100 micrograms per ml. readings were impossible owing to the formation of precipitates.

Five milliliters of buffer solution (pH 4.8) and 2 ml. of a fresh solution containing equal volumes of freshly prepared 0.1% ferrous sulfate and 0.5% sodium potassium tartrate were added to 5 ml. of test solution. A violet color rapidly developed in the presence of tannins and the per cent transmittance was determined on the spectrophotometer at 550 mμ, the wave length of minimum transmittance and therefore maximum sensitivity. The concentration of tannins was read from a standard curve prepared from solutions of tannic acid of known concentration.

Hydrogen-ion control was necessary because the reaction was specific only for tannic acid and tannins within the range 4.1 to 5.5 pH. Even in this range nordihydroguaiaretic acid in the

Table II. Effect of Water-Soluble Compounds on Determination of Nordihydroguaiaretic Acid by the Molybdate Method

Weight of Leaf Extract Extracted with 100 Ml. of H_2O	Apparent N.D.G.A.[a] Content of Water Extract (Determined Spectrophotometrically)	Average Calculated Value for X	Part of Apparent N.D.G.A. Due to Water-Soluble Compounds (Interference)	Amount of Interference Calculated on Basis of 1.5 Grams of Leaf Extract	Error Due to Water-Soluble Compounds in 5-Gram Leaf Sample
Grams	*Gram*	*Gram*	*Gram*	*Gram*	%
0.77	0.054	0.047	0.007	0.014	0.28
0.79	0.059	0.047	0.012	0.023	0.46
1.55	0.068	0.047	0.021	0.020	0.40
3.20	0.084	0.047	0.037	0.017	0.34
			Av.	0.018	0.37

[a] Apparent N.D.G.A. = grams of N.D.G.A. + interference expressed as grams of N.D.G.A.

amounts normally found in the leaf interfered by producing a yellow color that amounted to an error of approximately 0.2%, which had to be deducted. Creosote bush tannins gave a blacker color with the reagent than tannic acid and a slight error was probably introduced on this account.

The total tannin content (corrected for nordihydroguaiaretic acid) of creosote bush extract was found to be only 0.4 to 0.6% and of this approximately 0.1% was water-soluble and was therefore included in a previous correction. Inasmuch as tannic acid gives only one half the molybdate color intensity given by an equal amount of nordihydroguaiaretic acid, values would be only 0.15 to 0.25% high owing to the presence of water-insoluble tannins.

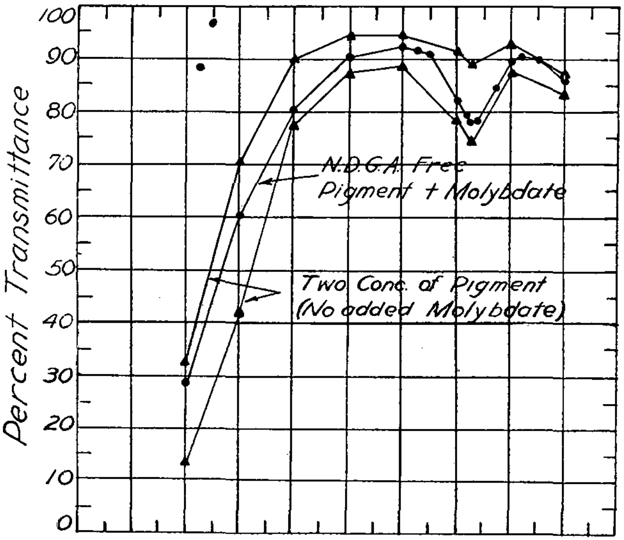


Figure 2. Effect of Molybdate Reagent on Transmittance Curves of Creosote Bush Pigments

Possibility of Reactions between Pigments and Molybdate Reagent. Waller (*10*) isolated small amounts of a yellow and an orange flavonol from creosote bush leaf. Because some flavonols like quercetin contain *o*-dihydroxybenzene groups, there was a possibility that these compounds might react with molybdate reagent.

It was found that flavonols could be extracted from the leaf with petroleum ether. Waller (*10*) had developed tests to distinguish between the creosote bush flavonols and nordihydroguaiaretic acid and these indicated that only small amounts of nordihydroguaiaretic acid were present. In the extract only 0.15% was found, an amount which could reasonably be accounted for by the presence of traces of nordihydroguaiaretic acid. There was, therefore, no evidence of interference due to flavonols extractable with petroleum ether. This was further confirmed by the work of Horn (*4*), whose partial elucidation of the structures of the yellow and orange pigments did not indicate the presence of *o*-hydroxy groups.

The flavonols extracted were assumed to be a mixture of the orange and yellow compounds, as they gave color reactions similar to those given by alcohol extracts known to contain the mixture.

The flavonols were not the only pigments in the creosote bush leaf, as evidenced by its green color. A method for preparing a mixture of all the pigments free of nordihydroguaiaretic acid took advantage of the fact that many phenolic compounds oxidize in basic solution. Apparently, the easily oxidized tautomeric quinoid form is attacked, resulting in cleavage of the ring structure and the formation of various colored products. The reaction depends on the presence of atmospheric oxygen.

A sample of leaf was extracted with benzene and the solvent-free extract was completely dissolved in dilute sodium hydroxide. Oxygen gas was bubbled through the red solution for 15 minutes in order to facilitate oxidation. Carbon dioxide was bubbled through the solution until a green amorphous material precipitated (pH about 7.8). The precipitate was filtered, washed with sodium bicarbonate solution, dissolved in alcohol, and tested with molybdate reagent. A negative test was obtained, indicating the absence of nordihydroguaiaretic acid and related compounds.

Alcoholic solutions of the original extract and the precipitated pigments were compared over the visible spectrum on the spectrophotometer. The shapes of the curves as shown in Figure 2 were very similar and showed no change before and after the removal of the nordihydroguaiaretic acid. This indicated that the treatment necessary for its removal had no effect on the leaf pigments. Therefore, as the pigments were unchanged, and the nordihydroguaiaretic acid–free extract gave no reaction with molybdate, neither the pigments nor any of the compounds associated with them in the precipitate interfere with the molybdate reaction for nordihydroguaiaretic acid. The effect of pigments can, therefore, be corrected through the use of a blank determination.

Possibility of Reactions between Other Compounds and Molybdate Reagent. In addition to the compounds already mentioned, Waller (*10*) isolated potassium chloride, sucrose, a wax, a phytosterol, a crude rubber, and a volatile oil. These compounds were tested with molybdate and failed to react. Possibly other unknown compounds were included in the group extracted with water or in the precipitate containing the pigments, but the fact that no important interference was observed in either case proves that none of these compounds interferes.

In order to test the specificity of the reaction, over 70 compounds representative of various functional groups were tested with molybdate after solution in appropriate solvents. The groups included were both aliphatic and aromatic—aromatic: hydrocarbons, halides, esters, aldehydes, ketones, nitro compounds, amines, and amino acids; aliphatic: ethers, mono-, di-, and trihydric alcohols, saturated and unsaturated acids, hydroxy and di- and tricarboxy acids, nitriles, amides, and oximes; also urea, purines, compounds containing nitrogen in the ring, alkaloids such as strychnine, brucine, cinchonine, atropine, and cocaine, phenols, fats, sterols, proteins, carbohydrates such as polysaccharides, gums, and sugars, lactones, glucosides, saponins, flavonols, terpenes, and sulfur-containing compounds.

The only compounds that gave a reaction were those containing *o*-phenolic groups; the color produced was in every case an orange. Phenolic compounds in which the phenolic groups were not in the ortho position such as phenol, *p*-cresol, picric acid, hydroquinone, phloroglucinol, and resorcinol gave no reaction.

DETERMINATION OF NORDIHYDROGUAIARETIC ACID IN CREOSOTE BUSH

A 5.00-gram sample of ground air-dried creosote bush leaf is weighed and extracted in a Soxhlet apparatus with 95% ethyl alcohol for at least 4 hours or until the alcohol in the Soxhlet tube gives a negative test for nordihydroguaiaretic acid with molybdate reagent. The alcohol is evaporated to a volume of about 50 ml. and the extract is cooled and allowed to stand for 2 or 3 hours. The alcohol-insoluble material which settles is filtered into a 200-ml. volumetric flask and the original flask and filter paper are washed until a negative test is obtained.

The contents of the flask are diluted with alcohol to volume and a 5-ml. aliquot is diluted with alcohol to 50 ml. in a volumetric flask. Four 5-ml. aliquots are removed from the 50-ml. dilution and transferred to small flasks.

Ten milliliters of distilled water are added to two of the flasks to serve as blanks and 10 ml. of 1% ammonium molybdate solution are added to the remaining two flasks to develop the orange color. The solutions are swirled and transferred to cuvettes for reading on the spectrophotometer at 500 mμ. The results in micrograms per milliliter can be read from the standard curve and can be calculated as percentage of nordihydroguaiaretic acid.

Water-insoluble tannic acid is estimated by the method given above and one half the obtained value is deducted from the total. A correction of 0.4% is applied to compensate for the interference of water-soluble *o*-dihydroxybenzene compounds.

Table III. Precision of Molybdate Method

	Sample Weight, Grams						
	5		5	8			
	Analyst						
	A	A	A	B			
	X^a	X	Y^a	Z^a			
	N.D.G.A., %						
					Av.	Max. Deviation of % N.D.G.A.	Precision, % $\left(\frac{1/2\text{ Max. \% Dev.}}{\text{Av. \% N.D.G.A.}} \times 100\right)$
1^b	9.13	8.95	9.10	9.36	9.13	0.41	2.2
2	7.98	8.00	8.20	8.28	8.11	0.30	1.8
3	9.57	9.61	9.50	9.47	9.54	0.14	0.7

[a] Date determined. X, Y, Z refer to different days.
[b] 1, 2, 3 are samples from different regions of Rio Grande Valley.

Calculations.

$$\%\,\text{N.D.G.A.} = \frac{\gamma/\text{ml. (from curve)} \times 2000\text{ (dilution factor)} \times 100}{1{,}000{,}000\text{ (convert to grams)} \times 5\text{ (wt. of sample)}} - \tfrac{1}{2}\text{ water-insoluble tannin (\%)} - 0.4\%\text{ (interference of water-soluble compounds)}$$

Or, condensed:

$$\%\,\text{N.D.G.A.} = \frac{\gamma/\text{ml.}}{25} - \tfrac{1}{2}\text{ water-insoluble tannin (\%)} - 0.4\%$$

The precision of the method is about ±2%, as is shown in Table III.

The determination of precision is based on checks run by the same analyst on the same and on different days and on comparison with the results of another analyst who used a different original sample weight. The method has been used for routine analysis of more than 150 samples with comparable reproducibility.

SUMMARY

The method as outlined is the only accurate method known for the determinaton of nordihydroguaiaretic acid in creosote bush leaf. It is rapid, easily mastered, and requires no special skills, and is, therefore, suited for routine analytical determinations. The method should be applicable to systems low in other *o*-dihydroxybenzene compounds and for the determination of added nordihydroguaiaretic acid in fats, oils, and various food products. The only disadvantage of the method is the limited interference by structurally similar compounds. Approximate corrections can be made for these compounds

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Automatic Steam-Distillation Apparatus

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A steam-distillation apparatus particularly applicable to Kjeldahl estimations is described. Its value lies in freeing the analyst during the distillation. It should save time and labor when large numbers of estimations are necessary.

DURING recent years many modifications have been made in the standard Kjeldahl steam-distillation apparatus (*1–4*). Equipment designed to carry out the distillation directly from the digestion flask has eliminated the necessity for a quantitative transfer. Alternatively where an aliquot has been transferred to the distilling apparatus, the automatic emptying device has proved most useful. To avoid an increase in the sample volume, arising from condensation during steam distillation, some investigators have surrounded the distillation vessel by a vacuum jacket or located it in the steam trap or even in the boiler itself. Experience gained with such modified equipment has been applied to the design of the apparatus described below, in which the more fragile components are enclosed within a robust exterior and a device is incorporated whereby when a desired

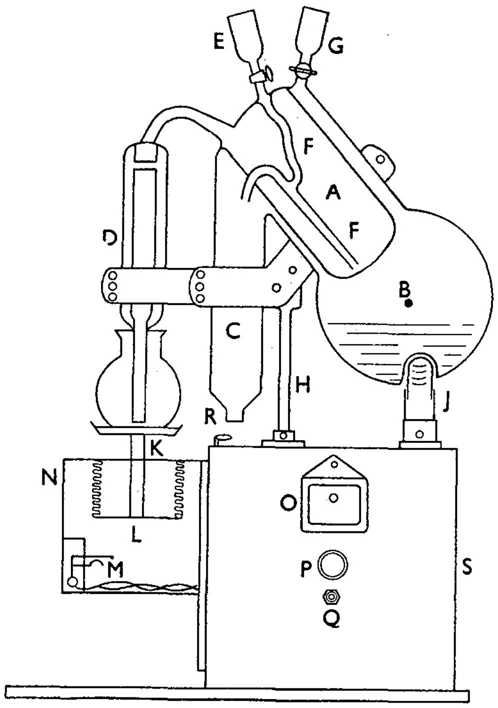

Figure 1. Automatic Steam-Distillation Apparatus

volume of distillate has been received, the heating circuit is automatically broken and an audible warning is given.

DESCRIPTION OF APPARATUS

The apparatus is shown diagrammatically in Figure 1. The glass section is made up of four main portions, the distillation flask, *A*, the steam boiler, *B*, the steam trap, *C*, and the condenser, *D*.

A is situated inside the neck of *B* and the sample is introduced by means of the tap funnel, *E*, and passes through the tube, *F*. After test the distillation vessel empties itself through an open side arm of *F* into *C*. *B* can be charged with water through a tap funnel, *G*. Steam enters *A* through *F*, distilling the sample into *D*. An aluminum clamp and stand, *H*, support the whole glass section of the apparatus.

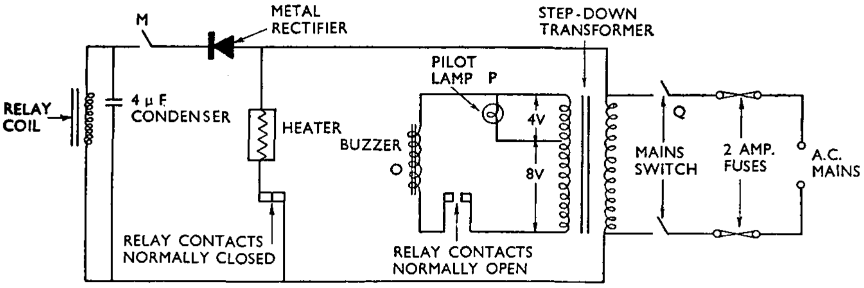

Figure 2. Electrical Circuit

The nonglass section of the apparatus consists essentially of a wooden box, *S*, fitted at one end with vertical metal runners down which a second smaller box, *N*, can slide when a release, *R*, is operated. A platform, *L*, supported by four springs, carries an aluminum pan, *K*. *L* can be depressed to close the contact switch, *M*, which operates a relay breaking the current to the electrical heater, *J*, and operating the buzzer, *O*.

The aluminum clamp embracing *B* has been omitted from Figure 1 to allow the details of the distillation vessel to be more clearly noted. The details of the electrical circuit are shown in Figure 2.

Receiver. The capacity of the receiver should be suited to the volume of distillate required. The present apparatus, designed mainly for routine Kjeldahl distillations, has been constructed for distillate volumes of about 50 ml. and the standard carbon dioxide flask of 150-ml. capacity with a wide neck has been found suitable. Only flasks of approximately equal weight have been selected as receivers and the lighter the flask, consistent with capacity, the greater the volume of distillate which can be received.

PROCEDURE

B is half-filled with distilled water which is brought to the boil. With *G* and *C* open, the sample is introduced into *A* through *E*. In the case of Kjeldahl distillations excess sodium hydroxide

Table I. Specifications for Apparatus

Reference Letter	Designation	Description and Dimensions
A	Distilling vessel	Pyrex, 5.75 cm. internal diameter × 17 cm.
B	Steam boiler	Pyrex, 7.6 cm. internal diameter × 17 cm. sealed to 1-liter round-bottomed flask. Heater well 3.2 × 3.2 cm. is made in boiler
C	Steam trap	Pyrex, 3.2 × 16.5 cm. drawn out at bottom to 0.95 × 3.8 cm. tube fitted with rubber tube and screw clip
D	Condenser	Pyrex, double surface 3.2 cm. internal diameter × 16.5 cm., drawn out at bottom to 0.95 × 10 cm. tube ground obliquely
E	Sample funnel	Pyrex tap funnel, capacity about 30 ml.
F	Sample and steam tube	Pyrex, 0.6 × 11.4 cm. Forked sections, one attached to *E* and one entering distillation vessel from *C*
G	Boiler funnel	Pyrex tap funnel, capacity about 30 ml.
H	Clamp and stand	Aluminum clamp 0.16 cm. thickness × 3.8 cm. width embracing condenser steam trap and neck of steam boiler. The stand, made from 1.25 × 20 cm. brass rod, is screwed to clamp between steam trap and boiler, and fits into an adjustable collar on top of *S*
J	Heating unit	2.5-cm. diameter Sindanyo (hard asbestos cement) bored rod grooved at top into a spiral, carrying heating element which consists of 8.2 meters of 30 standard wire gage Nichrome wire having a resistance of 150 ohms suitable for 240 volts a.c. Rod of heating unit enters *S* through metal collar and its height is adjustable
K	Sprung pan	Aluminum, 0.16 cm. thickness × 5.75 cm. diameter bored to reduce weight and mounted on aluminum rod 0.64 cm. diameter × 10.1 cm.
L	Sprung platform	Tufnol, 0.16 × 5.1 × 7 cm. suspended at each corner by springs made from 28-gage spring piano wire. Springs are made to extend 5 cm. when a weight of 110 grams is placed on *K*
M	Contact switch	Closed by pressure of *L*. Switch can be raised or lowered abou 2.5 cm. in Tufnol slide and locked into place by grub screw. A small metal rod is located under actual contact points to prevent *L* going beyond switch after making contact
N	Platform box	Tufnol, 8.3 cm. deep × 13.3 × 13.3 cm. Box slides up and down runners fixed to end of *S*
O	Buzzer	Normal bell type operated by stepdown transformer from the mains
P	Lamp	Panel operating lamp
Q	Switch	Double-pole toggle switch
R	Platform box release	Brass. When operated *N* can be held fast or allowed to slide as required
S	Box	Plywood, 12.7 cm. deep, 27.3 cm. long, 26.7 cm. high. Base 1.25 × 22.9 × 45.7 cm. One end wall is made of Sindanyo and carries runners for *N*. A slot in the Sindanyo wall allows wiring from *M* to travel up and down with *N*

solution is introduced into the distillation vessel, followed by aqueous washings of the funnel.

With N at the bottom of its runners, the receiving flask containing 10 ml. of 4% boric acid and 3 drops of indicator is placed on K. N is then slid up its runners until R engages and holds it in position. The bottom of the condenser tube should now be just below the surface of the liquid in the receiver. If necessary both H and the rod of J can be adjusted in their base collars until the correct height is attained. The taps on E and G and the screw clip on C are closed.

Dry steam enters the sample liquid through the branch of F and distillation ensues. Because A is surrounded by a steam jacket, very little increase in volume occurs. As the volume of distillate increases, K is depressed until M closes, operating O and switching off J.

In the construction, the ratio of spring extension of L to the height which the distillate will rise in the receiver (allowing adequate volume for complete distillation) can be so arranged that at least the last 5 ml. of the distillate enter the receiver after the bottom of the condenser tube has come above the surface of the distillate. Larger volumes of distillate can be received by lowering M in its adjustable slot. R allows N to slide down its runners and the receiver can then be removed from the pan and its contents titrated.

Steam distillations, not requiring the interception of gases as in the Kjeldahl distillation, may be carried out with N at the bottom of its runners. Any desired volume of distillate, within limits, can be collected.

The salient features in the design and use of the apparatus may be summarized as follows:

The glass section of the apparatus can be made in one piece if desired. The ground joint between the distillation head and the condenser is provided merely for convenience.

Increase in volume due to condensation of steam within the distillation vessel is reduced to a minimum.

The steam boiler is heated rapidly and effectively by electricity with very little heat loss. When, however, the circuit is automatically broken on receipt of the desired volume of distillate, the cooling of the element is rapid and the distillation vessel empties itself in about 20 seconds.

The single rod stand and clamp are neat and permit rapid adadjustment. The apparatus can be adjusted to receive, within limits, any desired volume of distillate. The operator is free to carry out other duties, knowing that the receiver cannot overflow if neglected and that audible warning will be given at the end of the experiment. The apparatus is best suited to employment in banks of four or six units when large numbers of distillations are required. The device for controlling the volume of distillate and time of distillation may well suggest to the reader similar laborsaving devices in his own laboratory.

ACKNOWLEDGMENT

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Calcium in High-Purity Sodium Salts

Determination of Microgram Amounts by the Oxine-Oxalate Method

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As little as 0.1 mg. of calcium can be determined in high-purity sodium chloride and sodium nitrate by separating the calcium with 8-hydroxyquinoline (8-quinolinol), followed by an oxalate precipitation and titration with 0.01 *N* potassium permanganate.

ONLY two chemical methods for calcium in sodium salts could be found by the authors in the literature (*1, 4*). Both methods depend upon the precipitation of calcium oxalate from concentrated salt solutions. The Association of Official Agricultural Chemists (*1*) describes a method for calcium in sodium chloride of "average" purity. Shuman and Berry (*4*) modified this procedure and "calibrated" it by using empirical corrections for varying amounts of calcium in high-purity sodium chloride (above 99.90%). For example, correction factors of +0.006 to −0.003% were added in the case of samples containing 0.02 to 0.2% calcium. As a 50-gram salt sample is usually taken for analysis, this means corrections of +3 mg. to −1.5 mg. of calcium for 10 to 100 mg. of calcium analyzed. However, Shuman and Berry reported good accuracy for calcium above 0.02% using these correction factors.

Tanne (*5*) found that low results were obtained for calcium and magnesium in proportion to the amount of salt taken for analysis and ascribed this fact to "something which complexed with calcium and magnesium, but did not react with the oxalate and phosphate ions." In another paper (*6*) he attributed the low recoveries of magnesium to water-insoluble magnesium hydroxide which was formed on hydrolysis. The low results are more understandable in view of the work of Maljaroff and Gluschakoff (*2*), who determined the solubilities of calcium oxalate in the concentrated salt solutions of ammonium, sodium, and magnesium. They found that 36 mg. of calcium oxalate would dissolve in 1 liter of 10% sodium chloride at 18° to 20° C.

The method described below eliminates the undesirable effect of concentrated sodium salt solutions on the solubility of calcium oxalate. As little as 0.1 mg. of calcium can be separated from the sodium salt by precipitation as 8-hydroxyquinolate (calcium oxinate) at pH 9.5 to 10 along with 8-hydroxyquinoline (8-quinolinol, oxine). After the oxine–calcium oxinate is ashed, calcium is precipitated as oxalate in the absence of high salt concentrations, and measured by titrating with 0.01 *N* potassium permanganate. The method assumes the absence of large amounts of cations which would be separated as oxinates and included in the calcium oxalate precipitation.

PROCEDURE

Solutions Required. 8-Hydroxyquinoline solution, 2.5% in 5% acetic acid.

Ammonium oxalate solution, 0.5 *N*.

Potassium permanganate solution, 0.01 *N*.

Method. Dissolve enough sodium salt in water (up to 50 grams) to give 0.1 to 5 mg. of calcium and make the solution slightly acid with hydrochloric acid. Adjust the volume to at

Table I. Recovery of Calcium from 100 Ml. of 20% Solutions of Sodium Nitrate and Sodium Chloride by the Oxine-Oxalate Method

Calcium Added		Calcium Recovered[a]				No. of Detns.	Av. Deviation from Mean	
		From NaNO₃		From NaCl				
Mg.	%	Mg.	%	Mg.	%		Mg.	%
0.09	0.0005	0.07	0.0004	0.08	0.0004	7	±0.02	±0.0001
0.18	0.0009	0.17	0.0009	0.20	0.0010	5	±0.04	±0.0002
0.28	0.0014	0.28	0.0014	0.26	0.0013	9	±0.03	±0.0002
0.37	0.0019	0.33	0.0017	0.38	0.0019	4	±0.03	±0.0002
0.46	0.0023	0.47	0.0024	0.45	0.0023	5	±0.02	±0.0001
0.92	0.0046	0.94	0.0047	0.94	...	2	±0.02	±0.0001
4.60	0.0230	4.65	0.0233	4.68	...	3	±0.07	±0.0004

[a] After subtracting blank values. Blank was 0.02 mg. Ca for $NaNO_3$, and 0.03 mg. Ca for NaCl.

Table II. Analysis of Calcium in Different Grades of Commercial Sodium Salts

Description of Sample	Ca + Mg, Mfrs.' Analysis[a]	Sample	Calcium Found
	%	*Grams*	%
Merck's NaCl No. 41748	0.01	50	0.0020
Baker's c.p. NaCl No. 6648	0.003	50	<0.0005
Baker's reagent grade NaNO₃ No. 12644	0.0003	50	<0.0005
Baker's c.p. NaNO₃ No. 22445	0.005	50	<0.0005

[a] Analyses by J. T. Baker and Merck chemical companies are: Calcium and magnesium are precipitated together in an ammoniacal solution with oxalate and phosphate. The precipitate is filtered, ignited, weighed, and reported as a single value.

least 100 ml. to give an approximately 20% sodium salt solution. Add 15 ml. of 2.5% oxine solution, and precipitate the oxine-calcium oxinate by slowly adding ammonium hydroxide and adjusting to pH 9.5 to 10 using a pH meter. Avoid an excess of ammonium hydroxide, as this may redissolve some of the voluminous precipitate. Allow the precipitate to stand for at least 3 hours, filter through ashless retentive filter paper, taking care that a minimum of cold water is used in transferring the precipitate, and wash once with a 5-ml. portion of cold water. Allow the bulky precipitate to drain completely, transfer the filter paper and precipitate to a platinum crucible, and dry thoroughly on a moderate hot plate or in a drying oven.

If the oxine precipitation was made in the presence of nitrate, great care should be exercised in ashing, for the gases formed during initial charring may expel the wad of filter paper from the crucible. The filter paper should be charred from the top to the bottom of the crucible, and when the burning has started, the crucible should be removed from the flame and the filter paper allowed to burn by itself. Ashing should be completed over a burner until a clear melt is obtained. Cool the melt, make it slightly acid with hydrochloric acid, and transfer to a 50-ml. beaker. Any acid-insoluble material should be removed by filtration.

The calcium is precipitated as oxalate from an acetic acid medium (*3*). Make the hydrochloric acid solution slightly ammoniacal and then slightly acid to methyl red with glacial acetic acid. Heat the solution to boiling, add 1 to 3 ml. of 0.5 *N* ammonium oxalate depending upon the amount of calcium present, and allow the precipitate to settle (at least 30 to 40 minutes). Filter the calcium oxalate through a sintered-glass microfilter tube using suction, and wash with small quantities of hot water. After attaching a clean suction flask to the filter tube, dissolve the precipitate by pouring hot 10% sulfuric acid through the filter. Transfer the solution to the beaker in which the original precipitation was made, heat to about 65° C., and titrate with 0.01 *N* potassium permanganate. A correction for the oxine reagent is unnecessary, as it contains less than 0.004% calcium.

RESULTS

Table I shows the recoveries of 0.1 to 5 mg. of calcium from 100 ml. of 20% solutions of sodium nitrate and of sodium chloride.

The values given in Table I were obtained by two independent analysts. Of 35 determinations made, only 6 exceeded an average deviation of ±0.04 mg. of calcium. The results indicate that whereas small amounts of calcium are lost in the range 0.1 to 0.4 mg., the average deviation is not prohibitive in estimating 0.1 mg. of calcium. This is equivalent to 0.0005% calcium, which value is 40 times more sensitive than hitherto reported results.

Two grades of commercial sodium nitrate and of sodium chloride were analyzed for calcium by the oxine-oxalate procedure. The results are reported in Table II.

To check the calcium analysis of the high-purity sodium salts, 0.28 mg. of calcium was added to 50-gram salt samples. Calcium was determined by the oxine-oxalate procedure, and the results are reported in Table III.

DISCUSSION OF PROCEDURE

Before arriving at the oxine-oxalate method for calcium in high-purity sodium salts, an attempt was made to precipitate calcium oxalate without a preliminary separation from the sodium salts. It was found that a minimum of 3 mg. of calcium in 20 grams of salt (0.015%) could be determined. This value was in accord with the smallest amount measurable by Shuman and Berry and is confirmed in Table IV, which shows the recoveries of various amounts of added calcium from 50 ml. of a 40% sodium nitrate solution (20 grams of total sodium nitrate) by a direct precipitation of calcium oxalate from an acetic acid medium (pH ca. 4.2).

Table III. Recoveries of 0.28 Mg. of Calcium Added to 50-Gram Samples of Sodium Salts

Salt	Calcium Found		Recovery of 0.28 Mg. Calcium Added
	In salt alone	In salt + 0.28 mg. calcium added	
	Mg.	*Mg.*	*Mg.*
Merck's c.p. NaCl No. 41748	1.03	1.29	0.26
Baker's c.p. NaCl No. 6648	0.03[a]	0.27	0.24
Baker's reagent grade NaNO₃ No. 12644	0.03[a]	0.28	0.25

[a] Figures represent blank values for respective salts.

Table IV. Recovery of Added Calcium from 50 Ml. of 40% Sodium Nitrate Solutions by Direct Precipitation as Calcium Oxalate

Calcium Added	NaNO₃ Present	Calcium Recovered
Mg.	*Grams*	*Mg.*
0.09	20	0.04
0.46	20	0.03
0.92	20	0.63
4.60	20	4.58
0.46	None	0.48
0.00	20	0.03

The method of Marsden (*3*) has a 2% accuracy for 0.1 to 5 mg. when calcium is precipitated as oxalate in 20 ml. of acetic acid solution. Apparently there are two factors which cause low results: the concentration of sodium salts on the solubility of calcium oxalate as reported by Maljaroff and Gluschakoff, and the effect of the increased volume of solution from which calcium oxalate is precipitated.

The mechanism of the oxine separation is not clear. Calcium is probably precipitated as the oxinate and is occluded, coprecipitated, or absorbed by the flocculent oxine precipitate. Undoubtedly the solubility of calcium oxinate would be prohibitive in determining microgram quantities of calcium were it not for the beneficial effect of the large excess of precipitated oxine.

The low results reported for less than 0.4 mg. of calcium may be ascribed in part to operative errors in transferring the precipitates, ashing, etc., but it is more probable that this is caused by the solubility of the calcium oxinate–oxine precipitate in the water wash solution. Tracer experiments with radiocalcium (Ca^{45}) showed that 3 mg. of calcium (microgram amounts of this carrier could not be used because of low specific activity) were precipitated quantitatively as calcium oxinate–oxine from a 20% sodium nitrate solution, but that about 5% calcium was lost in two washings. Therefore, a minimum amount of washing is advisable.

Because magnesium can be precipitated under the same conditions as calcium, it is entirely possible that magnesium can be estimated in the filtrate from the calcium oxalate precipitation.

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Determination of Unsaturation by Microhydrogenation

Method and Apparatus

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An apparatus and method for quantitative microhydrogenation are described. The simple design of the apparatus is made possible by use of magnetic stirring to agitate the reaction mixture. Data are presented to indicate the precision and accuracy of the method.

QUANTITATIVE hydrogenation as a means of measuring unsaturation has several advantages over the halogenation methods commonly used. This method is not subject to the errors resulting from the presence of two or more conjugated double bonds or from complex molecular structure. Except for choosing the correct catalyst for determining aromatic or aliphatic unsaturation, conditions for hydrogenation do not have to be empirically adjusted to the material being analyzed, as do those for halogenation. In general, hydrogenation is more reliable, for it eliminates the necessity of previous knowledge of the molecular structure.

Another important advantage is that a high catalyst-to-sample ratio can be used. This aids in obtaining complete hydrogenation and in overcoming the effects of poisoning the catalyst by small amounts of sample impurities. Halogenation micromethods are seldom used because of the many inherent errors.

Several authors have described hydrogenation microapparatus that have given reliable results. Among them are Johns and Seiferle (*1*), whose relatively simple apparatus was mounted on a plywood board and shaken as a single unit to agitate the reaction mixture. The apparatus of Prater and Haagen-Smit (*3*) was also mounted rigidly and rocked by an eccentric driven by a motor. It had two completely symmetrical systems, which were connected; one served as the reaction system and the other as the compensator. When no temperature compensation was necessary, two determinations could be run simultaneously.

