A novel method for the spectrophotometric determination of Cu(II) using salicylaldehyde acetoacetic acid hydrazone as the reagent. Application to water, alloys and grape leaves

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Abstract. Salicylaldehyde acetoacetic acid hydrazone (SHZN) reacts with copper (II) to form a highly stable 1:1 complex in a medium pH 2 at room temperature. The complex has maximum absorption at 425 nm with a molar absorptivity coefficient of 22.5×10^4 L mol^{-1} cm^{-1} and stability constant 9.947×10^8. A visible spectrophotometric method using SHZN was developed and the reaction conditions (pH, stability of the complex, amount of reagent required) were optimized and corresponding linearity range (0.0499 – 0.4994 µg/mL), Sandell sensitivity (0.0002824 µg cm^{-2}) and tolerance limit of various foreign ions were reported. The method was simple, sensitive and accurate and was successfully applied for the determination of copper (II) in synthetic water samples, grape leaves and alloy samples.

Keywords: Copper (II), Salicylaldehyde acetoacetic acid hydrazone, Spectrophotometry, Synthetic water samples, Grape leaves, Alloys.

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1. Introduction

Copper is an essential metal for plants, microorganisms, animals and human beings to perform specific biological functions. Copper is often added to fertilizers to serve as a supplement to plants. The failure to supply adequate amounts of copper leads to a variety of biochemical and physiological disorders in plants [1, 2]. In humans, it not only facilitates the conversion of iron to haemoglobin but also stimulates the growth of red blood cells. Copper is an integral part of certain digestive enzymes. Copper deficiency [3, 4] results in bodily weakness, digestive disturbances and impaired respiration. The effects of deficiencies vary depending on the nature of the living being, the factors affecting metal concentrations and the level of deficiency [5]. As a toxicant at elevated levels of biologically available form, it produces a physiological response. However, excess copper because of its potential incorporation in component organisms of food webs is of concern [6, 7].

Hence there is a need for rapid and sensitive methods for the analytical determination of copper. When appropriate complexing agents are employed, spectrophotometric methods usually provide inexpensive, rapid and sensitive procedures for the metal determinations. Many such already reported complexing agents [8-17] for the determination of copper and respective draw backs are listed in the **Table 1**. The aim of this article is to propose a rapid, selective and sensitive method for the determination of trace amounts copper (II) by employing salicylaldehyde acetoacetic acid hydrazone as complexing agent.

2. Experimental

2.1. Materials and Methods
All chemicals and reagents used were of analytical reagent grade obtained from Merck, India.

Table 1: Comparison with the reported methods

Reagent	λ_{max} (nm)	Beer's law range	Molar absorptivity $L\ mol^{-1}cm^{-1}$	M:L	Remarks	Ref. No.
2,2′-dipyridyl-2-pyridylhydrazone	448	Less than 1 ppm	3.8×10^4	1:1	Severe interferences, poor sensitivity and expensive reagent	8
2, 4-Dihydroxy benzo phenone benzoic acid hydrazone	410	0.31 - 2.20 µg/mL	1.55×10^4	1:1	Poor sensitivity	9
Benzil- α-monoxime isonicotinoyl hydrazone	346	0.50 - 5.08 mg/mL	1.2×10^4	1:1	Poor sensitivity	10
2, 5-dihydroxy aceto phenone benzoic acid hydrazone	400	0.30 - 6.00 µg/mL	1.1×10^4	1:1	Poor sensitivity	11
3-Methoxy, 4-hydroxy benzaldehyde 4-bromophenyl hydrazone	462	2.0 - 4.0 µg/mL	2.0520×10^4	1:1	Extraction step, poor sensitivity	12
2-Pyridine Carboxaldehyde Isonicotinyl Hydrazone	352	0.01—1.5 mg/mL	5.2×10^4	1:2	Poor sensitivity, high reagent consuming	13
α-(2-Benzimidazolyl) -α′,α″-(*N*-5-nitro-2-pyridyl hydrazone)	410	0 - 2.5 µg/mL	3.81×10^4	1:2	Extraction step, complex is unstable in aqueous medium, high reagent consuming	14
Benzothiazole-2-aldehyde-2-quinolylhydrazone	523	0.35 - 2.95 µg/mL	7.5×10^4	1:2	Extraction Step, poor sensitivity, high reagent consuming	15
5-(2-benzothiazolylazo)-8-hydroxyquinoline,	667 and 800	0.2 – 3.7 µg/mL	1.80×10^9	1:1	Complicated procedure, poor sensitivity	16
1-phenyl-1,2-propanedione-2-oxime-i-guanylhydrazone	405	0.78 - 51.00 µg/mL	5.6×10^3	1:1	Poor sensitivity	17

Buffer solutions were prepared by employing 1M HCl and 1M CH_3COONa (pH 1-3) and 0.2M acetic acid and 0.2M sodium acetate (pH 3.5-7.0). 0.1 M Cu(II) solution was prepared by dissolving required amount of copper (II) sulphate in 100 mL of distilled water and was standardized by standard procedure [18].

