THE IRON REAGENTS

G. FREDERICK SMITH CHEMICAL COMPANY
867 McKinley Avenue
Columbus, Ohio 43223

ANALYTICAL CHEMICALS SINCE 1928

THE IRON REAGENTS

Third Edition

1980

Published by

G. FREDERICK SMITH CHEMICAL COMPANY
867 McKinley Avenue
Columbus, Ohio 43223

TABLE OF CONTENTS

PREFACE

The first edition of *The Iron Reagents*, written by Diehl and Smith, was published in 1960 and Fourteen thousand copies were distributed in only four years. After its revision in 1965 by McBride and Cryberg, an additional 20,000 copies were distributed. Now several new reagents have been introduced and this new information is featured in this edition. Some of the procedural material for the older reagents has been eliminated in order to allow coverage of newer and less familiar procedures. Even so, a complete bibliography of the pertinent literature is included.

The author of this edition is pleased to acknowledge the assistance of Norita Mohamed, who performed the literature search, and the contributions of Professor Alfred A. Schilt, who contributed some of the material and reviewed the manuscript prior to publication.

Loren McBride

SECTION 1

INTRODUCTION

Iron is the fourth most abundant element in the crust of the earth and is widely distributed in nature. Due to its many commercial uses, its effects in trace amounts on manufactured products, and its vital role in living organisms, the determination of iron is one of the most common determinations performed in chemical analysis.

Numerous procedures have been developed for the determination of iron in various samples and concentration ranges, some for trace levels as low as a fraction of a part per billion. It is to reagents for the low concentration levels that this monograph addresses its attention.

With but one exception, the reagents discussed herein were developed as a consequence of the tireless and inspired research efforts of Professor Francis H. Case of Temple University. Analytical studies and applications for the compounds synthesized by Professor Case were carried out by Professors G. F. Smith, Harvey Diehl and A. A. Schilt, with their students and associates.

The iron reagents described in this monograph are suitable for a number of different determination procedures and methods. All enable determination of iron in the ppm range in aqueous media, some are practical for the determination of iron in the ppb range by extraction into an immiscible solvent, others are suitable for the simultaneous determination of iron and copper, another allows the determination of iron in strong alkalis, and still another is ideally suited to the determination of iron in acids. Some of the reagents can also serve in the indirect determination of reducing agents and certain anions.

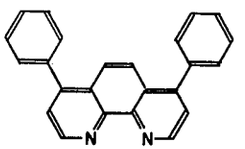
In so much as all of the reagents have been studied for sensitivity, interferences, pH effects, solvent effects and specificity as well as applications, it should be possible to adapt at least one of the reagents to apply to nearly any circumstance for which the determination of iron in the parts per billion or parts per million ranges is desired.

All the reagents, buffers, reducing agents, etc. that are available from the G. Frederick Smith Chemical Co. are denoted by GFS and are listed in the back of the book.

SECTION II

BATHOPHENANTHROLINE

4,7-Diphenyl-1,10-phenanthroline



$C_{24}H_{16}N_2$ Mol. Wt.: 332.41
Molar Absorptivity of
Fe (bathophenanthroline)$_3^{++}$ in
70 per cent water-30 per cent
ethyl alcohol: 22,140 at 543 nm
isoamyl alcohol: 22,350 at 533 nm
nitrobenzene: 23,300 at 538 nm
G. Frederick Smith Chemical Company,
Catalog Item No. 108

4,7-Diphenyl-1,10-phenanthroline is a sensitive and highly specific reagent for iron. It was first prepared by Case[1] and investigated as an analytical reagent by Smith, McCurdy and Diehl.[2] The common name Bathophenanthroline was assigned to 4,7-diphenyl-1,10-phenanthroline because the absorption maximum of the ferrous derivative lies at a longer wave length than that of 1,10-phenanthroline, that is, 533 nm compared with 510 nm, a bathochromic shift.

Not only is the molar absorptivity of the ferrous bathophenanthroline ion (22,350) greater than that of ferrous 1,10-phenanthroline (11,100) but it can also be extracted from aqueous solutions with certain immiscible solvents, such as isoamyl alcohol or *n*-hexyl alcohol. Two important advantages are gained from this: the iron in large samples can be easily concentrated into a small volume for measurement and the solutions of the necessary reagents can be freed from iron, thus reducing the blank to zero.

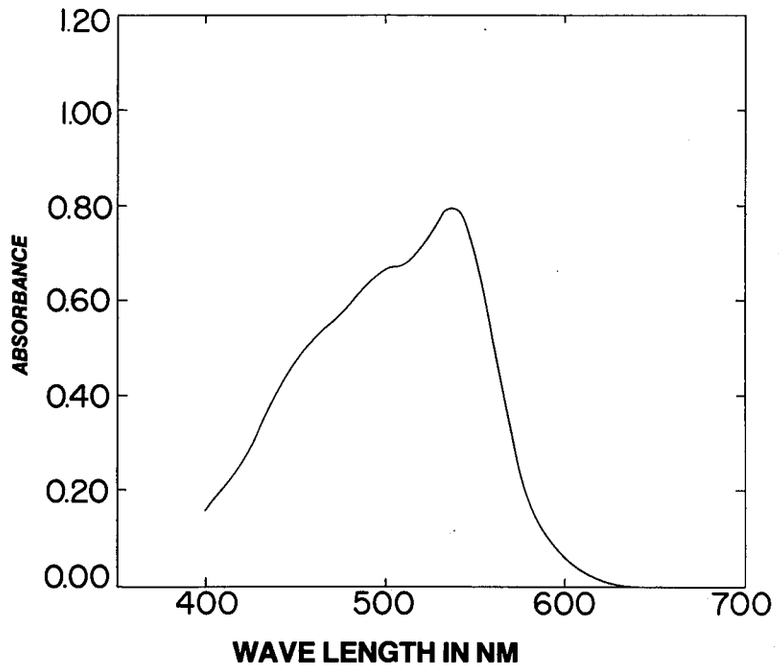


Fig. 1. Absorption spectrum of Fe(bathophenanthroline)$_3^{++}$ in isoamyl alcohol. 2.08 P.p.m. of iron. 1-cm cell.

All the iron in the sample must be in the ferrous form. The reduction of any ferric iron is most conveniently brought about by adding hydroxylammonium chloride.

The bathophenanthroline ferrous ion is stable over the pH range 2 to 9 but forms most rapidly in slightly acid solution, pH 4. If the reaction is carried out in a slightly acid solution, one buffered with acetate, copper does not interfere.

The absorption spectrum of the ferrous-tris(bathophenanthroline) ion is shown in Fig. 1. At the wave length of maximum absorption, 533 nm the molar absorptivity of the complex is 22,350. In water-ethyl alcohol solution, the wave length of maximum absorption is 543 nm and the molar absorptivity is 22,140.

The color is stable for long periods of time.

The oxidation-reduction potential of the ferrous (bathophenanthroline)$_3^{++}$ couple was determined by Smith and Banick[3] using potential buffered solutions of vanadate and vanadyl ions, present in equimolar concentrations, and dissolved in various strengths of sulfuric acid. By this method E_0 (hydrogen scale) was found to be 1.13 volts.

Applications. The general procedure for the determination of iron with bathophenanthroline was worked out by Smith, McCurdy and Diehl[2], who applied it to the determination of iron in raw and treated municipal water supplies. A number of subsequent papers have dealt with iron content of waters.[11,15,16,17,18,35,53,54,55,56,57,58,59] Iron in gold[20,60], beryllium[21], bismuth[10], tungsten[4,7,9], molybdenum[5,9], vanadium[7], chromium[7], titanium[7,67], niobium[7,9], uranium[7,8], gallium[62,64], arsenic[62], aluminum alloy[55], plutonium[66], nickel alloy[68], copper[2,7,8,9,50,51], zirconium[61], phosphates[61], alums[63], and salt[65] has been determined using bathophenanthroline. The iron present in aluminum oxide is important in the properties of crystals grown for lasers[23]. Bathophenanthroline has been investigated many times for the determination of iron and iron binding capacity in serum[12,13,24,25,26,27,28,29,30,32,33,34,69,70,71,72,73,74], in urine[36,37,38,48,75], in wine[38,76], in oil and fuels[39,77], in culture media[40], in plant tissues[78-80], in plasma[41], in butterfat[81,82], and in starting materials and intermediates for polymers and fibers[83]. In addition, several papers reported studies of the determination of ferrous in the presence of ferric ion[14,42,84,85], of ferrous iron in soils[43], and of ferric iron in ammonium salts[86], and sodium chloride[65]. Bathophenanthroline has also been used to determine, indirectly, the amount of certain reducing agents. It is said to more sensitive than many other reagents used in the determination of tocopherols[44,45], and corticoids[46]. It has also been used for the determination of hydrogen peroxide[47] and ferrocyanide[31]. Iron-bearing tissues have been stained using bathophenanthroline[48].

Specificity. Interferences. The familiar anions, chloride, sulfate, nitrate, acetate and perchlorate, do not interfere in the determination of iron with bathophenanthroline. If copper is present, certain anions such as iodide, thiocyanate, cyanide, thiosulfate, sulfide, and phosphate may cause precipitation in the aqueous solution upon addition of bathophenanthroline, but such precipitates do not interfere with the quantitative extraction of the ferrous-bathophenanthroline compound. Certain less common anions such as oxalate[4], citrate[4], lactate[5], fluoride[4,5] pyrophosphate[5] and phosphate[4] can interfere. This problem can be obviated by reducing the ferric iron in a strongly acidic solution by means of ascorbic acid, the acid reduction method, followed by the addition of bathophenanthroline and pH adjustment to the normal working range of 4 to 6 in the given order. Tartrate causes low results unless the extraction is made into chloroform in the presence of perchlorate[5,6]. Cyanide interferes by forming dicyano-bisbathophenanthroline Iron (II). Molybdates, tungstates and antimony chloride hydrolyze with formation of precipitates under the conditions of the determination; however, this problem can be solved by addition of tartrate to complex these metals, followed by a chloroform extraction of the bathophenanthroline-iron complex[5,6,7].

The following metal cations do not interfere: Li, Na, K, Be, Mg, Ca, Sr, Ba, Ce(IV), Ce(III), Pr(III) and the rare earths in general, Th, Ti, Zr, V

as vanadate and vanadyl, Cr(III), W, U, Mn, Fe(III), Ru(III), Os, Ni, Pd, Pt, Ag, Zn, Cd, Hg(I), Hg(II), B, Al, Ga, Tl(I), Sn(II), Sn(IV), Pb. Tellurium and selenium are reduced to the metal by hydroxylammonium chloride. Cobalt forms a light yellow color but this is not extracted from acid solution. When sodium hydrosulfite is used as the reducing agent an extractable brown compound is formed with cobalt which causes a significant error[4]. At a pH of 4 copper combines with bathophenanthroline to form the extractable cuprous-monobathophenanthroline ion. This complex is colorless and does not interfere unless insufficient bathophenanthroline is present to complex both the iron and copper present[2,7]. In the presence of large amounts of copper separation prior to determination can be performed by extraction of ferric chloride into ethyl acetate[8], or perhaps more conveniently by complexing the copper with thiourea[9]. Hair and Newman[51] precipitate the copper as cuprous thiocyanate and following filtration determine the iron in an aliquot of the filtrate. Manganese also interferes by competition for bathophenanthroline. Addition of excess bathophenanthroline overcomes this difficulty.

Reduction of Ferric Iron. If all iron present is in an uncomplexed state, hydroxylamine hydrochloride is the reducing agent usually used. Ascorbic acid, hydrazine hydrochloride or hydroquinone are also satisfactory if purified to free them from traces of iron contamination. In the presence of citrate, oxalate, tartrate or fluoride, reduction is incomplete with these reducing agents at pH of 4. Sodium hydrosulfite ($Na_2S_2O_4$) is however, satisfactory as a reducing agent even in the presence of the above mentioned ions. The iron contaminant in sodium hydrosulfite may be removed by the addition of bathophenanthroline followed by extraction into chloroform; however, the solution thus purified is not stable and should not be stored more than one-half hour, according to Gahler and coworkers[7]. Booth and Evett[10] used stannous chloride as the reducing agent added to a strongly acidic solution, followed by addition of citrate and EDTA to complex other metals present, then addition of buffer to raise the pH to the desired level. Penner and Inman[9] and Cluley and Newman[6] recommend ascorbic acid for the reduction of iron at a low pH. Thioglycolic acid has been used by Tetlow and Wilson[11] to both dissolve particulate iron and reduce any ferric iron present to the ferrous state.

Extraction. Isoamyl, *n*-amyl or *n*-hexyl alcohol may be used for the extraction. The distribution coefficient of the iron (II) complex of bathophenanthroline between these alcohols and water could not be readily determined because of the great solubility of the complex in the alcohols. However, the complex is more completely removed by one extraction with *n*-hexyl alcohol than by similar treatment with isoamyl or *n*-amyl alcohol. This is probably the consequence of *n*-hexyl alcohol being the least water-soluble alcohol of the group. Under ordinary conditions two extractions with isoamyl alcohol are sufficient to completely recover the iron. *n*-Hexyl alcohol may be preferable where the volume of sample is large and minute

amounts of iron are present. The reagent bathophenanthroline as well as its ferrous complex is extracted quantitatively from essentially neutral aqueous solution by the immiscible alcohols.

Nitrobenzene may also be used to effect the extraction of the ferrous bathophenanthroline ion. The molar absorptivity is somewhat greater in nitrobenzene than in the alcohol solvents, 23,300 at the wave length of maximum absorption, 538 nm. The turbidity which occasionally develops in the isoamyl alcohol extracts, particularly when perchlorate is present, does not appear in nitrobenzene. Repeated extractions can be made with ease if necessary, inasmuch as the nitrobenzene is the lower phase. Although nitrobenzene has a slight yellow color, its absorbance is zero at 538 nm and no correction needs to be made for it. After separation of the water and nitrobenzene layers the nitrobenzene solution is diluted with ethanol to the final volume; the ethanol renders miscible any droplets of water carried along with the nitrobenzene and has no effect on the intensity of the color.

Other solvents which have been used to extract the ferrous complex from the aqueous phase are octyl alcohol[12], chloroform[4,5], a chloroform-ethanol mixture[7,13] trichloroethane and isoamyl acetate[14]. In the determination of ferrous iron in the presence of ferric iron[14], Clark found that isoamyl acetate was indeed the only satisfactory solvent with which to carry out the extraction. Chloroform or chloroform-ethanol mixtures are recommended by several workers [4,7,13].

PROCEDURES FOR THE DETERMINATION OF IRON

REAGENTS. BATHOPHENANTHROLINE SOLUTION. Dissolve 0.0334 g of 4,7-diphenyl-1,10-phenanthroline (GFS) in 50 ml of ethyl alcohol and dilute with 50 ml of iron-free water. This is a 0.001 *M* solution. (GFS)

NITROBENZENE. Use reagent grade nitrobenzene.

ISOAMYL ALCOHOL. (GFS) Reagent grade isoamyl alcohol can be used directly, but redistilled is preferred.

IRON-FREE WATER. Distilled water may be passed through a column of monobed ion-exchange resin for extra protection, if desired.

NITRIC ACID. Use redistilled nitric acid. (GFS)

PERCHLORIC ACID. Use double-distilled perchloric acid. (GFS)

AMMONIUM HYDROXIDE. Reagent grade ammonium hydroxide may be used, or anhydrous ammonia may be distilled into iron-free water. (GFS)

HYDROCHLORIC ACID. Use redistilled hydrochloric acid. (GFS)

ETHANOL. Use reagent grade ethyl alcohol.

SODIUM ACETATE SOLUTION. (GFS) Dissolve 50 g of sodium acetate trihydrate in 100 ml of distilled water. Put in a separatory funnel and add 2 ml of 10% hydroxylammonium chloride, 3-4 ml of bathophenanthroline solution and 10-20 ml of isoamyl alcohol. Shake thoroughly and then allow the layers to separate. Draw off the lower layer and repeat the extraction to insure complete removal of iron. This stock solution may be used as is or diluted to 10% or 20% as needed.

HYDROXYLAMMONIUM CHLORIDE SOLUTION. Dissolve 50 g of hydroxylammonium chloride in 100 ml of distilled water, transfer to a separatory funnel and add 3-4 ml of bathophenanthroline solution and 10-20 ml of isoamyl alcohol. Shake thoroughly, and then allow the layers to separate. Draw off the lower layer, and repeat the extraction to insure complete removal of iron. This stock solution may be used as is or diluted to 10% or 20% as needed.

STANDARD IRON SOLUTION. 10.0 μg Fe per ml, 1.00 μg Fe per ml. Prepare two standard iron solutions, containing respectively 10.0 μg. and 1.00 μg. of iron per ml, starting with ferrous ethylenediammonium sulfate tetrahydrate or electrolytically prepared iron metal. If the salt is used proceed as follows. Weigh carefully 0.0684 g of ferrous ethylenediammonium sulfate tetrahydrate and transfer to a 1-liter volumetric flask. Add iron-free water to dissolve the salt, add 2.5 ml of concentrated sulfuric acid, dilute exactly to the mark with iron-free water, and mix well. Pipet 100.0 ml of this solution into a second 1-liter volumetric flask, add 2.5 ml of sulfuric acid, dilute to the mark with iron-free water, and mix well. The first of these solutions contains 10μg of iron per ml; the second contains 1μg of iron per ml.

Alternately, make up the standard iron solution starting with electrolytic iron. Weigh accurately about 0.10 g of iron and transfer to a 500-ml conical flask. Dissolve the iron in a mixture of 5 ml of concentrated sulfuric acid and 30 ml of water. Transfer the solution to a 1-liter volumetric flask and dilute to the mark with iron-free water. Pipet 10.0 ml of this solution into another 1-liter volumetric flask, add 2.5 ml of concentrated sulfuric acid and dilute to the mark with iron-free water. Because the electrolytic iron comes in small pieces and it is impractical to weigh exactly 0.1000 g, this final solution will contain only approximately 1 μg of iron per ml but the exact value will be known from the weight taken and the two dilutions made.