The apparatus described here is more simply constructed and much more easily assembled than the two just mentioned, for a stationary reaction vessel is used and agitation is produced by magnetic stirring. This type of agitation for hydrogenation was first described by Weygand and Werner (*4*) and more recently by Zaugg and Lauer (*5*), whose semimicroapparatus was designed for both Grignard and hydrogenation determinations. Although Weygand and Werner's apparatus is the simplest yet described, the manometer-buret assembly with rack and pinion device for leveling bulb adjustment described here makes possible more accurate volume measurements. This part of the apparatus is adapted from the Soltys' active hydrogen apparatus sold by Arthur H. Thomas Company. The ball joint connection gives the apparatus greater flexibility, and the detachable reaction vessel with standard-taper joint is easily accessible for cleaning.

APPARATUS

Figure 1 shows the apparatus and hydrogen purification train.

The purification train consists of a standard micro electric combustion furnace, *A*, heated to approximately 750° C. so that the platinum star contact catalysts, *C*, in the 8-mm. inside diameter quartz combustion tube, *B*, will effectively remove the oxygen from the hydrogen gas. The water so formed is absorbed by indicating Drierite in the inner tube of the Prater (*2*) semimicro absorption tube, *D*. If the hydrogen gas is wet, it is advisa-

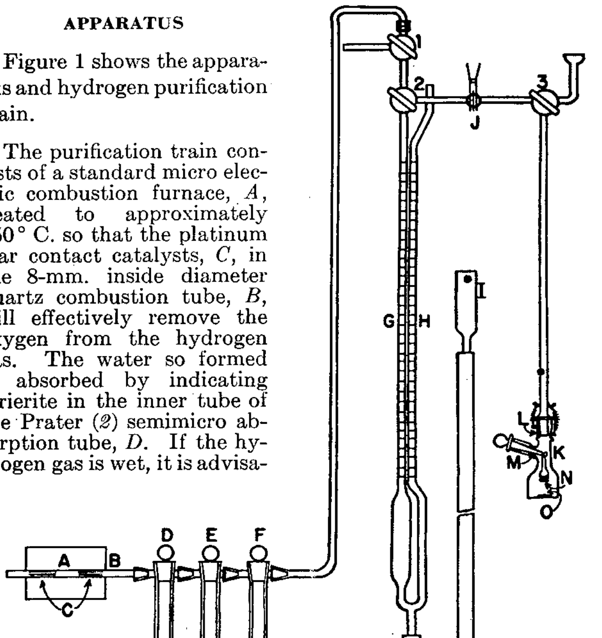

Figure 1. Hydrogenation Microapparatus

ble to place a drying tube before the combustion tube to remove the moisture and thus prolong the life of the absorbent in *D*.

Tubes *E* and *F* are used to saturate hydrogen with the solvent to be used in the reaction vessel. One inch (2.5 cm.) of the solvent is placed in *E*, and the inner tube of *F* is filled with glass wool to prevent mechanical carry-over of the solvent.

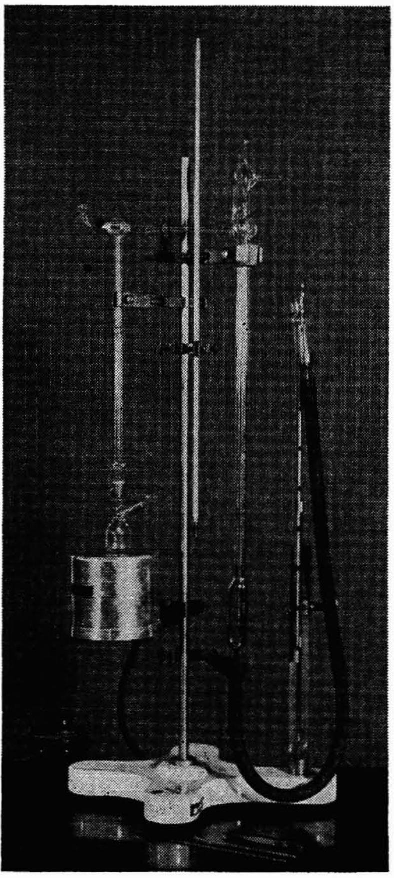

Figure 2. Hydrogenation Assembly and Accessories

All the 7/15 standard-taper ground joints in the hydrogen train are sealed with de Khotinsky cement. The purification train is attached to stopcock 1 of the apparatus by a glass tubing with a Tygon tubing connector. Buret *G* and manometer *H* have parallel and coinciding graduations, with 0.02-ml. intervals from 0 to 7 ml. Mercury is leveled in *G* and *H* by raising or lowering leveling bulb *I* with a rack and pinion device shown in Figure 2. The reaction unit is connected to the buret by the 12/2 ball joint, *J*, held in place by a suitable clamp. The 20-ml. reaction flask, *K*, is connected by a 14/20 ground-glass joint, *L*, and held in place by two steel springs. Side arm and stopper *M* are made from 10/12 ground joints. The stopper, which extends into the neck of the flask, has a groove near the tip and perpendicular to its long axis. Sample cup *N* is hung on the stopper and dropped by turning *M*. *N* is made by drilling an aluminum rod about 6 mm. in diameter and 8 mm. long so that its volume is about 0.1 ml. The Nichrome wire handle is attached through two holes drilled on opposite sides of the cup. The stirring bar, *O*, is made by sealing fine iron filings in a glass tube about 3 mm. in outside diameter and about 15 mm. long. The cup attached to stopcock 3 facilitates cleaning the tube leading to the reaction vessel. Stopper *M* and joint *L* are greased carefully before each analysis, and the stopcocks and ball joint are lubricated frequently to prevent loss of hydrogen.

Figure 2 shows the apparatus, including the magnetic stirrer assembly, support stand with rack and pinion, and other necessary equipment. The thermometer (range, 0° to 100° C.: graduated in 0.1°) is hung between the buret and reaction vessel. All temperature readings are made to the nearest 0.1°.

CATALYST

One gram of palladium chloride is dissolved in 25 ml. of distilled water and added to a 50-ml. water suspension of 6 grams of activated carbon. This mixture is transferred to a pressure bottle and hydrogenated under 35 pounds' pressure (2.46 kg. per sq. cm.) in a Burgess-Parr apparatus for 0.5 hour. The suspension is transferred to a Büchner funnel, the solvent is drawn off, and the catalyst is washed with water until the washings are free of hydrochloric acid. Volatile and flammable wash liquids should be avoided because the palladium catalyst may ignite the liquid. The catalyst is dried at room temperature in a vacuum oven for 16 to 20 hours, then stored in a desiccator over phosphorus pentoxide.

METHOD

Operation of the hydrogen purification train is started by causing hydrogen to flow from a pressure tank through the train and through stopcock 1, where it is vented to the atmosphere. The hydrogen pressure is adjusted to approximately 1 pound by the tank pressure-reducing valve, and the flow is adjusted to 25 to 35 ml. per minute. Furnace *A* is turned on, and after it has reached operating temperature, the hydrogen flow is stopped by turning stopcock 1. The gas in the train is under the same positive pressure as that in the reducing valve.

Four milliliters of redistilled acetic acid, about 20 mg. of catalyst, and the stirring rod are placed in the reaction flask. The aluminum cup containing a weighed sample equivalent to 3 to 5 ml. of hydrogen is suspended from the stopper in the reaction vessel by grasping the wire handle with tweezers and lowering the cup into the flask so that the handle rests in the groove of stopper *M*. Reaction flask *K* is attached to the apparatus and held in place with the springs. The burette is filled with mercury to stopcock 2, which is then turned to close the burette.

Stopper *M* is loosened, and the reaction unit is swept with hydrogen by turning stopcocks 1, 2, and 3 to allow the hydrogen to flow through the unit at a rate of 25 to 35 ml. per minute. After 15 minutes, *M* is seated firmly, stopcock 2 is turned to connect the buret to the purification train, and the buret is filled with hydrogen. Stopcock 1 is given a 1/8 turn to close all openings, and stopcock 2 is turned so that the buret is connected with the reaction unit. The hydrogen in the buret is placed under positive pressure by raising the leveling bulb, and the excess pressure is released by momentarily opening stopcock 3. The acetic acid and catalyst are stirred for 5 minutes after diminution of the gas volume ceases. The magnetic stirrer is moved from under the reaction flask, and the apparatus is allowed to equilibrate for 15 minutes before the volume, temperature, and pressure are read and recorded.

The cup containing the sample is dropped into the flask by turning stopper *M*, and the reaction mixture is stirred vigorously for 15 minutes after hydrogen uptake has ceased (about 30 minutes), as indicated by the manometer. Throughout the analysis, the mercury levels in the buret and manometer should not differ by more than 1 cm. The magnetic stirrer is removed, and after the apparatus is equilibrated for 15 minutes, the final volume, temperature, and pressure are recorded.

CALCULATION

V_i = buret reading before reaction of sample
T_i = temperature before reaction of sample
P_i = pressure before reaction of sample
V_f = buret reading after reaction of sample
T_f = temperature after reaction of sample
P_f = pressure after reaction of sample
V_t = total gas volume of the reaction unit plus the volume of the buret above the zero mark
P_{si} = vapor pressure of solvent at temperatures T_i
P_{sf} = vapor pressure of solvent at temperatures T_f
$V_t + V_i \times 273/T_i \times (P_i - P_{si})/760 = V_i^\circ$ = volume of H_2 at S.T.P. before reaction
$V_t + V_f \times 273/T_f \times (P_f - P_{sf})/760 = V_f^\circ$ = volume of H_2 at S.T.P. after reaction
Wt. of sample$/(V_i^\circ - V_f^\circ) \times 22{,}400$ = hydrogen number = weight of sample that will react with 1 mole of hydrogen

RESULTS

This method with palladium catalyst has been used successfully for determining the unsaturation of a number of fatty acids and their esters. Table I shows the hydrogen numbers obtained for some of these materials. The differences between the calculated and found values are less than 1 for all samples except methyl oleate. For this sample, hydrogenation gave an average value (298.7) midway between the calculated value (296.5) and that obtained by halogenation (300.7). The precision of the method is

Table I. Hydrogen Numbers for Some Unsaturated Acids and Esters

Sample	Hydrogen Number Found	Average	Calculated
Maleic acid	115.0 116.5 116.9 115.7 115.8 115.9 116.7 115.5 115.1 115.4	115.9[a]	116.1
Methyl oleate	299.9 297.4	298.7	296.5
Methyl linoleate	147.2 147.6 146.0	146.9	147.2
9,11-Linoleic acid	140.4 141.2	140.8	140.2
10,12-Linoleic acid	140.4 139.5	140.0	140.2
Methyl linoleate (alkali isomerized)	146.4 147.0 146.6	146.7	147.2
10-Hendecenamide	184.2 183.6	183.9	183.3

[a] Standard deviation = 0.6.

shown by the standard deviation of 0.6 for the ten values for maleic acid. The accuracy of the method is indicated by the close agreement between the calculated and found values in Table I. The confidence limits for the mean (115.9 ± 0.5) calculated from the maleic acid analyses brackets the theoretical value and shows that there is no significant inherent error in the method.

In general, the values are 0.2 to 0.5% below those calculated, which means that slightly more than the theoretical amount of hydrogen is consumed during the reaction. This indicates that there is a slight loss of hydrogen by diffusion during the analysis. Further evidence is that the hydrogen volume slowly but continuously diminishes if the apparatus is allowed to stand for several hours. After more data are obtained, it may be possible to correct for this apparent loss by subtracting a blank from the volume of hydrogen used, but probably this will not be greater than 0.01 ml.

The apparatus has been used only in a room in which the temperature is kept under close control. If such a room is not available, it would be advisable to house the apparatus in a cabinet, in which a relatively uniform temperature can be maintained.

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Simplified Partition Chromatographic Procedures

Resolution of Sulfonamides, Sulfones, and Their Metabolic Products from Biological Materials

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Apparatus suitable for partition chromatography, on narrow filter-paper strips or strings of various materials, using both ascending and descending solvent flows, is described. The feasibility of using these smaller columns and the resulting advantages are set forth. The use of these apparatus for the resolution of a sulfonamide mixture from the plasma of a chick fed a mixture of sulfonamides in a proprietary preparation is reported. The use of benzene-water and benzene-cyclohexane-water to resolve *p,p'*-sulfonyldianiline, related compounds, and metabolic products from solutions and biological fluids is reported.

PARTITION chromatography on filter paper is a relatively new technique. Although it was originally used by Consden, Gordon, and Martin (*4*) for the separation of amino acids, it has subsequently been employed for the separation of minute amounts of a wide variety of materials such as peptides, organic acids, sugars, antibiotics, anthocyanins, creatine, vitamin B_6, flavine nucleotides, sulfonamides, and other substances. The literature on many applications is reviewed by Consden (*3*).

In this paper details are given for the construction of two types of apparatus which have given satisfactory separations of mixtures of sulfonamides and sulfones from aqueous solutions and biological fluids. Use of either type of apparatus effectively permits the conservation of solvents, reagents, filter paper, and to a degree, test substances. Provision is made in one of the pieces of equipment for chromatographing labile compounds. The width of the filter paper is narrower than that previously reported (*5*, *6*, *11*) or even recommended by some (*3*, *9*). However, strips of this width are more easily handled, particularly when wet with solvents, yet are adaptable to the same types of separations described for paper of the more conventional size. The narrow strips have an additional advantage over wide strips because they may be used with simply constructed, all-glass apparatus. The compounds studied are localized in bands which may occupy a smaller area than spots.

It has been found possible to do partition chromatography in one dimension not only on narrow strips of filter paper, but also on strips or strings of materials other than filter paper: plain and mercerized cotton, glass wool, and thin asbestos paper. These materials may be considered to form internally supported chromatographic columns of small cross section. They are as much columns as glass tubes filled with silica gel or starch, and in many cases will accomplish the same separations but on a micro scale.

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APPARATUS

Type I. Devices for Ascending Solvent Columns. FILTER PAPER. The apparatus for filter paper in which the solvents travel upward by capillary forces is shown in Figure 1.

Two or more rimless test tubes, with the lips adjacent to the pouring spout, are attached with cellulose tape to a 1-liter graduated cylinder. Wicks of filter paper carry the individual solvents in the solvent combination from the external reservoirs, so formed, down the inner walls of the cylinder from which the solvents evaporate to maintain a saturated atmosphere. A straightened, large-size paper clip is skewered through a No. 12 cork and an ointment jar lid or inverted No. 14 rubber stopper. One half inch (1.24 cm.) of the clip is then bent against the cork and the part above the stopper is fashioned into a hook. Midway between upper and lower edges of the cork 10 to 12 pins are inserted and their heads snipped off. The pins hold the strips of filter paper (Whatman No. 1, 3 to 6 mm. × 34 cm., machine cut, with 2-mm. holes punched 6 mm. from either end). Lang-Levy pipets (*1*) calibrated to deliver 0.003 to 0.01 ml. are used to apply measured volumes of test solutions to the filter paper about 2 to 4 cm. above the level to which the paper will hang in the solvents. The cylinder is filled to the 100-ml. mark with the solvent combination, and the papers, with weights attached for tautness, are suspended in the cylinder until the solvents front (observed as boundary of visible dampening) has ascended to the pins or slows to a halt. Either event may occur in 3 to 48 hours, the time being somewhat dependent on the particular solvent combination.

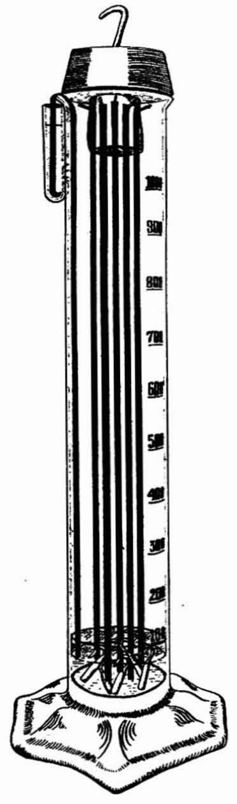

Figure 1. Apparatus for Ascending Filter-Paper Chromatograms

STRINGS. To employ partition chromatography on string, the two essentials are to keep the strings taut and separated from each other. Thus, using a 1-liter, graduated cylinder with attached tubes as for filter-paper columns, one merely inserts a rack on which a continuous length of string (making 20 to 30 "columns") has been strung, and covers with an inverted Petri dish. The rack, made of glass in one piece, is a 20- to 25-cm. rod (6 to 8 mm. in diameter) with each end fused to the center of an edge-notched, concave disk. The strings may be ordinary mercerized cotton, white wrapping cord rinsed in boiling water, or glass twine. The test solutions are applied at a point on the columns 2.5 cm. above the level to which they will stand in the solvents combination. Because the strings of mercerized cotton present a smaller cross section than the strips of filter paper, smaller amounts of solutes in the test solutions must be used. Otherwise the bands of substances whose R_F values are in a narrow range will overlap.

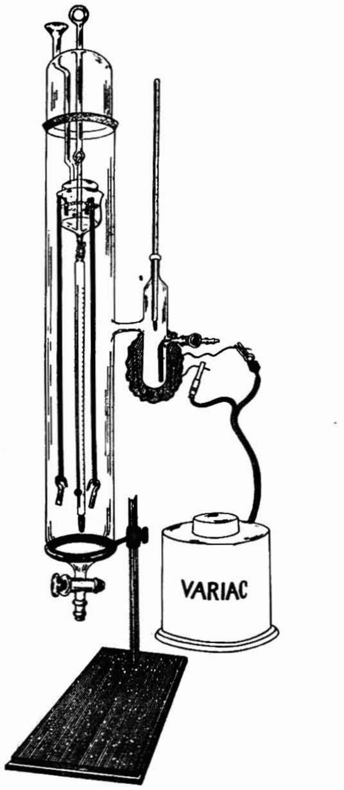

Figure 2. Apparatus for Descending-Solvent Chromatographic Columns

$$R_F \text{ value } (4) = \frac{\text{movement of band}}{\text{movement of advancing front of liquid}}$$

Type II. Device for Descending-Solvent Columns. The apparatus for filter paper or strings in which the final direction of solvent movement is downward is illustrated in Figure 2.

This apparatus is essentially a chamber constructed of Pyrex (7.5 × 50 cm.) with a bell top (12.5 cm. high) resting on a ground-glass seal. Above the bell top are a hook and a funnel. Underneath the hook is suspended a solvents reservoir with an outward-projecting rim, and below this is a thermometer. The stem from the funnel leads into the center of the reservoir. In the solvent reservoir is fitted a glass ring with inward-pointing spikes (Figure 3), on which are hung paper strips or strings. Alternatively a 3-cm. length of glass tubing 30 mm. in outside diameter, to which are tied paper strips or strings, may be used. This method is illustrated in Figure 4. The strips or strings are weighted by the glass crooks. One or more solvent boilers are connected to the main chamber. The boilers are heated by coiled resistance wire in which the current is regulated by a variable transformer, and insulated with asbestos tape and cement. Gases may be bubbled into the solvent boilers through the capillary tubing to obtain a solvent-saturated inert gas atmosphere. The solvent boilers are more efficient than pools of solvents or solvent-saturated wicks in bringing the atmosphere in the chamber to a saturated equilibrium and are particularly advantageous with low-boiling solvents.

SPECIAL PROCEDURES

Two-dimensional chromatography is possible under certain circumstances on the narrow filter-paper strips. Substances which have a common R_F value in one solvent combination, and which are separated from other test substances by a considerable distance of blank strip, may be rechromatographed in a second set of solvents on the remainder of the strip left after the other substances have been excised. For example, in butyl alcohol saturated with 3% ammonia, sulfadiazine has an R_F value of 0.17 (*9*), sulfathiazole 0.38 (*9*), *p,p'*-sulfonyldianiline (DDS) 1.0, and β-(*p*-sulfanilylanilino)-ethanol 1.0. To separate the two sulfones it is necessary to shorten the strip to within 2.5 cm. less than the distance traveled by the sulfones and to rechromatograph in a second set of solvents. Benzene, saturated with water, is satisfactory for the resolution of these two sulfones.

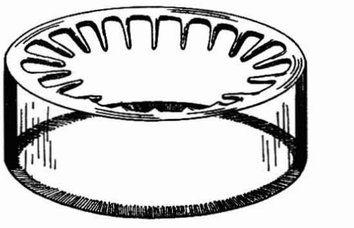

Figure 3. Glass Ring with Spikes for Securing Filter-Paper Strips

Illustrative of the types of compounds which may be separated by this procedure and apparatus are the sulfonamides. Although Shepherd (*9*) has detailed a method for their separation from aqueous solution and urine by paper chromatography, the experimental procedure given below is a variation of his technique which also permits the separation of sulfonamides from plasma.

EXPERIMENTAL

Resolution and Identification of Sulfonamides from Chick Plasma. APPARATUS. The apparatus for ascending filter-paper chromatograms (Figure 1) is preferred for this separation. With modifications the other two pieces of equipment can be used.

Lang-Levy pipets (*1*), 0.003-ml. and 0.01-ml. capacity.

1-Ml. pipets or medicine droppers.

Guillotine paper cutter.

Whatman No. 1 filter paper strips, 3 × 34 cm. Accurately cut strips in this size may be readily trimmed in a guillotine-type paper cutter. The blade of the cutter should be wiped free of oil, and the uncut filter paper protected from dust contaminations by wrapping paper or poster board. Many thicknesses of filter paper can be cut simultaneously and the resulting strips will remain bonded together until pulled apart. This is an advantage in that papers from the same cut can be selected for one experiment.

SOLUTIONS. Standards. Stock solutions of each of the sulfonamides likely to be encountered are prepared by suspending 0.1 gram of each sulfonamide in approximately 50 ml. of water, adding sufficient concentrated ammonia to effect solution, and diluting to volume with distilled water.

Chromatographing Solvents. The butyl alcohol–ammonia solution of Shepherd (*9*) is prepared by adding 40 ml. of butyl alcohol to 10 ml. of concentrated ammonia and making to 100 ml. with distilled water. The solution is shaken to form a temporary emulsion and poured into the cylinder. Best results with the chromatography of substances in viscid materials such as plasma have been obtained by adding 0.1 gram of nonaethyleneglycol monostearate (a nonionic surface-active agent, manufactured by the Glyco Products Company, Brooklyn 2, N. Y.) to the butyl alcohol ammonia, although this is unnecessary when the sulfonamides are in aqueous solutions or urine.

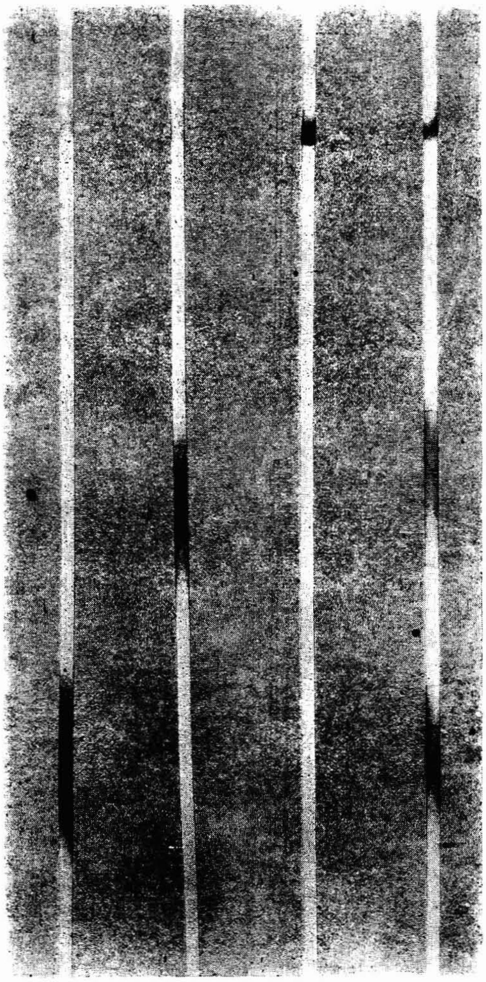

Figure 4. Positions Taken by Sulfadiazine (Lowest Band), Sulfathiazole (Middle Band), and DDS (Uppermost Band) in Butyl Alcohol–3% Ammonia

Positions remain constant, whether substances are alone or in mixture.

Kühnau's reagent (*8*) is made by dissolving 1 gram of *p*-dimethylaminobenzaldehyde in 100 ml. of 3% hydrochloric acid.

To make the nitrite solution, 0.1 gram of sodium nitrite is added to 100 ml. of glacial acetic acid containing 1 drop of concentrated hydrochloric acid. A fresh solution is prepared whenever a batch of filter papers is to be treated.

Coupling reagent (*2*) is prepared by dissolving 0.1 gram of *N*-(1-naphthyl)ethylenediamine dihydrochloride in 100 ml. of 95% butyl alcohol. It is stored in a brown bottle and renewed when discolored.

Preparation of Sample. A 40-gram chick is fed about one third of a 0.5-gram proprietary tablet (Triplex tablet, manufactured by the Eli Lilly Company, Indianapolis, Ind.) containing 0.167 gram of sulfadiazine, 0.167 gram of sulfamerazine, and 0.167 gram sulfathiazole. After 3 hours blood is drawn from the heart into a test tube containing potassium oxalate and centrifuged. The plasma, separated from the cells, is used.

Experimental Procedure. On individual filter-paper strips 0.003- and 0.01-ml. aliquots of the sulfonamide solutions, mixtures of the sulfonamide solutions, or plasma samples are applied. The filter papers, after a partial drying at room temperature, are placed in the cylinder containing 100 ml. of the butyl alcohol–3% ammonia (plus the surface-active agent when the plasmas are to be chromatographed), and left for 16 or more hours, or until the visible dampening is observed to be near the pins. The filter papers are removed from the cylinder and allowed to dry. If the ends of the papers are attached side by side at 1-cm. intervals to a length of cellulose tape they may be hung to the window of a hood for treatment by the color-developing agents and then mounted on graph paper.

When the paper strips are dry, the *p*-dimethylaminobenzaldehyde is applied to representative strips as desired; a 1-ml. pipet or medicine dropper is used to deliver a quantity sufficient to saturate the paper but not to cause "running" of the reagent. This reagent reacts with the sulfonamides and other primary aromatic amines to yield yellow to orange compounds which will indicate the positions of the bands. The *N*-(1-naphthyl)ethylenediamine which forms more intensely colored compounds when coupled with diazotized sulfonamides is then applied only to the sections of the other filter papers which encompass the bands. This is accomplished by adding, by pipet, the acetic acid solution containing sodium nitrite, drying for 2 minutes before an open hood, and applying the *N*-(1-naphthyl)ethylenediamine reagent. The colors, which quickly form, should then be protected from light except when viewing. An x-ray film viewer or a contrasting background will enable one to identify the fainter bands. If the bands are fused, it indicates that too much sulfonamide was applied to the strip and lower concentrations must be used.

Experimental Results. Each sulfonamide is found to migrate the same distance whether alone, in aqueous mixture, or in plasma. When fluids from undrugged animals are used, no bands are produced with either color-developing reagent. Sulfadiazine and sulfamerazine, because their R_F values are so close (0.17 and 0.30, respectively, *9*), tend to form overlapping bands unless very small quantities are used. However, if the aliquot contains about 0.25 microgram of these drugs, satisfactory resolution may be accomplished.

Resolution and Identification of Sulfones and Sulfonamides. Junge and Smith (*7*) have reported a synergistic action of sulfadiazine and *N*-propyl-*p,p'*-sulfonyldianiline against tuberculosis in guinea pigs. In anticipation of a need by clinicians to separate these two classes of compounds, mixtures of sulfones and sulfonamides have been chromatographed. It has been found, using butyl alcohol–3% ammonia, that *p,p'*-sulfonyldianiline (DDS), *N*-propyl-*p,p'*-disulfonyldianiline, and β-(*p*-sulfanilylanilino)-ethanol all migrate to a greater distance than sulfathiazole, which in turn migrates farther than sulfamerazine and sulfadiazine. The sulfones precipitate in sharp, well-defined bands in butyl alcohol–3% ammonia, but are coincident at the same level. Figure 4 is illustrative of the behavior of sulfadiazine, sulfathiazole, and DDS.

However, the sulfones may be resolved by a solvent combination composed of equal parts of benzene and cyclohexane saturated with water. The β-(*p*-sulfanilylanilino)ethanol remains stationary, the propyl compound migrates the total distance of the solvent excursion, and DDS migrates an intermediate distance. The mono-*N*-substituted sulfones and DDS give a pale rust color with nitrous acid, purple with Bratton and Marshall reagents, and yellow to orange with *p*-dimethylaminobenzaldehyde. It has been found that urine, plasma, and ethyl acetate extracts of plasma of rabbits fed DDS may be chromatographed using the identical procedure for pure solutions. These fluids yield one and sometimes two bands (*10*) in addition to the band characteristic of the parent compound in benzene-water. When DDS is added to urine and plasma from undrugged rabbits, only one band is discernible.

DISCUSSION

The two general types of apparatus and methods described in this paper can be adapted to accomplish most of the separations which have been claimed for partition chromatography. Both types are time-saving compared to apparatus handling only one or two paper strips, inasmuch as up to 20 runs may be made at one time in the same apparatus. The narrow strips of filter paper or strings possess the following advantages over the wide sheets of paper:

They may be mounted on graph paper to facilitate plotting R_F values.

Cotton and glass string have strength enough to resist tearing when wet.

Glass string will not disintegrate in the presence of reagents that would destroy paper.

The free use of many filter-paper strips, which is facilitated by the simplicity of these procedures, often obviates the need for two-dimensional chromatography. The same effect is obtained by selecting, for each substance in a mixture, a solvent combination that isolates an individual compound from its fellows and combining the results from several chromatograms into a completed analysis.

Uniformity of solvent excursion in any single run compares favorably with that obtained on wide strips of filter paper, so that the prediction of location of bands of colorless substances may be confidently made after one of two strips run simultaneously has been treated with color-developing agents. Appropriate sections of the second strip may then be cut and treated for quantitative analysis of the test substances.

Partition chromatography on filter paper is a most convenient method for studying the metabolic fate of sulfones and sulfonamides, and possibly many other drugs as well. It is a guide to isolation procedures (*10*) and can reveal the presence of unsuspected metabolites. The behavior (R_F values) of a compound in various solvent combinations serves to characterize it, being precise and supplementary to the more widely used physical constants such as melting and boiling points, absorption spectra, and indexes of refraction and rotation.

ACKNOWLEDGMENT

The author acknowledges the counsel and suggestions of M. X. Sullivan, Edward S. Josephson, Francis L. Schmehl, G. Robert Coatney, M. I. Smith, and Ernest L. Jackson. Arthur Robbins assembled the glassware shown in Figure 2. Mary King Boucher drew the illustrations.

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Submerged Bulblet Methods in Microchemistry

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An improved method for the determination of vapor pressure and/or vapor pressure lowering in various systems is described. The value of such data in a variety of microchemical investigations is discussed, and experimental results supporting this evaluation are cited for the following measurements: vapor pressure, decomposition pressure, phase studies, solubility, purity (of solute or solvent), molecular weight (as a function of concentration), and association.

A NEW method for the microdetermination of molecular weights, based on a measurement of the vapor pressure lowering that occurs when a known weight of the test substance is dissolved in a known weight of solvent at the latter's boiling point has recently been described (*6*). In the course of a further investigation it has proved possible to simplify the determination considerably; in particular, by entirely eliminating the use of a comparison tube and interpolation. It is then possible to achieve a generalized apparatus and technique applicable to a variety of microdeterminations. This paper describes the improved apparatus and technique, indicates the applications for which they are fitted, and discusses the limits of accuracy of which they are capable.

APPARATUS

The apparatus now proposed is basically similar to that previously described (*6*); but the addition of several auxiliary devices has greatly enhanced the flexibility of the equipment. In Figure 1 a diagrammatic sketch of the apparatus is presented.

H is a constant-temperature jacket insulated with sheet asbestos. Observation ports are cut in this lagging, so that the shank and the body of bulblet *L* may be observed. The fabrication and use of these bulblets have been fully described (*6*). The bulblet is supported by a hook in a glass rod, and may be raised from, or lowered into, the manometric fluid at *K* by sliding the glass rod through the lubricated sleeve, *F*.

