Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2011, 2 (4): 176-183

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Spectrophometric determination of copper (II) in Biological samples by using 2–acetylpyridine 4–methyl –3-thiosemicarbazone (APMT)

K. Vasudeva Reddy[1,2], D.Nagarjuna Reddy[2], S.VidyasagarBabu[2] and K. Hussain Reddy[2]

[1]Dept. of Chemistry, Govt. College for Women, Chintamani, Karnataka
[2]Dept. of Chemistry, Sri Krishnadevaraya University, Anantapur, AP, India

---

ABSTRACT

2-Acetylpyridine-4-methyl-3-thiosemicarbazone (APMT) is proposed as a new sensitive reagent for spectrophotometric determination of Copper (II). APMT reacts with Copper (II) in the pH range 5 – 8 to form yellow coloured complex. The absorbance value of Cu (II) - APMT complex was measured at different intervals of time at 380 nm, to ascertain the stability of the complex. It was observed that the colour development was instantaneous and stable for more than 48 h. The system obeyed Beer's law upto 2.54 µg mL^{-1} of opper(II). The molar absorptivity and Sandell's sensitivity of the species is 1.475 X 10^4 L mol^{-1} cm^{-1} and 4 X 10^{-3} µg cm^{-2} at 380 nm. The composition of the Copper (II) complex with APMT was studied by Job's continuous variation and molar ratio method. Various biological samples and certified reference materials have been tested for the determination of Copper for evaluating the accuracy of the developed method. The results of the proposed method are in agreement with AAS method.

Keywords copper, APMT, Biological samples and Spectrophotometry.

INTRODUCTION

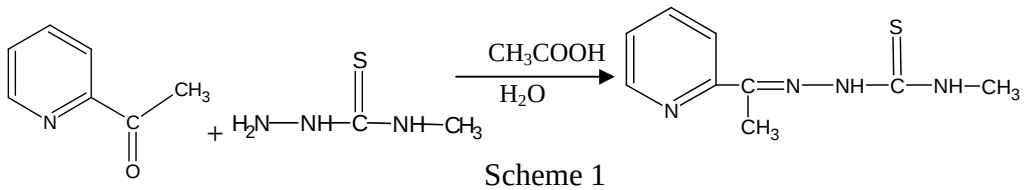
Copper is widely distributed in foods of plants and animal origin. Trace amounts of copper in various substances may be vital, objectionable or perhaps indicative of contamination or malfunction. Copper traces promote rancidity and off-flavors in foods and beverages. Its determination in biological samples such as blood, liver tissue, hair etc., can be of considerable significance in medical diagnosis and biochemical research. Chronic copper poisoning causes gastrointestinal catarrh and haemochromatosis. Copper is also a constituent of several pharmaceutical preparations. Hence rapid and sensitive methods for its determinations are in great demand. A nuber of spectrophotometric methods have been developed in recent years for the detetmination of Copper .Among the various organic reagents employed thiosemicarbazones

occupy a significant place[1-4]. Some of the methods using thiosemicarbazones are less sensitive or require the use of solvent extraction [5-8]. The present method is senstive and selective for the determination of Copper in aqueous DMF medium by complexing with 2-Acetylpyridine-4-methyl-3-thiosemicarbazone (APMT).

MATERIALS AND METHODS

***Apparatus*:**
A PerkinElmer Lambda 25 range 190-1100 nm, band width 1 nm UV-VIS spectrophotometer with 1.0 cm quartz cell was used for absorbance studies. An Elico LI-120 digital pH meter was used for pH adjustment.

***Reagents*:**
The reagent 2-Acetylpyridine-4-methyl-3-thiosemicarbazone was prepared by condensing with 2- Acetyl pyridine with 4-methyl 3-thiosemicarbazide as reported earlier [9]. The structure of regent as follows. (Scheme 1).



Scheme 1

A 0.01 M solution was prepared by dissolving 208 mg of APMT in 100 ml of dimethyl formamide (DMF).

Stock standard copper (II) solution was prepared by dissolving 0.1997 g of copper acetate monohydrate in double distilled water containing 1 ml of conc. sulfuric acid, and made up to the mark in 100 ml standard flask.. The solution was standardized titrimetrically [10] by a known method." The working solutions were obtained by diluting the stock solution to the requisite concentrations with double distilled water

1.0 mol L^{-1} sodium acetate and 1.0 mol L^{-1} acetic acid solutions were prepared in double distilled water. Suitable portions of these solutions were mixed to get the desired pH.

