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Selective Kinetic Spectrophotometric Determination of Copper(II) in Food and Medicinal Leafy Samples

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ABSTRACT

A simple, sensitive and fairly selective kinetic spectrophotometric method has been developed for the determination of copper(II) using 2,3,4-trihydroxyacetophenonephenylhydrazone (THAPPH) as an analytical reagent. The metal ion has formed 1:2 (M:L) bluish green coloured complex with THAPPH in hydrochloric acid-potassium chloride buffer of pH 2.5. Beer's law was obeyed in the range 0.04-0.64 µg/ml of Cu(II) at λ_{max} of 385 nm. The sensitivity of the method was calculated in terms of molar absorptivity ($1.0053 \times 10^5 \text{ L. mol}^{-1} \text{ cm}^{-1}$) and Sandell's sensitivity ($0.0006265 \mu\text{g cm}^{-2}$), which explains the method is more sensitive. The standard deviation (0.0024), relative standard deviation (0.47%), confidence limit (± 0.0017), and standard error (0.0008) found that the developed method has more precision and accuracy. The stability constant was calculated by Ausmu's (2.35×10^{11}) and Edmonds & Birnbaum's (2.44×10^{11}) methods at room temperature. The interfering effect of various cations and anions was also studied. The reliability of the method was assured by analyzing the standard alloys, Brass (BCS 5g) H.T. Bronze (BCS 10g) and Phosphor-Bronze (BCS 7a). The proposed method was successfully employed for the determination of copper(II) in food and medicinal leafy samples and inter compared the experimental values using atomic absorption spectrophotometer and statistically evaluated using F-test.

Keywords: Copper(II), 2,3,4-trihydroxyacetophenonephenylhydrazone, kinetics spectrophotometry, standard copper alloys, food and medicinal leafy samples.

INTRODUCTION

Copper is one of the several metal ions that play an important role in the biological system. It has a vital role during cell respiration, in the blood of invertebrate animals and in the formation

of haemocyanin[1]. Apart from the biological utility of the copper, it also finds applications in industries[2]. It is used in the electrical industry as fine wires, commutator bars and high conductivity tubes. In addition to this, it is an important pollutant in the environment resulting from the industrial discharge in the form of particulate or soluble copper waste from electroplating, chemical and textile industries. Copper occurs naturally in most vegetables, meat and grains. The study of copper in food items is of great concern, since it plays a definitive role in the intrinsic mechanism regulating vital biological processes[3,4]. A wide variety of clinical disorders have been associated with a dietary deficiency of copper which respond to copper therapy. They include anemia, depressed growth, bone disorders, depigmentation of hair or wool, abnormal wool growth, neo-natal ataxia, heart failure and gastro-intestinal disturbances[5,6].

Cu(II) forms stable complexes with a number of common organic reagents[7-12]. In view of this, separation and determination of copper from associated elements is indispensable. The determination of small quantities of copper in biological materials has posed considerable difficulties and often led to inaccurate results because of the presence of number of interfering elements. Moreover, the methods of determination are not highly sensitive. Hence, the uses of kinetic methods have attained significant importance in the analysis of trace quantities of copper[13-17].

The use of hydrazones in inorganic analysis has been reviewed extensively for the determination of Cu(II)[18-23]. In the present study, here in we report 2,3,4-trihydroxyacetophenonephenylhydrazone (THAPPH) as a good analytical reagent for the selective kinetic spectrophotometric determination of Cu(II) in standard alloys, food and medicinal leafy samples. The proposed method when compared with other spectrophotometric methods (Table 1)[18-28] was found to be more sensitive and selective.

EXPERIMENTAL SECTION

Apparatus:

Determination of metal ion concentration was carried out by Shimadzu 2450 UV-VIS spectrophotometer equipped with 1.0 cm quartz cell and the pH of solution was tested with an Elico LI-120 digital pH meter. A Perkin-Elmer 170-30 atomic absorption spectrophotometer was used for the comparison of results.