When 15 to 20 ml. of a pure solvent, together with a few glass beads, are heated in *H* over a small free flame and under reflux from condenser *E*, the inner chamber, *J*, rapidly comes to a temperature which, for a given solvent, closely corresponds—according to the vapor pressure curve of the solvent—to the pressure in *H*. This pressure may be adjusted via pinchclamp 2; it is stabilized by the 3-liter ballast flask, *D;* and it may be measured with barometer *A*. By suitable adjustment of the pressure in this system the inner chamber and its contents can be brought to a temperature determinable with an accuracy of at least 0.1° C. and variable within a range from about 25° C. below the boiling point of the solvent to 5° to 10° C. above this boiling point (if *A* has a working length of 1000 mm.). This range is often sufficient, for the data secured are generally precise enough to sustain a reasonably extended extrapolation. There is no need to shield the equipment from occasional drafts; and the temperature control is easy and precise over fairly long periods of time. The results are unaffected by fluctuations in barometric pressure.

At *K* there is a 2-cm. layer of concentrated phosphoric acid, prepared by mixing phosphoric anhydride with 85% phosphoric acid in the proportion 1 to 2 by weight. With the use of this concentrated acid the aqueous tension over the sealing medium is reduced to a satisfactorily low value—a pivotal consideration if the comparison bublet and interpolation, formerly required to compensate for the pressure rise due to back-diffusion of water vapor, are to be eliminated. In the previous work 85% phosphoric acid was employed, and a long-period upward drift in the observed vapor pressures was (erroneously) attributed to slow establishment of temperature equilibrium. The present use of a more concentrated acid eliminates the long-term pressure drift practically completely, so that the observed vapor pressure of the solution soon becomes constant and can be compared directly with the vapor pressure of the pure solvent in the outer jacket. For work with aqueous solutions phosphoric acid is no longer satisfactory and its replacement with dioctyl sebacate has been suggested (*3*). Trials made with this sealing fluid were attended with limited success.

When the sealing fluid stands at equal levels inside and outside the shank of bulblet *L* the pressure in *J* must be equal to the pressure in *L*; and the difference between this pressure and that prevailing in *H* may be determined with mercury manometer *B* and/or dibutyl phthalate manometer *C*. Thus, inaccuracy due to the determination of the vapor pressure lowering, Δp, as a small difference between much larger values is avoided by using the manometer(s) to register directly the difference between the vapor pressure of the pure solvent, boiling in the outer jacket, *H*, and a pressure equivalent to the vapor pressure of the same solvent over the solution in *L*. Values of Δp too large to be measured with manometer *C* are read with mercury manometer *B* with the left arm of this manometer connected to *H* through the right branch of stopcock 1.

If, as in the determination of vapor pressures, the absolute value of the pressure in *L* is desired, rather than the pressure difference, *B* may be used as a closed-end manometer after its left arm has been evacuated through the left branch of stopcock 1. The attachments to the left of manometers *A* and *B* make it possible to check and, if necessary, to improve the vacuum in the reference arms of the manometers (*5*).

The inner chamber, *J*, is also connected through tube *M* with the one-way valve, *N*, consisting of a 0.5-cm. layer of mercury resting on a 10-mm. fine-porosity sintered disk. Through this valve gas can pass from *M* to *O*, but not from *O* to *M*. *O* contains powdered anhydrous barium oxide, which serves as a desiccant, and is connected, through a short sleeve of rubber tubing, with trap *P*. The free branch of (specially constructed) stopcock 6 is connected, through rubber tubing and a guard tube packed with potassium hydroxide, with a good water aspirator. A similar guard tube is also attached to stopcock 5. The various components of this equipment are assembled with the aid of lengths of heavy-walled rubber tubing.

The ground joint, *G*, is lubricated with sirupy phosphoric acid and is further sealed against the atmosphere by pouring a little mercury into the annular space around the upper part of the male member of the joint. If a quantitative recovery of the solvent volatilized from *L* is not required it may be more convenient to replace this joint with a rubber stopper bearing similar attachments; the rest of the apparatus remains the same.

PROCEDURE

The use of this equipment is described for the most difficult case—the determination of apparent molecular weight as a function of concentration—and the simplifications possible in other studies are indicated in the discussion of these applications.

The same solvent to be used in the bulblet is introduced into the outer jacket, and the desired temperature is secured by appropriate adjustment, through pinchclamp 2, of the pressure in the jacket. Trap *P* is evacuated and weighed to the nearest milligram against a counterpoise of similar weight and design, and is then attached to the line. A dry ice bath, preferably held under somewhat diminished pressure, is set around the lower part of *P*. A bulblet of known weight, which contains weighed quantities of solute and solvent, is introduced into the inner chamber, and the apparatus is assembled as shown in Figure 1.

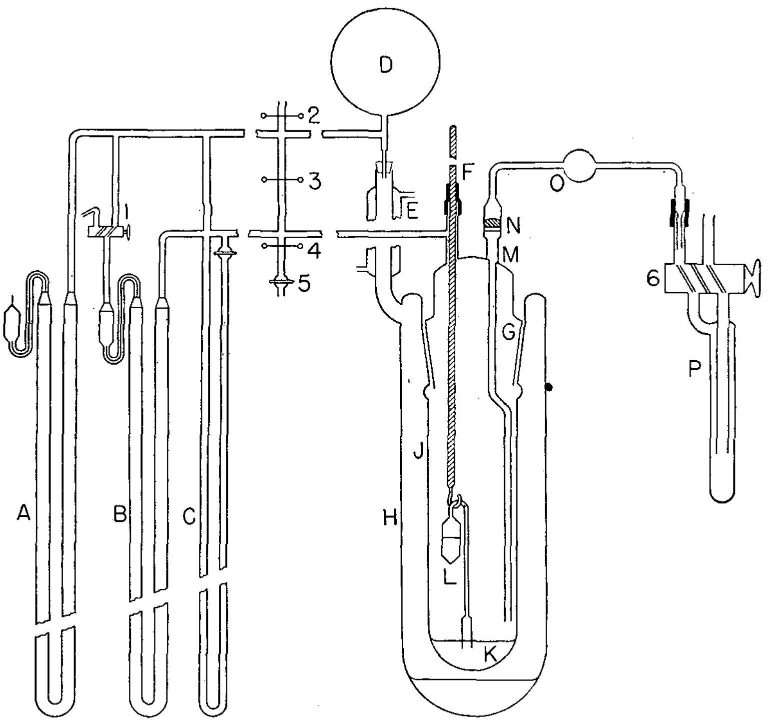

Figure 1. Diagram of Apparatus

With the solvent in the outer jacket boiling briskly, with pinchclamp 2 shut, and with valves 4 and 5 wide open, Apiezon-greased stopcock 6 is cautiously rotated toward the position shown, until gas begins to bubble through the mercury at *N*. A continuous stream of dry air is thus drawn through the equipment, from 4 and 5 toward *P*, so that any solvent distilled from *L* is swept through *N*, dried at *O*, and collected at *P*.

The opening through stopcock 5 is now very gradually diminished until the pressure in the inner chamber, which may be followed on manometers *B* and/or *C*, falls far enough to permit solvent vapor to escape from *L* at a rate of about 10 bubbles a minute. These conditions are maintained for about 0.5 hour, during which time all the air is swept from *L* by the stream of solvent vapor, while the latter is condensed in *P*. Then the opening through stopcock 5 is very cautiously widened, so that the pressure in chamber *J* gradually increases until bubbles no longer emerge from *L*. With occasional readjustment of the setting of this stopcock to prevent any further evolution of solvent vapor, the air current is continued for 5 to 10 minutes to permit recovery of practically all the solvent vapor previously distilled from *L*. Pinchclamp 4 and stopcock 6 are then closed simultaneously, and the inner chamber is allowed to come to temperature equilibrium over a period of 20 to 30 minutes. The pressure in *J* is adjusted occasionally, through pinchclamp 4, to maintain the sealing fluid at the same height inside and outside the shank tube of the bulblet. The vapor pressure lowering is determined directly from the reading on manometer *C*.

While the system is coming to temperature equilibrium stopcock 6 is rotated through 180° from the position shown; and, with the dry ice bath still in place, the trap is evacuated briefly (about 30 seconds) without disturbing the pressure equilibrium in *J*. Stopcock 6 is then shut, the line to the aspirator is detached, and trap *P* is separated from *O* at the connecting sleeve. The one-way valve at *N* prevents the entrance of air to the reduced pressure zone in *J*. *P* is wiped and, after it has come to room temperature, is reweighed against the counterpoise. The gain of weight of *P* represents the weight of the solvent collected therein, and by subtracting this quantity from the total weight of solvent initially present in *L* an approximate figure for the weight of the solvent present at the time of the pressure measurement is obtained.

P is now restored to its position in the train and stopcock 6 is cautiously opened to the aspirator until bubbling at *N* is observed. Pinchclamp 4 is then removed at once and small adjustments in the position of stopcock 5 are made, so that once again a stream of air is drawn through the apparatus while the pressure in *J* is held low enough to allow vaporization of solvent from *L*. When the solution in *L* has been sufficiently concentrated, the manipulations of the previous cycle are repeated and the new weight of *P* and the new value of Δp are recorded. It is thus possible to continue the experiment through five or six determinations made on increasingly concentrated solutions.

Because the recovery of solvent in *P* may not be 100% complete, a check on the weight of the volatilized solvent is desirable. Therefore, after the last reading has been secured, the rod supporting the bulblet is raised until the latter is clear of the pool of sealing fluid. The shank of the bulblet will still be sealed by a thin (approximately 1-mm.) layer of this liquid, but when air is cautiously admitted through 5 the film is ruptured. The bulblet should then be removed from *J* at once; and, when it has been washed and dried as described (*6*), it may be reweighed against a counterpoise of similar design. The loss of weight of the bulblet in the course of the experiment may then be compared with the gain in weight of *P*. Ordinarily there is a discrepancy of a few milligrams, more solvent usually having been lost from the bulblet than is recovered in the trap. The loss of weight of *L* is taken as correct, and any discrepancy found is proportioned among the various weight readings according to the quantity of solvent volatilized at each stage of the proceedings. Thus the approximate values for the quantity of solvent remaining in *L* at each point in the experiment, based simply on the changes in weight of *P*, may be refined so that the probable error in the weight of solvent left in *L* never surpasses 1 to 2%. An additional (marginal) correction for the weight of the solvent in the vapor phase at the time of the pressure measurement should be made whenever only small amounts of solvent are present in *L* (*6*).

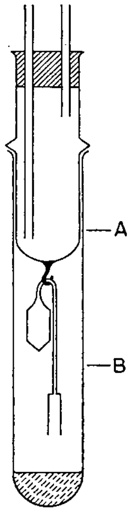

Figure 2. Refilling Bulblet

Solvent recovery was satisfactory when benzene or carbon tetrachloride was used with phosphoric acid as a sealing fluid, and when water was used with dioctyl sebacate (the barium oxide having been removed). Recovery was seriously incomplete when toluene was used with phosphoric acid. Thus, although satisfactory pressure readings may be secured with toluene, this solvent is not recommended for determinations involving a running series of indirect weight measurements.

In making the calculation of the apparent molecular weight at each concentration the weight of the solute, *g*, and the weight of solvent, *G*, corresponding to each reading of the vapor pressure lowering, Δp, are known. Furthermore, the vapor pressure of the pure solvent at the temperature of the measurement, p_0, will have been read from barometer *A*, and the molecular weight of the solvent, *M*, is known. Consequently, the apparent molecular weight of the solute, *m*, at each concentration can be calculated from the formula:

$$m = \frac{p_0 - \Delta p}{\Delta p} \times \frac{g}{G} \times M$$

When only small quantities of sample are available, a number of determinations may often be made with the same weighed quantity of solute in the same bulblet, simply by distilling fresh solvent into the latter whenever necessary. Refilling may be accomplished in about 15 minutes with the apparatus shown in Figure 2. The depleted bulblet is hung from the hook at the bottom of the cold-finger and the solvent in the larger tube is heated so that the reflux level stands alternately first at *A* and then at *B*. If the sample is a nonvolatile one, substantially the same results are secured with the refilled bulblet as in the original trial(s).

Because of the limited strength of the (blown glass) apparatus, experiments have not been made at solvent pressures exceeding approximately 1.3 atmospheres. However, the delicacy of these measurements for determinations in which only a small mole fraction of solute is present—e.g., for low solubilities, high molecular weights, and/or extremely small samples—could be greatly enhanced if the equipment were made to sustain and measure much higher pressures.

MATERIALS

Conductivity water and reagent grade organic solvents were used throughout. The samples investigated were commercial C.P. grade materials with the following exceptions: The sample of M-238, a hydroxyalkylnaphthoquinone, and the sample of benzanthracene were analytical specimens made available through the courtesy of L. F. Fieser and O. M. Friedman, respectively. A sample of $CaCl_2.4NH_3$ was prepared in situ from C.P. anhydrous calcium chloride. Although supposed to be of C.P. quality, the test sample of 1-naphthol subsequently showed evidence of the presence of impurities.

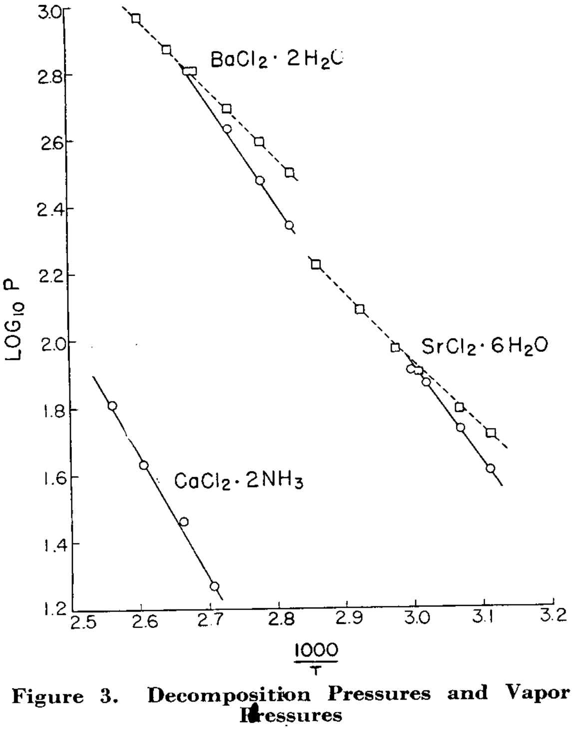

Figure 3. Decomposition Pressures and Vapor Pressures

APPLICATIONS

Measurement of Vapor Pressure. The isoteniscopic determination of simple vapor pressure was first described by Smith and Menzies (*9*), and during preliminary testing of the present equipment the vapor pressures of a number of organic solvents—e.g., benzene, toluene, carbon tetrachloride, etc.—were determined over a considerable range of temperatures. The temperature of the determination was deduced from the vapor pressure of the (dissimilar) solvent in jacket *H*, as read from barometer *A*; and an air pressure equivalent to the vapor pressure in *L* was read from manometer *B*, used with its reference arm evacuated. There was no need to collect at *P* the solvent volatilized from *L*.

The vapor pressure values secured were accurate to approximately 1 mm. The quantity of sample required for the determination need not exceed 0.1 gram, as the air may be purged from *L* completely yet economically, by sweeping out the bulblet with solvent vapor evolved at relatively low temperatures, and hence at low pressures.

Measurement of Decomposition Pressures (of Solids). Simple submerged bulblet techniques do not appear to have been previously applied to determinations of the decomposition pressures of solids, though the measurements may be made as described above. Though such methods do not provide extremely precise data, they can provide values free of the gross inaccuracies, due to adsorbed moisture and/or air, which have so often afflicted purely static measurements (*2*).

In Figure 3 decomposition pressures determined with barium chloride dihydrate, strontium chloride hexahydrate, and calcium chloride diammoniate are shown in circles, the solid lines representing the literature values in each case (*2*, *8*). Dioctyl sebacate was the sealing fluid in these experiments. The determinations were generally accurate to ca. 1 mm.; and the maximum deviation was 3 mm. A few tenths of a gram of the solid is a sufficient sample. The establishment of equilibrium is known to be rapid in the cases considered; and no doubt the results would be less satisfactory for systems that reach equilibrium more slowly. Nevertheless, any system that can be studied successfully by transpiration methods should be susceptible to this approach.

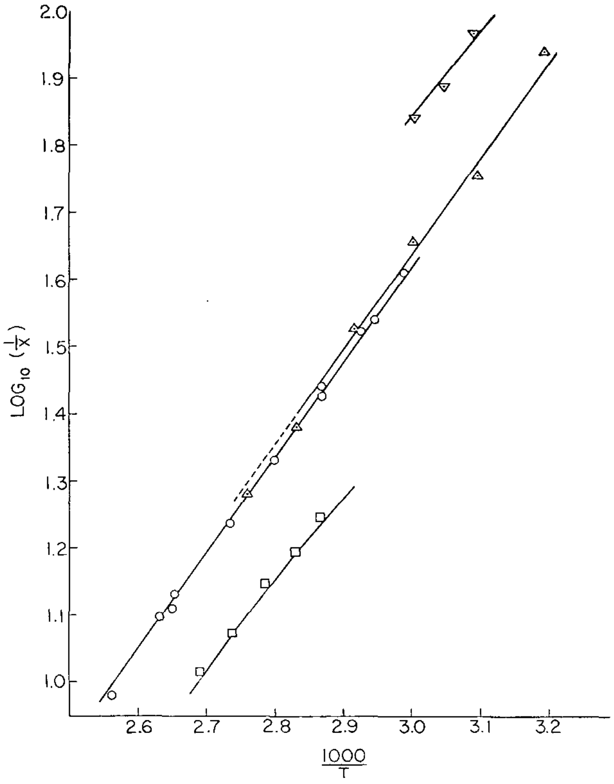

Figure 4. Solubility as a Function of Temperature

□ Boric acid in water
○ Anthracene in toluene
△ Anthracene in benzene
▽ Sulfur in carbon tetrachloride

Phase Studies. The broad outlines of phase diagrams, particularly with regard to phase transitions of solid hydrates, can be sketched from trials made with ca. 0.5 gram of sample, as indicated by the studies of the hydrates of barium chloride and strontium chloride. In Figure 3 the dotted lines represent literature values (*2*) for the vapor pressures of saturated aqueous solutions of the respective hydrates; and the solid lines indicate the accepted values for the decomposition pressures of the higher hydrates involved. The intersections of the lines represent transition points (lower hydrates formed). The experimental points are observed to define the theoretical lines well. Even though the saturated solutions do not obey the simple form of Raoult's law (neglecting ionization), the curves obtained may still be used to locate sharp discontinuities and/or intersections indicating phase transitions.

Determination of Solubility. An approach to the microdetermination of solubilities through measurement of the vapor pressure lowering in the saturated solution does not appear to have been previously attempted, although such a procedure is not without advantages. This possibility is of particular interest because there appear to be no generally satisfactory methods for the semiquantitative microdetermination of solubilities. Methods based on observation of *schlieren* (*4*) and microscopic examination (*1*) are basically qualitative, capillary methods (*4*) are semiquantitative but tedious and useful mainly at or near room temperature, and other methods require larger samples or elaborate apparatus.

A quantity of solute estimated to be sufficient to saturate 0.1 to 0.2 ml. of the solvent at the maximum temperature of the experiment is introduced into the bulblet, and 0.2 to 0.3 ml. of the solvent is added. The vapor jacket is charged with the same solvent, the apparatus is assembled as usual, and the pressure in the jacket is set at an appropriate (low) value. The air is swept from the bulblet, and after a pause to establish equilibrium, the pressure in the jacket, p_0, is read from barometer *A* and the value of Δp is taken from manometer *B* or *C*. The temperature of the experiment is fixed by the value of p_0, and if x is taken as the mole fraction of the solute, then $1/x = p_0/\Delta p$.

The pressure in the jacket is now increased to raise the temperature; and the pressure in the inner chamber is gradually elevated, so that little or no distillation from the bulblet occurs. When an apparent equilibrium has been attained (in about 40 minutes) the new values of p_0 and Δp are recorded. In this case the solubility equilibrium has been approached from the undersaturated side. Now, without altering the pressure in the jacket, the pressure in the inner chamber is slightly diminished, so that solvent vapor can escape from the bulblet, and after a few minutes the pressure in the inner chamber is raised until a new equilibrium is attained. In this case the equilibrium is approached from the oversaturated side. The ordinarily concordant values obtained indicate that a true equilibrium has been reached in both instances. If a small vibrator (buzzer) is set against the rod supporting the bulblet the approach to equilibrium may be accelerated.

Such trials establish the mole fraction of solute at different temperatures. The molecular weight of the solvent is known, and if the molecular weight of the solute is also known, the mole fractions are readily converted to gram solubilities. The molecular weight of the solute may be determined by the methods outlined below.

The experimental results obtained in several systems that obey Raoult's law fairly well are shown in Figure 4. The lines represent the literature values for the systems involved (*7*). The accuracy of the measurements depends on the value of p_0 at the temperature of the determination, and on the intrinsic solubility of the substance; but an accuracy of at least 5% can generally be maintained when dealing with moderately soluble substances which obey Raoult's law within this margin.

Only enough of the sample to saturate approximately 0.1 ml. of the solvent is required; and the solubility curve over a range of 25° to 30° C. in the neighborhood of the boiling point of the solvent can be drawn from results secured with the one sample. A reasonable extrapolation to provide data at other temperatures is probably permissible. No weighings, micro or otherwise, are involved.

The validity of the apparent equilibrium is checked by the successive approaches from under- and oversaturation. The method assumes the applicability of Raoult's law, but this can be checked, with the same sample, by a determination of the apparent molecular weight as a function of concentration. If the result is independent of concentration, then that value (which may, of course, be only an apparent molecular weight of a substance that is almost completely associated or dissociated) can be used in converting the mole fractions to gram solubilities. If the apparent molecular weight varies with concentration the prognosis is not favorable.

Though of recognized value, the characterization of pure substances by their solubility curves has not played its maximum role in organic chemical research, probably because the necessary data have not been available easily and with economy of material. Such data are made readily accessible by the present procedure;

and in this application the validity of Raoult's law is of no consequence, because the temperature dependence of the vapor pressure lowering in a saturated solution of a substance is in itself a valuable characterization.

Purity Determination. Assay of Solute Purity. Purity determinations based on a measurement of apparent solubility at constant temperature but as a function of the weight ratio of solute to solvent have been described by Webb (*11*) and others. Such an assay is particularly valuable when the sample does not show a well-defined melting point. The techniques usually employed in this work, involving as they do a number of individual solubility determinations with careful temperature control and many weighings, are exacting and time-consuming, and require considerable amounts of sample. By the method described some estimate of the degree of purity can be secured rapidly, without any microweighings and with only sufficient sample to saturate 0.5 to 1.0 ml. of the solvent at its boiling point.

A roughly weighed sample of the solid is placed in a weighed bulblet together with a (doubly distilled) solvent of high purity. (The purity of the solvent can be checked by the method described below.) The vapor jacket is charged with the same solvent; and the pressure in the jacket is set at approximately 760 mm. for the duration of the run. The bulblet with its contents is weighed, the equipment is assembled, the air is purged from the bulblet, and the solvent so volatilized is recovered in trap *P*. After a pause to establish equilibrium the weight of the solvent collected in the trap and the value of Δp read from manometer *B* or *C* are recorded. The vapor pressure lowering at a definite solution concentration is thus established.

After a further concentration of the solution the new value of Δp and the weight of the solvent in the trap are again noted. Continuing in this way a progressive diminution of the solvent present, a plot of vapor pressure lowering as a function of the ratio of solute to solvent is secured. Ideally this curve takes the form of *A* in Figure 5. An experimental curve secured with commercial c.p. anthraquinone in benzene, shown as *B* in Figure 5, indicates the presence of a maximum of ca. 0.2% impurity. A sample of the anthraquinone intentionally adulterated with 3.3% by weight of anthracene gave the results embodied in curve *C*. A fairly clear indication of the purity of the solute is apparent.

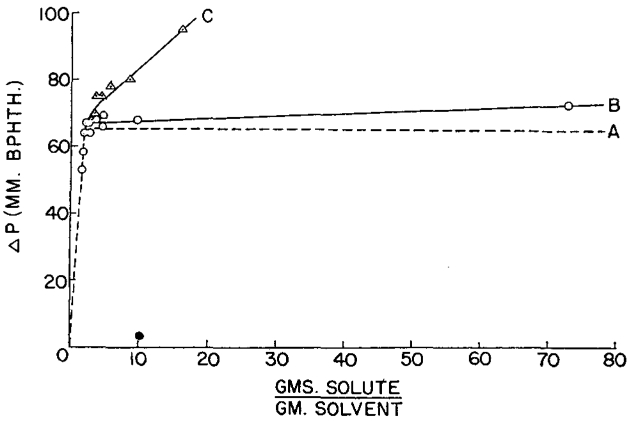

Figure 5. Purity and Solubility

This indication is not seriously weakened by deviations from Raoult's law, because the relative rather than the absolute values of Δp provide the critical criterion. When Raoult's law does apply fairly well, and when the solubility of the major component is known or can be estimated from the (interpolated) point at which the plateau begins, a semiquantitative evaluation of purity may be secured. However, the considerable scatter of the experimental points and the occasional appearance of a supersaturation maximum at the beginning of the plateau tend to mask the more subtle indications provided by more exact data.

Assay of Solvent Purity (Nonvolatiles Only). A portion of the solvent is weighed into a bulblet of known weight. The specimen of the same solvent with which the outer jacket is charged should be reasonably pure—extreme purity is unnecessary because any nonvolatiles here are not concentrated by evaporation. With the liquid in the vapor jacket boiling under a pressure of approximately 760 mm. the major portion of the solvent sample is distilled from the bulblet in the usual way. The vapor pressure of the residual solvent in the bulblet is then compared with the vapor pressure of the same solvent in the outer jacket, to find Δp, after which the bulblet is withdrawn, washed, wiped, and weighed.

From the observed vapor pressure lowering the mole fraction of nonvolatiles in the concentrated solution may be calculated; and when the ratio of the initial and final weights of sample is known, the mole fraction of nonvolatile impurity in the original material may be found. Thus, when 0.835 gram of toluene adulterated with 0.00025 gram of anthracene was evaporated until only 0.027 gram of the solution remained in the bulblet, a vapor pressure lowering (relative to pure toluene) of 51.5 mm. of dibutyl phthalate was observed. From these data the mole fraction of impurity in the original specimen was calculated as 1.7×10^{-4} (theoretical value 1.55×10^{-4}).

This mode of evaporation is a conspicuously clean one, and the vapor pressure measurement permits reliable estimation of quantities of impurities too small to be weighed conveniently with the ordinary analytical balance.

Molecular Weight Determination. The previously discussed (*6*) advantages inherent in this approach to the microdetermination of molecular weights are retained in the present technique. In the simplest form of this determination the usual measurements of p_0 and Δp are made with a bulblet of known weight, containing a weighed quantity of solute and an indeterminate amount of solvent. The bulblet is then removed and weighed, to secure a weight for the solvent present at the time of the measurement. With the weights of solvent and solute, the molecular weight of the former, and the vapor pressure readings, the molecular weight of the solute may then be calculated.

However, this method presents a further possibility which is, as far as the author knows, unique in a method for the microdetermination of molecular weight: A convenient determination of the apparent molecular weight as a function of concentration may be made in the course of one experiment and using only one weighed sample. This determination, described in the section on procedure, is important whenever there is any reason to suppose that the solution(s) involved may not behave ideally. Under such conditions deviations due to the nonideality of the solution cannot be evaluated on the basis of a single measurement; but when, as in the present instance, the concentration dependence of molecular weight is established, the error can be estimated or corrected or, failing this, it is at least made patent that the apparent value is not meaningful.

The results of the studies of molecular weight are given in Table I.

The sample weights were 5 to 15 mg. in most instances, although, for example, the molecular weight of anthracene in toluene was determined with a 0.71-mg. sample. Where only one result is cited for a given system the direct weighing technique was used. Multiple values were secured by successive measurements on the same sample in increasingly concentrated solutions. The results marked "Re" were obtained when the bulblet used in the immediately preceding trials was re-examined after refilling with a fresh charge of solvent. The bracketed values for the theoretical "molecular weight" of benzoic acid in benzene were calculated from the data of Wall and Rouse (*10*).

In most instances the determinations were accurate to approximately 2% and the observed values did not vary appreciably with concentration. However, in three cases appreciable systematic variations are perceptible.

Table I. Molecular Weight Determinations

Solute	Solvent	Concentration, G. of Solute per 100 G. of Solvent	Molecular Weight of Solute	
			Observed	Theoretical
Anthracene	Benzene	2.95	178	178.2
		4.98	176	
M-238	Benzene	5.31	409	418.4
		6.75	409	
		9.40	407	
		14.7	414	
Benzanthracene	Benzene	2.88	226	228.3
		4.81	225	
p-Dibromobenzene	Benzene	2.99	232.5	235.9
		4.22	236.5	
		5.96	244	
		9.18	251	
		15.3	251	
		5.10	253 Re	
1-Bromonaphthalene	Toluene	5.30	212	207.1
Anthracene	Toluene	1.01	183	178.2
Anthraquinone	Toluene	2.89	204	208.2
Diphenyl	Carbon tetrachloride	2.98	151	154.2
		4.90	150	
1-Naphthol	Carbon tetrachloride	1.585	153.7	144.2
		2.00	158.8	
		2.77	168.2	
		4.10	186.3	
		2.295	166.2 Re	
Boric acid	Water	14.3	61.4	61.8
Benzoic acid	Benzene at 80.3° C.			122.1
		4.17	207	(211)
		5.46	211	(215)
		8.24	220	(219)
		10.35	224	(222)
		13.23	227	(224)
		∞	239[a]	(244)
		7.18	220 Re	(218)
	Benzene at 70.0° C.	7.19	223.5	(222.5)
		9.68	226	(225)
		16.45	231.5	(229)

[a] Extrapolated.

The *p*-dibromobenzene variation was tentatively attributed not to any nonideality of the more concentrated solutions, but rather to progressive volatilization of the sample as the solution was concentrated. Indeed, when the bulblet was refilled and rerun the result was distinctly higher than those previously obtained at comparable dilutions. However, although sample volatility is a critical limiting factor in the applicability of this method, a reasonable approximation to the correct value can often be deduced when, as in the present case, the sample is not excessively volatile and the results of the first few trials are not in serious disagreement.

The trends observed in the trials with 1-naphthol and benzoic acid cannot be assigned wholly to volatilization losses, for the values secured on refilling the respective bulblets were only slightly higher than those initially obtained at comparable concentrations. Therefore these cases represent real departures from Raoult's law. In the case of 1-naphthol the relation of molecular weight to concentration is not a simple one, and is insufficiently defined by the experimental results to permit an unequivocal extrapolation to zero concentration. The molecular weight is defined only as lying between 130 and 145.

In the case of benzoic acid, the extrapolation to zero concentration down a line of steep and uncertain slope is also unsatisfactory. However, there can be little doubt that in concentrated solution this type of carboxylic acid is present almost exclusively as the dimer. If, then, the apparent molecular weights are plotted against the reciprocal concentration (see Figure 6) and extrapolated to $1/c = 0$ the molecular weight of the dimer is established, and the molecular weight of the monomer is calculable.

Furthermore, with such data on the concentration dependence of apparent molecular weight the association or dissociation characteristics in a given system may be assayed, provided that the solute's "true" molecular weight is known or has been determined. Table I indicates that the benzoic acid values are in harmony with the data of Wall and Rouse (*10*). The dissociation constant of dimerized benzoic acid in benzene at 80.3° C. may be calculated from the work of Wall and Rouse as 0.0157, whereas the present measurements lead to values of 0.019, 0.0215, 0.015, 0.012, and 0.011—average 0.016. The good agreement of the experimental average with the theoretical value is probably attributable, at least in part, to the cancellation of small and oppositely directed errors (the presence of traces of moisture and the occurrence of small volatilization losses). The theoretical value of the equilibrium constant at 70.0° C. is 0.0101, whereas the present experimental data yield the values 0.0090, 0.0093, and 0.0068—average 0.0084. Here there is a substantial discrepancy that is probably traceable to the fact that when the material is almost completely associated a small error in the determination of the apparent molecular weight is greatly magnified in the calculation of the dissociation constant (*10*).

Undoubtedly more precise methods are to be preferred when there is a plentitude of time and material; but when only a minimal (15-mg.) sample is available practically significant results may be obtained by this method more readily than would otherwise be the case.

