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The spectrophotometric determination of dissolved oxygen and other uses of 4,7-dihydroxy-1, 10- phenanthroline

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The spectrophotometric determination of dissolved oxygen and other uses of 4,7-dihydroxy-1,10-phenanthroline

by

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I. INTRODUCTION

The organic compound 1,10-phenanthroline has been used since 1934 as a reagent for the spectrophotometric determination of small amounts of iron. In the seventy-six years since the first synthesis of 1,10-phenanthroline, several hundred substituted 1,10-phenanthrolines have been synthesized. Many of these substituted phenanthrolines have found application in the spectrophotometric determination of metal ions, and in the form of derivatives of certain multivalent metals, as high-potential, reversible oxidation-reduction indicators.

The present work with 4,7-dihydroxy-1,10-phenanthroline was initiated in an attempt to make an ion selective electrode which would respond to iron. Membranes were prepared in which the iron derivative of 4,7-dihydroxy-1,10-phenanthroline was incorporated into a phenol-formaldehyde resin (Bakelite). The attempt failed but a considerable amount of knowledge of the chemistry of 4,7-dihydroxy-1,10-phenanthroline was uncovered.

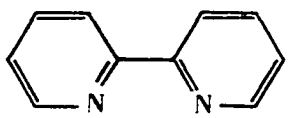
Certain features of the chemistry of 4,7-dihydroxy-1,10-phenanthroline make this compound unique among the phenanthrolines. It forms a derivative with ferrous iron which is stable in highly alkaline solution, quite in contrast to the parent compound. The ferrous derivative can in fact be used for the spectrophotometric determination of

iron in strong alkalies. Again, the formal reduction potential of the ferric dihydroxyphenanthroline-ferrous dihydroxyphenanthroline couple is -0.06 volt, much below the range +0.83 to +1.33 volt in which the iron couples with all other phenanthrolines fall. Exploitation of such differences is the basic theme of this dissertation.

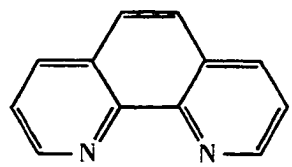
In this dissertation, the chemical properties of 4,7-dihydroxy-1,10-phenanthroline and its iron derivatives are described in detail and applications of these properties to chemical analysis are reported. The synthesis and the purity of 4,7-dihydroxy-1,10-phenanthroline have been improved. Evidence confirming the structure previously assigned to the compound has been adduced from the NMR spectrum. That 4,7-dihydroxy-1,10-phenanthroline is much more reactive than 1,10-phenanthroline has been demonstrated by a study of its reaction with formaldehyde and its sulfonation. The effect of pH on the reduction potential of the ferric-ferrous couple has been studied. Three distinctly different ferric compounds have been found. A method has been developed using the ferrous derivative for the spectrophotometric determination of dissolved oxygen. And a method has also been developed for removing iron from concentrated solutions of sodium hydroxide by extracting the ferrous derivative of 4,7-dihydroxy-1,10-phenanthroline into dimethylsulfoxide.

II. HISTORY OF 1,10-PHENANTHROLINE AND OF HYDROXY-1,10-PHENANTHROLINES

Phenanthroline chemistry began with the synthesis of 2,2'-bipyridine (I) by the Austrian chemist Fritz Blau (5) in 1888. The discovery that this compound forms an intensely red compound with ferrous iron led to studies on the nature of the iron compound, which were reported in 1889 (6). Blau



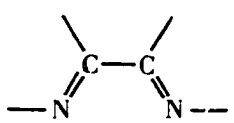
I 2,2'-Bipyridine
(α,α'-Bipyridyl)



II 1,10-Phenanthroline

conceived of 1,10-phenanthroline (II) as a bridged analog of 2,2'-bipyridine and succeeded in synthesizing it in 1898 (7). As he expected, it also formed an intensely red compound with ferrous iron, and in a brilliant investigation, he showed that the ferric compound was blue in color and that in both the ferric and ferrous compounds three molecules of the phenanthroline were combined with one atom of iron. He isolated numerous derivatives of these red and blue entities carrying a variety of negative radicals, he found that the ferric compounds were sensitive to light, and he showed that the ferric and ferrous compounds constituted a perfectly reversible, strikingly colored, oxidation-reduction system.

The work of Blau on 2,2'-bipyridine and 1,10-phenanthroline attracted little interest for many years although Alfred Werner (73), in working out the stereochemical aspects of his theory of coordination chemistry, did make use of the octahedral arrangement of the three molecules of 2,2'-bipyridine about the iron atom in the ferrous compound. The first use of a phenanthroline compound in chemical analysis appears to be the application of 2,2'-bipyridine to the determination of iron in biological materials by the British biochemist R. Hill (40) in 1930. Interest in the phenanthrolines really began with the publication of Walden, Hammett and Chapman (72) which described the use of the ferric-ferrous-1,10-phenanthroline couple as a reversible, high-potential oxidation-reduction indicator. This work attracted a great deal of attention and in particular there was soon coined, by Gleu (32), the terms ferriin and ferroin to designate the oxidized and reduced forms of the indicator. From these came quickly the generic term ferroin group for the atomic grouping responsible for the union with iron.



The use of 1,10-phenanthroline for the colorimetric determination of iron began with the practically simultaneous publication in 1937-1938 of three papers by different groups

of workers in the United States: Saywell and Cunningham (the work having been suggested and fostered actually by G. Frederick Smith) (58); Hummel and Willard (41); and Fortune and Mellon (27). G. Frederick Smith, Professor of Chemistry at the University of Illinois, devised a satisfactory synthesis of 1,10-phenanthroline and made the material available commercially. He studied various substituted phenanthrolines, devised applications of them in chemical analysis, and vigorously promoted their use. He persuaded Francis Case, Professor of Organic Chemistry at Temple University, to synthesize other phenanthrolines. For the thirty years 1935 to 1965, the chemistry of the phenanthrolines is the chemistry of G. Frederick Smith and coworkers, a fact that is readily appreciated from examination of the various reviews (8,14,23,24,59,64) which have appeared.

The principal uses in analytical chemistry of 1,10-phenanthroline and substituted 1,10-phenanthrolines has been as oxidation-reduction indicators and as spectrophotometric reagents for metals.

The oxidation-reduction indicator action takes advantage of the soluble, highly colored ferric and ferrous derivatives of 1,10-phenanthroline, $Fe(phen)_3^{3+}$-$Fe(phen)_3^{2+}$. The colors are respectively blue and red and the formal reduction potential is +1.06 volt. The reduction potentials of the corresponding couples with substituted phenanthrolines range

from around +0.8 volt (the methyl derivatives) to +1.3 volt (the 5-nitro derivative). The formal reduction potential of the corresponding couple with 4,7-dihydroxy-1,10-phenanthroline was found by Poe, during his work for the M.S. degree (54) to have the astonishingly low value of -0.33 volt. Further findings on this indicator are reported in Chapter VII of the present dissertation.

Substitution into the 1,10-phenanthroline molecule proved to have rather dramatic effects on the properties of the metal derivatives and made for great improvements as reagents for spectrophotochemical analysis. Substitution in certain positions increased the specificity, introduction of phenyl groups rendered the metal derivatives extractable into solvents immiscible with water, and in general lengthening the conjugation in the molecule increased the molar extinction coefficient of the metal derivative.

Two of the most important of the many substituted 1,10-phenanthrolines are 2,9-dimethyl-1,10-phenanthroline (neocuproine) and 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline). The presence of methyl groups at positions 2- and 9- prevents the formation of a colored compound with iron(II), undoubtedly because of steric hindrance which prevents the three organic molecules from entering the six positions about the atom of iron. On the other hand, 2,9- compounds do form colored compounds with copper(I); only two molecules of the phenanthroline combine

with the copper, the four nitrogen atoms are distributed at the corners of a regular tetrahedron about the copper atom, and steric hindrance plays no part. 2,9-Dimethyl-1,10-phenanthroline is a specific reagent for copper.

Introduction of phenyl groups at positions 4- and 7- in the phenanthroline molecule greatly increases the molar absorptivity of the metal derivatives, thus for ferrous iron the values at the wavelengths of maximum absorption are 11,110 and 22,350, respectively, for 1,10-phenanthroline and bathophenanthroline. The iron(II) derivative of bathophenanthroline is highly soluble in iso-amyl alcohol and other organic solvents and can be extracted with these solvents which makes it possible to concentrate the iron in a much smaller volume for spectrophotometric measurement. This increase in sensitivity makes it possible to determine iron at the parts-per-billion level (20). Extraction of tris(4,7-diphenyl-1,10-phenanthroline)iron(II) into an immiscible solvent also provides a method for the removal of trace quantities of iron from solutions of the necessary reagents for carrying out the determination, an advantage of great importance.

The introduction of hydroxy groups at positions 4- and 7- also markedly alters the properties of the phenanthroline molecule. The iron(II) derivative of 4,7-dihydroxy-1,10-phenanthroline is stable in strongly alkaline media, quite in contrast to the iron(II) derivatives of other

phenanthrolines. The synthesis of 4,7-dihydroxy-1,10-phenanthroline was reported first, in 1946, by H. R. Snyder and H. E. Freier (66). A method for purifying the material was found by Schilt, Smith and Heimbuch who also reported that the compound could be used for the spectrophotometric determination of iron in strongly alkaline media. The purification was made by recrystallization from hydrochloric acid. Poe improved the synthesis of 4,7-dihydroxy-1,10-phenanthroline, showed that the composition of the hydrochloride, the form in which the material is marketed, is highly variable, and used the iron derivative as a visual oxidation-reduction indicator in the titration of sodium hydrosulfite with potassium ferricyanide (54).

The acid dissociation constants of 4,7-dihydroxy-1,10-phenanthroline were reported by James and Williams (42), who evidently did not determine the constants themselves, but refer the reader to a paper by Pflaum and Brant (53). No mention is made of 4,7-dihydroxy-1,10-phenanthroline in the latter paper, and the reader is left wondering where the values reported by James and Williams came from. A value for the third acid dissociation constant of 4,7-dihydroxy-1,10-phenanthroline, obtained from ultraviolet absorption data, is reported in Part IV of this dissertation.

The stability of tris(4,7-dihydroxy-1,10-phenanthroline)-iron(II) in highly alkaline solutions has been attributed to

the strong electron-donating effect of the hydroxy groups, which are in the anionic form in highly alkaline solutions (11). The high electron density on the iron atom discourages attack by negatively-charged hydroxide ion.

Because of the strong electron-donating effect of the hydroxy group, it would be expected that other hydroxy-substituted 1,10-phenanthrolines would have properties similar to those of 4,7-dihydroxy-1,10-phenanthroline. To date at least thirty-nine such compounds have been prepared. Many of these compounds were prepared in the course of a search for compounds with anti-malarial activity. A large number were synthesized by Francis Case and coworkers at Temple University (15,75). The complete list is presented in Table II-1.

Of the compounds listed in Table II-1, the reduction of the iron derivatives of only two have been reported. Hale and Mellon (34) reported 0.71 volt for the reduction potential of the iron derivative of 3-carbethoxy-4-hydroxy-1,10-phenanthroline. George, Hanania, and Eaton reported -0.13 volt (pH $\geq$ 10) for the reduction potential of the iron derivative of 4,7-dihydroxy-1,10-phenanthroline (31).

Taylor and Schilt prepared the mixed-ligand compounds bis(4,7-dihydroxy-1,10-phenanthroline)-1,10-phenanthroline iron(II) trihydrate and 4,7-dihydroxy-1,10-phenanthroline-bis(1,10-phenanthroline)iron(II) trihydrate (68), but failed

Table II-1. Hydroxy-1,10-phenanthrolines

Compound as a substituted 1,10-phenanthroline	Type of information[a] (Literature reference)
One hydroxy group	
2-Hydroxy-	A (39,75)
2-Hydroxy-4-methyl-	A (10,14)
2-Hydroxy-4-methyl-3,6(?)-dinitro-	A (10)
4-Hydroxy-	A (14,61,66,67); B (34); C((34); D (37); E (52); F (36)
4-Hydroxy-2-methyl-	A (38,50); G (46)
4-Hydroxy-3-carbethoxy-	A (14,61,66); B (34); C (34); D (37)
4-Hydroxy-3-carboxy-	A (14,61,66)
4-Hydroxy-5-methoxy-	A (14,66,75)
4-Hydroxy-3-phenyl-	A (14,16)

[a]A - synthesis; B - colorimetric study; C - reduction potential; D - formation constant of iron compound; E - pH markers in isoelectric focusing; F - NMR spectrum; G - infrared spectrum; H - determination of copper; I - determination of iron; J - acid dissociation constants; K - Mossbauer spectrum; L - decomposition of metal derivatives by acid or base; M - catalyst; N - kinetics of oxidation of ferrous derivative with peroxydisulfate; O - mixed-ligand compounds; P - solubility of perchlorate.

Table II-1 (Continued)

Compound as a substituted 1,10-phenanthroline	Type of information[a] (Literature reference)
One hydroxy group (Continued)	
4-Hydroxy-3,5-diphenyl-	A (14,16)
4-Hydroxy-7-butoxy-	A (2)
4-Hydroxy-7-ethoxy-	A (2)
4-Hydroxy-7-dimethylamino-	A (2); G (33)
4-Hydroxy-3-carbethoxy-5-methoxy-	A (14,66)
4-Hydroxy-3-carboxy-5-methoxy-	A (14,66); E (52)
4-Hydroxy-2-methyl-5-methoxy-	A (49)
4-Hydroxy-2-methyl-5-methoxy-6-nitro-	A (49)
4-Hydroxy-3-carboxy-5,6-dimethoxy-	A (75)
4-Hydroxy-2,9-dimethyl-	A (15); H (25)
4-Hydroxy-2-methyl-9-phenyl-	A (15); H (25)
4-Hydroxy-2,9-dimethyl-7-methoxy-	A (15); H (25)
4-Hydroxy-2,7,9-trimethyl-	A (15); H (25)
4-Hydroxy-2-phenyl-7-methoxy-9-methyl-	A (15); H (25)

Table II-1 (Continued)

Compound as a substituted 1,10-phenanthroline	Type of information[a] (Literature reference)
One hydroxy group (Continued)	
4-Hydroxy-2-trifluoromethyl-5-methoxy	A (21)
5-Hydroxy-	A (48,75); B (26); E (52)
7-Hydroxy-2-methyl-	A (39)
7-Hydroxy-2-acetoxy-9-methyl-	A (39)
Two hydroxy groups	
2,4-Dihydroxy-	A (75)
2,4-Dihydroxy-5-methoxy-	A (75); B (26)
4,5-Dihydroxy-	A (75); B (26); E (52)
4,5-Dihydroxy-2-methyl-	A (75)
4,7-Dihydroxy-	A (14,66); B (26); C (31); E (52); G (29,65); I (29,60); J (42); K (18); L (11,13); M (17,57); N (11); O (68); P (12)
4,7-Dihydroxy-3,8-dicarbethoxy-	A (14,54,66)
4,7-Dihydroxy-3,8-dicarboxy-	A (14,66)

Table II-1 (Continued)

Compound as a substituted 1,10-phenanthroline	Type of information[a] (Literature reference)
Two hydroxy groups (Continued)	
4,7-Dihydroxy-3,8-diphenyl-	A (14,16)
4,7-Dihydroxy-2,9-dimethyl-	A (15); H (25)
4,7-Dihydroxy-2-methyl-9-phenyl-	A (15); H (25)
5,6-Dihydroxy-	A (14,75); B (26)
Three hydroxy groups	
4,5,6-Trihydroxy-3-carboxy-	A (75)

to determine the reduction potentials of the iron(III,II) couples. Mixed-ligand compounds such as these, and the simple tris-chelated iron derivatives of other hydroxyphenanthrolines should provide a host of indicators with reduction potentials lower than 0.8 volt.

A series of hydroxyphenanthrolines containing methyl and phenyl groups at positions 2- and 9- were prepared by Case (15) and applied to the spectrophotometric determination of copper in alkaline solutions by Dunbar and Schilt (25). The most sensitive of these was 2,9-dimethyl-4,7-dihydroxy-1,10-phenanthroline, the molar absorptivity of the copper(I) compound in 4 M sodium hydroxide being 11,500 at 400 nm.

Taking advantage of their acid-base characteristics and their similarity to amino acids, Nakhleh, Samra, and Awdeh (52) reported the use of the iron derivatives of five hydroxy-1,10-phenanthrolines as pH markers in isoelectric focusing, a technique similar to gel electrophoresis used for the identification of amino acids and proteins by their isoelectric points.

That many other uses of hydroxyphenanthrolines are possible is evident from the widely varied uses to which 1,10-phenanthrolines have been put. Under 1,10-Phenanthroline in any index of Chemical Abstracts is found references to applications as enzyme inhibitors, catalysts, precipitants, fungicides, fluorometric reagents, drying

agents in paints, flavor stabilizers in milk, masking agents, and electroplating agents.

III. SYNTHESIS, PURITY AND IDENTITY OF 4,7-DIHYDROXY-1,10-PHENANTHROLINE

Purification of 4,7-dihydroxy-1,10-phenanthroline is accomplished by recrystallization from concentrated hydrochloric acid and the compound is marketed as the hydrochloride. The amount of hydrochloric acid carried appears to be variable. Thus a knowledge of the amount of hydrochloric acid present in the compound is necessary before solutions of known concentration can be prepared. The amount of hydrochloric acid associated with 4,7-dihydroxy-1,10-phenanthroline can be determined by alkalimetric titration as shown by D. P. Poe (54). The same result can be obtained by a gravimetric determination of chloride. The accuracy of the alkalimetric titration was improved by performing the titration in a nonaqueous solvent mixture.

Determinations of hydrochloric acid present in several preparations of 4,7-dihydroxy-1,10-phenanthroline during the course of this work indicated that the 4,7-dihydroxy-1,10-phenanthroline was contaminated by an impurity, possibly 3,8-dicarboxy-4,7-dihydroxy-1,10-phenanthroline or 3-carboxy-4,7-dihydroxy-1,10-phenanthroline, both of which are intermediates in the synthesis of 4,7-dihydroxy-1,10-phenanthroline. In an attempt to avoid this contamination, an improved method for the hydrolysis and decarboxylation of 3,8-dicarbethoxy-4,7-dihydroxy-1,10-phenanthroline was devised.

Snyder and Freier (66) assigned the name 4,7-dihydroxy-1,10-phenanthroline to their product on the basis of the procedure followed during the synthesis. Correlation of the proton magnetic resonance spectrum of 4,7-dihydroxy-1,10-phenanthroline with the spectra of related compounds indicate that the hydroxy groups do occupy positions 4- and 7-.

A. Improvement in the Alkalimetric Titration of 4,7-Dihydroxy-1,10-phenanthroline

1. Experimental work

4,7-Dihydroxy-1,10-phenanthroline was prepared by the method of Snyder and Freier (66) as improved by Poe (54). The final product was recrystallized from concentrated hydrochloric acid as recommended by Schilt, Smith, and Heimbuch (60). Alkalimetric titrations in aqueous solutions were performed by dissolving about 0.10 g. of 4,7-dihydroxy-1,10-phenanthroline in standard sodium hydroxide and titrating the resulting solutions with standard hydrochloric acid. An atmosphere of nitrogen was maintained over the alkaline solutions to prevent absorption of carbon dioxide. The pH of the solutions was measured with a Corning No. 476022, high-alkalinity glass electrode and a saturated calomel electrode in conjunction with a Beckman Zeromatic SS-3 pH meter. The pH meter was calibrated against NBS buffers of pH 6.86, 9.18, and 12.45, prepared as described by Diehl (22) and corrected for temperature as described by Bates (3).

The alkalimetric titration was also carried out in a mixture of 2-propanol and dimethylsulfoxide. Exactly 0.0997 g. of 4,7-dihydroxy-1,10-phenanthroline was suspended in 15.00 ml. of 0.0941 N potassium hydroxide in 2-propanol. Dimethylsulfoxide was added until the solid dissolved. An atmosphere of nitrogen was maintained over the solution to prevent absorption of carbon dioxide. The potential measurement system was the same as that described in the preceding paragraph, except that the saturated calomel electrode was replaced with a glass-sleeve type s.c.e. in which the internal filling solution had been replaced by a saturated solution of tetramethylammonium chloride in 2-propanol (28).

2. Results and discussion

The titration curves resulting from titration of 4,7-dihydroxy-1,10-phenanthroline (recrystallized from hydrochloric acid) in aqueous sodium hydroxide and in potassium hydroxide in 2-propanol/dimethylsulfoxide appear in Figures III-1 and III-2, respectively. Data and calculations are tabulated in Table III-1.

It has been shown by Poe (54) that the amount of hydrochloric acid present in 4,7-dihydroxy-1,10-phenanthroline is equivalent to the difference between the number of milli-equivalents of alkali used to dissolve the sample and the

Figure III-1. Titration of 4,7-dihydroxy-1,10-phenanthroline in an aqueous solution of sodium hydroxide with 0.1 N hydrochloric acid

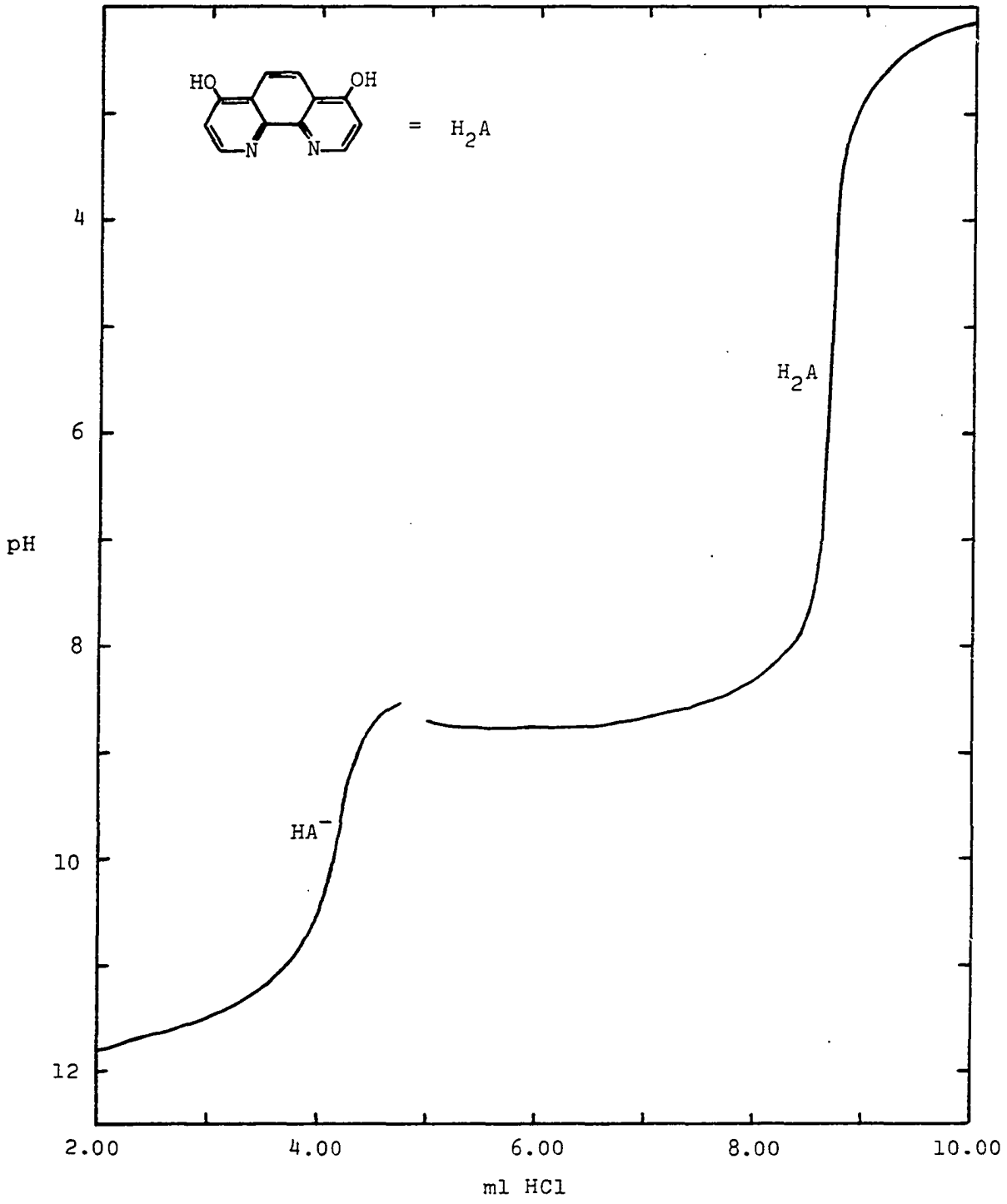

HO
OH
N
N
= H_2A
4
6
8
10
12
pH
H_2A
HA^-
2.00
4.00
6.00
8.00
10.00
ml HCl

Figure III-2. Titration of 4,7-dihydroxy-1,10-phenanthroline in a solution of potassium hydroxide in 2-propanol/dimethylsulfoxide with 0.1 N hydrochloric acid in 2-propanol

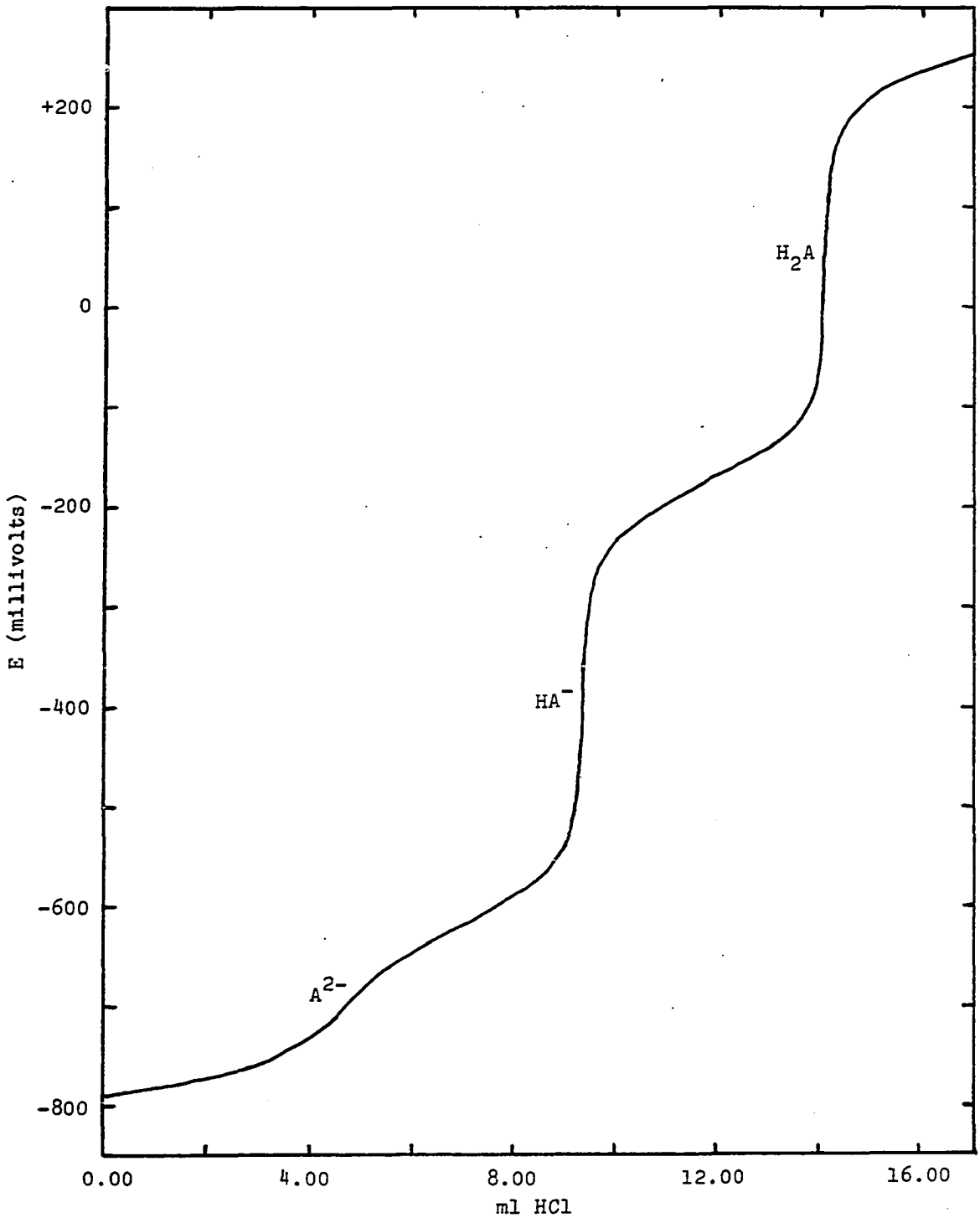

+200
0
-200
-400
-600
-800
E (millivolts)
H_2A
HA^-
A^{2-}
0.00
4.00
8.00
12.00
16.00
ml HCl

Table III-1. Alkalimetric titration of 4,7-dihydroxy-1,10-phenanthroline

	Recrystallized Material	Recrystallized Material	Unrecrystallized Material
Solvent	Water	IPA/DMSO	Water
Weight of 4,7-diOHphen (g.)	0.0981	0.0997	0.0868
Alkali added (meq.)	1.007	1.482	0.906
First end point (meq.)	0.413	0.904	0.502
Second end point (meq.)	0.859	1.362	0.902
HCl associated with 4,7-diOHphen (meq.)	0.148	0.120	0.004
4,7-diOHphen (mmoles)	0.446	0.458	0.400
Wt. HCl, calc. (g.)	0.0054	0.0044	0.0001
Wt. 4,7-diOHphen, total (g.) - Wt. HCl (g.)	0.0927	0.0953	0.0867
Mol. wt. 4,7-diOHphen, calc.	208	208	217
Percent HCl	5.5	4.4	0.1
Moles HCl/mole 4,7-diOHphen	0.33	0.26	

number of milliequivalents of acid added at the second equivalence-point when all of the 4,7-dihydroxy-1,10-phenanthroline has been precipitated.

The sharpness of the first equivalence-point was greatly enhanced by carrying out the titration in a mixture of 2-propanol and dimethylsulfoxide. An additional point of inflection appeared in this case, corresponding to the neutralization of the excess potassium hydroxide. This inflection-point unfortunately was not sharp, and could not be located with acceptable accuracy.

B. Presence of a Carboxy Compound in 4,7-Dihydroxy-1,10-phenanthroline and Improvement in the Hydrolysis of 3,8-Dicarbethoxy-4,7-dihydroxy-1,10-phenanthroline

Analysis of the data given in Table III-1 indicates the presence of some acidic impurity (in addition to hydrochloric acid) in the recrystallized material. The high equivalent weight obtained for the unrecrystallized material indicates the presence of some nonacidic impurity. The variable results obtained for the amount of hydrochloric acid present suggests inhomogeneity of the final product. This is not at all surprising, since the product contained some fairly large aggregates of crystals.

