

Determination of Copper(II) Present in Water Samples using 2-Hydroxy-N'-(1-(2-hydroxyphenyl)ethylidene)benzohydrazide - A Versatile Complexing Reagent and Evaluation of Antibacterial Activity of the Metal-ligand Complex

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ABSTRACT

2-hydroxy-N'-(1-(2-hydroxyphenyl)ethylidene)benzohydrazide (2-HAPSH) is identified as a sensitive and selective analytical reagent for the spectrophotometric determination of copper (II) at pH 6.0 to form a yellow colored metal-ligand (M-L) complex (1:2 ratio). The M-L complex showed maximum absorbance (λ_{max}) at 380 nm. Beer's law is obeyed in the range of 0.0125-0.126 $\mu\text{g mL}^{-1}$, with a correlation coefficient 0.99981 which indicates the linearity between the two variables. The molar absorptivity and sandell's sensitivity of the complex are found to be $1.335 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.0022 \mu\text{g cm}^{-2}$, respectively. The interfering effects of various cations and anions are also studied. The optimized method is successfully applied for the determination of copper(II) in natural and synthetic water samples. The obtained results are in agreement with AAS method. In addition, the extracted Cu(II)-2-HAPSH (M-L) complex showed excellent antibacterial activity against Gram positive bacteria, *B. subtilis*.

Key words: Spectrophotometry, copper, 2-HAPSH, metal-ligand complex, synthetic water samples, AAS, antibacterial activity.

Introduction

Copper, apart from its importance in industry, plays an important role in biological systems as well. It differs from the majority of the trace elements in that it is a physiological constituent of both plants and animals. It plays a vital role in the cell respiration of invertebrate animals and in the formation of haemocyanin. In spite of the fact that copper is an essential micronutrient required to all life forms but at the same time it is toxic when taken in large quantities and it is the third most abundant trace metal in the body after iron and zinc. Excessive copper intake can cause nausea, vomiting, abdominal pain and cramps, headache, dizziness, weakness, diarrhea and a metallic taste in the mouth (associated with water containing copper concentrations greater than 6 mg/L) [1-2]. An excessive accumulation of copper in the human liver and also animals is a characteristic of Wilson's disease which produces neurological and psychiatric defect [3] and deficiency of copper causes diseases such as anemia. The major source for the copper is various types of water and

food samples. Thus, there is a great demand and need to develop a rapid, simple, sensitive, selective and inexpensive method for the determination of Cu(II) present in various types of water samples. For this purpose, the frequently adopted and reported methods of analytical techniques are ICP-MS, ICP-OES, X-ray fluorescence, spectrophotometry, spectrofluorometry, AAS and other similar techniques. Among these, the spectrophotometric methods are preferred as they are simple, more convenient, cheaper, suitable for automation and have comparable sensitivity. Accordingly, a number of spectrophotometric or complexing reagents have been reported for the determination of copper (II). But, most of them suffer from one or more disadvantages such as low sensitivity, low ability of complexation and use of expensive complexing reagent or difficulties in their preparation. The use of chelating ligands containing nitrogen, oxygen and sulfur has received great attention in analytical studies and also in structural studies of metal complexes [4].

Hydrazones are extensively used for the spectrophotometric determination of metal ions for the past few decades. The extensive literature survey reveals that only a few hydrazones of carbonyl compounds have been used for the

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spectrophotometric determination of Cu(II) [5-18]. However, the reported spectrophotometric methods [5-13] suffer from one or more disadvantages such as severe interferences, less sensitivity, less selectivity and difficulty in the preparation of reagent etc. However, none has been reported on spectrophotometric determination of Cu(II) present in synthetic and natural water samples using *o*-Hydroxyacetophenonesalicylhydrazone (2-HAPSH) as a sensitive analytical reagent. In the present study the authors developed a rapid, more sensitive and selective spectrophotometric method for the determination of copper (II) using *o*-Hydroxyacetophenonesalicylhydrazone (2-HAPSH). Later, the present method is applied successfully for the determination of Cu(II) present in various types of water samples collected in and around Kadapa town, Andhra Pradesh, India.

