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Spectrophotometric determination of copper(II) in ground water and food samples by using N'-(1-(pyridin-2-yl)ethylidene)isonicotinohydrazide

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ABSTRACT

A *simple spectrophotometric method has been developed for the determination of copper(II) by using N'-(1-(pyridin-2-yl)ethylidene)isonicotinohydrazide (ACPINH) as a sensitive and selective analytical reagent. It forms a greenish yellow colour metal-ligand (M-L) complex (1:2) at pH 6.0. The Cu(II)-ACPINH complex shows maximum absorbance (λ_{max}) at 365 nm, while the ligand shows maximum absorbance at 284 nm. The method obeys Beer`s law in the concentration range of 0.0636 -0.636 µg $L^{-1}$ with linear regression 0.99867. The molar absorptivity coefficient and Sandell`s sensitivity of the complex are found to be 10.52x10^4 L $mol^{-1}cm^{-1}$ and 0.01962 µg cm^{-2}, respectively. Proposed method is successfully applied for the determination of Cu(II) in water and food samples collected in and around Kadapa. The experimental values are good agreement with the values obtained from AAS method.*

Keywords: ACPINH, ACPINH-Cu complex, water samples, food samples, spectrophotometry, AAS.

INTRODUCTION

Copper is an important micro-nutrient for all living forms. As a natural element in the earth's crust, copper exists in most of the world's surface water and groundwater, although the actual concentration of copper in natural water varies geographically. Drinking water can comprise 20-25% of dietary copper [1]. The World Health Organization recommends a minimal acceptable intake of approximately1.3mg/day [2]. Copper play a crucial role in the functioning of organs and metabolic processes in human beings [3, 4]. In addition to being an essential nutrient for humans, copper is vital for the health of animals and plants, plays an important role in agriculture. Copper(II) ions are widely distributed in biological systems, a significant amount of research has centered on the search for relatively simple copper(II) complexes which may display some of the properties of the metalloproteins [5]. In both humans and animals, the major target organs for copper deficiency are the blood and hematopoietic system, the cardiovascular system, connective tissue and bone, the nervous system and the immune system [6-8]. The excess concentration is harmful to human beings, causes metallic taste, ptyalism, nausea, vomiting, epi-gastric burning and diarrhoea. The accumulation of copper in the human liver and animals is a characteristic of Wilson's disease which produces neurological and psychiatric defects [9-11]. Several compounds are known to react with the metal ions to give coloured complexes and have been employed for the quantitative extraction and spectrophotometric determination of metals at trace levels. Schiff bases play an important role in inorganic chemistry as they easily

form stable complexes with most transition metal ions. The development of the field of bioinorganic chemistry has increased the interest in Schiff base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species. In view of their applicability in various fields, hydrazones, a member of the Schiff base family with tri-atomic >C=N–N< linkage, takes the forefront position in the development of coordination chemistry [12]. Huge number of methods has been reported on the determination of Cu(II) using various ligands by spectrophotometry. Though, the reported spectrophotometric methods [13-20] suffer from one or more disadvantages such as reproducibility, less sensitivity and severe interferences etc., (Table.1). Nevertheless, none has been reported on spectrophotometric determination of Cu(II) in water and food samples using N'-(1-(pyridin-2yl)ethylidene)isonicotinohydrazide (ACPINH) as an analytical reagent. Herein, we developed a simple and highly efficient spectrophotometric method for the determination of Cu(II) in water and food samples using N'-(1-(pyridin-2yl)ethylidene)isonicotinohydrazide (ACPINH) as sensitive and selective analytical reagent.

Table 1

S. No.	Reagent Name	λ_{max}	pH	Beer's law	ε	Remarks	Ref
1	3- methoxy-4- hydroxy benzaldehyde-4-bromo phenyl hydrazone	462	4.0	0.2.-4.0	2.052×10^4	i) Extraction ii) Time consuming	13
2	2,4-Dihydroxy benzophenone benzoic hydrazone	-	4.0	0.31–2.20	0.2×10^4	Less sensitive	14
3	2,2-Dipyridyl-2-pyridyl hydrazone	448	11.9-12.6	Upto1.0	0.4×10^4	Interference of mutual ions and expensive reagent	15
4	2,5-Dihydroxy acetophenone benzoic hydrazone	400	5.0	0.3–6.00	1.1×10^4	Poor selectivity, less sensitive and more reagent consumption	16
5	2,4-Di-hydroxy benzophenone isonicotinyl hydrazone	-	2.0	0.063 - 2.550	0.2×10^4	Less sensitive and more reagent consumption	17
6.	2,4-Dihydroxy acetophenone isonicotinyl hydrazone	-	2.0	0.063 - 2.550	0.2×10^4	Less sensitive, more reagent consumption	17
7.	p-Methyl isonitroso aceto phenone phenyl hydrazone	510	7.0	0.1 - 1.0	0.628×10^4	Less sensitive	18
8.	Di-acetyl monoxime benzoyl hydrazone	-	Basic medium	0.25 - 2.0	1.36×10^4	Less sensitive	19
9.	2,3,4-trihydroxy acetophenone phenyl hydrazone	385	2.5	0.04 - 0.64	10.053×10^4	More acidic(pH 2.5)	20
10.	ACPINH	365	6.0	0.0636-0.636	10.52×10^4	Highly sensitive, selective, simple and rapid	PM

