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Sulfonated derivatives of 1, 10-phenanthroline

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SULFONATED DERIVATIVES OF 1,10-PHENANTHROLINE

by

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Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1961

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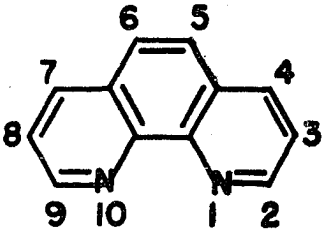
INTRODUCTION

The synthesis of 1,10-phenanthroline



was first reported by Blau (1) who prepared it during the course of his work on nitrogen ring compounds related to nicotine (2,3). The red compound formed by the reaction of 1,10-phenanthroline and ferrous iron is widely used as a colorimetric method for iron. The ferrous compound is also used as an oxidation-reduction indicator in acid solution; particularly in cerate oxidimetry. The color change upon oxidation is from red to blue, both the ferrous and ferric compounds containing three molecules of 1,10-phenanthroline to one atom of iron.

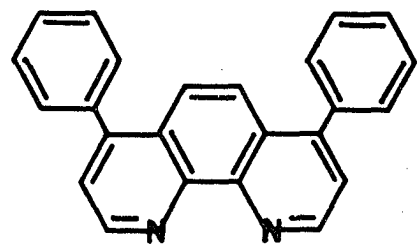
Other metals form compounds with 1,10-phenanthroline. Of particular importance is the yellow orange cuprous compound formed by the union of two molecules of 1,10-phenanthroline with one atom of copper. Many other metals form colorless compounds with 1,10-phenanthroline.

Some of the characteristics of 1,10-phenanthroline are: it can be crystallized out of water as the monohydrate; it is very soluble in ethanol but difficultly soluble in cold water; because of its basic nature, the compound readily

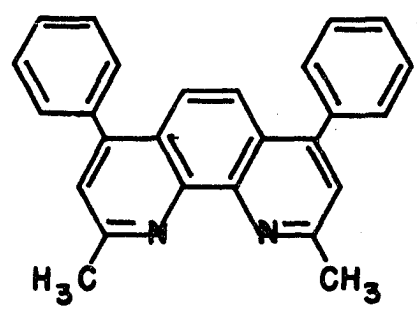
forms salts with mineral acids; and its ferrous derivative forms an insoluble perchlorate.

Direct substitution in the 1,10-phenanthroline molecule is accomplished relatively easily in the case of nitration (4). However, only the mononitro compound has been prepared owing to the difficulty in adding a second nitro group. Direct substitution by sulfonation has never been reported in the literature.

The initial paper of Smith et al. (5) on bathophenanthroline (4,7-diphenyl-1,10-phenanthroline),



a highly sensitive reagent for the spectrophotometric determination of iron, has been followed by several other papers detailing applications of the reagent to various materials bearing traces of iron (6). In a like manner, bathocuproine (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline),



originally proposed by Smith and Wilkins (7), is finding

numerous applications (8) because its unqualified specificity and high sensitivity make it the superior reagent for the spectrophotometric determination of traces of copper.

The difficulty encountered in the use of these reagents in water solution is their low solubility in water. Both are soluble in alcohols and also in water as the hydrochlorides, but in the neutral solutions needed for maximum color development with the metals, the excess reagent tends to precipitate, rendering the solutions turbid. Even this is all right if the procedures of the original authors are followed, for Smith and his co-workers extract the metal derivatives into amyl or hexyl alcohol and in these solvents make the final photometric measurements. The advantages of such extraction are considerable, for a significant concentration can be effected and the blank can be reduced to essentially zero by extracting from the various reagent solutions any iron and copper present. Workers with heavy loads of routine work, however, find the extraction a handicap, but in omitting it, that is, in making the photometric measurement on the water solution, are plagued with the turbidity difficulty.

Trinder (9) solved this problem by sulfonating bathophenanthroline and later Zak (10) followed with a sulfonation of bathocuproine. In both cases, the sulfonation was made by briefly boiling the starting material with chlorosulfonic acid, followed by dilution and neutralization. This solution

of the sulfonated material was used directly for the determination of iron and copper. No attempt was made to isolate, purify, identify, characterize, or further study the properties of the sulfonated materials.

It was the purpose of this work to sulfonate the three materials; bathophenanthroline, bathocuproine, and 1,10-phenanthroline, and to isolate the sulfonic acids in pure form in order to identify and characterize them and further to determine the nature of their metal derivatives, their sensitivities, their use in analysis in the presence of perchlorate, oxidation reduction indicator properties, and other analytical properties.

BATHOPHENANTHROLINEDISULFONIC ACID AND BATHOCUPROINEDISULFONIC ACID

Apparatus and Reagents

Apparatus

Spectrophotometric data were secured using a Cary Model 12 recording spectrophotometer and a Beckman DU spectrophotometer using 1 cm. matched cells. pH measurements were made using a Beckman Model G pH meter with a Beckman No. 30740 amber bulb glass electrode. Potential measurements were made with a Leeds and Northrup No. 7552 potentiometer. Infrared spectra were recorded using a Perkin-Elmer Model 21 spectrophotometer. The thermobalance used was one built in this laboratory.

Reagents

The starting materials, bathophenanthroline and bathocuproine, were obtained from the G. Frederick Smith Chemical Company, Columbus, Ohio. The commercial chlorosulfonic acid used was first subjected to vacuum distillation to insure the absence of iron and copper. The Amberlite IR-120 used was a reagent grade material. The water used was first distilled and then passed through a monobed ion exchange resin. All other chemicals used were reagent grade or of high purity.

Synthesis and Study of Properties

Synthesis

Because of the structural similarity of bathophenanthroline and bathocuproine, the same technique was used in sulfonating both materials.

One gram of bathophenanthroline or bathocuproine starting material and 10 ml. of freshly distilled chlorosulfonic acid were vigorously stirred (glass coated magnetic stirring bar) for 20 hrs. at 25°. The mixture was cooled in ice and 100 ml. of deionized water was carefully added. The mixture was then heated in a boiling water bath until a clear solution was obtained. Water and hydrochloric acid were removed by heating under reduced pressure, leaving the excess sulfuric acid and the sulfonated material.

Concentrated ammonium hydroxide was added until in excess, and the resulting solution then evaporated to dryness on a steam plate. The residue was pulverized and treated with 200 ml. of 95% ethanol. This mixture was heated to boiling, stirred for 30 min. and the ammonium sulfate filtered off using suction. The filtrate was evaporated to dryness on a steam plate. The residue was dissolved in 100 ml. of deionized water and the solution passed through a column of Amberlite IR-120 (a strong cation exchange resin) in the hydrogen form. The resulting acidic solution was partially neutralized with high purity sodium bicarbonate

and the carbon dioxide formed expelled by boiling while the solution was still slightly acidic. The pH of the solution was then adjusted to 8.5 with dilute sodium hydroxide. The solution was evaporated on a hot plate and the residue pulverized.

Yield Based on a disulfonated product in both cases, a yield of 97% was obtained for both bathophenanthroline and bathocuproine.

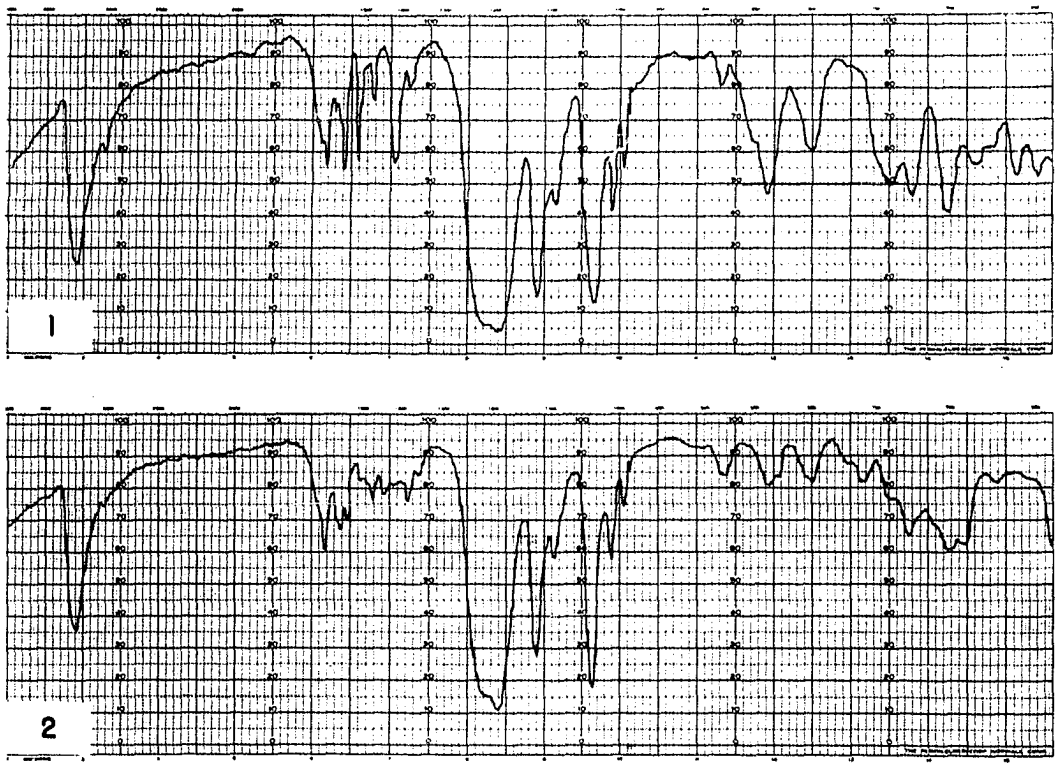
Purity Infrared spectra were run on the sulfonated compounds using the potassium bromide pellet technique; the spectra shown in Fig. 1. Owing to the nature of the compounds, no melting points were determined.

Ultimate analysis Found for disodium bathophenanthrolinedisulfonate: C 53.87%, H 2.85%, N 5.05%, S 11.87%, Na 8.77% (Huffman Microanalytical Laboratory, Wheatridge, Colorado) giving an empirical formula of $C_{24.2}H_{15.3}N_{2.0}S_{2.0}Na_{2.1}$; disodium bathophenanthrolinedisulfonate: $C_{24}H_{14}N_2S_2Na_2O_6$.

Found for disodium bathocuproinedisulfonate: C 52.41%, H 3.69%, N 4.57%, S 10.40%, Na 8.11% (Huffman Microanalytical Laboratory) giving an empirical formula of $C_{26.9}H_{26.0}N_{2.0}S_{2.0}Na_{2.2}$; disodium bathocuproinedisulfonate is $C_{26}H_{18}N_2S_2Na_2O_6$.

Equivalent weight The equivalent weights of both sulfonated compounds were determined by passing water

Fig. 1. Infrared spectra of disodium bathophenanthroline-disulfonate (spectrum 1) and of disodium bathocuproinedisulfonate (spectrum 2).


1
2

solutions of weighed samples of the sodium salts, dried at 110°, through a column of IR-120 in the hydrogen form and subsequently titrating with standard sodium hydroxide; the titration curves are given in Fig. 2. In order to check the quantitative conversion of the salts to the hydrogen form, following their titration several of the solutions were recycled through the column and retitrated. The conversion was found to be quantitative on each passage.

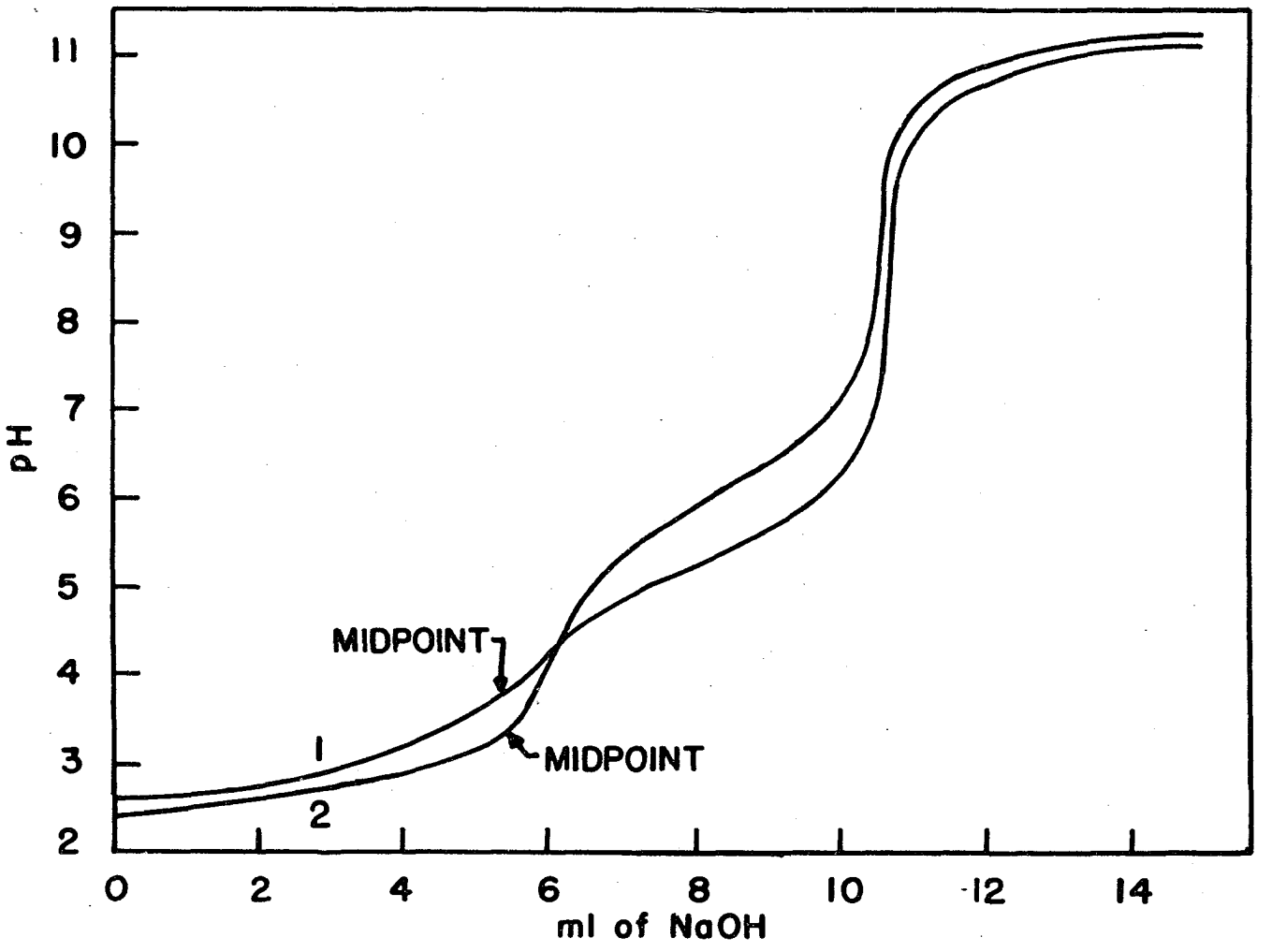
The equivalent weights found for disodium bathophenanthrolinedisulfonate were: 263.0, 264.5 (first preparation), 264.5 (second preparation); calculated for $C_{24}H_{15}N_2(SO_3Na)$ 434.4; for $C_{24}H_{14}N_2(SO_3Na)_2$ 536.5/2 or 268.2; for $C_{24}H_{13}N_2(SO_3Na)_3$ 638.5/3 or 212.8.

The equivalent weights found for disodium bathocuproinedisulfonate were: 278.0 (first preparation), 273.4, 273.0 (second preparation); calculated for $C_{26}H_{19}N_2(SO_3Na)$ 462.5; for $C_{26}H_{18}N_2(SO_3Na)_2$ 564.6/2 or 282.3; for $C_{26}H_{17}N_2(SO_3Na)_3$ 666.6/3 or 222.2.

Properties In the acid forms, both compounds are extremely hygroscopic and exist as syrupy liquids. The sodium salts of both compounds are tan hygroscopic solids, extremely soluble in water. Upon drying at 110° for 2 hrs., neither salt showed change in weight up to 275° (thermobalance) at which time the experiment was concluded.

Bathocuproine gives a light blue fluorescence under ultraviolet light but bathophenanthroline does not fluoresce.

Fig. 2. Titration of bathophenanthrolinedisulfonic acid (curve 1) and of bathocuproinedisulfonic acid (curve 2).


pH
11
10
9
8
7
6
5
4
3
2
MIDPOINT
MIDPOINT
1
2
0
2
4
6
8
10
12
14
ml of NaOH

Upon sulfonation, bathophenanthroline fluoresces light blue under ultraviolet light.

It proved impossible to prepare ammonium salts of the sulfonated materials of stoichiometric composition. The free acids were neutralized with excess concentrated ammonium hydroxide and dried on a steam plate at approximately 110°. The nitrogen present in the ammonium form was then determined by distillation with fixed alkali and titration. The nitrogen was found to be a great deal less than that predicted for an ammonium salt.

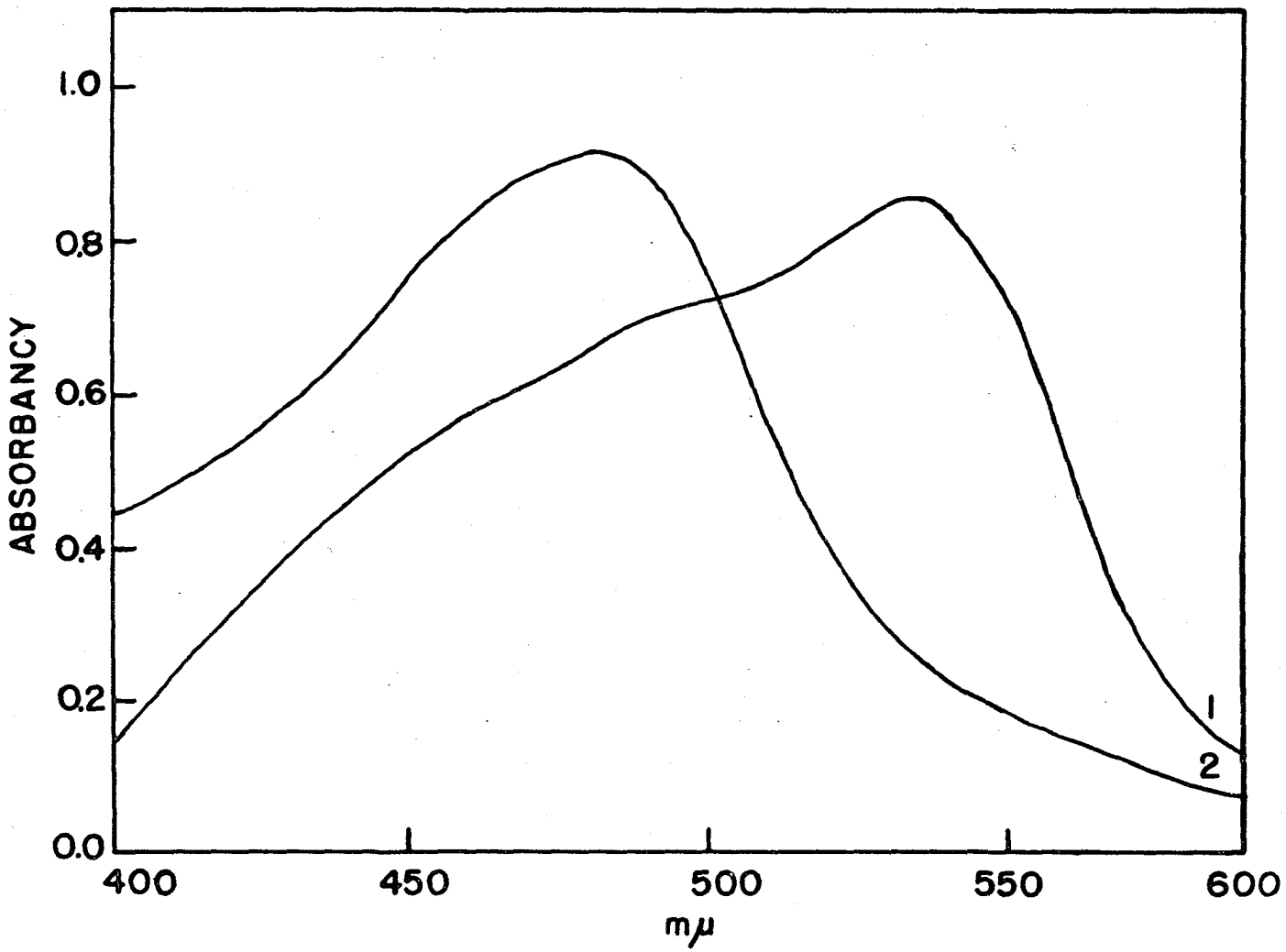
Ferrous Trisbathophenanthrolinedisulfonate and Cuprous Bisbathocuproinedisulfonate

Spectrophotometric constants and necessary conditions

Absorption spectra A solution of the ferrous derivative of bathophenanthrolinedisulfonate was prepared in the following way. To a 100 ml. volumetric flask was added 1.0 ml. of 3.77×10^{-3} M ferrous sulfate, 1 ml. of 10% hydroxylammonium chloride, 20 ml. of 1.5×10^{-3} M disodium bathophenanthrolinedisulfonate, and 2 ml. of 10% sodium acetate. The flask was diluted to the mark with deionized water and the spectrum recorded using a Cary Model 12 recording spectrophotometer and 1 cm. cells. The spectrum is shown in Fig. 3.