Assuming that no water was present in the unrecrystallized material (it had been heated at 300° for two hours

before being placed in a desiccator over anhydrous magnesium perchlorate), the data fit well with the proposition that some 3,8-dicarbethoxy-4,7-dihydroxy-1,10-phenanthroline remained following hydrolysis in ten percent potassium hydroxide. If the carbethoxy compound remained unchanged during the decarboxylation step, a molecular weight greater than 212 (calculated for 4,7-dihydroxy-1,10-phenanthroline) would be obtained on titration. The molecular weight calculated for the unrecrystallized material was 217. The low molecular weight (208) obtained for the recrystallized material indicated the presence of an acidic impurity, probably introduced by hydrolysis of the carbethoxy compound in hot, concentrated hydrochloric acid.

The work described below was performed in the hope that complete hydrolysis of 3,8-dicarbethoxy-4,7-dihydroxy-1,10-phenanthroline would occur in hot, concentrated hydrochloric acid, ultimately yielding a product of 4,7-dihydroxy-1,10-phenanthroline free of a carboxy impurity.

1. Experimental work

A mixture of 2.35 g. of 3,8-dicarbethoxy-4,7-dihydroxy-1,10-phenanthroline and 100 ml. of concentrated hydrochloric acid was stirred magnetically and heated to boiling in a 400-ml. beaker. The solid dissolved after a few minutes. The solution was evaporated to 50 ml. whereupon an off-white suspension was obtained which did not dissolve upon addition

of more hydrochloric acid. Heating was continued for two hours, and the mixture was allowed to cool while stirring overnight. The solid was collected on a medium porosity sintered glass funnel and washed several times with water. The filter cake was slurried with water and filtered again. After three more washings with water, the wet solid was placed in a vacuum oven at 75° for two hours. The off-white product weighed 1.72 g., 87 percent yield.

The product resulting from the acid hydrolysis was heated in an electric muffle at 310°-320° for two hours. This decarboxylated material, 4,7-dihydroxy-1,10-phenanthroline, was titrated as described in the preceding section.

2. Results and discussion

The molecular weight of the 4,7-dihydroxy-1,10-phenanthroline resulting from hydrolysis of the dicarbethoxy compound in concentrated hydrochloric acid and subsequent decarboxylation at 310° was determined by alkalimetric titration to be 222. This represents an even greater nonacidic impurity than was found in the product prepared by the original method of Snyder and Freier (66) in which the 3,8-dicarbethoxy-4,7-dihydroxy-1,10-phenanthroline is hydrolyzed in ten percent potassium hydroxide.

Although a purer product was not obtained, hydrolysis of 3,8-dicarbethoxy-4,7-dihydroxy-1,10-phenanthroline in concentrated hydrochloric acid is superior to hydrolysis

in ten percent sodium hydroxide, because fewer steps are involved, the procedure is less time-consuming, and the product is more easily separated from the reaction medium.

C. NMR Spectrum of 4,7-Dihydroxy-1,10-phenanthroline

1. Experimental work

Spectra were obtained using an Hitachi Perkin-Elmer R-20B High Resolution NMR Spectrometer, a 60-megahertz instrument.

The spectrum of 4,7-dihydroxy-1,10-phenanthroline was run in a solution of sodium deuteroxide in deuterium oxide plus dimethylsulfoxide-D_6. Sodium-2,2-dimethyl-2-silapentane-5-sulphonate (Tier's salt) was used as an internal reference.

2. Results and discussion

The spectra of 1,10-phenanthroline, 2,9-dimethyl-1,10-phenanthroline and 4,7-dihydroxy-1,10-phenanthroline are shown in Figure 3. Peak assignments and coupling constants are tabulated in Table III-2. That the absorption pattern centered at 9.22 ppm in the spectrum of 1,10-phenanthroline disappears in the spectrum of 2,9-dimethyl-1,10-phenanthroline, while the positions of the other peaks remain essentially unchanged, is good evidence for assigning

Figure III-3. NMR spectra of three phenanthrolines

From top to bottom:

1,10-phenanthroline

2,9-dimethyl-1,10-phenanthroline

4,7-dihydroxy-1,10-phenanthroline

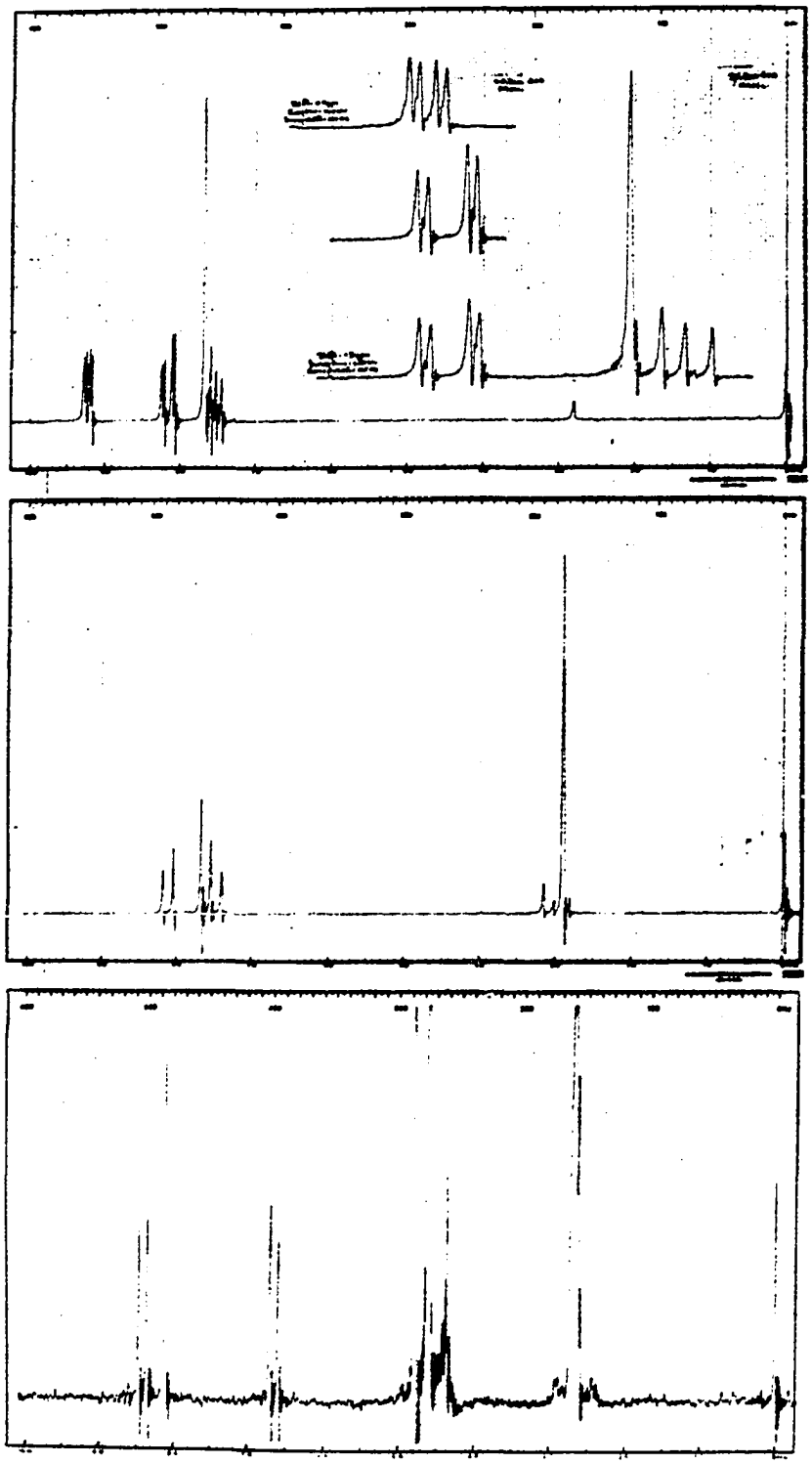


Table III-2. NMR absorption of three phenanthrolines

Compound	Chemical Shift, δ (ppm)	Pattern[a]	Assignment	Coupling Constants (Hz)
1,10-Phenanthroline	7.55	AMX	$H_{3,8}$	$J_{2,3} = J_{8,9} = 4.3$
	7.67	singlet	$H_{5,6}$	$J_{3,4} = J_{7,8} = 7.8$
	8.16	AMX	$H_{4,7}$	$J_{2,4} = J_{7,9} = 1.7$
	9.22	AMX	$H_{2,9}$	
2,9-Dimethyl-1,10-phenanthroline	2.90	singlet	methyl	$J_{3,4} = J_{7,8} = 8$
	7.46	AB	$H_{3,8}$	
	7.67	singlet	$H_{5,6}$	
	8.11	AB	$H_{4,7}$	
4,7-Dihydroxy-1,10-phenanthroline	6.64	AB	$H_{3,8}$	$J_{2,3} = J_{8,9} = 6$
	8.10	singlet	$H_{5,6}$	
	8.41	AB	$H_{2,9}$	

[a]Pattern designations defined by R. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds", John Wiley and Sons, New York, 2nd Edition, 1967. Page 120.

the peak at 9.22 ppm to the protons at positions 2 and 9. Corsini, Louch, and Thompson have also found that protons ortho to the heterocyclic nitrogen in 8-hydroxyquinolines absorb in this region (19). Absorption by protons at positions 2,9- and 3,8- is shifted upfield by about 0.9 ppm in 4,7-dihydroxy-1,10-phenanthroline relative to their positions in 1,10-phenanthroline and 2,9-dimethyl-1,10-phenanthroline.

D. Conclusions

The alkalimetric titration of 4,7-dihydroxy-1,10-phenanthroline has been improved by performing the titration in a nonaqueous solvent mixture of 2-propanol and dimethylsulfoxide. The author doubts, however, that the preparation of solutions of standard acid and base in nonaqueous solvents and the use of a modified reference electrode is justified by the slight increase in accuracy which is gained by performing the titration in a nonaqueous solvent. Titrations of 4,7-dihydroxy-1,10-phenanthroline before and after recrystallization from concentrated hydrochloric acid showed the presence of an impurity in the final product. An attempt to improve the purity of 4,7-dihydroxy-1,10-phenanthroline by performing the hydrolysis of 3,8-dicarbethoxy-4,7-dihydroxy-1,10-phenanthroline in concentrated hydrochloric acid proved unsuccessful. By comparing the NMR

spectrum of 4,7-dihydroxy-1,10-phenanthroline with the spectra of 1,10-phenanthroline and 2,9-dimethyl-1,10-phenanthroline peak assignments have been made, and the structure of 4,7-dihydroxy-1,10-phenanthroline has been substantiated by NMR evidence.

IV. ULTRAVIOLET ABSORPTION SPECTRUM AND THIRD ACID DISSOCIATION CONSTANT OF 4,7-DIHYDROXY-1,10-PHENANTHROLINE

Value for the acid dissociation constants of 4,7-dihydroxy-1,10-phenanthroline in 50:50 water:dioxane solutions were reported by James and Williams (42). These authors evidently did not determine the constants themselves, but refer the reader to another paper, by Pflaum and Brant, for the original measurements (53). No mention of 4,7-dihydroxy-1,10-phenanthroline is made in the latter paper, and the origin of the values reported by James and Williams remains unknown.

In this section the effect of pH on the ultraviolet absorption spectrum of 4,7-dihydroxy-1,10-phenanthroline in aqueous solutions is presented, and a new value for the third acid dissociation constant, designated as pK_{NH^+} by James and Williams, is estimated from ultraviolet absorption data.

A. Experimental Work

1. Preparation of solutions and recording of spectra

A stock solution of 5.00×10^{-4} M 4,7-dihydroxy-1,10-phenanthroline was prepared by dissolving 0.1116 g. of the hydrochloride, 95 percent 4,7-dihydroxy-1,10-phenanthroline,

in water containing 10 ml. of 0.10 M sodium hydroxide and 74.6 g. of potassium chloride, and diluting to one liter to give an ionic strength equal to one.

Buffer solutions from pH 9.1 to 11.5 were prepared by adding 1.0 M sodium hydroxide to 1.0 M ammonium chloride until the desired pH was obtained. Measurements of pH were made with a Beckman Zeromatic SS-3 pH meter in conjunction with a Corning 472022 high-alkalinity glass electrode and a saturated calomel electrode. Calibration was made against NBS buffers of pH 6.86, 9.18 and 12.45.

Absorption spectra were obtained with a Cary 14 recording spectrophotometer. Individual absorbance measurements were made with a Beckman DU or Beckman DU-2 quartz spectrophotometer. Cuvettes of fused silica with a 1.00-cm. path length were used.

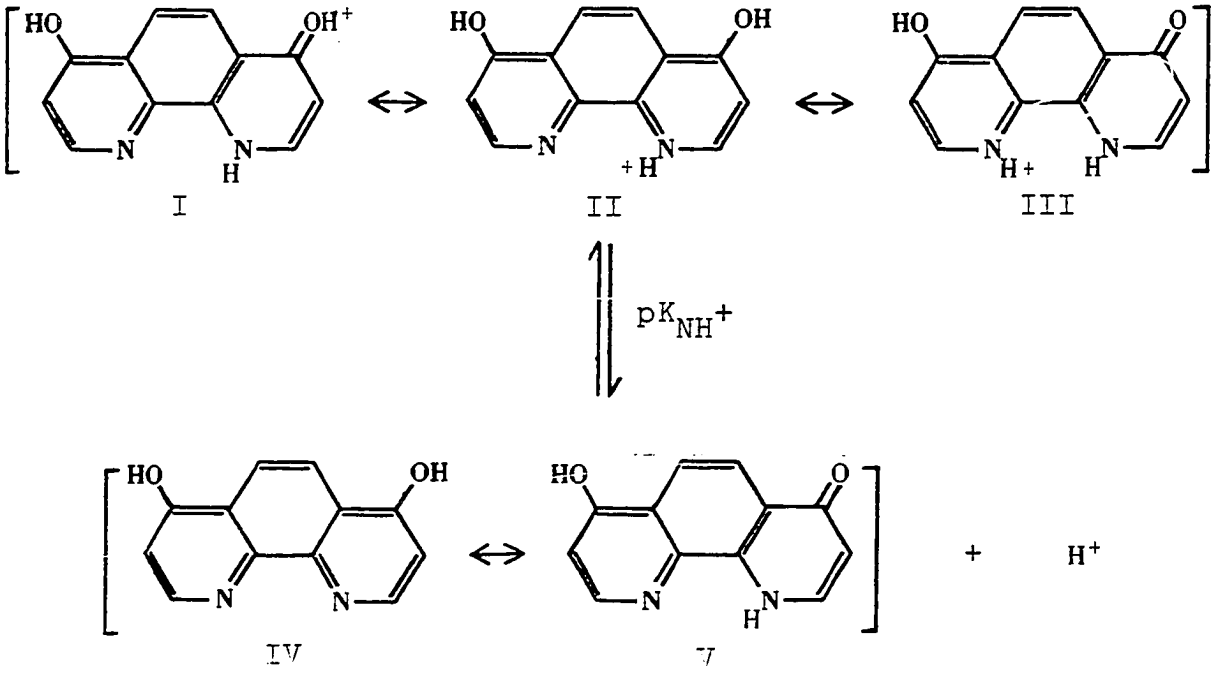
2. Ultraviolet absorption of 4,7-dihydroxy-1,10-phenanthroline as a function of pH

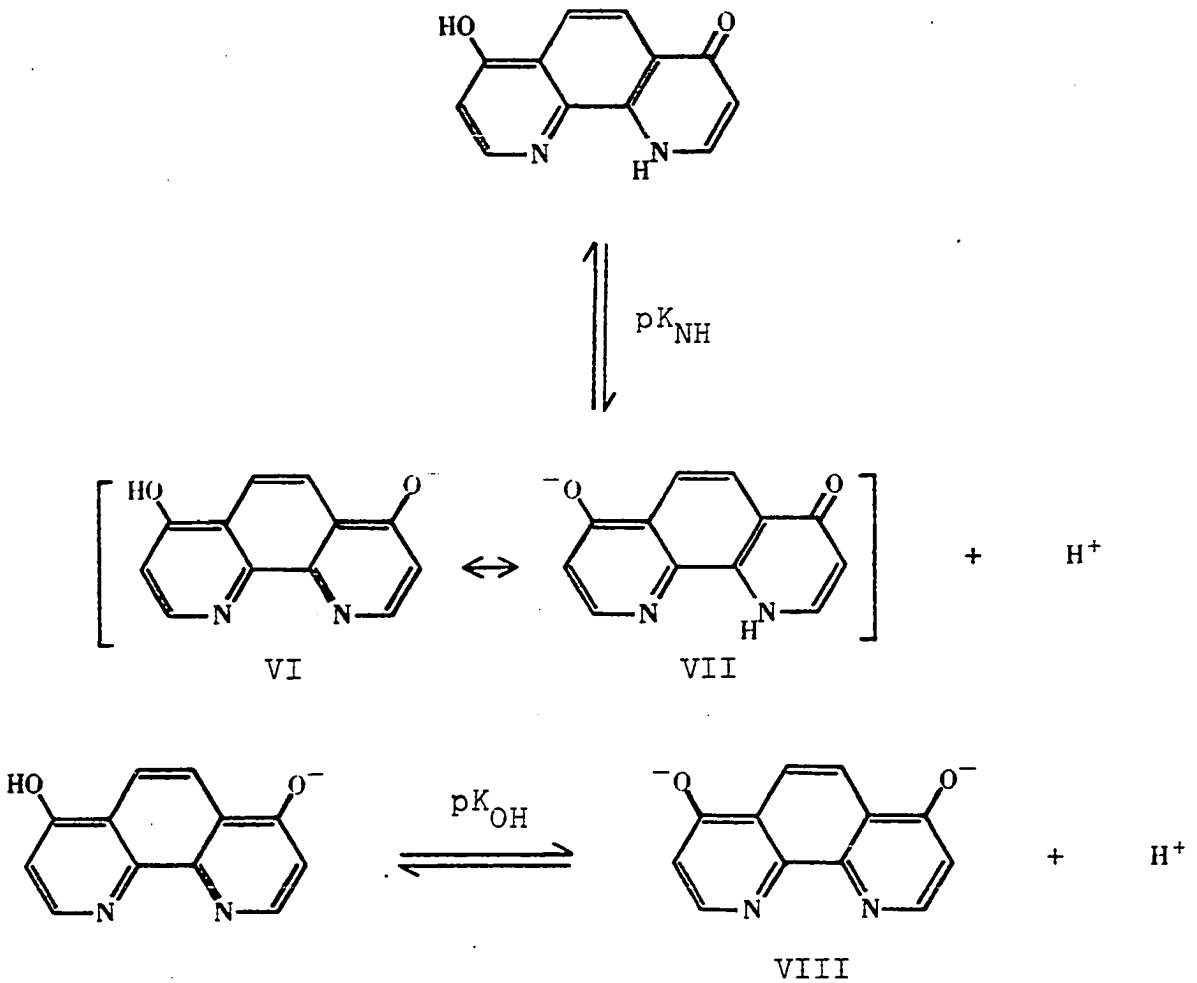
To each of thirteen 50-ml. volumetric flasks was added 5.00 ml. of 5.00×10^{-4} M 4,7-dihydroxy-1,10-phenanthroline, 10 ml. of buffer solution or the necessary volume of sodium hydroxide to adjust the pH to the desired value, and 1.0 M potassium chloride to a volume of 50.00 ml. The ultraviolet absorption spectrum of each solution was run between 250 and 400 nm. against a blank of distilled water. The pH of each solution was measured after the spectra were recorded.

B. Results and Discussion

1. Acid and base forms of 4,7-dihydroxy-1,10-phenanthroline

The acid dissociation constants of 4,7-dihydroxy-1,10-phenanthroline reported by James and Williams were: pK_{OH} = 2.55 ± 0.05 and 7.28 ± 0.03, pK_{NH^+} = 11.5 ± 0.1. The pK value for the immonium proton is much higher than any previously reported for a substituted 1,10-phenanthroline (63, 59, p. 43). The acidic and basic forms of 4,7-dihydroxy-1,10-phenanthroline may be represented as





Of the structures I, II and III, structures II and III appear more likely inasmuch as the positive charge on a ring nitrogen atom allows for better dissipation of the charge in the resonating electron system. Further, structure II is preferred over structure III because of the electrostatic repulsion developed by protonating both nitrogen atoms simultaneously.

Structure V has been shown to be the actual structure of the neutral compound by interpretation of infrared spectra (29,65).

In Part V of this dissertation it is shown that the ultraviolet spectrum of 4,7-dihydroxy-1,10-phenanthroline below 400 nm. is almost identical to the spectrum of the iron(III) derivative in the pH range 9.1 to 5 M sodium hydroxide. One proton dissociates over this pH region. The existence of VII in the iron derivative is highly unlikely, therefore, the proton corresponding to the third acid dissociation constant must be the hydroxy proton in structure VI.

In view of these observations, the designation of the pK_a values reported by James and Williams might be better written as $pK_{NH^+} = 2.55 \pm 0.05$, $pK_{NH} = 7.28 \pm 0.03$, and $pK_{OH} = 11.5 \pm 0.1$.

2. Third acid dissociation constant of 4,7-dihydroxy-1,10-phenanthroline

The ultraviolet absorption spectrum between 250 and 400 nm. of 4,7-dihydroxy-1,10-phenanthroline is shown in Figure IV-1. The two isosbestic points, at λ = 274 nm. and 347 nm., fall at the same pH indicating the existence of only two distinct absorbing species in solution. The two species most likely correspond to the mono- and binegative anions, structures VI and VIII in the preceding section.

For a value for the third acid dissociation constant, pK_{OH} can be taken as the pH of a solution which would produce an absorbance midway between that of the acidic form and that

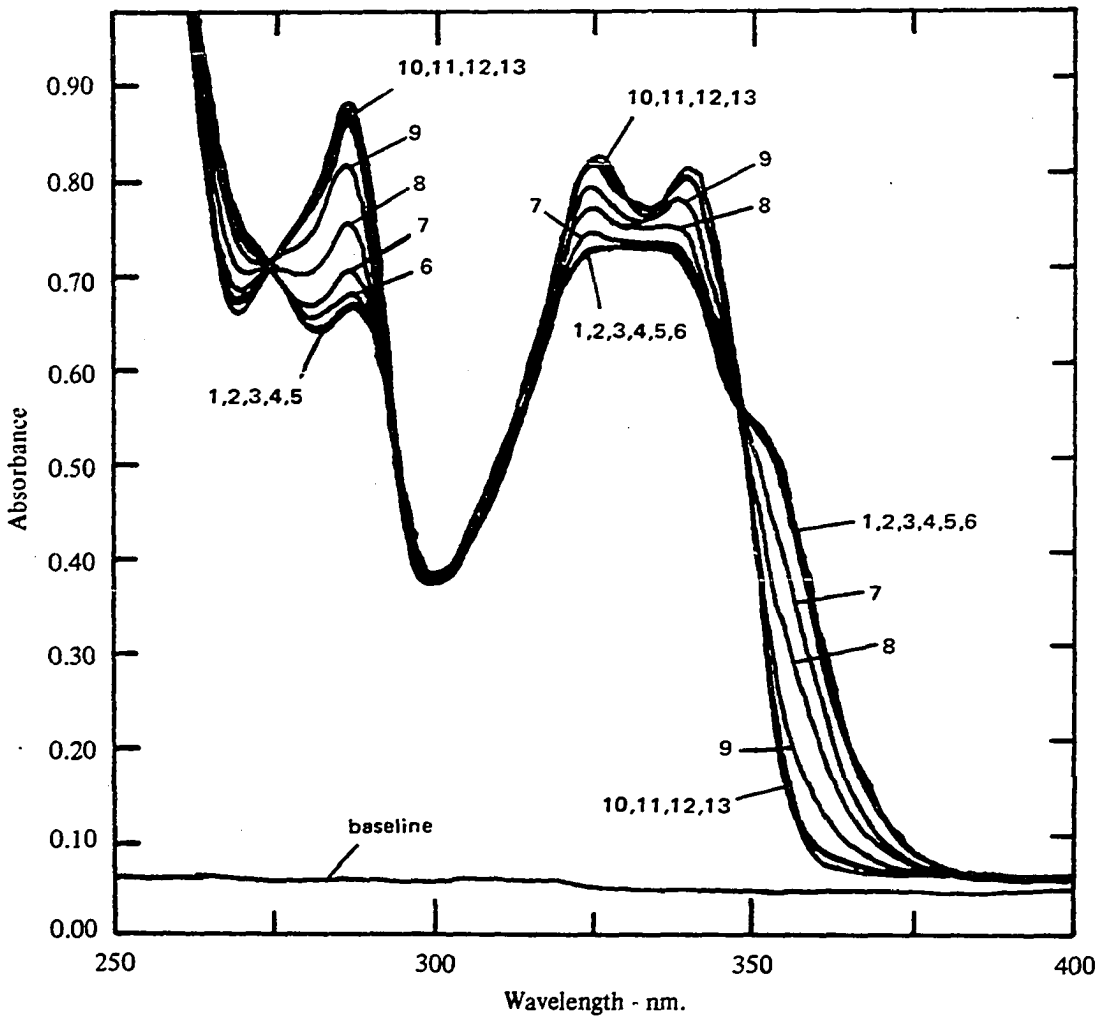
Figure IV-1. Effect of pH on the ultraviolet absorption spectrum of 4,7-dihydroxy-1,10-phenanthroline

Concentration of 4,7-dihydroxy-1,10-phenanthroline:

5.00×10^{-5} M

Ionic strength: 1.0

Curve	pH or [NaOH]
1	9.10
2	9.59
3	10.12
4	10.62
5	11.08
6	11.30
7	12.02
8	12.55
9	13.05
10	13.66
11	1.0 M NaOH
12	2.0 M NaOH
13	5.0 M NaOH


0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Absorbance
250
300
350
400
Wavelength - nm.
10,11,12,13
9
8
7
6
1,2,3,4,5
10,11,12,13
9
8
7
1,2,3,4,5,6
1,2,3,4,5,6
7
8
9
10,11,12,13
baseline

of the basic form. The greatest change in absorbance on passing from the acidic form to the basic form occurs at λ = 286 nm. By visual estimation at this wavelength the mid-point falls at pH = 12.7, that is pK_{OH} = 12.7. This value is believed to be accurate to within 0.2 pK units, and is referred to aqueous solutions of 4,7-dihydroxy-1,10-phenanthroline having an ionic strength equal to one and a temperature of approximately 25°.

C. Summary

Over the range of alkalinity, pH 9.1 to 5.0 M sodium hydroxide, over which 4,7-dihydroxy-1,10-phenanthroline is appreciably soluble, the forms in which 4,7-dihydroxy-1,10-phenanthroline is present in solutions are the mononegative anion (VI) and the binegative anion (VIII). The value of the third acid dissociation constant corresponding to the interconversion of these two forms, as determined from the ultraviolet absorption spectrum recorded as a function of pH, is $pK_{OH} = 12.7 \pm 0.2$.

V. ATTEMPTED PREPARATION OF THE IRON(II) DERIVATIVE OF 4,7-DIHYDROXY-1,10-PHENANTHROLINE IN SOLID FORM. DETERMINATION OF 4,7-DIHYDROXY-1,10-PHENANTHROLINE IN THE PRESENCE OF IRON

In the course of his work for the M.S. degree, Poe (54) prepared iron derivatives of 4,7-dihydroxy-1,10-phenanthroline in solid form for use in the preparation of ion selective membrane electrodes. The preparation of the iron compounds presented unexpected difficulties, pure products were not obtained and the materials were not properly characterized. In the present work, two further attempts were made to prepare the iron(II) derivative of 4,7-dihydroxy-1,10-phenanthroline in solid form. Satisfactory preparations were not obtained but in the course of the work, a method was developed for the determination of the iron in such compounds and also a method was developed for the determination of 4,7-dihydroxy-1,10-phenanthroline in the presence of iron.

A. Experimental Work

1. Iron(II) derivative of 4,7-dihydroxy-1,10-phenanthroline in solid form

a. Preparation 1 An amount of 4,7-dihydroxy-1,10-phenanthroline weighing 0.607 g. (2.72 mmoles) was dissolved in 80 ml. of 0.1 N sodium hydroxide in the cathodic compartment of an electrolytic cell. Oxygen was purged from

the solution by a stream of nitrogen and then 0.348 g. (0.89 mmole) of ferrous ammonium sulfate, dissolved in 5 ml. of water, was added. Any iron(III) present was reduced at a platinum cathode using a 12-volt battery charger as power source. The dark-red solution was drained into a 30-ml. medium-porosity, Pyrex Büchner funnel and mixed with deaerated dilute sulfuric acid. The precipitate was filtered off and dried under a stream of nitrogen. The product was stored under reduced pressure over anhydrous magnesium perchlorate.

b. Preparation 2 A sample of 4,7-dihydroxy-1,10-phenanthroline weighing 0.508 g. was dissolved in 50.00 ml. of 0.1007 N sodium hydroxide. The resulting solution was transferred to a specially designed 125-ml. conical flask to permit formation of the iron derivative and filtration of a precipitate in an inert atmosphere. Oxygen was purged from the solution by passage of a stream of nitrogen. A stoichiometric amount of ferrous ammonium sulfate hexahydrate, 0.296 g., was dissolved in 15 ml. of water and added to the 4,7-dihydroxy-1,10-phenanthroline. The resulting red solution was heated at 90° for one hour. The solution was cooled in an ice bath and 21.5 ml. of 0.1942 N perchloric acid was added. The resulting red precipitate was filtered off, washed with water, and allowed to dry under a steam of

nitrogen. The entire apparatus was placed in a vacuum desiccator over anhydrous magnesium perchlorate. The product was a microcrystalline brick-red solid.

2. Determination of iron in solid iron(II)-4,7-dihydroxy-1,10-phenanthroline preparations

The amount of iron present in Preparations 1 and 2 was determined by a modification of the method of Schilt, Smith, and Heimbuch (60). Samples of the solid iron derivatives weighing 10 to 20 mg. (weighed on a micro balance) were dissolved in alkaline solutions containing 10 ml. of 10 M sodium hydroxide (iron-free, preparation described in Part IX), 2 ml. of 0.01 M 4,7-dihydroxy-1,10-phenanthroline and 4 ml. of sodium hydrosulfite (200 mg./ml.) were added, and the mixture was diluted to 50.00 ml. with deionized water. These solutions were diluted by adding 5.00-ml. aliquots to 9 ml. of 10 M sodium hydroxide, 2 ml. of 4,7-dihydroxy-1,10-phenanthroline, and 4 ml. of sodium hydrosulfite (200 mg./ml.), and diluting to 50.00 ml. with deionized water. A series of standards containing known amounts of iron were prepared containing the same amounts of reagents. Absorbance measurements were made at 520 nm. with a Beckman DU quartz spectrophotometer using 10-mm. cuvettes.

