THE COPPER REAGENTS: CUPROINE, NEOCUPROINE, BATHOCUPROINE

THE G. FREDERICK SMITH CHEMICAL COMPANY
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THE COPPER REAGENTS: CUPROINE, NEOCUPROINE, BATHOCUPROINE

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PREFACE TO THE SECOND EDITION

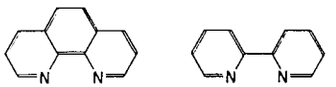
In the years since the original edition of this monograph was published, many novel and interesting applications have been made of the reagents cuproine, neocuproine and bathocuproine. Moreover, several new and especially promising cuproine type reagents have recently been introduced that afford extended or enhanced applicability to various samples and matrices. This edition retains most of the material from the original monograph. It also includes details of new applications and new reagents. Much detail has to be excluded from a booklet of this size, so an attempt was made to include a complete bibliography of recent literature to make amends.

The Authors

SECTION I

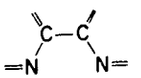
INTRODUCTION

The reaction of 1,10-phenanthroline and of 2,2′-bipyridine and their de-



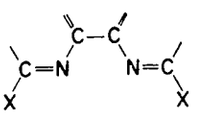
rivatives with the ferrous ion, yielding intensely red, soluble compounds, has proved of great value in analytical chemistry. Although the greatest interest in this reaction has been in the colorimetric determination of iron, other properties of the colored ions formed have been of great service too. The color change from blue to red of the oxidation-reduction couple Fe(1,10-phenanthroline)$_3^{+++}$ – Fe(1,10-phenanthroline)$_3^{++}$ furnishes a high potential oxidation-reduction indicator of great utility. The insolubility of tris (1,10-phenanthroline)ferrous perchlorate has provided a satisfactory procedure for the gravimetric determination of perchlorate.

The trivial name ferroin was coined for the ferrous 1,10-phenanthroline ion the oxidized and reduced forms of the couple being ferriin and ferroin, respectively. The terms ferroin reaction and ferroin group are used more generally for the color reaction of the ferrous ion with any 1,10-phenanthroline or 2,2′-bipyridine or for any compound having the atomic grouping



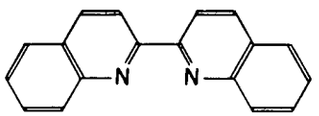
which is responsible for the reaction.

Quite early it was observed that the ferroin reaction failed with those compounds bearing substituent groups on the carbon atoms adjacent to the ring nitrogen atoms. It was only considerably later, however, that it was observed that the compounds which failed to give the ferroin reaction did produce colors with cuprous copper. The reaction was first discovered with 2,2′-biquinoline and was subsequently found for the 2,9-dialkyl-1,10-phenanthrolines. These substances are specific for copper and by analogy the terms cuproine reaction and cuproine group have been employed for the color reaction and the responsible atomic grouping.

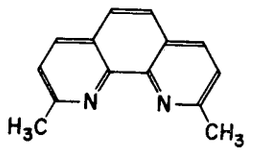


Less happily the term cuproine has come to designate 2,2′-biquinoline rather than its colored cuprous derivative in contrast to ferroin which is used exclusively for the metal derivative.

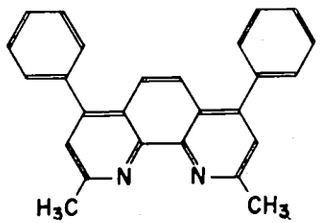
The names neocuproine and bathocuproine were coined later and refer to the reagents 2,9-dimethyl-1,10-phenanthroline and 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline, respectively. Sulfonated bathocuproine has been used as a common name for 2,9-dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonic acid disodium salt.



2,2′-Biquinoline
Cuproine



2,9-Dimethyl-1,10-phenanthroline
Neocuproine



2,9-Dimethyl-4,7-diphenyl-1,
10-phenanthroline Bathocuproine

These reagents are highly specific chromogens for copper for they react with no other metals to give colored products. The copper is present in the colored derivatives of these reagents in the univalent state. Two molecules of the reagent are associated with each copper atom, both nitrogen atoms of each molecule being bound to the copper so that two five-membered rings are formed. The four covalent bonds thus formed to the copper atom are directed toward the apexes of a regular tetrahedron about the copper atom so that the planes of the two five-membered rings lie at right angles to each other.

Fortunately, the copper compounds of three of these four cuproine reagents are not only intensely colored but are soluble in certain solvents immiscible with water. Thus, the copper may be concentrated by extraction into an immiscible solvent and a considerable increase in sensitivity achieved. Even more important the extraction process may be used to remove any copper in the distilled water and the solutions of the reagents used in the analysis, reducing the blank to zero and improving the certainty with which the determination of copper can be made. The sulfonated compound is water soluble and is used only in aqueous solution.

Hydroxylammonium chloride is usually chosen as the reducing agent and ammonium acetate as the agent for suitably buffering the solution. Other reagents can be used, however. Similarly, though isoamyl alcohol has usually been chosen as the extracting liquid, other higher alcohols can be used. Because the molar absorptivity of bathocuproine is the largest of the three, bathocuproine can be used for the determination of smaller amounts of copper than can be handled with cuproine or neocuproine. Curiously, though, as a visual qualitative test cuproine is the best of the four by a slight margin, its purple color being more easily detected by eye on a white background than the yellow or orange-yellow of the other three. The molar absorptivities and working ranges of the reagents are summarized in Table I.

Although copper is not an abundant element, not even among the first twenty in the earth's crust, its availability in large deposits and its excellent metallurgical properties have brought it into widespread use: in coinage, plumbing, electrical wire, and structural alloys. Its determination is thus a matter of considerable importance and excellent methods for its determination in macro amounts have been devised, notably the iodometric and electrodeposition methods (see for example, *Quantitative Analysis,* by Harvey Diehl, Oakland Street Science Press, Ames, Iowa, 1970). The determination of small amounts of copper is almost equally important for traces of copper are essential to the well-being of the animals, and the small amounts which find their way into commercial products from natural sources or from piping and containers often play significant roles, as in the deterioration of food products on storage. Of the numerous methods which have been proposed for the determination of small amounts of copper, the colorimetric methods based on cuproine, neocuproine, and bathocuproine are the best in being both sensitive and specific. Small amounts of copper are almost always associated with iron, and not the least of the merits of the cuproine reagents is their immunity to disturbances by even large quantities of iron. The 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine reagent (PDT) can play a dual role, that of determining both iron and copper in a single sample with good sensitivity for each. Details are presented in Section VII.

These compounds are not easy to synthesize and their prices are high although dropping as their use increases and experience is gained in their manufacture. Colorimetric reagents, however, go a long way and the cost per determination is negligible, particularly in comparison with the value of time saved over older methods. Most of the reagents used in the procedures contained in this monograph are available from the company and are so noted ⊛. A list of these may be found in the Appendix, Section VIII.

TABLE I. DATA RELATIVE TO THE USE OF THE COPPER REAGENTS

Beer-Lambert Law: $A = \varepsilon bc$
A = Absorbance
b = Length of light path

ε = Molar absorptivity
c = Concentration in moles per liter

$A = \log \frac{I_o}{I}$

T = Transmittance

$T = 100 \log \frac{I}{I_o}$

Best working range: A = 0.7 to A = 0.1 (T = 20% to T = 80%)

μg. = microgram (0.000,001 g.)

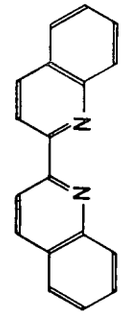
1 μg. per ml. = 1 part per million (p.p.m.)

	Cuproine	Neocuproine	Bathocuproine	Sulfonated Bathocuproine
Molar absorptivity	6,220	7,950	14,160	12,500
Solvent	Isoamyl alcohol	Isoamyl alcohol	n-Hexyl alcohol	Water
Wave length of maximum absorption, nm	546	454	479	483
Concentration in moles per liter corresponding to A = 0.1 (T = 80%) A = 0.7 (T = 20%)	 1.62×10^{-5} 11.2×10^{-5}	 1.26×10^{-5} 8.81×10^{-5}	 7.07×10^{-6} 49.5×10^{-6}	 8.0×10^{-6} 56.0×10^{-6}
Concentration in mg. per liter (= μg. Cu per ml.) corresponding to A = 0.1 (T = 80%) A = 0.7 (T = 20%)	 1.0 7.1	 0.80 5.6	 0.44 3.1	 0.50 3.5
Beer's law found to hold over the range, μg. per ml.	up to 40	up to 10.6	up to 10	up to 10
Recommended amounts of copper in final volume of 25.0 ml., μg.	25 to 170	20 to 130	10 to 75	10 to 80

SECTION II

CUPROINE

2,2'-Biquinoline



$C_{18}H_{12}N_2$

Mol. Wt.: 256.3

Molar Absorptivity of $Cu(cuproine)_2^+$ in isoamyl alcohol: 6220 at 546 nm

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That 2,2'-biquinoline fails to give a color reaction with ferrous iron was discovered by Smirnoff[1] in 1921. Later Willink and Wibaut[2] observed the same behavior of 6,6'-dimethyl-2,2' bipyridine,, a related compound having the same functional grouping. These materials do, however, yield stable compounds with cobalt and with cupric and cuprous copper, the cuprous derivatives having an intense purple color. Breckenridge, Lewis and Quick[3] who discovered this made a thorough study of the cuprous-biquinoline system, applying it to the detection and colorimetric determination of copper. After a considerable interval, during the World War II years, the method was reinvestigated by Hoste[4] who confirmed the Breckenridge findings on the great specificity of the reagent and found that the color could be extracted into immiscible solvents. Hoste assigned to 2,2'-biquinoline the common name Cuproine because of the analogy of the reagent and its reaction with the cuprous ion to the ferroin reaction of the ferrous ion with 1,10-phenanthroline. Subsequently the reagent received considerable attention and was applied to the determination of copper in a variety of materials.

The deep purple cuprous compound is obtained when cuprous salts in water, acetic acid, alcohol or dioxane solutions are mixed with solutions of the base. The tendency to form the purple color is very strong, as the color is produced when metallic copper comes in contact with an alcohol solution of the base and also when cupric compounds stand in contact with alcohol for some time. The color is not noticeably affected by exposure to sunlight over a long period, by boiling for several hours, or by bubbling air through the solution for several days.

The absorption spectrum of cuprous biquinoline is given in Fig. 1. The maximum in the absorption spectrum occurs at 546 nm.

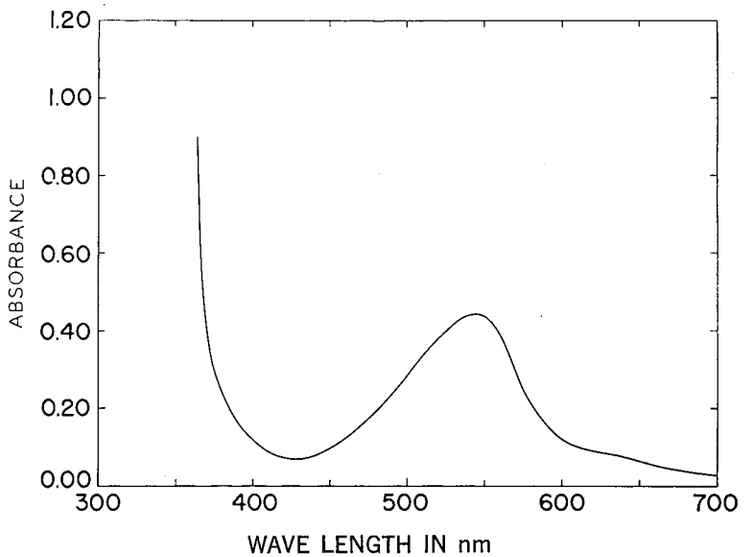

Fig. 1. Absorption spectrum of Cu(biquinoline)$_2^+$; 4.0 p.p.m. of copper in isoamyl alcohol.

The molar absorptivity in isoamyl alcohol is 6220 at 546 nm. The color conforms to Beer's law over the range 0 to 40 μg. per ml.

The biquinoline cuprous compound is a two to one compound, Cu-(biquinoline)$_2^+$. Although the compound could not be isolated the intensity of the color is not changed by the addition of more than two molecules of biquinoline per atom of copper.

The compound is not stable in highly acid solution, but it is formed at any pH from 2 to 9.

A 1 to 1 mixture of dimethylformamide is an excellent solvent in which to carry out the copper-biquinoline reaction[12,13]. The dimethylformamide serves as reducing agent. The purple color formed is due to the same two to one compound discussed above. The wave length of maximum absorption is 545 nm and the molar absorptivity 6450. A second absorption maximum occurs in this solvent at a wave length of 350 nm with a molar absorptivity of 52,000. The purple cuprous compound is also formed in acrylonitrile, the absorption maximum falling at 540 nm[14].

Synthesis. 2,2′-Biquinoline was prepared by Smirnoff using the Freidlander synthesis, namely, condensation of quinolylmethyl ketone and o-aminobenzaldehyde or of biacetyl and o-aminobenzaldehyde. A more convenient method is that of Wibaut and coworker[2] who hydrogenated quinoline in the presence of a nickel catalyst; this method was also used by Breckenridge.[3] The yield is reported to be 50 per cent.

Properties. 2,2′-Biquinoline is a white, crystalline solid, melting at 196°. It is insoluble in water but soluble in alcohol, isoamyl alcohol, isoamyl acetate, acetic acid, dioxane, acetonitrile and carbon tetrachloride. It is very soluble in dimethylformamide.

2,2′-Biquinoline shows three absorption maxima in the ultra-violet between 315 and 340 nm[12].