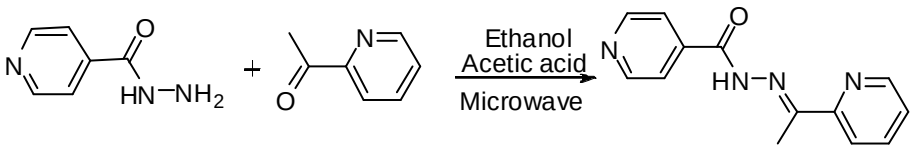
EXPERIMENTAL SECTION

Apparatus: A Double beam UV-Visible spectrophotometer (Shimadzu model UV-1800) with a 1.0 cm quartz cell is used for absorbance studies and pH meter (Systronics model 3305) is used for measurement of pH respectively. Flame atomic absorption spectrophotometer (Shimadzu model No: AA-6300) is used for the determination of Cu(II).

Reagents and solutions:
All the chemicals used are of analytical reagent grade or the highest purity available (Aldrich ACS or Merck pro-analysis grade). DMF and double distilled water are used though out the experiment.

Preparation of N'-(1-(pyridin-2-yl)ethylidene)isonicotinohydrazide (ACPINH):
The reagent is prepared according to the known procedure reported in the literature [21].

Microwave Method: Isonicotinic acid hydrazide [7.3 mmol, 1.0 g] and 2-acetyl pyridine [7.3 mmol, 1.0 g] are dissolved in 10.0 mL of ethanol and then 5% glacial acetic acid [2.5 mL] is added into a 100-mL beaker. The beaker is placed in a domestic microwave oven at 200 watts for 30-45 sec. The progress of the reaction is monitored by TLC. After completion of the reaction, the reaction mixture is cooled to RT. The formed crude product is washed twice with cold ethanol and dried. Finally, the obtained product is re-crystallised from hot ethanol.



Scheme: Preparation of N'-(1-(pyridin-2-yl)ethylidene)isonicotinohydrazide

Preparation of the standard solution of Cu(II):
The stock solution has been prepared by dissolving 2.497 g (0.01M) of copper sulphate in double distilled water (DDW) and the solution is made up to 1.0 L and standardised by iodometry [22]. This stock solution is diluted further, wherever necessary, with double distilled water.

Preparation of ACPINH ligand solution:
In a 100 ml volumetric flask 0.240 g of ACPINH (0.01M) ligand is dissolved in DMF and made up to the mark with DMF. This stock solution is further diluted to required concentration.

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

Acetic acid and sodium acetate buffer solution of pH 6.0 is prepared by mixing 25.0 mL of 0.2 M acetic acid and 475 mL of 0.2 M sodium acetate solutions by volume. The pH of the above buffer solution is measured by a pH meter and finally adjusted suitably.

General analytical Procedure for the Determination of Cu(II):
In a 10 mL standard flask, 1mL of standard copper sulphate aqueous solution of required concentration, 2.0 mL of ACPINH solution of required concentration and 4.0 mL of buffer (pH 6.0) are taken and diluted up to the mark with double distilled water. The contents of the flask are mixed well and the absorbance is measured at 365 nm against ligand blank, prepared similarly except Cu(II).

Preparation of natural water samples:
Different water samples are collected from the bore wells located 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 is 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 is filtered and quantitatively transferred into a 25.0 mL calibrated flask and made up to the mark with double distilled water [23].

Preparation of food samples:
Food samples are collected from various villages around Kadapa, A.P. India. The samples are cleaned and dried in open air, protecting them from mineral contamination. The dried sample is pulverised in a mortar for the purpose of analysis, to a convenient size. One gram of powdered sample is taken in a 100 mL beaker and digested with 10.0 mL of concentrated Nitric acid and Hydrochloric acid followed by 10.0 mL of double distilled water [24]. Then filter the solution by using Whatmann No. 41 filter paper. The filtrate is taken in a 10.0 mL standard flask and makes the volume up to the mark with double distilled water and analysed as per the general procedure.

