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The reaction of iron (II) with tripyridyl-s-triazine

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THE REACTION OF IRON(II) WITH TRIPYRIDYL-S-TRIAZINE

by

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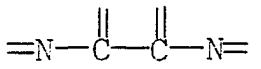
INTRODUCTION

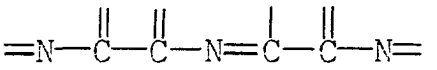
The determination of small amounts of iron has been greatly simplified by the introduction of 1,10-phenanthroline, 2,2'-bipyridine and 2,2',2''-terpyridine as colorimetric reagents. The reaction of this type of compounds with iron(II) to yield highly colored compounds was first observed by Blau in 1898 (1). On reexamination of this reaction in 1931, Walden, Hammett and Chapman (2) found that the red tris(1,10-phenanthroline)iron(II) ion could serve as an oxidation-reduction indicator in certain titrations.

The first published application of this reaction of 1,10-phenanthroline to the colorimetric determination of iron was in 1937 when Saywell and Cunningham (3) described a method for the determination of iron in fruit juices. Shortly thereafter, Hummell and Willard (4) proposed a somewhat similar method for the determination of iron in biological materials. A critical study of the use of 1,10-phenanthroline as a colorimetric reagent for iron was undertaken by Fortune and Mellon (5) who concluded that the method possesses great advantages owing to its sensitivity and freedom from interference by most of the common ions. Since this early work procedures for the determination of iron in a wide variety of materials using various 1,10-phenanthrolines and polypyridines have been described.

The functional group present in 1,10-phenanthroline and 2,2'-bipyridine which is responsible for the reaction with iron(II) and various other metal ions has the structure



and is generally termed the ferroine group (6). Similarly, the group in 2,2',2''-terpyridine

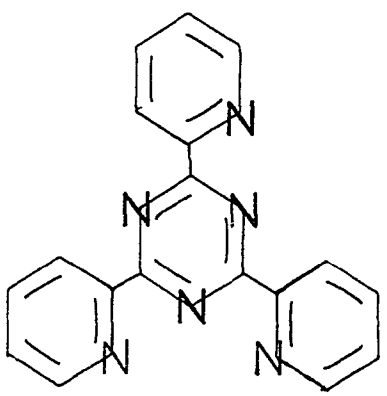


is known as the terroine functional group. The various compounds containing these groups are termed ferroine and terroine reagents.

A great number of substituted 1,10-phenanthrolines and polypyridines have been prepared and their metal derivatives studied primarily through the efforts of Professors G. Frederick Smith and Francis Case. One result of these investigations has been to show that, in general, the sensitivity of these various compounds toward iron can be greatly increased by substituting phenyl groups para to the ring nitrogen atoms. Thus, the molar extinction coefficient of the iron derivative of bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) is 22,400, over twice that of the corresponding derivative of 1,10-phenanthroline. A similar sharp increase in sensitivity is observed with 2,6-bis(4-phenyl-2-pyridyl)-4-phenylpyridine compared to 2,2',2''-terpyridine (7).

Bathophenanthroline was first introduced by Case, Smith, McCurdy and Diehl (8, 9) as an extremely sensitive reagent for the determination of iron in water. The general method has since been adopted for the determination of trace amounts of iron in various other materials. In addition to its sensitivity, the tris(4,7-diphenyl-1,10-phenanthroline)-iron(II) ion is extractable into certain organic solvents. This is a distinct advantage since it allows the various reagents used in the determination to be rendered iron free and the extraction may also serve as a concentration step. The preparation of bathophenanthroline is not simple; consequently, the reagent is rather expensive. There is, therefore, a need for a supersensitive iron reagent that can be prepared with greater ease.

The work now to be described is an investigation of 2,4,6-tris(2'-pyridyl)-s-triazine (TPTZ)



as a colorimetric reagent for iron. This compound, which contains the terroine functional group, is one of a series of

amino and pyridyl substituted symmetrical triazines recently synthesized by Professor Case. The absorption spectra of these compounds and their iron(II) derivatives have been studied (10) in an attempt to correlate their properties with analogous members of the bi- and terpyridine systems. No other work on 2,4,6-Tris(2'-pyridyl)-s-triazine has been reported. The first part of this investigation is a study of the reaction of TPTZ with iron(II). Following this, procedures are described for the determination of iron in various materials.

THE REACTION OF IRON(II) WITH 2,4,6-TRIS(2'-PYRIDYL)-S-TRIAZINE

Reagents and Apparatus

Reagents

2,4,6-Tris(2'-pyridyl)-s-triazine The synthesis of 2,4,6-tris(2'-pyridyl)-s-triazine (TPTZ) was accomplished by Professor Francis Case of Temple University (11). To supplement the amount of TPTZ obtained from Professor Case, more material was prepared following essentially the Case procedure. A quantity of 2-cyanopyridine weighing 93 g. and 1.6 g. of sodium hydride were heated for 5 hours under nitrogen at 160 to 165°. The mixture was stirred for the first two hours. After cooling, the brown residue was removed from the flask, crushed and extracted with benzene in a Soxhlet extractor. The benzene extract was evaporated to dryness yielding a tan material. After repeated recrystallization from water containing a small amount of ammonium hydroxide and decolorizing carbon, 27 g. of practically white TPTZ was obtained. This product was finally recrystallized from benzene. Melting point: 242 to 243.5°. Reported by Case: 244-245°. Solutions of the reagent used in this work were prepared by dissolving the compound in a few drops of hydrochloric acid and diluting with water.

Standard iron solutions A quantity of electrolytic iron weighing 0.5585 g. was dissolved in 20 ml. of hydrochloric acid and diluted to exactly 1 liter in a volumetric flask to give a solution 1.000×10^{-2} M in iron. The various iron solutions used in the following work were prepared from this solution, or solutions prepared in a similar way, by quantitative dilution.

Hydroxylammonium chloride A 10% solution was prepared by dissolving 100 g. of the salt in 900 ml. of water. To remove iron, 10 ml. of 0.001 M TPTZ and 1 g. of sodium perchlorate were added and the solution extracted with nitrobenzene.

Sodium acetate A 10% solution was prepared by dissolving 100 g. of sodium acetate in 900 ml. of water. Iron was removed by adding 10 ml. of 10% hydroxylammonium chloride, 10 ml. of 0.001 M TPTZ and 1 g. of sodium perchlorate and extracting the solution with nitrobenzene.

Sodium acetate-acetic acid buffer A solution 2 M in sodium acetate and 2 M in acetic acid was prepared by dissolving 164 g. of sodium acetate and 115 ml. of acetic acid in water and diluting to 1 liter. Iron was removed in the same manner as with the 10% sodium acetate solution.

Sodium perchlorate A 10% solution was prepared by dissolving 100 g. of the salt in 900 ml. of water. Iron was removed by adding 10 ml. of 10% hydroxylammonium chloride and 10 ml. of 0.001 M TPTZ and extracting with nitrobenzene.

1,10-Phenanthroline A quantity of 1,10-phenanthroline weighing 1.0 g. was dissolved in 1 liter of water by heating to give a 0.1% solution.

Ferroine reagents The various ferroine reagents titrated in nonaqueous solution were obtained from Professors G. Frederick Smith and Francis Case.

Deionized water Distilled water was passed through a column of Amberlite MB-3 ion exchange resin.

All other chemicals used were reagent grade or high purity organic solvents.

Apparatus

Spectrophotometric measurements All absorption spectra were recorded using a Cary Model 12 spectrophotometer with 1 or 2 cm. silica cells. All individual absorbancy measurements were obtained using a Beckman Model DU spectrophotometer with 1 cm. Corex cells or 5 cm. silica cells.

pH measurements A Beckman Model G or Model H-2 pH meter equipped with a glass-calomel electrode system was used for all pH measurements, and nonaqueous titrations. The calomel electrode used in the nonaqueous titrations was the sleeve type.

Spectrophotometric Constants and Necessary Conditions

Experimental work

Preliminary experiments showed that TPTZ reacts with iron(II) to form a violet colored, water soluble compound in weakly acidic solutions. Attempts to extract this compound into isoamyl alcohol, n-hexyl alcohol, benzene, chloroform, and ethyl acetate in the presence of chloride, iodide, acetate and perchlorate anions were unsuccessful, but it was found that the compound is extractable into nitrobenzene in the presence of perchlorate or iodide.

Absorption spectra A solution of the iron(II) derivative of TPTZ was prepared by adding 3.0 ml. of 0.001 M TPTZ, 2.0 ml. of 10% hydroxylammonium chloride and 10 ml. of 10% sodium acetate to 5.00 ml. of a 2.000×10^{-4} M iron solution contained in a 50-ml. volumetric flask. The solution was diluted to volume and the absorption spectrum shown in Figure 1 was recorded. To obtain the absorption spectrum of the compound in nitrobenzene, a similar solution was prepared in a 125-ml. separatory funnel and 2 g. of sodium perchlorate added. The solution was extracted three times using 10 ml. portions of nitrobenzene, the extracts combined in a 50-ml. volumetric flask and diluted to volume with 10 ml. of ethanol and nitrobenzene. The absorption spectrum obtained for this solution is shown in Figure 2.

Figure 1. Absorption spectrum of the iron(II) derivative of TPTZ in aqueous solution

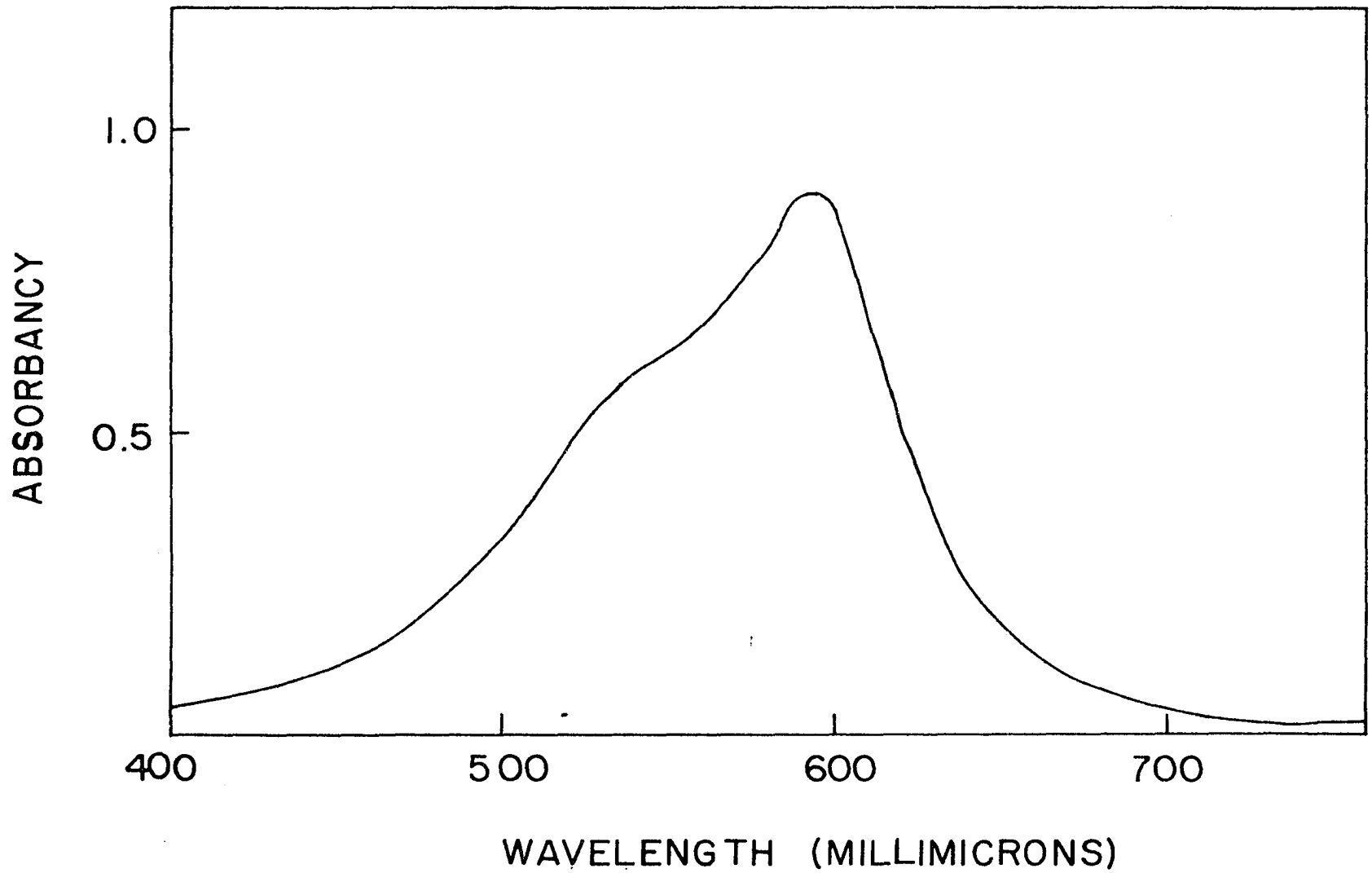
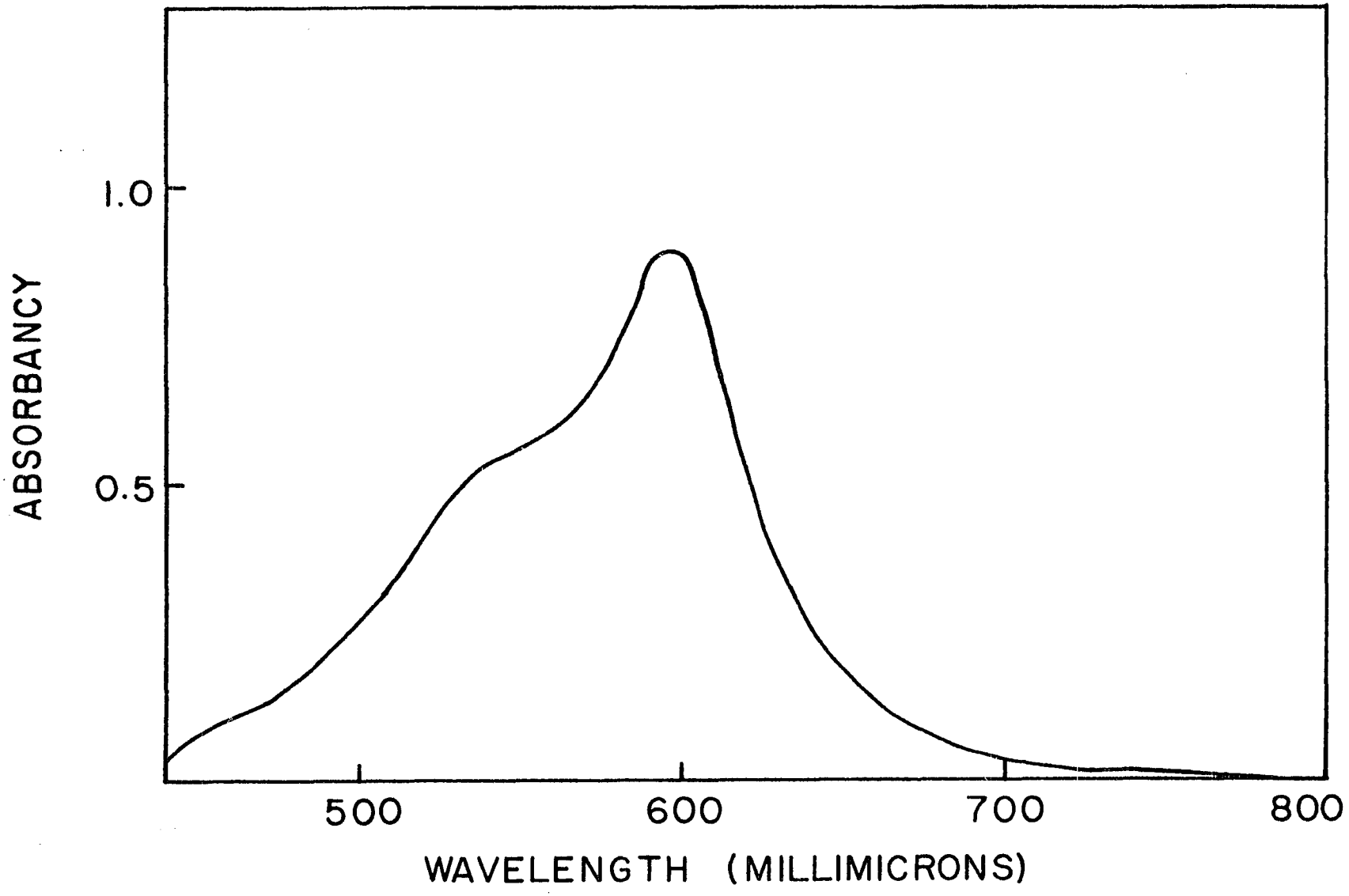

1.0
0.5
ABSORBANCY
400
500
600
700
WAVELENGTH (MILLIMICRONS)