Specificity. Interferences. Effects of Diverse Ions. CATIONS. 2,2′-Biquinoline is specific for copper. Breckenridge, Lewis and Quick[3], Hoste[4-8] and later, Guest[9] found that none of following cations gave a color reaction with the reagent: NH_4^+, Li, Na, K, Rb, Cs, Be, Mg, Ca, Sr,Ba,Al,La,Ce(III), Ce(IV), Nd, Sm, Ti(IV), Zr, Th, V(V), Nb, Ta, Cr(II), Cr(III), Mo(VI), W(VI), UO_2^{++}, Mn, Fe(II), Fe(III), Ru, Co(II), Rh, Ir, Ni, Pd, Pt, Ag, Au, Zn, Cd, Hg(I), Hg(II), Ga, In, Tl, Ge, Sn(II), Sn(IV), Pb, As(III), As(V), Sb(III), Sb(V), Bi, Se(IV), Se(VI), Te(IV), Te(VI). The titanous ion gives a pale green color which cannot be confused with the purple color of the cuprous-biquinoline compound. Some ions, of course, interfere because of their own color, nickel, for example; none of these are extracted into isoamyl alcohol

Tests with a great many of the common cations indicated that 1000 times as much metal as copper could be present without interfering. Iron, although it gives no perceptible color with 2,2′-biquinoline, interferes by reducing the transmittance, thus giving high values for copper[3]; for equal amounts of iron and copper, the transmittance is 1 per cent lower than with copper alone. For this reason Breckenridge[3] recommended removing the iron by precipitation with bromine water and ammonia followed by a reprecipitation. Later workers successfully used tartaric acid to tie up the iron in a non-interfering, complex ion.

ANIONS. The color of the cuprous biquinoline cation is not affected by large amounts of most anions: acetate, borate, bromide, chloride, chlorate, perchlorate, tartrate, nitrate, sulfate, and phosphate. Cyanide, thiocyanate, iodide and oxalate do interfere[8]. Citric acid has been employed as a masking reagent for iron and other heavy metal ions[21].

In the presence of both chromium(III) and citrate, low results are obtained for copper according to Irving and Tomlinson[22], who found that the three species form a kinetically inert ternary complex. Interference can be overcome by adding iron(II) or other transition cation to displace copper from the ternary complex[21,22].

Qualitative Test for Copper. The smallest amount of copper which gives a perceptible color with 2,2′-biquinoline is 8×10^{-9} g., or approximately 1 part in 1,000,000,000 (Breckenridge[3]). Hoste[4] claims only 1 part in 10^6 as the dilution limit. Our own experience indicates that 0.008 μg. of copper in 1 ml. of water gives a detectable color on a white spot plate.

Other cations than copper may be present in the ratio of 5000 to 1 of copper without affecting the sensitivity, unless they possess sufficient color of their own to interfere, cobalt and neodymium, for example.

Some metals, such as mercury, gold, selenium, and tellurium, give precipitates on reduction with sulfite. Such precipitates should be removed prior to the addition of the reagent, particularly if the quantity of copper to be detected is small. Ferric ion gives a brown precipitate with sulfite. This may be prevented by the addition of fluoride or phosphate.

The sensitivity of the detection of copper with biquinoline may be improved by extracting the colored copper compound with an immiscible solvent. The disturbing effects of colored ions are eliminated and a concentration effected by using a volume of solvent small compared to the water. Amyl alcohol is most suitable for the purpose.

In all tests, blanks should be run on water and reagents. Even "analytical reagent" chemicals often contain sufficient copper to give a response to this test.

PROCEDURE. To one drop of the solution to be tested, in a neutral or faintly acid condition, add a few crystals of potassium bisulfite, a drop of 6N acetic acid and 3 drops of reagent. A purple color indicates the presence of copper.

Alternatively, the test may be carried out on 10 drops solution, extracting with 5 drops of amyl alcohol. The limit of dilution when carried out in this manner is 5×10^6 and even 10,000 times as much foreign cation as copper may be present. Iron should be tied up with either fluoride or phosphate.

Extraction. The purple cuprous biquinoline compound is soluble in isoamyl alcohol, benzyl alcohol, hexyl alcohol, benzene, carbon tetrachloride, chloroform, ethyl acetate and amyl acetate. These solvents allow extraction of the color from aqueous solution, a matter of considerable importance for both qualitative and quantitative analysis. The partition coefficient is greatest in isoamyl alcohol and this solvent is recommended; as determined by Hoste, Eeckhout and Gillis[8], the partition coefficient is 1680. Simple consideration shows that one extraction is sufficient even with a volume ratio of water to isoamyl alcohol of 10.

A shaking time of 1 minute is sufficient to establish equilibrium if the pH is between 4.4 and 7.5. It was shown by Guest[9] that the extraction of the copper varies with the shaking time if the pH falls outside these limits.

Trichloroethyl alcohol has been suggested as the extracting solvent[20] but there seems to be no particular advantage in its use.

For the determination of very low concentrations of copper, in sea water, for example, in which the concentration is of the order of 30 μg. of copper per liter, it is desirable to use very large samples, as much as 1 liter. In this situation, two other factors are important to the extraction process. The solubility of the extracting solvent in water and the efficiency of the extraction. The solubility of isoamyl alcohol is 2.67 g. per 100 ml. at 22°, too great for large samples. Riley and Sinhaseni[19] recommend n-hexyl alcohol, the solubility of which is only 0.5 g. per 100 ml.

The efficiency of the cuproine extraction of copper decreases markedly with the copper concentration. Riley and Sinhaseni[19] found that although 1 p.p.m. of copper could be extracted with 99 per cent completeness by a single extraction, at a level of 0.01 p.p.m. two extractions were necessary to separate 97 per cent. The final procedure they recommend for copper in sea water calls for three extractions, with 8, 3 and 3 ml. of hexyl alcohol (containing the cuproine reagent) and intervening addition of hydroxylammonium chloride.

Stability of Color. As stated above, in the second paragraph, the colored copper-2,2′-biquinoline compound is stable toward sunlight, boiling, and atmospheric oxidation. Russel and Hart[18], for example, found no change in absorbance of a copper-2,2′-biquinoline solution on 1000 hours exposure to strong light. Fading has, however, been reported and has been ascribed to oxidation of the cuprous compound either by air or by an oxidizing impurity in the isoamyl alcohol used for the extraction. No trouble is experienced if an excess of reducing agent is present; Riley and Sinhaseni[19] recommend the addition of a bit of hydroquinone to the n-hexyl alcohol used in their procedure.

Applications to the Colorimetric Determination of Copper. (Working directions follow this section). That 2,2′-biquinoline would be useful for the determination of copper in a wide variety of materials because of its extraordinary specificity was implied in the original work of Breckenridge, Lewis and Quick[3]. Hoste, after extending the method by the extraction technique[4], applied the method specifically to plant material[5,7], to water[7,10], steel[7,8], animal tissue and blood plasma[8], and lamp black[8]. The method is ideal for the determination of copper in the range 1 to 10 μg., the amount of copper of interest in physiological and agricultural chemistry.

The work on the determination of copper in steel by Hoste and coworkers[7,8] was confined to the analysis of synthetic mixtures of iron and copper to which were added salts containing the elements commonly alloyed with iron: nickel, chromium, manganese, titanium, molybdenum, cobalt, vanadium and tungsten. For amounts of copper corresponding to 0.01 to 0.1 per cent the results were good. Guest[9] made a more extensive study of copper in metallurgical materials: cast iron, steel, zinc and aluminum alloys, bauxite, clay, and spelter, with copper varying from 0.01 to 2.5 per cent. Results on Bureau of Standards samples and other materials were highly satisfactory.

The method was also applied to the determination of a variety of alloys and synthetic mixtures low in iron by Pflaum, Popov and Goodspeed[12,13] who prefer to use as solvent, however, a 1 to 1 mixture of water and dimethylformamide.

Elwell[22] applied cuproine successfully to determine copper in a variety of steels, ferro-alloys, pure cobalt, and pure nickel, containing 0.001 to 1 per cent copper. Chromium interfered, however the low results could be eliminated either by prior reduction to chromium(II) or by adding a large excess of ferrous iron. Subsequent studies by Irving and Tomlinson[21] demonstrated that the chromium interference is due to a ternary complex between copper, chromium and citrate ions. Addition of excess ferrous iron displaces copper from the ternary complex.

For the determination of copper in plant material Hoste[7,8] employed both dry and wet ashing with about equal success. The dry ashing was carried out very slowly, 24 hours at a temperature below 450° to prevent melting the ash. The wet ashing, employing a mixture of sulfuric acid, perchloric acid and nitric acid, was much faster and less troublesome and was the procedure adopted for the routine determination of copper in some 500 samples of sugar beet.

The determination of copper in soil and in plant material with cuproine was also studied by Cheng and Bray[14].

The determination of traces of copper in gelatin is of considerable importance in the manufacture of photographic emulsions. Several methods of carrying out this determination were investigated by Russell and Hart[18] who concluded that the cuproine and neocuproine methods were about equivalent in most respects and superior to other reagents. The procedure finally recommended calls for wet oxidation of the gelatin with a mixture of nitric acid, sulfuric acid, and perchloric acid and extraction of the copper-biquinoline compound with isoamyl alcohol.

The merits of cuproine make it ideal as a reagent for copper in geochemical prospecting. Almond[16] of the United States Geological Survey adapted it to the rapid determination of copper in soils and rocks. The sample was first fused with potassium bisulfate. The melt was taken up in hydrochloric acid, the solution filtered, and the color developed by the addition of hydroxylammonium chloride, a buffer solution consisting of sodium acetate and sodium potassium tartrate, and of cuproine dissolved in isoamyl alcohol. The isoamyl alcohol layer was then separated for comparison with a standard series (field use) or for photometric measurement. Convenient, portable apparatus was devised for carrying out the fusion and other operations in the field.

The determination of copper in silicate rocks was also studied by Riley and Sinhaseni[19] who found considerably higher values on opening up the rocks with nitric and hydrofluoric acid than with the pyrosulfate fusion. This is probably because the fusion process attacks only the sulfide and oxide minerals in the sample and does not dissolve the copper present as silicate. Riley and Sinhaseni insured complete dissolution of the copper by evapo-

rating away the nitric and hydrofluoric acids and fusing the residue with potassium bisulfate. Their procedure, the working details of which are given below, was tested on the granite and diabase rocks used as the basis of a collaborative study of the precision of methods of analysis of silicate rocks. The results were in considerably better agreement than spectrographic values reported earlier (L. H. Ahrens, *Quantitative Spectrochemical Analysis of Silicate*, Pergamon Press, London, 1954, pp. 24 to 30). Thus, for granite G1 the cuproine methods gave 15.9 p.p.m. of copper with a coefficient of variation of 2.5 per cent, the spectrographic method 5 to 20 p.p.m.; and for diabase W1, the cuproine method gave 121 p.p.m. with a coefficient of variation of 3.5 per cent compared with 44 to 130 p.p.m. by the spectrographic method.

The direct determination of copper in limestone with cuproine presents no difficulty. Fiskell[17] used a direct procedure (extraction with isoamyl alcohol) for this analysis and also a method using Dowex 1 to eliminate the calcium and magnesium, an operation which would seem to be unnecessary.

The determination of the copper in sea water presents a more difficult problem than the determination of copper in lake, river or potable water because of the smaller amounts of copper present, of the order of 30 μg. per liter. The cuproine methods are still applicable. Because the colored compound can be extracted into immiscible solvents, large samples, up to 1 liter, can be used and the blank on the reagents (buffer and hydroxylammonium chloride) can be reduced to zero by treating the solutions with cuproine and extracting with isoamyl alcohol. The problems particular to this analysis are mentioned above under Extraction. Working details of the n-hexyl alcohol extraction method of Riley and Sinhaseni[19] are given below. This method was shown to be free of any salt error for the increments in absorbance per μg. of copper added were the same whether the extraction was made from sea water or distilled water. The reproducibility of the method was excellent: surface water from the Irish Sea (chlorinity 18.83°/$_{00}$) 26.0, 26.6, 27.0, 27.0, 27.4, 28.0, ave. 27.0 μg. of copper per liter; sea water from the English Channel (chlorinity 19.36°/$_{00}$), ave. 19.0 μg. of copper per liter (coefficient of variation 2.5 per cent).

Cuproine was applied also to the determination of copper in mineral oils by Hackett[11]. The copper was first extracted from the oil with an alcohol solution of hydrochloric acid and the copper was stripped from this extract by neutralization, reduction, addition of cuproine and extraction with chloroform. The method yields satisfactory results on amounts of copper from 10 μg. to 100 μg. in oil samples weighing 10 to 100 g. and is ideal in speed and simplicity. The procedure is given below; see also the neocuproine method of Zall, McMichael and Fisher, pages 20 and 22.

Chioffi and Osti used cuproine to determine copper in distilled liquor[25].

The solubility of the cuprous-biquinoline compound in various organic solvents provides a means of determining copper in such solvents. This is a great convenience and may be quite useful; for example traces of copper in acrylonitrile affect the rate at which it polymerizes when treated with certain catalysts. The cuprous-biquinoline compound is formed directly on the addition of biquinoline to acrylonitrile so that copper can be determined directly with acrylonitrile as the solvent[15]. The maximum color is developed at an apparent pH between 3.0 and 5.5, the absorption maximum falls at 540 nm, and no interference is observed from iron up to 10 times as much as the copper present or by a hundred-fold excess of cyanide. This appears to be a useful technical method and one which could undoubtedly be applied to other organic liquids.

Procedure for the Determination of Copper. REAGENTS. CUPROINE SOLUTION. 0.1 PER CENT IN ETHYL ALCOHOL OR 0.1 PER CENT in ISOAMYL ALCOHOL. Dissolve 1 g. of 2,2′-biquinoline, in 1 liter of ethyl alcohol. If the "empirical" extraction method (see below) is to be used, dissolve 1 g. of 2,2′-biquinoline in 1 liter of isoamyl alcohol. For the procedure for copper in sea water given below prepare the reagent solution by dissolving 0.3 g. of 2,2′-biquinoline in 1 liter of n-hexyl alcohol which has been distilled from sodium hydroxide.

WATER. Distilled water often contains detectable quantities of copper and the water used should be checked by a blank determination. The water used should be redistilled or de-ionized by passage of distilled water through a monobed of cation and anion exchange resins.

ISOAMYL ALCOHOL. (or N-HEXYL ALCOHOL) Redistill isoamyl alcohol before using, inasmuch as some commercial grades contain impurities which cause the color of copper-biquinoline to fade[8]. A more exhaustive purification may even be called for with some grades of isoamyl alcohol[18]. Shake 800 ml. of isoamyl alcohol with 100 ml. of a 10 per cent solution of sodium metabisulfite, separate the layers, and dry the alcohol layer by contact with anhydrous magnesium sulfate. Filter and distill, collecting the fraction boiling over the range 128° to 132°.

