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A rapid extractive spectrophotometric determination of Cu (II) in biological, geological and pharmaceutical samples using o-hydroxyacetophenone isonicotinoylhydrazone

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ABSTRACT

A rapid extractive spectrophotometric method is developed for the determination of copper (II) using o-Hydroxyacetophenoneisonicotinoylhydrazone (OHAPINH) as one of the sensitive analytical reagent. OHAPINH reacts with Cu(II) at pH 4.0 to form an yellowish brown colored complex, which can be extracted with dichloromethane (DCM). It is observed that the colour development is instantaneous and stable for more than 96 h. Beer's law is obeyed in the range of 0.2-2.2 µg /mL of Cu(II), with a correlation coefficient 0.9998. The molar absorptivity (ε) and Sandell's sensitivity of the complex are 4.8×10^4 $L.mol^{-1}.cm^{-1}$ and 0.0006 µg/mL, respectively. The metal to reagent ratio is found to be 1:2 by the method of Job's continuous variation, mole ratio, Asmus and slope ratio methods. The optimized method has been successfully applied for the determination of copper (II) in the presence of diverse ions and also in biological, geological and pharmaceutical samples. It is also observed that the obtained results are in agreement with ICP-MS and AAS methods. The proposed method is selective, sensitive and reproducible.

Keywords: *Copper(II), o-Hydroxyacetophenone isonicotinoylhydrazone, plant materials, minerals, pharmaceuticals, extractive spectrophotometry*

INTRODUCTION

Copper has received considerable attention owing to its uses in metallurgy and chemical industries. Besides, it is an essential micronutrient required by all life forms. Despite the fact that, copper is the third most abundant trace metal in the body after iron and zinc. The total amount of copper in the body is only 75-100 mg [1]. Copper is present in every tissue of the body, but is stored primarily in the liver, with fewer amounts found in the brain, heart, kidney and muscles [2]. Cu is involved in a variety of biological processes viz., embryonic development, mitochondrial respiration, regulation of hemoglobin levels as well as hepatocyte and neuronal function and it also keeps thyroid gland functioning normally [3-4]. Several reviews highlighted the participation of Cu in a myriad cellular activities and physiological processes such as cellular respiration, iron metabolism, biosynthesis of neurotransmitter, and free radical detoxification [5-9].Therefore, it is worth recalling that Cu is vital for normal healthy functioning of organisms. 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) [6]. The excessive accumulation of copper in the human liver and also animals is a characteristic of Wilson's disease which produces neurological and psychiatric defect [10] and deficiency of copper causes diseases such as anemia. Hence, there is a great need to develop, a rapid, simple, sensitive, selective and inexpensive method for the separation and determination of Cu(II) form its associated metal ions in biological, geological and pharmaceutical samples. For the determination of copper at micro levels there are several frequently adopted methods using analytical techniques such as, ICP-MS, ICP-OES, X-ray fluorescence, spectrophotometry, spectrofluorometry, AAS and other such techniques. Among these, the spectrophotometric methods are preferred as they are cheaper, suitable for automation and have comparable sensitivity. A number of spectrophotometric reagents have been used for the determination of copper (II), but a very few are used for the separation and determination of it.

Hydrazones are extensively used for the spectrophotometric determination of metal ions for the past few decades. The extensive literature studies reveal that only a few hydrazones of carbonyl compounds have been used for the spectrophotometric determination of Cu(II) [11-23]. However, the reported spectrophotometric methods[11-18] suffer from one or more disadvantages such as severe interferences, less sensitivity, less selectivity and difficulty in the preparation of reagent etc (Table.1). Nevertheless, none has been reported on extractive spectrophotometric determination of Cu(II) by *o*-Hydroxyacetophenoneisonicotinoylhydrazone (OHAPINH) in biological, geological and pharmaceutical samples.

Table 1. Comparison of present method with other reported spectrophotometric methods

Reagent	λ max (nm)	pH	Linear range	ε L mol^{-1}cm^{-1}	M:L	Ref. No.
Phenylpyruvicacid-2-quinolylhydrazone	450	10.5	≥2.5 µg/mL	3.15×10^4	-	11
2-Thiophenealdehyde-2-benzothiazolylhydrazone	422	5.1	Up to 1.20 µg/mL	4.4×10^4	-	12
2,2-dipyridyl-2-Pyridyl hydrazone	448	-	Less than 1ppm	3.8×10^4	1:1	13
(Z)-2-thiophenaldehyde 2-pyridylhydrazone	435	8.5	0.017 - 1.20 µg/mL	-	1:3	14
1-phenyl-1,2-propanedione-2-oxime-i-guanylhydrazone	405	-	0.78 - 51.0 µg/mL	5.6×10^3	1:1	15
N,N'-Oxalylbis (salicylaldehyde hydrazone)	422	2.0	0.4-1.8 µg/mL	0.92×10^4	1:1	16
α-(2-Benzimidazolyl)-α',α"-(N-5-nitro-2-pyridylhydrazone)-toluene	410	6.0	0–2.50 µg/mL	3.81×10^4	1:2	17
p-methylisonitrosoaceto phenonehydrazone	510	7.0	0.1–1.0 mg/mL	6.28×10^3	-	18
o-Hydroxyacetophenone isonicotinoylhydrazone (OHAPINH)	420	4.0	0.2-2.2 µg/mL	4.8×10^4	1:2	Present method

In the present study the authors developed a rapid, more sensitive and selective extractive spectrophotometric method for the determination of copper (II) using *o*-Hydroxyacetophenoneisonicotinoylhydrazone (OHAPINH). Later the method is applied successfully for the determination of Cu (II) in biological, geological and pharmaceutical samples in microgram quantities.

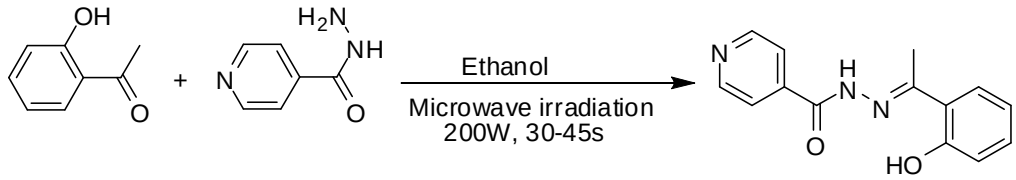
MATERIALS AND METHODS

Apparatus and Reagents

UV-Vis spectrophotometer (Shimadzu Model UV-1800) with a 1.0 cm quartz cell is used for absorbance studies. Systronics digital pH meter is used for pH adjustment. Results are compared with the data generated for the same using inductively coupled plasma mass spectrometer (ICP-MS) (Perkin Elmer Élan DRCII ICP-MS Toronto, Ontario, Canada), flame atomic absorption spectrophotometer (Shimadzu model No. AA-6300). All the reagents and solvents are of standard analytical grade and used without further purification.

Preparation and characterization of o-Hydroxyacetophenoneisonicotinoylhydrazone (OHAPINH)

2-Hydroxy acetophenone [1 gm, 7.3 mmol] and Isonicotinic hydrazide[1 gm, 7.3 mmol] are dissolved in 10 mL of ethanol is added into a 100-mL beaker. The beaker is placed in a domestic microwave oven at 200 watts for 30-45s. The progress of the reaction is monitored by TLC. After completion of the reaction, the reaction mixture is cooled to RT and then washed twice with cold ethanol. Finally, the obtained crude product is recrystallized from ethanol. The structure of the synthesized compound is confirmed by ^{1}H NMR and Mass Spectral data.