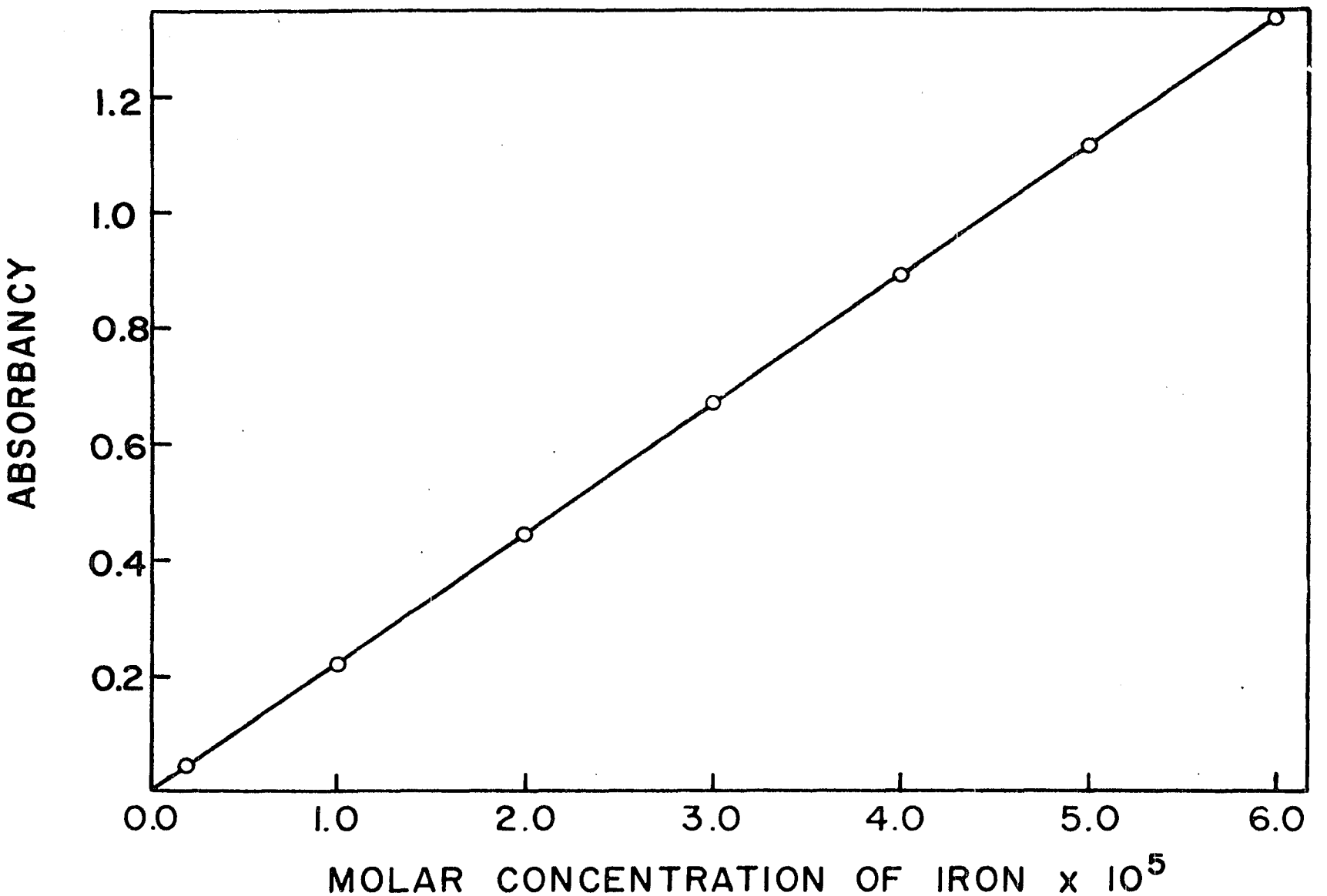
Figure 2. Absorption spectrum of the iron(II) derivative of TPTZ in nitrobenzene


ABSORBANCY
1.0
0.5
500
600
700
800
WAVELENGTH (MILLIMICRONS)

Sensitivity and stability in aqueous solution A series of solutions containing excess TPTZ and an iron concentration varying from 0 to 6 x 10^{-5} M was prepared by pipetting aliquots of a 1.000 x 10^{-4} M iron solution into 50-ml. volumetric flasks and adding 5.0 to 10.0 ml. of 0.001 M TPTZ to each solution. The molar ratio of TPTZ to iron was greater than three in each solution. Two ml. of 10% hydroxylammonium chloride was added to reduce any iron(III) and each solution was buffered by the addition of 10.0 ml. of a 2 M sodium acetate-2 M acetic acid solution. After dilution to volume, the absorbancy of each solution was determined at 593 mμ using 1 cm. cells. The solution to which no iron was added was used as a reagent blank and the absorbancy of this solution was subtracted from the absorbancy of each of the other solutions. A plot of absorbancy vs. concentration is shown in Figure 3. The absorbancy of each of these solutions was measured again 32 hours later and no significant change observed.

Sensitivity and stability in nitrobenzene To determine the molar extinction coefficient of the iron(II) derivative of TPTZ in nitrobenzene, a series of solutions of the complex was prepared in the following way. Various volumes ranging from 0 to 15 ml. of a 1.000 x 10^{-4} M iron solution were placed in 125-ml. separatory funnels and 2.0 ml. of 10% hydroxylammonium chloride, 5.0 ml. of 0.001 M TPTZ, 5.0 ml. of a 2 M sodium

Figure 3. Beer's law plot for the iron(II) derivative of TPTZ in aqueous solution

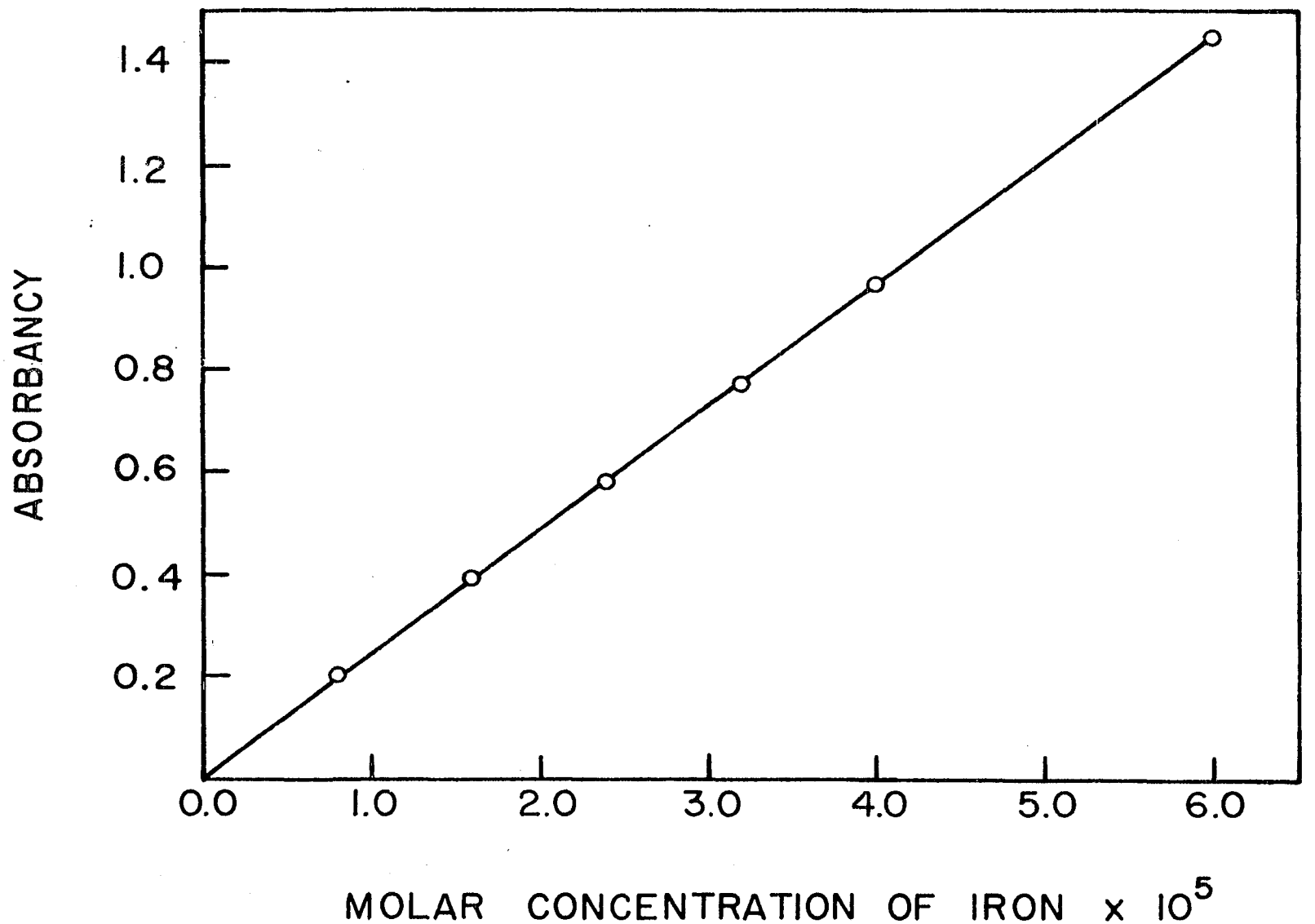

ABSORBANCY
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.0
2.0
3.0
4.0
5.0
6.0
MOLAR CONCENTRATION OF IRON x 10^5

acetate-2 M acetic acid buffer and 5.0 ml. of 10% sodium perchlorate were added to each funnel. Each solution was extracted with one 10 ml. portion of nitrobenzene followed by two extractions using 5 ml. portions. The extracts were collected in a 25-ml. volumetric flask, diluted to volume with ethanol and the absorbancy determined at 595 mμ using 1 cm. cells. The absorbancy of each solution was corrected for the reagent blank by subtracting from it the absorbancy of the solution to which no iron was added. A plot of absorbancy vs. concentration is shown in Figure 4. After 12 hours, the absorbancy of each of the solutions was again determined and no significant change was observed.

Effect of pH in aqueous solution A series of solutions 2.00×10^{-5} M in iron and containing excess TPTZ, hydroxylammonium chloride and varying amounts of hydrochloric acid or ammonium hydroxide was prepared. The pH of each solution was measured and its absorbancy determined at 593 mμ using 1 cm. cells. A plot of absorbancy vs. pH is shown in Figure 5.

Effect of pH on extraction To determine the effect of pH on extraction of the iron(II) derivative of TPTZ a series of solutions containing 5.00 ml. of 5.00×10^{-5} M iron, excess TPTZ, hydroxylammonium chloride and sodium perchlorate was prepared. After dilution to 50 ml., the pH of each solution was adjusted to some definite value with hydrochloric acid or ammonium hydroxide.

Figure 4. Beer's law plot for the iron(II) derivative of TPTZ in nitrobenzene


ABSORBANCY
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1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.0
2.0
3.0
4.0
5.0
6.0
MOLAR CONCENTRATION OF IRON x 10^5

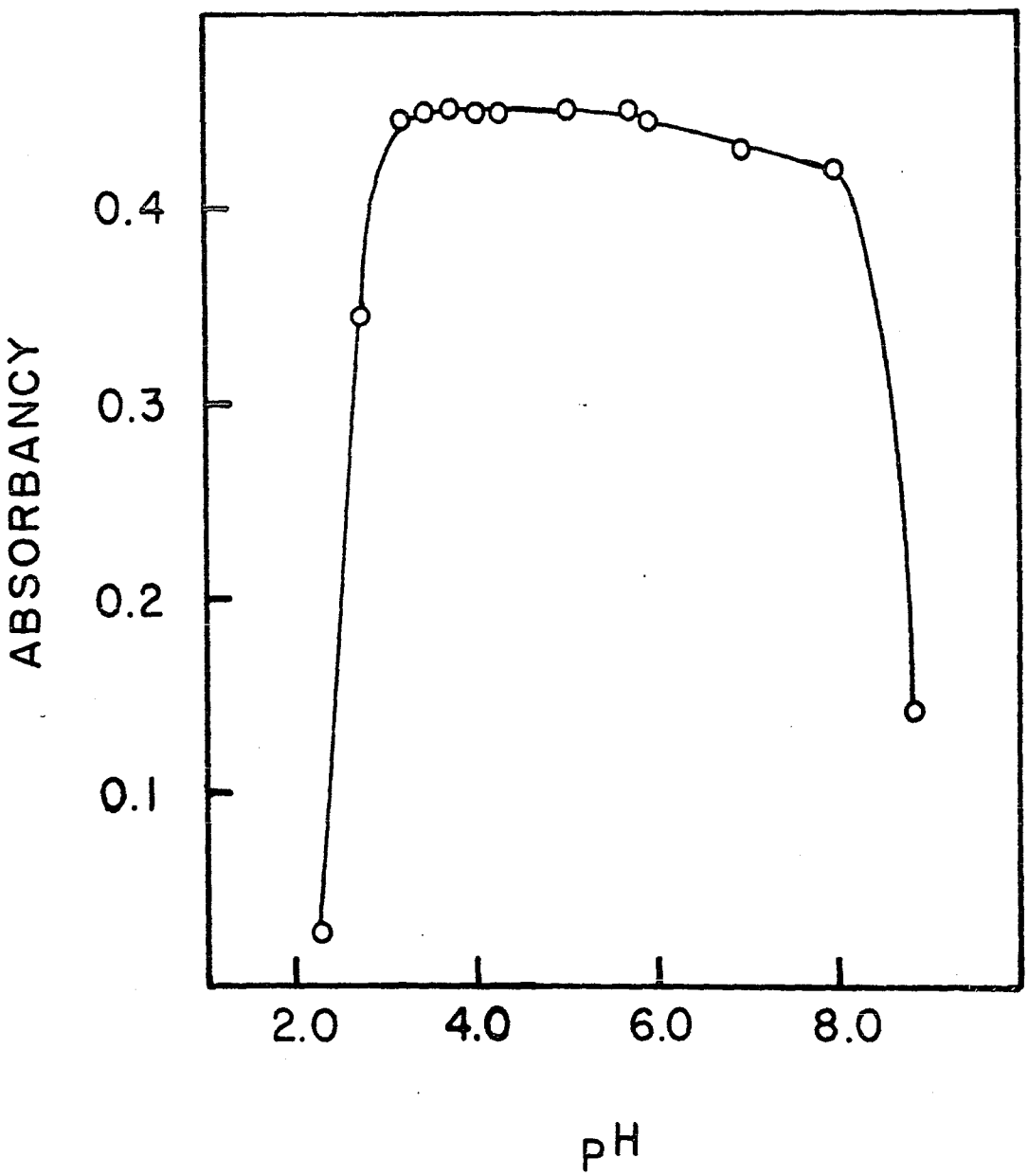


Figure 5. Effect of pH on color formation in aqueous solution

The solutions were transferred to 125-ml. separatory funnels and each solution extracted three times using 4.0, 2.0 and 2.0 ml. portions of nitrobenzene. The combined extracts of each solution were diluted to volume in a 10-ml. volumetric flask with ethanol and the absorbancy determined at 595 mμ using 1 cm. cells. A plot of absorbancy vs. pH is shown in Figure 6.

Results and discussion

The molar extinction coefficients for the iron(II) derivative of TPTZ in aqueous and nitrobenzene solutions at the wavelength of maximum absorption were calculated by the method of least squares. The values found are 22,600 at 593 mμ for the aqueous solutions and 24,100 at 595 mμ for the nitrobenzene solutions. Conformity to Beer's law is indicated in both systems. The shift in the absorption maximum to a longer wavelength and increase in molar extinction coefficient observed for the iron derivative of TPTZ in nitrobenzene resemble those found for tris(1,10-phenanthroline)-iron(II) perchlorate in nitrobenzene by Margerum and Banks (12).

The pH range over which the color is completely formed is from 3.4 to 5.8 in aqueous solution. With extraction into nitrobenzene the pH range is slightly greater, 2.7 to 7.0. While this pH range is not as wide as for many of the 1,10-phenanthroline and polypyridine reagents, a suitable pH can be easily obtained by use of an acetate buffer.