HYDROXYLAMMONIUM CHLORIDE. 10 PER CENT AQUEOUS SOLUTION. Dissolve 10 g. of hydroxylammonium chloride, in 100 ml. of redistilled or de-ionized water. If appreciable amounts of copper are present in the solution add a few ml. of 2,2′-biquinoline solution and then 10 ml. of isoamyl alcohol. Shake, then allow the liquids to separate and draw off the aqueous layer. Repeat the extraction until copper is absent.

SODIUM ACETATE BUFFER SOLUTION. 10 PER CENT. Dissolve 100 g. of hydrated sodium acetate, $NaC_2H_3O_2 \cdot 3H_2O$ in 1 liter of redistilled or de-ionized water. If the solution contains copper, add 2,2′-biquinoline and isoamyl alcohol and extract as described above for hydroxylammonium chloride.

PROCEDURE. Two procedures are given below for extracting the copper-biquinoline compound. In Method A the copper is completely extracted from the water layer by repeated extraction with isoamyl alcohol and the combined extracts diluted to a given volume. In Method B a single extraction is made, the slight loss in copper being offset by the increase in sensitivity owing to the smaller volume.

METHOD A. Pipet the sample or aliquot of such size as to contain 25 to 250 μg. of copper into a separatory funnel. Add 5 ml. of 10 per cent hydroxylammonium chloride solution. Adjust the pH to 5 to 6 with dilute ammonia (1:3) using pH paper. Add 2 ml. of 0.1 per cent cuproine solution and 10 ml. of isoamyl alcohol. Shake for 1 minute and allow the layers to separate. Drain off the lower layer into a second separatory funnel and transfer the alcohol layer to a 25-ml. volumetric flask. Repeat the extraction with 10 ml. of isoamyl alcohol, adding the alcohol layer to the first extract. Dilute to 25.00 ml. with isoamyl alcohol. Measure the absorbance of the solution at a wave length of 546 nm. Obtain the amount of copper from a calibration curve prepared by running known amounts of copper through the same procedure.

METHOD B. Pipet the sample or aliquot of such a size as to contain 2.5 to 50 μg. of copper. Add 5 ml. of 10 per cent hydroxylammonium chloride solution. Adjust the pH to 5 to 6 with dilute ammonia (1:3) using pH paper. Dilute to approximately 50 ml. with water. Add 5.00 ml. of 0.1 per cent cuproine in isoamyl alcohol. Shake vigorously for 3 minutes. Allow the liquids to separate and draw off the lower layer. Transfer the isoamyl alcohol layer to a centrifuge tube and centrifuge for 1 minute to free it of suspended droplets of water. Measure the absorbance of the clear purple solution at 546 nm, using in the reference cell a blank on the water and reagents used. Obtain the amount of copper from a calibration curve prepared by running known amounts of copper through the same procedure.

Determination of Copper in Plant Material. WET ASHING-CUPROINE METHOD (HOSTE, EECKHOUT AND GILLIS[8]). Weigh into a micro Kjeldahl flask 0.25 to 0.50 g. of dried plant powder. Add 5 ml. of a mixture of 1 part of redistilled, concentrated sulfuric acid, 1 part of 70 per cent vacuum distilled perchloric acid and 3 parts of redistilled concentrated nitric acid* . Heat the mixture over a low flame, drawing off the vapors with a water aspirator. The consecutive actions of the nitric acid, perchloric acid, and sulfuric acid can be clearly followed. Cool the flask and residue, dilute with a little water and transfer to a

separatory funnel. Complete the determination using Method A or Method B above.

*The combination of acids recommended here is more potent than necessary for the destruction of organic matter in plant material. The sulfuric acid may be omitted without loss of efficiency in the destruction of organic matter, a mixture of nitric acid and perchloric acid being sufficient; see G. FREDERICK SMITH, The Liquid Fire Reaction, *Anal. Chim. Acta*, 8, 397 (1953). Also see The Wet Chemical Oxidation of Organic Compositions, G. FREDERICK SMITH CHEMICAL CO., Columbus, Ohio, 1965.

Determination of Copper in Water (HOSTE, HEIREMANS AND GILLIS[7,10]). Pipet 10 ml. of the water to be analyzed into a separatory funnel. Add 500 mg. of hydroxylammonium chloride and 10 ml. of isoamyl alcohol containing 0.01 per cent of cuproine. Shake the mixture for 3 minutes and allow the layers to separate. Measure the absorbance of the isoamyl alcohol layer at a wave length of 546 nm using in a reference cell a blank run in identical fashion on doubly or triply distilled water. Obtain the amount of copper from a calibration curve obtained in similar fashion on known amounts of copper: for example, 2, 4, 8, 12, 16 and 20 μg. per 100 ml. of water.

Determination of Copper in Sea Water (RILEY AND SINHASENI[19]). Clean the glassware to be used in this analysis by setting them aside overnight filled with a mixture of equal parts of concentrated nitric and sulfuric acids. Empty and rinse well, concluding with a rinse of de-ionized water.

Filter the sample through a fine sintered-glass funnel. Transfer 900 ml. of the filtrate to a 1-liter separatory funnel. Add 10 ml. of 10 per cent hydroxylammonium chloride and 10 ml. of 10% sodium acetate buffer solution. Add 8 ml. of a n-hexyl alcohol solution of 2,2′-biquinoline mentioned above under reagents. Shake the mixture for 5 minutes and allow the liquids to separate. Draw the lower aqueous layer off into another separatory funnel. Add 2 ml. of hydroxylammonium chloride solution and extract again with a further 3 ml. of the n-hexyl alcohol solution of 2,2′-biquinoline. Separate the phases and extract again with 3 ml. of the 2,2′-biquinoline solution. Combine the n-hexyl alcohol extracts in a 10-ml. volumetric flask. Add 0.5 ml. of 1 per cent solution of hydroquinone in ethyl alcohol. Dilute to the mark with n-hexyl alcohol and mix. Measure the absorbance at 540 nm in a 4-cm. cell. Determine the reagent blank in the same manner with water distilled from a silica still. Prepare a calibration curve with 10.0 and 20.0 μg. of copper added to 900 ml. of metal-free water.

Procedure for the Determination of Copper in Silicate Rock (RILEY AND SINHASENI[19]). Weigh accurately 0.6 to 1 g. of the finely powdered rock into a platinum crucible. Add 2 ml. of concentrated nitric acid and 15 ml. of 40 per cent hydrofluoric acid. Set the covered crucible aside overnight on a water bath. Evaporate to dryness on a water bath. Fuse the residue with 1.5 to 2 g. of fused potassium bisulfate at a dull red heat for 5 minutes. Dissolve the melt in 100 ml. of water containing 1.5 ml. of concentrated hydrochloric acid, warming as necessary. Cool, transfer the solution to a 250-ml. volumetric flask, dilute to the mark and mix. Pipet an aliquot of this solution of such volume as to contain not more than 80 μg. of copper in a 250-ml. separatory funnel, add 5 ml. of hydroxylammonium chloride solution and 25 ml. of 10% sodium acetate buffer solution. Add 6 ml. of 2,2′-biquinoline solution in n-hexyl alcohol. Shake for two minutes, allow the phases to separate, and draw off the lower aqueous layer into a second separatory funnel. Add 2 ml. of hydroxylammonium chloride and 2.5 ml. of n-hexyl alcohol and repeat the extraction. Make a third extraction with 2 ml. of n-hexyl alcohol reagent. Combine the three extracts in a 10-ml. volumetric flask, add 0.5 ml. of a 1 per cent solution of hydroquinone in ethyl alcohol and dilute to the mark with n-hexyl alcohol. Measure the absorbance at 540 nm in a cell of suitable length. Determine the copper in the reagents and water by running a blank. Prepare a calibration curve using 5, 10, 25, and 50 μg. of copper.

Procedure for Copper in Metals and Ores (GUEST[9]). Weigh accurately 1 g. of the ore into a 250-ml. beaker. Add 10 ml. of concentrated hydrochloric acid and 10 ml. of water. Boil the solution for 10 minutes and cool. Add 5 ml. of concentrated nitric acid and 5 ml. of concentrated sulfuric acid. Evaporate the solution to dryness. Dissolve the soluble salts in 5 ml. of concentrated hydrochloric acid and 50 ml. of water. Bring the sample to boil and digest below boiling for 15 minutes. Filter off the insoluble residue on Whatman No. 40 paper and wash the residue with dilute hydrochloric acid (1:10). If the residue is suspected to contain copper, ignite and then fuse or sinter with sodium peroxide. Acidify the melt and add to the main solution. Dilute the filtrate to an appropriate volume in a volumetric flask.

Transfer a suitable aliquot to a 100-ml. beaker and add 5 ml. of 10 per cent hydroxylammonium chloride solution and 5 ml. of 10 per cent tartaric acid. Adjust the pH to 5 to 6 with dilute ammonium hydroxide (1:1). Transfer the solution to a 60-ml. separatory funnel, keeping the total volume to 40 ml. Add by pipet 10.0 ml. of 0.02 per cent cuproine in isoamyl alcohol. Shake the mixture for 1 to 2 minutes. Allow the layers to separate and draw off the lower, aqueous layer. Transfer the alcohol layer to a 1.5-cm. centrifuge tube and centrifuge for 1 minute to clear up any cloudiness which may be present. Measure the absorbance at 546 nm.

NOTES. If the ions which give insoluble chlorides are present, filter off the precipitated chlorides before carrying out the extraction.

The presence of moderate amounts of nitrate does not affect the results.

Procedure for Copper in Alloyed Steels (ELWELL[21]). Dissolve an accurately weighed 0.5-g. sample of the steel millings in 5 ml. of concentrated hydrochloric acid (special alloys require the use of nitric, sulfuric, hydrofluoric, or mixture of acids). Cool, oxidize with a slight excess of concentrated nitric acid, and evaporate to dryness. Do not bake. Add a few drops of concentrated hydrochloric acid, and evaporate as before. Cool, extract the residue with warm water and add a few drops of 20% by volume hydrochloric acid. Dilute with water to about 25 ml. and add 25 ml. of SO_2-saturated water. Boil for 5 minutes, cool, add 15 ml. of 50% citric acid solution, transfer to a 100-ml. calibrated flask and dilute with water to the calibration mark.

Transfer a 10.0-ml. aliquot to a 100-ml. beaker. If sample contains chromium and is low in iron, add 200 mg. of $FeSO_4 \cdot 7H_2O$. Carefully add dilute ammonium hydroxide until the pH is between 5 and 6. Add 2 ml. of 5% sodium acetate solution and transfer to a 50-ml. separatory funnel, keeping the total volume to 17.5 ml. Add 10.0 ml. of the cuproine reagent (50 mg. of cuproine in 100 ml. of amyl alcohol). Shake vigorously for 2 minutes, allow layers to separate for 5 minutes, draw off lower layer, and dry the inside of the stem with filter paper. Transfer the colored organic layer, via a dry filter paper, into a 1-cm. cell, and measure the absorbance at 546 nm.

Determination of Copper in Mineral Oils with Cuproine (HACKETT[11]). Weigh a sample of 10 to 100 g. of the oil and transfer to a separatory funnel of suitable size. Dilute with an equal volume of petroleum ether (b.p. 60-80°) or n-heptane. Carry along a blank determination starting it at this point with the same volume of petroleum ether. Add 10 ml. of alcoholic-hydrochloric acid prepared by mixing 10 ml. of concentrated hydrochloric acid with 90 ml. of ethyl alcohol. Shake and allow the layers to separate. Run the acid layer into a 100-ml. separatory funnel. Add a further 10 ml. of acid to the oil and repeat the extraction procedure. Make a final wash with 10 ml. of ethyl alcohol, combining the two extracts and the wash in the 100-ml. separatory funnel. Add 10 ml. of 30 per cent sodium acetate, 10 ml. of 10 per cent hydroxylammonium chloride, and 5 ml. of 0.1 per cent cuproine. Add 10 ml. of chloroform and shake well. Allow the layers to separate and run the chloroform layer into a 50-ml. volumetric flask. Repeat the extraction with a further 5 ml. of chloroform, at a wave length of 546 nm. Obtain the amount of copper by reference to a calibration curve and subtract the amount of copper in the blank from that in the sample.

Prepare a calibration curve by carrying through known amounts of copper obtained by pipetting various volumes of standard copper solution to give between 0 and 100 μg. of copper.

Applications to the Colorimetric Determination of Anions and the Indirect Determination of Potassium. Spectrophotometric determination of anions by solvent extraction of the ion pairs formed between copper (I) chelate cations and the anions was proposed by Yamamoto and coworkers[23] in 1969. Perchlorate or tetraphenylborate is readily extracted with the copper (I) chelate of cuproine into chloroform or chlorobenzene. Nitrate ions are selectively extractable into methyl isobutyl ketone in the form of ion pairs with the copper (I) chelate of neocuproine (see next section for general description of neocuproine). Phthalate ions can be extracted and determined using either

system. Selectivity in these determinations is primarily dependent on the extraction solvent employed. Certain solvents, e.g. isoamyl alcohol, can extract any and all salts of the copper (I) species. Other solvents extract none. Masking reagents and prior separations must also be employed in practical applications to avoid the effect of various interfering substances. Calibration curves for perchlorate, nitrate, tetraphenylborate, and phthalate are linear in the range 10^{-6} to 10^{-5} **M** for each anion in aqueous solution. Anionic interferences can be largely eliminated by addition of silver (I) or mercury (II) salts. The indirect determination of potassium by means of the tetraphenylborate procedure is applicable in the range of 20 to 200 μg. of potassium.

Determination of Perchlorate (YAMAMOTO, OKAMOTO AND TAO[23]). Pipet 2 ml. of 2×10^{-3} **M** copper sulfate, 2 ml. of 0.1 **M** hydroxylamine sulfate, 5 ml. of acetate buffer solution (equal volume mixture of 0.2 **M** sodium acetate and 0.2 **M** acetic acid) and 2-10 ml. of perchlorate sample solution into a separatory funnel. Adjust the pH to 3.8-6.7 and dilute with water to 25 ml. Add by pipet exactly 10 ml. of 1×10^{-3} **M** cuproine-chloroform or -chlorobenzene solution and shake for 5 minutes. After separation of the two layers, run off the extract into a small glass tube. Add 0.5 g. of anhydrous sodium sulfate and shake vigorously to remove the trace amount of water. Measure the absorbance of the extract at 550 nm in a 10-mm cell against a reagent blank as reference.

