PHENANTHROLINE AND SUBSTITUTED PHENANTHROLINE INDICATORS

Their Preparation, Properties, and Applications to Analysis

G. FREDERICK SMITH, Ph.D.
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and

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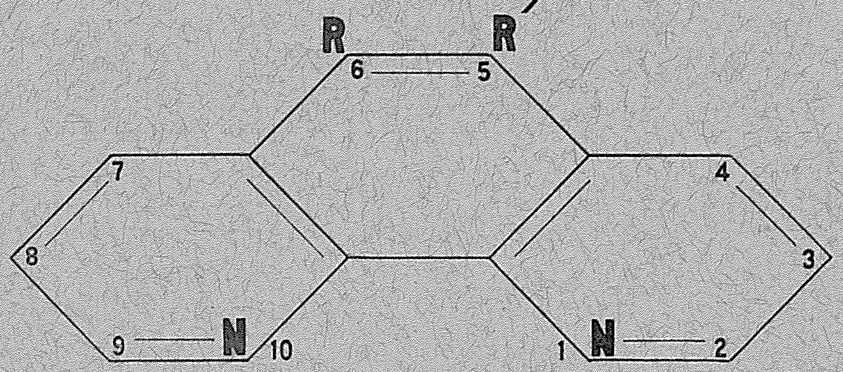
Director of Chemical Research, Textile Division
U. S. Rubber Company
Hogansville, Georgia



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PREFACE

The publication of the booklet entitled "Ortho-Phenanthroline" by the senior author of the present work, appeared in 1935, approximately two years after the first published application of 1,10-phenanthroline in analysis. Since the first introduction of the ferrous phenanthroline ion as an oxidation-reduction indicator through the studies of this remarkable reagent by Professors Walden, Hammett, and their associates, many additional applications have been added yearly. The first substituted 1,10-phenanthroline compound, namely 5-nitro-1,10-phenanthroline, also described by the latter investigators has been followed by the preparation of a considerable group of mono- and di-substituted derivatives, the properties of which are to be described.

The booklet "Ortho-Phenanthroline" was favorably received and a second printing of it was made in response to an extensive demand. During the past decade many new uses for the phenanthroline indicators have been described. Developments in the use of tetravalent cerium in volumetric analysis owes most of its, at present extensive applications, to the adaptability of the phenanthroline indicators in determining the equivalence point in these cerate oxidations. The determinations of glycerol in the soap industry as well as a whole new series of volumetric procedures in the determination of organic compounds including the sugar industry have been made practicable through the use of the phenanthroline indicators. Many uses in the field of photometric quantitative analysis with 1,10-phenanthroline as the color producing ingredient have been developed. The determination of iron in wine and fruit juices, iron in blood, tissues, and other biological materials have become routine control analytical procedures.

The fields of the analysis of metallurgical materials, special steel analysis, and the ceramic industries including cement and lime, draw repeatedly upon the use of the phenanthroline indicators in the development of new analytical procedures in their research and control laboratories. Many additional uses for the various phenanthrolines can be predicted for the future. Their applications as special precipitants have yet to be developed as well as widely extended applications in fields previously mentioned.

The present work's description of a series of new substituted phenanthrolines and their synthesis was developed as a result of the fact, as shown by Professors Walden and Hammett, that substitutions at the 5 and 6 position do not prevent the formation of the complex ferrous-1,10-phenanthroline cation, but at the same time materially alter the properties of the resultant product. The newly prepared substituted phenanthrolines, with the exception of 5-methyl-1,10-phenanthroline and possibly the 5-nitro-6-methyl-1,10-phenanthroline, are of limited analytical utility as a result of the new properties associated with their synthesis. The study of these products, however, is not without significance in the field of conjecture of theoretical aspects and may be suggestive of some future application in related fields of study.

In the present work the authors have drawn heavily upon the valuable published studies of the various research workers in the field and it is hoped that the assembly of information will be a stimulant to further investigations on the part of others.

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February, 1944

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FOREWORD

To F. Blau is given the credit for the discovery that 1,10-phenanthroline forms a highly colored system with iron in aqueous solution. Following this original observation in 1898, little seems to have been done for three decades to use the fact analytically. In 1931 the results of R. P. Chapman's work were published on the application of the iron-phenanthroline complex as a redox indicator.

This system possesses very desirable analytical properties, among which the following are noteworthy: intense color, good contrast in hue in the oxidized and reduced forms, high sensitivity, ready reversibility, stability, reaction in acidic media, and high redox potential. The various derivatives of the parent compound which have been studied show some variation in these characteristics.

It seems to be accepted that 1,10-phenanthroline reacts with iron to form a five-membered chelate ring involving the metal and the cyclic N—C—C—N group. This reaction is very effective for using certain compounds of this type as color-forming reagents. Thus, 1,10-phenanthroline is excellent for determining small amounts of ferrous iron. The reagent is water-soluble, colorless, stable, and sensitive; in addition, it reacts stoichiometrically, gives a stable and desirable hue, is little influenced by pH change between 2 and 9, and is relatively selective. It is a serviceable reagent for copper, as well as iron.

The related compounds, 2,2′-bipyridyl and 2,2′,2″-terpyridyl, have similar, desirable properties. However, there are important differences. For example, the iron complexes are not useful redox indicators in hot acid, as is that with 1,10-phenanthroline. Also the terpyridyl is the preferable reagent for determining cobalt colorimetrically. In general, this type of chelate complex appears unusually promising in analytical chemistry.

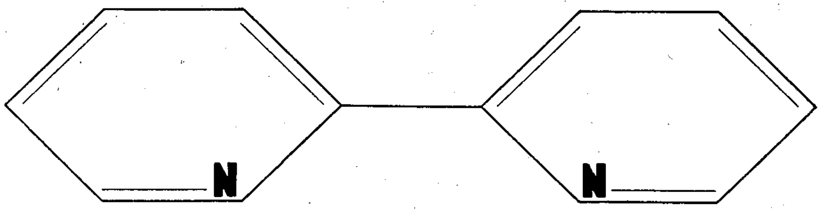
M. G. MELLON.

M. G. MELLON
Purdue University

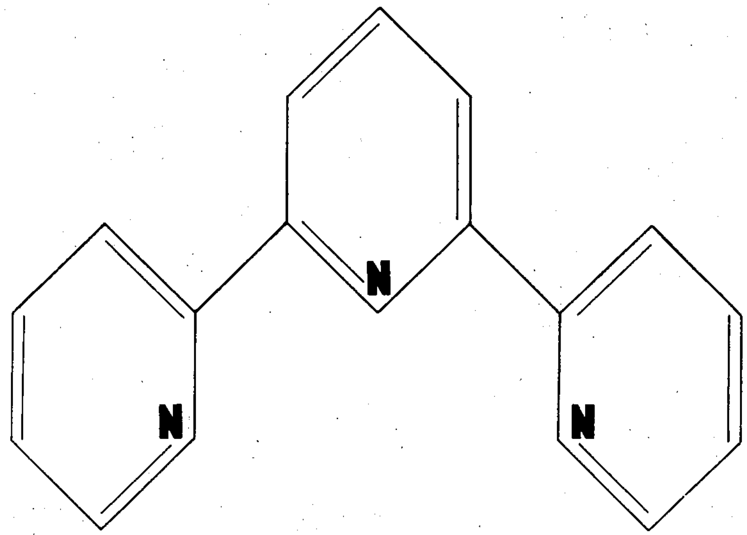
INTRODUCTION

The active functional group $=N-\overset{\|}{C}-\overset{\|}{C}-N=$, through which bipyridyl, terpyridyl, and 1,10-phenanthroline owe their capacity to form complex cations of the chelate ring type when reacted with ferrous and other ions, represents probably the most important functional group of all organic analytical chemical reagents in this category.

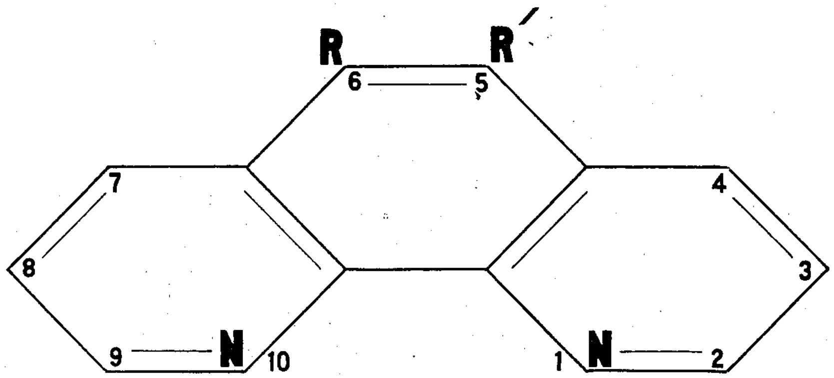
The structural formulae for the three types of compounds at present studied as analytical reagents are the following:



Bipyridyl



Terpyridyl



1,10-Phenanthroline

In the case of the last formula above, the various modified forms thus far studied are represented as follows:

R and R′ = H	forms 1,10-Phenanthroline
R = H and R′ = NO_2	forms 5-Nitro-1,10-Phenanthroline
R = H and R′ = CH_3	forms 5-Methyl-1,10-Phenanthroline
R = H and R′ = Cl	forms 5-Chloro-1,10-Phenanthroline
R = H and R′ = Br	forms 5-Bromo-1,10-Phenanthroline
R = CH_3 and R′ = NO_2	forms 5-Nitro-6-Methyl-1,10-Phenanthroline

The five and six position substitutions may be reversed and identical compounds result.

The metal ions other than ferrous iron which form complex cations with these compounds are Cd^{++}, Cu^{++}, Zn^{++}, Co^{++}, Ni^{++}, Cr^{++}, and Ru^{++}. In the case of the ferrous complex all the resulting compounds are red (purple in the case of terpyridyl) except with the ruthenium complex which is yellow. In all other cases the resulting complex cations are practically, if not entirely, colorless. Other metal ions may also form such complex cations which have not as yet been studied.

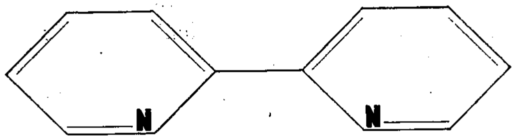
The most important application of these highly colored complex cations involves their use as oxidation-reduction indicators. The reduced form of the metal ion complex is colored intensely red or a slightly modified shade (the exception being the ruthenium complex). In the oxidized form the color resulting is a faint blue of about one-tenth the color intensity of the reduced form (exception, the ruthenium complex, the oxidized form of which is colorless).

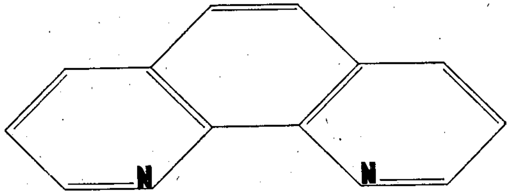
In the field of volumetric oxidation-reduction reactions employing the dichromate ion or tetravalent cerium as oxidant, the use of this type indicator is practically indispensable. This results from the fact that the phenanthroline type of complex is remarkably resistant to the destructive action of acid and to the presence of small excess quantities of oxidizing agents. The color intensity is adequate to counteract the green of the chromic ion and color from other ions which may be present, such as copper, nickel and ferric iron. In addition, the phenanthroline types of indicators are reversible in their transition from the ferrous to ferric forms. Repeated reversals of this equilibrium, almost indefinitely, result in no apparent diminution in color intensity. The potential at which these two forms may be converted from one to the other may be varied over a wide range, depending upon the strength of acid employed.

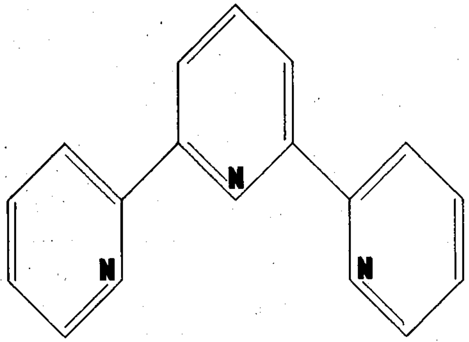
The great popularity in the present day use of cerate volumetric procedures was in great measure due to the development of the phenanthroline type of indicators. Previously the potentiometric equivalence point determination was required in most such cases. By the use of proper acid concentrations, the use of dichromate as oxidant (that is in such oxidations as that of ferrous iron) is entirely practicable in conjunction with the phenanthroline type of indicators although this fact is not generally known. These conditions will be defined in the subsequent material of this booklet. In the case of the use of 0.01 to 0.005 N permanganate solutions as oxidant, the equivalence point may be greatly intensified by use of the ferroin indicators. Micro-titration of iron is an example of such application.

By the use of perchloric acid solutions the quantitative determination of oxalic acid (and indirectly that of the determination of calcium) can be carried out at room temperature using perchloratoceric acid as oxidant and nitro-ferroin as indicator. This is a great advantage over the necessity of completing such oxidations at the boiling temperature as is required when sulfuric acid solutions are oxidized, using permanganate as oxidant. The reverse titration of perchloratoceric acid by use of standard oxalic acid solutions is equally effective. This reaction is particularly of great value in the case of the determination of organic compounds for which reactions a time interval for oxidation with excess perchloratoceric acid in perchloric acid solutions is required, followed by the titration of excess reagent. Since oxalic acid solutions are known to be stable in storage they are to be preferred for these determinations as compared to a standard ferrous solution which is not stable upon storage.

The use of bipyridyl, 1,10-phenanthroline, and terpyridyl, as color producing reactants in the determination of iron and other ions, is now well established and is almost universally employed in the analysis of wine, in biological materials such as blood, tissues, and feces, and in the micro-determination of iron in general inorganic analysis. These procedures will be taken up in full in the subsequent discussion.







The use of 1,10-phenanthroline and substituted phenanthrolines as precipitants for certain cations and anions by the addition of the phenanthrolinium ferrous sulfate ion has not been studied extensively. Data will be given in the subsequent material suggestive of further useful applications in this field. The determination of ferrous in the presence of ferric ions by use of 1,10-phenanthroline, in the presence of perchloric acid, is the reverse application. The separation of perchlorate from chlorate ions is another possible application in which ferroin solutions may serve as precipitant. Also the separation of tungstate ions from molybdate is a possibility. Solubility data is available for some of these applications and will be included in the material of this booklet.

The extremely valuable experimental studies in the applications to analysis of the materials described in this work, which were due to Professors Walden and Hammett of Columbia University, Professor Willard of the University of Michigan, and Professor Mellon at Purdue University, is here most gratefully acknowledged. Without their contributions, the position these methods represent, of supreme importance to quantitative analysis, would not have been, as yet, a matter of practical realization.

SECTION 1

ORGANIC CHEMISTRY OF 1,10-PHENANTHROLINE AND ITS DERIVATIVES

Historical. Organic Chemistry has made tremendous advances in the past sixty years. The Organic Chemist, in his zealous search for new compounds and new phenomena has left behind a trail of those seemingly useless substances we sometimes refer to as ''laboratory curiosities.'' It is unfortunate that the useful members of this host of organic compounds must frequently remain without practical application for so long a period of time. Striking indeed is the case of sulfanilamide. It was first synthesized by Gelmo *(1)* in 1908, but its chemotherapeutic activity was unknown until about 1935 *(2)*. To the analytical chemist it is, perhaps, even more amazing that although the application of the ferroin type indicator was first reported in 1931 by Walden, Hammett, and Chapman *(3)*, a phenanthroline derivative capable of forming a ferroin indicator had been reported forty-two years previously *(4)*.

Gerdeissen (loc. cit.) in his paper on the synthesis of 2-methyl-(1,10)-phenanthroline failed to mention the ferroin reaction, that is, the formation of the intensely red ferrous complex. To one who has worked with the 1,10-phenanthrolines it is almost inconceivable that he could have failed to observe this phenomenon. The ferroin reaction is so sensitive that in practise precautions must be taken to prevent discoloration of 1,10-phenanthroline crystals by iron rust in the laboratory atmosphere; the moist product is readily discolored by a ''stainless'' steel spatula.

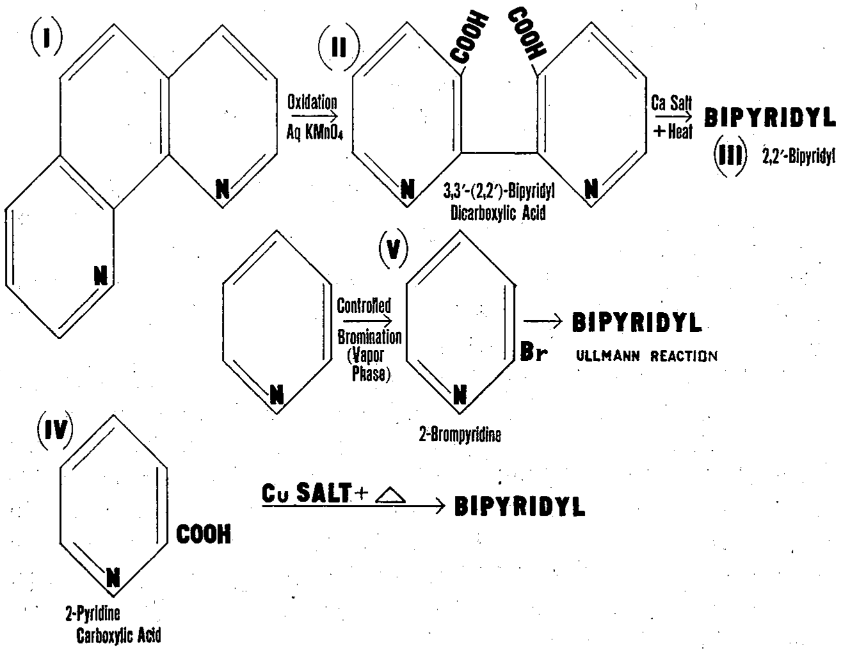
The formation of a ferroin type complex was first reported by Blau *(5,6)*. It is interesting to note that the 2,2′-bipyridyl complex with ferrous iron was known before that of 1,10-phenanthroline. Blau's interest in the metal complexes of 2,2′-bipyridyl finally led him to the synthesis of 1,10-phenanthroline *(7)* itself. The similarity in the structure of these compounds will be discussed in a later section.

---

(1) Gelmo, *J. prakt. Chem.*, (2) 77, 372 (1908).
(2) Zinsser, *Chem. and Eng. News*, 21, 1159, (1943).
(3) Walden, Hammett, and Chapman, *J. Am. Chem. Soc.*, 53, 3908 (1931).
(4) Gerdeissen, *Ber.*, 22, 245 (1889).
(5) Blau, *Ber.*, 21, 1077 (1888).
(6) Blau, *Monatsh.*, 10, 375 (1889).
(7) Blau, *Monatsh.*, 19, 666 (1898).

In two of his papers *(5,6)* Blau discusses the formation of various metal complexes. A somewhat detailed study of the ferrous complex of 2-methyl-(1,10)-phenanthroline *(5)* has since been reported by Pfeiffer and Christeleit *(8)*.

The Structure of 1,10-phenanthroline. The molecular structure of 1,10-phenanthroline follows from its methods of synthesis which will be discussed later. An interesting proof of the structure is illustrated by the following series of reactions:



Oxidation of 1,10-phenanthroline (I) with aqueous permanganate readily gives the corresponding 3,3′-(2,2′)-bipyridyldicarboxylic acid (II). This is decarboxylated by conversion to the calcium salt and heating to form the well known 2,2′-bipyridyl (III). The structure of the latter is indicated by its synthesis from either α-picolinic acid (IV) or 2-bromopyridine (V).

Although the molecular structure of 1,10-phenanthroline is evident from the above discussion, the position of the double bonds has not been proved. Resonance no doubt takes place but the bond structure as indicated in (I) is most probable for the following reasons:

---

(8) Pfeiffer and Christeleit, *J. prakt. Chem.*, 151, 127 (1938).

(1) The 5- and 6-positions appear to be equivalent because of the identity of the chlorophenanthrolines obtained from 5- and 6-chloroquinoline *(9)*. This would require a symmetrical structure as indicated in (I).

(2) Construction of the molecular model indicates that the symmetrical structure involves the least strain. The model, incidentally, also points to a planar molecule.

(3) The ease with which 1,10-phenanthroline (and particularly the methyl derivative) is ruptured at the 5,6-positions indicates that the molecule is unsaturated at this point. This is in agreement with the structure (I).

(4) The structure (I) agrees with the Freas rule.

Chemical Properties of 1,10-phenanthroline and its Derivatives. (a) *Salt formation.* The presence of two pyridine rings in the molecule would lead one to expect 1,10-phenanthroline to be basic. Actually, this is the case. The two tertiary nitrogen atoms readily add mineral acids to form 1,10-phenanthrolinium salts which are very soluble in water and other polar solvents. In this respect it is quite similar to pyridine and quinoline which readily react with acids to form pyridinium and quinolinium salts.

The basic nature of 1,10-phenanthroline can further be illustrated in an interesting manner by adding a few of the crystals to a dilute solution of ferric chloride. The phenanthroline gradually goes into solution as the hydrochloride while the hydrous ferric oxide is precipitated. Contrary to what one might expect, the blue, oxidized form of the ferroin indicator is *not* formed under these conditions.

The basicity of the nitrogen atoms is greatly affected by substitution in the 5- and 6-positions. The presence of a methyl group in the 5(6)-position increases the basicity; electronegative substituents such as nitro, bromo, and choro lower the basicity. Substitution apparently has the same effect as in ammonia, and examination of the structure (I) indicates that 5- and 6-positions are in vinylogous relation to the nitrogen atoms. The 5-bromo-6-nitro derivative is insoluble in strong mineral acids. In passing it is interesting to observe that electronegative substituents in the 5(6)-position results in a ferroin of lower potential than that of the parent base.

Picric acid in equimolecular proportion forms monopicrates with 1,10-phenanthroline and most of its known derivatives. These are usually high melting, nicely crystalline compounds of definite composition which have been useful as confirmatory derivatives.

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(9) Richter and Smith, *J. Am. Chem. Soc.*, 66, 396 (1944).

(b) *Substitution reactions.* The pyridine rings in 1,10-phenanthroline maintain their well-known property of resistance to aromatic substitution. Substitution, therefore, undoubtedly takes place in the only two benzenoid positions, namely the 5- and 6-positions.

Nitration *(10,11)* is effected with relative ease. The mononitro derivative is obtained in about 60 per cent yield, by heating the parent base in a mixture of nitric and sulfuric acids.

A dinitro derivative cannot be prepared by direct nitration under any known conditions. The entrance of a second nitro group into either of the pyridine rings or in *ortho* (the remaining benzenoid position) relation to the first would be unlikely. Increasing the temperature, strength of the nitric acid, or the use of a nitrate with stronger sulfuric acid merely results in the mononitro derivatives and eventual rupture of the molecule.

Sulfonation does not take place under any known conditions.

The formation of a tribromo derivative of undetermined structure by heating the parent compound with aqueous bromine under pressure has been claimed *(12)* but the authenticity of this source is questionable. Little has been reported about the direct halogenation of 1,10-phenanthroline.

(c) *Oxidation.* Aqueous permanganate readily breaks the 5,6-double bond as already noted. The 5-methyl derivative is, as one would expect by analogy with the corresponding 9(10)-methylphenanthrene, more sensitive to oxidation than the parent compound. No 5-carboxylic acid can be obtained by oxidation of the 5-methyl compound with permanganate.

The 5(6)-amino derivative obtained by reduction of the corresponding nitro compound is very sensitive to oxidation and although it forms a ferrous complex the use of this ferroin as an indicator is precluded because of its instability in oxidizing solutions. In postulating new derivatives for indicator purposes it is important to consider stability under strong oxidizing conditions.

(d) *Hydrate formation.* 1,10-phenanthroline and most of its known derivatives form stable monohydrates. These are definite compounds of characteristic melting point which lose their water of hydration at a characteristic temperature. Infra-red studies indicate that the formation of a monohydrate by 1,10-phenanthroline takes place by virtue of hydrogen bonding to the nitrogen atoms.

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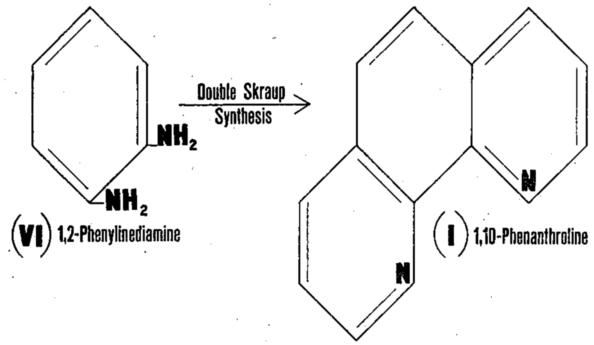
(10) Hammett, Walden, and Edmonds, *J. Am. Chem. Soc.*, 56, 1092 (1934).

(11) Marvel and Pepple, private communication.

(12) N. V. Nederlandsche Kininefabriek. French Patent No. 804,454.

Synthesis of 1,10-phenanthroline. A consideration of the structural formula (I) suggests at least two general methods of synthesis: (1) The molecule may be looked upon as derived from 1,2-phenylenediamine (VI) (ortho-phenylenediamine and hence the more common name ortho-phenanthroline), or (2) it may be considered as being derived from 8-aminoquinoline (VII).

The first consideration would require, synthetically, two Skraup reactions, both on the same molecule, and each leading the formation of a condensed pyridine ring from each of the two amino groups:



(VI) 1,2-Phenylinediamine

(I) 1,10-Phenanthroline

Blau *(5)* reported a yield of 30 per cent of 1,10-phenanthroline from 1,2-phenylenediamine by this method. It is significant that no one has since reported success in duplicating the yield he reported. More recently, C. R. Smith *(13)* reported the failure of this synthesis. In a study at the University of Illinois the authors found that although some 1,10-phenanthroline is indeed formed, the desired reactions are accompanied by so many side reactions as to make the isolation of the pure base very difficult and the method impractical. A consideration of the chemical properties of 1,2-phenylenediamine, e.g., its high reactivity and sensitivity to oxidation would indicate that it is a poor starting material to be employed under the drastic conditions required by a Skraup synthesis. The method is, then, only of theoretical interest and of no preparative value.

The second consideration, derivation from 8-aminoquinoline (VII) is synthetically successful *(7,13)* and of preparative value *(14)*.

The 8-aminoquinoline is obtained by reduction of the corresponding nitro compound, which, in turn, is synthesized from 2-nitroaniline by the method of Skraup in nearly quantitative yield.

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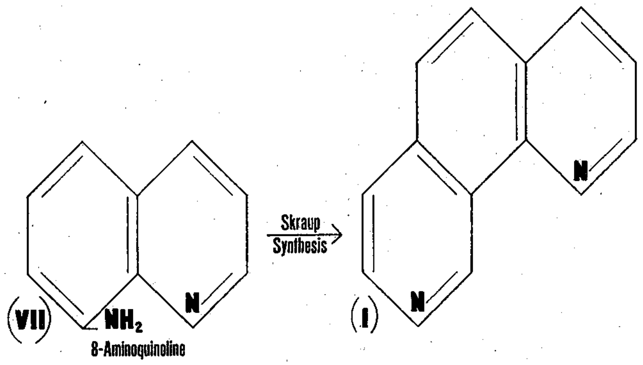
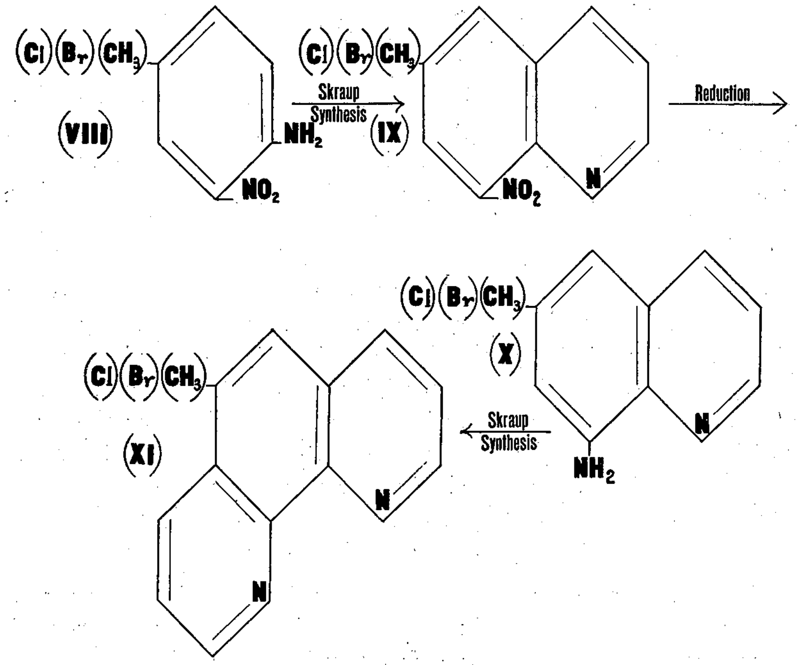
(13) Smith, *J. Am. Chem. Soc.*, 52, 397 (1930).

(14) Smith and Getz, *Chem. Rev.*, 16, 113 (1935).



Synthesis of the 5(6)-chloro, bromo, and methyl derivatives. Application of the Skraup synthesis to suitably substituted 2-nitroanilines (VIII) followed by reduction of the resulting 8-nitroquinolines (IX) and application of a second Skraup synthesis to the 8-aminoquinolines so formed (X) is the basis for the synthesis of these derivatives (9):



It is clear, that from the standpoint of orientation at least, 2-nitroanilines substituted in either the 4- or 5-positions could be employed

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because these positions later become identical in 1,10-phenanthroline. Almost any of the usual methods for reducing the nitro group to amino may be employed in the step (IX)-(X) and several different procedures will be illustrated under the preparation of these derivatives.

SECTION 2

THE PREPARATION OF MATERIALS

Notes on the Method of Skraup. The Skraup synthetical method enables the preparation of quinoline and many of its derivatives from primary aromatic amines. (The phenanthrolines may be considered as quinoline derivatives.) For a discussion of the mechanisms which have been proposed for the reactions involved, the reader is referred to the more advanced texts in Organic Chemistry *(15)*.

In general, the amine is heated with a mixture of glycerol, sulfuric acid as a dehydrating (condensing) agent, and an oxidizing agent, for example, nitrobenzene or arsenic pentoxide. The heating of a mixture of these different chemical types might naturally be expected to result in many different chemical reactions, which is, in practice, unfortunately the case. In addition to the desired reactions carbonization always takes place, some polymerization undoubtedly occurs, and oxidation of the starting amine tends to lower the yield. Unless the proportions of reactants and the conditions are carefully controlled these reactions will sometimes take place with explosive violence.

The following general procedure was developed *(9)* for the synthesis of the nitroquinoline intermediates and 1,10-phenanthrolines. It was found to give an optimum yield with a minimum of carbonization and difficulty in controlling the reactions involved.

Mass relations:

One (1) mole of the aromatic amine.

Four (4) moles of dry glycerol (300 per cent excess).

Three-fourths (0.75) mole of arsenic pentoxide (50 per cent excess).

A weight of sulfuric acid equal to 50-60 per cent of the weight of glycerol employed.

For best results the amine should be pure. Although the reactions will normally proceed in spite of rather impure starting material the impurities may give rise to side reactions and by-products which make it difficult to isolate the desired product in good yield.

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(15) e.g. "Organic Chemistry. Lectures for the Advanced Student" by Reynold C. Fuson. Edwards Brothers, Ann Arbor, Mich., 1939, p. 278.

It is essential that the glycerol be dry. This is accomplished by passing a current of dry air (calcium chloride tube) through the glycerol for three hours at 170-180 degrees C.

The arsenic pentoxide should be relatively free of iron and it is preferable to employ a C. P. grade.

Standard reference texts *(16)* usually recommend a proportion of sulfuric acid which is too high and which leads to excessive carbonization. There is, further, no advantage in using sulfuric acid of higher strength than the usual concentrated acid (Sp. Gr. 1.84). Experiments were conducted employing 10 and 20 per cent oleum and the yield was not improved. In some cases, the use of oleum resulted in the formation of excessive amounts of tar. It is important, however, that fresh sulfuric acid (Sp. Gr. 1.84) be employed and that it has not been diluted by exposure to atmospheric moisture for any length of time. Under no condition must any of the acid be added after the reaction mixture has gotten up to temperature or serious loss of yield will result.

It is apparent that any fraction or multiple of the quantities given above may be employed as long as the original molecular proportions are maintained.

In general, the amine, glycerol, and arsenic pentoxide are weighed into a *dry* three-necked flask of appropriate size. This is then equipped with a glycerol sealed stirrer in the center, a thermometer dipping into the liquid in one side arm, and a pyrex condenser fitted with a calcium chloride drying tube in the remaining arm. With the stirrer in rapid motion the sulfuric acid is added in a thin stream through the condenser at such a rate as to maintain the temperature below 130 degrees. When the addition of acid is complete (15-20 min.) the drying tube is replaced and the temperature held at 130-135 degrees C. for one hour. Usually, the heat of reaction will suffice for about the first half hour and then heat is applied carefully with an electric cone heater or an oil bath, but never directly with a burner. After an hour at 130-135 degrees there is usually little danger of the reaction getting out of control; the applied heat is then increased and the mixture allowed to reflux actively for six more hours. If the heat is applied indirectly the stirrer may be stopped after the second hour as there is little danger of the material "caking" due to localized heating. During the refluxing stage the reaction mixture reaches a maximum temperature and gradually drops as the reaction proceeds for several hours.

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(16) For example, see Houben Weyl, "Die Methoden der Organischen Chemie," Part 2, Vol. I., p. 369. Georg Thieme, Leipzig (1911).

The reaction mass, at the conclusion, is always a black, tarry product which becomes rather viscous on cooling to room temperature. The method of isolation and purification varies somewhat with the product being prepared and will be discussed separately under the preparation of each derivative.

Preparation of 1,10-phenanthroline. The preparation of this product in quantity has been adequately discussed *(14)*. For the synthesis of the base on a laboratory scale the following procedure may be applied:

8-nitroquinoline. A mixture of 138.1 g. (1 mole) of 2-nitroaniline (m. p. 70-71 degrees Eastman), 368.2 g. (4 moles) of glycerol, 172.4 g. (0.75) mole of arsenic pentoxide and 220.0 g. of sulfuric acid is treated according to the general procedure. The reaction mass, after cooling to room temperature, is poured into three liters of water, 30 g. of decolorizing carbon added and the mixture warmed and stirred for one hour. The cold filtrate is neutralized slowly with 1:1 aqueous ammonia and the crude product, which is suitable for the next step, is filtered. A small portion recrystallized from methanol melts at 91-92 degrees.