3. Determination of 4,7-dihydroxy-1,10-phenanthroline in the presence of iron

a. Preparation of solutions and recording of spectra

Preparation of the various solutions required (5.00×10^{-4} M 4,7-dihydroxy-1,10-phenanthroline and buffers) the measurement of pH, recording of spectra, and measurement of absorbance accurately, are described in Part IV, Experimental Work.

A solution of ferric perchlorate 1.000×10^{-2} M was prepared by dissolving 0.5591 g. of electrolytic iron in 20 ml. of 72 percent perchloric acid and diluting to 1,001.1 ml. with deionized water. All standard solutions of iron of lower concentrations were prepared by diluting aliquots of this standard solution and were used on the same day as prepared.

b. Ultraviolet absorption spectrum of 4,7-dihydroxy-1,10-phenanthroline in the presence and absence of iron

Two sets of six solutions containing 5.00×10^{-5} M 4,7-dihydroxy-1,10-phenanthroline and 1.67×10^{-5} M ferric perchlorate were prepared at each pH unit from 9 to 14. One set of six solutions contained 2.0 ml. of sodium hydrosulfite (200 mg./ml.) per 50 ml. The ultraviolet absorption spectra of the solutions containing no hydrosulfite were similar to the spectra of 4,7-dihydroxy-1,10-phenanthroline at corresponding values of pH, Figure IV-1. Absorbance of

the solutions containing hydrosulfite was greater than 2 from 250 to 380 nm., and the absorption spectra were not obtained.

A series of five solutions were prepared containing 5.00×10^{-5} M 4,7-dihydroxy-1,10-phenanthroline and 0.1×10^{-5} M ferric perchlorate. The pH of each solution was adjusted to 9.5. The ultraviolet absorption spectra are shown in Figure V-1.

In an effort to find a wavelength at which the free reagent and the iron derivative exhibit the same molar absorptivity, i.e., an isosbestic point, two sets of five solutions were prepared containing varying amounts of 4,7-dihydroxy-1,10-phenanthroline. One set of solutions contained 1.00×10^{-5} M ferric perchlorate. The ultraviolet absorption spectra are shown in Figure V-2.

For the determination of 4,7-dihydroxy-1,10-phenanthroline in the solid iron derivatives, 15-to-20 mg. samples of the iron derivatives were weighed to the nearest 0.1 mg., dissolved in buffer of pH 2.5, and diluted to 100 ml. with the same buffer. Dilutions of the resulting solutions were made by diluting 5.00-ml. aliquots to 50.00 ml. with 1.0 M potassium chloride. The absorbance of these solutions at 327 nm. being too high, 15.00-ml. aliquots plus 5 ml. of pH 9.5 buffer were diluted to 25.00 ml. with 1.0 M potassium chloride. The absorbances of solutions 6-10, Figure V-2, were used to plot a standard curve, which appears in Figure V-3.

Figure V-1. Ultraviolet absorption spectra of solutions containing 4,7-dihydroxy-1,10-phenanthroline and varying amounts of iron(III)

Concentration of 4,7-dihydroxy-1,10-phenanthroline: 5.00×10^{-5} M

pH: 9.5

Ionic strength: 1.0

Curve	Concentration of iron(III)
1	0.10×10^{-5} M
2	0.50×10^{-5} M
3	1.00×10^{-5} M
4	2.00×10^{-5} M
5	5.00×10^{-5} M

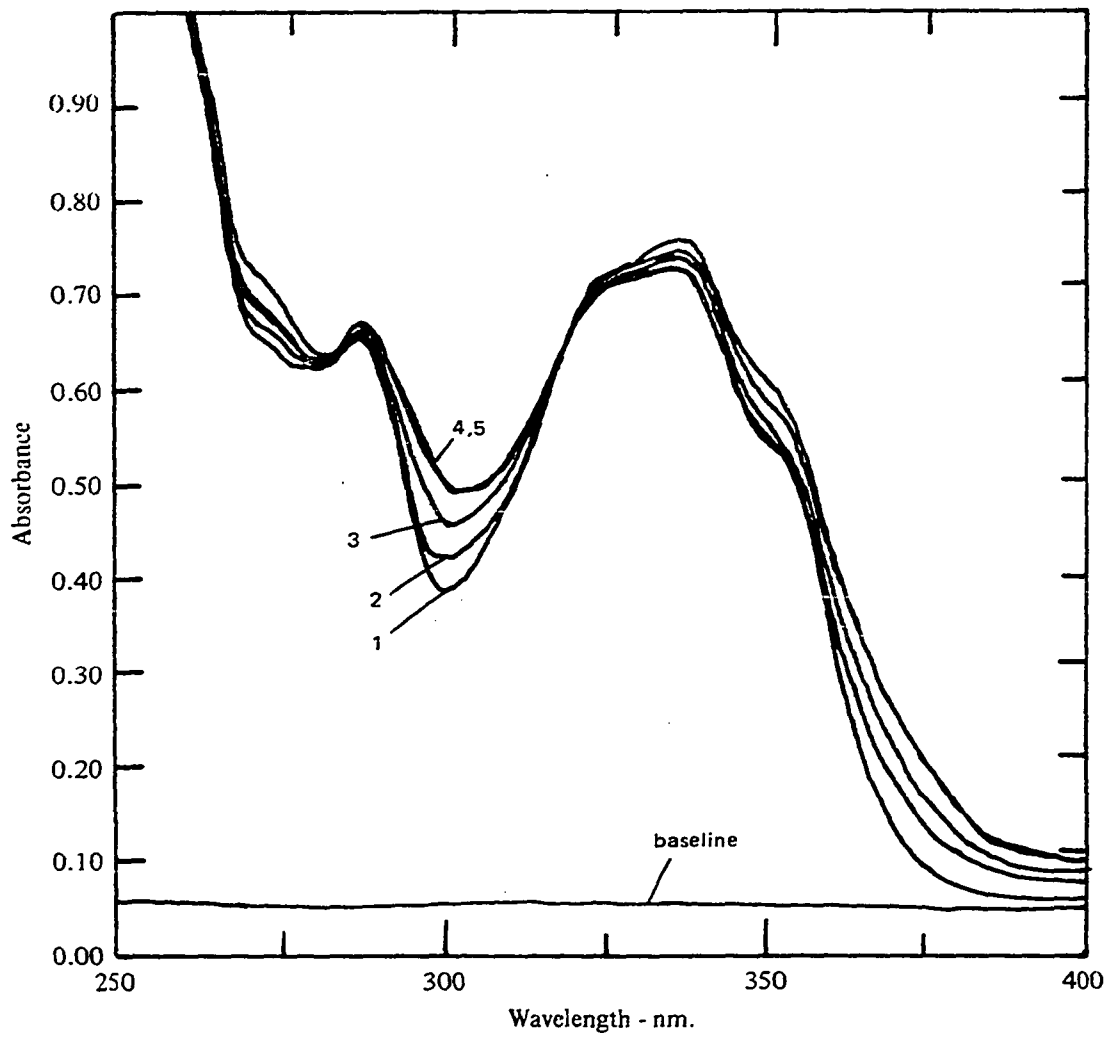

0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Absorbance
4,5
3
2
1
baseline
250
300
350
400
Wavelength - nm.

Figure V-2. Ultraviolet absorption spectra of 4,7-dihydroxy-1,10-phenanthroline in the presence and absence of iron(III)

Ionic strength: 1.0

pH: 9.5

Curve	Concentration of 4,7-diOHphen	Concentration of iron(III)
1	1.00×10^{-5} M	
2	2.00×10^{-5} M	
3	3.00×10^{-5} M	
4	4.00×10^{-5} M	
5	5.00×10^{-5} M	
6	1.00×10^{-5} M	1.00×10^{-5} M
7	2.00×10^{-5} M	1.00×10^{-5} M
8	3.00×10^{-5} M	1.00×10^{-5} M
9	4.00×10^{-5} M	1.00×10^{-5} M
10	5.00×10^{-5} M	1.00×10^{-5} M

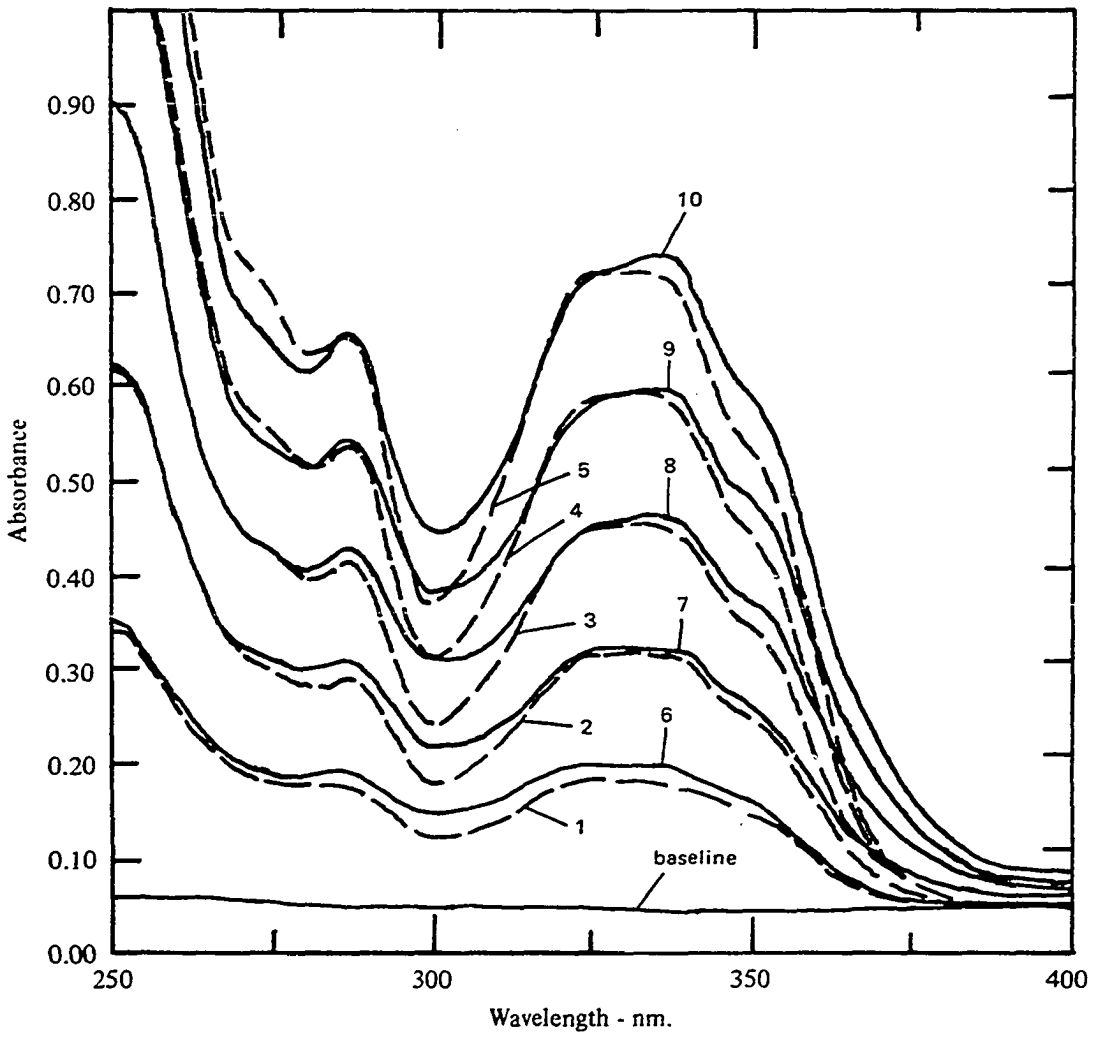

0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Absorbance
250
300
350
400
Wavelength - nm.
10
9
8
5
4
3
7
2
6
1
baseline

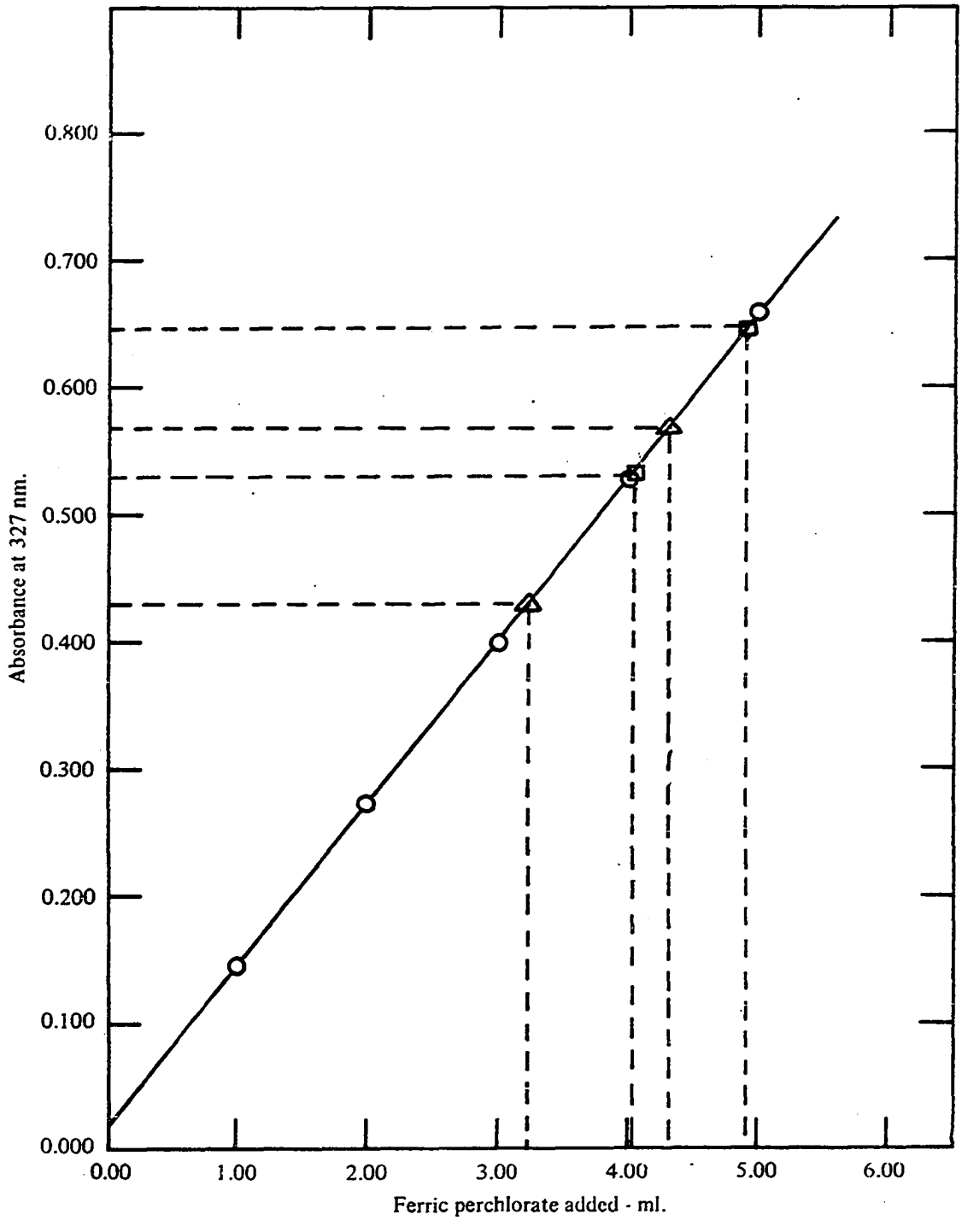
Figure V-3. Calibration curve for the determination of 4,7-dihydroxy-1,10-phenanthroline

Concentration of ferric perchlorate: 1.00×10^{-4} M

○ - Standard solutions

△ - Preparation 1

□ - Preparation 2


0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Absorbance at 327 nm.
0.00
1.00
2.00
3.00
4.00
5.00
6.00
Ferric perchlorate added - ml.

B. Results and Discussion

The method developed for the determination of iron in the solid preparations of the iron(II)-4,7-dihydroxy-1,10-phenanthroline compound was a straight forward application of the earlier use of 4,7-dihydroxy-1,10-phenanthroline for the determination of iron in strong alkalies. No particular difficulties were encountered and the error in the method is probably not greater than 1 to 2 percent, the error in spectrophotometric methods in general.

The determination of 4,7-dihydroxy-1,10-phenanthroline in solutions obtained by dissolving the solid preparation in alkali, that is, in the presence of iron, is based on the observation that at certain wavelengths in the ultraviolet the absorption of 4,7-dihydroxy-1,10-phenanthroline is independent of the form in which it is present, that is, whether or not bound to iron. As will be seen from an examination of the spectra presented in Figure V-2, the ultraviolet absorption spectra of 4,7-dihydroxy-1,10-phenanthroline in the presence and in the absence of iron(III) are almost identical. The ultraviolet absorption spectra in the presence of iron(II) was not studied because of the overwhelming absorption in the ultraviolet by hydrosulfite, the presence of hydrosulfite being necessary to insure the retention of the iron in the ferrous form.

The pH of 9.5 was chosen for the determination of 4,7-dihydroxy-1,10-phenanthroline because the broad absorption band centered at 332 nm. remained unchanged between pH 9 and 10. Variation of the amount of iron present in a solution of 4,7-dihydroxy-1,10-phenanthroline produced isosbestic points at λ = 284 nm. and 325-330 nm. (Figure V-1). By varying the concentration of 4,7-dihydroxy-1,10-phenanthroline with and without iron, it was found that the isosbestic point around 327 nm. was reproducible, except in solutions in which the concentration of 4,7-dihydroxy-1,10-phenanthroline was less than 3×10^{-5} M; these solutions showed no isosbestic point (Figure V-2).

The absorbance at 327 nm. of a solution of 4,7-dihydroxy-1,10-phenanthroline (3×10^{-5} to 5×10^{-5} M) buffered at pH 9.5 therefore is independent of the amount of iron(III) present, and these conditions were used for the determination of 4,7-dihydroxy-1,10-phenanthroline in the solid iron derivative.

The results obtained in the analyses made on Preparations 1 and 2 of the iron(II)-4,7-dihydroxy-1,10-phenanthroline compound are shown in Table V-1. In neither preparation is the ratio of iron to phenanthroline even close to the 1-to-3 ratio expected. A large excess of 4,7-dihydroxy-1,10-phenanthroline is present and it is apparent that the red iron(II) compound was decomposing rapidly to yield the free

Table V-1. Iron and 4,7-dihydroxy-1,10-phenanthroline in the solid iron derivatives

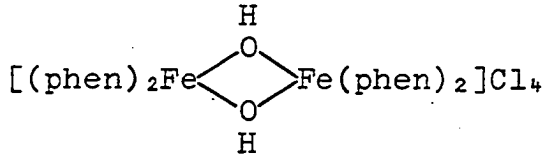
Preparation	Iron, percent	4,7-diOHphen, percent	Moles Fe/moles diOHphen (ave.)[a]
1	3.35 3.30	74.5 74.0	1/5.8
2	2.73 2.75	89.3 91.6	1/8.7

[a]Moles Fe/moles diOHphen = (percent Fe)(212)/(percent diOHphen)(55.85).

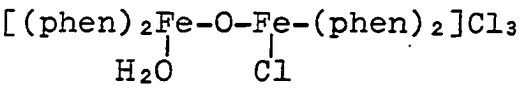
phenanthroline during the acidification made to precipitate the compound. It was observed also that the red, solid, iron(II) compound when heated in contact with acid decomposed to yield a light yellow solid, presumably the free phenanthroline. It has been observed too, by Burgess and Prince (13), in a study of the effect of substituents in the phenanthroline molecule on the acid fission for the iron(II) derivatives, that at 20°, the 4,7-dihydroxy-1,10-phenanthroline compound exhibited the largest first order rate constant for dissociation.

VI. NATURE OF THE OXIDIZED AND REDUCED FORMS OF THE IRON DERIVATIVES OF 4,7-DIHYDROXY-1,10-PHENANTHROLINE

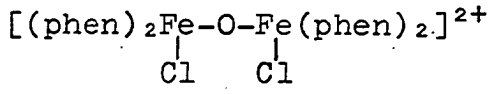
In his first paper on 1,10-phenanthroline, Fritz Blau reported that 1,10-phenanthroline forms a highly colored compound with ferrous iron in which the ratio of phenanthroline to iron is three to one (7). Among other findings, Blau reported that oxidation of tris(1,10-phenanthroline)iron(II) yields a blue compound, tris(1,10-phenanthroline)iron(III), which is unstable except in highly acidic solutions, and that direct addition of 1,10-phenanthroline to salts of iron(III) results not in the blue three-to-one compound, but in a brown compound. Blau reported color reactions which indicated that the ratio of phenanthroline to iron in this brown compound was also three to one. Later workers, however, have firmly established that the brown compound contains two molecules of phenanthroline to one atom of iron. Gaines, Hammett, and Walden (30) isolated the brown solid as the chloride and reported a composition corresponding to the double hydroxy-bridged species I. Later investigators have suggested structures II and III on the basis of magnetic data and infrared spectra. Reduction potential and pH measurements of aqueous solutions favor structure I (43).



I



II



III

The compound 4,7-dihydroxy-1,10-phenanthroline undergoes essentially the same reactions with iron as does 1,10-phenanthroline, with the exception that these reactions occur only in alkaline solution. Both the 3:1 and 2:1 derivatives of iron(III) are observed. Unlike the corresponding derivatives of 1,10-phenanthroline, the 3:1 and 2:1 compounds can be interconverted by adjusting the pH of the solution. Furthermore, the 2:1 compound exists in two different, colored forms depending on pH.

A. Experimental Work

1. Apparatus and chemicals

Absorbance measurements were made on a Beckman DU spectrophotometer. Absorption spectra were recorded on a Cary 14 Recording Spectrophotometer. Spectrosil cuvettes with 1.00-cm. path length were used throughout.

Equipment and methods for the measurement of pH were the same as those described in Part III.

2. Visible absorption spectra of the gray, amber, and purple compounds at various pH

In 10 ml. of 3.16 M sodium hydroxide was dissolved 0.0227 g. of 4,7-dihydroxy-1,10-phenanthroline. After dissolution, 10.5 ml. of 1.00×10^{-2} M ferric perchlorate was added from a graduated cylinder. The excess iron was filtered off as ferric hydroxide, and the filtrate was diluted to 50.00 ml. 5.00-ml. aliquots of this solution were pipeted into a 50-ml. beaker containing 5 ml. of deionized water, the pH was adjusted to values from 6 to 14 with HCl or NaOH, and the resulting solutions were diluted to 25.00 ml. After the visible absorption spectra (Figure VI-1) had been recorded, the pH of each solution was measured. After fourteen days the absorption spectrum of each solution was recorded again (Figure VI-2), and the pH measured again.

The spectra of the iron compound in highly alkaline solutions were also obtained. Into each of five 25-ml. volumetric flasks were added aliquots of 1.0 M or 10.0 M sodium hydroxide, 10.00 ml. of 3.37×10^{-3} M 4,7-dihydroxy-1,10-phenanthroline, and 1.00 ml. of 3.363×10^{-3} M ferric perchlorate. The solutions were diluted to volume and the visible absorption spectra were recorded after one hour

Figure VI-1. Absorption spectra of the amber and purple iron(III) derivatives of 4,7-dihydroxy-1,10-phenanthroline

Curve	pH	Color of solution
7	7.25	purple
8	7.76	purple
9	8.93	purple
10	9.90	---
11	10.67	amber
12	11.90	amber
13	12.90	amber
14	13.80	amber

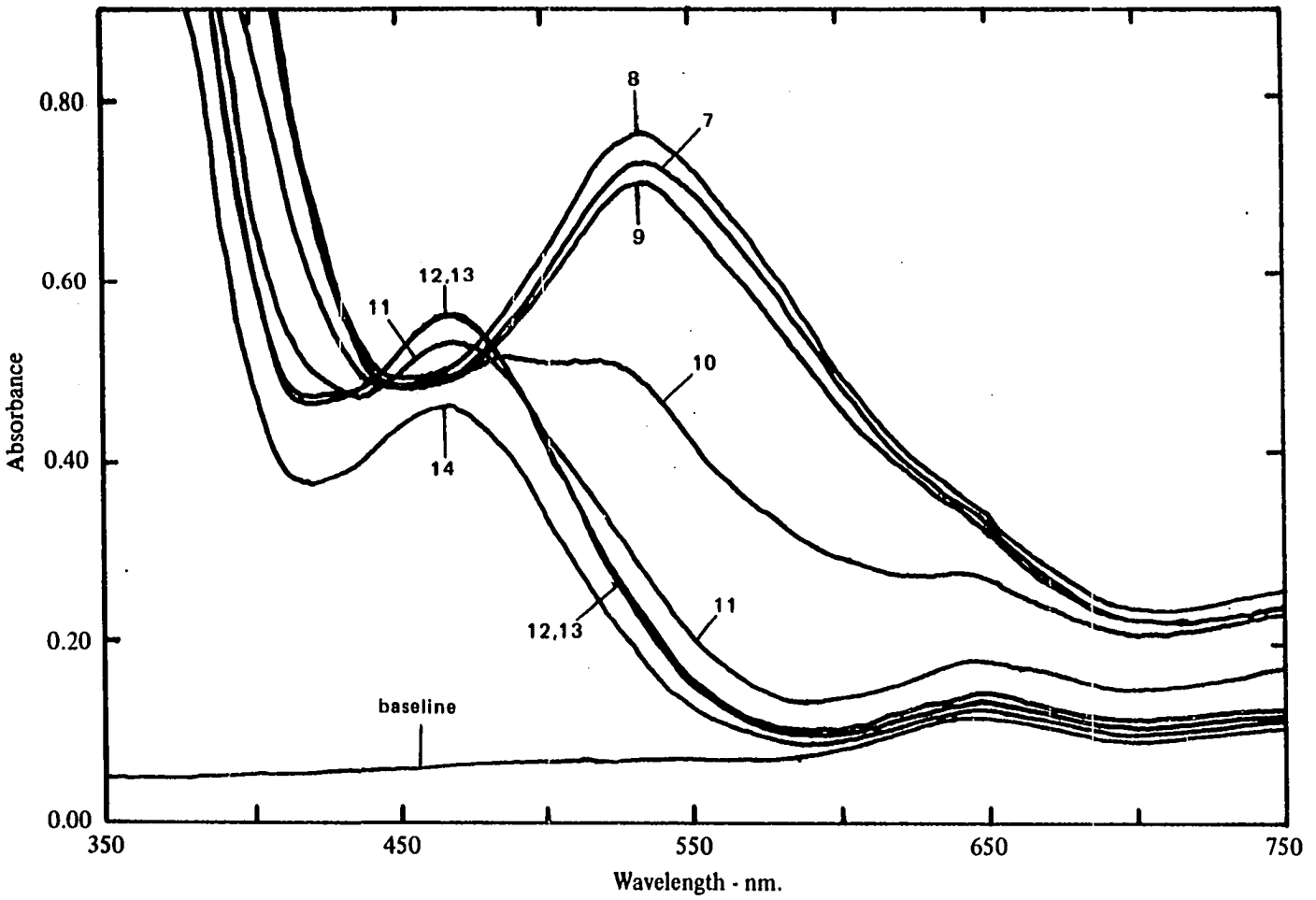
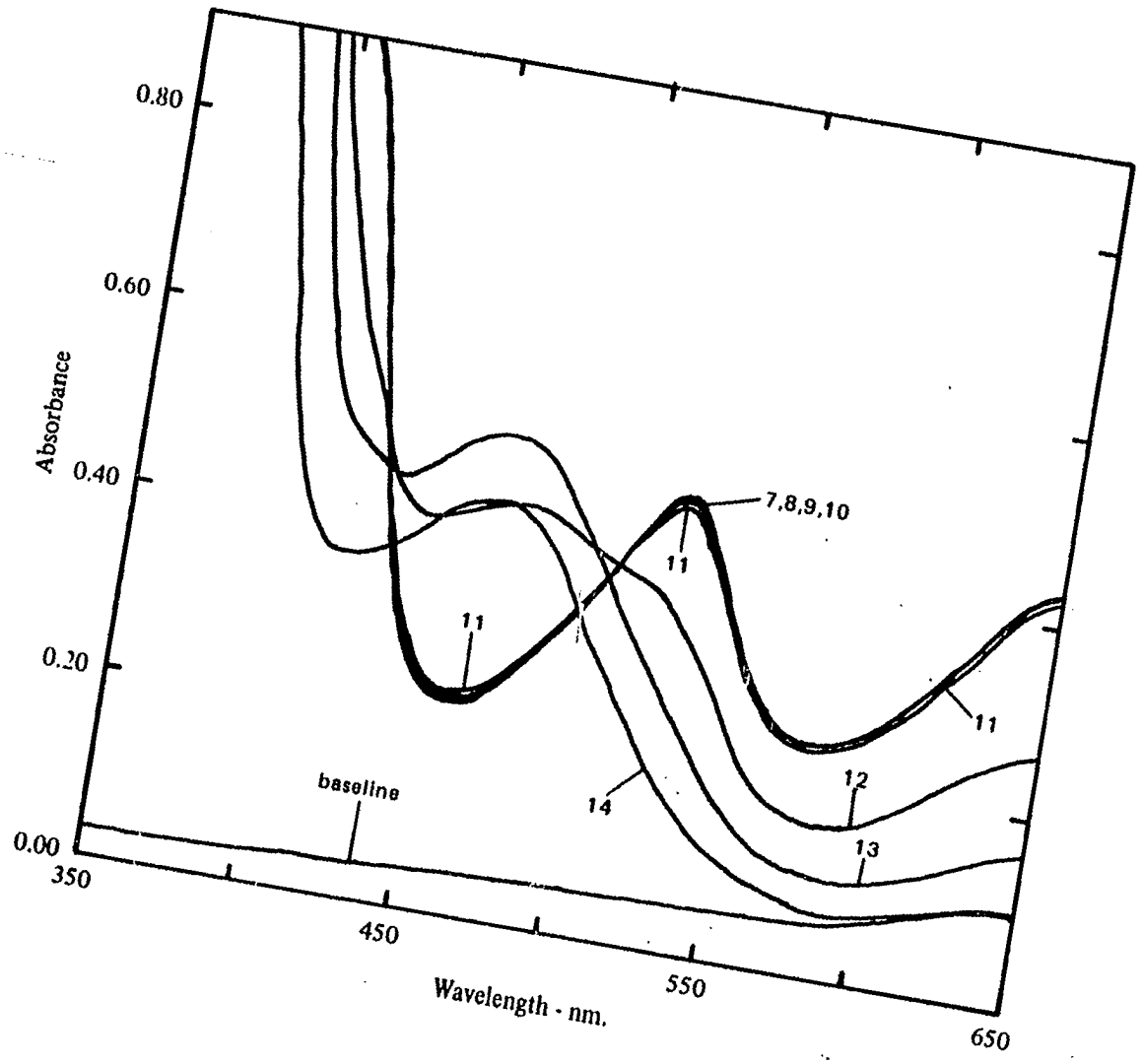

0.80
0.60
0.40
0.20
0.00
Absorbance
350
450
550
650
750
Wavelength - nm.
8
7
9
12,13
11
10
14
11
12,13
baseline

Figure VI-2. Absorption spectra of the amber and gray iron(III) derivatives of 4,7-dihydroxy-1,10-phenanthroline.

