Spectrophotometric Determination of Copper(II) in Biological Samples by Using 2-Acetylfuran Thiosemicarbazone as Chelating Reagent

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2-Acetylfuran thiosemicarbazone (AFT) has been proposed a selective reagent for the spectrophotometric determination of copper. 2-Acetylfuran thiosemicarbazone reacts with Cu(II) in the pH range 6.0-8.0 to form yellow coloured complex was measured at different intervals of time at 365 nm, to ascertain the stability of complex. The system obeyed Beer's law upto 0.915 µg mL^{-1} of copper(II). The molar absorptivity and Sandell's sensitivity of Cu(II)-2-acetylfuran thiosemicarbazone is 3.33×10^4 Lmol^{-1} cm^{-1} and 2.0×10^{-3} µg cm^{-2} at 365 nm. The method has been applied for the determination of copper in alloys and in biological samples.

Key Words: Copper, 2-Acetylfuran thiosemicarbazone, Spectrophotometry.

INTRODUCTION

Trace amounts of copper in various substances may be vital, objectionable or perhaps indicative of contamination or malfunction. Copper traces promote rancidity and off-flavours 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. Colorimetric and atomic emission or absorption methods are most commonly used. However, colorimetric methods are generally preferred, as they involve less expensive instrumentation and afford better sensitivity when appropriate chromogenic reagents are employed.

EXPERIMENTAL

A UV-VIS 160 A Shimadzu spectrophotometer with quartz cells of 1 cm path length was used. An elico L1-120 digital pH-meter was used for pH measurements.

All reagents used were of analytical grade unless otherwise stated. 2-Acetylfuran (4.0 mL, 0.0396 mol) in 5 mL of methanol, thiosemicarbazide (3.6 g, 0.0396 mol, dissolved in 10 mL of hot water) were taken in a round bottom flask. Suitable quantity (-2 mL) of glacial acetic acid was added to the reaction mixture and refluxed with stirring for 4 h. Pale

yellow coloured product was separated out on cooling the reaction mixture. It was collected by filtration and washed several times with hot water and 50 % cold methanol. This compound was recrystallized from methanol and dried in vacuum. Yield 3.5 g; m.p. 135-138 ºC (**Scheme-I**).
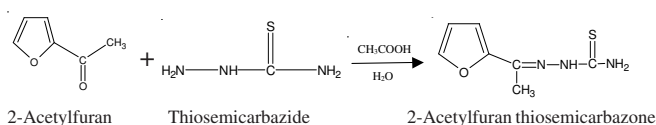
Scheme-I

A 0.01 M solution was prepared by dissolving 183 mg of 2-acetylfuran thiosemicarbazone 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 100 mL in standard flask. The solution was standardized titrimetrically by a known method[1]. 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 2-acetylfuran thiosemicarbazone

reagent solution and made up to the mark with distilled water. The absorbance of the complex was measured against the reagent blank at 365 nm.

RESULTS AND DISCUSSION

Characterization of 2-acetylfuran thiosemicarbazone: The compound was characterized by IR and ^{1}H NMR spectral data. Infrared spectrum of AFT shows bands at 3471 (m), 3378 (m), 3148 (s), 1592 (s), 1510 (s), 1481 (s), 1369 (w), 1233 (m), 700 (m) and 619-590 cm^{-1} corresponding to ν(N=H) (asym and sym), ν(C-H) aromatic stretch, ν(C=N) stretching (Schiff base), ν(C-H) aromatic ring, ν(C-H) of furan ring, ν(N-H) stretch (primary amide), ν(C=S), ν(C-H) bend (aromatic) and ν(C-O) -furan ring vibrations. ^{1}H NMR spectrum of AFT (CDCl$_3$ + DMSO-d_6) showed signals at 2.26-2.27(3H, S), 6.46-7.88 (m) due to furan ring protons.

pKa values of 2-acetylfuran thiosemicarbazone: The absorption spectra of 2-acetylfuran thiosemicarbazone were recorded at different pH values. The compound shows single absorption maximum at 310. In alkaline medium, the intensities of these bands are high, suggesting the formation of conjugated structure of the compound in solution.