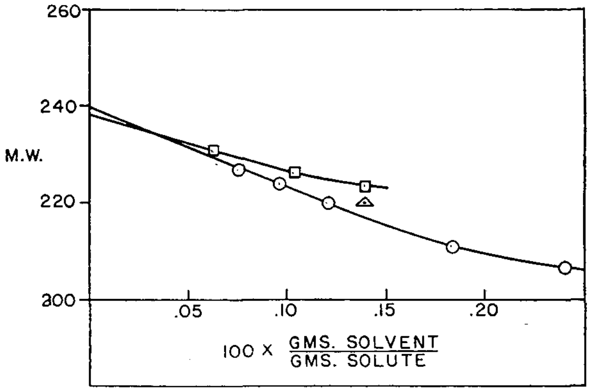

Figure 6. Apparent Molecular Weight of Benzoic Acid in Benzene

⊙ Run at 80.3° C.
△ Refilled bulblet, run at 80.3° C.
□ Run at 70.0° C.

ACKNOWLEDGMENT

The suggestion that submerged bulblet techniques might be applied to measurement of the decomposition pressures of solid hydrates was made to the author by G. P. Baxter.

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Determination of Free Sulfur in the Atmosphere

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A sensitive and specific field test for elementary sulfur aerosol was developed for use in a study of toxic effects of air-borne sulfur. Adaptation of Feigl's spot test for elemental sulfur has resulted in a method capable of determining concentrations as low as 0.05 p.p.m. of sulfur to within 90% accuracy, using 1 cubic foot of air. The procedure is based upon the formation of thallium polysulfide by sulfur and thallous acetate when treated with hydrogen sulfide in the pores of filter paper. Thallous acetate papers prepared this way may be stored several weeks for field use. Aerosol samples are analyzed by spotting the portion of the paper through which the gases have been passed with a drop of pyridine, immersing the paper in hydrogen sulfide, washing with acid, and comparing the polysulfide spot with standards. Studies, yet unpublished, have revealed that for amorphous and crystalline forms of the aerosol causing eye irritation, the threshold values are 0.2 and 8 p.p.m., respectively.

A SEARCH of the literature revealed a great need for a sensitive method for the determination of atmospheric free sulfur in connection with a study of the irritating and toxic effects of sulfur aerosols. The first reports in this field, which appeared in Italian journals (*5*, *8*), indicated that sulfur miners are subject to pneumoconiosis (fibroid induration and pigmentation of the lungs) as a result of their exposure to sulfur dust. American sulfur is mined by the Frasch process, whereas Italian sulfur must be removed from the hillsides by tunneling. Although a study of sulfur workers in the United States (*4*) revealed no lung lesions that could be attributed to the inhalation of sulfur dust, private communications have described a dry, persisting eye irritation apparently caused by air-borne sulfur.

Preliminary investigation of sulfur aerosols in the laboratories of Stanford Research Institute indicates that sulfur, even in extremely low concentrations, can cause eye irritation. Therefore high sensitivity is required of the analytical method used to study this irritation. Various existing micromethods (*3*, *6*, *7*, *9*) were investigated for possible application to the problem but were found unsatisfactory because of a lack in accuracy, sensitivity, or specificity.

EXPERIMENTAL

Preliminary investigation of Feigl's spot test for elemental sulfur (*2*) proved it to be the most promising. According to Feigl, when sulfur in solution contacts black thallous sulfide in the pores of filter paper, the red-brown thallium polysulfide ($2Tl_2S.Tl_2S_3$) is formed. In place of thallous sulfide paper the authors used thallous acetate paper because it proved to be more stable and gives more reproducible results. After the sample is collected on the acetate paper it is moistened with pyridine and immersed in hydrogen sulfide to permit the simultaneous formation of polysulfide (where free sulfur is present) and sulfide. The black thallous sulfide differs from the red-brown polysulfide not only in color but also in chemical properties. It may be removed from the paper by washing with dilute mineral acid and the intensity of the unchanged polysulfide color may be observed against a white background.

REAGENTS AND APPARATUS

The paper chosen for its ability to retain extremely small aerosol particles is Chemical Warfare Service Type 6 filter material, untreated. No. 1 Whatman filter paper, 7 cm. in diameter, is the best substitute if the other is not available. The papers are prepared by soaking them in a 0.5% aqueous solution of thallous acetate, then suspending them from a horizontal glass rod, and drying thoroughly at 80° to 105° C. in an electric oven. When stored in covered glass containers the prepared papers remain stable, with a slow decrease in sensitivity, for several weeks or more. The reagents necessary for completing an analysis are: pyridine, reagent grade; nitric acid, 0.5 *N*; and hydrogen sulfide in wide-mouthed Mason jars or similar containers.

An eye dropper that will drop 40 or preferably more drops of pyridine per ml. is required.

Standard solutions of sulfur in pyridine. After the number of drops of pyridine per milliliter delivered by the eye dropper is determined, solutions are prepared which will contain 1.0, 1.2, 1.4, 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, 120, 140, 160, 180, and 200 micrograms of sulfur per drop. The lower limit of detectability of sulfur in one drop of pyridine is 1 microgram, when the dropper delivers 43 drops per ml.

Atomizer of the DeVilbiss No. 15 type, containing no rubber parts that will contact the pyridine to be used in it.

Sampling device (*1*). A holder for the thallous acetate paper which will permit drawing air through only a portion of the paper, of approximately the size wet by one drop of pyridine from the calibrated eye dropper.

Gas meter.

Vacuum pump.

PROCEDURE

A sheet of thallous acetate paper is clamped in the holder of the sampling device; the gases to be tested are drawn through by a vacuum pump which exhausts to the atmosphere, and the volume is measured by a wet-test meter or orifice-type gas meter after passing through the paper. The paper is then removed from the holder and that portion through which the air has passed is sprayed with pyridine from the atomizer. The paper is allowed to dry and may be stored in a covered glass container. Greatest accuracy and sensitivity are obtained by carrying out the analysis within a few hours after collecting the sample.

A similarly prepared thallous acetate paper is spotted with one drop of each standard solution required to cover the range of sulfur content expected in the sample (the spots should not overlap at the edges), then allowed to dry thoroughly. The test spot and standard papers are suspended on a glass rod and sprayed with pyridine from the atomizer until slightly, but uniformly, damp. They are then allowed to dry almost completely and are placed in a jar of hydrogen sulfide for about 30 seconds. After the papers are removed, the remaining pyridine is allowed to evaporate. The papers are then washed in a large beaker of 0.5 *N* nitric acid to remove the thallous sulfide. In order to wash out the acid, thus delaying fading of the spots, the papers are transferred, by means of glass rod, to a container of distilled water. When the papers are removed from the water they are placed on a white porcelain surface and the intensity of the test spot is compared with the standards to determine the quantity of sulfur present in the sample.

INTERFERENCES

If the air contains considerable dust and very low concentrations of sulfur, it may be helpful to run the same sampling and testing process on paper not impregnated with thallous acetate to determine the extent of interference by dust. Any dark oils that may be collected can be removed from the paper by washing in alcohol or benzene after the initial spraying and drying of pyridine, without affecting the accuracy or sensitivity of the test. The presence of small quantities of foreign gases in the air should not interfere with the test. Selenium will behave analogously to sulfur, forming the polyselenide.

ACCURACY OF METHOD

When air containing known amounts of sulfur aerosol, in concentrations ranging from 0.5 to 1.0 p.p.m., was subjected to this analysis, a series of 15 determinations averaged 101% of the theoretical concentrations, with an average deviation of 8.7% and a maximum deviation of 25%.

APPLICATION

Using this method of analysis, preliminary investigation of threshold concentrations causing eye irritation in a series of individuals was made. For the amorphous form of the aerosol, which was formed by the instantaneous vaporization of an aqueous suspension of sulfur dropped on a hot plate in an especially constructed chamber, the threshold was in most cases 0.2 to 1.0 p.p.m. This preparation of amorphous sulfur resulted in particles averaging 2 microns in diameter, which were observed not to crystallize for several days. Subjecting the same individuals to atmosphere containing finely ground crystalline sulfur, approximately 25 microns in particle diameter, 8 to 10 p.p.m. were required to produce similar irritation.

ACKNOWLEDGMENT

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Colorimetric Determination of Beta-Pyridylcarbinol

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A rapid method for the colorimetric determination of β-pyridylcarbinol (3-pyridinemethanol) in pharmaceutical preparations is described. With the α-position of the molecule unsubstituted, this pyridine derivative produces a purple color with a maximum absorption at a wave length of 560 millimicrons, when reacted with cyanogen bromide and *p*-aminoacetophenone in acid medium. This color is used for photometric measurements. The colored compound was isolated and its analysis indicates that it is a hydrobromide of a polymethine dye. Data are presented showing the effect of various factors which have a bearing upon the development and the intensity of the color. A precision of ±2% has been obtained over the specified range of concentration.

BECAUSE β-pyridylcarbinol (3-pyridinemethanol) showed a distinct vasodilating action in clinical investigations, a method for its determination in pharmaceutical preparations was desired. Investigational work was therefore undertaken for the purpose of developing a suitable assay method.

For the colorimetric determination of compounds with pyridine ring structure, the Koenig reaction (*6*) has been used widely in its various modifications. This reaction is based upon the opening of the pyridine ring with cyanogen bromide, followed by conjugation of the carbon chain with an aromatic amine to form a so-called polymethine dye (*4*).

Various investigators have studied the applicability of a number of aromatic amines to this reaction for the quantitative estimation of nicotinic acid. Swaminathan (*7*) used aniline, von Euler *et al.* (*2*) used 2-naphthylamine, and Bandier and Hald (*1*) introduced *p*-methylaminophenol, while Harris and Raymond (*3*) and Kodicek (*5*) suggested *p*-aminoacetophenone.

It was thought that β-pyridylcarbinol, its α-position being unsubstituted, would react similarly to nicotinic acid when subjected to the cyanogen bromide reaction. When aniline was used as aromatic amine, a reddish color developed but was difficult to reproduce, as various shades from yellowish to red were observed. No color resulted with *p*-methylaminophenol.

On the other hand, *p*-aminoacetophenone yielded a yellowish color which turned purple upon standing. It was observed that this reaction could be accelerated by heating.

Efforts therefore were centered upon investigation of the cyanogen bromide reaction with *p*-aminoacetophenone for its usefulness for quantitative determinations.

Waisman and Elvehjem (*8*) have described the mechanism of the Koenig reaction for nicotinic acid. In the case of β-pyridylcarbinol it is believed that an analogous reaction takes place, except that the hydrobromide of the polymethine dye is formed (Figure 1).

Nicotinic acid will yield a yellow color with either one of the aromatic amines tested in the reaction, whereas β-pyridylcarbinol will give a purple color only with *p*-aminoacetophenone.

PREPARATION OF POLYMETHINE DYE FROM β-PYRIDYLCARBINOL

To 10 grams β-pyridylcarbinol 100 ml. of a solution of 30 grams of *p*-aminoacetophenone in ethanol were added. The mixture

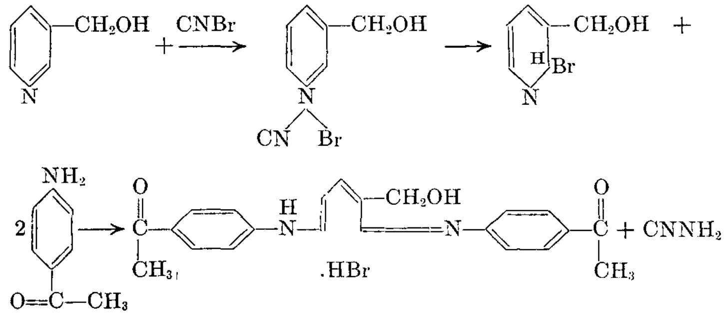

Figure 1. Cyanogen Bromide Reaction with *p*-Aminoacetophenone

was heated in a water bath until completely dissolved, 100 ml. of an aqueous 20% solution of cyanogen bromide and 5 ml. of concentrated hydrochloric acid were added, and the mixture was heated with constant swirling in a water bath for about 10 minutes. The dye was allowed to crystallize overnight in the refrigerator. It was filtered off through a Büchner funnel and thoroughly washed with water, ethanol, and ether. The dye was recrystallized from methanol-ether and again washed with water, ethanol, and ether. It was dried at 70° C. in vacuo. It consisted of purple crystals, which melted at approximately 150° under decomposition using the Kofler micro melting point hot stage.

The elementary analysis indicated an empirical formula of $C_{22}H_{23}N_2O_3Br$, which corresponds to the hydrobromic acid salt of the polymethine dye:

	Calculated, %	Found, %
C	59.9	59.9
H	4.8	5.2
N	6.35	6.2
Br	18.1	16.2

The slightly low values for bromine might be due to the fact that the solution was acidified with hydrochloric acid and thus some hydrochloride may have been formed.

The authors' belief that the colored compound is the hydrobromide, rather than a compound in which the hydrogen bromide has added to a double bond, is based on the following observations:

A saturated aqueous solution of the dye had a pH of 2.9.

Upon addition of silver nitrate solution to a saturated aqueous solution of the dye, silver bromide was precipitated.

The salt could be thrown out of an alcoholic solution by the addition of ether, in which it is insoluble.

PROCEDURE

Apparatus and Reagents. Instrument. A Klett-Summerson photoelectric colorimeter with filter No. 56. Calibrated test tubes of 12.5-mm. diameter which accompany this instrument.

Volumetric Flasks. Pyrex volumetric flasks, lifetime red, of 25-ml. capacity are fitted with hooks of heavy copper wire which permit rapid suspension of the flasks in the constant temperature bath.

Standard Solution. One hundred milligrams of β-pyridylcarbinol tartrate, recrystallized from methanol (melting point 148° C.) are weighed into a 1000-ml. volumetric flask and dissolved in 50 ml. of 0.1 *N* sodium hydroxide. The solution is diluted to the mark with water. This solution will contain 100 micrograms of β-pyridylcarbinol tartrate per ml., equivalent to 42.1 micrograms of β-pyridylcarbinol.

Cyanogen Bromide Reagent. A 4% aqueous solution, prepared by dissolving cyanogen bromide crystals, Eastman, in water. (It is stored in a refrigerator and used only if colorless.)

p-Aminoacetophenone Reagent. *p*-Aminoacetophenone, Eastman, is recrystallized from a warm saturated solution in 95% ethanol by addition of about 3% distilled water. After cooling in the refrigerator, the almost colorless crystals are filtered off and dried in vacuo at room temperature. A 5% solution in 95% ethanol, when stored in refrigerator, will keep for one week.

0.2 *N* hydrochloric acid.

0.1 *N* sodium hydroxide.

Preparation of Sample. Tablets. Twenty tablets are ground to a fine powder. An aliquot of the tablet mass, equivalent to about 40 mg. of β-pyridylcarbinol, is weighed into a 100-ml. beaker and dissolved in 50 ml. of 0.1 *N* sodium hydroxide. The solution is quantitatively transferred with water to a 1000-ml. volumetric flask and the flask is filled to the mark with water. After sufficient shaking the solution is filtered through an 11-cm. filter paper and the first 100 ml. of the filtrate are discarded. Aliquots of the remaining filtrate are used for the assay.

Ampoules. A portion of the ampoule solution, equivalent to about 40 mg. of β-pyridylcarbinol, is measured from a microburet into a 1000-ml. volumetric flask, 50 ml. of 0.1 *N* sodium hydroxide are added, and the flask is filled to the mark with water.

For colorimetry, the developed color of the unknown sample solution is compared against a standard solution of similar concentration and both are run in duplicate.

The blank solution consists of a 2-ml. aliquot of the sample solution plus the *p*-aminoacetophenone reagent and is acidified with 0.2 *N* hydrochloric acid in the same manner as the sample solution. No cyanogen bromide is added to the blank.

Two-milliliter aliquots of the sample solution and of the standard solution are delivered into individual 25-ml. volumetric flasks from microburets. These solutions are diluted with 11.5 ml. of water and 0.5 ml. of *p*-aminoacetophenone reagent is added to each flask. From a microburet 1.0 ml. of cyanogen bromide reagent is added, the content is well mixed, and the flask is immediately placed in a constant temperature bath of 80° C. Exactly 2 minutes after immersion of the first flask, it is removed and placed in an ice bath for another 2 minutes. Upon removal from the ice bath 10 ml. of 0.2 *N* hydrochloric acid are added, bringing the volume to 25 ml. Each successive flask is treated in exactly the same manner; accurate timing is maintained throughout the series. Having been mixed again, the flasks are set aside for their timed interval. The photoelectric colorimeter is set to 0 galvanometer divisions with the blank solution contained in a Klett test tube.

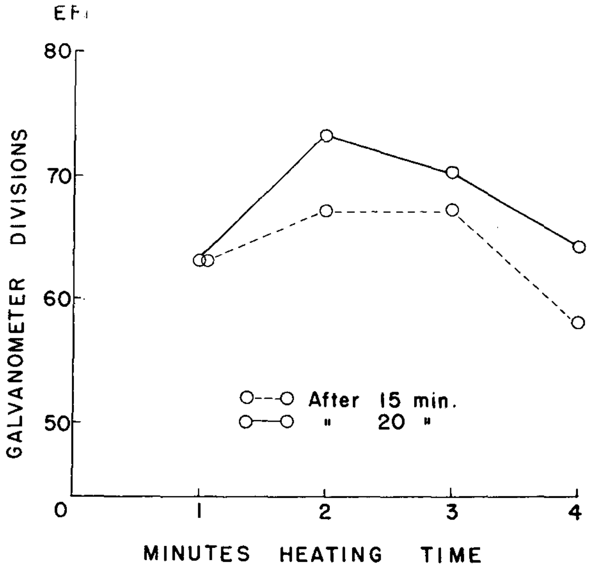

Figure 2. Effect of Heating Time at 80° C. upon Color Intensity

At exactly 20 minutes after their immersion into the bath of 80° C., each successive solution is read against this zero setting.

The results are calculated by direct comparison of the galvanometer readings obtained from the sample solution as against those obtained from the standard solution.

DISCUSSION

A study was made of all factors that might have a bearing upon the reproducibility of the results, such as temperature, preparation of blank and standard, pH, concentration of reagents used, duration of reaction time, and stability of color produced.

Effect of Heating Time. The effect of different temperatures upon development of the color reaction was investigated. As can be seen from Figure 2, a maximum color intensity was obtained by immersion of the volumetric flask for 2 minutes in a bath at 80° C.

Preparation of Blank. *p*-Aminoacetophenone reagent imparted a slight yellow color to the blank. This color was not intensified by the addition of the colorless cyanogen bromide reagent. It was therefore thought advisable to omit the cyanogen bromide reagent from the blank and to include an aliquot of the sample solution instead, in order to compensate for any measurable color of such a solution.

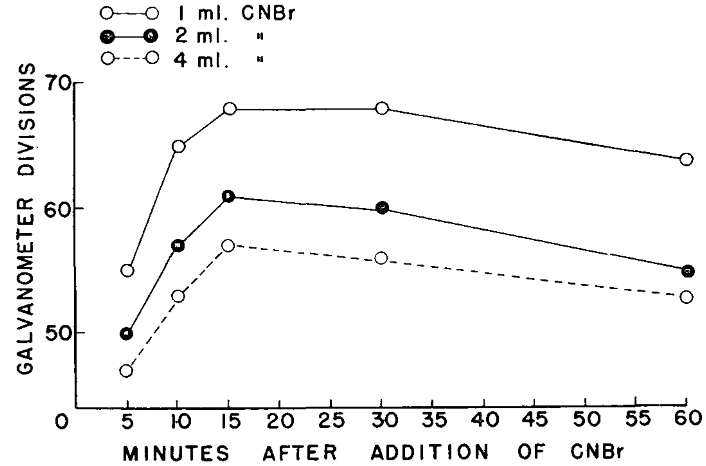

Figure 3. Effect of Variation in Amount of Cyanogen Bromide

Preparation of Standard Solution. β-Pyridylcarbinol is a liquid and therefore a derivative with a sharp melting point was considered preferable as a standard. The tartrate had previously been used for the preparation of tablets (W. Lauter, Hoffmann-LaRoche, Inc.). This salt can easily be recrystallized from methanol. The colorless crystals have a melting point of 148° (corrected), are stable, and were found suitable to prepare standard solutions.

Effect of Variation in Amount of Cyanogen Bromide. With 1 ml. of cyanogen bromide solution, the color reached a maximum intensity after 15 minutes, was stable for another 15 minutes, and subsequently decreased in absorbance (Figure 3). With 2 and 4 ml. of cyanogen bromide solution, respectively, the intensity, after 15 minutes, was lower and showed a proportionate tendency to fade. One milliliter of cyanogen bromide reagent was therefore chosen for the procedure.

Effect of Variation in Amount of *p*-Aminoacetophenone. In Figure 4 concentration was plotted against galvanometer divisions. With 0.5 ml. of *p*-aminoacetophenone reagent, a straight line was obtained. However, when 1.0 and 2.0 ml. of this reagent were added, the curves showed a deviation from linearity. Therefore, a quantity of 0.5 ml. of *p*-aminoacetophenone was adopted.

Effect of pH. Using a Beckman spectrophotometer, the absorption curves of the polymethine dye at pH 3.4 and 1.0 were plotted (Figure 5). While the maximum absorption of the solution of pH 3.4 was found at 540 mμ, it shifted to 560 mμ at pH 1.0. The curve at pH 1.0 covered a narrower band than the one at pH 3.4. This made a pH of 1.0 advantageous for use with the Klett-Summerson instrument, as its filters cover an absorption band of approximately 50 mμ. A pH adjustment to 1.0 was therefore held desirable.

Stability of Color Produced. Figure 6 shows the results when different aliquots of a standard solution were subjected to the procedure described. A maximum absorbance was reached after 15 minutes and remained stable for the next 15 minutes, but fading took place thereafter. It was therefore decided to select a time of exactly 20 minutes after addition of the cyanogen bromide reagent for galvanometer readings.

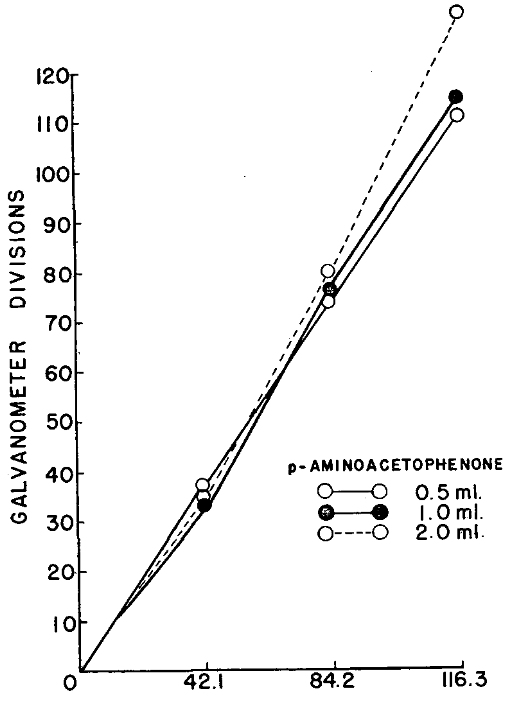

Figure 4. Effect of Variation in Amount of *p*-Aminoacetophenone

Calibration Curve. A plot of absorbance against concentration was found to be linear and passed through the origin. Beer's law was obeyed over the entire suitable range of concentration. With the Klett-Summerson instrument this range was held to the limits of 30 to 150 micrograms.

As variations in the reagents used as well as in temperature and timing exert a distinct influence upon the slope of the calibration curve, it was found advisable to run standards along with the sample solution in each determination.

Reproducibility of Results. Replicate determinations on one sample of tablets, prepared to contain approximately 25 mg., and one sample of ampoules, prepared to contain 50 mg. per ml. as active ingredient, were run on different days.

The results obtained on typical routine samples of these preparations are given in Table I. They indicate a reproducibility of ±2%.

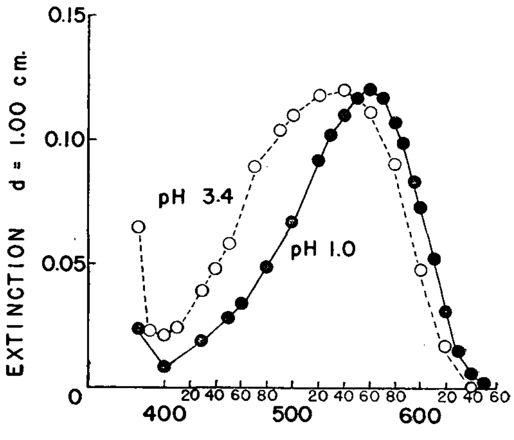

Figure 5. Absorption Spectrum of Color Developed

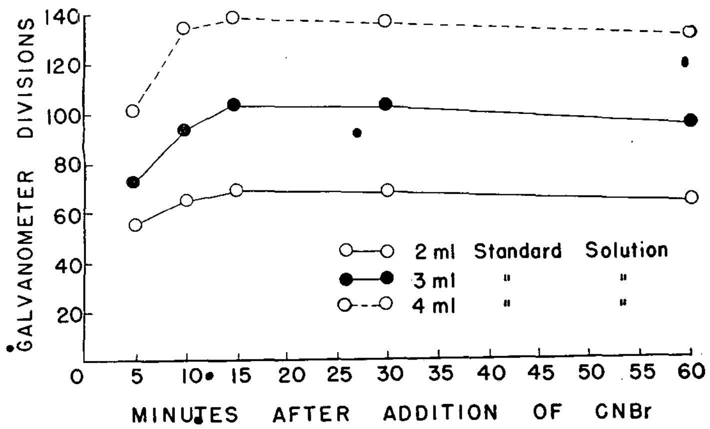

Figure 6. Stability of Color

Table I. Reproducibility of Results

	Tablets		Ampoules	
Assay	Found, mg./tablet	Deviation from mean, %	Found, mg./tablet	Deviation from mean, %
1	26.3	+0.8	51.3	0.0
2	26.4	+1.2	51.4	+0.2
3	26.1	0.0	51.4	+0.2
4	26.1	0.0	50.2	−2.2
5	26.1	0.0	52.0	+1.4
6	25.7	−1.6	51.3	0.0
7	26.1	0.0	51.9	+1.2

Interference of Ingredients in Pharmaceutical Preparations. TABLETS. No ingredients generally used in the manufacture of tablets gave a purple color under the conditions of the test.

AMPOULES. Various ingredients used as preservatives and antiseptics in the usual concentration for ampoule solutions were tested for their possible interference in the reaction. Phenol, *o*-cresol, the esters of *p*-hydroxybenzoic acid, chlorobutanol, and benzyl alcohol did not produce a color when subjected to the test.

It was therefore concluded that the method described can successfully be used for the assay of β-pyridylcarbinol in pharmaceutical preparations.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Alexander Motchane for the spectrophotometric measurements, to Al Steyermark for performing the elementary analyses, and to Morton Schmall for the preparation of the polymethine dye.

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RECEIVED January 21, 1949. Presented before the Analytical Group, AMERICAN CHEMICAL SOCIETY, North Jersey Section Meeting-in-Miniature, January 10, 1949.

NOTES ON ANALYTICAL PROCEDURES . . .

Source of Error in Estimating Small Amounts of Parathion

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IN THE course of work carried out in this laboratory on the analysis for parathion (*o,o*-diethyl *O*-*p*-nitrophenyl thiophosphate) residues on fruits, vegetables, and foilage by the method of Averell and Norris (*1*), a possible source of error was found. Examination of various graces of benzene from several manufacturers showed that there is present in all samples tested, in varying amounts, an impurity which gives a magenta color identical with that given by parathion. Inasmuch as benzene is the solvent recommended for stripping parathion residues from plant material, this solvent may constitute a common source of error.

The average results obtained by analyzing 200-ml. samples of benzene by the Averell and Norris procedure are shown in Table I, expressed in terms of micrograms of apparent parathion.

All readings were taken with a Klett-Summerson photoelectric colorimeter, glass-cell model, with a filter having its transmittance peak at 550 mμ.

Transmittance was set at 100% by use of a blank containing all reagents. This blank did not show any color development.

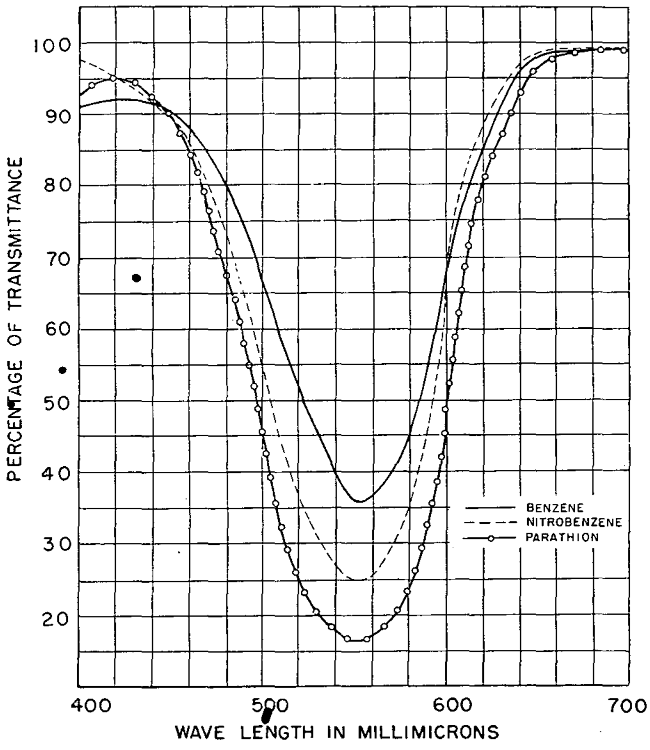

Figure 1. Transmittance–Wave-Length Curves

Table I. Apparent Parathion Content of Benzene

Grade of Benzene	Manufacturer	Apparent Parathion *γ/200 ml.*	Apparent Parathion after Distillation *γ/100 ml.*
C.P. thiophene-free			
1-lb. bottles	1	11.0	0
5-gal. drums	2	93.0	0
1-lb. bottles	3	6.9	0
Purified (99–100%), 5-gal. drums	2	68.8	0
Technical, 5-gal. drums	1	13.3[a]	2.4[a]

[a] This sample was turbid. Actual color was very faint.

Readings were taken 10 minutes after addition of the coupling agent, as in routine parathion determinations. The color, however, continued to deepen and did not become stable for several hours.

Because all samples of benzene developed some color, distillation was tried to remove the impurities. The results obtained after a simple distillation, in which 10% of the benzene was discarded as forerun and tailings, are given in Table I. The impurities were not appreciably volatile on the steam bath.

Further tests indicated that the impurity is probably a nitro aromatic compound, possibly nitrobenzene formed during the acid wash to remove thiophene. For these tests the sample of benzene from manufacturer 2 was chosen, as it had shown the highest percentage of impurity. When the reduction with zinc and hydrochloric acid was omitted, about 20% of the color was developed, an indication that the impurity was primarily a nitro compound with small amounts of an amine.

Nitrobenzene, *m*-nitrophenol, *p*-nitrotoluene, and *o*-nitrotoluene were accordingly tested, and in each case a magenta color was developed which to the eye was identical with the true parathion color. Comparative transmittance–wave-length curves were then plotted for a sample of benzene containing the impurity and for pure nitrobenzene. These data were obtained with a Beckman spectrophotometer, and are shown in Figure 1. Absorption maxima in both cases fall about 555 mμ, which corresponds to the parathion maximum.

Although the results set forth in this paper are not important where small quantities of benzene are involved, the analysis of spray residues requires the use of 200 ml. or more of benzene, and in such cases the error introduced may be appreciable. It would therefore seem that C.P. benzene should be distilled and wherever possible a control analysis be used as recommended (*1*).

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Device for Control of Still-Head Pressures during Isothermal Distillation

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TO DECREASE pressure surges due to intermittant application of cooling to the still head during distillation, Booth and McNabney (*2*) developed the "anticipator," a complex mechano-electrical device, which, as its name suggests, anticipates pressure changes and thereby administers the cooling in a manner that eliminates the surge characteristic. By means of this device distillations utilizing liquid nitrogen as the still-head coolant were accomplished with very small pressure variants.

McNabney (*3*) has outlined a proposed modification to this anticipator for use especially with dry ice–acetone still-head cooling. As the modification had not previously been tried out, it was made in this laboratory and tested. This article describes the construction of this modified anticipator and its operation.

The modified anticipator is made entirely of soda-lime soft glass. All parts are fused together, and the entire piece is mounted on a wooden plank, after which it is fused to the still-head line of the distillation apparatus. Tube *A* is made of 7-mm. glass tubing, *B* and *D* of 20 mm. tubing, and *C* of 5-mm. tubing. Stopcock *E*, used as a throttling valve, is a 2-mm. bore capillary stopcock. Reservoir *F* is connected below *D* by means of an ordinary stopcock. Length *J* is just over 760 mm., while length *T* is slightly greater, so that the bottom of *D* is at least 760 mm. from the center of *B*. At *H* a platinum wire is sealed in and the electrical contact between it and the adjustable probe, *G*, made of 26-gage nickel wire, is made through a 6-volt relay, to energize a small pump which supplies the coolant to the still head.