8-aminoquinoline. A slurry of the crude 8-nitroquinoline obtained above in 500 cc. of dilute (1:4) hydrochloric acid is stirred during the gradual addition of 175 g. of iron dust. The mixture is warmed on the steam bath and stirred for one hour. After cooling and making alkaline with 10 per cent aqueous sodium hydroxide the crude 8-aminoquinoline is extracted with hot benzene. The benzene is removed by distillation and the product purified by distillation in vacuo and/or recrystallization from ligroin. Yield 70-75 g. m.p. 70-72 degrees.

1,10-phenanthroline. The general procedure is applied to a mixture of 36.0 g. (0.25 mole) of 8-aminoquinoline, 92.1 g. (1.0 mole) of glycerol, 43.7 g. (0.19 mole) of arsenic pentoxide and 50.0 g. of sulfuric acid. The reaction mass is poured into 750 cc. of water, neutralized in the cold with 1:1 ammonia and the black syrupy residue extracted with boiling benzene. After removal of the benzene the product is purified by distillation in vacuo and/or crystallization. The monohydrate, m.p. 99-100 degrees, crystallizes from ethanol-water mixture or moist benzene. The anhydrous compound, m.p. 117 degrees, may be obtained by recrystallizing from benzene-petroleum ether mixture after distilling off the water of hydration. Yield of monohydrate 25-27 g.

Preparation of 5(6)-nitro-(1,10)-phenanthroline. A mixture of one gram of 1,10-phenanthroline m.p. 117 degrees, 5 ml. of sulfuric acid (Sp. Gr. 1.84) and 3 ml. of nitric acid (Sp. Gr. 1.42) is held at 120 degrees for two hours. The yellow solution is poured onto 50 g. of cracked ice and neutralized in the cold with 30 per cent sodium hydroxide. After filtering, the dry product is recrystallized from 95 per cent ethanol. Yield 0.75 g. or 60 per cent of the theory.

Preparation of 5(6)-methyl-(1,10)-phenanthroline. (8-nitro-6-methylquinoline.) The general procedure is applied to a mixture of 152.1 g. (1 mole) of 3-nitro-4-aminotoluene (m. p. 114-115 degrees Eastman), 368.2 g. (4 moles) of glycerol, 172.4 g. (0.75 mole) of arsenic pentoxide and 220.0 g. of sulfuric acid. The reaction product is poured into 3 liters of water, neutralized in the cold with 1:1 aqueous ammonia, and the crude 8-nitro-6-methylquinoline recovered by filtration. A portion recrystallized from ethanol melts at 121-122 degrees.

8-amino-6-methylquinoline. The crude nitro derivative above is warmed with 400-450 cc. of 95 per cent ethanol in a five-liter flask to effect solution as far as possible. The flask is then fitted with a two-hole rubber stopper carrying a long water-cooled condenser and an inlet tube for hydrogen sulfide. Fifty ml. of conc. aqueous ammonia are added through the condenser and hydrogen sulfide gas is passed into the mixture slowly with occasional shaking. The nitromethylquinoline gradually goes into solution, and the heat of the reaction is sufficient to cause the alcohol to reflux gently. After one hour the mixture is warmed on the steam bath and more hydrogen sulfide added occasionally. At the end of two hours the reaction is complete.

The reaction mixture is poured slowly into two liters of ice-cold, dilute (1:3) hydrochloric acid and the alcohol removed by distillation. The remaining liquid is neutralized with solid sodium carbonate and the 8-amino-6-methylquinoline obtained in fairly pure form by distillation with steam. Yield 70-80 g. The white crystals tend to become yellow on exposure to air; m.p. 60-62 degrees from petroleum ether (30-60 degrees fraction).

5(6)-methyl-(1,10)-phenanthroline. A mixture of 47.4 g. (0.3 mole) of 8-amino-6-methylquinoline (dried over conc. sulfuric acid in a vacuum dessicator), 51.7 g. (0.225 mole) of arsenic pentoxide, 110.5 g. (1.2 mole) of glycerol and 60 g. of sulfuric acid is allowed to react according to the general procedure. The reaction product is poured into 800 cc. of water, warmed with 8 g. of decolorizing carbon and the cold filtrate neutralized with 1:1 aqueous ammonia. The tarry residue is extracted with boiling benzene. The clear benzene extract is dried over solid potassium hydroxide, decanted, and the residue, after removal of the solvent, distilled in vacuo. The fraction 280-282 degrees/13 mm. is recrystallized from benzene-petroleum ether, m. p. 5-methyl-(1,10)-phenanthroline 114 degrees, yield 38.2 g.

Preparation of 5-methyl-6-nitro-(1,10)-phenanthroline. A solution of 1.00 g. of 5-methyl-(1,10)-phenanthroline (m.p. 114 degrees), 5 cc. of conc. sulfuric acid and 3 cc. of conc. nitric acid is held at 120 degrees for two hours. The yellow nitration mixture is poured onto 50 g. of cracked ice and neutralized in the cold with 30 per cent aqueous sodium hydroxide. The precipitated 5-methyl-6-nitro-(1,10)-phenanthroline is filtered, dried, and crystallized from 95 per cent ethanol, m. p. 268-270 degrees, yield 0.98 g. or 80 per cent of the theoretical.

Preparation of 5(6)-chloro-(1,10)-phenanthroline. (8-nitro-6-chloroquinoline.) The general procedure is applied to a mixture of 172.5 g. (1 mole) of 4-chloro-2-nitroaniline (m. p. 115-116 degrees Eastman), 368.2 g. (4 moles) of glycerol, 172.4 g. (0.75 mole) of arsenic pentoxide and 220 g. of sulfuric acid. The reaction product at room temperature is poured into 3 liters of water and neutralized in the cold with 1:1 aqueous ammonia. The crude 8-nitro-6-chloroquinoline is filtered and used without further purification in the next step. A portion recrystallized from ethanol melts at 159 degrees.

8-amino-6-chloroquinoline. The crude, dry nitro compound above is dissolved in 550 ml. of conc. hydrochloric acid. Stannous chloride dihydrate 677 g. (3 moles) is added gradually with stirring, and the mixture is heated on the steam bath for two hours. After dilution with water and neutralizing with 30 per cent sodium hydroxide the 8-amino-6-chloroquinoline is obtained as fairly pure needles by distillation with steam, m. p. 73 degrees (from petroleum ether 60-90 degrees fraction), yield 96-100 g. (54 per cent based on the 4-chloro-2-nitroaniline taken).

5(6)-chloro-(1,10)-phenanthroline. A mixture of 35.7 g. (0.2 mole) of 8-amino-6-chloroquinoline, 73.7 g. (0.8 mole) of glycerol, 34.5 g. (0.15 mole) of arsenic pentoxide and 42 g. of sulfuric acid is treated according to the general procedure. The reaction mass is poured into 700 cc. of cold water, neutralized in the cold with aqueous ammonia and the black residue extracted with boiling benzene. The benzene

extracts are concentrated and the crude 5(6)-chloro-(1,10)-phenanthroline precipitated by the addition of petroleum ether (60-90 degrees). The product is recrystallized from benzene-petroleum ether and forms fine white needles, m.p. 123 degrees (anhydrous), yield 24.0 g. or 56 per cent of the theoretical.

Preparation of 5(6)-bromo(1,10)-phenanthroline. (8-nitro-6-bromoquinoline.) The general procedure is applied to a mixture of 217.0 g. (1 mole) of 4-bromo-2-nitroaniline (obtained in practically quantitative yield from 2-nitroaniline by the method of Fuchs *(17)*, m. p. 112-113 degrees, 368.2 g. (4 moles) of glycerol, 172.4 (0.75 mole) of arsenic pentoxide, and 220 g. of sulfuric acid. The crude 8-nitro-6-bromoquinoline is isolated in the same manner as described above for 8-nitro-6-chloroquinoline. The crude product is suitable for the reduction. A portion recrystallized from 95 per cent ethanol melts at 170 degrees.

8-amino-6-bromoquinoline. The crude, dry 8-nitro-6-bromoquinoline obtained above is reduced with stannous chloride exactly as described for the reduction of 8-nitro-6-chloroquinoline. The product is obtained in pure form by steam distillation, m. p. 78 degrees (from petroleum ether), yield 136-142 g.

5(6)-bromo-(1,10)-phenanthroline. The general procedure is applied to a mixture of 22.3 g. (1.1 mole) of 8-amino-6-bromoquinoline, 36.8 g. (0.4 mole) of glycerol, 17.2 g. (0.075) mole of arsenic pentoxide and 26 g. of sulfuric acid. The tarry reaction mixture is poured into 500 cc. of cold water and neutralized in the cold with 1:1 aqueous ammonia. The semi-solid precipitate is extracted with boiling benzene and the clear extract concentrated. On cooling and adding a small amount of water the mono-hydrate crystallizes. The needles from moist benzene meet at 86 degrees. The anhydrous 5(6)-bromo (1.10)-phenanthroline may be obtained by recrystallizing the benzene-extract residue from chloroform. Yield of pure 5(6)-bromo-(1,10)-phenanthroline 11.8 g. (46 per cent) m. p. 119 degrees.

Structure of the tri-(1,10)-phenanthroline ferrous ion. Although 1,10-phenanthroline forms stable complexes with a variety of metal ions, e. g., Co^{++}, Cu^{++}, Ni^{++}, Zn^{++}, Cd^{++}, and Cr^{+++}, the ferrous complex is of greatest interest to the analytical chemist. The tri-(1,10)-phenanthroline ferrous ion forms salts with various anions, some of which, notably the perchlorate, periodate, and persulfate, are only

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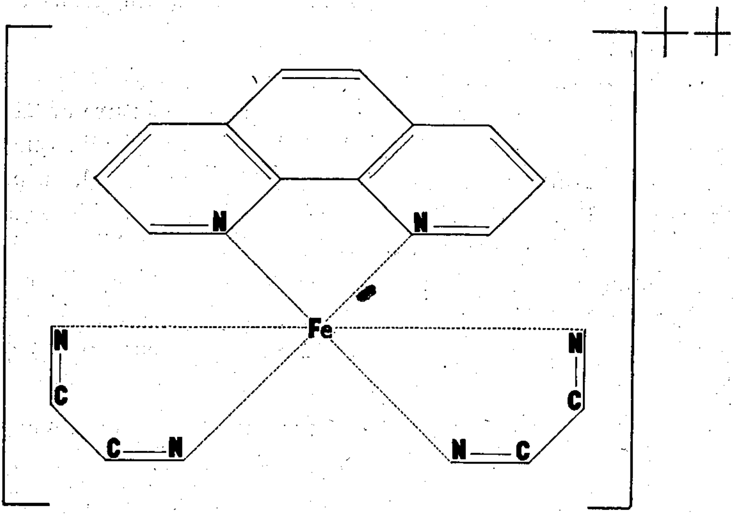
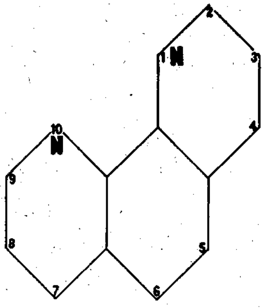
(17) Fuchs, *Monatsh.*, **36**, 139 (1915).

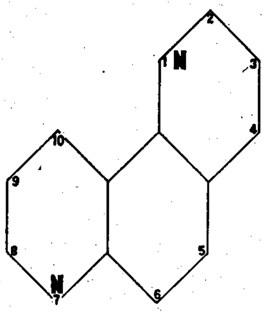
sparingly soluble in water. Analysis of ferroin salts indicates the formula $Fe(C_{12}H_{10}N_2)_3^{++}$ for the tri-(1,10)-phenanthroline ferrous ion. The structure below is most generally accepted:

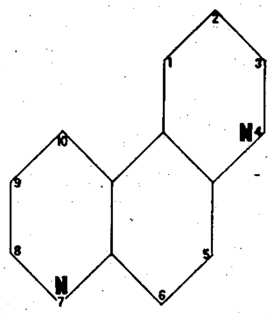


The phenanthroline molecules are held in very strong and stable attachment to the iron by means of secondary (Werner) valence forces.

Isomeric phenanthrolines. In addition to 1,10-phenanthroline two structurally isomeric phenanthrolines derivable from phenylenediamines are recognized in chemical abstracts. These are the 1,7- and 4,7-compounds, generically related to the 1,3- and 1,4-phenylenediamines respectively.



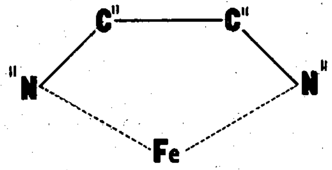




In consulting the early German literature on these compounds the differences in nomenclature and in numbering the ring systems is

sometimes confusing *(18)*. Ortho or 1,10-phenanthroline was known as "X-Phenanthroline" in the earlier literature.

The question has sometimes been raised as to whether the isomeric phenanthrolines have been investigated from the standpoint of indicator applicability. An examination of the structural formula of the tri-(1,10)-phenanthroline ferrous complex indicates that the iron atom forms, with each phenanthroline molecule, a five-membered ring of the type:



Each of these rings is undoubtedly planar, because, as previously pointed out, the 1,10-phenanthroline molecule itself appears to be planar.* If it is recalled that ring systems of more than six members are formed with difficulty, and usually only under very special conditions, together with the evidence for the planar nature of the ring involving the iron atom, it will be apparent that the formation of a ferrous complex by either the 1.7- or 4,7-isomer would be sterically unlikely. Actually neither of these compounds gives the ferroin reaction.

Compounds other than 1,10-phenanthroline and its derivatives which give the ferroin reaction. The first compound reported to give the ferroin reaction, 2,2′-bipyridyl, often incorrectly referred to as *a,a′*-dipyridyl, has already been mentioned. The structure of this molecule is quite similar to that of 1,10-phenanthroline:

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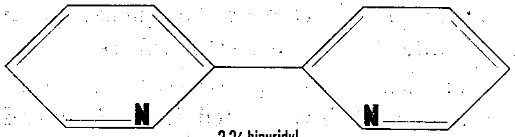
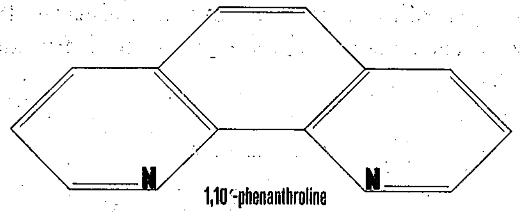
(18) For example, in "Beilstein's Handbuch der Organischen Chemie" the 1,10-, 1,7-, and 4,7-phenanthrolines are referred to as the 4,5-, 1,5-, and 1,8- compounds respectively.

* Further evidence that this ring is planar is indicated by the fact that 2,2′-bipyridyl (III) forms a stable ferrous complex whereas the 3,3′-(2,2′)-bipyridyldicarboxylic acid (II) does not form a complex at all. In (III), the -N-C-C-N- system can assume a planar arrangement because of the free rotation of the pyridine rings about the common 2,2′-pivot bond. In the dicarboxylic acid (II) the -N-C-C-N- grouping cannot lie in one plane because of restricted rotation due to the steric interference of the carboxyl groups. The fact that optically active isomers of (II) cannot be isolated is not an argument against restricted rotation in this case since the molecule is not unsymmetrically substituted and optically active isomers of (II) do not exist.

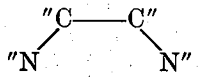


2,2'-bipyridyl



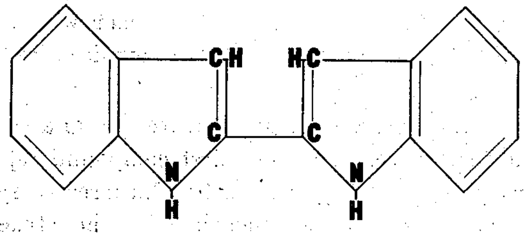
1,10'-phenanthroline

It will be observed that each molecule contains the grouping



which seems to be peculiar to the ferroin type complex.

A second compound, 2,2'-bi-indolyl, m.p. 302-303 degrees, has been reported to give a characteristic red color with ferrous iron. No study of this possible complex has been reported.



2,2'-BI INDOLYL

It is apparent that the -N-C-C-N- grouping in itself is not sufficient for the formation of a useful ferroin indicator since such compounds as 1,2-ethylenediamine do not give the characteristic red color with ferrous iron.

SECTION 3

THE PREPARATION OF BIPYRIDYL AND TERPYRIDYL

The preparation of 2,2'-bipyridyl was first described by Blau *(5)* following the pyrolysis of copper picolinate and this interesting compound was further studied in the form of tripyridyl ferrous bromide hexahydrate by Werner *(19)*. Later 2,2'-bipyridyl was prepared by methods of dehydrogenation by Meyer and Hoffman-Meyer *(20)*, by Hein and co-workers *(21)*, and by Wibaut and Willink *(22)*. The process of Hein and Retter *(21)* was employed by Morgan and Burstall *(23)* as best suited. The process involves the dehydrogenation of pyridine by anhydrous ferric chloride at 500 deg. C. and at 50 atmospheres pressure. An iron autoclave and mechanical stirrer with a stainless steel lining was employed and a charge of 8,000 g. of pyridine and 1,524 g. of anhydrous ferric chloride were allowed to react for a period of 36 hours.

The main product thus obtained was found to be bipyridyls (approximately 50 per cent of theory) and at least 20 other by-products consisting of the following type materials:

1. Gases (H_2, N_2, NH_3, saturated hydrocarbons and ethylene) in small amounts.

2. Neutral oil (b.p. 90-130 deg. C.) found to be nitrogenous and low in yield.

3. Alkylpyridines (pyridine: mono- and di-methyl, ethyl, and propylpyridines).

4. Bipyridyls and bipiperydyls (2,2'-bipyridyl, main product, 524 g.) including 3,4', 2,3', and 2,4' (3,3' and 4,4' absent). Alkylated 2,2'-bipyridyls were obtained and react with ferrous salts to form highly colored complex ions. (4,4'-bipyridyl gives a deep blue color with zinc and acetic acid which may be of some value as a reagent for zinc.) All the members of this group are volatile with steam from alkaline solution.

5. Terpyridyls. These are not volatile with steam from alkaline solution and are recovered from the residue after steam distillation

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(19) Werner, Ber., 45, 434 (1912).

(20) Meyer and Hoffman-Meyer, *J. prakt. Chem.*, 102, 287 (1921).

(21) Hein and Retter, *Ber.*, 61, 1790 (1928); Hein and Schwedler, *Ber.*, 68, 681 (1935).

(22) Wibaut and Willink, *Rec. trav. chim.*, 50, 287 (1931).

(23) Morgan and Burstall, *J. Chem. Soc.*, 20, (1932).

of group 4 substances by treating with a large excess of caustic soda and extracting with benzene. A crop of 55 g. of 2,2′2″-terpyridyl was obtained from a total of 225 g. of group 5 products. Other isomeric (2,2′X″) terpyridyls were isolated, some of which gave intense purple colorations with ferrous salts.

6. Resinamines (appreciable yields).

7. Insoluble residue (carbon, combustibles, and metallic iron).

The treatment of the products obtained from the reactions in the autoclave, after making them alkaline with caustic soda, consisted in steam distillation (the gaseous products and neutral oils ignored). The group 3 products were separated first, followed by group 4 products. When the steam distillate failed to give characteristic test with ferrous sulfate the residue was treated as indicated under 5 (terpyridyls) above).

For the reaction of sodium on pyridine the work of Smith *(24)* should be consulted.

A summary of the dehydrogenation reaction of Hein and Retter *(21)* as carried out by Morgan and Burstall *(23)* is given as follows:

Pyridine Used	Anhy. Ferric Chloride	Pyridine Recovered	Alkylated Pyridines	Dipyridyls	Terpyridyls	Resin-Amines
g.	*g.*	*g.*	*g.*	*g.*	*g.*	*g.*
8000	1524	5630	245	794	225	512

TABLE I

Properties and Physical Constants of the Isomeric Bipyridyls and Derivatives

Data by C. R. Smith, *J. Am. Chem. Soc.*, **46**, 414 (1924).

Bipyridyl Form	Literature Ref.	m.p. °C.	b.p. °C.	m.p. as Picrate °C.	Sol'y in Water
Alpha-Alpha	(25)	69.5	272.5	158	Only slightly
Alpha-Beta	(26)	Liquid	287-289	149.5	Insoluble
Beta-Beta	(27)	68	291-292	232	All proportions
Gamma-Gamma	(28)	114	305	213	Sol. in hot water
Beta-Gamma	(29)	61	297	215	V. sol. in cold water
Alpha-Gamma	(30)	Liquid	280-282	208	Sl. sol. in hot water

(24) Smith, *J. Am. Chem. Soc.*, **46**, 414 (1924).
(25) Blau, *Monatsh*, **10**, 378 (1889).
(26) Skraup and Vortmann, *ibid.*, **3**, 599 (1882).
(27) Skraup and Vortmann, *ibid.*, **4**, 591 (1883).
(28) Wibaut and Dingemanse, *Rec. trav. chim.*, **42**, 240 (1923).
Weidel and Russo, *Monatsh.*, **3**, 851 (1882).
(29) Meyer and Meyer, *J. prakt. Chem.*, **102**, 287 (1921).
(30) Smith, *J. Am. Chem. Soc.*, **46**, 414 (1924).

New Procedures in the Preparation of Bipyridyl. The application of the Ullman reaction (aromatic halide coupling reaction using metallic copper), in the experience of most investigators, gives such poor yields of bipyridyl (2-bromopyridine reacting with copper in the presence of cymene) as to discourage its use on a practical basis. Alpha-bromopyridine is not readily available at a favorable price and its manufacture is covered by patent *(31)* by the Dow Chemical Company.

As a substitute for the preparation of bromopyridine by direct halogenation by the Wibaut and Hertog method *(31)*, its preparation from 2-aminopyridine prepared as described by Wibaut and Dingemanse *(28)* or by Chichilabin and Zeide *(32)* is a possibility. From either source the 2-bromopyridine by application of the Ullmann reaction forms bipyridyl through the intermediate formation of the free pyridyl radical which couples to complete the synthesis *(33)*.

The studies of Kharasch and co-workers *(34)* concerning the mechanism of Grignard reactions, particularly the effect of the metallic halides, has led to the study of new procedures in the expectation that the yield of finished product would be more encouraging than that found for the Ullmann reaction.

The Grignard Reaction in the Synthesis of Bipyridyl from 2-Bromopyridine. By this procedure, the best of several modifications, 2-bromopyridine and cobaltous chloride are caused to react with ethyl magnesium bromide.

Experimental Procedure. The entire Grignard reaction, consisting in two parts, the preparation of the Grignard reagent and the coupling reaction, was carried out in a 3-necked flask fitted with a separatory funnel in one arm, a sealed stirrer in the center, and a reflux condenser and drying tube in the remaining opening. Provision was made for the application of external heat using an electric cone heater controlled by use of a variac.

Preparation of Ethyl Magnesium Bromide. Three grams of magnesium (0.1 mole for ethyl bromide plus excess) was placed in the reaction flask. To this was added a few milligrams of iodine crystals.

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(31) Process of Halogenating Pyridines, U. S. Patent 1,977,662, Oct. 23, 1934, Wibaut and Hertog.

(32) Chichilabin and Zeide, *J. Russ. Phys. Chem. Soc.*, **46**, 1216 (1914).

(33) Wibaut and Overhoff, *Rec. trav. chim.*, **47**, 761 (1928).

(34) Kharasch and co-workers, *J. Am. Chem. Soc.*, **65**, 493, 495, 498, 501 (1943).

(A suggestion by Dr. A. B. Spradling resulting in the investigation of the Grignard reactions. Herzog and Wibaut, *Rec. trav. chim.*, **55**, 122 (1936) were unable to prepare pyridylmagnesium bromide.)

Through the separatory funnel was added 10.9 g. (0.1 mole) of ethyl bromide. After the vigorous initial reaction had subsided, the mixture was boiled gently on the heating cone for 15 minutes. Ethyl bromide was then added to react with any excess magnesium metal which remained.

Reaction of Ethyl Magnesium Bromide with 2-Bromopyridine in the Presence of Cobaltous Chloride. To the cooled reaction mixture from the ethyl magnesium bromide preparation, a mixture of 16 g. (0.1 mole) of 2-bromopyridine and 1.3 g. (0.01 mole) of anhydrous cobaltous chloride in dry ether was added at such a rate as to obtain moderate refluxing of the ether. After the initial reaction was finished, the mixture was heated for two hours. The final isolation of 2,2′-bipyridyl was then carried out. By transferring the reaction ingredients to a steam distillation apparatus, acidifying with hydrochloric acid and applying steam, the organic products other than the bipyridyl were removed. The reaction mixture was then made alkaline using caustic soda and again steam distilled until the distillate no longer gave a test with ferrous ion. A small excess of hydrochloric acid was added to the distillate to prevent loss of bipyridyl by volatilization and the volume diminished by evaporation. After the addition of caustic soda the bipyridyl was extracted using ether and the product isolated by evaporation of the ether solution. The final product (1.5 g. or 19 per cent of theory) had a melting point of 70 deg. C.

The preparation of Bipyridyl from 1,10-Phenanthroline. The Wibaut and Willink (22) decarboxylation of 3,3′-dicarboxy-2,2′-bipyridyl, prepared as described by Smith *(24)* would serve as a satisfactory method for the preparation of bipyridyl, provided the 1,10-phenanthroline starting material could be made at a suitable cost. If the same reactions could be made to apply to the treatment of the insoluble 1,10-phenanthroline ferrous perchlorate complex, the procedure could be made useful in the recovery of the phenonthrolinium ferrous ion from solutions of 1,10-phenanthroline otherwise too dilute to make recovery practical by any other procedure, thus converting it ultimately to the more costly reagent bipyridyl.

The preparation of 3,3′-Dicarboxy-2,2′-Bipyridyl from 1,10-Phenanthroline. The quantities of materials employed in molecular ratio were as follows:

1 mole of 1,10-phenanthroline monohydrate
3 moles of potassium permanganate (one-third mole excess)
2 moles of sodium hydroxide
2 moles of silver nitrate.

Following these proportions, 8 g. (0.04 mole) of 1,10-phenanthroline was suspended in 800 ml. of water; 3.2 g. (0.08 mole) of sodium hydroxide was added; 19 g. (0.12 mole) of potassium permanganate was slowly added in the form of its crystals. After fifteen minutes the solution was heated to boiling and kept at this temperature for two hours. Throughout the entire operation the mixture was thoroughly agitated using a mechanical stirrer. Finally the excess permanganate was reduced by adding sucrose until no further permanganate color remained as tested by applying a drop of the reaction mixture to an area of filter paper and examining the spot test fringe for a purple border.

The voluminous precipitate of manganese dioxide was filtered out and the filtrate concentrated on the steam bath. The concentrate was acidified, using acetic acid and the carbon dioxide thus evolved was displaced from solution by stirring and digestion; 13.6 (0.08 mole) of silver nitrate was dissolved in the minimum required quantity of water added slowly with constant stirring. The resulting precipitate was filtered and transferred to a considerable volume of hot water in a precipitation flask and saturated with hydrogen sulfide. The silver sulfide was filtered and the filtrate treated with norite decolorizing carbon. After boiling to expel hydrogen sulfide the solution was filtered and the resulting colorless solution was concentrated to obtain the crystals of 3,3′-dicarboxy-2,2′-bipyridyl. The crystals may be recrystallized from alcohol-water as solvent. The yield is 85 per cent of theoretical.

Oxidation of 1,10-Phenanthroline Ferrous Perchlorate. The oxidation procedure previously described was applied without change to this compound. The yield was approximately the same per cent.

Decarboxylation of 3,3′-Dicarboxy-2,2′-Bipyridyl. Four grams of the dicarboxylic acid was mixed with 20 grams of biphenyl. This mixture was placed in a reaction flask and heated to 250-275 deg. C., using a Woods' metal bath. After maintaining this temperature for 15 minutes the reaction was assumed complete. The mixture was treated with a large excess of dilute hydrochloric acid before it has cooled enough to solidify and finally filtering out the biphenyl which separated upon cooling. The last small amount of biphenyl was removed by steam distillation. The flask contents were then made alkaline with sodium hydroxide and again steam distilled until the distillate gave no further color with ferrous ions. After acidifying the distillate with dilute hydrochloric acid it was concentrated to a small volume. The concentrate was made alkaline with sodium hydroxide and the bipyridyl extracted with ether. Evaporation of the solvent yielded 1.2 g. of the bipyridyl or a 47 per cent yield.

The Preparation of Ruthenium Bipyridyl Hexahydrate. The preparation of 2,2′-bipyridyl ruthenous chloride was described by Burstall *(35)* as follows:

> "Ruthenous chloride (6.0 g.) and 2,2′-bipyridyl (50 g.) were stirred and slowly heated; reaction started at 250 deg. C., the temperature rose to 260 deg. C. and the black ruthenium trichloride became dark brown and crystalline, the change being accompanied by a considerable increase in the quantity of insoluble product. The mixture was stirred at 250-260 deg. C. for three hours; the cooled melt was then extracted with hot benzene until no more solid dissolved. The yellow brown residue (17 g.) was dissolved in water, filtered from insoluble material including a little ruthenium and evaporated to the crystallizing point. The bright red leaflets were collected, crystallized from a small quantity of hot water and dried over sulfuric acid. (Found: C—48.4; H—4.6; N—11.35; Cl—10.0, 9.9; Ru—13.5, 13.4; H_2O—14.4 per cents.) $(C_{30}H_{24}N_6Ru)Cl_2 \cdot 6H_2O$ requires C—48.1; H—4.8; N—11.2; Cl—9.5; Ru—13.6; H_2O—14.4 per cents. 2,2′-bipyridyl ruthenous chloride hexahydrate forms well defined bright red leaflets easily soluble in water and alcohol but insoluble in acetone, chloroform, benzene, and petroleum ether. Slow crystallization gave large, deep ruby red rhombs. The chloride was unchanged by boiling, concentrated hydrochloric acid or 40 per cent caustic alkali. Concentrated sulfuric acid gave an orange yellow solution which became green on warming and concentrated nitric acid furnished a deep green solution. Hydrogen peroxide and chlorine were without action at room temperature. Water of crystallization was eliminated at 200 deg. C. in a vacuum, but the salt was not decomposed below 300 deg. C."

$Ru(dipyridyl)_3(OH)_2 \cdot 8H_2O$ is a strong enough base to displace NH_3 from ammonium salts. It is made by treatment of the concentrated aqueous solution of the chloride with excess of freshly precipitated silver oxide until free from halogen. Filter and evaporate over caustic soda.

The Improved Preparation of 5-Nitro-1,10-Phenanthroline. As previously described *(10,11)* the direct nitration of 1,10-phenanthroline by the use of concentrated sulfuric and nitric acids at 120 deg. C. results in the preparation of 5-nitro-1,10-phenanthroline in a yield of 60 per cent of theoretical. The use of fuming sulfuric acid *(10,11)* and concentrated nitric acid and higher temperatures did not result in the introduction of a second nitro group. However, by using fuming sulfuric acid and concentrated nitric acid and allowing the temperature to exceed 120 deg. C. it is found that the yield of 5-nitro-1,10-phenanthroline can be forced to approximately 100 per cent of theory as compared with the previously realized maximum yield of 60 per cent. This great increase in efficiency, coupled with the formation of a product almost insoluble in water serves to contribute to the marketing of the finished product at a greatly reduced price.

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(35) Burstall, *J. Chem. Soc.*, (1936), 173.

The Preparation of Ferroin, Nitro-Ferroin and Methyl-Ferroin Solutions. The three most practical forms in which the phenanthroline and substituted phenanthrolines are employed as oxidation-reduction indicators is in the form of their 0.01 N and 0.025 N solutions as complex phenanthrolinium ferrous ions and substituted phenanthrolinium ferrous ions, all of which are divalent and of an intensely red color. The 1,10-phenanthrolinium ferrous ions, as well as the 5-methyl-1,10-phenanthrolinium ferrous ions and their corresponding oxidized forms as ferric complexes, are completely stable in mineral acid solutions of all ordinarily encountered strengths. This makes their use either as oxidation-reduction indicators, or as precipitants for qualitative analysis, or for quantitative separations and determinations, most satisfactory. This is particularly true in their use as indicators because of their rather moderate oxidation potentials when compared with the 5-nitro-1,10-phenanthrolinium ions, and hence their more general applicability. The bipyridyl ferrous and ferric complex ions have an even lesser degree of stability in acid solutions than the 5-nitro-1,10-phenanthrolinium ferrous and ferric ions.

TABLE II

Data Concerning the Preparation of Complex Bipyridyl, 1,10-Phenanthroline, 5-Methyl-1,10-Phenanthroline and 5-Nitro-1,10-Phenanthroline Ferrous Sulfate Solutions

Organic Base Material	Molecular* Weight	Organic Base Required in Grams		g. $FeSO_4 \cdot 7H_2O$ Required		Sol'n in H_2O Dilution to
		0.01N	0.025N	0.01N	0.025N	
Bipyridyl ($C_{10}H_8N_2$)	156.18	4.6854	11.7135	2.7802	6.9505	1000 ml.
1,10-Phenanthroline ($C_{12}H_8N_2 \cdot H_2O$)	198.216	5.9465	14.8662	2.7802	6.9505	1000 ml.
5-Methyl-1,10-Phenanthroline ($C_{12}H_7N_2CH_3$)	194.226	5.8268	14.5670	2.7802	6.9505	1000 ml.
5-Nitro-1,10-Phenanthroline ($C_{12}H_7N_2NO_2$)	225.20	6.7560	16.8900	2.7802	6.9505	1000 ml.

* Atomic weights used from the official table for 1943 (C—12.01; N—14.008; H—1.008; 0—16).

The process of the conversion of all four of the organic molecules to their corresponding red complex ions, involves the simple procedure of mixing together the proper weights of reagents as shown in Table II and adding water, followed by continuous stirring until the interaction is complete, which is indicated when all the lesser soluble organic base compound has dissolved. The resulting solutions are then diluted to the required volumes as shown in Table II and are ready for use. All are stable upon storage over long extended periods.

