Spectrophotometric Determination of Ultramicro Amounts of Copper with 1,5-Diphenylcarbohydrazide

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▶ Efforts to obtain a more sensitive reagent for determination of copper in biological materials showed that 1,5-diphenylcarbohydrazide gives an extremely sensitive color reaction with copper in basic solution. The spectrophotometric sensitivity of this reagent is 0.0004 γ of copper per sq. cm., and its molar absorbance index is 158,800 at 495 mμ. The color reaction follows Beer's law over the concentration range from 0.01 to 0.26 p.p.m. As the rate of the reaction is dependent upon pH, the copper solution must be buffered precisely. Of the foreign ions investigated, only chromium(III) interfered at the level of concentration of the copper. The ethanolic solution of the reagent is stable for approximately 1 hour.

A SENSITIVE colorimetric reagent that has long been used for the determination of chromate in weakly acidic solution is 1,5-diphenylcarbohydrazide (*sym*-diphenylcarbazide) (*2–4, 14*). This reagent has also been used to detect molybdate and mercury(I) and (II) ions in acidic solution (*5, 6*). In basic solution 1,5-diphenylcarbohydrazide is a highly sensitive colorimetric reagent for copper. This paper describes a spectrophotometric method that can be used to determine as little as 0.005 p.p.m. of copper(II) ion with the reagent.

The level of sensitivity exhibited by 1,5-diphenylcarbohydrazide makes it superior in this respect to any colorimetric copper reagent currently known. Consequently, it would seem that 1,5-diphenylcarbohydrazide is particularly well suited to the determination of the trace amounts of copper in biological materials. Diethyl dithiocarbamate, dithizone, and bicyclohexanoneoxalyldihydrazone, the usual reagents for this purpose, are inadequate in most cases. Diethyl dithiocarbamate, in addition to lacking the desired sensitivity, forms interfering colors with iron and nickel. Dithizone itself is colored, reacts with many metals, and requires a complex extraction process over a narrow pH range if it is to be used selectively. Bicyclohexanoneoxalyldihydrazone gives inaccurate results below a concentration of 0.7 p.p.m. Table I compares the sensitivity of 1,5-diphenylcarbohydrazide with that of other compounds frequently used for the microdetermination of copper.

MECHANISM OF REACTION

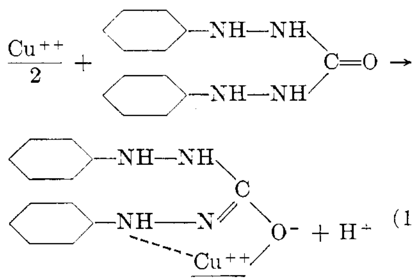
Caseneuve (*2–4*) in 1900 first observed that 1,5-diphenylcarbohydrazide gave a violet color with semimicro amounts of copper(II) ion in weakly acidic solution. He proposed that the active reagent in this reaction and in the reaction with chromate ion was the oxidation product of 1,5-diphenylcarbohydrazide, the diphenylcarbazone. Feigl and Lederer (*6*) later proposed that Caseneuve's reaction with copper yielded an inner metallic complex salt, with the copper attached at the two β-nitrogen positions.

The authors have found that in alkaline solution the 1,5-diphenylcarbohydrazide reacts with copper(II) ion at concentrations of about 0.10 p.p.m. to give an intense orange color. At higher copper concentrations (about 5 p.p.m.) the color changes to a red-violet. It was thought at first that this color might be the result of an oxidation reaction. However, the failure of 1,5-diphenylcarbohydrazide to give this color when oxidized in basic solution with either hydrogen peroxide or sodium hypochlorite indicated that a copper chelate was being formed.

In further experiments pure diphenylcarbazone and oxidized 1,5-diphenylcarbohydrazide failed to give a color reaction with copper(II) ion in basic solution, indicating that the 1,5-diphenylcarbohydrazide is the active chelating reagent here, and not the diphenylcarbazone.

The continuous variations technique, applied to determine the mole ratio with which the 1,5-diphenylcarbohydrazide combines with copper, showed that the ratio was 2 to 1.