Determination of Tetraphenylborate (YAMAMOTO, OKAMOTO AND TAO[23]). Pipet 2 ml. of 5×10^{-3} **M** copper sulfate, 2 ml. of 0.1 **M** hydroxylamine sulfate, 5 ml. of acetate buffer (see above) and 2-10 ml. of the tetraphenylborate solution into a separatory funnel. Adjust the pH to 3.8-5.0 and dilute with water to 25 ml. Complete the determination in the same manner as described above for perchlorate.

Determination of Potassium (YAMAMOTO, OKAMOTO AND TAO[23]) Place the sample solution containing 20-200 μg. of potassium in a centrifuge tube, adjust the pH to 4.0-5.0 with an acetate buffer solution, add 1.00 ml. of 1.0×10^{-2} **M** sodium tetraphenylborate, and dilute to 10 ml. with water. Age about 30 minutes. Separate the precipitate by centrifugation. Pipet 1 ml. of the supernatant into a separatory funnel. Determine the resulting tetraphenylborate equivalent to a specific amount of potassium by the above procedure described for the determination of tetraphenylborate.

SECTION II. BIBLIOGRAPHY. CUPROINE

(2,2′-Biquinoline, 2,2′-Biquinolyl)

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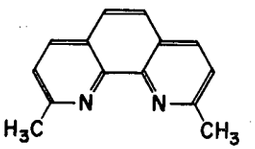
SECTION III

NEOCUPROINE

2,9-Dimethyl-1,10-phenanthroline



$C_{14}H_{12}N_2$

Molecular Weight: 208.25
Molar Absorptivity of
$Cu(neocuproine)_2^+$ in isoamyl alcohol.
7950 at 454 nm

G. Frederick Smith Chemical Company,
Catalog No. 154

2,9-Dimethyl-1,10-phenanthroline was first studied and employed in the colorimetric determination of copper by Smith and McCurdy[1]. They assigned to it the trivial name Neocuproine.

Neocuproine is a specific reagent for copper. It does not form a colored compound with ferrous iron and no other cation is known with which it forms a colored compound extractable with isoamyl alcohol.

Neocuproine reacts with cuprous copper to produce a bright orange, soluble compound. The cupric ion is conveniently reduced with hydroxylammonium chloride, the excess of which need not be removed. The colored compound is formed over the pH range of 3 to 10. The neocuproine is conveniently added as an alcohol-water solution, although other solvents such as amyl alcohol may be employed. The colored compound is completely extracted by n-amyl alcohol, isoamyl alcohol, n-hexyl alcohol, or chloroform, and the final spectrophotometric measurement is conveniently made on the extract.

The combining ratio of neocuproine and copper is two to one in both water and amyl alcohol as shown by Smith and McCurdy[1] by an application of the method of continuous variations. The bonds to the quadricovalent cuprous ion are disposed toward the apexes of a tetrahedron about the copper atom so that the planes of the two phenanthroline molecules are at right angles to each other.

The absorption spectrum of the cuprous neocuproine compound is shown in Fig. 2. The maximum in the absorption curve occurs at a wave length of 454 nm. No absorption occurs over the region 600 to 800 nm, there is a minimum in the absorption curve at 354 nm. The system conforms to Beer's law over the range 0.15 to 10.6 μg. per ml. of copper (1 μg./ml. = 1 p.p.m.) The color is stable indefinitely if a good grade of isoamyl alcohol is employed.

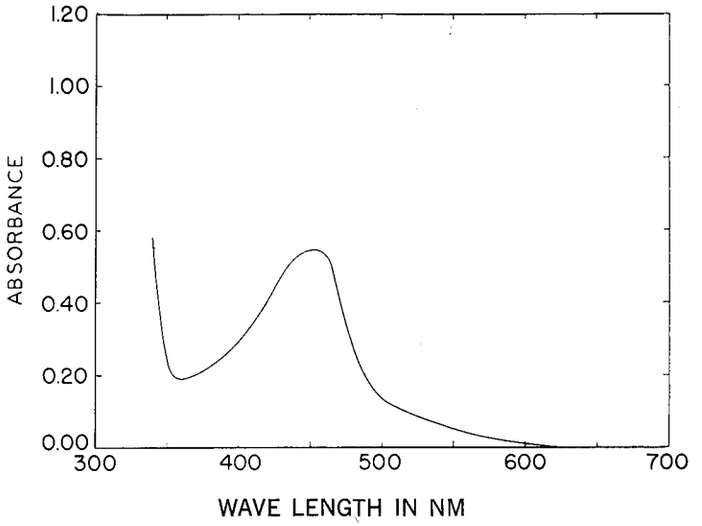


Fig. 2. Absorption spectrum of $Cu(neocuproine)_2^+$; 4 p.p.m. of copper in isoamyl alcohol.

Preparation. The preparation of 2,9-dimethyl-1,10-phenanthroline has been described by Case[2]. The synthesis involves two Skraup reactions, the first involving the condensation of o-nitroaniline and crotonaldehyde diacetate in sulfuric acid solution with arsenic pentoxide as the oxidizing agent. The 2-methyl-8-nitroquinoline thus formed is reduced to the corresponding aminoquinoline in alcohol with stannous chloride. A second Skraup reaction, using crotonaldehyde under the same conditions, gives 2,9-dimethyl-1,10-phenanthroline with an overall yield of about 7 per cent.

Properties. 2,9-Dimethyl-1,10-phenanthroline is obtained in anhydrous form when crystallized from benzene and as the hemihydrate when recrystallized from water. The hemihydrate melts at about 142°C. The anhydrous form melts at 167-168°C. The commercial material normally contains a small amount of water and melts at about 160°C. Both forms are slightly soluble in cold water and soluble in ethyl, amyl and hexyl alcohols, chloroform and benzene.

Neocuproine hydrochloride is finding increasing use as it is soluble in water and readily adaptable in automatic analyzer procedures. The colored complex from the hydrochloride with copper(I) is still extractable into immiscible solvents.

Specificity. Interferences. Effects of Diverse Ions. Cations. Smith and McCurdy[1] found no cations other than cuprous copper which would form a colored compound with neocuproine extractable into isoamyl alcohol. This rather astonishing specificity was confirmed by Luke and Campbell[3] who tested the reagent on 56 metals.

Satisfactory results for copper were obtained in the presence of 50 μg. of the metals except beryllium. When the latter was present, very low results for copper were obtained, owing presumably to adsorption of the copper or the copper neocuproine compound on colloidal beryllium hydroxide. By adding the hydroxylammonium salt and sodium citrate before the neutralization, the difficulty caused by the beryllium is eliminated and the method is made completely specific for copper.

Chromium(III) in amounts above 2 mg. cause low results for copper, according to Frank, Goulston and Deacutis[9], but not when iron is present or when chromium is reduced with sulfur dioxide. They recommend that the chromium be volatilized as chromyl chloride by fuming the solution with perchloric acid while adding hydrochloric acid dropwise. The A.S.T.M. procedure[10] for the determination of copper in stainless steels, however, calls simply for oxidizing the chromium to chromate with boiling perchloric acid dihydrate (72% $HClO_4$) and reducing with sulfur dioxide. Chromium interference studies by Irving and Tomlinson[11] have shown the cause of chromium interference to be the tying up of copper in a kinetically inert ternary copper(I)-chromium(III)-citrate complex. Addition of excess iron(II) displaces copper from the ternary complex, thus eliminating chromium interference.

Anions. The common anions chloride, sulfate, nitrate, perchlorate, tartrate, citrate and acetate do not interfere. An orange-yellow precipitate occurs with large amounts of nitrate, perchlorate and the halides (particularly iodide) upon the addition of neocuproine to an aqueous copper solution but causes no interference, as the colored compound is quantitatively extracted in all cases. Anions such as periodate, nitrate, thiocyanate and ferricyanide, which either react with hydroxylamine or give a yellow colored solution, may be suitably eliminated to adjust conditions to the test. Pyrophosphate and phosphate ions do not interfere, although for excessive amounts two or three extractions may be required to collect all the copper.

The effects of large amounts of anions were investigated by Gahler[4]. At least 10 ml. of 70 per cent perchloric acid may be used, the resulting ammonium perchlorate being without effect on the determination. Complete recovery of copper resulted with 5 ml. of 85 per cent phosphoric acid but only 96 per cent recovery with 10 ml. of phosphoric acid. Large amounts of sodium fluoride (1.5 g.) and ammonium chloride (15 g.) caused no interference. Only a trace of cyanide, less than 1 mg., may be present. Sulfide in amounts less than 0.1 mg. greatly reduces the recovery of copper. On the other hand, columbate, molybdate, tantalate, tungstate and vanadate do not interfere.

Extraction. Extraction with a chloroform-ethyl alcohol mixture appears to offer some advantages over extraction with isoamyl alcohol.[4] Chloroform is readily obtained pure and need not be redistilled. It separates from the aqueous layer quickly and being more dense than water is somewhat more convenient when several extractions are to be made and the organic layer collected.

The absorption maximum occurs at 457 nm in chloroform-ethyl alcohol[4], slightly different from the 454 nm maximum in isoamyl alcohol or hexanol. A small amount of alcohol must be present at the time of extraction with chloroform for maximum development of the color. The composition of the solvent does not affect the colored system, provided that a minimum of 2 ml. of ethyl alcohol is present in 25 ml. of chloroform. A solution containing less than 2 ml. of ethyl alcohol usually is turbid. If no ethyl alcohol is present, very little color forms in the chloroform layer.

One extraction with 10 ml. of isoamyl alcohol, hexanol or chloroform is sufficient to extract the copper compound completely. A second extract, in the nature of a wash, is recommended.

Qualitative Test for Copper. By visual examination of a drop of water containing cuprous ion and neocuproine on a spot plate, as little as 0.04 μg. of copper may be detected. Using a microdrop of n-hexyl alcohol as extractant, the limit of identification is 0.03 μg. of copper. The limit of concentration in the former case is 1 to 1,250,000, in the latter case 1 to 1,660,000. Neocuproine is as sensitive as dithizone for the detection of copper.

Applications to the Determination of Copper. The original paper of Smith and McCurdy[1] dealt primarily with the basic chemistry of neocuproine and its use in the determination of copper. The paper indicates that the method should be suitable for the determination of copper in almost any composition of matter; it applied it specifically to the determination of copper in iron and steel. Subsequent publications have extended the applications of neocuproine as reviewed in the following paragraphs. In general the method is remarkable in its sensitivity, specificity, rapidity and simplicity. At least four samples and blanks can be run in forty-five minutes and the precision is that of any first class colorimetric method. The separations of copper involving hydrogen sulfide or thiocyanate, characteristic of older methods for copper in complex materials, are obviated.

The size of the sample should be chosen so that 0.15 to 10 μg. should be present in each milliliter of final extract, for the color conforms to Beer's law over this range. For any given material a preliminary determination may be necessary to establish the copper content approximately. Then, a sample of proper size may be taken or a larger sample may be used at the start and a suitable aliquot taken.

Somewhat more precise results can be secured by making the spectrophotometric measurement with a copper solution of known concentration in the comparison cell rather than the pure solvent (isoamyl alcohol or chloroform)[9].

It is necessary in determining copper in steel to add a masking reagent to hold the iron in solution when the pH is raised. Citrate is most effective for this purpose, about 6 g. of sodium citrate being required for each gram of iron. Fluoride, pyrophosphate, oxalate, malonate and tartrate were studied[1] but were found inferior to citrate.

Smith and McCurdy[1] reported results on synthetic mixtures and steels covering the range of 0.012 to 0.17 mg. of copper in the presence of 1 g. of iron. The maximum error was of the order of 4 μg. of copper corresponding to 0.0004 per cent copper. The method for the determination of copper in steel was studied more extensively by Gahler[4] who applied it to the determination of copper in cast iron and a variety of alloy steels containing chromium, molybdenum, nickel, tungsten, vanadium, cobalt, silver and lead, and to ferrotungsten and tungsten ores. The range covered, broadened by appropriately varying the size of the sample or by taking aliquots, was 0.02 to 1 per cent copper.

A fine application of neocuproine was made by Luke and Campbell[3] of the Bell Telephone Laboratories to the determination of 0.1 to 1 p.p.m. of copper in the highly pure germanium used as semiconductors. The procedure is the original procedure of Smith and McCurdy[1] but using chloroform as the extracting liquid as recommended by Gahler[4].

The chloroform extraction was employed also by Fulton and Hastings[8] who worked out procedures for determining copper in aluminum and in lead-tin solders with neocuproine. The method for aluminum was checked against NBS Sample 87, British Chemical Standard 198a, and other pure and alloyed aluminum samples. The results were excellent. For checking the method for copper in solder, known amounts of copper were added to NBS 127 and 53c. The results again were good.

Neocuproine has been applied with success also to the determination of 0.005 to 0.4 per cent of copper in metallic titanium[9].

The method has also been applied to the direct determination of 0.01 per cent copper in tungsten[6]. It is necessary to complex the tungsten by converting it to phosphotungstic acid and adding a little citrate before neutralizing with sodium acetate.

The reagent is fine also for the direct determination of copper in beverages and water[5]. It is recommended that organic matter be destroyed by digestion with nitric and perchloric acids when the sample contains sugar.

One application of neocuproine which has tremendous advantages over older methods is the direct colorimetric determination of copper in fuel oil and other petroleum products devised by Zall, McMichael and Fisher[8]. A solution of neocuproine in isopropyl alcohol is added to the oil and chloroform is added as a mutual solvent. The reaction to form the copper-neocuproine color is almost instantaneous and the photometric measurement is made directly on the single phase solution so obtained using as a natural color blank a sample of the same size treated with the solvents but no reagent. No preliminary treatment of the sample, such as ashing or acid extraction, is necessary. Some attention must be paid to the possible interference by additives which may be in the oil.

Neocuproine has also proven advantageous for the photometric determination of copper in silicate rocks (Bodart[12]), plutonium metal (Lindsay and Plock[13]), and gallium and arsenic binary mixtures (Knizek and Pecenkova[14]).

A two-fold increase in sensitivity without loss in specificity in the determination of copper can be achieved, according to Luke[15], by extracting the copper neocuproine complex and then converting it to the copper diethylammonium diethyldithiocarbamate complex. The conversion is rapid and simple, involving merely the addition of 0.1 g. of the carbamate reagent to the chloroform extract of the copper(I) neocuproine complex.