In operation, the mercury is set by means of the leveling bulb *F*, so that the desired pressure head will result, the level in *B* being near the center. As shown in Figure 1, the mercury level in the three columns is set for atmospheric pressure distillation; for distillation at pressures less than atmospheric the level in the three columns has to be adjusted so that when a vacuum is attained, the level in *A* will be in the middle of *B* and the difference in height between that level and those in *C* and *D* gives the pressure at which distillation is to be carried out. As the still-head pressure increases the mercury will be forced down in *A* and up in *C* and *D*, contact will be made with the adjustable probe, and the still-head cooling agent will be injected into the still head. As the still head cools, the mercury will rise in *A*, owing to dropping pressure, and fall in *C* and *D*, thereby breaking the 6-volt circuit and stopping the cooling pump. At this equilibrium point, *D* which has fallen less rapidly than *C*, forces the mercury in that column up and thereby starts it slightly in advance of the building pressure in *A*. By this means future contacts will be in advance of the full pressure—i.e., anticipate full pressure. In a similar manner *D*, rising more slowly than *C*, will tend to deplete that column and make it fall sooner, anticipating the pressure drop. The rate at which the mercury flows into *D* will necessarily control the sensi-

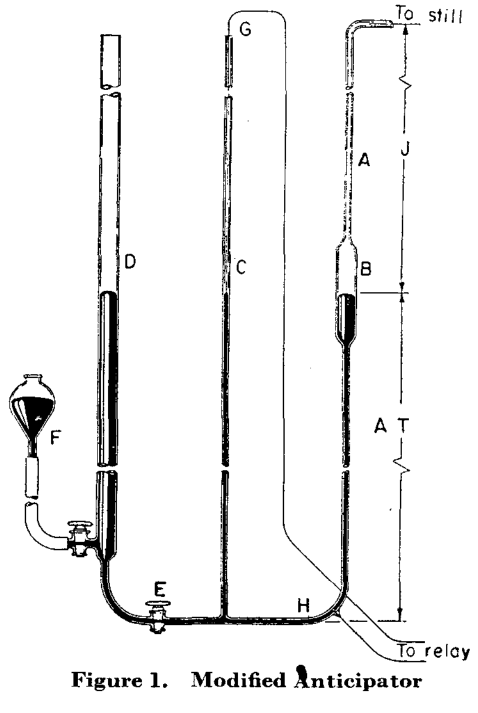

Figure 1. Modified Anticipator

tivity of the device; this rate is controlled by manual setting of stopcock *E*.

This type of regulator has the advantage of easy operation with a positive force opposing the pressure of the still head. Elimination of the automatic stopcock of systems such as those described by Booth and Bozarth (*1*) greatly simplifies the adjustments necessary during distillation. Although the greatest accuracy of pressure control with this type of regulator is in the region around atmospheric pressures, distillations at as low as 250 mm. are accomplished easily. The control at atmospheric pressure for distillations lasting as long as 8 hours, for 50 to 75 ml. of sample, gave control within ±2 mm., and the resulting fractions proved pure.

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An Improved Tensimeter-Still

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IN STUDIES of high-boiling derivatives of lactic acid, need arose for a device which would serve as a simple low-pressure still and also make possible accurate vapor pressure—i.e., boiling point—measurements in the low-pressure region—viz., 0.02 to 10 mm. of mercury.

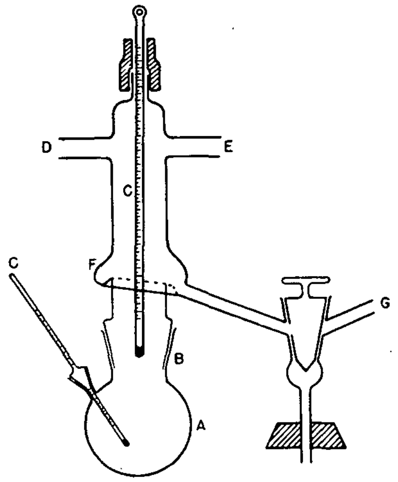

Figure 1. Tensimeter-Still

A. Boiler, 500- or 1000-ml. flask with thermometer side arm
B. 45/50 ground-glass joint
C. Thermometers
D. 12-mm. connection to vacuum gage
E. 12-mm. connection to trap and pump
F. Alembic, formed from 40-mm. tubing
G. Connection to pump

Several types of stills were tried before the design described in this paper was evolved. The improved still has been in use for more than 2 years and has proved reliable and simple to operate over the desired range. It has been used to obtain vapor pressure information on a wide variety of lactic acid derivatives (*10*).

Various means have been used to overcome difficulties in obtaining accurate boiling point measurements at low pressures. The "automanometer still" of Hickman and Weyerts (*8*) exposes the distilland to a relatively high pressure drop between still pot and condenser, for it is precisely by measuring the pressure drop across one or more orifices that this still gains its usefulness. It is operable down to a condenser pressure of perhaps 0.5 mm. of mercury.

On the other hand, the tensimeter-hypsometer of Hickman, Hecker, and Embree (*7*) offers negligible pressure drop but operates on total reflux, so that distillation is impossible. This device, moreover, in the present authors' hands failed to operate satisfactorily above 2 mm., because of excessive bumping. It is stated to be operable down to 0.03 mm. of mercury.

At pressures below 0.01 to 0.03 mm., noncondensable gases diffuse against the distillate vapor stream and vitiate boiling point measurements (*7*). Hence it is futile to attempt such measurements at extremely low pressures, even though some substances can be distilled at such pressures in the tensimeter-still. If at approximately 0.03 mm. the boiling point of the distilland is too high to permit distillation without decomposition, it is advisable to use a molecular still, where permanent gases are pumped away efficiently and distillate molecules have a short path to the condenser.

The tensimeter-still is similar in appearance to commercial equipment that has been described recently (*2*); the latter, however, is recommended for use in the range 0.001 to 0.1 mm., and is stated to have a separation efficiency of less than one theoretical plate.

The present still gains its usefulness from vigorous agitation of the distilland, obtained by rocking the entire still to and fro. The agitation minimizes or eliminates superheating and inhomogeneities in the distilland, and thus facilitates accurate boiling point measurements and equilibrium distillation. The separation efficiency is at least one theoretical plate.

APPARATUS AND METHOD

The tensimeter-still (Figure 1) consists of a 500- or 1000-ml. round-bottomed short-necked flask fitted with a thermometer side arm and a 45/50 ground-glass joint. The joint is attached to a wide upright condenser with an alembic take-off located so that the vapor path is as short as possible. The condenser bears a thermometer and connections for a vacuum gage and pump. To maintain vapor-liquid equilibrium and avoid bumping, the still is suspended from the side tubes near the top of the condenser and rocked to and fro by a crank powered by a stirrer motor. The crank is attached at the ground joint after the flask is connected and the joint is well wrapped with asbestos tape. The joint is lubricated with silicone-type grease to eliminate the sticking sometimes encountered with ordinary grease.

In operation as a tensimeter, the rocker is started, and heat is applied with a mantle heater, while vacuum is maintained. When boiling begins, the connection to the pump is throttled down, and the pressure is adjusted to the desired value by means of a slow leak. Heating is continued until the sample is distilling gently. The heat input must be adjusted so that the difference between liquid and vapor temperatures is a minimum, indicating minimum superheating and pressure drop between flask and condenser. At several millimeters this difference is usually 1° C. or less, but at low pressures may become several degrees. To assist in condensing the vapors, a strip of moist cloth is sometimes wrapped around the condenser. The stopcock makes it possible to operate on total reflux (usually necessary on small samples).

The boiling point is taken as the vapor temperature at the prevailing pressure. To make certain that the system is in equilibrium, the boiling is continued until the vapor temperature remains constant. The pressure is then adjusted to another value, and the process repeated. It is possible to cover the range 0.03 to 10 mm. in a few hours. The data thus obtained are adequate for the preparation of the usual log *P vs.* 1/*T* charts.

For the pressure measurements, a triple-range McLeod gage covering the ranges 0 to 0.2, 0 to 1.0, and 0 to 20 mm. of mercury is used. This gage is useful because of its high precision at lower pressures, where the boiling point changes rapidly with pressure—for example, a pressure change of 0.1 mm. of mercury at 4 mm. changes the boiling point of butyl phthalate about 1° C. At 0.04 mm. a pressure change of only 0.003 mm. changes the boiling point 1° C. Hence if the boiling points are to be correct to 1° C., the pressure gage must have high precision at low pressures; this high precision is obtained with the triple-range gage. To keep condensable vapors out of the gage a trap cooled with solid carbon dioxide is used.

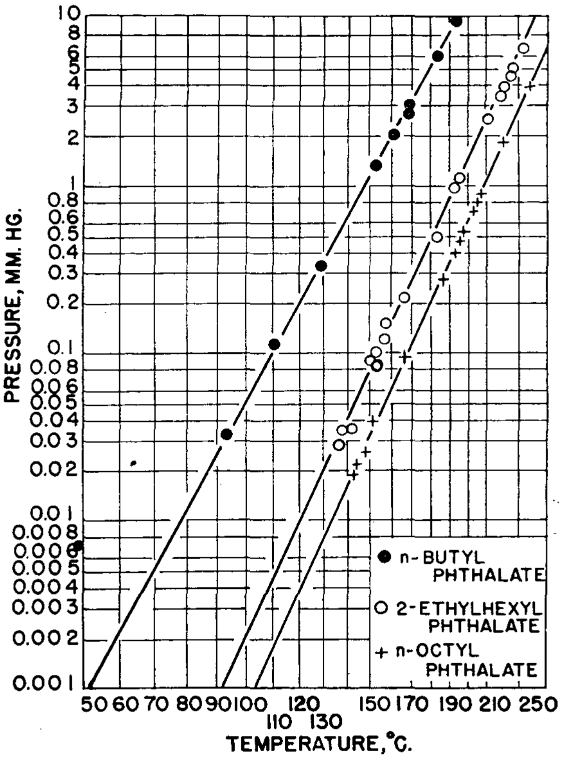

Figure 2. Vapor Pressure of Phthalates

The McLeod gage measures only the pressure of the permanent gases present. If the distilland has been well stripped of solvents and other light fractions, and if it is not undergoing decomposition during distillation, the McLeod measurements are reliable.

RESULTS AND DISCUSSION

The charts give boiling point curves for three common plasticizers (Figure 2) and for two potential plasticizers made from lactic acid (Figure 3), plotted as log P *vs.* $1/T$, ° K. Each curve is linear, and although the experimental points go down only to 0.02 to 0.03 mm. of mercury, the curves have been extrapolated to 0.001 mm. The information in the literature (*1, 3, 5, 7, 11*) on the vapor pressures of these common plasticizers is compared with these data in Table I. The observed values were taken from the boiling point curves, and the literature values from similar published curves. The experimental points define a straight line that is in good agreement with published values; the observed boiling points of 2-ethylhexyl phthalate may be somewhat low, as this was a commercial plasticizer sample. As a measure of the agreement to be expected, it may be pointed out that six publications in the period 1930 to 1946 give the boiling point of butyl phthalate at 3 mm. as 166° to 170° C. (*1, 3–5, 7, 11*).

Extrapolation of the lines in Figure 2 gives 52° and 92° C. at 0.001 mm. for butyl and 2-ethylhexyl phthalates, respectively. The literature (direct determination, *7, 9*) gives 56° and 96° C.

It is thus evident that the tensimeter-still can provide reliable vapor-pressure data over the range 0.03 to 10 mm. of mercury and that a linear extrapolation of considerable usefulness may also be made to 0.001 mm.

Several factors tend to introduce errors into low-pressure measurements of boiling points, especially when the distilland is not adequately agitated: Evolution of vapor begins before the distilland reaches the boiling point, resulting in too low vapor temperature (*6*); the distilland becomes superheated because of poor heat transfer, resulting in too high vapor temperature; and in the distillation of a multicomponent distilland the surface layer becomes leaner in the more volatile components, resulting in nonequilibrium distillation, superheated vapor, and poor fractionation. The first two factors are important in tensimeter measurements because they tend to cause erroneous vapor-temperature observations. Fortunately, they have opposite effects and their net effect is thus reduced. In the low-pressure distillation of a mixture, all three factors must be considered, though it is believed that the first is of minor importance. The effects of the second and third factors, however, are additive and may be large, thus causing serious errors in observed boiling points. Their effect is minimized or virtually eliminated, however, by vigorous agitation of the distilland throughout the distillation. This is conveniently accomplished in the tensimeter-still by the reciprocating motion of the still imparted by the motor-activated crank. It is believed that the satisfactory results obtained in the present work were possible with equipment of this general type only because efficient agitation of the distilland was achieved.

The agreement of these tensimeter data with vapor-pressure information obtained by other means [pendulum tensimeter (*7*), direct weighing and molecular dew (*9*)] indicates that a recent suggestion that "low pressure tensimeters relying on residual gas to measure the vapor pressure must be highly inaccurate in this range (0.15 mm.)" (*6*) is hardly valid. This suggestion was made on the basis of an observation during a distillation under conditions much different from those in the tensimeter-still.

As a still, the device is useful in the preparation of many high-boiling compounds, provided a low fractionating efficiency is sufficient—that is, the boiling points of the fractions to be separated are fairly wide apart. The preparation of dicarboxylic acid esters of various lactates (*10*) may be used to illustrate the use of the tensimeter-still in distilling organic compounds. After the crude

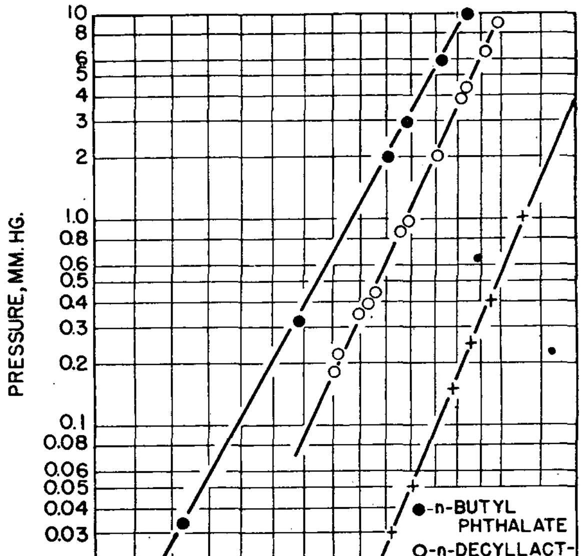

Figure 3. Vapor Pressures of Lactic Acid Derivatives

Table I. Comparison of Boiling Points with Published Values

	Boiling Points at Various Pressures					
	4 mm. of Hg		0.4 mm. of Hg		0.04 mm. of Hg	
Plasticizer	Obsd.	Lit.	Obsd.	Lit.	Obsd.	Lit.
Butyl phthalate	174	172	132	133	96	97
2-Ethylhexyl phthalate	220	225	177	179	141	141
n-Octyl phthalate	237	238	191	192	153	...

reaction mixture has been obtained, the lower boiling reagents are distilled from the product in a Vigreux still, and then the residue is distilled at low pressure in the tensimeter-still. The product thus obtained may be redistilled to obtain vapor-pressure data; if the reaction is clean-cut, however, the distillation and vapor-pressure determinations may be effected simultaneously. The reliability of the boiling point measurements indicates that the tensimeter-still has a separating efficiency of at least one theoretical plate.

ACKNOWLEDGMENT

The authors wish to thank Harry John of this laboratory for his suggestions and for the construction of the McLeod gage.

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Rapid Colorimetric Estimation of Phenol

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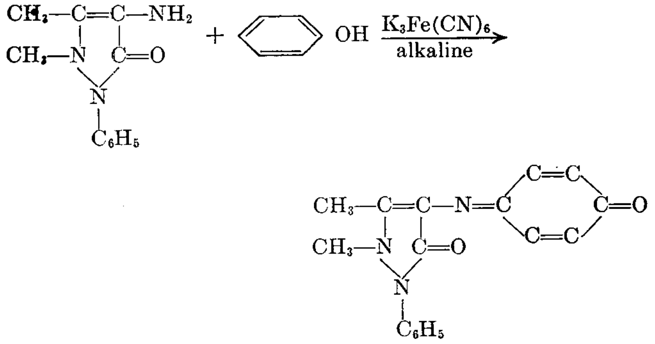
A PAPER describing the use of 4-aminoantipyrine as a reagent for the quantitative estimation of phenolic fungicides (*5*) prompts the report of a similar procedure for the estimation of phenol. The procedure, which has been in use in this laboratory for nearly 3 years, was developed when a method for the very rapid determination of small quantities of phenol in salt brines was required. In the range 0.2 to 2 p.p.m., phenol can be estimated either in water or in concentrated salt brines within ±10%.

A number of procedures have been developed for the determination of phenol in both low and high concentrations. In low concentrations the methods of Gibbs (*4*), Folin and Dennis (*3*), Houghton and Pelly (*6*), and Stoughton (*8*) have found the most use. Recently Lykken, Treseder, and Zahn (*7*) have modified Stoughton's procedure and applied it to the determination of phenols in petroleum and allied products. The main advantages of the present method are the ease and rapidity with which a determination can be made and the fact that it can be applied without modification to a concentrated salt brine containing only a few tenths of a part per million of phenol.

The color reaction is based on the condensation of 4-aminoantipyrine with phenols in the presence of alkaline oxidizing agents. The formation of the antipyrine dyes which result has been represented by Emerson (*2*), who discovered the reaction, as taking place according to the following example:



REAGENTS

4-Aminoantipyrine. The compound, which is a stable material, was prepared from antipyrine according to the procedure outlined by Eisenstaedt (*1*). A 2% stock solution was prepared by dissolving the purified compound in distilled water.

Potassium ferricyanide, 2% aqueous solution.

Ammonium hydroxide, 2 *N* solution.

Phenol. Freshly distilled, water-white phenol was used to prepare the standard solutions containing 10 and 100 p.p.m.

PROCEDURE

Transfer 50 ml. of the sample to a 50-ml. tall-form Nessler tube and add 0.3 ml. of the 2% 4-aminoantipyrine solution and 1 ml. of 2 *N* ammonium hydroxide. Mix the contents of the Nessler tube thoroughly, add 1 ml. of the 2% potassium ferricyanide solution, and mix again. Compare the reddish color produced by the sample in a Fischer nesslerimeter or similar suitable apparatus with standards prepared from phenol solutions of known concentration. Simple visual comparison of the colors can also be employed, with some sacrifice in accuracy.

Where the phenol content of a salt brine is to be determined, sodium chloride may be added to the standard phenol solutions, so that the known and unknown solutions contain approximately the same amount of sodium chloride. However, in most cases no difference in the intensity of the colors produced in salt or water solutions is noted and this added precaution is not necessary. Both in the presence and in the absence of salt in the solutions, the colors develop almost immediately, so that the samples need not stand more than 2 minutes before the readings are taken.

DISCUSSION OF RESULTS

With the apparatus used, the most sensitive color range for a 50-ml. sample was found between 0.2 and 2 p.p.m. of phenol. Samples of greater concentration may be used by properly diluting them before the analysis. Table I shows typical results.

Assuming an excess of 4-aminoantipyrine, the color reaction with phenol will occur over a rather wide range of concentrations for the base and oxidizing agent. However, maximum sensitivity is dependent upon the amount of each reagent used. The color reaction requires that the solution be slightly basic; and, although sodium hydroxide or sodium carbonate can be used,

Table I. Phenol Content of Aqueous Solutions

Sample	Phenol Present *P.p.m.*	Phenol Found *P.p.m.*	Error %
1	3.3	3.1	6.1
2	2.2	2.1	4.5
3	1.3	1.3	0.0
4	8.8	8.1	8.0
5	4850	4600	5.2
6	4850	4700	3.1

Emerson found that ammonia gave a more sensitive reaction with phenol (*2*). Gottlieb and Marsh (*5*) found that deviations of 0.5 pH unit in either direction from the limits they specified (10.4 to 10.6 pH) caused changes in both the intensity and stability of the colors produced with the phenolic fungicides which they studied. If the solution to be analyzed is acid or alkaline, it should be carefully neutralized before the analysis is attempted.

Standards can be used for 2 to 3 hours without serious variations in the intensity of color, although a very gradual change in the shade of the color may be noted. For precise work the standards should be made up within 1 to 1.5 hours of the time that they are to be used. Direct sunlight or strong artificial light has a bleaching effect on the color.

The reaction is a general one for phenols. Cresols, xylenols, naphthols, etc., each produce a characteristic color. Compounds like the above, if present, will interfere with the phenol determination.

It is imperative that the solutions be clear and colorless. Where the solutions are cloudy filtration can be used. For colored solutions the phenol can be distilled from the solution before analysis; the use of colored filters has been suggested but has not been studied. The use of the spectrophotometer is being studied at present. The use of this instrument might also prove useful in the analysis of mixtures of different phenols, each with its characteristic color. Iron salts in the brines caused interference due to reaction with the potassium ferricyanide. This difficulty can be obviated by substituting potassium persulfate for potassium ferricyanide as the oxidizing agent, but the colors develop at a slower rate and are not as stable. Potassium persulfate is inferior to potassium ferricyanide where rapid control tests are being made.

In practice, the method has been used extensively for quickly determining if the phenol content of a salt brine was below certain limits. In this case two standards were prepared, one representing the tolerable limit and one containing a slightly higher concentration of phenol.

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Application of Thermistors to Cryoscopy

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TEMPERATURE-sensitive elements available for cryoscopic measurements include resistance thermometers, thermocouples, and liquid-filled thermometers. The relative advantages of resistance thermometry with respect to sensitivity, precision, stability, and absence of temperature lag are well known. A recent article (*7*) has shown the adaptability of platinum resistance thermometers to automatic recording devices for industrial use. It was noted that high cost, fragility, size, and extremely sensitive electrical measuring equipment have been factors preventing the widespread usage of platinum resistance thermometers. It is the aim of the authors to bring attention to the use of Thermistor temperature-sensitive elements (*1*, *4*, *5*, *8*) in freezing point determinations. Despite publication in the Instrumentation Column of this journal (*5*) of information concerning the development and suggested uses of these thermally sensitive electrical resistors, their application to cryoscopic techniques, especially at low temperatures, does not appear to have received the attention merited.

Subsequent to the preparation of this paper, an article by Richards and Campbell (*6*) on the use of Thermistors for measuring the freezing point of solutions and soils, was called to the authors' attention. Richards and Campbell used the same type of Thermistor element for freezing points in the region of 0° C. They measured a standard deviation of 0.0015° C. in seven determinations of the ice point over a period of one week, and a smaller deviation with another Thermistor unit of the same type in four measurements during a 2-month period. A calibration curve was constructed for the 0° to −5° C. range using a standard thermometer.

Thermistors of extremely small size can be obtained and are thus available for measurement of semimicroquantities. They have been used to measure intravenous blood temperatures of animals (*2*). Although small in size, the elements are inexpensive and rugged.

The specific property of the Thermistor which recommends it for cryoscopy is the large, negative, temperature coefficient of resistance. The temperature coefficient for a representative type Thermistor is −4% per ° C. at 25° C. The resistance of a Type 14B Thermistor used in the authors' laboratory is 2235 ohms at 25° C., indicating a change of 89 ohms per degree at

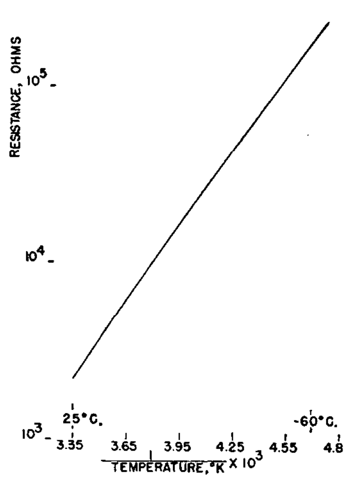

Figure 1. Logarithm of Resistance of Type 14B Thermistor as a Function of Inverse Absolute Temperature

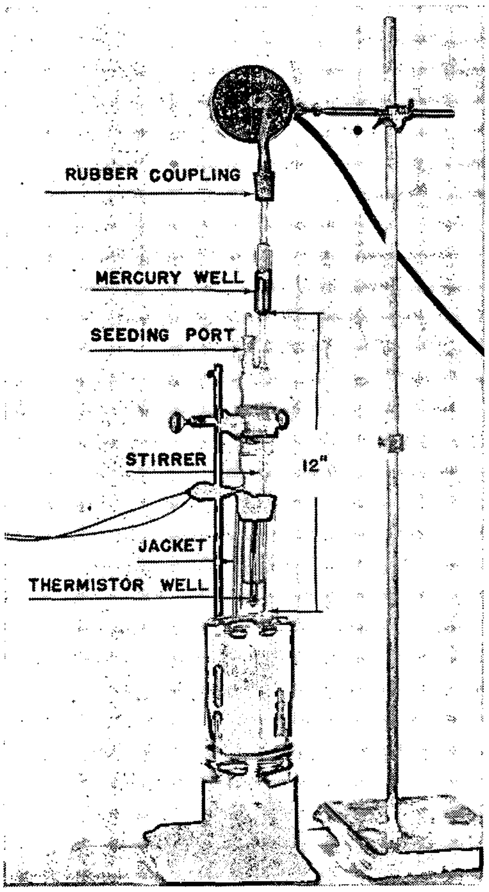

Figure 2. Freezing Point Apparatus

this temperature. As the temperature coefficient is negative, and the plot of logarithm of resistance against reciprocal of absolute temperature is approximately a straight line, both the absolute and relative sensitivities increase as temperature decreases. In the neighborhood of −30° C. the Type 14B has a resistance of approximately 3 × 10^4 ohms, and the temperature coefficient is almost 2 × 10^3 ohms per ° C. At −60° C. the resistance value approaches 2 × 10^5 ohms, and the temperature coefficient approaches 10^4 ohms per ° C., compared to a temperature coefficient of 0.1 ohm per ° C. for platinum at all temperatures. The resistance value of a typical platinum thermometer is 25 ohms at 0° C. It can readily be seen that lead and contact resistance corrections are unimportant in resistance measurements with a Thermistor, and ordinary electrical circuit components can be used in obtaining data of precision equal to those attainable with platinum resistances using the most sensitive accessories.

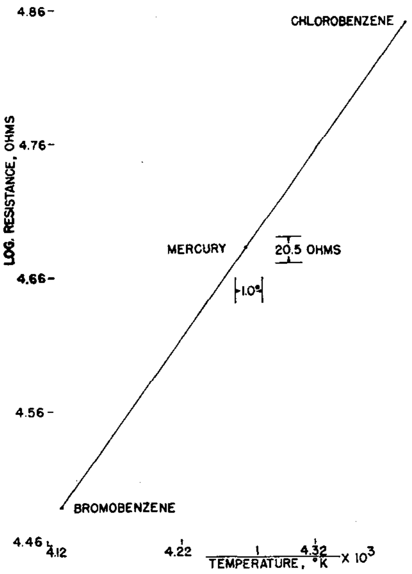

Figure 3. Logarithm of Resistance–Inverse Absolute Temperature Relation for Short Temperature Range

THERMISTOR RESISTANCE THERMOMETRY

The properties and uses of Thermistors have been discussed in detail (*1*, *4*, *8*). This paper describes the experimental equipment and the results obtained in the application of one type of these versatile instruments for the accurate determination of freezing points over an extended range at low temperatures.

The problem at hand was to construct a simple cryostat for determinations in the range from room temperatures to −60° C. which would be capable of precision to at least 0.05° C. Thermistors were considered for reasons already mentioned, and the Type 14B was selected as having the optimum resistance values for measurement on an ordinary Wheatstone bridge circuit (see Figure 1). The Type 14B is one of several types designed by the Western Electric Company primarily for thermometry; the Thermistor bead is enclosed in the slightly enlarged end of a solid glass cylinder having two tinned wire terminals brought out axially at the opposite end. The glass cylinder is approximately 2 inches (5 cm.) long and 0.10 inch in diameter. The thermal time constant of this element is about 2 seconds when immersed in liquid. The leads were soldered to lengths of No. 29 B. & S. copper wire, insulated, and placed in a thermometer well in the cryostat (Figure 2). The Thermistor bead was covered by 1 ml. of mercury in the bottom of the well to increase heat conduction and reduce time lag.

The 8-ml. liquid samples were agitated by a motor-driven glass stirrer having a displacement of 2 cm. and operated at 200 ± 20 strokes per minute. A Queen-Gray No. 8529, 4-decade Wheatstone bridge and a Leeds & Northrup Type 2420C lamp-scale galvanometer with a sensitivity of 0.014 μa. per mm. were used to measure resistance. The maximum operating voltage for the bridge was 1.5 volts in order to minimize heating of the thermal element. With 10,000-ohm fixed bridge resistance arms, the temperature rise of the element at 0° C. (calculated on the basis of the reported heat dissipation constant of 5 mw. per ° C.) is 0.011° C. At −30° C. the temperature rise is less than 0.01° C. Freezing of the mercury in the Thermistor well did not interfere with freezing point determinations. The small volume and low heat of fusion of the mercury allow the heating effect of its freezing to be dissipated quickly. Samples freezing more than 1° C. below mercury were run without difficulty.

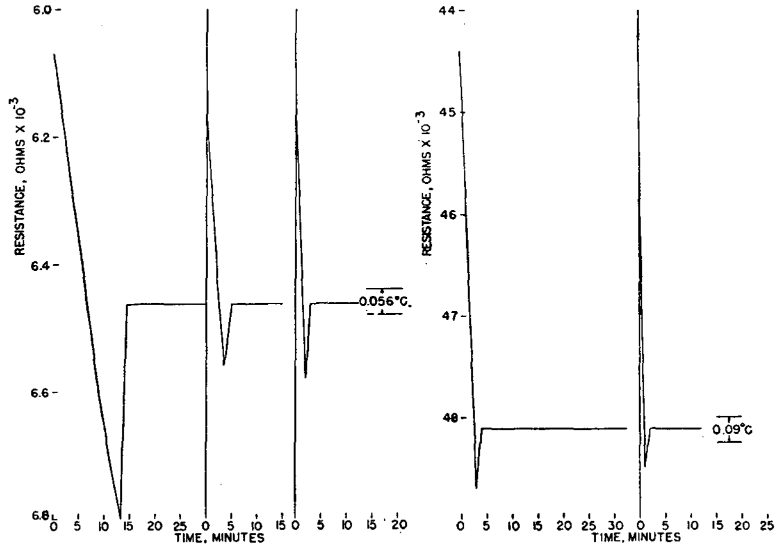

Figure 4. Freezing Curves of Water (*Left*) and of Mercury (*Right*)

The Thermistor element was calibrated at the freezing points of water, carbon tetrachloride, bromobenzene, mercury, chlorobenzene, and chloroform. Points above 0° C. were obtained by immersing the cryostat in constant-temperature water baths regulated to ±0.02° C.

The distilled water was boiled before using, the bromobenzene was a Bureau of Standards freezing point sample, the chlorobenzene was redistilled from C.P. material, and the mercury was triple-distilled material washed successively with nitric acid, potassium hydroxide, and water, and vacuum-dried. The carbon tetrachloride and chloroform samples were C.P. grade, used as received.

The linearity of the $\log R$ vs. $1/T$ relationship over short temperature ranges is indicated in Figure 3. Where resistance readings are made to ±1 ohm, and temperature readings accurate to 0.05° C. are desired, a linear interpolation over a 15° C. range is permissible. Figure 4 gives time-temperature curves obtained with water and mercury, which indicate the precision of the data obtained to be of the order of 0.01° C. or better. The abscissa for each curve in Figure 4 is started from zero to avoid confusion on the time base. The curve illustrated in each case is only that portion of the run immediately adjoining the crystallization points and has no reference to the time at which the experiments were started. The accuracy of measurement obviously depends upon the care observed in calibration. The three determinations with water were made over a 2-month period, and the freezing points agree within 1 ohm. The temperature coefficient of resistance at 0° C. is 250 ohms per ° C., indicating a difference of less than 0.01° C. and good stability of the Thermistor element.

FURTHER APPLICATION OF THERMISTORS TO CRYOSCOPY

An obvious application of Thermistors is to the analysis of mixtures where very small temperature differentials must be measured. This technique has been described by Glasgow, Streiff, and Rossini (*3*) and elsewhere.

An indirect application of Thermistors to freezing point measurement in this laboratory was the use of a Type V611 disk Thermistor as a temperature regulator in the water bath used to calibrate the 14B above 0° C.

