Non-Extractive Spectrophotometric Determination of Copper in Leafy vegetable samples Using 2, 4-Dihydroxyacetophenone Acetoylhydrazone (DAAH)

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Abstract

A very simple, highly selective and non-extractive spectrophotometric method for the trace amounts of copper(II) has been developed. 2,4-Dihydroxyacetophenone acetoylhydrazone(DAAH) has been proposed as a new analytical reagent for the direct non-extractive spectrophotometric determination of copper(II). The reagent reacts with copper(II) in acidic medium(pH 4.0) to form a pale yellow coloured 1: 1 (M : L) complex. The reaction is instantaneous and the complex shows maximum absorbance at 370 nm. The molar absorptivity and Sandell's sensitivity are found to be 1.02×10^4 L mol^{-1} cm^{-1} and 0.623 μg cm^{-2} respectively. The system obeys Beer's law in the range, 0.2- 2.0 μg/ml of copper(II). The method is highly selective for copper and successfully used for determination of copper in alloys.

Keywords: Spectrophotometric determination, 2,4-dihydroxyacetophenone acetoylhydrazone, Alloy and steel samples.

1. Introduction

Hydrazones are potential and interesting analytical reagents [1-3]. These reagents are frequently used for the spectrophotometric determination of metal ions. However, 2,4-dihydroxyacetophenone acetoylhydrazone (DAAH) is not so far used for the spectrophotometric determination of copper. Therefore, it is of interest to develop spectrophotometric method for the determination of copper using DAAH. Copper is widely utilized in electrical industries and in making industrially useful alloys. Hence determination of copper in alloys has been regarded as an interesting research activity. In the light of the above, and in continuation of our previous work [4-6], herein we report synthesis, characterization and spectrophotometric properties of DAAH. The developed DAAH method is applied for the determination of copper(II) in leafy vegetables..

2. Materials And Methods

Materials

2,4-Dihydroxyacetophenone and acetohydrazide were procured from Merck, India. Ethanol of AR grade, Merck and used as received. Solvent like N, N-dimethylformamide used after distillation.

Synthesis of reagent 2, 4-Dihydroxyacetophenone Acetoylhydrazone(DAAH)

2,4-Dihydroxyacetophenone (1.52 g, 0.01 mol, dissolved in 5 ml of ethanol) and acetohydrazide (0.74g, 0.01 mol dissolved in 3 ml of ethanol) were mixed in a clean round bottom flask. Suitable quantity(10 ml) of ethanol was added to the reaction mixture and refluxed with stirring for 3 hrs. The pale brown coloured product was separated out on cooling. It was collected by filtration, washed several times with hot water. This compound was recrystallized from methanol and dried in vacuum. The yield was found to be 78 % ; m.p.