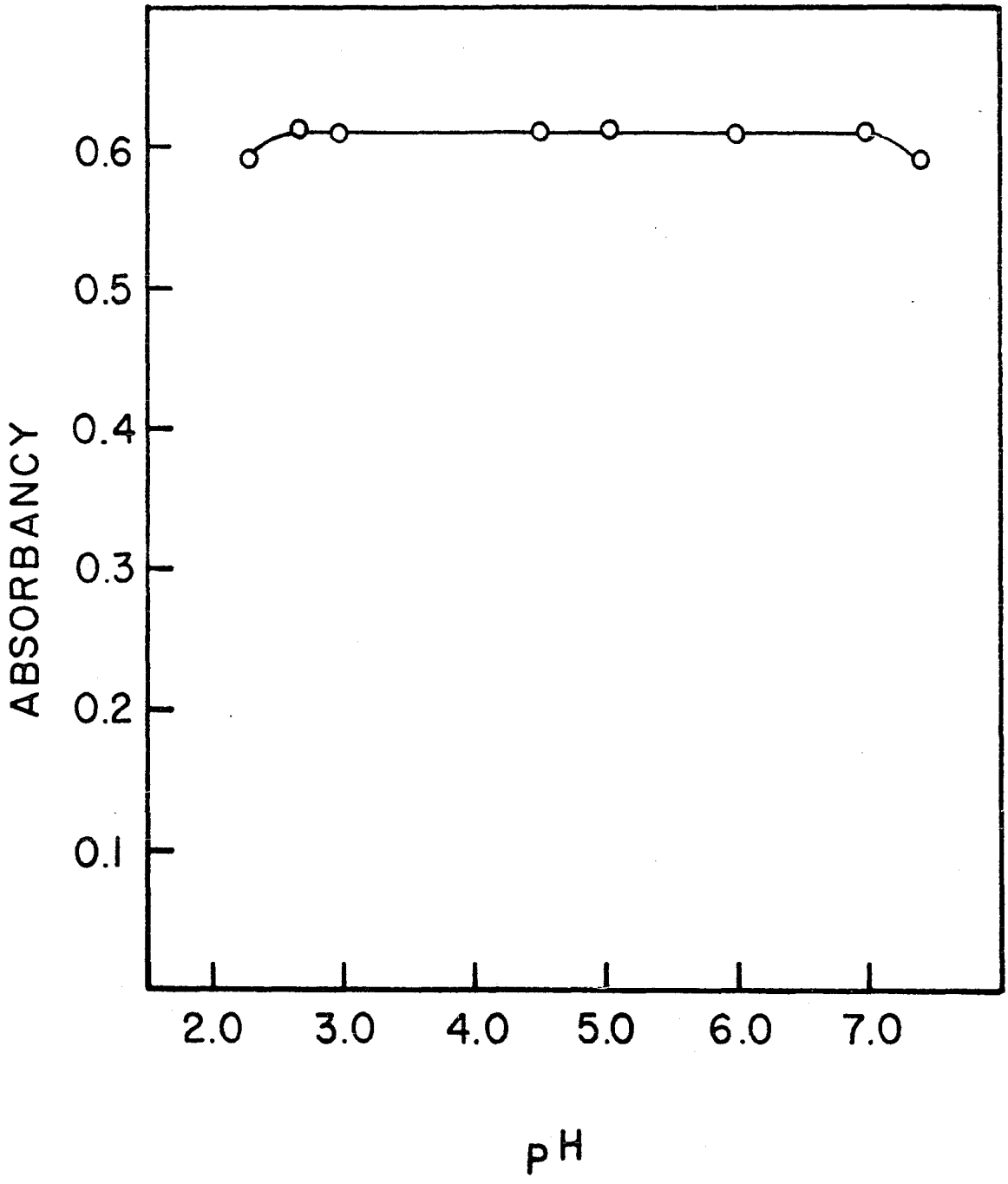


Figure 6. Effect of pH on extraction of the iron(II) derivative of TPTZ

Study of Interferences

Experimental

To test various ions for possible interference in the reaction of iron(II) with TPTZ, a series of solutions was prepared in the following way. A 10.00 ml. portion of a solution 1.000×10^{-4} M in iron was pipetted into a 50-ml. volumetric flask containing a solution of the ion to be tested. TPTZ was added in various amounts as shown in Table 1 followed by 1.0 ml. of 10% hydroxylammonium chloride and 5.0 ml. of 20% sodium acetate. The solution was then diluted to volume and its absorbancy determined at 593 mμ. The solutions containing the various ions to be tested were prepared from the reagent grade compounds listed in Table 1. In testing the possible interference of silver and chloride ions, hydroxylammonium sulfate was substituted for the chloride salt. To test the possible interference of sodium or acetate ions, a solution containing sodium acetate was compared with a solution containing no sodium acetate, the pH being adjusted with dilute ammonium hydroxide.

Results

The results obtained in this study of interferences are shown in Table 1. The method of calculating relative error is essentially the same as that proposed by Fortune and Mellon (5). The apparent concentration of iron in the solution to

Table 1. Effect of various ions on color formation

Ion	Concentration p.p.m.	Source	Relative error %	TPTZ added mmoles x 10^2
Cu^{++}	1.3	$Cu(NO_3)_2$	+0.7	0.5
Cu^{++}	2.5	$Cu(NO_3)_2$	+1.4	0.7
Cu^{++}	6.3	$Cu(NO_3)_2$	+4.8	0.9
Co^{++}	1.2	$CoSO_4$	+0/9	0.5
Co^{++}	2.4	$CoSO_4$	+1.8	0.7
Co^{++}	4.8	$CoSO_4$	+3.6	1.1
Ni^{++}	2.7	$Ni(ClO_4)_2$	+0.4	0.8
Ni^{++}	5.3	$Ni(ClO_4)_2$	+1.5	1.3
Ni^{++}	10.6	$Ni(ClO_4)_2$	+2.7	2.3
Zn^{++}	99.4	$ZnCl_2$	+0.2	10.0
Mn^{++}	110	$MnSO_4$	+0.2	2.0
Cr^{+++}	10.4	$K_2Cr_2O_7$-SO_2	+0.6	0.5
Cr^{+++}	20.8	$K_2Cr_2O_7$-SO_2	+2.4	0.5
Be^{++}	73.0	$Be(ClO_4)_2$	0.0	0.5
Al^{+++}	100	$AlCl_3$	0.0	0.5
Mg^{++}	100	$MgSO_4$	0.0	0.5
Ca^{++}	100	$CaCO_3$-HCl	0.0	0.5
Sr^{++}	99	$Sr(ClO_4)_2$	+0.2	0.5
Ba^{++}	101	$BaCl_2$	+0.2	0.5
Cd^{++}	100	$Cd(NO_3)_2$	-0.7	5.0
Hg^{++}	100	$HgCl_2$	precipitate	0.5
Bi^{+++}	100	$Bi(NO_3)_3$	precipitate	0.5
Sn^{++}	100	$SnCl_2$	-0.2	0.5
Pb^{++}	101	$Pb(NO_3)_2$	+0.2	2.0
Th^{++++}	120	$Th(NO_3)_4$	+0.2	0.5
UO_2^{++}	115	$UO_2(C_2H_3O_2)_2$	+0.4	0.5
Li^{+}	1020	LiCl	0.0	0.5
K^{+}	1067	KCl	+0.4	0.5
NH_4^{+}	1033	NH_4Cl	0.0	0.5
Na^{+}	5600	$NaC_2H_3O_2$	-0.2	0.5
Ag^{+}	102	$AgNO_3$	precipitate	0.5
CN^{-}	500	NaCN	very large	0.5
PO_4^{---}	528	KH_2PO_4	+0.2	0.5
F^{-}	502	NaF	+0.2	0.5
$C_2H_3O_2^{-}$	14400	$NaC_2H_3O_2$	-0.2	0.5
Br^{-}	556	NaBr	+0.2	0.5
I^{-}	497	KI	0.0	0.5
NO_3^{-}	504	KNO_3	+0.2	0.5

Table 1. (Continued)

Ion	Concentration p.p.m.	Source	Relative error %	TPTZ added mmoles x 10^2
NO_2^-	500	KNO_2	large	0.5
$SO_4^=$	512	K_2SO_4	0.0	0.5
ClO_4^-	524	$NaClO_4$	0.0	0.5
ClO_3^-	548	$NaClO_3$	+0.2	0.5
$S_2O_5^{--}$	538	$Na_2S_2O_5$	0.0	0.5
SCN^-	507	KSCN	+0.2	0.5
$S_2O_3^{--}$	528	$Na_2S_2O_3$	+0.4	0.5
BO_3^{---}	545	H_3BO_3	0.0	0.5
BrO_3^-	499	$KBrO_3$	0.0	0.5
MoO_4^{--}	34	$(NH_4)_2MoO_4$	very large	2.0

which the ion to be tested was added, c_2, is found from

$$c_2 = c_1 \frac{A_2}{A_1}$$

where c_1 and A_1 refer to the concentration of iron and absorbancy of the solution containing no interfering ion and A_2 is the absorbancy of the solution containing the possible interference. Relative error is given in percent by the expression

$$\frac{c_2 - c_1}{c_1} \times 100.$$

Of the various ions tested, Cu^{++}, Co^{++}, Ni^{++}, Cr^{+++}, Ag^+, Hg^{++}, Bi^{+++}, MoO_4^{--}, CN^-, $C_2O_4^{--}$ and NO_2^- were the only

ions found which interfere seriously. With Cu^{++}, Co^{++} and Ni^{++} the interference is due to the formation of colored compounds with TPTZ. A precipitate is formed in the presence of Ag^{+}, Hg^{++}, and Bi^{+++}, and with most of the other ions, the interference is obviously due to the color of the ion or to the formation of stable complexes with iron. In the presence of most of the transition metals, if an excess of TPTZ is not present the color development is retarded.

Nature of the Iron(II) Derivative of TPTZ

Spectrophotometric titration

A spectrophotometric titration of TPTZ with iron(II) was carried out to determine the combining ratio of the reactants in the following way. A series of solutions containing 5.00 ml. of 1.000×10^{-3} M TPTZ, 1.0 ml. of 10% hydroxylammonium chloride, 5.0 ml. of 10% sodium acetate and varying quantities of a 2.50×10^{-4} M iron solution was prepared in 50-ml. volumetric flasks. After dilution to volume, absorption spectra for the various solutions were recorded and the absorbancy values at 593 mμ were used for the titration curve shown in Figure 7. A combining ratio of 2 TPTZ to 1 Fe is indicated by this titration.

Continuous variations study

The method of continuous variations was also used to determine the combining ratio of TPTZ and iron(II). A series of

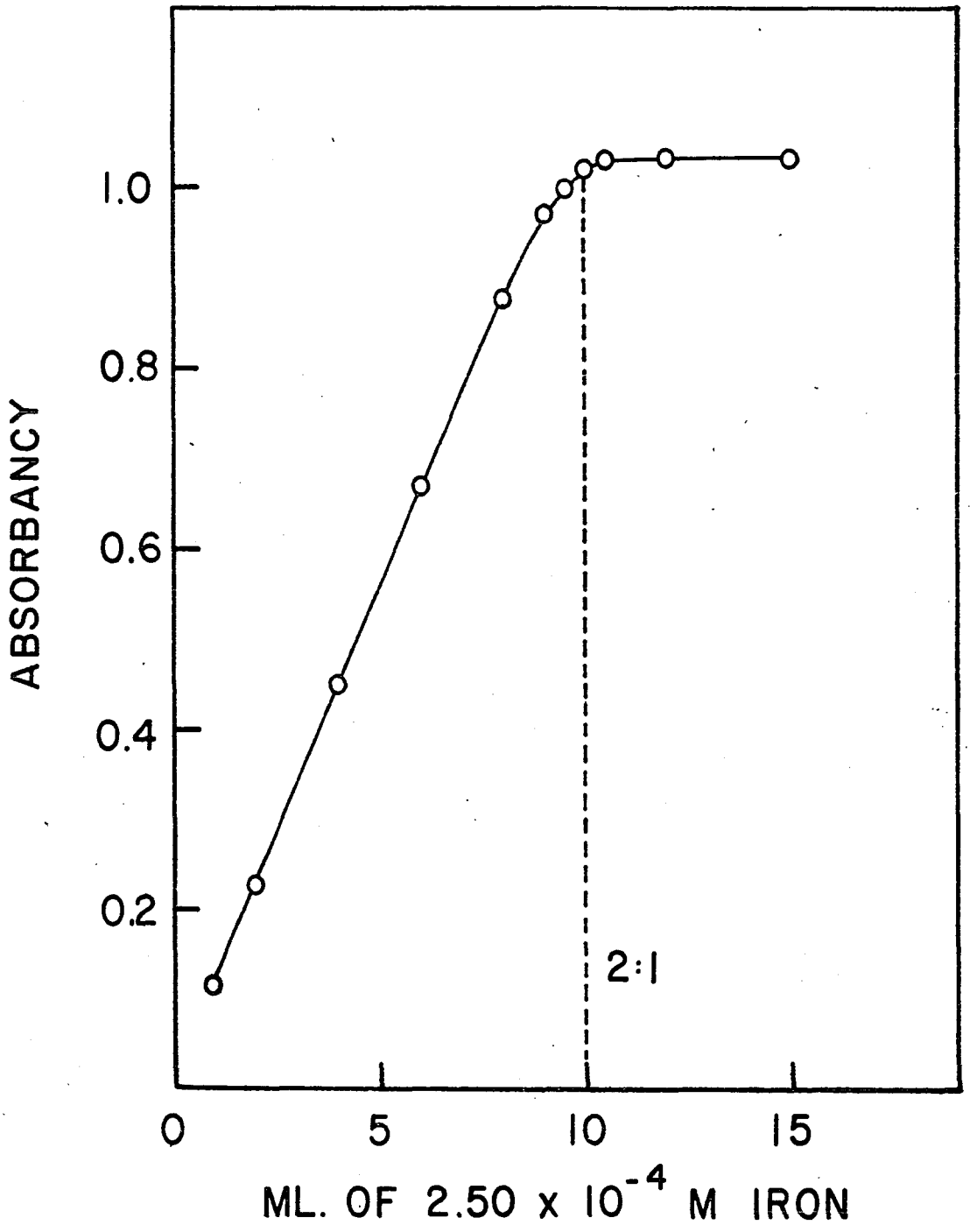


Figure 7. Spectrophotometric titration of TPTZ

solutions of the complex was prepared in which the concentration of TPTZ was varied from 0 to 2 x 10^{-4} M. The molar concentration of iron(II) plus TPTZ was also held constant at 2 x 10^{-4}. Hydroxylammonium chloride was added to reduce any iron(III) and sodium acetate served as a buffer. Absorption spectra were recorded for the various solutions and the values at 593 mμ were used for the plot of absorbancy vs. mole fraction of TPTZ shown in Figure 8. The results also indicate a combining ratio of 2 TPTZ to 1 Fe(II).

Preparation and analysis of the iron(II)-TPTZ-iodide compound

Preparation Equivalent amounts of TPTZ (0.001 mole) and ferrous ammonium sulfate (0.0005 mole) were dissolved in 300 ml. of water containing 1 g. of hydroxylammonium chloride and 1 ml. of hydrochloric acid by heating. The solution was then cooled and ammonium hydroxide added to adjust the pH to 4.8. Unreacted TPTZ was removed by filtration and 10 g. of potassium iodide added to the heated filtrate. On cooling, the iron(II)-TPTZ-iodide compound was precipitated as small crystals. These crystals were removed by filtration, dried and extracted with ethyl ether in a Soxhlet extractor for 30 hours to remove any potassium iodide. The compound, which is not soluble in ethyl ether, was then dried at 115° for 6 hours.

Analysis for iron Samples of the compound weighing approximately 50 mg. were dissolved in water containing a few