The pKa values were determined by recording the UV-visible spectra of micro molar (1×10^{-4}) solutions of reagent at various pH values and by taking the arithmetic mean of the values obtained from the measurement at different wavelengths determined spectrophotometrically using phillips and merit method[2]. The values of deprotonation of 2-acetylfuran thiosemicarbazone are 3.0 (pK$_1$) and 6.4 (pK$_2$) (**Scheme-II**).

Scheme-II
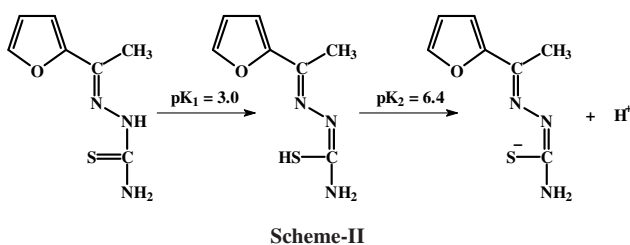
Analytical procedures for various samples

Analysis of leafy samples[2,4]: 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 % 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 ºC is reached in the course of about 3 h 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[2-5]: 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 0.5 h. After the disappearance of the froth, 6 mL of 1:1 nitric acid and perchloric acid were added. The contents were digested for 1 h 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 1 M nitric acid and made upto the volume in a 50 mL volumetric flask.

Analysis of alloys samples: 100 mg of alloy sample was dissolved in aqua regia 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-acetylfuran thiosemicarbazone

Absorption spectrum: The absorption spectrum of the copper(II)-AFT complex was studied over the wavelength 300-600 nm. The complex exhibited absorption maxima at 365 nm. Absorption due to the reagent at this wavelength was negligible. Therefore the wavelength of 365 nm used in all absorbance measurements (Fig. 1).
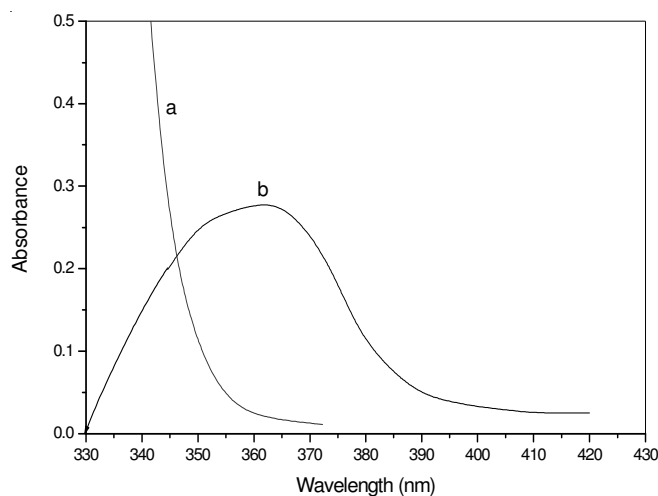

Fig. 1. Absorption spectra of; a) AFT *vs.* water blank; b) Cu(II)-AFT complex *vs.* AFT solution; [Cu(II)]=8 × 10^{-6} M, [AFT] = 1.2 × 10^{-4} M, pH = 6.5.

Effect of pH on Cu(II)-AFT complex: The effect of pH on the formation of Cu(II)-AFT complex was studied to find out the optimum pH for copper(II) determination. The pH studies were carried out using HCl-KCl (pH 1.0-2.6), CH$_3$COONa-CH$_3$COOH (pH 3.4-6.5) and NH$_4$Cl-NH$_4$OH (pH 7.0-11.0) buffers.