RESULTS AND DISCUSSION

Copper(II) reacts with ACPINH and forms a greenish yellow coloured complex at pH 6.0 in aqueous DMF. The coloured complex shows maximum absorbance at 365nm, against the ligand blank. Hence, a detailed study has been undertaken for the determination of Cu(II) using ACPINH by spectro-photometric method. The optimised method is successfully applied for the determination of copper in food and water samples alone or in presence of diverse ions.

Absorption spectra of ligand (ACPINH) and Cu(II)-ACPINH complex: The absorption spectrum of the ligand (ACPINH) is recorded against the solvent blank initially. The absorption spectrum of Cu(II)-ACPINH complex is

recorded against the ligand blank. The absorption spectrum of both complex and ligand are shown in fig.1. From the absorption spectra, it is clear that, the ligand shows maximum absorption at 284 nm, where as the complex shows maximum absorption at 365 nm. Therefore, all the spectral measurements are carried out at this wavelength.

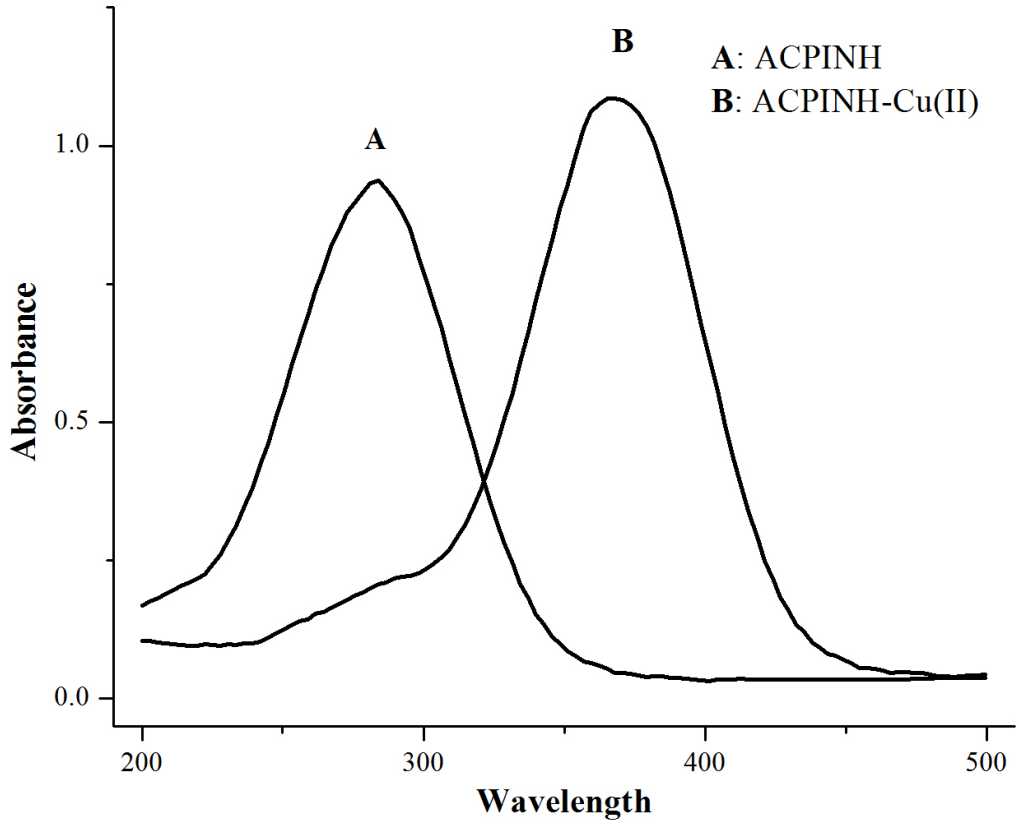


Fig.1. A) Absorption spectrum of ACPINH Vs. blank (B) Absorption spectrum of Cu(II)-ACPINH complex Vs. Ligand blank

Effect of pH:

The effect of pH on the formation of Cu(II)-ACPINH-complex is studied to evaluate the optimum pH. Buffers of various pH is prepared by hydrochloric acid-sodium acetate (pH 1.0 - 3.5) buffer, sodium acetate-acetic acid (pH 3.0 - 6.0) buffer and ammonium acetate (pH 7.0) buffer. The pH studies are carried out by taking 1.0 mL of copper(II) solution ($1x10^{-4}$M), 2.0 mL of ACPINH ($1X\ 10^{-4}$M) and 4.0 mL buffer solution in a 10 mL standard flask and made up to the mark. Measured the absorbance of the Cu(II)-ACPINH complex against reagent blank at 365 nm. The determination of Cu(II) with ACPINH has been studied over the pH range 1.0 - 7.0 and is observed that at pH 6.0 absorption is maximum, so it is considered as the optimum pH.