A solution of the cuprous derivative of bathocuproinedisulfonate was also prepared. To a 100 ml. volumetric

Fig. 3. Absorption spectra of ferrous trisbathophenanthrolinedisulfonate (spectrum 1) and cuprous bisbathocuproinedisulfonate (spectrum 2).


ABSORBANCY
1.0
0.8
0.6
0.4
0.2
0.0
400
450
500
550
600
mμ
1
2

flask was added 6.0 ml. of 1.25×10^{-3} M copper sulfate, 1 ml. of 10% hydroxylammonium chloride, 20 ml. of 2.78×10^{-3} M disodium bathocuproinedisulfonate, and 2 ml. of 10% sodium acetate. The spectrum was then recorded using 1 cm. cells and is shown in Fig. 3.

Sensitivity A series of solutions containing excess disodium bathophenanthrolinedisulfonate and an iron concentration varying from 0 to 6.45×10^{-5} M was prepared by transferring aliquots of a 6.455×10^{-4} M iron solution into 50 ml. volumetric flasks and adding 2 ml. of 10% hydroxylammonium chloride, 10 ml. of 1.66×10^{-3} M disodium bathophenanthrolinedisulfonate, and 5 ml. of 10% sodium acetate. After dilution to volume, the absorbancy of each solution was determined at 535 mμ, the absorption maximum, on the Beckman DU spectrophotometer using 1 cm. cells. A solution containing the reagents with the exception of the iron was used as a blank. A plot of absorbancy vs. concentration is shown in Fig. 4. The metal derivative was observed to conform to Beer's law over the concentration 0 to 3.6 p.p.m. iron. From the slope of the line, a value of 22,140 was found for the molar extinction coefficient of the ferrous derivative.

In order to determine the sensitivity of bathocuproinedisulfonic acid toward copper, a series of solutions containing excess disodium bathocuproinedisulfonate and a copper concentration varying from 0 to 1.25×10^{-4} M was

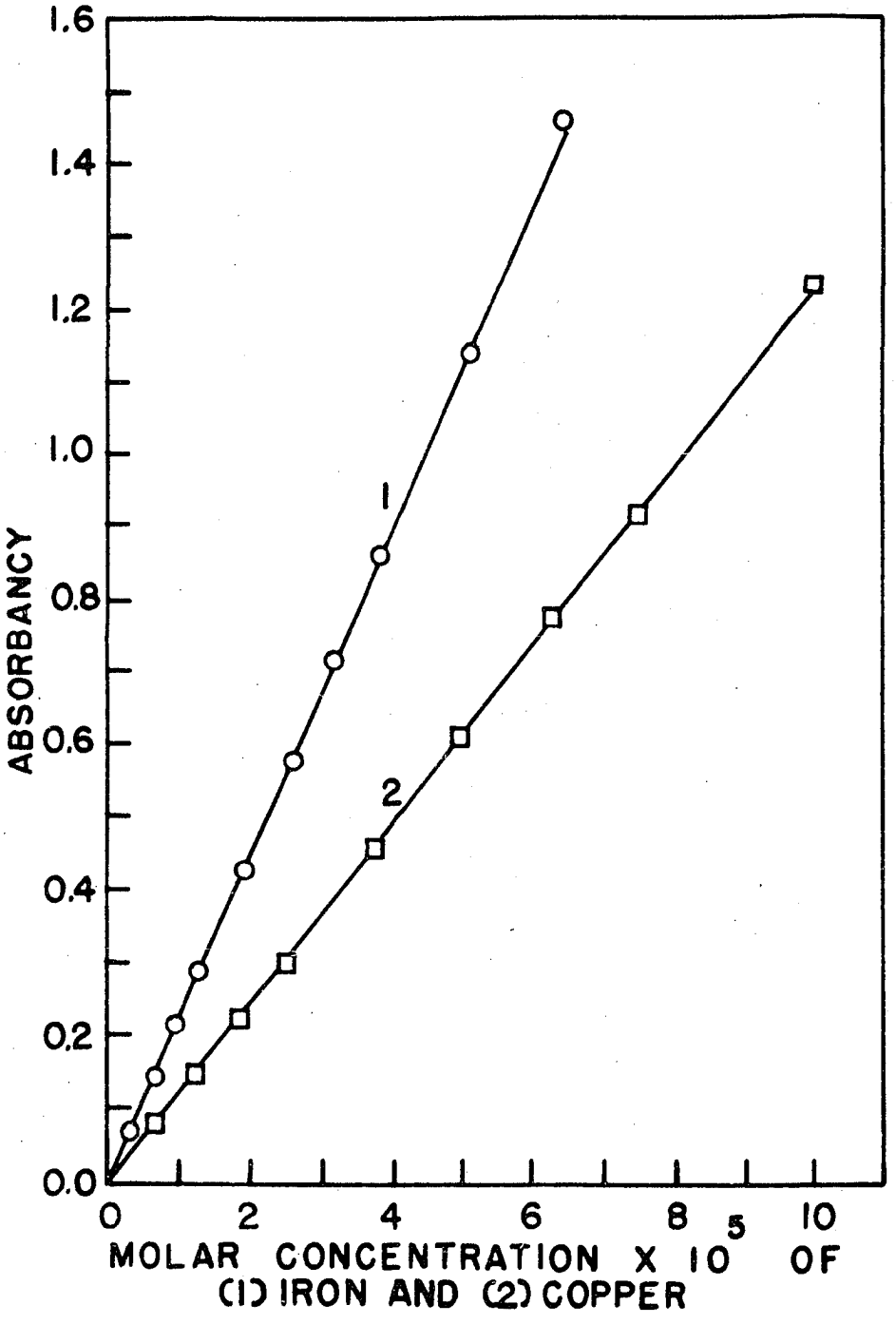


Fig. 4. Beer's law plot for the ferrous derivative of bathophenanthrolinedisulfonate (curve 1) and the cuprous derivative of bathocuproinedisulfonate (curve 2).

prepared using aliquots of a 1.252×10^{-3} M copper solution transferred into 50 ml. volumetric flasks and adding 2 ml. of 10% hydroxylammonium chloride, 10 ml. of 2.78×10^{-3} M disodium bathocuproinedisulfonate, and 5 ml. of 10% sodium acetate. After dilution to volume, the absorbancy of each solution was determined at 483 mμ, the absorption maximum, using 1 cm. cells and a reagent blank; the results are plotted in Fig. 4. Conformity to Beer's law from 0 to 6.35 p.p.m. copper was observed. A molar extinction coefficient of 12,250 was found for this derivative from the slope in Fig. 4.

Effect of pH upon stability A series of solutions were prepared in 100 ml. volumetric flasks and iron added so that the final solutions would be 3.77×10^{-5} M in iron and would contain 1 ml. of 10% sodium sulfite and 20 ml. of 1.587×10^{-3} M disodium bathophenanthrolinedisulfonate. The solutions were then adjusted from an initial acidic pH to various pH values using dilute hydrochloric acid and sodium hydroxide. One hour after preparation, the absorbancies of the resulting solutions were read at 535 mμ using 1 cm. cells and a reagent blank. The pH of the solutions were then read with a Beckman Model G pH meter; the resulting plot of absorbancy vs. pH is shown in Fig. 5.

A second series of solutions in 100 ml. volumetric flasks were prepared, each solution was 7.41×10^{-5} M in copper and contained 1 ml. of 10% sodium sulfite and 10 ml.

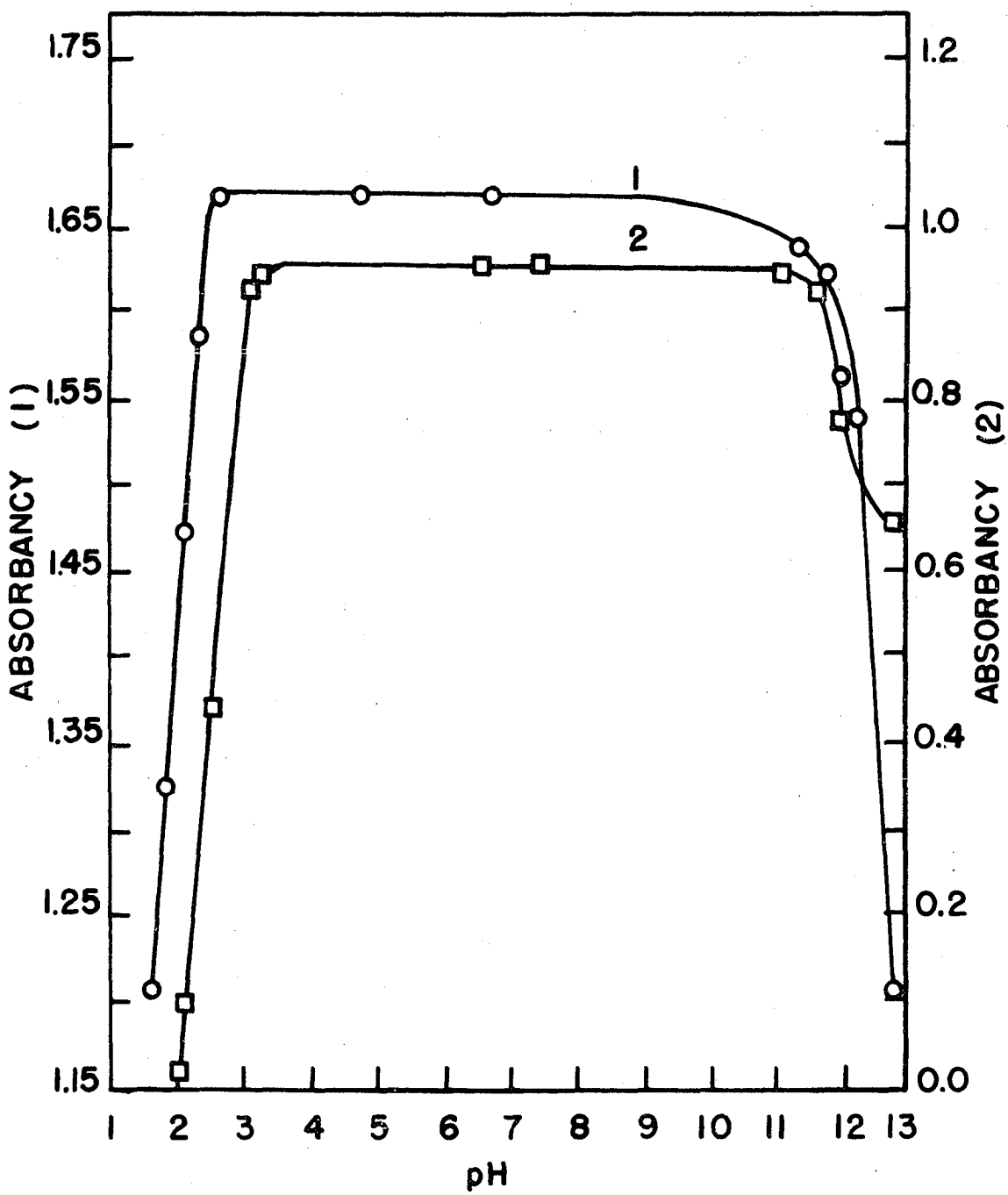


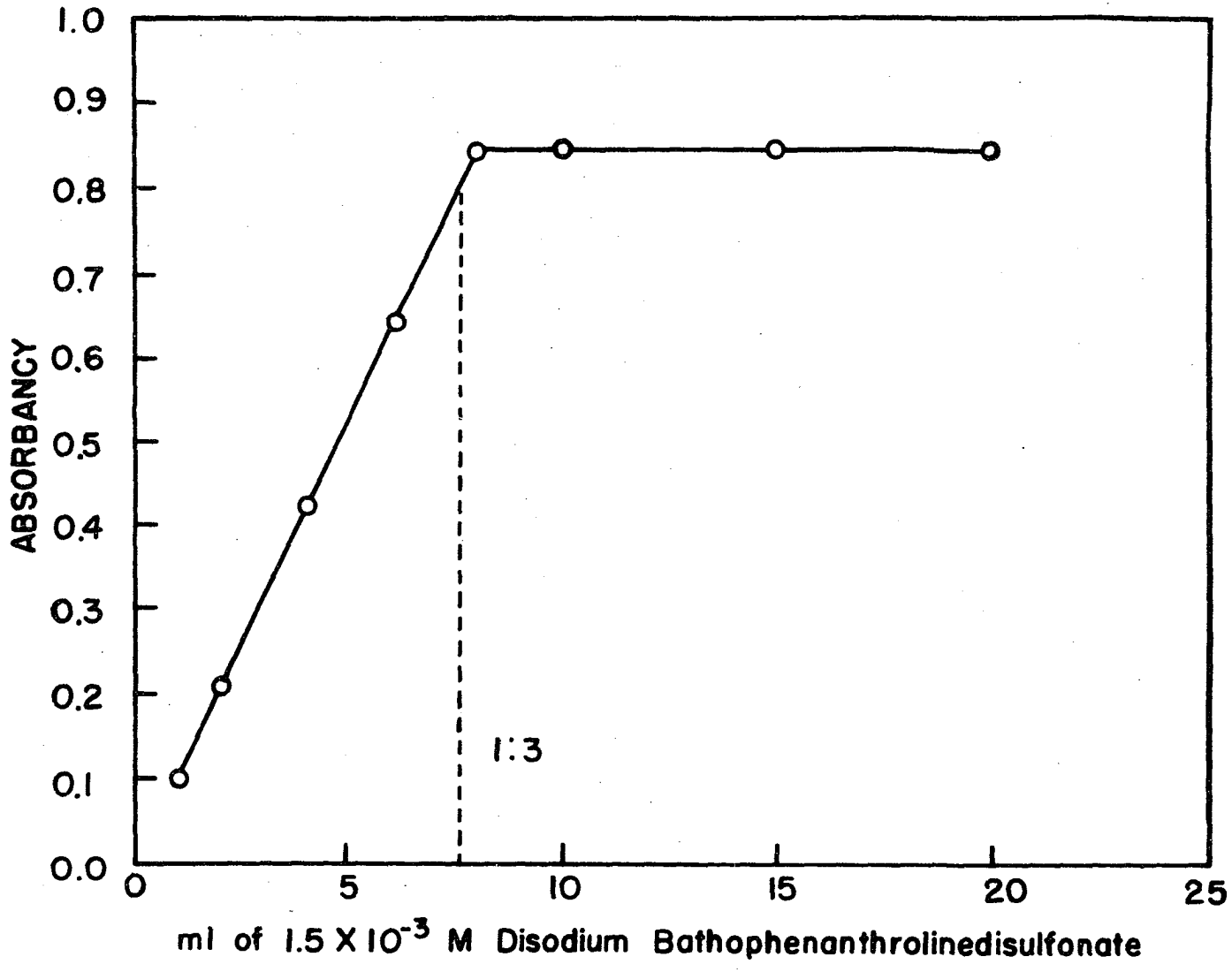
Fig. 5. Effect of pH upon the stability of the ferrous derivative of bathophenanthrolinedisulfonate (curve 1) and the cuprous derivative of bathocuproinedisulfonate (curve 2).

of 4.05 x 10^{-3} M disodium bathocuproinedisulfonate. The pH of each solution was adjusted to a different value and the solutions were diluted to volume and allowed to stand for 1 hr., at which time the absorbancy was read at 483 mμ. The pH of each solution was determined; the resulting plot of absorbancy vs. pH is shown in Fig. 5.

Combining ratios The combining ratio of the ferrous and bathophenanthrolinedisulfonate ions was determined by a spectrophotometric titration in which the iron concentration was held constant (3.77 x 10^{-5} M) in a series of 100 ml. volumetric flasks, while various amounts of a standard solution of disodium bathophenanthrolinedisulfonate (prepared from the salt dried for 1 hr. at 110°) were added, giving final concentrations of the sulfonated reagent ranging from 0 to 3.0 x 10^{-4} M. The color was developed in the usual way, adding 1 ml. of 10% hydroxylammonium chloride and 2 ml. of 10% sodium acetate to each solution. The absorbancies were read at 535 mμ on the Cary Model 12 recording spectrophotometer using 1 cm. cells and a reagent blank; the results of the titration are shown in Fig. 6. The combining ratio of the ferrous to the bathophenanthrolinedisulfonate ion was found to be 1:3.17 as calculated from the sharp break in the titration curve.

The combining ratio of the cuprous and bathocuproinedisulfonate ions was determined in the same way. The copper concentration in the solutions (in 100 ml. volumetric flasks)

Fig. 6. Spectrophotometric titration of iron.

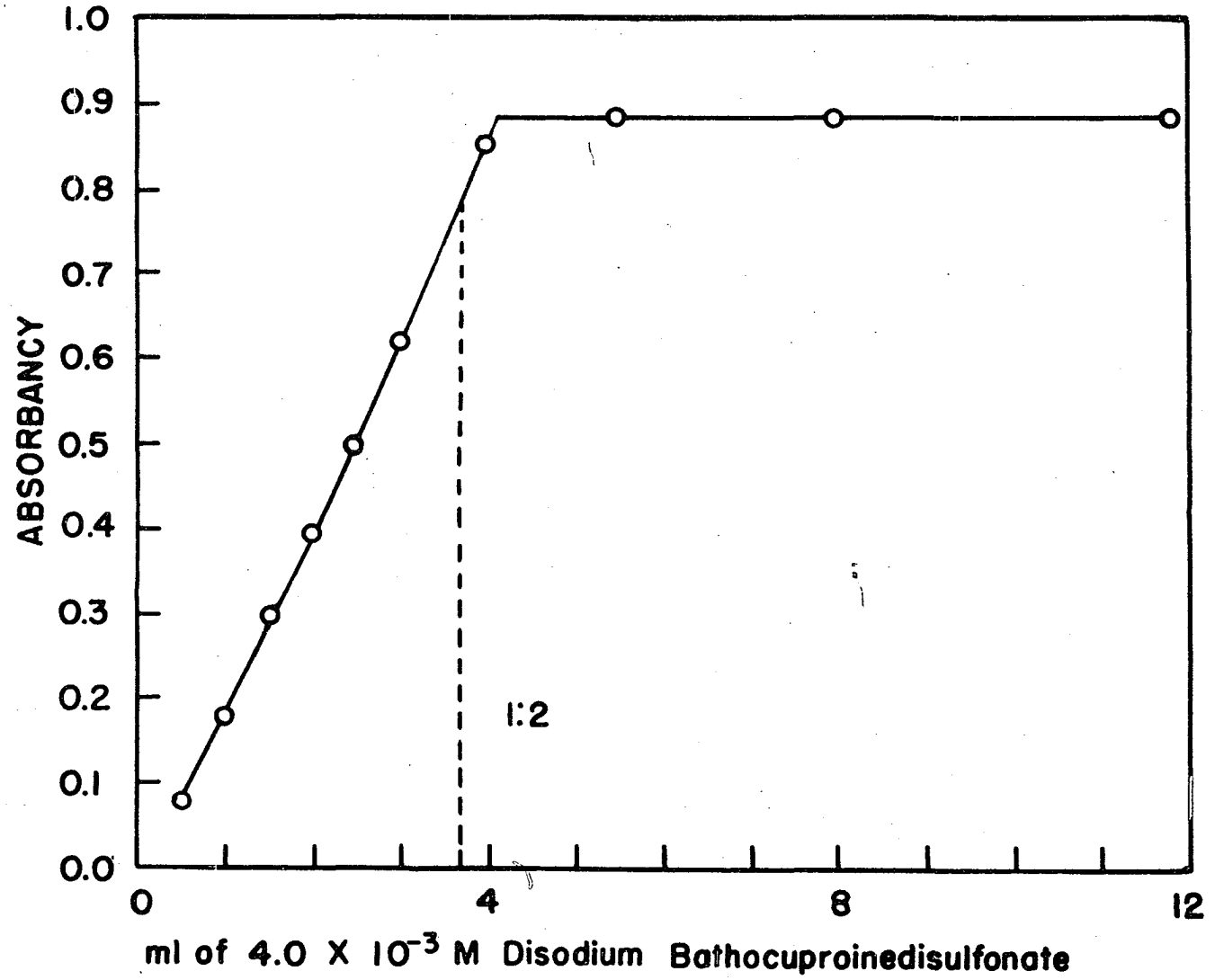

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
ABSORBANCY
1:3
0
5
10
15
20
25
ml of 1.5 X 10⁻³ M Disodium Bathophenanthrolinedisulfonate

was held constant at 7.4×10^{-5} M while the concentration of the disodium bathocuproinedisulfonate in the solutions ranged from 0 to 4.8×10^{-4} M. The colors were developed and the absorbancies read at 483 mμ, giving the curve shown in Fig. 7 which indicates a combining ratio of 1:2.24.