***General procedure*:** An aliquot of the metal solution was taken in 25 ml standard flask contains 10 ml buffer solution of pH=6.0 and 1.5 ml of APMT reagent solution and made up to the mark with distilled water. The absorbance of the complex was measured against the reagent blank at 380 nm.

RESULTS AND DISCUSSION

Analytical procedures for various samples

Analysis of leafy samples [11,13]

Dry ashing method was used in the analysis of organic samples. A 10 g of dried leafy sample was taken in a silica dish. The sample was heated over a low burner until the material chars. The charred mass was moistened with 1 : 1 HNO_3. Occasionally a 20 percent solution of magnesium nitrate was used for this purpose, particularly if the ash content is very low. Again evaporated to dryness, and transferred to a muffle furnace. The temperature to about 500^0C is reached in the course of about 3 hours and continued to heat at that temperature was raised until the ash is white. The dish was cooled and the ash was dissolved in a 5 ml portion of 1: 1 HCl. Finally add water amounting to about twice the volume of acid added. If an insoluble residue remains, filter on a small paper and wash on the paper with 1: 4 HCl. The solution was diluted to 50 ml in a standard flask. Aliquots of this solution were taken then treated as mentioned above in analysis of leafy vegetable samples.

Analysis of biological samples[12,13]

A 2 – 5 g of dried fish and sheep liver samples were taken in a 250 ml beaker 6 ml of concentrated nitric acid was, added and gently heated for half-an-hour. After the disappearance of the forth, 6 ml of 1 : 1 nitric acid and perchloric acid were added. The contents were digested for one hour and repeatedly treated with 6 ml portions of nitric acid and perchloric acid mixture until the solution becomes colourless. The acid solution was evaporated to dryness and the resulting white residue was dissolved in minimum volume of 1M nitric acid and made upto the volume in a 50 ml volumetric flask.

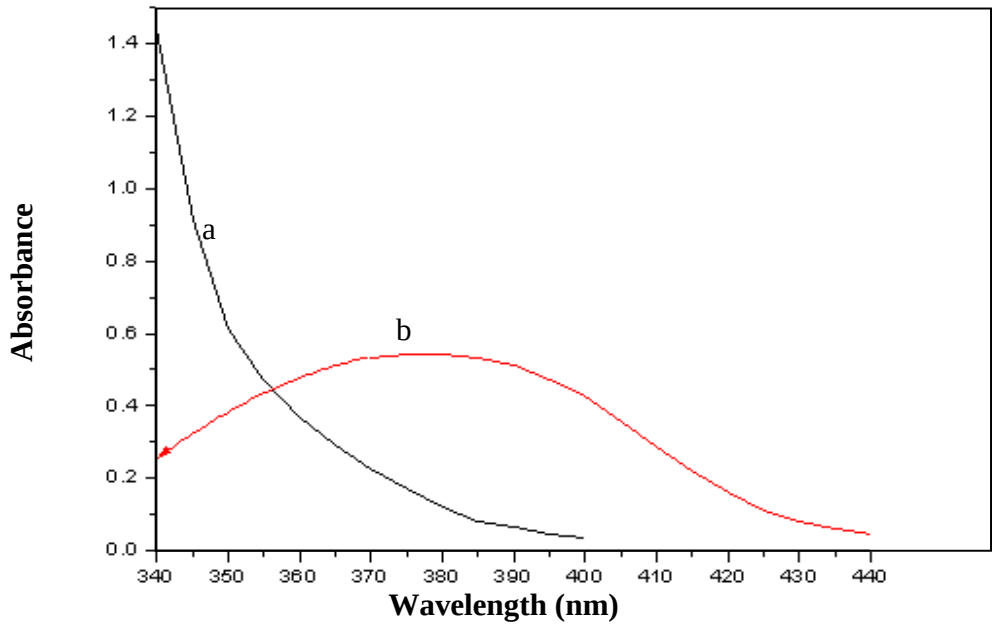


Fig: 1 Absorption Spectra of a) APMT Vs Water blank b) Cu (II) – APMT complex Vs APMT solution [Cu (II)] = 4 x 10^{-5} M , [APMT] = 6 x 10^{-4} M , pH = 6.0