Effect of the ligand Concentration:

The effect of the ligand concentration has been studied by taking 1.0 mL of $1x\ 10^{-4}$M copper(II) solution and 4.0 mL buffer solution (pH 6.0) into a set of 10 mL standard flasks. To these solutions 2.0 mL of the ligand solution of concentration ranging from $0.25\ x\ 10^{-4}$ M to $3.0\ x\ 10^{-4}$ M is added to each of the standard flask and the contents are made up to the mark with water and mix well. The absorbance is measured at 365 nm, against their corresponding ligand blanks. From the results, it is clear that 2 moles of ligand is necessary for the maximum recovery of Cu(II). The results are plotted in the graph (Fig.3).

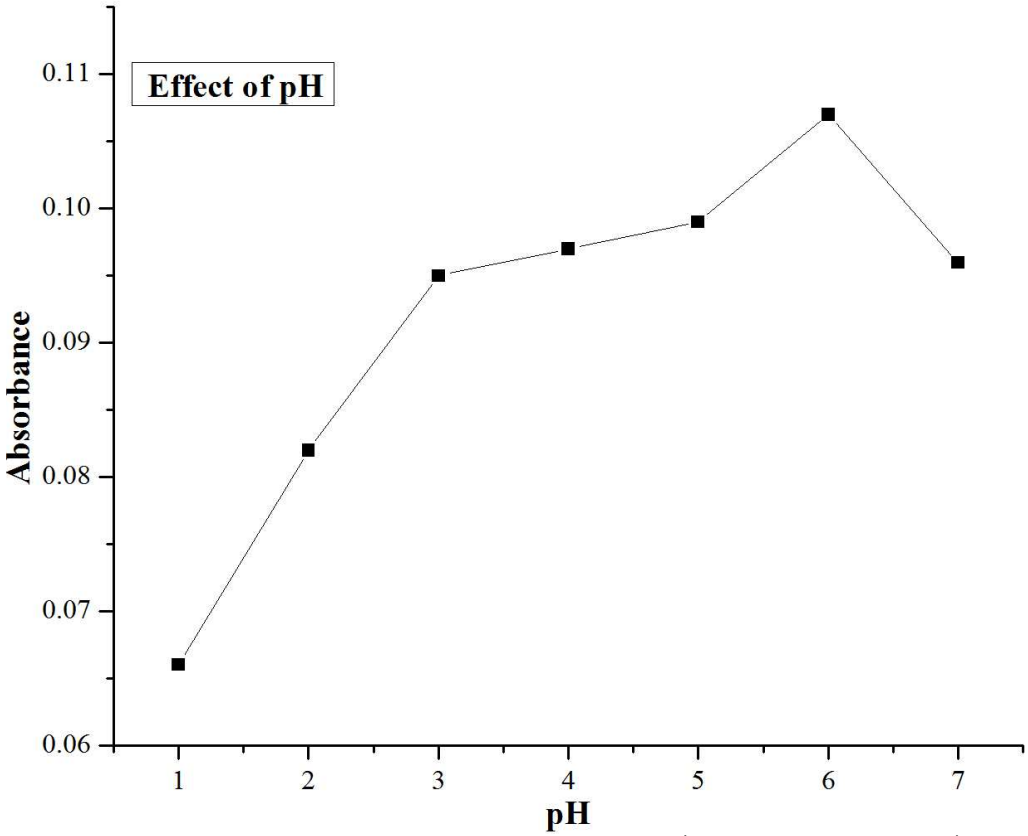


Fig.2. Effect of pH on absorbance of Cu(II)-ACPINH complex: Cu(II)=1.0 mL of 1x10^{-4} M, ACPINH=2.0 mL of 1x10^{-4} M, λmax= 365nm