Scheme 1: Preparation of *o*-Hydroxyacetophenoneisonicotinoylhydrazone (OHAPINH)

Characterization data of OHAPINH

Colorless solid; Yield: 1.73 gm (92%); mp 232-234^{o}C.; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.80 (s, 1H, -OH), 11.10(s, 1H, -NH), 8.40 (d, 2H, *J*=4.0 Hz, arom H), 7.5 (d, 2H, *J*=6.8 Hz, arom H), 7.18(d, 1H, *J*=6.8 Hz, arom H), 6.90 (t, 1H, *J*=7.2 Hz, arom H), 6.64-6.42 (m, 2H, arom H), 2.18 (s, 3H, -CH_3); MS (ESI): $(M+H)^+$ 256.10.

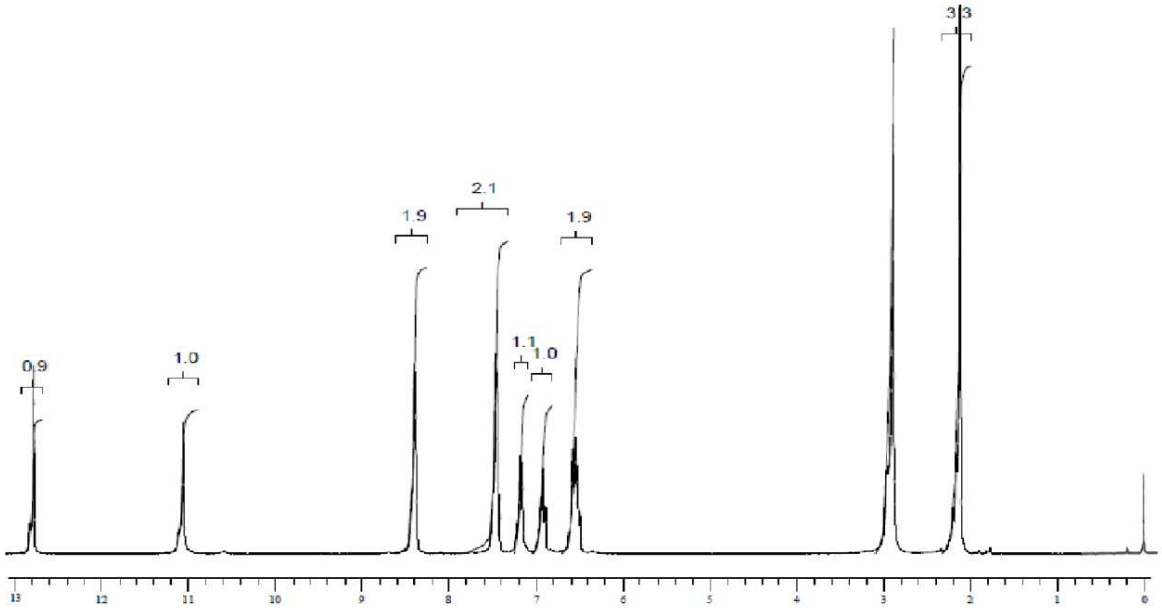


Figure 1. ^{1}H-NMR spectrum of *o*-Hydroxyacetophenoneisonicotinoylhydrazone (OHAPINH)

Preparation of standard solution of Copper (II) (0.025 M)

The stock solution has been prepared by dissolving 6.242 g of copper sulphate pentahydrate ($CuSO_4.5H_2O$) in double distilled water (DDW). The solution is made up to 1.0 L and the solution standardized by iodometry [24]. This stock solution is diluted further, whenever necessary with DDW.

Preparation of OHAPINH reagent solution (0.025 M)

In a 100-mL volumetric flask, 0.641g of OHAPINH reagent is dissolved in methanol. Further, the solution is made up to the mark with methanol. This stock solution is further diluted to required volume with methanol whenever necessary. The working standard solutions are prepared by accurate dilution.

Preparation of buffer solutions

Acetic acid and sodium acetate buffer solution of pH 4.0 is prepared by mixing 0.2 M acetic acid and 0.2 M sodium acetate solutions in 3:7 ratio by volume. The pH of the above buffer solution is measured by a pH meter and finally adjusted suitably.

General analytical procedure

To an aliquot of a solution of copper (II) in the range of 1×10^{-3} to 1×10^{-5} mol/L, 3 mL of pH 4.0 buffer and OHAPINH reagent solution are added to a separatory funnel and water is added to make up the volume to 10.0 mL.

Then the mixture is shaken with 10 mL of methanol saturated dichloromethane for one minute and allowed to stand for a few minutes. The absorbance of the organic layer separated out has been measured against the reagent blank.

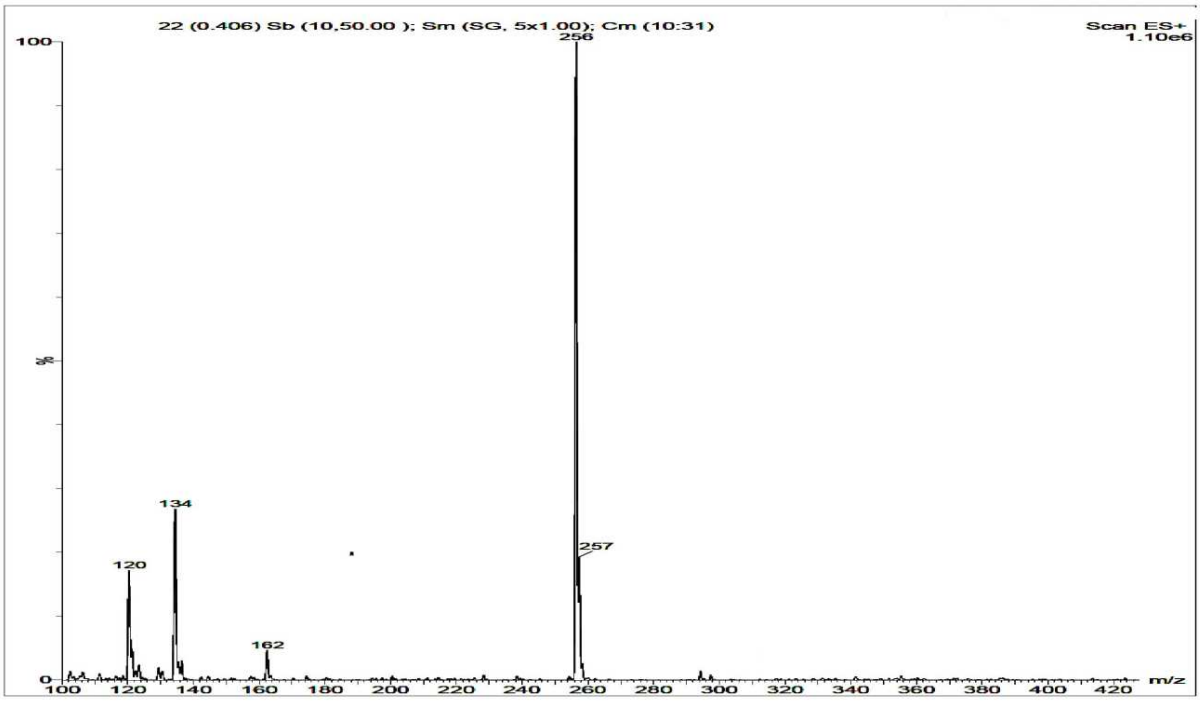


Figure 2. Mass spectrum of *o*-Hydroxyacetophenoneisonicotinoylhydrazone (OHAPINH)