ACKNOWLEDGMENT

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Spectrochemical Determination of Lanthanum in Praseodymium Metal

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IN THE course of an investigation of the properties of the rare earth elements at low temperatures, a study was made of a sample of praseodymium reported by the supplier to contain 1.3% iron and to be "essentially free from other associated metals." Qualitative spectrographic studies, however, indicated the presence of more than a trace of iron, calcium, silicon, aluminum, neodymium, and, especially, lanthanum. The quantitative determination of certain of the rare earths, including neodymium, can be conveniently made by spectrophotometric means. However, this method is not applicable in the case of lanthanum, because its ion exhibits no suitable absorption band. The present paper describes a spectrographic method of analysis for lanthanum.

An extensive review of the literature has appeared in a recent paper (*3*).

It was decided to use the copper spark method (*4*) of spectrographic analysis, because it requires only a very small sample and avoids the formation of cyanogen bands which mask many important lanthanum lines.

An obvious method of attack would have been to prepare a series of mixtures containing known amounts of lanthanum and praseodymium and to compare a solution of the praseodymium metal with this series. Although both pure lanthanum and praseodymium oxides were on hand, only a small amount of the latter was available, and it was thus necessary to use it sparingly. Furthermore, preliminary work here had indicated that the lanthanum content in the sample was so high that the praseodymium might not serve as a satisfactory internal standard. Therefore, it was decided to add an internal standard. The choice of manganese for this purpose was based on the usual considerations (*1*).

The possibility of interference was carefully considered in the light of the qualitative spectrographic investigations already made. Using the M.I.T. Wave Length Tables (*5*), it was found that the 3438.974 Å. Mn(II) line is free of interference from the elements known to be present in the praseodymium sample. The 4086.714 Å. La(II) line lies between the two praseodymium lines at 4086.24 Å. and 4087.206 Å. However, these do not interfere, because they are far enough from the lanthanum line to be resolved by the spectrograph used.

EXPERIMENTAL METHOD

Equipment. The equipment used included a grating spectrograph, multisource unit, and comparator-densitometer, equipped with a voltage regulator, supplied by the Applied Research Laboratories.

Photometric Procedure. Eastman spectrum analysis No. 2 film was processed in the ARL-Dietert film-developing machine maintained at 70° F. Films were developed with mechanical agitation for 4 minutes in Eastman D-19 developer, immersed in a 3% acetic acid solution short stop for 30 seconds, and fixed in Eastman x-ray fixing bath for 4 minutes.

The film was calibrated by use of the two-step filter, preliminary curve method (*2*). A gamma of 1.4 was obtained. Background corrections were made by subtracting the background intensity from the total intensity (*6*).

Preparation of Solutions. Stock solutions of lanthanum chloride and manganese chloride were prepared containing 0.00414 gram of lanthanum and 0.1316 gram of manganese per ml. The lanthanum chloride solution was prepared by dissolving in 0.5 N hydrochloric acid a weighed quantity of pure lanthanum oxide which had been previously ignited to constant weight to remove moisture and carbon dioxide. [The lanthanum and praseodymium oxides used in these studies were obtained from Adam Hilger and Sons, Ltd. They were reported to be of high purity (>99.75%). Qualitative spectrographic analysis confirmed the general correctness of these analyses.] The manganese chloride solution was made by dissolving a weighed quantity of Baker's C.P. manganese chloride tetrahydrate in water.

Preliminary studies indicated that the praseodymium sample might contain 30 to 40% lanthanum. Accordingly, four standards were prepared to cover the range from 28.2 to 42.3% lanthanum in the praseodymium sample under study. These standard lanthanum-manganese solutions were prepared by adding to 1 ml. of internal standard 0.40, 0.50, 0.55, and 0.60 ml., respectively, of the lanthanum chloride solution. The resulting mixtures were first diluted to 15 ml. with water and then mixed with an equal volume of 0.5 N hydrochloric acid solution. These dilutions were used because it was found that with more concentrated solutions much of the sample was often lost during excitation, owing to sputtering. The excess hydrochloric acid seemed to improve the reproducibility. The only reason deemed assignable to this phenomenon is that the hydrochloric acid may remove whatever oxide exists on the copper electrode and thus may decrease any tendency of the spark to become localized.

The stock solution of the praseodymium sample was made by dissolving a sufficient quantity of impure praseodymium metal in 0.5 N hydrochloric acid to yield a solution containing 0.0147 gram of the sample per ml. The solution used for analysis was prepared by adding 0.40 ml. of the stock solution to 1 ml. of the internal standard solution, diluting to 15 ml., and mixing with an equal volume of 0.5 N hydrochloric acid.

The electrodes were cut from 0.25-inch copper rods, as described by Fred, Nachtrieb, and Tomkins (*4*). To decrease oxidation before use, the electrodes were stored in a jar with their tips immersed in benzene and were always used within 12 hours after they were cut.

A hot plate was used to evaporate the solutions on the electrodes. It was found that about 0.0286 ml. was required to cover the tip of an electrode.

EXPERIMENTAL RESULTS

With each of the four standard lanthanum-manganese solutions, 21 spectra (three films containing seven each) were made, and the ratio of intensities of the 4086.714 Å. La(II) line to the 3438.974 Å. Mn(II) line was determined in each case. The average value of this ratio was found for each standard solution and a log-log plot of the results made. The working curve so obtained was linear.

From a determination of the intensity ratio for a solution of the praseodymium metal containing a known amount of added manganese chloride, the lanthanum content of the praseodymium can be calculated with the help of the working curve. Treating an average of five repeat determinations as a single result, the lanthanum contents found were: 38.5, 37.4, 39.7, 37.5, 33.7, and 35.3%, or a mean value of 37.0%. The average deviation from the mean corresponds to a variation of 1.7% in the lanthanum content of the praseodymium metal, or 4.6% in the analysis of the sample.

As a check of the accuracy of the analysis, a synthetic known to contain 38% lanthanum—i.e., very nearly the same amount as that found in the sample—was made up from pure lanthanum and praseodymium chlorides. This solution differed from that containing the praseodymium metal sample only in being free of minor impurities such as iron, neodymium, etc. Portions of the solutions of the synthetic sample and the praseodymium metal sample, each containing the same percentage of manganese as that used in the analysis of the praseodymium metal, were compared by photographing spectra of both on the same film. Seven exposures were made for each sample. The ratio of the intensities of the 4086.714 Å. La(II) line to the 3438.974 Å. Mn(II) line was 0.56 ± 0.04 for the synthetic sample and 0.55 ± 0.05 for the metal sample. This agreement was taken as proof of the essential correctness of the analysis.

Subsequent chemical and spectrophotometric analyses of the impure praseodymium metal gave the following results: iron 1.6%, silicon 0.9%, praseodymium 54.8%, neodymium 3.5%, unaccounted for 39.2%. The rare earths were determined by the spectrophotometric method of Rodden (*7*), care being taken first to reduce the iron to the ferrous state. The major portion (>90%) of the unaccounted for balance was found to precipitate as an oxalate in acid solution. Because spectrophotometric analysis failed to show absorption bands for any other rare earth in the range 380 to 1000 mμ, and qualitative spectrographic studies had shown gadolinium to be absent, it was presumed that this unidentified balance was lanthanum. This is in general agreement with the spectrographic results.

ACKNOWLEDGMENT

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Determination of Carbon by Wet Combustion

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FOR situations where a Van Slyke manometric apparatus cannot be provided, a scheme has been devised to use the Van Slyke–Folch combustion mixture (*3*) in the analysis of organic solids and relatively nonvolatile liquids. A sample of 10 to 12 mg. is heated with combustion solution in a stream of carbon dioxide–free air, and the carbon dioxide evolved is absorbed in sodium hydroxide. The carbonate thus formed is precipitated as barium carbonate and determined acidimetrically. A determination requires about 45 minutes.

The efficiency of the apparatus has been tested by analyzing pure samples of several compounds. The results were accurate to ±0.05 mg. of carbon. Halogens, nitrogen, and sulfur do not interfere; however, compounds that decompose to release hydrocyanic acid will give low carbon values.

In comparison with other methods for the determination of carbon, this procedure has the advantage of an inexpensive apparatus which can be assembled quickly and does not require much space. In addition, only one weighing is required for each determination. The Van Slyke–Folch combustion solution has been used in other procedures (*1*, *2*), but in these the carbon dioxide or barium carbonate has been determined gravimetrically.

REAGENTS

Combustion Solution. Pour 60 ml. of 30% fuming sulfuric acid into a flask containing 40 ml. of 85% phosphoric acid, and add 10 grams of chromium trioxide and 1 gram of potassium iodate. Heat to 140° to 150° C. and swirl or stir for 1 to 2 minutes. Cool and store in small glass-stoppered bottles. If fuming sulfuric acid is not available, use 20 grams of phosphorus pentoxide, 85 ml. of 95% sulfuric acid, and 15 ml. of 85% phosphoric acid with the stated quantities of chromium trioxide and potassium iodate.

Sodium Hydroxide. Dissolve 5 grams of sodium hydroxide in 20 ml. of water, add 1 ml. of 1 F barium chloride, and centrifuge. Dilute to 250 ml.

Barium Chloride Reagent, 1 F in barium chloride and 0.001 F in hydrochloric acid.

APPARATUS

The Kraissl tube, A, shown in Figure 1 is packed with 20 to 30-mesh Ascarite or soda lime, and 95% sulfuric acid is placed in the bubble counter, B. The 15-ml. centrifuge tube, M, is connected to head D by means of a 14/20 ₸ joint. The stopcock, E, is lubricated with phosphoric acid, but grease may be used in the other stopcocks. A plug of glass wool moistened with distilled water is placed in tube F to remove any hydrofluoric acid. The disperser, K, is made from a short length of Zircofrax tubing (12-7P.89, Carborundum Company) sealed to Pyrex tubing and closed at one end with a glass plug. The disperser inlet tube should be moistened with a drop of glycerol, so that it will move freely in the rubber stopper. The bottle, *H*, contains 0.5 *F* sodium hydroxide which is protected by a soda-lime tube. A 15-ml. centrifuge tube serves as the absorber, *G*.

In Figure 2 the soda-lime tube, *S*, is made from 7.5 cm. of 10-mm. tubing.

PROCEDURE

Weigh a 10- to 12-mg. sample into tube *M*. Moisten the ground surface on head *D* with 85% phosphoric acid, then attach *M*. Before attaching tube *G* to the assembly, allow 2 to 3 ml. of the sodium hydroxide to flush out the section below pinchclamp *J*. By adjusting stopcock *L* maintain air flow through the apparatus at 3 to 4 bubbles per second. After 5 minutes open *J* and slowly admit sodium hydroxide to within 2 to 3 cm. of the top of *G*. Pour 5 to 6 ml. of combustion solution into cup *N* and reduce the air flow to 1 to 2 bubbles per second. Stopcock *C* must be closed before stopcock *E* is opened to admit the combustion fluid to tube *M;* otherwise bubbles of the viscous liquid will be formed in *D* and combustion mixture will be blown up into the outlet tube. When most of the fluid has entered *M*, close stopcock *E* and open stopcock *C*.

With the air flow at 3 to 4 bubbles per second, warm the upper portion of the solution in *M*, then the lower part. Do not apply the heat strongly in one spot, as the reaction may proceed suddenly and evolve a large quantity of carbon dioxide. When gas evolution begins, observe bubble chamber *B*. If reverse flow starts, close *C* for a few seconds to prevent flow of carbon dioxide into *A*. Continue heating until the solution has boiled vigorously for 5 minutes. Cease heating and fill *M* to within 2 to 3 mm. of the inlet tube with 95% sulfuric acid. Close *C* during this process. Do not admit air through *E* while adding the acid. After reopening *C*, allow the air flow to continue at 3 to 4 bubbles per second for 3 minutes. Slide the disperser inlet tube up through the stopper until the disperser is well above the solution, then close stopcock *L*. Detach the rubber tubing from the disperser inlet tube, and fill this tube with carbon dioxide-free distilled water by means of a long capillary dropper. Carefully draw most of the water through the disperser but do not admit air. After removing the stopper, quickly rinse the bottom of the stopper, the outside of the disperser, and the walls of tube *G* with carbon dioxide-free distilled water.

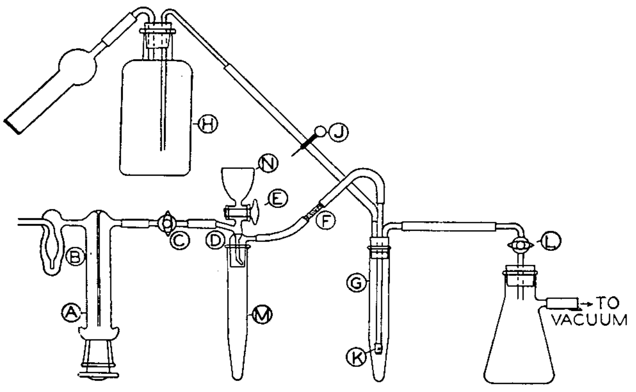

Figure 1. Apparatus for Determination of Carbon

With a dropper, inject 1 to 1.5 ml. of 1 *F* barium chloride reagent into *G*. If the reagent is injected forcefully, stirring is not necessary. Immediately stopper the tube with a rubber stopper which is fitted with a piece of 0.5-mm. capillary tubing and place it in a bath of boiling water for 3 minutes. Replace the stopper with a rubber centrifuge tube cap, then centrifuge until the solution is clear.

Moisten tube *R* of the vacuum siphon so that it moves freely through the rubber stopper. Mount centrifuge tube *G* in a buret clamp and replace the cap with the stopper assembly of the vacuum siphon. While applying suction push tube *R* down as the liquid level recedes until the tip is just above the precipitate.

Table I. Results of Carbon Analysis by Wet Combustion Method

	Milligrams of Carbon	
	Present	Found
Sodium oxalate	2.92	2.95
	2.15	2.16
	1.87	1.84
	1.86	1.88
	1.69	1.68
	1.88	1.92
Potassium hydrophthalate	4.51	4.54
	6.29	6.26
	5.33	5.33
	5.61	5.61
β,β′-Dichloroethyl sulfide	2.73	2.76
Adipic acid	5.84	5.85
	5.38	5.43
	5.20	5.20
	5.47	5.51

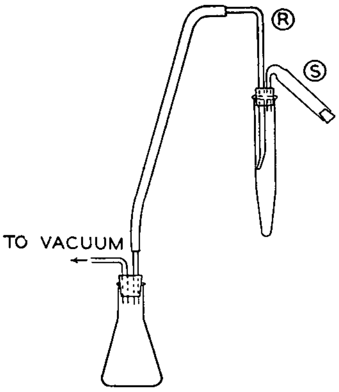

Figure 2. Vacuum Siphon

Add 1 drop of 0.2% phenolphthalein, then add 0.05 *F* hydrochloric acid, dropwise and with stirring, until the color is light pink. Add 8 to 10 drops of 1 *F* barium chloride reagent and 1 drop of 1% Aerosol OT. Stir; if necessary, add 0.05 *F* hydrochloric acid until the solution is colorless. Centrifuge; then discard the solution with a dropper.

Rinse down walls of the tube with 1 to 2 ml. of boiled distilled water. Add 1 drop of phenolphthalein and 3 to 4 drops of 1 *F* barium chloride. Decolorize, if necessary, with 0.05 *F* hydrochloric acid, then add 1 drop of 0.1% methyl orange. Add standard 0.2 *F* hydrochloric acid with stirring until a pink color appears. Heat the tube in a water bath, stir, and add about 0.1 ml. excess acid after a permanent pink is obtained. Transfer the solution to a 50-ml. flask and boil gently for 1 minute. Cool and titrate to the phenolphthalein end point with standard 0.05 *F* sodium hydroxide.

NOTES AND DATA

Tube *F* may be omitted if it is known that the sample does not contain fluorine. A clean tube and filling should be used for each run because absorbed sulfur trioxide fumes reduce the efficiency of absorption of hydrofluoric acid.

Considerable time is saved by neutralizing the base adsorbed on the barium carbonate instead of washing with water. An excess of barium chloride reagent is provided to prevent solution of the precipitate during the addition of acid. The time of neutralizing the adsorbed base and washing the precipitate should not exceed 3 to 4 minutes; otherwise an appreciable amount of carbon dioxide may be absorbed. The final titration is made to a phenolphthalein end point, inasmuch as the solution is initially adjusted to that pH.

Blank analyses gave less than 0.01 mg. of carbon.

Combustions performed with ammonium chloride and with biuret proceeded smoothly and produced no unusual or sudden gas evolution.

Some typical analytical results are given in Table I.

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Portable Electronic Instrument for Polarographic Measurements and Amperometric Titrations

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SEVERAL manual arrangements have been described (*1, 2, 4–6*), in which polarographic currents are measured directly by means of a galvanometer. However, because of the presence of a galvanometer, these arrangements lack the ruggedness indispensable in industrial routine work. Polarographic currents are also determined by measuring the potential drop on the terminals of a known resistance connected in series with the polarographic cell (*3, 5*). The potential drop is measured with a potentiometer and a sensitive galvanometer is used as zero instrument. The principle of this method of measurement is involved in the instrument described in the present paper; the potential drop is determined by means of a vacuum tube voltmeter. The output meter of the vacuum tube is a commercial microammeter which can be calibrated in current units. This arrangement thus combines ruggedness with the rapidity of a direct-reading method.

According to a procedure previously suggested (*7*), one determines a diffusion current by taking current readings at two preselected differences of potential, V_1 and V_2. Voltage V_1 corresponds to a point in the lower flat part of the investigated wave, voltage V_2 to a point in the upper flat part of the wave. The variation of the polarographic current between V_1 and V_2 is taken as equal to the diffusion current of the wave being investigated.

CIRCUIT DIAGRAM AND OPERATION

The schematic diagram of the unit is shown in Figure 1.

The direct current power is supplied by a transformer-rectifier unit having its output voltage stabilized by a two-stage gas tube regulator.

The preselected differences of potential to be applied to the polarographic cell are derived from a set of six potentiometers $(r_5 + r_6), (r_7 + r_8) \ldots\ldots (r_{15} + r_{16})$. Each set, with coarse and fine

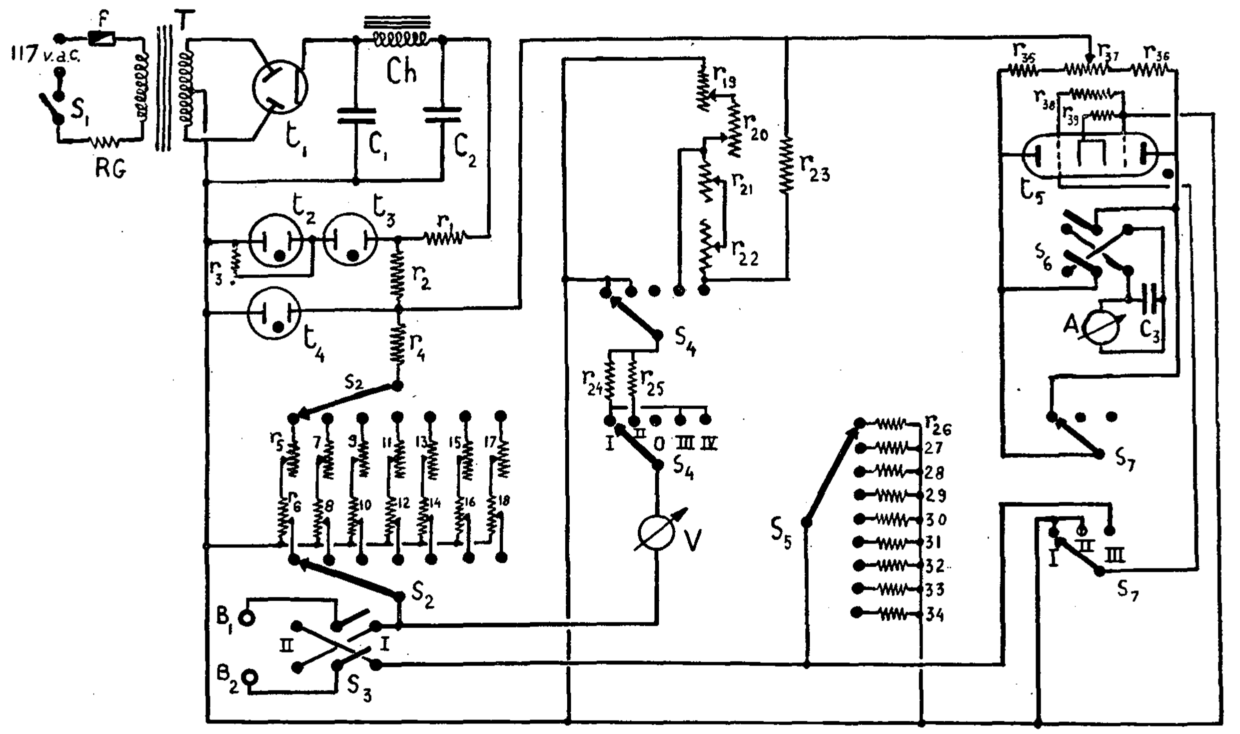

Figure 1. Schematic Diagram of Instrument

A. 0–25 microammeter (Simpson)
B_1B_2. Binding posts (B_2 connected to dropping mercury electrode)
Ch. Filter choke, 10 henrys, 125 ma.
C_1, C_2. Electrolytic condensers, 10 + 10 microfarads, 450 volts
C_3. Electrolytic condenser, 1000 microfarads, 25 volts
T. Power transformer, 700 volts (center tap), 125 ma., 5 volts, 6.3 volts
t_1. Full-wave rectifier tube 5 U 4
t_2t_3. Gas-filled regulators, VR 105
t_4. Gas-filled regulator, VR 150
t_5. Twin-triode amplifier 6 N 7
$r_1 = r_2$. 5000 ohms, adjustable, 25 watts
r_3. 100,000 ohms, 2 watts
r_4. 15,000 ohms, wired, 10 watts
$r_5 = r_7 = r_9 = r_{11} = r_{13} = r_{15} = r_{17}$. 1000 ohms, wired potentiometer
$r_6 = r_8$. 100 ohms, wired potentiometer
r_{10} = 150 ohms, wired potentiometer
$r_{12} = r_{14}$. 200 ohms, wired potentiometer
$r_{16} = r_{18}$. 300 ohms, wired potentiometer
$r_{19} = r_{21}$. 20 ohms, wired potentiometer
$r_{20} = r_{22}$. 125 ohms, wired potentiometer
r_{23}. 15,000 ohms, 10 watts
r_{24}. 8200 ohms (2 watts) + 5000 ohms (adjustable, 10 watts)
r_{25}. 18,000 ohms (2 watts) + 5000 ohms (adjustable, 10 watts)
r_{26}. 910 ohms, 1 watt
r_{27}. 1800 ohms, 1 watt
r_{28}. 2700 ohms, 1 watt
r_{29}. 3900 ohms, 1 watt
r_{30}. 5600 ohms, 1 watt
r_{31}. 8200 ohms, 1 watt
r_{32}. 12,000 ohms, 1 watt
r_{33}. 18,000 ohms, 1 watt
r_{34}. 30,000 ohms, 1 watt
$r_{35} = r_{36}$. 5000 ohms, adjustable, 10 watts
r_{37}. 400 ohms, wired potentiometer
r_{38}. 100,000 ohms, 1 watt
r_{39}. 820 ohms, 2 watts
RG. Clarostat line ballast resistance, type O
V. 0–100 microammeter (Simpson)
S_1. Power switch
S_2. Selector switch, 2 circuits, 9 positions
S_3. Reversing switch, position I for study of cathodic wave, position II for study of anodic wave
S_4, S_7. Selector switch, 2 circuits, 6 positions
S_5. Selector switch, 1 circuit, 12 positions
S_6. Reversing switch
f. 2-amp. fuse

adjustments, has a 0- to 3-volt range. By means of switch S_2 any of these preselected differences of potential can readily be applied to the polarographic cell connected in B_1B_2. Switch S_3 reverses the polarity of the difference of potential applied to the cell and thus makes the instrument suitable for the study of cathodic and anodic waves.

A 0–100 microammeter connected in series with adjustable resistance r_{24} or r_{25} is used as a voltmeter to measure the difference of potential applied to the cell. Full-scale deflection corresponds, respectively, to 1 and 2 volts. Different circuit combinations are selected by switch S_4. In position 0 the voltmeter is not in circuit; position I, 1-volt full-scale deflection; position II, 2-volt full-scale deflection. In position III a 1-volt compensating voltage adjustable by potentiometers r_{19} and r_{20} is applied and the meter reads from 1 to 2 volts. Position IV corresponds to readings from 2 to 3 volts; the compensating voltage of 2 volts is adjusted by potentiometers r_{21} and r_{22}.

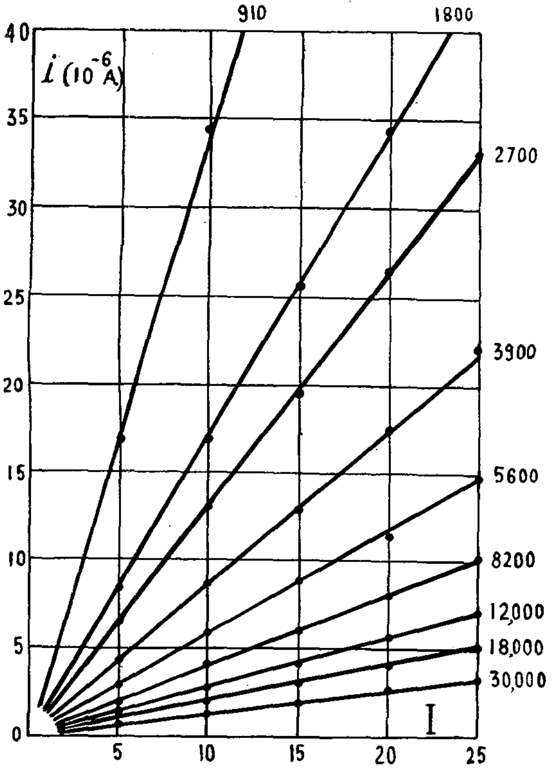

Figure 2. Polarographic Current as Function of Output Meter Deflection *I* for Different Sensitivities

Values indicated are those of resistance connected in series with polarographic cell

Switch S_5 selects a stepwise variable resistance, r_{26} to r_{34}, connected in series with the polarographic cell. The voltage on the terminals of this resistance is applied to a direct current one-stage amplifier. This amplifier is of a bridge type, minimizing the influence of the fluctuations in the voltage of the power supply. The output meter is a 0–25 microammeter shunted by a 1000-microfarad damping condenser.

Switch S_7 selects different circuit combinations. Position I is used during the heating period of the instrument. The output meter, *A*, of the amplifier is short-circuited. In position II *A* is brought to zero by means of potentiometer r_{37}, both grids of tube t_5 being at the same potential. In position III the instrument is ready for measurement.

In the case of two adjacent waves, the diffusion current of the first wave can be compensated by means of potentiometer r_{37}.

The instrument was assembled in a commercial amplifier-type cabinet and constitutes a portable unit. The set of six potentiometers, $(r_5 + r_6) \ldots . (r_{15} + r_{16})$, and the potentiometers, $r_{19} \ldots . r_{22}$, are located on the back panel of the instrument. One set of potentiometers $(r_{17} + r_{18})$ for the determination of a wave point by point is mounted on the front panel.

Characteristics of Circuit. The full-scale deflection of the 0–25 microampere output meter corresponds to a drop of potential of about 0.085 volt across the adjustable resistance, $r_{26} - r_{34}$. Because this drop of potential is by no means negligible, a flattening of the wave results. However, the magnitude of the diffusion current is not affected.

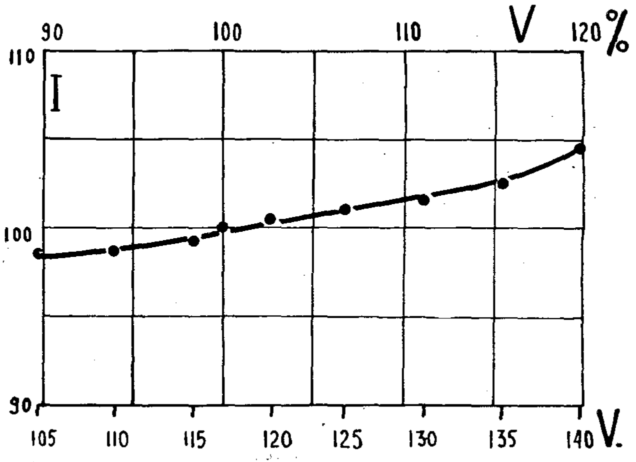

Figure 3. Variation of Output Meter Deflection *I* as Function of Line Voltage *V*

I expressed in %, *V* in volts and %

The linearity of the amplifier is shown by Figure 2. The full-scale sensitivity varies in nine steps from 3.2 to 85 microamperes.

Figure 3 shows that fluctuations of ±10% in the line voltage cause a relative error on the polarographic current not exceeding ±2%.

SUMMARY

An electronic instrument for polarographic measurements is described. A one-stage direct current amplifier is used for current measurements. The alternating current line is the only power supply. Variations of ±10% in the 117-volt nominal voltage result in changes of ±2% in current reading. A diffusion current is determined by two readings at preselected values of the difference of potential applied to the polarographic cell. A set of six potentiometers makes possible the simultaneous determination of at least three diffusion currents. The study of a wave point by point is also possible. The instrument can conveniently be used for amperometric titrations.

The instrument has been in constant use at the University of Oregon since February 1948.

ACKNOWLEDGMENT

The design and construction of the instrument described in this paper were carried out at the suggestion of Pierre Van Rysselberghe for use in a research project on the polarographic study of corrosion phenomena under contract between the University of Oregon and the Office of Naval Research.

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Stabilization of Biotin Solutions by Acid

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IN THIS laboratory and elsewhere (personal communications) it has been observed that dilute aqueous solutions of biotin kept in the refrigerator, as used for bacterimetric work, may rapidly lose their biological potency. In view of the reported stability of biotin toward aeration, as well as its relative resistance to heating, in acid or alkaline solutions (*1*), the authors have made some observations on the effect of acidity on the long-term stability of aqueous biotin solutions.

Using the ampoules of the General Biochemicals Company, which contain 25 micrograms per ml. of water, nonsterile solutions containing 1 microgram per ml. were prepared in ordinary distilled water, and in 10^{-3} *N*, 10^{-2} *N*, 10^{-1} *N*, and 1 *N* hydrochloric acid. The glass-stoppered 25-ml. flasks containing these solutions were stored at approximately 4° C. and opened only at the time of testing. For the potency determinations appropriate dilutions were compared with solutions made from freshly opened vials, for their power to cause growth of *Streptococcus faecalis* 9790 in the buffered medium of Toennies and Gallant (*2*), using the described bacterimetric technique.

The following results were obtained. In the first series of tests the standards contained 2 micrograms of biotin per 10 ml. of finished medium—i.e., more than the minimal requirement. In the other determinations several levels of standard, up to 0.5 microgram, and test solutions were used.

	Time, Months			
	3	6.5	9.5	18
Medium	Biological activity, % of fresh standard			
H_2O	~ 25	0	0	
10^{-3} *N* HCl	~100	~100	112	102
10^{-2} *N* HCl	~100	~100	108	103
10^{-1} *N* HCl	~100	~100	98	89
N HCl	~100	~100	73	44

The results indicate the advisability of making stock dilutions of biotin in 0.001 *N* or 0.01 *N* hydrochloric acid rather than in water. The data also attest to the stability and relative uniformity of the commercial aqueous solutions sealed in ampoules.

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Convenient Melting Point Apparatus

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A VERY simple, yet accurate melting point apparatus was developed to meet two basic requirements: to cover a wide range of temperatures from that of the room to about 500° C. and to be rapid and clean in use, avoiding hot liquid baths or calibrated metallic blocks. These two needs were met admirably in an apparatus which consists of only two concentric test tubes, a thermometer, and cork stoppers. Although this is similar to arrangements previously described (*2*, *4*, *7*), the exclusion of all capillary tubes while retaining a double air bath resulted in a remarkably accurate and efficient apparatus.

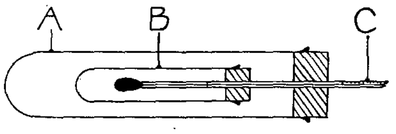

Figure 1. Apparatus

As shown by Figure 1, the apparatus consists of a double air bath of two test tubes, *A* and *B*, held concentric by means of cork stoppers and the thermometer, *C*. The entire unit is held in a horizontal position by means of a buret clamp. The sample, whose melting point is to be determined, is placed directly on the thermometer bulb, as Stahl (*7*) suggested, and heat is applied evenly along the outer edge of the larger tube by means of a Bunsen burner. The thermometer is cleaned after each use by wiping the melted sample off with a cloth. If any traces remain, they may be removed with acetone. The corks are grooved to prevent internal pressure.