GENERAL PROCEDURE. Carry several solutions through the process together; for example, two samples of the unknown to be analyzed, three or four standards, and a blank. Once a calibration curve has been established, further standards need not be run except as an occasional check. For the standards, use various amounts of the standard iron solution containing 1.00 μg of iron per ml, for example: 1.00, 2.00, 5.00, 10.0 and 15.0μg of iron. For the sample, choose a volume which will contain between 1 and 15μg of iron.

Pipet the sample or standard iron solution into a 60 ml separatory funnel. If the sample taken was less than 10 ml add sufficient iron-free water to bring the volume to 10 ml. Use 10 ml of iron-free water for the blank. To each sample add 2 ml of 10 per cent hydroxylammonium chloride solution and add 4 ml of 10 per cent sodium acetate solution. If the original sample of the water had been acidified with hydrochloric acid when taken, add an additional 4 ml of sodium acetate solution. Add 4 ml of 0.001 *M* bathophenanthroline and mix. Add 6.0 ml of isoamyl alcohol, stopper the funnel, and shake the mixture well. After the liquids have cleanly separated into two layers, draw off and discard the lower aqueous layer. Shake away any of the aqueous layer remaining in the separatory funnel. Drain the isoamyl alcohol layer into a 10 ml volumetric flask. If more than 10 ml are required to fill the cell of the colorimeter to be used later, use a 25 ml volumetric flask rather than a 10 ml flask at this point. Wash

out the separatory funnel with 2 to 3 ml of ethyl alcohol added from a pipet in such a manner that the upper stopper of the funnel and the walls of the funnel are uniformly washed at least twice by a film of alcohol as it drains from the top to the bottom. Transfer this wash alcohol to the volumetric flask. Dilute the solution in the flask to the mark with ethyl alcohol and mix by shaking. At this point the solution in the volumetric flask should be clear with no turbidity.

Measure the absorbance of the solution at 533 nm. Plot the data obtained on the standards after subtracting the absorbance of the blank, plotting absorbance against concentration. Use the calibration curve to obtain the amount of iron in the sample analyzed.

Nessler Tube Procedure for Iron in the Range 0.01 to 0.1 ppm Carry several solutions through the process together; for example, two samples of the unknown, a blank, and three or four standards. The colorimetric comparison is later to be made in Nessler tubes, and for routine work a series of standards can be made and preserved for a long period.

Pipet 100 ml of the water to be tested into a 125 ml separatory funnel. For the standards pipet the desired volumes, for example, 1.00, 2.00, 3.00, ..., 10.0 ml of the standard iron solutions containing 1μg of iron per ml into 100 ml of iron-free water in a 125 ml separatory funnel. Add 2 ml of iron-free, 10 per cent hydroxylammonium chloride solution. Add 4 ml of iron-free, 10 per cent sodium acetate solution; if the sample taken contained any free acid, add additional sodium acetate solution. Add 4 ml of 0.001 *M* bathophenanthroline and mix well. Add 10 ml of isoamyl alcohol. Shake the mixture well and then allow it to stand for 5 minutes. Draw off the aqueous layer into a second 125 ml separatory funnel. Add to this 10 ml of isoamyl alcohol, shake well and allow to stand until the layers have separated. Draw off and discard the aqueous layer. Transfer both colored isoamyl alcohol extracts to a 50 ml volumetric flask, rinsing both separatory funnels with alcohol, and mix thoroughly. Transfer to a 50 ml Nessler tube. Carry out the comparison by looking down through the full length of the tubes toward a sloping, white, reflecting background such as provided in the conventional Nessler rack.

Inasmuch as unknown and standards were diluted to the same volume, the unknown contains the same weight of iron as that in the standard which it matches. From this weight and the volume of sample taken, calculate the concentration of iron in the sample in ppm.

Determination of Iron in Raw and Treated Waters. Untreated, or raw, well water will normally contain from one-tenth of a part to five parts per million of iron. For this range, the general procedure given above, using a 1 to 10 ml sample of the water, is applicable. Municipal water plants generally supply city water mains with a product that is sufficiently free from iron to fall within the range covered by the Nessler tube procedure given above. A sample of 100 ml is used.

Sampling of Raw Water. It is important to sample well water at the source and to perform the analysis immediately after taking the sample. If the latter is not possible, fill the sample bottle completely full to help retard oxidation of iron by contact with atmospheric oxygen. If an appreciable length of time is likely to elapse between sampling and analysis, add 2 ml of iron-free hydrochloric acid to the sampling bottle before sampling to ensure that no precipitation of iron occurs.

Procedure for the Determination of Iron in Wine. DIRECT EXTRACTION METHOD OF BANICK AND SMITH[38]. In this procedure, carry a blank through the same operations omitting the wine. Pipet into a 100 ml beaker: (1) 5.00 ml of wine, (2) 5.0 ml of sodium acetate-acetic acid buffer (approximately 10 per cent sodium acetate adjusted to pH 4 by the addition of acetic acid), (3) 2.0 ml of 10 per cent hydroxylammonium chloride, (4) 2.0 ml of 0.001 *M* bathophenanthroline, and (5) (for red wines only, omit for white wines) 2.0 ml of 95 per cent ethanol. Heat the mixture to a gentle boil and boil for one minute. Transfer the solution while still hot to a 60 ml glass-stoppered separatory funnel. Add 6 ml of isoamyl alcohol and shake for 30 seconds, allow the mixture to stand for three minutes to permit the liquids to separate, and then draw off the lower, aqueous layer. Rinse the original beaker with 1 ml of 95 per cent ethanol and two 5 ml portions of water, transferring the rinsings to the separatory funnel containing the isoamyl alcohol layer. Add 10 ml of buffer solution (pH4) and shake the mixture for 30 seconds. Allow five minutes for the liquid phases to separate and draw off the lower layer. Transfer the isoamyl alcohol layer quantitatively to a 10-ml volumetric flask and dilute to the mark with 95 per cent ethanol. Measure the absorbance of this solution at a wave length of 533 nm. Obtain the amount of iron present from a calibration curve prepared in the same manner using appropriate volumes of a standard iron solution. Subtract the amount of iron in the blank and convert the net weight of iron in μg per 5.00 ml of sample to parts per million of iron for reporting.

Procedure for the Determination of Iron in Serum. METHOD OF PETERSON[24]. Pipet 1 to 2 ml of serum or plasma into a 15 ml centrifuge tube, the volume depending upon the anticipated amount of iron. Dilute to a volume of 6 ml with iron-free water and mix. Add 2 ml of a solution containing 20 g of redistilled trichloroacetic acid and 1 g of thioglycolic acid per 100 ml. Mix and allow to stand 5 to 10 minutes. Place in a water bath at 90 to 95°c for 10 to 15 minutes and then centrifuge. Decant the supernatant liquid into a 20 ml glass-stoppered test tube or a 25 ml glass-stoppered graduated cylinder. To the precipitate in the centrifuge tube add 2 ml of water and 0.5 ml of the trichloroacetic acid-thioglycolic acid reagent and mix. Return this tube to the water bath at 90 to 95°C for 5 to 10 minutes. Remove, centrifuge, and decant the supernatant liquid into the first supernatant liquid. Add 2 ml of saturated sodium acetate solution to the combined supernatant liquid, bringing the pH of the solution to 4.0 to 5.0. Add 2 ml of 0.0025 *M* bathophenanthroline in isoamyl alcohol, 0.5 ml of the reagent being required for each microgram of iron present. Add isoamyl alcohol to a total volume of 6 ml, stopper the tube and shake vigorously. Pipet the isoamyl alcohol layer into a spectrophotometer cell and measure the absorbance at 533 nm. Carry standards containing 2 to 4 μg of iron through the same procedure.

Procedure for the Determination of Iron in Serum. METHOD OF PETERS, GIOVANNIELLO, APT AND ROSS[27]. Pipet 2.0 ml of serum or plasma (fresh or stored, citrated, oxalated, or heparinized) into a test tube. Add 3.0 ml of 0.2 *M* hydrochloric acid and 1 drop of an 80 per cent solution of thioglycolic acid. Mix and allow to stand 30 minutes at room temperature. Add 1.0 ml of a 30 per cent solution of redistilled trichloroacetic acid. Mix with a stirring rod and allow to stand 15 to 30 minutes at room temperature, covered with a paraffin film. Centrifuge for about 15 minutes at high speed. Pipet 4.0 ml of the supernatant liquid into a colorimeter cell. Add 0.5 ml of saturated sodium acetate solution and 2.0 ml of bathophenanthroline solution prepared by dissolving 0.020 g of 4,7-diphenyl-1, 10-phenanthroline in 100 ml of a mixture consisting of three parts of isopropyl alcohol and one part of isoamyl alcohol. Mix well and measure the absorbance at 535 nm.

Carry known amounts of iron, obtained by measuring out portions of the standard solution described under General Procedure, through the determination and prepare a suitable calibration curve.

Micro Procedure for the Determination of Iron in Serum. METHOD OF FORMAN[13]. Pipet 100 μl of serum into a 400 μl micro-test tube and add 50 μl of 0.4 *M* hydrochloric acid and 1 drop of 80 per cent thioglycolic acid. Mix and allow to stand 5 minutes. Add 50 μl of 30 per cent trichloroacetic acid. Mix well with a micromixer centrifuge for 3 minutes at 10,000 rpm. Transfer 180 μl of the clear supernatant liquid to a micro-test tube and add 25 μl of potassium acetate (50 g per 100 ml) and 20 μl of .025 % bathophenanthroline in isopropyl alcohol. Mix thoroughly. After 10 minutes, extract with 200 μl of ethanol-chloroform solvent (1:4). Shake and centrifuge. Transfer the lower layer to a micro cell and measure the absorbance at 535 nm. Carry blank and standards through the same procedure.

Procedure for the Determination of Iron in Urine. METHOD OF SEVEN AND PETERSON[49]. Pipet 5 to 10 ml of urine into a 250 ml conical flask, add 0.75 ml of concentrated sulfuric acid and 5 ml of concentrated redistilled nitric acid. Insert a reflux head and digest on a hot plate at a temperature of about 250°c until the thick brown fumes almost disappear and the remaining solution is nearly colorless. Cool partially and wash down the reflux head and the walls of the flask with iron-free water. Add 5 ml of 30 per cent hydrogen peroxide through the reflux head and heat until the gas evolution stops. Cool partially and add 2 ml of 30 per cent hydrogen peroxide. Heat for 1 hour at an intermediate temperature not over 250°, making certain that the liquid condensed is being returned to the boiling solution. Cool, wash down the reflux head and remove it. Wash the walls of the flask until at least 20 ml of iron-free water has been added. Add 0.2% potassium permanganate dropwise (usually 1 to 3 drops) until a faint pink color persists. Add 3 ml of 10% hydroxylammonium chloride. Mix the solution and add 15 ml of saturated sodium acetate. Mix and add 4.00 ml, pipetted carefully, of 0.0025 *M* bathophenanthroline in isoamyl alcohol and immediately close the flask with a rubber or glass stopper. Shake vigorously until no further red color development is apparent, usually 60 to 90 seconds. When alcohol and aqueous phases have completely separated, aspirate the aqueous layer and discard. Close the flask immediately. Draw off enough isoamyl alcohol-bathophenanthroline solution to fill a cuvette and measure the absorbance at a wave length of 533 nm.

Carry a blank through the same procedure.

Procedure for the Determination of Iron in Urine. METHOD OF COLLINS AND DIEHL[36]. Run a reagent blank along with the samples in exactly the same manner. Pipet 50.0 ml of the urine into a 250 ml conical flask. Add 25 ml of nitric acid and 10 ml of perchloric acid. Place a reflux head on the flask, heat to fumes of perchloric acid and continue the digestion for 10 minutes. If a suitable hood is not available for the wet

ashing use a glass fume eradicator. After the flask and contents have cooled to room temperature, rinse the reflux head with water, and wash down the sides of the flask. Heat the solution to boiling to dissolve the precipitate of ammonium perchlorate and to remove chlorine. While still hot, transfer the solution to a 125 ml separatory funnel and add 2.0 ml of 10% hydroxylammonium chloride and 5.0 ml of 0.001 *M* bathophenanthroline. Place a small piece of Congo Red paper in the solution and add dropwise ammonium hydroxide until the paper turns red. Complete the adjustment of pH by adding 5.0 ml of 10% sodium acetate solution. After the solution has cooled to room temperature add 4.0 ml of nitrobenzene and shake vigorously for 1 minute. Allow the phases to separate, and gently swirl to dislodge any droplets of nitrobenzene clinging to the upper walls of the funnel. Collect the nitrobenzene layer in a 10 ml volumetric flask, and repeat the extraction two more times using 2.0 ml portions of nitrobenzene. Dilute the combined extracts to exactly 10 ml with ethanol and mix. Determine the absorbance of the solution at 538 nm using 1 cm cells. Use a mixture of nitrobenzene and ethanol (4:1) as the reference solution. Correct the absorbance of the unknown solution by subtracting from it the absorbance of the reagent blank.

Prepare a calibration curve by pipeting various volumes from 0 to 25 ml of the standard iron solution (1 μg Fe per ml) into 125 ml separatory funnels. Add 10 ml of 10% ammonium perchlorate , 2.0 ml of 10% hydroxylammonium chloride, 5.0 ml of 0.001 M bathophenanthroline and 8.0 ml of 10% sodium acetate solution, and proceed with the extraction as directed in the preceding paragraph. Use the extract from the solution to which no iron was added as the reagent blank, and subtract its absorbance from the absorbance of each of the other solutions. Prepare a plot of absorbance vs. concentration.

SECTION II. BIBLIOGRAPHY
BATHOPHENANTHROLINE
(4,7-Diphenyl-1,10-Phenanthroline)

1. F. H. Case, *J. Org. Chem.*, **16,** 1541 (1951)	Synthesis of substituted 1,10-phenanthrolines
2. G. Frederick Smith, W. H. McCurdy, Jr., and H. Diehl, *Analyst*, **77,** 418 (1952)	Colorimetric determination of iron in raw and treated water.
3. G. Frederick Smith and Wm. M. Banick, Jr., *Talanta*, **2,** 348 (1959).	Oxidation-reduction potential of the iron-bathophenanthroline complex.
4. R. H. A. Crawley and M. L. Aspinal, *Anal. Chim. Acta.*, **13,** 376 (1955).	Determination of iron in tungsten.
5. D. J. B. Galliford and E. J. Newman, *Analyst*, **87,** 68 (1962).	Determination of iron in molybdenum.
6. H. J. Cluley and E. J. Newman, *Analyst*, **88,** 3 (1963).	Determination of iron using bathophenanthroline.
7. A. R. Gahler, R. M. Hamner, and R.C. Shubert, *Anal. Chem.*, **33,** 1937 (1961).	Determination of iron in vanadium, chromium, titanium, niobium, tantalum, uranium, tungsten metals, alloys, and compounds.
8. R. J. Guest and F. P. Roloson, *Can. Dept. of Mines and Tech. Surveys, Mines Branch, Radioactivity Div., Topical Reports*, TR-137/57, (1956).	Determination of iron in uranium bearing materials.
9. E. M. Penner and W. R. Inman, *Talanta*, **9,** 1027 (1962).	Determination of iron in niobium, tantalum, molybdenum and tungsten metals.
10. E. Booth and T. W. Evett, *Analyst*, **83,** 80 (1958).	Determination of iron in metallic bismuth.
11. J. A. Tetlow and A. L. Wilson, *Analyst*, **89,** 442 (1964).	Determination of iron in boiler feedwater.
12. B. Zak, J. W. Landers and L. A. Williams, *Am. J. Med. Technol.*, **26,** 51 (1960).	Simultaneous determination of iron and copper in serum.
13. D. T. Forman, *Tech. Bull., Registry of Med. Technologists*, **34,** 93 (1964) *Amer. J. Clin. Pathol.*, **42,** 103 (1964).	Determination of iron in serum.
14. L. J. Clark, *Anal. Chem.*, **34,** 348 (1962)	Determination of iron(II) in the presence of iron(III).
15. American Society for Testing Materials, ASTM Standard (1958), Part 10, p. 1244.	Determination of iron in boiler water.
16. W. G. Knapp, *Anal. Chem.*, **31,** 1445 (1959).	Determination of iron in high purity water.
17. A. L. Wilson, *Analyst*, **89,** 389 (1964).	Determination of iron in boiler feedwater.
18. A. L. Wilson, *Analyst*, **89,** 402 (1964).	Determination of iron in boiler feedwater.

19. D. R. Gere and C. E. Melvan, *J. Inorg. Nucl. Chem.*, **25,** 1507 (1963).	Water associated with the tris (bathophenanthroline) ferrous ion on extraction from water to chloroform, nitrobenzene and 1-decanol
20. M. Miyamoto, *Bunseki Kagaku*, **9,** 753 (1960).	Determination of iron in high purity gold.
21. E. N. Pollock and L. P. Zopatti, *Anal. Chim. Acta.*, **28,** 68 (1963).	Determination of iron in high purity beryllium.
22. F. Nakashima and K. Sakai, *Bunseki Kagaku*, **10,** 94 (1961).	Stability constant of the ferrous-bathophenanthroline ion.
23. R. C. Chirnside, J. Cluley, R. J. Powell and P. M. C. Profitt, *Analyst*, **88,** 851 (1963).	The determination of small amounts of iron in sapphire and ruby.
24. R. E. Peterson, *Anal. Chem.*, **25,** 1337 (1953).	Determination of iron in serum.
25. P. Trinder, *J. Clin. Pathol.*, **9,** 170 (1956)	Sulfonation of bathophenanthroline and use of the resulting product to determine iron in serum.
26. G. R. Kingsley and G. Getchell, *Clin. Chem.*, **2,** 175 (1956).	Determination of iron in serum.
27. T. Peters, T. J. Giovanniello, L. Apt, J. F. Ross, and A. P. Trakas, *J. Lab. Clin. Med.*, **48,** 280 (1956).	Determination of iron in serum.
28. B. Zak and N. Ressler, *Anal. Chem.*, **28,** 1158 (1956).	Simultaneous determination of copper and iron in serum.
29. N. Ressler and B. Zak, *Am. J. Clin. Pathol.*, **28,** 549 (1957).	Simultaneous determination of iron and copper in serum.
30. B. Zak, *Clin. Chem. Acta*, **3,** 328 (1958).	Simultaneous determination of iron and copper in serum.
31. M. Avron and N. Shavit, *Anal. Biochem.*, **6,** 549 (1963).	Determination of ferrocyanide using bathophenathroline.
32. D. Kok and F. Wild, *J. Clin. Pathol.*, **13,** 241 (1960).	Determination of iron in serum.
33. T. Matsubara, *Igaku To Seibutsugaku*, **60,** 162 (1961).	Determination of serum iron.
34. R. N. Beale, J. O. Bostrom and R. F. Taylor, *J. Clin. Pathol.*, **14,** 488 (1961).	Determination of iron binding capacity of serum.
35. G. J. Lewis and E. D. Goldberg, *J. Marine Research*, **13,** 183 (1954).	Determination of iron in sea water.
36. P. F. Collins and H. Diehl, *Anal. Chem.*, **31,** 1692 (1959).	Determination of iron in urine after wet oxidation with perchloric acid.
37. J. F. Goodwin, *Anachem. Conference*, Detroit, Michigan, October 23, (1964).	Determination of iron in urine by a non-ashing technique.
38. W. M. Banick and G. Frederick Smith, *Anal. Chim. Acta.*, **16,** 464 (1957).	Determination of iron in urine.
39. J. S. Forrester and J. L. Jones, *Anal. Chem.*, **32,** 1443 (1960).	Determination of iron, nickel and vanadium in petroleum oils.