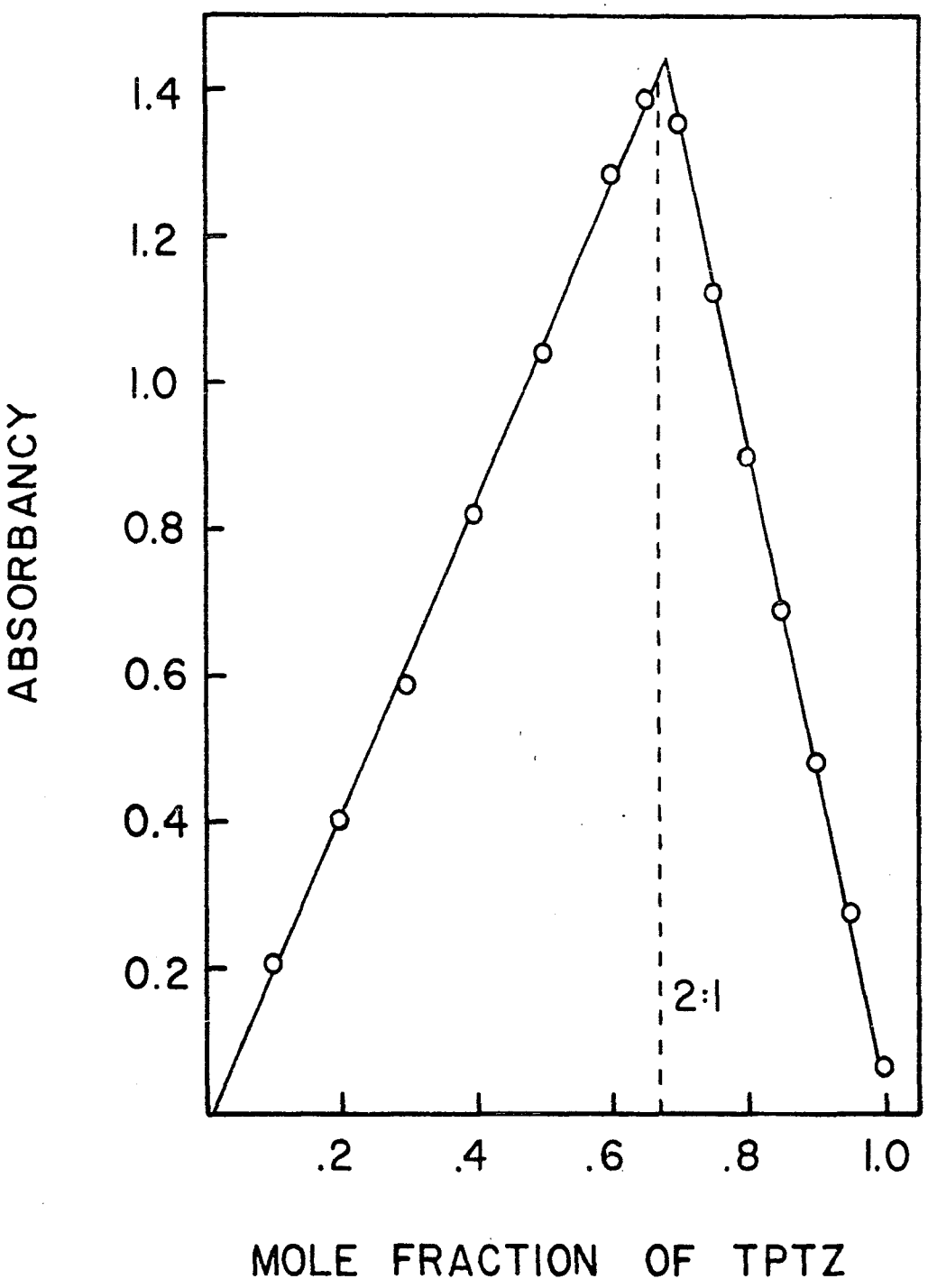
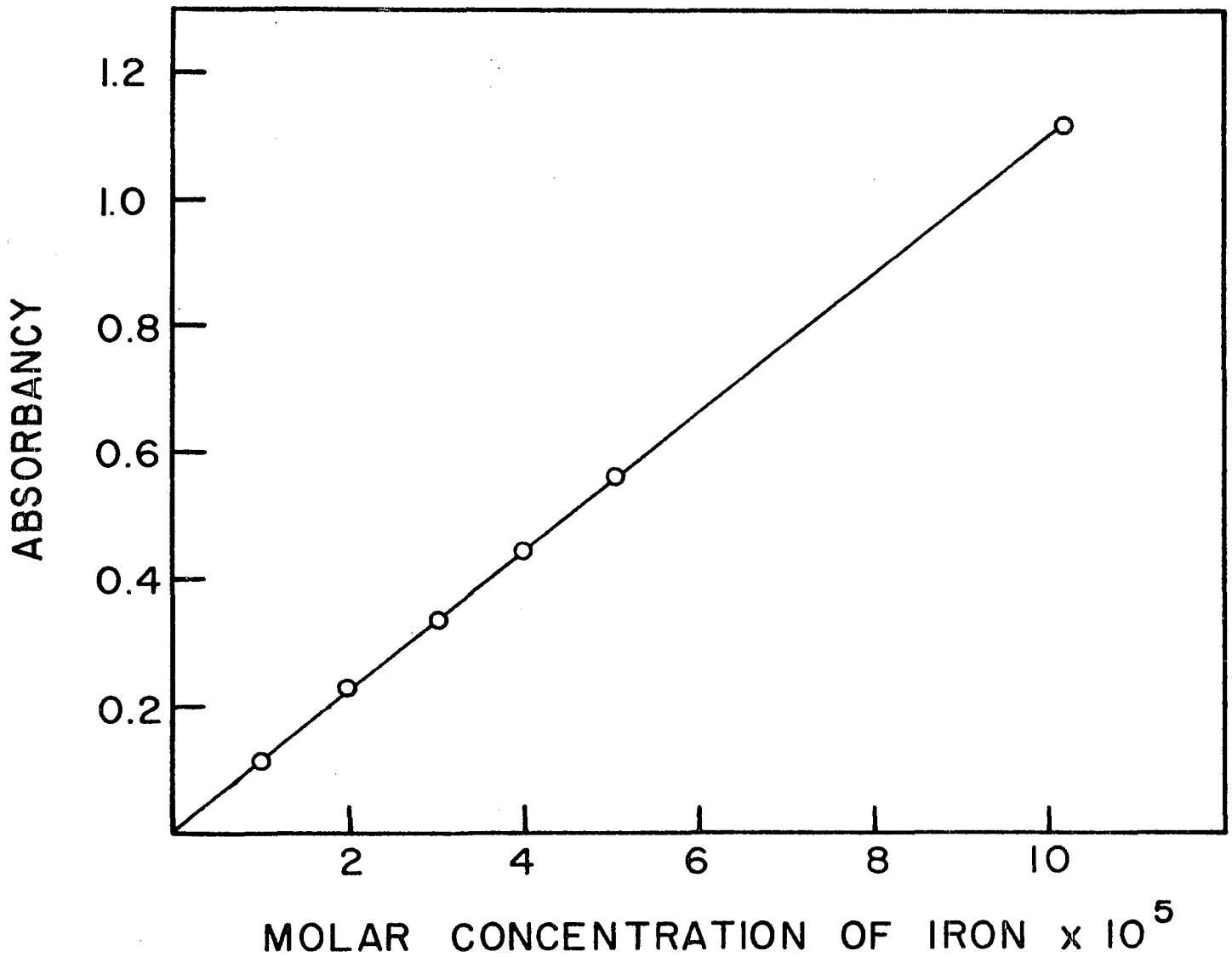


Figure 8. Continuous variations study of iron(II)-TPTZ

drops of sulfuric acid. After dilution to volume in 250-ml. volumetric flasks, 25.0 ml. aliquots of these stock solutions were wet ashed with nitric and perchloric acids and excess perchloric acid was removed by fuming with sulfuric acid. The solutions were diluted and heated to boiling to remove any chlorine and to dissolve iron(III) sulfate. After cooling, 2.0 ml. of 10% hydroxylammonium chloride and 10 ml. of 0.1% 1,-10-phenanthroline were added to each solution followed by sufficient ammonium hydroxide to neutralize the solutions to congo red. Each solution was diluted to volume in a 100-ml. volumetric flask and the absorbancy determined at 510 mμ. A series of standards was prepared in a similar way only omitting the wet ashing step to obtain the calibration curve shown in Figure 9. Iron found: 6.09, 6.10, 6.15%. Calculated for $Fe(TPTZ)_2I_2$: 5.98%.

Analysis for TPTZ Solutions containing the compound and excess iron(II) were prepared by pipetting 25.0 ml. of the previously prepared stock solutions into 100-ml. volumetric flasks and adding 20 ml. of a 1.00×10^{-3} M iron solution, 1.0 ml. of 10% hydroxylammonium chloride and 5.0 ml. of 10% sodium acetate. Each solution was diluted to volume and the absorbancy determined at 593 mμ. To obtain the calibration curve a series of solutions containing known amounts of TPTZ, excess iron(II), hydroxylammonium chloride and sodium acetate was prepared. The absorbancy of each standard was measured

Figure 9. Calibration curve for the determination of iron with 1,10-phenanthroline


ABSORBANCY
1.2
1.0
0.8
0.6
0.4
0.2
2
4
6
8
10
MOLAR CONCENTRATION OF IRON x 10^5

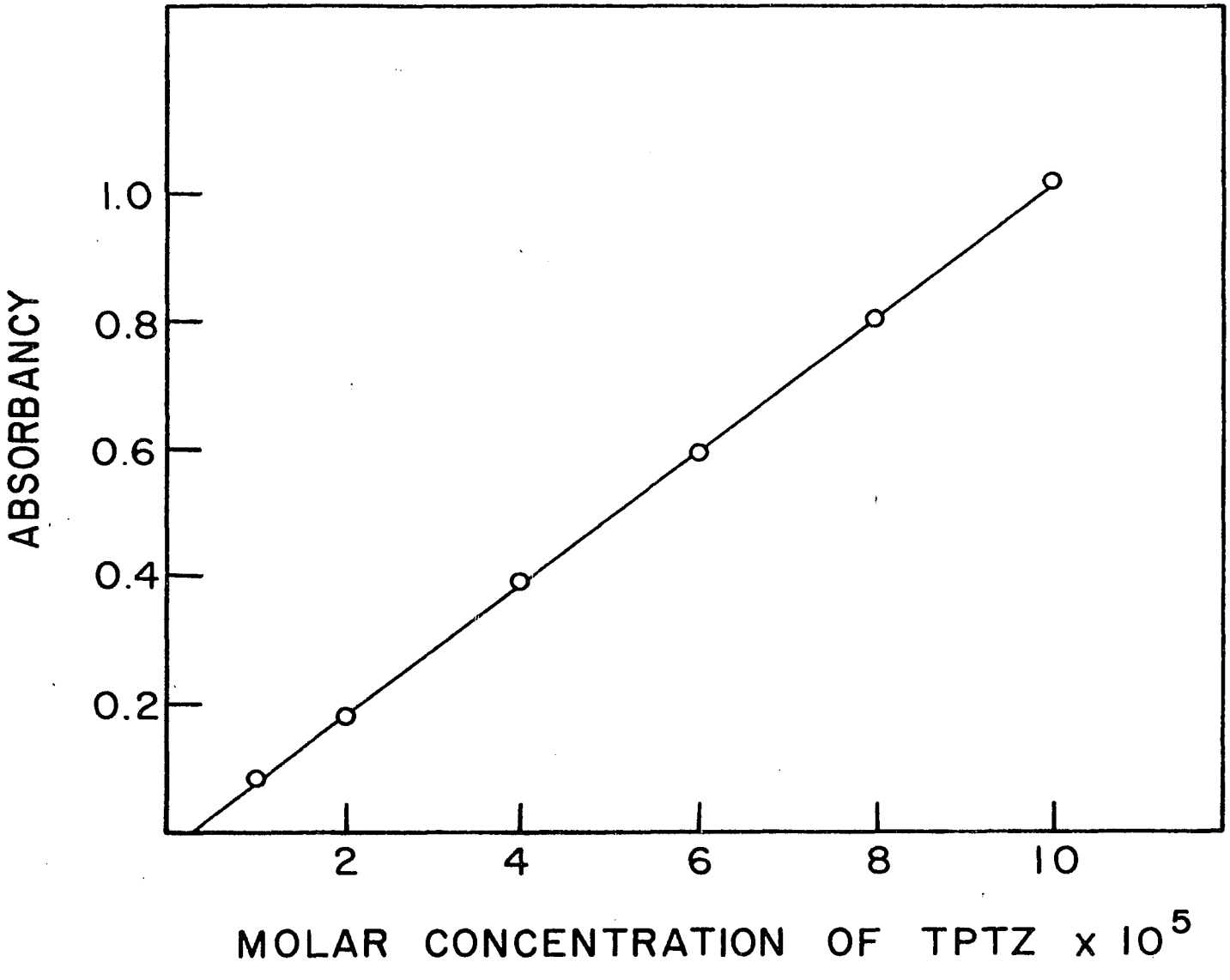
at 593 mμ and a plot of absorbancy vs. concentration of TPTZ is shown in Figure 10. TPTZ found: 66.4, 66.3, 66.7%. Calculated for $Fe(TPTZ)_2I_2$: 66.86%.

Analysis for iodide Samples weighing approximately 150 mg. were dissolved in water and passed through a column of Amberlite IR-120 cation exchange resin in the H^+ form to give a colorless solution containing hydriodic acid. The solutions were neutralized with dilute sodium hydroxide and titrated with 0.00996 M silver nitrate using eosin as the indicator. The silver nitrate was standardized against reagent grade potassium iodide also using eosin. Iodide found: 27.07, 26.88, 26.91%. Calculated for $Fe(TPTZ)_2I_2$: 27.17%.

Nonaqueous titrations

A series of nonaqueous titrations of various ferroine reagents and iron(II) derivatives was carried out in an attempt to gain some insight into the structure of the iron(II) derivative of TPTZ. The titrant employed was 0.00968 M perchloric acid (against potassium acid phthalate) in glacial acetic acid to which a small quantity of acetic anhydride had been added. Nitromethane, acetonitrile, and acetic acid were used as solvents. The bis(2,4,6-tris(2'-pyridyl)-s-triazine)iron(II) perchlorate was prepared by passing a solution of the iodide salt previously described through a column of Amberlite IRA-400 in the perchlorate form and evaporating the solution so obtained to dryness. Crystals were obtained and these were dried

Figure 10. Calibration curve for the determination of TPTZ


ABSORBANCY
1.0
0.8
0.6
0.4
0.2
2
4
6
8
10
MOLAR CONCENTRATION OF TPTZ x 10^5

at 115° for 24 hours. The compounds titrated, solvents used and results are given in Table 2 and the titration curves shown in Figures 11-18.

Discussion

A combining ratio of 2 TPTZ to 1 Fe(II) is shown by both the spectrophotometric titration and the continuous variations study. The empirical formula calculated from the analyses of the iodide salt of the complex is $Fe(TPTZ)_{1.94}I_{1.94}$.

In the titration of the amino and pyridyl substituted symmetrical triazines, it was observed that the number of equivalents of acid consumed corresponds to the number of pyridyl groups in every case. Therefore it appears reasonable to attribute the basicity of the compounds to the pyridyl nitrogens.

In the case of 1,10-phenanthroline, when the nitrogen atoms become bonded to iron(II), they can no longer be titrated. If this is also true with the symmetrical amino and pyridyl substituted triazines, it follows from the above results that in the iron(II) derivative of TPTZ, two pyridyl groups of each ligand are involved in the complexing since only one of the three can be titrated. On the basis of this evidence a structure is proposed in which two ligands, arranged perpendicularly to each other, surround the iron(II)

Table 2. Nonaqueous titrations of various ferroine reagents

Compound Titrated	Solvent	Result
1,10-phenanthroline	acetonitrile	One endpoint corresponding to one equivalent per mole
tris(1,10-phenanthroline)-iron(II) perchlorate	acetonitrile	No end point
4,6-diamino-2(2'-pyridyl)-s-triazine	acetic acid-nitromethane (1:10)	One endpoint corresponding to one equivalent per mole
2-amino-4,6-bis(2'-pyridyl)-s-triazine	acetic acid-nitromethane (1:12)	One endpoint corresponding to two equivalents per mole
2-amino-4,6-bis(4'-ethyl-2'-pyridyl)-s-triazine	acetic acid-nitromethane (1:12)	One endpoint corresponding to two equivalents per mole
2-amino-4,6-bis(4'-phenyl-2'-pyridyl)-s-triazine	acetic acid-nitromethane (5:2)	One endpoint corresponding to two equivalents per mole
2,4,6-tris(2'-pyridyl)-s-triazine	nitromethane	Two endpoints: the first corresponding to one equivalent per mole, the second to a total of three equivalents per mole
bis(2,4,6-tris(2'-pyridyl)-s-triazine)iron(II) perchlorate	nitromethane	One endpoint corresponding to two equivalents per mole