Charge on metal derivatives The following technique was used to determine whether ferrous trisbathophenanthrolinedisulfonate and cuprous bisbathocuproinedisulfonate carry a positive or negative charge. A 3% agar solution containing approximately 4×10^{-6} moles of the metal derivative under study and 10% potassium chloride was poured into a U tube until the material filled the bottom of the tube and extended into both arms. Above this solidified layer in both arms of the tube was placed a 3% agar solution containing 10% potassium chloride. Saturated potassium chloride solution was placed above this second layer and platinum wire electrodes were inserted in the salt solution.

The entire assembly was placed in an ice bath and 100 ma of D.C. current was passed through the tube. After a few minutes it was observed that in the case of both metal derivatives, the colored derivatives migrated through the agar toward the positive electrode, thus showing that the charge on both ferrous trisbathophenanthrolinedisulfonate and on cuprous bisbathocuproinedisulfonate is negative.

Fig. 7. Spectrophotometric titration of copper.


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
ABSORBANCY
1:2
0
4
8
12
ml of 4.0 X 10⁻³ M Disodium Bathocuproinedisulfonate

Interfering ions

Effect on the determination of iron The effect of various ions on the spectrophotometric determination of iron was determined on solutions containing 6.5×10^{-7} moles of iron and 9.5×10^{-6} moles of bathophenanthrolinedisulfonate in a total of 50 ml. The order of addition of the reagents was always the same; first the interfering ion, second the iron, next 2 ml. of 10% hydroxylammonium chloride, then the sulfonated reagent, and finally 3 ml. of 10% sodium acetate buffer. The effect of the various ions on the resulting spectrophotometric determination of the iron present is shown in Table 1.

The method of calculating the relative error is essentially that of Fortune and Mellon (11). The apparent concentration of iron in the solution to which the ion to be tested was added, c_2, is found from

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

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

$$\% \text{ Relative Error} = \frac{c_2 - c_1}{c_1} \times 100.$$

Table 1. Effect of various ions on the determination of iron with bathophenanthrolinedisulfonate

Ion	Interfering ion concentration p.p.m.	Added as	Relative error % of 0.726 p.p.m. Fe taken
Cu^{++}	0.9	$CuSO_4$	+2.2
Cu^{++}[a]	9.4	$CuSO_4$	+3.2
Co^{++}	1.2	$CoSO_4$	+1.1
Co^{++}[a]	5.8	$CoSO_4$	0.0
Ni^{++}	1.4	$NiSO_4$	+1.1
Ni^{++}[a]	5.5	$NiSO_4$	0.0
Zn^{++}	13.8	$ZnSO_4$	+0.3
Mn^{++}	25.3	$MnSO_4$	-1.1
Cr^{+++}	5.8	$K_2Cr_2O_7$	+1.6
Cr^{+++}	29.2	$K_2Cr_2O_7$	+4.7
Be^{++}	14.6	$Be(ClO_4)_2$	+2.2
Al^{+++}	121.3	$Al_2(SO_4)_3$	Prec.
Mg^{++}	58.7	$MgSO_4$	+0.3
Ca^{++}	189	$Ca(C_2H_3O_2)_2$	-1.1
Sr^{++}	571	$Sr(NO_3)_2$	0.0
Cd^{++}	35.5	$Cd(NO_3)_2$	0.0
Sn^{++++}	307	$SnCl_4$	Prec.
Th^{++++}	546	$Th(NO_3)_4$	+0.3
UO_2^{++}	653	$UO_2(NO_3)_2$	+1.6
Li^{+}	627	LiCl	0.0

[a]Solutions containing large excess (2.5×10^{-5} moles) of bathophenanthrolinedisulfonate.

Table 1 (Continued)

Ion	Interfering ion concentration p.p.m.	Added as	Relative error % of 0.726 p.p.m. Fe taken
ClO_4^-	17780	$NaClO_4$	+2.7
CN^-	536	KCN	No color
PO_4^{---}	1122	KH_2PO_4	0.0
F^-	543	NaF	+1.1
$C_2H_3O_2^-$	15320	$NH_4C_2H_3O_2$	0.0
Br^-	895	KBr	-2.3
I^-	1040	NaI	-0.8
Cl^-	5180	KCl	-0.8
NO_2^-	873	$NaNO_2$	-2.0
SO_4^{--}	3470	$(NH_4)_2SO_4$	0.0
ClO_3^-	818	$KClO_3$	-0.8
SCN^-	1200	NaSCN	-1.7
$S_2O_3^{--}$	1074	$Na_2S_2O_3$	Prec.
BO_3^{---}	1800	$Na_2B_4O_7$	+0.3
BrO_3^-	1380	$KBrO_3$	+0.3
MoO_4^{--}	93.2	Na_2MoO_4	0.0
$C_6H_5O_7^{---}$	1627	$H_3C_6H_5O_7$	-1.7
$S_2O_8^{--}$	1626	$(NH_4)_2S_2O_8$	-9.7

Of the various ions tested, copper, aluminum, tin (IV), thiosulfate, dichromate, cyanide, and persulfate were the only ions found which interfere seriously.

Effect on the determination of copper The same method was used to study possible interfering ions as was used in the previous case. Solutions were prepared by adding first the ion under study, second 1.9×10^{-6} moles of copper, next the hydroxylammonium chloride, then 1.4×10^{-5} moles of bathocuproinedisulfonate, and finally the sodium acetate buffer. The effect was reported as relative error in the amount of copper taken, Table 2.

Of the various ions tested, aluminum, tin (IV), uranyl, dichromate, cyanide, thiocyanate, thiosulfate, and persulfate were the only ions found which interfere seriously.

Formal reduction potentials

Method The formal reduction potentials of ferrous trisbathophenanthrolinedisulfonic acid were determined in both 1 M sulfuric acid and 1 M perchloric acid using a Leeds and Northrup No. 7552 potentiometer with a platinum indicator electrode and a saturated potassium chloride calomel reference electrode.

The method used to determine the formal reduction potentials of the metal derivative was to titrate potentiometrically a nearly equimolar mixture of ferrous iron and the ferrous derivative with a cerate solution; both titrant

Table 2. Effects of various ions on the determination of copper with bathocuproinedisulfonate

Ion	Interfering ion concentration p.p.m.	Added as	Relative error % of 2.41 p.p.m. Cu taken
Fe^{++}	5.8	$FeSO_4$	+0.2
Fe^{++}	57.8	$FeSO_4$	+1.2
Co^{++}	1.2	$CoSO_4$	0.0
Co^{++}	5.9	$CoSO_4$	+1.4
Ni^{++}	1.4	$NiSO_4$	0.0
Ni^{++}	6.9	$NiSO_4$	+1.4
Zn^{++}	126	$ZnSO_4$	-0.3
Mn^{++}	316	$MnSO_4$	0.0
Cr^{+++}	5.8	$K_2Cr_2O_7$	-0.2
Cr^{+++}	29.2	$K_2Cr_2O_7$	+3.1
Be^{++}	14.6	$Be(ClO_4)_2$	+1.1
Al^{+++}	90	$Al_2(SO_4)_3$	Prec.
Mg^{++}	67	$MgSO_4$	0.0
Ca^{++}	170	$Ca(C_2H_3O_2)_2$	-0.2
Sr^{++}	571	$Sr(NO_3)_2$	-2.1
Cd^{++}	360	$Cd(NO_3)_2$	-0.2
Sn^{++++}	351	$SnCl_4$	Prec.
Th^{++++}	525	$Th(NO_3)_4$	-0.2
UO_2^{++}	555	$UO_2(NO_3)_2$	+6.1
Li^{+}	642	LiCl	-0.2
ClO_4^{-}	16550	$NaClO_4$	0.0
CN^{-}	550	KCN	No color

Table 2 (Continued)

Ion	Interfering ion concentration p.p.m.	Added as	Relative error % of 2.41 p.p.m. Cu taken
PO_4^{---}	1284	KH_2PO_4	-0.3
F^-	696	NaF	-1.2
$C_2H_3O_2^-$	15660	$NH_4C_2H_3O_2$	-1.2
Br^-	1000	KBr	0.0
I^-	840	NaI	-0.6
Cl^-	5130	KCl	-2.1
NO_2^-	882	$NaNO_2$	-0.2
SO_4^{--}	3060	$(NH_4)_2SO_4$	-2.1
ClO_3^-	905	$KClO_3$	-1.1
SCN^-	975	NaSCN	-16.5
$S_2O_3^{--}$	1330	$Na_2S_2O_3$	+3.2
BO_3^{---}	1940	$Na_2B_4O_7$	-0.3
BrO_3^-	1028	$KBrO_3$	-0.3
MoO_4^{--}	81	Na_2MoO_4	-0.2
$C_6H_5O_7^{---}$	1627	$H_3C_6H_5O_7$	-0.8
$S_2O_8^{--}$	1567	$(NH_4)_2S_2O_8$	-15.4

and solution titrated contained a 1 M concentration of the acid desired. Two sharp breaks were obtained in each of the two titrations and the horizontal portion of the curve corresponding to the titration of the ferrous trisbathophenanthrolinedisulfonic acid was sufficiently broad (because of the large amount taken) to permit locating the mid-point with certainty. The mid-point of the first horizontal portion of the curve was taken to represent the formal reduction potential of the ferric-ferrous couple in 1 M sulfuric and 1 M perchloric acids.

Formal reduction potential in 1 M sulfuric acid

To 100 ml. of deionized water was added 0.3280 g. of disodium bathophenanthrolinedisulfonate and 10 ml. of an approximately 0.05 M ferrous sulfate solution. To this solution was added 110 ml. of 2 M sulfuric acid solution and the resulting solution was potentiometrically titrated to 100% past the second titration curve break using approximately 0.05 M sulfatoceric acid prepared from ceric hydroxide and being 1 M in sulfuric acid. The results of the titration are shown in Fig. 8.

The formal reduction potential found, after correcting to the hydrogen scale, was 1.09 volts.

Formal reduction potential in 1 M perchloric acid

To 100 ml. of deionized water was added 0.6370 g. of disodium bathophenanthrolinedisulfonate and 20 ml. of an approximately 0.05 M ferrous perchlorate solution. To this solution was

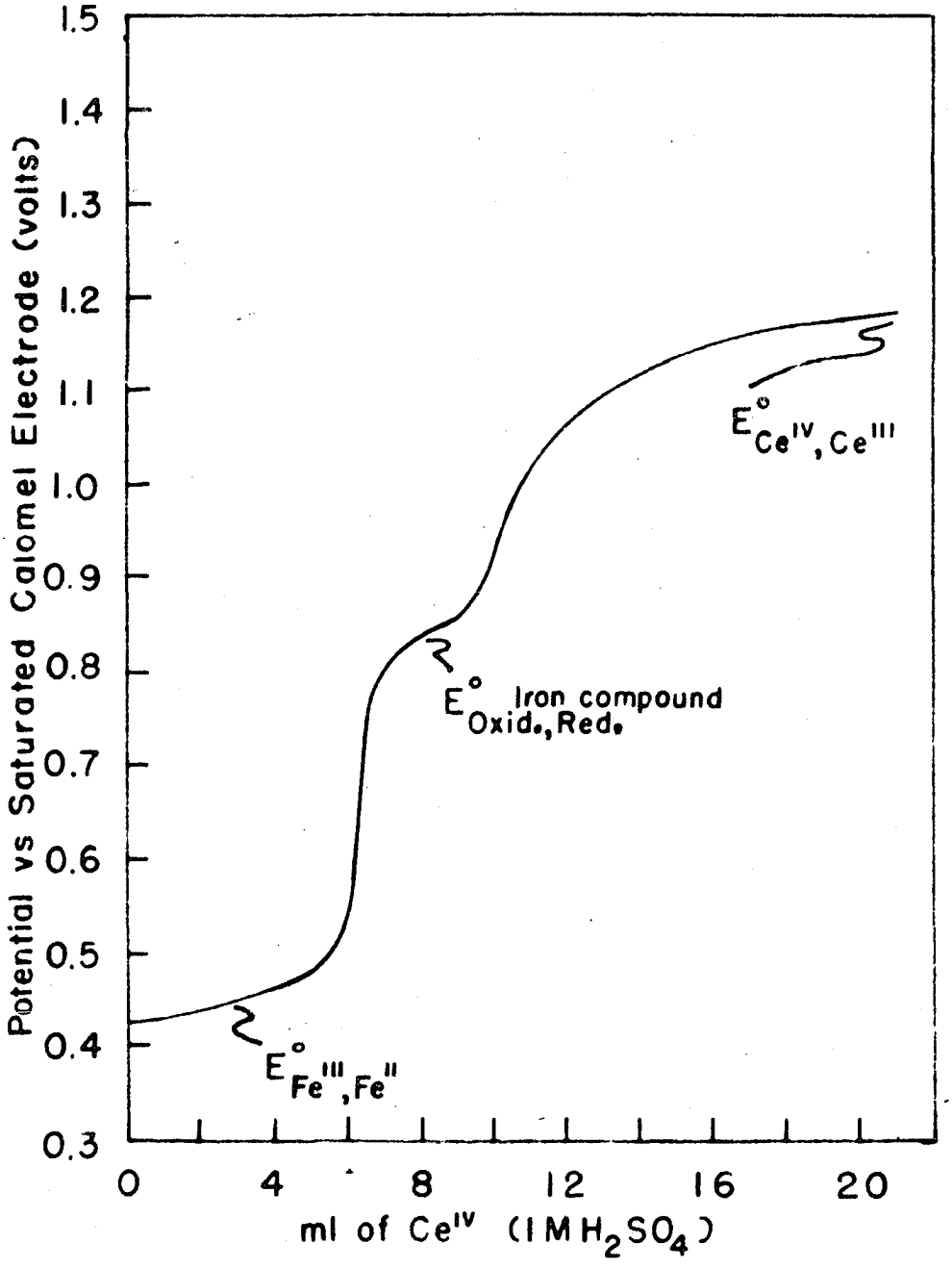


Fig. 8. Potentiometric titration of ferrous iron and ferrous trisbathophenanthrolinedisulfonic acid in 1 M sulfuric acid.

added 120 ml. of 2 M perchloric acid solution and the resulting solution was titrated using approximately 0.05 M perchloratoceric acid titrant prepared by heating ceric hydroxide with perchloric acid and anodically oxidizing the resulting solution to the cerate state. The resulting titrant was made 1 M in perchloric acid. The results of the titration are shown in Fig. 9.

The formal reduction potential vs. the hydrogen electrode, taken from the resulting titration curve, was 1.01 volts.

Potentials at color changes Two different techniques were used to determine the potentials at the color changes of the indicator. During the titration the potential was recorded at the point where the red reduced form of the iron derivative, as determined visually, had completely changed to the green oxidized ferric derivative.

The second technique used to determine the potential at the color change was to measure the potential of a mixture of ferrous iron and a small amount of the indicator after being titrated to the green oxidized form using cerate titrant 1 M in the desired acid. This technique was repeated on a number of solutions and the values averaged. The two techniques were found to agree quite well.

Ferrous trisbathophenanthrolinedisulfonic acid was found to give a sharp color change, corresponding to a potential vs. hydrogen of 1.15 volts in 1 M sulfuric acid and of 1.14 volts in 1 M perchloric acid.

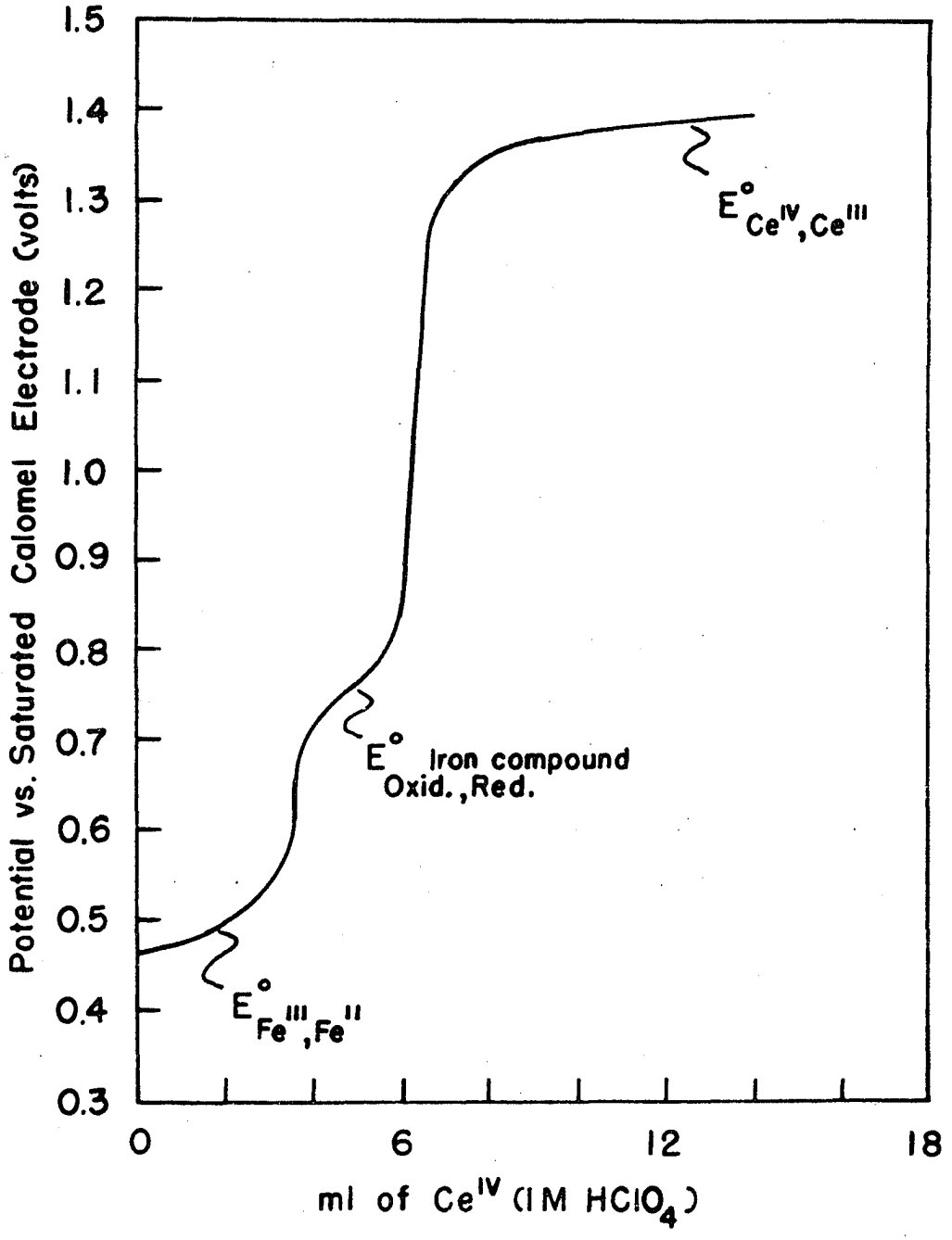


Fig. 9. Potentiometric titration of ferrous iron and ferrous trisbathophenanthrolinedisulfonic acid in 1 M perchloric acid.

Discussion

The problems involved in the synthesis of bathophenanthrolinedisulfonic acid and bathocuproinedisulfonic acid were essentially three-fold: a relatively mild sulfonation reaction was required in order that products would be obtained which were consistently sulfonated in the same substitution positions; secondly, owing to the high cost of the starting material, high yields were desirable; and thirdly a method for separating the organic sulfonated material from the inorganic salt produced in the reaction was needed.

By conducting the sulfonation at 25° over an extended period of time, a consistent product was produced. Several shorter intervals of sulfonation resulted in incomplete sulfonation.

The yields obtained for both bathophenanthrolinedisulfonic acid and bathocuproinedisulfonic acid were very good.

Many different techniques were tried in an attempt to isolate the sulfonated materials in pure form. The usual methods used for precipitating organic sulfonic acids from solution were uniformly unsuccessful because of the extremely high solubility of the compounds in water. Ion exchange techniques also proved unsatisfactory. The technique used, that of extracting the ammonium salt of the sulfonated material into 95% ethanol, appears satisfactory.

Contamination from iron and copper during the synthesis

must be avoided since no method of removing the contamination resulting from the formation of derivatives of these ions with the sulfonated reagents was found. This metal ion contamination comes primarily from the chlorosulfonic acid, unclean glassware, and the base used for the final neutralization of the sulfonated reagents.

The infrared spectra shown in Fig. 1 indicate the disodium bathophenanthrolinedisulfonate to be slightly purer than the disodium bathocuproinedisulfonate. Both compounds were tested and found to be free of chloride and sulfate contamination.