40. P. A. Seamer, *Nature*, **184,** 636 (1959).	Estimation of microgram quantities of iron in culture medium.
41. A. Quiroz M., C. Dias T., and R. B. Bradfield, *Arch. Venezolanos Nutric.*, **10,** 107 (1960).	Determination of iron in plasma.
42. G. F. Lee and W. Stumm, *J. Am. Water Works Assoc.*, **52,** 1567 (1960).	Determiation of ferrous iron in the presence of ferric iron.
43. J. L. Walker and G. D. Sherman, *Soil Science*, **95,** 325 (1962).	Determination of total ferrous iron in soils.
44. C. C. Tsen, *Anal. Chem.*, **33,** 849 (1961).	Determination of tocopherol using bathophenanthroline.
45. D. R. Erickson and W. L. Dunkley, *Anal. Chem.*, **36,** 1055 (1964).	Determination of tocopherol in milk and milk lipids.
46. N. M. Mazarella, *J. Biol. Chem.*, **225,** 239 (1957).	Microdetermination of corticoids.
47. R. Bailey and D. F. Boltz, *Anal. Chem.*, **31,** 117 (1959).	Spectrophotometric determination of hydrogen peroxide.
48. P. B. Hukill and F. A. Putt, *J. Histochem. Cytochem.*, **10,** 490 (1962).	A specific stain for iron bearing tissues.
49. M. J. Seven and R. E. Peterson, *Anal. Chem.*, **30,** 2016 (1958).	Determination of iron in urine.
50. H. Diehl and E. B. Buchanan, Jr., *Talanta*, **1,** 76 (1958).	Determination of iron in copper.
51. R. P. Hair and E. J. Newman, *Analyst*, **89,** 42 (1964).	Determination of iron and lead in the presence of copper.
52. F. Nakashima and K. Sakai, *Bunseki Kagaku*, **10,** 89 (1961).	Determination of iron in water.
53. A Sturla, *Acqua. Ind.*, **7,** 17 (1965).	Determination of reactive and total iron in industrial water.
54. F. J. Pocock, *Amer. Chem. Soc., Div. Water, Air, Waste Chem.*, **Preprints 6,** 59 (1966).	Colorimetric measurement of iron in high purity water.
55. B. G. Stephens and H. A. Suddeth, *Anal. Chem.*, **39,** 1479 (1967).	Determination of iron in sea water and aluminum alloy.
56. S. Takagi and K. Kumai, *Bunseki Kagaku*, **16,** 958 (1967).	Determination of microamounts of iron in water.
57. E. F. McFarren and R. J. Lishka, *Amer. Chem. Soc., Div. Water Waste Chem.*, **Preprints 7,** 120 (1967).	Evaluation of laboratory methods for the analysis of inorganics in water.
58. A. Rodriquez Cid, *Doc. Invest. Hidrol.*, **12,** 255 (1972). (Span.)	Determination of iron and manganese in public water supplies.
59. T. Tanaka, K. Hiiro, and A. Kawahara, *Fresenius' Z. Anal. Chem.*, **275,** 15 (1975).	Poly (vinyl chloride) film impregnated with bathophenanthroline for rapid colorimetric determination of ferrous iron in water.
60. Z. Marczenko, K. Kasiura, and M. Krasiejko, *Chem. Anal.* (Warsaw), **14,** 1277 (1969).	Determination of iron in high purity gold.

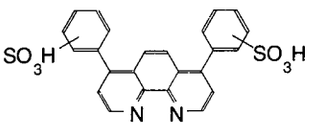
61. F. H. Lohman, D. F. Kuemmel, and E. M. Sallee, *Anal. Chem.*, **31,** 1739 (1959). — Determination of iron in phosphate and zirconium salts.
62. M. Knizek and A. Galik, *Z. Anal. Chem.*, **213,** 254 (1965). — Determination of iron in high-purity gallium arsenide, gallium, and arsenic.
63. I. G. Shafran and A. V. Petlakh, *Tr., Vses. Nauch. Issled. Inst. Khim. Reactiviv Osobo Chist. Khim. Veshchesty.* No. **28,** 42 (1966). — Determination of 0.05 ppm admixture of iron in alums.
64. Y. Ishihara, M. Koga, and H. Komuro, *Bunseki Kagaku*, **15,** 372 (1966). — Determination of iron in high-purity gallium.
65. H. Vahemets and G. Avarsoo, *Tartu Riikliku Ulikooli Toim.*, **289,** 106 (1971). — Determination of iron (III) in sodium chloride solution.
66. L. H. Anderson and E. Halloff, *U. S. Nat. Tech. Inform. Serv., PB Rep.* **1972,** No. 209509. — Spectrophotometric determination of iron in plutonium.
67. R. Dewolfs and F. Verbeek, *Fresenius' Z. Anal. Chem.*, **269,** 349 (1974). — Spectrophotometry of iron in titanium dioxide.
68. Y. Toita and H. Onishi, *Bunseki Kagaku*, **24,** 201 (1975). — Determination of iron in nickel-base alloys.
69. B. Paucnik, *Farm. Vestnik* (Ljubljana), **15,** 24 (1964). — Determination of serum iron.
70. V. Torjescu, M. Valeanu, E. Ceausu, and A. Mirea, *Viata Med.*, **16,** 335. — Determination of serum iron.
71. J. Bouda, *Vnitr. Lek.*, **15,** 1125 (1969). — Determination of serum iron and binding capacity of iron.
72. J. Bouda, *Clin. Chim. Acta*, **23,** 511 (1969). — Determination of iron-binding capacity.
73. R. Haeckel, *Z. Klin. Chem. Klin. Biochem.*, **11,** 301 (1973). — Mechanized determination of serum iron concentration and iron binding capacity with the Beckman Analyzer DSA 560.
74. P. Leflon and R. Plaquet, *Ann Biol. Clin.* (Paris), **32,** 97 (1975). — Determination of serum iron and total binding capacity of transferrin by a simple automatic technique without dialysis.
75. L. I. Idel'son and L. A. Appollonova, *Unifitsirovannye Metody Klin. Lab. Issled.*, **4,** 11 (1972). (Russ). — Determination of iron in urine.
76. O. Colagrande and A. Del. Re, *Ind. Agr.*, **7,** 206 (1969) (Ital.). — Determination of total iron and iron (II) in wines.
77. F. R. Short, H. C. Eyster, and W. G. Scribner, *Anal. Chem.*, **39,** 251 (1967). — Spectrophotometric determination of iron in high-temperature hydrocarbon jet fuels.
78. L. I. Lastorgueva, *Fiziol. Rast.*, **15,** 1093 (1968). — Spectrophotometric determination of iron in plant material.
79. D. E. Quinsland and D. C. Jones, *Talanta*, **16,** 282 (1969). — Microdetermination of iron in plant tissue.
80. A. DiFinizio, *Com. Naz. Energ. Nucl.* 1969, **RT/CHI (69)** 24. — Spectrophotometric determination of iron in plant samples.

81. G. Mrowetz and H. Klostermeyer, *Z. Lebensm.-Unters. Forsch.*, **153,** 348 (1973). — Determination of iron and copper in butterfat from one digest. Combined inverse polarographic-photometric method.
82. H. Timmen and J. Bluethgen, *Z. Lebensm.-Unters. Forsch.*, **153,** 283 (1973). — Estimation of copper and iron in butterfat from one decomposition. Photometric method.
83. J. Majewska and A. Milosz, *Przegl. Wlok.*, **27,** 615 (1973). — Monitoring iron content in starting materials and intermediates used in the production of rayon, polyacrylonitrile, and polyester fibres.
84. E. N. Pollock and A. N. Miguel, *Anal. Chem.*, **39,** 272 (1967). — Determination of iron (II) in the presence of thousand-to-one ratio of iron (III).
85. T. Mizuno, *Talanta*, **19,** 369 (1972). — Determination of traces of iron (II) in the presence of iron (III).
86. Z. Gregorowicz and T. Suwinska, *Zesz. Nauk. Politech. Slask., Chem.* No. **53,** 53 (1970). — Determination of trace amounts of ferric ions in some ammonium salts by kinetic methods and with bathophenanthroline.

SECTION III

BATHOPHENANTHROLINEDISULFONIC ACID

4,7-Diphenyl-1,10-phenanthrolinedisulfonic Acid



$C_{24}H_{14}N_2(SO_3H)_2$
F. W.: 492.5
$C_{24}H_{14}N_2(SO_3Na)_2$
F. W.: 536.5
Molar Absorptivity of ferrous complex 22,140 at 535 *nm*
G. Frederick Smith Chemical Company, Catalog Item No. 286

One difficulty encountered in the use of bathophenanthroline in water solution is its low solubility in water. The reagent is soluble in alcohol and also in water as the hydrochloride, but in the neutral solutions needed for maximum color development with iron, the excess reagent tends to precipitate, rendering the solutions turbid. This turbidity causes no trouble if the iron derivative, together with the excess reagent, is extracted into isoamyl alcohol for the measurement of absorbance as prescribed in the operating procedure of the original publication of Smith, McCurdy and Diehl[1]. The extraction is an extra step, however, and burdensome when the work load is heavy. Trinder at the Royal Infirmary, Sunderland, County Durham, solved the problem by treating bathophenanthroline with chlorosulfonic acid; the resulting sulfonated derivative was not isolated but the water soluble product retained the sensitivity of bathophenanthroline toward iron and was free from the turbidity difficulty. Isolation and further characterization of the sulfonated product were accomplished by Blair and Diehl[8] and Cryberg and Diehl[9].

Properties of Bathophenanthrolinedisulfonic Acid. The nature and characteristics of the sulfonation product of bathophenanthroline were worked out in detail by Blair and Diehl[8]. The material was isolated as the disodium salt. An ammonium salt of stoichiometric composition could not be obtained. The disodium salt is light tan in color when prepared free from contamination by iron. It is extremely soluble in water and somewhat hygroscopic. It can be dried at 110° and in fact shows no loss in weight up to 275° (thermobalance study). It shows a light blue fluorescence under ultraviolet light. The neutralization titration of bathophenanthrolinedisulfonic acid takes place in two steps, with the respective acid dissociation constants being pK=2.83 and pK_2=5.20.

Properties of the Ferrous Derivative. Bathophenanthrolinedisulfonic acid reacts with ferrous iron to produce an intense red color. The ab-

sorption spectrum of this colored ion was measured on a solution of ferrous sulfate containing also hydroxylammonium chloride, sodium acetate, and a ten-fold excess of bathophenanthrolinedisulfonic acid, see figure, at the wavelength of maximum absorption, 535 nm. The molar absorptivity is 22,140.

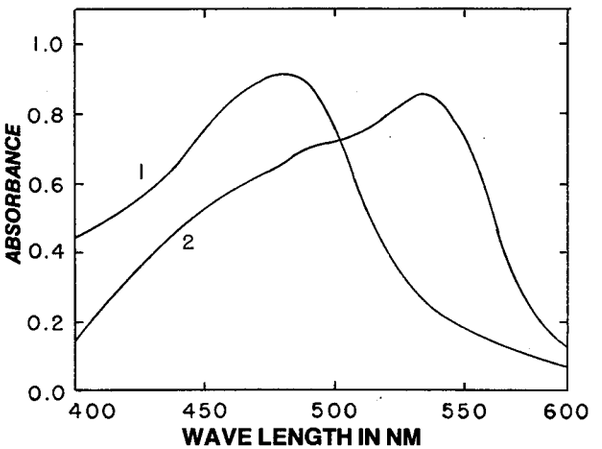


Figure 2. Absorption spectra of cuprous di(bathocuproindisulfonic acid), curve I and of ferrous tris(bathophenanthrolinedisulfonic acid) curve 2. From Blair and Diehl[8].

The combining ratio of the ferrous ion and bathophenanthrolinedisulfonic acid was determined by a spectrophotometric titration, the iron concentration being held constant in a series of solutions and the concentration of reagent varied. Straight lines were obtained for the rising and horizontal portions of the curve and the sharp break fell at the ratio Fe:bathophenanthrolinedisulfonic acid = 1:3.17.

The pH range over which ferrous tris(bathophenanthrolinedisulfonic acid) is stable was determined by preparing a series of solutions, each solution having the same amount of iron, sodium sulfite, and an excess of bathophenanthrolinedisulfonate; the pH was adjusted with hydrochloric acid or sodium hydroxide and after 1 hour the absorbance of each solution was measured. The optimum pH is in the range 3–7, with loss of intensity outside the range 2.5–11.

Applications. The initial application of sulfonated bathophenanthroline by Trinder[2] was to the determination of iron in serum and this remains a major use[3–6,11–20]. Several of the above cited papers deal with the simultaneous determination of iron and copper in serum [3-5]. Determination of iron in urine[7], biological fluids[22], liver biopsy[23], commercial ATP preparations[24], and protein [25] are some of the other uses primarily in the clinical chemistry field. Additionally, nutritionists may use it in the determination of dietary iron[26] and industrial chemists may apply it to numerous

other substances such as starting materials and intermediates in rayon or polyester production[27]. Five articles deal with the automation of the iron determination[11,15,17,21,28] and one with the indirect determination of ferrocyanide[10]. Blair and Diehl[8]studied the use of ferrous tris(bathophenanthrolinedisulfonate) as an oxidation–reduction indicator in the cerium (IV) titration of ferrous iron.

Interferences. Studies by Blair and Diehl[8] indicate that the determination of iron with bathophenanthrolinedisulfonic acid is relatively free of interferences from ions of copper, cobalt, nickel and chromium. Aluminum, tin and thiosulfate may interfere by precipitation. Cyanide and persulfate ions will inhibit the color formation. The other common ions studied showed little or no effect. A table of the Blair and Diehl study may be found in the reference[8] or in an earlier edition of this monograph.

Reagents. BATHOPHENANTHROLINEDISULFONATE SOLUTION. Dissolve 0.10 g of disodium bathophenanthrolinedisulfonate in iron–free water and dilute to 100 ml. REDUCING AGENTS, BUFFER, IRON–FREE WATER, REAGENT ACIDS AND BASES, see page 11.

TRICHLOROACETIC ACID. Reagent grade trichloroacetic acid should be distilled before use. Redistilled acid is available .

FERROUS TRISBATHOPHENANTHROLINEDISULFONIC ACID. Dissolve 0.039 g of ferrous ammonium sulfate and 0.16 g of disodium bathophenanthrolinedisulfonate in 10 ml of water. 0.01 *M*.

General Procedure for the Determination of Iron. Several solutions should be carried through simultaneously, perhaps two of unknown, four standards and a blank. Once a calibration curve is established, only an occasional standard need be run. For best results the iron should be in the 0.5 – 5 ppm range in the final solution. Measure the slightly acidic or neutral sample into a suitable volumetric flask and add 2 ml of 10% hydroxylammonium chloride, 2 ml of 0.1% sodium bathophenanthrolinedisulfonate and 5 ml of 10% sodium acetate solution, shake, dilute to the mark with distilled water and mix again. Measure the absorbance at 535 nm. Several more-specialized procedures follow.

Procedure for the Determination of Iron in Yeast. METHOD OF BLAIR AND DIEHL[8]. Transfer a weighed sample of about 2 g of the yeast to a 250-ml conical flask. Add 5 ml of concentrated sulfuric acid and insert a reflux head in the neck of the flask. Carry along simultaneously a blank, starting with the sulfuric acid. Char the yeast by heating the mixture for 15 minutes on a hot plate. Cool, and then add 20 ml of a mixture of equal volumes of 72% perchloric acid and 70% nitric acid. Replace the reflux head and boil the mixture in such a fashion that the water and nitric acid are expelled in about 15 minutes and perchloric acid begins to condense on the walls of the flask. Continue to reflux smoothly, without undue escape of perchloric acid, for 10 minutes. Cool the mixture, remove the reflux head, and wash it and the flask with approximately 30 ml of deionized water.

To this solution add 5 ml of a 10% solution of hydroxylammonium chloride and 10 ml of a 0.1% solution of disodium bathophenanthrolinedisfulfonate. Add ammonium hydroxide until the pH of the solution reaches 7 to 8 as shown by pH paper. Bring the pH to between 4 and 5 by the dropwise addition of perchloric acid. Transfer the solution to a 100–ml volumetric flask, dilute to the mark with deionized water, and mix. Measure the absorbance in a 1-cm cell at 535 nm.

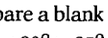
Prepare a calibration curve following the procedure just given, starting with various volumes of a standard iron solution, prepared by dissolving electrolytic iron in sulfuric acid, diluting, and aliquoting, and again diluting as appropriate. The calibration curve should be linear over the range 0 to 3.6 ppm of iron.