If the copper(II) ion were bonded to the nitrogen atoms of the 1,5-diphenylcarbohydrazide, the chelate would be positively charged. On the other hand, if the copper were being bonded to the oxygen of the enol form of the reagent, the chelate would be neutral. These are the only two possibilities for a stable five- or six-membered ring. "Moving boundary" experiments carried out by the authors revealed that the 1,5-diphenylcarbohydrazide–copper chelate in basic solution is uncharged. Therefore, the following reaction is proposed:



(1)

Further support for the above structure is found in the fact that the rate of the color reaction is dependent on the hydronium ion concentration, increasing at pH values above 7 (Figure 2). This could be interpreted as indicating the necessity of the loss of the α-hydrogen before chelation could occur.

REAGENTS AND APPARATUS

Triple-Distilled Water. Because the double-distilled water available in the laboratory was not sufficiently pure, it was redistilled in borosilicate glass stills. It has been reported that water passed through ion exchange resins is not sufficiently pure for reactions of this sensitivity, so this method of purification was not used.

Buffer Solutions. Clark and Lubs buffer solutions within the pH range from 8.0 to 9.6 were prepared in triple-distilled water.

Solutions of Various Ions. Reagent grade chemicals were used to prepare aqueous solutions of the various ions.

Standard Copper Solutions. A stock standard solution (500 γ per ml.) was prepared by dissolving 1.9640 grams of copper sulfate pentahydrate in triple-distilled water and diluting to 1000 ml. From this stock solution diluted standards containing 0.01 to 0.26 γ per ml. were prepared.

1,5 - Diphenylcarbohydrazide Reagent. A 0.1% solution of 1,5-diphenylcarbohydrazide (Eastman Kodak Co., Rochester, N. Y.) was prepared in redistilled 95% ethyl alcohol.

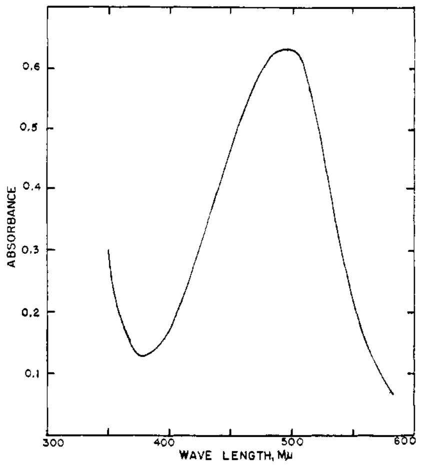


Figure 1. Absorption curve for 1,5-diphenylcarbohydrazide–copper chelate

Obtained with 0.26 p.p.m. of copper and 0.1% reagent solution; pH, 9.6; 5-minute color development

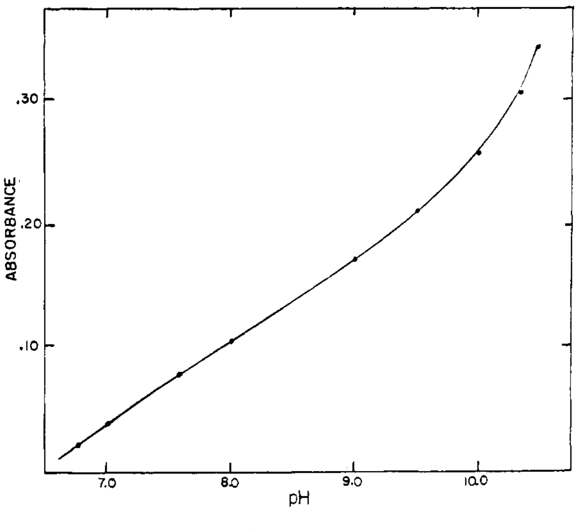


Figure 2. Effect of pH on color development

Glassware. Borosilicate glassware was used throughout the experiment. The glassware was washed with hydrochloric acid and rinsed thoroughly with triple-distilled water after each use.

Spectrophotometer. A Beckman Model DU spectrophotometer with 1-cm. Corex absorption cells was employed.

PROCEDURE

The following procedure gave optimal color development and was adopted for use in all determinations.

A 1-ml. aliquot of the copper solution containing 0.01 to 0.26 γ was added to a 4-ml. cuvette and buffered at pH 9.0 with 1 ml. of the buffering solution. This solution was diluted to 3 ml., 0.2 ml. of the reagent solution was added, and the solution was thoroughly stirred. The absorbance was then read between 4 and 7 minutes at 495 mμ. Because some copper ion was always found to be present, reagent blanks had to be carried through the procedure in all determinations.

