Modified Colorimetric Determination of Copper with Sodium Diethyldithiocarbamate in the Presence of Hydroxylamine

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Introduction

Sodium diethyldithiocarbamate was at first used by Callan and Henderson⁽¹⁾ in the colorimetric determination of a small amount of copper. He was followed by several workers who had studied this colorimetric determination, and among them Conn *et al*⁽²⁾ made a special study of the various colorimetric determinations of copper being used up to that period. He pointed out the experimental defect of each method of determination and recommended the use of the sodium diethyldithiocarbamate method as the most accurate. This method is to be followed after isolating the copper with hydrogen sulphide to avoid interference from other elements usually present in the sample to be tested. He reported that hydrogen sulfide separation is superior to the tedious and time-consuming electrolytic separation recommended by Grendel.⁽³⁾ Yet unfortunately it was also reported unsatisfactory.⁽⁴⁾

Upon these results Sylvester and Lampitt⁽⁵⁾ attempted the colorimetric determination of copper in milk with this same reagent after having extracted the copper with chloroform as dithizonate. But Nydahl⁽⁶⁾ proposed the same method as Sylvester and Lampitt in determining copper in fats, using carbon tetrachloride in place of chloroform as an extractant. Thus this reagent, producing an intense golden yellow coloration when put into contact with any amount of copper in the solution, has been used as highly sensitive reagent in the determination of minute quantities of the element.

However it has one drawback in colorimetry in that, when extracted with some other organic solvents, it does not variate in degrees of coloration in direct proportion to the amount of copper present. To solve this difficulty organic solvents such as ether,⁽⁷⁾ chloroform,⁽⁵⁾ carbon tetrachloride,⁽⁸⁾ amyl alcohol⁽⁹⁾ and isoamyl alcohol⁽¹⁰⁾ were proposed but none were satisfactory.

Such being the case, color comparison must be undertaken by a series of standard solutions treated in the same way or by the use of a Lovibond tintometer at the loss of accuracy and sensitivity of the reagent. But McFarlane⁽⁹⁾ reported that the use of a Duboscqu colorimeter would, to some extent, produce color variations proportional to the amount present. Of course that is when the copper is extracted with amyl alcohol. However, unfortunately the results obtained by some workers could,⁽¹⁰⁾⁽¹¹⁾ not prove this statement. Naturally the degree of coloration in the original aqueous solution strictly conforms with the Lambert-Beers' law as it does in the case of amyl alcohol and carbon tetrachloride solution being used as an extractant, provided that the specially made filter⁽¹²⁾ is used. The use of such a filter, however, is not generally possible. On the other hand Nydhal explained in his report that his method in practice agrees with the law if shaken successively two or three hundreds times with carbon tetrachloride. However, the best accuracy cannot be expected. Though a highly sensitive reagent is used for the determination, this drawback is unavoidable being probably due to the difficulty of transferring the colored products completely into solvents.

This defect was, however, finally abolished by the application of a new method tried by the author using carbon tetrachloride as an extractant in the presence of hydroxylamine. The object of this report will therefore be to give some details on this new method.

Procedure Discussed

Color reaction Though sodium diethyl-

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(1) T. Callan and J.A.R. Henderson, *Analyst*, **54**, 650 (1929)

(2) L.W. Conn, A.H. Johson, H.A. Trebler and V. Karprenko, *Ind. Eng. Chem. Anal. Ed.*, **7**, 15 (1935); R.W. Thatcher, *J. Am. Chem. Soc.*, **55**, 4524 (1933)

(3) F. Grendel. *Phrm. Weekblad.*, **67**, 913, 1343 (1930); *Z. Anal. Chem.* **97**, 47 (1934)

(4) Walker. *J. Assoc. Offici. Agr. Chem.*, **13**, 426 (1930)

(5) N.D. Sylvester and L.H. Lampitt. *Analyst*, **60**, 377 (1935)

(6) F. Nydahl, Z. *Anal. Chem.*, **116**, 315 (1939)

(7) S.L. Tompsett, *Biochem. J.*, **29**, 480 (1929)

(8) L.A. Haddock and N. Evers, *Analyst*, **57**, 495 (1932)

(9) McFarlane, *Biochem. J.*, **26**, 1022 (1932); *Z. Anal. Chem.*, **101**, 71 (1935)