Procedure for the Determination of Iron in Serum. METHOD OF TRINDER[2]. Dissolve 160 mg of disodium bathophenanthrolinedisulfonate in 20 ml distilled water. Add 1 ml of thioglycolic acid and add sufficient water to bring the volume to 100 ml.

Transfer 2 ml of serum to a 15-ml cylindrical centrifuge tube and add 2.5 ml of water and 1.5 ml of 20% trichloroacetic acid. Mix by lateral shaking. Cover the tube with an aluminum cap and heat for 10 minutes in a water-bath maintained at 90° to 95°. After 5 minutes mix the contents of the tube by lateral shaking and mix again just before removal from the water-bath. Cool and centrifuge for a few seconds to dislodge droplets of condensed water and shake the tube to mix the contents. Centrifuge (still covered with the aluminum cap) at 4,000 r.p.m. , radius 6 in, for 15 minutes. Transfer 4 ml of the clear supernatant liquid to a test tube. Prepare a blank by heating a mixture of 3 ml of water and 1 ml of 20% trichloroacetic acid at 90° to 95° in a test tube covered with an aluminum cap. Heat the mixture for 10 minutes and cool. To each tube add 0.2 ml of iron reagent, 0.6 ml of 40% sodium acetate, and 0.4 ml of 1 to 1 sulfuric acid in that order, mixing the contents of the tubes after each addition. Measure the absorbance in a 20-mm cell, setting the instrument at zero with the blank, and making the measurement at 535 nm. Use a green filter if a photoelectric colorimeter is used for the measurement.

In a similar manner prepare a calibration curve by treating 3 ml of 20% trichloroacetic acid, and so on. The quantities of iron used in the standards correspond to serum iron values of 52.5 to 420 μg per 100 ml and if 20-mm cells are used to obtain the readings, the corresponding absorbances are 0.1 to 0.8.

Ferrrous tris(bathophenanthrolinedisulfonic acid) as an oxidation-reduction indicator. Because the ferrous derivative of bathophenanthrolinedisulfonic acid does not form an insoluble perchlorate it has a distinct advantage over the corresponding 1,10-phenanthroline and 5-nitro-1,10-phenanthroline iron complexes as an oxidation-reduction indicator, with the exception that the redox potential is 0.15 v lower than 5-nitro-1,10-phenanthroline. The formal reduction potential was measured by Blair and Diehl[8] in both 1 *M* sulfuric acid and 1 *M* perchloric acid. The formal reduction potential is about the same as that of 1,10-phenantholine, around 1.1 volt on the hydrogen scale. As an oxidation-reduction indicator ferrous tris(bathophenanthrolinedisulfonic acid) is very advantageous in cerate oxidimetry inasmuch as it is soluble in solutions containing perchlorate, whereas the other ferroin indicators are only sparingly soluble. The color change is vivid and the end-point sharp. The indicator was tested by Blair and Diehl[8] in the analysis of three standard iron ores. The indicator was used later by Miller and Diehl in the analysis of iron ores prepared at the G. Frederick Smith Chemical Company for analysis by students. Briefly the method involves placing the ore in solution with a mixture of perchloric and phosphoric acids, reducing the iron by passage through an amalgamated zinc reductor, and titrating the ferrous iron with sulfatoceric acid.

Procedure for the Determination of Iron in Iron Ore by Titration with Four-Valent Cerium Using Ferrous Tris(bathophenanthrolinedisulfonic Acid) as Indicator. MODIFIED METHOD OF BLAIR AND DIEHL[8]. Weigh accurately into a 500-ml conical flask 0.3 to 0.35 g of the iron ore. For the standardization of the oxidizing agent, weigh accurately about 0.22 to 0.24 g of electrolytic iron and transfer it to a 500-ml conical flask. Carry ore and electrolytic iron through the following procedure in identical fashion. Add 20 ml of a mixture of equal volumes of 70% perchloric acid and 85% phosphoric acid. Swirl the mixture until all particles are wetted and free from the bottom of the flask. Place a reflux head in the neck of the flask and heat, preferably on a gas-fired or electrically-heated hot plate. Boil the solution gently until the ore has dissolved and only a fine, white residue remains. The solution may be pink in color owing to the presence of trivalent manganese. The dissolution will be complete in five to ten minutes but a few additional minutes of boiling will cause the liquid refluxing on the walls of the flask to wash down any solid particles and insure the complete dissolution of the sample. Cool the mixture and remove the reflux head rinsing it with water. Dilute with water to a volume of 100 to 125 ml. Pass the solution through an amalgamated zinc reductor  (amalgamated zinc column about 2.5 cm in diameter by 22 cm long, 500-ml suction flask as receiver) previously well washed with 2% sulfuric acid. Adjust the flow to a rate just somewhat faster than the drops can be counted. Use suction if necessary. In manipulating the reductor during the washing, during the passage of the iron-bearing solution and during the subsequent washing do not draw air into the zinc column, in order to avoid the formation of hydrogen peroxide. Transfer the iron-bearing solution quantitatively to the reductor, washing with 2% sulfuric acid and allowing the level of the liquid in the reductor to drop to the top of the zinc column after each washing. Finally wash the column with five 15 ml portions of 2% sulfuric acid. Add 6 drops of 0.002 *M* ferrous tris(bathophenanthrolinedisulfonic acid) and titrate with 0.1 N sulfatoceric acid to the color change from pink to light green.

Prepare the 0.002 *M* ferrous tris(bathophenanthrolinedisulfonic acid) indicator by dissolving 0.078 g of ferrous ammonium sulfate and 0.32 g of disodium bathophenanthrolinedisulfonate in 100 ml of water and mixing well.

Indirect Determination of Ferrocyanide Using Iron and Bathophenanthrolinedisulfonic Acid. The iron in ferrocyanide and in ferricyanide is so tightly bound that it does not react with 1,10-phenanthroline or other phenanthroline reagents. Ferrocyanide is oxidized by ferric iron and the ferrous ion so produced will react with the phenanthrolines. The oxidation is complete and indirectly ferrocyanide can be determined by measuring the intensity of the ferrous-phenanthroline color. The method using bathophenanthrolinedisulfonic acid, devised by Avron and Shavit[10], is based on an earlier method, Krogman and Jagendorf, *Plant Physiol.*, 32, 373 (1957), using the same chemistry, but employing 1,10-phenanthroline. As expected, Avron and Shavit found the bathophenanthrolinedisulfonic acid method about twice as

sensitive as the 1,10-phenanthroline method. Ferricyanide is used as a so-called "electron acceptor" in enzymology and a measurement of the extent of the reduction is of importance, for example, in the photolysis of water by cell-free preparation of photosynthetic tissue (Hill reaction).

Ferricyanide does not produce a color so that ferrocyanide can be determined accurately even in the presence of a large excess of ferricyanide. The decrease in intensity of the color of ferricyanide itself (at 420 nm) has been used in biological studies but is far less sensitive than even the 1,10-phenanthroline method.

Citric acid is added to hold the ferric iron in solution at the pH at which the determination is made. Variation of pH in the range 2.5 to 6.5 does not affect the intensity or stability of the color developed; at high pH the intensity of the color slowly increases with time. Exposure to light causes an increase in the intensity of the color and the cuvette in which the reaction (and the absorbance measurement) is carried out should be kept in the dark.

In the procedure given below a high concentration of sodium acetate is specified to permit freedom in the acid content of the sample.

As much trichloroacetic acid as 6% may be present in the sample without affecting the results.

Procedure for the Indirect Determination of Ferrocyanide. METHOD OF AVRON AND SHAVIT[10]. Place the sample, of such size as to contain 0.002 to 0.1 μmole of ferrocyanide, in a 1-cm cuvette of 3 ml capacity. Fill the cuvette to 2.10 ml with water. Prepare, just before use, a mixture of 0.3 ml of 25% sodium acetate, 0.3 ml of 0.2 *M* citric acid, 0.15 ml of 0.0033 *M* ferric chloride, and 0.15 ml of 0.33% bathophenanthrolinedisulfonate. Add 0.90 ml of this mixture to the cuvette and mix. Allow the mixture to stand 5 minutes. Measure the absorbance at 535 nm using as reference a blank (stopped at time zero when working with biological material). Calculate the result using the relation[a]

$$(\text{Absorbance})\,(0.145) = \mu\text{moles of ferrocyanide}$$

All volumes may be reduced to one third those given, the final volume in the 1 cm cuvette then being 1.0 ml, and as little as 0.001 μ mole of ferrocyanide can be determined.

[a]*Assumes a value of 20,500 for the molar absorptivity*

SECTION III. BIBLIOGRAPHY. BATHOPHENANTHROLINEDISULFONIC ACID. (4,7-Diphenyl-1,10-Phenanthrolinedisulfonic Acid)

1. G. Frederick Smith, W. H. McCurdy Jr. and Harvey Diehl, *Analyst*, **77,** 418 (1952). — Determination of iron using bathophenanthroline.
2. P. Trinder, *J. Clin. Pathology*, **9,** 170 (1956). — Treatment of bathophenanthroline with chlorosulfonic acid to give a sulfonated product which is water soluble. Determination of iron in serum.
3. B. Zak, *Clin. Chem. Acta*, **3,** 328 (1958). — Simultaneous determination of iron and copper in serum.
4. J. W. Landers and B. Zak, *Am. J. Clin. Pathol.*, **29,** 590 (1958). — Simultaneous determination of iron and copper in serum.
5. B. Zak, J. W. Landers and L. A. Williams, *Am. J. Med. Technol*, **26,** 51 (1960). — Simultaneous determination of iron and copper in serum.
6. R. Callahan, *Clin. Chem.*, **9,** 487 (1963). — Determination of iron in serum.
7. J. F. Goodwin, Anachem. Conference, Detroit, Michigan, October 23, 1964. — Colorimetric estimation of urinary iron using a non-ashing technique.
8. D. Blair and Harvey Diehl, *Talanta*, **7,** 163 (1961). — Preparation, characterization and application of bathophenanthrolinedisulfonic and bathocuproinedisulfonic acids.
9. R. L. Cryberg and Harvey Diehl, *Proc. Iowa Acad. Sci.*, **70,** 184 (1963) — Sulfonation of bathophenanthroline and bathocuproine.
10. M. Avron and N. Shavit, *Anal. Biochem.*, **6,** 549 (1963). — The determination of ferrocyanide.
11. B. Zak and E. Epstein, *Clin. Chem.*, **11,** 641 (1965). — Automated determination of serum iron.
12. M. Keler-Bacoka and A. Stojanovski-Bubanj, *Farm. Glas.*, **25,** 363 (1969). — Comparison of iron contents in native and deproteinized serum by two spectrophotometric methods.
13. B. Zak, E. S. Baginski, E. Epstein, and L. M. Weiner, *Clin. Toxicol.*, **4,** 621 (1971). — Determination of serum iron.
14. R. E. Megraw, A. M. Hritz, A. L. Babson, and J. J. Carroll, *Clin. Biochem.*, **6,** 266 (1973). — Single tube technique for serum total iron and iron-binding capacity.
15. J. Burnichon and J. Pre, *Feuill. Biol*, **15,** 45 (1974). — Continuous-flow automatic direct determination of blood iron and latent binding capacity.
16. E. Swiatek and L. Tomaszewski, *Diagn. Lab.*, **10,** 253 (1974). — Simple micromethod of determination of serum iron and binding capacity.
17. B. Brozovic and Y. Purcell, *J. Clin. Pathol.*, **27,** 222 (1974). — Automated micromethod for measuring iron concentration in serum.
18. F. Watanabe, M. Itoh, and Y. Yamaguchi, *Rinsho Ragaku*, **3,** 460 (1975). — A one-tube method for determination of the serum iron concentration and unsaturated iron-binding capacity.

19. A. Roguljic, G. Ivankovic, and S. Hrdina, *Farm Glas.*, **32,** 357 (1976). — Determination of iron in serum.

20. R. N. Beale, J. O. Bostrom, and R. F. Taylor, *J. Clin. Pathol.*, **14,** 488 (1961). — Determination of serum transferrin iron and of serum latent iron-binding capacity. No heating or precipitation is involved.

21. R. W. Bide, *Anal. Biochem.*, **30,** 271 (1969). — Automated estimation of low levels of iron in biological fluids.

22. M. Y. Tsao and R. P. Beliles, *Amer. J. Clin. Pathol.*, **59,** 160 (1973). — Modified micromethod for determination of iron in tissue.

23. M. Barry and S. Sherlock, *Lancet*, **1,** 100 (1971). — Measurement of liver-iron concentration in needle-biopsy specimens.

24. W. H. Harrison, R. M. Gray, and T. DeCloux, *Biochim. Biophys. Acta*, **192,** 525 (1969). — Determination of iron in commercial ATP preparations.

25. R. H. Wickramasinghe, *Clin. Biochem.*, **7,** 88 (1974). — Investigation of acid-labile protein-iron.

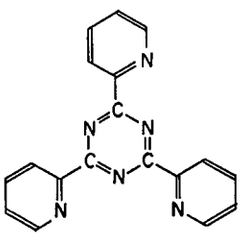
26. M. I. Davies, K. Bush, and I. Motzok, *J. Ass. Off. Anal. Chem.*, **55,** 1206 (1972). — Determination of iron in low iron diets by colorimetric and atomic absorption procedures.

27. J. Majewska and A. Milosz, *Irzegl. Wlok.*, **27,** 615 (1973). — Monitoring iron content in starting materials and intermediates used in production of rayon, polyacrylonitrile and polyester fibrel.

28. H. G. Gisenwiener, *Z. Klin. Chem. Klin. Biochem.*, **13,** 21 (1975). — Determination of iron with the Centrifichem system.

SECTION IV

2,4,6-TRIPYRIDYL-S-TRIAZINE
TPTZ



$C_{18}H_{12}N_6$
F.W.: 242.25
Molar Absorpitivity of
Fe $(TPTZ)_2^{++}$ in water: 22,600 at 593 nm; of Fe $(TPTZ)_2(ClO_4)_2$ in nitrobenzene: 24,100 at 595 nm
G. Frederick Smith Chemical Company, Catalog Item No. 291

2,4,6-Tripyridyl-s-triazine (2,4,6-tripyridyl-1,3,5-triazine) (TPTZ) reacts with ferrous iron to yield an intense violet color which is eminently suited to the spectrophotometric determination of iron. This reagent has the same high sensitivity toward iron as bathophenanthroline and bathophenanthrolinedisulfonic acid (molar absorptivities of the order of 22,000); like bathophenanthroline it forms a ferrous derivative which can be extracted into immiscible solvents (in this case as the perchlorate into nitrobenzene); it is highly specific for iron; and it is relatively easy to prepare. 2,4,6-Tripyridyl-s-triazine is the best of a number of pyridyl substituted triazines first synthesized by Case and Koft[1]. The fundamental chemistry of its reaction with iron and the applications of it to the determination of iron in water, wine, urine, silicates, refractories and limestone was worked out by Collins, Diehl and Smith[2,3,4]. It has since been applied to the determination of iron in serum[5-10] in alcohol beverages[11], in sodium products[12], in waters[13-15] and in nonferrous alloys and metals[30]. Other applications include the determination of tocopherols[16], EDTA[17], ruthenium[18], periodate[19], sulfur dioxide[20] and cobalt[21]. A number of further studies of the properties and uses of TPTZ in analysis have been reported[22-29].

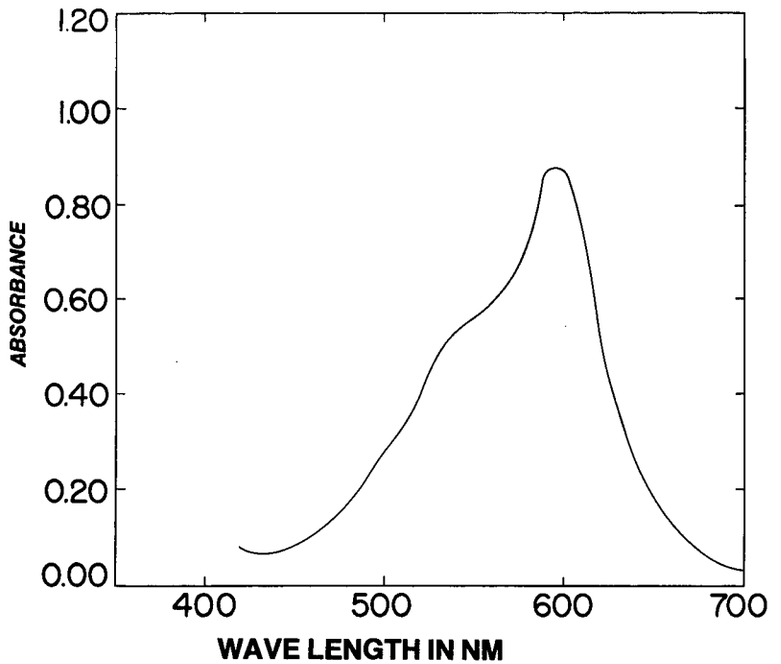


Figure 3. Absorption spectrum of Fe (2,4,6-tripyridyl-s-triazine)$_2$(ClO$_4$)$_2$ in nitrobenzene. (2.08 Ppm of iron, 1-cm cell.)

The absorption spectrum of Fe $(TPTZ)_2^{++}$ in nitrobenzene is shown in Fig. 3. The molar absorptivity is 22,600 in water solution and somewhat greater, 24,100, in nitrobenzene. The absorption peaks lie at essentially the same wave length in the two solvents, 593 nm in water and 595 nm in nitrobenzene. The spectra in both solvents show a shoulder on the short wave length side of the absorption maximum as do most of the ferrous derivatives of the ferroin reagents, that is, of the 1,10-pnenanthrolines, the 2,2'-bipyridines, and the 2,2',2''-terpyridines. In both water and nitrobenzene the color conforms to Beer's law over the useful range of spectrophotometers, up to about 6×10^{-5} *M* iron in this case. The formaton constant of Fe $(TPTZ)_2^{++}$ was found by Crichton[22], using the spectrophotometric method, to be log K= 10.83.

The color is stable, no change having been observed[2] in a water solution for 32 hours or in a nitrobenzene solution in 12 hours.