Figure 11. Titration curve of 1,10-phenanthroline

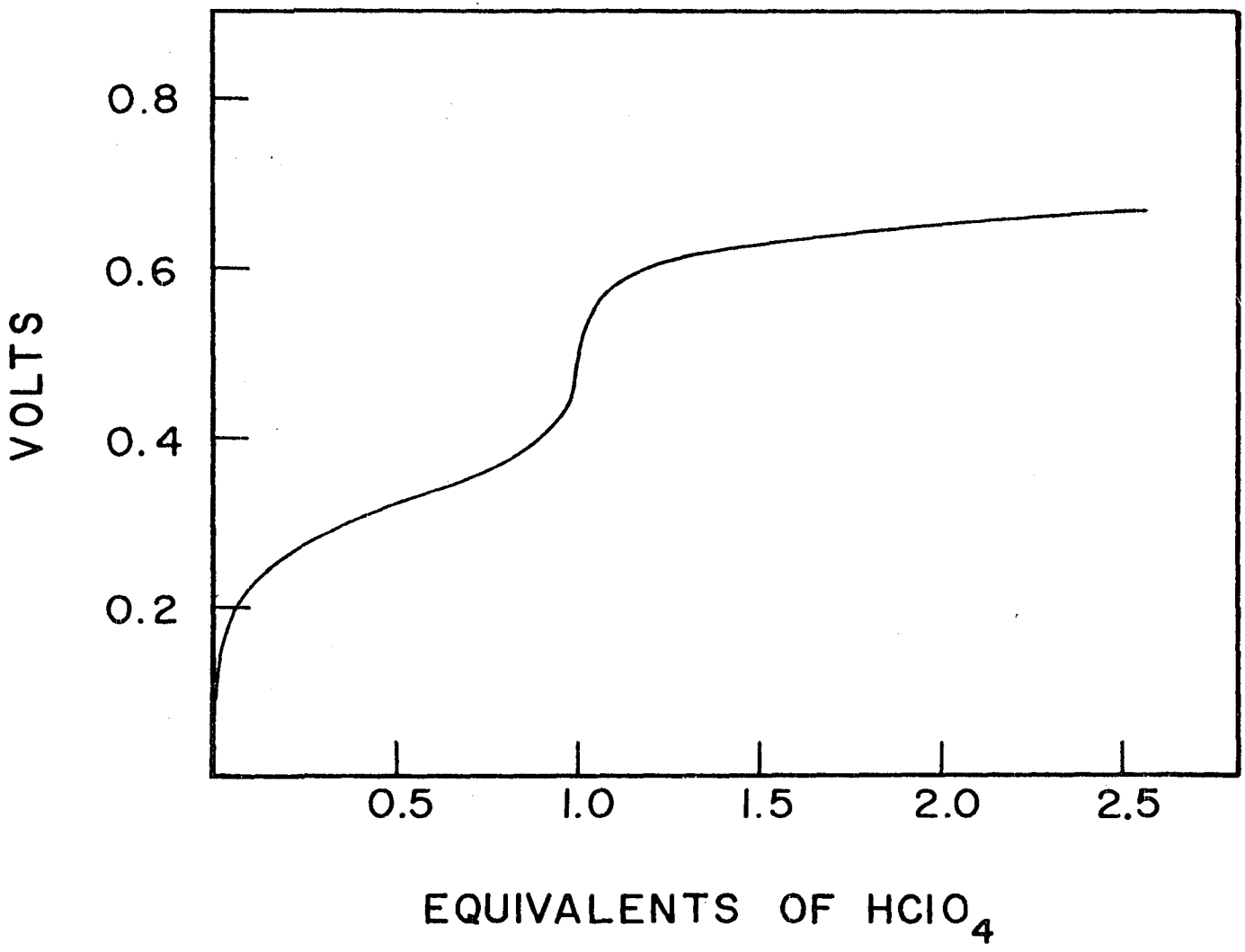

VOLTS
0.8
0.6
0.4
0.2
0.5
1.0
1.5
2.0
2.5
EQUIVALENTS OF HClO4

Figure 12. Titration curve of tris(1,10-phenanthroline)-iron(II) perchlorate

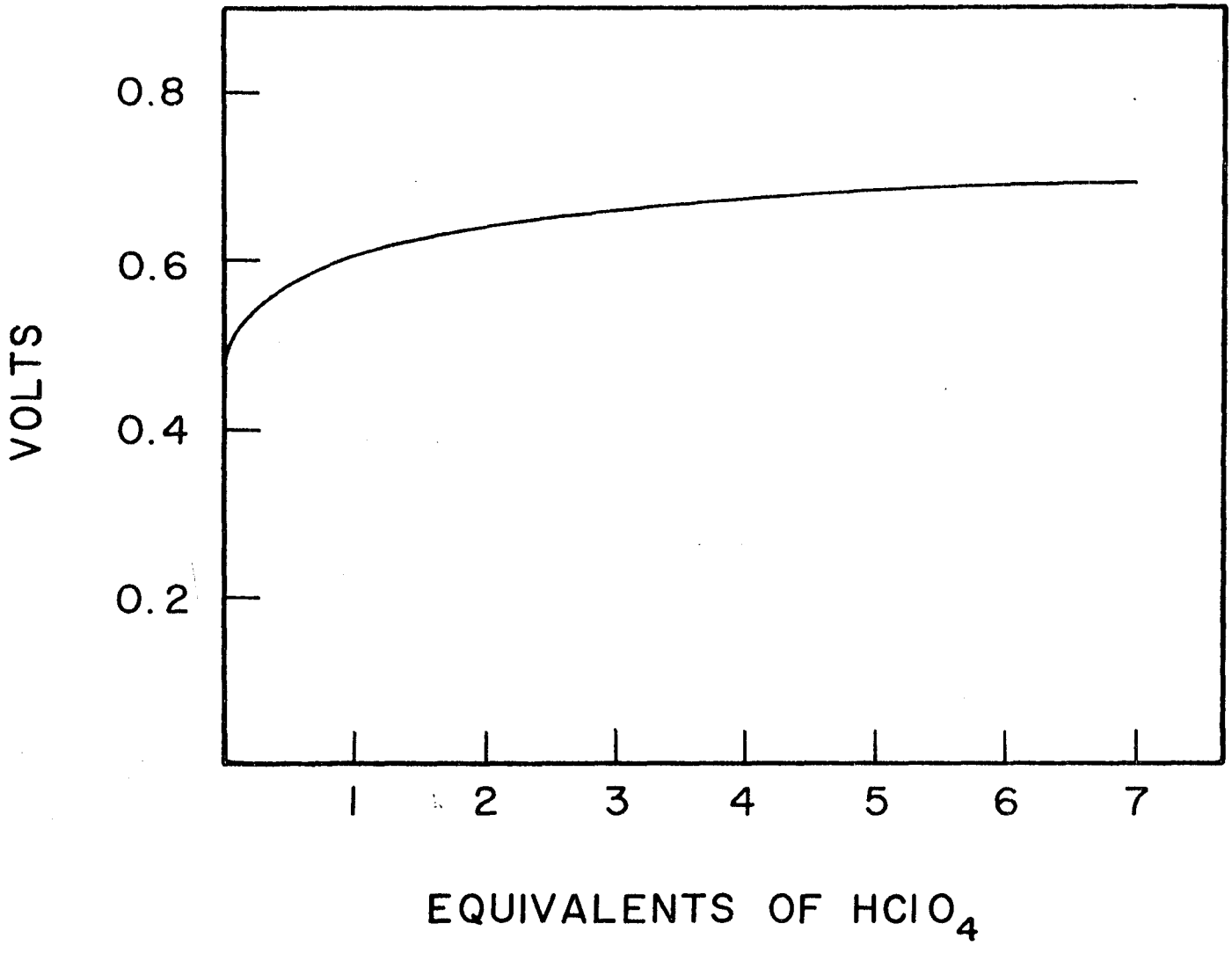

0.8
0.6
0.4
0.2
VOLTS
1
2
3
4
5
6
7
EQUIVALENTS OF $HClO_4$

Figure 13. Titration curve of 4,6-diamino-2(2'-pyridyl)-s-triazine

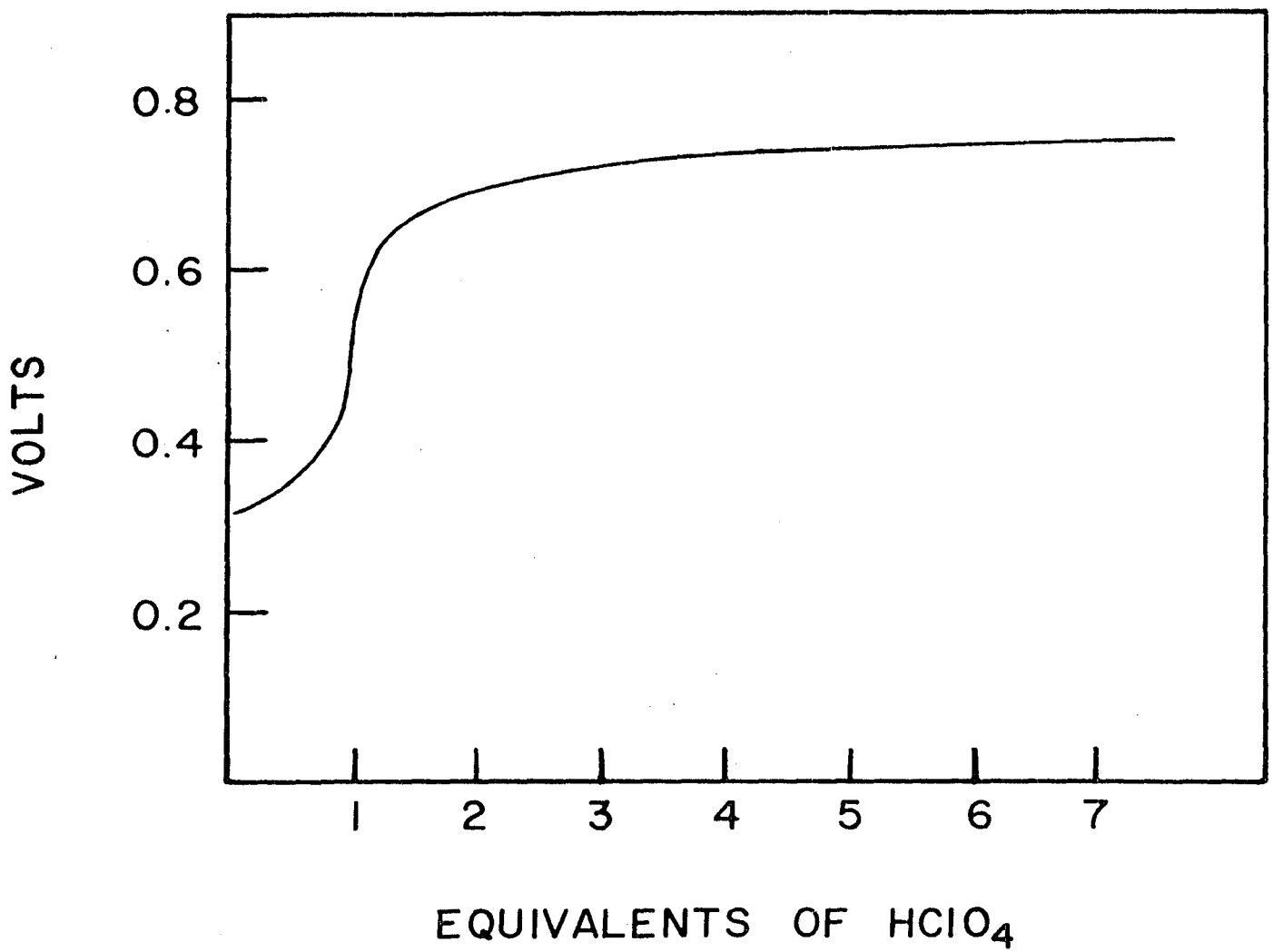

0.8
0.6
0.4
0.2
VOLTS
1
2
3
4
5
6
7
EQUIVALENTS OF $HClO_4$

Figure 14. Titration curve of 2-amino-4,6-bis(2'-pyridyl)-s-triazine

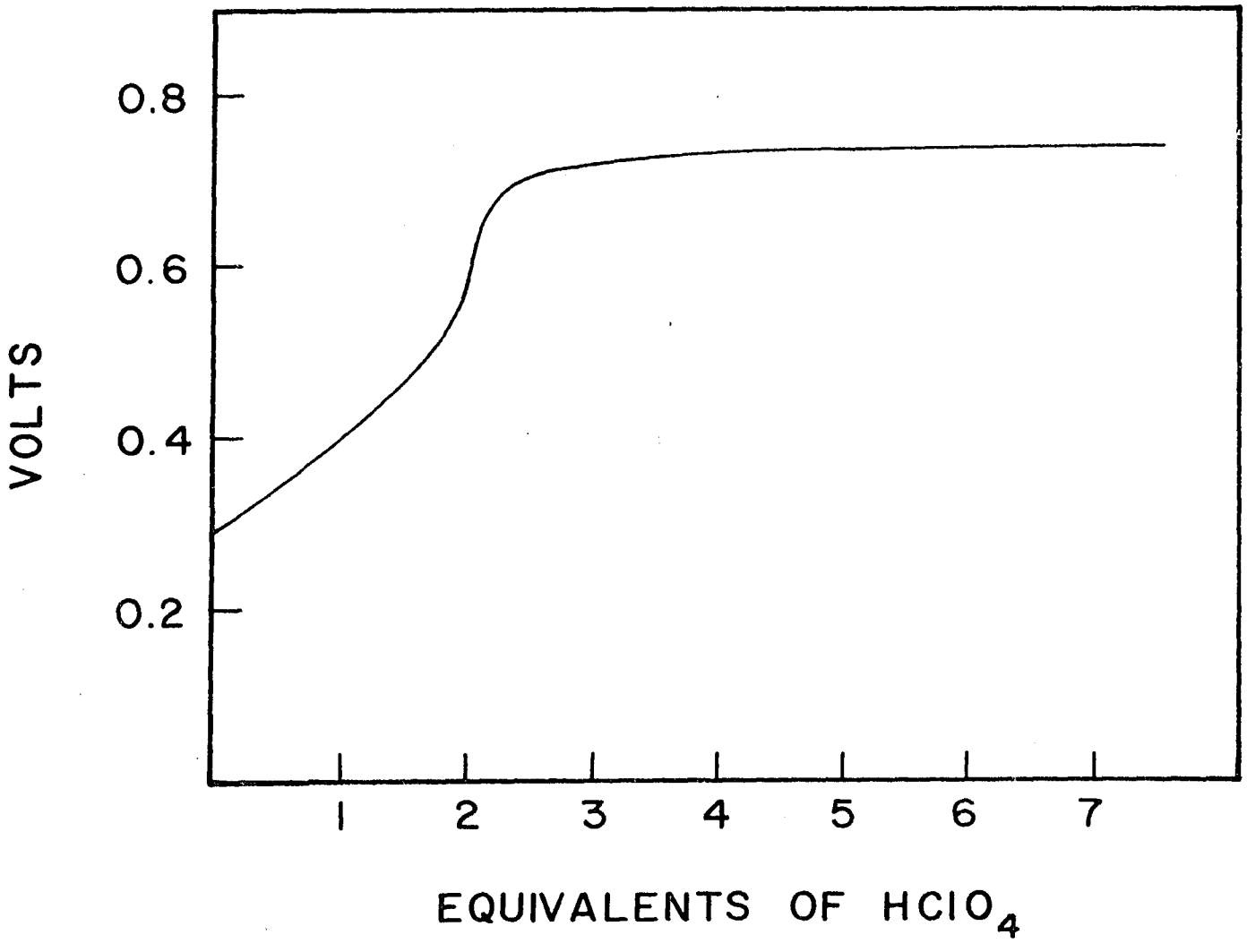

0.8
0.6
0.4
0.2
VOLTS
1
2
3
4
5
6
7
EQUIVALENTS OF HClO4

Figure 15. Titration curve of 2-amino-4,6-bis(4'-ethyl-2'-pyridyl)-s-triazine

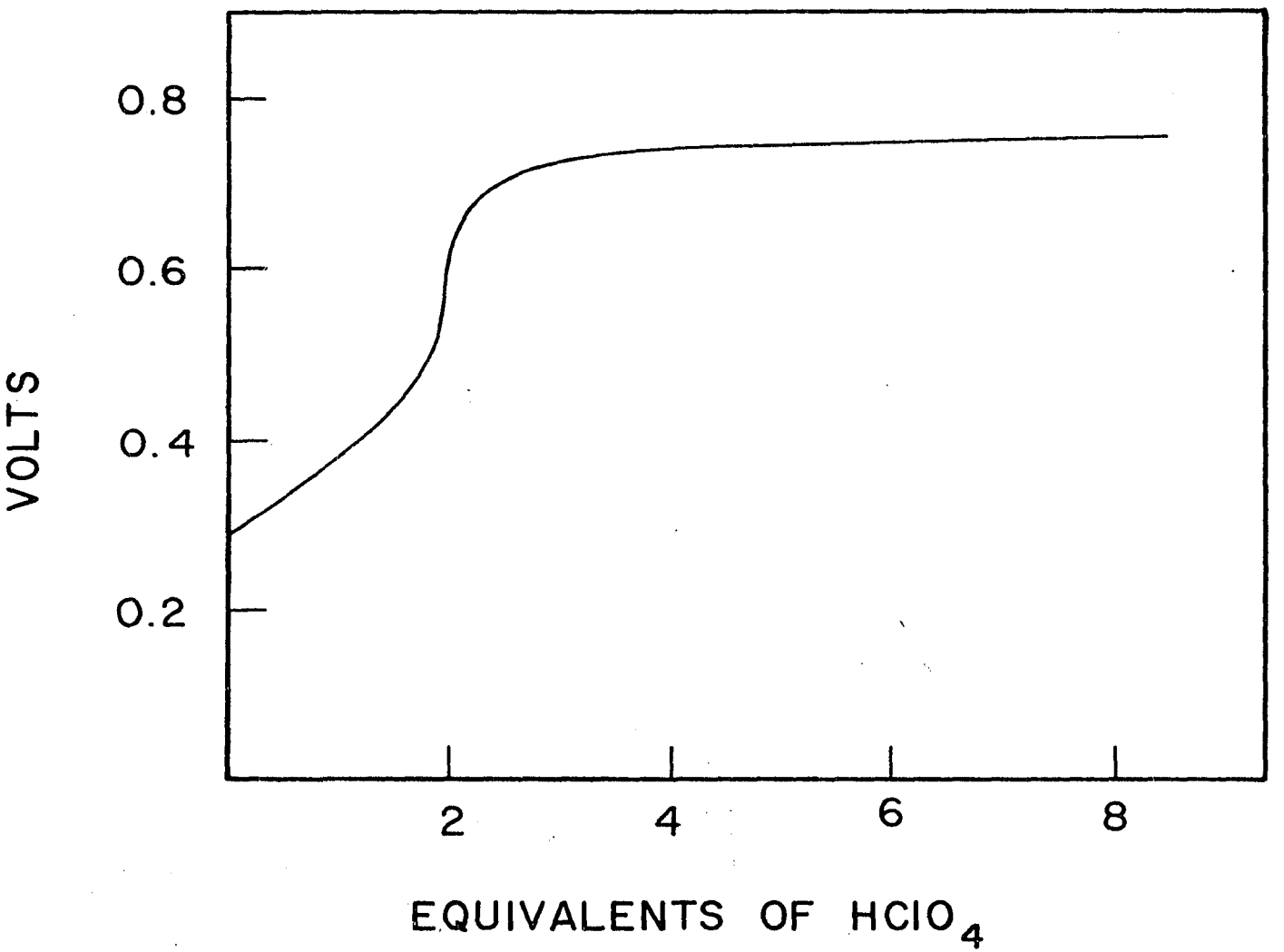

0.8
0.6
0.4
0.2
VOLTS
2
4
6
8
EQUIVALENTS OF HClO4

Figure 16. Titration curve of 2-amino-4,6-bis(4'-phenyl-2'-pyridyl)-s-triazine

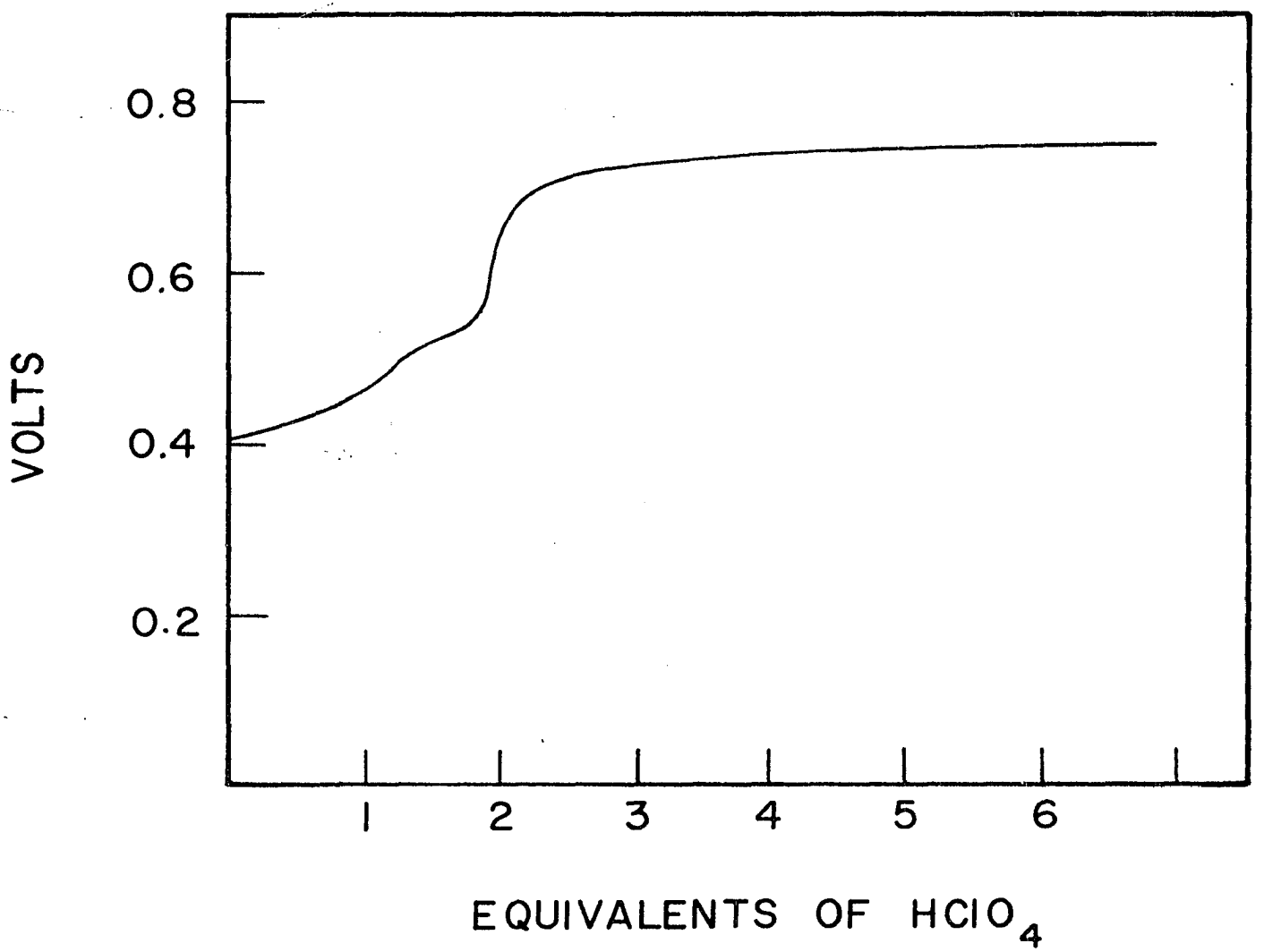

0.8
0.6
0.4
0.2
VOLTS
1
2
3
4
5
6
EQUIVALENTS OF HClO4

Figure 17. Titration curve of 2,4,6-tris(2'-pyridyl)-s-triazine

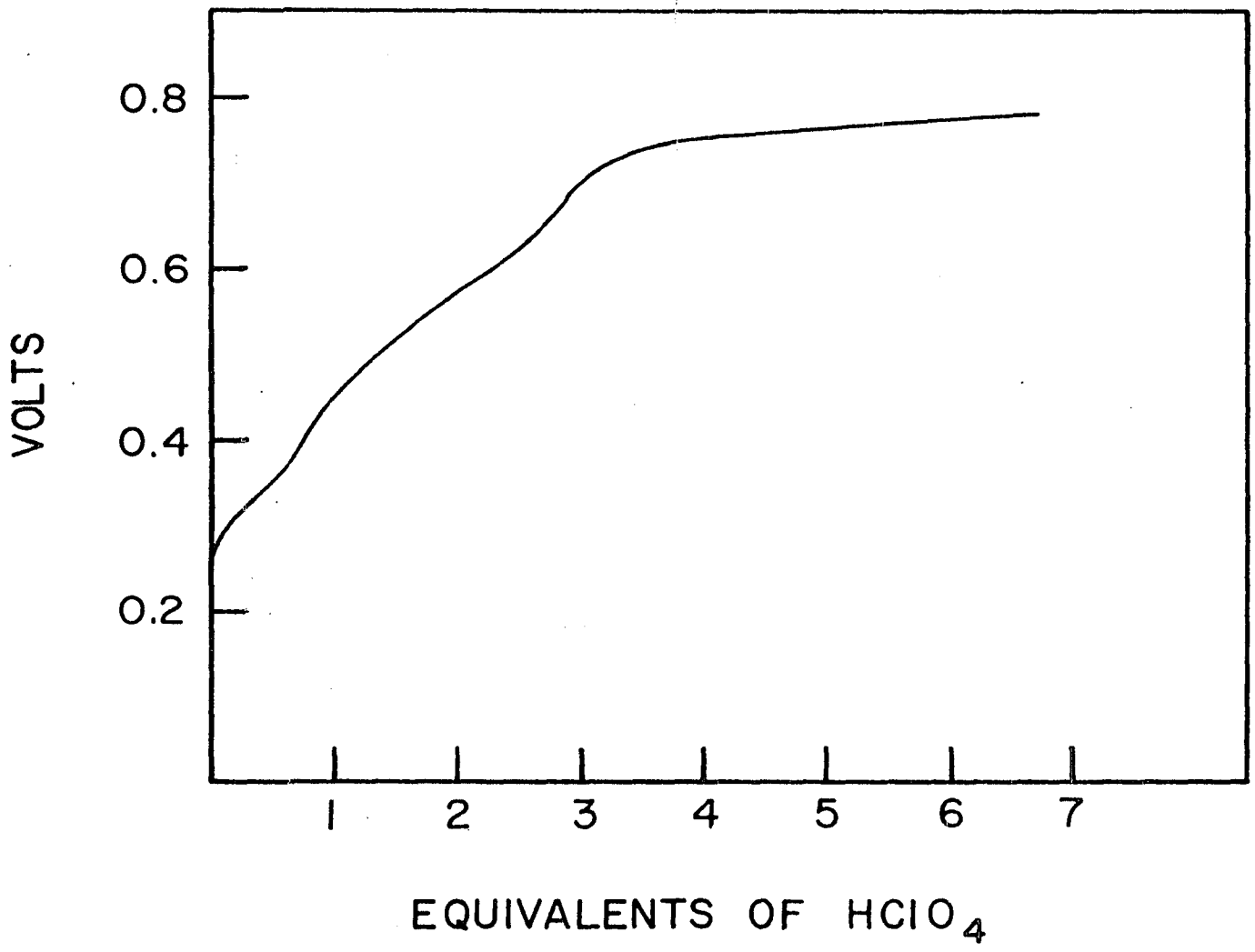
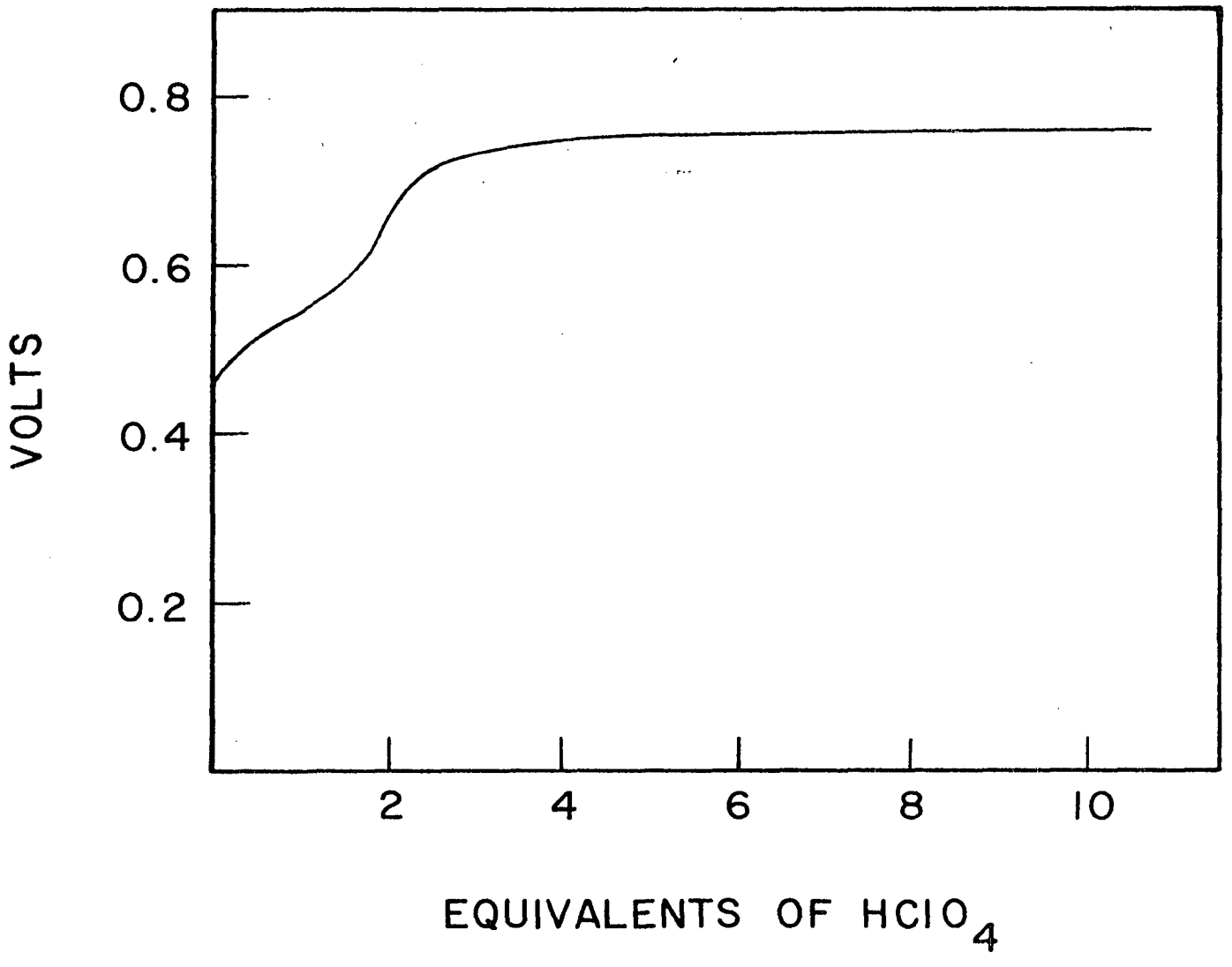
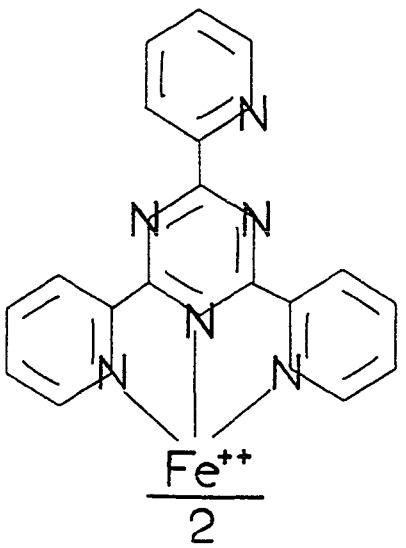

0.8
0.6
0.4
0.2
VOLTS
1
2
3
4
5
6
7
EQUIVALENTS OF $HClO_4$

Figure 18. Titration curve of bis(2,4,6-tris(2'-pyridyl)-s-triazine)iron(II) perchlorate


0.8
0.6
0.4
0.2
VOLTS
2
4
6
8
10
EQUIVALENTS OF HClO4

ion. Three nitrogen atoms of each ligand are bonded to the iron(II) as



and the three nitrogen atoms of one ligand occupy octahedral positions 2, 3 and 4 and of the second, positions 1, 5 and 6.

APPLICATIONS

Iron is an ubiquitous element and its determination in a wide variety of materials is of considerable importance. The work previously described indicated that 2,4,6-tris-(2'-pyridyl)-s-triazine (TPTZ) should be a valuable reagent for the determination of small amounts of iron due to its sensitivity and ease of preparation; therefore, various applications have been studied. In this section, methods using TPTZ for the determination of iron in wine, water, siliceous materials, urine and blood are described.

The Determination of Iron in Wine

Previous work

The determination of iron is important in the production of wine since it is necessary to keep the iron content low. If the amount of iron exceeds 5 to 10 parts per million, a turbidity develops on standing due to the formation of insoluble iron compounds.

The various colorimetric methods for the determination of iron in wine have been reviewed by Deibner and Bouzigues (13) and they conclude that the methods utilizing 1,10-phenanthroline are superior. The procedure of Saywell and Cunningham (3) employs wet ashing with perchloric and sulfuric acids and the determination is completed by the use of 1,10-phenanthroline. Deibner and Bouzigues (14) have utilized

1,10-phenanthroline following a wet ashing procedure using hydrogen peroxide. A determination of iron in wine has been described by Banick and Smith (15) in which bathophenanthroline is utilized as the color forming reagent and the wet ashing step is eliminated. The bathophenanthroline is added directly to the wine sample and the iron(II) bathophenanthroline complex is extracted into isoamyl alcohol.

Samples for analysis

Wine samples were obtained through retail outlets and analyzed for iron by the following procedure which is a modification of that by Saywell and Cunningham.