The studies were carried out keeping the 2.0 mL of 2 × 10^{-4} mol L^{-1} copper(II) solution and 5.0 mL of 1 × 10^{-3} mol L^{-1} AFT solution constant and varying the pH values from 1.0 to 10.0 using suitable buffer solutions, keeping the volume constant adjusted to 25 mL with double distilled water. The absorbances of the complex were measured at 365 nm, using their corresponding reagent blanks. The plot between pH and

its absorbance is shown in Fig. 2. From the graph, the maximum absorbance value shown pH at 6.0. Hence, sodium acetate-acetic acid buffer is used for further studies, keeping 6 as the optimum pH.
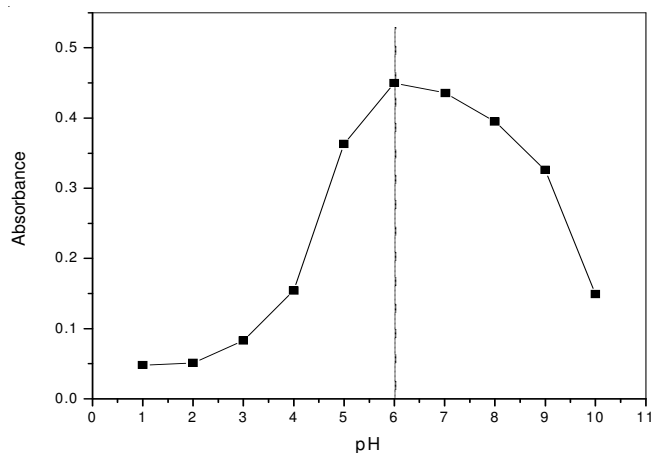

Fig. 2.   Effect of pH on the absorbance of Cu (II) - AFT complex [Cu (II)] = 1.6×10^{-5} M, [AFT] = 2×10^{-4} M, wavelength = 365 nm

Validity of Beer's law: A calibration graph suggests Beer's law obeyed within the range of 0.076-0.915 ppm of copper at 365 nm. The straight line obeys equation $A_{365} = 0.46623$ C + 0.0142. The molar absorptivity and sandell's sensitivity of the method is 3.33×10^4 L mol^{-1}cm^{-1} and 0.002 µg/ cm^2 of Cu(II), respectively (Fig. 3).
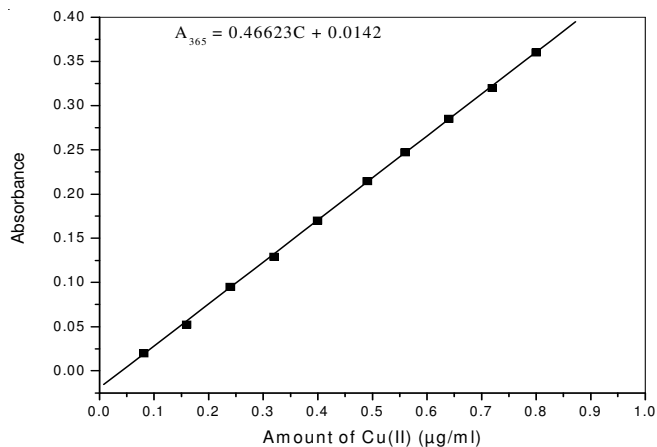

Fig. 3.   Calibration plot for Cu (II) determination pH = 6.5, [AFT]=6×10^{-4} M, Wavelength = 365 nm.

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 8×10^{-6} mol L^{-1} copper(II) solution and 10.0 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 1×10^{-2} 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 365 nm, against their corresponding reagent blanks. From the data revealed that a 15-fold molar excess of the reagent is required for maximum colour development.

Determination of the composition of Cu(II)-AFT complex: Spectrophotometric investigation of the metal complex was made to obtain the composition of the complex. Job's method of continuous variation and molar ratio methods were employed to elucidate the composition of the complex.