DAAH
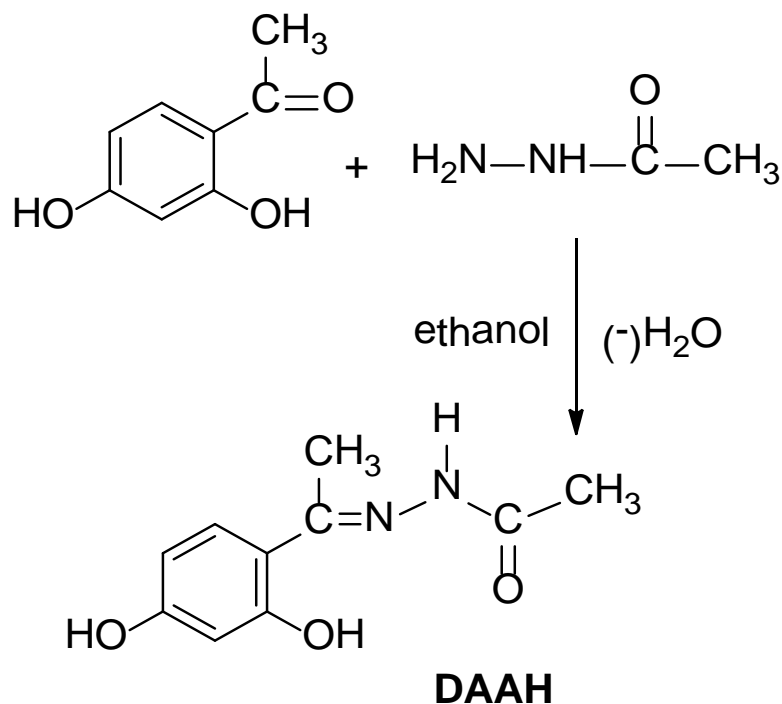
Preparation of reagent solution

The reagent solution (0.01M) was prepared by dissolving 0.052gm of the compound in 10 ml of dimethylforamide (DMF) in 25-ml volumetric flask. The reagent solution was found to be stable for 10hrs.

Preparation of Copper (II) solution

A 1×10^{-2} M stock solution of divalent copper was prepared by dissolving requisite quantity (0.20 g) of $Cu(CH_3COO)_2$ H_2O in doubly distilled water containing few drops of glacial CH_3COOH and made up to mark in 100- ml volumetric flask. The stock solution was standardised gravimetrically [7]. Dilute solutions were prepared from this stock solution. Solutions of large number of inorganic ions, complexing agents were prepared from their analaR grade (or) equivalent grade water soluble salts.

Procedure

An aliquot of the solution containing copper in optimum concentration range , 10ml of buffer solution (pH 4.0) and 1ml of 0.01 M reagent solution were combined in 25ml volumetric standard flask and resulting solution was diluted to the mark with distilled water. The absorbance of the solution was measured at 370 nm against reagent (DAAH) blank. The measured absorbance was used to compute the amount of copper from predetermined calibration plot.

Preparation of Leafy Vegetable Sample

The established optimized conditions of extractive spectrophotometric method were applied to leafy vegetables, for the determination of copper(II) content. The leafy vegetables analyzed were brought from the local market during the month of January. The samples were cleaned and dried in open air, protecting them from mineral contamination. The dried sample was pulverized in a mortar for the purpose of analysis, to a convenient size. Ten grams of each powdered sample was taken into a silica crucible, heated to oxidize the organic matter, and ashed at 550 °C in a muffle furnace for 4-5 h.

The ash was dissolved by heating with 10 mL 2N hydrochloric acid and filtered through an acid-washed filter paper (Whatman no. 41), and then the residue was washed with hot water. The filtrate and washings were collected in a 25 mL volumetric flask and finally made up to the mark with double-distilled water.

Procedure

An appropriate aliquot was analyzed for copper(II) by the recommended procedure using DAAH from the leafy vegetable was extracted and absorbance values are measured against reagent blank prepared under the similar conditions at 370nm . The process was repeated four times for each sample, and the results obtained were confirmed by direct atomic absorption spectrometer. The results obtained are presented in (Table 4).

Apparatus

A Perkin – Elmer (Lamda 25), UV – Visible spectrophotometer equipped with 1.0- cm (path length) quartz cell and ELICO model LI- 610 pH meter were used in the present study.

3. Results and Discussion

Characterization of reagent 2,4-Dihydroxyacetophenone acetoylhydrazone (DAAH)

The newly synthesized reagent 2,4-Dihydroxyacetophenone acetoylhydrazone (DAAH) is characterized using IR, NMR and Mass spectral data.

IR spectra

Infrared spectrum (Fig 1) shows sharp strong peak at 3461 cm^{-1} may be assigned for the stretching vibrations of –OH and 3235 cm^{-1} may be assigned for the stretching of secondary –NH groups. The sharp peaks appeared at 1662 cm^{-1} may be assigned for stretching vibrations of amide –C=O group. The band at 1627 cm^{-1} may be assigned for plane bending vibration of –NH group, band at 1581 cm^{-1} may be assigned for streching vibration of azomethine –C=N group, band at 1451 cm^{-1} may be assigned for asymmetric bending vibration of -CH group, band at 1296 cm^{-1} may be assigned for stretching vibration of –CN group, band at 1269 cm^{-1} may be assigned for stretching vibration of -CN group (coupled), band appeared at 751 cm^{-1} is assigned for oop bending of aromatic –CH group and band at 695 cm^{-1} may be assigned for stretching vibrations of – OCN deformation.