To a 25.0 ml. wine sample contained in a 250-ml. conical flask fitted with a reflux head was added 25 ml. of nitric acid. After the initial reaction had subsided, the solution was evaporated to near dryness and the wet ashing completed by digesting with an additional 50 ml. of nitric acid and 5 ml. of perchloric acid until white fumes of perchloric acid appeared. The solution was then cooled, the sides of the flask and reflux head were washed down with water and 3 ml. of sulfuric acid added. The solution was evaporated to near dryness to remove the perchloric acid, cooled, diluted with water and heated to boiling. After cooling, 2.0 ml. of 10% hydroxylammonium chloride and 5.0 ml. of 0.01 M 1,10-phenanthroline were added and the solution neutralized to congo red

paper with ammonium hydroxide. The solution was transferred to a 50-ml. volumetric flask and diluted to volume with water. The absorbancy of the solution was measured at 510 mμ using 1 cm. cells and the iron content of the wine calculated by use of the calibration curve previously shown in Figure 9. A blank was carried through the entire operation. The results are shown in Table 3. All determinations were carried out in at least triplicate.

Determination of iron with TPTZ following wet ashing

Special reagents In addition to the reagents previously described, reagent grade nitric acid was distilled from an all glass still to remove traces of iron. Reagent grade perchloric acid was vacuum distilled.

Procedure Pipet a 3.00 ml. sample of wine into a 250-ml. conical flask fitted with a reflux head. Add 10 ml. of 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 a 2 M sodium acetate-2 M acetic acid buffer and 5.0 ml. of 0.001 M TPTZ. Transfer the solution to a 150-ml. beaker and neutralize 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 1 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 two 2.0 ml. portions of nitrobenzene. Collect the extracts in a 10-ml. volumetric flask, dilute to volume with ethanol and measure the absorbancy at 595 mμ using 1 cm. cells. Run a blank through the entire procedure and subtract its absorbancy from the absorbancy of each of the other solutions.

Results The iron content of the wines was calculated using the calibration curve shown in Figure 4 and the results are given in Table 3. All determinations were carried out at least in triplicate.

Direct determination of iron using TPTZ

It was attempted to develop a direct procedure with the elimination of the wet ashing step using TPTZ similar to the one of Banick and Smith using bathophenanthroline.

Procedure To a 3.00 ml. sample of wine contained in a 100-ml. beaker add 2.0 ml. of 10% hydroxylammonium chloride, 5.0 ml. of ethanol, 5.0 ml. of a 2 M sodium acetate-2 M acetic acid buffer and 5.0 ml. of 0.001 M TPTZ. Heat the solution to boiling for 5 minutes, cool and transfer to a 125-ml. separatory funnel. Wash the beaker with 20 ml. of ethanol and 1.0 ml. of 10% sodium perchlorate and add the

Table 3. Results in the determination of iron in wine

Wine	Wet ashing 1,10-phenanthroline		Wet ashing TPTZ		Direct TPTZ	
	Fe found mg./l.	Average deviation	Fe found mg./l.	Average deviation	Fe found mg./l.	Average deviation
Italian Swiss Colony California Sherry	2.54	.07			2.51	.01
Meier's Ohio State Tawny Port	3.79	.15			3.80	.04
Medoc Bordeaux Red Wine	7.53	.10	7.56	.04	6.44	.03
Virginia Dare White Wine	4.42	.01	4.48	.01	4.16	.08
Virginia Dare Red Wine	5.81	.02	5.82	.03	5.37	.11
Ambassador California Burgundy	4.73	.07	4.69	.01	4.24	.02
Richelieu California Port	5.15	.01	5.12	.02	4.68	.01
Homestead Piestengel Rhubarb Wine	2.27	.00	2.27	.01	2.28	.02

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 absorbancy of the solution at 595 mμ using 1 cm. cells. Run a blank through the entire procedure and subtract its absorbancy from the absorbancy of the sample solution.