RESULTS AND DISCUSSION

The Copper (II) reacts with *o*-Hydroxyacetophenoneisonicotinoylhydrazone (OHAPINH) in presence of sodium acetate-acetic acid buffer of pH 4.0 forming a yellowish brown coloured complex which can be extracted into methanol saturated DCM. The colored complex in DCM showed a maximum absorbance at 420 nm, when the spectrum of the complex has been recorded, against the reagent blank. The color of the complex is stable for a minimum of 96 h. Therefore, a detailed study of the extraction of copper(II) with OHAPINH has been undertaken in a view to develop the procedure for rapid and sensitive extractive spectrophotometric method for the determination of copper(II) when present alone or in presence diverse ions which are usually associated with copper in minerals, alloys, soils, pharmaceutical and also in biological samples.

Absorption spectra

The absorption spectra of the reagent and Cu(II)-OHAPINH complex are shown in figure 3. From the spectra, it is clear that the Cu(II)-OHAPINH complex showed maximum absorbance at 420 nm, whereas the reagent showed absorbance at 285 and 333 nm. Hence, the optimum wavelength is fixed at 420 nm. Therefore, all of the spectral measurements are carried out at this wavelength.

Effect of pH

The effect of pH on the formation of Cu (II)-OHAPINH complex has been studied to find out the optimum pH for Cu(II) determination. The pH studies are carried out by using standard buffer solution [hydrochloric acid-potassium chloride (pH 1.0-2.6), sodium acetate-acetic acid (pH 3.4-6.5)]. 1.0 mL of copper (II) solution (2.5×10^{-3} mol/L), 1.0 mL of OHAPINH (5.0×10^{-2} mol/L) solution and 3.0 mL of buffer solution are transferred to a separatory funnel and the contents are adjusted to 10.0 mL with double-distilled water. The formed yellowish brown colored Cu (II)-OHAPINH complex is extracted with 10mL of methanol saturated DCM. The absorbance of the organic phase separated out is measured at 420 nm against the reagent blank. The extraction of copper (II) with OHAPINH has been studied over the pH range 1.0-6.5 and is observed that extraction percentage of Cu (II) is maximum at pH 3.5-4.5 (Figure 4). Hence, all of the extractions have been carried out at pH 4.0 and considering it as the optimum pH.

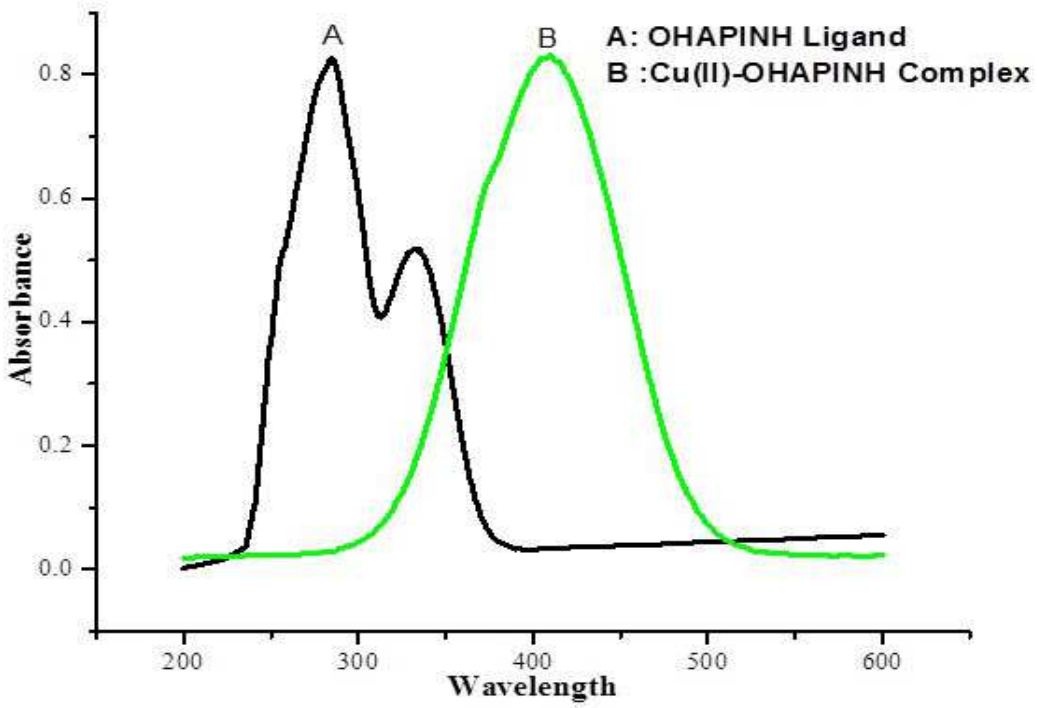


Figure 3. Absorption spectra of (A) OHAPINH ligand against solvent blank and (B) Cu(II)-OHAPINH Complex against reagent blank

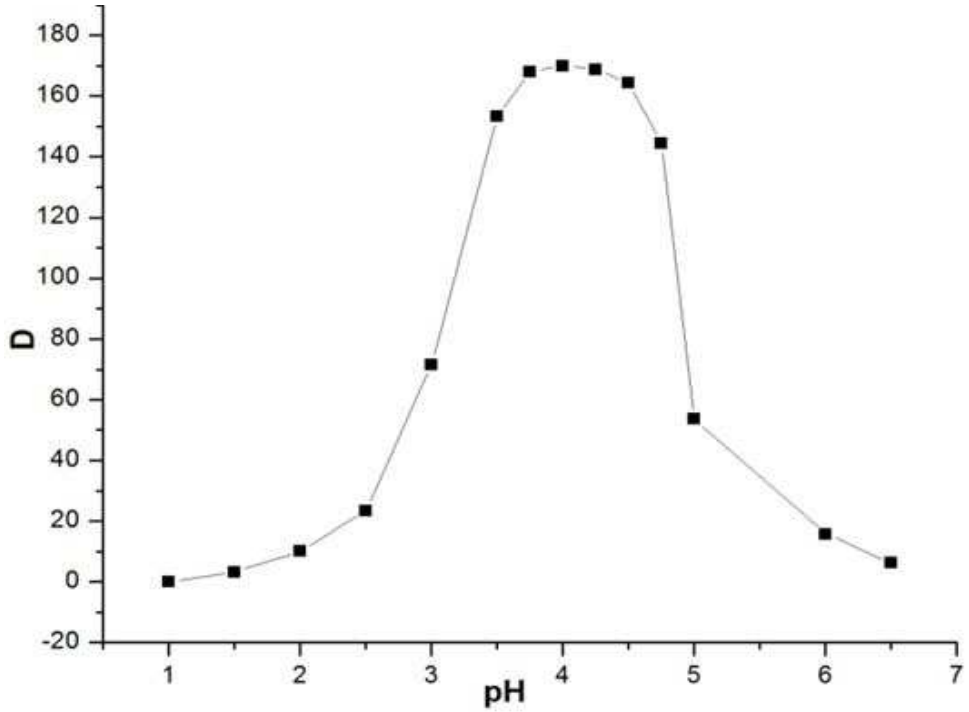


Figure 4. D (distribution ratio) vs Effect of pH on Cu(II)-OHAPINH complex. Cu(II), 1.0 mL of 2.5×10^{-4} mol/L; OHAPINH, 1.0 mL of 5.0×10^{-2} mol/L; λ_{max} 420 nm