Both total and partial immersion thermometers have been used with equal satisfaction. For routine work, the student grade thermometers with ranges up to 110°, 250°, or 310° C. are most useful; for higher values or greater accuracy, a thermometer extending to 530° C. or high quality A.S.T.M. instruments were employed. The size of test tubes *A* and *B* is not critical. Equally good results have been obtained with a 6-inch (1.8-cm. diameter, 15-cm. length) or an 8-inch (2.5-cm. diameter, 20-cm. length) outer tube, *A*, and a 4-inch (1.2-cm. diameter, 9.5-cm. length) or a 5-inch (1.5-cm. diameter, 12.5-cm. length) inner tube, *B*.

When taking a melting point, it has been found convenient to place the buret clamp on the larger stopper. This allows the test tubes to be put in position or removed quickly, eliminating the time between successive melting points, which otherwise would be needed to allow the tubes to become cool enough for reuse. The only precaution needed is to heat the larger test tube evenly and be sure that the tubes are held concentric. The actual point of melting is very easily seen through the clear tubes and the thermometer stem is completely unobstructed.

PERFORMANCE OF THE APPARATUS

In checking this instrument, two methods were employed: an absolute accuracy determination, and a comparison with the usual capillary method.

For the first, benzoic acid was employed as a standard, inasmuch as this substance is now accepted as a fixed point in thermometry, with a melting point of 122.362° ± 0.002° C. (*6*). Coleman and Bell C.P. acid was used, and was purified by recrystallizing from water, subliming, and finally fractional freezing by the procedure of R. S. Jessup (*5*). By this procedure, the acid was obtained as a 99.99% pure product, which was then used to calibrate an A.S.T.M. high softening point total immersion thermometer. The thermometer range was from 30° to 200° C. graduated every 0.5° C. For the calibration, cooling curves were determined, while the transition point was noted with a 10× magnifying lens, equipped with a cross hair, and so held in a stand as to prevent any error of parallax. The uncorrected melting point recorded on the thermometer was 121.5° C. with a stem temperature of 44° C. The corrected value was 122.4° C., in excellent agreement with the accepted value. The identical thermometer and magnifying system was then used with the air bath apparatus, and the melting point of the same purified benzoic acid was determined.

An average of six determinations gave an uncorrected melting point of 121.7° C. with a stem temperature of 37° C. which, when corrected, yielded the melting point of 122.6° C. The discrepancy between the cooling curve value and that of the present

Table I. Melting Point Comparisons

Substance	Melting Points Present Method	 Capillary tube method	 Literature value	Reference
Benzalaniline	49.3–49.5	50.5–51.0	56 (48)	(*3*)
Benzil	92.5–95.2	92.8–95.6	95	(*3*)
Acetyl *o*-toludine	109.5–110.0	109.0–110.5	112	(*1*)
Acetyl 2-naphthylamine	130.5–132.0	132.8–133.9	132	(*1*)
Cinnamic acid	132.8–133.0	132.5–133.2	133	(*3*)
m-Nitrobenzoic acid	138.8–139.4	141.6–143.5	140	(*1*)
p-Aminobenzenesulfonyl amide	162.8–163.5	162.8–163.8	163	(*1*)
Silver nitrate	211.5–212.0	212.0–213.0	212	(*3*)
Potassium chlorate	362.0–364.0	......	368	(*3*)
Potassium dichromate	398.0–399.0	......	398	(*3*)
Cupric chloride	496.0–500.0	......	498	(*3*)

method amounted to 0.2° C. which is about the accuracy with which the thermometer can be read.

Also investigated were the effects of the rate of heating, the uniformity of heating, and the position of the substance on the thermometer bulb. In the case of the benzoic acid, the rate of heating was found to have no effect on the result, other than to diminish the accuracy with which the thermometer temperature and sample could be observed when both were changing very rapidly. However, the melting point of 121.7° C. was checked when the acid was heated at a rate of 1° every 10 seconds and also at a rate of less than 1° every minute.

When the heat was applied uniformly below the thermometer bulb, over an area extending 4 cm. on either side of the bulb, the position of the sample on the bulb made no difference in the results. Under these conditions, the sample melted simultaneously over the entire bulb. On the other hand, when purposely only the extreme end of the large test tube was heated, the sample melted first at the tip of the bulb, and then progressively further up the bulb. In the latter case, the difference between the first and last temperatures of melting was as large as 6° C. The average of the lowest and highest values gave an uncorrected melting point of 122.0° C. for the benzoic acid. The single position of greatest accuracy was 7 mm. from the end of the thermometer, or approximately two thirds the length of the bulb.

The second comparative method of checking the apparatus is tabulated in Table I. These results were obtained by the use of a variety of thermometers, over a range of temperatures. In each case, however, the same thermometer was employed for the air bath and for the capillary tube method for any given compound. The values reported are the uncorrected melting points. This air bath method compares very favorably with the usual capillary tube, liquid bath method (*1*), but at the same time is less cumbersome, more rapid, and above all, has a greater range of temperatures covered.

The melting point ranges reported are those associated with the purity of a compound. The sample is so small that, even when melted, it remains on the top of the thermometer bulb, thus ensuring equilibrium conditions between the solid and liquid phases.

CONCLUSIONS

The melting point apparatus described possesses many advantages, mainly its use at high temperatures and quickness of operation. The accuracy is at least within 0.2° C. or as great as that of the thermometer used.

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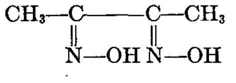
Received December 2, 1948.

CRYSTALLOGRAPHIC DATA

Contributed by Armour Research Foundation of Illinois Institute of Technology

24. Dimethylglyoxime (Diacetyl Dioxime)



$$\begin{array}{c} CH_3-\underset{\underset{\displaystyle N-OH}{\|}}{C}-\underset{\underset{\displaystyle N-OH}{\|}}{C}-CH_3 \end{array}$$

Structural Formula for Dimethylglyoxime

Excellent crystals of dimethylglyoxime can be obtained from alcohol-water mixtures (50-50) by cooling hot saturated solutions with agitation. Twinned crystals are very common and cleavage parallel to *b* is pronounced. The fibrous nature of the cleavage fragments and the fact that the highest refractive index is approximately parallel to *b* indicate that the molecules are oriented within the crystal so that their longest direction is approximately parallel to *b*.

Crystal Morphology (determined by W. C. McCrone).

Crystal System. Triclinic.

Form and Habit. Tablets flattened on 100 and usually elongated parallel to *b*. The principal forms are the three pinacoids. The brachydome {011} and macrodome {101} also appear occasionally.

Axial Ratio. $a:b:c = 0.950:1:0.703$.

Interfacial Angles (Polar). 100 ∧ 010 = 63°; 100 ∧ 001 = 73°; 011 ∧ 100 = 80°.

Crystal Angles. $\alpha = 125°$; $\beta = 91°$; $\gamma = 79°$.

Twinning Plane. 001.

Cleavage. 001 and 100.

Principal Lines

d	I/I_1	d	I/I_1
6.63	0.05	2.20	0.02
5.92	0.6	2.14	0.02
5.40	0.2	2.10	0.02
5.19	0.05	2.08	0.02
3.76	0.1	2.04	0.02
3.66	0.1	1.89	0.01
3.51	0.1	1.84	0.02
3.42	0.2	1.82	0.1
3.33	1.0	1.78	0.01
3.02	0.1	1.75	0.02
2.93	0.05	1.70	0.02
2.86	0.05	1.67	0.1
2.58	0.2	1.47	0.02
2.51	0.1	1.44	0.02
2.45	0.05	1.38	0.01
2.34	0.1		

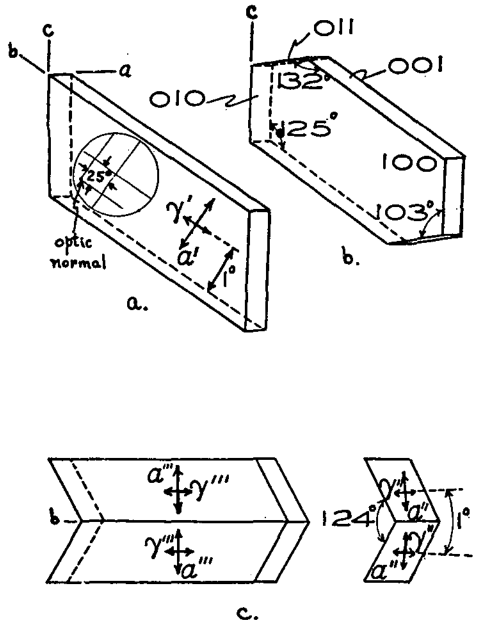


Figure 1. Orthographic Projections of Several Typical Crystals of Dimethlyglyoxime

a. Lying on 100, showing orientation of optic normal
b. Lying on 100, showing one additional form
c. Typical twin drawn lying on an edge

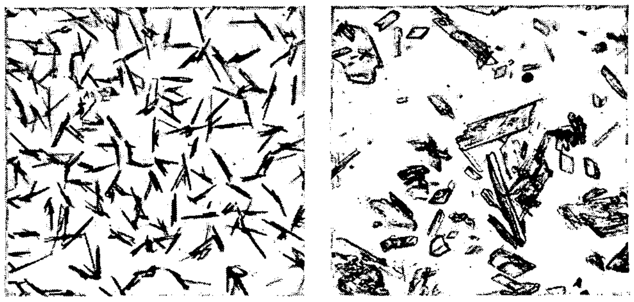


Figure 2. Dimethylglyoxime

Left. Sublimate
Right. Crystals from 50% aqueous ethanol

X-RAY DIFFRACTION DATA (determined by W. C. McCrone).
Cell Dimensions. a = 6.07 Å.; b = 6.39 Å.; c = 4.48 Å.
Formula Weights per Cell. 1.
Formula Weight. 116.12.
Density. 1.353 (pycnometer); 1.32 (x-ray).

OPTICAL PROPERTIES (determined by W. C. McCrone).
Refractive Indexes (5893 Å.; 25° C.). $\alpha = 1.40 \pm 0.01$. $\beta = 1.54 \pm 0.01$. $\gamma = 1.85 \pm 0.01$.
Optic Axial Angles (5893 Å.; 25° C.). $2V = 80°$.
Dispersion. $r > v$.
Optic Axial Plane. Approximately parallel to 100.
Sign of Double Refraction. (+).
Acute Bisectrix. Approximately parallel to b.
Extinction. 1° on 100 with γ' almost parallel to b.
Molecular Refraction (R) (5893 Å.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.59$. R (calcd.) = 29.7. R (obsd.) = 29.0.

FUSION DATA (determined by W. C. McCrone).
The literature melting point of dimethylglyoxime is 260–266° C. However, under the usual conditions of fusion between a cover glass and slide the sample sublimes completely before melting. The sublimate is made up of large rods and tablets elongated parallel to b and usually lying on 100.

SCIENTIFIC COMMUNICATIONS

Kinetics of Paper-Chromatogram Development

SEVERAL concepts and criteria have been proposed to put the valuable techniques of paper-chromatography on a quantitative basis. Well known among these are the R_F value as defined by Consden, Gordon, and Martin (*1*) and the expressions of Flood (*2*) and of Hopf (*3*) relating zone radii to the concentration. Nevertheless, it is becoming increasingly evident that kinetic studies on the process of paper chromatogram development can throw much light on the nature of this complex phenomenon (*4, 5*).

We have derived and confirmed a simple expression relating the motion of solute and colored zones to elapsed time for the paper disk chromatogram described by Rutter (*6*). In this useful technique, a narrow rectangular strip is cut in a circular disk of filter paper from its circumference to the center. The narrow strip is bent downward in a plane perpendicular to that of the horizontal disk. The mixture to be separated is deposited on the center of the disk or on the pendant strip, and when the latter is immersed in the eluting liquid, it acts as a wick to admit eluent to the sample. Eluent and colored zones soon diffuse outward and eventually develop in the form of concentric rings.

For the conditions under which solvent access occurs at a constant rate, we may write $v = ct$ where v = volume, t = time, and c = a constant.

At any instant, the liquid occupies a circular spot on the paper disk, for which the volume is given by:

$$v = \pi r^2 f d$$

where r = radius, d = thickness of paper, and f = accommodation factor.

Combining these and lumping all constants, we can write:

$$r_1{}^2 = at$$

If the transport of colored components follows the same relationship, but at different rates, then for successive zones, $r_2{}^2 = bt$, $r_3{}^2 = ct$, etc.

This parabolic relationship has been confirmed for various solvents and several binary mixtures of dyes. It holds for the maximal, minimal, and median zone radii, but obviously with different slope factors. Furthermore, because the R_F factor is defined as the ratio of the distance traversed by a colored zone to that traversed by the solvent, we have in this case:

$$R_F = \frac{r_2}{r_1} = \sqrt{\frac{b}{a}}$$

where r_2 = zone radius and r_1 = solvent radius.

This will hold at all times t for which there is a perceptible difference in the positions of the zones. As the slope factors a, b, etc., can be determined with considerable precision from a plot of the square law data, the R_F value so computed is more convincing than a result obtained from two zone positions.

A similar relationship appears to hold for separations accomplished in vertical rectangular strips in which the eluent is rising, although it is derivable from a different set of assumptions. Our measurements are not well enough along to decide upon its validity or usefulness.

Experimentally, it has been found convenient to mark off radial target points on the paper disk with fine pencil dots and in the square law sequence of intervals. Under these conditions, the various components should cross successive target positions in uniform time intervals. To avoid confusion when dealing with complex mixtures, it is useful to have a multipen chronograph for accurate timing and identification. As a substitute, we used a simple tapped voltage divider with push buttons to connect identifying potentials to a recording potentiometer. The chart drive was synchronous and each push button was labeled to represent the various components and solvent.

The principal limitation in precision seems to lie in deciding when a zone crosses a target point. Although this source of error is minimized by appropriate illumination and viewing, automatic recording is advantageous, especially in view of the long time periods involved. It has been found feasible to project minute points of light on the paper in the square law sequence and record the transmitted light photoelectrically. An increase in translucency of about 35% occurs as each target point is moistened by advancing solvent and decreases occur as colored zones cross the points in proportion to the absorbance.

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Received September 29, 1949.

A New Test for Fructose

The use of 3,5-dinitrosalicylic acid as a test for reducing sugars in urine has been reported by Sumner (*2*) and Short (*1*). Sumner used a slightly alkaline solution of his reagent and heated the urine sample with the reagent in boiling water for 5 minutes. Short reported that more reliable results were obtained if Sumner's reagent were made more strongly alkaline with sodium hydroxide. In both methods, a color developed with any of the reducing sugars.

The present investigation is part of a study to develop a systematic scheme for the qualitative analysis of a mixture of the common sugars: glucose, fructose, lactose, maltose, and sucrose.

Reagents. (1) Dissolve 2 grams of 3,5-dinitrosalicylic acid in 70 ml. of distilled water at 80° to 90° C., and add 10 ml. of 20% aqueous solution of sodium carbonate. When this mixture has cooled to 20° to 25° C., dilute to 100 ml. with distilled water. (2) Dissolve 1.5 grams of sodium hydroxide in enough distilled water to make 100 ml. of solution at 20° to 25° C.

Experimental Procedure. If the sample is a solid, dissolve 0.2 gram in 3 ml. of distilled water. If the sample is a liquid, concentrate it on a water bath until its specific gravity is approximately 1.1 at 20° C., and take 3 ml. of this concentrate. To the 3-ml. sample solution in a 30-ml. test tube, add 1 ml. of reagent 1 and 1 ml. of reagent 2. Close the test tube and shake well, then allow the mixture to stand at 20° to 25° C. Observe any color change which appears in 2 hours.

Some ketones will be found which are not soluble in water alone. In that event, shake the solid sample with 1 ml. of 95% ethyl alcohol, centrifuge, and decant. Place the clear decantate in a test tube and add water to it dropwise until a turbidity appears, then add 95% ethyl alcohol dropwise until the mixture clears with shaking. Use this solution as the test sample and treat it as described for samples soluble in water alone.

Sample	Color at End of 2 Hours	Minimum Time for Orange Color to Develop, Hours
Glucose	Yellow	3
Sucrose	Yellow	Remains yellow
Lactose	Yellow	13
Maltose	Yellow	13
Raffinose	Yellow	Remains yellow
Fructose	Red-orange	50 min.
Acetone	Orange	50 min.
Acetophenone	Orange	1.5
Benzophenone	Orange (pale)	2
Urine	Yellow	48

Only fructose, among the sugars tested, develops an orange color with the reagents in 2 hours under the conditions described. The test is not specific for fructose, however, because other ketones develop an orange color within 2 hours. The orange color developed by other ketones is not identical but may be confused with the red-orange color developed by fructose. The test is specific for fructose in mixtures with the sugars listed. The test is sensitive to 2 mg. of fructose per ml. of solution whether it is alone in solution or mixed with other sugars.

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Location of Colorless Chromatographic Zones with an Ultraviolet-Sensitive Multiplier Phototube

The difficulty of locating zones of colorless compounds on chromatographic columns has made work with such substances extremely difficult. The present work was undertaken to see if photoelectric cells, which have been used to locate zones in paper chromatography (*1*, *3–5*), could also be used to locate zones on regular cylindrical chromatographic tubes packed with solid adsorbents. It has been found that the position of a colorless compound can be readily determined by illuminating the column with a narrow slit of ultraviolet light and using an ultraviolet-sensitive phototube to measure the intensity of the scattered light as a function of the displacement of the light beam along the column. The presence of light-absorbing material is evidenced by a drop in photoelectric current.

A quartz lens (4 cm. in diameter; 14 cm. in focal length) was used to focus the beam of ultraviolet light emerging from the monochromator of a Beckman Model DU spectrophotometer on a quartz chromatographic tube 24 mm. in diameter, packed with silica gel or alumina. An RCA 1P28 multiplier phototube was placed approximately 14 cm. away from the column and as near the path of the light beam as possible to measure the scattered light. The acceleration potential was 90 volts per dynode and a 45-volt anode supply was applied through a 100,000-ohm protective resistor in series with an RCA Type WV-84A direct current microammeter.

This apparatus has been used to locate the zones obtained in the chromatographic separation of anisaldehyde-benzophenone and benzophenone–methyl benzoate mixtures on both Eimer & Amend c.p. silicic acid and Alorco Grade A alumina (−200 mesh, screened from commercial −80 mesh), 4 or 8% diethyl ether in petroleum ether (Skellysolve F, boiling point 35° to 60° C.) being used as developer. The compounds were selected because of their differing spectral absorption maxima (methyl benzoate, 230 mμ; benzophenone, 254 mμ; anisaldehyde, 279 mμ; all in 95% ethanol); light of a wave length absorbed maximally by either member of a binary pair was satisfactory for detecting the location of both zones, for each compound absorbs appreciably at the wave length of the other's absorption maximum. With a 2-mm. monochromator slit width, empty regions of the column gave photocurrents ranging from about 15 μa. at 279 mμ to about 3 μ at 230 mμ; current decreases for the chromatographic zones ranged from as much as 8 μa. at the higher wave length to about 1 μa. at the shorter wave length. This simple setup, although effective, is inconvenient to use and cannot be employed to follow the movement of zones in a vertical column during development. Accordingly, work is beginning on the construction of a scanning device which will move a light beam rapidly over a vertical column and present the resulting information of reflectivity versus column location in a convenient form on the screen of a cathode-ray oscilloscope (*2*).

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RECEIVED July 9, 1949

BOOK REVIEWS

Laboratory and Workshop Notes. *Ruth Lang.* xii + 272 pages. Edward Arnold & Co., London, and Longmans, Green and Co., Inc., 55 Fifth Ave., New York, N. Y., 1949. Price, $5.25.

This is a compilation of papers printed during the past 25 years in the Laboratory and Workshop Notes Section of the *Journal of Scientific Instruments.* The selection was made by Lang for the Institute of Physics, which is to receive the royalties that accrue. The selection was necessarily rather arbitrary, but Lang has made a rough grouping: laboratory and workshop tools; clamps, supports, and agitators; soldering, brazing, and welding; glass manipulation and silvering; vacuum and pressure technique; electrical and magnetic devices; optical devices; and the handling of liquids and gases. Altogether some 180 articles have been reprinted. They vary from simple recipes and tricks—for example, cements, silvering, preparation of thermocouple—to ingenious solutions of really tough problems (slow constant flow of liquids, design of high pressure valves, maintenance of constant potential).

Lang has shown fine discrimination in her selection. None of the articles are trivial, most describe techniques which would not be immediately obvious to one skilled in the art, and many are fascinating reading. Paper and binding are second rate.

HARVEY DIEHL

Rapid Microchemical Methods for Blood and CSF Examinations. *F. Rappaport.* xviii + 404 pages. Grune and Stratton, 381 Fourth Ave., New York, N. Y., 1949. Price, $8.75.

This unusual volume will be of value largely because it includes the many and ingenious methods developed by the author and his collaborators. Its emphasis on microchemical techniques could be adopted much more widely and profitably than is customary in most clinical laboratories. Its style, arrangement, and make-up are not likely to appeal strongly to most of the laboratories to which it is addressed.

The book is devoted to presentation of methods for determining the usual clinically important chemical substances and some physical tests such as specific gravity, sedimentation rate, and hematocrit. Blood gases, anions, cations, nitrogen and its fractions including protein fractions, carbohydrates and their derivatives, ketone bodies, fats and lipides, bile pigments and liver function, enzymes, vitamins, sulfa drugs, and some miscellaneous materials are discussed. Many of the methods given are the standard ones, sometimes followed by a semimicro or micromodification. Not all of the methods are the most dependable of those available. A few are virtually obsolete—e.g., the pyroantimonate method for sodium, and the 1,2-naphthoquinone method for amino nitrogen—some are empirical, and many of the most advanced and convenient micromethods are omitted—e.g., the diffusion procedures of Conway. One method for determination of iron involves reaction of ferric ion with potassium iodide and steam distilling the released iodine, which is determined through the Kendall titration method. This odd procedure is balanced somewhat by later inclusion of the *o*-phenanthroline colorimetric method applied to serum iron only. Not all of the slow and inconvenient methods are offset by inclusion of a satisfactory alternative.

The make-up and proofreading of this volume are probably poorer than those of any volume previously read by the reviewer. Much that is of value in the book is obscured by poor setup of headings. The reader is constantly distracted by misspelled words, use of German spelling and word order, and inconsistency of spelling and arrangement. As one of many possible illustrations, the words ferricyanide and ferric cyanide are mixed indiscriminately throughout the volume, whereas ferrocyanide is never given as ferrous cyanide. Though numerous chemical errors occur, they are overshadowed by the errors that should have been corrected by a competent proofreader.

The author has included a reasonable number of references at the ends of the chapters, thus distinguishing this book from many of the clinical manuals. In common with most manuals, there is no indication at any point of the exact accuracy or reliability of any method, nor is any critical discussion included.

The sincere admirers of the author's many significant contributions to analytical techniques and procedures will share the reviewer's unhappiness that this volume was not more carefully compiled and produced. A well arranged volume of the author's own work would be inherently valuable if all the other methods were omitted. It is their inclusion in this volume that will constitute its chief claim to distinction.

PAUL L. KIRK

Textile Laboratory Manual. *Walter Garner.* x + 478 pages. National Trade Press, Ltd., Tower House, Southampton St., London W.C.2, England. Price, 30 shillings.

The author of this book set himself the task of "bringing together in one volume a range of analytical and testing methods sufficient to enable most of the problems likely to be encountered in a textile works laboratory, to be investigated." This objective is astoundingly well fulfilled. The title may mislead Americans, in that there is a substantial content of descriptive background and interpretive material. In most instances this adds a great deal to the value of the book—for example, the criteria for selecting a microscope for textile work will repay the cost of the book for many laboratories. In some cases, as in the discussion of hydrogen ion concentrations, the treatment is trivial.

Initial chapters discuss the characteristics of natural, modified, and synthetic textile fibers, and include tests for detection and assignment of damage. An unexciting and brief description of physical testing methods terminates with an interesting chapter covering textiles as thermal and sound insulators, including a discussion of resilience. The chapters on sizes, oils, color matching, and dyes are helpful. Textiles are so subject to ill-defined variations that one needs long experience in interpreting test results. It is therefore not surprising that the author, reporting on tests in which he has developed confidence, refers mainly to the literature of 15 years ago. This experience increases the book's value. It is unfortunate that there are so many minor errors and instances of obtuse writing. The book would be improved by careful editing.

Alfred Seely Brown

Modern Instrumental Analysis. *David F. Boltz,* editor. Vol. I. viii + 191 pages. Edwards Bros., Inc., Ann Arbor, Mich., 1949. Price, $3.

In this first of two volumes on the subject of instrumental analysis edited by Professor Boltz of Wayne University, six well qualified authors have written chapters on spectrochemical analysis (two chapters), mass spectrometry, optical instruments (a really large order for one brief chapter), electron diffraction, and x-ray diffraction. The basic principles of these methods are presented in rather brief and elementary form, but with clarity. In general, each author has endeavored to emphasize fundamental principles, constructional and operational characteristics of typical instruments, and practical applications in chemical analysis and research. However, no operating instructions for specific instruments or directions for laboratory exercises have been included and these omissions will be sadly missed in cases where the effort is being made to institute instrumental methods in teaching and research. Obviously a definite need for texts in this field has been felt by Boltz and his collaborators, and with this beginning perhaps ultimately a really authoritative text including laboratory experiments will be forthcoming. This small paper-bound volume is lithoprinted and the illustrations, especially the line drawings, are good.

G. L. Clark

Inorganic Quantitative Analysis. *Carroll Wardlaw Griffin.* xiv + 368 pp. Blakiston Company, 1012 Walnut St., Philadelphia, Pa., 1949. Price, $4.50.

This text covers briefly calibration of equipment; accuracy, precision, errors; acidimetry and alkalimetry; oxidation reduction processes (reagents: potassium permanganate, ceric sulfate, potassium dichromate, iodine, sodium thiosulfate); gravimetric analysis; coprecipitation; gravimetric estimation of chlorine, sulfur, phosphorus; analysis of limestone; two organic precipitants; volumetric precipitation; potentiometric titration; electroanalysis and analysis of brass; and colorimetric determination of manganese in steel. Supplementary material includes: care of platinum ware, list of reference works, and tables of ionization constants, solubility products, formula weights, specific gravity *vs.* composition of ammonia and acids, five-place logarithms, and atomic weights.

A liberal assortment of problems, review questions, and numerical illustrations is included.

A few shortcomings in the text, in the opinion of the reviewer, are: rather small emphasis on the principles of separation and upon physicochemical methods of analysis; very brief treatment of the important topic of extraction, in which the author has done original research (this topic is mentioned only as an adjunct to the theory of washing precipitates, pp. 13–14). Chromatographic adsorption is not mentioned. Under errors there is no reference to modern statistical methods of handling data, control chart methods, etc. In Chapter 7 on neutralization, the Brönsted concept is introduced briefly, after which the treatment is conservative in pre-Brönsted fashion and in greater detail than might be expected from the scope of the remainder of the book.

The book covers a short conventional selection of work for a year in the subject (two semesters). The author acknowledges great indebtedness to a number of the leading texts in the field, and the present volume appears to be a well balanced brief selection of the topics that are presented in greater detail in some of the longer texts, with emphasis on titration methods, gravimetric estimations, and electroanalysis. The election of the experiments and the general arrangement of the text follow well established teaching trends in this subject over the past 10 or 15 years. There is scant reference to published work other than texts subsequent to 1940.

The type is clear and easy to read and very few errors were noted. The illustrations, other than reproductions of photographs, are in general clear and well prepared.

N. H. Furman

RTB Methods of Analysis. *Chemists' Conference Committee, Richard Thomas and Baldwins, Ltd.* 1st ed. 112 pages. Richard Thomas and Baldwins, Ltd., 47 Park St., London, W.1, England, 1949. Price, 2 guineas.

The book, produced by the cooperative efforts of the chemists and metallurgists of Richard Thomas and Baldwins, Ltd., presents details of methods used in the company for analysis of metals and shown by their experience to be most reliable. Originality is not claimed for the procedures, which place on record the methods of analyses used by each section of the company for the stated primary purpose of benefiting the sponsor's own laboratories.

The book contains five parts: ferrous metals; nonferrous metals; raw materials and slags; miscellaneous methods; and an appendix containing data on preparation of standard solutions and other reference information likely to be required by a practicing analytical chemist. It includes methods for analysis of fluxes, refractories, water, pickling solutions, and fuels, and determination of tin coating weights as well as for analyzing metals for the elements usually covered in commercial specifications. Spectroscopic methods, despite their well established place in the analysis of metals, are not included in the book. The table of contents serves as an index also and lists the elements in each part alphabetically, so that pertinent information on a desired method can be located readily. The material in the book is well arranged and written in an easily comprehensible style. Unfortunately, some of the cross references are inaccurate.

This book should prove especially useful to students and to college graduates beginning their careers as analytical chemists, for it contains many practical hints to supplement their theoretical knowledge. It also should prove a valuable reference for the experienced chemist who has only an occasional metal sample to analyze. The chemist who is in daily contact with metal analysis will find it interesting as a means for comparing methods in common use in England with those he is using.

H. F. Beeghly

A Simplified Course in Elementary Qualitative Analysis. *Bruce E. Hartsuch*, editor. 192 + ix pages. D. Van Nostrand Co., 250 Fourth Ave., New York, N. Y. Price, $3.

This book has been written to review and extend the student's knowledge of the principles of general chemistry, to present the subject matter in a brief simplified manner easily understood by the beginner, to assist him in the use of theoretical tools, to direct him in a careful explicit way, training him to do dependable work, to guide him in making proper and significant operations, and to impress him with the importance of sound theoretical foundations on which to build manual operations.

The subject matter is presented in the light of the modern theory of ionization as modified by Debye, the laws of mass action and chemical equilibrium, and the electronic changes taking place during chemical reactions. Prefaced by a section on suggestions to beginning teachers, the book is divided into four parts. The first or theoretical part of the book deals with a discussion of solutions, ionization and disassociation, homogeneous and heterogeneous chemical equilibrium, amphoteric compounds, atomic structures and types of bonds, redox reactions, and hydrolysis. The laboratory parts of the book include studies of 21 cations and 11 anions. The actual manual operations are clearly outlined and are interspersed with pertinent questions which can be answered in the laboratory. Part IV is devoted to a glossary of signs and symbols and tables of commonly used solubilities, ionization constants, solubility products, the periodic system, test solutions, etc.

The course provides for the needs of the general student as well as for the student preparing to enter the profession of chemistry, though more laboratory work should be expected from the latter. It is advantageously presented from the instructor's standpoint.

W. A. Kirklin

CORRESPONDENCE

Analysis of Gases by Absorption and Combustion

Sir: An error of omission in our recent paper entitled "Analysis of Gases by Absorption and Combustion" [Anal. Chem., 21, 1105–16 (1949)] has been brought to our attention by Robert D. Schultz of the Applied Physics Laboratory at The Johns Hopkins University. The addition of potassium iodide was omitted in the directions for preparation of the potassium iodomercurate reagent which is used for the selective absorption of acetylenes. The correct procedure for preparing this reagent is as follows:

> Slowly stir 100 ml. of 50% potassium hydroxide solution into a solution containing 25 grams of mercuric iodide and 30 grams of potassium iodide in 100 ml. of distilled water. It is preferable to prepare the quantity needed each day from these two stock solutions.

It was also noted by Dr. Schultz that, in the preparation of cuprous chloride reagent as described in our paper, sodium chloride may precipitate from solution. Should this occur, it is our practice to withdraw and use the supernatant solution.

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Frequency Errors in Timing with Electric Clocks

Sir: The conclusions drawn by Craig, Satterthwaite, and Wallace in the article "Frequency Errors in Timing with Electric Clocks" [Anal. Chem., 20, 555 (1948)] are apparently too broad when they state that ". . . . the frequency errors revealed in this study are probably representative of those to be expected in using the line current available in any large American city." Some unfortunate errors have crept in on observations of time made by the use of local power line frequencies.

There is no criticism of the report on the system controlled by Pittsburgh as reported. However, we have found errors of much greater magnitude for the Philadelphia system, of which Baltimore and Washington are a part.