Analysis of alloys samples

100 mg of alloy sample was dissolved in aquaregia and evaporated on hot water bath to dryness. The residue was dissolved in minimum amount of dil. HCl and transferred into 50 -ml standard flask quantitatively. The contents were diluted to the mark with distilled water

Determination of Cu(II) using 2–acetylpyridine 4–methyl –3-thiosemicarbazone (APMT)

Absorption spectrum

The absorption spectrum of the Copper (II)-APMT complex was studied over the wavelength 300-600 nm. The complex exhibited absorption maxima at 380 nm, where the reagent blank had negligible absorbance. Therefore the wavelength of 380 nm used in all absorbance measurements (Figure 1)

Effect of pH on Cu(II)-APMT complex

The effect of pH on the formation of Cu (II)-APMT complex was studied to find out the optimum pH for copper(II) determination. The pH studies were carried out using hydrochloric acid-potassium chloride (pH 1.0- 2.6), sodium acetate-acetic acid (pH 3.4-6.5) and ammonium chloride-ammonium hydroxide (pH 7.0 - 11.0) buffers.

The studies were carried out keeping the 2.0 mL of 4 X 10^{-5} mol L^{-1} Copper (II) solution and 5.0 mL of 4 X 10^{-4} mol L^{-1} APMT solution constant and varying the pH values from 1.0 to 10.0 using suitable buffer solutions, keeping the volume constant adjusted to 25.0 mL with double distilled water. The absorbances of the complex were measured at 380 nm, using their corresponding reagent blanks. The maximum absorbance value shown pH at 6.0. Hence for further studies, keeping **6** as the optimum pH.

Validity of Beer's law

Beer's law was obeyed within the range of 0.25- 2.54 ppm of copper at 380 nm.. The straight line obeys equation A_{380} =0.2125 C+ 0.0234 (Fig .2) The molar absorptivity and sandell's sensitivity of the method is 1.475 x10^4 L $mol^{-1}cm^{-1}$ and 0.0043 µg/ cm^2 of cu (II) respectively. The standard deviation of the method for ten determinations of 1.27 µg/ml of Cu (II) was 0.0048.

Effect of reagent concentration on the absorbance of the metal complex

The effect of reagent concentration on the absorbance of the complex has been studied by using different solutions containing 1.0 ml of 4 X10^{-5} mol L^{-1} Copper (II) solution and10.0 ml of pH 6.0 buffer solution. To these solutions, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml of 6 X10^{-4} M reagent solution was added to get maximum colour formation. The total volume adjusted to 25.0 ml with double distilled water. The absorbances were measured at 380 nm, against their corresponding reagent blanks. From the data reveal that a 15-fold molar excess of the reagent is required for maximum colour development.

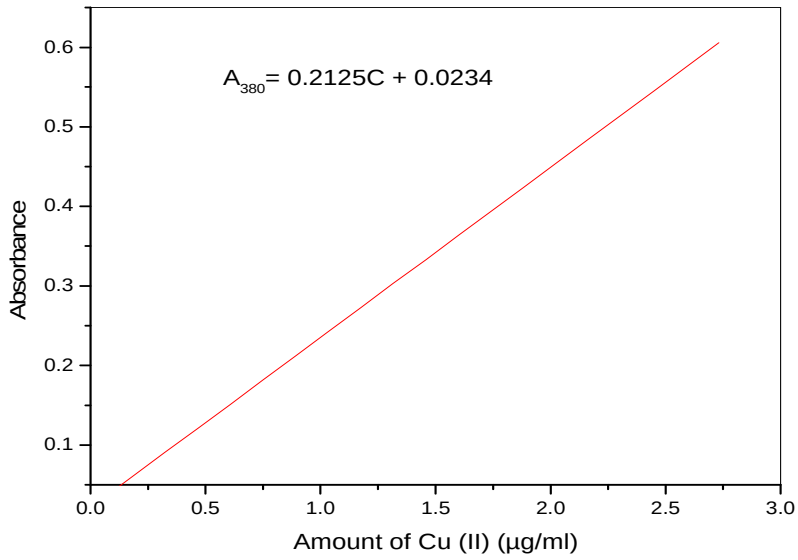
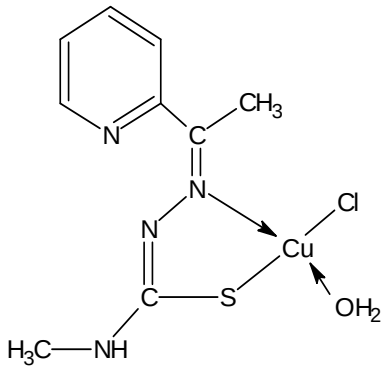


