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A Method for the Determination of Copper in Biological Materials Involving the Use of Sodium Diethyldithiocarbamate

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The method described for determining copper is based on a complexation reaction effected in acetic acid solution. The acetic acid not only effects complexation but also dissolves the complex and no intermediate extraction is therefore necessary. The sensitivity is higher than that for other methods based on diethyldithiocarbamate. Various metal ions interfere in the determination of copper using diethyldithiocarbamate and the elimination of these interferences is discussed.

The application of the method to the determination of trace amounts of copper in various biological materials, particularly foodstuffs, is discussed.

Many colorimetric methods have been described for the determination of trace amounts of copper, and those based on bathocuproine (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline)[1] and neocuproine (2,9-dimethyl-1,10-phenanthroline)[2] are considered to be specific. Extraction with dithizone (diphenylthiocarbazone) in acidic solution is extremely sensitive but not specific. The most widely used methods, however, are still based on dithiocarbamates,[3–5] including zinc dibenzyldithiocarbamate.[6]

Diethyldithiocarbamate reacts with copper to give a yellow - brown suspension in slightly acidic or alkaline solution and, as this suspension is not suitable for use in direct determinations, it has been customary to form the copper complex in aqueous solution and to extract this complex with an immiscible solvent (chloroform and carbon tetrachloride are considered to be the most satisfactory solvents for this purpose).

Zinc, cadmium, indium, magnesium, silver, lead, iron, cobalt and nickel form white or coloured complexes with diethyldithiocarbamate, and methods have been devised for the elimination of these interferences by using preferential complexation with EDTA,[5] citrate,[4] etc.

The procedure described in this paper is based on the use of glacial acetic acid to effect complexation and to effect dissolution of the copper complex, giving clear and strongly coloured solutions. The molar extinction coefficient varies with the concentration of the acetic acid in solution, but the determination is carried out when the molar extinction coefficient has reached its optimum value. The drawbacks associated with the use of aqueous suspensions are thereby overcome and an alternative to the usual procedure in which solvent extraction is necessary has been developed. The sensitivity is higher than for other methods based on diethyldithiocarbamate and the reproducibility is considerably enhanced. Interference from various metal ions (at reasonable concentrations) is eliminated by the use of EDTA.

Experimental

Reagents—

All reagents were of analytical-reagent grade.

Glacial acetic acid.

Sodium diethyldithiocarbamate, $(C_2H_5)_2NCSSNa.3H_2O$—The reagent is added in the solid form. It dissolves in the acetic acid solution and eventually decomposes, but this decomposition does not interfere in the reaction.

Ethylenediaminetetraacetic acid, $C_{10}H_{14}O_8N_2.2H_2O$—This reagent is added in the solid form.

Stock standard copper solution—A 0·3929-g amount of copper(II) sulphate ($CuSO_4.5H_2O$) was dissolved in water and the volume made up to 1 litre in a standard flask. This solution contains 100 μg ml^{-1} of copper.

Working standard copper solutions—Working standards containing 2·5, 5·0, 7·5 and 10·0 μg ml^{-1} of copper were prepared by serially diluting the stock standard solution when required. These working standard solutions are, however, stable.

ABSORBANCE MEASUREMENTS—

The absorbance measurements were made with a Beckmann, Model DB, spectrophotometer using 1-cm cuvettes. The reference cuvette was filled with copper-free distilled water in every instance.

METHOD

PROCEDURE—

Transfer a 10-ml aliquot of the copper solution (free from mineral acid) by pipette into a 50-ml calibrated flask and add 2·5 ml of copper-free distilled water from a burette, then add 30 ml of glacial acetic acid and swirl the contents of the flask in order to mix them. Then add about 10 mg of sodium diethyldithiocarbamate and again swirl the solution. Allow the flask to stand for 30 minutes after making the volume of the liquid up to the mark with glacial acetic acid.

Read the absorbance at 430 nm in a 1-cm cuvette in the Beckmann spectrophotometer.

REAGENT BLANKS—

Reagent blanks presented little difficulty; diethyldithiocarbamate dissolves readily in the acetic acid medium.