(10) E.J. Caulson, *J. Assoc. Offici. Agr. Chem.*, **20**, 178 (1937)

(11) D.L. Drabkin, *J. Assoc. Offici. Agr. Chem.*, **21**, 202(1938); L.A. Haddock and N. Evers, *Analyst*. **57**, 495 (1932); c.f. O.B. Winter, *J. Assoc. Offici. Agr. Chem.*, **19**, 359 (1936)

(12) D.L. Drabkin, sec foot-note (11)

dithiocarbamate is not exclusively active with copper, but reacts with such metals as Bi, Fe, Mn, Ni, Co, Cr, forming colored complexes and with Zn, Pb, Hg, Cd, Sb, Sn and Al becoming turbid, it nevertheless has been used for copper determination because of its high sensitivity. It is therefore necessary that copper be preliminarily separated from other metals to obtain the best results. After a comparative study of preliminary separation of copper, the author decided upon the use of dithizone in the basic solution containing some citrate after the Sandells' direction, details of which will be reported in the last communications. With the use of dithizone in carbon tetrachloride and the acid treatment with 0.01 N hydrochloric acid some of these interfering metals are avoided, for Sb, Sn, Al and Cr, do not react with dithizone and Pb, Zn and Cd perfectly separate into the acid solution being isolated from copper by treating the dithizonates with 0.01 N hydrochloric acid. The amount of Fe and Mn in this case is extremely small owing to the addition of citrate which prevents its reaction with dithizone. Even should they be present, 50 p.p.m. of each are permissible in a small amount of copper as 1 to 3 p. p. m.(5) Coloration with some other metals was studied, and E_γ was calculated from the results, meaning the extinction coefficient of 1cm. of the solution when 1 γ of the element present in 1 ml. of the solution is measured. (Table 1)

Table 1

Sensitivity of some elements to sodium diethyldithiocarbamate

Ions	Cu	Mn	Co	Ni	Bi	Zn	Pb	Fe	Ti
E_γ	0.201	0.0000	0.0168	0.0081	0.0018	0.0040	0.0001	0.0007	0.0000
Color-reaction	Golden yellow	Pink (unstable)	Dull green	Greenish yellow	Yellow (precipitated)	White turbidity	White turbidity	Deep brown (precipitated)	Yellowish white turbidity

E_γ was obtained in carbon tetrachloride solution extracted at pH 10.5 and the color reaction (in third column) means the coloration in the water solution when a considerable amount of the element is present.

These results indicated that the reagent is ultimately most sensitive to copper and that interference from other metals is generally negligible even though the acid treatment might not have been the best. If the coloration is produced at pH higher than 10.5, as much as 5 mg. Zn is permissible in 10 γ copper determination as it can be thought $Zn(NH_3)_4^{\cdot\cdot}$ forms with slightly excess ammonia. Therefore it is possible to determine copper in the presence of a considerable amount of Zn. But in this case, it is safer to make a color comparison immediately as it tends to become turbid on standing. It is easy to prevent the turbidity by lead with the addition of thiourea and in this case the extraction of the copper compound is easily made without the addition of hydroxylamine. Sylvester *et al* reported that 50 p.p.m. each of Co or Ni are permissible with 0.2 to 2.7 p.p.m. copper determination. It is evident from these results that serious interference is far from being encountered, because the amount of these elements in living plants are usually one hundredth to thousandths of the amount of copper.(14)

Likewise it is generally unnecessary to consider the interference of bismuth because of its rarity in biological matter and its lower sensitivity. Thus from the above study of interferences from other metals in copper determination, it can be concluded that the application of this color reaction to copper in biological matter can be successfully undertaken without serious interference.

Hydroxylamine in the extraction The degree of coloration into the aqueous solution is strictly proportional to the amount of copper present, but not when extracted with some organic solvents. It is thought that the rate of transference of colored matter into organic solvents is so small that complete transference is impossible. Thereupon this color reaction has not been widely used though superior in such respects as simplicity, high sensitivity and speed. To overcome these defects Haddok and Evers(8) recommended that extraction be repeated until the solution becomes colorless. Yet this process, it is said by many workers, is not in comformity with the Lambert-Beers' law. Only McFarlane reported that it comforms to the law when extracted with amyl alcohol though the range of concentration proportional to the degree of coloration is somewhat narrow. Nydahl added that this would comform with the law if shaken vigorously about three hundred times for each extraction, but accurate results cannot be expected in this case.