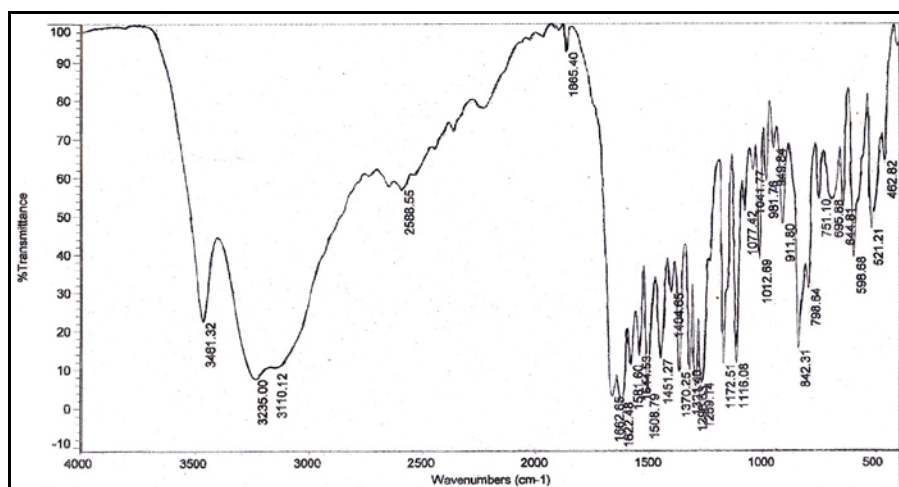**Figure 1: Infrared Spectrum of DAAH in KBr disc**

^{1}H – NMR spectra

The ^1H – NMR spectrum (Fig . 2)of DAAH (in DMSO –d_6) showed signals at (δ ppm) 2.02, 2.26, 6.25. 7 36, 9.80 and 10.76 due to -CH_3 protons, acetoyl [-$COCH_3$] protons, phenyl protons, imine protons, p-phenolic and o-phenolic protons respectively..
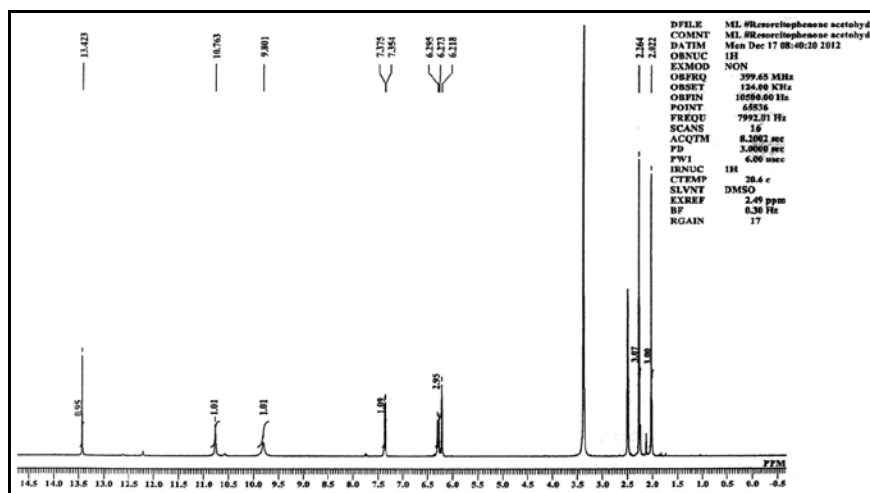