EXPERIMENTAL

Reagent Stability. Because of air oxidation, the ethanolic reagent solution was unsuitable for analytical purposes if allowed to stand for longer than 1 hour. The reagent can be preserved for somewhat longer periods if precautions are taken to isolate it from the air. Allen (*1*) gives information on the quality and shelf life of the reagent.

Color Reaction. SENSITIVITY AND CONFORMITY TO BEER'S LAW. The colored 1,5-diphenylcarbohydrazide–copper chelate in aqueous solution (pH 9.0) has a molar absorbance index of approximately 158,800 when read at 5 minutes and at its point of maximum absorbance, 495 mμ (Figure 1). The spectrophotometric sensitivity of the reagent, in micrograms per square centimeter as defined by Sandell (*13*), is 0.0004. The copper(II) complex in aqueous solution conforms to Beer's law over the concentration range of 0.01 p.p.m. (A_s = 0.010) to 0.26 p.p.m. (A_s = 0.200). This is also the optimal concentration range as shown by the method of Ringbom (*11*).

EFFECT OF pH. The rate of the color reaction is dependent upon the pH of the solution (Figure 2). Although the slope of the pH curve is a relatively steep one, the pH of the reaction could be fixed precisely with the Clark and Lubs buffer of sodium hydroxide and boric acid–potassium chloride. The largest deviation in pH encountered while using this buffer was ±0.01 pH unit, which corresponds to an error of ±2% in the determination. The most accurate results were obtained in the pH range from 8.0 to 9.5.

RATE OF COLOR DEVELOPMENT. It is particularly significant that the color development reaction follows Beer's law for only 8 minutes. During this time there is a gradual but constant increase in color. Beyond 8 minutes the color intensity continues to increase, but the reaction becomes erratic.

The reaction rate may be accelerated by heating above room temperature. Figure 3 illustrates how the reaction rate varies between 4° and 37° C. All readings for this graph were taken 5 minutes after the addition of the reagent.

REAGENT CONCENTRATION. As long as all of the copper ion in solution is complexed with the reagent, no significant color change occurs with the addition of small amounts of excess reagent. However, large excesses of reagent accelerate the rate of reaction to the point of inducing appreciable

Table I. Relative Sensitivities of Chromogenic Reagents for Microdetermination of Copper

	Analytical Wave Length, Mμ	Molar Absorbance Index	Literature Reference
Diethyl dithiocarbamate (aqueous)	440	8,000	(*10*)
Diethyl dithiocarbamate (amyl alcohol)	440	12,700	(*9, 10*)
2,2'-Biquinoline	540	5,490	(*7*)
2,9-Dimethyl-1,10-phenanthroline	454	7,950	(*15*)
4,7-Diphenyl-1,10-phenanthroline	420	12,140	(*16*)
2,9-Dimethyl-4,7-diphenyl-1,10-phenanthroline	480	14,100	(*17*)
Bicyclohexanoneoxalyldihydrazone	595	16,000	(*10*)
Oxalyldihydrazide	542	22,000	(*18*)
2-Carboxy-2'-hydroxy-5'-sulfoformazylbenzene (Zincon)	600	22,500	(*12*)
Dithizone	508	24,600	(*8*)
1,5-Diphenylcarbohydrazide	495	158,800	

error in the 4- to 7-minute readings. The optimum concentration of the reagent for the amounts of copper ion being determined was 0.2 ml. of 0.1% solution. Doubling or tripling this reagent concentration increased the reaction rate by as much as 15 to 20%.

Effect of Foreign Ions. Table II lists the effect of various anions and cations upon the rate or extent of color development. In determining interferences, aqueous solutions of the indicated concentrations were added to buffered solutions containing 0.1 p.p.m. of copper-(II) ion. An interference was defined for the purposes of this work as ±6% deviation from the value for the copper sulfate standard solution.

None of the ions tested gave a positive interference. In the few cases where a precipitate formed after color development, it was removed by centrifugation before making the spectrophotometric analysis. The only ion which interfered at the level of the copper concentration was chromium(III). The interference caused by the formation of precipitates of several of the metal hydroxides [iron(III), manganese(II), cadmium-(II)] could be prevented by the addition of citrate at 500 p.p.m. Mercury-(II) ion could be effectively masked at 10 p.p.m. by the addition of iodide. Molybdenum could be masked at higher concentrations with oxalate.