It is evident that room temperature sulfonation with chlorosulfonic acid has produced a disulfonated material in the case of both bathophenanthroline and bathocuproine. The parent 1,10-phenanthroline cannot be sulfonated under these conditions. It appears reasonable to assume then that the sulfonic groups have entered into the two phenyl rings in both cases. Sulfonation of a phenyl ring, like nitration, tends to hinder further substitution in the ring; therefore, it is safe to assume that one sulfonic group has substituted on each of the phenyl groups in the case of both bathophenanthroline and bathocuproine. Some efforts were made to determine the exact positions of the sulfonic acid groups on the phenyl rings, but were unsuccessful.

Various work has shown that 1,10-phenanthroline and its derivatives act as monoacidic bases, presumably because the

spatial arrangement permits only one proton to enter the space between the ring nitrogens. It would be expected that a proton from one of the two sulfonic acids would be transferred to the ring nitrogens giving the molecule a zwitter ion structure. The titration curves, Fig. 2, can be explained on this basis: the first titration break corresponds to the titration of one of the strongly acidic sulfonic acids, the second to the titration of the proton held by the ring nitrogens. The negative logarithm of the respective acid dissociation constants are:

	pK_1	pK_2
Bathophenanthrolinedisulfonic Acid	2.83	5.20
Bathocuproinedisulfonic Acid	2.65	5.80

The pK_2 of bathocuproinedisulfonic acid is larger than the pK_2 of bathophenanthrolinedisulfonic acid, presumably because the basic character of the former is enhanced by the presence of the 2- and 9-methyl groups.

The zwitter ion structure probably accounts for the failure in either case to obtain a diammonium salt on neutralization of the acids with ammonium hydroxide with subsequent evaporation and drying.

Since in the case of both iron and copper, the lower oxidation state is the form in which the derivatives of bathophenanthrolinedisulfonate and bathocuproinedisulfonate are formed with the metals, reduction of the metals must precede color formation; hydroxylammonium chloride in acid

solution was used almost exclusively to reduce the iron to the ferrous state and the copper to the cuprous state. The exception was the pH stability study in which sodium sulfite was used in place of hydroxylammonium chloride because erratic pH readings were found in basic solution when even small amounts of hydroxylammonium chloride were present.

The pH over which the metal derivatives are stable extends somewhat further into the alkaline region for the sulfonated materials, to nearly pH 11, in contrast to pH 9 for the metal derivatives of the parent compounds.

As would be predicted, the combining ratio found for the ferrous bathophenanthrolinedisulfonate derivative, 1:3.17, and that found for the cuprous bathocuproinedisulfonate derivative, 1:2.24, are in agreement with the known ratio of metal ions to parent compounds (1:3 and 1:2 respectively). The value found for the cuprous bathocuproinedisulfonate derivative was not as close to 1:2 as might be desired; possibly the material analyzed was not perfectly dry. The material is highly hydroscopic.

The evidence that both ferrous trisbathophenanthrolinedisulfonate and cuprous bisbathocuproinedisulfonate carry negative charges as shown by the migration of the compounds toward the positive electrode, is in agreement with the fact that the combining ratio of ferrous to bathophenanthrolinedisulfonate is 1:3 and that sulfonic groups are present. With six sulfonic groups present in the derivative, a net

charge of -4 would be expected on the derivative; a charge of -3 would be expected on the cuprous bisbathocuproinedisulfonate derivative composed of a 1:2 ratio of copper to sulfonated compound.

In the study of the effect of various ions upon ferrous trisbathophenanthrolinedisulfonate and cuprous bisbathocuproinedisulfonate, depending upon the concentration of foreign ion present, an approximate relative error of 2% was generally taken to represent possible experimental error and not interference from the ion in question.

In the determination of iron using bathophenanthrolinedisulfonate, interference from various ions was due to several factors. In the case of copper, interference was due to the increased color produced by the cuprous ion reacting with excess sulfonated reagent. Cyanide interfered by tying up the iron so that it did not produce the colored derivative with the sulfonated reagent. Aluminum, tin (IV), and thiosulfate all precipitated under the conditions of the experiment and thus interfered. Persulfate affected the color development of ferrous trisbathophenanthrolinedisulfonate while dichromate interfered by virtue of the fact that it is colored.

A large excess of bathophenanthrolinedisulfonate was added in the study of the effects of copper, cobalt, and nickel since it was likely that these form derivatives with the reagent, thus using up bathophenanthrolinedisulfonate.

As a result of their precipitation, aluminum, tin (IV), and thiosulfate interfere in the determination of copper using bathocuproinedisulfonate. Dichromate and uranyl were found to interfere due to the fact that they are colored, while cyanide, thiocyanate, and thiosulfate interfere with the color development of cuprous bisbathocuproinedisulfonate.

In order to check the techniques used for the determination of the formal reduction potentials of ferrous tris-bathophenanthrolinedisulfonic acid, the formal reduction potential of ferrous 1,10-phenanthroline was determined in 1 M sulfuric acid and found to be 1.06 volts which agrees with the value reported in the literature (12).

The formal reduction potentials in 1 M sulfuric acid found for iron and for cerium from the titration curve (Fig. 8) agreed with those values already reported in the literature (13,14). In 1 M perchloric acid, the formal reduction potential for iron (Fig. 9) agreed with that value already reported in the literature (13), however, the 1.63 volts found for the formal reduction potential of cerium at 100% past the second end-point was considerably lower than the 1.70 volts reported by Smith and Getz (13).

Since the presence of foreign anions can greatly reduce the reduction potential of cerium, efforts were made to prepare pure perchloratoceric acid following the procedure used by Smith and Getz (13). Pure ferrous perchlorate was prepared and used for the ferrous salt in order that the only anion

present would be perchlorate. A salt bridge of saturated ammonium perchlorate connected the saturated calomel with the solution; the bridge being removed at 100% past the end-point and a reading taken then. The purpose of this bridge was to prevent chloride from diffusing into the solution. No significant potential difference was observed with and without the bridge. Using the above procedure no significant change was observed in the formal reduction potential previously obtained for cerium in 1 M perchloric acid.

Since the formal reduction potential of iron, as determined by the potentiometric titration, agreed with the already established values, it wasn't felt that further work to duplicate the formal reduction potential of cerium already reported was necessary and it was concluded that the values found for the formal reduction potentials of the indicator was correct.

1,10-PHENANTHROLINE-5-SULFONIC ACID MONOHYDRATE AND 1,10-PHENANTHROLINE-3-SULFONIC ACID

Apparatus and Reagents

Apparatus

The apparatus and instruments used for this study were the same as those used in the study of bathophenanthroline-disulfonic acid and bathocuproinedisulfonic acid.

Reagents

The 1,10-phenanthroline starting material was obtained from the G. Frederick Smith Chemical Company, Columbus, Ohio. The 5-hydroxy-1,10-phenanthroline and 3-chloro-1,10-phenanthroline were obtained from Professor F. H. Case of Temple University. The water used was first distilled and then passed through a monobed ion exchange resin. All other materials were reagent grade or of high purity.

Synthesis and Study of Properties

Synthesis

To a three necked one liter round bottom flask was added 40 g. of 1,10-phenanthroline monohydrate and 200 g. of ammonium acid sulfate. The temperature was raised to 365-370° using a Meker burner and the mixture was stirred mechanically for 10 min. at this temperature. A water condenser was inserted in one of the necks in order to condense

volatilized 1,10-phenanthroline. The mixture was allowed to cool until almost solid at which time the thermometer was removed and 200 ml. of water was cautiously added.

Concentrated ammonium hydroxide was then added to the mixture in the flask until the solution was basic, and the resulting solution was evaporated to dryness on a steam plate. The solid residue was pulverized using a mortar and pestle, 400 ml. of chloroform was added and the slurry stirred for 15 min. after which it was filtered and the chloroform distilled leaving a 3 g. residue of unreacted 1,10-phenanthroline.

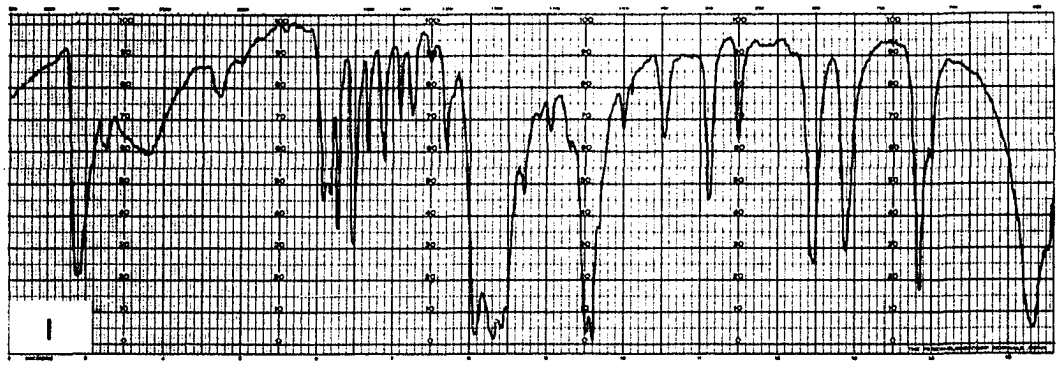
After the chloroform extraction, the residue of ammonium sulfate and ammonium salt of sulfonated 1,10-phenanthroline was dried briefly and stirred for 30 min. with 4 l. of 85% ethanol at a temperature of approximately 65°. While still hot the mixture was filtered using aspiration and 500 ml. of water was added to the filtrate. The resulting solution was heated to 65° and while hot was passed through an electrically heated column, 5 cm. x 20 cm., containing Amberlite IR-120 in the hydrogen form. The flow rate of the solution through the column was approximately 300 ml./min. Approximately 500 ml. of hot water was used to wash the solution through the column.

The solution was evaporated to 135 ml., allowed to stand overnight and then filtered by aspiration. The filtrate upon evaporation yielded approximately 12 g. of a

mixture of various sulfonated 1,10-phenanthrolines, sulfonated oxidation products of 1,10-phenanthroline, and possibly some mono and polysulfonated 1,10-phenanthrolines. The solid material left in the funnel following the filtration, was dissolved in approximately 1 l. of hot water and evaporated to 400 ml., cooled to room temperature for 2 hrs. and then filtered by aspiration. Approximately 13 g. of what later proved to be 1,10-phenanthroline-5-sulfonic acid monohydrate was filtered out at this point.

The filtrate was evaporated to 100 ml., cooled for 2 hrs. at room temperature and then filtered by aspiration. Approximately 4.5 g. of a mixture of 1,10-phenanthroline-5-sulfonic acid monohydrate and what was found later to be 1,10-phenanthroline-3-sulfonic acid remained in the funnel. The separation of these two compounds was accomplished by adding 300 ml. of methanol to the dried, pulverized material which was stirred for 1 hr. and then filtered by aspiration. Approximately 1.7 g. of 1,10-phenanthroline-3-sulfonic acid was recovered from the funnel; the filtrate containing 1,10-phenanthroline-5-sulfonic acid contaminated with a very small amount of 1,10-phenanthroline-3-sulfonic acid as shown by an infrared spectrum of the filtrate residue. Fig. 10 shows the infrared spectra of pure 1,10-phenanthroline-5-sulfonic acid monohydrate and 1,10-phenanthroline-3-sulfonic acid, obtained using the potassium bromide pellet technique. Throughout the separations, infrared data was used to

Fig. 10. Infrared spectra of 1,10-phenanthroline-5-sulfonic acid monohydrate (spectrum 1) and 1,10-phenanthroline-3-sulfonic acid (spectrum 2).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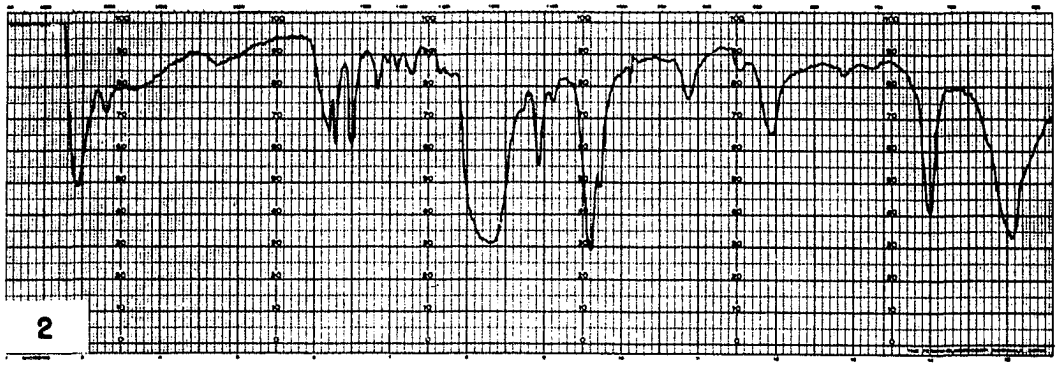
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determine the purity of the materials obtained. This was important in determining the degree of contamination of 1,10-phenanthroline-5-sulfonic acid with 1,10-phenanthroline-3-sulfonic acid and vice versa. There is a strong absorption peak at 12.45μ in the spectrum of 1,10-phenanthroline-5-sulfonic acid monohydrate which is absent in the spectrum of the 3-sulfonic acid material. 1,10-phenanthroline-3-sulfonic acid shows a strong peak at 12.0μ while the 1,10-phenanthroline-5-sulfonic acid monohydrate doesn't. These complementary absorption peaks thus provide a convenient check of contamination from each other.

Yield A yield of 30% conversion of the starting material was found for 1,10-phenanthroline-5-sulfonic acid while a yield of 4% was found for 1,10-phenanthroline-3-sulfonic acid. A highly water soluble residue composed chiefly of a mixture of disulfonated 1,10-phenanthrolines was recovered in a yield of 19%.

Ultimate analysis Found for 1,10-phenanthroline-5-sulfonic acid monohydrate: C 52.16%, H 3.60%, N 10.43%, S 10.20% (Huffman Microanalytical Laboratory, Wheatridge, Colorado) giving an empirical formula of $C_{11.7}H_{9.6}N_{2.0}S_{0.9}$; 1,10-phenanthroline-5-sulfonic acid monohydrate: $C_{12}H_{10}N_2SO_4$.

Found for 1,10-phenanthroline-3-sulfonic acid: C 53.63%, H 3.46%, N 10.29%, S 11.67% (Huffman Microanalytical

Laboratory) giving an empirical formula of $C_{12.2}H_{9.3}N_{2.0}S_{1.0}$; 3-sulfonated 1,10-phenanthroline: $C_{12}H_8N_2SO_3$.

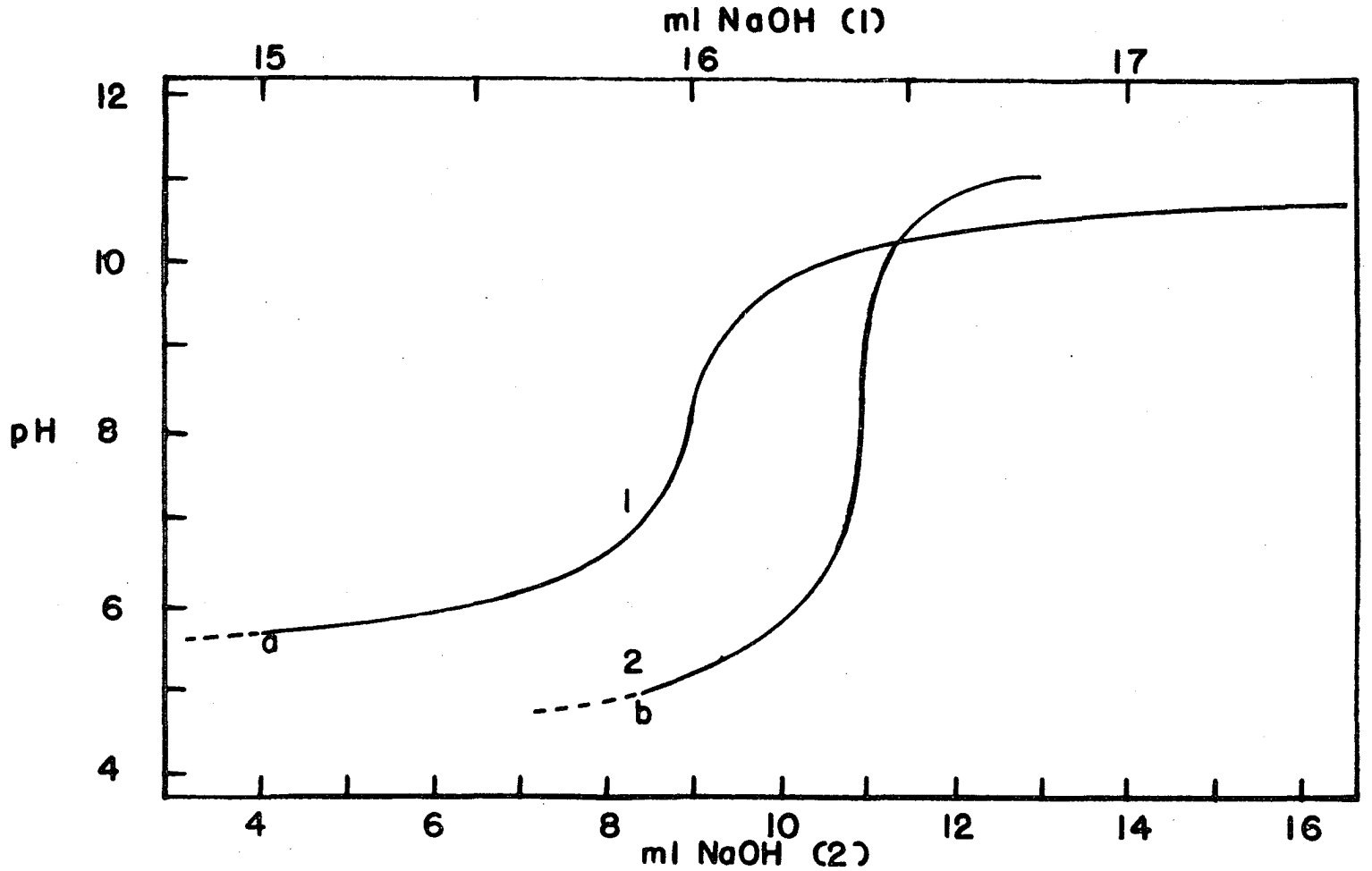
Equivalent weight Both compounds were dried for 2 hrs. at 110°. A sample of 1,10-phenanthroline-5-sulfonic acid monohydrate weighing 0.5016 g. was titrated with standard 0.1136 N sodium hydroxide. A sample of 1,10-phenanthroline-3-sulfonic acid weighing 0.3293 g. was also titrated. A Beckman Model G pH meter was used to measure pH. The resulting titration curves are shown in Fig. 11. Both 1,10-phenanthroline-5-sulfonic acid monohydrate and 1,10-phenanthroline-3-sulfonic acid are not very water soluble and the sulfonic acids dissolved only after the titrations had proceeded for some time. This is shown by the dotted lines in Fig. 11.

The equivalent weights found for 1,10-phenanthroline-5-sulfonic acid monohydrate were: 278.0, 277.8; calculated for $C_{12}H_7N_2(SO_3H)\cdot H_2O$, 278.3; for $C_{12}H_6N_2(SO_3H)_2\cdot H_2O$, 179.2.

The equivalent weights found for 1,10-phenanthroline-3-sulfonic acid were: 264.3, 265.8; calculated for $C_{12}H_7N_2(SO_3H)$, 260.3; for $C_{12}H_6N_2(SO_3H)_2$, 170.2.

Melting points Owing to the extremely high melting points of both compounds, a special apparatus was set up to determine the melting points of 1,10-phenanthroline-5-sulfonic acid monohydrate and 1,10-phenanthroline-3-sulfonic acid. The apparatus, similar to that designed by May (15), consisted of a 15 x 125 mm. test tube inserted in a

Fig. 11. Titration of 1,10-phenanthroline-5-sulfonic acid (curve 1) and of 1,10-phenanthroline-3-sulfonic acid (curve 2) showing points at which solid acids were completely dissolved (a and b).


ml NaOH (1)
15
16
17
12
10
pH 8
6
4
1
a
2
b
4
6
8
10
12
14
16
ml NaOH (2)

20 x 180 mm. test tube, both test tubes fitted with one hole rubber stoppers through which a 500° thermometer was inserted to extend the full length of the smaller inner tube. The rubber stoppers were slotted lengthwise so that upon heating the expanding gas could escape. The larger tube was wrapped with resistance wire and covered with asbestos except for a small window opening through which the bulb of the thermometer inside the inner test tube could be viewed while the apparatus was being electrically heated using a rheostat to control the temperature. The entire apparatus was placed in a horizontal position and a little of the sulfonated material was placed on the bulb of the thermometer. Upon heating, the material on the bulb melted, and the melting point was recorded.