Curve	pH	Color of solution
7	7.91	gray
8	7.88	gray
9	8.02	gray
10	8.87	gray
11	10.38	gray
12	11.83	---
13	12.90	amber
14	13.80	amber


0.80
0.60
0.40
0.20
0.00
Absorbance
350
450
550
650
Wavelength - nm.
baseline
7,8,9,10
11
11
11
12
13
14

(Figure VI-3), after one day (Figure VI-4), and again after heating at 80° for two hours.

3. Determination of combining ratios in the gray, amber, and purple compounds

A 3.37×10^{-3} M solution of 4,7-dihydroxy-1,10-phenanthroline was prepared by dissolving 0.3756 g. of the material, 95 percent 4,7-dihydroxy-1,10-phenanthroline, in a minimum amount of 0.1 M sodium hydroxide and diluting to exactly 500 ml.

A 3.363×10^{-3} M solution of ferric perchlorate was prepared by dissolving 0.1878 g. of electrolytic iron in 17 ml. of 72 percent perchloric acid, and diluting to exactly one liter with deionized water. The solutions were delivered from an automatically filling 10-ml. buret.

a. Amber compound To each of nineteen 25-ml. volumetric flasks were added 5 ml. of 1.0 M sodium hydroxide, aliquots of 3.37×10^{-3} M 4,7-dihydroxy-1,10-phenanthroline and 3.363×10^{-3} M ferric perchlorate such that the total volume of phenanthroline and iron solutions in each flask was 10.00 ml. Those solutions containing excess iron, indicated by the presence of a precipitate of ferric hydroxide, were filtered through Whatman No. 41 filter paper. All solutions were diluted to exactly 25 ml. The absorbance of each solution was measured at 470 nm. within several hours after preparation against a blank of pure water.

Figure VI-3. Absorption spectra of the iron(III)-4,7-dihydroxy-1,10-phenanthroline compound in solutions of sodium hydroxide. Spectra obtained one hour after preparation of solutions

Curve	Concentration of sodium hydroxide
1	0.2 M
2	0.5 M
3	1.0 M
4	2.0 M
5	4.0 M

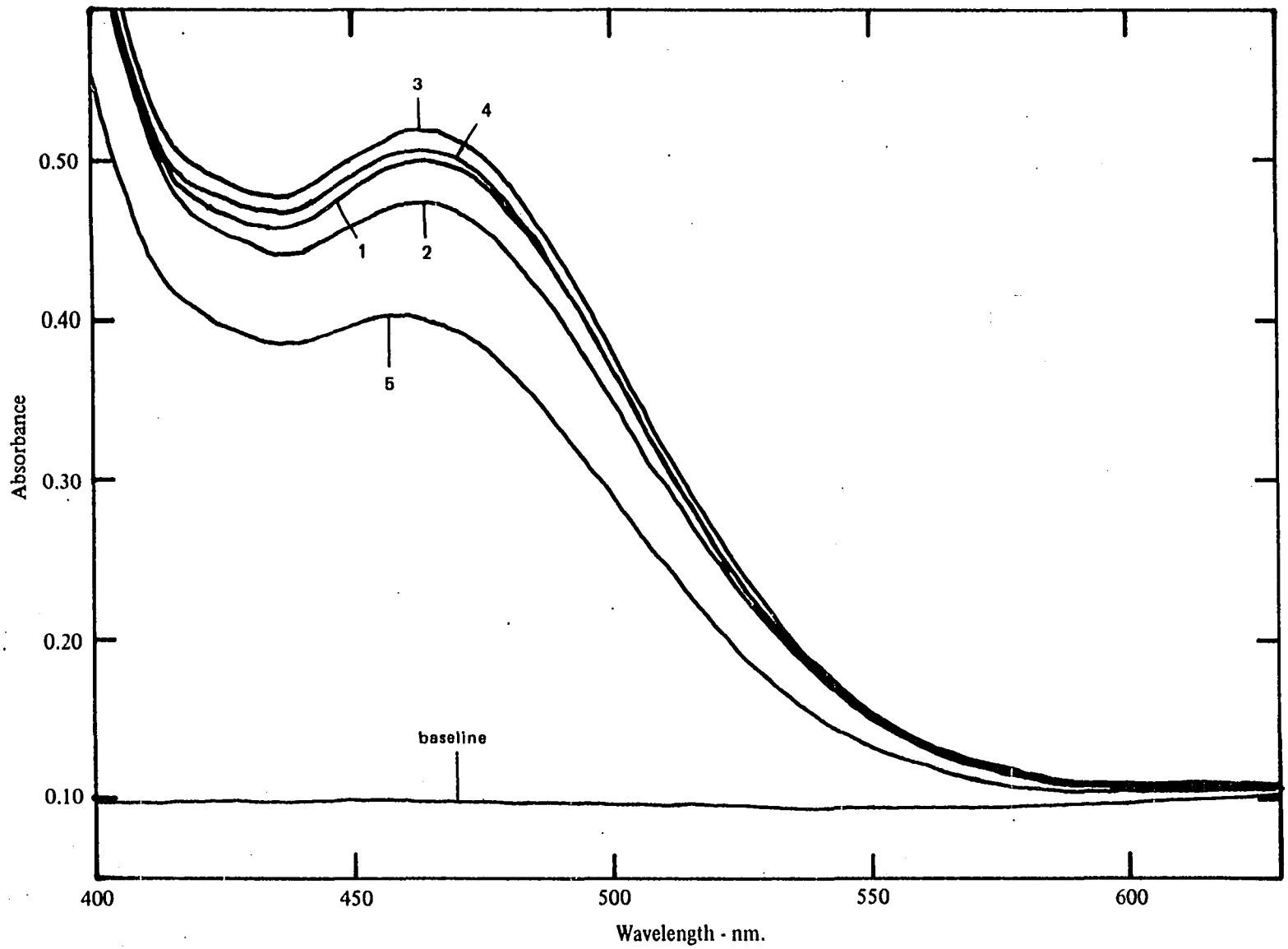
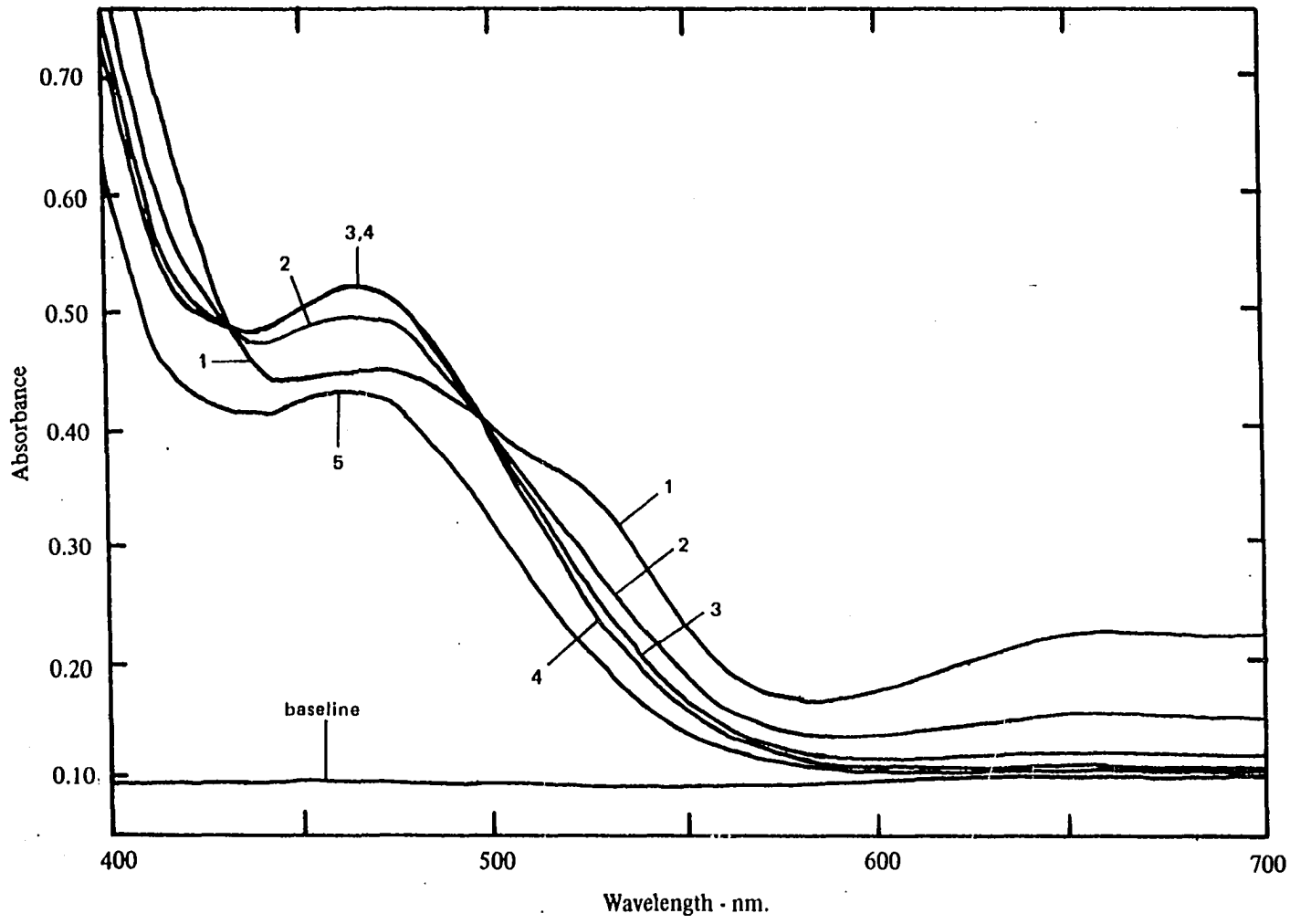

3
4
1
2
5
baseline
0.50
0.40
0.30
0.20
0.10
Absorbance
400
450
500
550
600
Wavelength - nm.

Figure VI-4. Absorption spectra of the iron(III)-4,7-dihydroxy-1,10-phenanthroline compound in solutions of sodium hydroxide. Spectra obtained on day following preparation of solutions

Curve	Concentration of sodium hydroxide
1	0.2 M
2	0.5 M
3	1.0 M
4	2.0 M
5	4.0 M


0.70
0.60
0.50
0.40
0.30
0.20
0.10
Absorbance
3,4
2
1
5
1
2
3
4
baseline
400
500
600
700
Wavelength - nm.

b. Gray compound To each of thirteen 25-ml. volumetric flasks were added 5 ml. of a buffer consisting of 2 M ammonium hydroxide and 2 M ammonium chloride, pH 9.4, aliquots of 3.37×10^{-3} M 4,7-dihydroxy-1,10-phenanthroline, and aliquots of 3.363×10^{-3} M ferric perchlorate, such that the volume of phenanthroline plus iron was equal to 8.00 ml. in each flask. The pH 9.4 buffer solution was tested for iron with 4,7-dihydroxy-1,10-phenanthroline and sodium hydrosulfite. None could be detected. The solutions were diluted to 25 ml. with deionized water, and were then heated in a water bath at 75° for 2 hours. A precipitate formed in all solutions. The absorbance of each solution was measured against a water blank at 526 nm. The liquid was drawn off with a disposable pipet, the precipitate having settled to the bottom of the flask.

c. Purple compound Two liters of a buffer solution containing 76 g. of sodium tetraborate decahydrate and enough hydrochloric acid to give a pH of 8.65 was prepared. To each of fourteen 25-ml. volumetric flasks were added 5 ml. of 5.0 M sodium hydroxide and aliquots of 3.37×10^{-3} M 4,7-dihydroxy-1,10-phenanthroline and 3.363×10^{-3} M ferric perchlorate, such that the total volume of phenanthroline plus iron in each flask was 8.00 ml. The solutions were heated in a hot water bath to promote formation of the iron compound and coagulation of ferric hydroxide. To

each solution was added just enough 10 M hydrochloric acid to cause precipitation of the purple compound. The excess acid was immediately neutralized with dilute sodium hydroxide to yield a purple solution. Then, 5.0 ml. of the borate buffer was added and the solutions were diluted to 25 ml. with deionized water. The solutions were centrifuged if any precipitate (ferric hydroxide) was present, and the absorbance of the supernatant liquid was measured at 533 nm. against a water blank.

4. Acid dissociation constant of the purple compound

Buffer solutions consisting of ammonium hydroxide and ammonium chloride were prepared over the range pH 6.5 to 12.0. A stock solution of the amber 2:1 compound was prepared by dissolving 0.0456 g. (2.04×10^{-4} mole) of 4,7-dihydroxy-1,10-phenanthroline in dilute sodium hydroxide. Twenty milliliters of 1.00×10^{-2} M ferric perchlorate (2.00×10^{-4} mole) was added. The excess iron precipitated as ferric hydroxide, and the solution was heated almost to boiling to promote formation of the iron compound and to make the ferric hydroxide more filterable. The concentration of sodium hydroxide at this point was 2 M. The ferric hydroxide was removed by filtering the warm mixture through Whatman No. 41 filter paper. The filtrate was diluted to exactly 100 ml. with 10 M sodium hydroxide and water, so that the final concentration of sodium hydroxide was 2 M.

Solutions for absorbance measurements were prepared by adjusting 5.00-ml. aliquots of the stock solution approximately to the desired pH, adding 2 ml. of the appropriate buffer, and diluting to 25.00 ml. with deionized water. This gave an ionic strength of approximately 0.4 in each solution. The absorbance of each solution was measured within thirty minutes after preparation on a Beckman DU spectrophotometer at 533 nm. The pH of each solution was then measured accurately on a pH meter which had been calibrated against NBS standard buffers.

B. Results and Discussion

Passage of oxygen through a solution containing sodium hydrosulfite and the red tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) ion results in the formation of a gray compound. If the pH of the solution is greater than 12, this gray compound is converted to an amber compound over a period of about one day. The rate of conversion from gray to amber increases with increasing alkalinity and increasing temperature.

The amber compound is formed directly on mixing solutions of 4,7-dihydroxy-1,10-phenanthroline and ferric iron at a pH greater than 12. At a pH less than 12, the amber compound is slowly converted to the gray compound.

A purple compound is obtained by adjusting the pH of a solution of the amber compound to below 9. The transition is rapidly reversible. The pH range in which the amber and purple forms are both present in solution is 9 to 10.5. Below pH 6.5 a purple compound precipitates.

Solutions of the purple compound are unstable, yielding the gray compound after several days at room temperature, or immediately when heated to boiling. An exception is that the purple compound is stable in solutions containing carbonate or bicarbonate below pH 9.

1. Effect of pH on the nature of the iron(III) compounds

The visible absorption spectra of the three compounds as a function of pH are shown in Figures VI-1 and VI-2. The spectra shown in Figure VI-1 were obtained within two hours after preparation of the solutions. Solutions 7, 8, and 9 (these numbers are roughly equal to the pH) were distinctly purple; 11, 12, 13, and 14 were amber. The spectra in Figure VI-2 were obtained on the same solutions fourteen days later. Solutions 7, 8, 9, 10, and 11 were gray at this time; 13 and 14 were amber.

The purple compound, thus, exists between pH 6.5 and 9. Both the purple and the amber compounds exist in solution between pH 9 and 11. The amber compound is present above pH 11.

The purple compound, and to some extent the amber compound, are unstable, spontaneously decomposing to yield the gray compound. The conversion of the purple compound to the gray compound is complete over the entire pH range over which the purple compound exists, up to pH 9. Between pH 9 and 11 the three forms, purple, amber, and gray, coexist in equilibrium. Above pH 11 and up to 2 M sodium hydroxide, the amber and gray compounds are in equilibrium (see Figure VI-4). The ferric compound exists completely as the amber compound in solutions in which the concentration of sodium hydroxide is 2 M or greater.

Conversion of the amber and purple compounds to the gray compound at pH below 12 is slow, occurring over a period of about one day. The conversion can be made to occur in a few minutes by heating solutions of the amber or purple compounds to boiling. Once formed, the gray compound cannot be converted back to the amber or purple compound, except by increasing the concentration of alkali, whereupon the amber compound is formed.

2. Combining ratios in the gray, amber, and purple compounds

In the determination of the combining ratio of an organic ligand with a metal ion in a colored compound by the method of continuous variations (71), the total number of moles of reacting species present, metal ion plus ligand,

is held constant as the ratio of concentrations of the two is varied. The formation of many compounds involving a metal ion and an organic ligand may be represented by the equation

$$M + nL = ML_n$$

in which M is the metal ion, L the organic ligand, and n the ligand number. The ligand number can be calculated from the maximum in a plot of absorbance vs. mole fraction of reacting species by the equation

$$n = X_L/X_M \qquad \text{(VI-1)}$$

in which X_L and X_M represent the mole fractions of ligand and metal ion, respectively. If solutions of L and M of the same concentration are used to prepare the solutions of the colored compound, equation (VI-1) may be rewritten

$$n = vol_L/vol_M \qquad \text{(VI-2)}$$

where vol_L and vol_M represent the volumes of solutions of L and M, respectively. Equation (VI-2) is applicable in the present study. The maximum in each case was taken as the point of intersection of the two straight-line portions of the curve.

a. Amber compound The studies on the amber compound were made in 0.2 M sodium hydroxide. It is now apparent that 2 M sodium hydroxide would have been a better medium, because the amber compound is stable indefinitely in it, Figure VI-4.

In 0.2 M sodium hydroxide the amber compound is partially converted to the gray compound on standing. However, the amber compound is formed first when iron(III) and 4,7-dihydroxy-1,10-phenanthroline are mixed at high pH, see Figure VI-3, and the partial conversion to the gray form occurs slowly, over a period of a day or so. Inasmuch as the absorbance measurements were taken within several hours after preparation of the solutions, it is safe to assume that essentially all of the compound was in the amber form.

Absorbance measurements were taken at the wavelength of maximum absorptivity, 470 nm. The maximum in the plot of absorbance vs. ml. of 4,7-dihydroxy-1,10-phenanthroline, Figure VI-5, occurred at 6.77 ml. of 4,7-dihydroxy-1,10-phenanthroline and 3.23 ml. of ferric perchlorate, which corresponds to n = 2.1. The combining ratio of 4,7-dihydroxy-1,10-phenanthroline to iron in the amber compound is thus two moles of 4,7-dihydroxy-1,10-phenanthroline to one mole of iron(III).

b. Purple compound The purple compound was prepared by acidifying solutions of the amber compound, and adjusting the pH to 8.6 by the addition of a borate buffer. The presence of tetraborate had no observable effect on the spectrum of the purple compound. Absorbance measurements were made immediately after preparation, since the purple compound is slowly converted to the gray compound. The

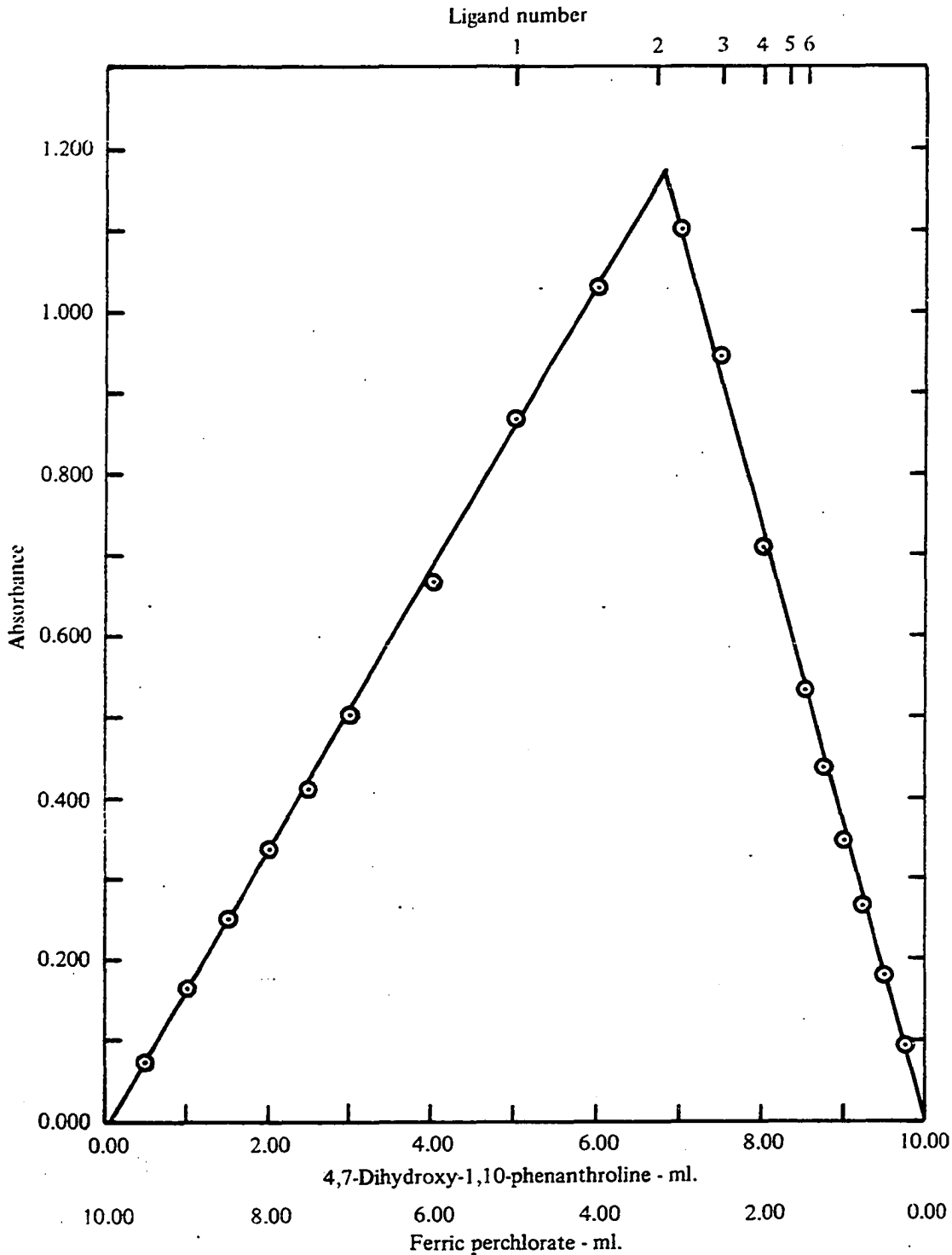
Figure VI-5. Combining ratio in the amber compound of iron(III) and 4,7-dihydroxy-1,10-phenanthroline. Method of continuous variations

Ferric perchlorate: 3.363×10^{-3} M

4,7-Dihydroxy-1,10-phenanthroline: 3.37×10^{-3} M

Sodium hydroxide: 0.20 M

Wavelength: 470 nm.


Ligand number
1
2
3
4
5
6
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Absorbance
0.00
2.00
4.00
6.00
8.00
10.00
4,7-Dihydroxy-1,10-phenanthroline - ml.
10.00
8.00
6.00
4.00
2.00
0.00
Ferric perchlorate - ml.

wavelength setting, 533 nm., corresponded to the wavelength of maximum absorptivity. The maximum in the plot of absorbance *vs.* ml. of 4,7-dihydroxy-1,10-phenanthroline, Figure VI-6, occurred at 5.44 ml. of 4,7-dihydroxy-1,10-phenanthroline and 2.56 ml. of ferric perchlorate, which corresponds to n = 2.1. The combining ratio of 4,7-dihydroxy-1,10-phenanthroline to iron in the purple compound is thus two moles of 4,7-dihydroxy-1,10-phenanthroline to one mole of iron(III).

c. *Gray compound* The gray compound was prepared by heating solutions of ferric perchlorate and 4,7-dihydroxy-1,10-phenanthroline buffered at pH 9.4. At this pH the iron compound is completely in the gray form (see Figure VI-2). Absorbance measurements were made at the wavelength of maximum absorptivity, 526 nm. The maximum in the plot of absorbance *vs.* ml. of 4,7-dihydroxy-1,10-phenanthroline, Figure VI-7, occurred at 6.00 ml. of 4,7-dihydroxy-1,10-phenanthroline and 2.00 ml. of ferric perchlorate, which corresponds to n = 3.0. The combining ratio of 4,7-dihydroxy-1,10-phenanthroline to iron in the gray compound is thus three moles of 4,7-dihydroxy-1,10-phenanthroline to one mole of iron(III).

3. *Acid dissociation constant of the purple compound*

That the transition between the amber and purple 2:1 compounds is rapidly reversible and is effected by the

Figure VI-6. Combining ratio in the purple compound of iron(III) and 4,7-dihydroxy-1,10-phenanthroline. Method of continuous variations

Ferric perchlorate: 3.363×10^{-3} M

4,7-Dihydroxy-1,10-phenanthroline: 3.37×10^{-3} M

pH: 8.6

Wavelength: 533 nm.

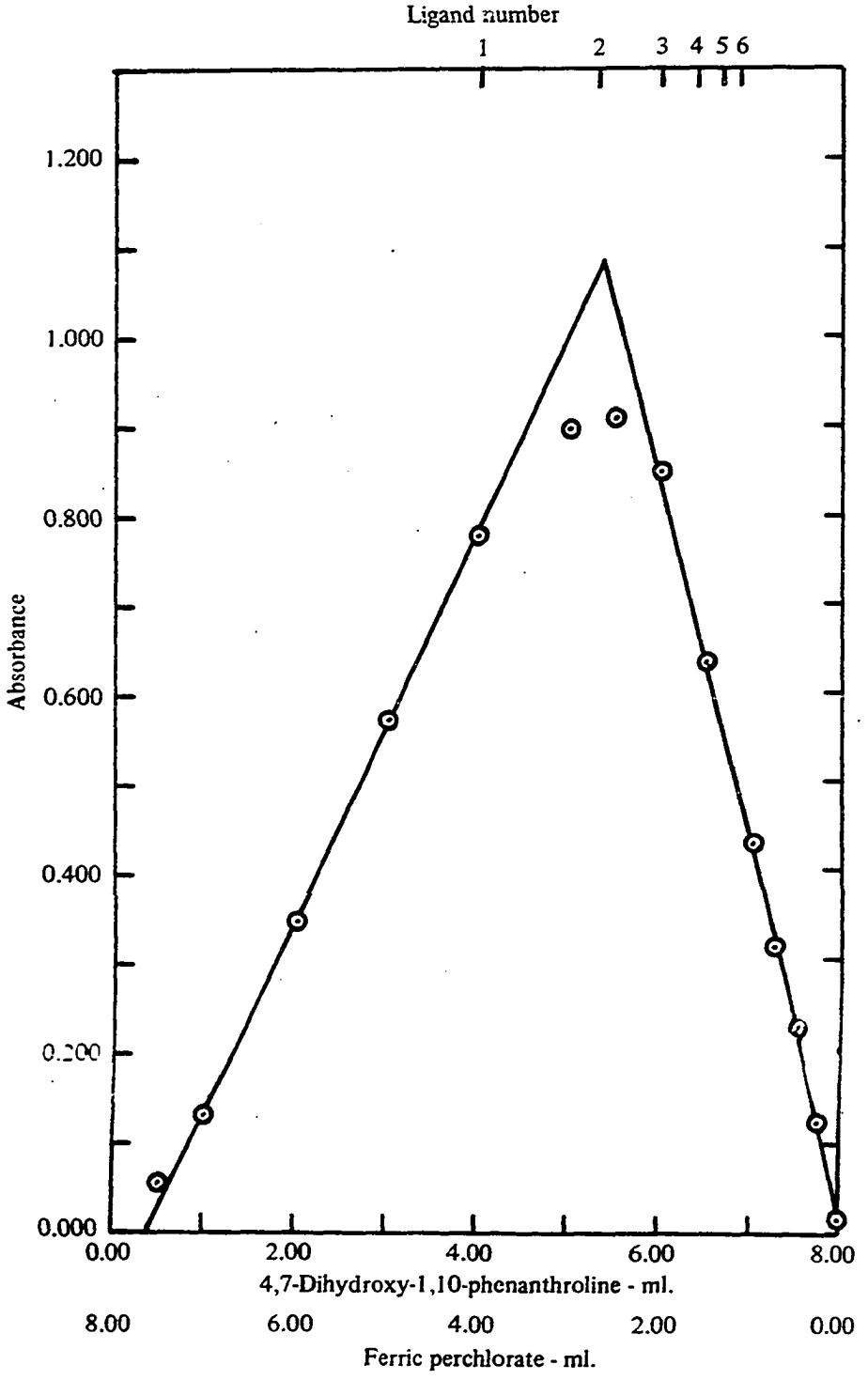

Ligand number
1
2
3
4
5
6
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Absorbance
0.00
2.00
4.00
6.00
8.00
4,7-Dihydroxy-1,10-phenanthroline - ml.
8.00
6.00
4.00
2.00
0.00
Ferric perchlorate - ml.