Reagents:

All the chemicals used were of the analytical grade unless specified here. 2,3,4-trihydroxyacetophenonephenylhydrazone was prepared by dissolving accurately weighed 25 g of phenylhydrazine hydrochloride and 30 g of sodium acetate in 250 ml of doubly distilled water. An alcoholic solution of 2,3,4-trihydroxyacetophenone (16 g) is added to the above solution and the mixture was heated on a hot water bath for about 30 minutes and was kept overnight. The pale yellow colored product of 2,3,4-trihydroxyacetophenonephenylhydrazone (THAPPH) obtained was filtered and was recrystallised using 30% ethanol in presence of animal charcoal. The purity of the reagent was checked by melting point (m.p. 146-147°C) and TLC.

The stock Cu(II) solution was prepared by dissolving accurately weighed 7.940 g of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in doubly distilled water containing a few drops of concentrated

sulphuric acid. The solution was made upto 1.0 L and standardised by iodometry²⁹. The buffer solution was prepared using 2.0 moles/L hydrochloric acid and 2.0 moles/L potassium chloride solutions in doubly distilled water and suitable portions of these solutions were mixed to get the desired pH using a pH meter.

General Kinetic Spectrophotometric Procedure:

1.0 ml of (1.5741×10^{-4} M) copper(II) solution is taken into a 25 ml standard flask. To this solution, 5.0 ml of (3.1482×10^{-4} M) 2,3,4-trihydroxy acetophenonephenylhydrazone (10-fold excess), 5.0 ml of hydrochloric acid-potassium chloride buffer of pH 2.5 and 1.5 ml of 0.6% v/v hydrogen peroxide solutions were added. The mixture was made upto the mark with doubly distilled water. The spectrum of the complex was recorded against the reagent blank at λ_{\max} of 385nm by fixed time method (25th minute).

Preparation of Copper alloy samples:

About 0.1g of each oven dried (110°C) standard alloys of copper such as Brass (BCS 5g), H.T. Bronze (BCS 10g) and Phosphor-Bronze (BCS 7a) were dissolved in 15ml of aqua regia. They were heated to near dryness and the nitrate was expelled from the residue, using 5ml of concentrated HCl. Each residue was twice extracted into doubly distilled water and made up to 100ml, separately²⁹. Suitable volumes of these solutions were taken and were analyzed for Cu(II) using the recommended general procedure.

Preparation of food and medicinal leafy samples:

The food and medicinal leafy samples analyzed were brought from the local agricultural fields. The samples were cleaned and dried in open air, protecting them from mineral contamination. The dried sample was pulverized in a mortar for the purpose of the analysis, to a convenient size. 10.0g of each powdered sample was taken in to a silica crucible, heated to oxidize the organic matter, and ashed at 550°C in a muffle furnace for 4 -5 hours. The ash was dissolved by heating with 10.0 ml of 2.0 moles/L hydrochloric acid and filtered through an acid washed filter paper (Whatman no.41), and then the residue was washed with hot water²⁹. The filtrate and washings were collected in a 25ml volumetric flask and to this 10 μ g/g of copper(II) was added and finally made up to the mark with doubly distilled water. An appropriate aliquots of the dilute solution was taken and its pH was adjusted to 2.5 and determined copper(II) by the above prescribed procedure.

RESULTS AND DISCUSSION

The effects of pH of the solution, concentration of the reagent, presence of hydrogen peroxide, temperature of the system and the presence of foreign ions, on the rate of catalysed oxidation of THAPPH by H₂O₂, in presence of copper(II) as catalyst, were studied in detail to establish suitable conditions for sensitive and selective spectrophotometric determination of copper(II) in alloys, food and medicinal leafy samples.

Absorption spectrum of the complex:

The absorption spectrum of the complex was recorded against the reagent blank shown in Figure-1. It was observed that the complex has λ_{\max} at 385 nm. The absorbance of the reagent at this wave length was minimum and hence further absorbance measurements of the complex were made at 385 nm.

Effect of pH:

A mixture containing 1.0 ml of copper(II) solution and 5.0 ml of reagent solution was treated with 1.5 ml of 0.6% v/v hydrogen peroxide as for the general procedure. The pH of the solution was adjusted to the required value by adding dilute hydrochloric acid-potassium chloride solution. Then the solution was made upto 25 ml with distilled water. The absorbance was measured at 385 nm against the reagent blank. The experiment was repeated for different pH values from 0.5-5.0. A plot (Figure-2) has drawn between pH and absorbance shows that the absorbance was maximum and constant in the pH range of 2.0-3.0. Hence, further studies were carried out at pH 2.5.