A method for determining submicrogram amounts of copper, devised by Bailey, Dagnall and West[16], involves separation of copper by chloroform extraction of its neocuproine complex from a citrate and E.D.T.A. medium. The extract is then shaken with a solution of 1,10-phenanthroline and Rose Bengal Extra in phosphate pH9 buffer to convert the neocuproine copper complex into a ternary copper-1,10-phenanthroline-Rose Bengal Extra complex. Fluorescence of the ternary complex is excited at 560 and measured at 570 nm. Fluorescent intensity is linear for 0.1 to 0.6 μg. of copper as measured.

Procedure for the Determination of Copper (SMITH AND McCURDY[1]). REAGENTS. NEOCUPROINE SOLUTION 0.1 PER CENT IN ETHYL ALCOHOL. Dissolve 1 g. of 2,9-dimethyl-1,10-phenanthroline,⊜ in 1 liter of ethyl alcohol.

WATER. Distilled water often contains detectable quantities of copper and the water used should be checked by a blank determination. De-ionized water obtained by passage of distilled water through a bed of Amberlite MB-1 is satisfactory.

HYDROXYLAMMONIUM CHLORIDE SOLUTION. 10 PER CENT AQUEOUS SOLUTION.⊜ Dissolve 10 g. of hydroxylammonium chloride,⊜ in 100 ml. of redistilled or de-ionized water. Transfer the solution to a separatory funnel and add 1 ml. of neocuproine solution. Extract with 10-ml. portions of chloroform until a colorless extract is obtained.

SODIUM CITRATE SOLUTION. Dissolve 300 g. of sodium citrate, $Na_3C_6H_5O_7 \cdot 2H_2O$, in 1 liter of water. Add 2 ml. of hydroxylammonium chloride solution and 1 ml. of neocuproine solution. Extract with 10-ml. portions of chloroform until a colorless extract is obtained.

STANDARD COPPER SOLUTION. 0.0100 MG. CU PER ML.⊜ Weigh accurately 0.1000 g. of pure copper and transfer to a 250-ml. conical flask. Add 5 ml. of nitric acid and 5 ml. of water and heat gently to dissolve the copper. Add 5 ml. of perchloric acid and evaporate to fumes of perchloric acid. Cool, dilute with water and transfer to a 1-liter volumetric flask. Dilute to the mark with water and mix. Pipet a 100-ml. aliquot of this solution to a second 1-liter volumetric flask. Dilute to the mark with water and mix. If exactly 0.1000 g. was weighed out initially this solution will contain 0.100 mg. of copper per ml.; if some other weight was used, label appropriately.

APPARATUS. The reaction and extraction are most conveniently carried out in separatory funnels of 60-ml. or 100-ml. capacity. A sharper separation of the immiscible phases will be obtained later if the usual stopcock of the funnel is replaced with one having a bore of only 0.5 mm. diameter.

The photometric measurements are best made on a spectrophotometer, for example, the Beckman DU or Beckman Model B. The measurements can be made, but with less sensitivity, on a filter photometer using a filter having a minimum in the absorption curve near 454 nm, the wave length of maximum absorption of the copper-neocuproine compound.

GENERAL PROCEDURE. A. EXTRACTION WITH ISOAMYL ALCOHOL. Transfer a sample of such size as to contain between 4 and 200 μg. of copper to a separatory funnel. Add 5 ml. of 10% hydroxylammonium chloride. If iron is present, add 20 ml. of 30 per cent sodium citrate solution for each gram of iron in the sample. Add 10 ml. of 0.1 per cent neocuproine. Add 1 g. of sodium acetate or more to bring the pH of the solution between 5 and 6. Extract the colored solution with a 10-ml. portion of distilled isoamyl alcohol⊜ . Separate the liquids, transferring the isoamyl alcohol layer to a 50-ml. volumetric flask. Repeat the extraction, again transferring the isoamyl alcohol to the volumetric flask. Dilute the extracts to 50.00 ml. with isoamyl alcohol. Measure the absorbance of the solution, preferably with a spectrophotometer, at a wave length of 454 nm.

Following the same procedure, prepare a calibration curve using various volumes of the standard copper solution.

GENERAL PROCEDURE. B. CHLOROFORM EXTRACTION. To the solution containing 20 to 200 μg. of copper in a separatory funnel add water to bring the volume to about 50 ml. Add 5 ml. of 10 per cent hydroxylammonium chloride solution to reduce the copper and 10 ml. of 30 per cent sodium citrate solution. Add sufficient ammonia to bring the pH of the solution to between 4 and 6 as indicated by pH paper. Add 10 ml. of 0.1 per cent neocuproine solution in ethyl alcohol. Add 10 ml. of chloroform ⊜ and shake the mixture for 1 minute. Allow the two layers to separate. Transfer the chloroform layer to a 50-ml. volumetric flask. Rinse the funnel and stem with two 1-ml. portions of chloroform. Repeat the extraction with 6 ml. of chloroform and rinse the stem as before. Dilute to exactly 50 ml. with ethyl alcohol. Measure the absorbance at 454 nm, preferably using in the reference cell a blank on the reagents which has been carried through the same treatment as the sample. Calculate the copper concentration by referring to an absorbance-concentration curve prepared by taking different volumes of a standard copper solution through the same procedure.

GENERAL PROCEDURE C. AQUEOUS SOLUTION. To a sample of acidic (pH4 or less) solution containing copper, add 5 ml. of 10% hydroxylammonium chloride. If iron is present in appreciable concentration, add also 10 ml. of 30% sodium citrate solution. Add 10 ml. of 0.1% neocuproine hydrochloride⊜ in water, and adjust the pH to 4-6 with ammonium hydroxide. Dilute to a convenient, exact volume and mix. Measure the absorbance at 454 nm, and refer to a suitably prepared calibration curve to determine the copper concentration. The final solution should contain 50 to 200 μg. of copper per 100 ml. for best results. If copper is present in very low concentration, an extraction procedure can be used to increase the sensitivity by a factor of 5 or 10. A 100-ml. sample may be extracted with 5 ml. of isoamyl alcohol, followed by a 3 ml. isoamyl alcohol wash and dilution to 10 ml., to obtain a concentration factor of 10.

Procedure for the Determination of Copper in Iron and Steel (METHOD OF GAHLER[4]). Dissolve the sample by any appropriate means, preferably with hydrochloric acid. Add sufficient nitric acid to oxidize the iron and evaporate to a small volume to remove the excess acid. If an aliquot is to be taken, transfer the solution to a volumetric flask and dilute to the mark with distilled water. Transfer the sample or the aliquot, containing up to 200 μg. (0.2 mg.) of copper, to a separatory funnel. Proceed as described in A or B above.

NOTES. Most alloy steels will dissolve in hydrochloric acid to which is added a little nitric acid. Ferrotungsten is best dissolved in hydrofluoric acid (platinum dish) and a little nitric acid added dropwise; sulfuric acid must then be

added and the solution evaporated to sulfuric acid fumes to eliminate the hydrofluoric acid. Tungsten ores of the Scheelite type are soluble in hydrochloric acid. A cobalt-molybdenum-tungsten steel can be dissolved by a preliminary treatment with hydrochloric acid, followed by digestion and evaporation to fumes with a mixture of sulfuric acid, phosphoric acid, perchloric acid and a little hydrofluoric acid.

Procedure for the Determination of Copper in Lead-Tin Solder (FULTON AND HASTINGS[7]). Dissolve 0.500 g. of solder in 25 ml. of hydrobromic acid. When the reaction ceases, add dropwise just enough hydrobromic acid-bromine mixture (prepared by mixing 180 ml. of 48 per cent hydrobromic acid and 20 ml. of bromine) to clear the solution. Cool, transfer to a 100-ml. volumetric flask, dilute to the mark and mix. Pipet an aliquot containing 40 to 200 μg. of copper to a separatory funnel. Proceed as described in A or B above.

NOTES. Add the hydrobromic acid-bromine mixture dropwise as the copper must be in the univalent state and any excess of oxidizing agent should be avoided. Sufficient hydrobromic acid must be present to keep the metals in solution when diluted to 100 ml. About 25 ml. of 48 per cent hydrobromic acid will keep 0.5 g. of solder in solution.

Procedure for the Determination of Copper in Aluminum (FULTON AND HASTINGS[7]). Weigh accurately 0.5 g. of aluminum and transfer the sample to a glass beaker. Add 5 ml. of concentrated hydrochloric acid and 5 ml. of water. Add a little nitric acid to aid the dissolution of the metal. Evaporate the solution until almost dry. Rinse the walls of the beaker with a little nitric acid, add 2 ml. of hydrofluoric acid, and heat until any silicon present dissolves and the solution is clear. Dilute to 25 ml. if necessary, filter through a medium-fine paper collecting the filtrate in a 100-ml. volumetric flask. Wash any residue and paper thoroughly with dilute hydrochloric acid (1 to 20). Dilute to 100.0 ml. and mix thoroughly. Transfer an aliquot containing 40 to 200 μg. of copper to a separatory funnel and proceed as above in A or B.

NOTES. High-silicon aluminum alloys may be dissolved with the aid of hydrofluoric acid. Some siliceous residue may remain but it will probably not contain copper.

High purity aluminum dissolves very slowly in hydrochloric acid. The addition of cupric ion or ferric ion noticeably hastens the dissolution of the aluminum. For samples of pure aluminum, add 1 ml. of ferric sulfate solution to aid the dissolution. The aluminum dissolves readily but reduces the iron which precipitates as a gray powder and dissolves with a few drops of nitric acid. Keep the nitric acid to a minimum to prevent oxidation of the copper.

Procedure for the Determination of Copper in Fuel Oil (ZALL, McMICHAEL AND FISHER[8]), REAGENT. NEOCUPROINE-HYDROQUINONE SOLUTION. Dissolve 1 g. of 2,9-dimethyl-1,10-prenanthroline⊖ and 1 g. of hydroquinone⊖ in 1 liter of isopropyl alcohol⊖.

PROCEDURE. Run a sample and a blank which serves to cancel the effect of any natural color in the sample. Treat both identically except to omit the neocuproine-hydroquinone solution from the blank. Pipet a sample of fuel oil, copper naphthenate or other petroleum product into a 25-ml. volumetric flask. If the copper content is high pipet the sample into a 100-ml. volumetric flask, dilute to the mark, mix and pipet an aliquot into a 25-ml. flask with chloroform. Mix thoroughly and allow to stand for 30 minutes. Measure the absorbance of the solution at 454 nm using the blank solution in the reference cell. Calculate the amount of copper by reference to a calibration curve prepared in identical fashion using known amounts of copper.

NOTES. Some organic inhibitors used in fuel oil, such as N-phenyl-1-naphthylamine, N,N′-tetramethyl-diaminodiphenyl-methane and other amino compounds (below 1 per cent), cause no interference. Some mercaptans interfere, causing low results. Mercaptobenzothiazole and its sodium salt, in concentrations below 0.1 per cent, cause low results. This interference can be overcome by a large excess of neocuproine. Butylzymate interferes seriously. Amounts of barium sulfonate and sodium sulfonate above 0.1 per cent retards the color development.

Applications to the Indirect Determination of Various Substances. (PERCHLORATE, NITRATE, GLYCINE, REDUCING SUGARS, AND URIC ACID.) A method for the determination of perchlorate ions using neocuproine was reported by Collinson and Boltz[17]. Perchlorate is extracted into ethyl acetate in the presence of copper(I) and neocuproine. The authors refer to the

extracted species as perchloratobis (2,9-dimethyl-1,10-phenanthroline) copper(I), however, no experimental evidence is presented in support of pentacoordinated copper(I), nor is precedent to be found in the literature for such an assignment, at least not for copper(I). It appears more likely that the extracted species is an ion association complex or salt of the bisneocuproine copper(I) cation and perchlorate anion. Following the extraction step, the final measurement to determine perchlorate is made on the extract either by spectrophotometry or atomic absorption spectrometry. Applicable to amounts of perchlorate up to 0.6 mg. and concentrations in the range of 0.5 to 5 p.p.m. of perchlorate, the method is capable of excellent precision. Relative standard deviations of 0.24% for the spectrophotometric and 0.43% for the atomic absorption measurements were obtained in the analysis of six solutions, each containing 3 p.p.m. of perchlorate. Nitrate, ammonium, magnesium, or aluminum should not be present in concentration greater than 10 p.p.m. Acetate, chloride, sulfate, phosphate, potassum, or iron(II) ions are tolerated at the 15 p.p.m. level.

Microamounts of perchlorate in biological fluids were determined by Weiss and Stanbury[18] using an adaptation of the method of Collinson and Boltz[17]. Greater selectivity and enhanced sensitivity were achieved through use of a preliminary separation by ion exchange. As little as 5 μg. of perchlorate in urine or serum can be accurately determined in 2 ml. of fluid.

Determination of nitrate in fresh water samples by extraction into methyl isobutyl ketone and subsequent photometric measurement of the ion pair between the neocuproine copper(I) complex cation and nitrate anion was described by Yamamoto, Okamoto, and Tao[19]. High results are caused by moderate amounts of chloride, bromide, iodide, or thiocyanate; these can be avoided by prior treatment with silver sulfate. Iron, aluminum, and cyanide ions cause low results. Interference from nitrite can be removed by boiling with sulfamic acid. Good precision for replicate determinations using 10-ml. samples containing 1.50 p.p.m. of nitrate was obtained.

The determination of reducing sugars, using neocuproine in place of the commonly used molybdate color reagent, was described by Brown[20]. Excellent results were obtained for samples containing 5 to 30 μg. of glucose. A modification of Brown's method, in which glycine replaces tartrate as complexing agent, was reported by Dygert, Li, Florida, and Thoma[21]. Their modification increased the precision, reduced the value of the blank, and made possible the extension of the range to cover from 5 to 125 μg. of glucose. Any substance that can reduce copper(II), alter the pH of the oxidant, or chelate copper can potentially interfere. A distinct advantage of the method over the Somogyi method[22] or its modified version[23] is its greater insensitivity to interfering substances. The effects of many interferences can be compensated for by including them in the standards. The relative standard deviation was less than 3% for determinations involving 10 to 90 μg. of glucose. Bittner and McCleary[24] have reported successful analysis of glucose in whole blood by a similar neocuproine method. An automated procedure for the determination of monosaccharides in serum using the Auto Analyzer has been described by Bittner and Manning[27].