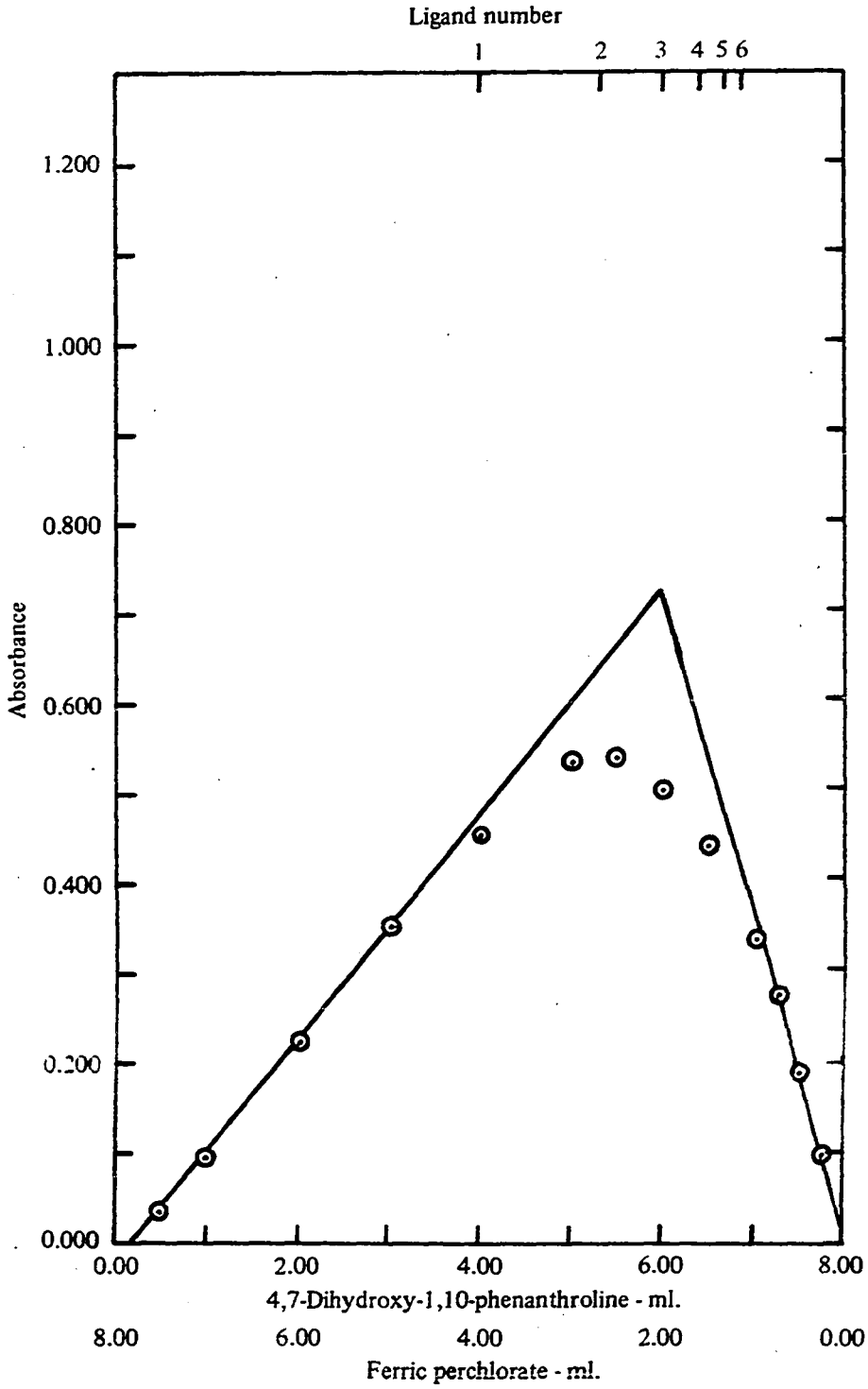
Figure VI-7. Combining ratio in the gray compound of iron(III) and 4,7-dihydroxy-1,10-phenanthroline. Method of continuous variations

Ferric perchlorate: 3.363×10^{-3} M

4,7-Dihydroxy-1,10-phenanthroline: 3.37×10^{-3} M

pH: 9.4

Wavelength: 526 nm.


Ligand number
1
2
3
4
5
6
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Absorbance
0.00
2.00
4.00
6.00
8.00
4,7-Dihydroxy-1,10-phenanthroline - ml.
8.00
6.00
4.00
2.00
0.00
Ferric perchlorate - ml.

addition or removal of hydrogen ions suggest that the purple compound may be considered as a weak acid, and the amber compound as its conjugate base. The dissociation of a weak acid may be represented by the equation

$$H_bA = bH^+ + A^{b-}$$

and the overall acid dissociation constant

$$K_a = K_{a,1}K_{a,2}\cdots K_{a,b} = \frac{[H^+]^b[A^{b-}]}{[H_bA]}$$

or in logarithmic form

$$pK_a = pH - (1/b)\log\frac{[A^{b-}]}{[H_bA]} .$$

The logarithmic term may be expressed in terms of absorbance and the equation rewritten

$$pH = pK_a + (1/b)\log\frac{A_{HA} - A_{mix}}{A_{mix} - A_A}$$

where A_{HA} = absorbance when compound is 100 percent protonated,

A_A = absorbance when compound is 100 percent dissociated,

A_{mix} = absorbance when H_bA and A^{b-} are both present in solution.

In a plot of $\log\frac{A_{HA} - A_{mix}}{A_{mix} - A_A}$ *vs.* pH, the intercept will occur where pH = pK_a. Also, if $K_{a,1} = K_{a,2} = \cdots = K_{a,b}$, the inverse of the slope will be equal to b, the number of protons involved in the dissociation reaction.

Plots of absorbance *vs.* pH and of $\log\frac{A_{HA} - A_{mix}}{A_{mix} - A_A}$ *vs.* pH are shown in Figures VI-8 and VI-9, respectively. The intercept on the latter occurred at pH = 9.77. Thus, the pK_a of the purple compound at a temperature of 23° and ionic strength of 0.4 is 9.77. The slope is equal to 0.88, or b = 1.1. The conversion of the purple form to the amber form thus involves the loss of one proton.

4. Supporting evidence for assignments of combining ratios

The combining ratios of 4,7-dihydroxy-1,10-phenanthroline with iron(III) in the gray, purple, and amber compounds can be inferred from the following observations.

a. Oxidation of the red ferrous compound in which the ratio of phenanthroline to iron is three to one always yields the gray compound, independent of pH and the nature of the oxidant. The analogous oxidation of tris(1,10-phenanthroline)iron(II) is a reversible one-electron oxidation which produces the blue tris(1,10-phenanthroline)-iron(III) ion. There is no obvious reason to expect the dihydroxy compound to behave differently. Oxidation of the

Figure VI-8. Absorbance of the amber and purple iron(III)-4,7-dihydroxy-1,10-phenanthroline compounds as a function of pH

Wavelength: 533 nm.

Ionic strength: 0.4

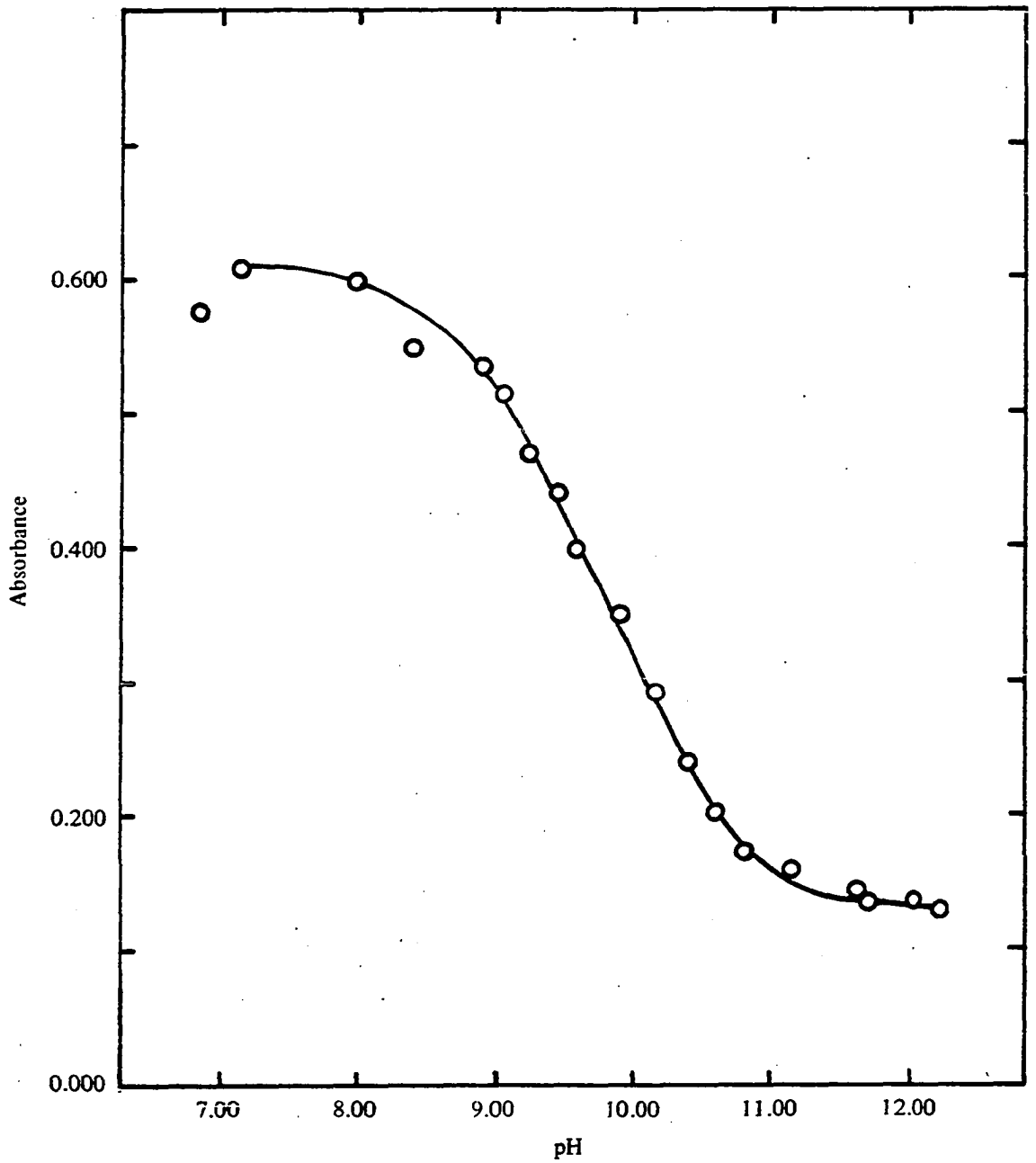
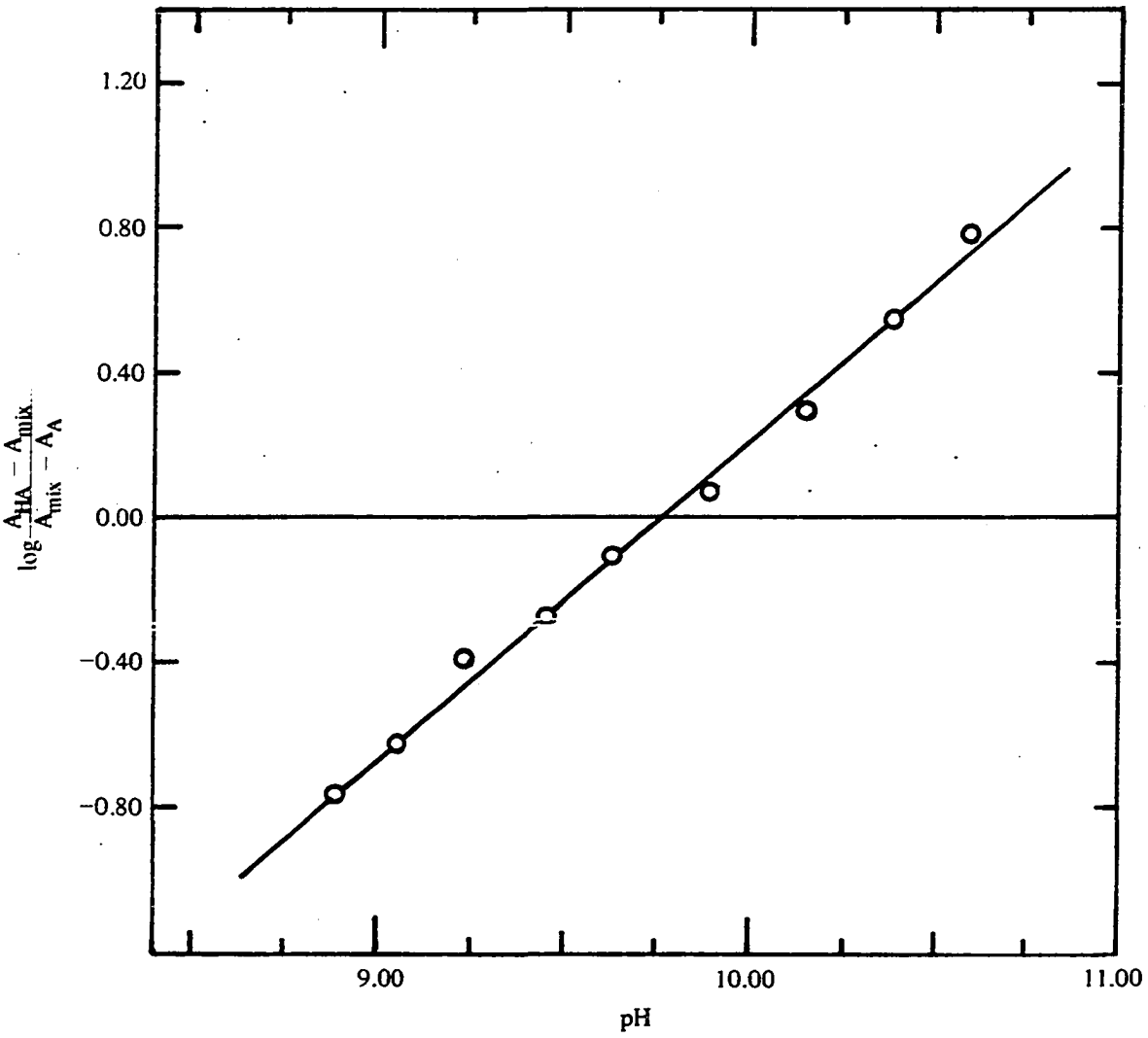

0.600
0.400
0.200
0.000
Absorbance
7.00
8.00
9.00
10.00
11.00
12.00
pH

Figure VI-9. Logarithmic treatment of the absorbance of the amber and purple compounds as a function of pH

$$\log \frac{A_{HA} - A_{mix}}{A_{mix} - A_{A}} \quad \underline{vs.} \quad pH$$

pK_a = pH at intercept


1.20
0.80
0.40
0.00
−0.40
−0.80
$\log \frac{A_{HA} - A_{mix}}{A_{mix} - A_A}$
9.00
10.00
11.00
pH

ferrous derivative of 4,7-dihydroxy-1,10-phenanthroline by either oxygen, potassium ferricyanide, or hydrogen peroxide results in the gray compound. Even in highly alkaline solutions, in which the amber 2:1 compound is stable, the gray 3:1 compound is formed first upon oxidation of the ferrous compound, and is then slowly converted to the amber 2:1 compound.

b. The gray compound may be formed in highly alkaline solutions by adding a very large excess of 4,7-dihydroxy-1,10-phenanthroline.

c. The amber compound is formed directly by mixing solutions of 4,7-dihydroxy-1,10-phenanthroline and iron(III), even in the pH range in which the gray compound is stable. Other phenanthrolines form 2:1 compounds when mixed directly with iron(III).

d. Interconversion of the amber and purple forms is rapidly reversible, whereas interconversion of the amber or purple compound with the gray compound is slow. The union or separation of a molecule of phenanthroline from the iron atom in ferrous compounds is slow; this appears to be true in the iron compounds of 4,7-dihydroxy-1,10-phenanthroline.

5. Structure of the amber and purple compounds

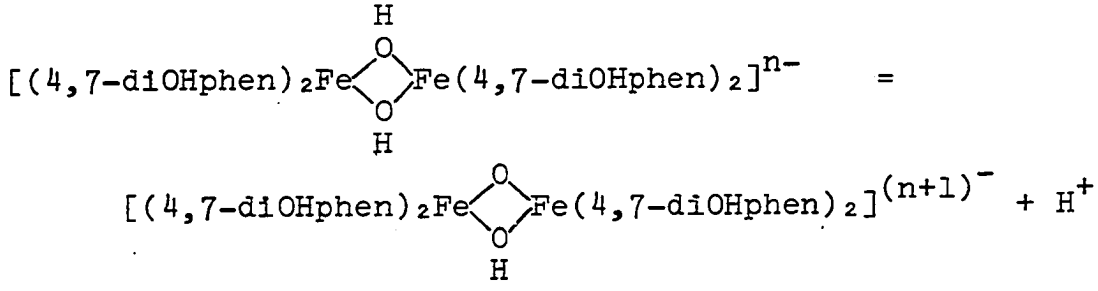
Because the transition between the purple and amber compounds involves only one proton, this proton is most likely associated with one of the bridging oxygen atoms

between the two iron atoms as shown in structure I in the introduction to this chapter. There would appear to be little difference in the pK_a values of similar protons on individual phenanthroline molecules about the same iron atom, and essentially no difference in the case in which the phenanthroline molecules are attached to different iron atoms in the same compound. If the transition between the purple and amber forms were due to ring protons, one would obtain a value of b (number of protons involved in the neutralization reaction) greater than one, or an indication of more than one inflection point, if the pK_a's were slightly different, in the plot of absorbance vs. pH, Figure VI-8. Only one inflection point is observed, and a value of b equal to 1.1 is obtained.

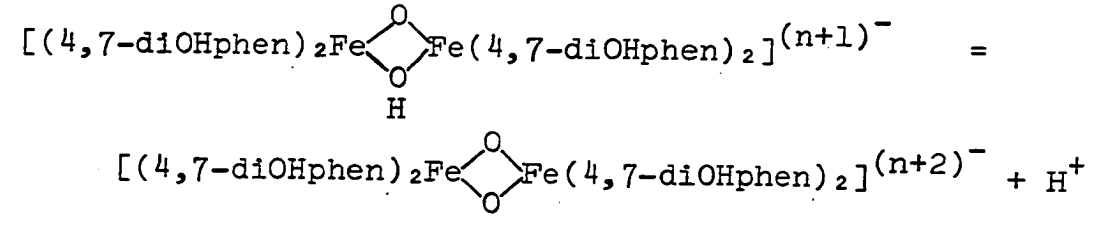
Further evidence that the transition between the purple and amber compounds does not involve dissociation of the hydroxy groups on the phenanthroline rings is found in Part V of this dissertation. The ultraviolet spectra of 4,7-dihydroxy-1,10-phenanthroline and its iron(III) derivative are almost identical from pH 9 to 5 M sodium hydroxide, and the estimated pK_a value of 12.7 for 4,7-dihydroxy-1,10-phenanthroline can also be applied to its iron(III) derivative. No change in the ultraviolet absorption spectrum of either compound occurs between pH 9

and 10, Figure IV-1, the pH region in which the pK_a of the purple compound falls.

Of the structures listed in the introduction to this part, only I is reasonable for the iron(III)-4,7-dihydroxy-1,10-phenanthroline compound. The absence of anionic ligands such as chloride precludes structures II and III. Perchlorate was present in the systems studied, but it is highly unlikely that such a weakly coordinating ligand would be favored over the hydroxide ion, especially in alkaline solutions. The transition from the purple to the amber form thus is



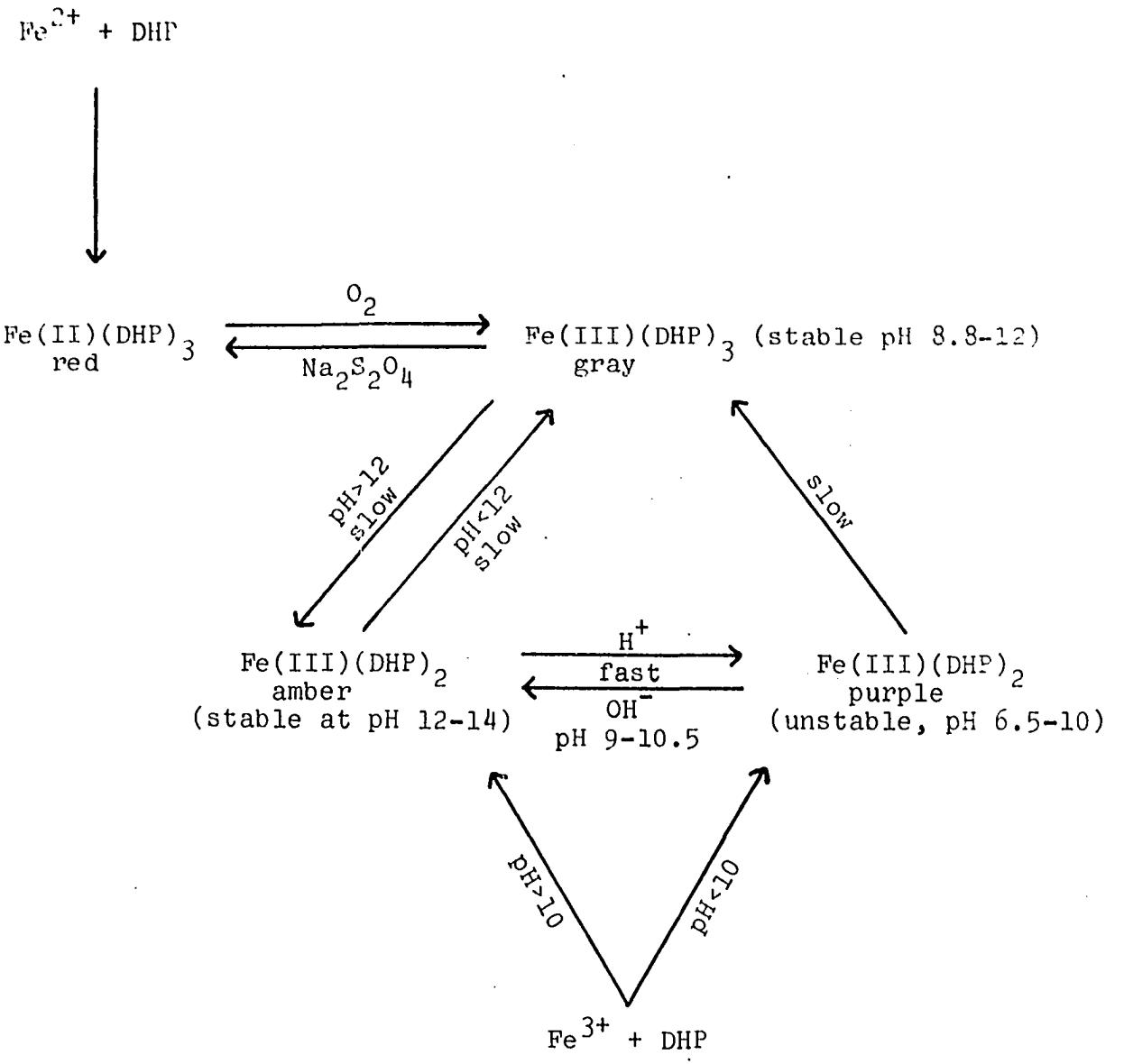
or



C. Summary

A summary of the chemistry of the iron-4,7-dihydroxy-1,10-phenanthroline compounds is given graphically in Figure VI-10. Three distinct iron(III) derivatives of 4,7-dihydroxy-1,10-phenanthroline exist, a gray, a purple, and an amber compound. The gray compound, formed by oxidation of tris-(4,7-dihydroxy-1,10-phenanthroline)iron(II), is isostructural with the ferrous derivative, having three molecules of 4,7-dihydroxy-1,10-phenanthroline per atom of iron, and is therefore called tris(4,7-dihydroxy-1,10-phenanthroline)-iron(III). The gray compound is stable between pH 8 and 12. Below pH 8 the gray compound is insoluble, and above pH 12 it is converted to an amber compound, in which the ratio of phenanthroline to iron is two to one. Once formed, the amber compound is converted to a purple compound by adjusting the pH to below 9. The purple compound precipitates below pH 6.5. Solutions of the purple compound are unstable, yielding the gray compound on standing for a day or so. The amber and purple compounds are isostructural, differing by only one proton. The acid dissociation constant of the purple compound is 9.77. The purple compound is probably the binuclear species bis(4,7-dihydroxy-1,10-phenanthroline)iron(III)-μ-dihydroxobis(4,7-dihydroxy-1,10-phenanthroline)iron(III), and the acidic group involved in the interconversion of the purple and amber compounds is a bridging hydroxy group.

Figure VI-10. Summary of the interrelations of the iron compounds of 4,7-dihydroxy-1,10-phenanthroline


Fe2+ + DHP
Fe(II)(DHP)3
red
O2
Na2S2O4
Fe(III)(DHP)3 (stable pH 8.8-12)
gray
pH>12
slow
pH<12
slow
slow
Fe(III)(DHP)2
amber
(stable at pH 12-14)
H+
fast
OH−
pH 9-10.5
Fe(III)(DHP)2
purple
(unstable, pH 6.5-10)
pH>10
pH<10
Fe3+ + DHP

VII. FORMAL REDUCTION POTENTIAL OF THE 4,7-DIHYDROXY-1,10-PHENANTHROLINE-IRON(III,II) COUPLE

The reduction potential of the 4,7-dihydroxy-1,10-phenanthroline-iron(III,II) couple was first measured by George, Hanania and Eaton (31), the value found being -0.13 volt on the hydrogen scale. This value was reported incidentally in a discussion of the reduction potentials of hemes and hemoproteins and is stated to be the result of unpublished work; no details are given other than the pH was greater than 10. Later Poe (54), in the course of work for the M.S. degree, measured the formal reduction potential of the system in 2 M sodium hydroxide and found $E^{o\prime} = -0.33$ volt. The value, of course, is astonishingly low, and Poe showed further that the system performed neatly as a highly colored and rapidly reversible oxidation-reduction indicator, suitable for titrations in alkaline solutions with standard ferricyanide.

In the present work, the formal reduction potential of the 4,7-dihydroxy-1,10-phenanthroline-iron(III,II) couple has been studied as a function of the concentration of alkali from pH 10 to 10 M sodium hydroxide. The formal potential of the parent system does not vary with pH over the pH range 3

$$\underset{\text{Ferriin}}{Fe(1,10\text{-phen})_3^{3+}} + e^- = \underset{\text{Ferroin}}{Fe(1,10\text{-phen})_3^{2+}}$$

$E^\circ = +1.06$ volt

to 10, the range over which the compounds involved are stable. In highly alkaline solutions, the hydroxy groups of 4,7-dihydroxy-1,10-phenanthroline would be dissociated and the iron derivatives thus carry six or so negative charges on the periphery of the molecule. Obviously, these charges have greatly altered the reduction potential and obviously also a more detailed knowledge of the change of the formal reduction potential with change in concentration of alkali becomes of interest.

The formal reduction potential at pH 10 to 10 M sodium hydroxide was measured by following the potential at a bright platinum foil electrode in appropriately buffered solutions of sodium hydrosulfite and tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) with standard potassium ferricyanide. The potential at the midpoint in the titration of the phenanthroline derivative was taken as the formal reduction potential.

A. Experimental Work

1. Reagents and solutions

Buffer solutions of pH 10 and 11 were prepared by adding 107 ml. and 227 ml. of 0.1 M sodium hydroxide, respectively, to 500 ml. of 0.05 M sodium bicarbonate. Buffer solutions of pH 12 and 13 were prepared by adding 60 ml. and 660 ml. of 0.2 M sodium hydroxide, respectively, to 250 ml. of 0.2 M potassium chloride (35).

Solutions of 0.05 M potassium ferricyanide were prepared by dissolving 3.3 to 3.4 g. of the anhydrous reagent-grade material in ca. 180 ml. of buffer. The pH of the resulting solutions were adjusted to the desired values by the addition of hydrochloric acid or sodium hydroxide, if necessary. The pH-adjusted solutions were then diluted to 200 ml.

2. Apparatus and calibration of apparatus

The potential of the solutions at a bright platinum foil electrode was measured against a saturated calomel electrode with a Beckman Zeromatic SS-3 pH meter. The millivolt scale of the pH meter was calibrated against a standard Weston cell, or alternatively by adjusting the reading to zero millivolts while shorting the input and reference terminals of the pH meter. The two methods of calibration led to the same results.

3. Procedure

Samples of 4,7-dihydroxy-1,10-phenanthroline (0.06-0.10 g.) and sodium hydrosulfite (0.04-0.06 g.) were dissolved in 50 ml. of the appropriate buffer solutions in a 150-ml. beaker. The solutions were kept under an atmosphere of nitrogen and were stirred magnetically. Electrodes, buret tip, and nitrogen inlet were inserted through holes in a sheet of Parafilm which was fitted tightly over the opening of the beaker. A stoichiometric amount of ferrous ammonium

sulfate hexahydrate or ferrous sulfate heptahydrate was dissolved in a minimum amount of water and added to each solution of 4,7-dihydroxy-1,10-phenanthroline. The pH was adjusted to the appropriate value, if necessary, with 0.1 M sodium hydroxide. Solutions of pH 13 or less were heated to boiling to insure complete formation of tris(4,7-dihydroxy-1,10-phenanthroline)iron(II). When cool, each solution was titrated with 0.05 M potassium ferricyanide prepared in a solution at the same pH.

B. Results and Discussion

The classical method of measuring the formal reduction potential of a couple consists of titrating a solution of the reduced form of the couple with an appropriate oxidant, or titrating a solution of the oxidized form with a suitable reductant. If the reaction has the same stoichiometry throughout the entire titration, and the oxidized and reduced forms of the couple are stable, the composition of the solution at the midpoint of the titration corresponds to the condition that the concentration of the oxidized form is equal to the concentration of reduced form. When this condition is met, the logarithmic term of the Nernst equation

$$E = E^\circ + \frac{RT}{nF} \ln \frac{[\text{oxidized}]}{[\text{reduced}]}$$

becomes zero, and the potential is equal to the standard reduction potential of the couple (E°) if the titration is

performed in one molar hydrogen ion and the members of the couple do not form slightly-dissociated ions with other components of the solution. The potential at the midpoint under any other conditions is called the formal reduction potential ($E^{\circ\prime}$), and the conditions are usually stated describing the composition of the solution.

The potential at a platinum electrode of a solution containing only a small amount of a couple is often not readily established. Thus, when the amount of material is limited, or the solubility low, a relatively large amount of a strong oxidizing agent or of a reducing agent is also added to the solution. Not only does this help to establish the potential at the electrode, but it insures that all of the compound being studied is in either the oxidized or the reduced form at the beginning of the titration.