Effect of hydrogen peroxide:

To different aliquots of solutions containing a constant volumes of Cu(II) and reagent at 5.0 ml of pH 2.5 buffer, in 25 ml standard flasks. To these solutions, further 1.5 ml of varying percentages of (0.1 to 1.0%) H₂O₂ solutions were added. The solutions were made upto the mark of the flasks and the experiment was carried out by fixed time method. The absorbances were measured at 385 nm, against the reagent blank. The plot (Figure-3) has drawn between percentage of hydrogen peroxide and absorbance shows that the absorbance was maximum at 0.6% v/v H₂O₂. Hence further studies were made at 0.6% v/v hydrogen peroxide.

Effect of reagent concentration:

To study the effect of reagent concentration, the volumes of copper(II) (1.0 ml of 1.574×10^{-4} M), hydrochloric acid-potassium chloride buffer (pH 2.5) and hydrogen peroxide (0.6% v/v) solutions were kept constant in 25 ml of standard flask and varied the concentration (0.5 to 8.0 ml of 3.1482×10^{-4} M) of reagent and made upto the mark with doubly distilled water. The absorbances were measured at 385 nm, against the reagent blank and the results were shown in Figure-4. From the Figure-4, it was clear that the addition of ten fold molar excess of the reagent solution was necessary for maximum colour development.

Applicability of the method:

Known aliquots of copper(II) solutions (0.2-1.6ml) of 1.574×10^{-4} M were treated with 5.0 ml of 3.1482×10^{-4} M reagent solution followed by 5.0ml of hydrochloric acid-potassium chloride buffer (pH 2.5) and 1.5 ml of 0.6% v/v hydrogen peroxide solutions. These solutions were made upto 25ml with distilled water. The absorbances of these solutions were measured at 385 nm for every five minutes against the reagent blank. The rate of the catalyzed reaction increases with increase in the concentration of copper. Plots for different amounts of copper(II) were drawn between the absorbance and time (Figure-5). Slope values ($\tan \theta$) were calculated for each curve. A graph was also plotted between $\tan \theta$ and concentration (Figure-6). A straight line was obtained in the range of 0.04-0.64 $\mu\text{g/ml}$ of copper. The determination was also carried out using fixed time method. A plot was drawn between the amount of copper(II) and its absorbance (Figure-7). In this method also, the determination range of copper(II) was found to be 0.04-0.64 $\mu\text{g/ml}$.

Sensitivity, precision and accuracy of the method:

The molar absorptivity of the Cu(II)-THAPPH complex was calculated as $1.0053 \times 10^5 \text{ lit. mol}^{-1} \cdot \text{cm}^{-1}$. The Sandell's sensitivity of the complex was found to be $0.00063 \mu\text{g} \cdot \text{cm}^{-2}$. These values reveal that the kinetic spectrophotometric determination of Cu(II) by THAPPH, in presence of

H₂O₂, was fairly sensitive when compared with other reported spectrophotometric determinations of Cu(II) (Table-1). The standard deviation (0.0024), relative standard deviation (0.47%), confidence limit (± 0.0017) and standard error (0.0008) of this method clearly indicate that the precision and accuracy of the method were good.

Effect of temperature:

The effect of temperature was studied by warming the reaction mixture containing 1.0 ml of 1.5741×10^{-4} M copper(II), 5.0 ml of pH 2.5 buffer, 5.0 ml of 3.1482×10^{-4} M reagent and 1.5 ml of 0.6% v/v hydrogen peroxide solutions on a water bath upto 60°C. The absorbance values at 25th minute for 30°C and 60°C were recorded as 0.507 and 0.511 respectively, and these values show that the variation was negligible. Hence, the experiment was carried out at room temperature.

Composition of the complex:

The composition of Cu(II)-THAPPH complex was determined by Job's continuous variation, molar ratio and Asmus' methods.