EXPERIMENTAL

Apparatus and Reagents

Double beam UV-Visible spectro photometer 2203 (Systronics, India) with 1 cm quartz cell is used for absorbance studies. Digital pH meter (Systronics, India) is used for pH adjustment. Atomic Absorption Spectrometer 2380 (Perkin-Elmer) is used for the comparison of results. The IR spectrum is recorded on Perkin-Elmer FT-IR spectrometer (Spectrum 2) using KBr optics. ¹H NMR spectrum is recorded on Jeol 400 MHz NMR Spectrometer (JNM-400) and Mass spectrum is recorded on Shimadzu-LCMS with ESI probe (LC-2010EV).

All the chemicals and solvents are used to prepare the ligand are of analytical grade made by merck unless otherwise stated.

Synthesis of ligand, 2-hydroxy-N'-(1-(2-hydroxyphenyl)ethylidene)benzohydrazide (2-HAPSH)

The 2-hydroxy-N'-(1-(2-hydroxyphenyl)ethylidene)benzohydrazide (2-HAPSH) is synthesized and recrystallized according to the reported literature procedure [19] as shown in scheme 1. Accordingly, in a 100-mL three necked RB flask, 1.0 g (7.3 mmol) of 2-Hydroxy acetophenone (**I**) and 1.1 g (7.2 mmol) of Salicyl hydrazide (**II**) are dissolved in 50 mL of ethanol. The reaction mixture is stirred at reflux temperature for 3 hours. The progress of the reaction is monitored by TLC. After completion of the reaction, the reaction mixture is cooled to RT and pale yellow colored

solid is obtained. Then the solvent is filtered-off and washed with cold ethanol. The formed product is recrystallized using warm ethanol as solvent. The obtained product is dried in vacuum oven at 50°C.

Characterization data of the ligand, 2-hydroxy-N'-(1-(2-hydroxyphenyl)ethylidene) benzohydrazide: Pale yellow colored solid; Yield: 1.92 g (98%); m.p. 241-243°C

IR (KBr, cm⁻¹) : Peaks at 3438, 3276, 3059, 2924, 1631, 1617 and 1584 indicates the presence of OH (str.), NH (str.), Aromatic-H (str.), Aliphatic-H (str.), C=O (str.), C=N (str.) and NH (bend) respectively.

¹H NMR (400 MHz, DMSO-d₆): δ 13.17 (s, 1H, -OH), 11.76(s, 1H, -NH), 11.55 (s, 1H, -OH), 7.99 (dd, 1H, *J*=1.6 Hz, *J*=7.60 Hz arom H), 7.66 (d, 1H, *J*=7.6 Hz, arom H), 7.46 (td, 1H, *J*=1.6 Hz, *J*=8.4 Hz arom H), 7.32 (t of d, 1H, *J*=1.6 Hz, *J*=8.4 Hz arom H), 7.03 (m, 2H, arom H), 6.92 7.03 (m, 2H, arom H), 2.44 (s, 3H, -CH₃)

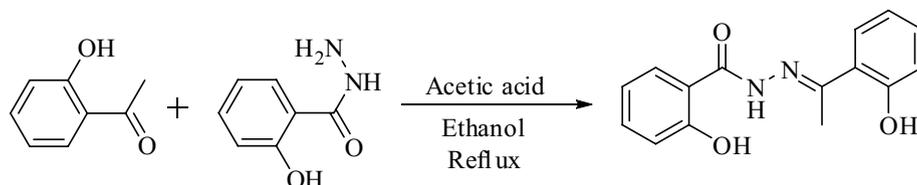
Mass (ESI): m/z (M+H)⁺ 270.80.

Preparation of the Standard solution of Copper (II):

In a 1000 mL volumetric flask 6.242 g of copper sulphate (CuSO₄.5H₂O) is dissolved in water and make up to the mark to get a stock solution of concentration of 2.5 X 10⁻² mol/L. This stock solution is standardized by iodometry [20]. According to the necessity, the stock solution is further diluted to the required volume with double distilled water. The working standard solutions are prepared by accurate dilution.

Preparation of buffer solutions: 1.0 mol/L sodium acetate and 1.0 mol/L acetic acid solutions are prepared in double distilled water. To get the desired pH, suitable portions of these solutions are mixed.

Adopted procedure: 1.0 mL of copper(II) solution of the required concentration, 4.0 mL of buffer solution (pH=6.0) and 1.0 mL of ligand, 2-HAPSH solution of the required concentration are taken in a 10.0 mL standard flask and the contents are make up to the mark with double distilled water. The absorbance of the solution is recorded against the reagent blank which is prepared under the same conditions.