In the case at hand the addition of sodium hydrosulfite (also called sodium hyposulfite and sodium dithionite) insured the reduction of all of the iron to the ferrous state. The titration in 1.0 M sodium hydroxide proceeded in two steps with clean-cut breaks at two equivalence points, corresponding respectively to the titration first of sodium hydrosulfite and second of the tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) ion, Figure VII-1. The potential at the midpoint of the first stage, -0.80 volt *vs.* s.c.e. (-0.55 volt on the hydrogen scale) does not agree at all with

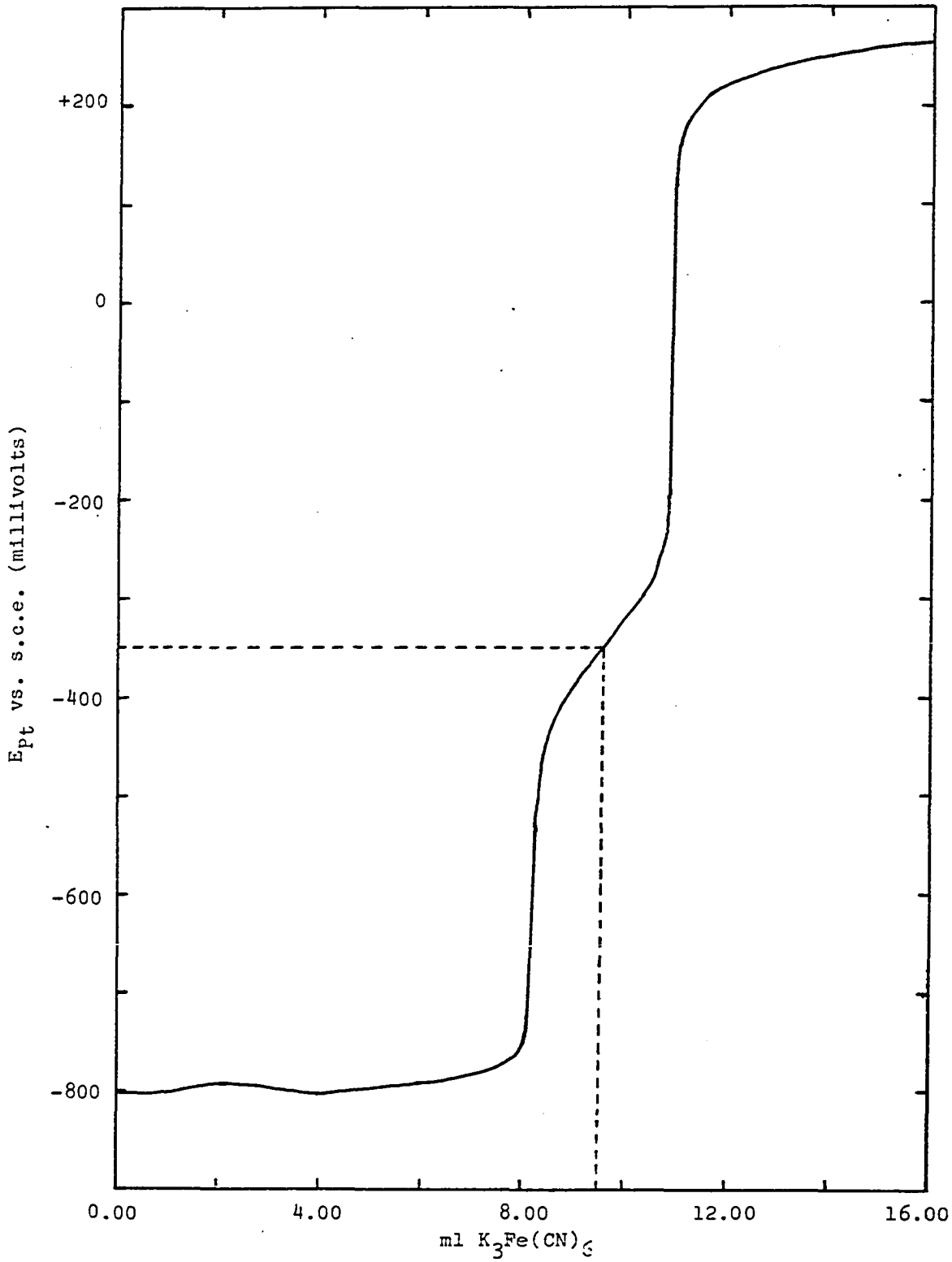
Figure VII-1. Titration of sodium hydrosulfite plus tris(4,7-dihydroxy-1,10-phenanthroline)-iron(II) in 1.0 M sodium hydroxide with standard potassium ferricyanide

Wt. sodium hydrosulfite: 0.0447 g.

Wt. ferrous ammonium sulfate hexahydrate: 0.0540 g.

Wt. 4,7-dihydroxy-1,10-phenanthroline: 0.0946 g.

Concentration of potassium ferricyanide: 0.0502 M (in 1.0 M sodium hydroxide)


+200
0
-200
-400
-600
-800
E_{Pt} vs. s.c.e. (millivolts)
0.00
4.00
8.00
12.00
16.00
ml $K_3Fe(CN)_6$

the value given in Latimer (44) for the formal reduction potential of the sulfite-hydrosulfite system in 1 M alkali:

$$2SO_3^{2-} + 2H_2O + 2e^- = S_2O_4^{2-} + 4OH^-, \quad E_B^\circ = -1.12 \text{ volts.}$$

This discrepancy is mentioned in Poe's M.S. thesis (54), in which is also presented a description of the use of the tris(4,7-dihydroxy-1,10-phenanthroline)iron(III,II) couple as a perfectly reversible visual oxidation-reduction indicator system with excellent color contrast in the titration of sodium hydrosulfite with potassium ferricyanide.

The potential at the midpoint of the second stage of the titration was taken as the formal reduction potential of the tris(4,7-dihydroxy-1,10-phenanthroline)iron(III,II) couple. The values obtained for the formal reduction potential in solutions of various pH are listed in Table VII-1.

The formal reduction potential was found to have a constant value and was fairly repreducible over the pH range 10 to 13, the average value being -0.063 volt. Formal reduction potentials measured in solutions of higher pH became more negative with increasing alkalinity and were not reproducible. Formal reduction potentials in solutions outside the pH range reported in the table could not be determined; at lower pH the oxidation of hydrosulfite with ferricyanide proceeded too slowly near the equivalence point, and in higher concentrations of alkali the potential drifted too greatly to be significant.

Table VII-1. Formal reduction potential of the tris(4,7-dihydroxy-1,10-phenanthroline)iron(III,II) couple as a function of pH

pH or [NaOH]	Formal reduction potential, volts (vs. NHE)	Temperature, °C
10	-0.064	28
11	-0.063	27
12	-0.053	27
13	-0.070	28
1.0 M NaOH	-0.105	28
	-0.115	
	-0.199	25
2 M NaOH	-0.33[a]	
3.2 M NaOH	-0.260	25

[a]Reference 54.

Inasmuch as the formal reduction potential remains essentially constant over the pH range 10 to 13, it appears that the hydrogen ion is not involved in the couple and thus that the oxidized and reduced forms are chemically the same except for the difference of one electron, that is, that the couple is expressed by:

$$Fe(III)(diOHphen)_3 + e^- = Fe(II)(diOHphen)_3$$

in which no commitment is made as to the charges carried by the ions although presumably the iron(III) ion carries a charge of -3, the iron(II) a charge of -4.

The irreproducibility and general decrease in the formal reduction potential with increasing alkalinity in solutions one molar and greater in sodium hydroxide arise from the instability of the tris(4,7-dihydroxy-1,10-phenanthroline)-iron(III) ion in highly alkaline solutions. It was observed from visible absorption spectra that an appreciable amount of tris(4,7-dihydroxy-1,10-phenanthroline)iron(III), formed by bubbling oxygen through a solution of the ferrous compound, decomposes in highly alkaline solutions to form an amber compound within several minutes following oxidation. The chemistry of the amber compound, in which the ratio of phenanthroline to iron(III) is two to one, is described in Chapter VI of this dissertation.

In general, the reduction potential of a couple which consists of slightly dissociated ions is related to the

formation constants of the slightly dissociated ions and the standard reduction potential of the unbound metal ions. The basic form of the Nernst equation describing the potential of the ferric-ferrous couple is:

$$E = E^\circ + \frac{2.303\ RT}{nF} \log \frac{[Fe^{3+}]}{[Fe^{2+}]} \qquad \text{(VII-1)}$$

$[Fe^{3+}]$ and $[Fe^{2+}]$ can be written in terms of the formation constants of the reactions:

$$Fe^{3+} + 3diOHphen = (diOHphen)_3Fe(III), \qquad K_{f,III} = \frac{[(diOHphen)_3Fe(III)]}{[Fe^{3+}][diOHphen]^3} \qquad \text{(VII-2)}$$

and

$$Fe^{2+} + 3diOHphen = (diOHphen)_3Fe(II), \qquad K_{f,II} = \frac{[(diOHphen)_3Fe(II)]}{[Fe^{2+}][diOHphen]^3} \qquad \text{(VII-3)}$$

Solving for $[Fe^{3+}]$ and $[Fe^{2+}]$ in equations VII-2 and VII-3, and substituting into equation VII-1:

$$E = E^\circ + \frac{2.303\ RT}{nF} \log \frac{K_{f,II}[(diOHphen)_3Fe(III)]}{K_{f,III}[(diOHphen)_3Fe(II)]} \qquad \text{(VII-4)}$$

At the midpoint of the titration, $[(diOHphen)_3Fe(III)] = [(diOHphen)_3Fe(II)]$, and

$$E = E^{\circ\prime} = E^\circ + \frac{2.303\ RT}{nF} \log \frac{K_{f,II}}{K_{f,III}} \qquad \text{(VII-5)}$$

Inserting values for the constants

$$E^{\circ\prime} = -0.06 \text{ volt},$$

$$E^{\circ} = +0.77 \text{ volt},$$

$$\frac{2.303RT}{nF} = 0.059 \text{ at } 25^{\circ}C,$$

there is obtained $\frac{K_{f,II}}{K_{f,III}} = 10^{-14}$, that is, the formation constant of tris(4,7-dihydroxy-1,10-phenanthroline)iron(III) is 10^{14} times greater than the formation constant of the corresponding iron(II) compound. It also follows that the concentration of unbound iron(II) at the midpoint of the titration is 10^{14} times greater than the concentration of unbound iron(III). Since the formal reduction potential becomes more negative with the formation of the amber two-to-one iron(III) compound in highly alkaline solutions, the formation of the amber compound effectively removes more ferric iron from solution than does the formation of the gray tris(4,7-dihydroxy-1,10-phenanthroline)iron(III) ion.

It is apparent that the values for the formal reduction potential of the tris(4,7-dihydroxy-1,10-phenanthroline)-iron(III,II) couple reported in Table VII-1 are valid only in the pH range 10-13. In more alkaline solutions the requirement that the concentration of tris(4,7-dihydroxy-1,10-phenanthroline)iron(III) equals the concentration of tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) at the midpoint of the titration is not met, and the values obtained are the reduction potentials of an unstable system composed

of the tris(4,7-dihydroxy-1,10-phenanthroline)iron(III,II) couple and an amber compound which is formed by the decomposition of tris(4,7-dihydroxy-1,10-phenanthroline)-iron(III).

C. Conclusion

The formal reduction potential of the tris(4,7-dihydroxy-1,10-phenanthroline)iron(III,II) couple has been measured and found to be constant over the pH range 10 to 13 and to have the value -0.06 volt. It has been shown that in this pH range the oxidized and reduced forms of the couple are chemically identical, except for the difference of one electron, and that hydrogen ion is not involved in the couple.

The irreproducibility and general decrease in the reduction potential at the mid-point in the titration of tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) in solutions greater than 1.0 M in sodium hydroxide has been shown to be due to the instability of tris(4,7-dihydroxy-1,10-phenanthroline)iron(III) in highly alkaline solutions, and that the values obtained under these conditions do not represent the formal reduction potential of the tris(4,7-dihydroxy-1,10-phenanthroline)iron(III,II) couple. The relative values of the formation constants of tris(4,7-dihydroxy-1,10-phenanthroline)iron(II), tris(4,7-dihydroxy-1,10-phenanthroline)iron(III), and an amber compound in which

the ratio of phenanthroline to iron is two to one have also been discussed.

VIII. SPECTROPHOTOMETRIC DETERMINATION OF DISSOLVED OXYGEN USING TRIS(4,7-DIHYDROXY-1,10-PHENANTHROLINE)IRON(II)

The extremely low formal reduction potential (-0.06 volt) of the tris(4,7-dihydroxy-1,10-phenanthroline)iron(III,II) couple, in conjunction with the intense color of the ferrous derivative and the reversible nature of the oxidation-reduction reaction which it shares with other iron-phenanthrolines, make tris(4,7-dihydroxy-1,10-phenanthroline)-iron(II) an excellent reagent for the spectrophotometric determination of dissolved oxygen in water. Addition of water containing dissolved oxygen to a solution of the ferrous compound should result in oxidation of the ferrous compound to a ferric compound and a corresponding decrease in absorbance in direct proportion to the amount of oxygen in the sample. In the development of such a method several questions need to be answered. Is oxidation of the reagent by oxygen quantitative, and if so, under what conditions? Can conditions be found under which the change in absorbance of the reagent solution is reproducible? What substances interfere, if any? Finally, can a simple method be devised to make it a practical method for the determination of dissolved oxygen?

In this chapter the working details for using the ferrous compound as a reagent for the determination of

dissolved oxygen are presented, followed by a discussion of the experiments involved in the development of the method, and finally a discussion of the accuracy and applicability of the method.

A. Experimental Work

1. Reagents

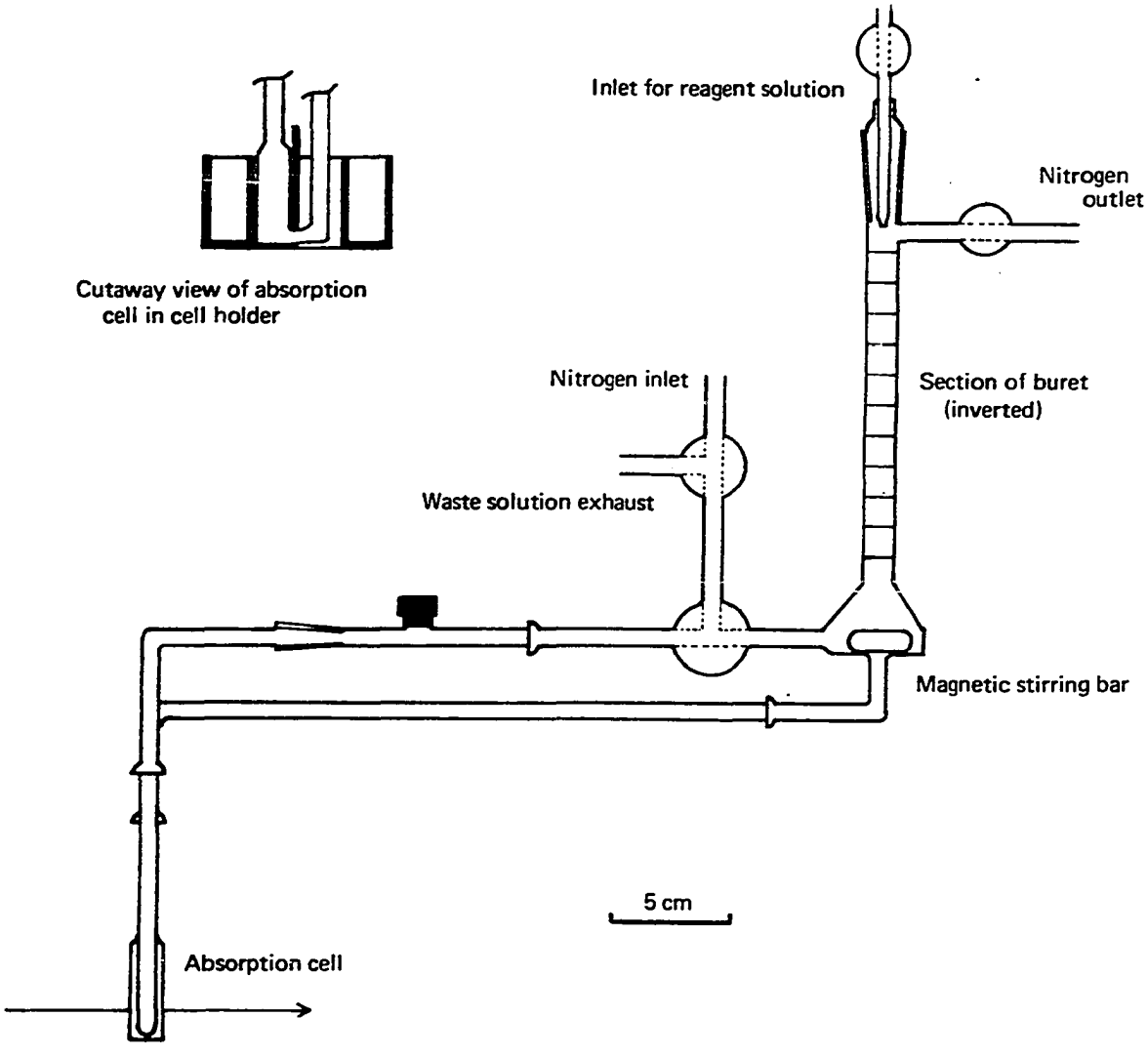
A stock solution of 4,7-dihydroxy-1,10-phenanthroline (available from the Hach Chemical Company, Ames, Iowa, U.S.A. 500.0), 6×10^{-4} M, was prepared by dissolving 0.15 g. of the hydrochloride in 1 liter of a buffer consisting of 2.0 M ammonium hydroxide and 0.1 M ammonium chloride stored in a Machlett buret under nitrogen.

A solution of iron(II) was prepared by dissolving 3.35 g. of ferrous ammonium sulfate, 1.5 g. of tartaric acid, and 1 ml. of concentrated ammonium hydroxide in 15 ml. of water. The solution was stored in a serum bottle covered with a rubber septum. The commercial nitrogen used contained a significant amount of oxygen; this oxygen was removed by bubbling the nitrogen through vanadium(II) sulfate stored over zinc amalgam (47) then through alkaline permanganate (to remove hydrogen sulfide formed in the vanadium(II) tower), and finally through water.

2. Apparatus

An all-glass flow-through cell system was constructed for use with a Beckman DU spectrophotometer, Figure VIII-1. A cell holder was altered to accept a 1.00-cm. cell with side-arm, the cell being inserted into the bottom of the holder (Figure VIII-1, inset). The two glass tubes rising from the sample compartment were painted black and were inserted through two holes in a strip of black latex rubber which covered the compartment and blocked out light from the room. Four ball-and-socket joints were included in the apparatus to permit movement of the cell in and out of the light path. Flexible rubber or synthetic tubing could not be used because they are permeable to oxygen. A rubber septum was used as a sample injection port and a Teflon-coated magnetic stirring bar was used to circulate the liquid through the system. The Teflon coating slowly released oxygen into the solution, causing a steady decrease in absorbance during use; this effect was eliminated by storing the stirring bar in alkaline sodium hydrosulfite solution for a week or two before placing it in the apparatus. A glass-covered stirring bar introduced so much suspended silica from wear on the glass vessel that it had to be rejected. The calibrated portion of the apparatus was a section of a 50-ml. buret, inverted. The volume of the apparatus when filled to the zero mark on the buret was

Figure VIII-1. Flow-through cell system


Cutaway view of absorption cell in cell holder
Inlet for reagent solution
Nitrogen outlet
Nitrogen inlet
Section of buret (inverted)
Waste solution exhaust
Magnetic stirring bar
5 cm
Absorption cell

determined. The total volume of solution circulating in the system original solution plus sample, could thus be determined. The reagent solution inlet was fitted with a short section of Tygon tubing, which formed an air-tight seal when the tip of the Machlett buret was inserted for filling the apparatus.

Absorption spectra were obtained on a Cary 14 recording spectrophotometer.

3. Procedure

The flow-through cell system was purged with nitrogen by passage of nitrogen for thirty minutes and was then filled with the phenanthroline solution. Enough iron(II) solution (3 to 5 µl.) was injected into the system through the rubber septum to bring the absorbance at 520 nm. to 0.7 to 0.8. A measured volume of the sample was injected through the septum from a 1-ml. tuberculin syringe. After the system had come to equilibrium (about 15 minutes) the absorbance was measured. After several samples had been injected, enough ammoniacal sodium hydrosulfite solution (5 g. per 100 ml.) was injected from a 50-µl. syringe to bring the absorbance back to 0.7 to 0.8. Within a few minutes the system was ready to use for more determinations.

B. Results and Discussion

The intensely red compound tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) is easily oxidized to a gray iron(III)

compound which is stable indefinitely in 0.2 to 4 M ammonia solution, and in general in the pH range 8.5 to 12. At higher pH this gray compound is converted to an amber compound. By application of the method of continuous variations (71), it was established that in the gray iron(III) compound three molecules of phenanthroline are combined with one atom of iron and that in the amber iron(III) compound two molecules of phenanthroline are combined with one atom of iron. For details see Part VI of this dissertation.

The absorption spectra between 350 and 650 nm. of the iron(II) and iron(III) compounds are shown in Figure VIII-2. The wavelength of maximum absorbance of the iron(II) compound, 520 nm., and molar absorptivity, ε = 14,800 liter$\cdot$mole$^{-1}\cdot$cm^{-1}, found were the same as reported earlier by Schilt and co-workers (60). These workers showed that the molar absorptivity of the iron(II) compound is independent of the concentration of alkali; in the present study this was shown to be true also in ammonia buffers as dilute as 0.2 M NH_4OH plus 0.1 M NH_4Cl (pH = 9.7). The molar absorptivity in all solutions agreed with the reported value. At 520 nm. the absorbance of the iron(III) compound in ammonia solution is constant over the pH range 8.5 to 12, Figure VIII-3, ε = 3300 liter$\cdot$mole$^{-1}\cdot$cm^{-1}. At higher pH values the absorbance of the iron(III) compound decreases owing to the formation of the amber iron(III) compound.

Figure VIII-2. Absorption spectra of the iron(II) and iron(III) derivatives of 4,7-dihydroxy-1,10-phenanthroline

——— Tris(4,7-dihydroxy-1,10-phenanthroline)iron(II), 4.89×10^{-5} M

····· Bis(4,7-dihydroxy-1,10-phenanthroline)iron(III)-μ-dihydroxobis(4,7-dihydroxy-1,10-phenanthroline)iron(III), 2.45×10^{-5} M

-·-·- Tris(4,7-dihydroxy-1,10-phenanthroline)iron(III), 5.00×10^{-5} M

----- 4,7-Dihydroxy-1,10-phenanthroline, 4.0×10^{-4} M

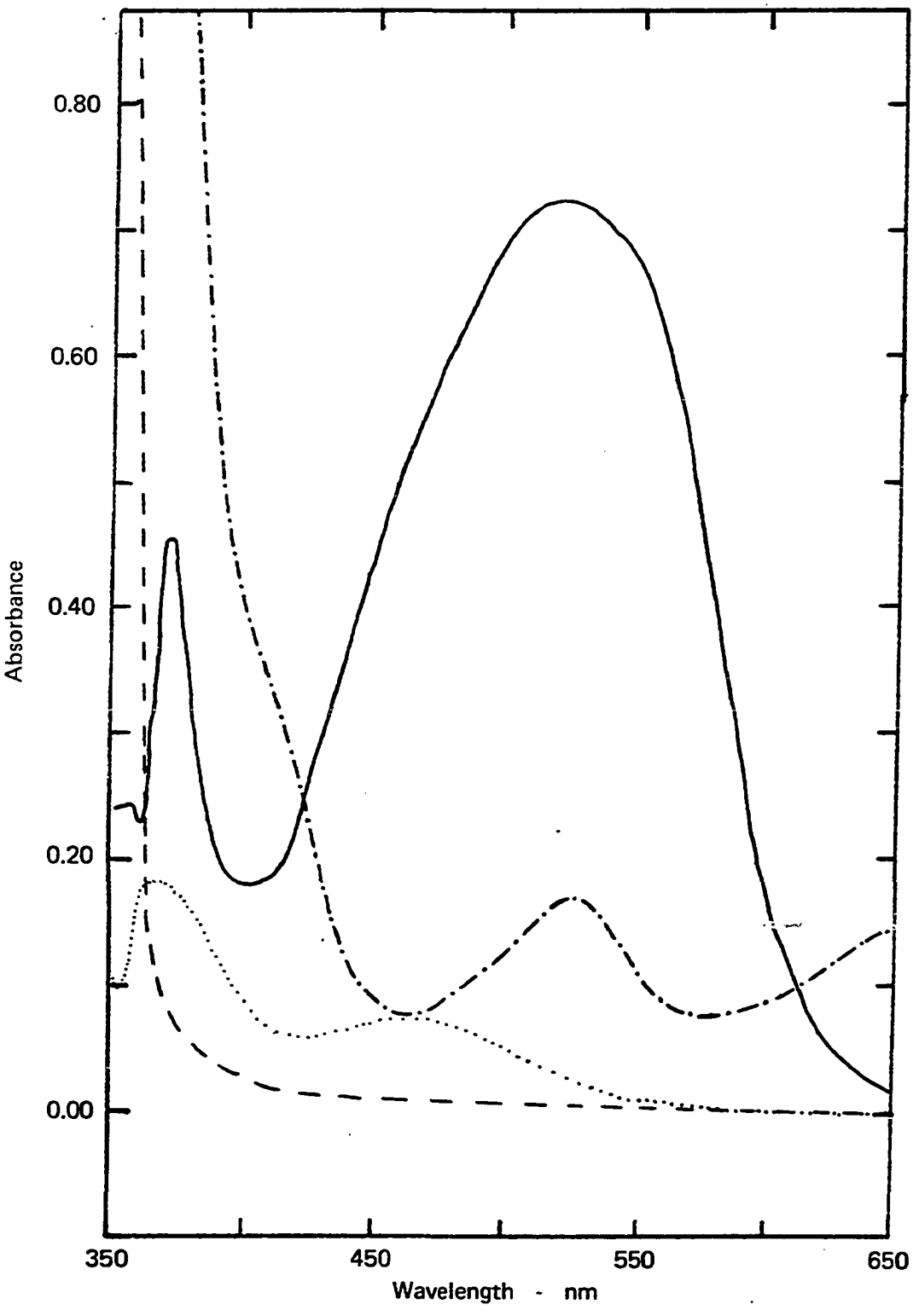
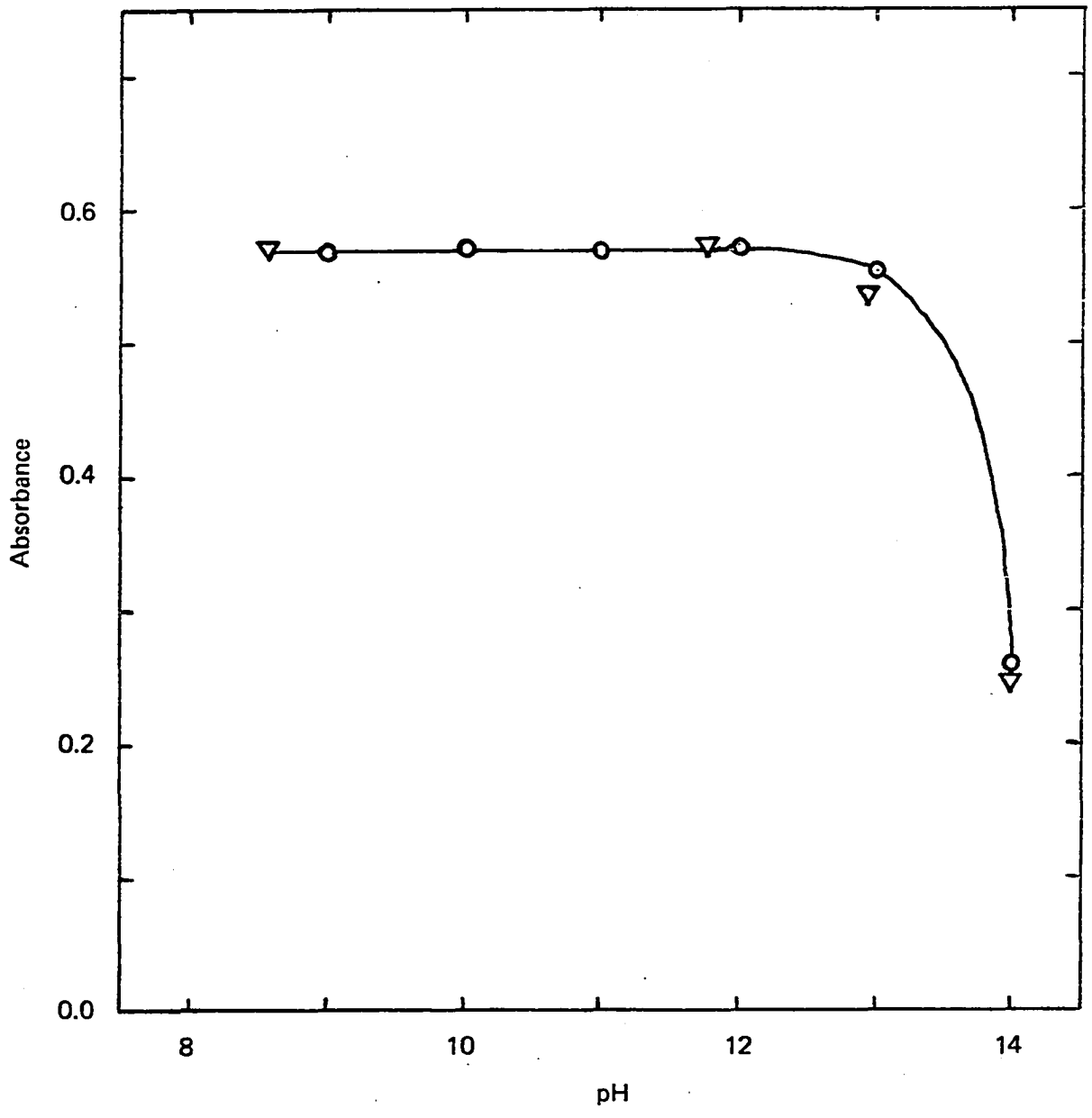

0.80
0.60
0.40
0.20
0.00
Absorbance
350
450
550
650
Wavelength - nm

Figure VIII-3. Absorbance of tris(4,7-dihydroxy-1,10-phenanthroline)iron(III) as a function of pH

O - Absorbance measured on same day as solutions were prepared

Δ - Absorbance measured two weeks after solutions were prepared


Absorbance
0.6
0.4
0.2
0.0
8
10
12
14
pH

A solution 2 M in ammonium hydroxide and 0.1 M in ammonium chloride was chosen to contain the reagent because both the oxidized and reduced forms of the iron-phenanthroline compound are stable and the molar absorptivities reproducible in it. To test the stability of the oxidation product in ammonia solutions and carbonate solutions, solutions containing equal concentrations of the ferrous compound plus sodium hydrosulfite were bubbled with oxygen until the red color disappeared, and the absorbance at 520 nm. was monitored over a 20-hour period. Figure VIII-4 shows that the oxidation product is stable in 0.5 to 4.0 M ammonium hydroxide plus 0.1 M ammonium chloride. Solutions containing the same concentration of iron in carbonate solutions gave higher absorbance readings which changed with time (Figure VIII-5). Solutions buffered with ammonium hydroxide (0.5 to 4.0 M) and ammonium chloride were chosen to contain the reagent on this basis.

Since ammonia is a volatile solute and it is necessary to purge the reagent solution with nitrogen, the loss of ammonia on purging a solution of ammonium hydroxide was investigated. One liter of a solution 1.0 M in ammonium hydroxide and 0.1 M in ammonium chloride was bubbled with nitrogen. At various time intervals aliquots were titrated in triplicate with standard hydrochloric acid, and the concentration of ammonia calculated, as presented in Table VIII-1. After two hours of bubbling with nitrogen,

Figure VIII-4. Stability of the oxidation product of the reaction of tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) with oxygen in ammonia buffers

Wavelength: 520 nm.