The electronic spectra of the ligand and its metal complexes were recorded on the Schimadzu UV-160 Spectrophotometer. pH measurements were made using ELICO pH meter Model L1-10, ELICO Private Limited, Hyderabad, India.

2.2. *Preparation of salicylaldehyde acetoacetic acid hydrazone (SHZN) [19]* (**Fig.1**)

Equimolar solutions of acetoacetic acid hydrazide and salicylaldehyde prepared in aqueous methanol were refluxed for two hours. The contents were cooled and the solid product obtained was filtered, washed with water and dried. The crude

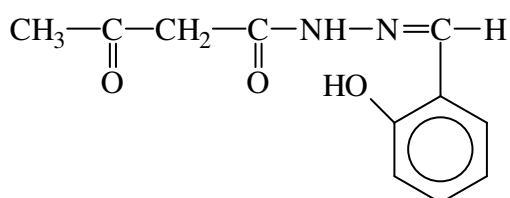product so obtained was recrystallysed from hot aqueous methanol. (Melting point: 220°C).

$$CH_3-\underset{\underset{O}{\|}}{C}-CH_2-\underset{\underset{O}{\|}}{C}-NH-N=C-H$$

Fig. 1. Structure of salicylaldehyde acetoacetic acid hydrazone

2.3. General experimental procedure

1 mL of standardized Cu (II) solution, 5 mL of buffer solution of appropriate pH and 1 mL of SHZN solution and 1 mL of dimethylformamide were taken in a 10 mL volumetric flask. The solution was diluted to the mark with distilled water. The absorbance of the solution was measured at 425 nm against the reagent blank solution.

3. Results and Discussions

In the present investigation Cu (II) interacts with the SHZN to form a greenish yellow colored complex in the media of pH 2. The color reaction was instantaneous and the intensity of the color remained constant for several hours. The color reaction was utilized for the micro determination of copper (II). The molar absorptivity and Sandell sensitivity were 22.5×10^4 L mol^{-1} cm^{-1} and 0.0002824 µg cm^{-2} respectively.

3.1. Optimal conditions for formation of the complex

The effect of pH (in the range 1-10) on the Cu(II)-SHZN system was studied and the results are presented in the **Fig. 2**. The absorbance of the greenish yellow colored complex solution in the media of pH 1 to 6 range from 0.197 - 0.446. Further in this pH range, absorbance due to metal ion and that of the reagent were negligible. Keeping in view the high absorbance and high reproducibility of the spectrum (**Fig. 3**), a media of pH 2 and a wavelength of 425 nm were chosen for the further investigation.

The experimental observations pertaining to the effect of reagent concentration on the color

reaction reveal that an optimum of 5-fold reagent concentration was required for the chromogenic reaction. Hence a 5-fold excess of the regent concentration was recommended for the further studies. However, it was found that presence of the excess of the reagent does not alter the absorbance of the reaction mixture.
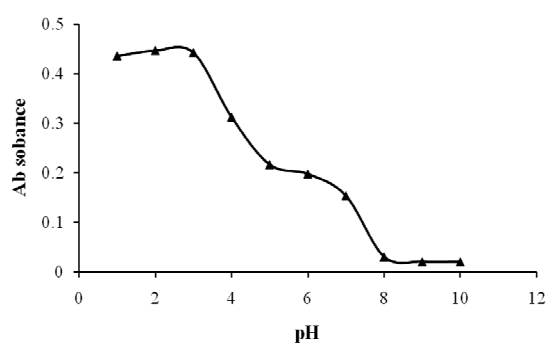