The pH range over which the violet compound is completely formed in water solution is 3.4 to 5.8. With extraction of the perchlorate into nitrobenzene the pH range is greater, 2.7 to 7.0. Although this pH range is not as wide as for many of the 1,10-phenanthroline and polypyridine reagents, a suitable pH can be obtained easily by the use of an acetate buffer. The violet Fe $(TPTZ)_2^{++}$ ion forms water soluble salts with the common cations which are not extracted from the water layer by isoamyl alcohol, *n*-hexyl alcohol, benzene, or chloroform , or ethyl acetate; this is true whether the anion present be sulfate, chloride, nitrate, iodide, acetate, or perchlorate. In the presence of perchlorate or iodide, the color is extracted rapidly and completely by nitrobenzene.

Specificity. Interferences. An extensive study was made[2] of the effects of various cations and anions on the colorimetric determination of iron with TPTZ. Of the ions tested only Cu^{++}, Co^{++}, Ni^{++}, Cr^{+++}, Ag^{+}, Hg^{++}, Bi^{+++}, MoO_4^{--}, CN^{-}, $C_2O_4^{--}$, and NO_2^{-} interfere signficantly. The interference of Co^{++}, Cu^{++}, and Ni^{++} is due to the formation of colored compounds with TPTZ; however, 2.5 ppm of Cu^{++}, 2.4 ppm of Co^{++} or 5.3 ppm of Ni^{++} results in a relative error of less than 2% in the determination of iron. A precipitate is formed in the presence of Ag^{+}, Hg^{++} and Bi^{+++} and the other ions retard color development or interfere due to the color of the ion. In the presence of most of the transition metals, if an excess of TPTZ is not present the color development is incomplete.

REAGENTS. 2,4,6-TRIPYRIDYL-S-TRIAZINE (TPTZ). 0.001 *M*. Dissolve 0.312 g of 2,4,6-Tripyridyl-s-triazine (GFS) in a few drops of hydrochloric acid and dilute to 1 liter with de-ionized water.

HYDROXYLAMMONIUM CHLORIDE. 10% Solution, Iron-Free. (GFS) Dissolve 10 g of hydroxylammonium chloride in 100 ml of water. Add 1 ml of 0.001 *M* TPTZ and 1 g of sodium perchlorate. Add 10 ml of nitrobenzene and shake the mixture for one minute. Allow the mixture to stand in a separatory funnel for a few minutes and draw off the lower nitrobenzene layer and discard. Store the water solution of the iron-free hydroxylammonium chloride in a glass bottle with a plastic cap with a polyethylene liner.

SODIUM ACETATE-ACETIC ACID BUFFER. 2 *M* SODIUM ACETATE-2 *M* ACETIC ACID, IRON-FREE. (GFS) Dissolve 16.4 g of sodium acetate and 11.5 ml of acetic acid in 100 ml of water. Add 1 ml of 0.001 *M* TPTZ, 1 ml of 10% hydroxylammonium chloride solution, and 1 g of sodium perchlorate. Add 10 ml of nitrobenzene and shake the mixture for one minute. Allow the mixture to stand in a separatory funnel for a few minutes and then draw off the lower, nitrobenzene layer and discard. Store the solution in a glass bottle with a plastic cap with a polyethylene liner.

SODIUM PERCHLORATE. 10% SOLUTION, IRON-FREE. Dissolve 10 g of sodium perchlorate (GFS) in 100 ml of water. Add 1 ml of 0.001 *M* TPTZ and 1 ml of 10% hydroxylammonium chloride solution. Add 10 ml of nitrobenzene and shake the mixture for 1 minute. Allow the mixture to stand in a separatory funnel for a few minutes and then draw off the lower nitrobenzene layer and discard. Store the solution in a glass bottle with a plastic cap with a polyethylene liner.

STANDARD IRON SOLUTION. 10.0 μg Fe/ml; 1.00 μg Fe/ml. Dissolve 1.000 g electrolytic iron (GFS) and dissolve in 50 ml of 20% sulfuric acid. Transfer quantitatively to a 1000 ml volumetric flask and dilute to the mark with iron free water. This solution is 1 mg Fe/ml. Dilute 10.0 ml to exactly 1 liter in a second volumetric flask for 10 μg Fe/ml. Dilute 50.0 ml of this to exactly 500 ml in another flask for 1 μg Fe/ml.

Procedure for the Determination of Iron in Burnt Refractories, Silicates and Argillaceous Limestone. METHOD OF COLLINS, DIEHL AND SMITH[2]. Weigh a sample of such size as to contain 3 to 5 mg of ferric oxide into a silver crucible and add 1.0 g of sodium carbonate and 1.0 g of sodium borate decahydrate. Mix thoroughly and then gently heat the crucible and contents over a Meker burner until the water of the sodium borate has been vaporized. Gradually increase the heat to melt the flux and continue heating until the sample has been completely decomposed. Rotate the crucible while cooling to cause the melt to solidify on the sides of the crucible. After the crucible has cooled to room temperature, add 10 ml of water and 5 ml of hydrochloric acid, cover with a watch glass and gently heat on a hot plate until the melt has dissolved. It may be necessary to add more hydrochloric acid to obtain complete dissolution of the residue (a precipitate of silver chloride and silica will remain which is later removed). Transfer the contents of the crucible to a 250-ml volumetric flask and dilute to the mark. Mix well and filter or centrifuge a portion of the solution to remove any suspended silver chloride and silica.

Pipet a 5.00 ml aliquot of this solution into a 50-ml volumetric flask; add 2.0 ml of 10% hydroxylammonium chloride solution, 5.0 ml of 0.001 *M* TPTZ and 10 ml of 2 *M* sodium acetate-2 *M* acetic acid buffer. Dilute the solution to exactly 50 ml and measure the absorbance at 593 nm using a 1-cm cell. Run a blank on the reagents and silver crucible in exactly the same manner.

Procedure for the Determination of Iron in a Silicate of Low Iron Content (For Example, Granite G-1)[2]. Fuse a 3.0 g sample with 5.0 g of sodium carbonate and 5.0 g of sodium borate decahydrate in a 50-ml silver crucible (or an iron-free platinum crucible prepared as described in the procedure for iron in glass on p. 35) and continue heating until a clear melt is obtained. Rotate the crucible while cooling and place it in a 600-ml beaker. Add 100 ml of hydrochloric acid and 200 ml of water and heat until the melt has been completely dissolved. Cool, remove the crucible with washing and dilute the solution to exactly 1 liter in a volumetric flask. Pipet a 25.0 ml aliquot of this solution into a 250 ml beaker, add 5 ml of hydrochloric acid and heat for several hours to precipitate silica. Cool the solution, transfer to a 250 ml volumetric flask and dilute to the mark. Filter a portion of this solution (no washing) to remove silica and silver chloride. Pipet a 15.00 ml aliquot of the filtered solution into a 50-ml volumetric flask; add 2.0 ml of 10 % hydroxylammonium chloride solution, 5.0 ml of 0.001 *M* TPTZ, and 10 ml of 2 *M* sodium acetate-2 *M* acetic acid buffer. Dilute the solution to exactly 50 ml and measure the absorbance at 593 nm using a 1-cm cell. Run a blank on the reagents and silver crucible in exactly the same manner.

Procedure for the Determination of Iron in a Silicate of High Iron Content (For Example, Diabase W-1)[2]. Mix 0.22 g of the sample with 1.0 g of sodium carbonate and 1.0 g of sodium borate decahydrate in a silver crucible (or an iron-free platinum crucible prepared as described in the procedure for iron in glass on p. 35) and fuse. Continue heating until the decomposition of the sample is complete (about 15 minutes), cool and add 20 ml of water and 10 ml of hydrochloric acid. Heat the crucible on a hot plate until the melt has completely dissolved (a residue of silica and silver chloride will remain), cool and dilute to exactly 500 ml in a volumetric flask. Pipet a 50.0 ml aliquot of this solution into a 500 ml volumetric flask, dilute to volume and centrifuge a portion of the final solution to remove silica and silver chloride. Place

15.00 ml of this solution in a 50-ml volumetric flask, add 2.0 ml of 10% hydroxylammonium chloride, 5.0 ml of 0.001 *M* TPTZ, and 10.0 ml of 2 *M* sodium acetate-2 *M* acetic acid and dilute to volume. Mix well and determine the absorbance of the solution at 593 nm. Run a blank through the entire procedure.

Procedure for the Determination of Iron in Glass and Glass Sand[2]. Free a platinum crucible of iron by repeated heating to 1000° to 1200° in a muffle furnace and leaching with hot hydrochloric acid. Weigh a sample of such size as to contain 0.5 to 1.0 mg of ferric oxide into the iron-free platinum crucible. Add 2 ml of water and 4 ml of hydrofluoric acid if the sample is a glass or 4 ml of hydrofluoric acid if the sample is glass sand. After the reaction has subsided, add 1 ml of perchloric acid and evaporate to dryness on a hot plate without boiling. Cool, add 2 ml of hydrofluoric acid and again evaporate to dryness. Place the crucible and contents in a 250-ml beaker, add 20 ml of hydrochloric acid and 50 ml of water and heat. If complete solution is obtained, cool, transfer the solution to a 250-ml volumetric flask, dilute to volume and continue the determination as directed in the next paragraph. If an insoluble residue remains, filter the solution into a 250-ml volumetric flask using a medium porosity filter paper. Wash first with dilute hydrochloric acid (1:100) and then with water. Ash the filter in a silver crucible (or in an iron-free platinum crucible prepared as described above). Add 1 g of sodium carbonate and 1 g of sodium borate decahydrate and heat until a clear melt is obtained. Cool to room temperature, add 5 ml of hydrochloric acid and 10 ml of water, cover with a watch glass and heat on a hot plate until the residue dissolves. A precipitate of silver chloride and silica will remain. Transfer the contents of the crucible to the 250-ml volumetric flask containing the filtrate and dilute to volume. After mixing, centrifuge or filter a portion of this solution to remove any silica and silver chloride.

Pipet a 25.0 ml aliquot of the solution into a 50-ml volumetric flask and add 2.0 ml of 10% hydroxylammonium chloride and 5.0 ml of 0.001 *M* TPTZ. Dropwise add ammonium hydroxide until the violet color of the iron derivative remains on mixing, add 10 ml of the 2 *M* sodium acetate-2 *M* acetic acid buffer and dilute to the mark. Measure the absorbance at 593 nm using a 1-cm cell. Run a blank on reagents and crucible in exactly the same manner.

Procedure for the Determination of Iron in Limestone[2]. Weigh a sample of such size as to contain 0.5 to 1.0 mg of ferric oxide into a 250-ml beaker. Cover with a watch glass, add 20 ml of water and 10 ml of hydrochloric acid and heat gently. After the reaction is completed, filter the solution into a 250-ml volumetric flask using a medium porosity paper. Wash the filter with dilute hydrochloric acid (1:100) and water. Place the filter in a silver crucible (or an iron-free platinum crucible prepared as described in the procedure for iron in glass above), ash, cool and add 1.0 g of sodium carbonate and 1.0 g of sodium borate. Heat gently at first and then more strongly to melt the flux and decompose the residue. Rotate the crucible while cooling to cause the melt to solidify on the sides of the crucible. After cooling, add 10 ml of water and 5 ml of hydrochloric acid and warm to dissolve the residue. After complete dissolution (a precipitate of silica and silver chloride will remain), transfer the contents of the crucible to the 250-ml volumetric flask containing the original filtrate. Dilute the solution to volume, filter or centrifuge a portion of the solution. Pipet a 25.0

ml aliquot of the solution into a 50-ml volumetric flask and add 2.0 ml of 10% hydroxylammonium chloride solution and 5.0 ml of 0.001 M TPTZ. Add dropwise ammonium hydroxide until the violet color of the iron derivative remains on mixing, add 10 ml of the 2 M sodium acetate-2 M acetic acid buffer and dilute to the mark. Measure the absorbance at 593 nm using a 1-cm cell. Run a blank on reagents and crucible in exactly the same manner.

Procedure for the Determination of Iron in Wine. METHODS OF COLLINS AND DIEHL[3]. Pipet various volumes of the standard iron solution into 125-ml separatory funnels to cover the range from 0 to 40 μg of iron. To each solution add 2.0 ml of 10%, iron-free hydroxylammonium chloride, 5.0 ml of 0.001 *M* TPTZ, 5.0 ml of 2 *M* sodium acetate-2 *M* acetic acid buffer, and 1.0 ml of 10% sodium perchlorate. Extract each solution three times using 4.0, 2.0 and 2.0 ml portions of nitrobenzene. Collect the extracts of each solution in a 10-ml volumetric flask, dilute to the mark with ethanol and determine the absorbance at 595 nm using 1-cm cells. This calibration curve may be used for any of the nitrobenzene extraction procedures.

Pipet 3.00 ml of the wine into a 250-ml conical flask (preferably one of Vycor) and insert a reflux head. Add 10 ml of concentrated nitric acid and 5 ml of perchloric acid and heat to fumes of perchloric acid. Cool the solution, add 20 ml of water and heat to boiling to remove any chlorine. After cooling the solution, add 2.0 ml of 10% hydroxylammonium chloride, 2.0 ml of sodium acetate-acetic acid buffer, and 5.0 ml of 0.001 *M* TPTZ. Neutralize the solution with ammonium hydroxide to pH 4 to 5 using a pH meter or pH indicating paper. Transfer the solution to a 125-ml separatory funnel, add 4.0 ml of nitrobenzene and shake vigorously for one minute. Allow the phases to separate and gently swirl to remove drops of nitrobenzene clinging to the upper walls of the funnel. Repeat the extraction using 2.0 ml portions of nitrobenzene. Collect the extracts in a 10 ml volumetric flask, dilute to the mark with ethanol and measure the absorbance at 595 nm using a 1-cm cell. Run a blank through the entire procedure and subtract the iron so found from that obtained in the analysis.

Alternatively, pipet 3.00 ml of the wine into a 100-ml beaker. Add 2.0 ml of 10%, iron-free hydroxylammonium chloride solution, 5.0 ml of ethanol, 5.0 ml of acetate buffer and 5.0 ml of 0.001 *M* TPTZ. Heat the solution to boiling for five minutes, cool and transfer to a 125-ml separatory funnel. Wash the beaker with 20 ml of ethanol and 1.0 ml of 10%, iron-free sodium perchlorate and add the washings to the separatory funnel. Extract the solution three times using one 4.0 ml and two 2.0 ml portions of nitrobenzene and collect the extracts in a 10-ml volumetric flask. Dilute to volume with ethanol and determine the absorbance of the solution at 595 nm using a 1-cm cell. Run a blank through the entire procedure and subtract the iron so found from that obtained in the analysis.

Procedure for the Determination of Iron in Treated Water and in Sea Water (parts per billion range). METHOD OF COLLINS AND DIEHL[4], Pipet 100.00 ml of the water into a 125-ml separatory funnel. Add 2.0 ml of 10% iron-free hydroxylammonium chloride solution, 2.0 ml of 10% iron-free sodium perchlorate solution, 5.0 ml of 0.001 *M* TPTZ, and 5.0 ml of 2 *M* sodium acetate-2 *M* acetic acid buffer. If the previous treatment of the sample has introduced much acid, neutralize with ammonium hydroxide to pH 4 to 5 prior to addition of buffer. Add 10 ml of nitrobenzene, shake for one minute, allow the phases to separate and gently swirl the funnel to dislodge any drops of nitrobenzene clinging to the upper walls. Drain the nitrobenzene layer into a 25-ml volumetric flask and repeat the extraction with another 10-ml portion of nitrobenzene. Dilute the combined extracts to 25.0 ml with ethanol. Measure the absorbance of the solution at 595 nm using a 5-cm cell and a mixture of nitrobenzene and ethanol (4:1) in the solvent cell. Run a reagent blank through the entire operation and subtract the absorbance found for it from that of the unknown solution.

Prepare a calibration curve following the above procedure, but using various volumes from 0 to 50 ml of the standard iron solution containing 50.0 μg Fe per liter.

It must be remembered that in the determination of iron in this range, the glassware used should be very thoroughly cleaned. It may be necessary to leach new glassware with nitric or hydrochloric acids before starting, rinsing thoroughly, and even then the first few determinations may be suspect.

Procedure for the Determination of Iron in Urine. METHOD OF COLLINS AND DIEHL. Pipet 50.0 ml of the urine into a 250-ml conical flask. Add 25 ml of redistilled nitric acid and 10 ml of 70% perchloric acid. Place a reflux head on the flask, heat to fumes of perchloric acid and continue the digestion for 10 minutes. If a suitable hood is not available for the wet ashing use a glass fume eradicator. Wash down the sides of the flask and reflux head with water and heat to boiling to remove chlorine. While still hot, transfer the solution to a 125-ml separatory funnel, cool the solution and add 2.0 ml of 10%, iron-free hydroxylammonium chloride and 5.0 ml of 0.001 *M* 2,4,6-tripyridyl-s-triazine. Place a small piece of Congo Red paper in the solution and add ammonium hydroxide dropwise until the paper turns red. Complete the adjustment of pH by adding 5.0 ml of 2 *M* sodium acetate-2 *M* acetic acid buffer. Dilute the solution to about 100 ml with water to dissolve the precipitate of ammonium perchlorate. Add 4.0 ml of nitrobenzene and shake vigorously for 1 minute. Allow the phases to separate, collect the nitrobenzene layer in a 10 ml volumetric flask and repeat the extraction two more times using 2.0-ml portions of nitrobenzene. Dilute the combined extracts to exactly 10.0 ml with ethanol, mix and determine the absorbance of the solution at 595 nm using a 1-cm cell. Use a mixture of nitrobenzene and ethanol (4:1) as the reference solution.

Prepare a calibration curve using solutions containing 0-20 μg of iron, following the same procedure.

Procedure for the Determination of Iron in Serum. METHOD OF CARAWAY[7]. Pipet 2.0 ml of serum into a small test tube, add 1 ml of 0.2*N* hydrochloric acid-1% ascorbic acid solution, mix, and allow to stand 5 minutes. Add 1 ml of 20% trichloroacetic acid made from redistilled, iron-free acid , and 1 ml of chloroform. Stopper and shake for 10 to 15 seconds. Centrifuge for 10 minutes. Carefully decant the supernatant liquid into another small test tube. Pipet 2.0 ml of the clear liquid into a 12-mm cuvette or test tube. In identical tubes place a blank and a standard.