Selection of solvent for the extraction

Different solutions with 1.0 mL of copper (II) solution (2.75×10^{-3} mol/L) and 1.0 mL of OHAPINH (5.5×10^{-2} mol/L) solution and 3.0 mL of pH 4.0 buffer are taken in a separatory funnel and the volume is brought to 10.0 mL

with double-distilled water. Then the metal ligand complex is extracted with various solvents like toluene, dichloro ethane, carbon tetrachloride, *n*-butanol, *n*-pentanol, ethyl acetate, *n*-amyl alcohol, chloroform and dichloromethane (DCM). Based on the obtained results (Table 2), DCM has been identified as a suitable solvent for the effective extraction of the Cu(II)-OHAPINH complex. Therefore, DCM is chosen for all further studies.

Table 2. Effect of solvents on the extraction of Cu (II)-OHAPINH complex*

Name of the solvent	Absorbance
Toluene	0.005
Dichloroethane	0.011
Carbon tetrachloride	0.125
n-Butanol	0.308
n-Pentanol	0.412
Ethyl acetate	0.598
n-Amyl alcohol	0.741
Chloroform	1.28
Dichloromethane	1.465

**Cu(II),1.0 mL of 2.75 × 10^{-3} mol/L; OHAPINH,1.0 mL of 5.5 × 10^{-2} mol/L; 3.0 mL of pH 4.0 buffer; λ_{max}420 nm.*

Effect of reagent concentration

The effect of the reagent concentration has been studied by using different solutions containing 1.0 mL of (1.0×10^{-3} mol/L) copper (II) solution and 3.0 mL of pH 4.0 buffer solution are taken in a set of equilibrium tubes. To each of these solutions, 1.0 mL of reagent solution containing varying concentrations ranging from known aliquots of 1.0 ×10^{-3} mol/L to 3.0 × 10^{-2} mol/L is added and the contents are made up to 10 mL with double distilled water. Then the solutions are extracted with 10mL of DCM, in each case and the absorbances of the organic phases are measured at 420nm, against reagent blank as shown in figure 5. From the results, it is clear that nearly 20~fold molar excess of reagent is necessary for the maximum recovery Cu (II).

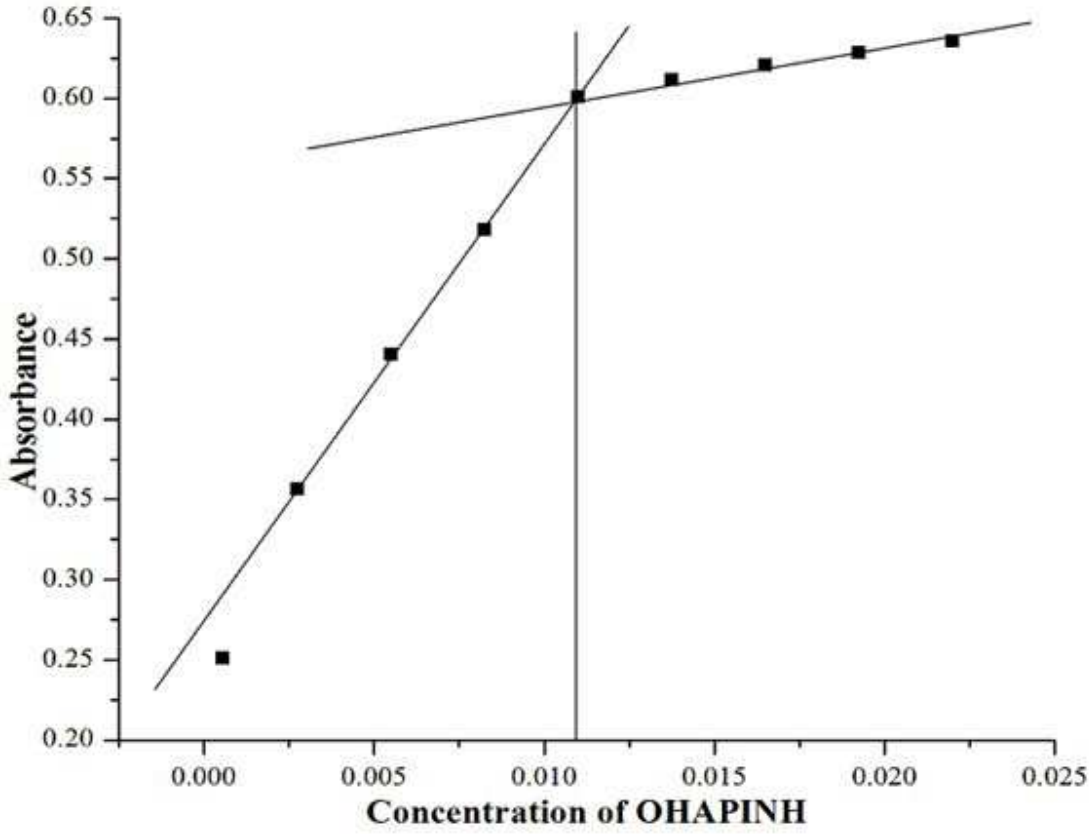


Figure 5. Effect of reagent concentration on extraction of Cu(II)

Validity of Beer's law, Molar absorptivity, Sandell's sensitivity and correlation coefficient 1.0 mL of (6.0×10^{-3} mol/L) OHAPINH and 3.0 mL of pH 4.0buffer solution are taken into a set of equilibrium tubes. To each of these solutions 1.0 mL of known concentration of Cu(II) solution in the range from 0.31×10^{-4} mol/L (0.2µg /mL) to 0.4×10^{-3} mol/L (2.6 µg /mL) is added and the contents are made up to 10 mL with double distilled water. Then the solutions are shaken with 10mL of DCM in each case. The absorbance of the organic layer is measured at 420nm against the reagent blank. A graph is plotted between the amount of Cu(II) and its absorbance as shown in figure 6. From the graph, it is clearly observed that a linear plot passing through the origin obeys the Beer's law in the range 0.2-2.2 µg /mL of Cu(II). The molar absorptivity (ε), Sandell's sensitivity and correlation coefficient of the complex have been calculated as 4.8×10^4 L.mol^{-1}.cm^{-1}, 0.0006 µg/mL and 0.9998 respectively.