The Preparation of the Phenanthroline Ferrous Perchlorate and Substituted Phenanthroline Ferrous Perchlorate Complex Salts. For use as indicators in oxidation-reduction reactions, the use of the relatively insoluble ferrous perchlorate complex salts of 1,10-phenanthroline and substituted phenanthrolines, has noteworthy advantages. The saturated solution of 1,10-phenanthroline ferrous perchlorate is approximately 0.001 molar. Thus one milliliter of such a solution is approximately equal to one drop (0.05 ml.) of a 0.025 molar solution of 1,10-phenanthroline ferrous sulfate ordinarily employed as indicator for a single oxidation-reduction reaction at 250-400 ml. volume of solution to be titrated. It is much more convenient to make a large volume of the saturated aqueous solution of the ferrous perchlorate complex by shaking an excess of the red salt with a liter of water, then allowing the excess complex perchlorate to settle and use the clear red "supernatant" liquid as indicator solution, employing one milliliter of the same rather than one drop of the equally effective 0.025 molar solution of the more soluble ferrous sulfate complex. By this scheme the routine analyst is not so likely to add 2 ml. of the 0.001 molar indicator solution as they are two drops of the 0.025 molar ferrous sulfate complex salt and a saving is thus accomplished. When the "supernatant" indicator solution thus prepared from the ferrous perchlorate complex is exhausted, it is a simple matter to add water as solvent and shake to saturation with the excess of perchlorate complex previously added to replenish the solution previously consumed. The ordinary indicator dropping bottle is provided with an approximately 1 ml. volume dropper tube, the full contents of which may be conveniently added for each titration rather than the addition of one drop of a 0.025 molar ferrous sulfate solution.

The 1,10-phenanthroline ferrous complex salt, $(C_{12}H_8N_2)_3 \cdot Fe(ClO_4)_2$, is obtainable commercially or is easily prepared. A solution of 1,10-phenanthroline ferrous sulfate prepared as previously described in connection with the data of Table II is made 0.001 to 0.01 molar in free perchloric acid whereby the relatively insoluble ferrous perchlorate complex is precipitated and may be filtered, washed with 0.001 molar perchloric acid and finally with a little pure water, and dried at a temperature under 100 deg. C.

The same principles apply to the use of 5-nitro-1,10-phenanthroline. In this case the ferrous perchlorate complex salt is only

approximately one-third as soluble as the previously described unnitrated compound.

The use of the ferrous perchlorate complex compounds as indicators is particularly appropriate when the solutions being titrated for quantitative determination have been previously acidified, using perchloric acid. In such case the use of one drop of the indicator in the form of its ferrous sulfate complex results in its precipitation as the ferrous perchlorate complex and this then has to be stirred excessively to return it to solution in the reaction mixture. This procedure is avoided by the use of the indicator in the form of its saturated aqueous solution of the perchlorate complex.

Desired Research Developments in the Field of the Complex Phenanthroline Type of Compounds. The following topics are suggestive of the various research developments now in demand, which projects are at present being actively investigated. Some of these projects have been studied and the working solution satisfactorily indicated, but as yet not completely investigated.

1. The study of the conditions necessary in the accomplishment of the double Skraup synthesis in the preparation of 1,10-phenanthroline starting with ortho-phenylene diamine. This procedure was claimed to be successful by some *(5,36)* and not successful by other investigators *(9,13)*.

2. The preparation of 8-nitroquinoline starting with quinoline.

3. The preparation of substituted 1,10-phenanthroline derivatives of both higher and lower oxidation potentials than those at present available. The preparation of 5-nitro-6-chloro-1,10-phenanthroline by Dr. Richter has been accomplished and its properties are now being studied. Its oxidation potential should be higher than that of 5-nitro-1,10-phenanthroline in the form of its ferrous complex. An even more desirable new type of substitution would be the preparation of 5,6-dimethyl-(1,10)-phenanthroline whose ferrous complex ion should have an oxidation potential approximately that of the bipyridyl ferrous complex (0.97 volts in molar sulfuric acid solution), and be a much more stable compound in the form of its ferrous or ferric ion complex.

4. The recovery of the organic base compound from the ferrous perchlorate complex compounds. The extremely insoluble nature of the various organic base materials in the form of their ferrous perchlorate complexes makes it attractive to be able to recover the parent compound from solution.

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(36) Hieber and Muhlbauer, *Ber.*, 61, 2149 (1928).

5. The preparation 2,2′-bipyridyl following the procedure of Hein and Schweidler *(21)*. This compound is in demand for use in the quantitative estimation of Vitamin E (tocopherol) by a procedure first described by Emmerie and Engel *(37)* and perfected by Mayer and Sobotka *(38)*, for which method 1,10-phenanthroline is said not to apply satisfactorily. A similar application might well be that of the determination of Vitamin C (ascorbic acid) based upon the same principle but for which determination other methods are available.

6. The study of the preparation and properties of the ruthenium complex compounds analogous to those of Burstall *(35)*, from 1,10-phenanthroline and its substitution products. Such complex ruthenium compounds are known to exist and their properties should be studied in somewhat the same manner as with the bipyridyl compound as described by Steigman, Birnbaum, and Edmonds *(39)*. The oxidation potential which they give for the bipyridyl ruthenous ion, 1.33 volts, is lower by 0.08 volt, a conclusion which can be drawn from the work of Hume and Kolthoff *(40)*. As will be shown in the form of its ferrous sulfate complex in a later section of this booklet the oxidation potential of 2,2′-bipyridyl is 0.97 volt. The substitution of the ruthenous ion for the ferrous ion has increased the oxidation potential 0.28 volt. By comparison 1,10-phenanthroline in the form of its ruthenous compound, therefore, should have an oxidation potential of 1.34 volts.

7. The formation of a terpyridyl ruthenous complex ion should lead to a more favorably colored ion than that of any previously made which are all yellow. The terpyridyl ruthenous ion should be red rather than yellow. The prediction of the oxidation potential cannot be made or the stability in hot acid solutions surmised by comparison with the bipyridyl ruthenous ion stabilities.

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(37) Emmerie and Engel, *Rec. trav. chim. Pays-Bas*, 57, 1351 (1938); 58, 283, 895 (1939).

(38) Mayer and Sobotka, *J. Biol. Chem.*, 143, 695 (1942).

(39) Steigman, Birnbaum, and Edmonds, *Ind. Eng. Chem.*, Anal. Ed., 14, 30 (1942).

(40) Hume and Kolthoff, *J. Am. Chem. Soc.*, 65, 1895 (1943).

SECTION 4

THE PHENANTHROLINES AND SUBSTITUTED PHENANTHROLINE METAL COMPLEX IONS AS OXIDATION-REDUCTION INDICATORS

THE DETERMINATION OF THEIR OXIDATION POTENTIALS

Early Work in the Field of Indicators for Use in Oxidation-Reduction Reactions. The study of oxidation-reduction indicators is of comparatively recent scientific interest. The color transforming indicator method of evaluating oxidation-reduction intensity was first placed upon a practical basis by W. Mansfield Clark *(41)*. The first such indicator system applied by Clark *(41)* was that of methylene blue-methylene white. The introduction of this color indicator method is to the oxidation-reduction systems what the acid-base indicators are in the study of hydrogen ion equilibria. A summary of the results from some two score investigations by Clark of similar systems, chiefly of interest in biological chemical studies, was compiled in 1934 *(42)*. The first great impetus towards the use of such systems in volumetric oxidation-reduction determinations in inorganic analyses was originated by Knop *(43)*, who applied the organic compound diphenylamine (which is colorless in reduced form and violet when oxidized) to the determination of iron by oxidation using dichromate. A great deal has been accomplished in the field opened up by Knop *(43)* as may be judged by reference to the data of Table III. A great deal remains to be done in providing indicators more evenly and closely graded with respect to their various characteristics. The grading of oxidation, provision of suitably vivid color change at their transition potentials, and increased stability in strong mineral acid solution are variables to be studeid. This is particularly true with regard to indicators applicable to the lower potential ranges.

It is important to note in connection with the use of the data in Table III that because of the relatively low color intensity of the oxidized form of the "ferroin type" of reagents, they are effectively one color indicators. The point of visible color change (red to almost colorless) corresponds to 90 per cent oxidation of the indicator when

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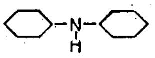
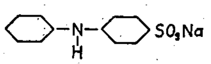
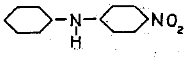
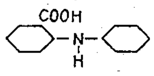
(41) Clark, *J. Wash. Acad. Sci.*, 10, 255 (1920).

(42) Clark, *Medicine*, 13, 207 (1934).

(43) Knop, *J. Am. Chem. Soc.*, 46, 263 (1924).

TABLE III

Oxidation-Reduction (Redox) Indicator Characteristics

Indicator	Formula	Color Change Upon Oxid.	Transition E.M.F. in Volts N H_2 Elec. as Ref.	Preparation of Solution	Literature Reference
Methylene Blue	$C_{16}H_{18}N_3ClS \cdot 3H_2O$	Blue to Colorless	0.53 pH=2.86	0.2 g. in 100 ml. of water	(41)
Diphenylamine		Colorless to Violet	0.76	1g. in 100 ml. conc.H_2SO_4 (0.006 M)	(43) (44)
Diphenylbenzidine		Colorless to Violet	0.76	1g. in 100 ml. conc. H_2SO_4 (0.003 M)	(44)
Ba-Diphenylamine Sulfonate (Na_2SO_4 used to pp't Ba as $BaSO_4$)		Colorless to Red-Violet	0.84	3.17 g. per l. of H_2O (0.005 M)	(45)
Sodium Diphenylbenzidine Sulfonate	Same as 2 with 10($NaSO_3$)$^+$ Substitutions	Colorless to Violet	0.87	1.35 g. per l. of H_2O (0.001 M)	(46)
2,2'-Bipyridyl Ferrous Sulfate	$(C_{10}H_8N_2)_3FeSO_4$	Red to Faint Blue	0.97	See Table II, p. 23	(47)
Eriogreen	Complex Triphenyl Methane Derivative	Red-yellow to Rose	0.99	0.1 g. per 100 ml. of water	(48)
Erioglaucine	Complex Triphenyl Methane Derivative	Red-Yellow to Rose	1.00	0.1 g. per 100 ml. of water	(48)
5-Methyl-1,10-Phenanthroline Ferrous Sulfate (Methyl Ferroin)	$(C_{12}H_7N_2CH_3)_3FeSO_4$	Red to Faint Blue	1.02	See Table II, p. 23	(9)
p-Nitro-Diphenylamine		Colorless to Violet	1.06	1 g. in 100 ml. conc.H_2SO_4	(10)
1,10-Phenanthroline Ferrous Sulfate (Ferroin)	$(C_{12}H_8N_2)_3FeSO_4$	Red to Faint Blue	1.06*	See Table II, p. 23	(9) (40)
N-Phenanthranilic Acid		Colorless to Pink	1.08	1.07 g. in 20 ml. of 5% Na_2CO_3 and dil. to 100 ml. (0.005 M)	(49)
5-Nitro-1,10-Phenanthroline Ferrous Sulfate (Nitro Ferroin)	$(C_{12}H_7N_2NO_2)_3FeSO_4$	Red to Faint Blue	1.25	See Table II, p. 23	(9) (40)
Ruthenium Tripyridyl Nitrate	$(C_{10}H_8N_2)_3Ru(No_3)_2$	Yellow to Colorless	1.25**	See Refer. (39) (35)	(39)

* This potential erroneously given as 1.14. See Reference (40).

** Reference (39) gives 1.33 volts. Reference (40) applies here also.

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(44) Kolthoff and Sarver, *J. Am. Chem. Soc.*, 52, 4179 (1930).

(45) Sarver and Kolthoff, *ibid.*, 53, 2902 (1930).

(46) Sarrer and Von Fisher, *Ind. Eng. Chem.*, Anal. Ed., 7, 271 (1935).

(47) Blau, *Monatsch.*, 19, 647 (1898).

(48) Furman and Wallace, *J. Am. Chem. Soc.*, 52, 2347 (1930).

(49) Syrokomsky and Stiepen, *ibid.*, 58, 928 (1936).

used in customary amounts for individual titrations. The reaction is as follows in one case:

$$[(C_{12}H_8N_2)_3Fe]^{+++} + e^- \rightleftarrows [(C_{12}H_8N_2)_3Fe]^{++} \qquad (E^\circ = 1.06 \text{ volts})$$

$$\underset{}{\text{(Ferriin)}} \qquad\qquad \text{(Ferroin)}$$

$$\text{or} \quad I_{ox} \quad + e^- \rightleftarrows \quad I_{red.}$$

The Nernst expression applied to this reaction results in the following:

$$E = E^\circ + 0.059 \log [I_{ox}]/[I_{red}].$$

Substituting for the logarithmic expression its value 0.9 the value of E is found to be:

$$E = 1.06 \text{ volts} + 0.056 \text{ volts} = 1.12 \text{ volts (rounded figures)}.$$

The result of these effects is to demand an oxidizing agent with a higher oxidation potential, for use in visual titrations using the ferroin type of indicators, than would be required in case a potentiometric titration were to be employed for the same oxidation. In the case of methyl ferroin in visual titrations the oxidation potential is 1.08 volts, nitro-ferroin 1.31 volts, and in the case of the bipyridyl ferrous complex the oxidation potential will be 1.03 volts as seen from subsequent data and correction for 90 per cent oxidation to acquire a visual end point.

The indicators, nitro-ferroin and bipyridyl ferrous sulfate, are not completely stable in both the oxidized and reduced forms in mineral acid solution. The oxidized form of these indicators, particularly, unlike the case of ferroin and methyl ferroin, is not completely stable. This fact does not preclude their use as redox indicators for their stability is ample to provide for individual titrations and reversal of the color change at the equivalence point. Nitro-ferroin is firmly established in routine laboratory applications *(50)*, particularly in the oxidation of oxalic acid, the indirect determination of calcium *(51)*, and in the titration of the cerate ion use in excess in the oxidation of organic compounds as described by Smith and Duke *(52)* and *(53)*. There is apparently no reason why the bipyridyl ferrous complex should not find ready application in like manner. Its use might be predicted to be advantageous in the case of dichromate oxidations because of its low oxidation potential.

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(50) Smith and Getz, *Ind. Eng. Chem.*, Anal. Ed., **10**, 191 (1938).

(51) Smith, "Cerate Oxidimetry," p. 82, G. Frederick Smith Chem. Co., Columbus, Ohio (1942).

(52) Smith and Duke, *Ind. Eng. Chem.*, Anal. Ed., **13**, 558 (1941).

(53) Smith and Duke, *ibid.*, **15**, 120 (1943).

The Role of Complex Formation in Relation to the Oxidation Potential of the Ferrous-Ferric System. The determination of the oxidation potential of the ferrous-ferric system under standard conditions, with the iron in various types of complex formation and the uncomplexed ionic condition are given in Table IV.

TABLE IV

The Oxidation Potential of the Ferrous-Ferric System in Various Type Complex Formations. (Including the Chromous-Chromic System)

Electrode Reaction	Potential in Volts Ref. H_2 Elec.	Remarks
$Cr^{+++} + e^- = Cr^{++}$	—0.41	Reaction carried out under H_2
$Fe(CN)_6^{---} + e^- = Fe(CN)_6^{----}$	0.36	
$FeF_6^{---} + e^- = Fe^{++} + 6F^-$	Ca 0.40	Estimated from failure of FeF_6^{---} to oxidize I^-
$[Fe(PO_4)_2]^{---} + e^- = Fe^{++} + 2PO_4^{---}$	0.61	1 M H_2SO_4 and 0.5 M H_3PO_4
1,10-Phenanthroline Cr^{+++} ion + e^- = 1,10-Phenanthroline Cr^{++} ion	Ca 0.77	The value of Ref. (10), an estimation
$Fe^{+++} + e^- = Fe^{++}$	0.771	Standard accepted value
Methyl Ferriin + e^- = Methyl Ferroin	1.02	Value determined as shown subsequently
Ferriin + e^- = Ferroin	1.06	Value of Hume and Kolthoff (40)
Nitro-Ferriin + e^- = Nitro-Ferroin	1.25	Value of Ref. (10) orig. est'd

From an examination of the values of Table IV it will be seen that the greatest change in oxidation potential due to complex formation is in that of the chromous-chromic system in its chelate ring complex of these ions with 1,10-phenanthroline. The oxidation potential has changed from —0.41 volt to approximately 0.77 volt or an increase of 1.18 volts. The transformation was studied by Hammett, Walden, and Edmonds *(10)* and described as follows:

> "The addition of phenanthroline to a solution of chromous acetate produces a red solution which undergoes a sharp and reversible change to reddish-violet with oxidation in acid solution presumably because of oxidation to a chromic compound. From the behavior of the oxidized compound with ferrous ion it appears that the potential of this system is not very different from the ferric-ferrous system. . . . The compounds have no practical value as indicators because the color intensity is not high and the color contrast at the end point is not great."

The 2,2′-bipyridyl complex with the chromous ion has been studied by Barbieri and Tetamanzi *(54)* with apparently no practical developments.

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(54) Barbieri and Tetamanzi, *Atti Accadi. Lincei*, **15**, 877 (1932).

The influence of the various other types of complex ions of Table IV shows an increase or decrease in oxidation potential of from —0.41 to a +0.48 volt. The larger alterations involve the chelate type complexes. The functional group in the phenanthroline type of chelate complexes $(=N-\overset{\parallel}{C}-\overset{\parallel}{C}-N=)$ bears a considerable speculative analogy to the functional group present in the dioxime type of chelate complex formation $(-N=\underset{|}{C}-\underset{|}{C}=N-)$. The former functional group forms an intensely red soluble complex with ferrous iron and the latter an intensely red insoluble complex with nickel.

The Determination of Indicator Oxidation Potential and the Distinction Between Molal and Formal Potentials. The experimental determination of the oxidation potential of the 1,10-phenanthroline ferrous ion in its conversion to the ferric ion form (Ferroin → Ferriin), accompanied by the vivid color change from deep red to faint blue, was first described by Walden, Hammett, and Chapman *(55)*, and then revised and corrected by Hume and Kolthoff *(40)*. The cell system employed was as follows:

$$\text{Gold}\left\{\begin{array}{c}\text{Quinhydrone—Sat'd}\\ H_2SO_4\text{—1 Molar}\end{array}\right\|\left.\begin{array}{c}FeSO_4\text{—0.002 Molar}\\ [C_{12}H_8N_2)_3Fe]SO_4\text{—0.002 Molar}\\ H_2SO_4\text{—1 Molar}\end{array}\right\}\text{Gold}$$

The left half of this cell was connected by means of a "bridge" to the titration reaction mixture of the right hand half of the cell. The "bridge" liquid was molar sulfuric acid and diffusion from the titration reaction cell into the bridge was prevented by use of a ground glass cap. The cell containing the mixture of ferrous sulfate and ferroin was oxidized, using 0.01 molar ceric sulfate (sulfatoceric acid) and the reaction potentials determined, using a suitable potentiometer.

This cell and titration assembly provided for the plotting of a titration curve relating oxidation potential and degree of oxidation for the simultaneous determination of the three following half-cell reactions:

(1) $Fe^{+++} + e^- \rightleftarrows Fe^{++}$ $E^\circ = 0.75$ volt (assumed)
(2) Ferriin + $e^- \rightleftarrows$ Ferroin $E^\circ = 1.14$ volts (determined)
(3) $Ce^{++++} + e^- \rightleftarrows Ce^{+++}$ $E^\circ = 1.44$ volts (assumed)

The conditions employed assume that the molal potentials of the first and third reactions were duplicated. Unfortunately this was not the case as shown by a further study of the system by Hume and Kolthoff *(40)*. By their revision of the data the value for the oxidation potential of the indicator reaction (reaction 2 above) was found to be 1.06 volts.

The necessity for the revision of the determination of the oxidation potential in the case of the ferriin-ferroin system as originally given in reference *(55)* and revised as in reference *(40)* was caused by the former investigator's failure to differentiate between "molal" and "formal" oxidation potentials.*

Some Comparisons of Formal and Molal Oxidation Potentials. Molal oxidation potentials are idealized reaction constants. Molal potentials may be evaluated by the use of three independent experimental procedures. The electrochemical method is most direct and results in the use of the least complicated experimental procedure. A second method involves the determination of reaction equilibrium constants. The third procedure is based upon the calculation of potentials using heat of reaction data. Molal potentials determined as a result of all three procedures should give identical results.

Formal oxidation potentials are practical rather than idealized constants *(56)*. Formal potentials rarely conform in magnitude to molal potentials *(57)*. The differences between conditions under which molal potentials and formal potentials are determined involve conformity to and deviation from the requirements classed as "standard state." Molal potentials require hydrogen ion concentrations to be calculated to include activity relationships; formal potentials ignore such corrections. Molal potentials include hydrolysis effects, formal potentials do not. Formal solutions from other circumstances do not conform strictly to the prerequisites of conditions of "standard state." The chief discrepancy probably consists in the formation of complex ions the dissociation constant of which is unknown.

It is the purpose of the present discussion to point out some of the wide discrepancies which result from the assumption that reaction constants for oxidation-reduction reactions can be even approximated from the use of molal potential values. The distinction between molal and formal oxidation potential data has been discussed with this point in view by Swift *(56)*:

> "These molal potentials are often of little practical value, and may be misleading, when one attempts to predict from the behavior of oxidizing and reducing agents in the relatively concentrated salt and acid solutions encountered in analytical chemistry. . . . These formal potentials could be calculated from the molal potentials (or the reverse) if there were available adequate data for the hydrolysis constants, for the dissociation constants of any complex ions which

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(55) Walden, Hammett, and Chapman, *J. Am. Chem. Soc.*, **55**, 2652 (1933).

*In this connection consult the work of the following references:

(56) Swift, "A System of Chemical Analysis," Prentice-Hall, 1939, pp. 49-50 and 540.

(57) Smith, *Trans. Ill. Acad. Sci.*, **36**, 132 (1943).

> may be formed, and, finally, for the activity coefficient of the reactants in these relatively concentrated solutions. Since such data are usually inadequate, it is advantageous to have the experimentally measured formal potentials."

The situation as it exists was one which may have prompted a Dutch chemist's warning that, "theory guides, experiment decides."

Selection of Reactants. Many oxidizing and reducing agents could be selected to illustrate the point of distinction between molal and formal potentials. Three commonly encountered reactants will be selected for illustration; namely, ferrous, dichromate, and vanadate ions.

Experimental Apparatus. A potentiometric titration assembly of the usual type consisted of a working cell (2 dry cells), a decade resistance box, a student potentiometer and tapping key, a moderately sensitive galvanometer and a standard Weston cell. The electrode pair used was a platinum wire and a saturated calomel half cell. By the use of a double throw switch the calibration of the galvanometer circuit can be made with reference to the standard Weston cell or the potential of the unknown reaction cell can be measured. The titration apparatus was provided with a mechanical stirrer and calibrated buret. Samples for determination were transferred to the reaction cell by the use of standard pipets. The accuracy claimed for the potentials determined is such that the value is approximate in the third place of decimals.

Descriptive Procedure. A description in detail will be given of a familiar reaction: $Cr_2O_7^{=} + 6Fe^{++} + 14H^{+} \rightarrow 2Cr^{+++} + 6Fe^{+++} + 7H_2O$. The half cell reactions involved are as follows:*

(1) $Cr_2O_7^{=} + 14H^{+} + 6e^{-} = 2\,Cr^{+++} + 7H_2O$ $\quad E^{\circ} = 1.36$ Volts

(2) $Fe^{+++} + e^{-} = Fe^{++}$ $\quad E^{\circ} = 0.771$ Volt

Reactants. $FeCl_3 \cdot 6H_2O$ (app. 0.2 mol.) dissolved in 162.2 ml. of reagent hydrochloric acid (sp. gr. 1.19) and diluted to 2,000 ml. making a solution 0.1 formal in ferric ion and formal in hydrochloric acid.

An approximately 0.01666 formal solution of $K_2Cr_2O_7$ in formal hydrochloric acid (0.1 N $Cr_2O_7^{=}$ in F HCl).

Formal hydrochloric acid for dilution purposes made by dilution of 81.1 ml. of reagent hydrochloric acid (sp. gr. 1.19) to 1,000 ml.

A 25.00 ml. portion of the ferric chloride solution after passing through a Walden silver reductor was transferred to a 400 ml. beaker and diluted to 250 ml. by addition of formal hydrochloric acid. This solution was then titrated, using the 0.1 N $K_2Cr_2O_7$ solution and the resulting potentials were plotted (Fig. 1 and Table V).

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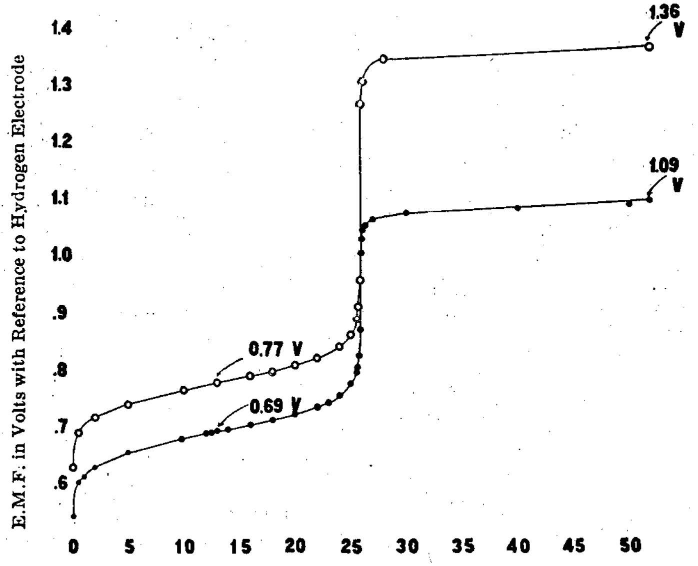
* The molal potentials (E°) are taken from Latimer "Oxidation Potentials," Prentise-Hall, 1938. Formal potentials will be indicated by the symbol $E^{\circ\prime}$.



Ml. Titrating Solution. (○), Calculated values. (●), Actual values.

FIG. 1. The titration of $FeCl_2$ in 1 FHCl solution by $Cr_2O_7^{=}$ in 1F HCl. (25.00 ml. 0.1 N Fe^{++} by 0.1 N $Cr_2O_7^{=}$).

TABLE V

The Potentiometric Titration of Ferrous Ions by Dichromate Ions in Formal Hydrochloric Acid Solution

$Cr_2O_7^{-}$ ml.	E.M.F.[a] Volts	$Cr_2O_7^{-}$ ml.	E.M.F. Volts	$Cr_2O_7^{-}$ ml.	E.M.F. Volts
0.00	0.5380	16.00	0.6981	25.85	0.8661
0.50	0.5907	18.00	0.7073	25.90	1.0064
1.00	0.6083	20.00	0.7181	26.00	1.0250
2.00	0.6255	22.00	0.7306	26.10	1.0403
5.00	0.6510	23.00	0.7386	26.30	1.0491
10.00	0.6748	24.00	0.7510	27.00	1.0586
12.00	0.6838	25.00	0.7713	30.00	1.0700
12.50	0.6858	25.50	0.7908	40.00	1.0778
13.00	0.6876	25.60	0.8008	50.00	1.0843
14.00	0.6906	25.70	0.8213	51.80	1.0925

[a] E.M.F. values are corrected to refer to hydrogen electrode as standard.

From the results of Table V the formal half-cell reactions involved are the following:

$$(1)\quad Cr_2O_7^{=} + 14H^+ + 6e^- = 2Cr^{+++} + 7H_2O \qquad E^{\circ\prime} = 1.09 \text{ Volts}$$

$$(2)\quad Fe^{+++} + e^- = Fe^{++} \qquad E^{\circ\prime} = 0.69 \text{ Volt}$$

The formal potential for reaction (1) is seen to be at variance with the molal potential to the extent of 20 per cent and for reaction (2) over 10 per cent. It is of interest to calculate the most important reaction constants for the reaction between ferrous and dichromate ions based upon molal potential data as compared to formal potentials. The constants calculated and the general formulation for each constant is as follows:

$$E_e\left(\begin{array}{l}\text{equiv.}\\ \text{point}\\ \text{potential}\end{array}\right) = \frac{bE^\circ_1 + aE^\circ_2}{a+b} \qquad -\log K_{\text{equil.}} = \frac{(E^\circ_1 - E^\circ_2)n}{.059}$$

$$[Red_2]/[Ox_2] = \sqrt[a+b]{\left(\frac{a'b}{ab'}\right)^a K}$$

Then

	Molal	Formal
E_e	1.276 Volts	1.033 Volts
K. equil.	1.26×10^{-60}	1.66×10^{-41}
$[Red_2]/[Ox_2]$	3.06×10^{-9}	1.65×10^{-6}

Formal Potentials of the Ferric-Ferrous and Dichromic-Chromic Systems at Various Concentrations. The determination of the ferric-ferrous formal potentials at 2, 3, and 4 formal strength of hydrochloric acid were similarly determined (as described in Table V and shown graphically in Figure 1. The same procedure gave also the formal potentials for the dichromic-chromic system at the same concentration of acid. In all cases the potentiometric titrational curves were just as smooth and precise and the formal potential values determined could be evaluated with an equal degree of precision. Duplicate titrational curves were obtainable at will and the formal potentials thus determined agreed to within ±0.01 volt. The same type experiments were carried out with the same systems in sulfuric acid solution except that the ferrous sulfate solutions in the various formal strengths of sulfuric were prepared from ferrous sulfate hexahydrate and therefore required no reduction before titration. The results appear in Table VI.

Choice of Oxidation-reduction Indicators for Use in the Determination of Iron by Dichromate. The oxidation-reduction indicator selected for the titration of iron by dichromate has generally been diphenylamine or diphenylamine sulfonic acid. These indicators are of quite low values in potentials which bring about their color change (0.76 volt and 0.84 volt respectively). For this reason phosphoric acid is always added to lower the oxidation potential of the ferric-ferrous system below the value ordinarily attained in either sulfuric or hydrochloric acid solutions. The use of these indicators is obviously not fully satisfactory. It would be reasoned that because of the presence of the green chromic ion in these titrations, a more vividly colored and more reversible indicator of the ortho-phenanthroline type would be used.

TABLE VI

The Formal Potentials of the Ferric-Ferrous and Dichromic-Chromic Systems in Sulfuric Acid and Hydrochloric Acid of Various Concentrations

Acid Used	$Cr_2O_7^{=}+14\ H^+ + 6e^- = 2Cr^{+++} + 7H_2O$		$Fe^{+++} + e^- = Fe^{++}$	
	E°′ Volts	E°	E°′ Volts	E°
1 F HCl	1.09(3)	1.36	0.68(8)	0.771
2 F HCl	1.10(7)		0.67(7)	
3 F HCl	1.19(0)		0.67(1)	
4 F HCl	1.15(0)		0.65(8)	
2 F H_2SO_4	1.10(9)		0.68(8)	
4 F H_2SO_4	1.14(7)		0.68(7)	
6 F H_2SO_4	1.30(0)		0.68(1)	
8 F H_2SO_4	1.34(6)		0.65(8)	

The fact that ferroin indicator is not employed in the determination of iron by dichromate is rather unexplained on the basis of the molal oxidation potential values given for the half cell reaction of the $Cr_2O_7^{=} \rightarrow 2Cr^{+++}$ (1.36 volts) and the ferriin-ferroin system (1.12 volts).* The difference between the molal and formal potentials given in Table VI indicates the failure of conditions to be suitable except in 6 to 8 formal sulfuric acid solution and unsatisfactory conditions at any strength of hydrochloric acid.

Influence of Acid Concentration on the Oxidation Potential of Indicators of the Phenanthroline Series. The indicator ferroin (1-10-orthophenanthroline ferrous sulfate) and substituted phenanthroline indicators such as 5-methyl-1-10-orthophenanthroline ferrous sulfate (methyl ferroin) are subject to very material changes in oxidation potential with changes in hydrogen ion concentration in solutions in which they are employed. These values have been determined in previous work (a report of which is subsequently given) and are summarized as given in Table VII.

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* The potential at the point of visual transformation.

TABLE VII

The Formal Oxidation Potentials of Ferroin and Methyl Ferroin Indicators

Reactions: Ferriin + e^- → Ferroin $E^\circ = 1.06$ Volts
Methyl Ferriin + e^- → Methyl Ferroin $E^\circ = 1.02$ Volts

Acid Conc.	Ferriin-Ferroin $E^{\circ\prime}$ in Volts	Methyl Ferriin-Ferroin $E^{\circ\prime}$ in Volts
1 Formal	1.06	1.02
2 Formal	1.03	1.00
3 Formal	1.00	0.96
4 Formal	0.96	0.93
6 Formal	0.89	0.86
8 Formal	0.76	0.70

From the data in Table VII it is observed that just as with the oxidation of iron by dichromate, wide changes in oxidation potential occur in the indicator system. This requires that both the variations should be taken into consideration when reaction conditions are to be selected. With this point in view the methyl ferroin indicator would be selected for dichromate titrations of iron because its oxidation potential is lower than that of ferroin. It would be predicted that satisfactory conditions prevail at 4 formal strength of sulfuric acid. A test case potentiometric titration of a mixture of ferrous and methyl ferroin ferrous ions in 4 formal sulfuric acid was titrated, using the dichromate ion in 4 formal sulfuric acid solution as oxidant. The values obtained are shown graphically in Figure 2. It will be observed that a satisfactory differential titrational procedure results.

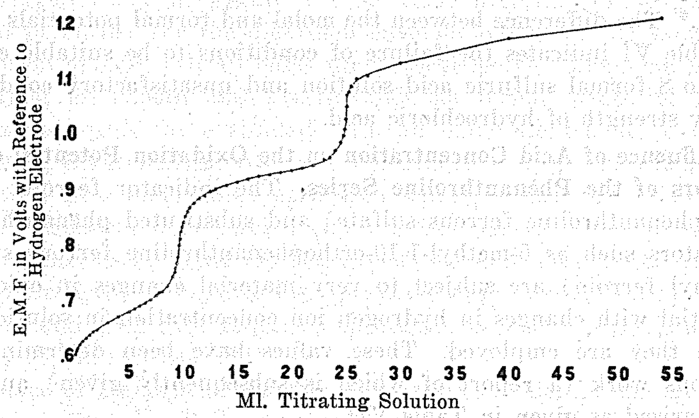

FIG. 2. The titration of 10.0 ml. of Fe^{++} solution plus 15.0 ml. of ferrous methyl 1,10-phenanthroline using $Cr_2O_7^{=}$. Solutions approximately 0.1 N and 4 F H_2SO_4 used throughout. $Fe^{+++} + e^- = Fe^{++}$ potential 0.69 volts, methyl ferriin + e^- = methyl ferroin 0.96 volts.