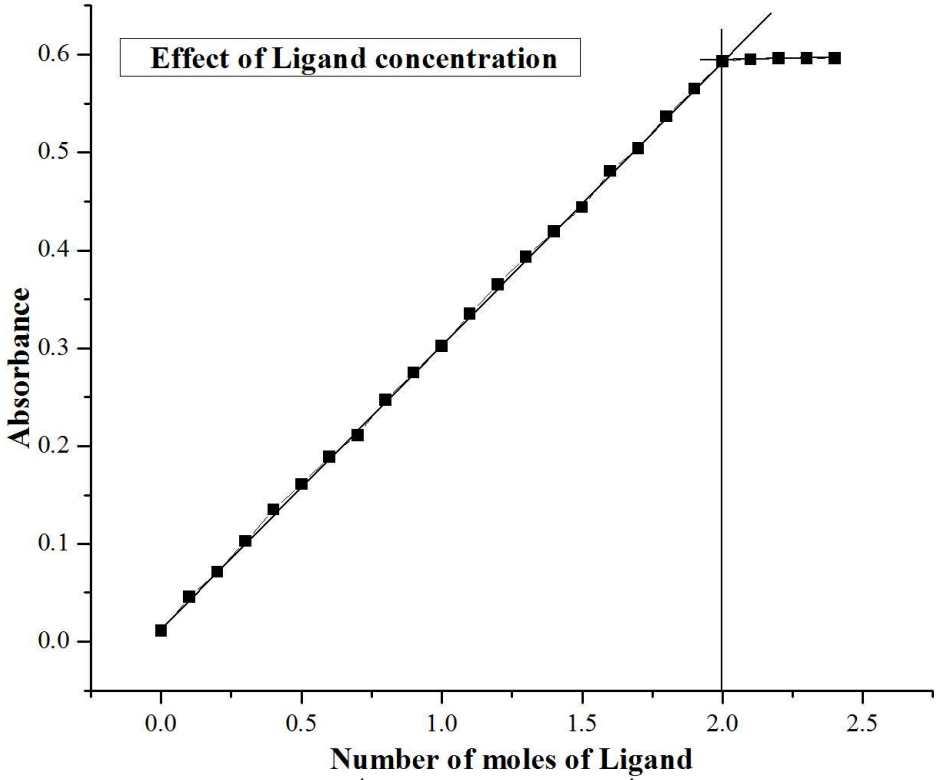


Fig.3. Mole ratio method: 2.0 mL of Ligand (1x10^{-4}M), 1.0 mL of Cu(II) of (1x10^{-4}M),4.0 mL of buffer (pH 6.0), λmax= 365nm

Validity of Beer`s law (Molar absorptivity; Sandell's sensitivity and correlation coefficient):
2.0 mL of ACPINH solution, 4.0 mL of buffer solution of pH 6.0, 1.0 mL of (1 x10^{-4} M) copper(II) solution, in the range of 0.0636 to 0.636 µg mL^{-1} are added in a 10 mL standard flask and made up to the mark with double distilled water and mix well and absorbance is determined at 365 nm against the blank. The correlation coefficient is 0.99867 which indicates the linearity between the two variables. The molar absorptivity and Sandell's sensitivity of the complex are found to be 10.52x10^4 L $mol^{-1}cm^{-1}$ and 0.01962 µg cm^{-2}, respectively.