Organic materials such as purines and pyrimidines inhibit the reaction. These must be destroyed with nitric and sulfuric acid before the spectrophotometric determination is carried out on biological materials.

Precision. Data were obtained on 0.1 γ of copper. The standard deviation on 20 replicate samples was 0.006 γ or 6%. This is an average deviation of 0.004 γ or 4%.

Solubility of Colored Complex. The 1,5-diphenylcarbohydrazide–copper complex is soluble in benzene and isoamyl alcohol, and these solvents may be used for extraction.

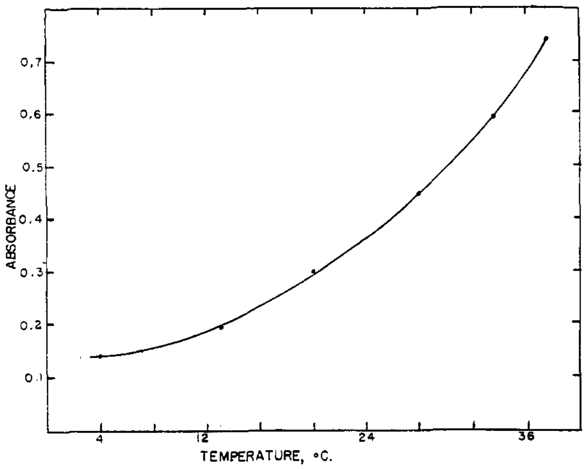


Figure 3. Effect of temperature on reaction rate

5-minute color development

Table II. Noninterfering Anions and Cations in Copper Determination with 1,5-Diphenylcarbohydrazide at 495 Mμ

Ion	Added As	Ion	Added As
1000 P.P.M.		500 P.P.M.	
F^-	NaF	Citrate	$Na_3C_6H_5O_7.2H_2O$
Cl^-	NaCl	$Cr_2O_7^{--}$	$K_2Cr_2O_7$
Br^-	NaBr	Li^+	LiCl
I^-	KI	Pb^{+++}	$Pb(NO_3)_2$
$B_4O_7^{--}$	$Na_2B_4O_7$	Ag^+	$AgNO_3$
NO_2^-	$NaNO_2$		
		100 P.P.M.	
NO_3^-	NH_4NO_3		
CrO_4^{--}	$(NH_4)_2CrO_4$	PO_4^{--}	$Na_3PO_4.12H_2O$
$Br_4O_7^{--}$	$Na_2Br_4O_7$	$P_2O_7^{-4}$	$Na_4P_2O_7.10H_2O$
WO_4^{--}	$Na_2WO_4.2H_2O$	$S_2O_3^{--}$	$Na_2SO_3.5H_2O$
CO_3^{--}	Na_2CO_3	Mg^{++}	$Mg(C_2H_3O_2)_2.4H_2O$
HCO_3^-	$NaHCO_3$	Al^{+++}	$Al(NO_3)_3.9H_2O$
AsO_4^{---}	$Na_2HAsO_4.7H_2O$	Zn^{++}	$ZnSO_4.7H_2O$
SO_4^{--}	Na_2SO_4	Sr^{++}	$SrCl_2$
SO_3^{--}	Na_2SO_3		
		10 P.P.M.	
$C_2H_3O_2^{--}$	$NaC_2H_3O_2.3H_2O$		
		MoO_4^{--}	$Na_2MoO_4.2H_2O$
$C_2O_4^{--}$	$NH_4C_2O_4$		
NH_4^+	$(NH_4)_2SO_4$		
		1 P.P.M.	
Na^+	NaCl	Fe^{+++}	$Fe(NO_3)_3.9H_2O$
K^+	KCl	Ce^{+4}	$Ce(SO_4)_2.4H_2O$
Ba^{++}	$Ba(C_2H_3O_2)_2.H_2O$		
		0.1 P.P.M.	
Ca^{++}	$CaCl_2.2H_2O$		
		Cd^{++}	$CdCl_2.2^1/_2H_2O$
		Mn^{++}	$MnSO_4.4H_2O$
		Co^{++}	$CoCl_2.6H_2O$
		Hg^{++}	$HgCl_2$

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