The reproducibility of the reagent blank was studied together with the other experiments described under Reproducibility. The results that are relevant are as follows:

Average absorbance of reagent blank in 1-cm cuvette	Over-all standard deviation, absorbance units	Degrees of freedom
0·013	$0{\cdot}0008_2$	19

The over-all standard deviation is reported as there was no significant difference between the between-batch and the within-batch standard deviations at the 95 per cent. confidence level.

REPRODUCIBILITY—

Aliquots of 10 ml of each of the working standards containing 2·5, 5·0, 7·5 and 10·0 μg ml^{-1} of copper were taken in quadruplicate and subjected to the treatment described under Procedure. Reagent blanks were also prepared in quadruplicate, 10·0 ml of copper-free distilled water being used in place of the copper solutions.

Absorbance measurements were made and the results of analytical interest were obtained by subtracting the mean absorbance of the reagent blank from the absorbance of the copper solution. The procedure was repeated on five different occasions and the results were analysed statistically. The between-batch standard deviations were not significantly larger (at the 95 per cent. confidence level) than the within-batch standard deviations. The results were pooled so as to obtain the over-all standard deviations reported in Table I.

TABLE I

REPRODUCIBILITY OF THE METHOD

Amount of copper in 50-ml flask/μg	Mean absorbance reading corrected for absorbance of reagent blank	Over-all standard deviation, absorbance units	Degrees of freedom
25	0·150	$0{\cdot}0013_2$	10
50	0·298	$0{\cdot}0017_3$	19
75	0·446	$0{\cdot}0022_6$	19
100	0·596	$0{\cdot}0028_4$	19

The mean absorbance of the blank solutions was 0·013 with a standard deviation of $0{\cdot}0008_2$.

CALIBRATION GRAPH—

In studying the reproducibility, it was shown that the between-batch standard deviation was not significantly larger than the within-batch standard deviation. The mean absorbance of the twenty readings obtained at each level (corrected for the absorbance of the reagent blank) was therefore used in order to plot the calibration graph.

The calibration graph was linear and passed through the origin, and can be represented by the equation

$$y = 0{\cdot}596 \times 10^{-2}\, x$$

where y is the absorbance reading obtained under the conditions described under Procedure and x is the amount of copper in micrograms in the 50-ml flask.

INTERFERENCES—

Several metal ions interfere in the determination of copper using diethyldithiocarbamate. However, the following modification of the method, involving preferential complexation of the interfering ions with EDTA, eliminates the interfering effects of iron, nickel, cobalt, manganese and mercury.

Transfer by pipette a 10-ml aliquot of the copper solution containing the interfering metal ion and free from mineral acid into a 50-ml flask, then add 2·5 ml of copper-free distilled water from a burette followed by 25 ml of glacial acetic acid. Add 50 mg of EDTA and swirl the flask in order to mix the contents. Allow the flask to stand for 15 minutes, then add 10 mg of sodium diethyldithiocarbamate, swirl the flask and allow it to stand for 15 minutes. Filter the suspension, if any, into a 50-ml calibrated flask through a small Whatman No. 41 filter-paper and carefully wash the flask and the residue on the paper with small volumes of glacial acetic acid. Finally, make the solution up to the mark with glacial acetic acid and allow it to stand for 15 minutes before reading the absorbance. The results are reported in Table II.

TABLE II

INTERFERENCE OF METAL IONS IN THE DETERMINATION OF 25 μg OF COPPER

	Copper found/μg	
Metal ion (100 μg)	In the absence of EDTA	In the presence of EDTA
Fe^{2+}	36·4	25·1
Fe^{2+}	34·2	25·3
Ni^{2+}	32·6	24·8
Co^{2+}	35·8	24·8
Mn^{2+}	25·6	25·0
Pb^{2+}	25·8	25·2
Hg^{2+}	26·2	25·4

In addition to the interfering metal ions mentioned above, certain other common constituents of food and other biological materials were also studied and the results are reported in Table III.

