

Non-Extractive Spectrophotometric Determination of Copper in Vegetable samples Using 2,4-Dihydroxyacetophenone acetylhydrazone (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 acetylhydrazone(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 $1.02 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.623 \mu\text{g cm}^{-2}$ respectively. The system obeys Beer's law in the range, 0.2- 2.0 $\mu\text{g/ml}$ of copper(II). The method is highly selective for copper and successfully used for determination of copper in vegetable samples.

KEYWORDS: Spectrophotometric determination, 2,4-dihydroxyacetophenone acetylhydrazone, molar absorptivity vegetable samples.

I.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 acetylhydrazone (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 presence in the atmosphere, soil and water even in traces can cause serious problems to all organisms due to the bio accumulation in the food chain especially can be dangerous to the human health. The copper is one of the persistent environmental contaminants which may be deposited on the surfaces and then adsorbed into the tissues of vegetables. Plants take up copper by absorbing them from deposits on the parts of the plants exposed to the air from polluted environment as well as contaminated soil. 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 various vegetable samples.

II.MATERIALS AND METHODS

A. 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.

B.Synthesis of reagent 2,4-Dihydroxyacetophenone acetylhydrazone(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. 243 – 245°C. The reaction route for the synthesis is shown in Fig 1.

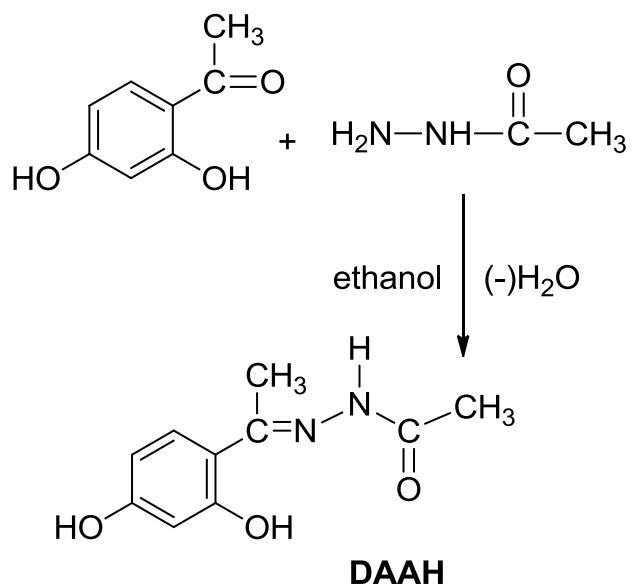


Fig1 Synthesis of ligand (DAAH)

C.Preparation of reagent solution

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

D.Preparation of copper(II) solution

A 1×10^{-2} M stock solution of divalent copper was prepared by dissolving requisite quantity (0.20g) of Cu(CH₃COO)₂ H₂O in doubly distilled water containing few drops of glacial CH₃COOH 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.

E.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.

F. Digestion procedure for vegetable samples

The aqua regia method involving 6 mL of HNO₃-HCl and 0.2 g samples using a conical beaker heated on a hot plate at 100 °C for 4 h was used to digest the soil samples (Hseu et al.2002; Arain et al. 2008). About 0.5 g dried vegetable samples were weighed in Pyrex flasks and treated with HNO₃-H₂O₂ (2:1) on hot plate at 100 °C for 3 h for decomposition of vegetable samples (Jalbani et al. 2007). The solutions were made up to 25 mL with distilled water and analyzed with F-AAS. The working calibration solutions were made up from 1000 mg/L certified standards and within the recommended linear ranges. The regression values (R^2) of the calibration curve was >0.999. The

concentration of Cu in the digested samples was determined at a wavelength of 370 nm using an air-acetylene flame. The analyses were conducted in triplicate and the results presented as mean \pm SD.

G. Apparatus

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

III.RESULTS AND DISCUSSION

A. 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.

1)IR spectra

Infrared spectrum (Fig 2) shows sharp strong peak at 3461 cm^{-1} may be assigned for the stretching vibrations of $-\text{OH}$ and 3235 cm^{-1} may be assigned for the stretching of secondary $-\text{NH}$ groups . The sharp peaks appeared at 1662 cm^{-1} may be assigned for stretching vibrations of amide $-\text{C=O}$ group. The band at 1627 cm^{-1} may be assigned for plane bending vibration of $-\text{NH}$ group, band at 1581 cm^{-1} may be assigned for stretching vibration of azomethine $-\text{C=N}$ group, band at 1451 cm^{-1} may be assigned for asymmetric bending vibration of $-\text{CH}$ group, band at 1296 cm^{-1} may be assigned for stretching vibration of $-\text{CN}$ group, band at 1269 cm^{-1} may be assigned for stretching vibration of $-\text{CN}$ group (coupled), band appeared at 751 cm^{-1} is assigned for oop bending of aromatic $-\text{CH}$ group and band at 695 cm^{-1} may be assigned for stretching vibrations of $-\text{OCN}$ deformation.