The Formal Potentials of the Vanadate-Vanadyl System. The same procedure as previously described was employed for the determination of the formal potentials for the half cell reaction, $VO_3^- + 4H^+ + e^- = VO^{++} + 2H_2O$ ($E^\circ = 1.10$ volts), in the various strengths of sulfuric acid from 1 F through 8 F. The results are shown in Table VIII.

TABLE VIII

The Formal Potentials of the Vanadate-Vanadyl System in 1 to 8 F H_2SO_4

Formal H_2SO_4	$E^{\circ\prime}$ in Volts	Formal H_2SO_4	$E^{\circ\prime}$ in Volts
1	1.02	5	1.17
2	1.07	6	1.20
3	1.10	7	1.25
4	1.14	8	1.30

The Graphical Representation of the Differential Titration of Ferrous and Ferroin Solution Mixtures Using Potassium Dichromate in Sulfuric Acid Solution. The data shown graphically in Figure 2 is repeated as one of the titration curves of Figure 3, and shows the differential titration of ferrous ions and ferroin ions in 4 formal sulfuric acid solution, using dichromate as oxidant. The data shown graphically in Figure 3 includes the same titration under four different conditions, namely, in 2, 4, 6, and 8 formal sulfuric acid solution. An examination of Figure 3 shows that the titration in 2 F H_2SO_4 gives a satisfactory "end point break" for the ferrous-ferric system but unsatisfactory for the ferroin-ferriin system. Both "end point breaks" are satisfactory at 4 F H_2SO_4 concentrations and the 6 F H_2SO_4 reverses the 2 F H_2SO_4 results giving a satisfactory end point break for the indicator system but not for the ferrous-ferric system. Finally at 8 F H_2SO_4 a simultaneous oxidation of both the ferrous and ferroin ion occurs. These observations are explained by the fact that while the formal oxidation potential of the ferric-ferrous system does not change with increase in acid strength the formal oxidation potential of the indicator rapidly decreases as shown in Table VII. The determination of the formal oxidation potentials of the various phenanthroline type of indicators will next be described. From the discussion immediately preceding the following conclusions can be drawn:

Formal versus molal oxidation potentials differ over such a wide range under normal variations in titrational environments that special cognizance of this situation is of prime importance. The cases cited in this discussion are typical, not isolated examples. The system of recording formal potentials and molal potentials in the same table of reaction constants as instigated by Swift (loc. cit.) should be extended and emphasis should be placed on the use of these formal potentials in preference to molal potentials as a guide to predicting suitable reaction conditions.

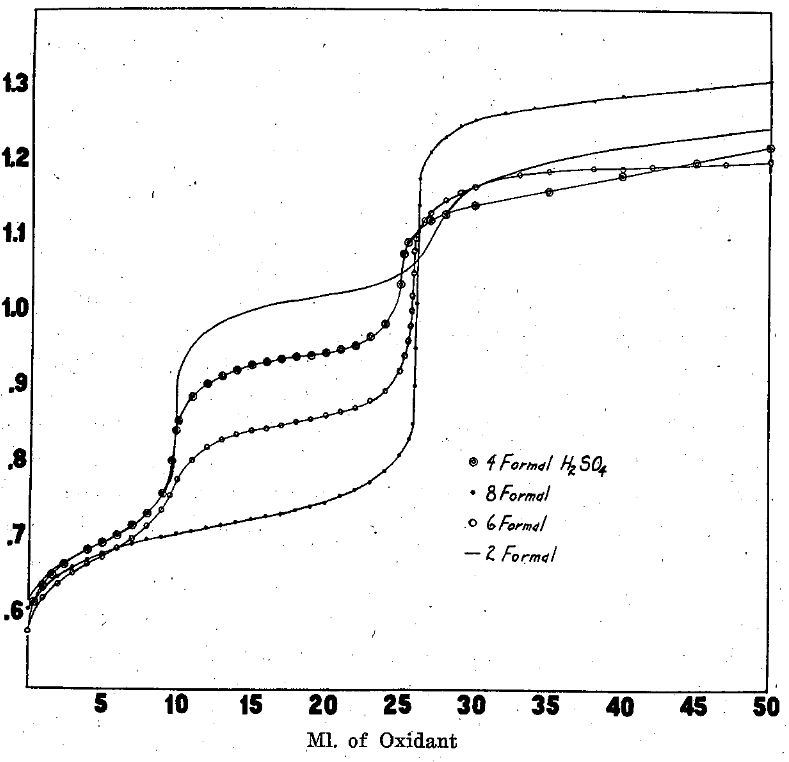

FIG. 3. The Differential Titration of Ferrous and 5-Methyl-(1,10)Phenanthroline Ferrous Ions in H_2SO_4 Solution Using Dichromate as Oxidant.

Further Determinations of the Indicator Oxidation Potential for the Phenanthroline and Substituted Phenanthroline Ferrous Complexes. The same type system as that previously described by Walden, Hammett, and Chapman *(55)* was used except that potassium dichromate in sulfuric acid solution was also employed as oxidant. The formal oxidation potentials ($E^{\circ\prime}$ values) employed as reference points are given in Table IX. The determination of the formal oxidation potentials in the case of 1,10-phenanthroline and 5-methyl-(1,10)-phenanthroline could be carried out by the titrational scheme described in connection with Figure 2. This is in accordance with the fact that

both the oxidized and reduced form of the indicator is stable in the formality of acid employed. For some of the phenanthrolinium ions the potential of the systems listed in Table IX were not sufficiently high to bring about the oxidation of the indicator. To use a perchloric acid solution medium to increase the ceric-cerous potential was not feasible since the indicator ions in this acid precipitate. In other cases equally insoluble sulfuric acid complex indicator ions form at higher acidities.

TABLE IX

Formal Oxidation Potential of Various Systems in Sulfuric Acid Solution

Potential Determined	$E^{\circ\prime}$ Values in Volts at Indicated F H_2SO_4 Solution				
	1F	2F	4F	6F	8F
$Fe^{+++} \longleftrightarrow Fe^{++}$	0.69	0.68	0.68	0.68	0.68
$Cr_2O_7^{=} \rightarrow 2Cr^{+++}$	......	1.11	1.15	1.30	1.35
$Ce(SO_4)_2 \rightarrow Ce^{+++}$	1.44	1.43	1.42	......	1.40

In these cases solutions of 0.01 or 0.025 normal sulfatocerate ion in solutions containing 1 to 8 g. molecules of sulfuric acid per liter were prepared by dissolving pure ammonium nitratocerate in concentrated sulfuric acid followed by gradual dilution to the proper volume. The substituted ferroin indicator solutions of 0.01 or 0.025 M concentrations were prepared by solution of the proper weight of indicator base (Table II) in 0.01 or 0.025 N ferrous ammonium sulfate solution. Measured portions of the cerate solution (25.00 ml.) were placed in 400 ml. beakers and an equal volume of sulfuric acid of twice the strength finally prepared then added. Dilution was then made to 200 ml. with sulfuric acid of the same desired strength.

A measured portion (50.00 ml.) of the ferroin or substituted ferroin complex was then added in one portion with vigorous stirring. The potential of the resulting solution was read at once, using a saturated calomel electrode and a bright platinum electrode as references. Any condition such as results from instability of the ferric phenanthrolinium ions was indicated by a gradual fall of potential which could be observed without difficulty. Such instability was more pronounced at higher acid concentrations and with ferric phenanthrolinium ions of highest electrode potential. The systems showed no appreciable change in potential during the time required for the reading of the potential of the first mixing.

The data obtained are found in Table X. By determination of potentials in many cases by both procedures, the values proved to be reliable within 0.02 volt. As a further test of accuracy, the values obtained in the case of either procedure duplicated those of Hume and Kolthoff *(40)* for the case of 1,10-phenanthroline ferrous sulfate. The assumption made by these authors *(40)*, that the oxidation potential of the 2,2′-bipyridyl ferrous sulfate complex is the same as for the 1,10-phenanthroline ferrous sulfate complex, is wrong as will be seen by consulting the values of Table X.

TABLE X

The Formal Oxidation Potential of Ferroin and Substituted Ferroin Indicators at Various Strengths of Sulfuric Acid

Indicator as $FeSO_4$ Complex	Sulfuric Acid Strength and Oxidation Potential in Volts						
	0.5 F	1F	2F	3F	4F	6F	8F
5-Nitro-(1,10)-Phenanthroline	1.26	1.25	1.22	......	1.17	1.12	1.01
5-Methyl-6-Nitro-(1,10)-Phenanthroline	......	1.23	......	......	......	......	......
5-Bromo-(1,10)-Phenanthroline	1.13	1.12	......	......	......	......	......
5-Chloro-(1,10)-Phenanthroline	......	1.12	1.10	......	1.04	0.97	......
1,10-Phenanthroline	......	1.06	1.03	1.00	0.96	0.89	0.76
5-Methyl-(1,10)-Phenanthroline	......	1.02	1.00	0.96	0.93	0.86	0.70
2,2′-Bipyridyl	......	0.97	......	......	0.92	......	......

In the use of the data of Table X the same qualifications as mentioned in connection with the data of Table III (page 28) apply.

The values of Tables V, VI, VII, VIII, and IX are shown graphically in Figure 4. From a study of Figure 4 it can be easily predicted under what conditions a given ferroin type indicator will be oxidized by the two oxidants, either dichromate or vanadate. For example at 1 F sulfuric acid concentrations neither of these two oxidants is capable of converting either ferroin or nitro-ferroin from their red form to the blue form. The ferroin indicator has practically the same oxidation potential as the vanadate ion at 1 F H_2SO_4 concentrations. At 2 F sulfuric acid concentrations either vanadate or dichromate will oxidize ferroin and methyl-ferroin but not nitro-ferroin. At five formal sulfuric acid concentrations all these three indicators would be oxidized.

If it is remembered that the oxidant should be at least 0.18 volt higher than the ion being oxidized for quantitative results it will be observed that the reaction of oxidation of methyl-ferroin would be quantitative at 4 F sulfuric acid concentrations but toward ferroin not quantitative without excess oxidant. At 6 F sulfuric acid nitro-ferroin would be quantitatively oxidized by an equivalent amount of dichromate but not using an equivalent amount of vanadate. Many other conclusions can be predicted and established by experiment. For example 2,2′-bipyridyl ferrous sulfate could be employed to carry out an iron titration in lower sulfuric acid strengths than would be the case using methyl ferroin as indicator.

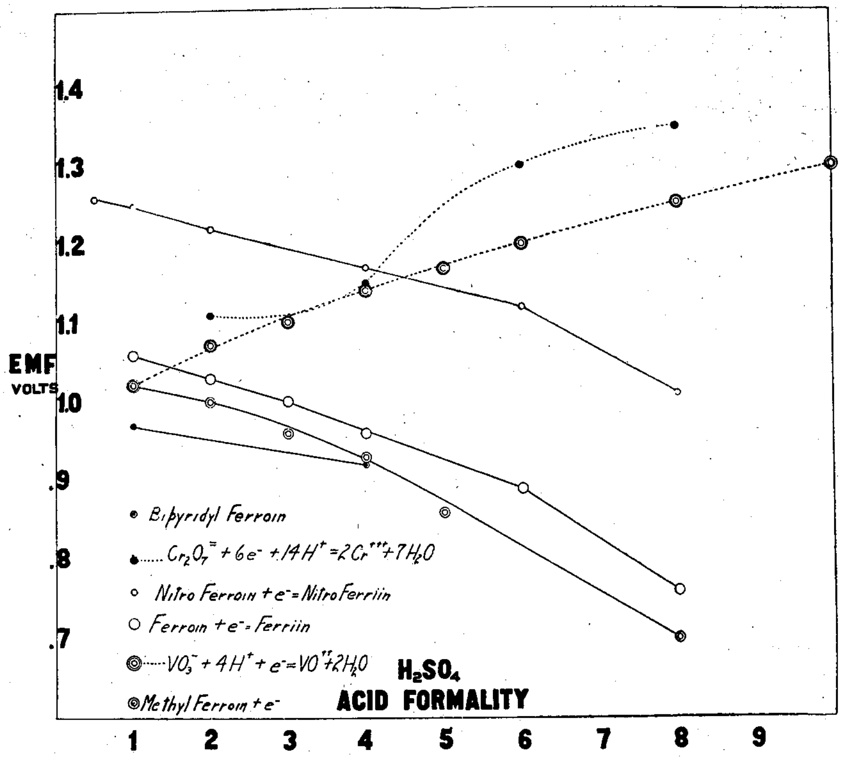

FIG. 4. The Single Electrode Potentials of Various Redox Systems as a Function of Acid Formality.

Two interesting review papers on the subject of oxidation-reduction indicators are those of Walden and Edmonds *(58)* and that of Whitehead and Wills *(59)*. The definition of an oxidation-reduction (redox) indicator is given by Kolthoff and Sandell *(60)*, as "A substance the oxidized form of which has a color different from the reduced form. The oxidation and reduction of the indicator should be reversible. In general, the action of an oxidation-reduction indicator does not depend upon the specific nature of the oxidant or

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(58) Walden and Edmonds, *Chem. Rev.*, 16, 81 (1935).
(59) Whitehead and Wills, *ibid.*, 29, 69 (1941).
(60) Kolthoff and Sandell, "Quantitative Inorganic Analysis," The MacMillan Co. (1943), p. 491.

reductant titrated but upon the relative positions of the oxidation potentials of the indicator and of the system titrated." It might be profitably added that the transition point for oxidation-reduction indicators often takes place at different potential levels as influenced by the concentration and nature of the acid solution being studied. A practical distinction should be made between "molal" and "formal" potentials in predicting the behavior of all reactants involved.

The indicator oxidation potential determinations reported above were reprinted in accordance with reference *(61)*.

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(61) Smith and Richter, *Ind. Eng. Chem.*, Anal. Ed., 16, 580 (1944).

SECTION 5

THE SOLUBILITY OF (1,10)-PHENANTHROLINE, 5 NITRO-, AND 5 BROMO- (1,10)-PHENANTHROLINE AND THEIR FERROUS PERCHLORATE COMPLEX SALTS

The determination of the solubilities of (1,10)-phenanthroline and substituted phenanthrolines are important in connection with the isolation of the materials during synthesis, and in the preparation of solutions for use in the formation of complex salts either of the soluble type as the ferrous sulfate complex or the insoluble perchlorate type. The bromo- and nitro- substituted phenanthrolines showed the most important solubility relationships as precipitants in quantitative separations (to be discussed in a subsequent section).

Solubility data herein reported followed a three-stage procedure:

1. Preparation of saturate solutions through the agitation of excess solute with the desired solvent at a definite temperature.

2. Dilution of measured volumes of saturated solutions to volumes suitable for spectro-photometric measurement.

3. The determination of the transmittancy at the predetermined wave length of maximum absorption.

The common ferrous complex phenanthrolines which are most insoluble are the perchlorate, periodate, dichromate, ferrocyanide, picrate, thiocyanate and persulfate salts. Solubility determinations of the perchlorate complexes over a considerable range of conditions were made in planning quantitative separations. The procedures may be readily extended to include other insoluble complex phenanthroline salts. A number of typical quantitative determinations will be devised and subsequently described.

Since the accuracy of the colorimetric determination of the phenanthroline's ferrous ion is of the order of three per cent the method of procedure about to be described is entirely adequate for precise determination of solubility.

Apparatus Employed. Transmittancy measurements were carried out using a General Electric Recording Spectrophotometer supplemented by the use of a Cenco-Sheard Spectrophotometer. The record-

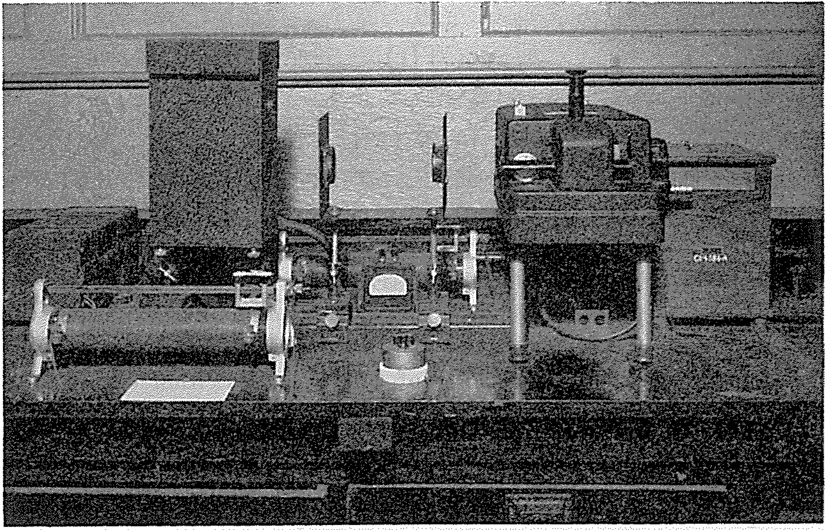

FIG. 5. The Cenco-Sheard Spectrophotometer

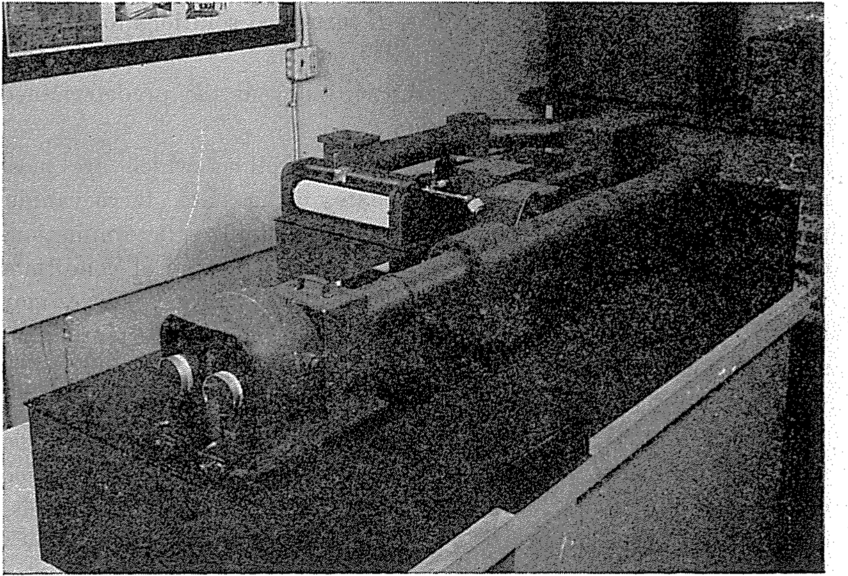

FIG. 6. The General Electric Recording Spectrophotometer

ing spectrophotometer* was employed only for the preparation of the calibration curves (transmittancy in the visual range 400-700 mμ). The Cenco-Sheard Spectrophotometer (Figure 5) was employed in determining the transmittancy at the wave length of maximum absorption.

A special type thermostat with equipment provided for rotating solubility tubes to the point of solution saturation equilibrium was used. Racks for storing tubes and saturated solutions were provided to allow separation of the solution and excess solute. The bath was cooled by running tap water, heated by a 100-watt electric lamp, and regulated by a mercury "make and break" circuit electronically sensitized and powered by the same 110-volt power line used for the mechanical working parts and the heating circuit. The temperature regulation was controlled to within 0.05 degree, a more accurate control being unnecessary.

Standard Iron Solution. The standard iron solution was prepared by weighing a quantity of $FeSO_4 \cdot 7H_20$ such that 1,000 grams of solution contained 1 gram of iron. Enough sulfuric acid was added to prevent hydrolysis. This was prepared on the day of its use to eliminate errors due to oxidation of the ferrous ion. Weight burets were used throughout.

(1,10)-Phenanthroline and Substituted Phenanthroline Reagents. Pure samples of (1,10)-phenanthroline, 5 bromo- and 5 nitro-(1,10)-phenanthroline were used to prepare standard ferrous complex solutions. These were made in the form of one-tenth per cent solutions. To facilitate dissolution of the organic base compound, a minimum

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* The Engineering College of the University of Illinois, because of the fact that its Electrical Engineering Department has the only General Electric Automatic Recording Photoelectric Spectrophotometer available in the State, has provided for the possible employment of this equipment by commercial interests. This service, dealing with studies of (1) color permanence of organic materials, (2) vitreous enamels, (3) photoelectric colorimetry, and (4) biological and physiological investigations is made possible in conformation to the University of Illinois Statutes (Art. 46, page 18, March 10, 1936) rule that:

"Commercial tests or investigations, for individuals, firms, institutions or corporations may be undertaken by the University of Illinois, when, in the opinion of the head of the department in which the tests would be conducted and of the dean of the college, it is desirable that the work be done. In general, such tests are justified when the results may be of scientific value, or when the necessary facilities do not exist elsewhere or are not readily accessible."

Departments using the services of the University of Illinois Electrical Engineering Department will please refer those commercial interests wishing this service direct to the Photometric Laboratories, Professor J. O. Kraehenbuehl in charge, 201 Electrical Engineering Annex, Urbana, Illinois. Costs and service regulations will be supplied on request. The apparatus is illustrated in Figure 6.

amount of ethanol was used, followed by dilution to the desired volume with iron-free distilled water.

The perchlorate complex salts were obtained from their respective ferrous sulfate complexes by adding excess of dilute perchloric acid which resulted in the precipitation of the desired reagent. The red insoluble ferrous perchlorate precipitates were filtered, washed with distilled water, and dried in preparation for the desired solubility determinations.

Solubility tubes consisted of test tubes of 25 mm. in diameter made into the form of ampules in preparation for introducing excess solute and solvent followed by closure by sealing the constricted throat of the ampule.

The Preparation of Calibration Graph. Solutions of (1,10)-phenanthroline ferrous sulfate from solutions containing 1, 2, 3, 4, 5, and 6 parts per million of iron were prepared. Weight burets were used to measure the amounts of iron taken and a slight excess of dye base was added. Time was allowed for complete color development. No adjustment of pH was necessary. The absorption spectra of these solutions was graphically recorded using the General Electric recording instrument with the results shown in Figures 7, 8, and 9. The same procedure was applied to the solutions of 5 nitro-(1,10)-phenanthroline ferrous sulfate and 5 bromo-(1,10)-phenanthroline ferrous sulfate. Since the latter two color complexes show appreciable change in color within 24 hours' storage, transmittancy measurements were made at once both for the preparation of the calibration graphs and for the determination of unknown amounts of iron.

From the graphical data thus obtained, calibration curves for the iron determination were prepared in which the logarithm of the reciprocal of the per cent of transmittancy (at the point of maximum absorption) were plotted against the milligrams of iron per liter (p.p.m.) present. The straight line functions, shown graphically in Figure 10, were obtained. By the use of this procedure the per cent transmittancy at the wave length of maximum absorption, [(1,10)-phenanthroline, 510 mμ, 5 bromo- and 5-nitro-(1,10)-phenanthroline, 512 mμ], can be determined using the Cenco-Sheard Spectrophotometer and from the graph, Figure 10, the concentration of iron present determined.

For the determination of the solubility of the parent dye bases the reverse procedure is applied. After suitable dilution of the saturated solution of the dye base an excess of ferrous iron is added and the red ferrous sulfate complex is then analyzed by the above

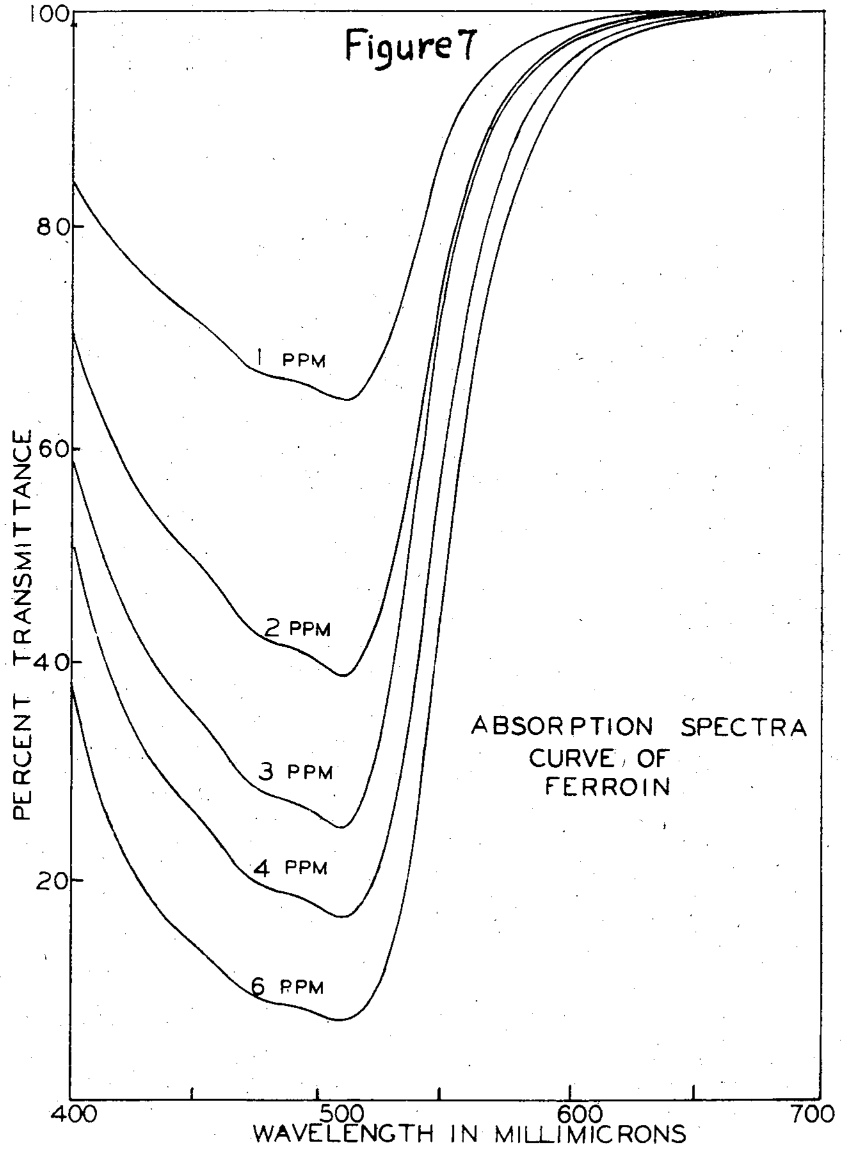
Figure 7

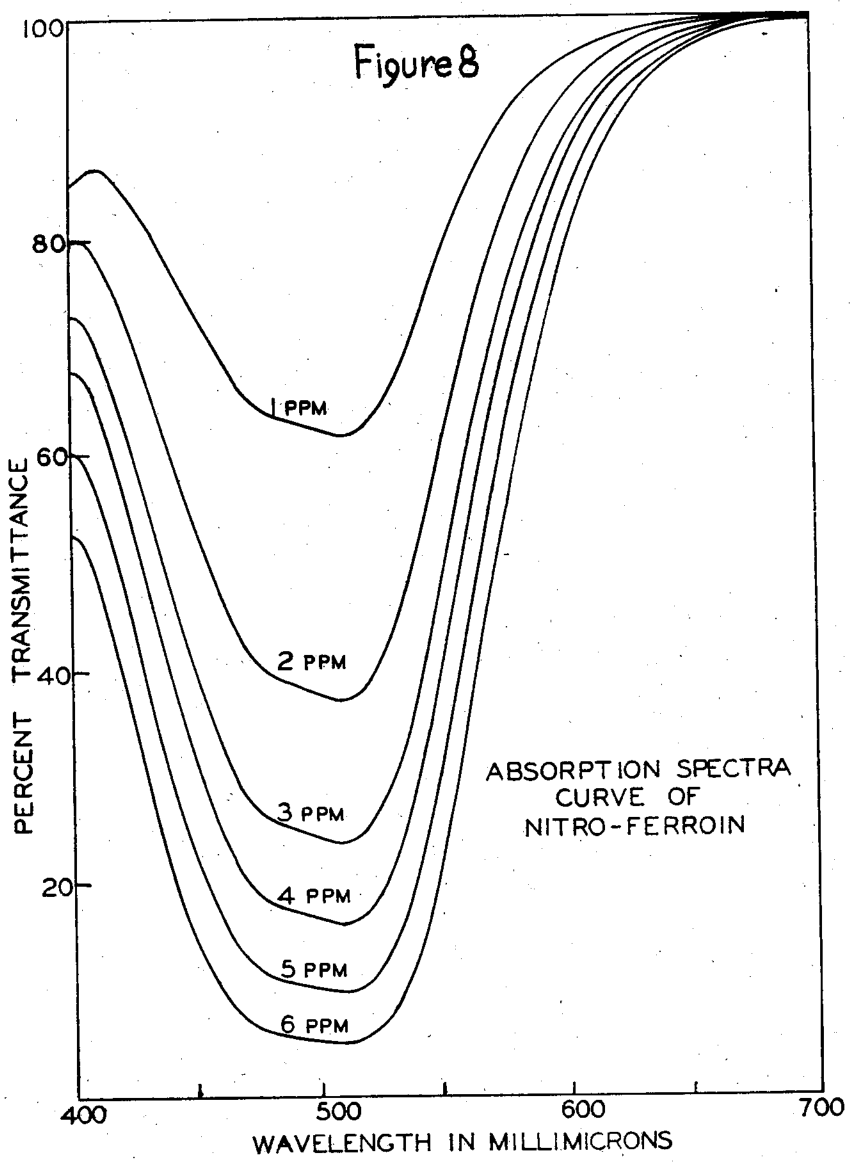

Figure 8

ABSORPTION SPECTRA CURVE OF NITRO-FERROIN

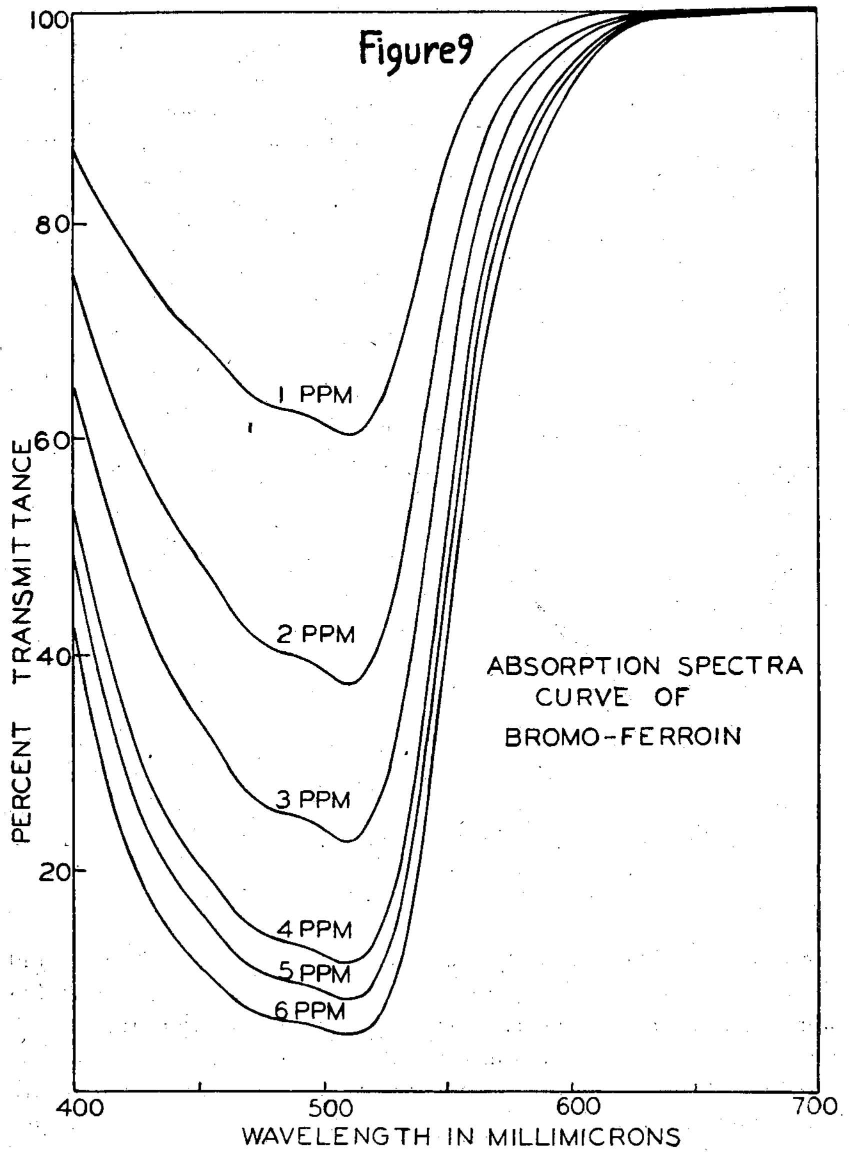

Figure 9

ABSORPTION SPECTRA CURVE OF BROMO-FERROIN described procedure and from the iron found the organic dye base present may be calculated.

The Solubility Determination. The general procedure was to place approximately 50 ml. of the selected solvent and a generous excess of solute in the solubility tubes. The ampule like necks were then sealed at the blast lamp. The reaction tubes were mounted on the revolving racks of the thermostat until equilibrium was attained at 25 degrees centigrade. The solubility tubes were then placed in a vertical position in racks within the thermostat and the solid and liquid phases allowed to separate as completely as possible. The tubes were opened and the nearly clear supernatant solution rapidly filtered, using a fritted glass filtering crucible. The saturated solution was then transferred to a 25-ml. pycknometer at 25 degrees centigrade and the weight of the known volume of solution determined. The contents of the pycknometer was then transferred to a volumetric flask for dilution, bringing its iron content within the range of 0.1 p.p.m. to 6 p.p.m. of iron (the range of the calibration curves, Figure 10). All determinations were carried out in duplicate. In the case of the pure dye bases the same procedure was employed except that the use of ferrous sulfate was required to develop the color and much more extensive dilutions were required.