Figure 2: ^1H-NMR Spectrum of DAAH in DMSO – d_6 medium

Mass spectra

Mass spectrum (Fig. 2) of DAAH shows signal at m/z 208 due to the formation of molecular ion. The peaks observed at m/z values of 193 and 165 are due to the loss of –CH_3 and -$COCH_3$ radicals respectively.
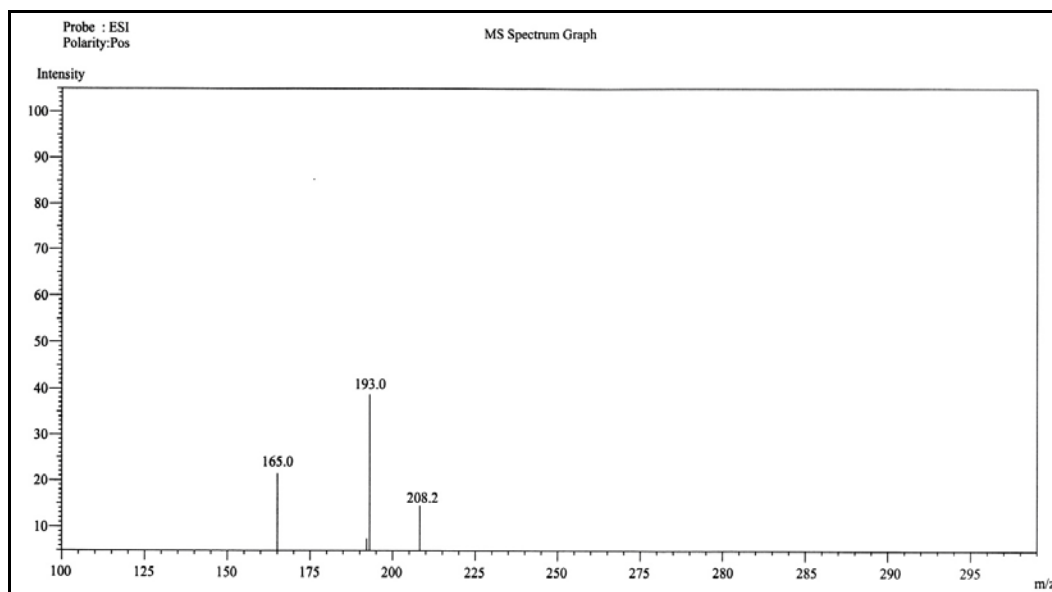

Figure 3: Mass spectrum of DAAH

UV-Visible spectra

Absorption spectra (Fig. 4) of 2 x 10 $^{-5}$M DAAH solution at different pH values were recorded and pKa values were determined spectrophotometrically using Phillip and Merritt method [8]. The bathochromic shift from 295 – 365 nm indicates that in solution on increasing pH the >C=0 group of the reagent (DAAH) is enolised and dissociated. The values of DAAH are 5.4 (pK_1) and 8.5 (pK_2) respectively. The pK_1 and pK_2 values are presumably due to keto – enol tautomerism and deprotonation of - NH group respectively.
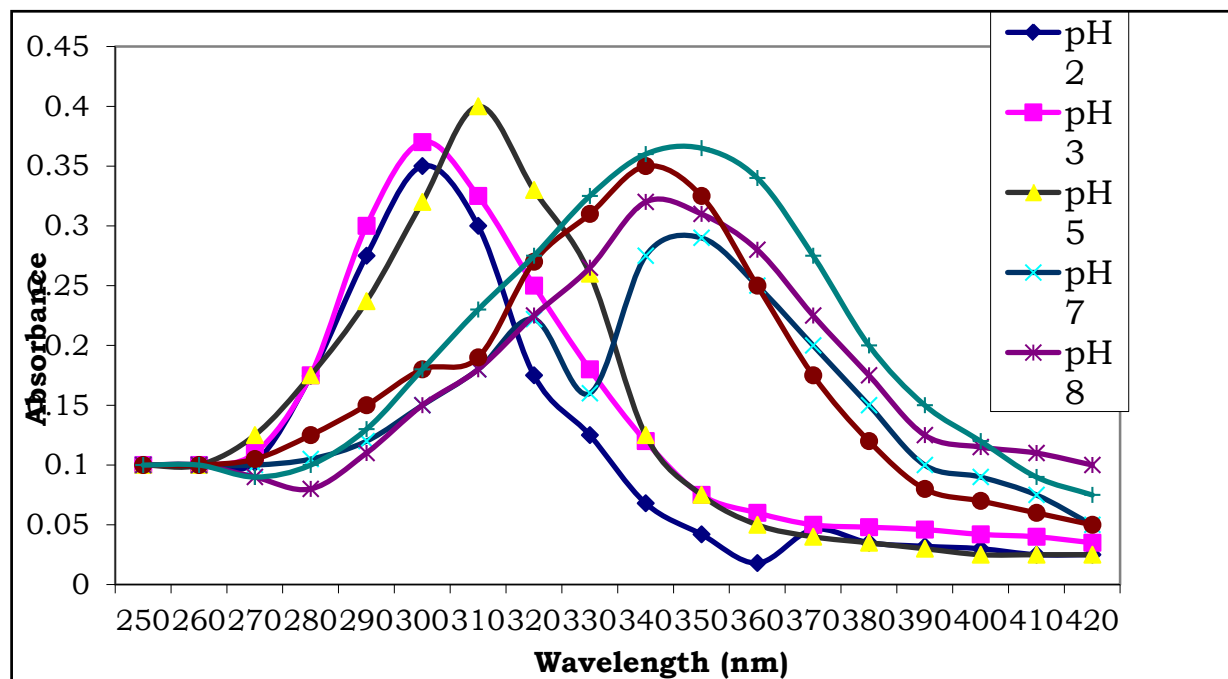
Figure .4. Absorption spectra of 2×10^{-5} M at different pH values