Scheme 1:
Preparation of 2-hydroxy-N'-(1-(2-hydroxyphenyl)ethylidene)benzohydrazide

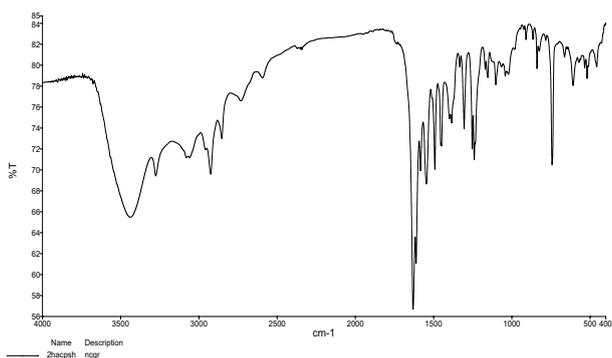


Fig. 1: IR Spectrum of 2-HAPSH

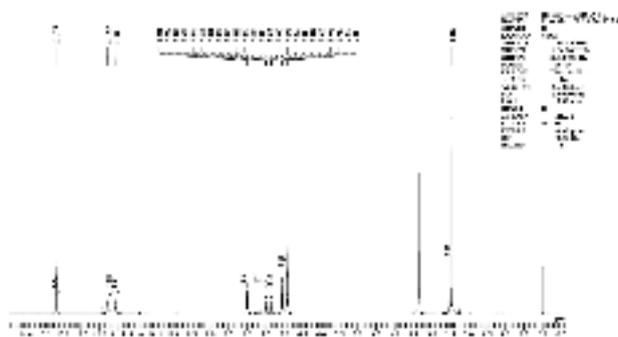


Fig. 2 : ¹H NMR Spectrum of 2-HAPSH

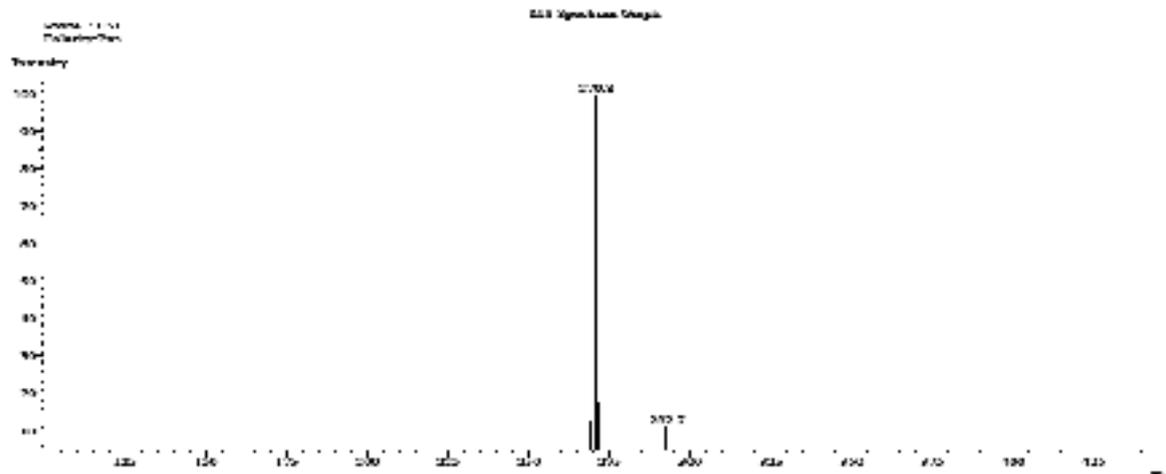


Fig. 3 : Mass Spectrum of 2-HAPSH

Results and Discussion

Absorption spectra of ligand, 2-HAPSH alone and Cu(II)-2-HAPSH complex (M-L complex): Initially, the absorption spectrum of the reagent (2-HAPSH) is recorded against the solvent blank. The absorption spectrum of Cu(II)-2-HAPSH (M-L) complex is recorded against the reagent blank.

The absorption spectra of both the M-L complex and reagent (ligand) are shown in Figure 4. From the absorption spectra, it is clear that the reagent have shown maximum absorptions both at 280 nm and 330 nm where as the M-L complex showed maximum absorbance at 380 nm.