An indirect photometric method for determining uric acid using neocuproine was reported by Bittner, Hall and McCleary[25]. Based upon the reduction of copper(II) by uric acid in slightly acidic medium to produce the deeply colored copper(I) neocuproine complex, the method is free of interference from glucose, creatinine, tyrosine, tryptophane, ascorbic acid, and cysteine. Lofland and Crouse [28] have described an automated procedure for the Bittner method[25] based on the use of the Auto Analyzer.

An automated procedure employing neocuproine in the enzymatic determination of serum uric acid, for either the Auto Analyzer or the Robot Chemist, was described by Morgenstern, Flor, Kaufman and Klein[26]. The difference in the amount of copper(I) formed by reaction of a copper(II), alkanolamine buffered solution with serum uric acid under precisely controlled conditions before and after uricase treatment of the serum is measured as the neocuproine complex. The difference is proportional to the true serum uric acid content. Very good agreement between uric acid values obtained by the automated method and by ultraviolet spectrophotometry was found.

Glycine can be determined in the complex glycine potassium trioxalatochromate(III) by an indirect procedure involving treatment with a suspension of copper phosphate, formation of soluble copper glycinate, and subsequent determination of copper with neocuproine[24].

Procedure for the Determination of Perchlorate (COLLINSON and BOLTZ[17]). REAGENTS. NEOCUPROINE REAGENT. Dissolve 0.4165 g. of 2,9-dimethyl-1,10-phenanthroline ⊖ in 1 liter of reagent grade ethyl acetate. PHOSPHATE BUFFER. Dissolve 34.0 g. of potassium dihydrogen phosphate in distilled water and dilute to 1 liter (pH 4.3). COPPER(II) SULFATE SOLUTION. Dissolve 0.3140 g. of reagent grade anhydrous copper(II) sulfate in distilled water and dilute to 1 liter. HYDROXYLAMINE SULFATE SOLUTION. Dissolve 50 g. of hydroxylamine sulfate ⊖ in 950 ml. of water. STANDARD PERCHLORATE SOLUTION. Dissolve 0.1232 g. of reagent grade anhydrous sodium perchlorate ⊖ in distilled water and dilute to exactly 1 liter. Dilute exactly 25 ml. of this stock solution to 100 ml. to obtain a standard solution containing 25.0 μg. of perchlorate per ml.

GENERAL PROCEDURE. Accurately measure a sample containing no more than 0.6 mg. of perchlorate ion, adjust the pH to 3-5, and dilute to 25 ml. Transfer 6 ml. of the copper(II) solution to a 25-ml. volumetric flask and add 5.0 ml. of sample solution, 2 ml. of the hydroxylamine sulfate solution and 5 ml. of the phosphate buffer solution. Dilute to volume with distilled water and mix thoroughly. Add exactly 10 ml. of the neocuproine reagent to a 60-ml. separatory funnel. Transfer solution from volumetric flask to separatory funnel. Use 1 ml. of buffer solution to rinse flask. Add rinsing to separatory funnel. Shake for 3 minutes to extract. Remove aqueous layer. Drain extract through cotton placed in stem of funnel and collect in small bottle. Measure the absorbance at 456 nm in a 1.00-cm. cell against a reagent blank in the reference cell. Refer absorbance reading to a calibration graph obtained using standard perchlorate solutions.

Procedure for the Determination of Reducing Sugars (DYGERT, LI, FLORIDA AND THOMA[21]). REAGENTS. SOLUTION A. Dissolve 40 g. of anhydrous sodium carbonate in 600 ml of distilled water; then add and dissolve 16 g. of glycine, followed by 0.450 g. of copper(II) sulfate pentahydrate ⊖. Dilute to 1 liter. SOLUTION B. Dissolve 0.12 g. of neocuproine hydrochloride ⊖ in 100 ml. of distilled water.

PROCEDURE. Pipet exactly one (or two) ml. of sample solution into a graduated test tube. Pipet into the same tube X ml. of Solution A and X ml. of Solution B. (Note: for 5-25 μg. of glucose, X is 1.0; 26-50 μg., X is 2.0; 51-75 μg., X is 3.0; 76-100 μg., X is 4.0; and for 101-125 μg. of glucose, X is 5.0) Mix the contents, cap the tubes, and place in a vigorously boiling water bath. Heat for 8 minutes if sample contains only glucose; heat for 12 minutes if larger saccharides are present. If a precipitate forms during heating, insufficient reagents have been used. After heating the specified time, cool the tube in running tap water. A trace of precipitate may form if cooled too much, but it redissolves upon warming to room temperature. Dilute the contents to an appropriate, exact volume (10 to 25 ml.) and measure the absorbance at 450 nm within one hour against water. Reading all tubes against water serves to keep one aware of the relative size of the blank. Run blank and standards the same way to prepare a suitable calibration curve.

SECTION III. BIBLIOGRAPHY, NEOCUPROINE (2,9-Dimethyl-1,10-phenanthroline)

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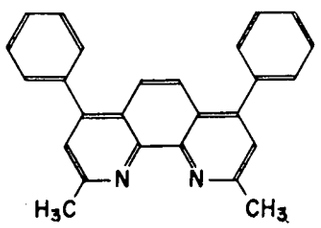
SECTION IV

BATHOCUPROINE

2,9-Dimethyl-4,7-diphenyl-1,10-phenanthroline



$C_{26}H_{20}N_2$

Molecular Weight: 360.44
Molar absorptivity of $Cu(bathocuproine)_2^+$:
in water: 13,900 at 479 nm
in n-hexyl alcohol: 14,160 at 479 nm

G. Frederick Smith Chemical Company,
Catalog No. 240

2,9-Dimethyl-4,7-diphenyl-1,10-phenanthroline is a highly specific and exceptionally sensitive reagent for the colorimetric determination of copper. It was given the trivial name Bathocuproine by the original investigators, Smith and Wilkins[1]. As with other 2,9-substituted phenanthrolines, bathocuproine does not form a chelate ring compound with iron. Just as the introduction of phenyl groups into the 4- and 7- positions of 1,10-phenanthroline greatly increases the molar absorptivity of the ferrous derivative, similar substitution into 2,9-dimethyl-1,10-phenanthroline (neocuproine) greatly increases the intensity of the color of the cuprous derivative; the molar absorptivity of the cuprous derivative is 14,160 in contrast to the 7,950 of the cuprous derivative of neocuproine.

The molar absorptivity of the cuprous derivative of bathocuproine varies somewhat with the solvent: 13,900 in water solution; 14,160 in n-hexyl alcohol; 14,200 in isoamyl alcohol. The absorption spectrum is shown in Fig. 3. The color system conforms to Beer's law over the range 1 to 10 p.p.m. of copper. The cuprous compound is oxidized slowly by air, about 0.05 per cent per hour at room conditions.

Preparation. 2-9-Dimethyl-4,7-diphenyl-1,10-phenanthroline was first synthesized by Case and Brennan[2]. 2-Methyl-8-nitro-4-phenylquinoline was prepared by a Doebner-Miller reaction from o-nitroaniline and phenyl propenyl ketone ($C_6H_5COCH=CHCH_3$) and reduced to the corresponding amino compound. The amine and phenyl propenyl ketone by a modified Skraup reaction then gave 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline.

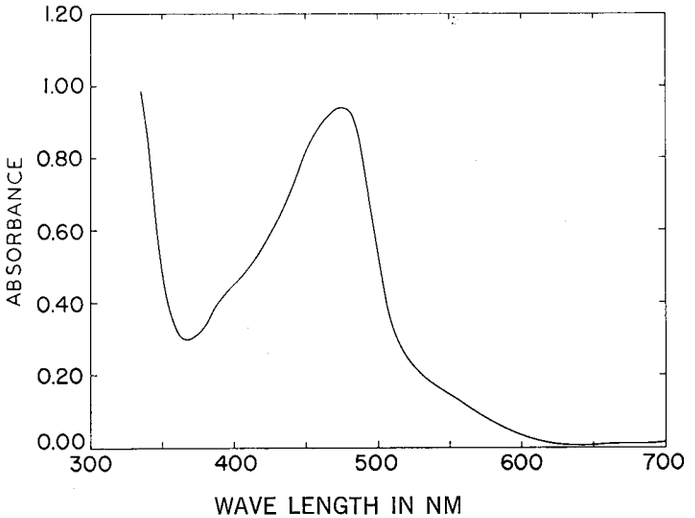


Fig. 3. Absorption spectrum of $Cu(bathocuproine)_2^+$; 4.0 p.p.m. of copper in isoamyl alcohol.

Specificity. Interferences. Effects of Diverse Ions. Cations. No detailed study of the interferences of cations has been reported but other similar reagents, for example, neocuproine, are known to be free of interference by practically all metals.

Anions. The common anions such as chloride, nitrate, perchlorate, phosphate, sulfate and citrate do not interfere[1].

Quantitative Determination of Copper. In the original paper dealing with bathocuproine Smith and Wilkins[1] worked out the general chemistry of bathocuproine and its use in the determination of copper. They applied the reagent specifically to the determination of copper in iron and indicated that the method could undoubtedly be applied to biological, medicinal and food products.

COPPER IN PULP AND PAPER. An extensive review of the methods of determining copper in pulp, paper, and pulping liquids was made by Borchardt and Butler[3] who came to the conclusion that bathocuproine was superior to all other methods in sensitivity and selectivity. The copper in such products is generally from a few tenths p.p.m. to 20 p.p.m. and bathocuproine is the ideal reagent following wet ashing of a suitable sample, for example, 1 g. of paper, by digesting with a mixture of nitric and perchloric acids.

COPPER IN WINE. The extraction of the copper-bathocuproine compound into isoamyl alcohol makes possible the direct determination of copper in wine. Banick and Smith[4], who worked out the method, found the direct extraction method gave results equally as precise and accurate as the wet ashing method at a considerable saving of time. Because of the low content of copper in wines, it is preferable to add a known amount of copper to the sample for analysis. This "seeding" technique is often used in gravimetric and radiochemical processes and serves as a check on the accuracy and precision of the results. In the wine analyzed by Banick and Smith[4] the results for copper by the wet ashing procedure were: 0.61, 0.67, 0.64, ave.

0.64 p.p.m. Cu; by the direct procedure: 0.62, 0.62, 0.66, 0.65, 0.60, ave. 0.63 p.p.m. Cu.

COPPER IN IRON. For the determination of 0 to 10 p.p.m. of copper in pure iron, Kammori, Shirai and Okubo[5] employed the bathocuproine photometric method of Smith and Wilkins[1]. Chloroform was used in place of n-hexyl alcohol as the extraction solvent. Ions of the following metals, each at 20 p.p.m. did not interfere: Al, As, B, Ca, Ce, Co, Cr, Nb, Ni, Mg, Mn, Mo, P, Pb, Si, Sn, Ta, Ti, V, W, or Zn.

COPPER IN SOILS, SEDIMENTS, AND ROCKS. Nowlan[6] recommended a bathocuproine photometric method for the determination of copper in soils, sediments, and rocks. The sample is decomposed with a mixture of nitric, perchloric, and hydrofluoric acids. Following the adjustment of pH and other solution conditions, copper(I) is extracted as the bathocuproine complex into isoamyl alcohol, and the absorbance of the extract is measured at 475 nm. A standard deviation of 0.84 p.p.m. of copper was given by duplicate analyses of 27 samples of stream sediments and rocks, in which copper concentrations ranged from 5 to 50 p.p.m.

Bathocuproine has also been utilized in the determination of copper in serum by Zak and Ressler[7] and by O'Leary, Novalis and Vosburgh[8].

General Procedure for the Determination of Copper (SMITH AND WILKINS[1]). REAGENTS. WATER. Use water redistilled from an all glass apparatus or deionized water prepared by passage of distilled water through a monobed resin such as Amberlite MB-1.

n-HEXYL ALCOHOL.◉ Distill a commercial grade of hexyl alcohol, discarding the first and last fractions.

ISOAMYL ALCOHOL.◉ Redistill a good grade of isoamyl alcohol.

BATHOCUPROINE SOLUTION. 0.01 M IN n-HEXYL ALCOHOL OR ETHYL ALCOHOL. Dissolve 0.36 g. of 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline ◉ in 100 ml. of redistilled n-hexyl alcohol. If the absorbance of the copper bathocuproine compound is to be measured on the aqueous solution and the extraction with hexyl alcohol omitted, prepare the reagent solution by dissolving 0.36 g. of the reagent in 100 ml. of ethyl alcohol.

HYDROXYLAMMONIUM CHLORIDE. 10 PER CENT AQUEOUS SOLUTION.◉ Dissolve 10 g. of hydroxylammonium chloride◉ in 100 ml. of redistilled water. Test 10 ml. of this solution for copper by adding a ml. of bathocuproine solution and a little ammonia. If a detectable amount of copper is present, treat the entire solution in a separatory funnel with bathocuproine in n-hexyl alcohol and a little ammonia. Shake vigorously for two minutes, allow the liquids to separate and withdraw the lower, copper-free solution to a reagent bottle.

AMMONIUM ACETATE. 10 PER CENT AQUEOUS SOLUTION.◉ pH 7.1. Dissolve 10 g. of ammonium acetate in 100 ml. of water. Test 10 ml. of the solution for the presence of copper by adding a little hydroxylammonium chloride solution and a little bathocuproine solution. If copper is present treat the entire solution with a few ml. of hydroxylammonium chloride solution and bathocuproine in n-hexyl alcohol. Shake vigorously with a few ml. of n-hexyl alcohol in a separatory funnel, allow the layers to separate and draw off the ammonium acetate solution to a reagent bottle.

BUFFER OF pH 4.0 Dissolve 41 g. of sodium acetate in 320 ml. of water. Add 170 ml. of glacial acetic acid. Adjust the solution to pH 4.0 using 10 per cent sodium hydroxide or dilute acetic acid as necessary and employing a pH meter to follow the adjustment. The final buffer is about 1.0 molar in sodium acetate and 5.5 molar in acetic acid.

BUFFER OF pH 7.1. Dissolve 39 g. of ammonium acetate in 500 ml. of water. This solution is then about 1.0 molar in ammonium acetate.

STANDARD COPPER SOLUTION. 5.00 μg. CU PER ML.◉ Weigh accurately 0.1000 g. of pure copper and transfer to a 250-ml. conical flask. Add 5 ml. of nitric acid and 5 ml. of water and heat gently to dissolve the copper. Add 5 ml. of perchloric acid and evaporate to fumes of perchloric acid. Cool, dilute with water and transfer to a 1-liter volumetric flask. Dilute to the mark with water and mix. Pipet 50.00 ml. of this solution to a second 1-liter volumetric flask. Dilute to the mark with water and mix. If exactly 0.1000 g. was weighed out initially this solution will contain 0.00500 mg. of copper per ml.; if some other weight was used, label appropriately.