Effect of reagent concentration

The data (Table .1) indicate that a 10- fold molar excess of reagent is sufficient for full colour development.

Table 1: Effect of reagent (DAAH) concentration on the absorbance of the complex at 370 nm

Cu(II) : DAAH	Absorbance
1 : 05	0.460
1 : 10	0.470
1 : 20	0.484
1 : 40	0.492
1 : 60	0.500

Effect of time

The absorbance of Cu(II) – DAAH complex was measured at different time intervals to ascertain the time stability of the complex .The absorbance of the Cu(II) complex was measured at 370 nm. The colour development is instantaneous and the absorbance of the complex remains constant for 3 hrs and there after showed gradual decrease in intensity with increasing time.

Effect of pH

The effect of pH on the colour intensity of Cu(II) – DAAH complex is studied . A plot between absorbance of the complex at 370 nm and pH is shown in Fig 5. The graph indicates that the complex shows maximum and constant absorbance in the pH range 3.5 – 4.5. Hence, buffer solution of pH 4.0 is chosen for subsequent studies.
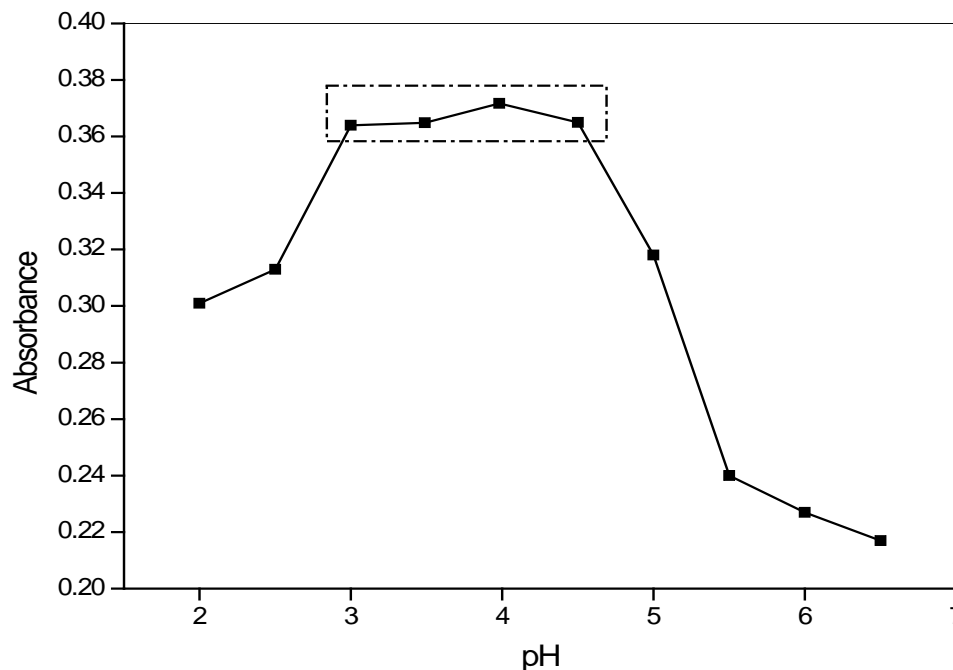
Figure 5: Effect of pH on the absorbance of Cu(II)-DAAH complex.