Fig: 2 Calibration plot for Cu (II) determination
pH =6.0, [APMT] = 6 x 10^{-4} M, Wavelength= 380 nm

Determination of the composition of Cu(II)-APMT complex

Job's method of continuous variation and molar ratio methods were employed to elucidate the composition of the complex. It was found to be 1:1. The stability constant determined by job's method was 1.3 x10^{6}

Based on the compositions of the complex determined in solution state, the structure tentatively assigned for the copper complex with 2–acetylpyridine 4–methyl –3-thiosemicarbazone (Structure 1).



Structure 1 Cu (APMT) Cl (H_2O) complex

Effect of diverse ions

The effect of foreign ions has been investigated. Many anions and cations do not interfere in the determination of copper (II) using APMT reagent. The effect of foreign ions was studied by measuring absorbance of the copper complex containing 0.38 µg/ml of copper in the presence of different associated anions and cations. An error of ±2% in the absorbance value was considered tolerable (Table 1)

Table.1: Tolerance limit of foreign ion in the determination of 1.27 µg/ ml of Cu (II)

Tolerance limit µg/ ml	Ion Added	Ion Added	Tolerance limit µg/ ml
Citrate	384	W (VI)	368
Tartarate	296	Co (II)	2.4[a]
Urea	288	Mn (II)	2.2
Iodate	254	Cr (III)	1.1
Bicarbonate	244	Pb (II)	0.42
Thiocyanate	232	Tl (III)	0.41
Sulphate	192	Hg (II)	0.40
Oxalate	176	Au (III)	0.39
Thiourea	152	Pt (IV)	0.39
Nitrate	124	Ni (II)	0.24
Acetate	118	Cd (II)	0.22
Phosphate	20	Fe (II)	0.22
Bromide	16	Pd (II)	0.21
Chloride	7.1	V (V)	0.20
Fluoride	4.0	Zn (II)	0.13[a]

[a] *Masked with 200 µg/ml of thiocyanate*

Applications of the developed method

Determination of copper (II) in leafy sample

Leafy vegetable samples like Thotakura (*Amaranthus gangeticus)*, Chukkaku (IRumex vesicarius), Tutikura (*Ipomoea reptuns*), Cauliflower green (*brassica deraceavar botntis*), Khesari (*Latyrus sativus*) and Medicinal leaves like Vepaku (*Azadirachta indica*) and Gaddi chamanti (*Tridax procombens L*) were analyzed for copper(II) using the proposed method. The results obtained are comparable with AAS method (Table 2).

Table 2 Determination of copper in leafy samples

Name of the samples	**Amount of copper[a] found (µg/g in dried leaves)**	
	AAS method	**APMT method**
Thotakura (*Amaranthus gangeticus*)	0.268	0.265
Chukkaku (IRumex vesicarius)	0.250	0.256
Tutikura (*Ipomoea reptuns*)	0.265	0.270
Cauliflower green (*brassica deraceavar botntis*)	0.296	0.289
Khesari (*Latyrus sativus*)	0.175	0.179
Medicinal leaves (Vepaku) (*Azadirachta indica*)	0.266	0.270
Gaddi chamanti (*Tridax procombens L*)	0.121	0.120

[a]. *Average of five determinations.*

Determination of copper(II) in biological samples

Biological sample like fish & sheep liver samples were analyzed for copper(II) using the proposed method. Aliquots of each solution were then treated according to the present procedure. The results obtained are given in Table 3.

