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CU(I) IN BLOOD SERUM: A SELECTIVE WAY OF DETERMINATION

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ABSTRACT

2,5-Dimethyl-4-(2-quinolylazo)phenol (DM-p-QAP), a new heterocyclic azo dye, has been prepared, characterised and proposed as a sensitive spectrophotometric reagent for copper(l). The metal interacts with 2,5-dimethyl-4-(2-quinolylazo)phenol to give a greenish yellow coloured 1:2 complex soluble in 40% ethanolic medium. The complex exhibits maximum absorbance at 452 nm in the pH 2.5. The Sandell's sensitivity of the coloured reaction is 0.00117 ng of Cu/cm^2 with a molar absorptivity of $5.43x10^4$ L $mol^{-1}cm^{-1}$. The reagent has been found to be fairly selective for the determination of copper(l) in presence of various interfering metal ions. The proposed method has been successfully applied to the selective determination of copper(l) in human blood serum. The precision and accuracy has been found to be satisfactory.

Key words: 2,5-dimethyl-4-(2-quinolylazo)phenol, Copper, Blood serum.

INTRODUCTION

Copper is an essential trace element that is vital to the health of all living things. The human body normally contains copper at a level of about 1.4 to 2.1mg per kg body weight. Copper is transported in the blood serum on a plasma protein called ceruplasmin [Bertini *et al*, 1998]. Cerruplasmin has been shown to catalyse the oxidation of iron(ll) by molecular oxygen and has also been identified as the essential plasma factor necessary for release of iron from perfuse liver in combination with transferrin, an iron protein of plasma. Copper deficiency can, therefore, cause anaemia because its presence in ceruplasmin makes stored iron available for haemopoiesis i.e. when a congenital in the homeostatic mechanism for copper exists,

the metal accumulates in liver, discrete areas of brain, the cornea of the eyes and other tissues causing hepatolenticular degeneration or Wilson's disease. Copper is closely related to estrogens metabolism and is required for women fertility and to maintain pregnancy. Imbalance can cause every conceivable female organ related difficulty such as premenstrual syndrome, ovarian cysts, infertility, miscarriage and many more. It affects men less than women in this area. Copper imbalances are highly associated with most psychological, emotional and often neurological conditions. These include memory loss, depression, anxiety, bipolar disorder etc. Hence, the determination of copper in human blood serum is highly important. There are numerous spectrophotometric reagents for the determination of copper. But there are very limited number of heterocyclic azo dyes which give colour reaction with Cu(l).

The present communication reports the analytical potentiality of 2,5-dimethyl-4(2- quinolylazo)phenol (DM-p-QAP), a new heterocyclic azo dye, in the selective and sensitive determination of Cu(l) in 40% ethanol medium. The method presented here is simple, rapid and highly selective and sensitive.

MATERIALS AND METHODS

(A) Reagents and Solutions

Stock solution of Cu(ll): A stock solution of Cu(ll) was prepared by dissolving appropriate amount of $CuSO_4.5H_2O$ (Merck: MW 249.67) in double distilled water.

Ligand solution: The ligand DM-p-QAP was prepared by the method of Deka *et al.,* 2014. A $1x10^{-3}$ M solution of the ligand was prepared by dissolving appropriate amount of the ligand in 100 mL ethanol. The solution is stable for more than a month.

Ascorbic acid solution: 2% solution (w/v) of ascorbic acid was prepared by dissolving appropriate amount of it in double distilled water. The solution was stored in amber coloured bottle. In no case a solution of older than two days was used.

Trichloroacetic acid solution: 10% solution (w/v) of trichloroacetic acid (TCA) was prepared by dissolving appropriate amount of it in double distilled water.

Dilute solutions of sodium hydroxide and sulphuric acid were used for pH adjustment.

Blood samples: Blood samples of healthy people were collected through a local hospital of Guwahati metropolitan city, Assam, India.

(B) Equipments

i) A UV-1700 Pharmaspec UV–Visible spectrophotometer (Shimadzu) with matched quartz cells of 10 mm path length were used for absorbance measurements.

ii) Eutech Cyberscan pH 310 digital pH meter with glass electrodes of 0-14 pH range were used for pH adjustments. Standardisation of the pH meter was carried out from time to time with standard buffer solutions.

iii) Pre calibrated pipettes, burettes, measuring flasks, conical flasks etc (Borosil make) were used for volume measurement.

(C) Protocol

To an aliquot containing 1.90 to 5.70 g of Cu(ll) ion, 2 mL of 2% ascorbic acid solution was added and allowed to stand for about 10 minutes. Sufficient excess of DM-p-QAP solution was added, pH adjusted in the range of 2.25 to 2.75 and the volume raised to 10 mL maintaining the ethanol concentration at 40% (v/v). The solution was heated for 5 minutes in a water bath and then allowed to cool it to room temperature. The volume of the solution lost due to heating was compensated by ethanol. The absorbance at 452 nm ($_{max}$) was recorded against corresponding reagent blank prepared in a similar way. The amount of Cu(l) present in the solution was calculated from the standard calibration curve drawn under similar conditions.