The melting point found for 1,10-phenanthroline-5-sulfonic acid monohydrate was approximately 477-483° while that for 1,10-phenanthroline-3-sulfonic acid was approximately 381-386°. Both compounds showed discoloration at this high temperature. When heated rapidly both compounds melted sharply and without discoloration.

Determination of structure 1,10-Phenanthroline-5-sulfonic acid monohydrate and 1,10-phenanthroline-3-sulfonic acid were both dried for 2 hrs. at 110° and then run on the thermobalance to determine if they contained water of hydration.

A 0.1676 g. sample of the 1,10-phenanthroline-5-sulfonic

acid monohydrate was used, giving the weight loss vs. temperature curve shown in Fig. 12. A weight loss corresponding to 1.03 H_2O was found. Sodium 1,10-phenanthroline-5-sulfonate was also run; no significant weight loss being found.

The work was repeated using 1,10-phenanthroline-3-sulfonic acid and sodium 1,10-phenanthroline-3-sulfonate; no significant weight loss was found over the temperature range 25-375°.

The position of substitution of the sulfonic group on the compound that we have previously designated as 1,10-phenanthroline-5-sulfonic acid was determined by fusing the sulfonated material with sodium hydroxide in a silver crucible, dissolving the melt in dilute sulfuric acid and adjusting the pH to 7 with sodium hydroxide. The resulting precipitate was extracted into isoamyl alcohol, washed with water, the solution evaporated to dryness and the resulting hydroxy-1,10-phenanthroline precipitated from hot ethanol. An infrared spectrum was run on the material using the potassium bromide pellet technique. The resulting spectrum was found to be exactly the same as that obtained using pure 5-hydroxy-1,10-phenanthroline supplied by Zacharias and Case (16) and synthesized so that the hydroxy group was necessarily in the five position.

The position of the sulfonic acid group in 1,10-phenanthroline-3-sulfonic acid was determined in the following way. The sulfonated compound was converted to the

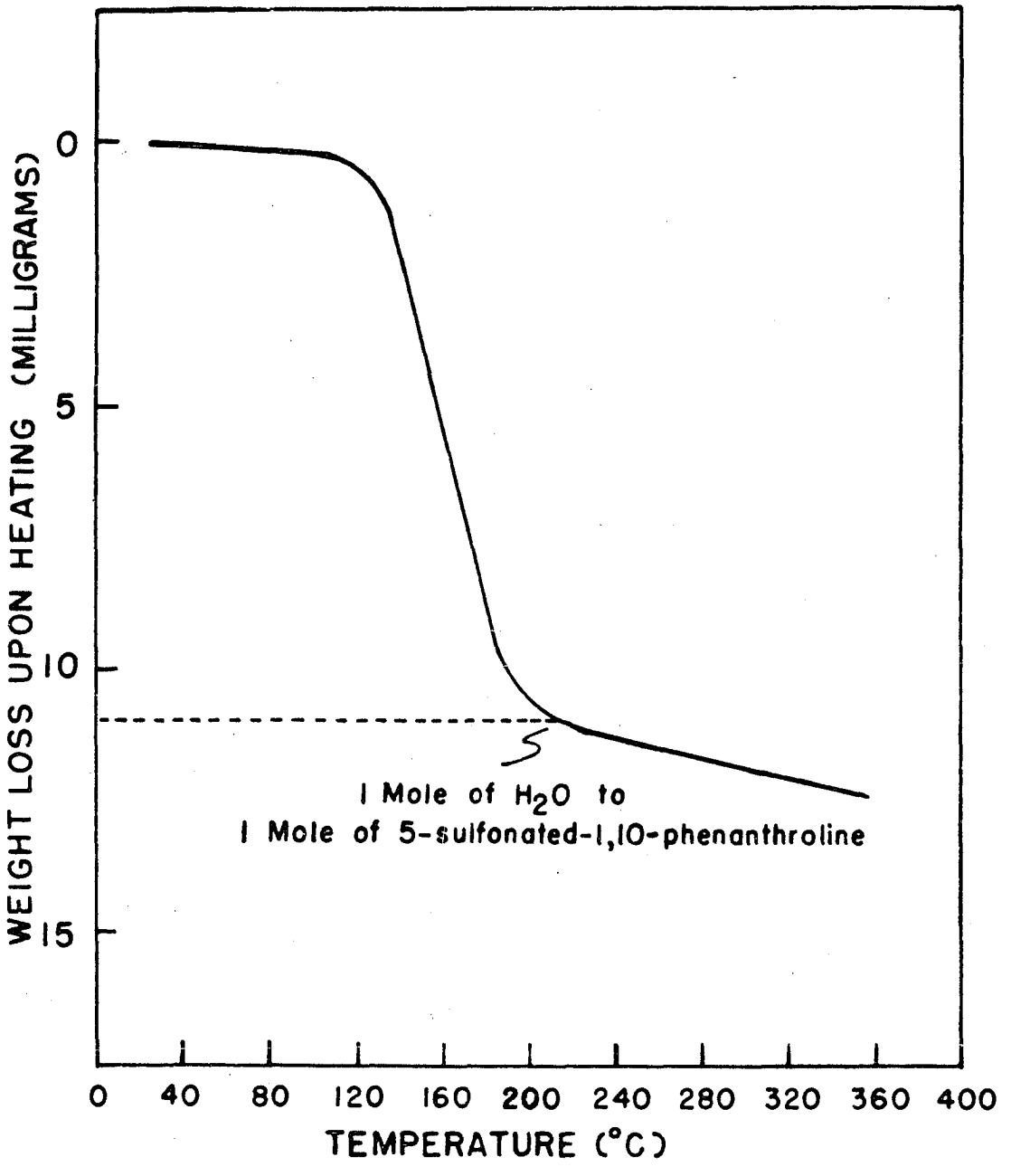


Fig. 12. Thermogravimetric curve of 1,10-phenanthroline-5-sulfonic acid monohydrate.

corresponding hydroxy compound using the same technique as that used to convert 1,10-phenanthroline-5-sulfonic acid to the hydroxy compound, with the exception that potassium hydroxide was substituted for the sodium hydroxide in the initial fusion. An infrared spectrum of the resulting hydroxy-1,10-phenanthroline indicated it to be different than 4-hydroxy-1,10-phenanthroline obtained from Case, prepared according to the method of Snyder and Freier (17). Since 3-hydroxy-1,10-phenanthroline had not been prepared, and since 3-chloro-1,10-phenanthroline was available from Case (18), the hydroxy-1,10-phenanthroline was converted to the corresponding chloro-1,10-phenanthroline utilizing a reaction used by Snyder and Freier (17) in preparing other chloro-1,10-phenanthroline derivatives.

To 0.13 g. of the hydroxy-1,10-phenanthroline converted from the sulfonated material was added 0.15 g. of phosphorous pentachloride and 0.25 ml. of phosphorous oxychloride. The mixture was heated for 2 hrs. at 130° in an oil bath. It was then cooled and approximately 25 ml. of water was added and the pH made 7 with sodium hydroxide. The mixture was extracted into chloroform and washed with water three times. The chloroform was evaporated and the residue taken up in hot benzene. The chloro-1,10-phenanthroline was precipitated from this and recrystallized once more from benzene.

The infrared spectrum of the dried product, using the potassium bromide pellet technique, was identical with the

spectrum obtained from the 3-chloro-1,10-phenanthroline obtained from Case, thus showing the sulfonated material to be 1,10-phenanthroline-3-sulfonic acid.

Properties

The water solubilities at 25° of both the acids and the sodium salts of 1,10-phenanthroline-5-sulfonic acid monohydrate and 1,10-phenanthroline-3-sulfonic acid were determined by preparing saturated solutions of the respective sulfonated materials in a constant temperature bath for several days, at which time the solid material was rapidly filtered off, the clear solution pipetted into flasks, and the colored derivative developed using excess ferrous iron. The concentration of the sulfonated material was then determined by spectrophotometrically measuring the absorbencies of the ferrous derivatives.

The water solubility of the 1,10-phenanthroline-5-sulfonic acid monohydrate at 25° was 0.518 g./l. while that of the sodium salt was 7.35 g./l. The solubility of 1,10-phenanthroline-3-sulfonic acid in water was found to be 0.920 g./l.; the sodium salt 4.39 g./l.

Both compounds are readily recrystallized as the acids from hot water to give crystalline cream colored needles. Both materials give a yellow fluorescence under ultraviolet light. The sodium salts give a purple fluorescence.

An attempt was made to prepare the ammonium salt of

1,10-phenanthroline-5-sulfonic acid. Excess concentrated ammonium hydroxide was added to the acid form and the solution was evaporated to dryness and heated for 10 hrs. at 120°. A Kjeldahl distillation was used to determine the amount of ammonium nitrogen present in 0.4154 g. of the dry salt. There was found 4.45% ammonia in contrast to 6.15% ammonia for a theoretical composition of one ammonium ion per 1,10-phenanthroline-5-sulfonate molecule.

Ferrous Tris 1,10-Phenanthroline-5-sulfonate and Ferrous Tris 1,10-Phenanthroline-3-sulfonate

Spectrophotometric constants and necessary conditions

Absorption spectra A solution of the ferrous derivative of 1,10-phenanthroline-5-sulfonate was prepared in the following way. To a 100 ml. volumetric flask was added 1.0 ml. of 9.43×10^{-3} M ferrous chloride, 1 ml. of 10% hydroxylammonium chloride, 4 ml. of 1.15×10^{-2} M 1,10-phenanthroline-5-sulfonate (prepared by dissolving the acid form of the material in water containing a small amount of ammonium hydroxide), and 1 ml. of 10% sodium acetate. The flask was diluted to the mark with deionized water and the absorption spectrum recorded with a Cary Model 12 recording spectrophotometer using 1 cm. matched cells; the resulting spectrum is shown in Fig. 13.

A solution of the ferrous derivative of 1,10-phenanthroline-3-sulfonate was also prepared. To a 100 ml. flask

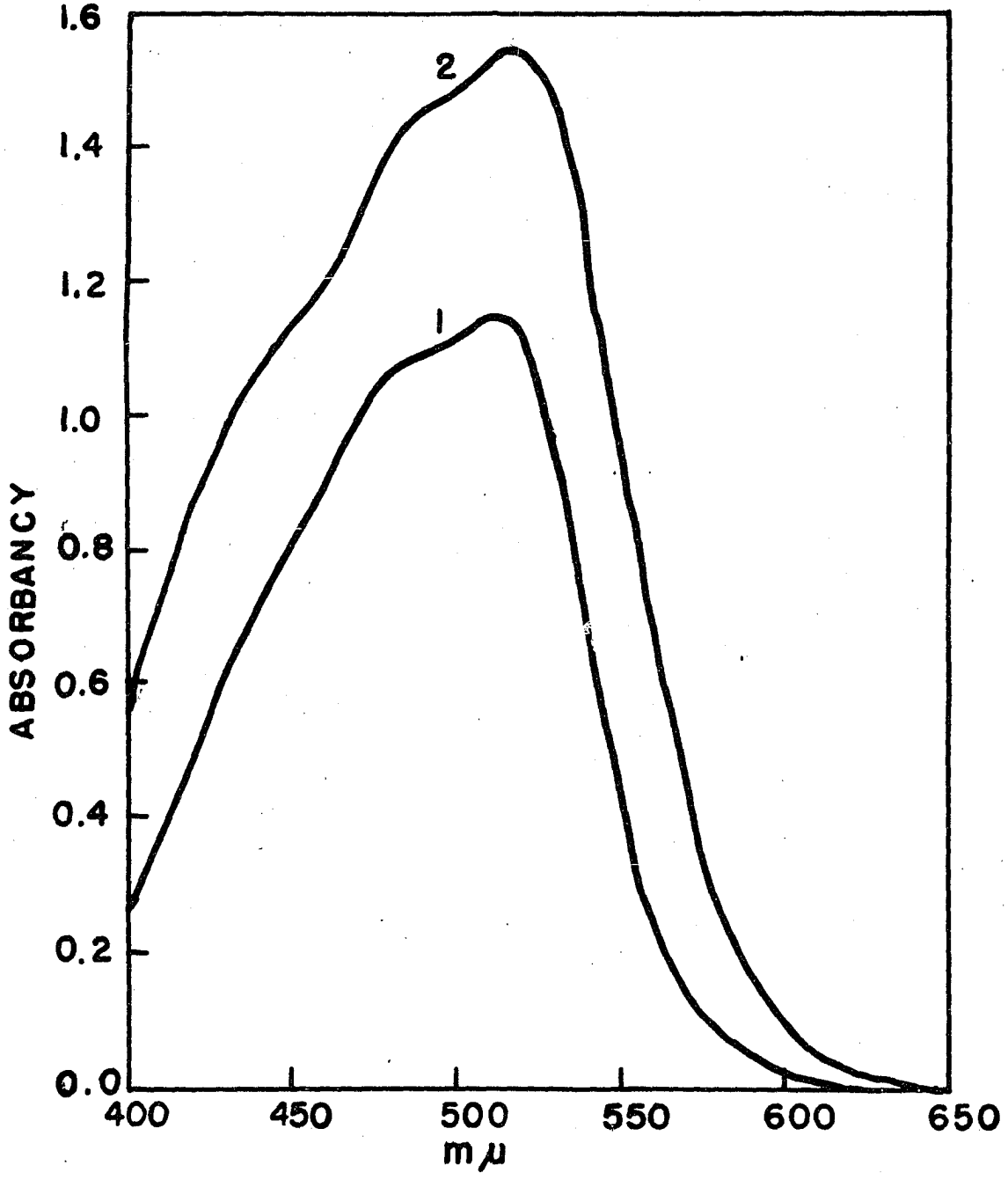


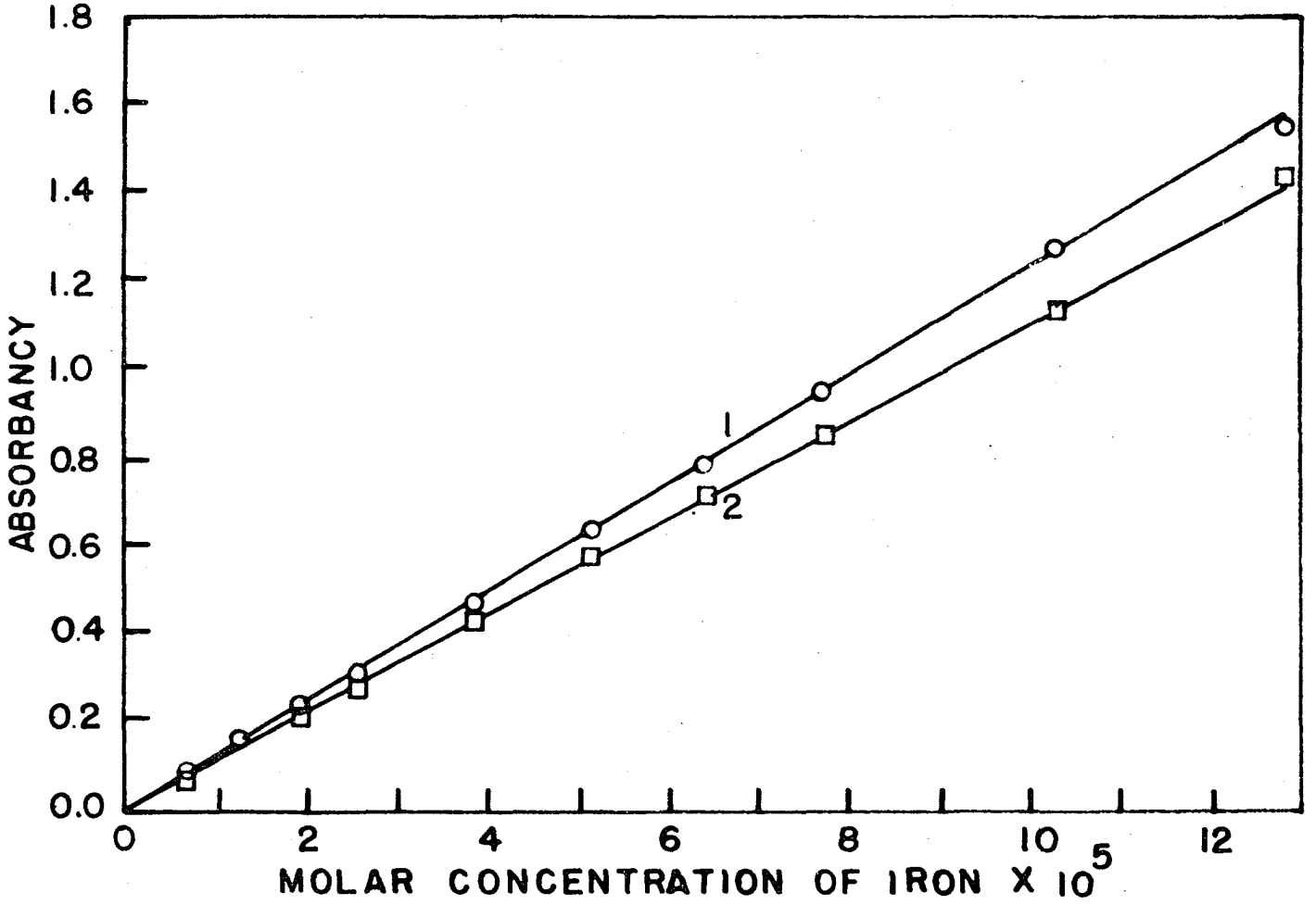
Fig. 13. Absorption spectra of the ferrous derivative of 1,10-phenanthroline-5-sulfonate (spectrum 1) and of the ferrous derivative of 1,10-phenanthroline-3-sulfonate (spectrum 2).

was added 2.0 ml. of 7.55×10^{-3} M ferrous chloride, 4 ml. of 10% hydroxylammonium chloride, 6 ml. of 1.22×10^{-2} M 1,10-phenanthroline-3-sulfonate (prepared by dissolving the compound in water using ammonium hydroxide to get into solution), and 5 ml. of 10% sodium acetate. The absorption was recorded using 1 cm. cells and is shown in Fig. 13.

Sensitivity A series of solutions containing excess 1,10-phenanthroline-5-sulfonate and an iron concentration varying from 0 to 1.29×10^{-4} M was prepared by transferring aliquots of a 6.45×10^{-4} M iron solution into 50 ml. volumetric flasks and adding 2 ml. of 10% hydroxylammonium chloride, 5 ml. of 6.18×10^{-3} M 1,10-phenanthroline-5-sulfonate, and 5 ml. of 10% sodium acetate. After dilution to volume, the absorbancy of each solution was determined at 512 mμ, the absorption maximum, using a Beckman DU spectrophotometer and 1 cm. cells. A solution containing all of the reagents except the iron was used as a blank. The resulting Beer's law plot is shown in Fig. 14. From the slope of the line, a molar extinction coefficient of 12,240 was found for the ferrous derivative.

The procedure for determining the sensitivity of 1,10-phenanthroline-3-sulfonate as a colorimetric reagent for iron was exactly the same as that just described, with the exception that 5 ml. of 6.34×10^{-3} M 1,10-phenanthroline-3-sulfonate was used as the colorimetric reagent and that the absorbancy readings were made at 517 mμ, the

Fig. 14. Beer's law plot for the ferrous derivative of 1,10-phenanthroline-5-sulfonate (curve 1) and for the ferrous derivative of 1,10-phenanthroline-3-sulfonate (curve 2).


1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ABSORBANCY
0
2
4
6
8
10
12
MOLAR CONCENTRATION OF IRON X 10^5
1
2

maximum for this derivative. The Beer's law plot for this system is shown in Fig. 14. The molar extinction coefficient found for this derivative from the slope in Fig. 14 is 10,840.

Both ferrous derivatives obey Beer's law over the concentration range, 0 to 7.2 p.p.m. of iron.

Effect of pH on stability To a series of 50 ml. volumetric flasks each containing two drops of concentrated hydrochloric acid, was added 1.0 ml. of 2.59 x 10^{-3} M iron, 1 ml. of 10% sodium sulfite, and 3 ml. of 6.18 x 10^{-3} M 1,10-phenanthroline-5-sulfonate. Using dilute hydrochloric acid and sodium hydroxide, the solutions were adjusted to various pH values. The solutions were diluted to 50 ml. with deionized water and allowed to stand for 1½ hrs. at which time the absorbancy values were read on the Beckman DU spectrophotometer at 512 mμ using 1 cm. cells and a reagent blank. The pH of the solutions were determined using a Beckman Model G pH meter; standardization of the instrument was done with various commercial buffers. The results of this stability study are shown in Fig. 15.

The same procedure was used to determine the stability of the ferrous 1,10-phenanthroline-3-sulfonate derivative with varying pH, with the exceptions that 3.0 ml. of 6.34 x 10^{-3} M 1,10-phenanthroline-3-sulfonate was used as the sulfonated reagent and that the absorbancy readings were made at 517 mμ. The results are shown in Fig. 15.