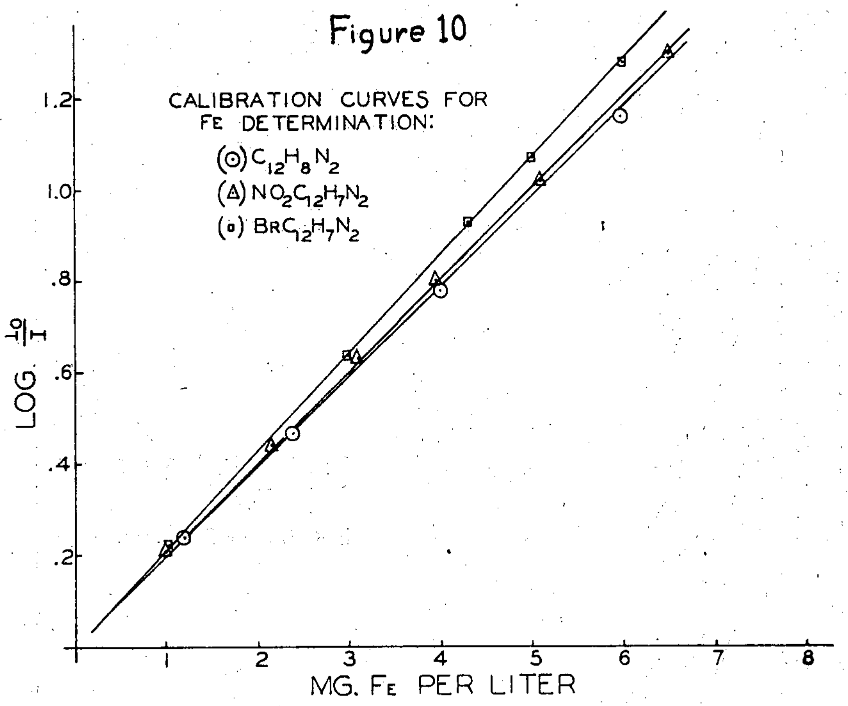
Figure 10

Sample Calculation. As an illustration the solubility of 6 bromo-(1,10)-phenanthroline in 0.0007 molar perchloric acid is experimentally determined.

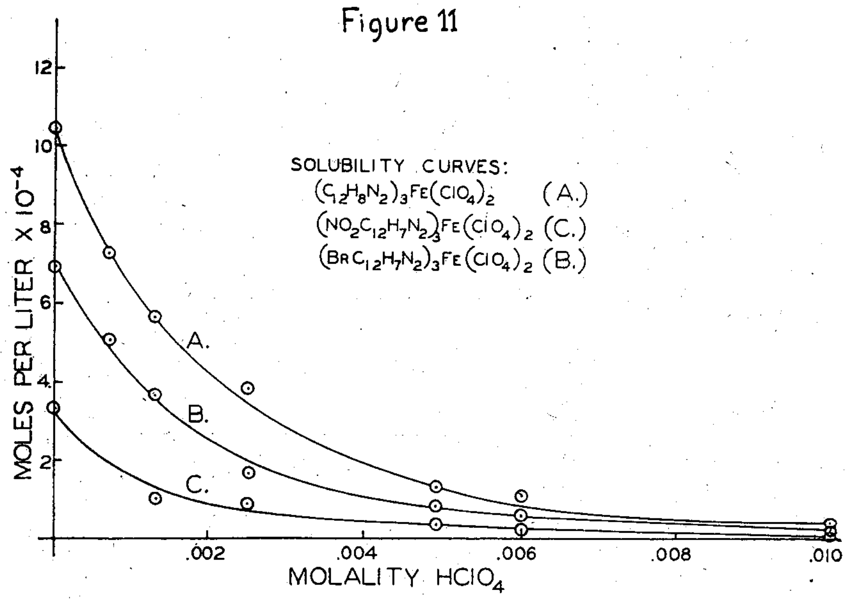
Figure 11

A solubility tube containing 0.0007 molar perchloric acid, saturated with 5 nitro-(1,10)-phenanthroline ferrous perchlorate, was removed from the constant temperature bath, filtered, and placed in a pycknometer of 25.018 ml. volume at 25 degrees centigrade (Solution A). This entire volume was weighed and transferred to a flask and diluted to 300 grams (Solution B). Solution B gave a transmittancy value of 31 per cent using the Cenco-Sheard Spectrophotometer and light source of wave length 512 mμ. The transmittancy factor (I/I_o) is 0.31 and log 1/T (i.e. log I_o/I) is 0.51. By reference to the calibration curve, Figure 10, this value was seen to correspond to 2.36 p.p.m. of iron or 2.36 mg. of iron per 1,000 g. of solution B. Since the total volume of solution B was 300 g. the total iron present was 0.3 x 2.36 mg. of iron or 0.708 mg. of iron in solution A. To find the concentration of iron in solution A, the total iron content was divided by the volume of solution A or 25.018 ml. This result when

TABLE XI

The Solubility of (1,10)-Phenanthroline, 5 Nitro-(1,10)-Phenanthrolines and Their Ferrous Perchlorate Complexes in Water and Various Strengths of Perchloric Acid

Solute	Solvent and Solubility in Moles per Liter						
		Molar Perchloric Acid Concentration					
Ferrous Perchlorate Complex of	H_2O	0.0007	0.0013	0.0025	0.0049	0.0061	0.0100
(1,10)-Phenanthroline	0.00105	0.00073	0.00055	0.00038	0.00013	0.00011	0.00005
	0.00102	0.00071	0.00057	0.00038	0.00014	0.00010	0.00005
5 Nitro-(1,10)-Phenanthroline	0.00033	....	0.00011	0.000086	0.000036	0.000022	0.000006
	0.00032	....	0.00009	0.000086	0.000036	0.000022	0.000006
5 Bromo-(1,10)-Phenanthroline	0.00067	0.00051	0.00035	0.00016	0.000079	0.000060	0.000024
	0.00072	0.00051	0.00038	0.00016	0.000073	0.000057	0.000027
(1,10)-Phenanthroline (uncomplexed)	0.0159	....	....	....	....	....	....
	0.0162	....	....	....	....	....	....
5 Nitro-(1,10)-Phenanthroline (uncomplexed)	0.000129	....	....	....	....	....	....
	0.000108	....	....	....	....	....	....
5 Bromo-(1,10)-Phenanthroline (uncomplexed)	0.00099	....	....	....	....	....	....
	0.00099	....	....	....	....	....	....

The solubility of (1,10)-phenanthroline monohydrate in absolute ethyl alcohol was found to be 2.78 moles per liter. That of 5 nitro-(1,10)-phenanthroline in absolute ethyl alcohol was found to be 0.042 moles per liter.

divided by the atomic weight of iron gave the molar solubility of the complex perchlorate of 5 bromo-(1,10)-phenanthroline.

$$0.000708/25.018 \times 1{,}000/55.85 = 5.07 \times 10^{-4} \text{ moles per liter.}$$

The results from the solubility determinations are given in Table XI.

The results from Table XI are shown graphically in Figure 11. The accuracy shown conforms to a maximum deviation of 5 per cent from the mean determined value which is satisfactory for colorimetric determinations in the range of 1×10^{-3} to 1×10^{-5} moles per liter. Since no foreign ions were introduced in the solubility determinations, it seems reasonable that at least the same accuracy was attained in the determination of the solubilities as was provided in the compilation of the data of Figure 10, in which case the close agreement of values to conformity with the application of Beer's Law lends confidence to the accuracy of the results as shown. More than a 95 per cent decrease in solubility is seen to occur with the ferrous perchlorate complexes in passing from pure water to 0.01 molar perchloric acid as solvent. The greatest decrease in solubility occurs in the case of the 5 nitro-(1,10)-phenanthroline. The use of this dye base for the isolation of ferrous in the presence of ferric iron, perchlorate, periodate, and persulfate in the presence respectively of chlorate, iodate, and sulfate is to be described in a subsequent section.

SECTION 6

THE MICRO-VOLUMETRIC DETERMINATION OF IRON USING BIPYRIDYL AS INDICATOR

The determination of iron by a micro-volumetric procedure involves the use of a 0.01 to 0.001 N solution of the particular reagent selected. Either by the use of titanous or permanganate solutions difficulty is encountered in their lack of stability even during short periods of storage. The use of a titanous solution obviates the requirement that ferrous solutions must be prepared, but requires that the titanous solution be stored in a neutral atmosphere and that titrations be carried out in the absence of air. Such dilute permanganate solutions require daily evaluation and the use of a reductor to prepare the iron solutions for titration. The use of 0.01 to 0.001 N dichromate solution as a substitute for permanganate proved to be completely unsatisfactory.

These observations caused Van Nieuwenburg and Blumendal *(61^a)* to employ ceric solutions for the determination as described in their studies *(61^a)* under the title "Cerimetric Titration of Small Amounts of Iron by Means of 2,2′-Bipyridyl as an Indicator."

Since in a solution of iron which has been reduced quantitatively to the ferrous state using the Walden Silver Reductor *(62)* the hydrochloric acid concentration is 0.5 to 1.0 M, there is introduced a complication in that the addition of an aqueous solution of a small trace of 2,2′-bipyridyl does not form the familiar red ferrous complex of the indicator compound. Since also the ferrous bipyridyl complex indicator ion forms best and most quickly at a pH of approximately 3.5 the difficulty was solved by adding the indicator (5 drops of 0.015 M solution in 1-1 conc. NH_4OH) to the strongly acid ferrous chloride solution in 50 ml. total volume. The addition in this manner produces enough of the ferrous bipyridyl complex to serve as an indicator and one drop of 0.002 N sulfatocerate solution decolorizes the solution at the equivalence point in the reaction.

Preparation of Indicator Solution. 0.25 g. of 2,2′-bipyridyl is dissolved in 50 ml. of water (this is a saturated solution at room temperature). Then add 50 ml. of concentrated ammonium hydroxide. Approximately 5 drops of this solution will do for approximately 50 ml. of ferrous solution. The remainder of the description of the procedure is given by direct quotation:

"**Procedure of Analysis.** In this way . . . titrations of . . . ferrous solutions containing from 0.1-10 mgr. Fe in 50 ml. N HCl by means of 0.002-0.015 N ceric sulfate in 1 N sulfuric acid solution . . . were possible. The end point is quite sharp within one drop, i.e. far better than with a titanous solution. Even when using ceric sulfate solutions only 0.001 N the end-point is still satisfactory (about two drops). When the ceric solution is guarded against direct sunlight it is quite stable, even in these extreme dilutions.

"Our silver reductor consisted of a tube of 20 cm. length and 7 mm. opening with a small funnel on top, and below a stopcock and a capillary tube passing into a small suction flask, connected to a pump to prevent oxidation by air.

"The silver sponge must always be kept under 1N HCl. When it has been used for some time, it is completely regenerated by inserting a rod of (pure) zinc and leaving it overnight. We found that one passage of ferric solution through the silver is quite sufficient for a complete reduction. As a rule our ferric solution had a volume of about 20 ml. It was passed through the reductor and rinsed with N HCl three times with 10 ml. each, so that the final volume to be titrated was about 50 ml.

"Some of the results are given below, Table XII:

TABLE XII

The Micro-Volumetric Determination of Iron Using Sulfatoceric Acid with 2,2′ Bipyridyl as Indicator

Fe Taken	0.0139 N Ce^{++++} Calculated	0.0139 N Ce^{++++} Found	Fe Found
mg.	*ml.*	*ml.*	*mg.*
9.79	12.61	12.53	9.73
9.79	12.61	12.56	9.75
9.79	12.61	12.52	9.72
4.89	6.30	6.27 (7)	4.87
4.89	6.30	6.22 (3)	4.87
	0.00139 N Ce^{++++}	0.00139 N Ce^{++++}	
0.489	6.30	6.10 (7)	0.474
0.489	6.30	6.12 (0)	0.475

"It will be observed, that the results obtained are always slightly under those calculated, and the more so as the amount of iron decreases. We are of opinion, that this is a nearly inevitable error due to the oxidation by air, when we remember that 50 ml. of our liquid can contain 0.42 mgr. dissolved oxygen, able to transform 3 mgr. ferrous iron into the ferric state.

"So if we want to use the method for quantities below one-half mgr. of iron it is advisable to work in a smaller volume. The reductor and the burette can be built to meet the requisites of carrying out the titration on a still smaller scale. In fact, we made some determinations of 0.1-0.3 mgr. Fe in a liquid volume of 5 ml. with a reductor of 10 cm. length and 0.4 cm. opening, a burette of 5 ml. and 0.00139 N ceric solution. We found 0.125-0.126-0.249-0.251 mgr. Fe instead

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(61a) Van Nieuwenburg and Blumendal, *Mikrochemie*, 18, 39 (1935).

(62) Walden, Hammett and Edmonds, *J. Am. Chem. Soc.*, 56, 350 (1934).

of 0.122 and 0.245 calculated. The slightly high results are probably due to the fact that the indicator is not altogether free from oxidizable impurities. Indeed we got still higher results when using a sample of 2,2′ bipyridyl which was colored slightly brown.

"On the whole we think that our results compare favorably with those of titanometric and similar older procedures under equally dilute conditions.

"We found it undesirable to use a sulfuric acid solution of ferrous iron. The color of the bipyridyl complexes is far better developed in hydrochloric acid. In 4N H_2SO_4 no color is obtained at all, unless large quantities of the expensive indicator are used. On the other hand it is not advisable to go below 0.5N HCl because then the reduction by means of silver tends to become incomplete."

The above reprinting was made with a change in a number of cases from the English form of spelling (color for colour, etc.) and with a somewhat altered table of data (Table XII). The term 2,2′-bipyridyl was substituted for the term alpha-alpha prime dipyridyl.

The authors feel that (1,10)-phenanthroline could probably be substituted for the use of 2,2′-bipyridyl without change in the procedure as far as the production of the indicator complex and its red color are concerned. This would mark a saving in reagent cost. It would involve an increase in the required potential to destroy the red complex at the equivalence point from 1.03 volts to 1.12 volts. Since the oxidation potential of sulfatoceric acid (the ceric ion in H_2SO_4 solution is 1.28 volts in molar hydrochloric acid) is not as high as in sulfuric acid solution a small indicator correction would be involved. The use of 5-methyl-1,10-phenanthroline would require no correction.

In case (1,10)-phenanthroline were to be used as indicator solution an improvement in producing the red complex would involve withdrawing a few milliliters of the ferrous chloride solution in N hydrochloric acid and neutralizing with N ammonium hydroxide to a pH of 3.5 (congo red paper as indicator). This would then be followed by the addition of 1-2 ml. of saturated aqueous solution of (1,10)-phenanthroline to produce the red indicator color. The solution thus prepared would then be combined with the major portion of the solution to be analyzed and the titration using ceric ion completed. For an accuracy of one part per thousand the indicator correction could probably be ignored. Better still, use the 5-methyl-1,10-phenanthroline.

SECTION 7

THE DETERMINATION OF IRON AND OF COPPER, USING 2,2′ BIPYRIDYL, 1,10-PHENANTHROLINE AND SUBSTITUTED 1,10-PHENANTHROLINES

Introduction. The determination of small concentrations of iron is of great importance in the wine industry, beer or distilled liquors, fruit juices and similar products. The determination of iron in biological materials is of importance and has been repeatedly studied. It likewise, together with traces of copper, is known to have an effect on the keeping qualities of dried milk products such as skim milk, whole milk, powdered ice cream mix, and powdered butter milk. Studies of the mineral constituents of plant material and certain foodstuff, such as corn starch, dried spinach, raisins, and prunes, involve the determination of iron. All of these problems involve the destruction of organic matter by either dry ashing or wet ashing methods. The author is of the opinion that as the excellency, rapidity, and accuracy of the wet ashing procedure becomes thoroughly familiar to control laboratories in general, the somewhat less desirable method of dry ashing at temperatures of 500 degrees centigrade and above will be practically eliminated. This fact, has, for example resulted in the development of the wet ashing procedure in the case of analyses of leather preparatory in the determination of iron and more important the determination of chromium. Former outmoded methods required the firing of the leather samples for analysis of the ash constituents in an electric muffle at temperatures above a red heat. This process was time consuming and required platinum ware. The new wet ashing procedure is complete in 30 minutes or less as compared with hours of heating by the old method.

Wet Ashing of Samples of Biological Materials, Foods and Other Commercial Products. It is not the scope of the present booklet to take up this general subject as a major method of procedure. The subject has been extensively treated in a former booklet, "Mixed Perchloric, Sulfuric and Phosphoric Acids and Their Application to Analysis" *(63)*. Section 2 of this booklet, pages 10 to 52, describes the wet ashing, using mixtures of HNO_3, $HClO_4$, H_2SO_4, and H_3PO_4 in pairs of two or combinations of three acids, some of which, those

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(63) Smith, "Mixed Perchloric, Sulfuric and Phosphoric Acids, etc.," G. Frederick Smith Chemical Company, 1935.

in particular containing phosphoric acids, are the most powerful solvents known to analytical chemistry. General considerations are discussed in detail and the Kahane procedures outlined as applied mainly to biological and physiological investigations. The method is described as applied to the determination of ash ingredients in plant materials such as sweet clover (roots and tops), alfalfa hay, red clover hay, alsike clover hay, timothy hay, redtop hay, wheat straw, corncobs, grain corn and soy beans; the determination of iron (and of chromium) in raw tanned and finished tanned leather. The destruction of organic matter in a score or more of organic chemical compounds for the determination of a wide variety of elements is described including arsenicals and medicinal iodine containing products. A general discussion of the destruction of organic matter in the case of a wide variety of cases where reagent organic chemicals are employed in analysis the excess of which must be removed. Cellulose and cellulose products, 8-hydroxy quinoline, cupferron, citric acid and tartaric acids are examples in point. The determination of silica in plant materials and the determination of various mineral components of biological materials such as liver, heart, and lung tissues are described. The destruction of organic matter containing lipides resist oxidation in the wet way but can be successfully manipulated. The methods, as sometimes popularly supposed, are not hazardous. They are as a unit efficient, rapid, and of superior accuracy. It can be said without fear of contradiction that dry ashing procedures are continuously being supplanted and wet ashing procedures mounting daily into analytical ascendency. As a single example of application of the procedure to analysis the following procedure has been described by S. H. Jackson *(64)* in a paper entitled "The Determination of Iron in Biological Materials." It is "a method that gives accurate results with whole rats, milk and milk products and a large number of both fresh and dried foods."

The dry ashing procedure was applied and compared with results by wet ashing. The method of Klumpp *(65)* or Farrar's method *(66)* used in connection with the determination of the iron by titration with the titanous ion was employed. The material was dried with a small amount of concentrated sulfuric acid (about 2 ml. per gram dry weight of sample) and ashed in a muffle at 500-550 degrees C. The ash was then taken up in hydrochloric acid, filtered and diluted to an appropriate volume. With phosphorus present interference was

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(64) Jackson, *Ind. Eng. Chem.*, Anal. Ed. 10, 302 (1938).
(65) Klumpp, *J. Biol. Chem.*, 107, 213 (1934).
(66) Farrar, *ibid.*, 110, 685 (1935).

met with the formation of pyrophosphates. To obviate this difficulty the acidified ash was warmed at 80 degrees C. for 18 hours by hydrolyzing the pyrophosphate to normal phosphate which does not interfere. The methods were applied to dried spinach and corn starch, pablum, farina, and milk powder, as well as the whole rat of which an aliquot of the entire treated ash was analyzed.

> "Four methods of ashing were tried: dry-ashing with addition of sodium carbonate, dry-ashing with addition of a small amount of sulfuric acid (Klumpp's method), dry-ashing after covering the sample with a layer of iron free calcium carbonate (Farrar's method), and wet-ashing using nitric, sulfuric and perchloric acids. Dry ashing with sodium carbonate was soon discarded because of poor checks obtained. Klumpp's and Farrar's methods gave fairly consistent checks and recoveries of added iron with an occasional erratic result. Wet ashing gave consistently good checks and iron recoveries. This solution consisted of 7.5 per cent glucose, 2.5 of urea, 100 mg. per cent of calcium phosphate, 100 mg. per cent of sodium chloride and 1.00 mg. per cent of iron added as a standard iron solution dissolved in hydrochloric acid. When 10 ml. aliquots of this solution were dried down and ashed, with Klumpp's method the best recovery was 54 per cent of the known iron content, while most of the recoveries were about 40 per cent. The recoveries from Farrar's calcium carbonate method were 80 to 87 per cent, much better than Klumpp's but still not good enough. When the glucose-urea was wet-ashed, the recoveries were 100 per cent, with an error of ± 1 per cent. Wet ashing was thus demonstrated to be the only acceptable method of those tested. The following method of analysis was finally adopted:
>
> "A convenient sized sample of material, containing at least 0.02 mg. of iron, was accurately measured and placed in a 300 ml. Kjeldahl flask. If the sample was a dry powder a few milliliters of water were added. Five milliliters of nitric acid (distilled to remove iron impurities) and 1 ml. of concentrate sulfuric acid was added and the flask was gently treated so that the solution was just boiling. Further 5 ml. additions of nitric acid were made as the solution began to clear, until the oxidation had practically ceased. One milliliter of perchloric acid was then added and the flask was heated slightly more vigorously until white fumes began to form; heating was stopped at this stage if the solution was colorless; otherwise it was continued until this point was reached. After cooling, the solution was diluted to about 25 ml. Usually the solution was clear, but if a precipitate of calcium sulfate was present, as with milk samples where there is a large proportion of calcium to iron, this was filtered off and the paper washed.
>
> "A drop of bromothymol blue was added to the acid ash solution and concentrated ammonium hydroxide added until the acid was neutralized. About 3 ml. excess ammonium hydroxide was then run in, a total of 8 ml. usually being required, and hydrogen sulfide was passed in until the solution became saturated. It was allowed to stand a few minutes and filtered by suction on a Jena glass filter, the filtrate being discarded. This type of filter would pick up a precipitate so finely dispersed as to appear only as a faint green coloration, if visible at all. Care was taken not to suck the precipitate dry as this might result in the loss of some iron due to oxidation to the soluble sulfate. Without washing, the precipitate was dissolved in 1.0 ml. of 1 to 1 hydrochloric acid. The Kjeldahl flask was rinsed into the filter once with 1 ml. of 1 to 1 hydrochloric acid and several

times with small amounts of water, and the whole sucked through the filter, which was washed several times with water. The combined filtrate and washings were boiled to remove excess hydrogen sulfide, and effect solution of any colloidal sulfur. After cooling, the solution was quantitatively transferred to an appropriate volumetric flask and the excess acidity neutralized with 25 per cent sodium hydroxide. The solution was left sufficiently acid to remain perfectly clear. It was then diluted to the mark."

The most recent applications of the wet-ashing procedure to the destruction of organic matter in which perchloric acid nitric acid and sulfuric acids are employed are those of Evans and St. John *(67)* "Determination of Total Sulfur in Feeds. Modified Nitric and Perchloric Acid Digestion Procedure" and that of J. B. Thompson *(68)* "Determination of Iron in Food Products."

The Colorimetric Determination of Ferric or Ferrous Iron by Use of Various Inorganic and Organic Color Producing Reagents. The determination of iron in small amounts has been repeatedly described using a long list of color producing reagents. Such a list is the following:

TABLE XIII

Color Producing Reagents for Use in the Determination of Ferrous or Ferric Iron

Reagents for Fe^{+++}	Reagents for Fe^{++}
KCNS	$K_3Fe(CN)_6$
$K_4Fe(CN)_6$	Dimethylglyoxime
7-indo-8 hydroxyquinoline-5-sulfonic acid	Pyramidone
Thioglycolic-acid (Mercaptoacetic Acid)	Alloxantin
Thiosalicylic acid (in acid solution)	
Salicylic acid	2-2′ bipyridyl
Salicylsulfonic acid	Terpyridyl
Acetylacetone	(1,10)-Phenanthroline
Pyrocatecol	Substituted (1,10)-Phenanthrolines
8 hydroxyquinoline	

For details concerning the applications of the reagents of Table XIII, other than those described in this booklet (the reagents with the functional group $=N\text{-}\underset{\|}{C}\text{-}\underset{\|}{C}\text{-}N=$), the text by Snell and Snell *(69)* should be consulted.

The methods involving the use of 2,2′-bipyridyl are numerous. The first application made of this reagent in the estimation of ferrous iron was that of Blau *(70)*. The same reagent was employed by Hill *(71)* in "A Method for the Extraction of Iron in Biological Materials." The separation of iron and copper using 2,2′-bipyridyl was carried out by Parker and Griffin *(72)*. The determination of iron in beer by the direct application of 2,2′-bipyridyl with no preliminary treatment of the sample is possible since iron in beer occurs only in the ferrous condition as shown by Gray and Stone *(73)*. This procedure has not been approved by the Official Association of Agricultural Chemists through their official referee covering the method. This method is similar to that of Bode *(74)*.

The "Photometric Determination of Copper and Iron in Distilled Liquors" was described by Gerber, Claasen and Boruff *(75)*. For this purpose the copper was estimated using the reagent diethyldithiocarbonate and the iron was determined using 2,2′-bipyridyl.

Other applications in the use of 2,2′-bipyridyl are those of the determination of vitamin E (tocopherol) by Emmerie and Engel *(37)* and perfected by Mayer and Sobotka *(38)* and that of Koenig, Schiefelbusch and Johnson *(76)* entitled "Chromagenic Reagent for Vitamin C Determination." The author is of the opinion that for most, if not all, the previously listed procedures in which the reagent 2,2′-bipyridyl has been employed could be improved through the use of 1,10-phenanthroline. This implication is substantiated from many sources in the published literature on procedures for the colorimetric determination of iron. Most of the published record in which 2,2′-bipyridyl has been employed grow out of the fact that this reagent was commercially available prior to the supply of 1,10-phenanthroline. Both reagents are now readily available and only the greater applicability of one or the other of these reagents is now the controlling factor either from the point of view of cost or active source of supply.

Report of Heavy Metals in Beer. The following report *(77)* is that of W. H. Harrison on the referee work of the A.O.A.C. on the subject of the determination of iron in beer. It is reprinted in part here for the reason that it furthers the adoption of a wet-ashing procedure employing perchloric acid and in addition that some of the collaborat-

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(67) Evans and St. John, *Ind. Eng. Chem.*, Anal. Ed., **16**, 630 (1944).

(68) Thompson, *ibid.*, **16**, 646 (1944).

(69) Snell and Snell, "Colorimetric Methods of Analysis," D. Van Nostrand (1936), Chapter XXVI.

(70) Blau, *Monatsh.*, **19**, 647 (1898).

(71) Hill, *Proc. Roy. Soc. London (B)*, **107**, 208 (1930).

(72) Parker and Griffin, *Can. J. Research*, **17**, 66 (1939).

(73) Gray and Stone, *Ind. Eng. Chem.*, Anal. Ed., **10**, 415 (1938).

(74) Bode, *Wochschr. Brau.*, **50**, 321 (1921).

(75) Gerber, Claasen and Boruff, *Ind. Eng. Chem.*, Anal. Ed., **14**, 364 (1942).

(37) Emmerie and Engel, *Rec. trav. Chem. Pays-Bos*, **57**, 1351 (1938); **58**, 283, 895 (1939).

(38) Mayer and Sobotka, *J. Biol. Chem.*, 143, 695 (1942).

(76) Koenig, Schiefelbusch and Johnson, *Ind. Eng. Chem.*, Anal. Ed., **15**, 181 (1943).

(77) Harrison, *J. Assoc. Official Agri. Chem.*, **24**, 215 (1941).

ing referees employed 2,2′-bipyridyl as color reagent. This reference will complete the review of the use of this color-producing reagent as applied to the photometric determination of iron.

"In choosing a method most suitable for the determination of iron in beer the Associate Referee kept in mind two main factors, simplicity and accuracy. The best method for the destruction of organic matter was found to be the wet-ashing procedure specifying perchloric and nitric acids. The hazard connected with the use of perchloric acid is eliminated by the predigestion of the sample by the nitric acid in the mixture and by the small quantity of perchloric acid used. This method of ashing, which was found to be very rapid, seems to eliminate entirely the error caused by the formation of iron pyrophosphate during dry ashing procedures. It is very important, however, that the acids used in the digestion mixture be practically iron free. Redistilled nitric acid and double-distilled perchloric acid are recommended. Results that were obtained on dry-ashed were satisfactory but not so constant as those similar samples wet ashed.

"The method selected for the determination of iron was that of Windsor *(78)* in which the iron thiocyanate color is developed in an acid medium of approximately 75 per cent methoxyethanol (methyl cellosolve). The color is quite stable in this medium and is approximately 85 per cent more intense than in a water medium.

Reagents

"(a) Nitric acid. Redistil through pyrex and store in pyrex bottles.

"(b) Perchloric acid. Double vacuum distilled 72 per cent $HClO_4$. Store in pyrex bottles.

"(c) Methyl cellosolve. Dissolve 80 grams of NH_4CNS in methyl cellosolve and dilute to 2 liters with the same solvent. A faint pink color may be noticeable after the reagent has been prepared but this will fade upon standing.

"(d) Standard iron solution. Contains the equivalent of 0.01 mg. of ferric iron per ml. Add small quantity of bromine water.

Preparation of Standards

"Make up standards by using water, concentrated HCl, and the methyl cellosolve in the following proportions:

Standard iron solution	X ml.
Water	5—X ml.
Concentrated HCl	1.25 ml.
Methyl cellosolve	18.75 ml.

"Pipet 25 ml. of thoroughly degassed beer into a 100 ml. extraction flask (pyrex No. 5160) and boil down to a thick syrup, slightly charred. (It is necessary to use a flask with a rather small base so that 2 ml. of $HClO_4$, which remains at the end of the digestion, will cover the entire bottom surface of the flask.) Add 20-25 ml. of HNO_3 and 2 ml. of $HClO_4$ (72 per cent). Cover the flask with a watch-glass and heat gently until the initial reaction begins, at which stage a fairly vigorous boiling with the evolution of brown oxides of nitrogen takes place. After the reaction has subsided again heat the contents of the flask to a slow boiling and continue the boiling until all the HNO_3 is driven off. (With certain beers, or when too rapid boiling is used, the procedure may not complete the oxidation. In this case the solution rapidly turns brown, or even black, depending upon how much material remains unoxidized. A few additional ml. of HNO_3 will produce a clear but not colorless solution (brown). Continue the heating to expel all the HNO_3 as evidenced by the evolution of copious fumes of $HClO_4$. (On cooling the residue should be colorless or at most a pale yellow.) If this is not the case, further heating with small additions of HNO_3 and $HClO_4$ should be used.

"After slight cooling, add 5 ml. of distilled water and warm the mixture until the precipitate salts are in solution. Allow the solution to cool to room temperature and add 18.75 ml. of the ammonium thiocyanate-methyl cellosolve reagent. Immediately after the color is developed, place the flasks and standards in a pan of circulating cold water, since heat causes a slow fading of the red color. Compare the color produced in the sample with that of a standard of almost similar color intensity by means of a colorimeter or photometer.

"In testing the accuracy of the method, analyses were made of samples of beer to which known quantities of iron as ferric chloride had been added. Table XIV shows the results:

TABLE XIV

Recoveries of Added Iron

(Weight of Beer 25 grams)

No.	Iron Added	Iron Found	Iron Recovered	
	mg.	*mg.*	*mg.*	*pct.*
1	……..	0.017	……..	……
2	……..	0.017	……..	……
3	0.005	0.022	0.005	100
4	0.005	0.021	0.004	80
5	0.010	0.028	0.011	110
6	0.010	0.026	0.009	90
7	0.015	0.034	0.017	113
8	0.015	0.035	0.018	120
9	0.020	0.036	0.019	95
10	0.020	0.035	0.018	90
11	0.025	0.043	0.026	104
12	0.025	0.042	0.025	100
Av.				100

"A comparison was made between the wet-ashing procedure described previously and the same method including a dry-ashing procedure. With the wet-ashing procedure, 50 ml. of beer was evaporated to dryness in a 200 ml. casserole and ignited to a carbon free-ash in a muffle at 1000 deg. F., oxygen being used as an accelerator. The ash was then dissolved in a mixture of approximately 2 ml. of concentrated hydrochloric acid and distilled water, warmed to facilitate solution, and filtered. The filtrate was evaporated to dryness, after which 1.25 ml. of concentrated hydrochloric acid was added and the color developed as described above. Results obtained by the two procedures on the same lot of beer are shown in Table XV.

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(78) Windsor, *Ind. Eng. Chem.*, Anal. Ed., 9, 453 (1937).

TABLE XV

Ashing Procedure Results (P.P.M. Iron Found)

No.	Dry-ashing Procedure	Wet-ashing Procedure
1.	0.70	0.68
2.	0.67	0.66
3.	0.65	0.64
4.	0.62	0.66
5.	0.62	0.68
6.	0.59	0.64
Av.	0.64	0.66

"In order to test the method further, two lots of beer that were known to differ widely in iron content were obtained. The individual bottles from each lot were opened, and the beer was poured into a large carboy, where it was mixed, rebottled (aluminum spot crowns used), and repasteurized. Samples from the two lots, together with sufficient quantities of double distilled perchloric acid and methyl cellosolve, were sent to collaborators, who were requested to analyze the samples by the wet-ashing procedure outlined here and by any other methods they may desire, for the purpose of comparison.

"(The referees were 10 in number and represented besides the A. O. A. C. referee the best known beer manufacturers and their associated canning industries. The 'Discussion' of the over all results are by the official referee and 'Recommendations' are quoted in full.)

Discussion

"The Association Referee believes that the results of the collaborative work are not, as a whole, a true test of the method. Some of the results are in excellent agreement, while others vary widely from the average values. It will be observed (see the original publication for tabular results) that the results obtained from the collaborators who used some kind of instrument for the final measurement of the color are much more favorable than those obtained by comparisons in Nessler tubes. Those who are interested in doing further work with this method may find it helpful to know the maximum color absorption for photometric work lies between 475 and 490 mμ. It is quite obvious that for most accurate work, the flocculent silica precipitate in the solution should be filtered off. The fading of the final iron thiocyanate color seems to be one of the most serious defects to the method. The method of ashing seems to be quite satisfactory.

"The results that have been reported on these samples by other methods emphasize the importance of a good method for the determination of iron in beer. Not only do these results differ widely from the results obtained by the wet-ash-methyl cellosolve method, but they vary widely among themselves.

Recommendations

"It is recommended:

"(1) That further studies be made of this method and of other methods that may be applicable to the determination of iron in beer and that further collaborative work be done.

"(2) That work be started on the determination of copper in beer."