PROCEDURE. Transfer such a quantity of the solution as to contain between 0.06 and 2 mg. of copper to a separatory funnel of 60- or 100-ml. capacity. Add 2 ml. of 10 per cent hydroxylammonium chloride solution. Add sufficient ammonium acetate solution to bring the volume to about 25 ml. The pH of the solution at this point should be about 7. Add 1 ml. of 0.01 M bathocuproine solution in n-hexyl alcohol. Add 5 ml. of n-hexyl alcohol and shake vigorously for 2 minutes. The orange color of bisbathocuproine copper(I) forms immediately. Allow the liquids to separate. After 5 minutes draw off the lower, aqueous layer and transfer the n-hexyl alcohol layer to a 10-ml. volumetric flask. Dilute to the mark and mix thoroughly. Measure the absorbance of the solution at 479 nm. Obtain the amount of copper present from a calibration curve prepared by running known amounts of copper through the same procedure.

Alternatively, the extraction step may be omitted and the photometric measurement made on the aqueous solution. A solution of bathocuproine in ethyl alcohol rather than in n-hexyl alcohol must then be used. Make the measurement at the same wave length, 479 nm but use a calibration curve prepared under the same conditions because the molar absorptivity is slightly lower in water than in n-hexyl alcohol.

Procedure for the Determination of Copper in Iron (SMITH AND WILKINS[1]). Weigh accurately a sample of such size as to contain from 0.06 to 2 mg. of copper. Dissolve the sample in hydrochloric acid and evaporate to a volume of a few milliliters. Add 1.5 g. of citric acid. Neutralize with ammonia to a pH of approximately 5, employing the green color of ferric citrate as indicator. Transfer the solution to a separatory funnel of 60- or 100-ml. capacity. Add 1 ml. of 0.01 M bathocuproine in n-hexyl alcohol and 5 ml. of 10 per cent hydroxylammonium chloride solution. Shake the solution for two minutes and then allow the phases to separate. Draw off the lower aqueous layer. Wash the n-hexyl alcohol layer with 5 ml. of ammonium acetate solution and 1 ml. of hydroxylammonium chloride solution. Draw off the aqueous layer and transfer the alcohol layer quantitatively to a 25-ml. volumetric flask. Dilute to 25.00 ml. with n-hexyl alcohol. Measure the absorbance at 479 nm. Obtain the amount of copper present from a calibration curve obtained by running known amounts of copper through the same procedure.

Procedure for the Determination of Copper in Iron (KAMMORI, SHIRAI, AND OKUBO[5]). Dissolve an accurately weighed 0.1-g. sample in 10 ml. of dilute hydrochloric acid (1:1 containing a few drops of hydrogen peroxide (30% by vol.). Cool and add 15 ml. of ammonium citrate (20% by wt.). Adjust the pH to approximately 6 with ammonium hydroxide. Add 2 ml. of bathocuproine solution (0.1 g. in 100 ml. of ethyl alcohol) and 2 ml. of hydroxylamine hydrochloride solution (10 g. in 100 ml. of water). After standing for 5 minutes, shake the solution with 5.00 ml. of chloroform for about 2 minutes. Measure the absorbance of the organic layer at 477 nm against chloroform.

Determination of Copper in Pulp, Paper and Pulping Liquors (BORCHARDT AND BUTLER[3]). Wet Digestion. Weigh accurately a sample of appropriate size (1 g. in the case of pulp and paper, 10 ml. of pulping liquors) and transfer the sample to a 100-ml. Kjeldahl or conical flask equipped with a reflux still head. Add 20 ml. of nitric acid, 5 ml. of 72 per cent perchloric acid, and a few Carborundum boiling chips. Warm the solution over a burner until most of the carbonaceous matter has been oxidized, and continue heating until all nitric acid has been removed and strong fumes of perchloric acid are evolved. Cool the solution and transfer to a 100 ml. separatory funnel. Pipet the following reagents into the solution in the order given: 2 ml. of 10 per cent hydroxylammonium chloride solution, 1.00 ml. of 0.01 M bathocuproine in n-hexyl alcohol, and 5.00 ml. of n-hexyl alcohol. Shake the solution for 2 minutes and then allow the layers to separate for 5 minutes. Discard the aqueous layer and transfer the n-hexyl alcohol solution with a pipet to the absorption cell. Measure the absorbance of the solution at a wave length of 479 nm against a reference solution carried through the digestion and color development steps.

NOTE. In the procedure of Borchardt and Butler[3] the n-hexyl alcohol is pipetted accurately into the separatory funnel and later the n-hexyl alcohol containing the colored copper-bathocuproine compound is pipetted from the funnel to the absorption cell. The procedure can also be carried out by transferring

the n-hexyl alcohol layer to a volumetric flask and making a second extraction with n-hexyl alcohol, the second portion acting as a wash. Dilution to volume is made with ethyl alcohol which dissolves any droplets of water accompanying the extracts.

Procedure for the Direct Determination of Copper in Wine (BANICK AND SMITH[4]). In the following procedure three solutions are carried along simultaneously: (a) a solution, containing the wine sample, on which the analysis is actually made, (b) a solution, containing the wine but to which no bathocuproine is added, which serves as a natural color blank to counteract the effect of any color originally present in the wine extracted by isoamyl alcohol (Note 1, below), and (c) a solution, containing all the reagents but no wine, which acts as a blank on the water and chemicals used.

Label separatory funnels of 60- or 100-ml. capacity (a), (b), and (c). In (a) and (b) place equal samples of the wine, conveniently a volume of 5.00 ml. To (a) and (c) add 5.00 ml. of a standard copper solution containing 5.00 μg. of copper per ml.

To each of (a), (b), and (c) add the following reagents in the order given: 5 ml. of sodium acetate solution, pH 4.0; 2.0 ml. of 10 per cent hydroxylammonium chloride; 1.0 ml. of 0.01 M bathocuproine in ethyl alcohol (a) and (c) only, sufficient water to bring the volume to about 20 ml.; 6 ml. of isoamyl alcohol. Shake each solution for 2 minutes and allow the layers to separate. Draw off the lower aqueous layer. Add 5 ml. of buffer of pH 7.1 and 5 ml. of water (Note 2). Shake for 1 minute. Allow 5 minutes for the layers to separate. Draw off the lower, aqueous layer and discard. Transfer the alcohol layer quantitatively to a 10-ml. volumetric flask. Dilute to the mark with ethyl alcohol. Measure the absorbance at a wave length of 479 nm of solution (a) with solution (b) in the reference cell. Measure the absorbance of solution (c) at the same wave length with water in the reference cell. Obtain the amount of copper in (a) and (c) from a calibration curve obtained by carrying known amounts of copper through the procedure. Subtract from the amount of copper found in (a) the copper found in the blank (c) and also the amount of copper present in the 5.00 ml. of standard copper solution added.

NOTE 1. Omission of a natural color blank (solution (b) in the above procedure) results in an error of about 1 per cent with most wines and an error of approximately 5 per cent with a red Burgundy wine.

NOTE 2. The extraction of copper bathocuproine from a wine is difficult if a buffer of pH 7.1 is used, owing to the formation of a black precipitate which gets into the isoamyl alcohol and interferes in the separation of the liquid phases and with the spectrophotometric measurement. The black precipitate does not form at pH 4.0. The treatment of the isoamyl alcohol extract with buffer of pH 7.1 is to insure that all of the copper is converted to the proper, colored compound.

Procedure for the Determination of Copper in Wine. Wet Ashing Procedure. (BANICK AND SMITH[4]). Transfer a 5-ml. sample of wine to a 250-ml., conical, Vycor flask containing 10 ml. of concentrated nitric acid and 5 ml. of 70 per cent perchloric acid . After the initial reaction has subsided, insert a refluxing still head . Place the flask on a hot plate, either in a hood or on a laboratory bench if a fume eradicator (*Anal. Chim. Acta*, 8, 400 (1953)) is used so that fumes are drawn off through a water aspirator. Apply a medium heat. Continue heating until all nitric acid has been expelled and dense fumes of perchloric acid have been evolved. Remove the flask from the hot plate, and cool under a stream of running water. Rinse and remove the refluxing still head and transfer the contents of the flask quantitatively to a 100-ml. beaker. Add 2 ml. of concentrated sulfuric acid. Cover the beaker with a cover glass supported by glass hooks. Heat in a fume hood and continue the evaporation to moist dryness. Add 3 drops of concentrated hydrochloric acid and 2 ml. of water. Heat gently to promote complete solution.

Transfer the solution quantitatively to a 60-ml. glass-stoppered separatory funnel. Add 5 ml. of ammonium acetate solution (pH 7.1). Pipet into the solution exactly 5 ml. of the standard copper solution containing 5.00 μg Cu per ml. Add 2 ml. of 10 per cent hydroxylammonium chloride and 1 ml. of 0.01 M bathocuproine. Add water to bring the volume to about 20 ml. Add 6 ml. of isoamyl alcohol. Shake for 1 minute and allow the two liquids to separate. Draw off the lower, aqueous layer and transfer the alcohol layer quantitatively into a 10-ml. volumetric flask. Dilute to the mark with ethyl alcohol. Measure

the absorbance at a wave length of 479 nm. Obtain the amount of copper from a calibration curve prepared by carrying known amounts of copper through the procedure of this paragraph. Subtract the amount of copper in the 5.00 ml. of standard copper solution added above. Preferably suitable blanks should be carried along with the determination.

SECTION IV. BIBLIOGRAPHY. BATHOCUPROINE

(2,9-Dimethyl-4,7-diphenyl-1,10-phenanthroline)

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4. W. M. Banick and G. Frederick Smith, *Anal. Chim. Acta*, **16**, 464 (1957).	The in situ determination of iron and copper in wine.
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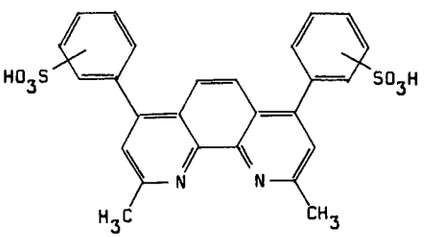
6. G. A. Nowlan, *U.S. Geol. Surv. Profess. Paper*, **525-D**, 189 (1966).	Determination of copper in soils, sediments, and rocks.
7. B. Zak and N. Ressler, *Clin. Chem.*, **4**, 43 (1958).	Determination of copper and iron in serum.
8. J. A. O'Leary, G. S. Novalis, and G. J. Vosburgh, *Obstet. Gynecol.*, **28**, 112 (1966).	Determination of copper in maternal serum.

SECTION V

BATHOCUPROINEDISULFONIC ACID

2,9-Dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonic Acid



$C_{26}H_{18}N_2(SO_3H)_2$
Mol. Wt.: 520.58

$C_{26}H_{18}N_2(SO_3Na)_2$
Mol. Wt.: 564.55

Molar Absorptivity of cuprous complex 12,250 at 483 nm.

G. FREDERICK SMITH CHEMICAL COMPANY, Catalog No. 294

On treating bathocuproine with chlorosulfonic acid, Zak and coworkers[1,2,3] obtained a sulfonated water-soluble derivative that retained its sensitivity and specificity for copper. The simple procedure of sulfonating bathocuproine greatly simplifies its application to aqueous systems. Addition of alcohol or an extraction step to avoid turbidity problems arising from the low solubility of excess reagent in water is obviated, and the number of possibilities for analyzing various matrices for copper is increased. To demonstrate its utility, Zak employed the sulfonated derivative to determine copper in serum[2].

Isolation of the disodium salt of bathocuproinedisulfonic acid in solid form was reported by Blair and Diehl[4]. They also extended the study of its use as a copper reagent by investigating possible interferences and by applying it to the determination of copper in dry yeast and in the presence of perchlorate ion.

The molar absorptivity of the orange cuprous derivative of sulfonated bathocuproine is 12,250 at 483 nm, the wavelength of maximum absorbance. Complete formation of the copper(I) complex occurs over the pH range 3 to 11. A combining ratio of 2.28 ligands per copper ion was found by Blair and Diehl[4], who concluded that the complex was a bischelate, attributing the disparity from the theoretical combining ratio of 2 ligands per copper to impurities.

Properties of Bathocuproinedisulfonic Acid. Neutralization of the acidic derivative occurs in two steps, corresponding to a dibasic acid with dissociation constants of $pK_1 = 2.65$ and $pK_2 = 5.80$[4]. The structure of the sulfonated derivative, although not precisely determined, is known to involve sulfonation in the phenyl groups[5]. The sodium salt is obtained as a tan powder. It is very soluble in water and is slightly hygroscopic.

Specificity and Interferences. The effects of various ions on the determination of copper were studied by Blair and Diehl[4]. A relative error greater than 2% in the determination of 2.41 p.p.m. of copper was caused by the presence of the following: 29 p.p.m. of chromium (added as $K_2Cr_2O_7$), 555 p.p.m. of UO_2^{+2}, 975 p.p.m. of thiocyanate, 1330 p.p.m. of thiosulfate, or

1570 p.p.m. of persulfate. Cyanide interfered quantitatively. Precipitates were given by 90 p.p.m. of aluminum and by 350 p.p.m. of tin(IV). No significant interference was found for iron(II), cobalt(II), or nickel(II) when each was present separately at 6 p.p.m.

Applications to the Determination of Copper. The first application of sulfonated bathocuproine was made by Zak and coworkers to determine copper in serum[1,2,3]. Blair and Diehl[4] determined copper in dry yeast after preliminary treatment with sulfuric acid followed by wet oxidation with a mixture of nitric and perchloric acids. Poillon and Dawson[6] employed the disodium salt in a study to ascertain the nature and valence states of prosthetic copper in the enzyme ascorbate oxidase. In another study[7], they used it to elucidate the role of prosthetic copper during ascorbate oxidation.

General Procedure for the Determination of Copper. REAGENTS. SULFONATED BATHOCUPROINE SOLUTION. Dissolve 0.1 g. of bathocuproinedisulfonic acid disodium salt ⊖ in 100 ml. of distilled water. OTHER REAGENTS. Refer to Section IV on bathocuproine for descriptions.