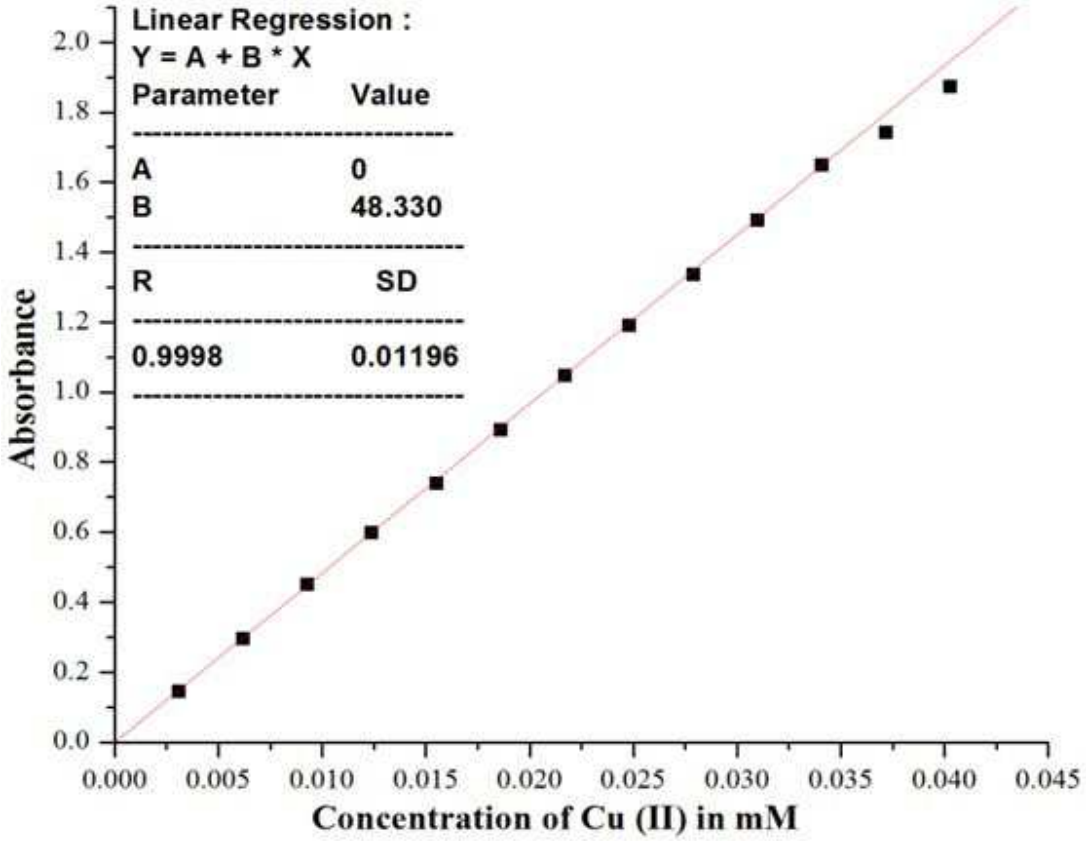


Figure 6. Applicability of Beer's law on Cu(II)-OHAPINH complex. pH 4.0; λ_{max} 420 nm

Determination of the composition of the Cu(II)-OHAPINH complex. The composition of the Cu(II)-OHAPINH complex has been studied by the method of Job's continuous variation, mole ratio, Asmus and slope ratio methods and the obtained results are discussed below**.** Job's method of continuous variation Equimolar solutions of copper (II) and OHAPINH (2.5×10^{-3} mol/L) are prepared. x mL of reagent and (1-x) mL of metal ion solutions are taken into a separatory funnel. Now the total volume in the separatory funnel including two solutions is 1.0 mL. Different amounts of the reagent and the metal ion are transferred into a set of separatory funnels such that the total volume of both solutions in each is 1.0mL. 3.0 mL of sodium acetate acetic acid buffer (pH 4.0) is added to each and the volume of the aqueous phase is brought to 10.0 mL with double-distilled water. Each of the above aqueous phases is shaken thoroughly with 10.0 mL of DCM for 1 minute. The absorbances of all the organic phases are recorded at 420 nm, against the reagent blank. A plot of absorbance Vs mole fraction of the metal is shown in figure 7. From the graph, it is clear that the composition of the metal-ligand complex is 1:2.

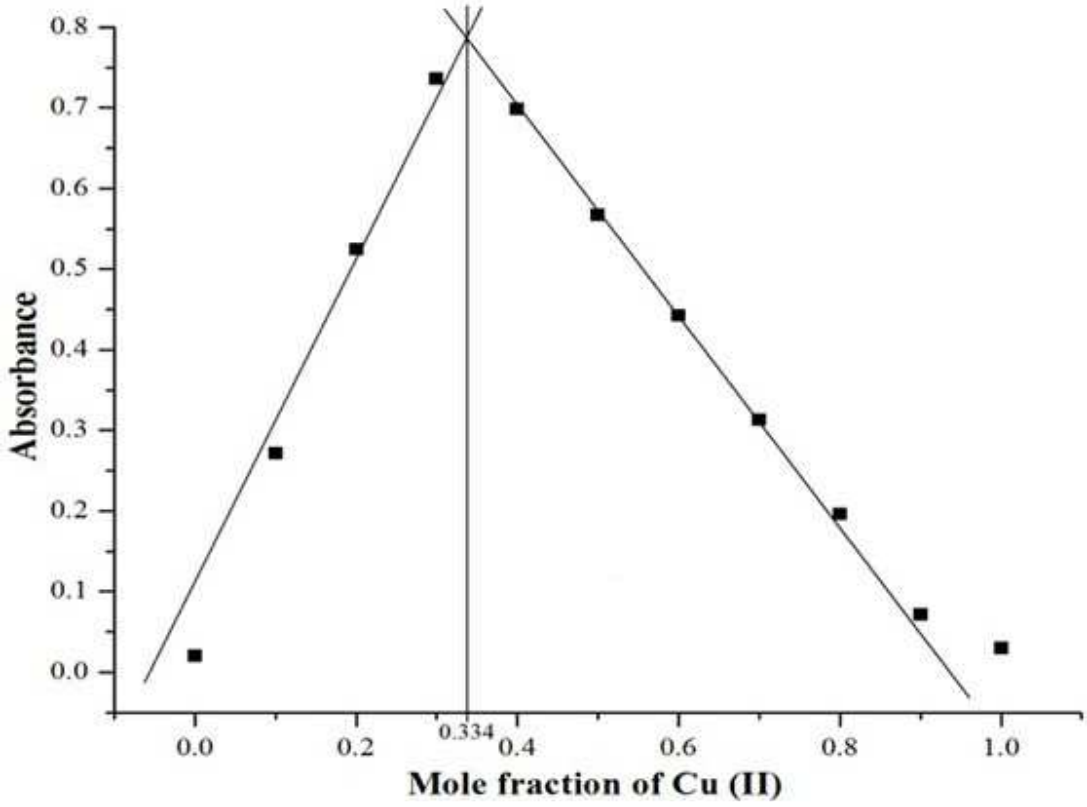


Figure 7. Job's method of continuous variation of Cu(II)-OHAPINH complex. Cu(II) or OHAPINH, 2.5 × 10^{-3} mol/L; pH 4.0; λ_{max} 420 nm