Concentration of iron: 5.00×10^{-5} M

Concentration of ammonium chloride: 0.1 M

Curve	Concentration of ammonium hydroxide
———	0.2 M
- - - - - - -	0.5 M
- • - • - • -	1.0 M
• • • • • • •	2.0 M
- • • - • • -	4.0 M

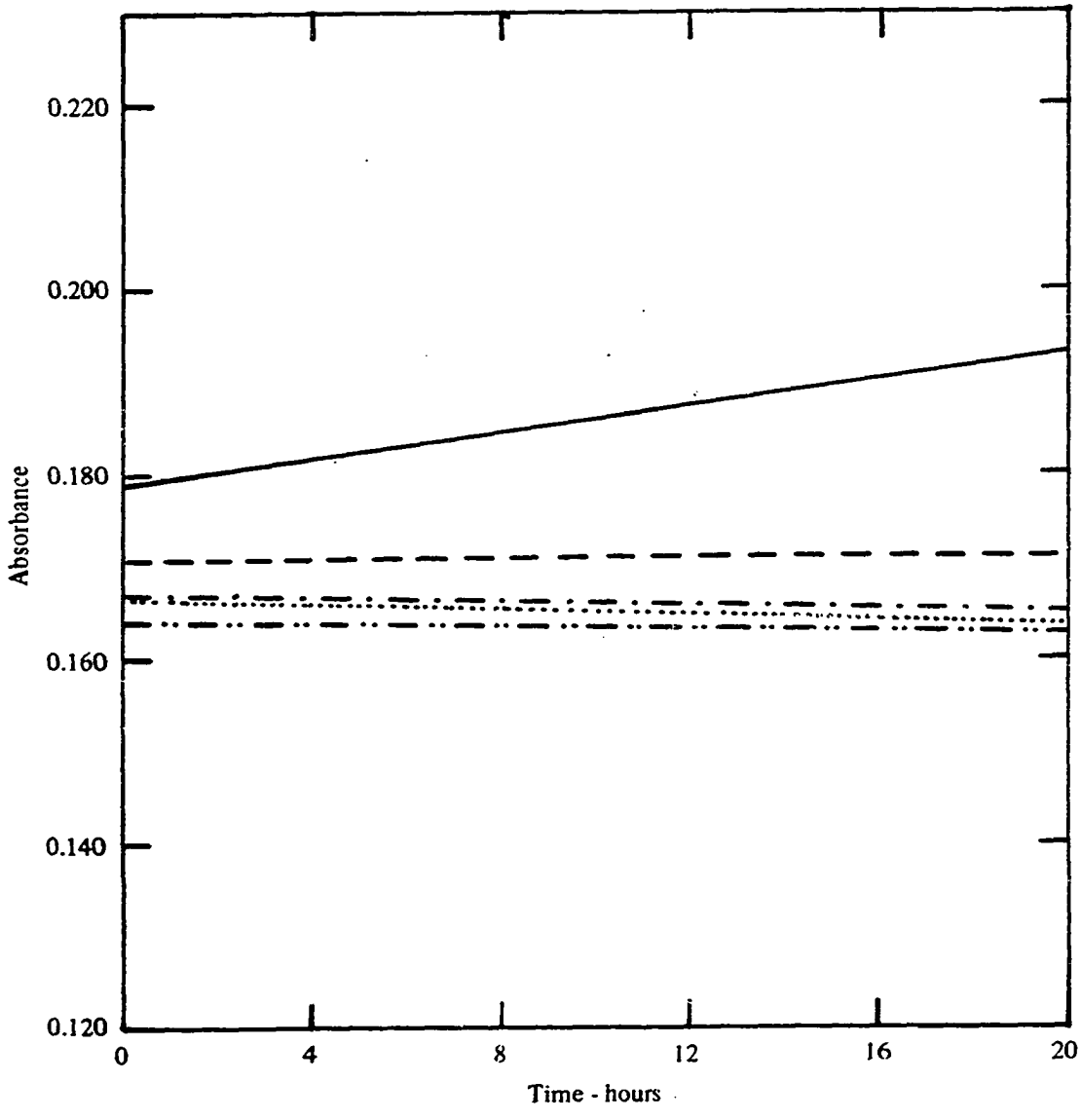

0.220
0.200
0.180
0.160
0.140
0.120
Absorbance
0
4
8
12
16
20
Time - hours

Figure VIII-5. Stability of the oxidation product from the reaction of tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) with oxygen in carbonate buffers

Wavelength: 520 nm.

Concentration of iron: 5.00×10^{-5} M

Concentration of potassium bicarbonate: 0.2 M

Curve	Concentration of potassium carbonate
———	0.2 M
- - - - - - -	0.5 M
-·-·-·-	1.0 M
·····	2.0 M

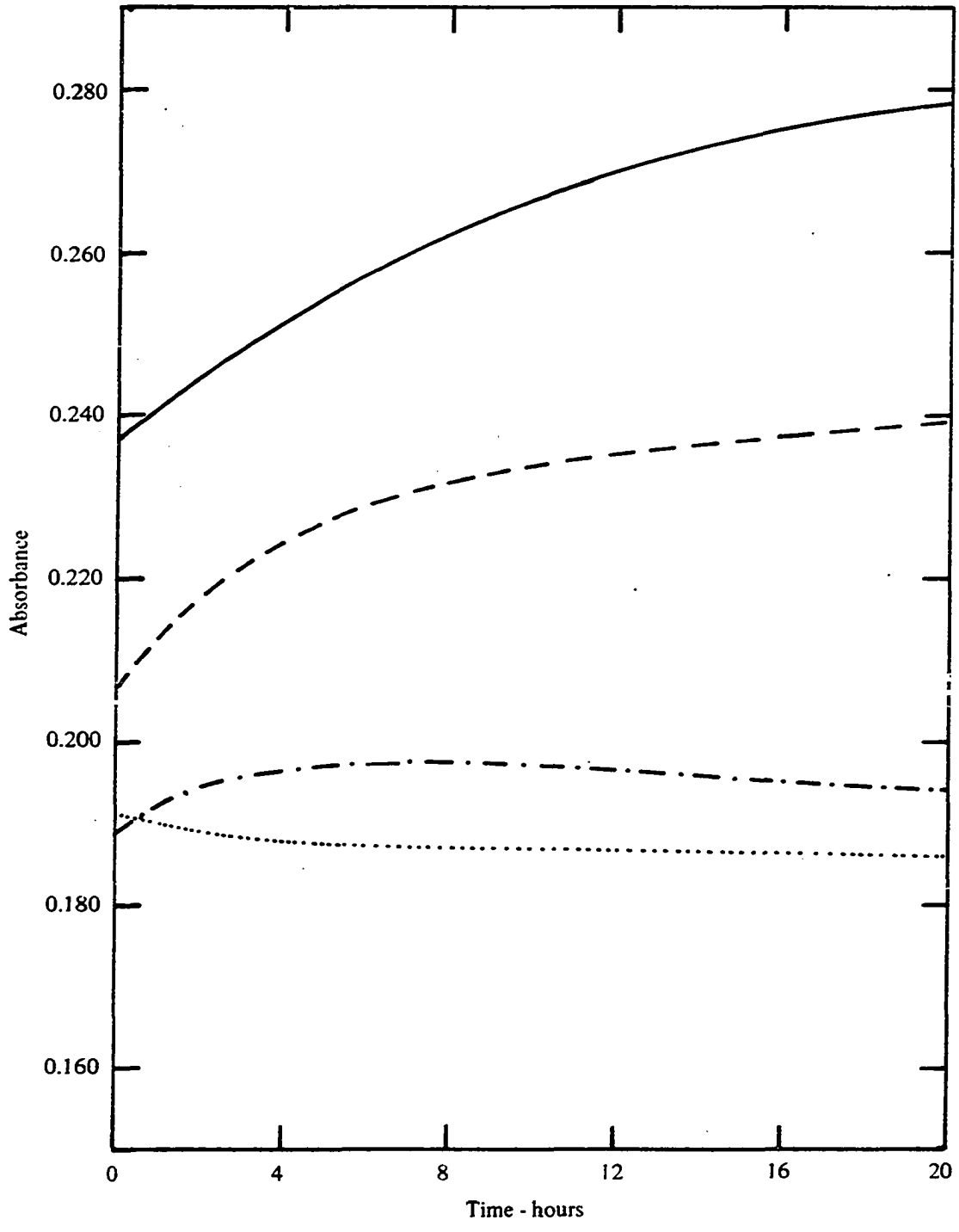

0.280
0.260
0.240
0.220
0.200
0.180
0.160
Absorbance
0
4
8
12
16
20
Time - hours

Table VIII-1. Loss of ammonia from a 1 M aqueous solution by bubbling with nitrogen

Temperature of room: 24°C

Atmospheric pressure: 754.0 mm. Hg

Flow rate of nitrogen: 10.0 cc./sec.

Normality of HCl: 0.0988

Time (min.)	Sample volume (ml.)	Volume of HCl (ml.)			Molarity of ammonium hydroxide (ave.)
0	4.00	39.85	40.01	40.14	0.988
15	4.00	39.71	39.92	39.81	0.983
60	4.00	39.09	38.89	38.90	0.963
120	4.00	38.00	37.81	37.69	0.935

the loss of ammonia was insignificant with respect to the requirements for obtaining a stable oxidation product.

Injection of a concentrated solution of ferrous ammonium sulfate into the solution containing the organic ligand resulted in the formation of a dense, gelatinous precipitate which could not be redissolved. Addition of tartrate to the solution prevented such precipitation. It was inferred from reference 60 that the amount of tartrate added would not interfere with the formation of tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) and this proved true.

The 1-ml. tuberculin syringe which was used to inject oxygen-containing samples into the system was calibrated by weighing 0.30-ml. portions of distilled water delivered from the syringe into clean, dry, weighed bottles with stoppers. The results, presented in Table VIII-2, indicate that the accuracy of the syringe was more than adequate.

Table VIII-2. Calibration of tuberculin syringe

Weight of 0.30 ml. of water (g.)
0.2981
0.3000
0.3000
0.3000
0.3005
0.3002
Average 0.2998

Standard deviation = 7.3×10^{-4} g. = 0.0007 ml.

Absorbance data obtained after injecting successive portions of water in equilibrium with air into the flow-through cell system charged as described above are reported in Figure VIII-6. Absorbance as measured was corrected for volume change using the relation $A_c = A_m(V_m/V_o)$, A_c

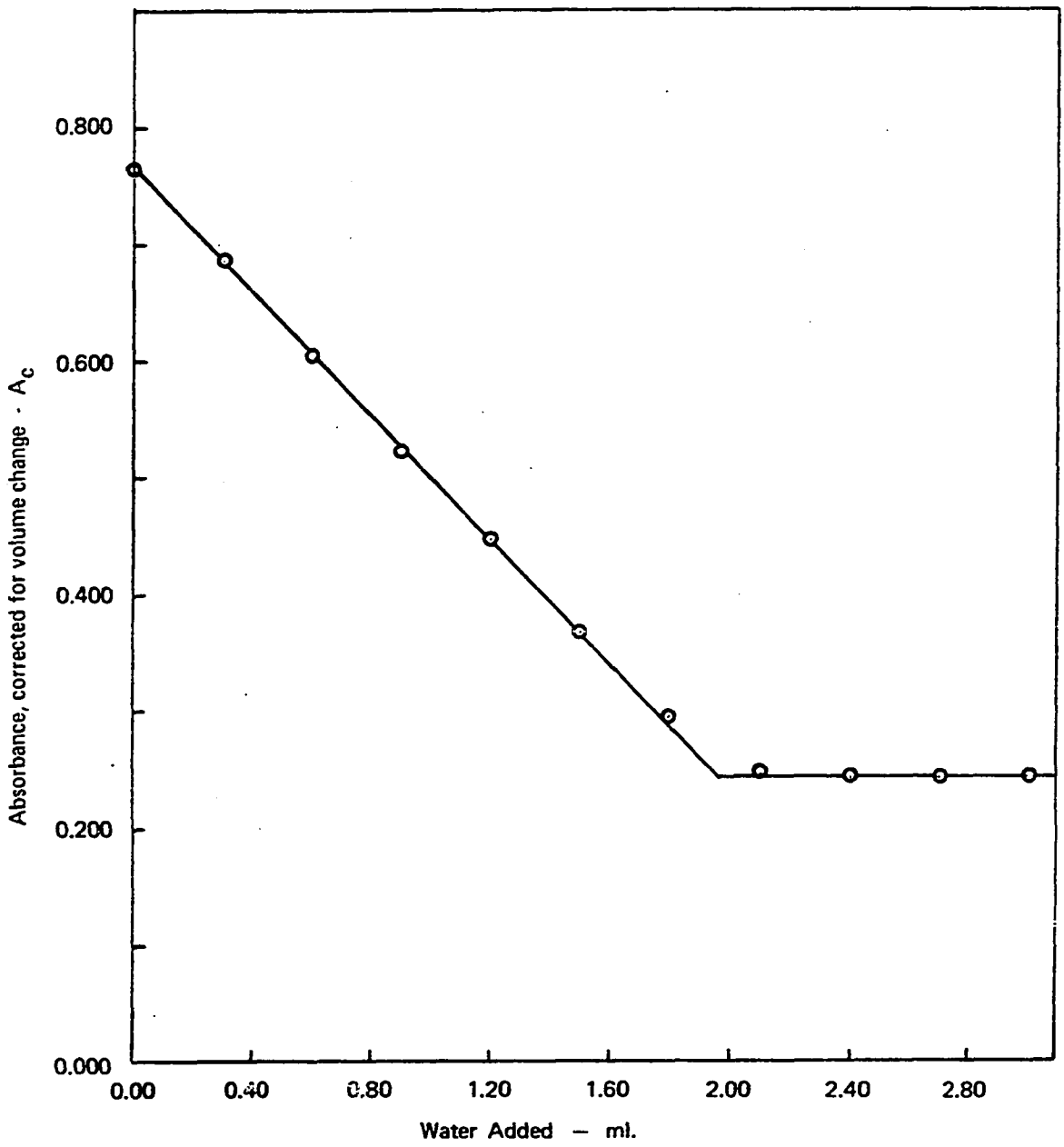
Figure VIII-6. Absorbance as a function of oxygen added as a solution in water in equilibrium with air. Absorbance corrected for volume change resulting from addition of liquid sample

Atmospheric pressure: 744 mm. Hg

Temperature: 26.5°

Initial volume: 40.6 ml.

Absorbance measured at 520 nm.


0.800
0.600
0.400
0.200
0.000
Absorbance, corrected for volume change - A_c
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80
Water Added — ml.

representing the corrected absorbance, A_m the absorbance measured, V_m the volume at the time of measurement, and V_o the initial volume.

Corrected absorbance was linear with oxygen added until the absorbance became very low, whereupon slightly high readings were obtained for A_c, the reaction being considerably slower in this region. No further change in A_c occurred with the addition of excess oxygen. The intersection of the two straight-line portions of the curve marked the equivalence point in the titration of the reagent with oxygen. This point, as calculated from the absorbance data and the molar absorptivities of the iron(II) and (gray) iron(III) compounds, occurred when one molecule of oxygen had been added per four atoms of iron oxidized:

$$4Fe(diOHphen)_3^{2+} + O_2 + 2H_2O = 4Fe(diOHphen)_3^{3+} + 4OH^-$$

The calculation was made assuming that the additivity relation

$$A_c = \varepsilon_{Fe(II)}l[Fe(II)] + \varepsilon_{Fe(III)}l[Fe(III)]$$

is valid (using [Fe(II)] and [Fe(III)] to represent the molar concentrations of the corresponding iron-phenanthroline compounds). Insertion of the end values and rearrangement (l = 1.00 cm) gives:

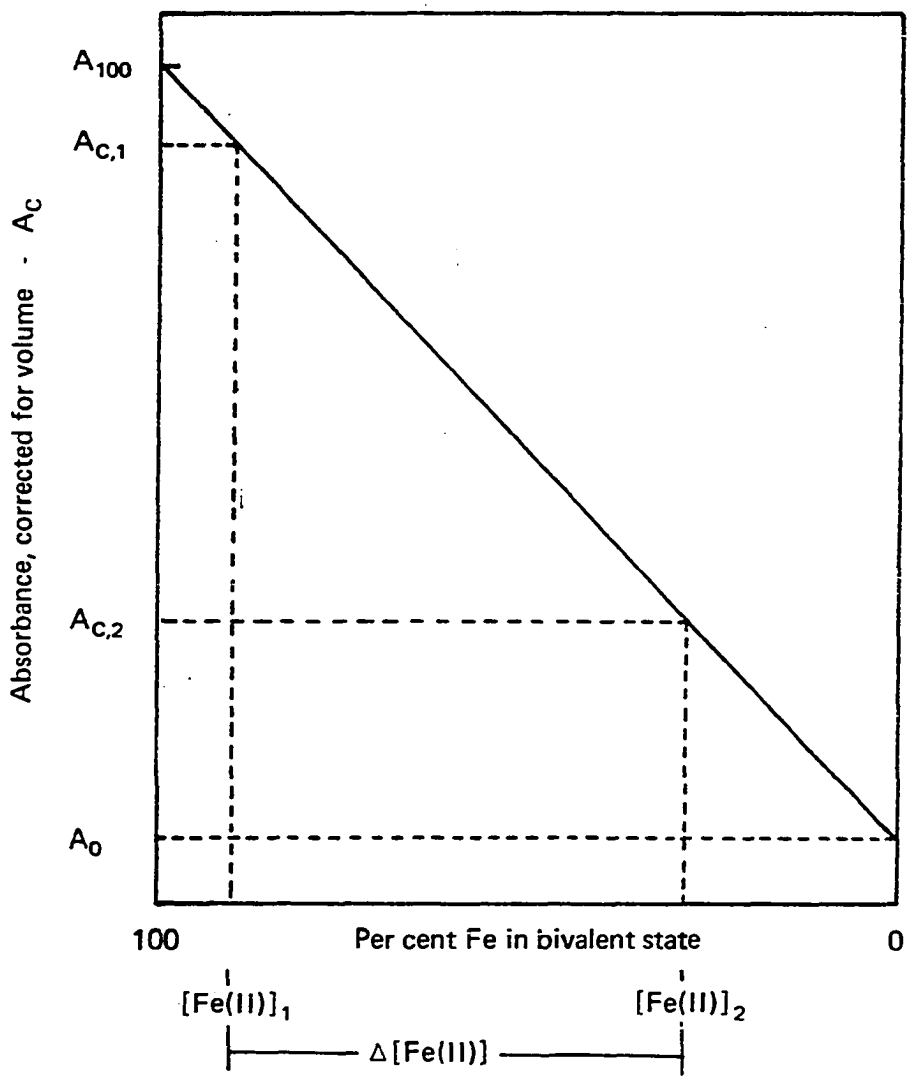
$$A_c = [Fe(II)](\varepsilon_{Fe(II)} - \varepsilon_{Fe(III)}) + \varepsilon_{Fe(III)}[Fe(II)]_{100}$$

(This is essentially merely linear interpolation; Figure VIII-7). The change in the concentration of iron(II) is given

Figure VIII-7. Linear interpolation of the absorbance of solutions containing the iron(II) and iron(III) derivatives of 4,7-dihydroxy-1,10-phenanthroline

A_{100}, absorbance when all of the iron is in the ferrous form, = $\varepsilon_{Fe(II)}[Fe(II)]$

A_o, absorbance when all of the iron has been oxidized, = $\varepsilon_{Fe(III)}[Fe(II)]_{100}$


A100
Ac,1
Ac,2
A0
Absorbance, corrected for volume - Ac
100
Per cent Fe in bivalent state
0
[Fe(II)]1
[Fe(II)]2
Δ[Fe(II)]

by:

$$[Fe(II)] = (A_{c,1} - A_{c,2})/(\varepsilon_{Fe(II)} - \varepsilon_{Fe(III)})$$

In general, very little iron(III) was present initially and $A_{c,1}$ was close to A_{100}. The data given in Figure VIII-6, using the initial and cut-off points, give [Fe(II)] = 4.55×10^{-5}; the initial volume being 0.0406 liter, the moles of iron changed was 1.85×10^{-6}.

The concentration of oxygen in the water (air-saturated at 26.5°C, 744 mm. Hg) was determined using the azide modification of the standard Winkler method (1) to be 7.60 ppm. The cut-off point fell at 1.96 ml. of water added and corresponding to 0.456×10^{-6} moles of oxygen. Thus, the ratio of moles of iron(II) changed to moles of oxygen was 3.98. Considering the precision of the spectrophotometric method, it may be reasonably concluded that four moles of tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) react quantitatively with one mole of oxygen.

In using the system for the determination of oxygen, the oxygen in ppm is given by:

$$O_2 \text{ in ppm} = \frac{(A_{m,1}V_{m,1} - A_{m,2}V_{m,2})(32.0)(10^3)}{(11{,}500)(4)(V_s)}$$

V_s being the volume of the oxygen-bearing sample added.

The effects of iron (ferrous and ferric) in the sample were determined. Ferric iron (20 ppm) caused no significant

interference. Ferrous iron did interfere significantly. On a sample containing 20 ppm iron(II) the spectrophotometric method gave 3.72 ppm O_2; the standard Winkler method, azide modification, gave 4.94 ppm O_2; the distilled water sample before the addition of iron as ferrous sulfate contained 7.99 ppm O_2.

The method was compared to the standard Winkler method, azide modification, for the determination of dissolved oxygen in distilled water, water from Lake LaVerne (on the ISU campus), and water from the Ames Municipal Water Works (Table VIII-3). Excellent agreement was obtained on distilled water and water from Lake LaVerne. The negative values obtained for the non-aerated water from Ames Water Works was a result of ferrous iron in the water as it comes from the wells. The discrepancy of about 0.5 ppm between the results by the proposed and the standard method for the aerated (but otherwise untreated) water was undoubtedly caused by ferrous iron still present. The discrepancy found on the finished water is left unexplained; curiously it was just about equal to the residual chlorine present.

No attempt was made to determine the effects of possible interferences other than iron. However, mild oxidizing agents, which interfere in the Winkler method (1,55,74) and in a more sensitive method using indigo carmine (9,45) may be assumed to interfere. The accuracy of the method in the

Table VIII-3. Comparison of spectrophotometric method with standard Winkler method, azide modification

	Oxygen in parts per million	
	Spectrophotometric method	Winkler method
Distilled water, air-saturated at 26.3°C, 736 mm. Hg	7.54 7.43 7.55	7.53 7.54 7.57
Lake LaVerne water, 5°C	12.21 11.86 11.75	12.10 12.52 12.02 11.96
Ames Water Works		
Nonaerated water	-0.54 -0.57	0.00 0.00
Aerated water	6.61 6.44	6.94 7.02 7.01 6.93
Finished water	7.23 7.53	7.97

absence of interferences is good, being limited only by the accuracy with which the sample is measured and by the accuracy of the spectrophotometric measurement, the latter yielding the greater errors. Very small volumes of sample are required (less than 1 ml. for water in equilibrium with

air), and in this respect the method is superior to the Winkler method, which requires samples on the order to 200-300 ml.

C. Use of Evacuated Ampoules for the Determination of Dissolved Oxygen

The idea of using evacuated ampoules containing an oxygen-sensitive reagent for the colorimetric determination of dissolved oxygen is not new[1]. The possibility of using evacuated Spectronic 20 cells containing solid tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) salts plus a water-soluble alkali was investigated.

1. Experimental work

Solid tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) sulfate (Preparation 1, Part V) weighing 0.0273 g. was thoroughly ground and mixed with 4.8082 g. of potassium carbonate one-and-one-half hydrate which had been passed through a 70-mesh sieve. Samples of this mixture weighing 100 (±2) mg. were placed in Spectronic 20 tubes with glass beads. Rubber stoppers (No. 00) in which had been inserted 4-inch lengths of 4-mm. Pyrex tubing were fitted tightly

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[1] CHEMetrics, Inc., 3 Brentwood Circle, Nitro, West Virginia 25143, manufactures disposable vacuum-reagent ampoules, CHEMets, for colorimetric determinations of dissolved oxygen and phenols in wastewater.

in the ends of the Spectronic 20 tubes. The ampoules were evacuated to <0.30 mm. Hg and refilled with nitrogen three times, and finally were evacuated a fourth time. The glass tubing was sealed with a flame. The glass tube was submerged in deaerated water and broken off near the stopper. The Spectronic 20 tube filled with a turbulent rush of water and the reagent dissolved instantly. After several inversions of the tube the absorbance at 520 nm. was measured on a Bausch and Lomb Spectronic 20 spectrophotometer by inserting the reagent ampoule in the cell holder.

2. Results and discussion

Absorbance readings obtained for deaerated water are presented in Table VIII-4. Absorbance values were much lower

Table VIII-4. Results obtained on filling evacuated reagent ampoules with deaerated water

Ampoule No.	Absorbance after elapsed time			Absorbance after addition of sodium hydrosulfite
	0 min.	15 min.	30 min.	
1	0.210	0.152		0.76
2	0.240	0.202		0.75
3	0.272	0.228		0.79
4	0.262	0.230	0.219	
5	0.317	0.289	0.192 (after shaking)	0.75
6	0.313			0.75

than the calculated value of about 1.0, based on the amount of reagent used and assuming no oxidation of the solid reagent. Results were also highly variable, and absorbance decreased with time. Addition of an excess of sodium hydrosulfite brought the absorbance up close to the expected value.

The data collected indicate that not all oxygen was excluded from the reagent ampoules, or that part of the solid reagent was oxidized before use. The presence of a small bubble in every water-filled ampoule was indicative of incomplete evacuation. Very small leaks around the rubber stopper would be enough to affect the results radically. In addition to these technical difficulties, a major shortcoming of the method is that the reagent must be weighed out accurately so that a reproducible absorbance is obtained in the absence of oxygen. After considering the expense and technical difficulties involved in improving the method, it was decided to drop this approach.

IX. REMOVAL OF AND DETERMINATION OF IRON IN CONCENTRATED SOLUTIONS OF SODIUM HYDROXIDE

When Schilt, Smith, and Heimbuch first introduced the use of 4,7-dihydroxy-1,10-phenanthroline as a reagent for the colorimetric determination of iron in strong alkali, they noted that commercial grades of sodium hydroxide invariably contain appreciable amounts of iron, and it is preferable to use the reagent in solution form in carefully measured amounts and to correct for the iron in these solutions by carrying through a blank determination (60). They also noted that iron may be removed from solutions of sodium hydrosulfite by extraction as tris(4,7-diphenyl-1,10-phenanthroline)-iron(II) into n-hexyl alcohol, that is, as the ferrous derivative of bathophenanthroline.

In the present investigation it was found that the sodium hydrosulfite commercially available from the J. T. Baker Chemical Company and the Hach Chemical Company contained only a small amount of iron, so small that the extraction step was unnecessary if the reagent was added in carefully measured amounts. The sodium hydroxide from the J. T. Baker Chemical Company contained large amounts of iron, so that even with careful control of the volume of sodium hydroxide used the blank was too large to permit a satisfactory analysis.

It has been shown by Trusell and Diehl that phenyl-2-pyridyl ketoxime can be used to extract iron from solutions greater than 1 M in sodium hydroxide into a mixture of isoamyl alcohol and ethyl alcohol (70). The work described below shows that iron can also be removed from concentrated solutions of sodium hydroxide by extraction of the ferrous derivative into an immiscible solvent.

A. Recommended Procedures

1. Recommended procedure for removal of iron from solutions of alkalies

To 500-1500 ml. of 10 to 16 M sodium hydroxide in a 2-liter separatory funnel, add 50 ml. of dimethylsulfoxide, 5 ml. of 0.01 M 4,7-dihydroxy-1,10-phenanthroline, and about 0.5 g. of sodium hydrosulfite. Shake vigorously and drain off the lower aqueous phase. Discard the upper phase. Repeat the extraction without the addition of sodium hydrosulfite. Finally, perform the extraction once more using dimethyl-sulfoxide only.

2. Procedure for the simultaneous estimation of iron in sodium hydroxide and sodium hydrosulfite

To two solutions of 1 to 10 M sodium hydroxide add 1.0 g. and 2.0 g. of sodium hydrosulfite, respectively. Add 2.00 ml. of 0.01 M 4,7-dihydroxy-1,10-phenanthroline to each solution, and dilute to exactly 100 ml. with the 1 to 10 M

sodium hydroxide. Measure the absorbance of each solution at 520 nm. in a 1.00-cm. cell against a blank of the 1 to 10 M sodium hydroxide. Extrapolate the absorbance to zero grams of sodium hydrosulfite and calculate the concentration of iron in the 1 to 10 M sodium hydroxide from the Beer-Lambert law, using 14,800 as the molar absorptivity if a spectrophotometer with moderately high resolution such as the Beckman DU is used. The use of low-resolution instruments such as filter photometers requires an independent determination of the molar absorptivity of tris(4,7-dihydroxy-1,10-phenanthroline)iron(II). Calculate the concentration of iron in the sodium hydrosulfite from the difference in the absorbances produced by the solutions containing 1 g. and 2 g. of sodium hydrosulfite, respectively.

B. Results and Discussion

Solutions of sodium hydroxide, following extraction of iron into dimethylsulfoxide, contained no iron detectable by addition of sodium hydrosulfite and 4,7-dihydroxy-1,10-phenanthroline. Some dimethylsulfoxide remained in the aqueous phase, as evidenced by its strong odor. Plots of absorbance vs. concentration of iron showed no deviation from Beer's law using the method of Schilt, Smith, and Heimbuch and iron-free sodium hydroxide containing dimethylsulfoxide.

A number of other immiscible solvents were tried with generally little success. No iron was extracted into non-polar solvents such as hexane, chloroform, and methyl-isobutyl ketone, and separation of the phases was poor. Precipitation of the sodium hydroxide occurred when alcohols containing three or more carbons were used. The iron-dihydroxyphenanthroline compound was extracted into ethanol, but a greater number of extractions was required to remove all of the iron from the sodium hydroxide than when dimethyl-sulfoxide was used. Removal of iron was complete after two extractions with ethyl cellusolve (2-ethoxyethanol), but a yellow color developed in the aqueous phase.

Results obtained for the simultaneous estimation of iron in sodium hydroxide (J. T. Baker Chemical Company) and sodium hydrosulfite (Hach Chemical Company) are presented in Table IX-1. Identical results were obtained for sodium hydrosulfite

Table IX-1. Iron in reagent sodium hydroxide and sodium hydrosulfite

Molar Concentration of Sodium Hydroxide	Weight of Sodium Hydrosulfite (g.)	Absorbance at 520 nm.
1.0	1.00 g.	0.030 0.030
1.0	2.00 g.	0.034 0.034

received from J. T. Baker Chemical Company. By extrapolation to zero grams of sodium hydrosulfite, the absorbance due to iron in 1.0 M sodium hydroxide was found to be 0.026, corresponding to a concentration of iron of 1.8×10^{-6} M, or 0.10 part per million. Sodium hydrosulfite produced an absorbance of 0.004 per gram of sodium hydrosulfite in 100 ml. of solution. Since the amount of sodium hydrosulfite called for in the procedure for the determination of iron as outlined by Schilt, Smith, and Heimbuch is 1.6 g. per 100 ml. of solution, the absorbance due to the iron in this reagent may be easily compensated for by adding it in solution form in carefully measured amounts, and by carrying through a blank determination. The absorbance produced by the iron in sodium hydroxide is much greater, and the use of iron-free sodium hydroxide is recommended for the most accurate results.