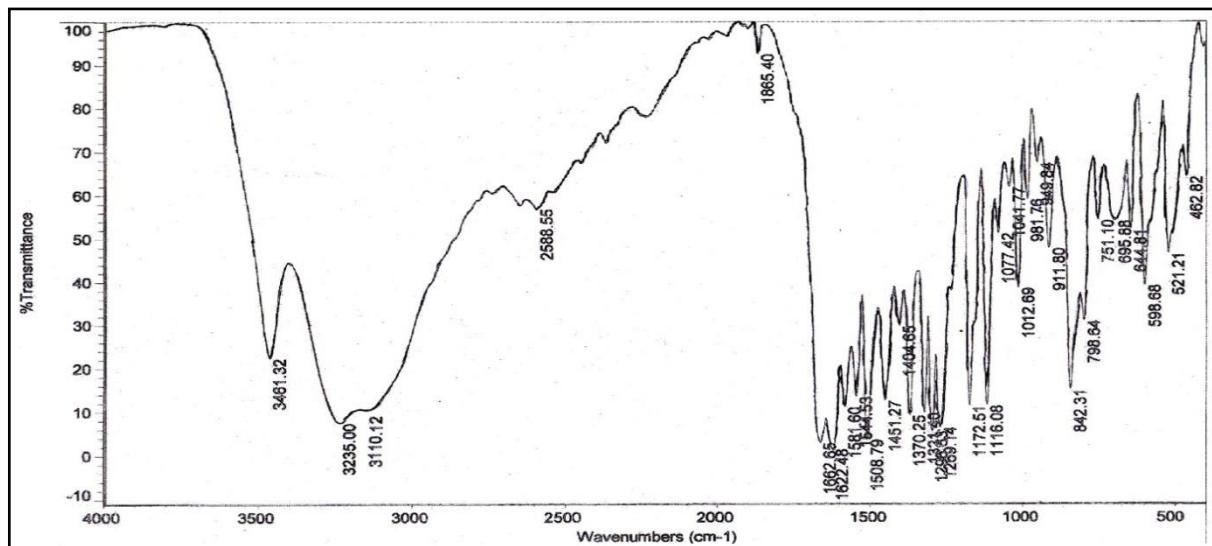


Fig.2. Infrared Spectrum of DAAH in KBr disc

2) ^1H – NMR spectra

The ^1H – NMR spectrum (Fig 3) of DAAH (in $\text{DMSO}-\text{d}_6$) showed signals at (δ ppm) 2.02, 2.26, 6.25, 7.36, 9.80 and 10.76 due to $-\text{CH}_3$ protons, acetoyl $[-\text{COCH}_3]$ protons, phenyl protons, imine protons, p-phenolic and o-phenolic protons respectively.

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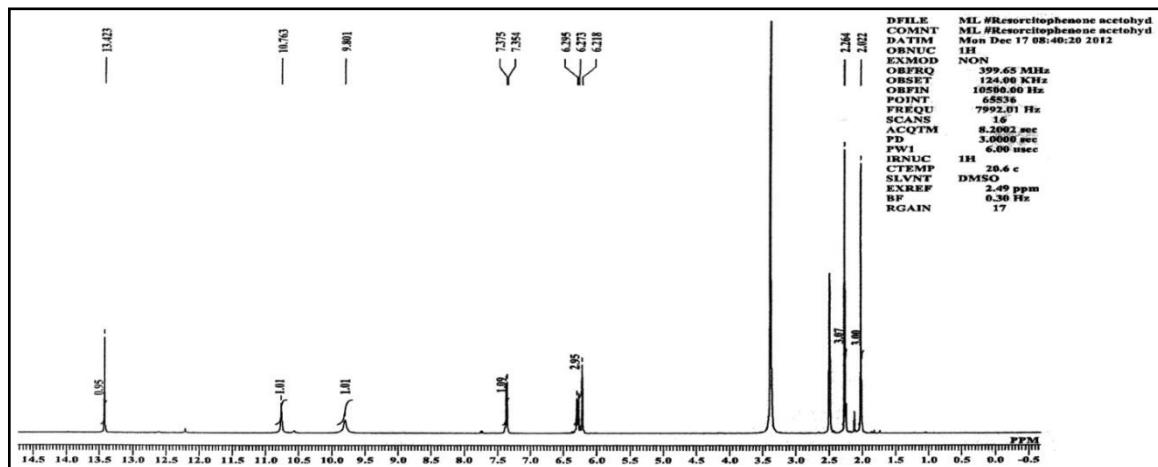