Effect of pH: The absorbance of the M-L complex is increased as the pH increases from pH 1.0 to 5.0 and after

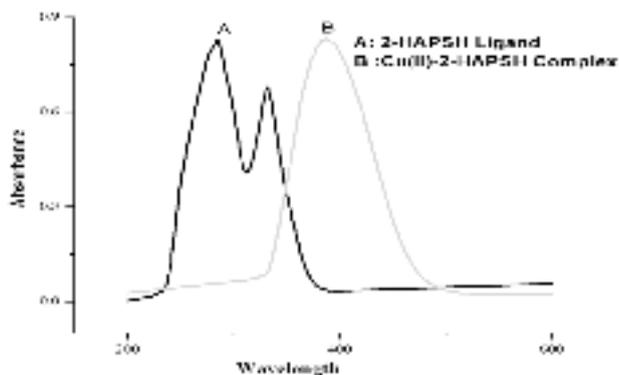


Fig. 4 : (A) Absorption spectrum of 2-HAPSH Vs. blank (B) Absorption spectrum of Cu(II)-2-HAPSH complex Vs. blank [Cu(II) = 1.0 mL of 1.0×10^{-4} M, 2-HAPSH = 1.0 mL of 1.0×10^{-4} M and pH = 6.0

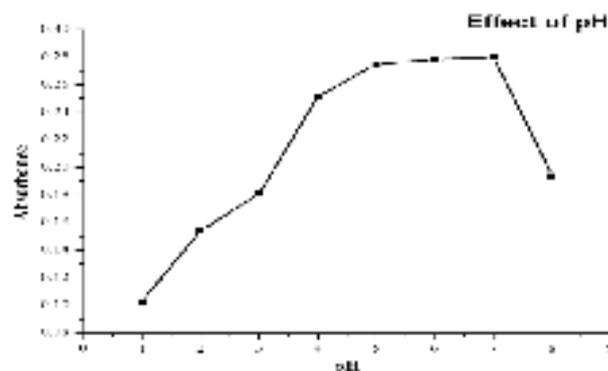


Fig. 5 : Effect of pH on absorbance of Metal-Ligand complex: Cu(II) = 1.0 mL of 1.0×10^{-4} M; ligand, 2-HAPSH = 1.0 mL of 1.0×10^{-3} M; λ_{max} = 380 nm

that it remains constant in the range of pH 5.0-7.0. Therefore, all the experiments are carried out at pH 6.0 and considering it as the optimum pH.

Effect of reagent concentration: The effect of reagent concentration on the formation of the Cu(II)-2-HAPSH complex (M-L complex) has been studied with the constant concentration of metal ion solution of 1.0×10^{-4} M concentration and the ligand. The results clearly indicate that a twofold molar (2.0×10^{-4} M) excess of reagent to that of the metal ion is sufficient for maximum color development of the Cu(II)- 2-HAPSH complex (M-L complex). Hence a twofold molar excess of the reagent is maintained for further studies (Figure 8a.).

Applicability of Beer's law: Beer's law is obeyed in the range of 0.0125 - $0.126 \mu\text{g mL}^{-1}$, with a correlation coefficient 0.99981 which indicates the linearity between the two

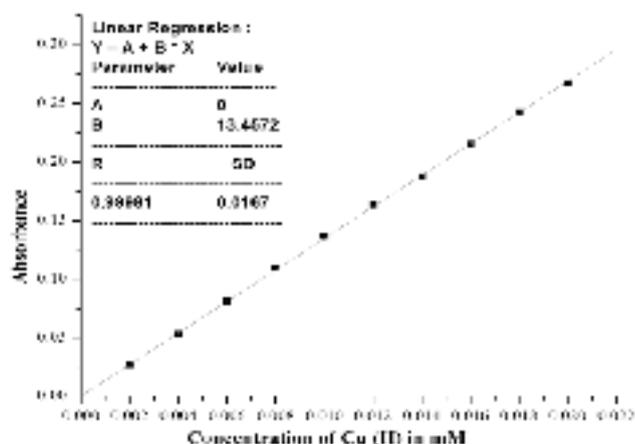


Fig. 6 : Applicability of Beer's law to Cu(II) - 2-HAPSH complex: Cu(II) = 1.0 mL of 0.10×10^{-4} - 1.0×10^{-4} M; ligand, 2-HAPSH = 1.0 mL of 2.0×10^{-4} M; pH = 6.0 and $\lambda_{\text{max}} = 380 \text{ nm}$