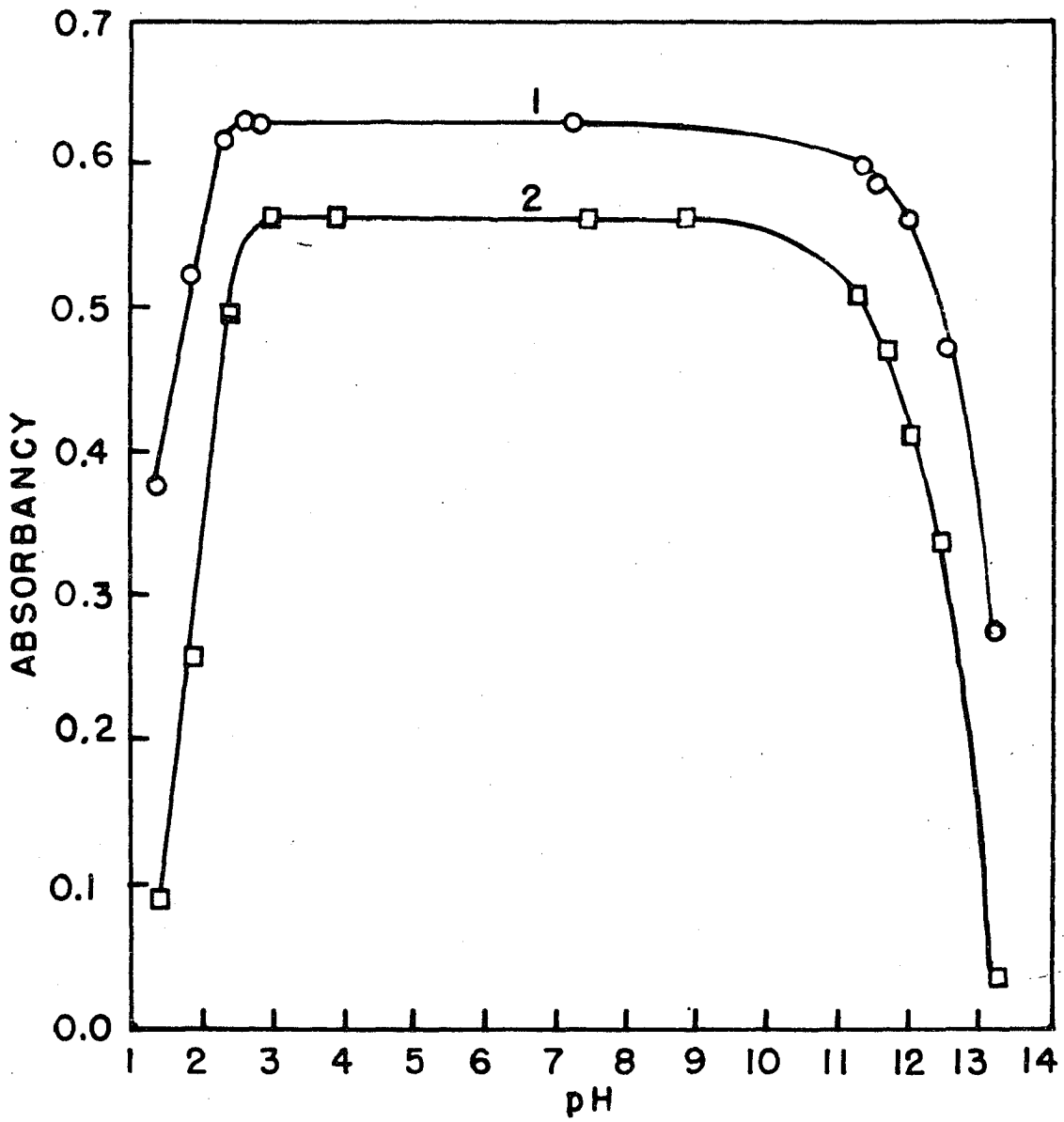


Fig. 15. Effect of pH upon the stability of the ferrous derivative of 1,10-phenanthroline-5-sulfonate (curve 1) and of the ferrous derivative of 1,10-phenanthroline-3-sulfonate (curve 2).

Combining ratios The combining ratio of the ferrous and 1,10-phenanthroline-5-sulfonate ions was determined by a spectrophotometric titration in which in 100 ml. volumetric flasks, the iron concentration was held constant at 9.43×10^{-5} M while the concentration of the 1,10-phenanthroline-5-sulfonate was varied from 0 to 5.76×10^{-4} M. The color was developed using 1 ml. of 10% hydroxylammonium chloride and 1 ml. of 10% sodium acetate. The absorbancies were read against a reagent blank at 512 mμ using 1 cm. cells and the Cary Model 12 recording spectrophotometer. The titration curve is shown in Fig. 16 and shows a combining ratio of ferrous to 1,10-phenanthroline-5-sulfonate of 1:3.06.

Essentially the same technique was used to determine the combining ratio of the ferrous and 1,10-phenanthroline-3-sulfonate ions as has just been described. The iron concentration was held constant at 1.51×10^{-4} M while the concentration of the 1,10-phenanthroline-3-sulfonate was varied from 0 to 1.22×10^{-3} M. The color was developed using 4 ml. of 10% hydroxylammonium chloride and 5 ml. of 10% sodium acetate.

The absorbancies were read at 517 mμ and the resulting titration curve is shown plotted in Fig. 17. The combining ratio of ferrous to 1,10-phenanthroline-3-sulfonate was found to be 1:3.15.

Charge on ferrous derivatives Following essentially the same procedure and utilizing the same apparatus as that

Fig. 16. Spectrophotometric titration of iron.

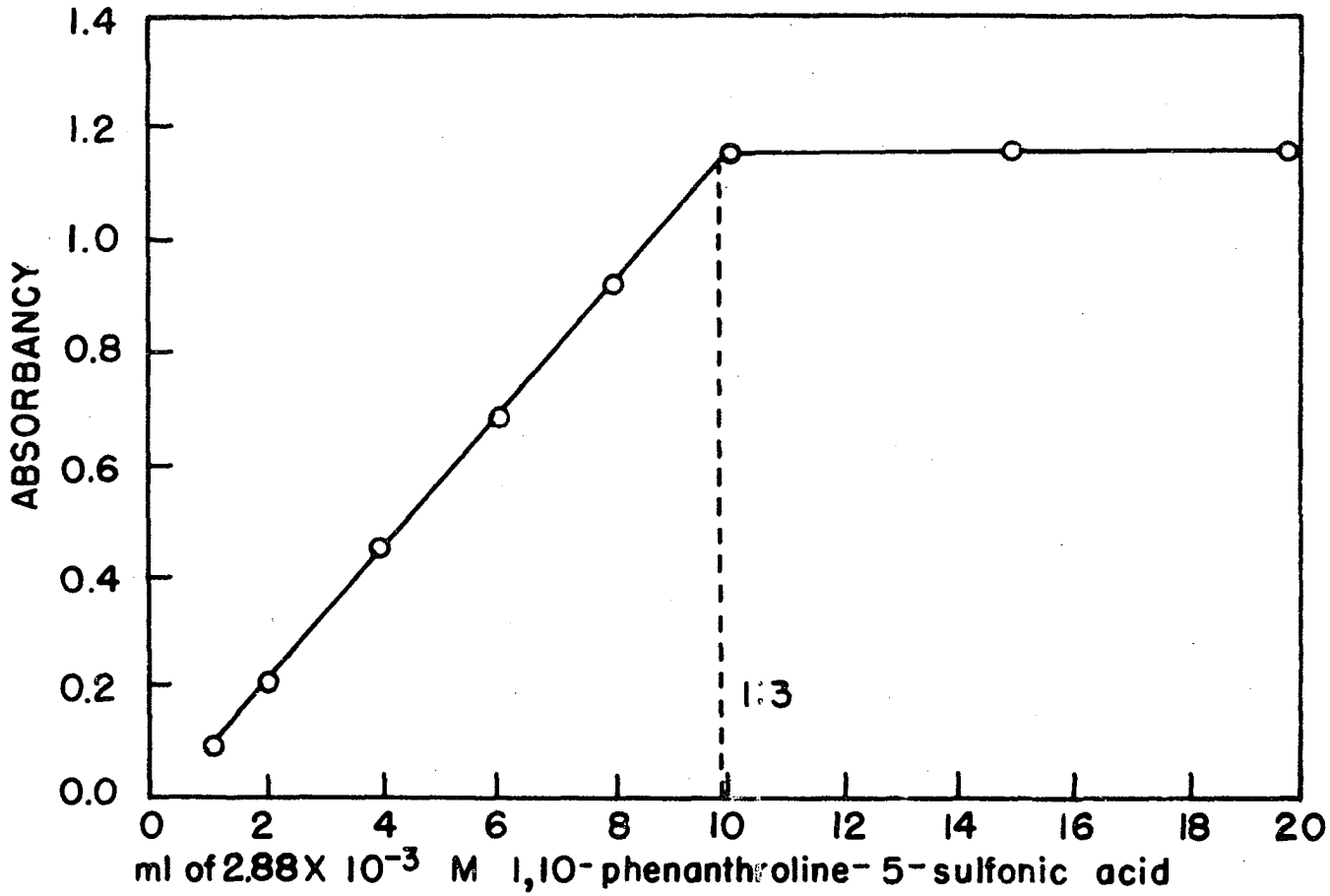
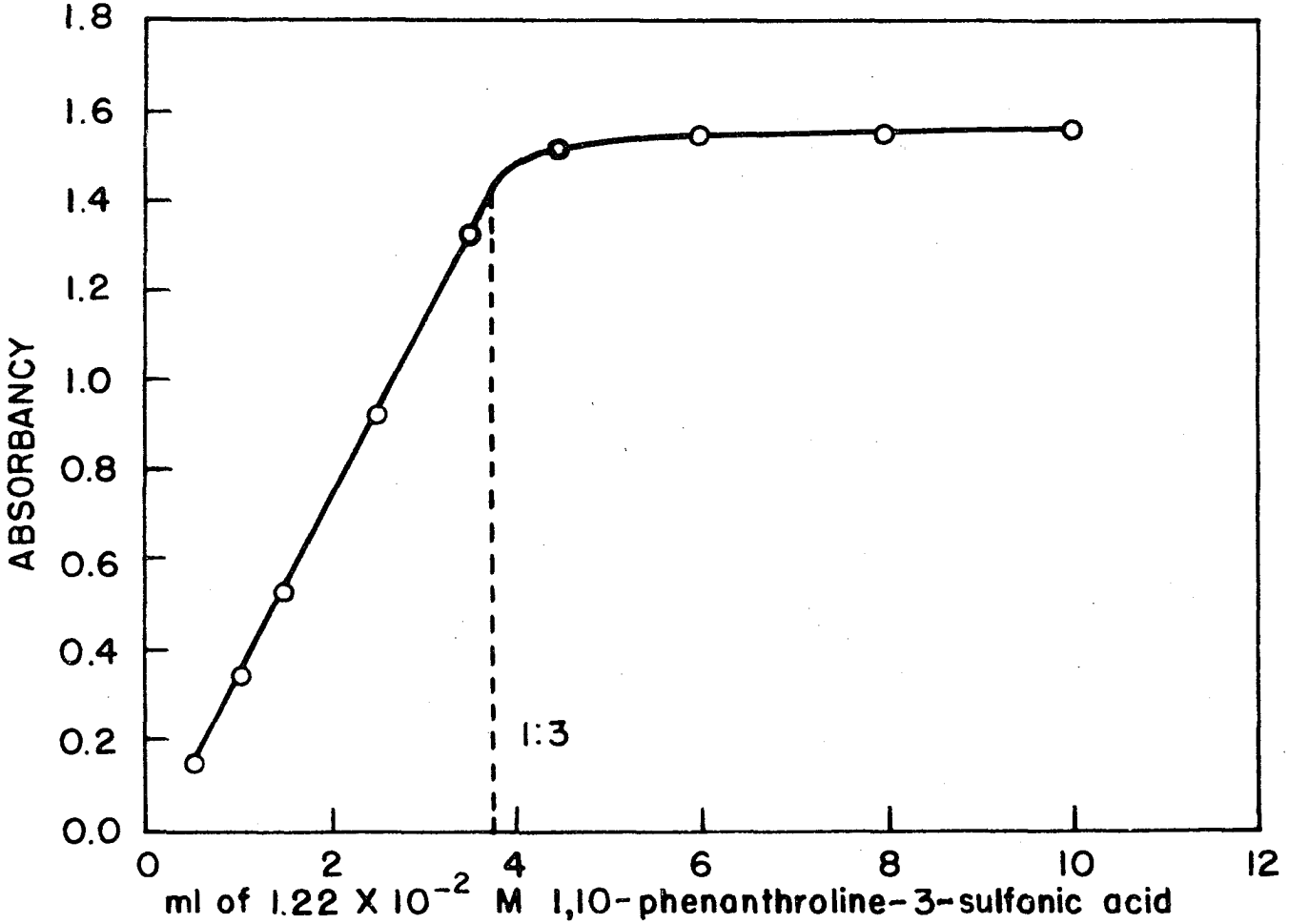

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ABSORBANCY
1:3
0
2
4
6
8
10
12
14
16
18
20
ml of 2.88 X 10⁻³ M 1,10-phenanthroline-5-sulfonic acid

Fig. 17. Spectrophotometric titration of iron.


1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ABSORBANCY
1:3
0
2
4
6
8
10
12
ml of 1.22 X 10^{-2} M 1,10-phenanthroline-3-sulfonic acid

previously described for determining the charge of ferrous trisbathophenanthrolinedisulfonate and cuprous bisbathocuproinedisulfonate to be negative; determination of the sign of the charge of ferrous tris 1,10-phenanthroline-5-sulfonate and ferrous tris 1,10-phenanthroline-3-sulfonate was attempted.

It was observed that in the case of the colored derivatives, mobility toward both the positive and negative electrodes was observed, as well as the apparent failure of most of the colored material to move at all. No significant conclusions can be drawn from the results other than the conclusion that the charges on the metal derivatives are theoretically negative one, and depending upon the degree of ionization of the sulfonic groups, the mobility is thus determined.

The ferrous derivative of 1,10-phenanthroline was run under the same conditions and as would be expected with a theoretical charge of positive two, the colored material moved rapidly to the negative electrode.

Formal reduction potentials

Method The formal reduction potentials of ferrous tris 1,10-phenanthroline-5-sulfonic acid and ferrous tris 1,10-phenanthroline-3-sulfonic acid in both 1 M sulfuric and 1 M perchloric acids were determined using the same procedure as that already described for determining the formal

reduction potentials of ferrous trisbathophenanthroline-disulfonic acid.

Ferrous tris 1,10-phenanthroline-5-sulfonic acid in 1 M sulfuric acid To 100 ml. of hot deionized water was added 0.4180 g. of 1,10-phenanthroline-5-sulfonic acid monohydrate and 20 ml. of approximately 0.05 M ferrous sulfate. The solution was cooled and 120 ml. of 2 M sulfuric acid was added. The resulting solution was titrated potentiometrically to 100% past the second endpoint using approximately 0.05 M sulfatoceric acid prepared from ceric hydroxide and being 1 M in sulfuric acid. The resulting potentiometric titration curve is shown in Fig. 18.

The formal reduction potential obtained from the curve, corrected to the hydrogen electrode, was 1.20 volts.

Ferrous tris 1,10-phenanthroline-3-sulfonic acid in 1 M sulfuric acid The formal reduction potential of ferrous tris 1,10-phenanthroline-3-sulfonic acid was determined using essentially the same procedure as the one just described. To 50 ml. of hot water was added 0.1587 g. of the sulfonated material and 10 ml. of approximately 0.05 M ferrous sulfate. The solution was cooled and 60 ml. of 2 M sulfuric acid was added and the resulting solution potentiometrically titrated. The titration was performed rapidly due to the fact that the ferrous derivative breaks up rapidly in the acid strength used. This can be observed by looking at the titration curve shown in Fig. 19 showing that only

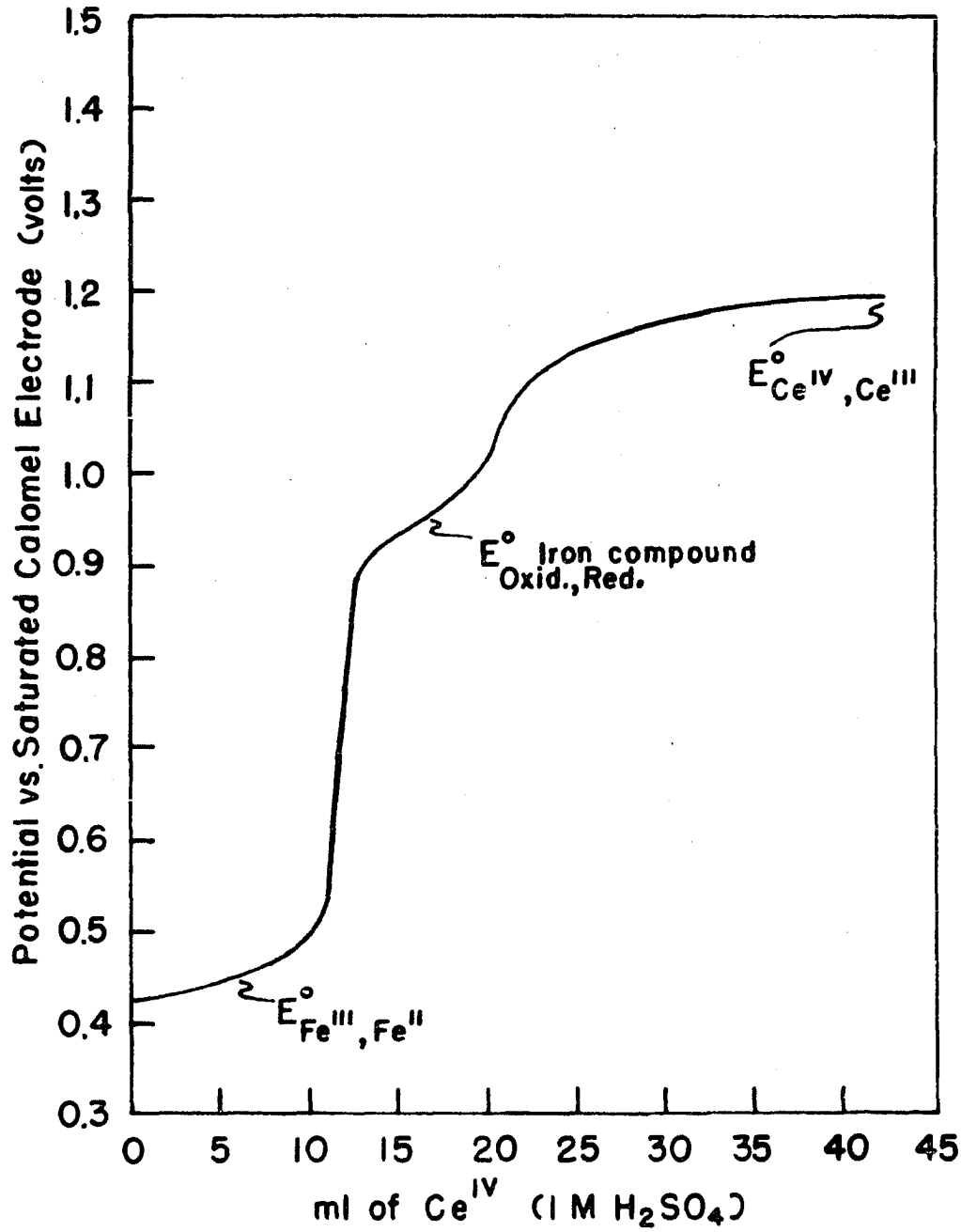


Fig. 18. Potentiometric titration of ferrous iron and ferrous tris 1,10-phenanthroline-5-sulfonic acid in 1 M sulfuric acid.