The use of 2,2′-bipyridyl as a reagent in microscopy is described by Polucktov and Nazarenko *(79)* in a paper entitled "The Use of Ferrous Bipyridyl Complexes in Chemical Microscopy." Ions identified by the formation of characteristic crystals were I^-, CNS^-, MnO_4^-, ReO_4^-, VO_3^-, $Fe(CN)_6^{+--}$, $Fe(CN)_6^{----}$, as well as nitroprusside, chloroplatinate and fluotantalate ions.

A thesis by P. S. Markunas *(80)* covers this subject in a more comprehensive manner under the title "Complex Cations in Microanalysis: The Application of 2,2′-Bipyridyl, 1,10-Phenanthroline and Nitro-(1,10)-Phenanthroline Complexes in the Microscopical Detection of Ions."

Fritz Feigl and L. I. Miranda, in a paper entitled *(81)* "A Selective Spot Reaction for Cadmium," show that cadmium may be quantitatively separated from other metals by precipitation from an ammoniacal solution by means of ferrous bipyridyl and iodide. This is a separation from other metals, particularly copper and zinc; (1,10)-phenanthroline serves the same purpose. This paper contains numerous references to further applications of these two complex forming precipitating reagents.

The Determination of Iron Using 1,10-Phenanthroline. The previous portions of this booklet section have dealt with the numerous applications of 2,2′-bipyridyl in analysis, particularly to the determination of iron. There can be no doubt that the companion reagent 1,10-phenanthroline is its superior in many ways as a color producing reagent for use in the determination of iron. For a better understanding of the possibilities the following treatment of the subject is reprinted in practically its original form following the excellent discussion of W. B. Fortune and M. G. Mellon *(82)*:

Determination of Iron with 1,10-Phenanthroline. A Spectrophotometric Study. It was shown by Walden, Hammett, and Chapman in 1931 that the complex ion formed with ferrous iron and (1,10)-phenanthroline has a high oxidation potential and may be used as an internal indicator in certain oxidimetric analytical procedures *(83)*. Previously Blau had given an extensive description of the properties of (1,10)-phenanthroline, along with the method of preparation *(7)*. Saywell and Cunningham reported recently that one can make a quantitative determination of iron in small concentrations in various fruit juices and other products by comparing the color of the (1,10)-phenanthroline complex with that of a series

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(79) Polucktov and Nazarenko, *J. App. Chem., U. S. S. R.*, **10**, 2105 (1937).

(80) Markunas, Diss. University of Illinois (1940).

(81) Feigl and Miranda, *Ind. Eng. Chem.*, Anal. Ed., **16**, 141 (1944).

(82) Fortune and Mellon, *ibid.*, **10**, 60 (1938).

(83, 3) Walden, Hammett and Chapman, *J. Am. Chem. Soc.*, **53**, 3908 (1931).

(7) Blau, *Monatsh.*, **19**, 647 (1898).

of standards *(84)*. Hummel and Willard *(85)* have applied the method to biological materials.

The purpose of the present paper is to present a critical study of various factors which may affect the formation of the colored complex, including a study of the effect of varying concentrations of fifty-five ions liable to be encountered in routine analysis.

Apparatus and Methods

In the determination of iron in fruit products, as carried out by Saywell and Cunningham *(84)*, the color comparisons were made in graduated test tubes and in a colorimeter. The development of the photoelectric spectrophotometer, described by Michaelson and Liebhafsky *(86)*, has provided a means of detecting very small color changes with a much higher degree of precision and accuracy than is possible with visual methods. A General Electric recording instrument was used in all transmittancy measurements in this work. The cells were 1.00 cm. thick, the "blank" in the reference beam of light being filled with a solution containing the same amount of hydroxylamine hydrochloride and (1,10)-phenanthroline as was used with the iron in the other cell.

All pH measurements were made with a "universal" potentiometer and glass electrode, as described by Mellon *(87)*.

The standard solution of iron was prepared by dissolving electrolytic iron wire in dilute hydrochloric, nitric, or perchloric acid. The solutions were then diluted to volumes such that 1.00 ml. contained 0.100 mg. of iron. A 0.10 per cent solution of (1,10)-phenanthroline was prepared by dissolving the monohydrate in doubly distilled, iron-free water. Saywell and Cunningham used an ethanol solution but the authors found that the reagent dissolved readily in water heated to about 80° C. It is important that the (1,10)-phenanthroline monohydrate be free from impurities. Certain contamination, at least, is evidenced by a pink coloration of the crystalline material, and a lowering of the melting point, stated by Smith (page 10) to be 99° to 100° C. A 10 per cent solution of hydroxylamine hydrochloride, used as a reducing agent for the iron, was prepared by dissolving the C. P. reagent in doubly distilled water. Solutions used in the determination of interfering cations were prepared from the chloride or nitrate salts of the metals; the anion solutions were prepared from the sodium or potassium salts.

In making up all colorimetric solutions used in this study, the following procedure was adopted: The required amount of the standard iron solution was measured out; 1.0 ml. of the hydroxylamine hydrochloric was added to reduce the ferric iron; the solution was diluted to approximately 75 ml.; an excess of (1,10)-phenanthroline solution was added, 5.0 ml. being used with iron concentrations up to 4.0 p.p.m. and 10.0 ml. with higher concentrations; and the resulting solution was diluted to 100 ml. The pH value was then determined and any desired adjustment in acidity was made. The volumes of acid or base required for this adjustment never amounted to more than 0.1 ml. of 6 *N* hydrochloric acid or 6 *N* ammonium hydroxide, thus keeping possible error from dilution sources below 0.1 per cent. Solutions of possible interfering ions were added before the (1,10)-phenanthroline color was developed.

Calculations of apparent error due to interference by ions were made from transmittancy measurements by means of Beer's law, $T_1 = T_2^{\frac{C_1}{C_2}}$, where C_2 is the concentration of iron in the standard solution and C_1 is the calculated concentration after the possible interfering ion has been added. These calculations were made by means of a special color slide rule. The error was calculated thus: $\frac{C_2 - C_1}{C_2} \times 100 =$ per cent "apparent error." An apparent error of 2 per cent was arbitrarily set as the limit of negligible interference in the case of the added ions. Since visual methods of color comparison often have a precision not less than 5 per cent, it was necessary to set a lower figure in order to provide for other possible factors.

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(84) Saywell and Cunningham, *Ind. Eng. Chem.*, Anal. Ed., 9, 67 (1937).

(85) Hummell and Willard, *ibid.*, 10, 13 (1938).

(86) Michaelson and Liebhafsky, *Gen. Elec. Rev.*, 39, 445 (1936). See Figure 6, page 46.

(87) Mellon, "Methods of Quantitative Analysis," page 413, New York, MacMillan Co., 1937.

The Color Reaction

The colored complex ion formed between the (1,10)-phenanthroline and the ferrous ion has been postulated by Blau *(7)* to be composed of three molecules of (1,10)-phenanthroline and one ferrous ion. The intensity of the color produced is determined by the amount of iron when there is an excess of the (1,10)-phenanthroline reagent present. It is this direct relationship between the iron and the intensity of the color which permits the use of the method.

The transmittancy curves for varying amounts of iron are shown in Figure 1. The peak of the absorption band is located at 508 mμ, with a secondary band shown at 474 mμ. Six parts per million of iron was found to be the maximum concentration which could be used with a 1.00-cm. cell. A concentration of 0.10 p.p.m. gave a minimum transmittancy of 95.5 per cent at this thickness. It would be possible to use a less or more highly concentrated solution in a color comparator where very thick or very thin layers of solution might be used for comparative purposes.

A study of the minimum amount of an 0.10 per cent solution of (1,10)-phenanthroline required to produce the maximum color revealed that 6.0 ml. of the reagent were required for every 5 p.p.m. of iron present. Any amount less than this did not produce complete development of the color. This study was made by keeping the amount of iron constant and varying the total amount of (1,10)-phenanthroline added.

Transmittancey curves for solutions wherein the color was fully developed and the pH varied from 2.0 to 9.0 were exactly superimposed upon one another, showing that over this pH range the color was not dependent on the pH of the solution.

The conformity of the colored solution to Beer's law was tested over the range from 0.10 to 6.00 p.p.m. of iron, the limits found applicable for a 1.00-cm. cell, by plotting 2 + log *T* against concentration. A straight line indicated very close conformity. Further tests were carried out by determining transmittancy curves on solutions in 1.00-cm. cells, diluting the solutions to exactly twice the original volume and again determining the transmittancy in 2.00-cm. cells. The two curves were superimposed on each other.

Accelerated, as well as ordinary, fading tests agreed well with Saywell and Cunningham's conclusions *(84)* that the color is stable for at least 15 days. On one test, the solutions were sealed in test tubes and exposed to varying degrees of light over a period of 11 days. The transmittancy curves on the original solutions and the same solutions after exposure were identical. In making the accelerated fading tests, the solutions were placed in glass-stoppered Pyrex bottles of about 60-ml. capacity after the transmittancy curves were determined. These bottles were then placed in an air thermostat at 30° C. at a distance of 60 cm. from a mercury arc lamp for a period of 100 hours. Redetermination of the trans-

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(7) Blau, Monatsch., 19, 666 (1898).

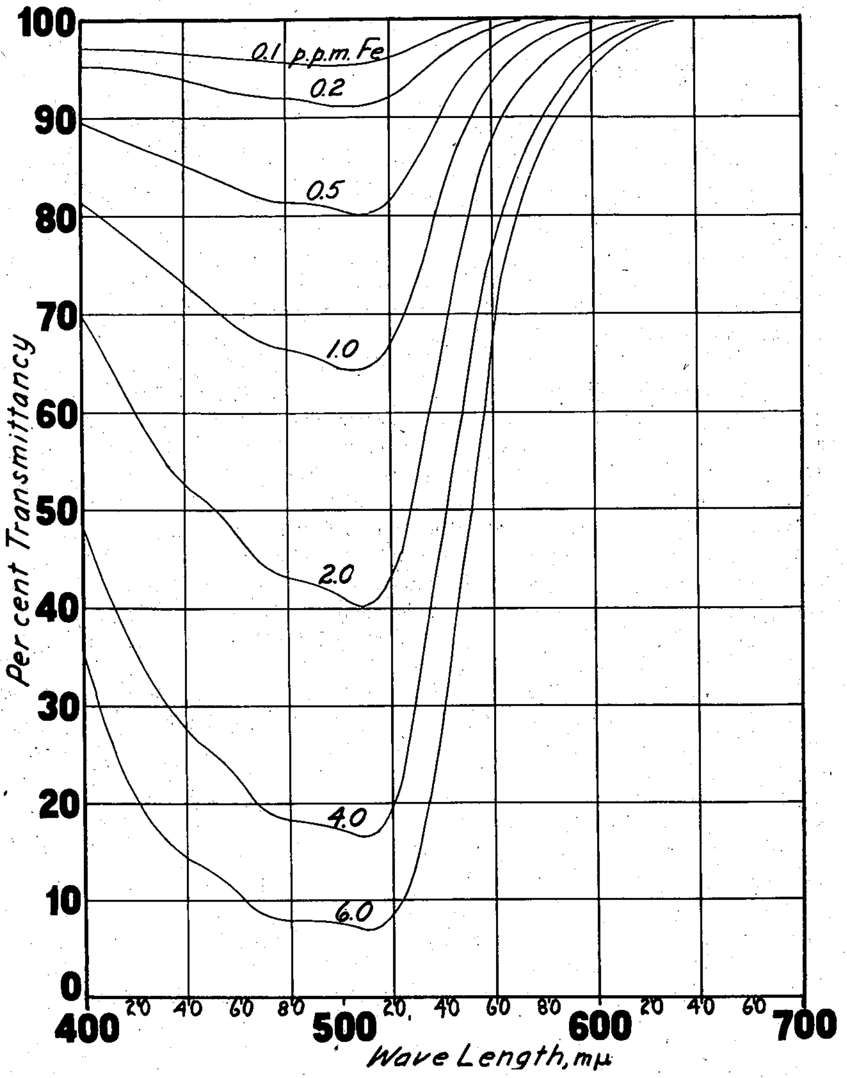

FIG. 12. Effect of Iron Concentration
pH Range 3.0-5.0

mittancy curves indicated no fading. These solutions were then set aside for 150 days, when the transmittancy curves again agreed well within experimental error.

Reducing Agents

Following the original work of Saywell and Cunningham, the authors used a 10 per cent solution of hydroxylamine hydrochloride as the reducing agent in all work on interfering ions and in concentration tests. Because of the relatively high cost of the reductant, it was decided to try certain other agents. A 10 per cent aqueous solution of sodium sulfite was tried with very unsatisfactory results. In slightly acid solution a brown colored complex was formed. The presence of the brown coloration proved to be a serious interference when the (1,10)-phenanthroline color was developed, causing errors varying from 4.5 to 37.5 per cent.

Sodium and potassium formates (analytical reagent) were found to be free from iron in appreciable quantities. However, determinations using these materials as reducing agents showed appreciable error. According to Mellor *(87a)*, this is probably due to the formation of a complex between the ferric ion and the formic acid formed in the solution.

Formaldehyde was used, but again a complex was formed between the iron and the reagent, causing appreciable error.

The hydroxylamine hydrochloride solution was found to be the most satisfactory for the reduction of the iron. The relatively small amounts used and the speed with which the iron was reduced are sufficient to warrant the use of this reagent.

Effect of Ions on Color Developed

Studies on the possible interference of ions were made in all cases on a solution containing 2.00 p.p.m. of iron. The iron was reduced with 1.0 ml. of a 10 per cent solution of hydroxylamine hydrochloride, the ion in question was introduced, the (1,10)-phenanthroline was added, and the solution was diluted to the mark. After mixing, the pH was adjusted with ammonium hydroxide or hydrochloric acid to a point within the range of applicable pH values shown in Tables XVI and XVII, and the transmittancy curves were determined. These curves were then compared with the standard for the same amount of iron without the added ions, and the per cent "apparent error" was determined as stated above.

It was decided that, with 2.00 p.p.m. of iron present, 500 p.p.m. of ion (250 times the concentration of iron in the solution) should be sufficient to test for interference. If this amount does not interfere, it is very improbable that the ion will interfere in any quantity.

EFFECT OF CATIONS. Of the large number of transmittancy curves obtained for cations, only certain representative ones are shown in Figure 13. They illustrate typical types of interference.

Table XVI gives the maximum concentration of ions and the applicable pH ranges which may be used without error in the determination of 2.00 p.p.m. of iron.

Potassium and sodium showed no interference when present in quantities as high as 1000 p.p.m. The following cations may be present in concentrations as high as 500 p.p.m. over the applicable pH range without interference: ammonium, arsenic (as arsenate or arsenite), barium, calcium, lead, lithium, magnesium, manganese (as manganous ion), and strontium.

(87a) Mellor, "Comprehensive Treatise on Inorganic and Theoretical Chemistry," Vol. XIV, p. 88, London, Longmans Green and Co., 1935.

Chromic ion changed the hue of the solution by absorbing somewhat in the red and violet. There was no apparent error in the minimum of the transmittancy curve with concentrations up to 50 p.p.m. However, at this concentration, the hue was quite different. At 20 p.p.m., there was very little change in hue and the solutions could be compared visually.

TABLE XVI

Effect of Cations

Ion	Added as	Maximum Concentration, p.p.m.	Maximum Interference, % Fe	Applicable pH Range
Aluminum	$AlCl_3$	500.0	None	2.0-3.0
		250.0	1.4	2.0-5.0
Ammonium	NH_4Cl	500.0	None	2.0-9.0
Antimony	$SbCl_3$	30.0	None	3.0-9.0
Arsenic	As_2O_5	500.0	None	3.0-9.0
Arsenic	As_2O_3	500.0	None	3.0-9.0
Barium	$BaCl_2$	500.0	None	3.0-9.0
Beryllium	$Be(NO_3)_2$	500.0	1.3	3.0-5.5
Bismuth	$Bi(NO_3)_3$	None	....	....
Cadmium	$Cd(NO_3)_2$	50.0	1.0[a]	3.0-9.0
Calcium	$Ca(NO_3)_2$	500.0	None	2.0-9.0
Chromium	$Cr_2(SO_4)_3$	20.0	None	2.0-9.0
Cobalt	$Co(NO_3)_2$	10.0	1.5	3.0-5.0
Copper	$Cu(NO_3)_2$	10.0	None	2.5-4.0
Lead	$Pb(C_2H_3O_2)_2$	500.0	None	2.0-9.0
Lithium	$LiCl$	500.0	None	2.0-9.0
Magnesium	$Mg(NO_3)_2$	500.0	None	2.0-9.0
Manganese	$MnSO_4$	500.0	None	2.0-9.0
Mercury	$HgCl_2$	1.0	None	2.0-9.0
Mercury	$Hg_2(NO_3)_2$	10.0	None	3.2-9.0
Molybdenum	$(NH_4)_6Mo_7O_{24}$	100.0	None	5.5-9.0
Nickel	$Ni(NO_3)_2$	2.0	None	2.5-9.0
Potassium	KCl	1000.0	None	2.0-9.0
Silver	$AgNO_3$	None	....	....
Sodium	$NaCl$	1000.0	None	2.0-9.0
Strontium	$Sr(NO_3)_2$	500.0	None	2.0-9.0
Thorium	$Th(NO_3)_4$	250.0	1.5	2.0-9.0
Tin	H_2SnCl_6	20.0	None	3.0-6.0
		50.0	None	2.5
Tin	H_2SnCl_4	10.0	None	2.0-6.0
		20.0	None	2.0-3.0
Tungsten	Na_2WO_4	10.0	Negligible	2.5-9.0
Uranium	$UO_2(C_2H_3O_2)_2$	100.0	None	2.0-6.0
Zinc	$Zn(NO_3)_2$	10.0	None	2.0-9.0
Zirconium	$Zr(NO_3)_4$	50.0	1.8	2.0-9.0
		100.0	2.2	3.0-9.0

[a] 15.0 ml. of (1,10)-phenanthroline in excess of the original amount added. Iron reduced with hydroxylamine hydrochloride in all tests.

Bismuth and silver must be completely absent from the solution because of the formation of precipitates. In each case, the ion causing the precipitate came from the (1,10)-phenanthroline solution. At concentrations up to 30 p.p.m. of antimonous ion, no interference was noted. Concentrations above this precipitated insoluble basic salts. Beryllium showed no interference in concentrations

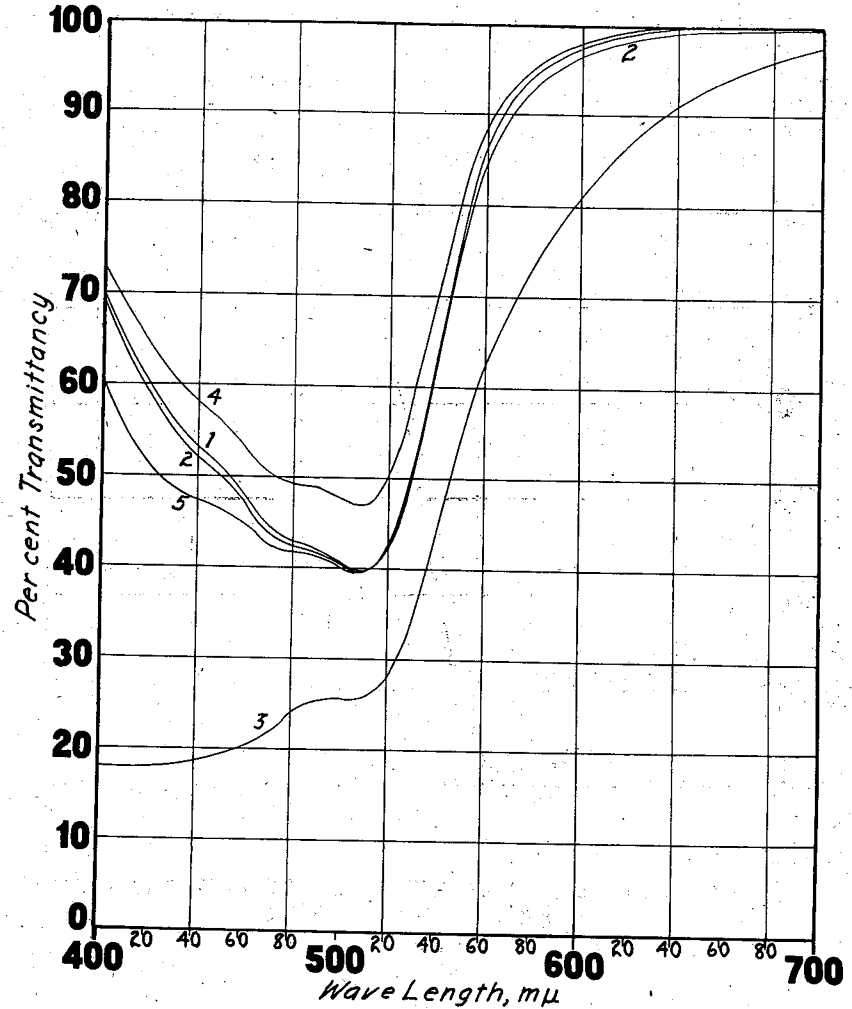

Fig. 13

1.0 ml. Hydroxylamine Hydrochloride
5.0 ml. (1,10)-Phenanthroline
2.0 p.p.m. Fe

1—Fe alone, pH 4.57
2—Fe plus 20 p.p.m. Cr^{+++}, pH 4.64
3—Fe plus 10 p.p.m. Cu^{++}, pH 5.86
4—Fe plus 20 p.p.m. Zn^{++}, pH 4.00
5—Fe plus 200 p.p.m. UO_2^{++}, pH 3.57

up to 50 p.p.m. when the pH was kept between 3.0 and 5.5. Above pH 5.5 the hydroxide formed, and below 3.0 a complex was formed with the (1,10)-phenanthroline. Cadmium, mercuric, and zinc ions also formed precipitates with the (1,10)-phenanthroline. With small amounts of these ions it was possible to prevent appreciable interference by adding excess (1,10)-phenanthroline. When 15 ml. excess of reagent were added to 50 p.p.m. of cadmium ion, the interference dropped to 1.0 per cent. Ten parts per million of zinc ion appeared to be the limiting concentration without appreciable error. One part per million was set as the maximum concentration of mercuric ion which could be present.

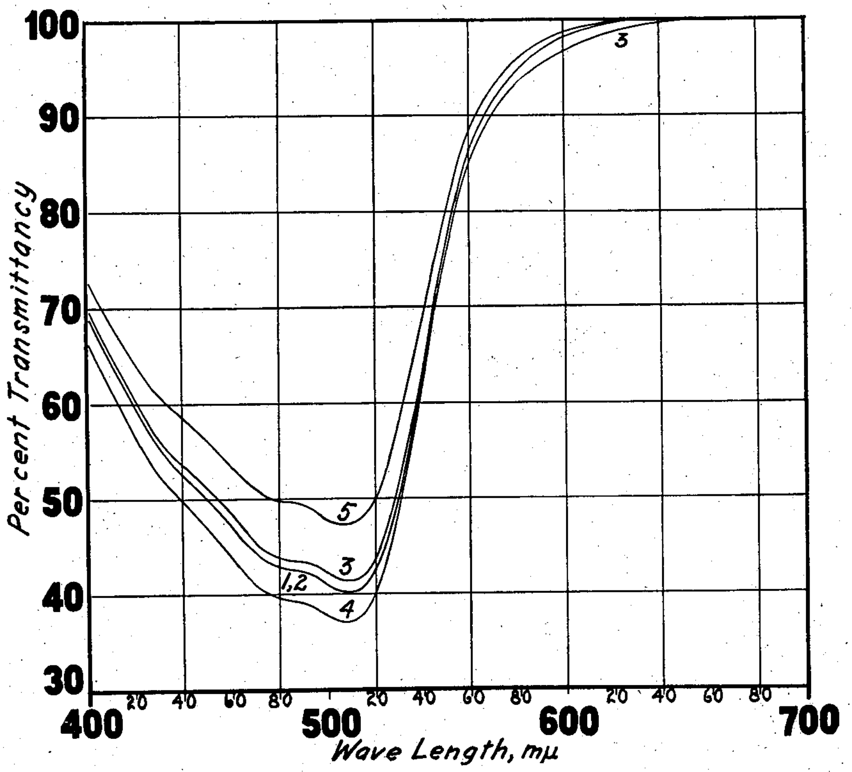

FIG. 14

1.0 ml. Hydroxylamine Hydrochloride
5.0 ml. (1,10)-Phenanthroline
2.0 p.p.m. Fe
1—Fe alone, pH 4.57
2—Fe plus 500 p.p.m. CNS^-, pH 3.45
3—Fe plus 20 p.p.m. $Cr_2O_7^{-2}$, pH 2.38
4—Fe plus 50 p.p.m. P_2O_5, pH 5.60
5—Fe plus 100 p.p.m. $P_2O_7^{-4}$, pH 4.35

pH was a very important factor in the interference of molybdenum, present as molybdate ion. At a pH of 4.0, a milky solution resulted with as little as 10 p.p.m. of molybdenum. Thirty parts per million caused serious error at a pH of 4.5, but did not interfere at a pH of 5.0. At pH values above 5.5, 100 p.p.m. of molybdenum could be present without appreciable interference.

Nickel ion produced a change in the hue of the solution. Apparently a complex was formed with the ferrous ion, the nickel ion, and the (1,10)-phenanthroline solution. At all wave lengths below 540 mμ the transmittancy was higher than normal, in proportion to the amount of nickel present, reaching a maximum difference from the normal at about 480 mμ. Two parts per million of nickel was found to be the maximum concentration which could be present without interference.

Tungsten, present as tungstate ion, formed a complex with the ferrous ion present and caused a fading effect, evidenced by an increase in transmittancy. Ten parts per million of tungsten caused an apparent change in hue of the solution, evidenced by a decrease in the transmittancy in the longer wave lengths. At a concentration of 5 p.p.m. no interference was noted.

Certain other ions were rather limited in the pH range over which they might be present without interference. These conditions are indicated in Table XVI.

EFFECT OF ANIONS. Representative curves illustrating typical types of anion interference are shown in Figure 14.

Table XVI gives a summary of the limiting concentrations and the applicable pH ranges of the common anions encountered in routine analysis.

No interference was shown in concentrations as high as 500 p.p.m. for the following anions over the applicable pH range: acetate, bromide, chlorate, chloride, citrate, iodide, nitrate, sulfate, sulfite, and thiocyanate.

pH was a very important factor in the study of the anion interferences. Oxalate and tartrate showed no interference in concentrations as high as 500 p.p.m., provided that the pH was kept above 6.0 for the oxalate and above 3.0 for the tartrate.

Cyanide ion interfered most seriously of the anions studied. Apparently it formed a complex with the ferrous ion, from which the (1,10)-phenanthroline was not strong enough to remove all of the iron. Transmittancy curves on solutions containing this ion always showed a fading effect. Ten parts per million of cyanide ion could be present with 2 p.p.m. of iron with a maximum interference over the applicable pH range of just 2 per cent.

Twenty parts per million of dichromate ion did not interfere with the quantitative color. However, amounts higher than this gave a change in hue which would prevent comparing these solutions with standards.

Nitrite ion did not interfere when the pH of the solution was kept above 2.5. At lower values nitrous acid was apparently formed and a brown coloration resulted.

Pyrophosphate ion in concentrations of 50 p.p.m. interfered to the extent of 1.0 per cent when the pH was kept at 6.0 or above. Even as little as 20 p.p.m. interfered when the pH dropped to 5.5.

Thiosulfate ion was rather unique in that any interference it showed was due to the precipitation of free sulfur. The amount of sulfur precipitated was directly dependent upon the time that the solution was allowed to stand before measuring the transmittancy. Five hundred parts per million of thiosulfate ion showed no interference, provided the transmittancy curves were determined within 5 to 10 minutes after the solution was prepared and the color developed.

The degree of interference of several of the other ions was directly influenced by the pH of the solution, as may be seen from Table XVII. In nearly every case, however, it is feasible to adjust the pH to a region where the interference is at a minimum.

TABLE XVII

Effect of Anions

Ion	Added as	Maximum Concentration, p.p.m.	Maximum Interference, % Fe	Applicable pH Range
Acetate	$NaC_2H_3O_2$	500.0	None	2.0-9.0
Tetraborate (as B_2O_3)	$Na_2B_4O_7$	500.0	None	3.0-9.0
Bromide	NaBr	500.0	None	2.0-9.0
Carbonate	Na_2CO_3	500.0	None	3.0-9.0
Chlorate	$KClO_3$	500.0	None	2.5-9.0
Chloride	NaCl	1000.0	None	2.0-9.0
Citrate	$C_6H_8O_7$	500.0	None	2.0-9.0
Cyanide	KCN	10.0	2.0	2.0-9.0
Dichromate	$K_2C_2O_7$	100.0	Change hue	....
		20.0	None	2.5-9.0
Fluoride	NaF	500.0	1.6	4.0-9.0
Iodide	KI	500.0	None	2.0-9.0
Nitrate	KNO_3	500.0	None	2.0-9.0
Nitrite	KNO_2	500.0	None	2.5-9.0
Oxalate	$(NH_4)_2C_2O_4$	500.0	None	6.0-9.0
Perchlorate	$KClO_4$	100.0	1.2	2.0-9.0
Phosphate (as P_2O_5)	$(NH_4)_2HPO_4$	20.0	1.4	2.0-9.0
Pyrophosphate	$Na_4P_2O_7$	50.0	1.0	6.0-9.0
		20.0	1.0	5.5-9.0
Silicate	Na_4SiO_3	100.0	None	2.0-4.5
		50.0	None	2.0-5.0
Sulfate	$(NH_4)_2SO_4$	500.0	None	2.0-9.0
Sulfite	Na_2SO_3	500.0	None	2.0-9.0
Tartrate	$(NH_4)_2C_4H_4O_6$	500.0	None	3.0-9.0
Thiocyanate	KCNS	500.0	None	2.0-9.0
Thiosulfate	$Na_2S_2O_3$	500.0	None[a]	3.0-9.0

[a] Transmittancy determined within 10 minutes after color developed. Iron reduced with hydroxylamine hydrochloride in all tests.

Discussion

The interference of some of the ions studied was probably due to the formation of iron complexes from which the (1,10)-phenanthroline was unable to remove all of the iron. Certain other interferences were due to the formation of an insoluble compound of the iron with the (1,10)-phenanthroline. In a few cases the interference was due to the color of the added ion, changing the hue of the solution itself.

While the limiting concentration of the iron was set as 6.0 p.p.m. in 1.00-cm. cell, through a process of dilution one could determine much larger quantities in an unknown sample.

One of the serious disadvantages often found in the colorimetric determination of iron—namely, color formation in an alkaline solution with a consequent precipitation of many of the metal hydroxides or hydrated oxides—was not present in this method, wherein pH had very little effect on the color itself and the solution could remain acidic throughout the determination. Any common indicator paper, such as nitrazine paper, could be used as a rough check on the pH of the solution before color comparison.

The great advantages of the method are its sensitivity and its freedom from interference by most of the common ions. The very low amounts of iron which can be quantitatively determined by this method permit its use not only for routine food and biological analyses, but also for determination of purity of inorganic reagents.

Summary

A spectrophotometric study indicates that Saywell and Cunningham's method of using (1,10)-phenanthroline for the determination of iron is very satisfactory for small amounts. It is more sensitive than the more common methods, the range being from 0.10 to 6.0 p.p.m. in a 1.00-cm. comparison cell.

Hydroxylamine is the best reductant studied. One-tenth milliliter of an aqueous 10 per cent solution is required to reduce each p.p.m. of iron completely from the ferric to the ferrous state.

Sodium sulfite, sodium formate, and formaldehyde are unsatisfactory as reductants, owing to the formation of complexes with the ferric iron.

Six milliliters of an aqueous 0.10 per cent solution of (1,10)-phenanthroline are required to produce the maximum color with 5.0 p.p.m. of iron.

The color reaction conforms to Beer's law over the entire range of concentrations studied. Visual comparison is thus applicable.

pH has no effect on the intensity of the color over the applicable range, 2.0 to 9.0.

The color is stable and does not change over a period of 6 months, including 100 hours under ultraviolet radiation during an accelerated fading test.

There are very few ions that seriously interfere with the production of the quantitative color reaction. A study of fifty-five possible interfering ions was made.

Color Reaction of 1,10-Phenanthroline Derivatives. Continuing with the description of the preceding pages is the reprinting of the paper by M. L. Moss, M. G. Mellon, and G. Frederick Smith *(87b)*:

Many examples of the influence of slight changes of structure on the behavior of organic reagents may be found among the benzene arsonic and arsinic acids, a score of which have been prepared and studied. Differences between 8-hydroxyquinoline and its derivatives, such as ferron, have also been reported. The oxidation potential of the ferrous (1,10)-phenanthroline complex is 1.06 volts compared with 1.25 for the corresponding nitro derivative, nitro-ferroin, and the range of high-potential, oxidimetric indicators is thus extended. Dimethylglyoxime, after 35 years of useful service as the nickel reagent, may soon be rendered obsolete by 1,2-cyclohexanedionedioxime.

Since reactions involving formation of the metal complexes appear to be susceptible to slight changes in the reagent used, it is of interest to study the effect of various substituents whenever possible. These effects are usually unpredictable. Accordingly, a comparison of 1,10-phenanthroline and five of its derivatives was made with respect to their reactions with iron, copper, and molybdenum.

Apparatus and Solutions

Transmittancy measurements were made with a General Electric recording spectrophotometer set for a spectral band width 10 mμ. pH values were measured with a glass electrode meter calibrated with Clark and Lubs buffers.

One-tenth per cent solutions of the following compounds were prepared by dissolution in 95 per cent ethanol and dilution with redistilled water: 1,10-phenanthroline and its 5-bromo, 5-chloro, 5-methyl, nitro (position in ring uncertain), and 5-nitro-6-methyl derivatives. The last two are practically insoluble in water

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(87b) Moss, Mellon and Smith, *Ind. Eng. Chem.*, Anal. Ed. 14, 931 (1942).

and require about 80 per cent alcohol for complete dissolution. The final alcohol content was 5 per cent by volume for the four other reagent solutions.

A standard ferric nitrate solution prepared from iron wire of reagent grade contained 0.01 mg. iron per ml. and sufficient nitric acid to prevent hydrolysis. Measured quantities of this solution were reduced with a 10 per cent solution of hydroxylamine hydrochloride to obtain known concentrations of ferrous iron. The merits of this reducing agent have been established (*82*).

Twenty per cent ammonium acetate solution was used as a buffer and pH adjustments were made with 6 *N* solutions of ammonium hydroxide and hydrochloric acid.