Fig. 2. Effect of pH on absorbance
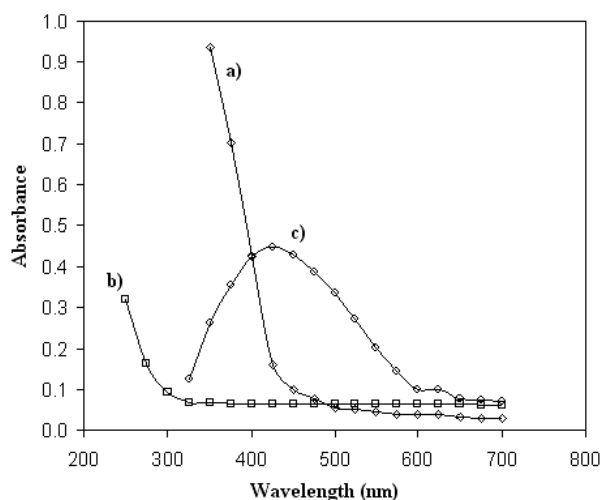[Cu(II)] $=2 \times 10^{-5}$ M; [SHZN]$= 2 \times 10^{-4}$ M

Fig. 3. Absorption spectra of a) SHZN against buffer blank, [SHZN]$= 2 \times 10^{-4}$ M; pH =2. b) Cu(II) against buffer blank, [Cu(II)] $=2 \times 10^{-5}$ M; pH =2. c) Cu(II)- SHZN against reagent blank, [Cu(II)] $=2 \times 10^{-5}$ M; [SHZN]$= 2 \times 10^{-4}$ M; pH =2.

3.2 Micro determination of the copper(II)-Construction of calibration plot

A series of solutions containing different concentrations of the metal ion and the other solutions as specified in the general experimental procedure were taken and the absorbance of the solutions were measured at 425 nm. A linear plot obtained between the concentration of the metal and the absorbance reveal that Cu (II) can be determined in the range 0.0499 – 0.4994 µg/mL. The results are shown in the **Fig. 4**. The calibration plot fits the equation $A = 0.899\,C + 0.002$ with a correlation coefficient 0.999. The method detection limit and limit of quantification were found to be 0.01441 µg/mL and 0.02883µg/mL respectively.
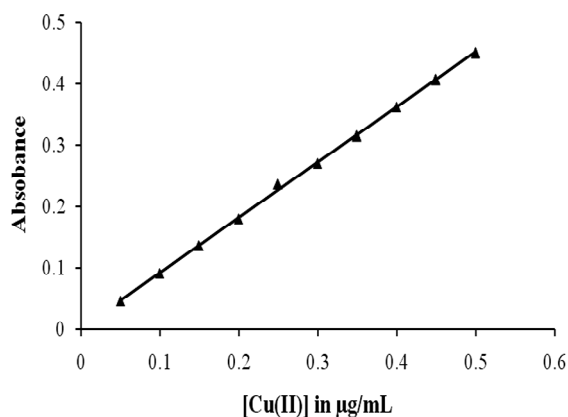

Fig. 4. Analytical determination of copper (II) [SHZN]= 2×10^{-4} M; λ_{max} = 425 nm; pH =2.

3.3. Characteristics of the complex

Job's method of continuous variation [20] was conducted to find out stoichiometry of the complex. Keeping the total volume constant, equimolar solutions of copper(II) and SHZN were taken in different volume ratios in 10 mL volumetric flasks.

The general experimental procedure was followed and a graph was plotted between the absorbance and mole fraction of the metal. The plot (**Fig. 5**) reveals a stoichiometry of 1:1 (M:L). The stoichiometry was further confirmed by mole ratio method [21]. The value of stability constant calculated from the Job's method was found to be 9.947×10^{8}.

The selectivity of the proposed method was examined by studying the effect of diverse ions on the absorbance of the experimental solution containing fixed amount (0.0635 ppm) of copper (II).
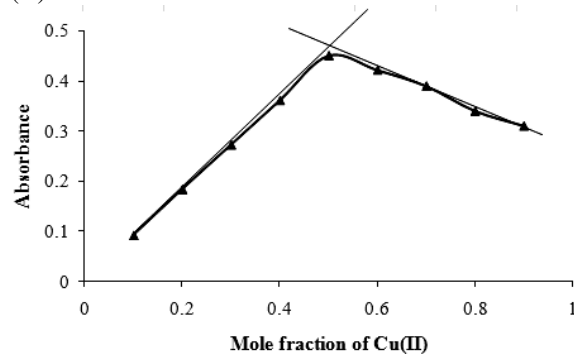

Fig. 5. Job's Method of continuous variation [Cu(II)] = [SHZN]= 2×10^{-4} M; λ_{max} = 425 nm; pH =2.

A deviation of ±1% in the absorbance value was considered as tolerance limit. The results are presented in **Table 2**. It can be seen from the table that large number of common ions did not interfere in the proposed method.