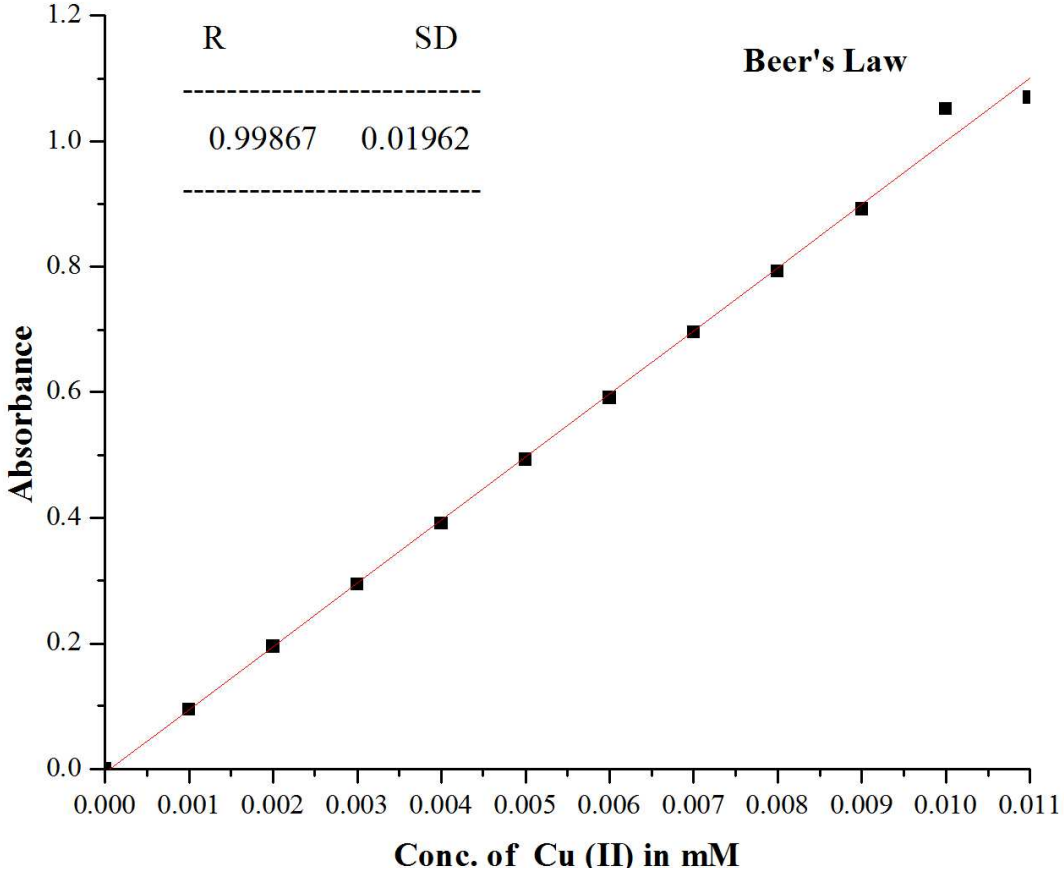


Fig.4. Beer's law : Ligand = 2.0 mL 0f 1x10^{-4} M, 1.0 mL of 0.001 - 0.010mM of Cu(II), 4.0 mL of buffer (pH 6.0), λ max= 365nm

Determination of the composition of the Cu(II)-ACPINH complex:
The composition of the Cu(II)-ACPINH complex has been studied with the help of Job`s continuous variation and mole ratio methods.

Job's method of continuous variation:
The stoichiometric ratio between Cu(II) and ACPINH is evaluated by Job's method of continuous variations. Equi-molar solutions of copper(II) and ACPINH (1x10^{-4} M) are prepared. X mL of the ligand and (5-X) mL of the metal ion solutions are taken into 10 mL standard flasks. Now the total volume in the flask including two solutions is 5.0 mL and 4.0 mL of buffer solution of pH 6.0 and made up to the mark with double distilled water. The absorbance of each solution is determined against their corresponding ligand blanks. A plot of absorbance Vs mole fraction of the metal is shown in fig7. From the graph, it is clear that at 365 nm the composition of the metal ligand complex is 1:2.

Mole Ratio method:
The effect of the ligand concentration has been studied by taking 1.0 mL of (1x 10^{-4}M) copper(II) solution and 4.0 mL buffer solution (pH 6.0) into a set of 10 mL standard flasks. To these solutions 2.0 mL of the ligand solution of concentration ranging from 0.25 x 10^{-4} M to 3.0 x 10^{-4} M is added to each of the standard flask and the contents are made up to the mark with water and mix well. The absorbance is measured at 365 nm, against their

corresponding ligand blanks. From the results, it is clear that 2 moles of ligand is necessary for the maximum recovery of Cu(II). The results are plotted in the graph (Fig.6).