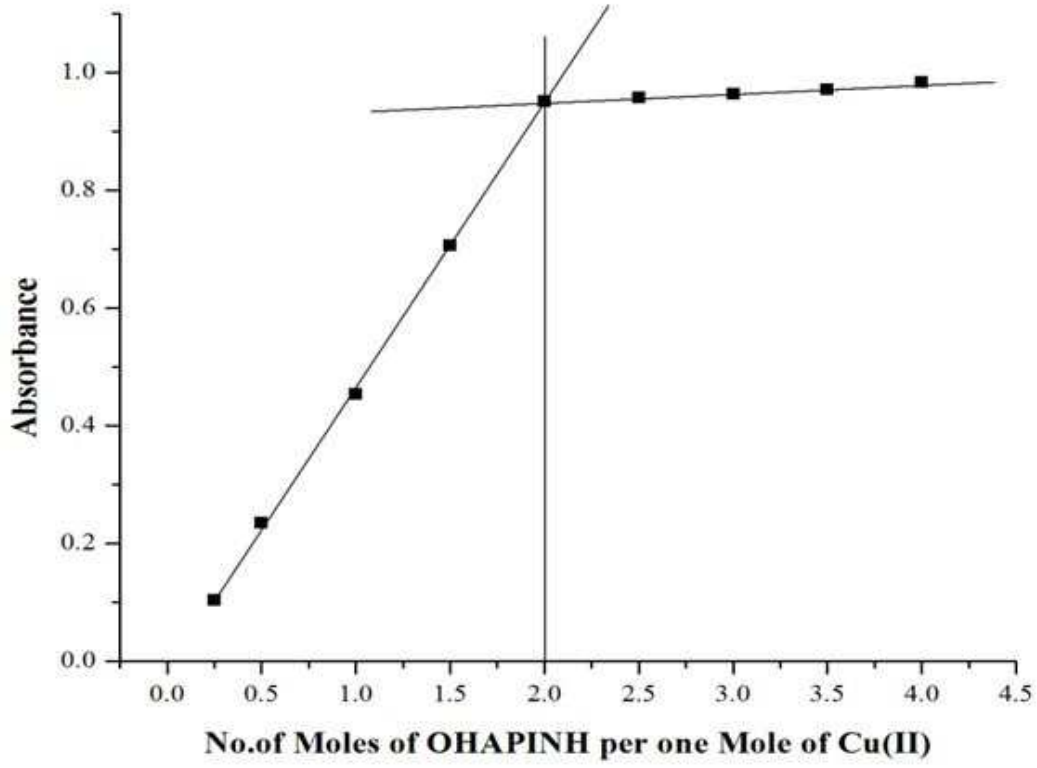


Figure 8. Mole ratio method of Cu(II)-OHAPINH complex Cu(II) or OHAPINH, 2.5 × 10^{-3} mol/L; pH 4.0; λ_{max} 420 nm

Mole ratio method

The amount of metal ion (1.0 mL of 2 × 10^{-3} mol/L) and volume of buffer (3.0 mL, pH 4.0) taken into equilibrium tubes are maintained constant and the reagent concentration is varied with a proportional increase from 0.25 to 3.0 moles of OHAPINH solution to that of metal ion taken in aqueous phase. The solutions are shaken with 10 mL of DCM and measured the absorbance of these organic phases at 420 nm, against their corresponding reagent blanks. A graph is drawn between absorbance and molar proportion of the reagent to that of metal ion (Figure 8). From the graph, it is observed that two moles of the reagent and one mole of the metal ion are participating in the complex formation, which is in good agreement with the results of Job's method of continuous variation.

Asmus method

1.0 ml of 1.0 x 10^{-3}moles/L Copper (II) solution and varying volumes (1-5 mL) of the reagent solution of concentration 1.0 x 10^{-3} moles/L are taken in a set of equilibrium tubes. To these solutions 3.0 mL of sodium acetate-acetic acid buffer (pH 4.0) and the volume is adjusted to 10 mL with double distilled water. The absorbances are measured at 420 nm using the reagent blank. The data are recorded and the plots are drawn between 1/*V*, $1/V^2$, $1/V^3$ and 1/*m* values (Figure 9) where '*V*' is the volume of reagent and `*m*' is the extinction modulus. Among the plots between 1/*m* and 1/*V*, $1/V^2$ and $1/V^3$ only the plot between 1/*m* and $1/V^2$ is a linear plot, indicating that the composition of the complex is 1:2 (M:L) and the instability constant of Cu (II)-OHAPINH has been calculated as 6.25×10^{-8} at room temperature.

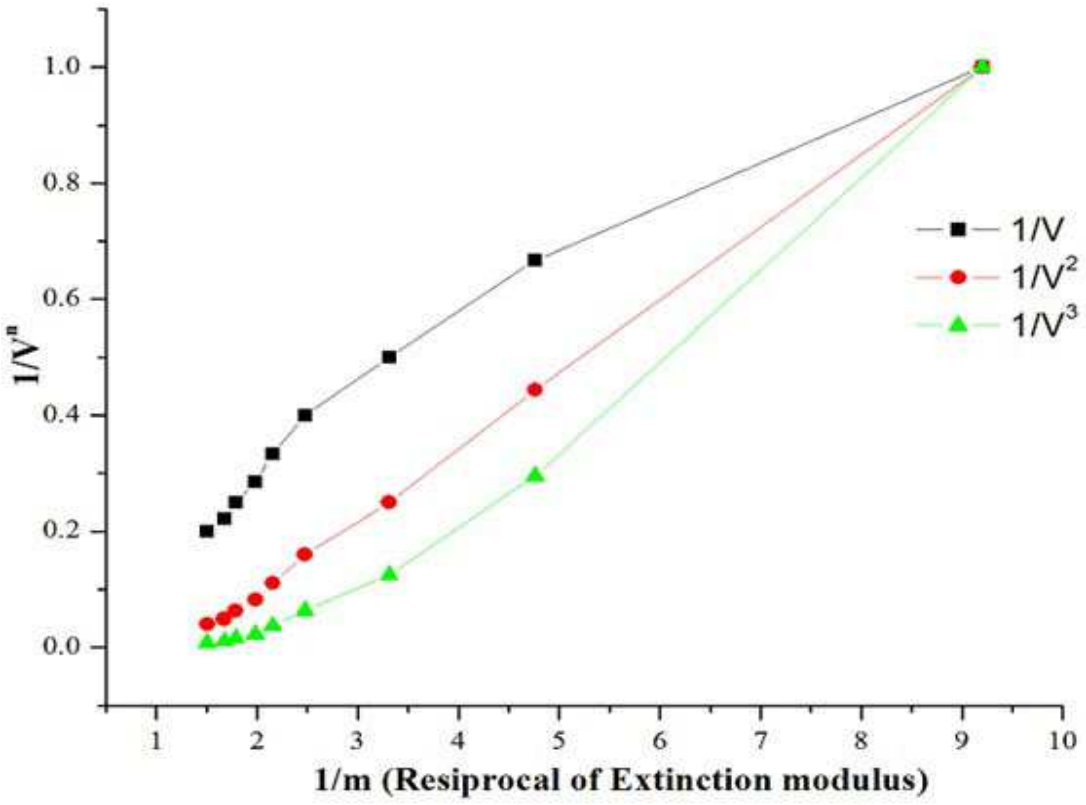


Figure 9. Asmus method of Cu(II)-OHAPINH complex.; Cu(II) or OHAPINH, 1.0 × 10^{-3} mol/L; pH 4.0; λ_{max} is 420 nm