The blank is prepared by mixing 1.0 ml of water, 0.5 ml of 0.2*N* hydrochloric acid-1% ascorbic acid and 0.5 ml of 20% trichloroacetic acid. The standard is prepared by mixing 1.0 ml of the iron standard (2.00 μg Fe per ml), 0.5 ml of 0.2*N* hydrochloric acid-1% ascorbic acid, and 0.5 ml of 20% trichloroacetic acid.

To each of the three solutions thus prepared add 0.2 ml of 0.1% TPTZ. Mix and add 0.2 ml of 40% ammonium acetate solution and mix again. Using the blank as a reference, measure the absorbance at 590 nm within five minutes if possible.

The final volume obtained is 2.4 ml. For instruments which require 3 ml of solution, 0.8 ml of 10% ammonium acetate may be substituted for the 0.2 ml of 40%.

The results may be calculated using the relation:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 200 = \mu\text{g Fe per 100 ml of serum}$$

For the microdetermination of serum iron, all volumes are reduced by a factor of 20 and the techniques of ultra micro-chemistry are applied.

Procedure for the Determination of Total Iron Binding Capacity of Serum (TIBC) METHOD OF CARAWAY[7]. Pipet 2 ml of serum into a small test tube, add 4.0 ml of ferric iron solution (5.0μg Fe per ml), mix, and allow to stand for 5 minutes. Add 0.5 g of anhydrous magnesium carbonate powder, stopper, and shake for 10 to 15 seconds. Allow the solution to stand for 30 minutes but remix 4 or 5 times during this interval. Centrifuge for 10 minutes. Pipet 2.0 ml of the clear supernatant liquid into a test tube and analyze for serum iron as described above.

The TIBC may be calculated using the relation:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 600 = \text{IBC in } \mu\text{g Fe per 100 ml of serum}$$

For the microdetermination of TIBC, all volumes are reduced by a factor of 20.

Procedure for the Determination of Microgram Quantities of EDTA in Urine. METHOD OF KRATOCHVIL AND WHITE[17]. REAGENTS. $Fe(TPTZ)_2^{++}$. $5x10^{-4}$ *M* in 0.1*M* sodium acetate-0.1*M* acetic acid. Dissolve 0.228 g of TPTZ in a few milliliters of deionized water containing several drops of concentrated hydrochloric acid. Add 50 ml of 1*M* sodium acetate-1 *M* acetic acid buffer. Add 0.0995 g of ferrous ethylenediammonium sulfate tetrahydrate and dilute to 500 ml.

STANDARD EDTA SOLUTION $1.00x10^{-4}$ *M* disodium dihydrogen ethylenediaminetetraacetate. Dissolve 0.3723 g of primary standard EDTA in deionized water, transfer quantitatively to a 1-liter volumetric flask, dilute to the mark and mix well. Transfer 10.0 ml of this solution to a 100 ml volumetric flask, dilute to the mark and mix.

Place the sample containing 0.1 to 1μ mole of EDTA in a 25 ml volumetric flask, add 2.00 ml of the $5x10^{-4}$*M* solution of $Fe(TPTZ)_2^{++}$, dilute to volume with deionized water and mix well. Measure the absorbance against a deionized water blank at 593 nm. A calibration curve is prepared using 0-10ml portions of the $1.00x10^{-4}$*M* EDTA solution in place of the sample. If any metals other than alkali metals are present, measure the absorbance as soon as possible after the addition of the $FE(TPTZ)_2^{++}$ solution and at 1 minute intervals for 2 or 3 minutes, and extrapolate back to zero time to obtain the amount of uncomplexed EDTA.

SECTION IV. BIBLIOGRAPHY
2,4,6-TRIPYRIDYL-S-TRIAZINE (TPTZ)

1. F. H. Case and E. Koft, *J. Am. Chem. Soc.*, **81,** 905 (1959). Synthesis of substituted 1,3,5-Triazines containing the ferroin group.
2. P. F. Collins, H. Diehl and G. F. Smith, *Anal. Chim.*, **31,** 1862 (1959). TPTZ as a reagent for iron. Determination of iron in limestone, silicates and refractories.
3. P. F. Collins and H. Diehl, *Anal. Chim. Acta*, **22,** 125 (1960). Determination of iron in wine.
4. P. F. Collins and H. Diehl, *J. Marine Research*, **18,** 152 (1960). Determination of iron in sea water.
5. B. Zak, G. A. Cavanaugh and L. A. Williams, *Chemist-Analyst*, **50,** 8 (1961). Spectrophotometric microdetermination of iron and copper in a single aliquot.
6. T. Matsubara, *Igaku To Seibutsugaku*, **60,** 103 (1961). Determination of iron in serum.
7. W. T. Caraway, *Clin. Chem.*, **9,** 188 (1963). Macro and micro methods for the determination of serum iron and iron-binding capacity.
8. D. S. Fischer and D. C. Price, *Clin. Chem.* **10,** 21 (1964). Determination of iron in serum.
9. M. London and J. H. Marymont, Jr., *Clin. Chim. Acta*, **12,** 227 (1965). Determination of serum iron.
10. G. Piccardi, M. Nyssen, and J. Dorche, *Clin. Chim. Acta*, **40,** 219 (1972). Determination of human serum iron-binding capacity and concentration without precipitating serum proteins.
11. M. K. Meredith, S. Baldwin, and A. A. Andreasen, *J. Ass. Offic. Anal. Chem.*, **53,** 12 (1970). Determination of iron in alcoholic beverages.
12. T. P. Steiner, Technicon Symp., 2nd., *N. Y. London*, **1965,** 186. Determination of trace amounts of iron in sodium products using an Auto Analyzer.
13. C. Hammerton, *Proc. Soc. Water Treat. Exam.*, **16,** 293 (1967). A sensitive and specific colorimetric method for iron in water.
14. B. G. Stephens and H. A. Suddeth, *Anal. Chem.*, **39,** 1478 (1967). Determination of iron in sea water and aluminum alloys. Extraction of iron (II) complexes into propylene carbonate.
15. R. Tremblay, R. St. Jean, and S. S. Kasatiya, *Environ. Lett.*, **7,** 275 (1974). Further evaluation of the Beckman DSA - 560 analyzer for the chemical analysis of water.
16. C. C. Tsen, *Anal. Chem.*, **33,** 849 (1961). Determination of tocopherols.
17. B. Kratochvil and M. C. White, *Anal. Chem.*, **37,** 111 (1965). Determination of EDTA
18. W. A. Embry and G. H. Ayres, *Anal. Chem.*, **40,** 1499 (1968). Spectrophotometric determination of ruthenium.
19. G. Avigad, *Carbohyd. Res.*, **11,** 119 (1969). Rapid, sensitive determination of periodate.
20. B. G. Stephens and H. A. Suddeth, *Analyst (London)*, **95,** 70 (1970). Spectrophotometric determination of sulfur dioxide by reduction of iron (II) and simultaneous chelation of the iron (II) with TPTZ.

21. M. J. Janmohammed and G. H. Ayres, *Anal. Chem.*, **44,** 2263 (1972). Spectrophotometric determination of cobalt.

22. D. Crichton, *Dissertation Abstracts*, **23,** 2677 (1963). Factors affecting the molar absorptivity of solutions of bis (TPTZ) Fe(II) perchlorates.

23. H. Diehl, E. B. Buchanan, Jr., and G. F. Smith, *Anal. Chem.*, **32,** 1117 (1960). Iron(II) derivatives of pyridyl substituted s-trazines.

24. T. B. Jones, E. A. McElhill, and J. O. Smith, *J. Chem. Eng. Data*, **7,** 227 (1962). Thermal stability of various organic compounds including 2,4,6-tripyridyl-s-triazine.

25. E. B. Buchanan, Jr., D. Crichton, and J. R. Bacon, *Talanta*, **13,** 903 (1966). Study of factors that influence the reaction of iron(II) with TPTZ.

26. A. Jarive, *Pharm. Biol.*, **7,** 1045 (1972). Colorimetric determination of ferrous iron by a simple, rapid method.

27. G. Nakagawa and H. Wada, *Talanta*, **20,** 829 (1973). Effects of auxilliary complex-forming agents on the rate of metallochromic indicator color change.

28. W. K. Dougan and A. L. Wilson, *Water Treat. Exam.*, **22,** 100 (1973). Absorptiometric determination of iron.

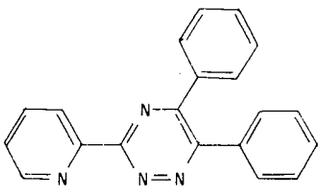
29. B. G. Stephens, H. L. Felkel, Jr., and W. M. Spinelli, *Anal. Chem.*, **46,** 692 (1974). Spectrophotometric determination of copper and iron subsequent to the simultaneous extraction of bis (2,9-dimethyl-1,10-phenanthroline)-copper(I) and bis(2,4,6-tris(2-pyridyl)-1,3,5-triazine) iron(II) into propylene carbonate.

30. S. Maekawa and K. Kato, *Bunseki Kagaku*, **16,** 482 (1967). Determination of iron in nonferrous metals and alloys.

SECTION V

PDT

3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine



$C_{20}H_{14}N_4$
F. W.: 310.34
Molar absorptivity of
$Cu(PDT)_2^+$ 7,990 at 488 nm
$Fe(PDT)_3^{+2}$ 23,500 at 555 nm
G. Frederick Smith Chemical Company,
Catalog No. 516

An outstanding reagent for the rapid, simultaneous determination of copper and iron, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine is conveniently referred to in abbreviated fashion as PDT[1]. This reagent and its sulfonated derivative are extremely sensitive as iron chromogens[2]. PDT serves best in procedures requiring separation or preconcentration of iron, because it forms a highly extractable iron(II) chelate[2].

To achieve maximum formation, extraction and stability of both the copper(I) and iron (II) chelates of PDT, the following conditions are satisfied in the simultaneous determination of copper and iron: (1) adjustment of the pH to between 3.5 and 6, (2) use of hydroxylamine hydrochloride in the aqueous phase as a reductant, (3) extraction of both complexes into isoamyl alcohol, and (4) use of ascorbic acid in the extract to protect against air oxidation of the copper(I) complex. The isoamyl alcohol extract containing both iron(II) and copper(I) is measured spectrophotometrically. Sodium cyanide is then added to rapidly and completely convert the copper PDT complex into a colorless cyanide complex, and the extract is once again measured spectrophotometrically. The copper content is directly proportional to the loss in absorbance, and the iron content is proportional to the final absorbance. Spectra of solutions treated as described are shown in Figs. 4 and 5.

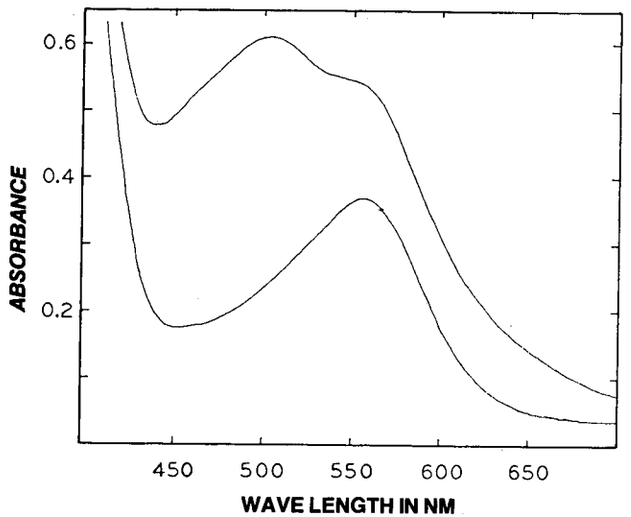


Figure 4. Absorption spectrum of a solution of iron(II) and copper(I) PDT complexes in isoamyl alcohol vs. air before (upper curve) and after addition of sodium cyanide (lower curve). Iron (II) and copper(I) concentrations are 1.37×10^{-4} and 4.48×10^{-4} *M*, respectively.

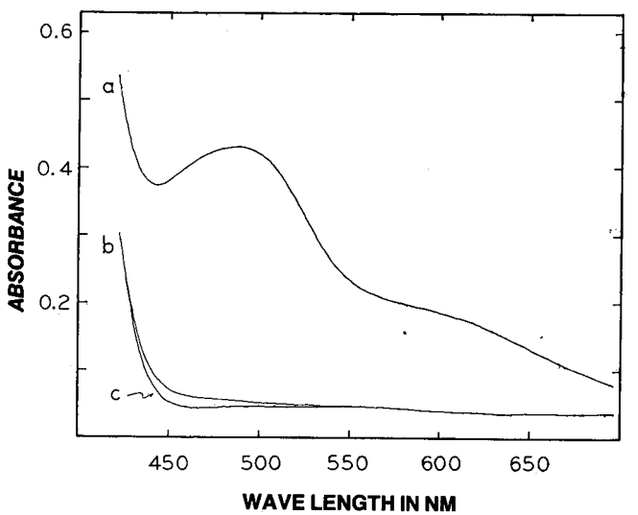


Figure 5. Absorption spectrum of 4.84×10^{-4} *M* copper(I)—PDT in isoamyl alcohol vs. air, curve a; after addition of sodium cyanide, curve b; spectrum of reagent blank, curve c.

By employing a 200-ml sample, extraction into 10 ml of isoamyl alcohol, and a 1-cm absorption cell, as little as 1 part per billion of iron and 4 parts per billion of copper can be detected (each based on an absorbance difference from the blank of 0.005 absorbance unit). Approximately 0.2 μg of iron and 0.8 μg of copper can thus be detected. Quantitative determinations, however, require about 80 times these amounts (16 μg of iron and 64 μg of copper) for optimum relative accuracy.

Interferences. The following ions are tolerated at 1000 ppm each: NH_4^+, Li^+, Na^+, K^+, Ag^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+}, Mn^{2+}, Zn^{2+}, Sn^{2+}, Cd^{2+}, Hg^{2+}, UO_2^{2+}, Al^{3+}, Th^{4+}, F^-, Cl^-, Br^-, I^-, IO_4^-, NO_3^-, ClO_3^-, BrO_3^-, OAc^-, SCN^-, SO_4^{2-}, $S_2O_8^{2-}$, SO_3^{2-}, PO_4^{3-}. At 500 ppm nitrite, oxalate or molybdate ions do not interfere. Offending ions in the iron determination and their approximate tolerance levels (given in parentheses) are Co^{+2} (50), Cr^{+3} (200) and Ni^{+2} (10). In the copper determination the ions that interfere and levels tolerated (given in parentheses) are thiosulfate (20), cobaltous (< 1) and nickel (50).

Applications. PDT has been applied to the simultaneous determination of copper and iron in water, milk, and wine[1]. Numerous other applications appear promising, especially in view of the demand for suitable methods for determining trace amounts of both iron and copper in foods, beverages, biological specimens, and various commercial products such as paper, petroleum and alloys. The presence of these metals can be of considerable significance in medical diagnosis and biochemical research, they are known to promote off-flavors in foods and beverages, and their presence in lubricating oils or other commercial products is indicative of wear, malfunction or contamination. An interesting application by Lundgren and Schilt[6] involves the coating of Amberlite XAD-2 with PDT for use in purification of various reagents and for the preconcentration of iron, copper, cobalt, nickel and zinc contained in samples prior to analysis. This coated resin is also available from the GFSCC.

General Procedure for Simultaneous Determination of Copper and Iron. (SCHILT AND TAYLOR[1]). REAGENTS. PDT SOLUTION. Prepare a 0.01 *M* solution by adding 8 drops of concentrated hydrochloric acid to 0.30 g of PDT followed by 100 ml of ethyl alcohol. ASCORBIC ACID SOLUTION. Prepare fresh daily by dissolving 0.5 g of the solid in 100 ml of ethyl alcohol. pH 4.5 BUFFER SOLUTION. Prepare by adding sufficient glacial acetic acid to 1 liter of 1 *M* sodium acetate to adjust the pH to 4.5. To remove trace amounts of iron and copper, add 1 g of hydroxylamine hydrochloride, 5 ml of 0.01 *M* PDT and extract with isoamyl alcohol until a colorless extract is obtained. SODIUM PERCHLORATE SOLUTION. Dissolve 100 g sodium perchlorate in 100 ml of distilled water. Remove traces of iron and copper by adding 0.2 g of hydroxylamine hydrochloride, 2 ml of 0.01 *M* PDT and extracting with isoamyl alcohol.

RECOMMENDED PROCEDURE. Pipet sample of sufficient size to contain 1-25 μg of Fe and/or 4-80 μg of Cu into separatory funnel and add 5 ml of pH 4.5 buffer, 2 ml of 10% hydroxylamine hydrochloride , 2 ml of 0.01 *M* PDT, and 2 ml of 50% $NaClO_4$ solution. Adjust the pH to 4.5 $\pm$1, if necessary, using pH indicating paper. Extract once with 6 ml and again with 2 ml of isoamyl alcohol. Combine the isoamyl alcohol extracts in a 10 ml volumetric flask, and alcohol dilute to volume with 0.5% ascorbic acid in ethanol. Measure the absorbance of the solution vs. a similarly

prepared blank at 488 nm; add 5-10 mg of sodium cyanide to the isoamyl alcohol solution, and measure the absorbances at 488 and 555 nm vs. the similarly treated blank. Make use of suitably prepared calibration curves or empirical equations to convert the loss in absorbance at 488 nm to concentration of Cu and the final absorbance at 555 nm to concentration of Fe.

Procedure for Analysis of Whole Milk. Pipet 25.00 ml of whole milk slowly into a heated crucible, at a rate sufficient to evaporate without frothing (approximately 1 drop per second). if sample weight is desired, determine the density of a second sample. After all moisture has been removed, raise the temperature slowly to approximately 450-500°C, avoiding loss of sample by foaming and swelling, and ignite at this temperature until a gray ash is obtained. Allow the crucible to cool, add 1 ml of concentrated nitric acid, evaporate to dryness, and ignite again at 450-500°C for 1 hr. Dissolve the resulting white ash in 10 ml of 1 *M* nitric acid, and transfer the resulting solution quantitatively into the 60 ml separatory funnel. Complete the analysis following the recommended procedure.