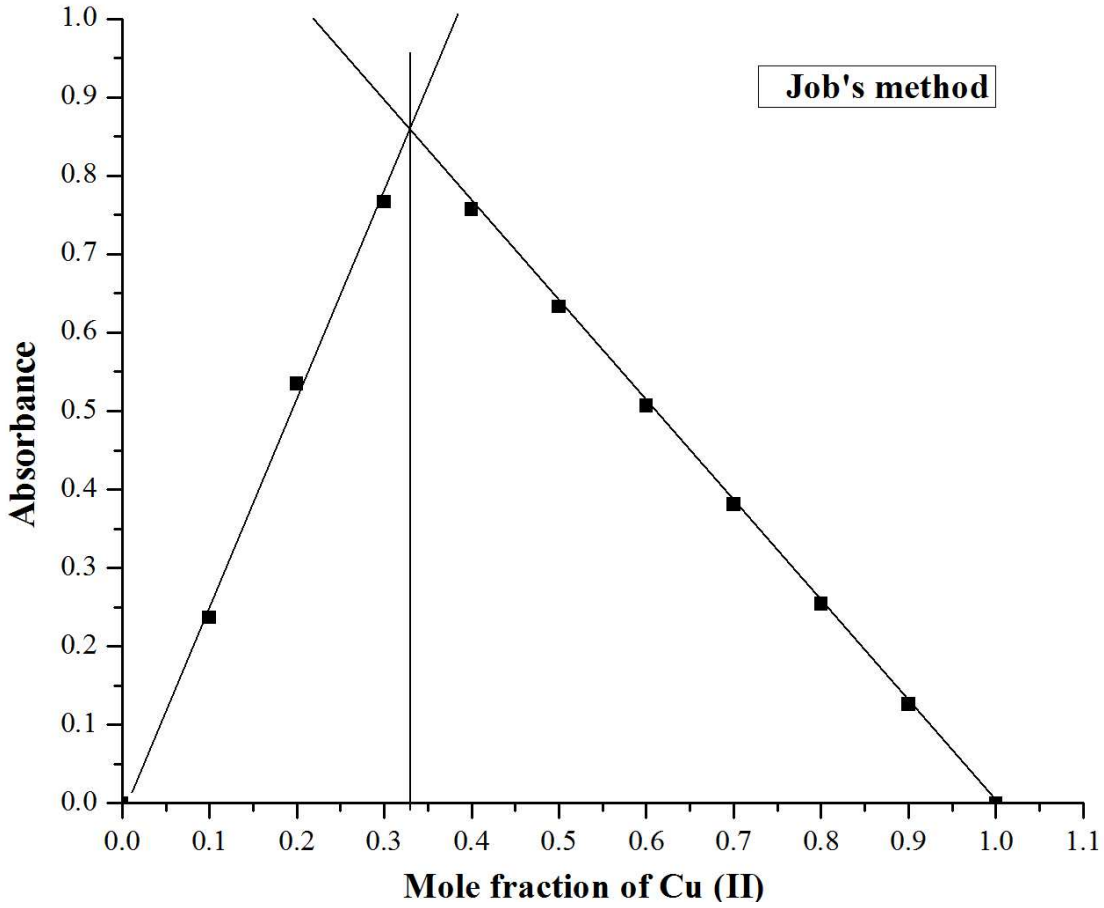


Fig.5. Job's plot of continuous variations for stoichiometric ratio between Cu(II) and ACPINH (1×10^{-4} M each); 4.0 mL of buffer pH 6.0, λ max =365nm

In addition to the above, different molar excesses of Cu(II) are added to the fixed amount of ACPINH and absorbance are measured according to the standard procedure. It is observed that the reagent and the metal molar ratio of 2:1. Based on the above two methods the composition of the Cu(II)-ACPINH complex is confirmed as 1:2 ratio.

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) ($1x10^{-4}$ M) and various amounts of each diverse ion being discussed. 4.0 mL of buffer (pH 6.0), 0.5 mL of copper solution and 0.5 mL of diverse ion are transferred to an equilibration tube 1.0 mL of DMF solution containing 240.12 µg ($1.0x10^{-4}$ M) of ACPINH ligand is added and absorbance is measured at 365 nm. A change of absorbance ± 0.01 is taken as tolerance limit for the interference.

The results are indicated in table 2 and interference of foreign ions effect is removed by one millilitre of 0.2% fluoride is used as a masking agent for Fe(II) and Fe(III). Interference due to Co(II), Ni(II), Zn(II), Pd(II) and Cd(II) can be suppressed by adding 1.0 mL of 0.2% citrate solution. Increasing the amounts of their corresponding masking agents proportionately can mask higher amounts of interfering ions.

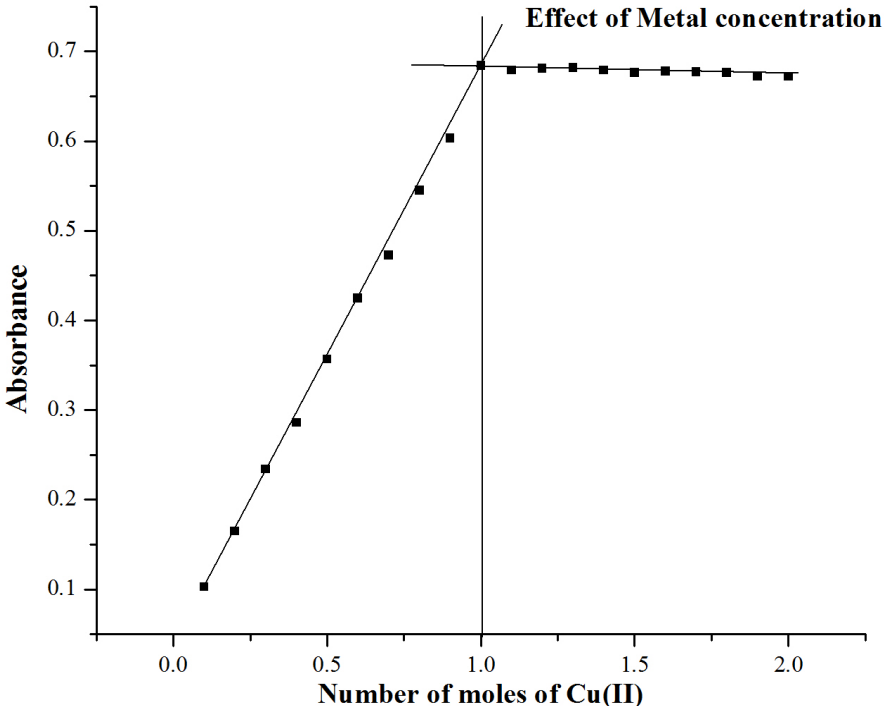


Fig.6. Mole ratio method: Metal variation, ACPINH=2.0 mL of 1.0x10^{-4} M, Cu(II)=1.0 mL of (0.1x10^{-4}M-1.0x10^{-4}M), 4.0 mL of buffer solution pH 6.0, λ max =365nm