Slope ratio method

Two series of mixtures of have been prepared by using 1.0×10^{-3}mol/L solutions of the reagent and Cu(II). (*i*) *Excess of metal ion:* In one series, the content of Cu(II) (1.0 mL of 1.0 ×10^{-3} mol L^{-1}) has been kept constant and to the samples varying volumes (0.1-1.0 mL of 1.0 ×10^{-3}mol L^{-1}) of the reagent solutions are added. To each solution, 3.0 mL of pH 4.0 buffer solution is added and finally the volumes are adjusted to 10 mL with double distilled water. This mixture is shaken with 10mL DCM for one minute. Absorbance values of the organic phases are measured at 420nm, against their corresponding reagent blanks. In this experiment, the D values increase with the concentration

of the reagent. A graph is drawn between log [reagent] Vs log D [Figure 10a].; (*ii*) *Excess of reagent:* In another series, the concentration of the reagent is fixed (1.0 mL of 1.0 $\times 10^{-3}$ mol L^{-1}) and to the samples varying volumes (0.1-1.0 mL of 1.0 $\times 10^{-3}$ mol L^{-1}) of Cu(II) solutions are added and the rest of the procedure is the same as described above. In this experiment, the D values decrease with the concentration of the metal ion in the aqueous phase. A graph is drawn between log [metal] and log D (Figure 10b). The above two plots indicate the formation of 1:2 metal-ligand complex, under experimental conditions.

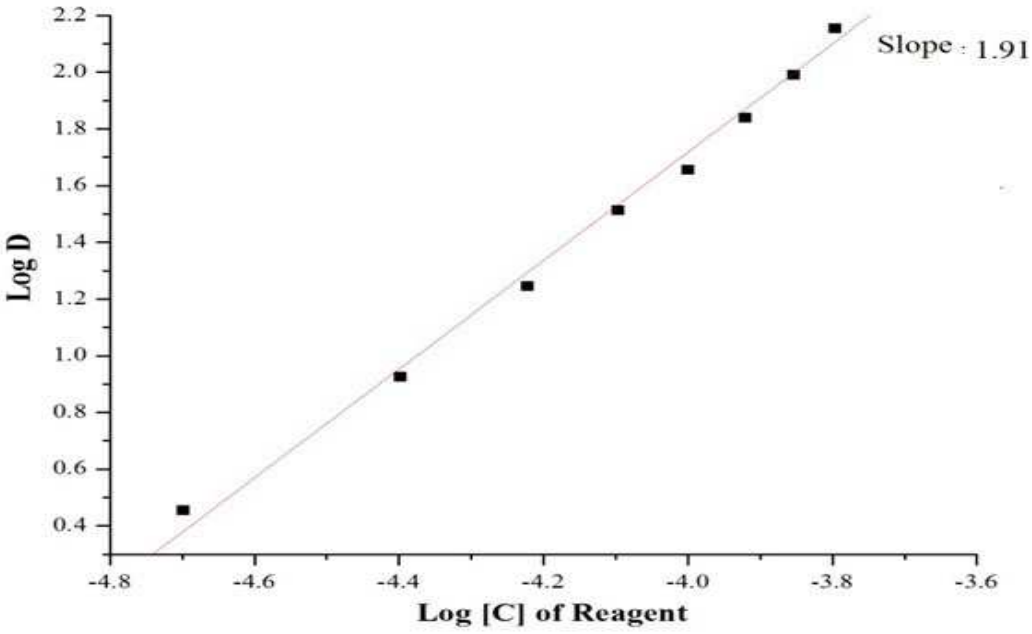


Figure 10a. Slope ratio method of Cu(II)-OHAPINH complex. Excess of metal ion: Cu(II): 1.0 mL of 1.0 $\times 10^{-3}$; OHAPINH: 0.1 - 1.0 mL of 1.0 $\times 10^{-3}$ mol L^{-1} ; pH: 4.0; λ_{max}: 420 nm

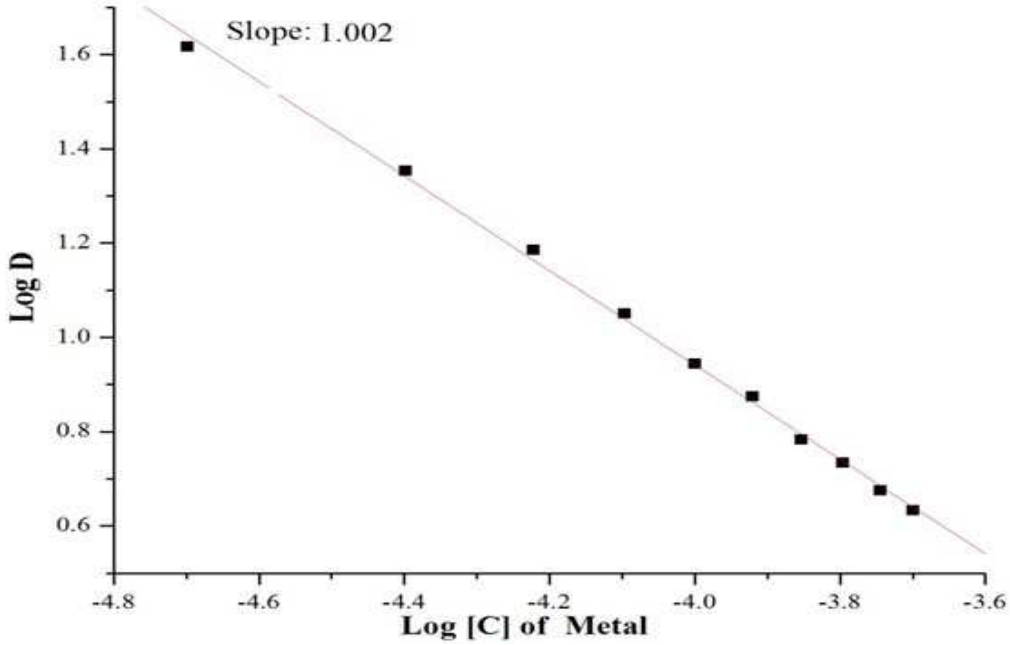


Figure 10b. Slope ratio method of Cu(II)-OHAPINH complex. Excess of reagent: Cu(II): 0.1 - 1.0 mL of 1.0 $\times 10^{-3}$ mol L^{-1}; OHAPINH: 1.0 mL of 1.0 $\times 10^{-3}$ mol L^{-1}; pH: 4.0; λ_{max}: 420 nm

From all the four methods mentioned above, it is evident that the composition of Cu(II)-OHAPINH complex is 1:2 (M:L).

Effect of foreign ions

The effect of diverse ions in the determination of copper (II) has been studied by using 63.6 µg of copper (II) (1.0×10^{-2} mol/L) and various amounts of each diverse ion being discussed. 3.0 mL of buffer (pH 4.0), 0.5 mL of copper solution and 0.5 mL of diverse ion are transferred to an equilibration tube. 1.0 mL of methanolic solution containing 254.4µg (1.0×10^{-3} mol/L) of OHAPINH reagent is added and extracted as usual. The absorbance is measured at 420nm. A change of absorbance ± 0.01 is taken as tolerance limit for the 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 2500 µg, whereas Mo(VI)and W(V) can be tolerated up to 2000 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 5000 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 biological, geological and pharmaceutical samples. The relative merits of *o*-Hydroxyacetophenoneisonicotinoylhydrazone as a highly sensitive spectrophotometric reagent for Cu(II) over those of other hydrazones listed in table.1.