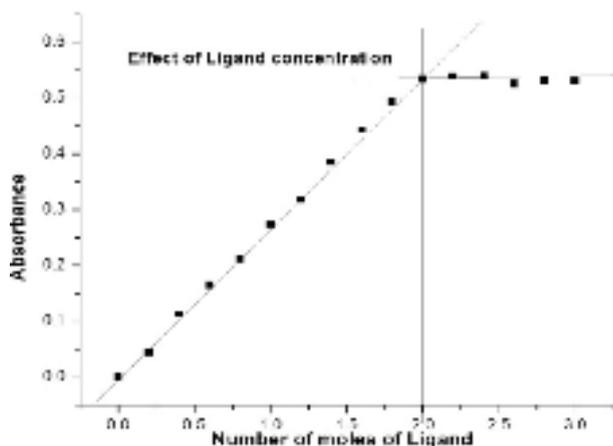


Fig. 8a : Molar ratio method-Ligand variation: Cu(II) = 1.0 mL of 1.0×10^{-4} M, 2-HAPSH = 1.0 mL of (0.25×10^{-4} - 3.0×10^{-4} M), pH = 6.0 and $\lambda_{\text{max}} = 380 \text{ nm}$

variables (Figure 6). The molar absorptivity and sandell's sensitivity of the complex are found to be $1.335 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.0022 \mu\text{g cm}^{-2}$, respectively.

Composition of the M-L complex: The composition of the M-L complex has been determined by continuous variation (Figure 7) and molar ratio method (Figure 8a & 8b). Based on the above two methods the composition of the Cu(II)- 2-HAPSH complex is confirmed as 1:2 ratio.

Composition of the Cu(II)-ligand, 2-HAPSH complex (M-L Complex): Job's method of continuous variation and mole ratio methods are employed to elucidate the composition of the M-L complex as discussed below.

Effect of foreign ions: The effect of foreign ions on complexation is studied by taking 1.0 mL of Cu(II) solution, 1.0 mL of required concentration of the foreign ion solution,

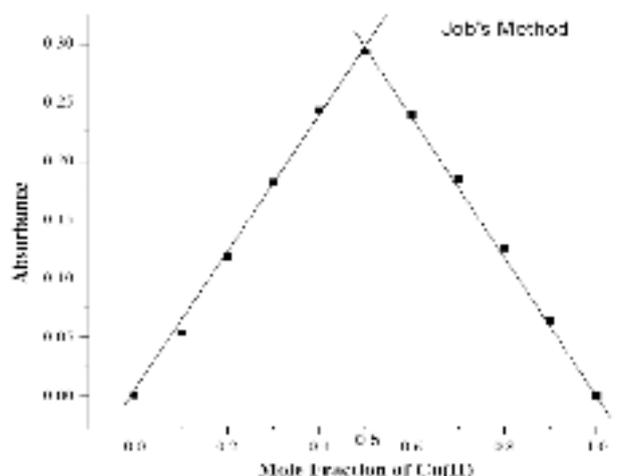


Fig. 7 : Job's method of continuous variation: [concentration of Cu(II) = 2-HAPSH] = 1.0 mL of 1.0×10^{-4} M, pH = 6.0 and $\lambda_{\text{max}} = 380 \text{ nm}$

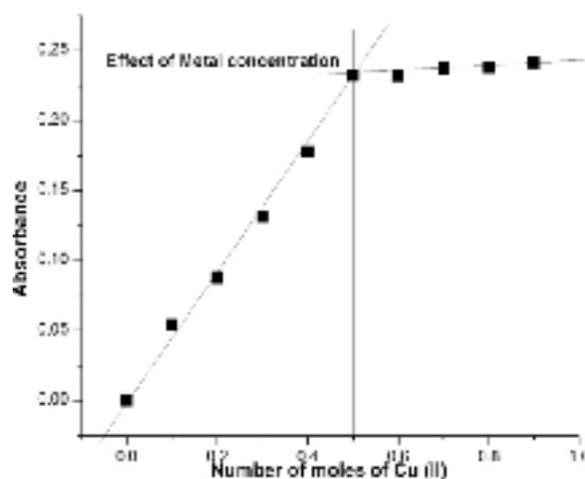


Fig. 8b: Molar ratio method: Metal variation 2-HAPSH = 1.0 mL of 1.0×10^{-4} M, Cu(II) = 1.0 mL of (0.1×10^{-4} - 1.0×10^{-4} M), pH = 6.0 and $\lambda_{\text{max}} = 380 \text{ nm}$