Table 2

Cations			Anions		
Foreign ions	Tolerance limit	remarks	Foreign ions	Tolerance limit	remarks
Ba (II)	5000 µg	Less interference	fluoride, bromide, iodide and chloride	5000 µg	Less interference
Ca(II), Mg(II) Pb(II), Mn(II) and Bi(III)	4000 µg	Moderate interference	nitrate, sulphate and thio-sulphate	4500 µg	Moderate interference
Al(III), Cr(III), Ag (I) and Sb(II)	3000 µg	Moderate interference	citrate, acetate and tartrate	4000 µg	Moderate interference
Zn(II), Pd(II) and Cd(II)	2000 µg	More interference	thio-cyanate and oxalate	100 µg	More interference
Co (II), Ni (II), Fe (II) and Fe(III)	1000 µg	More interference	EDTA	50 µg	More interference

From the above discussion, it is clear that copper can be separated from a number of associated metal ions usually present in water and food samples. The relative merits of ACPINH as a highly sensitive spectrophotometric ligand for copper(II) over those of other hydrazones.

Applications of the developed method:

The proposed spectrophotometric method is successfully applied for the determination of copper(II) in water and food samples collected from various villages in and around Kadapa.

Natural water samples:

The natural water samples are prepared according to the earlier repoted procedure. An aliquot of the solution is taken and the Cu(II) is determined by adopting the well optimized procedure. Results are reported in Table 3.

Table 3: Determination of Cu(II) in water samples collected from in and around Kadapa

S. No.	Sample no.	Cu(II) added (µg/mL)	AAS (µg/mL)	Present Method (µg/mL)	% of recovery
1	[a]Sample 1	2	1.99	1.99	99.50
2	[b]Sample 2	2	1.98	1.98	99.00
3	[c]Sample 3	2	2.85	2.82	141
4	[d]Sample 4	2	6.81	6.77	338.5
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]Sample8	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
11	[k]Sample 11	2	1.98	1.99	99.5
12	[l]Sample 12	2	2.0	2.01	100.5
13	[m] Sample 13	2	1.99	2.0	100

a-m :Sample collection sites in and around Kadapa town : [a]Ajad nagar, Kadapa: [b]Peddadarga, Kadapa; [c]Near sub jail, Kadapa; [d]Krishnapuram, Kadapa; [e]Yerramukkapally, Kadapa; [f]Near RIO office, Kadapa; [g]Near ZP office, Kadapa; [h]Patha kadapa, Kadapa; [i]Devuni kadapa, Kadapa; [j]Koparthy, Kadapa. [k]Royalgold colony, Kadapa; [l]Palempapaiah street, Kadapa;[m]NGO colony, Kadapa.

Food Samples

The food samples are prepared as per the earlier reported procedure. An aliquot of the solution is taken and the Cu(II) is determined by adopting the well optimized procedure. Results are presented in Table 4.

Table 4: Determination of copper(II) in food samples

S. No.	Sample	Copper added (µg/L)	Conc. of Cu(II) by AAS (µg/L)	Conc. of Cu(II) by Present method (µg/L)
1.	*Hibiscus cannabinus (Gongura)*	0	2.70	2.69
		100	102.72	102.70
		500	502.73	502.71
2.	*Coriandrum sativum (Kothimera)*	0	12.55	12.54
		100	112.56	112.54
		500	512.57	512.55
3.	*Amaranthus cruentus (Thotakura)*	0	30.24	30.23
		100	130.25	130.23
		500	530.25	530.24
4.	*Amaranthus graecizans (Cirraku)*	0	32.35	32.34
		100	132.37	132.36
		500	532.38	532.37
5.	*Rumex vesicarius (Chukaku)*	0	20.21	20.20
		100	120.21	120.20
		500	520.22	520.21
6.	*Spinacia oleracia (Palakura)*	0	11.80	11.78
		100	111.81	111.80
		500	511.83	511.82

In order to highlight the utility of the proposed method, it is used for the spectrophotometric determination of copper contents of natural water samples and food samples. The results are in good agreement with the values obtained from Atomic Absorption Spectroscopy.

CONCLUSION

We developed a simple, efficient and rapid spectrophotometric method for the determination of Cu(II) using highly sensitive and selective N'-(1-(Pyridin-2yl)ethylidene)isonicotinohydrazide as an analytical reagent. The proposed method offers advantages like good sensitivity, selectivity, reliability, reproducibility, less interference and immediate colour development. The present method is found to be quantitative comparable to other standard methods. The molar absorptivity of the complex (10.52×10^4 L $mol^{-1}cm^{-1}$) reveals that the ligand is fairly sensitive for copper(II) when compared with other hydrazones. A number of associated elements don't interfere in the determination of copper. Hence, ACPINH is highly useful ligand for the spectrophotometric determination of copper(II) present in low and trace levels from various natural water and food samples.

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