Jobs method of continuous variation: Equimolar solutions of copper(II) and the 2-acetylfuran thiosemi-carbazone (3×10^{-4} mol L^{-1}) were prepared. In a set of (nine) 25 mL volumetric flasks, 10 mL of buffer solutions and equimolar solutions of metal ion and reagent solutions in different volume proportions (keeping the volume at 10 mL) were added to each flask. The contents in each flask were made upto the mark with distilled water. The absorbance of the complex in each flask was measured at 365 nm wavelength against a corresponding reagent blank prepared under identical conditions. A plot between mole fraction of the reagent and the absorbance was made from which the composition of the complex was computed. From the Fig. 4, it is observed that 2 moles of reagent reacts with 1 mole of metal ion. Therefore the composition of the complex in solution is 1:2 (M:L).
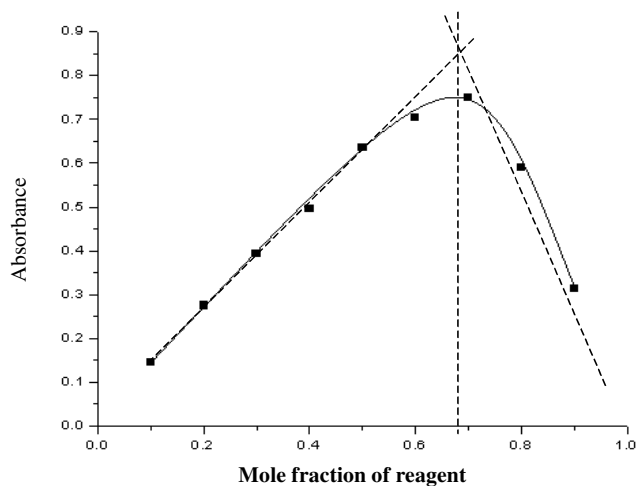

Fig. 4.   Job's curve Cu (II) = AFT = 3×10^{-4} (stock solution) wavelength = 365 nm, pH = 6.5

Molar ratio method: 10 mL of buffer solution, constant amount of metal ion (3.0 mL of 3×10^{-4} mol L^{-1}) and varying aliquots of the reagent (3×10^{-4} M) solutions were added (keeping the volume at 10 mL). The contents of each flask were made upto the mark with distilled water. The absorbance of the coloured complex in each flask was measured at 365 nm wavelength against the reagent blank prepared under identical conditions and a plot is drawn between the absorbance and the volume of the reagent, the composition of the complex was ascertained (Fig. 5). Molar ratio plot suggests the composition of the complex as 1:2 (M:L).

Based on the compositions of the complex determined in solution state, the structure tentatively assigned for the copper complex with 2-acetylfuran thiosemicarbazone (**I**).

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 2-acetylfuran thiosemi-carbazone. The effect of foreign ions was studied by measuring absorbance of the copper complex containing 0.38 µg/mL of

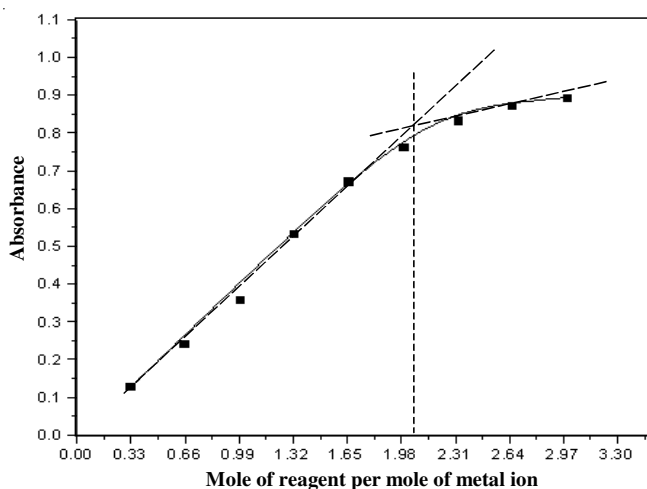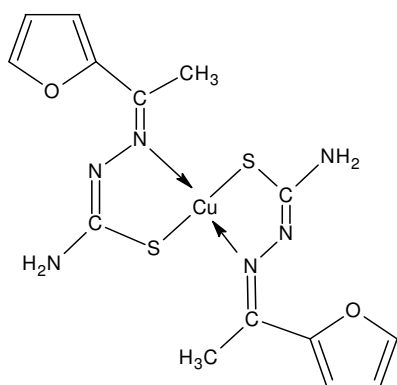Fig. 5. Molar ratio method of Cu(II)-AFT complex; Cu (II)= 3.6×10^{-5} M wavelength = 365 nm, pH = 6.5