Job's method of continuous variation:

The composition of the complex was studied by this method, using a series of solutions of Cu(II) and 2,3,4-trihydroxyacetophenonephenylhydrazone of same strength (1.0×10^{-4} M). Absorbance values of these solutions were measured at 385 nm against the reagent blank at 25th minute as explained earlier and a graph was drawn between the mole fraction of the reagent and its absorbance (Figure-8). From the Figure-8, the metal to ligand ratio of the complex (M:L) was found to be 1:2.

Molar ratio method:

To various 1.0 ml of 1.0×10^{-4} M copper(II) solutions in 25ml volumetric flasks, different volumes (0.5- 4.0 ml) of 1.0×10^{-4} M reagent solutions were added and these solutions were treated with 5.0 ml of pH 2.5 buffer and 1.5 ml of 0.6% v/v hydrogen peroxide solutions. The solutions were made upto the mark with doubly distilled water. The absorbances were measured at 385 nm against the reagent blank at 25th minute. A graph was plotted between the mole fraction of the reagent and its absorbance (Figure-9). In this method also, metal to ligand ratio of the complex was found to be 1:2.

Asmus' method:

To 1.0 ml of 1.0×10^{-4} M copper(II) solutions in different 25 ml standard flasks, varying volumes (0.5 to 4.0 ml) of 1.0×10^{-4} M reagent solutions were added. To these solutions, 5.0 ml buffer of pH 2.5 and 1.5 ml of 0.6% v/v hydrogen peroxide solutions were added and finally made upto 25 ml with distilled water. The absorbances of these solutions were measured at 385 nm against the reagent blank at 25th minute. A linear plot (Figure-10) was obtained between $1/m$ and $1/v^2$, which confirms the formation of a 1:2 complex between copper(II) and THAPPH. This method has good agreement with the above two methods.

Instability constant of Copper(II)-2,3,4-trihydroxyacetophenonephenylhydrazone complex:**Asmus' method:**

The instability constant was calculated from the data obtained in Table-10. From the intercept on y-axis of Figure-10, the instability constant of the complex was calculated making use of the

following equation.

$$\text{Intercept} = -(b_0/v)^n \cdot 1/k$$

Here the value of 'n' was 2 and the intercept obtained from graph is -0.68. The value of 'K' obtained by substitution was 2.35×10^{-11} at 30°C.

Edmonds and Birnbaum's method:

Absorbances of solutions containing the fixed amount of copper(II), buffer and H₂O₂, but different known amounts (0.2×10^{-5} - 1.0×10^{-5} M) of reagent solutions were measured after making up the volume to 25 ml with doubly distilled water. In each case, the reagent was always in excess of the metal ion. The absorbances were measured at 385 nm against the reagent blank. The instability constants were calculated by choosing different pairs of solutions, and the average value was found to be 2.44×10^{-11} at 30°C.

Effect of diverse ions:

The effect of diverse ions on copper(II) catalyst in the oxidation of 2,3,4-trihydroxyacetophenonephenylhydrazone by hydrogen peroxide was studied. The absorbances of solutions containing 1.0 ml of 1.5741×10^{-4} M copper(II), varying amounts of diverse ion, 5.0 ml of 3.1482×10^{-4} M reagent solution, 5.0 ml hydrochloric acid-potassium chloride buffer (pH 2.5) and 1.5 ml of 0.6% v/v hydrogen peroxide were measured at 385 nm (t =25th minute) against the reagent blank. The amount of diverse ion required to cause ± 2 per cent error, in the absorbance, was taken as tolerance limit. The tolerance limits of various ions were given in Table-2.

A number of metal ions such as Ca(II), Cd(II), Co(II), Mn(II), Mo(VI), Mg(II), Pb(II) and Zn(II) do not interfere in the determination. Al(III), Bi(III), Th(IV), V(V), Ag(I) and Cr(VI) interfere seriously. The interference of Al(III), Bi(III), Th(IV), Ti(IV), V(V) and W(VI) was prevented by using 1 ml of 2% sodium fluoride solution as a masking agent and Ag(I) and Cr(VI) were masked by using 2 ml of 1% sodium thiosulphate solution. Among the anions studied, citrate, EDTA, oxalate, phosphate and tartrate interfere seriously in the determination of copper(II), due to the formation of more stable compounds.