PROCEDURE. Transfer an accurately measured aqueous sample, containing 10 to 200 μg. of copper, to a 25-ml. volumetric flask. Add 2 ml. of 10% hydroxylammonium chloride, ⊖ 5 ml. of the sulfonated bathocuproine solution, and 5 ml. of 10% ammonium acetate ⊖ solution. Dilute to the calibration mark and mix. Measure the absorbance at 483 nm. Refer to a calibration curve prepared using known amounts of copper in the same procedure to convert absorbance to concentration or amount of copper.

Determination of Copper in Serum (ZAK[2]). Pipet 3 ml. of serum into a centrifuge tube, add 1 ml. of 1.4 M hydrochloric acid and heat in a near boiling water bath for 5 minutes. Cool, add 2 ml. of 10% trichloracetic acid, mix well and centrifuge at high speed for 15 minutes. Pipet 4 ml. of the clear filtrate into a small flask. Add and dissolve 10-15 mg. (a small spatula tipful) of solid ascorbic acid (amount not critical). Add by pipet 0.5 ml. of sulfonated bathocuproine solution (0.1 g. of bathocuproinedisulfonic acid disodium salt in 100 ml. of saturated sodium acetate). Determine the absorbance of the yellow complex at 485 nm against a blank prepared by substituting 3 ml. of metal-free water for the serum. Refer to a previously made calibration curve to determine the copper content from the measured absorbance.

Determination of Copper in Dry Yeast (BLAIR AND DIEHL[4]). Transfer a weighed sample of about 2 g. of the yeast to a 250-ml. conical flask. Add 5 ml. of concentrated sulfuric acid and insert a reflux head in the neck of the flask. Carry along simultaneously a blank, starting with sulfuric acid. Char the yeast by heating for 15 minutes, cool, and add 20 ml. of a mixture of equal volumes of 70% nitric acid and 72% perchloric acid. Replace the reflux head and boil the mixture in such a fashion that the water and nitric acid are expelled in about 15 minutes and perchloric acid begins to condense on the walls of the flask. Continue to reflux smoothly, without undue escape of perchloric acid for 10 minutes. Cool the mixture, remove the reflux head, and wash it and the inside of the flask with approximately 30 ml. of distilled water. Add 5 ml. of a solution 10% in hydroxylamine hydrochloride and 2% in citric acid. Next add 5 ml. of a 0.1% solution of disodium bathocuproine disulfonate. Adjust the pH to 4-5 with ammonium hydroxide. Dilute to exactly 100 ml. and measure the absorbance at 483 nm against the blank. Refer to a suitably prepared calibration curve to convert absorbance to copper content.

SECTION V. BIBLIOGRAPHY
BATHOCUPROINEDISULFONIC ACID

(2,9-Dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonic Acid)

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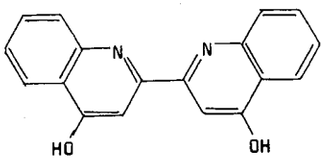
SECTION VI.

4,4′-DIHYDROXY-2,2′-BIQUINOLINE



$C_{18}H_{12}N_2O_2$

Mol. Wt.: 292.29

Molar absorptivity of $Cu(C_{18}H_{12}N_2O_2)_2^+$ in isoamyl alcohol: 6900 at 525 nm

G. FREDERICK SMITH CHEMICAL COMPANY, Catalog No. 549

NOTE: At press time this material is available in limited quantities only. It should become more readily available as experience is gained in the synthesis.

Closely related to cuproine and neocuproine and equally specific for copper, 4,4′-dihydroxy-2,2′-biquinoline has the unique ability of forming a highly stable, extractable copper(I) complex in strongly alkaline solutions. It affords the distinct advantage of enabling the direct determination of copper in strong bases without necessity of neutralization of the alkalinity and pH adjustment. Extractability into isoamyl alcohol of the purple copper(I) complex provides a significant preconcentration step that enhances the sensitivity of the copper determination. This outstanding reagent was literally custom synthesized by Case and Lesser[1] for the intended purpose of chelating copper in alkaline solution. A study of its properties and application to the determination of copper was reported by Schilt and Hoyle[2].

Absorption spectra of the copper(I) complex exhibit some small dependence on hydroxide ion concentration from 1 to 5 M but are essentially constant from 5 to 8 M hydroxide. When extracted into isoamyl alcohol from 7 to 12 M sodium hydroxide solutions, the copper(I) complex exhibits a constant wavelength of maximum absorbance at 525 nm and molar absorptivity of 6,900. Beer's law is followed up to a concentration of at least 10 p.p.m. of copper. No change in absorbance occurs on exposure to air for one hour, provided that an excess of hydroxylamine hydrochloride reductant is added to the extracted solutions. In stoppered flasks the color is stable for several weeks.

Specificity and Interferences. The effects of various ions on the determination of copper were studied by Schilt and Hoyle[2] who found that no metal ion other than cuprous formed a colored complex extractable into isoamyl alcohol. Of the ions studied only cyanide interfered seriously. Up to 100 p.p.m. of thiocyanate is tolerated. Sodium tartrate does not interfere and can be used as a masking reagent for iron and other heavy metal ions. Barium, strontium, manganese(II), oxalate, and fluoride ions precipitate from concentrated sodium hydroxide and do not interfere.

Applications. The reagent was applied by Schilt and Hoyle[2] to the determination of copper in sodium hydroxide, sodium metal and potassium metal. A relative standard deviation of 1.08% was obtained for 14 replicate determinations of 3.19 p.p.m. of copper in 12 M sodium hydroxide solutions. The general method should be suitable also for silicate and other refractory solids that yield water soluble products following sodium hydroxide or sodium carbonate fusions.

General Procedure for the Determination of Copper in Strong Bases (SCHILT AND HOYLE[2]). Weigh or measure a sample for analysis of sufficient size so that the hydroxide ion concentration of the final solution will be 7 to 12 M. If necessary, solid sodium hydroxide or saturated sodium hydroxide solution (19M) can be added along with the sample to attain the requisite hydroxide ion concentration. Transfer the measured sample to a separatory funnel, and add 2 ml. of 10% hydroxylamine hydrochloride ⊖ and 2 ml. of 0.004 M 4,4′-dihydroxy-2,2′-biquinoline ⊖ (0.12 g. of compound treated with 3 drops of 6M sodium hydroxide followed by 100 ml. of ethyl alcohol). Extract once with 5 ml. and again with 1 ml. of isoamyl alcohol. ⊖ Combine the extracts in a 10-ml. volumetric flask, add 1 ml. of 10% hydroxylamine hydrochloride in 1:1 water-ethanol solution, dilute to volume with ethyl alcohol, and measure the absorbance against a similarly prepared blank at 525 nm. Refer to a suitably prepared calibration curve to convert absorbance to copper content.

The extraction step can be omitted if high sensitivity is not necessary or if a simpler procedure is preferred. In this event, proceed as follows: Pipet a sample (6-8M in hydroxide) containing 5-100 μg. of copper into a 10-ml. volumetric flask. Add 1 ml. of 10% hydroxylamine hydrochloride solution, 2 ml. of 0.004M solution of the hydroxy cuproine reagent, and dilute to volume with 6M sodium hydroxide. Measure the absorbance at 528 nm against a similarly prepared blank.

Determination of Copper in Sodium Metal. Wash the sodium metal with anhydrous ether, and carefully slice off the oxide layer. Accurately weigh a clean 9-gram sample, and carefully dissolve it in 100 ml. of methanol. Evaporate the solution on a hot plate to a thick paste, carefully and slowly add 25 ml. of distilled water, and heat to boiling to drive off the remaining alcohol. Transfer the solution quantitatively to a 50-ml. volumetric flask, and dilute to volume with distilled water. Complete the determination using an aliquot of suitable size and the above recommended procedure.

Determination of Copper in Potassium Metal. Follow the above procedure as for determining copper in sodium metal, except with the following important modification: dissolve an accurately weighed 16- to 17-gram sample of the potassium in 100 ml of methanol, kept at 0°C in an ice bath, by carefully adding pea sized chunks one at a time to the cold methanol.

SECTION VI BIBLIOGRAPHY

(4,4′-Dihydroxy-2,2′-Biquinoline)

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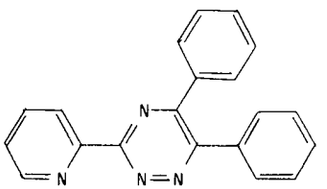
SECTION VII

PDT

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



$C_{20}H_{14}N_4$

Mol. Wt.: 310.34

Molar absorptivity of

$Cu(PDT)_2^{+}$ 7,990 at 488 nm

$Fe(PDT)_3^{+2}$ 23,500 at 555 nm

G. Frederick Smith Chemical Company, Catalog No. 516

An outstanding reagent for the rapid, simultaneous determination of copper and iron, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine is conveniently referred to in abbreviated fashion as PDT[1]. This reagent and its sulfonated derivative are extremely sensitive as iron chromogens[2,3,4]. PDT serves best in procedures requiring separation or pre-concentration of iron, because it forms a highly extractable iron(II) chelate[2]. The disodium salt of disulfonated PDT is readily soluble and forms a highly water soluble iron(II) chelate with a molar absorptivity of 27,900 at 562 nm[3]. Because it is free of solubility problems, the sulfonated PDT is particularly adaptable to automated procedures[3]. As yet, it has not been characterized as a copper reagent, so it will not be discussed further here.

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

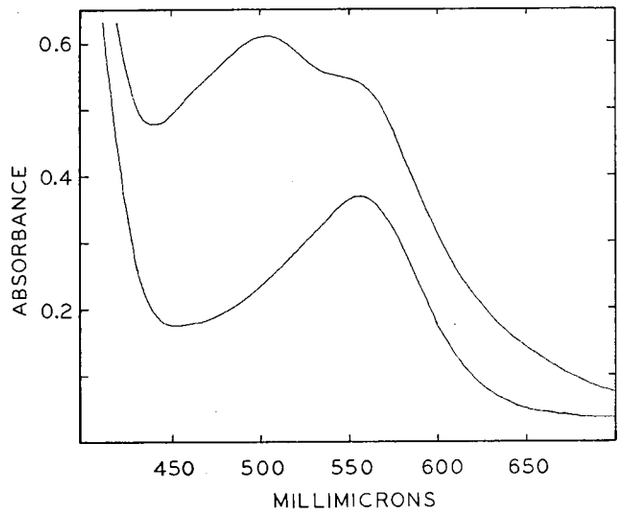


Figure 4. Absorption spectrum of a solution of iron(II) and copper(I) PDT complexes in isoamyl alcohol vs. air before (upper curve) and after addition of sodium cyanide (lower curve). Iron(II) and copper(I) concentrations are 1.37×10^{-4} and 4.48×10^{-4}M, respectively.

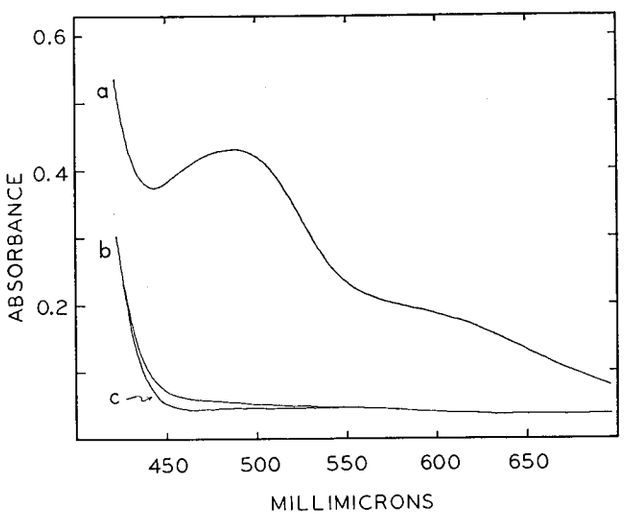


Figure 5. Absorption spectrum of 4.84×10^{-4}M copper(I)—PDT in isoamyl alcohol vs air, curve a; after addition of sodium cyanide, curve b; spectrum of reagent blank, curve c.

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

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

Applications. PDT has been applied to the simultaneous determination of copper and iron in water, milk, and wine[1]. Numerous other applications appear promising, especially in view of the demand for suitable methods for determining trace amounts of both iron and copper in foods, beverages, biological specimens, and various commercial products such as paper, petroleum and alloys. The presence of these metals can be of vital importance to physiological processes, their levels may be of considerable significance in medical diagnosis and biochemical research, they are known to promote off-flavors in foods and beverages, and their presence in lubricating oils or other commercial products is indicative of wear, malfunction or contamination.

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

RECOMMENDED PROCEDURE. Pipet sample of sufficient size to contain 1-25 μg. of Fe and/or 4-80 μg. of Cu into separatory funnel and add 5 ml. of pH 4.5 buffer, 2 ml. of 10% hydroxylamine hydrochloride ⊖, 2 ml. of 0.01 M PDT, and 2 ml. of 50% $NaClO_4$ solution. Adjust the pH to 4.5 ±1, if necessary, using pH indicating paper. Extract once with 6 ml. and again with 2 ml. of isoamyl alcohol. Combine the isoamyl alcohol extracts in a 10-ml. volumetric flask, and dilute to volume with 0.5% ascorbic acid in ethanol. Measure the absorbance of the solution vs. a similarly prepared blank at 488 nm; add 5-10 mg. of sodium cyanide to the isoamyl alcohol solution, and measure the absorbances at 488 and 555 nm vs. the similarly treated blank. Make use of suitably prepared calibration curves or empirical equations to convert the loss in absorbance at 488 nm to concentration of Cu and the final absorbance at 555 nm to concentration of Fe.

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

Procedures for the Analysis of Wine. DRY ASHING PROCEDURE. Pipet 2.00 ml. of wine into a crucible (determine density if sample weight is desired), evaporate to dryness, and ignite at 450-500°C to a gray ash. Allow to cool, add

1 ml. of concentrated nitric acid ⊖, evaporate, and ignite for 1 hr. at 450-500°C. Dissolve the cool, white ash in 10 ml. of 1M nitric acid, and transfer the solution quantitatively into a 60-ml. separatory funnel. Complete the analysis following the recommended procedure.

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

SECTION VII BIBLIOGRAPHY

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

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SECTION VIII

APPENDIX

G.F.S. Reagents for the Determination of Copper — **Catalog No.**