Table 2. Tolerance limit of diverse ions in the presence of 0.0635 ppm of Cu(II)

Anion added	Tolerance limit (ppm)	Cation added	Tolerance limit (ppm)
Citrate	2268.00	Bi(III)	250.78
Tartrate	1184.00	W(VI)	73.54
Iodide	1015.20	Mo(VI)	19.19
Thiosulphate	1985.44	Fe(III)	55.85
Oxalate	1056.00	Mn(II)	32.96
Bromide	479.46	Ag(I)	64.72
Thiourea	608.96	Al(III)	80.94
Nitrate	620.00	Pb(II)	82.88
Urea	480.00	Hg(II)	80.23
Acetate	354.00	Zn(II)	39.22
Thiocyanate	696.00	Fe(II)	22.34
Chloride	212.70	Ni(II)	51.12
Phosphate	379.88		
Fluoride	114.00		

Table 3. (a) Determination of copper (II) in synthetic water mixtures containing Cu(II)

Amount of Copper (µg/mL)	Amount of copper found (µg/mL)		Recovery (%)	RSD (%)
Taken	AAS Method	Proposed method*		
0.10	0.105	0.095±0.0020	95.00	2.96
0.25	0.256	0.246±0.0029	98.40	1.65
0.45	0.451	0.445±0.0046	98.89	1.43

*t value at 95% confidence level is 2.26.

(b) Determination of copper (II) in grape leaves

Sample	Standard value (µg/mL)	Value by proposed method	Relative error (%)
Grape leaves	35.1	34.72	− 1.08

(c) Determination of copper (II) in different alloy samples

Sample No.	Sample Identification	Composition	Percentage of copper(II)		Relative Error %
			Certified	Calculated	
1	Tin base white metal	Sn = 82.20%; Cu = 4.58%; Ni = 0.17%; Bi = 0.11%; Fe = 0.024%; Sb = 9.45%; Pb = 3.18%; Cd = 0.14%; Zn = 0.040%.	4.50	4.45	−1.11
2	Devard's alloy	Al = 45.00%; Zn = 5.00%; Cu = 48.91%.	48.91	48.20	−1.45
3	BCS 180/2	Cu = 68.12%; Fe = 0.68%; Ni = 30.15%; Mn = 0.75%; C = 0.05%; S = 0.006%; Co = 0.005%; Pb = 0.003%.	68.12	68.01	−0.16
4	BAS 106	Cu = 4.10%; Ni = 1.93%; Fe = 0.03%; Mn = 0.19%; Si = 0.29%; Mg = 1.81%; rest Al.	4.10	3.98	−2.93

3.4. Applications

The proposed method was employed for the determination of Cu(II) in synthetic water samples, grape leaves and alloy samples. The results are presented in the **Table 3 (a), (b) and (c).**

Deionized water samples were spiked with the known amounts copper (II) and the concentration was determined by employing the recommended procedure.

Fresh grape leaves were procured from the grape gardens located in surroundings of Hyderabad, Andhra Pradesh, India. The cleaned and dried leaves were finely powdered. The sample solution was prepared by the procedure described by Piper [22].

The analytical determination of copper was carried out by the recommended procedure after the addition of required volume of citrate solution to mask iron (II). The accuracy of the results was confirmed with Grape Research Institute, Hyderabad, A. P. India.

A known weight of the alloy sample was dissolved in a 10 mL of aquaregia, evaporated to minimum volume and extracted with 10 mL of 2M HNO_3. The resulting solution was diluted with distilled water to a suitable volume. This serves as stock solution. The experimental solution was prepared by pipetting out 10 mL of the stock solution into 100 mL volumetric flask and the solution was made up to the mark with distilled

water. The recommended procedure was followed by taking a known volume of sample alloy solution.

3.5 Features of the proposed method

The correlation coefficient of the 1:1 (M:L) Cu(II)–SHZN complex was found to be 0.999, which revealed an excellent linearity between the concentration and absorbance. The repeatability of the method was indicated by the relative standard deviation (RSD < 3%; n = 10). The recovery (95 to 99%) and relative error (1 to 3%) reported by the proposed method demonstrated the accuracy of the method. The selectivity of the proposed method was indicated by the fact that large number of common cations and anions did not interfere with the determination when present at significant levels.

4. Conclusions

Spectrophotometric method reported was rapid, simple, sensitive and selective for the micro determination of copper (II). Analytical parameters of the proposed method were evaluated and presented. The satisfactory application of the proposed method for the analytical determination of copper (II) in synthetic mixtures containing copper (II), grape leaves and alloy samples was also demonstrated in this article.

5. References

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