Adherence to to Beers law, Molar absorptivity, and Sandell's sensitivity

The system obeys Beer's law in the range of 0.2 - 2.0 μg/ml of copper (II). The calibration plot is shown in Fig 6. Molar absorptivity and Sandell's sensitivity of the method are found to be 1.02×10^4 L mol-1 cm-1 and 0.623 μg . cm^{-2} respectively.
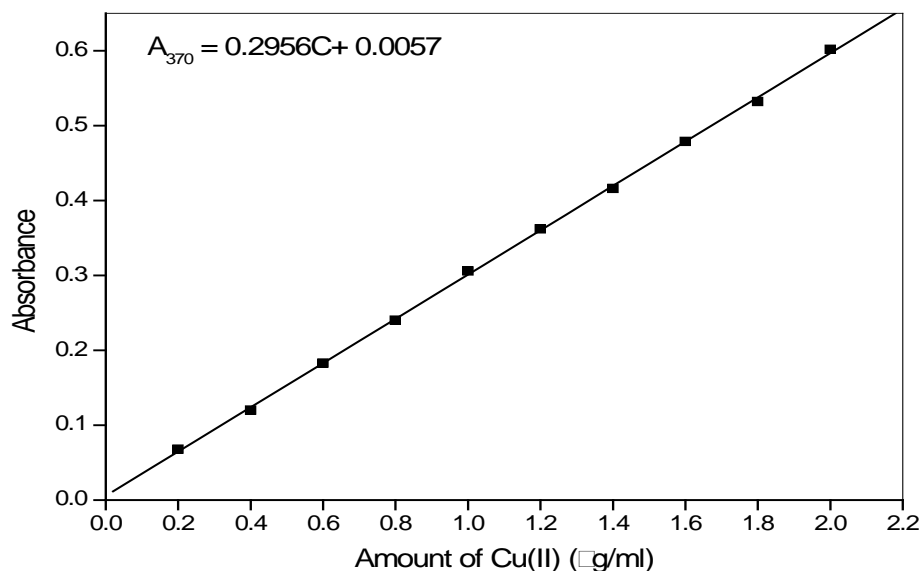
$$A_{370} = 0.2956C + 0.0057$$

Fig. 6. Calibration plot for Cu(II) determination

Precision

The precision of the method was checked by ten replicate analysis of sample each containing 2.12 ppm of copper. The standard deviation and relative standard deviation are found to be ± 0.0059 and ± 1.75% respectively.

Effect of foreign ions

The validity of the method was assessed by investigating the effect of various cations and anions on the determination of copper(II). The amount of foreign ion which brings about a change in absorbance by ± 2% was taken as its tolerance limit and results are given in Table 2.

Table 2. Tolerance limit of foreign Ions in the determination of 1.27 µg/ml of copper

Ion added	Tolerance limit µg/ml	Ion added	Tolerance limit µg/ml
Edta	615	Mg(II)	28
Tartrate	592	Zn(II)	26
Citrate	326	Ni(II)	24
Sulphate	307	Mn(II)	22
Iodide	252	Mo(II)	19
Bromide	240	Fe(II)	11
Thiourea	152	Hg(II)	8[a]
Chloride	142	Co(II)	2.3
Nitrate	124	Ag(I)	2.2
Fluoride	76	Cu(II)	1.9
Phosphate	30	Al(III)	0.5
Oxalate	18	Fe(III)	0.4[b]

A. Masked with 200 µg/ ml of iodide.

B. Masked with 450 µg/ ml of cyanide.

Larger amounts of Hg(II) and Fe(III) do not interfere in the presence of masking agents. Interference of molybdenum(IV) and iron(III) are masked with iodide and cyanide respectively.