Applications

The proposed extractive spectrophotometric method has been applied for the determination of Cu(II) in biological, pharmaceutical and geological samples as discussed below.

Biological samples

Plant materials are collected in and around Kadapa, A.P., India. The samples are cleaned and dried in open air, protecting them from mineral contamination. The dried sample is pulverized in a mortar for the purpose of analysis, to a convenient size. Few grams of each powdered and dried plant material is taken in a silica crucible, the organic matter is ignited at 550° C, in a muffle furnace for 4-5 h. The ash sample is then dissolved by heating with 10 mL of 2.0 M hydrochloric acid, filtered using Whatmann No. 41 filter paper and then washed with hot water. The filtrate and washings are collected in a 25 mL volumetric flask and finally, made up to the mark with double distilled water. An appropriate aliquot has been analyzed for copper (II) by adopting the procedure using OHAPINH. The process is repeated four times for each sample, and the results obtained are comparable with those values determined (Table 3).

Table 3. Determination of copper(II) in biological samples

Name of the sample	Amount of Cu(II) found (ppm)		SD	RSD%
	ICP-MS	Present method[a]		
Phyllanthus amarus [25]	21.6	21.40	0.141	0.658
Tinosporacardifolia (Guduchi) [26]	22.9	22.60	0.212	0.932
Eclipta alba (Bhringaraja) [26]	26.1	25.90	0.141	0.544
Satavari root (Asparagus racemosus) [27]	12.4	12.30	0.071	0.573
Amla (Emblica afficinalis) [27]	15.6	15.40	0.141	0.912
Flaxseed	12.3	12.20	0.071	0.577
Pterocarpus santalinus root	24.17	24.13	0.028	0.117
Pterocarpus santalinus leaf	3.40	3.38	0.014	0.417
Aloevera shoot	20.21	20.18	0.021	0.105
Aloevera root	19.17	19.14	0.021	0.111
Vitex nugando root	29.54	29.50	0.028	0.096
Jatropha gossipifolia leaf	6.05	6.01	0.028	0.469

[a]*Average of five readings*

Geological samples

About 100mg of each powdered and dried mineral sample is accurately weighed and dissolved in 2.5 mL of conc. hydrochloric acid and 0.5 mL of conc. nitric acid by gentle heating. 2.0 mL of conc. sulphuric acid is added and the

solution is fumed off to dissolve sulphur. Cooled, treated with water and the insoluble portion is filtered off. Now the filtrate containing copper (II) and other impurities are evaporated to dryness to remove free acid. After cooling, the residue is dissolved in 100 mL of water. An appropriate aliquot is analyzed for copper(II) as described above. The results obtained are comparable with those values determined (Table 4).

Table 4. Determination of copper(II) in geological samples

Name of the Sample	Composition	Percentage of Copper		S.D	RSD (%)
		Reported method	Present method[a]		
Chalcocite [28]	Copper-79.8%, Sulphur-20.15 %	79.8	79.77	0.021	0.027
Covellite [29]	Copper-66.00 % , Sulphur-33.50%	66.0	65.92	0.057	0.086
Libethenite [30]	Copper-66.55 %	66.55	66.51	0.028	0.043
Enargite [31]	Copper-48.00%, Arsenic-19 %	48.0	47.94	0.042	0.088
Tennentite [32]	Copper-40.00 %, Arsenic-20.37 %, Iron[b]-3.8%	40.0	39.93	0.049	0.124
Tetrahydrite[32]	Copper-34.80 %,Sb-29.64%, Iron[b]-10.2%	35.0	34.95	0.035	0.101

[a]Average of five readings
[b]Masked with 3% NaF

Pharmaceutical samples

The samples are treated separately with 10 mL of aqua-regia on a hot-plate, at a low temperature, to avoid violent spurting and the temperature of the hot-plate is increased to 300°C, followed by treatment with 1.0 mL of $HClO_4$ to decompose organic matter. The residue obtained is dissolved in 2.0 M HNO_3 and then slowly heated for 2 h to near dryness. Further the residue obtained is treated with 2.0 M HCl twice and heated to near dryness. Finally, the residue is dissolved in a minimum amount of double distilled water. The same solution is quantitatively transferred into a 25 mL volumetric flask and then made up to the mark with double distilled water. An appropriate aliquot is analyzed for copper(II) by the procedure described using OHAPINH. The results obtained are comparable with those values determined (Table 5).

Table 5. Determination of Cu(II) in pharmaceutical samples

Name of the Sample	Composition, Certified value mg/tablet	Amount of Cu(II) found (mg/tablet)		Present method	
		FAAS method	Present method	S.D	RSD (%)
A to Z NS (Alkem)	Elemental zinc 10 mg, Elemental manganese 2.0 mg, Elemental copper 0.9 mg, Elemental selenium 55 mcg	0.90	0.89	0.007	0.790
Revital (Ranbaxy)	Calcium-75mg,phosphorous-58mg, Ferrous fumarateb-17 mg, Magnesium-3mg, Zinc-10 mg, Iodine-0.1 mg, Manganese- 0.5 mg, Copper-0.5 mg, potassium-2.0 mg	0.50	0.49	0.007	1.428
Supradyn (Bayer)	Copper sulphate IP 3.39 mg (equivalent to elemental copper,0.86mg);Zinc Sulphatec IP 2.20 mg; Sodium borate IP 0.88 mg	0.86	0.85	0.007	0.827
Zincovit (Apex)	Zincc-22mg, Magnesium-18 mg, silica-1.0mg,Manganese-0.9mg, Copper-0.5mg, Boron-150 mcg, Selenium-50 mcg, Iodine-150 mcg, Chromium-25 mcg, Molybdenum-25 mcg	0.50	0.49	0.007	1.428
Vimgran (Sarabhai Chemicals, India)	Calcium carbonate USP 250 mg; Iron (II)sulphateb IP 35 mg; Potassium sulphate IP 10 mg; Copper sulphate IP 4.0 mg(equivalent to elemental copper, 1.00 mg); Manganese sulphate IP 6.6 mg; Magnesium oxide IP 10 mg	1.00	0.99	0.007	0.711
Fersolate (Glaxo, India)	Iron (II)sulphateb 195 mg Copper sulphate IP 2.6 mg (equivalent to elemental copper, 0.66 mg); Mangan- ese sulphate IP 2.6 mg	0.66	0.65	0.007	1.080

[a]Average of five readings
[b]Masked with 3% NaF
[c]Masked with 0.5% Thiosulphate
IP-Indian Pharmacopeia
USP-United State pharmacopeia

CONCLUSION

In summary, for the first time the authors developed a rapid extractive spectrophotometric determination of copper(II) using *o*-Hydroxy acetophenone isonicotinoylhydrazone (OHAPINH) 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 (4.8×10^4 $L.mol^{-1}.cm^{-1}$) reveals that the reagent is fairly sensitive for copper(II) when compared with other hydrazones. A number of associated elements do not interfere in the determination. Hence, OHAPINH is strongly recommended for the extractive spectrophotometric determination of Cu(II) at minor and trace levels from various biological, geological and pharmaceutical samples.

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