Results The iron content of the wines was calculated from the calibration curve shown in Figure 4 and the results are given in Table 3. All determinations were carried out at least in triplicate.

Recovery of iron To determine if all the iron was recovered using this direct procedure, the aqueous solutions remaining from the extraction of the Virginia Dare Red Wine were analyzed for iron using the wet ashing procedure employing TPTZ. The results are shown in Table 4.

Table 4. Results in the direct determination of iron in Virginia Dare Red Wine

Sample	Fe found by direct method mg./l.	Fe found in remaining solution mg./l.	Total Fe found mg./l.
1	5.47	0.26	5.73
2	5.43	0.35	5.78

Discussion

From the results shown in Table 3, it is apparent that the determination of iron in wine by the direct method using TPTZ usually gives values which are lower than those obtained by wet ashing procedures. This is presumably due to the presence of a very stable iron complex which is not broken by TPTZ. If this is the case, it may well be that the "complexed iron" is inactive in forming the turbidity in wine and that the results obtained for "uncomplexed iron" by this direct method are more useful to the wine producer than a knowledge of the total iron content. Various conditions, such as length of heating and ethanol concentration, were changed; but in no case was it possible to completely recover the iron from certain wines.

The iron which is not recovered by the direct method remains in the aqueous phase as may be seen in Table 4. The iron content of the Medoc Bordeaux Red Wine was also determined by the direct method of Banick and Smith and found to be 6.46 mg. of iron per liter. This is low compared to the values obtained by methods involving wet ashing and is practically the same as found by the direct TPTZ method.

The procedure employing preliminary wet ashing followed by determination of the iron with TPTZ does have distinct advantages over the usual 1,10-phenanthroline methods. A smaller sample may conveniently be used due to the increased sen-

sitivity of the reagent and the extractability of the iron derivative into nitrobenzene. Also, it is unnecessary to remove the excess perchloric acid as is the case in the determination employing 1,10-phenanthroline.

The Determination of Iron in Water

Background

The determination of iron in water is complicated by the wide range over which the iron content may vary. In many ground and surface waters, the iron content is greater than 0.1 parts per million and can be accurately determined by usual procedures employing reagents as 1,10-phenanthroline (16) and bathophenanthroline (9).

Sea water and treated water, on the other hand, usually contain much smaller quantities of iron and consequently, their analysis presents a greater problem. Furthermore, the iron in sea water is usually classified into two types, soluble and particulate. Experimentally, these two types are differentiated by use of membrane filters which have a known porosity. Although the iron content of sea water is small, it is intimately involved in marine growth.

Lewis and Goldberg (17) have used bathophenanthroline for the determination of soluble iron which is usually between

0 to 7 parts per billion. The advantages of bathophenanthroline over the reagents formerly employed are its greater sensitivity toward iron and the extractability of the iron derivative into immiscible organic solvents.

The following work was carried out to develop suitable procedures for the determination of iron in various waters using TPTZ.

Samples for analysis

Inasmuch as the iron content of sea water has a tendency to change on standing in containers because of adsorption of iron on the walls of the vessel, synthetic samples containing 10 parts per million of fluoride, 3% sodium chloride and varying amounts of iron were prepared. Samples of raw and treated water were obtained from the Municipal Water Works, Ames, Iowa.

Special reagents

In addition to the reagents previously described, the following are needed.

Sodium perchlorate-hydroxylammonium chloride This solution was prepared by dissolving 100 g. of hydroxylammonium chloride and 100 g. of sodium perchlorate in water and diluting to 1 liter. Iron was removed by adding 10 ml. of 0.001 M TPTZ and extracting with 10 ml. of nitrobenzene.

Acetic acid-sodium acetate buffer A solution 1 M in sodium acetate and 1 M in acetic acid was prepared by dissolving 82 g. of sodium acetate and 58 ml. of acetic acid in water and diluting to 1 liter. Iron was removed by adding 5 ml. of the sodium perchlorate-hydroxylammonium chloride solution and 10 ml. of 0.001 M TPTZ and extracting with nitrobenzene.

Determination of iron in sea and treated water

Difficulty was encountered in the first few experiments presumably owing to iron adsorbed on the glassware which is only slowly leached away. It is recommended that the separatory funnels and volumetric flasks be only rinsed with deionized water between determinations and not subjected to the usual cleaning methods. Pipets were cleaned in a dichromate-cleaning solution prepared from reagent grade chemicals.

Procedure Pipet 100.0 ml. of the water sample into a 125-ml. separatory funnel. Add 2.0 ml. of the sodium perchlorate-hydroxylammonium chloride solution, 5.0 ml. of 0.001 M TPTZ and 5.0 ml. of buffer. If previous treatment of the sample, such as wet ashing, has introduced much acid, neutralize with ammonium hydroxide to pH 4 to 5. Add 10 ml. of nitrobenzene, shake for 1 minute, allow the phases to separate and gently swirl the flask to dislodge any drops of nitrobenzene clinging to the upper walls of the funnel.

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 ml. with ethanol. Determine the absorbancy of the solution at 595 mμ using 5 cm. cells and a mixture of nitrobenzene and ethanol (4:1) in the solvent cell. Run a reagent blank through the entire operation and subtract its absorbancy from the absorbancy of the unknown solution.

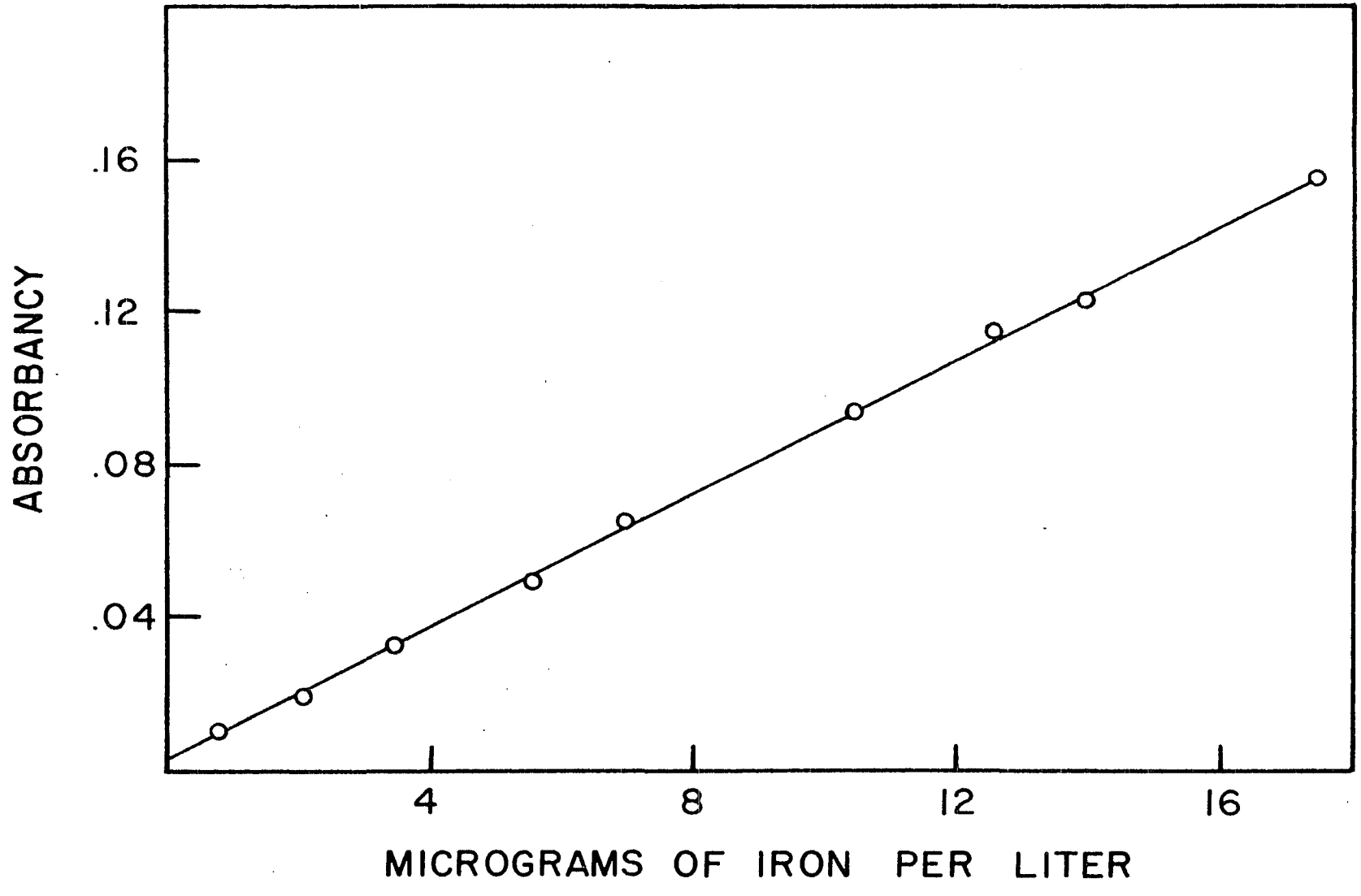
Calibration curve A 1.25×10^{-6} iron solution was prepared by successive dilutions of a 0.01000 M iron solution which had been prepared from electrolytic iron. Aliquots of this solution were diluted to 100 ml. and run, using the above procedure. The calibration curve is shown in Figure 19.

Results The results obtained for the iron content of the synthetic sea water samples are shown in Table 5. The results obtained for the treated water from the Ames Water Works were 7.1, 7.3, 7.4, 7.4 μg. of Fe/l.

Table 5. Results of the determination of iron in synthetic sea water samples

Fe added μg./l.	Fe found μg./l.	Absolute error μg./l.
0.70	0.70	0.00
3.49	3.40	0.09
6.98	7.35	0.37
10.47	10.4	0.1
13.96	14.2	0.2

Figure 19. Calibration curve for the determination of small amounts of iron in water


ABSORBANCY
.16
.12
.08
.04
4
8
12
16
MICROGRAMS OF IRON PER LITER

Determination of iron in untreated water

Procedure To a 3.00 ml. sample of the water contained in a 125-ml. separatory funnel add 2.0 ml. of the sodium perchlorate-hydroxylammonium chloride solution, 5.0 ml. of 0.001 M TPTZ and 5.0 ml. of the buffer. Extract the solution with 4.0 ml. of nitrobenzene followed by 2 extractions using 2.0 ml. portions of nitrobenzene. Dilute the combined extracts to volume in a 10-ml. volumetric flask with ethanol and determine the absorbancy of the solution at 595 mμ using 1 cm. cells. Use a mixture of nitrobenzene and ethanol (4:1) in the solvent cell. Run a blank through the entire procedure.

Results The results obtained for the untreated Ames water are shown in Table 6 and were found using the calibration curve previously shown in Figure 4. The samples were also analyzed for iron using a slight modification of the 1,10-phenanthroline procedure of Diehl and Smith (18) and these results are also presented in Table 6.

Table 6. Results of the determination of iron in untreated Ames water

Sample	Fe found TPTZ ppm	Fe found 1,10-phenanthroline ppm
1	6.18	6.23
2	6.24	6.23
3	6.18	6.21
Average	6.20	6.22

Discussion

Very small amounts of iron in water can be estimated with fair accuracy using TPTZ. This is possible because TPTZ is a very sensitive reagent for iron and the iron derivative can be extracted in nitrobenzene. In the recommended procedure a concentration factor of 4 is obtained by the extraction. The main precautions which must be taken in this determination are to remove iron from reagents and to condition the glassware by going through the procedure one or two times before carrying out the actual determination.

TPTZ can also be used for the determination of iron in water containing much larger amounts of iron as is common with many ground and surface waters.

The Determination of Iron in Siliceous Materials

Background

That the general quality of silicate rock analyses leaves much to be desired is readily apparent from the preliminary report of an extensive investigation undertaken by the United States Geological Survey, the Massachusettes Institute of Technology and the Geophysical Laboratory of the Carnegie Institute of Washington (19). Samples of a granite (G-1) and a diabase (W-1) have been analyzed for their various components by many different analysts using both chemical

and spectrochemical methods in an attempt to establish standard rock samples. The wide variation in results for most analyses among the various participating laboratories indicates a need for improved methods or greater care on the part of the analyst.

One of the main difficulties in determining the iron content of siliceous materials is completely decomposing the sample without loss of iron. Treatment with hydrofluoric acid often leaves a residue that is insoluble in hydrochloric acid so a fusion of the sample may be required. The possibility of loss of iron in fusions using platinum crucibles has been studied by Shell (20). During the fusion, iron is evidently reduced and alloys with the platinum. This alloyed iron is difficult to completely recover and affects subsequent analyses inasmuch as it may be partially released at a later time. To overcome this absorption of iron, Shell has suggested the use of silver crucibles employing a mixture of equal amounts of sodium carbonate and sodium borate as the flux.

Preliminary experiments which were carried out using platinum crucibles indicated that a serious loss of iron was occurring in some of the sodium carbonate fusions. Silver crucibles were prepared and used for all fusions in this work.

Silver crucibles

Crucibles were made by electroplating silver on 20 or 30 ml. nickel crucibles using an electrolyte containing 30 g. of silver nitrate, 36 g. of sodium cyanide and 112 g. of potassium nitrate per liter of solution. The crucibles were rotated during the plating and a current of 0.5 ampere was used. Silver anodes in the form of rods were cast from silver which had been purified by electrolytic deposition as loose dendritic crystals from a neutral silver nitrate solution. After 80 to 100 g. of silver had been deposited, the nickel crucible was dissolved away with hydrochloric acid. Several fusions of a mixture of sodium carbonate and sodium borate in the silver crucibles reduced the blank for iron determinations to a negligible value.

Procedures for the determination of iron

Burnt refractories, clays, argillaceous limestone

Weigh a sample containing 3 to 5 mg. of Fe_2O_3 into a silver crucible and add 1.0 g. of sodium carbonate and 1.0 g. of sodium borate decahydrate. After mixing thoroughly, gently heat the crucible and contents over a Meker burner until the water from the sodium borate has been vaporized. Gradually increase the heat to melt the flux and continue heating until the sample is completely dissolved. Rotate the crucible while cooling to cause the melt to solidfy on the sides of the crucible. When 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 residue 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 volume. After mixing well, 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, 5.0 ml. of 0.001 M TPTZ and 10 ml. of a 2 M sodium acetate-2 M acetic acid buffer. Dilute the solution to volume and determine the absorbancy at 593 mμ using 1 cm. cells. Run a blank on the reagents and silver crucible in exactly the same manner.

Glass and glass sand Weigh a sample containing 0.5 to 1.0 mg. of Fe_2O_3 into an iron free platinum crucible. (Iron may be removed from a platinum crucible by repeated heating to 1000-1200° in a muffle furnace and leaching with hot hydrochloric acid.) Add 2 ml. of water and 4 ml. of hydrofluoric acid if the sample is 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 following paragraph. If an insoluble residue remains, filter the solution into a 250-ml. volumetric flask using medium porosity filter paper. After washing first with dilute hydrochloric acid (1:100) and finally with water, ash the filter in a silver crucible. 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 complex remains on mixing, add 10 ml. of the 2 M sodium acetate-2 M acetic

acid buffer and dilute to volume. Determine the absorbancy at 593 mμ using 1 cm. cells. Run a blank on reagents and crucible in exactly the same manner.

Limestone Weigh a sample of limestone containing 0.5 to 1.0 mg. of Fe_2O_3 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. After washing the filter with dilute hydrochloric acid (1:100) and water, place it in a silver crucible, ash, cool and add 1.0 g. of sodium carbonate and 1.0 g. of sodium borate decahydrate. 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 and conclude the determination exactly as directed in the last paragraph in the procedure for the determination of iron in glass and glass sand.

Granite (G-1) 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 and continue heating until a clear melt is obtained. Rotate the crucible while cooling and place in a 600-ml. beaker. Add 100 ml. of hydrochloric acid and 200 ml. of water and heat until the residue is 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 volume. 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, 5.0 ml. of 0.001 M TPTZ and 10.0 ml. of a 2 M sodium acetate-2 M acetic acid buffer and dilute the solution to volume. Determine the absorbancy at 593 mμ using 1 cm. cells. Carry a blank through the entire operation.

Diabase (W-1) 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 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 residue is com-

pletely dissolved (a residue of silica and silver shloride 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 a 2 M sodium acetate-2 M acetic acid buffer and dilute to volume. Mis well and determine the absorbancy of the solution at 593 mμ. Run a blank through the entire procedure. All volumetric flasks and pipettes used in this determination were calibrated.