4.0 mL of sodium acetate acetic acid buffer (pH 4.0) and 2.0 mL of 2-HAPSH solution in a 10.0 mL standard flask. The total volume of the solution is brought to 10.0 mL with double distilled water. The experiment is repeated by changing the concentration of the diverse ion and the absorbance is measured at 380 nm. A change of ± 0.02 was taken as the tolerance limit for interference.

The results indicated that Ca(II), Mg(II), Pb(II) and Mn(II) do not interfere even when present up to 5000 μg . Interference due to Al(III) and Cr(III) can be tolerated up to 3000 μg , whereas Mo(VI) and W(V) can be tolerated up to 2500 μg only. Extraction of copper (II) is not possible in the presence of Co(II), Ni(II), Fe (II)/Fe(III), Zn(II), Pd(II), and Cd(II), due to their severe interference, even when present in trace amount. Anions such as fluoride, bromide, chloride, nitrate, sulfate, thiosulfate and acetate do not affect the extraction of copper (II), even when present up to 4000 μg . In the presence of thiocyanate, oxalate and EDTA, extraction of copper(II) is not possible. 1.0 mL of 0.2% fluoride has been used as a masking agent for Fe(III). Ni(II), Co(II), Zn(II) and Cd(II) do not interfere in the pH range studied. From the above discussion, it is clear that copper can be separated from a number of associated metal ions usually present in natural and synthetic water samples.

Applications: The proposed Spectrophotometric method has been applied for the determination of copper contents in natural and synthetic water samples.

Analysis of samples: In order to highlight the utility of the proposed spectrophotometric method, it is used for the determination of copper(II) contents in natural and synthetic water samples. The results are given in Tables 1 & 2.

Natural water samples: Different water samples are collected from in and around KADAPA, A.P. India. Each filtered water samples is evaporated nearly to dryness with a mixture of 5.0 mL of concentrated H_2SO_4 and 10.0 mL of concentrated HNO_3 in a fume cupboard and then cooled to room temperature. The residue was then heated with 10.0 mL of double distilled water, in order to dissolve the salts. The solution is cooled and neutralized with dilute NH_4OH in the presence of 1–2 mL of 0.01% (W/V) tartarate solution. The resulting solution was filtered and quantitatively transferred into a 25.0 mL calibrated flask and made up to the mark with double distilled water [21]. The aliquot was analyzed for Cu(II), using 2-HAPSH adopting the recommended procedure.

Preparation of Synthetic water samples

Metal ion solutions of Cu^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Mn^{2+} , Zn^{2+} , Hg^{2+} , Pd^{2+} , Cd^{2+} and Pb^{2+} are prepared from Merck - analytical grade stock standards of concentration 1000mg/l. The synthetic water solutions are then prepared by mixing the different metal ions as prescribed in the table.2. The aliquot is analyzed for Cu(II), using 2-HAPSH adopting the recommended procedure.

Antimicrobial activity

Table - 1
Determination of Cu (II) in water samples collected from in and around KADAPA town

S.No.	Sample no.	Cu added ($\mu\text{g/mL}$)	AAS ($\mu\text{g/mL}$)	Present Method ($\mu\text{g/mL}$)	% of recovery
1	^a Sample 1	2	1.96	1.95	97.5
2	^b Sample 2	2	1.98	2.01	100.5
3	^c Sample 3	2	2.05	1.99	99.5
4	^d Sample 4	2	1.94	1.93	96.5
5	^e Sample 5	2	1.99	1.97	98.5
6	^f Sample 6	2	2.05	2.01	100.5
7	^g Sample 7	2	1.93	1.91	95.5
8	^h Sample 8	2	2.09	2.08	104
9	ⁱ Sample 9	2	1.96	1.95	97.5
10	^j Sample 10	2	2.01	2.02	101

a-j :Sample collection sites in and around Kadapa town : ^aAlankhanpalli, Kadapa; ^bSrinivasa Engineering college, Kadapa; ^cR K Nagar, Kadapa; ^dJMJ college, Kadapa; ^eRIMS Hospital, Kadapa; ^fIndustrial area, Kadapa; ^gRailway Station, Kadapa; ^hDwaraka nagar, Kadapa; ⁱNagaraju peta, Kadapa; ^jYogi Vemana University, Kadapa.