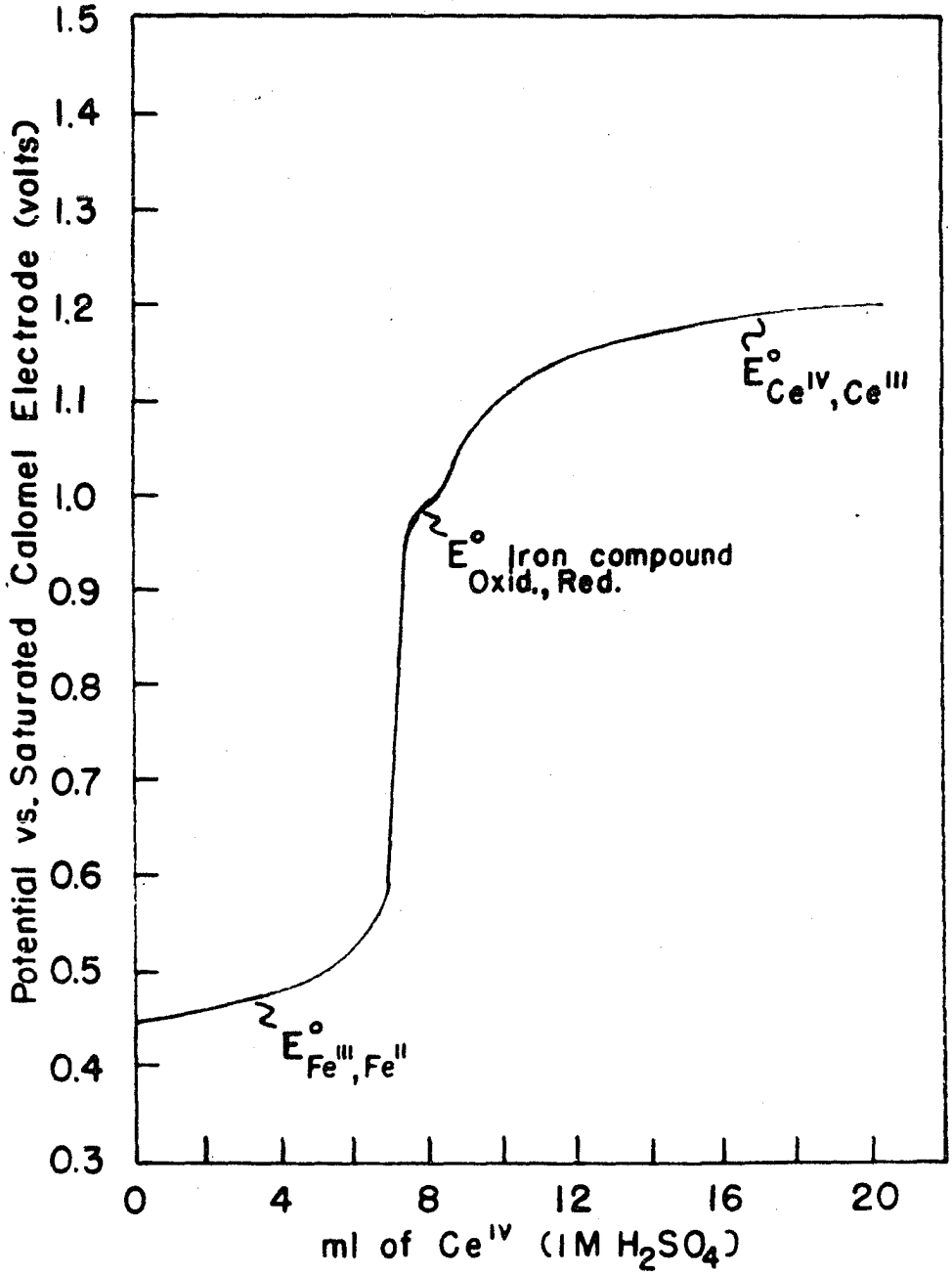


Fig. 19. Potentiometric titration of ferrous iron and ferrous tris 1,10-phenanthroline-3-sulfonic acid in 1 M sulfuric acid.

a small quantity of ferrous tris 1,10-phenanthroline-3-sulfonic acid was actually titrated due to the rapid dissociation of the ferrous derivative in the acid solution.

The formal reduction potential vs. hydrogen was found to be 1.23 volts.

Ferrous tris 1,10-phenanthroline-5-sulfonic acid in 1 M perchloric acid To 100 ml. of hot water was added 0.4180 g. of 1,10-phenanthroline-5-sulfonic acid monohydrate and 20 ml. of approximately 0.05 M ferrous perchlorate. The solution was cooled and 120 ml. of 2 M perchloric acid was added and the solution potentiometrically titrated using an approximately 0.05 M perchloratoceric acid titrant prepared by heating ceric hydroxide with perchloric acid and anodically oxidizing the resulting solution to the cerate state. The titrant was then made 1 M in perchloric acid. The titration curve is shown in Fig. 20.

The formal reduction potential found for ferrous tris 1,10-phenanthroline-5-sulfonic acid was 1.16 volts.

Ferrous tris 1,10-phenanthroline-3-sulfonic acid in 1 M perchloric acid To 43 ml. of hot water was added 0.175 g. of 1,10-phenanthroline-3-sulfonic acid and 7 ml. of approximately 0.05 M ferrous perchlorate. The solution was cooled and 50 ml. of 2 M perchloric acid and 50 ml. of 1 M perchloric acid were added and the solution titrated potentiometrically using approximately 0.05 M perchloratoceric acid in 1 M perchloric acid as titrant. The metal

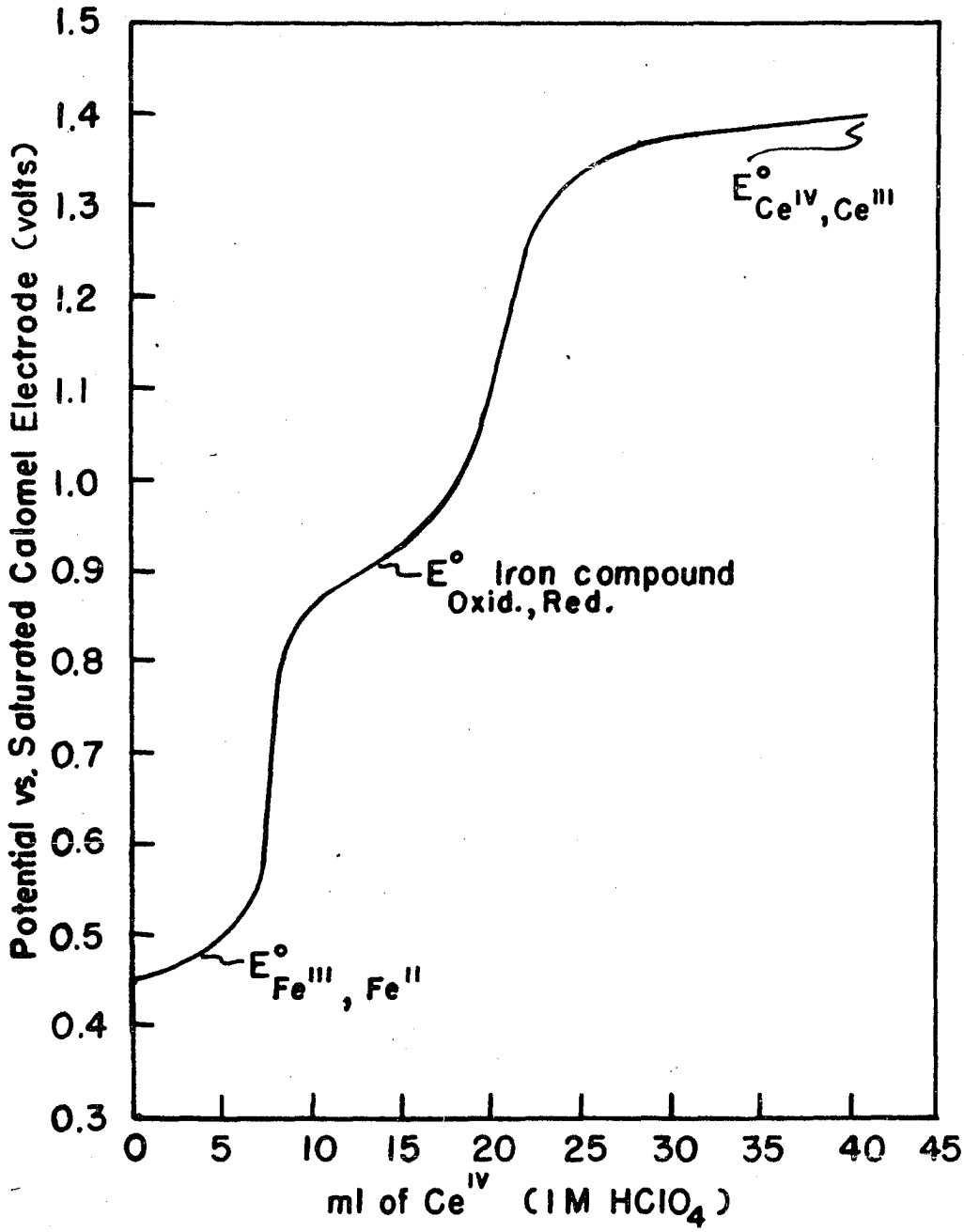


Fig. 20. Potentiometric titration of ferrous iron and ferrous tris 1,10-phenanthroline-5-sulfonic acid in 1 M perchloric acid.

derivative was very unstable toward dissociation. The results are shown in Fig. 21.

A value of 1.21 volts was obtained from the curve for the formal reduction potential vs. hydrogen of the ferrous compound.

Potential at color changes The same techniques were used as have been described previously for determining the potential at the color change of the reduced ferrous trisbathophenanthrolinedisulfonic acid to the ferric form.

Upon oxidation, the color change of ferrous tris 1,10-phenanthroline-5-sulfonic acid is from red to blue. This occurs at a potential vs. hydrogen of 1.26 volts in 1 M sulfuric acid and 1.26 volts in 1 M perchloric acid.

The color change of ferrous tris 1,10-phenanthroline-3-sulfonic acid upon being oxidized is from red to bluish-green. This occurs at a potential vs. hydrogen of about 1.26 volts in 1 M sulfuric acid and approximately 1.29 volts in 1 M perchloric acid.

Discussion

A number of different methods were used in attempting to sulfonate 1,10-phenanthroline. 1,10-Phenanthroline was boiled with chlorosulfonic acid, refluxed with fuming sulfuric acid with mercuric sulfate present, and finally refluxed with a mixture of sulfuric acid, potassium sulfate and mercuric sulfate. None of these techniques effected any

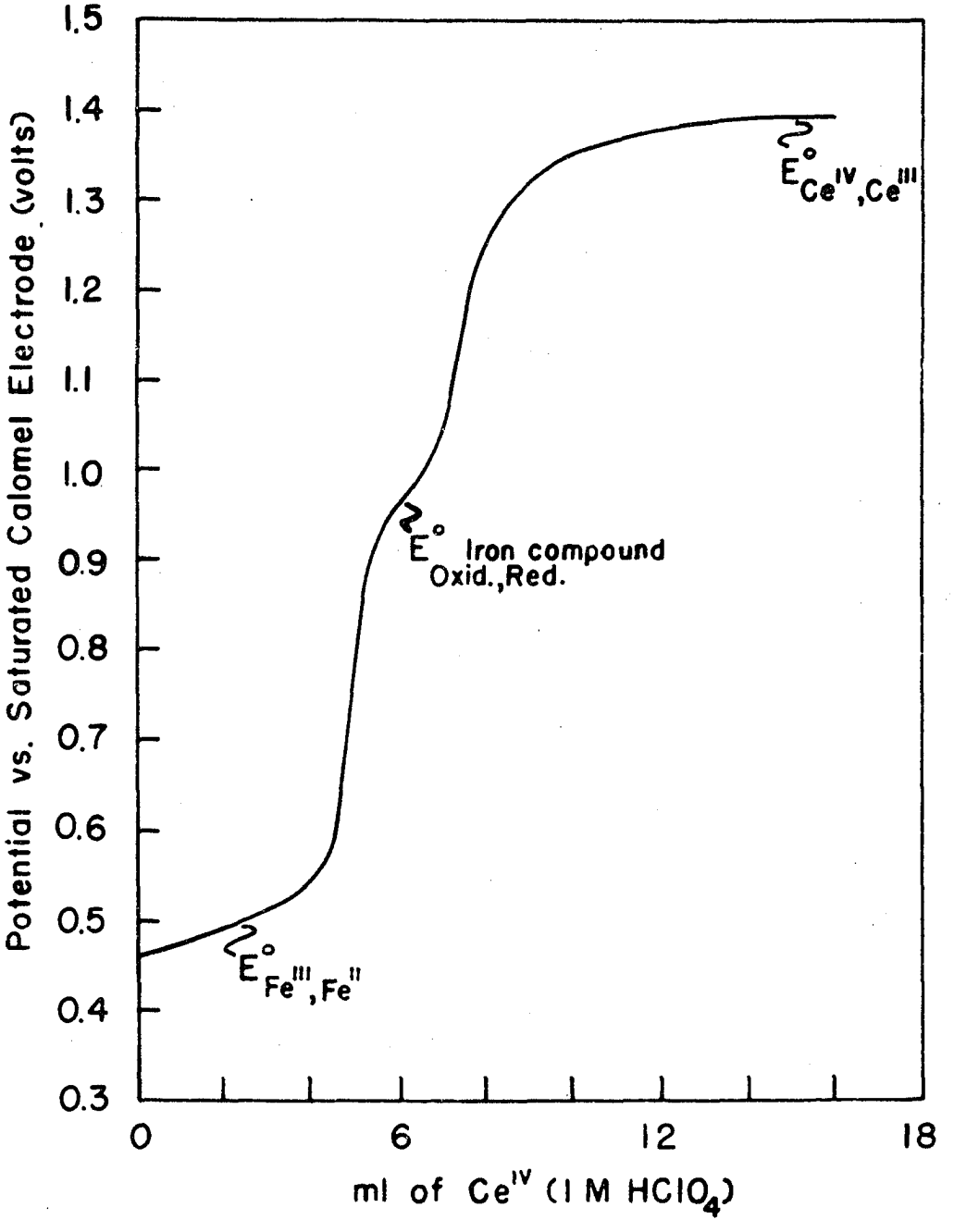


Fig. 21. Potentiometric titration of ferrous iron and ferrous tris 1,10-phenanthroline-3-sulfonic acid in 1 M perchloric acid.

apparent change upon the 1,10-phenanthroline.

Fusion with ammonium acid sulfate at 365-370° was successful and although it provides rather harsh conditions, it produced a variety of sulfonated compounds. It is feasible that the temperature-time conditions can be worked out to give higher yields of 1,10-phenanthroline-5-sulfonic acid.

Unsuccessful efforts were made to precipitate the monosulfonated material out of solution at varying pH. The final separation of the monosulfonated materials from the other products of the sulfonation reaction was made by making use of two properties of the monosulfonated 1,10-phenanthroline. The ammonium salts of the sulfonated 1,10-phenanthrolines are reasonably soluble in 85% ethanol while ammonium sulfate is relatively insoluble. This served to separate the sulfonated organic materials from the inorganic sulfates, while the further separation and purification of the monosulfonated 1,10-phenanthroline was accomplished by making use of the property that the acid form is less soluble in water than the corresponding salt. This can be explained on the basis of the zwitter ion structure of the acid form.

The conversion to the acid form was accomplished by passing a solution of the ammonium salt through a strong cation exchange column in the hydrogen form. The column was heated and the solutions were passed rapidly through the resin while hot because the acid form precipitates when

cool and would, if allowed to cool, precipitate in the column and cause it to clog.

An effort was made to isolate in pure form the various disulfonated and possibly polysulfonated 1,10-phenanthrolines. The sulfonated materials were placed on an alumina column as the sodium salts and eluted with 70% ethanol. The passage of the sulfonated material down the column was followed with ultraviolet light, making use of the differently colored fluorescence of each sulfonated material. Several different compounds were isolated and infrared spectra were obtained for three of the compounds; no extensive work being done with the compounds other than to observe some of their more obvious properties. They form red, ferrous derivatives which are a deeper red than the ferrous monosulfonated compounds. The ferrous derivatives are very unstable in acid solution. The sulfonated materials are very water soluble and a mixture of them upon titration gave an equivalent weight corresponding to 1,10-phenanthroline-disulfonic acid.

The titration curves of 1,10-phenanthroline-5-sulfonic acid and 1,10-phenanthroline-3-sulfonic acid show that as might be expected, the electrophilic sulfonic acid substituted in the pyridine ring of the 1,10-phenanthroline molecule as found in 1,10-phenanthroline-3-sulfonic acid, yields a compound whose nitrogens are less basic than is found in a compound such as 1,10-phenanthroline-5-sulfonic

acid where the sulfonic acid substitution is not in one of the pyridine rings. This is evidenced by the much larger break in the titration curve of the 3-sulfonated material as shown in Fig. 11. The larger break can be explained by the fact that 1,10-phenanthroline-3-sulfonic acid acts as a stronger acid since its ring nitrogens are less basic and its zwitter ion thus produces a stronger acid than does 1,10-phenanthroline-5-sulfonic acid.

The effect of the zwitter ion structure is easily seen as one examines the solubilities of the acids in water as compared to the solubility of their sodium salts. Sodium 1,10-phenanthroline-5-sulfonate is fourteen times more soluble than the acid form. The reason for this is no doubt due to the fact that the acid, existing as a zwitter ion, has much less salt like properties than sodium 1,10-phenanthroline-5-sulfonate and is thus less soluble in a polar solvent such as water. It would be expected that 1,10-phenanthroline-3-sulfonic acid would be more soluble than 1,10-phenanthroline-5-sulfonic acid, and in accordance, it was found to be nearly twice as soluble. Its sodium salt is only approximately five times more soluble than the acid form.

The zwitter ion no doubt also explains the instability of the ammonium salt.

As has been previously discussed concerning the formation of ferrous trisbathophenanthrolinedisulfonate, hydroxyl-

ammonium chloride is added to insure that the iron present is reduced to the ferrous state while the sodium acetate is added to buffer the solution at a favorable pH for maximum color development. The order of addition of reagents was always closely adhered to. That is: the iron present in slightly acid conditions; next the addition of the hydroxylammonium chloride reducing reagent; then the sulfonated 1,10-phenanthroline reagent; and finally the sodium acetate buffer.

Sulfonation causes an increased stability of the ferrous derivatives in more basic solution as shown in Fig. 15. The stability in acidic solution is decreased somewhat in the case of ferrous tris 1,10-phenanthroline-5-sulfonic acid as compared to ferrous tris 1,10-phenanthroline.

Ferrous tris 1,10-phenanthroline-3-sulfonic acid is quite unstable in acid solution. Sodium sulfite was used as the reducing agent in place of hydroxylammonium chloride in this study since it appeared impossible to get stable pH readings for basic solutions when even small amounts of hydroxylammonium chloride were present.

The formal reduction potentials of the ferrous derivatives of 1,10-phenanthroline-5-sulfonic acid and 1,10-phenanthroline-3-sulfonic acid in both 1 M sulfuric acid and 1 M perchloric acid were determined under non-ideal conditions. Two factors prevented obtaining completely stable readings in the potentiometric titrations. Ferrous

tris 1,10-phenanthroline-3-sulfonic acid is very unstable in 1 M acids and made steady readings of potential difficult. Ferrous tris 1,10-phenanthroline-5-sulfonic acid is much more stable in acid solution and dissociation of the derivative was not a problem.

The second factor has to do with the phenomenon of blue ferric tris 1,10-phenanthroline reverting back to the red ferrous derivative form upon standing. Both ferric tris 1,10-phenanthroline-5-sulfonic acid and ferric tris 1,10-phenanthroline-3-sulfonic acid undergo this reversion to the red ferrous form, but revert much more rapidly than ferrous tris 1,10-phenanthroline. In both cases this caused somewhat unstable readings at the second endpoint of the potentiometric titration. However, it wasn't felt that this was serious enough to cause error in the formal reduction potentials determined for the two ferrous compounds.

Some time was spent studying the phenomenon just described and the following information was found. The decreasing order of the rate of reduction of the ferric derivative to the ferrous derivative was found to be: 1,10-phenanthroline-5-sulfonic acid and 1,10-phenanthroline-3-sulfonic acid; bathophenanthrolinedisulfonic acid; and 1,10-phenanthroline. Light speeds the reaction but it will proceed in absolute darkness. Sodium bismuthate was used as an oxidizing agent in place of cerate and the same results were obtained. The reaction takes place in the absence of air.

The concentration of the acid present greatly affects the speed at which the reaction takes place; the higher the acid concentration, the slower the reaction. Recycling through a number of oxidation-reduction transitions results in a gradual loss of color and eventual disappearance of the original red ferrous derivative. In the case of 1,10-phenanthroline-5-sulfonic acid, the sulfonated material precipitates after continued recycling and an infrared spectrum on the precipitated material shows it to be identical with the starting material.

The author is cautious to suggest an explanation, but it is apparent that the ferric form of these reagents acts as an oxidizing agent upon standing, the kinetics of the reaction being a key to the phenomenon. Apparently light somewhat enhances the speed of the reaction, more so in the case of the 1,10-phenanthroline derivative than the others. It is interesting to note that the listing of compounds with regard to rate of reduction of their ferric derivatives is paralleled by the formal reduction potentials of the ferrous derivatives.

This effect in no way impairs the use of the ferrous derivatives of these compounds as oxidation-reduction indicators since the reversing reaction involved is reasonably slow and would occur only after the titration was completed.