The addition of hydroxlamine in the process of extraction newly tried by the author clearly solves this difficult problem. By its addition in making the extraction with carbon tetrachloride it comforms accurately with the law and brings about the best results by a short

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(14) F. Honcamp, "Handbuch der Pflanzenernährung und Düngenlehre", I. 261, Leipzig (1931)

shaking for 20 seconds. The addition of hydroxylamine had been found useful in several cases when tested with solvents which had been proposed. Yet among them carbon tetrachloride is the most advantageous owing to its larger density, lower solubility and lower vapor pressure which are properties convenient for practical use.

Table 2

Stability of the coloration in carbon tetrychloride

Time(min.)	Extinction Coefficient 0	30	60	80	100	180	24 hrs.
10.05 γ Cu$^{(a)}$	0.384	0.380	0.376	0.370	0.373	0.370	0.362
10.05 Cu$^{(b)}$	0.452	0.436	0.414	——	0.409	0.404	0.401
20.10 Cu$^{(a)}$	0.755	0.750	——	——	——	0.743	0.735

(a) extracted at pH 10.5 after adding 2 ml. of 4 % hydroxylamine and the final volume, 25 ml.

(b) the same condition as (a) except the addition of 6 ml. of 4 % hydroxylamine.

Another advantage is that it has no ill effect on the stability of the coloration, at the same time preventing any change in coloration.

It was found that no appreciable decrease was noticed after 24 hours standing. Yet the stability decreases slightly upon increasing the amount of hydroxylamine added, though its efficiency for extraction increases. For this therefore 2 ml. of 4 % hydroxylamine solution was found most satisfactory. Further advantage of its addition is found in diminishing the pinkish coloration due to the presence of manganese, if a little excess of ammonia than the recommended is added.

Table 3

Relation between the amounts of hydroxylamine and the stability of the coloration

4 % hydroxylamine added (ml.)		2	4	6	8
Extinction	0 min.	0.384	0.406	0.452	0.509
Coefficient	30 min.	0.380	0.396	0.436	0.459

Table 4

Efficiency of extraction at various pH (copper present 11.00 γ)

pH	3.0	6.7	7.2	8.6	9.7	10.5	10.9	11.5
Extinction Coefficient	0.430	0.440	0.457	0.451	0.454	0.464	0.434	0.414
Copper found (γ)	10.70	10.95	11.37	11.22	11.29	11.54	10.75	10.30

The extraction must be done under great precaution so that the separated carbon tetrachloride be free from droplets of aqueous solution during the procedure of drawing off from the separatory funnel, which brings about futile oxidation of the reagent in extracted solution. Oxidation of the reagent develops a certain cloudiness.

It was found that pH 7.2 to 10.5 was the the most suitable pH for speedy extraction and for ineffectiveness on the stability of the coloration. The smaller the pH of the aqueous phase the more necessary is the repetition of the extractive operation.

Though the effective period of the sodium diethyldithiocarbamate solution was reported to be one week or at the most two weeks,(15) it was found that it could be effective for a month if used in the presence of hydroxylamine and stored in an amber colored bottle.

Accuracy and reproducibility The addition of hydroxylamine has increased the sensitivity of the reagent as the extraction can be completely carried out. The results obtained on the various concentrations of copper standard solution will be shown in Table 5, indicating that it holds closely to the Lambert-Beers' law therefore rendering the color comparison much more simple.

These results have found that the range of error lies within 5 % according to the amounts of copper (5 γ to 20 γ) in 25 ml. carbon tetrachloride solution. But, whenever possible, the copper range selected should not be less than 0.2 p.p.m. in order to obtain greatest accuracy.