In the determination of copper in blood serum, the deproteinisation procedure has been adopted. Blood serum from the blood was separated by centrifuging approximately 5.0 mL of freshly drawn blood. 0.5 mL of this serum is taken in a centrifuge tube, followed by addition of 1.0 mL of 10% TCA and 2.0 mL of 2% ascorbic acid. The mixture was allowed to stand for about 15 minutes and then centrifuged again. Copper(l) content in 0.2 mL of the supernatant liquid is then determined by following the recommended procedure. The standard addition procedure was also applied to ascertain the accuracy of the method.

RESULTS AND DISCUSSION

Addition of ethanolic solution of DM-p-QAP to a very dilute solution of copper(l) forms a greenish yellow coloured complex. The complex precipitates out when the ethanolic concentration is less than 30%. Subsequent studies were, therefore, carried out in

40% ethanolic concentration. The complex is stable at least up to 25 hours under this condition.

Spectral behaviour of Cu(I) –DM-p-QAP complex and effect of pH : A series of solutions containing 1.0 mL of $1x10^{-4}$ M Cu(I) [in presence of ascorbic acid] and 1.0 mL of $1x10^{-3}$ M reagent were prepared and pH were adjusted at different levels in a total volume of 10 mL maintaining ethanolic concentration at 40% (v/v). Their spectra were taken against corresponding reagent blanks. The studies revealed that only one complex with maximum absorbance at 452 nm are formed at all pH values. It was also observed that at least six hours time is necessary for complete colour development at room temperature. However colour development completes when the solution is heated for 5 minutes in a water bath. Further studies were carried out under this condition. The volume loss due to heating was made up by ethanol. The complex was found to be quite stable and even up to 25 hours no change in absorbance was noticed. Plots of pH versus absorbance at the $_{max}$ value show that constant absorbance is exhibited in the pH range 2.25 to 2.75.

Effect of ethanol concentration : It has been found that when the ethanol concentration is below 30% the complex precipitates out. But the precipitate dissolves when the ethanol concentration is increased. No difference in absorbance at $_{max}$ was observed when measured at different ethanolic media. However, in subsequent studies the ethanol concentration was maintained at 40% (v/v). Further it was observed that 1.0 mL of 2% ascorbic acid is sufficient to reduce Cu(II) to Cu(I) present in 1.0 mL of $1x10^{-4}$ M solution of Cu(II) in two minutes. It was also observed that at least five times molar excess of DM-p-QAP to the metal is required for complete complexation.

Other physico-chemical characteristics of the Complex : The results obtained at the $_{max}$ for the Cu(I) – DM-p-QAP complex for validity of Beer's law, optimum concentration range, Sandell's sensitivity and molar extinction coefficient are summarized bellow in Table 1.

Effect of diverse ions : The effect of diverse ions in the determination Cu(I) has been investigated by preparing synthetic solutions containing 0.635 g/mL of Cu(I) and different amounts of diverse ions. The copper content in these solutions were determined by the recommended procedure mentioned earlier. It has been found

Table 1: Optical constants of Cu(I) – DM-p-QAP complex

1	$_{max}$	452 nm
2	Beer's law validity	0.0 - 0.635 ppm
3	Optimum concentration range	0.19 – 0.57 ppm
4	Sandell's sensitivity	0.00117 g of Cu(I) cm^{-2}
5	Molar extinction coefficient ( )	5.43 x 10^4 L mol^{-1} cm^{-1}
6	Molar composition	1:2

that F^-, Cl^-, Br^-, I^-, NO_3^-, SO_3^{2-}, SO_4^{2-}, PO_4^{3-}, BO_3^{3-}, oxalate, tartrate, citrate, and acetate did not interfere. Among the cations Na^+, K^+, NH_4^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Al^{3+}, Mn^{2+} Zn^{2+}, Ni^{2+}, Co^{2+}, Ag^+, Hg^{2+}, Cd^{2+},Fe^{2+},Fe^{3+}, Cr^{3+} did not interfere. However S^{2-}, $S_2O_3^{2-}$, thiourea, thiocyanate, NO_2^-, CN^- and EDTA interfered seriously.

Recovery of copper in blood serum : The standard addition procedures in the blood samples were tried by adding 0.32 ppm of copper. The recovery results are shown in Table 2 & Table 3. The recovery results confirmed the precision of this method.

There are only a few reagents for spectrophotometric determination of Cu(I). Two main specific reagents for spectrophotometric determination of Cu(I) are cuproine (2,2'-biquinolyl) and 1,10-phenanthroline [Sandell *et al*, 1978]. Majority of the other reagents for spectrophotometric determination of Cu(I) are derived from cuproine. Cuproine is more specific reagent for Cu(I) and though 1,10-phenanthroline is slightly more sensitive than cuproine, it is found to be more sensitive to Fe(II). The cuproine chromophore is present in cuproine and phenanthroline and their derivatives have been found to be specific for Cu(I). The complexes are cationic and can be extracted in different polar solvents.