Determination of composition of the complex

The composition of the complex (M : L = 1 : 1) was determined by Job's continuous variation method and molar ratio method . The plots are shown in Fig.7 and Fig. 8 respectively, The dissociation constant (α) and concentration (c) of the

reagent at intersecting point were used in the calculation of stability constant of the complex. Stability constant of the complex 1:1 (M : L) complex is given by 1-α /4α3c2. The predicted structure of Cu(II) – DAAH is given in Fig .9.
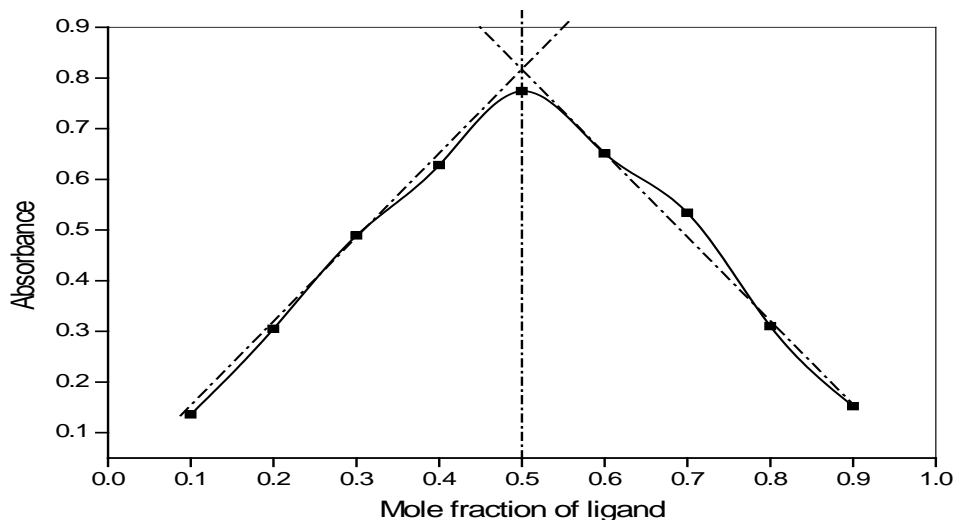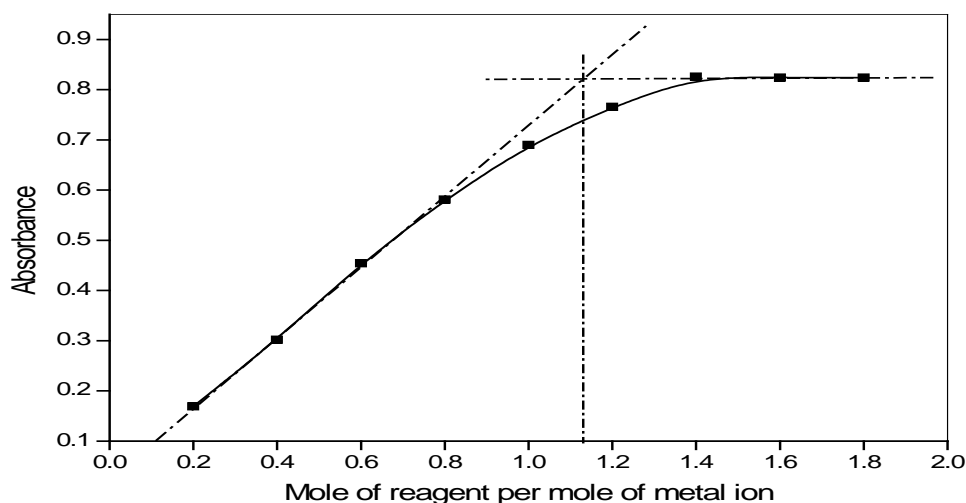

Figure 6. Job's curve
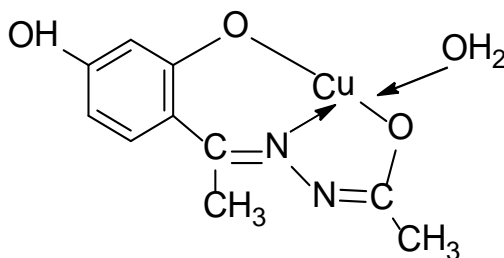

Figure .7. Molar ratio plot.