Table 3: Determination of copper liver samples

Liver sample	Amount of copper[a] found (µg/g in dried liver)	
	AAS method	APMT method
Sheep liver.	2.48	2.53
Fish liver	1.57	1.61

.[a] Average of five determinations

Determination of copper (II) in certified reference materials

The present method is applied for the determination of copper(II) in certified reference alloys like NKK-1021-Alloy and NBS-SRM – 54 D . A known aliquot of the sample solution was taken in a 25 – ml standard flask containing 10 ml of buffer solution of pH 6.0, and reagent 1.5ml 0.01 M APMT solution and made upto the mark with distilled water. The absorbance of the complex was measured at 365 nm against the reagent blank prepared under the similar experimental conditions. The results obtained are given in Table 4.

Table 4: Determination of copper in alloys

sample	Amount of copper [a] in µg/ ml		Error
	Certified	Found	
NKK-1021-Alloy[a]	3.05	3.08	+ 1.0 %
NBS-SRM – 54 D[b]	3.05	3.07	+ 0.6 %
BCS 180/2[c]	3.05	3.03	- 0.6 %
Devard's alloy[d]	3.05	3.09	+ 1.3 %

[a] Average of three determinations
a.Si - 5.56; Cu - 2.72; Zn - 1.76; Fe - 0.99; Mg - 0.29; Mn - 0.20; Ni - 0.14 and Cr -0.03%
b.Sn - 88.5; Sb - 7.04; Cu - 3.62; Pb- 0.62; As - 0.08; Bi - 0.04; Fe -0.03; Ag -0.003 and Ni – 0.002%
c. Cu-68.12; F-0.68; Ni-30.15; Mn-0.75; C-0.05; S-0.06; Co-0.005 and Pb-0.003%
d. Al-45; Zn-5 and Cu-48.91%

CONCLUSION

A thorough literature survey revealed that many thiosemicarbazones were used for the determination of copper (II). Studies upon the use of 2-Acetylpyridine-4-methyl-3-thiosemicarbazone (APMT) as an analytical reagent are limited. Hence, the present investigations were carried out with a view to test the potentiality of APMT as a complexing agent for Cu(II) and its subsequent determination by non- extractive spectrophotometry. The method has good sensitivity, compared with other existing spectrophotometric determination methods. Finally, the developed method can be conclusively declared apt for the determination of Cu(II) in leafy samples and biological samples.

Acknowledgement
Authors are thankful to Dr. K. Ramakrishna Prasad, Associate Professor, IISC, Bangalore for his help in recording IR and NMR spectra of reagent samples. Permission to K.Vasudeva Reddy by the Commissioner, Collegiate Education Department, Government of Karnataka is acknowledged

REFERENCES

[1] Singh,R.B; Garg, B.S;Sing, R.P. *Talanta*, **1975**, 25, 619.
[2] Asuero, A.G;Cano,J.M. *Analyst*, **1978,** 103,140.
[3] Singh,R.B; Ishii, H. *Crit. Rev. Anal. Chem.* **1991**, 22, 381.
[4] Reddy, K.J; Kular, J.R; Narayana, S.L; Ramachandraiah, C; Threveni, T; Reddy, A.V. *Environ. Monit. Assess*. **2007**, 124, 309.
[5] Bhatt, G.H; Patel, I.J; Desai, K.R. *J. Inst. Chem*., **1993**, 65, 190.
[6] Patel, I.J; Bhatt, G.H; Desai, K.R. *J. Inst. Chem*., 1995, 67, 120.
[7] Prasad, N.B.L; Hussain, R.K. *Indian J. Chem*., **2004**, 43(A), 111.
[8] Vidyasagar, B.S; Hussain, R.K. *J. Indian Chem. Soc.,* **2006**, 83 (1),20.
[9] VasudevaReddy, K; Nagarjuna, R.D; Hussain R.K. *J.Chem. Pharm. Res*., **2011**, 3(2):234-244
[10] Vogel, A.I., *"A Text Book of Quantitative Inorganic Analysis,"* 3rd edn., ELBS and Longman (**1975**) p. 325.
[11] Foster De Snell and Corneelia T. Snell. *"Colorimetric methods of analysis"*, (**1949**) 3rd edn, Vol. 11. pp 92.
[12] Hussain Reddy, K and Venkata Reddy, D, *Acta ciencia India*, (**1984**) Vol. XC, No. 4, 207.
[13] 13.Marczenko, Z., *Spectrophotometric determination of elements*, Wiley, New York, (**1976**) 241, 351 and 602.