Copper sulfate solution containing 0.05 mg. copper per ml. was prepared by dissolving copper wire in nitric acid, evaporating with sulfuric acid, and suitably diluting with redistilled water.

A standard molybdate solution, made by dissolving 1.503 grams of molybdic oxide in 10 ml. of 10 per cent sodium hydroxide solution and diluting to 1 liter, contained 1 mg. of molybdenum per ml.

A solution of chlorostannous acid contained 110 grains of C. P. "stannous chloride" and 170 ml. of concentrated hydrochloric acid per liter.

Determination of Iron

Color Reaction. Substitution of various groups in 1,10-phenanthroline does not materially affect the hue of the ferrous complexes except in the case of the nitro derivative which has a more purplish color than the others (Figures 15 and 16). A weak absorption band at 480 mμ characteristic of the other compounds is absent. An extra band at 440 mμ is exhibited by the 5-methyl but not by the 5-nitro-6-methyl derivative. The latter compound gives a slightly lower transmission for 2 p.p.m. of iron than the others, although the difference is too small to be significant. In more highly concentrated solutions—e.g., 0.025 *M* in iron—the nitro derivative appears visually to be the most sensitive. For solutions of 50 ml. containing 2 p.p.m. of iron, 2 to 3 ml. of reagent solution provide an excess for development of the maximum color and larger amounts do not affect the intensity, provided the acidity is not less than the lower limits specified in Table XVIII.

Effect of pH. In the determination of iron with 1,10-phenanthroline, pH variations between 3 and 9 do not influence the color. The derivatives studied are similar to the parent compound in this respect. Limits between which pH variations have no effect on the color reaction are shown in Table XVIII.

TABLE XVIII

Comparison of 1,10-Phenanthroline Derivatives

Reagent	Applicable pH Range	Wave Length of Maximum Absorption, Mμ	Molecular Extinction Coefficient
1,10-Phenanthroline	3-9	510	11,100
5-Chloro-1,10-phenanthroline	2.7-8.9	512	11,700
5-Bromo-1,10-phenanthroline	3-8	512	12,100
5-Methyl-1,10-phenanthroline	2.5-10	516	11,500
Nitro-1,10-phenanthroline	2.5-9.5	510	11,500
5-Nitro-6-methyl-1,10-phenanthroline	3-8	512	12,400

Effect of Iron Concentration. All the compounds are highly sensitive in detecting iron in low concentrations. In this respect they are a slight improvement over phenanthroline itself but not sufficiently so to commend their use. Beer's law is valid in the range 0.1 to 5 p.p.m. of iron for all the reagents studied. This is the optimum working range for determinations with a 1-cm. transmission cell.

Stability of the Color. Solutions of 50 ml. containing 0.1 to 5.0 p.p.m. of iron with an excess of both the color-forming reagent and hydroxylamine hydrochloride buffered with 0.2 gram of ammonium acetate were allowed to stand under ordinary laboratory conditions in glass-stoppered bottles. In 30 days there was no change in the case of the methyl derivative. After 3 months, decreases of several per cent in transmission at 400 mμ appeared. The nitro and bromo compounds changed appreciably in 24 hours, accompanied by slight fading. In 10 days, the nitromethyl compound underwent a change in hue, but the fading was only slight at the wave length of maximum absorption. The chloro compound darkened slightly, the transmission decreasing about 2 per cent in 2 weeks for concentrations greater than 1 p.p.m. The bromo compound faded overnight, producing a green suspension. A small amount of the green material precipitated on further standing. It was found to be soluble in ethanol and showed its maximum transmission at 560 mμ with absorption bands at 415 and 660 mμ. Solutions of ferrous complexes of each of the reagents studied showed some tendency to decrease in transmission in the red, thus becoming more green. The time required varied from 1 day in the case of the bromo derivative to 3 months in the case of the methyl derivative and the parent compound. Standards for visual comparison in the determination of iron with (1,10)-phenanthroline are reliable for at least 6 months, however (*82*). Blank solutions of the reagents containing no iron became noticeably darker at 400 mμ on standing 1 to 2 weeks.

Effect of Diverse Ions. The extent of interference by diverse ions is dependent only slightly on the reagent used. The effects of cobalt, copper, chromium, carbonate, orthophosphate, pyrophosphate, and cyanide were determined using the various reagents. These are representative of the ions which interfere with (1,10)-phenanthroline. In each case, the ion in question was added to the iron before reducing. Interference was found in all cases.

Determination of Copper

Ammoniacal cupric solutions containing 1,10-phenanthroline yield an insoluble, brown, complex cuprous salt when treated with hydroxylamine. If the copper concentration is sufficiently low, precipitation is delayed and the intensity of the color is determined by the amount of copper present. Precipitation can be avoided by adding an organic solvent miscible with water and conformity to Beer's law thereby achieved.

As a reagent for copper, no advantage over 1,10-phenanthroline was observed in any of its derivatives. The color reaction occurs immediately on the addition of hydroxylamine and the reddish-purple hue varies somewhat according to the reagent. A flocculent precipitate forms with several of the reagents, but this dissolves immediately on dilution with the solvent, while the hue undergoes a striking change from purple to orange. The optimum working range for a cell thickness of 1 cm. is 0.5 to 10 p.p.m. Beer's law is valid and the color is stable for 24 hours.

The phenanthrolines have no value as precipitants for copper.

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(82) Fortune and Mellon, *Ind. Eng. Chem.*, Anal. Ed., 10, 60 (1938).

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(82) Fortune and Mellon, *Ind. Eng. Chem.*, Anal. Ed., 10, 60 (1938).

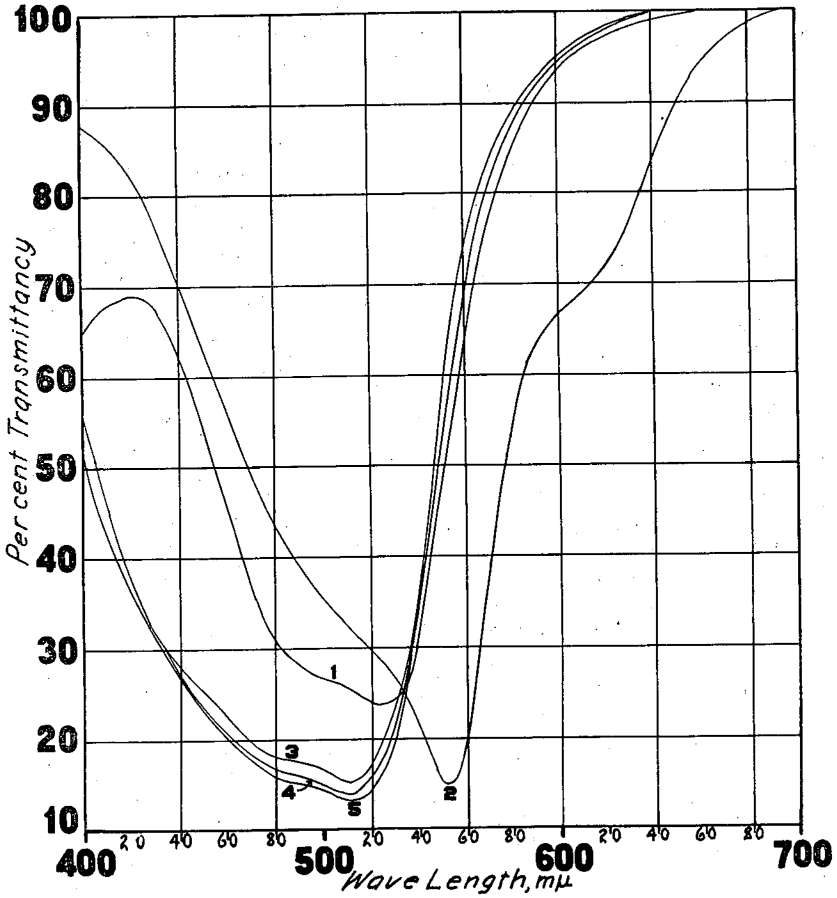

FIG. 15. Reagent for Iron

2 p.p.m. Fe, 2 cm. cell
1—2,2′ Bipyridyl
2—2,2′,2″ Terpyridyl
3—1,10-Phenanthroline
4—5-Cl-1,10-Phenanthroline
5—5-Br-1,10-Phenanthroline

Determination of Molybdenum

A sensitive color reaction involving reduction of molybdate ion in the presence of 2,2′-bipyridyl with chlorostannous acid has been described *(88)*. Development of the color was attributed to reaction of a "lower oxide of molybdenum" with bipyridyl.

1,10-Phenanthroline and its derivatives give a reddish-purple color similar to that obtained with bipyridyl. Bipyridyl is the least sensitive of the reagents tried. None of the reagents produced a color sufficiently stable to be useful for the determination of molybdenum. Various organic solvents miscible with water and present in concentrations of 50 per cent did not appreciably retard the fading.

Under the most favorable conditions for carrying out the test with the phenanthroline derivatives, fading proceeded at the rate of an incraese of 5 to 10 per cent in transmission within the first 5 minutes. Reproducibility is poor. Satisfactory transmission curves could not be obtained even in the short time of only 3 minutes required for operation of the recording instrument used.

In order to produce the color, the reagent must be added before the chlorostannous acid. Ten milliliters of reagent and 10 ml. of chlorostannous acid are suitable for developing a color with 0.5 mg. of molybdenum in 50 ml. of solution. Concentrated hydrochloric acid (0.5 ml.) prevents the color development if added before the reagent, but if added immediately after, 10 ml. may be present.

Although the reaction described may be used for detecting molybdenum, even in the presence of iron, it should be regarded as unsound for quantitative purposes.

Summary

A spectrophotometric study of the ferrous complexes of five derivatives of 1,10-phenanthroline indicates that these compounds may be used successfully as color-forming reagents for the determination of iron. Advantages over several methods in use were observed, including sensitivity and freedom from pH effects. With the exception of the methyl derivative, they do not form complexes of exceptional stability and are not preferred over the parent compound.

1,10-Phenanthroline and its derivatives form insoluble, highly colored, cuprous complexes which can be measured colorimetrically in the presence of suitable solvents. These reagents are not satisfactory precipitants for copper.

Molybdate ion in the presence of 1,10-phenanthroline, or its derivatives, may be reduced with chlorostannous acid to give a reddish hue capable of detecting 0.5 p.p.m. of molybdenum. The color is not sufficiently stable for quantitative purposes.

Colorimetric Determination of Copper with 1,10-Phenanthroline.

This topic was first published by M. L. Moss and M. G. Mellon *(89a)*. It is here reproduced in its unabridged form.

Tartarini *(89)* in a discussion of new color reactions involving cuprous salts, reported that 2,2′-bipyridyl and 1,10-phenanthroline form copper complexes which can be reduced with hydroxylamine to give highly colored cuprous compounds. The reaction is brought about by treating an ammoniacal solution of a cupric salt with the reagent and reducing. All cuprous salts containing ammonia or strong organic bases are either colorless or very pale. With a slight modification, the test with 1,10-phenanthroline can be used satisfactorily for the determination of copper.

Apparatus and Solutions

Transmittancy curves were determined with a General Electric recording spectrophotometer set for a spectral band width of 10 mμ.

A standard copper sulfate solution was prepared by dissolving copper wire in 1 to 1 nitric acid, fuming down with sulfuric acid, and diluting with redistilled water to a concentration of 0.05 mg. of copper per ml. Five milliliters of this solution diluted to 50 ml. give a copper concentration of 5 p.p.m.

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(88) Komerowsky and Poluektoff, *Microchim. Acta*, 1, 264 (1937).

(89) Tartarini, *Gazz. chim. ital.*, 63, 597 (1933).

(89a) Moss and Mellon, *Ind. Eng. Chem.*, Anal. Ed., 15, 116 (1943).

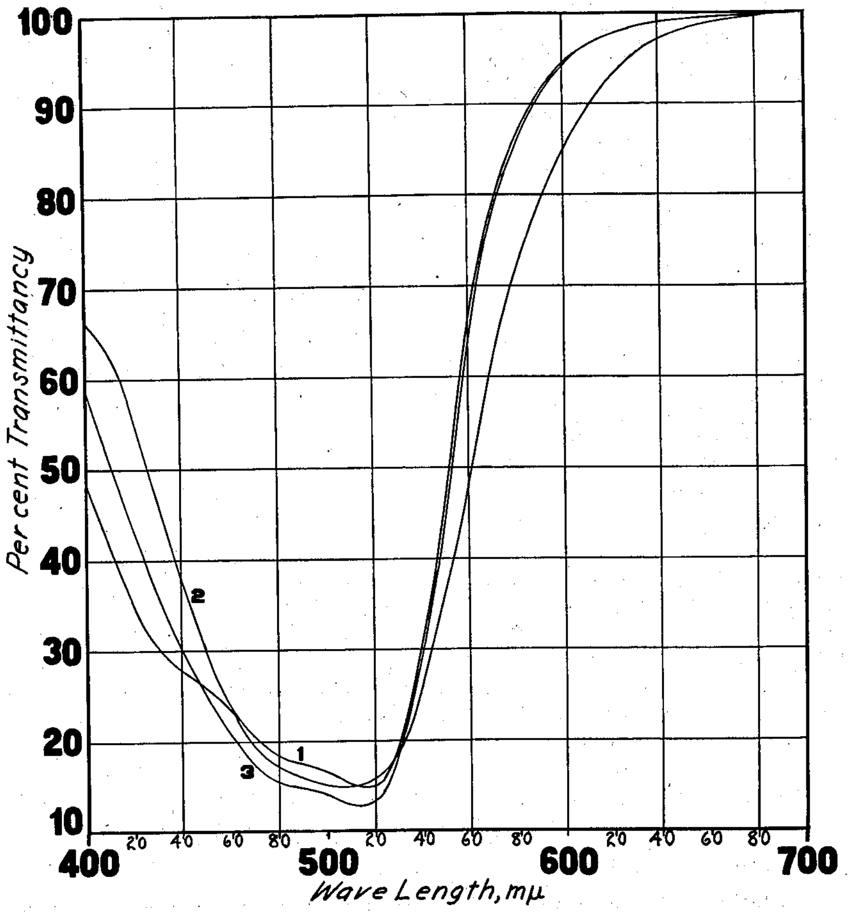

FIG. 16. Reagents for Iron

2 p.p.m. Fe, 2 cm. cell
1—5-Me-1,10-Phenanthroline
2—NO_2-1,10-Phenanthroline
3—5-NO_2-6 Me-1,10-Phenanthroline

A 0.1 per cent aqueous solution of 1,10-phenanthroline monohydrate was used as the color-forming reagent. The reducing agent was a 10 per cent solution of hydroxyalmine hydrochloride. Both of these solutions are used in the 1,10-phenanthroline method for iron *(82)*.

Commercial grades of the following solvents were used: acetone, ethanol, methanol, 2-propanol, dioxane, methyl Cellosolve, methyl Carbitol, ethyl Carbitol, chloroform, carbon tetrachloride, ethyl acetate, *n*-amyl alcohol, benzene, chlorobenzene, and ethyl ether.

pH adjustments were made with 6 *N* hydrochloric acid and 6 *N* ammonium hydroxide.

Standard solutions of the anions studied were prepared from the alkali metal salts. Nitrates, chlorides, and sulfates were used for the cations. Each solution contained 10 mg. of the ion in question per ml.

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(82) Fortune and Mellon, *Ind. Eng. Chem.*, Anal. Ed., 14, 931 (1942).

The Color Reaction

The order in which the reagents are added is of primary importance, the two following conditions being necessary: copper must be in the form of the ammonia complex and the reagent must be added before the hydroxylamine. Ammonia concentration is a critical factor but is easily controlled by neutralizing the solution to litmus and adding a measured quantity of 6 *N* ammonium hydroxide.

Formation of a complex cuprous salt such as $Cu(C_{12}H_8N_2)X$ is responsible for the orange color *(89)*. The time required for precipitation to occur in aqueous solutions varies with the copper concentration. Precipitation is prevented entirely by the presence of certain alcohols or other solvents miscible with water. Other undesirable properties of aqueous solutions of the complex which can be avoided by addition of a solvent are instability of the color, nonconformity to Beer's law, and change of hue with copper concentration.

Forty per cent methyl Carbitol is most satisfactory of the solvents tried, although ethyl Carbitol can also be used. Fading occurs within 2 days with dioxane, methyl Cellosolve, or the alcohols. With methyl Carbitol, the slight change in 2 days is not measurable by visual means. Acetone reacts with the hydroxylamine.

Extraction of the color with any of the following solvents is not feasible: *n*-amyl alcohol, benzene, chlorobenzene, chloroform, carbon tetrachloride, ether, and ethyl acetate.

The 5-chloro, 5-bromo, 5-methyl, nitro, and 5-nitro-6-methyl derivatives of 1,10-phenanthroline have no advantage over the parent compound from the standpoint of sensitivity or stability of the colored complex. 2,2′-bipyridyl is less sensitive and 2,2′,2″-terpyridyl does not react. In contrast, all of these compounds are about equally effective for determining ferrous iron *(82, 90)*.

EFFECT OF CONCENTRATION OF REAGENTS. The effects of concentrations of phenanthroline, ammonia, and hydroxylamine are not independent of one another. The effect of ammonia concentration, for example, is different for different concentrations of hydroxylamine. These variables can be easily controlled, however. For solutions with a final volume of 50 ml., 2 ml. of 6 *N* ammonium hydroxide, 10 ml. of 0.1 per cent phenanthroline solution, and 5 ml. of 10 per cent hydroxylamine hydrochloride are sufficient for copper concentrations up to 10 p.p.m. Concentration of methyl Carbitol is not critical. Twenty milliliters in 50 ml. of solution are recommended, but less may be used if precipitation of dissolved salts is likely to occur.

Buffering with ammonium acetate offers no advantage because the required amount of free ammonia must be present before reducing the copper, in which case proper adjustment of pH will have been made.

EFFECT OF COPPER CONCENTRATION. For a cell thickness of 1 cm., determinations of copper can be made conveniently in the range 0.1 to 10 p.p.m. as shown in Figure 17. All solutions contained 2 ml. of ammonium hydroxide, 8 ml. of reagent, 5 ml. of hydroxylamine hydrochloride solution, and 20 ml. of methyl Carbitol in 50 ml. The solution in the back cell was identical with the solution measured, except for copper, thereby canceling any adverse effects due to impurities in the reagents. Methyl Carbitol and several of the other solvents were found to contain considerable copper. Beer's law is valid at 435 mμ throughout

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(89) Tartarini, *Gazz. chim. ital.*, 63, 597 (1933).
(82) Fortune and Mellon, *Ind. Eng. Chem.*, Anal. Ed., 10, 60 (1938).
(90) Moss and Mellon, *Ind. Eng. Chem.*, Anal. Ed., 14, 862 (1942).

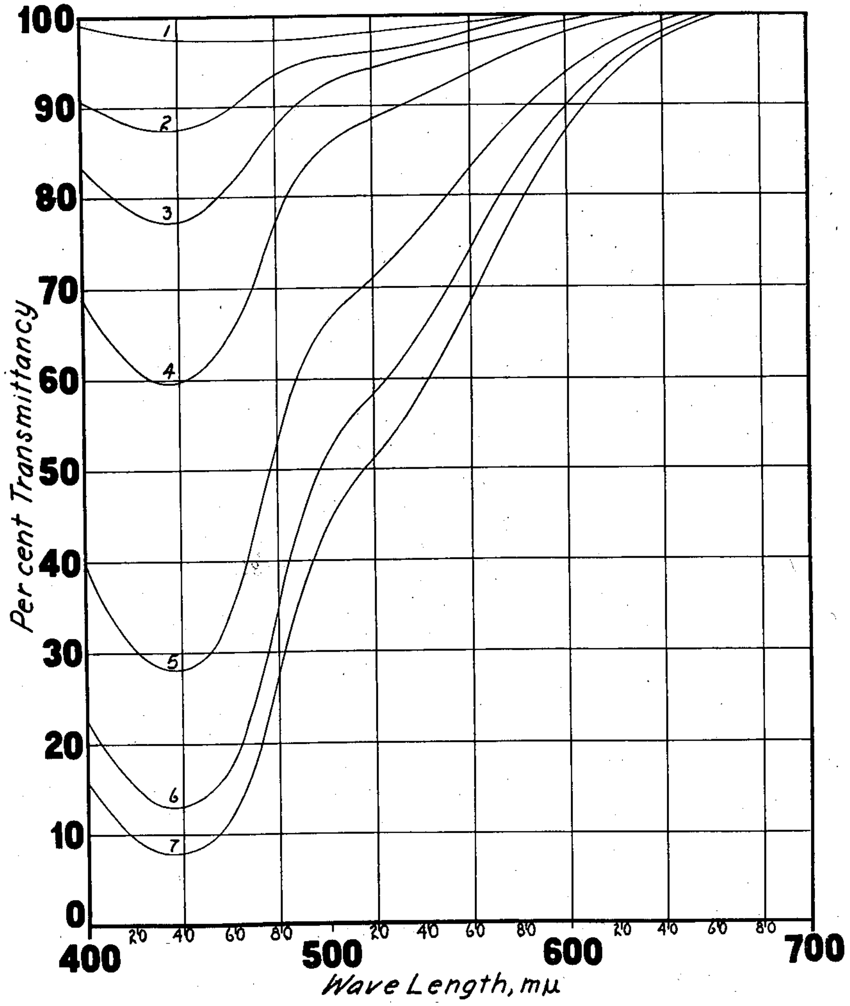

FIG. 17. Effect of Copper Concentration

1—0.1 p.p.m. Cu
2—0.5 p.p.m. Cu
3—1.0 p.p.m. Cu
4—2.0 p.p.m. Cu
5—5.0 p.p.m. Cu
6—8.0 p.p.m. Cu
7—10.0 p.p.m. Cu

the range of concentrations susceptible to measurement. The molecular extinction coefficient at 435 mμ is 7030. For visual work, standards such as the solutions represented in Figure 1 are reliable for at least 24 hours.

EFFECT OF DIVERSE IONS. The phenanthroline method for copper is comparatively free from interference by the anions. Few cations interfere aside from those which precipitate in the presence of ammonia.

In determining the extent of interference for the various ions, the following procedure was adopted: A measured volume of solution containing the ion in question was added to 5 ml. of the copper sulfate solution containing 0.05 mg. of copper per ml. After neutralizing to litmus with 6 *N* ammonium hydroxide or hydrochloric acid, the following were added in order: 2 ml. of 6 *N* ammonium hydroxide, 10 ml. of reagent solution, 1 ml. of hydroxylamine solution, and 20 ml. of methyl Carbitol. The solution was then diluted to 50 ml. with redistilled water and the transmission curve run. Ammonium acetate was added in a number of cases without any apparent advantage. If interference was pronounced, smaller quantities of the ion were used successively until an estimate of the limiting permissible concentration could be made, 2 per cent error being set arbitrarily as a reasonable tolerance. These values are summarized in Table XIX.

TABLE XIX

Effect of Diverse Ions

Ion	Added as	Present	Error	Amount Permissible
		P.p.m.	%	*P.p.m.*
Ba^{++}	$Ba(NO_3)_2$	500	3	300
Be^{++}	$Be(NO_3)_2$	100	0	100
Cd^{++}	$Cd(NO_3)_2$	10	2	10
Co^{++}	$Co(NO_3)_2$	10	6	5
Ni^{++}	$Ni(NO_3)_2$	5	2	5
Sr^{++}	$Sr(NO_3)_2$	500	10	100
Zn^{++}	$Zn(NO_3)_2$	10	2	10
CO_3^{--}	Na_2CO_3	500	3	300
CHO_2^-	HCO_2H	500	3	300
$C_2O_4^{--}$	$(NH_4)_2C_2O_4$	500	3	300
$C_3H_5O_3^-$	$CH_3CHOHCO_2H$	500	3	300
$C_4H_4O_6^{--}$	$(NH_4)_2C_4H_4O_6$	500	3	300
$C_6H_5O_7^{---}$	$(HO)C(CO_2H)(CH_2CO_2H)_2$	500	3	300
$C_7H_5O_2^-$	$C_6H_5CO_2Na$	500	3	300
$C_7H_5O_3^-$	$C_6H_4(OH)CO_2Na$	500	3	300
Cn^-	KCN	2	14	0
$Cr_2O_7^{--}$ (Cr)	$K_2Cr_2O_7$	25	2	25
$P_2O_7^{--}$	$Na_4P_2O_7$	500	4	300
SCN^-	KSCN	500	6	250
$S_2O_3^{--}$	$Na_2S_2O_3$	30	18	5
VO_3^-	KVO_3	500	14	100

For solutions containing 5 p.p.m. of copper, the following ions may be present in concentrations of at least 500 p.p.m. without causing an error greater than 2 per cent: ammonium, calcium, lithium, magnesium, potassium, sodium, acetate, arsenate, arsenite, bromide, chlorate, chloride, fluoride, iodate, iodide, molybdate, nitrate, nitrite, orthophosphate, perchlorate, periodate, silicate, sulfate, sulfite, tetraborate, and tungstate.

The following cations precipitate: aluminum, antimony, bismuth, cerium, chromium, cobalt, iron, lead, manganese, mercury, silver, titanium, thorium, uranium, zirconium, and beryllium (in concentrations exceeding 100 p.p.m.).

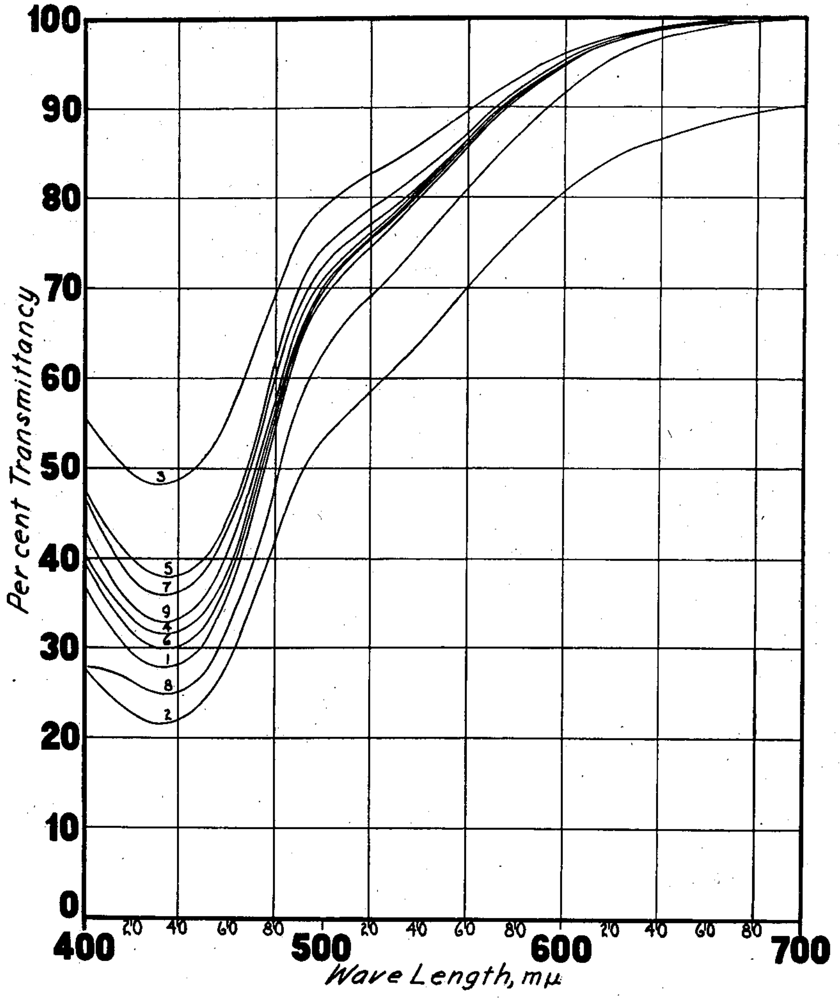

FIG. 18. Effect of Diverse Ions

1— 5 p.p.m. Cu alone
2—500 p.p.m. Be^{++}
3— 50 p.p.m. Cd^{++}
4— 10 p.p.m. Co^{++}
5— 10 p.p.m. Ni^{++}
6—500 p.p.m. $P_2O_7^{==}$
7— 30 p.p.m. $S_2O_3^{=}$
8— 50 p.p.m. $Cr_2O_7^{=}$
9— 2 p.p.m. CN^{-}

Cadmium, cobalt, nickel, and zinc interfere presumably by complex formation with the reagent. These metals also interfere in the determination of iron with 1,10-phenanthroline *(82)*.

Among the anions, serious interference was encountered only with cyanide and thiosulfate. These should be absent. Large amounts of dichromate and metavanadate also interfere. Transmission curves for several solutions containing interfering ions are shown in Figure 18.

Discussion

Since the color reaction must be carried out in ammoniacal solution, iron must be removed irrespective of the fact that it, also, gives an intense color with 1,10-phenanthroline. This separation can be made by careful precipitation with ammonia as directed by Mehlig *(92)*. When the filtration has been completed, copper is present as the ammonia complex and, if the concentration is high enough to give a measurable color, no advantage over the ammonia method *(92)* can be gained by using 1,10-phenanthroline. If the concentration is too low, however, the proposed method may be used with good results for concentrations greater than 0.1 p.p.m.

In using the method for copper ores, results were calculated by the following procedure.

Instead of comparing the transmittancy of the unknown solution with a standard, the change in transmittancy produced by adding a known amount of copper to the unknown was determined. The unknown concentration, *x*, was then calculated according to Beer's law expressed as follows:

$$T_x = T_{a+x}^{\left(\frac{x}{a+x}\right)}$$

$$x = a \log T_x / \log \frac{T_{a+x}}{T_x}$$

in which T_x is the transmittancy of an aliquot of the sample and T_{a+x} the transmittancy of a similar aliquot containing a small additional quantity of copper, *a*. Uncertainties arising from the presence of other constituents, turbidity, extraneous color, or other resources, except presence of copper in the reagents, are thus compensated. Foster, Langstroth, and McRae have applied this principle successfully to the spectrographic determination of lead *(91)*.

1,10-Phenanthroline is somewhat less sensitive as a copper reagent than is sodium diethyldithiocarbamate. The latter reagent forms an insoluble copper salt requiring use of gum arabic or extraction with a suitable solvent. Methyl Carbitol is not satisfactory for preventing precipitation with the diethyldithiocarbamate method nor can gum arabic be used with the phenanthroline method.

Recommended Procedure

TREATMENT OF SAMPLE. Prepare a solution of the sample by appropriate means to contain 0.01 to 0.5 mg. of copper (see *92* for copper ore and mattes).

If necessary to remove any metals precipitated by ammonia, make the solution just basic to litmus by adding 15 *N* ammonium hydroxide, filter into a conical flask, and wash the precipitate with 3 *N* ammonium hydroxide until the washings emerge colorless. Remove the flask, wash the precipitate into the original con-

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(82) Fortune and Mellon, *Ind. Eng. Chem.*, Anal. Ed., **14**, 931 (1942).

(91) Foster, Langstroth, and McRae, *Proc. Roy. Soc.* (London), *A*153, 141 (1935).

(92). Mehlig, *Ind. Eng. Chem.*, Anal. Ed., 7, 387 (1935); 13, 533 (1941).

tainer, and dissolve the material with a minimum amount of concentrated sulfuric acid. Then repeat the precipitation and washing with ammonium hydroxide as before, and catch the filtrate in the same conical flask. Evaporate the solution to 15 ml.

MEASUREMENT OF DESIRED CONSTITUENT. Neutralize the solution to litmus with 6 *N* hydrochloric acid or ammonium hydroxide and add the following in order: 2 ml. of 6 *N* ammonium hydroxide, 10 ml. of 0.1 per cent 1,10-phenanthroline solution, 1 ml. of 10 per cent hydroxylamine hydrochloride solution, and 20 ml. of methyl Carbitol.

Dilute to 50 ml. with water, mix well, and measure or compare the color by any of the usual means. A blue filter such as Corning No. 556 is recommended for filter photometers.

Summary

The intense, brown color of cuprous-phenanthroline complex may be used as the basis of a colorimetric method for the determination of copper. Beer's law is valid for concentrations from 0.5 to 10 p.p.m. of copper, at least, this being the range most suitable for measurements with a 1-cm. transmission cell. The colored system is stable for at least 24 hours.

Of the metals whose compounds are soluble under the conditions used, only cadmium, cobalt, nickel, and zinc interfere seriously. Metals which precipitate with ammonia are removed during the course of the procedure. Among the anions studied, only cyanide, dichromate, and thiosulfate interfere appreciably.

COLORIMETRIC DETERMINATION OF COBALT WITH TERPYRIDYL

This determination of cobalt has been described in detail by Moss and Mellon *(92)*. A complete reprinting of this paper is not possible here because of limited space. While terpyridyl is an excellent reagent for iron according to usual methods of appraisal, it is not particularly sensitive for cobalt, although it can be used over a wider range (0.5-50 p.p.m.). The liberal tolerance in pH is significant (2-10). Beer's law is valid. Interference of most of the common metals except Cu, Ni, and Fe, is not serious. Cyanide and dichromate should be absent. The color is of limited stability and reference standards should be made daily.

The original paper should be consulted for details.

SECTION 8

THE DETERMINATION OF IRON IN WINE AND VARIOUS FRUIT PRODUCTS, PREPARATION OF SAMPLE BY WET-ASHING USING PERCHLORIC ACID AND COLORIMETRIC ESTIMATION USING 1,10-PHENANTHROLINE

For the determination of the iron content of grapes and wine the published work of Byrne, Saywell, and Cruess *(93)* and Saywell and Cunningham *(84)* are authoritative and the following material is a discussion of their study with quotations from references 93 and 84.

> "In all wine-making regions of the world wine often becomes hazy or cloudy owing to the formation of a finely divided colloidal precipitate caused by the presence in the wine of an excess of iron salts. The problem is of considerable economic importance in California and is one to which much attention has been given in this laboratory." (Fruit Products Division, University of California, Berkeley.)

Iron casse (cloudiness due to precipitated iron compounds) develops in wine when the iron content is 10 p.p.m. or even less and this tendency to cloudiness varies with the type of wine and its processing. The iron content of 59 samples of 16 grape juice varieties of California was found to average 8.6 p.p.m. (maximum 23, minimum 1.5). The iron content of the juice decreases strikingly during fermentation. The iron content (from 16 varieties of California grapes) showed an average of 9 p.p.m. in the juice and the wines therefrom 1.9 p.p.m. The wines made from the fermentation of the skins gave 1.7 p.p.m., while the iron content of the whole grape was 11.9 p.p.m. "It was not determined whether the iron was utilized by the yeast in its tissues, or precipitated as an insoluble salt."