Fig.3. ^1H -NMR Spectrum of DAAH in $\text{DMSO}-\text{d}_6$ medium

3) Mass spectra

Mass spectrum (Fig. 4) 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 $-\text{CH}_3$ and $-\text{COCH}_3$ radicals respectively.

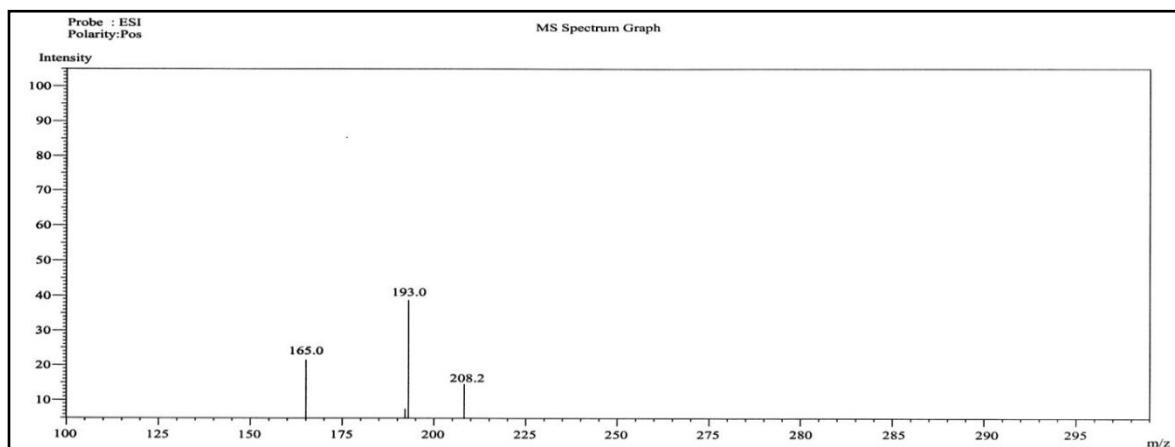


Fig4. Mass spectrum of DAAH

4) UV-Visible spectra

Absorption spectra (Fig 5) of $2 \times 10^{-5}\text{M}$ DAAH solution at different pH values were recorded and pK_a 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 $>\text{C}=\text{O}$ 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 $-\text{NH}$ group respectively.