By use of a differential frequency recorder described in the August 1949 issue of the *National Bureau of Standards Technical News Bulletin*, it is possible to obtain a continuous, automatically plotted graph of drift in frequency of the commercial power line with respect to a crystal controlled standard frequency accurate to one part in 5×10^7. By choosing suitable coordinates, the graph can be made to indicate the drift of the "time error."

A detailed study of some of these graphs shows frequent errors in a 10-minute interval three times greater than the maximum error ever recorded as reported by Wallace and his associates.

The cause of the discrepancy between Wallace's observations and our own lies, I believe, in the difference between the total generating capacity of the two power nets concerned. When two or more alternating current generators are connected in a parallel line, and one of the generators fails to contribute its share of the load and tends to turn over at an electrical frequency lower than that of the line, that generator becomes in effect a motor, loading the line rather than feeding it. This load is dissipated by causing the "slow" generator (acting as a motor) to continue rotating at an electric frequency very nearly equal to the line. Conversely, when one generator tends to rotate at an electric frequency greater than that of the line, it attempts to supply enough power to the line to place all other generators on the line in the "motor" category and bring their speed up to its own. There are automatic cutouts to remove a generator from the line altogether if its phase drifts too far from that of the line.

Thus, in a well monitored net all generators rotate at the same electric angular velocity. For the frequency of the net to increase or decrease, the inertia of all armatures must be overcome, and all armatures will accelerate or decelerate together; and the greater the total inertia of the armatures on the line, the more difficult it will be for the net to accelerate. Hence, it follows that other factors being equal, the larger the power net supplying the city, the greater will be the frequency stability.

In the article, the authors did not state that their intervals were adjoining; they merely stated that the intervals were "not overlapping." If the intervals were not adjoining, it would be necessary to have a very precise method of sampling in order that they might have a true representation of conditions.

By use of our apparatus we did not have to meet the problem of sampling, because our intervals were all adjoining.

H. A. Bowman

Time Section
National Bureau of Standards
Washington, D. C.

Sir: Bowman's observations concerning frequency errors in timing with electric clocks in the Washington area are significant in that they indicate the magnitude of such errors for another area and another power network. It is obvious that our conclusions were too broad when we stated that the frequency control in other metropolitan areas was probably comparable with that in the Pittsburgh area.

Although there seems to be no doubt that frequency errors in this locality are less than in the areas supplied by the Philadelphia power network, in my opinion the cause of the difference is not definitely established. Bowman is of the opinion that the difference in the total generating capacity of the two systems is responsible for difference in quality of frequency control. Undoubtedly this is a factor. Perhaps it is the major factor. However, the character of the industrial load borne by the Pittsburgh system is of such a nature as at least partially to nullify the capacity factor. I have recently learned that the Pittsburgh system has an electronically operated automatic system for frequency control, whereas the system servicing Washington is, to the best of my knowledge, controlled manually. This may also contribute to the more precise control of frequency here.

The time intervals chosen in our study were all adjoining.

W. E. Wallace

University of Pittsburgh
Pittsburgh, Pa.

Electron Microscope Society of America

F. A. HAMM, ***General Aniline & Film Corporation, Easton, Pa.***

THE seventh annual meeting of the Electron Microscope Society of America was held at the National Bureau of Standards, Washington, D. C., on October 6, 7, and 8, 1949. The attendance was the largest to date, with some 250 people attending. E. U. Condon, director, National Bureau of Standards, and F. O. Schmitt, president, Electron Microscope Society of America, opened the meeting with a few introductory remarks.

L. A. Chambers, Camp Detrick, Frederick, Md., who served as chairman of the committee on local arrangements, and R. G. Picard, Radio Corporation of America, Camden, N. J., who served as program chairman, deserve much credit for planning this successful meeting. The customary Friday evening banquet was held at the Wardman Park Hotel. For the first time in the history of the society, no after-dinner speeches were given. Incumbent president F. O. Schmitt introduced president-elect R. W. G. Wyckoff, National Institutes of Health, Bethesda, Md., who will begin his term as president of the society in January 1950.

The program of this meeting is abstracted below; only those papers of general interest to the readers of ANALYTICAL CHEMISTRY are included. Consequently the papers dealing with biological problems are omitted. The first session consisted of contributed papers dealing primarily with instrumentation.

Temperature of Electron Microscope Specimens. G. D. SCOTT AND FRANCES DAWES, University of Toronto, Toronto, Ontario, Canada.

Although tremendous thermal energies may be generated (10^{-4} calories per second) in a strongly electron absorbing specimen, a good portion of the heat is dissipated by radiation to the walls of the instrument. Stable materials such as alumina or sodium chloride can be made to melt. The Formvar substrate absorbs little infrared radiation, so that it absorbs heat only where contact with the specimen is made. Thus, the very high temperatures experienced by electron microscope specimens under strong illumination by electron beam must be taken into consideration.

Vibration Studies Related to Electron Microscopy. F. A. HAMM AND F. C. SNOWDEN, General Aniline & Film Corp., Easton, Pa.

An oscillographic trace due to a voltage generated piezoelectrically by a phonograph (crystal) pickup can be used to detect and measure vibration, and as a guide for properly locating an electron microscope where building vibration is a problem. Furthermore, this scheme makes it possible to correlate qualitatively image shifts with vibration amplitude. Because building vibrations are usually only periodically prohibitive, the scheme described can be used as a guide for high resolution electron microscopy. The circuit and calibration procedure were briefly described.

Performance Tests on the RCA Model EMC Electron Microscope. S. G. ELLIS, RCA Laboratories, Princeton, N. J.

It is not possible to increase the resolution of this instrument beyond about 50 Å., primarily because the angular aperture of illumination cannot be further lowered. This value is not ordinarily realized, but by mechanically modifying the specimen stage (to prevent thermal drift) and by improving the objective lens asymmetry, a resolution of 50 Å. is possible. The normal wide angle cone of rays from the projector causes reflection of extraneous electrons onto the photographic plate. Insertion of a projector aperture is therefore desirable because image contrast is improved.

Testing of Electron Microscope Objectives. JAMES HILLIER AND HENRY FROULA, RCA Laboratories, Princeton, N. J.

Virtually all electron microscope objective pole pieces exhibit some astigmatism. By photographing a test object (carbon black) defocused by known amounts in the range $+100\mu$ to -100μ a series of Fresnel fringes is recorded. A chart illustrating the width (and number) of these fringes versus known object displacement, but having used a well corrected objective pole piece, is then prepared. The image at the same magnification, recorded with the uncorrected lens being tested, is compared with the chart. One direction through the image must be in sharp focus, the nature of the fringe along the perpendicular direction is then used to assign a value (in microns) to the lens asymmetry. An asymmetry of less than a few microns is a prerequisite to high resolution.

Technique for Comparison of Identical Surface Areas in Light and Electron Microscopes. ERNEST F. FULLAM AND RUTH H. PETCHER, Knolls Laboratory, General Electric Co., Schenectady, N. Y.

This technique facilitates the correlation of light and electron micrography of the same metal surface. An acute angle is made near the area of interest by means of a sharp needle. The initial nitrocellulose replica is then replicated by a thin double film of beryllium and quartz. This double film is necessary because the final replica is supported by a specimen screen having a large hole in the center to eliminate masking due to specimen screen wires. The first organic film is removed with solvent vapor. The acute angle near the important structure aids in its location and in the interpretation of shadows.

A Centerable Condenser Aperture for the RCA Model B Electron Microscope. F. F. MOREHEAD, American Viscose Corp., Marcus Hook, Pa.

The alignment of this instrument is simplified by employing a mechanical arrangement for centering the condenser aperture while the instrument is in operation. A pair of spring tension adjustment screws represents the essential feature of the mechanism. The time required for alignment is greatly reduced.

Permanent Magnet Lenses. JOHN H. REISNER, RCA Victor Division, RCA, Camden, N. J.

Permanent magnets made with Alnico V iron have been made to exert the same magnetic flux as the present electromagnetic lenses used in commercial instruments. Stray magnetic fields which often adversely affect the resolution in high magnification images are eliminated in this new system. Focusing is done by varying the accelerating potential of the electron beam. Several configurations utilizing more than one magnet afford a variety of possibilities in lens design.

Beam Deflection Focusing Device. HAROLD T. MERYMAN, Naval Medical Research Institute, Bethesda, Md.

Two coils with polarities reversed are placed inside the specimen chamber above the specimen holder. The angular aperture of illumination to the specimen is increased during focusing, so that the smaller depth of focus enables the operator to focus more critically. This technique appears to be superior to the electrostatic method because in the latter method the focusing deflection plates are very close to the electron beam, so that traces of dirt may sometimes charge up and affect the beam.

Motion Pictures of Electron Microscope Images. JOHN H. L. WATSON AND LUTHER E. PREUSS, Edsel B. Ford Institute for Medical Research, Detroit 2, Mich.

Motion pictures (8-mm. film) of phenomena occurring in the electron microscope were illustrated for the first time at a meeting of this kind. The opportunities afforded are numerous, especially in determining the effect of the electron beam on the specimen. The three-dimensional aspect and the speed with which the frames are recorded make the interpretation of the image easier.

The melting and subsequent growth of tungsten oxide and sodium chloride crystals were illustrated. Radiation-chemical effects rather than purely thermal effects may also be studied. The movie camera is mounted on a tripod in front of the front viewing window. The image in the electron microscope is reflected to the camera by tilting the final viewing screen.

The second session was devoted exclusively to papers on sectioning.

Improved Sectioning Technique for the Electron Microscope. RICHARD F. BAKER AND DANIEL C. PEASE, University of Southern California, Los Angeles, Calif.

These authors have slightly modified their earlier technique, which was really the first one reported [*Proc. Soc. Exptl. Biol. Med.*, **67**, 470 (1948)] in which an ordinary hand microtome was used to cut sections for the electron microscope. The superiority of osmic acid as a fixative for general use was discussed. A new procedure for sharpening knives on a rotating glass plate with fine alumina abrasives was also described.

Ultramicrotomy by a New Method. SANFORD B. NEWMAN, EMIL BORYSKO, AND MAX SWERDLOW, National Bureau of Standards, Washington, D. C.

Three new significant aspects pertinent to thin sectioning were described in this paper: the embedment of the biological sample in a plastic monomer (*n*-butyl methacrylate) followed by polymerization; the advancing of the embedded specimen by thermal expansion of the supporting brass block rather than by the usual mechanical mechanism; and the sharpening of the cutting knife by means of stropping on leather in conjunction with a small amount of diamond dust and subsequently with rouge.

Some Refinements of the Rotary Microtome Modified for Ultrathin Sectioning. JAMES HILLIER, RCA Laboratories, Princeton, N. J., AND MARK GETTNER, Sloan-Kettering Institute, New York, N. Y.

Several refinements of the Spencer Model 820 rotary microtome as applied to cutting sections in the 0.05- to 0.1-micron range were described. These instrument modifications may be outlined as follows: The advancing mechanism is motor driven to eliminate vibration and irregularities normally introduced in hand operation. A spring mechanism is used to support the horizontal member of the advancing mechanism so that static friction during the horizontal travel is reduced. The instrument thus modified and used in conjunction with the reservoir has been used to cut 0.02-micron sections with no detectable variations in thickness. Uniform compression of the specimen during cutting is apparently the secret to eliminating distortion.

Serial Sections for Electron Micrography. MARK GETTNER, Sloan-Kettering Institute, New York, N. Y., AND JAMES HILLIER, RCA Laboratories, Princeton, N. J.

Tissue sections sufficiently thin to be examined in the electron microscope are difficult to see and manipulate. Friction between the knife and section usually causes the section to roll up or fold over. To eliminate this and thus to permit serial sectioning with subsequent ease of manipulation, a reservoir was attached to the cutting knife. This is filled with an alcohol-water mixture whose meniscus approaches the knife edge. This scheme causes each section to float (flat) on the liquid surface, and the sections are joined in a head to tail fashion because each section is pushed onto the liquid surface by the leading edge of the succeeding section. The flat sections are then handled in the usual way. A truly serial sectioning technique is sometimes necessary for tissue structure determination.

A Technique for Preparation of Grease or Solid Samples in Greaselike Media for Examination with the Electron Microscope. A. Y. MOTTLAU, Standard Oil Development Co., Linden, N. J.

This technique precludes the removal of water-soluble soaps fundamental to some greases. The older techniques caused removal of this component. The procedure is essentially that of imbibition of a thin film of grease onto a Formvar coated specimen screen, followed by extraction of the oil (carrier) in naphtha. The resultant specimen illustrates the nature of the solid particles as they occurred in the original grease.

Samples of catalysts or powdered pigments can be conveniently dispersed and prepared for electron microscopy by using a similar technique. A white petrolatum is deliberately added in preparing the smear for imbibition. It is then subsequently removed as before.

Replication of Frozen Liquids by Vacuum Evaporation. HAROLD T. MERYMAN, Naval Medical Research Institute, Bethesda, Md.

One of the prime considerations in the interpretation of electron microscope images of specimens, which under normal circumstances contain liquids, has to do with the effect of drying out, before or during examination in the instrument. This technique is a step in the direction of evaluating this loss in liquid or, better, of examining the specimen still containing the original liquids. The replication of biological specimens in their original wet state is an important application of the procedure described in this paper. Ice crystals, formed under liquid nitrogen, have been studied rather exhaustively.

Measurement of Cellulose Particle Length by the Electron Microscope. F. F. MOREHEAD, American Viscose Corp., Marcus Hook, Pa.

This paper described a technique for measuring crystallite length in highly crystalline and amorphous areas in viscose rayon. A new technique for casting extremely thin films of viscose permits the detection of "skin" and "core" areas. Apparently zinc ions cross link with the cellulose xanthate to give a skin effect. The crystallites are smaller (75 Å.) on the surface than they are in the core (150 Å.). These crystallite lengths were compared with those observed on fibers after acid hydrolysis plus ultrasonic disintegration. Good agreement was found.

Reproducibly Representative Specimen Fields in Electron Microscopy. ROBERT C. BACKUS AND ROBLEY C. WILLIAMS, University of Michigan, Ann Arbor, Mich.

This paper described a new technique for preparing electron microscope specimens that can be used for quantitatively assaying a specimen having one or more components. A number distribution count is based on a reference component (polystyrene latex spheres) whose assay is known. Tiny droplets (100 cu. μ or greater) are sprayed with clean air (20 to 40 pounds pressure per square inch) onto a substrate. The droplets dry very rapidly. An aqueous ammonium acetate solution is used to aid in the dispersion; it is completely volatile.

Determination of the Weight of the Bushy Stunt Virus Particle by Electron Microscopy. ROBLEY C. WILLIAMS AND ROBERT C. BACKUS, University of Michigan, Ann Arbor, Mich.

This scheme is unique in that the magnification of the electron microscope does not have to be calibrated, and molecular weights as low as 70,000 can be determined. The method of counting the number of particles is based on the procedure described in the previous paper. The particle may have any configuration; its size must be sufficiently large so that it can be resolved. By determining the dry weight of the particle (virus) in a known volume, it is easy to calculate the weight of a single particle. The weight of a bushy stunt virus particle so determined checks well with data determined in a variety of ways.

Electron Metallography of Cast Irons. CHARLES H. GEROULD, Dow Chemical Co., Midland, Mich.

The nature of the graphite in normal cast iron as compared with that in under- and overtreated cerium- and magnesium-treated cast irons was related to the physical properties of these various samples. The nodular structure of graphite is readily detected by a two-step replica technique. Cleaning the etched metal surfaces by stripping collodion from the surface improves the quality of the replica.

Quantitative Metallography with the Electron Microscope. F. K. IVERSON AND ALFRED L. ELLIS, International Harvester Co., Chicago, Ill.

The usefulness of the electron microscope in corroborating and improving light microscopical data pertaining to structure in the 0.5μ range was demonstrated. Almost round carbide structures in an annealed steel were counted by means of a replica technique. Based on a calibration against light microscopical data, only ten plates of five different exposures each are necessary for an accuracy of ±0.5% of the mean. Fewer exposures are required for slightly poorer accuracy. This method was used to determine quantitatively the carbide content of steels, followed by relating this content to the physical properties of the steel.

Electron Metallography of Cemented Carbides. W. L. GRUBE, General Motors Corp., Detroit, Mich.

This application is somewhat similar to that described in the previous paper in that the electron microscope was used as an analytical tool in studying the microstructure of carbide tools, with special emphasis on the cemented carbides. The physical properties of these carbides, not completely resolved in a metallograph, are well exhibited by replicas in the electron microscope. These properties may then be correlated with the high speed tool performance.

Electron Diffraction Studies of Thin Iron and Chromium Films. LELAND L. ANTES, University of Texas, Austin, Tex.

The rate of air oxidation of thin films of iron, chromium, and nickel was followed by means of electron diffraction, the RCA EMU electron microscope having been set up as a diffraction camera. The effect of thinness of the metal film on the rate of oxidation was demonstrated.

Electron Microscopy of Enamel and Dentin. DAVID B. SCOTT AND RALPH W. G. WYCKOFF, National Institutes of Health, Bethesda, Md.

This paper was primarily concerned with the structure of the dentin as illustrated by shadowed collodion replicas of etched ground sections of teeth. Apparently human tooth dentin consists of relatively large fibers within tubules with collagen in the matrix. These fibers may contain other finer fibers.

Studies of the Action of Sodium Fluoride on Human Enamel by Electron Microscopy and Electron Diffraction. DAVID B. SCOTT AND RALPH W. G. WYCKOFF, National Institutes of Health, Bethesda, Md., AND ROBERT G. PICARD, RCA Victor Division, Camden, N. J.

Calcium fluoride forms gradually on the surface of human tooth enamel if the enamel is immersed in a 2 to 4% sodium fluoride solution. The normal apatite diffraction pattern is gradually replaced by the calcium fluoride pattern. This calcium fluoride can be washed off with water, but this becomes more difficult as the immersion time (in sodium fluoride) increases. The effect of fluoride ion on enamel could not be related to clinical data.

The Molecular Configuration in Seed Globulin Crystals. C. E. HALL, Massachusetts Institute of Technology, Cambridge, Mass.

This paper illustrated the application of the electron microscope in the determination of structure of macromolecules present in extremely small protein crystals. Preshaded silica replicas of crystalline edestine exhibited the molecular configuration from which the face-centered cubic nature of the crystal was derived. The unit cell dimension is 114 Å. (dry state), which was corroborated by x-ray diffraction data.

The Friday afternoon session consisted of a series of invited papers on complementary methods of microscopy.

Recent Developments in Conventional Microscopic Equipment and Techniques. A. H. BENNETT, American Optical Co., Stamford, Conn.

Three modifications of the standard light microscope represented the main thesis of this paper. They were the phase contrast microscope, the polanret microscope, and the fluorescent microscope. Although these optical systems are no longer new, the theory and particular applications were reviewed in a comprehensive way.

Recent Developments of the Optical Microscope. DAVID S. GREY, Polaroid Corp., Cambridge, Mass.

A new family of microscope objectives developed primarily in England and this country was discussed from the standpoint of aberrations, depth of focus, resolving power, etc. For example, the adaptation of Schwarzschild aplanatic mirrors for use in light microscope objectives has afforded high numerical apertures with good apochromatic correction between 220 and 800 mμ. This system has admirably lent itself for use in the color translating ultraviolet microscope. The optics and mechanical features of this more recent development at the Polaroid Corporation were described.

An Approach to X-Ray Microscopy. PAUL H. KIRKPATRICK, Stanford University, Stanford, Calif.

This paper traced the development of the idea of an x-ray microscope from Roentgen's time to the present day x-ray microscope which has resolved structures on the order of 1μ. The importance of the x-ray microscope stems primarily from the fact that x-rays have much more penetrating power than electrons. Furthermore, the resolving power is independent of wave length and is on the order of 70 Å. theoretically. Elliptical metal-coated glass mirrors are used instead of glass lenses so as to minimize absorption and astigmatism. The critical angle of incidence limits the numerical aperture, which in turn limits the resolution. The system is filled with helium, x-rays of wave length on the order of 2 to 3 Å. are used, and the image is focused on a fluorescent screen just as in the electron microscope. Magnifications of 100× have been realized.

Electron Microscope Research in Europe. E. G. RAMBERG, RCA Laboratories, Princeton, N. J.

There are two spheres of electron microscopy in Europe—the German and all the other countries in combination. Apparently there is little or no interchange of ideas between these two spheres of influence. Brücke in Mosbach is leading the way in so far as instrumental developments are concerned. The new A.G. electrostatic microscope is unique in that the gun and condenser are combined and focusing is done by varying the accelerating voltage. The special instrument built at the Philips Institute in Delft for the Royal Dutch Shell Corp. was also described. It is essentially a combination electron diffraction camera and electron microscope limited to 3000 diameters. A description of the British laboratories was also included.

The Saturday morning session was devoted exclusively to the application of electron microscopy to biological problems.

The last session consisted of papers dealing with a variety of topics.

Use of Polystyrene Latex 580 G, Lot 3584, in Electron Microscopy. CHARLES H. GEROULD, Dow Chemical Co., Midland, Mich.

The results of a survey from 16 different laboratories concerning the size distribution of Dow latex spheres were presented.

The consensus of opinion suggests that this material serves a useful purpose as an internal standard if it is used properly. Precautions in using the latex were critically reviewed.

Particle Size and Density of Dow Latex 580 G Measurements with the Ultracentrifuge. D. Gordon Sharp, Duke University, Durham, N. C.

Sedimentation velocity studies of the Dow Latex 580 G spheres corroborate the size and uniformity data compiled by the electron microscopists. Furthermore it was proved that this latex does not hydrate; hence it may also be used as an internal standard in ultracentrifugation studies of materials that do hydrate (viruses). This independent check on the size distribution of this Dow latex is a significant contribution.

Apparent Size of Small Semiopaque Objects. Raymond A. Kern and S. F. Kern, Lilly Research Laboratories, Indianapolis, Ind.

This paper disclosed an interesting proposed explanation for the apparent differences in size and shape of certain objects in certain types of specimens. For example, various investigators have reported elongated and nonuniformly sized Dow polystyrene latex spheres (580 G), which are presumably true spheres of very uniform size. The authors speculate that some unshadowed particles (including the Dow latex) charge up negatively and affect the beam as a lens. Thus, the final image appears larger than it should be. Shadowed specimens would dissipate this charge to the substrate wherever contact is made. Elongation would therefore result from some portions of the specimen not being shadowed. Specimen contamination as related to image size was also discussed.

A Removable Intermediate Lens for the RCA Model EMU. James Hillier, RCA Laboratories, Princeton, N. J.

This new development is a very significant contribution for several reasons. Magnifications between about 1000 and maximum instrument magnification are possible without modifying the objective or projector lens pole pieces. Spherical aberrations manifest as projector lens distortion are minimized. Within limits of about 5 kv., one value of magnification is independent of accelerating voltage.

The lens is placed directly above the projector lens and only minor changes in the projector lens circuit are necessary.

Optical Method for the Thickness of Thin Films. G. D. Scott, University of Toronto, Toronto, Ontario, Canada.

This paper described the application of Tolansky's (S. Tolansky, "Multiple Beam Interferometry," Clarendon Press, New York, 1948) method to the determination of thickness of thin films normally used as electron microscope specimens. The author of this paper has modified the original technique so that it may be applied to very small and very thin (10 Å.) films with improved precision.

A Technique for Studying the Electron Microstructure of Steel. D. M. Teague and L. H. Zimmerman, Chrysler Corp., Detroit, Mich.

This paper was divided into two parts. The first was devoted to a discussion of the Joint Committee on Electron Microstructure of Steel. The membership and aims of this group were outlined. Standardization of techniques and terminology are the chief goals. The second portion was used to describe a modified Formvar replica technique as applied to steels. The use of a monolayer of soap on the metal surface facilitates removal of the plastic replica.

Recent Advances in Electron-Microscopical Resinography. M. C. Botty, T. G. Rochow, and F. G. Rowe, American Cyanamid Co., Stamford, Conn.

The application of electron microscopy to the study of single-phase and polyphased resin systems was described with emphasis placed on specimen preparation and micrograph interpretation. Replication of molded or fractured surfaces reveals information concerning the compatibility, distribution, size, shape, etc., of the component units. These units are believed to be the macromolecular units. Correlation between structure and physical properties is possible.

Continuous Observations on the Formation of Metallic Films in the Electron Microscope. R. S. Sennett and T. A. McLauchlan, University of Toronto, Toronto, Ontario, Canada.

This was probably the first report on a continuous observation on the formation of thin evaporated metallic films in situ. The first deposition of condensed metal vapor (silver, gold, cadmium, etc.) in the electron microscope under operating conditions always occurs with the formation of a large number of small nuclei. Additional evaporation causes these nuclei to increase in size rather than in number.

The Analyst's Calendar

Philadelphia Analytical and Microchemical Groups

The Analytical and Microchemical Group of the Philadelphia Section, American Chemical Society, has arranged the following program. Albert B. Sample, John S. Sharpe Research Foundation, Bryn Mawr Hospital, Bryn Mawr, Pa., is secretary of the group.

December 6	Radiochemistry, a New Tool for Analytical Chemistry. Clement J. Rodden
February 7	Microchemistry in the Petroleum Industry. Harry Levin
March 8	Pharmaceutical Control. Charles M. Mitchell Microbiological Analysis. Robert J. Ferlauto

The March meeting will be held in the new Smith, Kline & French Laboratories, and after the formal program the laboratories of the Research and Control Departments will be open for inspection.

International Microchemical Congress

The Austrian Society for Microchemistry is planning the first International Microchemical Congress in Graz, Austria, birthplace of microchemistry, July 2 to 6, 1950.

A group of microchemists in the United States has proposed to the Mayor of Graz that a street be named in honor of Friedrich Emich, famous microchemist, especially in commemoration of the tenth anniversary of his death, in 1950. The Austrian Society of Microchemists, whose present chairman is Hans Lieb, successor of Fritz Pregl at the University of Graz, proposes also to erect a stone tablet at the Felix Dahn Platz, near which the new Chemical Institute of the Technische Hochschule will be built. A monument in honor of Emich is to be placed in the entrance hall of the Technische Hochschule at a later date.

The Division of Analytical Chemistry of the American Chemical Society has been invited to participate in the undertaking and to send representatives. It is expected that Fritz Feigl, now in Brazil, will attend the international meeting in Graz and will arrange for the cooperation of chemists in South American countries.

American Council of Commercial Laboratories. Miami, Fla., December 5 to 7

Third Symposium on Analytical Chemistry. Louisiana State University, Baton Rouge, La., January 30 to February 2, 1950

Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. William Penn Hotel, Pittsburgh, Pa., February 15 to 17, 1950

Chemical Institute of Canada, Analytical Division. Royal Connaught Hotel, Hamilton, Ontario, March 2 and 3, 1950. Second annual symposium

Third Annual Summer Symposium. Ohio State University, Columbus, Ohio, June 16 to 17, 1950

AIDS FOR THE ANALYST . . . . .

Automatic Apparatus for Feeding Liquid at Low Rate.

M. Z. Fainman, Standard Oil Company (Indiana), Whiting, Ind.

An apparatus for delivering liquid at the rate of 1 ml. per hour (Figure 1) can be constructed without difficulty from readily available component parts.

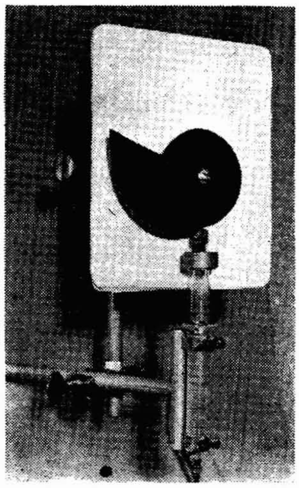

Figure 1. Apparatus for Delivering 1 Ml. of Liquid per Hour

The driving mechanism is a Telechron motor movement, Model IM210, which makes one shaft revolution per day. For protection from laboratory fumes, the motor is enclosed in a case through which only the shaft protrudes. The end of the shaft is fitted with an adapter bearing a cam, which in turn operates against a roller fastened to the top of a syringe piston.

By suitable design of the cam, a 10-ml. syringe can be made to deliver amounts of material ranging from 0.1 to 3 ml. per hour. By modification of the rate of shaft rotation, size of syringe, and design of cam, a wide range of delivery rates may be obtained for either continuous or fixed-increment delivery.

The template for preparation of the cam is made by drawing a circle at the center of a sheet of polar-coordinate paper, each 15° interval corresponding to 1 hour. The distance of thrust corresponding to 1 ml. on the syringe is carefully measured, and the distance corresponding to the volume desired at each increment of time is then plotted, using the periphery of the circle as the base line. To obtain a cam corresponding to continuous delivery, a smooth curve is drawn, which is used as the template (Figure 2, *A*). For incremental delivery the curve is composed of an arc plus the next increment at the appropriate time (Figure 2, *B*).

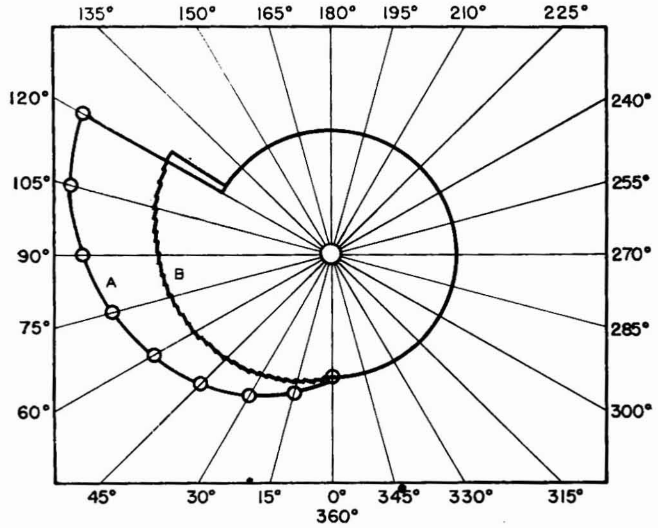

Figure 2. Curves for 10-Ml. Syringe with Thrust of 0.5 Cm. per Ml. and 1-Revolution-per-Day Motor

A. Continuous delivery of 1 ml. per hour
B. Incremental delivery of 0.1 ml. per 0.25 hour

The curve is transferred to 0.3-cm. (0.125-inch) sheet Bakelite, which is cut and polished to conform to the curve. In the case of a cam for incremental delivery, the sharp points are rounded to ensure smooth functioning.

Simple Liquid Circulating System.

Norman F. Floyd, The Lankenau Hospital Research Institute, and Institute for Cancer Research, Philadelphia, Pa.

An inexpensive system for circulating liquids (hot or cold) through condensers can be easily devised in the laboratory from materials usually available.

Figure 1 shows the system as set up for laboratory use.

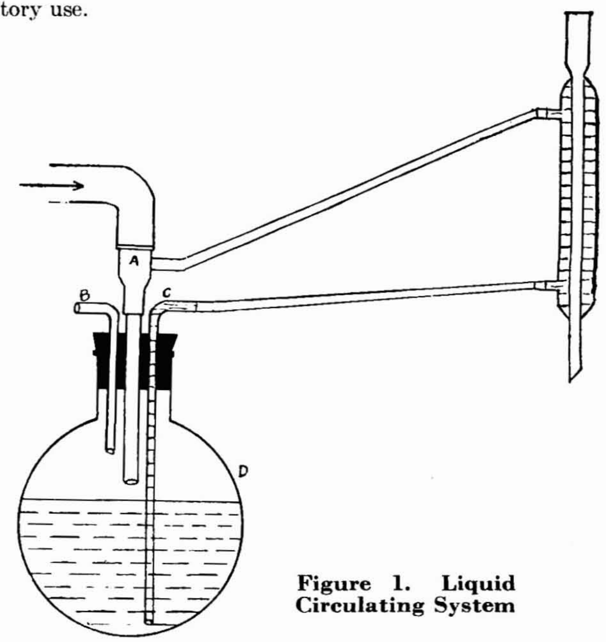

Figure 1. Liquid Circulating System

An ordinary water aspirator, *A*, is inserted in a vented, *B*, rubber stopper containing a glass tube, *C*, that reaches to the bottom of the liquid reservoir, *D* (a flask or bottle). The top opening of the condenser is connected to the side arm of the aspirator and the bottom opening to *C*. Air pressure, from a suitable source, is forced through the aspirator until sufficient vacuum is developed at its side arm to circulate the liquid through the condenser. The liquid returns to the reservoir through the aspirator. Ice and water, with or without added salt, can be used for 0° C. or lower, and a Glas-Col heating mantle or immersion of the reservoir in a temperature-controlled bath will serve for higher temperatures. This system is used in this laboratory for reactions employing low boiling solvents.