TABLE III

EFFECT OF CONSTITUENTS OF BIOLOGICAL MATERIALS ON THE DETERMINATION OF 25 μg OF COPPER

Substance added (50 mg)	Copper found/μg
$CaCO_3$	24·8
$MgSO_4$	24·6
$Ca_3(PO_4)_2$	24·6
$Al_2(SO_4)_3$	24·9
$(NH_4)_2SO_4$	25·0
NaCl	25·1

BEHAVIOUR OF THE COPPER - DIETHYLDITHIOCARBAMATE COMPLEX IN ACETIC ACID SOLUTION AND DETERMINATION OF THE OPTIMUM CONDITIONS FOR THE DETERMINATION OF COPPER

Preliminary studies were carried out on the behaviour of the complex in the acetic acid medium. In these experiments, a standard solution containing 10 μg ml^{-1} of copper

in 90 per cent. *V/V* glacial acetic acid was used, which was prepared by dilution of the stock standard solution with glacial acetic acid.

A 10-ml volume of the solution containing 100 μg of copper was transferred by pipette into a 50-ml calibrated flask and a sufficient amount of copper-free distilled water was added so as to adjust the final strength of the acetic acid to the required value. This solution was then examined as described under Procedure.

The absorbance readings obtained at each acetic acid concentration, corrected for the absorbance of the reagent blank, were used to calculate the molar extinction coefficients.

RESULTS—

The molar extinction coefficient of the copper - diethyldithiocarbamate complex varied with the concentration of glacial acetic acid in the solution. It was observed that at glacial acetic acid concentrations up to about 50 per cent. *V/V*, the solutions were turbid owing to the poor solubility of the complex in the medium, and that the molar extinction coefficient increased for glacial acetic acid concentrations in the range 60 to 78 per cent. *V/V*, reaching a broad peak with a maximum at 75 per cent. *V/V*, corresponding to a value of 18 935 $l\ mol^{-1}\ cm^{-1}$. The variation of molar extinction coefficient for glacial acetic acid concentrations in the range 72 to 78 per cent. *V/V* was very small. The molar extinction coefficient then decreased, reaching a fairly constant value at glacial acetic acid concentrations in the range 90 to 96 per cent. *V/V*. Details of these results are given in Table IV.

TABLE IV

VARIATION OF MOLAR EXTINCTION COEFFICIENTS WITH GLACIAL ACETIC ACID CONCENTRATION

Glacial acetic acid in solution, per cent. *V/V*	Corrected absorbance reading at 430 nm corresponding to 100 μg of copper in 50 ml	Calculated molar extinction coefficient
60	0·480	15 253
62	0·506	16 080
64	0·530	16 840
66	0·554	17 605
68	0·570	18 073
70	0·584	18 555
72	0·592	18 810
74	0·596	18 935
76	0·596	18 935
78	0·588	18 685
80	0·578	18 365
82	0·560	17 790
84	0·534	16 965
86	0·510	16 206
88	0·485	15 410
90	0·473	15 030
92	0·473	15 030
94	0·479	15 220
96	0·473	15 030

On the basis of the results, it was decided to carry out the determination of copper at a glacial acetic acid concentration of 75 per cent. *V/V*.

The absorption curve under optimum conditions, *i.e.*, in 75 per cent. *V/V* glacial acetic acid, showed a maximum at 430 nm and a minimum at 372 nm.

APPLICATION OF THE METHOD TO FOOD AND OTHER BIOLOGICAL MATERIALS

Treatment of liquid samples, e.g., *water and spirituous liquors, containing* 0·1 *p.p.m. or more of copper*—For colourless liquid samples, evaporate 50 ml of the sample and adjust the final volume to 10 ml with copper-free distilled water.

For coloured liquid samples, evaporate 50 ml of the liquid to dryness in a platinum dish and oxidise the residue with a few drops of concentrated nitric acid, heating the dish to complete the oxidation. Finally, evaporate the oxidised liquid to dryness and take up the residue in 10 ml of copper-free distilled water.

Treatment of solid samples containing 5 p.p.m. or more of copper—Ash 1 g of well sampled material in a platinum dish, dissolve the residual ash in dilute hydrochloric acid and evaporate the solution to dryness. If the residue is yellow in colour, dissolve it in 2 ml of water and evaporate the solution to dryness, then repeat this step. This should remove any colour. Take up the residue in 10 ml of copper-free distilled water.

Alternatively, wet oxidise 1 g of well sampled material using Middleton and Stuckey's method,[7] remove any excess of nitric acid, and add 10 ml of copper-free distilled water.

Determination of copper—To the final 10-ml volume of the sample treated as described above, add 2·5 ml of copper-free distilled water and 25 ml of glacial acetic acid and proceed as described under Interferences.

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