APPLICATIONS

The present method was applied for the determination of Cu(II) in standard alloys, food and medicinal leafy samples.

Estimation of copper in brass and bronze:

The standard alloy samples were prepared by adopting the given earlier procedure. An aliquot of the solution was taken and the copper(II) was determined by adopting recommended procedure. Results were presented in Table-3.

Determination of copper(II) in food and medicinal leafy samples:

The food & medicinal leafy samples were prepared by adopting the given earlier procedure. An aliquot of the solution was taken and the copper(II) was determined by the adopting the recommended procedure. Results were presented in Table-4 & 5.

Table-1. Comparison of Characteristic Features of Various Reported Methods with Present Method for the Spectrophotometric Determination of Copper(II)

Reagent	pH	λ_{\max} , nm	Beer's law range $\mu\text{g.ml}^{-1}$	Molar absorptivity $\epsilon \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1}$	M:L	Remarks	Reference
Diacetylmonoxime benzoylhydrazone	Basic medium	-	0.25-2.0	1.36	-	Less sensitive	18
2,5-Dihydroxyacetophenonebenzoic hydrazone	Acidic medium	400	0.3-6.0	1.1	1:1	Less sensitive	19
p-Methyl isonitrosoaceto phenone phenyl hydrazone	7.0	510	0.1-1.0	0.628	-	Less sensitive	20
2,4-Dihydroxy benzophenone benzoic hydrazone	4.0	-	0.31-2.20	0.00002	-	Less sensitive and stable	21
2,4-Dihydroxy acetophenone isonicotinyl hydrazone	2.0	-	0.063-2.550	0.00002	-	Less sensitive, more reagent consumption	22
2,2-Dipyridyl-2-pyridylhydrazone	11.9-12.6	-	0.0-1.0	0.00004	-	Interference of mutual ions and expensive reagent	23
Thiosemicarbazone of 2-carboxy benzaldehyde	-	346	0.5-5.0	1.2	1:1	Less sensitive	24
2,4-Dihydroxy acetophenone thiosemicarbazone	5.0	390	0.8-5.5	1.5	1:1	Less sensitive	25
2,4-Dihydroxy-5-bromo acetophenone thiosemicarbazone	6.0	420	0.1-2.7	0.145	1:2	Very poor sensitivity	26
Isonitrosoprophenonethiosemicarbazone	10	390	0.5-6.0	0.583	1:2	Less sensitive	27
7-Methyl-2-chloroquinoline-3-carbaldehyde thiosemicarbazone	4.0	400	5.0	0.03434	-	Mo(IV) interference, very poor sensitive	28
2,3,4-trihydroxy acetophenone phenyl hydrazone (THAPPH)	2.5	385	0.04-0.64	10.053	1:2	Highly sensitive	Present method

Table-2. Effect of Foreign ions: Amount of Copper(II) = 0.4 µg/ml

Foreign ion	Added Salt Formula	Tolerance limit (µg/ml)
Ag(I)	AgNO ₃	None
Al(III)	Al(NH ₄)(SO ₄) ₂ .12H ₂ O	None
Be(II)	BeSO ₄ .4H ₂ O	4.0
Bi(III)	Bi(NO ₃) ₃ .5H ₂ O	None
Ca(II)	CaCl ₂	10.0
Cd(II)	(CH ₃ COO) ₂ Cd.2H ₂ O	10.0
Co(II)	CoCl ₂ .7H ₂ O	20.0
Cr(VI)	K ₂ Cr ₂ O ₇	None
Fe(II)	(NH ₄) ₂ (SO ₄).FeSO ₄ .6H ₂ O	8.0
Fe(III)	NH ₄ Fe(SO ₄) ₂ .12H ₂ O	8.0
Hg(II)	HgCl ₂	20.0
Mn(II)	MnSO ₄ .H ₂ O	10.0
Mo(II)	(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	10.0
Mg(II)	MgSO ₄ .7H ₂ O	20.0
Ni(II)	(NH ₄) ₂ SO ₄ .NiSO ₄ .6H ₂ O	20.0
Pb(II)	(CH ₃ COO) ₂ Pb.2H ₂ O	10.0
Th(IV)	Th(NH ₄) ₄ .6H ₂ O	None
Ti(IV)	K ₂ TiO(C ₂ O ₄) ₂ .2H ₂ O	None
U(VI)	UO ₂ (CH ₃ COO) ₂ . H ₂ O	4.0
V(V)	NH ₄ VO ₃	None
W(VI)	Na ₂ WO ₄ .2H ₂ O	None
Zn(II)	ZnSO ₄ .7H ₂ O	20.0
Zr(IV)	ZrO(NO ₃) ₂ .2H ₂ O	None
Ascorbate	NaC ₆ H ₇ O ₆	10.0
Bromide	KBr	20.0
Chloride	NaCl	10.0
Citrate	Na ₃ C ₆ H ₅ O ₇ .2H ₂ O	None
EDTA	Na ₂ .EDTA	None
Fluoride	NaF	20.0
Iodide	KI	10.0
Oxalate	(NH ₄) ₂ C ₂ O ₄ .H ₂ O	None
Phosphate	NH ₄ H ₂ PO ₄	None
Sulphate	K ₂ SO ₄	20.0
Tartrate	(NH ₄) ₂ C ₄ H ₄ O ₆ .4H ₂ O	None
Thiocyanate	NH ₄ SCN	10.0
Thiosulphate	Na ₂ S ₂ O ₃ .5H ₂ O	20.0