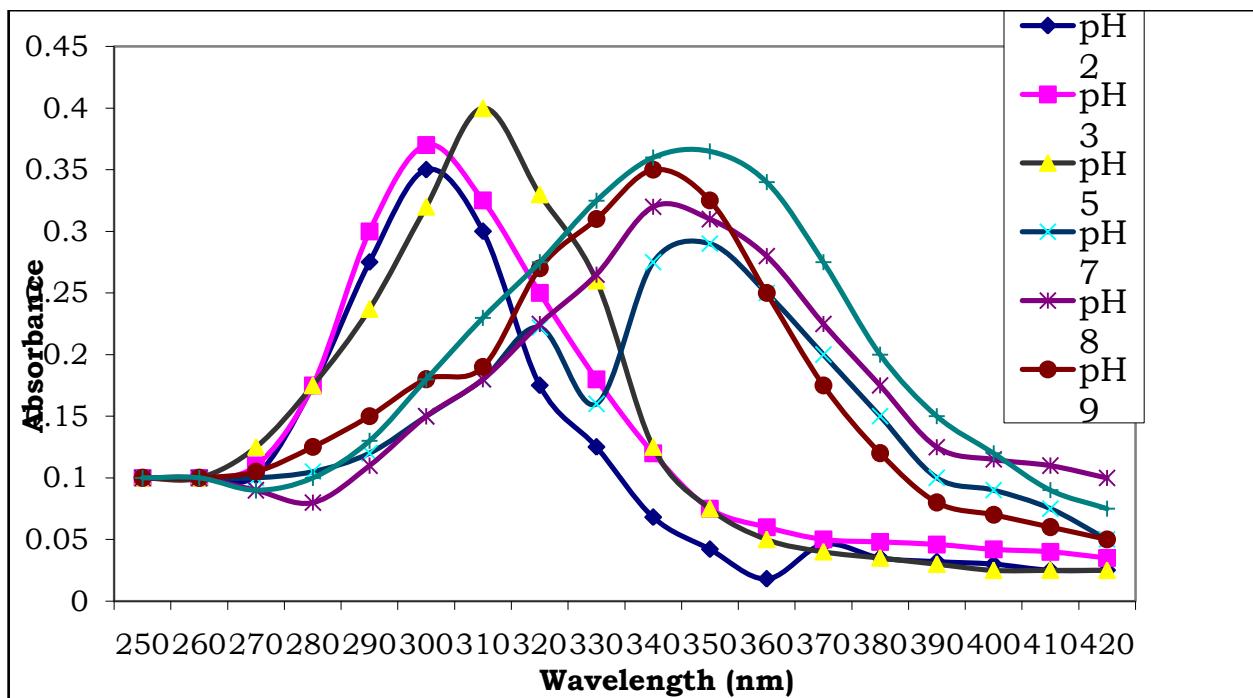


Fig. 5. Absorption spectra of 2×10^{-5} M of DAAH at different pH values

B .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.

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

C. 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.

D. Effect of pH

The effect of pH on the colour intensity of Cu(II) – DAAH complex is studied . A plot between absorbance at of the complex at 370 nm and pH is shown in Fig 6. 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.

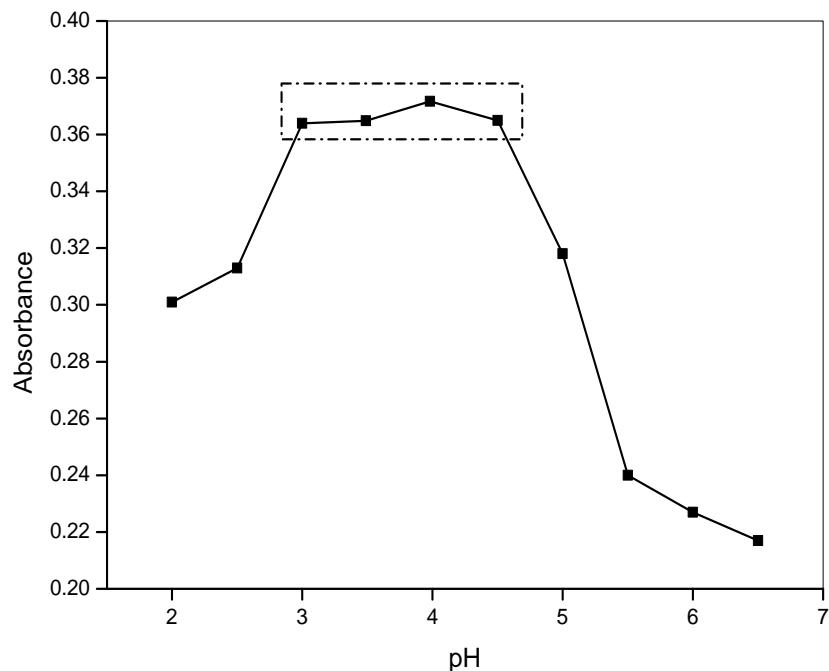


Fig: 6. Effect of pH on the absorbance of Cu(II) – DAAH complex

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

The system obeys Beer's law in the range of 0.2 - 2.0 $\mu\text{g}/\text{ml}$ of copper (II). The calibration plot is shown in Fig.7. Molar absorptivity and Sandell's sensitivity of the method are found to be $1.02 \times 10^4 \text{ L mol}^{-1}\text{cm}^{-1}$ and $0.623 \mu\text{g} \cdot \text{cm}^{-2}$ respectively.