Fig .9. Structure of Cu – DAAH complex

Various physico – chemical and analytical characteristics of copper complex are summarized in Table 3.

Table 3: Physico – Chemical and Analytical Characteristics of Cu(II) Complex with DAAH

S. No.	Characteristics	Results
1	λ_{max} (nm)	370
2	pH range (optimum)	3.0 – 4.5
3	Mole of reagent required per mole of metal ion for full colour development	5 fold
4	Time stability of the complex (in hrs)	2
5	Beer's law validity range (µg/ml)	0.2 – 2.0
6	Molar absorptivity (lit mol^{-1}cm^{-1})	1.02×10^4
7	Specific absorptivity (ml g^{-1}cm^{-1})	0.160
8	Sandell's sensitivity µg of Cu(II) cm^{-2}	0.623
9	Composition of the complex as obtained in Job's and molar ratio methods	1 : 1
10	Stability constant of the complex	1.08×10^5
11	Mean absorbance	0.230 ± 0.0005
12	Standard deviation in the determination of 1.27 µg/ml of Cu(II) for ten determinations	0.0059
13	Relative Standard deviation (RSD) %	1.75
14	Y–intercept	0.0057
15	Angular coefficient	0.5912
16	Detection limit (µg/ml)	0.0523
17	Determination limit (µg/ml)	0.156

Applications

The amount of copper present in certain leafy vegetable samples was determined by the developed method. The amount of the metal ion in leafy vegetable samples given in Table 4.

Table 4: Determination of Copper(II) in leafy vegetable samples using DAAH

Name of the sample	Amount of copperII) added (µg/g)	Amount of cooper(ii) found[a] (µg/g)		SD	RSD(%)
		AAS method	Present method		
Cucumber (Cucumis sitivas)	10.00	10.52	10.51	0.03	0.28
Green peas (Pisum sativam)	10.00	11.15	11.14	0.05	0.43
Fresh bean (Dolichos lablab)	10.00	10.88	10.87	0.04	0.37
White raddish (Raphanus sativus)	10.00	14.21	14.20	0.06	0.42

a= average of four replicants. n = 4

4. Conclusion

A comparison of Spectrophotometric methods [9 - 14] for the determination copper is given in

Table 5. The data suggest that the present method seems to rank among the sensitive methods.

Table 5: Comparison of Spectrophotometric Methods for the Determination of copper (II)

S.No	Name of the reagent	λ_{max}(nm)	pH range	Determination of Cu(II) (µg/ml)	ε $x10^4$ (L mol^{-1}cm^{-1})	Ref
1	Eosin-Perindopril	535	-	-	6.55 X 10^3	9
	Ramipril	535	-	-	4.0 X 10^3	
2	3-{2-[2-(2-hydroxy imino-1-methyl-propylidene amino) -ethyl-imino-butan-2-one oxime	570	alkaline		0.16 X 10^4	10
3	1-phenyl-1-hydrazonyl-2 -oximino propane-1,2-dione	345	9.4		0.35x10^3	11

4	Acetophenone-p-chloro – phenyl thiosemicarbazone	4.0-9.0	0.25-6.35	-	$5.5x10^3$	12
5	Cefixime	336	1.015-1.822	-	$8.29X10^3$	13
6	N-(o-hydroxy benzilidine – 4-methyl aniline) arabanoze	-	5.8 – 6.8	1.0 – 20.0	$0.89X10^3$	14
7	2,4-Dihydroxyacetophenone acetoylhydrazone	370	4.0	0.2-2.0	$1.0 X 10^4$	PM

PM – Present method

The present method is simple, rapid and more sensitive than other reported methods for determination of copper. It is successfully applied for the determination of copper in alloy samples.

5. Acknowledgement

6. References

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