Results and discussion

Various National Bureau of Standards samples and the two rock samples from the Geological Survey were analyzed and the results are shown in Table 7. No definite values for the iron content of the granite and diabase have as yet been established. In the preliminary report published in 1950, the averages and standard deviations of the results from 24 laboratories are: Granite (G-1), % Fe = 1.44, σ = .231; Diabase (W-1), % Fe = 7.88, σ = .18. These values for the iron content expressed as % Fe_2O_3 are 2.06 and 11.27. Later work reported by Goldich and Oslund (21) gave an average value of 1.86% Fe_2O_3 for the granite and 11.09% Fe_2O_3 for the diabase.

Preliminary analyses of the granite were carried out using 150 mg. samples. Fe_2O_3 found: 1.70, 1.93, 1.73, 1.82%. The poor precision obtained was attributed to inhomogeneity of the sample inasmuch as much more precise results had been obtained by a similar procedure on Bureau of Standards samples of burnt refractories, etc. On examination of the material, it was noted that black particles are scattered throughout the sample which are attracted to a magnet. Presumably these particles are magnetite and the distribution through the mass is not absolutely uniform. In subsequent analyses of the granite, larger samples, 3 g. were taken and the results are given in Table 7.

For many of the samples analyzed the iron content as determined by the various TPTZ procedures is lower than the average reported by the Bureau. The possibility that iron was present in the precipitate of silica and silver chloride was checked in the case of the NBS 76 burnt refractory and no significant amount of iron was found. The range of values obtained for each sample by the various analysts as shown in the certificates is often quite large; this indicates the difficulty of accurate silicate analysis. The procedures used for determining the iron content of silicates usually employ lengthy separations which require the use of relatively large amounts of reagents and reagent grade chemicals often contain appreciable amounts of iron. In the proposed

Table 7. Results on the determination of iron in siliceous materials

Sample no. and description	% Fe_2O_3 found	Reported av. value	Range of reported values
NBS 76 Burnt Refractory	2.11, 2.11, 2.11, 2.12, 2.08 Average: 2.11	2.38	2.22 to 2.50
NBS 77 Burnt Refractory	0.82, 0.81, 0.82 Average: 0.82	0.90	0.79 to 1.39
NBS 78 Burnt Refractory	0.71, 0.71, 0.70 Average: 0.71	0.79	0.70 to 1.17
NBS 1a Argillaceous	1.59, 1.57 Average: 1.58	1.63	1.57 to 1.69
NBS 88 Dolomite	0.083, 0.083, 0.084 Average: 0.083	0.084	0.082 to 0.086
NBS 97 Flint Clay	0.92, 0.92, 0.93, 0.92 Average: 0.92	0.98	0.92 to 1.01
NBS 98 Plastic Clay	1.97, 1.97, 1.99, 1.97, 2.00 Average: 1.98	2.05	2.00 to 2.11
NBS 81 Glass Sand	0.074, 0.076, 0.075, 0.074 Average: 0.075	0.073	0.067 to 0.077
NBS 91 Opal Glass	0.073, 0.074, 0.073, 0.074 0.073, 0.076. Average: 0.074	0.081	0.070 to 0.095
NBS 93 Borosilicate Glass	0.078, 0.078, 0.079 Average 0.078	0.076	0.07 to 0.078
G-1 Granite	1.85, 1.85, 1.84, 1.85 Average: 1.85	See text	1.29 to 2.99
W-1 Diabase	10.91, 10.94, 10.87 Average: 10.91	See text	10.70 to 12.19

TPTZ methods, most of the reagents can be easily freed of iron by virtue of the extractability of the iron derivative of TPTZ into nitrobenzene. This reduces the blank to almost zero. These methods also have the advantage that no tedious separations are necessary in the analysis of silicates usually encountered.

The Determination of Iron in Blood Serum

Background

The concentration of non-hemoglobin iron in blood serum is of clinical interest as evidenced by the many methods for its determination which have been devised. Since the non-hemoglobin iron is present as various protein complexes, the usual methods involve extraction of the iron from the protein material, the amount of iron extracted varying with the method used. Vannotti and Delachaux (22, p. 64) list four forms of serum iron which are differentiated as follows: Iron fraction A is obtained by dilution of the serum with water and subsequent protein removal with trichloroacetic acid. Iron fraction B can only be split off by the addition of hydrochloric acid. The extraction is dependent upon the concentration of the acid and the nature and quantity of serum proteins. Fraction C can only be quantitatively estimated after ashing of the hydrochloric acid and fraction D can only be obtained, together with fractions A, B and C, by total incineration of the untreated serum.

Trinder (23) has proposed a method for determining iron in blood serum using sulfonated bathophenanthroline after extraction of the iron with trichloroacetic acid. The fraction of the iron extracted does not correspond exactly to any of those listed by Vannotti and Delachaux since the mixture of serum and trichloroacetic acid is heated for several minutes. Evidently the total amount of non-hemoglobin iron is not determined by this method but the values found are of use.

TPTZ has about the same sensitivity toward iron as bathophenanthroline and the following work was undertaken to determine if TPTZ could be used in a similar procedure for the determination of serum iron.

Serum sample

Serum was prepared from pig blood by allowing the blood to clot for 24 hours. The serum that had separated was then removed, centrifuged and analyzed for iron by a slight modification of Trinder's method.

Special reagents

In addition to the reagents previously described, the following are required.

Sodium acetate A saturated sodium acetate solution was prepared and iron removed by adding 1 g. of hydroxylammonium chloride, 1 g. of sodium perchlorate and 5 ml. of 0.001 M TPTZ and extracting with nitrobenzene.

Trichloroacetic acid reagent A 20% solution was prepared by dissolving 20 g. of redistilled trichloroacetic acid in 80 ml. of water and adding 1 g. of thioglycollic acid.

Procedure

To a 2.00 ml. serum sample contained in a 15 x 125 mm. test tube add 2.50 ml. of water and 1.50 ml. of the trichloroacetic acid reagent. Cover the tube with an aluminum foil cap and shake gently to mix the contents. Heat for 5 minutes at 90 to 95° in a water bath, mix, and continue heating for another 5 minutes. Centrifuge for 15 minutes and pipet 3.00 ml. of the clear supernatant liquid into a test tube. Add 1.00 ml. of water, 0.20 ml. of 0.1% TPTZ and 0.80 ml. of saturated sodium acetate. Mix and determine the absorbancy of the solution at 593 mμ. Carry a blank through the entire operation.

Calibration curve

Various amounts from 0.50 to 3.00 ml. of a solution containing 2.776 μg. of iron per ml. were placed in test tubes, each solution diluted to 3.00 ml. with water and 1.0 ml. of the trichloroacetic acid reagent added. Following the addition of 0.20 ml. of 0.1% TPTZ and 0.80 ml. of saturated sodium acetate, the absorbancy of each solution was determined at 593 mμ. A plot μg. of iron vs. absorbancy is shown in Figure 20. Using the above procedure for the determination of iron, the concentration determined by use of this plot is in μg. of iron per ml. of serum.

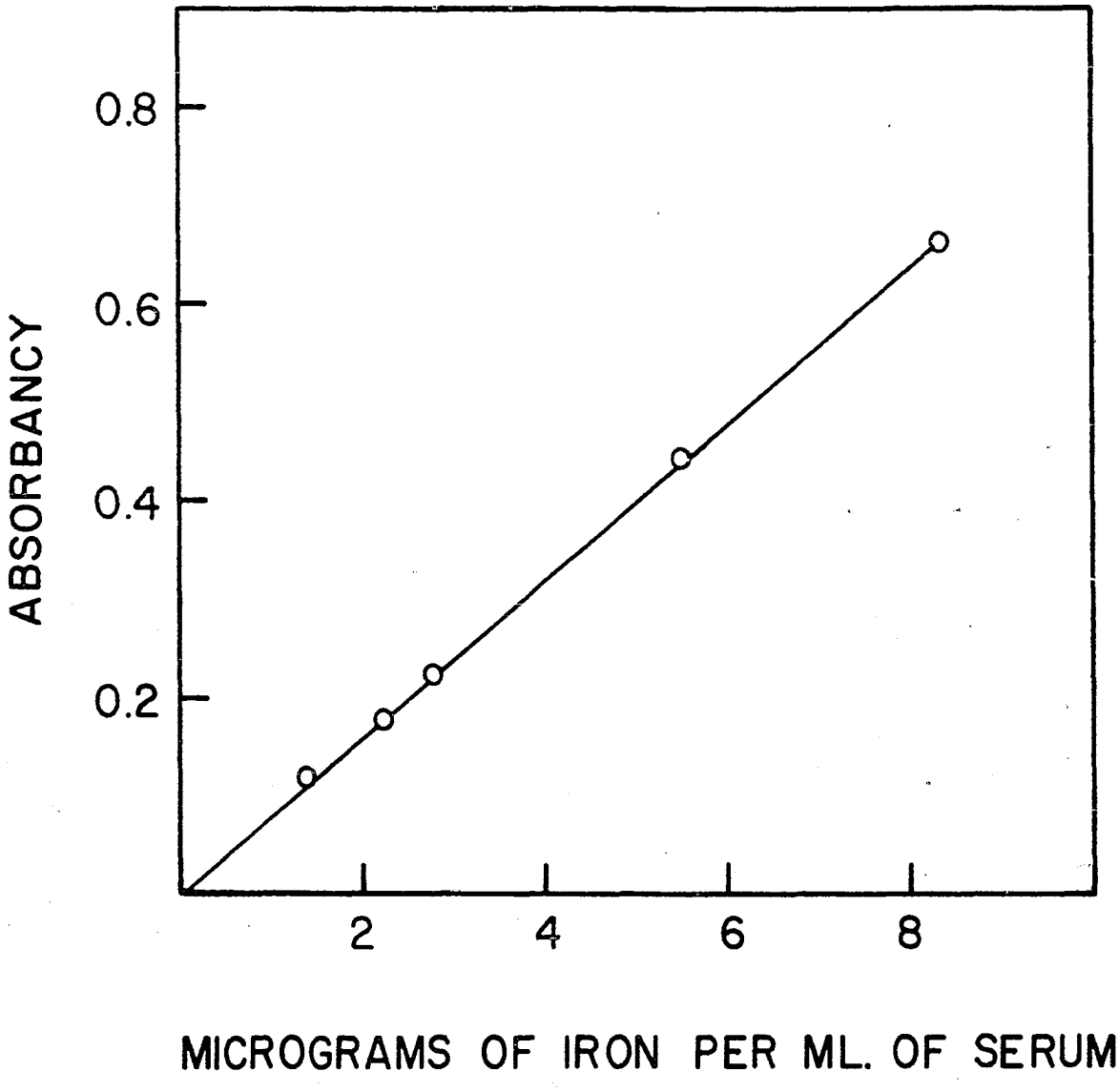


Figure 20. Calibration curve for the determination of iron in blood serum

Results

The iron content of the serum found using Trinder's method was 1.41, 1.45, 1.47 and 1.43 μg. Fe/ml.; average, 1.44. Using the TPTZ procedure, the values found were 1.42, 1.43, 1.44 μg. Fe/ml.; average, 1.43. These results indicate that the same fraction of non-hemoglobin iron is determined by both methods and with about the same accuracy.

The Determination of Iron in Urine

Background

The iron content of normal urine is very low; approximately 0.04 to 0.2 mg. of iron is excreted from the human body in this way during a 24 hour period (24). In the case of certain kidney disorders, however, the iron in urine is increased (22, p. 28) and therefore a knowledge of the concentration of iron in urine is of clinical interest.

Seven and Peterson (25) have recently proposed a method using bathophenanthroline for the determination of iron in urine. The sample is ashed using nitric and sulfuric acids and hydrogen peroxide. After ashing, the determination is completed by forming the iron derivative of bathophenanthroline and extracting into isoamyl alcohol. The procedure is quite lengthy and the following work was carried out in an attempt to devise a faster method using TPTZ.

Special reagents

In addition to the reagents previously described, the following are required.

Nitric acid Reagent grade nitric acid was doubly distilled from an all glass still to remove traces of iron.

Perchloric acid Reagent grade perchloric acid was vacuum distilled.

Ammonium hydroxide Iron free ammonium hydroxide was prepared by distilling anhydrous ammonia into deionized water.

Procedure

Rinse all apparatus with hydrochloric acid (1:1) followed by deionized water to remove iron. To a 50 ml. sample contained in a 250-ml. conical flask add 25 ml. of nitric acid and 10 ml. of perchloric acid. Place a reflux head on the flask and heat the solution to fumes of perchloric acid. Continue the digestion for an additional 15 minutes and cool. Wash down the sides of the flask and reflux head with water and heat to boiling to remove chlorine. Cool the solution, add 2.0 ml. of 10% hydroxylammonium chloride, 5.0 ml. of 0.001 M TPTZ and 2.0 ml. of 2 M sodium acetate-2 M acetic acid buffer. Adjust the pH to 4.0 to 4.5 with ammonium hydroxide and transfer the solution and precipitate of ammonium perchlorate to a 125-ml. separatory funnel. Dilute the solution to about 100 ml. and extract three times using 4.0, 2.0 and 2.0 ml. portions of nitrobenzene. Collect the extracts in a

10-ml. volumetric flask, dilute to volume with ethanol and determine the absorbancy of the solution at 595 mμ using 1 cm. cells. Run a reagent blank and subtract the absorbancy of this solution from the absorbancy of the sample solution.

Recovery of iron

Various quantities ranging from 5 to 15 ml. of a solution containing 0.694 μg. of iron per ml. were added to urine samples and analyzed by the above procedure.

Results and discussion

The results obtained for the urine samples to which iron was added are shown in Table 8. The calibration curve previously shown in Figure 4 was used to calculate the results. The iron content of this urine was very low, 0.6 μg. iron per 50 ml., and the precision is in the order of 0.1 μg. of iron. In those abnormal cases where the iron content is higher, the relative accuracy should be much greater.

No difficulty was experienced in the wet ashing of the urine using nitric and perchloric acids. The ashing step requires only 30 to 45 minutes which is considerably faster than the ashing proposed by Seven and Peterson. The precipitate of ammonium perchlorate completely dissolves when the solution is diluted to 100 ml. and causes no difficulty.

Table 8. Recovery of iron added to urine

Absorbancy	Fe added µg.	Fe found µg.	Fe originally present in 50 ml. of urine µg.
.027	0	0.61	0.61
.028	0	0.67	0.67
.171	3.47	4.02	0.55
.329	6.94	7.60	0.66
.473	10.41	11.05	0.64

SUMMARY

2,4,6-Tris(2'-pyridyl)-s-triazine (TPTZ) reacts with iron(II) to form an intensely colored, water soluble derivative which can be extracted into nitrobenzene from an aqueous solution containing perchlorate or iodide. The color is completely formed over the pH range of 3.4 to 5.8 in aqueous solution and when the extraction into nitrobenzene is used, the permissible pH range is increased to 2.7 to 7.0

The molar extinction coefficient of the bis(2,4,6-tris-(2'-pyridyl)-s-triazine)iron(II) ion is 22,600 at 593 mμ in aqueous solution. In nitrobenzene, the perchlorate salt has a molar extinction coefficient of 24,100 at 595 mμ. Thus, TPTZ is a very sensitive reagent for iron.

The combining ratio of TPTZ with iron(II) is 2 to 1 as shown by a spectrophotometric titration, a continuous variations study and by the analysis of the iodide salt of the complex. A series of nonaqueous titrations was carried out and the results indicate that only two of the three pyridyl groups are involved directly in forming the iron(II) derivative. A structure for the compound has been proposed.

The extractability of the iron(II) derivative into nitrobenzene is advantageous since it provides a simple way to remove iron from the reagents used in a determination as well

as serving as a concentration step.

A study of interfering ions was carried out and of the ions tested only Cu^{++}, Co^{++}, Ni^{++}, Cr^{+++}, Ag^{+}, Hg^{++}, Bi^{+++}, MoO_4^{--}, CN^{-}, $C_2O_4^{--}$ and NO_2^{-} interfere significantly. The interference of Co^{++}, Cu^{++} and Ni^{++} is due to the formation of colored compounds with TPTZ; however, 2.5 p.p.m. of Cu^{++}, 2.4 p.p.m. of Co^{++} or 5.3 p.p.m. 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.

The determination of iron in wine with TPTZ has been studied and a suitable procedure for this analysis developed. Attempts to eliminate the wet ashing step and directly determine the iron gave low results. However, the results obtained by this direct method may be of greater significance to the wine producer than the results for total iron obtained after a preliminary wet ashing of the wine.

A procedure has been developed for the determination of very small amounts of iron as are present in sea and treated water using TPTZ. This reagent may also be used for the determination of iron in ground and surface water which usually contain larger amounts of iron.

Various rocks, clays, glass and glass sand, burnt refractories and limestones have been analyzed for iron by various procedures which were developed using TPTZ. Silver crucibles were made and used for the necessary fusions.

A procedure for the determination of iron in urine has been devised making use of a wet ashing with nitric and perchloric acids to decompose the sample. The determination is completed by forming the iron(II) derivative of TPTZ and extracting it into nitrobenzene. This procedure requires considerably less time than those previously proposed.

TPTZ can be used to determine iron in blood serum. The total amount of iron is probably not determined by the procedure used, but the results obtained for the fraction of iron determined are of clinical interest.

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