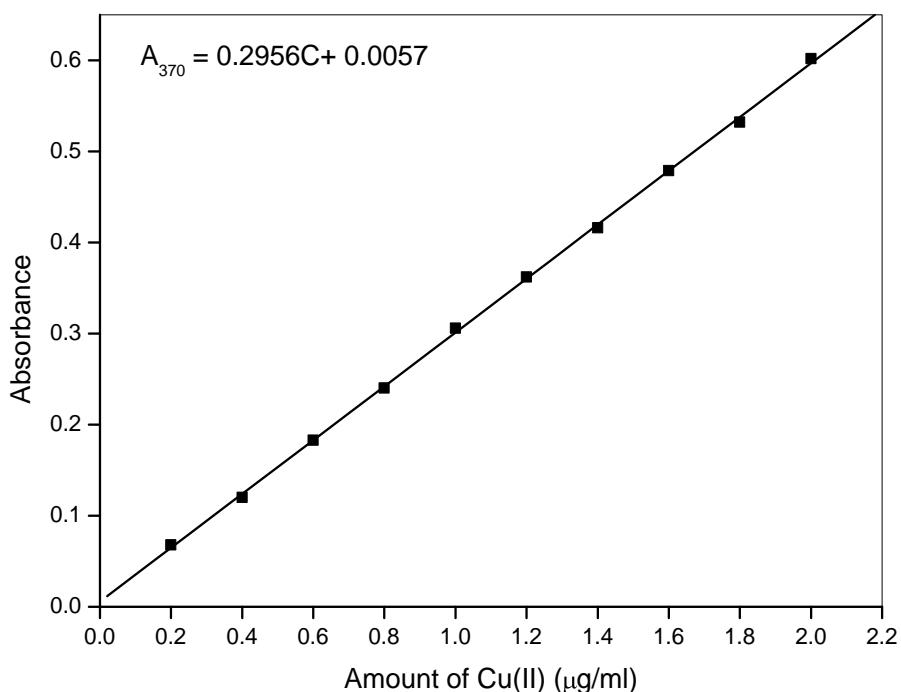


Fig.7. Calibration plot for Cu(II) determination

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F. 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 $\pm 1.75\%$ respectively.

G. 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 $\pm 2\%$ 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 $\mu\text{g}/\text{ml}$ of copper

Ion added	Tolerance limit $\mu\text{g}/\text{ml}$	Ion added	Tolerance limit $\mu\text{g}/\text{ml}$
Edta	15	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 $\mu\text{g}/\text{ml}$ of iodide.

^b Masked with 450 $\mu\text{g}/\text{ml}$ 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.

H. 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 6 and Fig 7 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 $1-\alpha /4\alpha^3 c^2$. The predicted structure of Cu(II) – DAAH is given in Fig 8 and the Various physico – chemical and analytical characteristics of copper complex are summarized in Table 3.

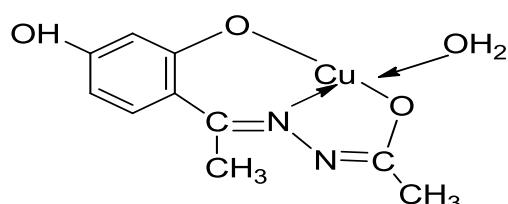


Fig 8. The structure of Cu-DAAH Complex

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Table 3. The Physico – chemical and analytical characteristics of Cu(II) – DAAH complex

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 ($\mu\text{g/ml}$)	0.2 – 2.0
6	Molar absorptivity ($\text{lit mol}^{-1}\text{cm}^{-1}$)	1.02×10^4
7	Specific absorptivity ($\text{ml g}^{-1}\text{cm}^{-1}$)	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 $\mu\text{g/ml}$ 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 ($\mu\text{g/ml}$)	0.0523
17	Determination limit ($\mu\text{g/ml}$)	0.156

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IV.APPLICATIONS

The present method was successfully applied for the determination of copper in various vegetable samples and the results were presented in Table 4.

Table 4: Determination of Cu(II) (mean \pm SD, n = 3) in vegetable samples

Sample	Amount of copper* found in $\mu\text{g/g}$	
	F-AAS method	DAAH
Tomato	5.3 \pm 0.32	5.6 \pm 0.41
Cabbage	8.0 \pm 0.26	8.4 \pm 0.35
Spinach	15 \pm 0.40	16 \pm 0.42

* Average of three determinations

VII.CONCLUSION

After reviewing the above mentioned studies it may be concluded that monitoring and assessment of copper concentrations in different vegetables market as well as production sites require more extensive studies for assessing the risk of health hazards to the human beings. It is therefore suggested that regular monitoring of heavy metals in plant tissues is essential in order to prevent excessive build-up of copper in the human food chain and this method is the one of the sensitive method to for the determination of the copper in the various vegetable samples.

VIII. ACKNOWLEDGEMENT

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