The addition of a small amount of citric acid to white wines usually prevents iron casse and is the standard preventative measure in France. The stability of red wines, such as claret and Burgundy types, is apparently unaffected by the concentration of iron normally encountered in California wines.

Determination of Iron in Wine with Wet-Ashing of Samples using Sulfuric and Perchloric Acids. The wet-ashing method of Leavell and Ellis *(94)* has been successfully employed with the usual ashing

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(93) Byrne, Saywell and Cruess, *Ind. Eng. Chem.*, Anal. Ed., 9, 82 (1937).
(84) Saywell and Cunningham, *ibid.*, 9, 66, (1937).
(94) Leavell and Ellis, *Ind. Eng. Chem.*, Anal. Ed., 6, 4 (1934).

times of 3 to 7 hours for wines and many fruit juices reduced to periods of 2 to 10 minutes for each sample.

Mercaptoacetic acid was employed by Leavell and Ellis *(94)* but "because of the difficulty in color comparison with concentrations lower than 2 or 3 p.p.m. and occasional fading of the mercaptoacetic acid colored complex, it appeared desirable to have a method of equal or greater sensitivity and accuracy, with a higher degree of precision." The color producing medium selected was that of 1,10-phenanthroline.

"The authors (Saywell and associates) have observed that the color, when once formed in slightly acid to neutral solutions, is very stable and standards have been kept for over 4 months without exhibiting differences from freshly prepared standards. Solutions containing a final iron concentration of 1 to 10,000,000 give color of sufficient intensity for colorimetric comparison. For iron concentrations of the same magnitude, the intensity of the color is proportional to the iron content. Concentrations greater than 20 or 25 p.p.m. may be conveniently diluted by one half, or smaller samples may be taken for analysis.

"As the iron in the strongly acid solution remaining from the wet-ashing is in the ferric condition, reduction to the ferrous condition before the addition of the 1,10-phenanthroline is necessary. Several reducing agents were studied, the best being sodium hypophosphite or hypophosphorous acid, and sodium formate, which were discontinued because of the considerable iron content of these products and the necessary correction factor. The use of a concentrated solution of iron-free hydroxylamine hydrochloride has been adopted.

"Numerous experiments were made to ascertain the most satisfactory procedure and conditions for the addition of the reagents. As a result it was observed that a treatment of warm acid solution (remaining from the wet-ashing) with hydroxylamine, followed by the addition of 1,10-phenanthroline and neutralization with concentrated ammonium hydroxide, using Congo red indicator paper, gave a quantitative development of the color due to the formation of ferrous, 1,10-phenanthroline complex. An excess of 1 or 2 drops of ammonium hydroxide is permissible.

"Pyrex test tubes, 25 × 150 mm., are calibrated to contain 10 and 15 ml. Using such tubes the entire procedure of wet-ashing, development of color and dilution may be carried out in the same test tube without possible error of transfer and at a considerable saving of time. Iron free concentrated sulfuric acid, 60 or 70 per cent perchloric acid, 10 per cent aqueous hydroxylamine hydrochloride solution, 1.5 per cent solution of 1,10-phenanthroline in ethyl alcohol, and concentrated ammonium hydroxide are required.

"A primary standard iron solution is prepared by dissolving 1 gram of pure iron in dilute sulfuric acid and concentrated nitric acid. Oxides of nitrogen must be expelled by boiling to fumes of sulfur trioxide. The solution is then diluted to one liter. Other standard solutions may be prepared by diluting 20, 10, and 5 ml. of the primary standard to 1 liter each, these standards containing 20, 10, and 5 p.p.m. of iron respectively. Further standards may be prepared as desired. All standards should be made acid with sulfuric acid.

"**Procedure.** Samples of 2 ml. of a fruit juice or wine are digested with 1 ml. of concentrated sulfuric acid and 0.7 to 1 ml. of 60 to 70 per cent perchloric acid in the Pyrex test tubes over a free flame. It is convenient to hold the tubes in a wire clamp and agitate the contents vigorously during the heating and digesting. Digestion to a clear water-white liquid will occur ordinarily in 2 to 10 minutes. If combustion is not complete in this time, an additional 0.5 to 1 ml. of perchloric acid should be used and digestion continued. When digestion is complete, 1 ml. of distilled water is added. This is followed by the addition of 1 ml. of the 10 per cent hydroxylamine hydrochloride solution, shaking and the addition of 0.5 ml. of the 1.5 per cent 1,10-phenanthroline solution. Concentrated ammonium hydroxide (about 2 ml.) is added until a light red or pink color develops, a small piece of congo red indicator is dropped into the solution and the addition of the ammonium hydroxide is continued until the Congo red paper is distinctly pink. In the meantime the full color of the ferrous 1,10-phenanthroline complex will have developed. The solution is then diluted with distilled water to the graduation mark on the test tube indicating a volume of 10 ml. and thoroughly mixed. If the color intensity of the solution is greater than that of the highest standard available, the unknown solution may be diluted to the 15 ml. graduation mark on appropriate calculation made. Determinations may conveniently be made on a series of samples, and the reagents added consecutively from measuring pipets or burets.

TABLE XXI

Iron Content of Wine by Various Procedures

Method	Iron Content p.p.m.					
	Muscat	Sherry	Port	Sauterne	Riesling	Claret
Stugard *(95)*..........................	3.3 3.1	3.1 3.5	3.5 3.2	4.8 4.9	7.4 7.8	3.9 3.8
Mercaptoacetic Acid *(94)*....	3.2 3.4	2.8 2.9	3.1 3.3	5.4 5.0	7.1 7.8	3.7 4.0
1,10-Phenanthroline *(84)*....	3.4 3.5	3.3 3.3	3.2 3.2	5.2 5.3	7.4 7.3	3.9 4.0
Original Wine......................	....	....	....	3.8 3.8	....	2.9 2.9
Original + 2.5 p.p.m.	....	....		6.3 6.3	....	5.4 5.4
Original + 5.0 p.p.m.	....	....		8.7 8.8	....	7.8 7.9

"Standards should be prepared by the same procedure. A blank determination on the distilled water and reagents used should be made. For purposes of comparison with a colorimeter, standards containing 2.5, 5.0, 7.5, 10, and 15 p.p.m., respectively, have been convenient. For routine plant purposes where many determinations are being made, much time may be saved by having permanent standards of 1, 2, 4, 6, 8, 10, 12, and 15 p.p.m., respectively, in a rack so that it is only necessary to compare the solutions in similar tubes. The iron content can be easily estimated to within 1 p.p.m. or better in this manner. For material such as dried fruits, in which the iron content may be up to 40 to 60 p.p.m., it is desirable to weigh out a small sample of approximately 0.5 gram on a small piece of wax paper and ash the entire sample. One may also weigh out a larger sample, prepare a water solution or suspension of it, and use a suitable aliquot for ashing. In general the authors (Saywell and associates) have

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(95) Stugard, *Ind. Eng. Chem.*, Anal. Ed., 3, 39 (1931).
(94) Leavell and Ellis, *ibid.*, **6**, 46 (1934).
(84) Saywell and Cunningham, *ibid.*, **9**, 67 (1937).

used original concentrations of up to 20 p.p.m. with a total of 0.04 mg. of iron present in each determination. Volumetric flasks and long tubes of small diameter for color comparison may also be used.

"A series of determinations by the method as described and by previously published procedures are given in Table XXI."

SECTION 9

DETERMINATION OF IRON IN BIOLOGICAL MATERIALS. THE USE OF 1,10-PHENANTHROLINE

This topic has been discussed by Hummel and Willard *(85)* and is reprinted in its entirety.

"For several years 1,10-phenanthroline has been used in the Research Laboratory of the Children's Fund of Michigan as a satisfactory reagent for the determination of iron in foods, feces, blood stroma, and other types of biological materials. Since numerous requests have been made for the details of the procedure followed, it seems desirable to record the method in full.

"The small amount of iron present in some biological materials precludes the use of the classical gravimetric or titration methods, various colored compounds of iron have therefore been adapted to colorimetric determination. Several of these procedures have been investigated by the authors, but have not been found satisfactory. The presence of large amounts of phosphate in biological materials, especially certain foods and feces, interferes with methods involving ferric ion, and has necessitated the use of tedious modifications to avoid this interference. For this reason attention has been turned to the color reactions of ferrous iron, which does not form a stable complex with pyrophosphate.

"The colored complex formed by ferrous iron with 1-10-phenanthroline, which was originally observed by Blau *(70)*, has been used for the determination of iron in various types of materials *(71)*. The orange-red color is quantitatively proportional to the concentration of iron within the pH range 2.5 to 8.0, and has been used therefore in both titration *(62)*, and colorimetric methods. The colors have much the same characteristics, with the advantage that the latter reagent is less expensive and more readily available.

"The method described herein was originally devised for use in the colorimeter, but has since been adapted to the Cenco-Sheard-Standard photelometer. The latter instrument possesses certain advantages over the colorimeter, since the use of light filters widens the range of accuracy. The relation of density of color to the concentration of iron is determined at the outset of the experiment which obviates making up a standard simultaneously with the unknown.

REAGENTS

"**Standard Iron Solution.** Dissolve 1 gram of electrolytic iron in 10 per cent sulfuric acid and dilute to 1 liter. Dilute 1 to 10 for use; 1 cc. of diluted standard corresponds to 0.1 mg. of iron.

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(85) Hummel and Willard, *Ind. Eng. Chem.*, Anal. Ed., 10, 13 (1938).

(70) Blau, *Monatsh.*, 19, 647 (1898).

(62) Walden, Hammett, and Edmonds, *J. Am. Chem. Soc.*, 56, 350 (1934).
complexes formed by iron with a,a'-dipyridyl *(70, 71)* and 1,10-phenanthroline

(70) Blau, *Monatsh.*, 19, 647 (1898).

(71) Hill, *Proc. Roy. Soc.* (London), B 107, 208 (1930).

"**Hydroquinone.** Dissolve 1 gram of hydroquinone in 100 cc. of sodium acetate-acetic acid buffer solution with a pH of 4.5. Keep the solution in the refrigerator and discard as soon as any color develops.

"**Sodium Acetate.** 0.2 M, M, and 2 M are convenient concentrations to have available.

"**1,10-Phenanthroline.** Dissolve 0.5 gram of 1,10-phenanthroline monohydrate in 100 cc. of distilled water, and warm to effect solution.

PROCEDURE

"The material to be analyzed (foods and feces dried at 60 degrees to 80 degrees C. were used) is ashed overnight in an electric muffle furnace at 450 degrees to 500 degrees C. The ash is dissolved in the smallest possible amount of dilute hydrochloric acid (1 to 3) and the solution is filtered into a 100-cc. volumetric flask, if the first ashing is incomplete, the paper and residue are re-ashed, after thorough washing with distilled water, and the ash is dissolved as before and filtered into the same flask. The solution is then made to volume, and an aliquot selected for analysis which will fall within the range of accuracy of the colorimeter or the photelometer—i.e., 0.2 to 0.5 mg. or 0.01 to 0.70 mg. of iron, respectively.

"Similar aliquots of the above unknown solution are measured into both a 25 cc. volumetric flask and a test tube, and 2 M sodium acetate solution is added from a buret to the test tube until the color corresponding to pH 3.5 is reached, using 5 drops of La Motte indicator bromophenol blue. The unknown solution in the 25 cc. volumetric flask is adjusted to pH 3.5, using the same amount of 2M sodium acetate, followed by the addition of 1 cc. of 1 per cent hydroquinone solution and 1 cc. of 1,10-phenanthroline solution. After thorough mixing, the solutions are allowed to stand for 1 hour to assure complete conversion of the iron to the ferrous 1,10-phenanthroline complex, and then made to volume and read in either the colorimeter or the photelometer.

"If the colorimeter is to be used, a series of standards containing from 0.2 to 0.5 mg. of iron is prepared simultaneously with the unknown. Since the color becomes yellow with dilution, it is not feasible to read lower concentrations in the colorimeter. Accurate results are rarely obtained if the unknown varies from the standard by more than 25 per cent, this confirms the observation of other investigators *(96-97)*.

"The procedure for the determination of iron recorded herein differs from that of Saywell and Cunningham *(84)* in several respects. The large quantity of calcium and phosphorus present in metabolic materials necessitates a concentration of acid sufficient to prevent the precipitation of calcium phosphate, which carries down most of the iron as ferric phosphate. Since the ferrous 1,10-phenanthroline complex is not stable in the presence of strong acid, the pH range 3.0 to 4.0 was selected, this acidity permits maximum color development, yet prevents any precipitation. A solution of hydroquinone in a sodium acetate-acetic acid buffer of pH 4.5 has been used effectively to reduce the iron to the ferrous state. Sodium hydrosulfite was also tried as a reducing agent, but was abandoned because it occasionally caused the solutions to become turbid. Since accurate measurement of the hydroquinone solution can be made, and equal quantities used for standard or unknown, traces of iron in the reagent do not cause appreciable error.

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(96) Bandemer and Schaible, *ibid.*, 16, 317 (1944).
(97) Sanford, Sheard and Osterberg, *Am. J. Clin. Path.*, 3, 405 (1933).
(84) Saywell and Cunningham, *Ind. Eng. Chem.*, Anal. Ed., 9, 82 (1937).

"**Results.** The solutions may be read with equal accuracy within the concentration range 0.2 to 0.5 mg. of iron for the colorimeter, and 0.01 to 0.07 mg. for the photelometer. The two instruments were used interchangeably in obtaining the results presented in Tables XXII and XXIII.

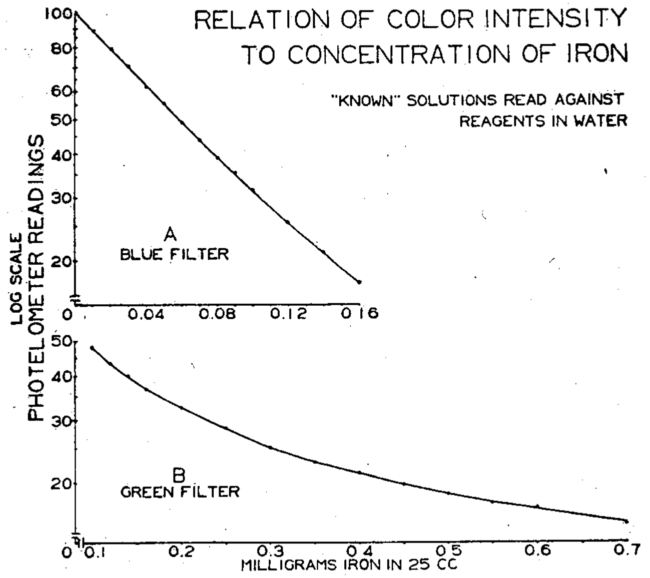

FIG. 19.

"The smooth curves shown in Figure 24 were obtained by measuring the color developed by a series of standards in the photelometer. Curve A is obtained from the concentration range 0.01 to 0.16 mg. of iron, using a blue filter, when the solutions are read against a solution of the reagents in sodium acetate-acetic acid buffer pH 4.5 set at 100. The effect of traces of iron present in the reagents as impurity is eliminated by using them as the standard instead of distilled water. Curve B is obtained in the same manner for the concentrations 0.1 to 0.7 mg. of iron, using a green filter.

"Table XXII shows the recovery of known amounts of iron added to ash solutions. In order to check the accuracy of the dry-ashing method, in some cases the iron was added to the food before ashing. The recoveries are for the most part within the accuracy assigned to colorimetric methods, and dry-ashing gives satisfactory results.

"Since the principal difficulty encountered in colorimetric methods for iron in biological materials especially has been the interference of pyrophosphate, this action was investigated by adding amounts of sodium pyrophosphate representing from 10 to 50 mg. of phosphorus to standard iron solutions. The color was developed in the usual manner, and, after standing from ten minutes to twenty-four hours, was read against iron standards containing no pyrophosphate. The results shown in Table XXIII indicate that if the determinations are allowed to stand 30 minutes, the error caused by fairly large amounts pyrophosphate is within normal limits.

TABLE XXII

Recovery of Iron Added to Foods Before and After Ashing

Sample	Iron				
	Determined	In Ash*	Recovered	Added	Recovered
	mg.	*mg.*	*mg.*	*mg.*	*Pct.*
1	0.335	0.234	0.101	0.100	101.0
2	0.250	0.165	0.085	0.0862	98.6
3	0.300	0.165	0.135	0.135	100.0
4	0.435	0.236	0.199	0.200	99.5
5	0.296	0.194	0.102	0.100	102.0
6	0.333	0.194	0.139	0.135	103.0
7	0.284	0.199	0.085	0.0862	88.6
8	0.330	0.199	0.131	0.135	97.0
9	0.374	0.167	0.207	0.200	103.5
10	0.370	0.199	0.171	0.172	99.4
11	0.254	0.167	0.087	0.0862	100.9
12	0.339	0.167	0.172	0.172	100.0
13	0.293	0.196	0.097	0.100	97.0
14	0.293	0.196	0.097	0.100	97.0
15	0.390	0.196	0.194	0.200	97.0
16	0.400	0.196	0.204	0.200	102.0
17	0.495	0.196	0.299	0.300	100.0
18	0.250	0.098	0.152	0.150	101.3
19	0.305	0.098	0.207	0.200	103.5
20	0.305	0.098	0.198	0.200	99.0
21	0.350	0.098	0.252	0.250	100.8
22	0.344	0.098	0.246	0.250	98.4

*Ashes analyzed separately by the same method. Samples 1 to 12: Iron was added to ash solution. Samples 13 to 22: Iron was added to food before ashing.

"The influence of some of the elements which occur in traces in biological materials was investigated. Two milligrams each of lead, zinc, aluminum, mercury, arsenic, fluorine, and iodine, 0.6 mg. of tin, and 0.2 mg. of copper were added to standard iron solutions, and the iron was determined in the usual manner. Since copper reacts with 1,10-phenanthroline in amounts over 0.2 mg. and tin precipitates at a pH of 3.5 if more than 0.6 mg. is present, quantities which rarely occur in biological materials, smaller concentrations of these elements were used. No interference was observed.

"Preliminary experiments have shown that 1,10-phenanthroline may be used for the determination of available iron in foods in much the same manner as 2,2′-bipyridyl *(4)*; it does not form the colored complex when added to purified hematin.

"None of the elements found in traces in biological materials except copper in amounts over 0.2 mg. and tin in amounts over 0.6 mg. interferes with the determination of iron by this method.

"**Possible Sources of Error in the Determination of Iron Using 1,10-Phenanthroline.** A study of the possible sources of error in the methods previously described for the determination of iron using 1,10-phenanthroline has been given by Bandemer and Schaible *(98)*. The results of their work is given by reprinting their summary of results.

TABLE XXIII

Iron Recovered in Presence of Varying Amounts of Phosphorus*

Time of Standing	Phosphorus Added				
	10 mg.	20 mg.	30 mg.	40 mg.	50 mg.
	Iron Recovered				
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
10 minutes	0.202	0.199	0.189	0.163	0.161
30 minutes	0.200	0.199	0.199	0.196	0.192
1 hour	0.200	0.199	0.199	0.205	0.196
3 hours	0.202	0.198	0.202	0.196	0.195
24 hours	0.201	0.196	0.200	0.200	0.195

*All samples contained 0.2 mg. of iron.

"Sodium Citrate was found more satisfactory than the acetate in adjusting the reaction for the development of the color of the iron-1,10-phenanthroline complex.

"If the pH was adjusted before the introduction of 1,10-phenanthroline, the rate of color development was influenced by such factors as the time intervals between the additions of reagents, temperature of the solutions, type and amount of phosphate present, amount of citrate and length of time the solutions stood before reading in the photometer. If the sodium citrate was added after the hydroquinone and 1,10-phenanthroline at temperatures above 20 degrees C., these factors did not adversely affect the recovery of iron. Under these conditions, maximum color developed when the solutions stood only 30 minutes.

"For samples of similar materials of approximately the same size, it was found expedient to use an average volume of citrate rather than to titrate each sample individually."

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(98) Bandemer and Schaible, *Ind. Eng. Chem.*, Anal. Ed., 16, 317 (1944).

SECTION 10

(1,10) AND 5-NITRO-(1,10)-PHENANTHROLINE AND ITS FERROUS SULFATE COMPLEX AS PRECIPITANTS IN THE MICRODETERMINATION OF VARIOUS METAL CATIONS*

The phenanthroline and substituted phenanthrolines and their ferrous sulfate complexes may be used as precipitants for a considerable number of metal cations. The cost in terms of these reagents makes a macrochemical operation involving their use as precipitants of somewhat questionable practicability. Such applications would demand a satisfactory procedure for the recovery of the reagents for repeated use. For microchemical operations the methods are entirely feasible. The following described procedures illustrate a few only of a large number of possibilities. The methods are subject to many suitable variations. The centrifugal separation of the precipitates involved and the estimation of the cations found by measurement of the volume of the precipitate is but one possible procedure. The determinations could be worked out by finding the weight of the precipitate or by dissolving the precipitate followed by titration with a standard oxidant, or the solution of the precipitate could be evaluated by determination of the color produced. In the determinations described, the 5-nitro-1,10-phenanthroline ferrous sulfate complex or the uncomplexed base is used as precipitant. The various substituted phenanthrolines or bipyridyl or terpyridyl all have possibilities. Non-aqueous solvents might be used with considerable advantage. The very high molecular weight of the precipitates involved, results in a high degree of precision for both gravimetric and centrifugal volumetric methods of measurement. Colorimetric and oxidimetric estimations of the precipitates are in some ways equally attractive.

The excellence of the method of attack about to be described, brings to the foreground the urgent need for a readily applicable procedure by which the dye bases may be recovered from solutions of the various reagents, as the ferrous complexes, or from solutions of the precipitates. Such a recovery procedure is now under investigation and the method will be published in due time but is not available for inclusion in this booklet. The use of the phenanthroline type of ferrous complexes as masking reagents for such separations as aluminum from iron and other possible separations, depends upon the availability of a simple procedure to recover the rather costly parent dye base. Once this problem has been solved, the realm of applications of these remarkable reagents to quantitative analysis in procedures, such as those mentioned and those about to be described, is, to those skilled in analytical manipulations, not difficult to visualize.

The Separation and Microdetermination of Ferrous Iron in the Presence of Ferric Iron, Fluorides, Sulfates and Phosphates. The procedure depends upon the precipitation of ferrous iron by the use of a slight excess of 1,10-phenanthroline in the presence of fluorides, sulfates, and phosphates followed by the addition of an excess of perchloric acid. The insoluble ferrous perchlorate complex of 1,10-phenanthroline is formed and the amount of ferrous iron present is estimated by measurement of the volume of the precipitant following centrifugation. The fact that fluorides, sulfates, and phosphates do not interfere adds to the value of the procedure since hydrofluoric, sulfuric, and phosphoric acids or mixtures of the same may be used as solvents for the sample analyzed. An insoluble silicate may therefore be dissolved in a mixture of hydrofluoric and sulfuric acids in an inert atmosphere and the ferrous iron might be conceivably determined by the described procedure applied directly on the solution thus prepared.

Apparatus. Standard Goetz tubes used for the determination of phosphorus in steel on a macro-basis following centrifugation, were employed. Their 100-ml. capacity was no disadvantage although far too large, but the trunnion cups used as their support and the International Equipment Company centrifuge with which they are employed is none the less suitable, and was at hand. The capillary tubes of the Goetz apparatus for determination of phosphorus in steel was of far too great capacity and these were replaced by much smaller capillaries. The total capacity of the capillary tube was found to be 2×10^{-5} ml. One millimeter of the capillary had a volume of 8×10^{-7} ml. and their total length was approximately 25 ml.

The remainder of the equipment required is standard laboratory apparatus including a suitable weight buret to increase precision.

Solutions. 1,10-phenanthroline. 1.485 g. of the monohydrate dissolved in 100 g. of solution (.025M).

$FeSO_4 \cdot 7H_2O$. 0.5 g. per 100 g. of solution. (1 mg. of iron per g. of solution.) Enough H_2SO_4 was added to prevent hydrolysis.

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* Data from the Thesis by J. E. DeVries, Analytical Applications of Modified Phenanthrolinum and Related Ions. University of Illinois, 1944.

TABLE XXI

The Determination of Ferrous Iron Using 1,10-Phenanthroline and Perchloric Acids as Precipitant

No.	Material Deter-mined	Weight Taken	Material Added	Ration of Ions	Volume Ppt. ml. x10^{-6}	Weight Anion Found	Weight of Precipi-tant	Number of Samples
1	$FeSO_4 \cdot 7H_2O$	0.25	......	......	3.10	....	0.20	4
2	$FeSO_4 \cdot 7H_2O$	0.50	......	......	5.50	....	0.30	4
3	$FeSO_4 \cdot 7H_2O$	0.75	......	......	8.00	....	0.40	4
4	$FeSO_4 \cdot 7H_2O$	1.00	......	......	11.20	....	0.50	4
5	$FeSO_4 \cdot 7H_2O$	1.25	......	......	14.40	....	0.60	4
	Fe^{++}		$Fe_2(SO_4)_3$					
6	Fe^{++}	0.10	0.3	1-2	5.6	0.101	0.30	4
7	Fe^{++}	0.10	0.6	1-6	5.6	0.101	0.30	4
8	Fe^{++}	0.10	0.9	1-9	5.6	0.101	0.30	4
9	Fe^{++}	0.05	0.9	1-18	3.2	0.06	0.30	4
	$FeSO_4 \cdot 7H_2O$		NaF					
10	$FeSO_4 \cdot 7H_2O$	0.75	3.0	1-4	8.8	0.77	0.40	2
11	$FeSO_4 \cdot 7H_2O$	0.50	5.0	1-10	5.7	0.52	0.30	3
12	$FeSO_4 \cdot 7H_2O$	0.50	10.0	1-20	5.6	0.51	0.30	3
13	$FeSO_4 \cdot 7H_2O$	0.50	20.0	1-30	5.7	0.52	0.30	3
			K_2SO_4					
14	$FeSO_4 \cdot 7H_2O$	0.50	5.0	1-10	5.6	0.51	0.30	2
15	$FeSO_4 \cdot 7H_2O$	0.50	10.0	1-20	5.6	0.51	0.30	2
16	$FeSO_4 \cdot 7H_2O$	0.50	15.0	1-30	5.6	0.51	0.30	2
17	$FeSO_4 \cdot 7H_2O$	0.50	20.0	1-40	6.4	0.57	0.30	2
			$NaH_2PO_4 \cdot H_2O$					
18	$FeSO_4 \cdot 7H_2O$	0.50	5.0	1-10	5.6	0.51	0.30	2
19	$FeSO_4 \cdot 7H_2O$	0.50	10.0	1-20	5.6	0.51	0.30	2
20	$FeSO_4 \cdot 7H_2O$	0.50	15.0	1-30	5.6	0.51	0.30	2
21	$FeSO_4 \cdot 7H_2O$	0.50	20.0	1-40	5.6	0.51	0.30	2

$Fe_2(SO_4)_3 \cdot 9H_2O$. 1.5 g. per 100 g. of solution. (3 mg. of iron per g. of solution.)

NaF. 1 g. per 100 g. of solution.

$K_2SO_4 \cdot$0.2 g. per 100 g. of solution.

$NaH_2PO_4 \cdot H_2O$. 2 g. per 100 g. of solution.

$HClO_4$. 1 molar solution.

TABLE XXII

Minimum Precipitant Requirements and Calibration Data for the Determination of ClO_4^-, IO_4^-, $S_2O_8^{-2}$, and MoO_4^{-2} Anions

No.	Sample	Sample Weight	Precipi-tant ml. 0.025 M. Sol'n	Vol. Ppt. ml. x 10^{-6}	Samples
		mg.			
1	$KClO_4$	0.50	0.25	0.00	2
2	$KClO_4$	0.50	0.35	3.20	5
3	$KClO_4$	0.50	0.50	11.30	7
4	$KClO_4$	0.50	0.75	14.40	7
5	$KClO_4$	0.50	0.85	14.40	5
6	$KClO_4$	0.50	1.00	15.20	5
7	$NaIO_4$	1.00	0.20	2.40	3
8	$NaIO_4$	1.00	0.30	6.40	3
9	$NaIO_4$	1.00	0.35	8.40	3
10	$NaIO_4$	1.00	0.50	9.60	3
11	$NaIO_4$	1.00	0.75	11.40	3
12	$NaIO_4$	1.00	1.00	12.20	3
13	$K_2S_2O_8$	0.50	0.25	0.00	3
14	$K_2S_2O_8$	0.50	0.35	6.80	3
15	$K_2S_2O_8$	0.50	0.50	10.40	3
16	$K_2S_2O_8$	0.50	0.75	13.00	3
17	$K_2S_2O_8$	0.50	1.00	13.60	3
18	Na_2MoO_4	0.50	0.10	5.60	3
19	Na_2MoO_4	0.50	0.20	15.64	3
20	Na_2MoO_4	0.50	0.30	18.40	3
21	Na_2MoO_4	0.50	0.40	17.60	3
22	Na_2MoO_4	0.50	0.50	18.40	3
23	Na_2MoO_4	0.50	0.60	19.20	3

Illustrative Procedure. From a weight buret of 0.3 g. of 1,10-phenanthroline solution (0.006 millimoles for precipitating iron, and excess) was added to the Goetz tube in which the capillary was previously filled with water. To this was added 0.3 g. of ferric sulfate solution (equivalent to 0.9 mg. of iron) and 0.1 g. of ferrous sulfate solution (equivalent to 0.1 mg. of iron or 0.002 millimoles). To the resulting red complex was added 0.5 ml. of 1N perchloric acid (approximately 100 times excess for the reaction). After centrifuging for 4 minutes at 2,000 r.p.m. the height of the precipitate was 7 mm. which is equivalent to 5.6×10^{-6} ml., corresponding to 0.101 mg. of iron.

This procedure was applied to ferrous sulfate alone, varying the size of the sample, and then in the presence of ferric sulfate up to 20 times excess compared to ferrous iron, sodium fluoride up to 30 fold excess, potassium sulfate 30 fold excess, and sodium dihydrogen phosphate up to a 40 fold excess. The results of these separations are given in Table XXI.

TABLE XXIII

The Determination of Perchlorate, Persulfate and Periodate in the Presence of Chlorate, Sulfate and Iodate 5-Nitro, 1,10-Phenanthroline Ferrous Sulfate as Precipitant

Determination of	Weight Sample	Substance Added	Weight Substance Added	Ratio of Ions	Volume Ppt. ml. $v10^{-6}$	Weight Found	No. Samples
	mg.		*mg.*			*mg.*	
$KClO_4$	0.375	$KClO_3$	3.0	1-8	10.3	0.370	5
	0.375	$KClO_3$	6.0	1-16	10.4	0.370	3
	0.375	$KClO_3$	7.5	1-20	10.4	0.370	3
	0.375	$KClO_3$	15.0	1-40	10.4	0.370	3
$K_2S_2O_8$	0.40	K_2SO_4	2.0	1-5	9.60	0.40	4
	0.20	K_2SO_4	2.0	1-10	1.60	0.20	4
	0.20	K_2SO_4	4.0	1-20	1.60	0.20	4
$NaIO_4$	0.50	KIO_3	2.5	1-5	4.00	0.51	4
	0.50	KIO_3	5.0	1-10	4.20	0.51	4
	0.50	KIO_3	10.0	1-20	4.00	0.50	5

The first 5 values of Table XXI were used to make the calibration graph shown in Figure 24. The values in the remainder of Table II obtained from this graph.

Data for similar calibration graphs and for the determination of minimum precipitant requirements in the case of the precipitation of perchlorate, periodate, persulfate, and molybdate are given in Table XXII. In all cases 5-nitro-1,10-phenanthroline was used as precipitant.

The data of Table XXII was used to determine the minimum amount of precipitant required for complete precipitation of the various anions, ClO_4^-, IO_4^-, $S_2O_8^{-2}$, and MoO_4^{-2}.

The determination of perchlorates, periodates, and persulfates in presence respectively of chlorate (reduced to chloride in the presence of perchlorate using SO_2), iodate, and sulfate, in increasing ratios were then carried out and the results are shown in Table XXIII. The separation of molybdate from tungsten failed.

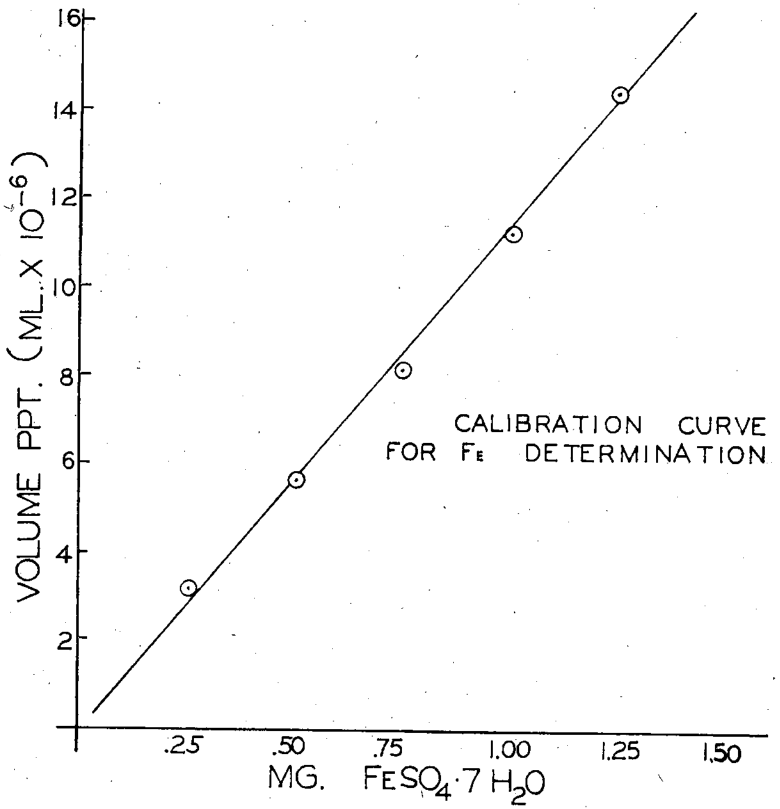

FIG. 20.