Procedures for the Analysis of Wine. DRY ASHING PROCEDURE. Pipet 2.00 ml of wine into a crucible (determine density if sample weight is desired), evaporate to dryness, and ignite at 450-500°C to a gray ash. Allow to cool, add 1 ml of concentrated nitric acid , evaporate, and ignite for 1 hr. at 450-500°C. Dissolve the cool, white ash in 10 ml of 1*M* nitric acid, and transfer the solution quantitatively into a 60 ml separatory funnel. Complete the analysis following the recommended procedure.

WET ASHING PROCEDURE. Pipet a 2.00 ml sample into a 125 ml conical flask, add a 10 ml mixture of equal volumes perchloric acid (70%) and concentrated nitric acid , and heat gently until vigorous evolution of brown fumes subsides. Continue heating more strongly until dense white fumes of perchloric acid completely fill the flask. Allow to cool, add 20 ml of water, and boil briefly to expel any chlorine. Add sufficient concentrated ammonium hydroxide (approximately 4 ml) to adjust to pH 4.5 $\pm$1 using pH indicating paper. Transfer the solution quantitatively to a 60 ml separatory funnel, and complete the analysis following the recommended procedure. Additon of the 50% $NaClO_4$ solution maybe omitted, because the sample solution contains sufficient perchlorate salt.

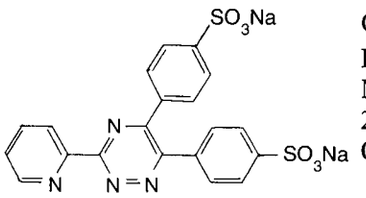
SECTION V BIBLIOGRAPHY
PDT
3-(2-Pyridyl)5,6-diphenyl-1,2,4-triazine)

1. A. A. Schilt and P. J. Taylor, *Anal. Chem.*, **42,** 220 (1970). — Simultaneous determination of iron and copper.
2. A. A. Schilt, *Talanta*, **13,** 895 (1966). — Some new chromogens for iron, cobalt and copper.
3. F. H. Case, *J. Org. Chem.*, **30,** 931 (1965). — Synthesis of hydrazidines and triazines containing the ferroin group.
4. C. L. Kamra and G. H. Ayres, *Anal. Chim. Acta*, **78,** 423 (1975). — Spectrophotometric determination of ruthenium.
5. C. D. Chriswell and A. A. Schilt, *Anal. Chem.*, **46,** 992 (1974). — Spectrophotometric determination of iron in acids and acidic solutions by an extraction formation reaction.
6. J. L. Lundgren and A. A. Schilt, *Anal. Chem.*, **49,** 974 (1977). — Purification of reagents, preconcentraton of trace metals from dilute solutions, group separation of metal ions, analysis of sea water, determination of trace metals in reagent grade chemicals.

SECTION VI

PDTS

3-(2-Pyridyl)-5,6-bis (4-phenylsulfonic acid)-1,2,4-Triazine disodium salt.



$C_{20}H_{14}N_4O_6S_2Na_2$
F. W. 514.46
Molar absorptivity of $Fe(PDTS)_3$
25,400 at 560 nm
G. Frederick Smith Chemical Company
Catalog Item No. 569

In 1970, the first published work with the sulfonated derivative of PDT appeared under the authorship of L. L. Stookey[1] who coined the trivial name "ferrozine", which later became a trademark of the Hach Chemical Co. Because of its attractive price and slightly higher sensitivity than previously available sulfonated, water-soluble iron reagents, it attracted considerable attention resulting in a number of applications and additional studies.

Interferences. Ions that interfere in the determination of iron include Cu^{+1}, Co^{+2}, Cr^{+3}, Ni^{+2}, CN^- and NO_2^-. Oxalate in concentrations over 500 ppm also will interfere. Copper interference may be overcome by determining the copper simultaneously or by adding cyanide to the sample after determination and measuring the absorbance again for a blank value[2]. Cyanide and nitrite interferences can also be overcome by boiling the sample with an acidic reducing agent solution.

Applications. In addition to the determination of iron in potable water[1], other applications published for the use of PDTS in the determination of iron include iron in wine[2], plant material[3], and in serum or blood[4-15]. A number of these also include determination of iron-binding capacity and automated procedures. PDTS has been applied also to the determination of ascorbic acid[16], sulfur dioxide[17], ruthenium and osmium[18], copper and cobalt[19], and the simultaneous determination of iron and copper[20].

General Procedure for the Determination of Iron. REAGENTS. 0.01 *M* PDTS solution. Dissolve 0.515 g PDTS in water and dilute to 100 ml.

Reducing agent. Iron-free 10% hydroxylammonium chloride is prepared as described on p. 12.

Buffer solution. Iron-free 10% sodium acetate is prepared as described on p. 11.

Acidic reducing solution. 500 ml of iron-free 10% hydroxylammonium chloride is combined with 500 ml of 6 *M* iron-free hydrochloric acid and 5.15 g of PDTS.

Standard Iron solutions. 10.0 μg Fe/ml; 1.00 μg Fe/ml is prepared as described on p. 12.

Pipet a sample sufficient to contain 5-100 μg of iron into a 100 ml volumetric flask. Add 2 ml of 0.01 *M* PDTS, 2 ml of 10% hydroxylamine hydrochloride and 5 ml of 10% sodium acetate buffer. Dilute to the mark and mix well. Measure the absorbance of the solution vs. a similarly treated blank at 560 nm.

Procedure for the Determination of Iron in Potable Water. METHOD OF STOOKEY[1]. Pipet 50.00 ml of freshly drawn water sample into a 125 ml Erlenmeyer flask and add 2 ml of acidic reducing solution. Heat on a hot plate and hold at boiling for 10 minutes. A glass stirring rod should be used to prevent bumping. Cool to 20°C and transfer quantitatively to a 50 ml volumetric flask. Add 1.00 ml of pH 5.5 buffer (400 g of ammonium acetate and 350 ml of concentrated ammonium hydroxide diluted to 1l) and dilute to the mark with demineralized water. Mix thoroughly and allow one minute for full color development. Measure absorbance at 562 nm and compare with a calibration curve prepared from standard solutions treated in the same manner. Note that the blank is kept small by extraction of iron impurities from the buffer solution and the reducing agent by utilizing the extraction procedure in section V. These are modifications in Stookey's original procedure to avoid the high blanks resulting from using unextracted reagents.

SECTION VI. BIBLIOGRAPHY
PDTS

1 L. L. Stookey, *Anal. Chem.*, **42,** 779 (1970). Ferrozine — a new spectrophotometric reagent for iron.

2. A. Mosandl, *Mitt. Hoeheren Bundeslehr-Versuchsanst. Wein Obstbau, Klosterneuburg,* **23,** 27 (1973). Determination of iron in wine.

3. C. H. Chen and J. Lewin, *J. Sci. Food Agrie.*, **23,** 1355 (1972). Colorimetric determination of iron in plant material.

4. P. Carter, *Anal. Biochem.*, **40,** 450 (1971). Spectrophotometric determination of serum iron.

5. J. P. Persijn. W. Van der Slik, and A. Riethorst, *Clin. Chim. Acta*, **35,** 91 (1971). Determination of serum iron and latent iron-binding capacity.

6. D. Plant, E. Miller, J. Mac Queen, and R. Blanchard, *Amer. J. Med. Technol.*, **38,** 82 (1972). Determination of iron in serum.

7. P. Carter, *Mikrochim. Acta*, **1972,** 410. Automated determination of serum iron and iron binding capacity.

8. F. Bellina, G. Giordani, and P. Lo Greco, *Friuli Med.*, **28,** 449 (1973). Spectrophotometric method for the determination of serum iron without deproteinization.

9. J. M. White and H. A. Flashka, *Clin. Chem.*, **19,** 526 (1973). Automated procedure for serum iron and iron binding capacity.

10. A. Manasterski, R. Watkins, E. S. Baginski, and B. Zak, *Z. Klin. Chem. Klin. Biochem.*, **11,** 335 (1973). Microdetermination of haemoglobin iron.

11. E. Horak and F. W. Sunderman, Jr., *Ann. Clin. Lab. Sci.*, **4,** 87 (1974). Accurate spectrophotometric method for serum iron and iron-binding capacity without deproteinization or centrifugation.

12. I. K. Tan, C. Y. Khoo, and K. S. Chua, *Adv. Autom. Anal., Technicon Int. Symp.*, **1974,** 57. Estimation of serum iron, serum total iron binding capacity and urine iron by the Auto Analyzer I System.

13. R. Ruutu, *Clin. Chim. Acta*, **61,** 229 (1975). Determination of iron and unsaturated iron binding capacity in serum.

14. E. Horak, D. C. Hohnadel, and F. W. Sunderman, Jr., *Ann. Clin. Lab. Sci.*, **5,** 303 (1975). Modified method for analysis of serum iron.

15. Y. Narikawa and T. Yoshino, *Rinsho* **19,** 649 (1975). Serum iron determination by the Fe^{++} Kit-S.

16. B. Jaselskis and J. Nelapaty, S. J., *Anal. Chem.*, **44,** 379 (1972). Spectrophotometric determination of microamounts of ascorbic acid in citrus fruits.

17. A. Attari and B. Jaselskis, *Anal. Chem.* **44,** 1515 (1972). Determination of sulfur dioxide.

18. S. K. Kundra, M. Katyal and R. P. Singh, *Current Sci.* **44,** 548 (1975). Determination of ruthenium(III) and osmium (VIII).

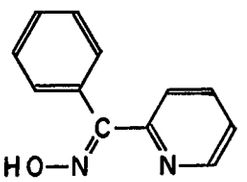
19. S. K. Kundra, M. Katyal and R. P. Singh, *Anal. Chem.*, **46,** 1605 (1974). Determination of copper and cobalt.

20. H. Y. Yee and J. F. Goodwin, *Clin. Chem.*, **20,**188 (1974). Simultaneous determination of iron and copper in a single aliquot of serum.

SECTION VII

PHENYL-2-PYRIDYL KETOXIME

syn-Phenyl-2-pyridyl Ketoxime



$C_{12}H_{10}N_2O$
F. W.: 198.22
Molar absorptivity of
$[Fe(Phenyl\text{-}2\text{-}pyridyl\ Ketoxime)_3]^{+}$
in isoamyl alcohol: 15,600 at 550 nm
G. Frederick Smith Chemical Company,
Catalog Item No. 295

Phenyl-2-pyridyl ketoxime exists in two stereoisomeric forms, *syn* and *anti*[1]. The lower-melting (*syn*-) isomer was found by Tschugaeff[2] to form colored complexes with metals. It is a white crystalline solid which is soluble in benzene, chloroform, dioxane and alcohols, sparingly soluble in cold ethanol, and insoluble in water. The solutions are stable if protected from sunlight, otherwise they take on a yellow color.

Phenyl-2-pyridyl ketoxime reacts with ferrous ion to form a red, water soluble complex which exhibits a single absorption peak in the visible region at 545 nm. The complex is formed in neutral or basic solutions, even as alkaline as saturated sodium hydroxide. The red complex can be extracted into either isoamyl alcohol or chloroform, provided that the concentration of base (NaOH or equivalent) is 1 *M* or greater. A single extraction with isoamyl alcohol and phenyl-2-pyridyl ketoxime will suffice to remove the iron quantitatively if a few milliliters of ethyl alcohol are also added. In isoamyl alcohol, the absorption maximum is at 550 nm. Solutions of the iron compound in isoamyl alcohol show no change in absorbance over a 24 hour period of standing, but aqueous solutions give rise to precipitate formation within a few hours. Chloroform solutions are noticeably unstable in the light.

The method of continuous variations, employed to determine the combining ratio of phenyl-2-pyridyl ketoxime to iron, indicated that three molecules of the oxime coordinate to each ion of iron[3].

Interferences. Phenyl-2-pyridyl ketoxime forms colored complexes with a number of the transition elements in neutral and alkaline solutions. Iron(III) as well as iron(II) forms a red, copper(I) an orange, copper (II) a green complex, and cobalt(II), manganese(II), palladium(II), platinum(II) and uranyl ions all produce yellow complexes. All of these metal derivatives except platinum and uranyl can be extracted into chloroform. As phenyl-2-pyridyl ketoxime forms colored extractable complexes with many transition elements, high specificity toward iron is not expected, but the tolerance to the transition metals closely associated with iron is

sufficiently great for its application to be useful. Cluley and Newman[5], found that recoveries of iron were slightly low in the presence of equal weights of cobalt, copper, manganese and nickel. No interference was found in the presence of cerium(III), mercury(I), aluminum, calcium and zinc when the extraction procedure is followed. Low results were obtained in the presence of large amounts of cadmium, lead and magnesium. The following anions do not interfere: chloride, sulfate, nitrate, perchlorate, acetate, citrate, orthophosphate, and borate. Arsenate, fluoride, molybdate, oxalate and tartrate interfere if present in relatively high concentrations.

Applications. In addition to its use for the determination of iron[3-6], phenyl-2-pyridyl ketoxime has been employed as a colorimetric reagent for the determination of palladium[7], gold[8], and rhenium[9]. Its unique advantage over other iron reagents described in this monograph is its applicability in strong base, enabling determination of iron in caustic samples without need of neutralization and pH adjustment. In this regard it is similar to 4,7-dihydroxy-1,10-phenanthroline which was the first iron reagent shown to be directly applicable to caustic solutions[10].

Procedure for the Determination of Iron in Strong Alkalies. METHOD OF TRUSELL AND DIEHL[3]. REAGENTS. PHENYL-2-PYRIDYL KETOXIME. 0.2%. Dissolve 2 g of phenyl-2-pyridyl ketoxime in 1 liter of 0.1 *M* hydrochloric acid.

SODIUM HYDROSULFITE. 10% SOLUTION, IRON-FREE. Prepare fresh each day. Dissolve 2 g of sodium hydrosulfite in 10 ml of water, add 5 ml of 0.2% phenyl-2-pyridyl ketoxime and 5 ml of 10 *M* sodium hydroxide, iron free, and allow to stand 10 minutes. Extract the iron compound thus formed into 10 ml of isoamyl alcohol and 5 ml of ethyl alcohol.

SODIUM HYDROXIDE. 10 *M*, IRON-FREE. Dissolve 400 g of sodium hydroxide in 800 ml of water. Add 10 ml of sodium hydrosulfite and 50 ml of 0.2% phenyl-2-pyridyl ketoxime. Allow the mixture to stand for 10 minutes and then extract the red compound thus formed into 100 ml of isoamyl alcohol and 25 ml of ethyl alcohol. Dilute the colorless, aqueous phase to 1 liter.

STANDARD IRON SOLUTION. 5×10^{-4} *M*. Dissolve 0.5585 g of electrolytic iron in a few ml of hydrochloric acid and dilute to 1 liter. Pipet 50.0 ml of this solution into a 1-liter volumetric flask and dilute to the mark with distilled water.

Procedure for Oxidized Iron in Strong Alkalies. Weigh a sample of such size as to give a solution 4 to 5 *M* in alkali when dissolved in 250 ml of water. Dissolve the sample in deionized water and dilute to 250 ml. Pipet 25.0 ml into a 100 ml beaker and add 2.0 ml of 10% sodium hydrosulfite and 5.0 ml of 0.2% phenyl-2-pyridyl ketoxime. Heat the mixture 5 minutes on a steam plate and transfer to a separatory funnel. Add 5 ml of ethyl alcohol and 10 ml of isoamyl alcohol, shake, and allow the layers to separate. Draw off the aqueous layer. Transfer the alcohol layer to a 25 ml volumetic flask, dilute to the mark with isoamyl alcohol, and measure the absorbance at 550 nm.

Prepare a calibration curve by adding known amounts of the standard iron solution, up to 10 ml, to 25 ml of 10 *M* iron-free sodium hydroxide. Add 2.0 ml of 10% sodium hydrosulfite, 5.0 ml of 0.2% phenyl-2-pyridyl ketoxime, dilute to approximately 50 ml and heat on a steam plate for five minutes. Transfer the solution to a separatory funnel and extract the red iron compound into 5 ml of ethyl alcohol and 10 ml of isoamyl alcohol. Draw off the aqueous layer. Transfer the alcohol layer to a 25 ml volumetric flask, dilute to the mark, and determine the absorbance at 550 nm.

Procedure for Iron in Glass Sand. METHOD OF TRUSELL AND DIEHL[3] as modified by Cluley and Newman[4]. Dry the sample for two hours at 110°. Weigh 100 mg into a platinum crucible which has been previously freed of iron by alternate ignition to red heat and treatment with hot hydrochloric acid. Add 1 g of an equimolar mixture of sodium carbonate and sodium tetraborate, and blend the contents thoroughly. Fuse in the usual manner. After cooling, dissolve the melt in 10 ml of water. Transfer the solution to a small beaker with 25 ml of 10 *M* sodium hydroxide, treat with 2 ml of 10% sodium hydrosulfite and 5 ml of 0.2% phenyl-2-pyridyl ketoxime and heat on a steam plate five minutes. Transfer the solution to a separatory funnel and extract with a mixture of 5 ml of ethyl alcohol and 10 ml of isoamyl alcohol. Transfer the alcohol layer to a 25 ml volumetric flask, dilute to the mark, and measure the absorbance at 550 nm.

Procedure for Iron in Ethylene Amines. METHOD OF CHERNIN AND SIMONSEN[5]. Introduce a 10 ml portion of the sample (less if the iron content is greater than 20 ppm) into a 50 ml graduated mixing cylinder. Dilute to 25 ml with distilled water, mix and transfer the solution to a 10 ml beaker. Add 2 ml of 10% iron-free sodium hydrosulfite and allow the mixture to stand a few minutes. Add 5 ml of 0.2% phenyl-2-pyridyl ketoxime and heat for 10 minutes in a hot water bath (80-100°). Cool and transfer the solution to a separatory funnel. Add 15 ml of isoamyl alcohol-ethanol (15:2), shake and separate. Filter the alcohol layer into a 25 ml volumetric flask and rinse the paper with isoamyl alcohol. Dilute to the mark, mix, and measure the absorbance at 588 nm against a reagent blank. A calibration curve is prepared by adding known amounts of iron to 10 ml samples of iron-free ethylene amine and treating in the same manner.