Ligand and M-L compounds are tested using disk diffusion method against both gram-positive bacterial strains: *Basillus Subtilis* (MTCC 106), *Staphylococcus aureus* (MTCC 737) and gram-negative bacterial strains: *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109) [21-24]. Chloromphenicol is used as positive control, and DMSO is used as negative control. The susceptibility is assessed on the basis of diameter of zone of inhibition against gram-positive and gram-negative strains of bacteria. Inhibition zones are measured and compared with the controls. The bacterial zone of inhibition values are given in Table 3, and

minimum inhibitory concentration, mg/mL of M-L complex is given in Table 4. The M-L Complex exhibited promising antibacterial activities against both gram-positive and gram-negative human pathogenic bacterial strains.

From the data, it is confirmed that Cu (II)-2-HAPSH (M-L complex) complex showed excellent antibacterial activity against gram +Ve bacteria, *B.Subtilis*, which is more than that the standard drug, Chloramphenicol as shown in Table 3. It is found ligand (2-HAPSH) alone shoed low antibacterial activity with both Gram +Ve and Gram -Ve strains

Table - 2
Determination of Cu(II) in Synthetic water samples

Samples	Metal 1 (2µg/mL)	Metal 2 (2µg/mL)	Metal 3 (2µg/mL)	Metal 4 (4µg/mL)	AAS (µg/mL)	Present Method (µg/mL)	% of recovery
1	Fe	Co	Ni	Cu	3.97	3.95	98.75
2	Co	Ni	Mn	Cu	3.99	3.96	99.00
3	Fe	Zn	Ni	Cu	3.96	3.93	98.25
4	Cd	Pb	Ni	Cu	3.98	3.97	99.25
5	Hg	Pd	Mn	Cu	3.99	3.98	99.50

Table - 3
In vitro antibacterial activities of Ligand (2-HAPSH) and M-L complex (Cu(II) - 2-HAPSH complex)

Name of compound	Concentration (µg/mL)	Diameter of Zone of Inhibition (mm)			
		Gram +Ve bacteria		Gram -Ve bacteria	
		<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>K. eumoniae</i>
2-HAPSH ligand	100	6	5	2	3
	200	11	9	5	7
Cu-2-HAPSH complex (M-L complex)	100	15	11	8	6
	200	19	14	13	10
Chloromphenicol	100	16	16	17	18
DMSO control		-	-	-	-

Positive control: Chloromphenicol
Negative control: DMSO

Table - 4
Minimum inhibitory concentration (MIC), µg/mL of ligand and M-L complex

Name of compound	Minimum inhibitory concentration (µg/mL)			
	Gram +Ve bacteria		Gram -Ve bacteria	
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>
2-HAPSH ligand	60	80	100	90
Cu-2-HAPSH complex (M-L complex)	4.5	5	30	40
Chloromphenicol	7.0	6.5	6.25	6.0

Conclusion

In summary, the authors developed a rapid spectrophotometric determination of copper(II) present in various types of water samples using 2-hydroxy-N'-(1-(2-hydroxyphenyl) ethylidene)benzohydrazide (2-HAPSH) as an analytical reagent. The proposed method offers advantages like good sensitivity, selectivity, reliability, reproducibility, less interference and immediate color development. The developed method is found to be quantitative comparable to other standard methods. The results show good agreement with the standard method. The molar absorptivity value of the complex ($1.335 \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1}$) reveals that the reagent is fairly sensitive for copper (II) when compared with other hydrazones. A number of associated elements don't interfere in the determination. Hence, 2-HAPSH is highly useful reagent for the spectrophotometric determination of Copper(II) present in low and trace levels from various natural water samples and synthetic mixtures. Further, the M-L complex exhibited excellent antibacterial activity against gram +Ve bacteria, B.Subtilis, which is more than that the standard drug, Chloramphenicol.

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