Examples of heterocyclic azo dye as sensitive and selective chromogenic reagent are very rare. Baruah *et al.* 1981 reported 4-(2-quinolylazo)phenol, p-QAP, a heterocyclic azo dye, as a highly sensitive reagent for Cu(I). The reagent is also highly selective due to the fact that no other metal complex of p-QAP absorbs at this wave length and the complexes of other metal ions are unstable at 40% ethanolic concentration. The reagent has been employed successfully for selective determination of copper in alloys, various blood serum, milk samples, alcoholic

Table 2: Male Blood Serum Analysis

Sample	Natural Cu present (ppm)	Amount of Cu added (ppm)	Total Cu present (ppm)	Total Cu found (ppm)	Error %
	0.266	0.32	0.586	0.578	-1.36
Sample 1	0.273	0.32	0.593	0.590	-0.50
	0.266	0.32	0.586	0.578	-1.36
	0.273	0.32	0.593	0.590	-0.50
Sample 2	0.279	0.32	0.599	0.597	-0.33
	0.279	0.32	0.599	0.597	-0.33
	0.266	0.32	0.596	0.590	-1.00
Sample 3	0.266	0.32	0.596	0.590	-1.00
	0.266	0.32	0.596	0.597	+0.16
	0.273	0.32	0.593	0.590	-0.50
Sample 4	0.273	0.32	0.593	0.590	-0.50
	0.279	0.32	0.599	0.590	-1.01

Table 3: Female Blood Serum Analysis

Sample	Natural Cu present (ppm)	Amount of Cu added (ppm)	Total Cu present (ppm)	Total Cu found (ppm)	Error %
	0.279	0.32	0.599	0.590	-1.50
Sample 1	0.279	0.32	0.599	0.590	-1.50
	0.273	0.32	0.593	0.590	-.50
	0.279	0.32	0.599	0.597	-0.33
Sample 2	0.273	0.32	0.593	0.590	-0.50
	0.279	0.32	0.599	0.590	-1.50
	0.273	0.32	0.593	0.590	-0.50
Sample 3	0.273	0.32	0.593	0.590	-0.50
	0.279	0.32	0.599	0.590	-1.50

beverages and soft drinks. The added advantages of this ligand are that colour formation is instantaneous and extraction is not necessary. The present study reports another heterocyclic azo dye 2,5-dimethyl-4-(2-quinolylazo)phenol, DM-p-QAP, as a highly sensitive chromogenic reagent for Cu(I). The reagent is highly selective too as no other metal complex of DM-p-QAP absorbs at this wave length and other metal complexes of DM-p-QAP exhibit intense colour only at alkaline medium.
Molar absorptivity of the colour system has been compared with some other reagents known for this purpose in Table 4.

Table 4: Sensitivities of the colour reactions of various reagents with Cu(I)

Sl no	Reagent	Sandell's sensitivity (g Cu(I)/cm^2)	Reference
1.	Cuproine/isoamyl alcohol (2,2′-Biquinolyl)	0.0098/546 nm 0.0012/358 nm	Sandell *et al*, 1978
2.	Neocuproine/isoamyl alcohol (2,9-dimethyl-1,10-phenanthroline)	0.0080/454 nm	Sandell *et al*, 1978
3.	Bathocuproine/isoamyl alcohol (2,9-dimethyl-4,7-diphenyl-1, 10-phenanthroline)	0.0044/479 nm	Sandell *et al*, 1978
4.	Neocuproine and Rose Bengal/$CHCl_3$	0.0010/570 nm	Bailey *et al*,1966
5.	2,3,8,9-Dibenzo-4,7-dimethyl-5, 6-dihydro-1,10-phenanthroline	0.00799/554 nm	Ackermann *et al*, 1968
6.	4,4′-Dihydroxy-2,2′-biquinolyl/ isoamyl alcohol	0.0092/525 nm	Schilt *et al*, 1969
7.	3,3′-Dimethylene-4,4′ -diphenylbiquinolyl/isoamyl alcohol	0.0065/555 nm	Uhlemann *et al*, 1968
8.	6,7-Dimethyl-2,3-di(2-pyridyl) -quinoxaline and methyl orange/$C_2H_4Cl_2$	0.0021/418 nm	Stephen *et al*, 1967
9.	6-Methyl-2-pyridylphenylketoxime	0.0057/430 nm	Pamberton *et al*, 1969
10.	Ferrozine-3-(2-pyridyl)- 5,6-bis(4-phenylsulphonic acid)-1,2,4-triazine	0.0148/470 nm	Kundra *et al*, 1974

(Cont...)

11.	3-(2-Pyridyl)-5, 6-diphenyl-1,2,4-triazine (PDT)	0.0794/488 nm	Schiff *et al*, 1970
12.	4-(2-Quinolylazo)phenol (p-QAP)	0.0012/440 nm	Barua *et al*, 1981
13.	2,5-Dimethyl-4-(2-quinolylazo) phenol (DM-p-QAP)	0.00117/452 nm	Present work

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