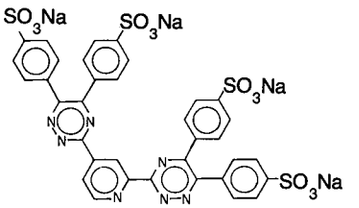
SECTION VII. BIBLIOGRAPHY
PHENYL-2-PYRIDYL KETOXIME

1. E. H. Huntress and H. C. Walker, *J. Am. Chem. Soc.*, **70,** 3702 (1948). — Preparation of phenyl-2-pyridyl ketoxime and separation of syn- and anti-isomers.
2. L. A. Tschugaeff, *Ber*, **39,** 3382 (1906). — Palladium and platinum compounds of phenyl-2-pyridyl ketoxime
3. F. Trusell and H. Diehl, *Anal. Chem.*, **31,** 1978 (1959). — Determination of iron in strong alkalies. Extraction of iron from highly alkaline solutions. Determination of "oxidized" iron in the presence of metallic iron.
4. H. J. Cluley and E. J. Newman, *Analyst*, **88,** 3 (1963). — The determination of iron with phenyl-2-pyridyl ketoxime.
5. R. Chernin and E. R. Simonsen, *Anal. Chem.*, **36,** 1093 (1964). — Spectrophotometric determination of iron in ethylene amines.
6. K. Mallik and B. Sen, *Anal. Chim. Acta.*, **23,** 225 (1960). — Spot test for iron.
7. B. Sen., *Anal. Chim. Acta.*, **21,** 881 (1959). — Spectrophotometric determination of palladium.
8. B. Sen., *Anal. Chim. Acta.*, **21,** 35 (1959). — Spectrophotometric determination of gold.
9. J. Guyon and R. K. Murmann, *Anal. Chem.*, **36,** 1058 (1964). — Spectrophotometric determination of rhenium.
10. A. A. Schilt, G. F. Smith and A. Heimbuch, *Anal. Chem.*, **28,** 809 (1956). — Spectrophotometric determination of iron in highly alkaline solutions and in glass sand.

SECTION VIII

2,4-BDTPS

2,4-Bis(5,6-diphenyl-1,2,4-triazin-3-yl)-pyridine Tetrasulfonic Acid Tetrasodium Salt



$C_{35}H_{19}N_7(SO_3Na)_4$
F. W. : 949.85
Molar absorptivity of
Fe(II) chelate: 32,200 at 565 nm
Cu(I) chelate: 9,700 at 460 nm
G. Frederick Smith Chemical Company
Catalog No. 664

One of the most sensitive chromogenic reagents for iron developed to date from among ferroin type compounds, 2,4-BDTPS is readily soluble in water and ideally suited for the determination of trace amounts of iron as well as for simultaneous determination of copper and iron. Its solubility, rapidity of reaction with iron(II) and copper(I), the stability of the complexes once formed, freedom from troublesome interferences, and selectivity of its chromogenic reactions should make it the reagent of choice for use in automated procedures or for samples that are unusually small or limited in availability.

Synthesis. The parent, unsulfonated compound 2,4-bis(5,6-diphenyl-1,2,4-triazin-3-yl)-pyridine (2,4-BDTP) was synthesized in 1970 by Case[1]. Evaluation of the chromogenic properties of 2,4-BDTP by Schilt, Chriswell, and Fang[2] revealed its outstanding properties, although severely limited in practical application due to its extremely low solubility in water. Subsequent studies by Traister and Schilt[3] resulted in the synthesis and isolation of the water-soluble, sulfonated derivative together with evidence of the formula and structure of 2,4-BDTPS.

Properties. Spectra of the iron(II) and copper(I) chelates of 2,4-BDTPS are shown in Figure 6, where Curve A is that of the reagent blank, Curve B of 2.96×10^{-5} *M* copper(I), and Curve C of 1.46×10^{-5} *M* iron(II). Suitable wavelength pairs for the simultaneous determination of iron and copper are 460 and 563 nm or 600 and 563 nm. Absorbances are additive and follow Beer's law. A concentration of 1.6×10^{-7} *M*(0.009 ppm) iron and/or 5×10^{-7} *M* (0.033 ppm) copper can be detected, based on an absorbance of 0.005 in a 1 cm cell.

The effect of pH on completeness of color formation of the iron(II) complex is shown by Curve A in Figure 7 and for copper(I) by Curve B. Careful control of pH permits color formation by iron(II) and avoids that by copper(I). Another method that permits measurement of absorbance

due only to iron(II) is that devised by Schilt and Taylor[4] which involves rapid destruction of the copper(I) chelate once formed without immediate effect on the iron(II) chelate by treatment with solid sodium cyanide.

Relatively few substances interfere in the spectrophotometric determination of iron and copper. Cyanide and pyrophosphate interference can be eliminated by preliminary acidification and boiling. Chromium(III), cobalt(II), and nickel(II) cause positive errors but can be tolerated at or below 21, 7, and 3 ppm levels, respectively.

Applications. A number of analytical applications for 2,4-BDTPS have been developed and tested by Traister and Schilt[3]. These include simultaneous determination of iron and copper in blood serum, selective determination of iron in blood serum, determination of iron in beer, and determination of iron and copper in drinking water.

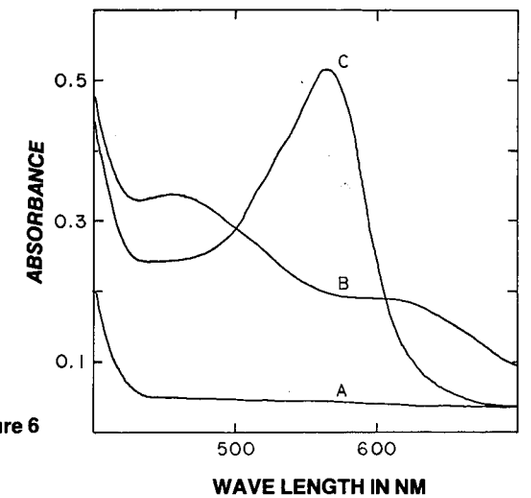


Figure 6

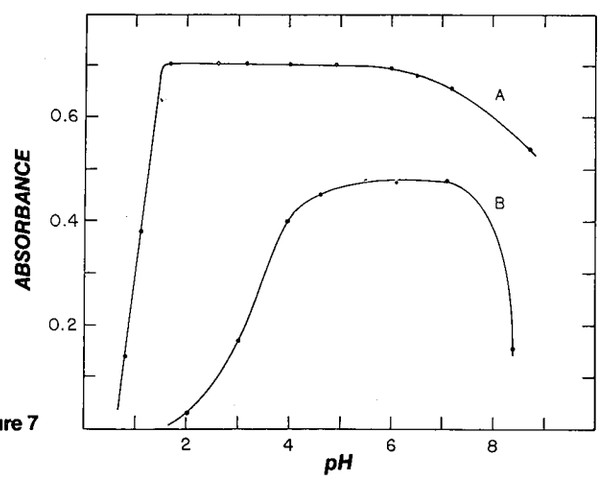


Figure 7

Procedure for Simultaneous Determination of Iron and Copper in Blood Serum. Pipet 0.50 ml of serum sample into a 13 x 100 mm test tube, add 0.50 ml of extractant-reductant solution (0.2 *M* HCl containing 5 g/l $H_2NOH{\cdot}HCl$), mix, and let stand approximately 10 minutes. Add 0.50 ml of protein precipitant solution (12% trichloroacetic acid), mix, stopper the tube, and centrifuge until supernatant is clear. Transfer 1.00 ml of the supernatant to a second tube, add 0.30 ml of 0.005 *M* 2,4-BDTPS reagent and 0.20 ml of acetate buffer solution (2.5 *M* in NH_3 and 0.3 *M* in NH_4OAc) (to adjust to pH 5-6), mix well, and allow to stand for 10 minutes for maximum color development. Measure the absorbance at 563 and also at 460 nm. Carry a reagent blank through the procedure utilizing 0.50 ml of distilled water in place of the sample. Subtract the absorbances of the blank from those of the sample. From the corrected absorbances of the sample calculate the concentrations of each metal by simultaneous solution of the two linear equations, coefficients for which having been determined by carrying standards through the same procedure as unknowns. For convenience and simplicity a suitably prepared nomograph can serve effectively in place of the calculations.

Procedure for the Selective Determination of Iron in Blood Serum. Follow the same procedure as the above (for the simultaneous determination of iron and copper) except in place of the 0.20 ml of the acetate buffer add 0.20 ml of tartrate buffer (1 *M* disodium tartrate) to adjust the pH to 2.5-3.5. Heat the mixture for 15 minutes at 40°C, cool, and measure the absorbance at 563 nm. Carry a reagent blank and standard through the same procedure. Calculate the concentration of iron in the unknown from the measured absorbance corrected for the blank or refer to a suitably prepared calibration curve.

Procedure for the Determination of Iron and Copper in Water. Pipet a sample of appropriate size (1-20 ml containing 4-40 μg of Fe and/or 16-160 μg of Cu) into a 50 ml conical flask, add 3 drops of 6 *M* hydrochloric acid, and bring to near boiling on a hot plate. Cool and transfer the contents of the flask quantitatively to a 25 ml volumetric flask. If only dissolved iron, free of particulate matter, is to be determined the sample need not be acidified and heated but can be delivered directly into the 25 ml volumetric flask. Add 1 ml of 0.005 *M* 2,4-BDTPS reagent and 1 ml of 10% hydroxylamine hydrochloride solution, mix thoroughly, add 1 ml of 10 *M* ammonium acetate (to adjust to pH 5-7), and dilute to volume with distilled water. Measure the absorbance at 563 and also at 460 nm. Carry a reagent blank and standard through the same procedure to obtain suitable corrections and calibration data. Calculate the concentration of each metal or determine them from a suitable nomograph.

As an alternate method[4] for the simultaneous determination measure the absorbance at 463 nm before and 1-15 minutes after adding 10-30 mg of sodium cyanide to the contents of the flask. The iron concentration is directly proportional to the final absorbance, and the decrease in absorbance on adding sodium cyanide is linear with copper concentration. Calibration curves are simpler to prepare and use in this than the above method.

Procedure for the Determination of Iron in Beer. Pipet a 25 ml sample of degassed beer into a 125 ml flask and evaporate to near dryness on a hot plate. Cool and add 5 ml of an equal volume mixture of concentrated nitric acid (70%) and

perchloric acid (70%). Heat gently until dark brown fumes cease to evolve. Continue heating at a higher temperature unitl the solution becomes colorless and dense white fumes of perchloric acid fill the flask. To the cooled flask add 5 ml of distilled water, 1 ml of 0.005 *M* 2,4-BDTPS reagent, 2 ml of 10% hydroxylamine hydrochloride, and 3 ml of 1 *M* disodium tartrate. Adjust the solution to pH 2.5-3.1 with concentrated ammonium hydroxide (requires approximately 0.5 ml). Heat the solution at 40-60°C for 15 minutes to produce complete color development. Cool to room temperature, transfer the solution quantitatively to a 25 ml volumetric flask, and dilute to volume with distilled water. Measure the absorbance at 563 nm. Carry a reagent blank and standards through the same procedure. Make use of a suitably prepared calibration curve or empirical equation to convert absorbance to concentation.

SECTION VIII. BIBLIOGRAPHY

1. F. H. Case, *J. Heterocylic Chem.*, **7,** 1001 (1970).
2. A. A. Schilt, C. D. Chriswell, and T. A. Fang, *Talanta*, **21,** 831 (1974).
3. G. L. Traister and A. A. Schilt, *Anal. Chem.*, **48,** 1216 (1976).
4. A. A. Schilt and P. J. Taylor, *Anal. Chem.*, **42,** 220 (1970).

SECTION IX

NEW IRON REAGENTS

Recently Introduced or Currently under Investigation

In recent years a number of new and promising chromogenic reagents for the determination of iron and other important transition metal ions have been synthesized by Professor F. H. Case at Temple University and characterized for their chelation properties and analytical applications by Professor A. A. Schilt and students at Northern Illinois University. Most of these await further specialized study by individuals interested in applying them to their specific types of samples and analytical problems. A listing of the reagents and pertinent references is included here to direct attention to their interesting possibilities and their commercial availability from the G. Frederick Smith Chemical Company.

Unsulfonated Iron Chromogens. The following three compounds exhibit properties that are highly suitable for their use as extraction reagents as well as spectrophotometric reagents for iron:

Compound and GFS Item No.	Molar Absorptivity of Fe(II) Chelate	References
2,4-BIS(5,6-DIPHENYL-1,2,4-TRIAZIN-3-YL)-PYRIDINE (2,4-BDTP), **No. 663**	32,000 at 563 *nm*	1,2
3-(4-PHENYL-2-PYRIDYL)-5,6-DIPHENYL-1,2,4-TRIAZINE (PPDT), **No. 661**	28,700 at 561 *nm*	3,4,5
3-(4-PHENYL-2-PYRIDYL)-5-PHENYL-1,2,4-TRIAZINE (PPT), **No. 727**	34,800 at 560 *nm*	5,6

Sulfonated Iron Chromogens. The following are water-soluble reagents, free of solubility problems, and suitable for spectrophotometric determination of trace quantities of iron and for automated procedures:

Compound and GFS Item No.	Molar Absorptivity of Fe(II) Chelate	References
3-(4-PHENYL-2-PYRIDYL)-5,6-DIPHENYL-1,2,4-TRIAZINE-TRISULFONIC ACID TRISODIUM SALT (PPDTS), **No. 662**	30,700 at 563 *nm*	6,8
3-(4-PHENYL-2-PYRIDYL)-5-PHENYL-1,2,4-TRIAZINE DISULFONIC ACID DISODIUM SALT (PPTS), **Item No. 728**	32,900 at 565 *nm*	9

SECTION IX. REFERENCES

1. F. H. Case, *J. Heterocyclic Chem.*, **7,** 1001 (1970).
2. A. A. Schilt, C. D. Chriswell, and T. A. Fang, *Talanta*, **21,** 831 (1974).
3. A. A. Schilt, *Talanta*, **13,** 895 (1966).
4. F. H. Case, *J. Org. Chem.*, **30,** 931 (1965).
5. A. A. Schilt and W. C. Hoyle, *Anal. Chem.*, **39,** 114 (1967).
6. F. H. Case, *J. Heterocyclic Chem.*, **10,** 353 (1973).
7. A. A. Schilt, T. A. Yang, J. F. Wu, and D. M. Mitzki, *Talanta*, **24,** 685 (1977).
8. G. L. Traister and A. A. Schilt, *Anal. Chem.*, **48,** 1216 (1976).
9. A. A. Schilt and M. DiTusa, unpublished work nearing completion.

GFS Chemical Co. Products Mentioned in Text with GFS Product Item Number.

767	Amberlite XAD - 2 with PDT Coating
811	Ammonium Hydroxide
3	Ammonium Perchlorate
108	Bathophenanthroline (4,7-Diphenyl-1,10-phenanthroline)
1086	Bathophenanthroline, 0.02% in 3 to 1 Isopropyl - Isoamyl Alcohol
812	Bathophenanthroline, 0.0025 *M* in Isoamyl Alcohol
813	Bathophenanthroline, 0.0025 *M* in 95% Ethanol
286	Bathophenanthroline, Sulfonated, Sodium Salt
665	Bathophenanthroline, Sulfonated, Ferrous Sulfate Solution
663	2,4-Bis(5,6-Diphenyl-1,2,4-Triazin-3-yl)-Pyridine(2,4-BDTP)
664	2,4-Bis(5,6-Diphenyl-1,2,4-Triazin-3-yl)-Pyridine Tetrasulfonic Acid Tetrasodium Salt (2,4-BDTPS)
26	Ceric Sulfate, Solution 0.1 N (Sulfato-Ceric Acid)
816	Chloroform
550	Ferrous Ammonium Sulfate
41	Ferrous Ethylenediammonium Sulfate
1087	n-Hexyl Alcohol
625	Hydrochloric Acid, 37%, Reagent
504	Hydrochloric Acid, 6 Molar, Redistilled
382	Hydrochloric Acid, 1 N
143	Hydroxylammonium Chloride, Reagent
1083	Hydroxylammonium Chloride, 10%, Iron-free
1543	Hydroxylammonium Chloride, 20%, Iron-free
226	Iron, Electrolytic
1082	Isoamyl Alcohol
626	Nitric Acid, Reagent
63	Nitric Acid, Redistilled
66	Perchloric Acid, 70%, Reagent
661	3-(4-Phenyl-2-Pyridyl)-5,6-Diphenyl,-1,2,4-Triazine (PPDT)
662	3-(4-Phenyl-2-Pyridyl)-5,6-Diphenyl,-1,2,4-Triazine Trisulfonic Acid Trisodium Salt (PPDTS)
295	Phenyl-2-Pyridyl Ketoxime
727	3-(4-Phenyl-2-Pyridyl)-5-Phenyl-1,2,4-Triazine (PPT)
728	3-(4-Phenyl-2-Pyridyl)-5-Phenyl-1,2,4-Triazine Disulfonic Acid, Disodium Salt (PPTS)
516	3-(2-Pyridyl)-5,6-Diphenyl-1,2,4-Triazine (PDT)
569	3-(2-Pyridyl)-5,6-Diphenyl-1,2,4-Triazine Sulfonated Sodium Salt
656	Sodium Acetate, Reagent
1084	Sodium Acetate, 10%, Iron-free
1542	Sodium Acetate, 20%, Iron-free
385	2 M Sodium Acetate - 2 M Acetic Acid Buffer, Iron-free
558	Sodium Hydrosulfite
630	Sodium Hydroxide
92	Sodium Perchlorate, Hydated
1085	Standard Solutions - Iron 1 μg/ml
544	Standard Solutions - Iron 10 μg/ml
545	Standard Solutions - Iron 1 mg/ml
562	Stannous Chloride
628	Sulfuric Acid, Reagent
390	Trichloroacetic Acid, Redistilled
291	2,4,6-Tripyridyl-s-Triazine (TPTZ)