I: Cu(AFT)$_2$ complex

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
EFFECT OF DIVERSE IONS IN THE DETERMINATION OF 0.38 µg/mL OF COPPER(II)

Ion Added	Tolerance limit µg/ mL	Ion Added	Tolerance limit µg/ mL
Tartarate	711	Cd (II)	54.0
Iodate	609	Mn (II)	26.0
Phosphate	470	Pb (II)	25.0
Citrate	384	Zn (II)	16.0
Bromide	381	Co (II)	14.0
Nitrate	298	Ni (II)	14.0
Urea	288	Cr (VI)	12.0
Bicarbonate	244	Hg (II)	4.00
Thiocyanate	232	Fe (III)	2.40[a]
Sulphate	230	Tl (III)	0.40
Chloride	170	Pt (IV)	0.39
Acetate	142	Au (III)	0.39
Fluoride	94	Fe (II)	0.22
Oxalate	18	Pd (II)	0.21
Thiourea	15	Ag (I)	0.21
		V (V)	0.20

[a]Masked with 70 µg/mL of fluoride

Applications of the developed method: The proposed method was applied for the determination of copper(II) in leafy

vegetable samples, biological samples and certified reference materials.

Determination of copper(II) in leafy sample: Leafy vegetable samples like thotakura (*Amaranthus gangeticus*), chukkaku (*Rumex vesicarius*), tutikura (*Ipomoea repens*), Cauliflower (*Brassica oleracea varbotrytis*), khesari (*Lartyrus sativus*) and medicinal leaves like neem (*Azadirachta indica*) and gaddi chamanti (*Tridax procumbens*) 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 Leaf samples	Amount of copper[a] found (µg/g in dried leaves)	
	AAS method	AFT method
Thotakura (*Amaranthus gangeticus*)	0.268	0.272
Chukkaku (*Rumex vesicarius*)	0.250	0.254
Tutikura (*Ipomoea repenss*)	0.265	0.269
Cauliflower green (*Brassica oleracea varbotrytis*)	0.296	0.291
Khesari (*Latryrus sativus*)	0.175	0.180
Medicinal leaves neem (*Azadirachta indica*)	0.266	0.269
Gaddi chamanti (*Tridax procumbens L*)	0.121	0.122

[a]Average of five determinations.

Determination of copper(II) in animal samples: Animal sample like fish and 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 IN LIVER SAMPLES

Liver sample	Amount of Copper [a] found (µg/g in dried liver)	
	AAS method	AFT method
Sheep liver	2.48	2.51
Fish liver	1.57	1.60

[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 1.5 mL of 0.01 M 2-acetylfuran thiosemicarbazone 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.

Composition of NKK-1021-alloy: 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 %. **Composition of NBS-SRM-54 D:** 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%. **Composition of BCS 180/2:** 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 %. **Composition of Devard's alloy:** Al-45; Zn-5 and Cu-48.91%. **Composition**

TABLE-4
DETERMINATION OF COPPER IN ALLOYS

Sample	Copper (%)		Error (%)
	Certified	Found*	
NKK-1021-Alloy[a]	2.72	2.69	- 1.10
NBS-SRM–54 D[b]	3.62	3.64	+ 0.50
BCS 180/2[c]	68.12	68.4	+ 0.40
Devard's alloy[d]	48.91	48.75	- 0.30
Tin base white metal [e]	4.58	4.54	- 0.87

*Average of three determinations.

of tin base with metal: Sn-82.2; Cu-4.58; Ni-0.17; Bi-0.11; Fe-0.024; Sb-9.45; Pb-3.18; Cd -0.14 and Zn-0.40 %.

Conclusion

The literature survey revealed that many thiosemicarbazones were used for the determination of copper(II). Studies upon the use of 2-acetylfuran thiosemicarbazone as an analytical reagent are limited. Hence, the present investigations were carried out with a view to test the potentiality of 2-acetylfuran thiosemicarbazone 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.

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