Consideration of the use of ferrous tris 1,10-phenan-

throline-5-sulfonic acid as an oxidation-reduction indicator in either sulfuric or perchloric acid is important since: the material is quite stable in acid solution; it is highly colored and thus little titrant is needed to produce a color change; the end point is very sharp; the derivative is soluble in solutions containing perchlorate; and an increased formal reduction potential is observed over that obtained for ferrous tris 1,10-phenanthroline.

Ferrous tris 1,10-phenanthroline-3-sulfonic acid does not function too satisfactorily as an oxidation-reduction indicator since it is not very stable in acid solution.

APPLICATIONS

In order to demonstrate the applicability of these new sulfonated reagents, three applications of analytical significance were made. The use of the ferroin type ferrous compounds as oxidation-reduction indicators in perchloric acid solution is unsatisfactory owing to the insoluble nature of the products formed with perchlorate. Since the perchlorate ion in no way interferes with the analytical uses of the sulfonated compounds as described in the studies on interference, the analysis of iron in various iron ores was carried out using ferrous trisbathophenanthrolinedisulfonic acid as the oxidation-reduction indicator. The titrations were performed using perchloratoceric acid as the titrant, the sample being titrated being present in a mixture of sulfuric, phosphoric, and perchloric acids.

Perchloric acid in conjunction with one or more other acids is widely used for the "wet oxidation" of various organic materials in order that metals such as iron and copper can be subsequently determined. Ordinarily in the colorimetric determination of iron or copper, the perchloric acid must be driven off by prolonged heating in the presence of sulfuric acid before the analysis can be performed; or the iron or copper must be extracted into an immiscible solvent as an extractible metal derivative.

By using bathophenanthrolinedisulfonate for the

colorimetric determination of iron and bathocuproinedisulfonate for copper, the procedure is greatly simplified. Since both reagents are water soluble and since the presence of the perchlorate ion does not interfere with either determination, the organic material is merely decomposed, neutralized, the color developed, and the concentrations of iron and copper determined colorimetrically.

The determination of iron and copper in yeast was selected to illustrate the advantages of using these two sulfonated reagents.

Determination of Iron in Iron Ores Using Ferrous Trisbathophenanthrolinedisulfonic Acid as Indicator

Samples for analysis

Two of the samples selected for analysis of iron were ore samples supplied by the National Bureau of Standards (NBS No. 29a and NBS No. 26). The results of the analysis of the NBS No. 26 sample was reportedly based on a very limited number of determinations and is not certified by the National Bureau of Standards. The third iron ore was provided by Lerch Brothers, Incorporated, Hibbing, Minnesota as a 1953 standard.

The samples, along with the electrolytic iron used for standardization, were dried for 2 hrs. at 110°.

Procedure

Samples of the iron ores weighing 0.2 to 0.3 g. were

transferred to 500 ml. conical flasks. To each flask was added 15 ml. of a mixture of equal volumes of 70% perchloric acid and 85% phosphoric acid (19). Reflux heads (20) were inserted into the necks of the flasks and the flasks were heated on a hot plate until the ore had dissolved. The flasks and contents were cooled and 70 ml. of water was added to each. The reflux heads were replaced by stirring rods and the solutions boiled for 4 min. The solutions were cooled and 30 ml. of dilute sulfuric acid (1:1) was added to each flask. The iron present in each flask was reduced by passage through an amalgamated zinc reductor, the reductor being washed with dilute sulfuric acid (1:20). Care was taken not to allow any air to be drawn into the zinc column during the washing operation. As soon as the contents of a flask were passed through the reductor, four drops of 0.00375 M ferrous trisbathophenanthrolinedisulfonate indicator was added, and with vigorous stirring supplied by a magnetic stirrer, the ferrous iron was titrated with 0.1 N perchloratoceric acid. The end point in all cases was marked by a very sharp change from red to green.

The perchloratoceric acid was standardized against electrolytic iron ignited in moist hydrogen (obtained from the G. Frederick Smith Chemical Company, Columbus, Ohio) in exactly the same manner.

Results

The results obtained on the three iron ores are reported in Table 3.

Table 3. Determination of iron in various ores by cerate titration using ferrous trisbathophenanthroline-disulfonic acid

Sample	Iron found %	Average and standard deviation	Iron reported %
NBS No. 29a	69.30	69.30	69.54
	69.36	σ = 0.07	
	69.26		
	69.21		
	69.37		
NBS No. 26	58.23	58.24	58.62[a]
	58.32	σ = 0.08	
	58.33		
	58.13		
	58.22		
Lerch Brothers, Incorporated, 1953 Standard[b]	58.03	58.04	58.19
	58.03	σ = 0.04	
	57.98		
	58.08		
	58.08		
Electrolytic iron (Standardization)		Normal concentration of cerate solution	
		0.07350	
		0.07338	
		0.07323	
		0.07335	
		0.07327	
		Avg. 0.07335	
		σ = 0.00010	

[a]Based on a small number of determinations and not certified.

[b]Lerch Brothers, Incorporated, Hibbing, Minnesota.

Determination of Iron in Yeast

Samples for analysis

As a check on the use of bathophenanthrolinedisulfonate as a colorimetric reagent for the determination of iron in the presence of perchlorate, a commercial yeast (Fleischmann's "Active Dry") was selected as a suitable and homogeneous material requiring wet ashing.

Procedure

A sample of yeast weighing 2 g. was transferred to a 250 ml. conical flask. Five ml. of concentrated sulfuric acid was added and a reflux head was placed in the neck of the flask. A blank determination was carried along simultaneously starting with the sulfuric acid. The yeast was charred by heating the mixture on a gas hot plate for 15 min. The mixture was cooled and then 20 ml. of equal parts by volume of 70% perchloric acid and 70% nitric acid was added. The reflux head was replaced and the mixture was heated in such a fashion that the water and nitric acid were expelled in about 15 min. and perchloric acid began to condense on the walls of the flask. This smooth refluxing of perchloric acid without undue escape of perchloric acid was continued 10 min. The mixture was then cooled and the reflux head and flask washed with approximately 30 ml. of deionized water.

To this solution was added 5 ml. of a 10% solution of

hydroxylammonium chloride and 10 ml. of a 0.1% solution of disodium bathophenanthrolinedisulfonate. Ammonium hydroxide was added until the pH of the solution reached 7 to 8 as shown by pH paper. The pH was then brought to between 4 and 5 by the dropwise addition of perchloric acid. The solution was cooled, transferred to a 100 ml. volumetric flask, diluted to the mark with deionized water, and mixed. Using a Beckman DU spectrophotometer, the absorbancy vs. a reagent blank was measured in a 1 cm. cell at 535 mμ.

The concentration of iron in the various solutions was determined using the calibration curve (Fig. 4) already previously described in the section dealing with the sensitivity of bathophenanthrolinedisulfonate toward iron. Known amounts of iron were added to certain samples of the yeast before digestion.

Results

The results on the yeast and on the spiked samples are summarized in Table 4.

Determination of Copper in Yeast

Samples for analysis

The same supply of yeast was used for the determination of copper as was used for the determination of iron.

Procedure

The same procedure of wet ashing was used for this

Table 4. Determination of iron in dry yeast with bathophenanthrolinedisulfonate

Sample number	Iron added mg.	Iron found mg.	Iron in yeast p.p.m.[a]
1	none	0.100	50.0
2	none	0.102	51.0
3	none	0.101	50.5
4	none	0.101	50.5
5	none	0.101	50.5
6	none	0.099	49.5
		Avg. 0.101	Avg. 50.3

			Iron recovered mg.	Error mg.
7	0.036	0.139	0.038	+0.002
8	0.036	0.143	0.042	+0.006
9	0.036	0.138	0.037	+0.001
10	0.072	0.172	0.071	-0.001
11	0.072	0.169	0.068	-0.004
12	0.072	0.173	0.072	0.0

[a]Based on a sample weight of 2 g.

determination as that previously described, with the exception that standard copper was used to spike the samples instead of iron previous to the ashing.

To the solution following wet ashing was added 5 ml. of a solution 10% in hydroxylammonium chloride and 2% in citric acid. Then 5 ml. of a 0.1% solution of disodium bathocuproinedisulfonate was added. Ammonium hydroxide was added until the pH reached 7 to 8 as indicated by pH paper. The solution was then brought to pH 4 to 5 by the dropwise addition of dilute perchloric acid. The solution was diluted to exactly 100 ml. and the absorbancy measured at 483 mμ.

Using the calibration curve for copper (Fig. 4), the copper content of each solution was determined. The results are summarized in Table 5.

Discussion

The titration of iron in the presence of perchloric acid using perchloratoceric acid as the titrant and ferrous trisbathophenanthrolinedisulfonic acid as the indicator proved successful. The endpoints were extremely sharp, requiring a very small amount of titrant to convert the red ferrous compound to the green ferric form. Excellent precision was found for the analysis of three samples.

Noticeably lower values were found for NBS No. 26 than that value reported by the National Bureau of Standards. It should be kept in mind that this particular sample is not

Table 5. Determination of copper in dry yeast with bathocuproinedisulfonate

Sample number	Copper added mg.	Copper found mg.	Copper in yeast p.p.m	
1	none	0.053	26.6	
2	none	0.053	26.6	
3	none	0.052	26.0	
4	none	0.053	26.7	
5	none	0.052	26.0	
6	none	0.051	25.6	
		Avg. 0.052	Avg. 26.2	
			Copper recovered mg.	Error mg.
7	0.079	0.125	0.073	-0.006
8	0.079	0.129	0.077	-0.002
9	0.079	0.126	0.074	-0.005
10	0.158	0.203	0.151	-0.007
11	0.158	0.201	0.149	-0.009
12	0.158	0.202	0.150	-0.008

certified by the Bureau since it was analyzed only a small number of times.

The only difficulty encountered in the iron ore analyses was the tendency of a flocculent white precipitate to form in the solution being titrated at the point where the perchloratoceric acid touched the solution. This precipitate, which dissolved immediately, was observed to be caused by the interaction of the phosphoric acid with the titrant. This minor difficulty was overcome by the use of phosphoric acid in the concentration described in the procedure and by the use of a magnetic stirrer to speed up mixing.

The use of ferrous trisbathophenanthrolinedisulfonate as an oxidation-reduction indicator in the presence of perchlorate was successfully demonstrated by the determination of iron in iron ore using a perchloratoceric acid titrant.

The analysis of iron and copper in yeast following a decomposition of the yeast with perchloric acid was shown to be successful, using the two sulfonated colorimetric reagents, bathophenanthrolinedisulfonate and bathocuproinedisulfonate respectively.

In decomposing the yeast for the subsequent determination of iron and copper, various mixtures of acid solvents were used. A mixture of nitric and perchloric acids failed to completely decompose the yeast as evidenced by the low, erratic results found for iron. A mixture of sulfuric, nitric, and perchloric acids proved better, but still left

a little undecomposed material. The procedure utilizing a preliminary ashing of the yeast with hot concentrated sulfuric acid apparently leaves the material in a form in which the final mixture of sulfuric, nitric, and perchloric acids can readily complete the decomposition. A periodic-perchloric acid decomposition (21) proved unsuccessful in completely decomposing the yeast.

The point in the decomposition where the nitric acid has completed its oxidation and the perchloric acid begins its more vigorous attack is marked by an exothermic reaction and it is best to slow the heating just prior to this reaction in order to keep from expelling any of the sample from the flask.

Ammonium hydroxide was used to neutralize the solvent acids since sodium hydroxide normally contains too high a concentration of iron which would contaminate the samples and blank. Due to the limited solubility of ammonium perchlorate, the volume that the solution is diluted to is a consideration. The procedure described allows for sufficient volume that solubility is not a problem.

In the determination of iron and copper in yeast, the final pH is adjusted to between 4 and 5 since calcium phosphate originally present in the yeast precipitates at a higher pH and thus interferes with subsequent spectrophotometric determinations. When determining copper, citric acid is added to the hydroxylammonium chloride since the iron

present in the yeast tends to precipitate as the hydroxide in the warm solutions when the pH becomes too high upon the addition of ammonium hydroxide for neutralization. The citric acid helps to hold the iron in solution and was found to prevent the formation of the hydroxide which is only slowly dissolved at more acidic pH values.

Due to the relatively low concentration of copper in yeast as compared to the higher concentration of iron, the error in the iron determination caused by the copper present is very small and can be neglected.

The decomposition of yeast and subsequent determination of iron using bathophenanthrolinedisulfonate and copper using bathocuproinedisulfonate was successful, no interference being found by leaving the perchloric acid in the solvent mixture and thus the time was saved that is normally required to evaporate the perchlorate off in the presence of sulfuric acid.

SUMMARY

4,7-Diphenyl-1,10-phenanthroline (bathophenanthroline) and 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (bathocuproine), highly sensitive but water-insoluble colorimetric reagents for iron and copper respectively, have been sulfonated and water-soluble products of equal sensitivity obtained. Sulfonation was effected at 25° by simple treatment with chlorosulfonic acid. Upon neutralization with ammonia, separation of the ammonium salts from ammonium sulfate was effected through the solubility of the ammonium salts in ethanol. The products were ultimately isolated as the sodium salts, the yields being 97% in both cases. The products proved to be disulfonic acids as shown by ultimate analysis and equivalent weight determinations.

4,7-Diphenyl-1,10-phenanthrolinedisulfonic acid and 2,9-dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonic are zwitter ion acids as shown by the titration curves obtained on neutralization. Two breaks are obtained in the titration curve of each acid, the negative logarithm of the respective acid dissociation constants being:

	pK_1	pK_2
Bathophenanthrolinedisulfonic acid	2.83	5.20
Bathocuproinedisulfonic acid	2.65	5.80

In the free acid in each case, the proton of one sulfonic acid is transferred to the cyclic nitrogen atom (1,10-phenanthroline and numerous substituted 1,10-phenanthrolines

all function only as monoacidic bases), leaving one sulfonic acid free for titration as a strong acid (pK_1). The proton on the nitrogen atoms is titrated only at higher pH (pK_2).

Because of the ease with which sulfonation was achieved and in view of the great difficulty in sulfonating the parent, unsubstituted 1,10-phenanthroline, it has been concluded that in each material one sulfonic acid group has entered each of the phenyl rings.

Infrared spectra of the sodium salts of both of the sulfonated products were obtained using the potassium bromide pellet technique. The sulfonated materials were observed to fluoresce under ultraviolet light. The sodium salts were found to be very hygroscopic but upon drying at 110° showed no water of hydration upon further heating.

The absorption spectrum of the red ferrous derivative of bathophenanthrolinedisulfonate was obtained in water solution, using hydroxylammonium chloride to reduce the iron to the ferrous state and sodium acetate to buffer the solution prior to color development. A value of 22,140 was found for the molar extinction coefficient at 535 mμ, the wavelength of maximum absorption. The system conformed to Beer's law over the range of 0 to 3.6 p.p.m. of iron.

The absorption spectrum of the orange, cuprous derivative of bathocuproinedisulfonate was obtained in a similar way. The molar extinction coefficient found was 12,250 at 483 mμ, the wavelength of maximum absorption. The system

conformed to Beer's law over the range 0 to 6.35 p.p.m. of copper.

The combining ratio of 4,7-diphenyl-1,10-phenanthrolinedisulfonate with iron was found to be three to one, and of 2,9-dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonate with copper was two to one, spectrophotometric titrations being employed in both cases for this measurement.

Ferrous trisbathophenanthrolinedisulfonate was found to be stable over the pH range 2.7 to 10.5; cuprous bisbathocuproinedisulfonate over the pH range 3.5 to 11.0.

That both metal derivatives carried a negative charge was shown by their migration behavior in solution.

The effect of a large number of cations and anions upon the determination of iron using bathophenanthrolinedisulfonate was studied. It was found that copper, aluminum, tin (IV), thiosulfate, dichromate, cyanide, and persulfate interfere. A similar study of the determination of copper using bathocuproinedisulfonate showed that aluminum, tin (IV), uranyl, dichromate, cyanide, thiocyanate, thiosulfate, and persulfate interfere. The extent of these interferences was measured with some precision.

The formal reduction potential of the ferric-ferrous-trisbathophenanthrolinedisulfonic acid couple was determined in both 1 M sulfuric and 1 M perchloric acids by potentiometric methods. Values of 1.09 volts and 1.01 volts vs. the hydrogen electrode were obtained, respectively, for the acid

solutions.

In addition to the increased solubility in water that sulfonation provides, sulfonation causes the metal derivatives to be water soluble in the presence of the perchlorate ion. This is in contrast to the metal derivatives of the unsulfonated materials and other ferroin type reagents and is important in the determination of iron and copper in plant and animal material following wet ashing with perchloric acid.

When wet ashing organic material for the subsequent determination of iron using perchloric acid in conjunction with other acids, the perchloric acid must be removed by evaporation with sulfuric acid if the iron is to be determined colorimetrically without extraction. Since the presence of the perchlorate ion does not interfere with the use of these sulfonated reagents, the removal of the perchloric acid is not necessary, and time is saved. The iron and copper content of yeast was determined by a wet oxidation involving perchloric acid and the iron was successfully spectrophotometrically determined using disodium bathophenanthrolinedisulfonate and the copper successfully determined using disodium bathocuproinedisulfonate; perchlorate ion being present in great excess in both cases.

This solubility of ferrous trisbathophenanthrolinedisulfonic acid in perchloric acid solution is also

advantageous when it is used as an oxidation-reduction indicator, for titrations may be performed in perchloric acid solutions. Thus, the indicator can be used for the titration of iron with perchloratoceric acid following dissolution of an iron ore in perchloric (plus phosphoric) acid. The color change, red to green, is sharp and vivid. Satisfactory results were obtained with it in the analysis of standard iron ores.

Sulfonation of 1,10-phenanthroline was achieved, but the drastic procedure of fusion with ammonium acid sulfate at 365° was necessary. Separation of the sulfonation products from ammonium sulfate was accomplished through the solubility of the ammonium salt of the material in 85% ethanol. The sulfonated material was then converted to the acid form by passage through a strong cation exchange resin in the acid form. By utilizing differences in solubilities, several sulfonated materials were isolated.

Two cream colored crystalline materials were isolated, and by titration and ultimate analysis, shown to be monosulfonated-1,10-phenanthrolines. One of the compounds was converted to 5-hydroxy-1,10-phenanthroline by fusion with sodium hydroxide and its structure thus proved to be 1,10-phenanthroline-5-sulfonic acid. The other crystalline compound was converted to the hydroxy and then to the corresponding chloro compound using phosphorous pentachloride and phosphorous oxychloride. The infrared spectrum of this

chloro-1,10-phenanthroline was identical with that of a 3-chloro-1,10-phenanthroline material, thus showing the compound to be 1,10-phenanthroline-3-sulfonic acid. 1,10-Phenanthroline-5-sulfonic acid was isolated as a monohydrate, melting point 477-483° in 30% yield; 1,10-phenanthroline-3-sulfonic acid was obtained as the anhydrous acid, melting point 381-386°, in 4% yield. Both acids fluoresce under ultraviolet light.

The solubilities of the acid and sodium salt forms of both compounds was determined and the solubility of 1,10-phenanthroline-5-sulfonic acid monohydrate in water at 25° was found to be 0.518 g./l. while that of its sodium salt was 7.35 g./l. The solubility of 1,10-phenanthroline-3-sulfonic acid was found to be 0.920 g./l.; the sodium salt 4.39 g./l.

The absorption spectra of the ferrous derivatives of the two materials was determined. The molar extinction coefficient of ferrous 1,10-phenanthroline-5-sulfonate was found to be 12,240 at 512 mμ, the wavelength of maximum absorption; the system conformed to Beer's law over the range 0 to 7.2 p.p.m. of iron. Ferrous 1,10-phenanthroline-3-sulfonate was found to have a molar extinction coefficient of 10,840 at 517 mμ. and to conform to Beer's law over the range 0 to 7.2 p.p.m. of iron.

The combining ratio of ferrous iron to each sulfonated material was shown to be one to three by spectrophotometric

titration. The ferrous derivative of 1,10-phenanthroline-5-sulfonate was found to be stable over the pH range of 2.5 to 11; the ferrous derivative of 1,10-phenanthroline-3-sulfonate over the pH range of 3 to 10.

The formal reduction potential of both ferrous derivatives was determined in both 1 M sulfuric acid and 1 M perchloric acid. The formal reduction potential vs. hydrogen for ferrous tris 1,10-phenanthroline-5-sulfonic acid in 1 M sulfuric acid was found to be 1.20 volts; in 1 M perchloric acid, 1.16 volts. The formal reduction potential vs. hydrogen for ferrous tris 1,10-phenanthroline-3-sulfonic acid in 1 M sulfuric acid was 1.23 volts; in 1 M perchloric acid, 1.21 volts.

Thus, sulfonation raises the formal reduction potential of the ferrous derivative considerably above that of the ferrous derivative of the parent 1,10-phenanthroline which has a formal reduction potential vs. hydrogen in 1 M sulfuric acid of 1.06 volts. In addition, sulfonation causes the ferrous derivative to be soluble in the presence of the perchlorate ion.

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