X. REACTION OF 4,7-DIHYDROXY-1,10-PHENANTHROLINE WITH FORMALDEHYDE

The investigations of the chemistry and applications of 4,7-dihydroxy-1,10-phenanthroline which are described in this dissertation were begun with the objective of incorporating the iron(II) and iron(III) derivatives of 4,7-dihydroxy-1,10-phenanthroline into a phenol-formaldehyde resin in an effort to make an ion selective, membrane electrode. The initial objective has been obscured in the work of clearing up the chemistry of 4,7-dihydroxy-1,10-phenanthroline and of following up promptly the possibilities which the work offered of making useful applications to chemical analysis. That the initial proposal was at least feasible is shown in this part of this dissertation in which I show that 4,7-dihydroxy-1,10-phenanthroline does react with formaldehyde and therefore can very well participate along with phenol in making a bakelite-type resin.

A. Experimental Work

1. Recording of spectra

Infrared spectra were obtained by the KBr-pellet technique and using a Beckman IR-18A infrared spectrophotometer.

NMR spectra were obtained using a Perkin-Elmer Hitachi R-20B 60-MHz spectrometer, or a Varian Aerograph A-60 60-MHz spectrometer. Tier's salt was used as an internal reference.

2. Reaction of 4,7-dihydroxy-1,10-phenanthroline with formaldehyde

In a 100-ml. boiling flask was placed 0.4 g. of 4,7-dihydroxy-1,10-phenanthroline and 5 ml. of 37 percent formaldehyde. Sodium hydroxide (1 M) was added dropwise until the phenanthroline dissolved. The flask was fitted with a water-cooled condensor and heated in an oil bath at 85° for two hours. A yellow solid formed during the reaction. The reaction mixture was transferred to a 600-ml. beaker containing 100 ml. of water, heated almost to boiling, and acidified with 2 M hydrochloric acid, as indicated by pH paper. The light yellow solid was collected on a medium-porosity, Pyrex, Buchner funnel, transferred to a watch glass, and heated at 115° for 30 minutes. The compound did not melt below 300°.

3. Reaction of tris(4,7-dihydroxy-1,10-phenanthroline)-iron(II) with formaldehyde

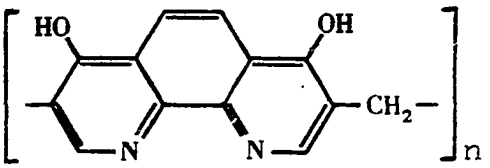
A mixture of 2 ml. of 37 percent aqueous formaldehyde and 0.18 g. of tris(4,7-dihydroxy-1,10-phenanthroline)-iron(II) (Preparation 1, Part V) was heated with stirring in a nitrogen atmosphere in a 10-ml. pear-shaped flask with

reflux condenser. The mixture was maintained at 70° overnight, after which time some solid still remained. Several drops of ammonia were added, and the reaction was discontinued after six hours of heating at 120°. The reflux condensor was removed and the black mixture was heated at 65° for three hours. The temperature was then slowly increased to 125° over a period of ten hours, and heating at 125° was continued overnight.

About 0.10 g. of the solid black residue was dissolved in alkaline cyanide solution, reprecipitated by addition of HCl, and filtered as a finely crystalline yellow material. An NMR spectrum of the solid dissolved in a solution of sodium deuteroxide in deuterium oxide showed no absorption. The reprecipitation was repeated, adding hydroxylamine hydrochloride before adding hydrochloric acid. The NMR spectrum of the recrystallized material showed some absorption.

B. Results and Discussion

The reaction of phenol with formaldehyde results in the polymer bakelite in which the phenol molecules are joined into a chain with methylene linkages ortho and para to the hydroxy groups. 4,7-Dihydroxy-1,10-phenanthroline would be expected to react with formaldehyde in the same manner, the product being:



The infrared spectrum of 4,7-dihydroxy-1,10-phenanthroline has been reported and interpreted by Smith (65). The infrared spectrum of the product of the reaction of 4,7-dihydroxy-1,10-phenanthroline with formaldehyde obtained in this work differed significantly from that of the starting material, especially in the 1000-1650 cm^{-1} region. In particular, the band at 1610 cm^{-1} in the spectrum of 4,7-dihydroxy-1,10-phenanthroline (due to C-C ring vibrations) appeared at lower energy (1580 cm^{-1}, broad) in the spectrum of the reaction product. Bands at 2853 and 2926 cm^{-1}, due to stretching of the methylene C-H bond, were not detected. This does not, however, mean that the desired product was not obtained, because this portion of the spectrum is dominated by absorption due to N-H stretching of the immonium ion (65, page 146).

Absorption data from the NMR spectra of the products from the reaction of formaldehyde with 4,7-dihydroxy-1,10-phenanthroline and with tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) are presented in Table X-1. Assignments of chemical shifts were referred to water at 4.70 ppm (δ).

Table X-1. NMR absorption data of the products of reactions of formaldehyde with dihydroxyphenanthrolines.

Compound + Formaldehyde	δ,ppm	Type	Estimated relative intensity	Assignment
4,7-Dihydroxy-1,10-phenanthroline	4.60	singlet	1	methylene
	7.95	singlet	1	5,6-
	8.37	singlet	1	2,9-
	8.25	singlet	0.3	?
Tris(4,7-dihydroxy-1,10-phenanthroline)-iron(II)	2.8	broad		methylene
	7.7	broad		?

A doublet at 6.64 ppm occurs in the NMR spectrum of 4,7-dihydroxy-1,10-phenanthroline; this peak is assigned to the protons at positions 3,8-, Table III-2. The disappearance of the doublet in the spectra of the reaction products, and the appearance of singlets at 4.60 ppm in the product of the reaction of formaldehyde with 4,7-dihydroxy-1,10-phenanthroline indicate substitutions at positions 3- and 8- on the phenanthroline rings. The singlet at 4.60 ppm seems to be reasonable for a methylene linkage between two phenanthroline molecules.

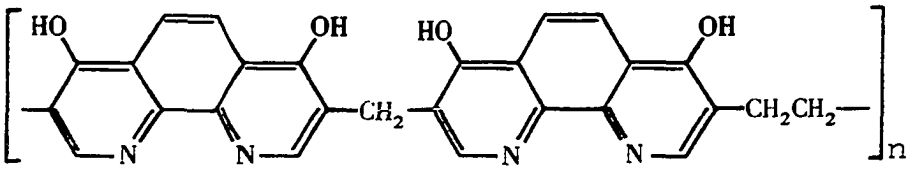
Results of analyses for carbon, hydrogen and nitrogen of two preparations of the product of the reaction of 4,7-dihydroxy-1,10-phenanthroline with formaldehyde (Galbraith

Laboratories, 2323 Sycamore Drive, Knoxville, Tennessee 37921) are presented in Table X-2. The empirical formulae were calculated on the basis that oxygen made up the balance of the molecule, and that there were exactly two nitrogen atoms per phenanthroline entity.

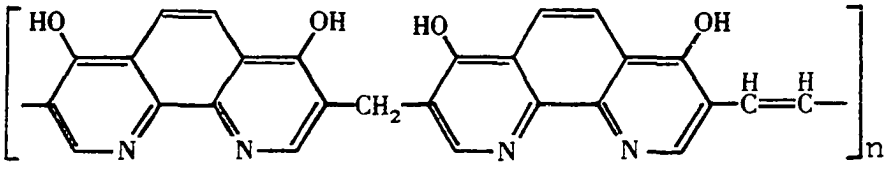
Table X-2. Analysis of the product of the reaction of 4,7-dihydroxy-1,10-phenanthroline with formaldehyde

Preparation	Percent C	Percent H	Percent N	Empirical formula
1	61.96	4.19	10.65	$C_{13.58}H_{11.03}N_{2.00}O_{3.82}$
2	64.02	3.95	11.12	$C_{13.44}H_{9.95}N_{2.00}O_{3.29}$
Calculated for 4,7-dihydroxy-1,10-phenanthroline	67.92	3.77	13.21	$C_{12}H_8N_2O_2$
Calculated for $(-4,7\text{-diOHphen-}CH_2-)_n$	69.64	3.57	12.50	$C_{13}H_8N_2O_2$

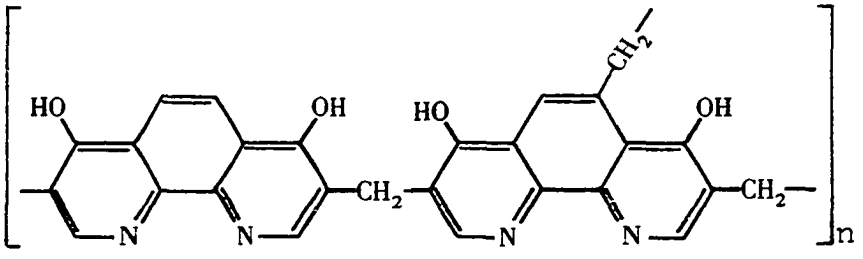
The result that there are 13.5 carbon atoms per unit is surprising. Several possible structures corresponding to this result are shown below.



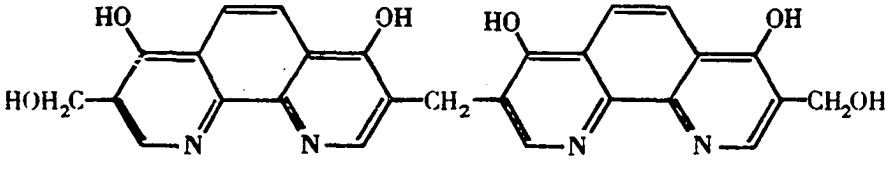
I $C_{13.5}H_9N_2O_2$



II $C_{13.5}H_8N_2O_2$



III $C_{13.5}H_{8.5}N_2O_2$



IV $C_{13.5}H_{10}N_2O_3$

Assuming that some water was present, the empirical formulae of preparations 1 and 2 may be rewritten

Preparation 1: $C_{13.58}H_{7.39}N_{2.00}O_{2.00}\cdot 1.82H_2O$

or $C_{13.58}H_{9.39}N_{2.00}O_{3.00}\ 0.82H_2O$,

Preparation 2: $C_{13.44}H_{7.37}N_{2.00}O_{2.00}\cdot 1.29H_2O$

or $C_{13.44}H_{9.37}N_{2.00}O_{3.00}\cdot 0.29H_2O$

Structure IV appears to be the best fit with respect to its empirical formula, but there is no evidence in the infrared spectrum of O-H stretching, and only one peak in the NMR spectrum occurs in the range expected for methylene protons, whereas two such peaks would be expected for structure IV. Of structures I, II, and III, structure II has the best-fitting empirical formula on the basis of the number of hydrogens present. However, since percent oxygen was obtained by difference, and removal of oxygen from the formula as water also involves removal of hydrogen, a choice between structures I, II, and III is difficult to make.

There appears little doubt that 4,7-dihydroxy-1,10-phenanthroline reacts with formaldehyde.

XI. SULFONATION OF 4,7-DIHYDROXY-1,10-PHENANTHROLINE

There are many examples in the chemical literature where sulfonation of an organic compound has resulted in increased solubility of the compound in water. One particular case involves the sulfonation of bathophenanthroline. The insolubility of bathophenanthroline prevents direct measurement of the color produced with ferrous iron in aqueous solutions. The problem can be overcome by extracting the ferrous derivative into isoamyl alcohol (with concomitant increase in sensitivity because of the increase in concentration made possible by the smaller volume of the isoamyl alcohol). Workers performing routine analyses for very small amounts of iron desire a highly sensitive reagent like bathophenanthroline, but prefer to skip the time-consuming extraction step. The synthesis of bathophenanthrolinedisulfonic acid by Trinder (69), and improvements by Blair and Diehl (4) and Cryberg and Diehl (20) provided such workers with a highly sensitive reagent for iron which is very soluble in aqueous solutions.

The insolubility of 4,7-dihydroxy-1,10-phenanthroline below pH 9 prohibits its use as a reagent for the determination of iron in acid solution, and, as the iron derivative, as an oxidation-reduction indicator in acidic solutions. An acid-soluble derivative of 4,7-dihydroxy-1,10-phenanthroline would quite possibly extend the use of

phenanthroline indicators in acidic solutions to include titrations in which an indicator having a low reduction potential is necessary. In the present work the sulfonation of 4,7-dihydroxy-1,10-phenanthroline was attempted. As hoped, reaction with hot, fuming sulfuric acid produced an acid-soluble sulfonic acid derivative of 4,7-dihydroxy-1,10-phenanthroline.

A. Synthesis

To 0.75 g. of 4,7-dihydroxy-1,10-phenanthroline in a 100-ml. round-bottom flask with a standard-taper fitting was added 10 ml. of fuming sulfuric acid (20 percent sulfur trioxide). The flask was fitted with a water-cooled condenser and heated in an oil bath at 130° for 12 hours. The cool flask was immersed in an ice bath and the contents neutralized by the dropwise addition of concentrated ammonium hydroxide. The solution was acidified with hydrochloric acid, diluted to about 50 ml. with deionized water, and filtered to remove any insoluble material. Sodium hydroxide was added to the filtrate until a pH of 9 was reached, the solution was heated to boiling, and 0.5 M barium chloride was added until no further precipitation of barium sulfate occurred. The barium sulfate and barium carbonate were filtered off and the filtrate was passed through a 4.5 x 60 cm. glass column containing 500 ml. of wet Amberlite IR-120 strong-acid cation exchange resin,

20-50 mesh, in the hydrogen form. The eluate was collected until 2 ml. of the eluate added to 5 ml. of 5 M sodium hydroxide containing sodium hydrosulfite and ferrous iron no longer developed a red color. The bulk of the eluate was evaporated by heating on an electric hot plate while stirring and bubbling nitrogen through the solution, and the last 50 ml. was evaporated to dryness on a steam-heated hot plate. The solid residue of sulfonated material weighed 1.03 g.

B. Characterization

1. Properties

The sulfonated material was a tan, crystalline solid that did not melt below 300°. It was dried at 110-120° for two hours without apparent decomposition. It was very soluble in water at any pH, the solubility being increased by the addition of alkali.

2. Infrared spectrum

Infrared spectra of 4,7-dihydroxy-1,10-phenanthroline and of the sulfonated material were obtained by the KBr-pellet technique using a Beckman IR-18A infrared spectrophotometer.

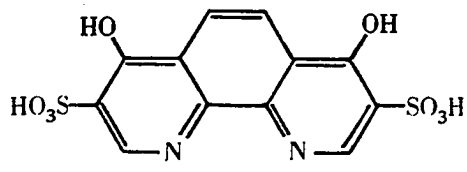
Two strong bands in the infrared spectrum of the sulfonated material were found at 1200 cm^{-1} (8.4 μ, broad) and 1050 cm^{-1} (9.6 μ, sharp). Bands at these positions were

reported for bathophenanthrolinedisulfonic acid and bathocuproinedisulfonic acid (20). Nakanishi (51) states that the sulfonic acid group produces strong absorption in the regions 1260-1150 cm^{-1} (asymmetric stretching of the $-SO_2-$ group) and 1080-1010 cm^{-1} (symmetric stretching of the $-SO_2-$ group). The bands are not present in the infrared spectrum of 4,7-dihydroxy-1,10-phenanthroline. In addition, moderately strong absorption was found in the O-H stretching region in the spectrum of the sulfonated material (3580 cm^{-1}, sharp; 3430 cm^{-1}, broad). These bands do not appear in the infrared spectrum of 4,7-dihydroxy-1,10-phenanthroline.

3. NMR spectrum

The NMR spectrum of a solution of the sulfonated material in deuterium oxide containing sodium deuteroxide was obtained on a Perkin-Elmer Hitachi R-20B NMR spectrometer, a 60 MHz instrument. The water peak at δ = 4.70 ppm was used as a reference.

The sulfonated material produced two singlets of equal intensity at δ = 7.99 ppm and 8.78 ppm. By analogy to the NMR spectrum of 4,7-dihydroxy-1,10-phenanthroline, Figure III-3 and Table III-2, these peaks are produced by absorption by protons at positions 5,6- and 2,9-, respectively, which leads to the structure of the sulfonated material



in which at least one of the protons from the sulfonic acid groups is attached to a ring nitrogen atom.

Evidence based on the procedure followed in the synthesis, the infrared spectrum, and the NMR spectrum lead directly to the conclusion that the sulfonated material is 4,7-dihydroxy-1,10-phenanthroline-3,8-disulfonic acid, but titration data and the mass spectrum indicate other possibilities.

4. Acidimetric titration

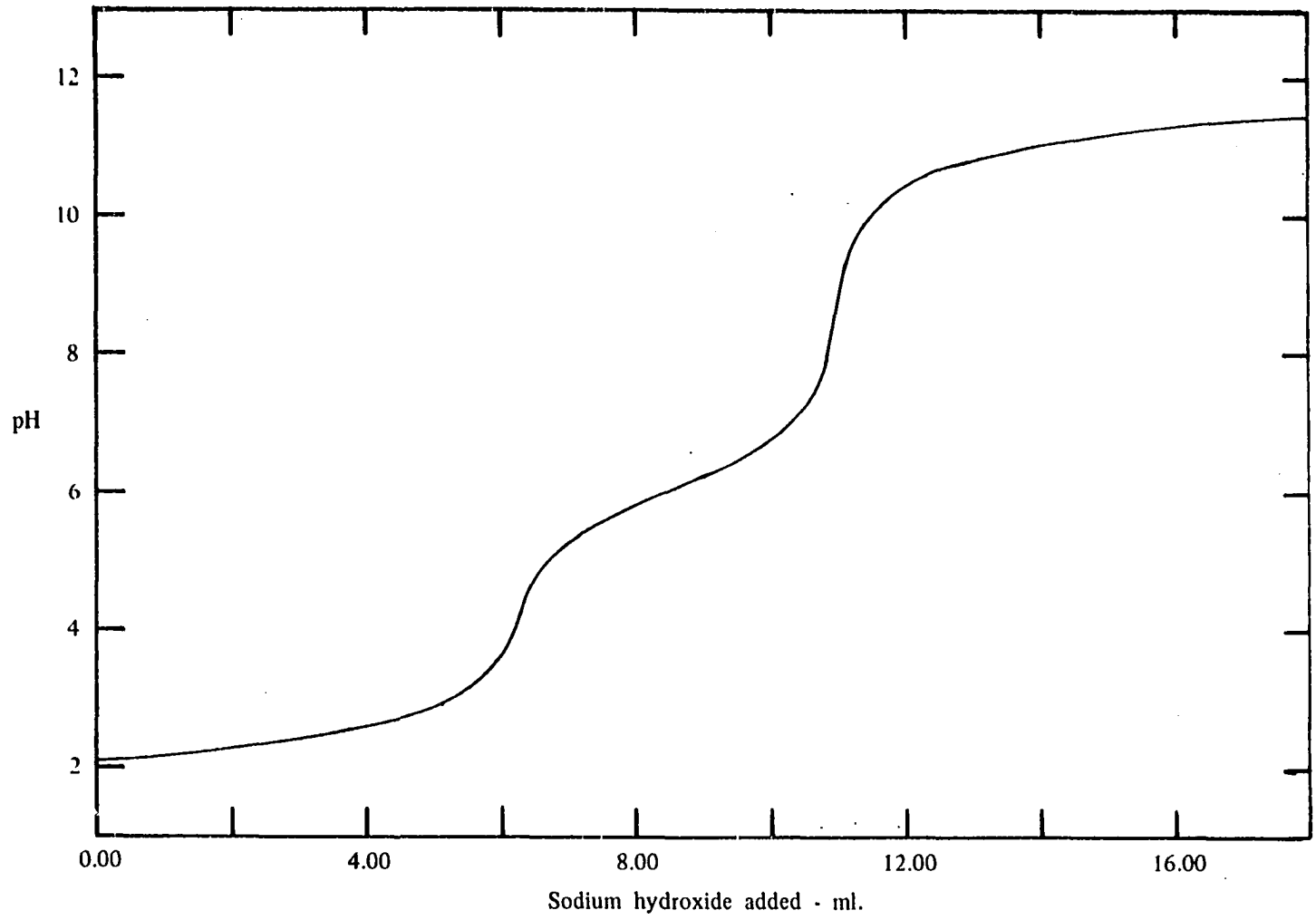
A sample of the sulfonated material, 0.3163 g., was dissolved in 50 ml. of 0.1 M potassium chloride (to keep ionic strength approximately equal to 0.1) and titrated with 0.1006 N sodium hydroxide. The pH was followed with a Beckman Zeromatic SS-3 pH meter in conjunction with a Corning No. 476022 Triple Purpose glass electrode and a saturated calomel electrode, calibrated against NBS standard buffers of pH 4.01, 6.86, and 9.14 (temperature = 32°). The titration curve is shown in Figure XI-1.

Figure XI-1. Acidimetric titration of sulfonated 4,7-dihydroxy-1,10-phenanthroline

Weight of sulfonated material taken: 0.3163 g.

Concentration of sodium hydroxide: 0.I006 N

Temperature: 32°


12
10
8
6
4
2
pH
0.00
4.00
8.00
12.00
16.00
Sodium hydroxide added - ml.

End points occurred at 6.21 ml. and 10.96 ml. of sodium hydroxide added, yielding equivalent weights of 506 g. and 287 g., respectively. The equivalent weight based on the amount of sodium hydroxide taken between the two end points is 662 g. That a larger amount of sodium hydroxide was used to reach the first end point than was used to proceed from the first to the second end point was probably due to the presence of some hydrochloric acid. A test for chloride, performed by adding a few drops of 0.25 M silver nitrate to an acid solution of a few milligrams of the sulfonated material, produced a definite white turbidity which settled and darkened on standing. The most reliable value for the equivalent weight of the material then is the value based on the amount of sodium hydroxide taken between the two end points, 662 grams/equivalent.

5. Mass spectrum

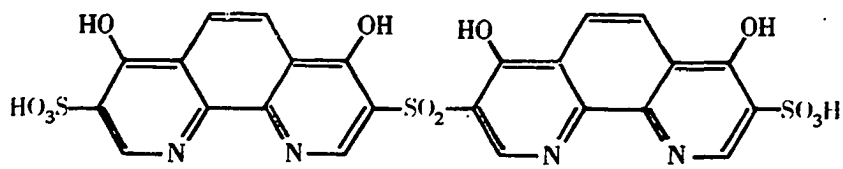
The mass spectrum of the sulfonated material was obtained on an Atlas CH 4 mass spectrometer. The most significant peaks (and relative intensities) in the spectrum at masses greater than 30 were 35 (17), 36 (17), 37 (17), 38 (17), 48 (17), 64 (17), 80 (17), 125 (1.5); peaks above 200 mass units were of very low intensity: 212, 213, 234, 236, 268, 270, 282, 287, 361.

The very intense peaks at 80, 64, and 48 mass units were probably due to SO_3, SO_2, and SO, respectively. The peak at

212 mass units was no doubt due to the 4,7-dihydroxy-1,10-phenanthroline entity. No further interpretation of the mass spectrum could be made.

C. Conclusions

Infrared and mass spectra definitely indicate that a derivative of 4,7-dihydroxy-1,10-phenanthroline carrying sulfonic acid groups was prepared. The NMR spectrum indicates substitution at positions 3- and 8-. The equivalent weight obtained from titration data, 662 grams/equivalent, does not agree at all with the molecular weight of the expected product 4,7-dihydroxy-1,10-phenanthroline-3,8-disulfonic acid, 372 grams/mole. The possibility that the sulfone



was formed seems likely, since the molecular weight of it is 646 g., which can be brought up to the determined value of 662 g. by the addition of one mole of water. The NMR spectrum of the sulfone would be essentially the same as that of the simpler disulfonic acid. No evidence for the presence of the sulfone derivative could be found in the infrared and mass spectra.

XII. SUMMARY

The history of the chemistry of 1,10-phenanthroline has been reviewed. In particular, a complete tabulation has been made of the 1,10-phenanthrolines bearing the hydroxyl group and the literature identified as to the information supplied, synthesis, chemistry, and applications to chemical analysis.

The determination of 4,7-dihydroxy-1,10-phenanthroline in the hydrochloride, the form in which the material is obtained and marketed, by titration with standard base has been improved by performing the titration in a nonaqueous solvent consisting of 2-propanol and dimethylsulfoxide. Analysis of various preparations of 4,7-dihydroxy-1,10-phenanthroline coupled with related information has revealed the presence of an impurity, probably a carboxy derivative of 4,7-dihydroxy-1,10-phenanthroline, an intermediate in the synthesis. An improved method of hydrolyzing 3,8-dicarbethoxy-4,7-dihydroxy-1,10-phenanthroline has been devised.

The NMR spectrum of 4,7-dihydroxy-1,10-phenanthroline and related compounds has been obtained and the spectra interpreted. Confirmation has thus been obtained that the compound is 4,7-dihydroxy-1,10-phenanthroline.

The ultraviolet absorption spectrum of 4,7-dihydroxy-1,10-phenanthroline has been obtained as a function of alkalinity over the range pH 9.1 to 5.0 M sodium hydroxide.

From the absorbance data, the third acid dissociation constant of 4,7-dihydroxy-1,10-phenanthroline has been found to be $pK_{OH} = 12.7 \pm 0.2$ (ionic strength 1.0; 25°).

Two unsuccessful attempts have been made to prepare the solid iron(II) derivative of 4,7-dihydroxy-1,10-phenanthroline by acidifying an alkaline solution of the compound. It was found that tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) rapidly decomposes in acidic media.

A direct spectrophotometric method has been developed for the determination of iron in solid preparations of the iron(II)-4,7-dihydroxy-1,10-phenanthroline compound.

A method has been developed for the determination of 4,7-dihydroxy-1,10-phenanthroline in the presence of iron(III). The method takes advantage of an observation that the absorption at certain wavelengths in the ultraviolet by 4,7-dihydroxy-1,10-phenanthroline is independent of the form in which the molecule is present, that is, whether free or bound to iron. The method has been found useful for the analysis of preparations of the iron(II)-4,7-dihydroxy-1,10-phenanthroline compound.

It has been shown that three iron(III) compounds of 4,7-dihydroxy-1,10-phenanthroline exist, a gray, a purple, and an amber compound. The gray compound is made of three molecules of 4,7-dihydroxy-1,10-phenanthroline and one atom of iron; it is formed by the oxidation of the iron(II)

compound of 4,7-dihydroxy-1,10-phenanthroline with oxygen and other oxidizing agents. The purple and amber compounds are made up of two molecules of 4,7-dihydroxy-1,10-phenanthroline and one atom of iron, and are interrelated by one proton; they are formed by the direct union of the phenanthroline and the ferric ion. The gray and amber compounds can be interconverted by adjustment of pH, the reaction being rather slow and irreversible.

The combining ratios of 4,7-dihydroxy-1,10-phenanthroline and iron in the gray, purple, and amber compounds have been established from visible absorption data by using the method of continuous variations.

The acid dissociation constant of the purple compound has been calculated from visible absorption data and found to be 9.77 at 23° and an ionic strength of 0.4. It has been shown that the acidic proton is not part of the phenanthroline molecule, and that the purple compound is probably the binuclear species bis(4,7-dihydroxy-1,10-phenanthroline)iron(III)-μ-dihydroxobis(4,7-dihydroxy-1,10-phenanthroline)iron(III).

The formal reduction potential of the tris(4,7-dihydroxy-1,10-phenanthroline)iron(III,II) couple has been found to be constant over the pH range 10 to 13 and to have the value -0.06 volt. It has been shown that in this pH range the oxidized and reduced forms of the couple are

chemically identical, except for the difference of one electron and that the hydrogen ion is not involved in the couple.

It has been shown that tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) reacts rapidly, quantitatively and stoichiometrically with oxygen. The reaction has been applied to the spectrophotometric determination of dissolved oxygen in water.

It has been shown that four molecules of tris(4,7-dihydroxy-1,10-phenanthroline)iron(II) are oxidized by each molecule of oxygen and that the oxidation product, tris(4,7-dihydroxy-1,10-phenanthroline)iron(III) is stable in a solution containing ammonium hydroxide and ammonium chloride. It has been found that the loss of ammonia from such solutions by bubbling nitrogen is negligible and thus that an ammonium hydroxide-ammonium chloride buffer may be used in the reagent in the spectrophotometric determination of dissolved oxygen.

Apparatus for carrying out the spectrophotometric determination of dissolved oxygen has been devised employing an all-glass flow-through cell system in conjunction with a spectrophotometer. Deaerated reagent solution is delivered to the system from a Machlett buret. The sample is injected into the system through a rubber septum from a tuberculin syringe, and reagent and reagent plus sample is circulated through the absorption cell with a magnetic stirring bar.

The method for the determination of dissolved oxygen has been compared to the Winkler method, currently the standard method for the determination of dissolved oxygen. Excellent agreement between the two methods was obtained on distilled water and on surface water from Lake LaVerne, Iowa State University. The methods yielded different results on raw water from the Ames municipal water plant owing to iron(II) in the raw water.

A method for the removal of iron from concentrated solutions of sodium hydroxide by extraction of the ferrous derivative of 4,7-dihydroxy-1,10-phenanthroline has been devised, employing dimethylsulfoxide as an immiscible solvent. The iron-free sodium hydroxide thus prepared is superior to reagent grade sodium hydroxide for the preparation of standard solutions used in the determination of iron with 4,7-dihydroxy-1,10-phenanthroline. A procedure for the simultaneous estimation of iron in sodium hydroxide and sodium hydrosulfite has also been developed.

A product has been isolated from the reaction of 4,7-dihydroxy-1,10-phenanthroline with formaldehyde and shown by analyses for carbon, hydrogen and nitrogen, and by infrared and NMR spectra to contain the methylene linkage joining phenanthroline molecules and the probable presence of hydroxymethyl groups. It has thus been shown that incorporation of 4,7-dihydroxy-1,10-phenanthroline into a phenol-formaldehyde resin by co-polymerization is feasible.

A sulfonated derivative of 4,7-dihydroxy-1,10-phenanthroline has been prepared which is soluble in acidic solutions. Interpretation of infrared, NMR, and mass spectral data showed that the compound contains sulfonic acid groups at positions 3- and 8- on the phenanthroline molecule. Titration data, however, indicate that the simple sulfonated derivative, 4,7-dihydroxy-1,10-phenanthroline-3,8-disulfonic acid, was not obtained, but a sulfone-sulfonic acid derivative.

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