In practical use a blank test should be frequently made on all material used. In this case when cells of 50 mm. thickness were used the readings correspond to 0.9 γ copper. When its method is compared with the extractive

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(15) J. Grant and A. Mezgy, *Analyst*, **61**, 401 (1936)

Table 5

Accuracy and conformability to the law

Copper present (γ)	Accuracy: Extinction Coefficient (50 mm. thickness)	Accuracy: Copper found (γ)	Diff. (γ)	Conformability: Extinction Coefficient (average)	Conformability: E_γ (Calculated)
2.01	0.081	2.02	+0.01	0.081	0.203
5.03	0.197	4.90	−0.13		
	0.193	4.80	−0.23		
	0.208	5.17	+0.14	0.201	0.201
	0.191	4.75	−0.28		
	0.215	5.35	+0.32		
10.05	0.392	9.83	−0.22		
	0.396	9.95	−0.10		
	0.386	9.60	−0.45	0.401	0.201
	0.401	9.98	−0.07		
	0.408	10.14	+0.09		
	0.422	10.50	+0.45		
20.10	0.820	20.40	+0.30		
	0.800	19.90	−0.20	0.801	0.203

titration or mixed colored method with dithizone,(13) both tedious and requiring skillful operation and a considrable blank factor (owing to the color of the reagent itself), the new method is speedier and more accurate owing to its complete extraction of copper by the addition of hydroxylamine.

Reagent and Apparatus

Apparatus Separatory funnel: Approximately 30 ml. capacity. Silica dish or platinum dish; These apparatus should be free from copper by washing with distilled nitric acid.

Reagent Water; Redistilled in glass apparatus. Hydrochloric acid (1;1): It is preferable to use freshly distilled hydrochloric acid showing constant boiling point. Ammonia saturated: Saturate redistilled water with ammonia gas generating from ammonia aqua on heating by the addition sodium hydroxide. Sodium diethyldithiocarbamate, 0.1 per cent solution; Dissolve 0.5 g. of the reagent in 500 ml. of redistilled water, stored preferably in amber colored bottle. Hydroxylamine, 4 per cent solution; Dissolve 4 g. of its hydrochloride salt in 100 ml. of redistilled water. Carbon tetrachloride: Shake with one tenth of its volume of concentrated sulfuric acid and then wash with redistilled water to remove the latter. After that purify by distillation in usual way.

Procedure

The aliquot portion of the solution containing copper is treated as follows: If copper was concentrated with organic reagent such as dithizone it should be ignited to destroy all organic matter in silica or platinum dish. Then add 1 ml. of hydrochloric acid (1;1) and by means of a glass rod over the interior of the dish so that all the cupric oxide will be dissolved. At this point if copper is less than approximately 10 γ hardly any color is noticeable. Add 10 ml. of water, transfer into the separatory funnel and rinse thoroughly with some water. Thus the solution to be tested is ready for the extraction.

Make the solution alkaline with ammonia to litmus paper and add the excess of 0.2 ml. of ammonia solution. Next add 2 ml. of 4 per cent hydroxylamine solution and 10 ml. of 0.1 per cent sodium diethyldithiocarbamate solution in the order named. It becomes instantly golden yellow due to the copper. Shake vigorously with 7 ml. of carbon tetrachloride and draw it off into a 25 ml. volumetric flask after leaving it to settle. Leave a few drops of the carbon tetrachloride to prevent mixing of the aqueous solution and rinse the stem of the funnel with 2 ml. of carbon tetrachloride. In this extraction the stem should be perfectly dried. Then shake again in the same manner as before. Usually two shakings is sufficient to entirely extract the colord matter. Then fill to the mark with carbon tetrachloride and measure the extinction coefficient with Pulfrich photometer using S 43 filter ($434\,\mathrm{m}\mu$). If the final volume is reduced to less than 25 ml., then less than 5 γ of copper can be determined with fair results.

Conclusion

The addition of hydroxylamine proposed by the author has removed entirely all defects in the determination of copper with sodium diethyldithiocarbamate as pointed out by previous workers.

Its use has given satisfactory results superior to other methods in the following respects: (a) Speedy and complete extraction of copper diethyldithiocarbamate complex with carbon telrachloride, (b) Normal effect on the stability of coloration and the effective period of the reagent remakably increased and (c) increase of accuracy in color comparison by complete

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(13) E.B. Sandell, *Ind. Eng. Chem. Anal. Ed.*, **9**, 464 (1937)

extraction of the colored matter so that it comforms with Lambert-Beers's law.

By the adoption of this method, copper can be determined in a short while after bringing the sample into solution. The experiments already related have proved that it is one of the most accurate methods in comparison with other methods which have been used. At the present time when the role played by copper in biological matter is given greater attention this new method will fulfill its mission in being of invaluable importance in this respect. A new colorimetric method of copper in biological matter adapting both the dithizone extraction and this new method will be reported in the near future by the author.

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