Table-3. Determination of Copper(II) in Standard alloy Samples

Name of the sample	Reported composition	Amount of Cu(II) found ^a (%)		SD		RSD%		F-test
		Present method	AAS method	Present method	AAS method	Present method	AAS method	
Brass (BCS 5g)	Cu(67.4), Zn(28.6), Sn(1.09), Pb(2.23), Fe(0.30), Ni(0.333) and P(0.01)	66.89	66.93	0.031	0.029	0.046	0.043	1.14
H.T. Brass (BCS 10g)	Cu(60.8), Zn(32.05), Sn(0.21), Pb(0.23), Fe(1.56), Mn(1.36), Ni (0.16) and Al (3.34)	60.2	60.08	0.027	0.026	0.045	0.043	1.09
Phosphor-Bronze (BCS 7a)	Cu(88.4), Sn(10.1), Pb (0.02) Fe(0.01), P(1.31) and Sb (0.07)	88.0	88.06	0.021	0.024	0.024	0.027	1.26

a: average of five replicates

Table-4. Determination of Copper(II) in Food Samples

Botanical name of the sample	Amount of Cu(II) found ^a (µg/g)		SD		RSD%		F-test
	Present method	AAS method	Present method	AAS method	Present method	AAS method	
<i>Amaranthus gangeticus</i>	12.11	12.14	0.0024	0.0021	0.019	0.017	1.25
<i>Colcasia antiquorum</i>	12.02	11.98	0.0022	0.0019	0.018	0.016	1.26
<i>Rumex vesicarius</i>	10.34	10.32	0.0026	0.0024	0.025	0.023	1.18
<i>Limonia acidissima</i> L	15.98	16.01	0.0019	0.0020	0.012	0.012	1.00
<i>Lagenaria vulgaris</i>	11.09	11.08	0.0016	0.0018	0.014	0.016	1.31
<i>Eluesine coracana</i>	10.33	10.34	0.0030	0.0032	0.029	0.032	1.14

a: average of five replicates'

Table-5. Determination of Copper(II) in Medicinal Leafy Samples

Botanical name of the sample	Amount of Cu(II) found ^a , (µg/g)		SD		RSD%		F-test
	Present method	AAS method	Present method	AAS method	Present method	AAS method	
<i>Azardirecta indica</i>	10.45	10.46	0.0037	0.0032	0.035	0.031	1.27
<i>Asclepis curassvica</i>	14.04	14.10	0.0042	0.0038	0.029	0.027	1.15
<i>Myrtis suaveolens</i>	12.22	12.00	0.0024	0.0025	0.019	0.021	1.22
<i>Lawsonia inermis</i>	15.01	15.12	0.0022	0.0023	0.015	0.015	1.00
<i>Pongamia pinnata</i>	14.83	14.90	0.0036	0.0038	0.024	0.026	1.17

a: average of five replicates

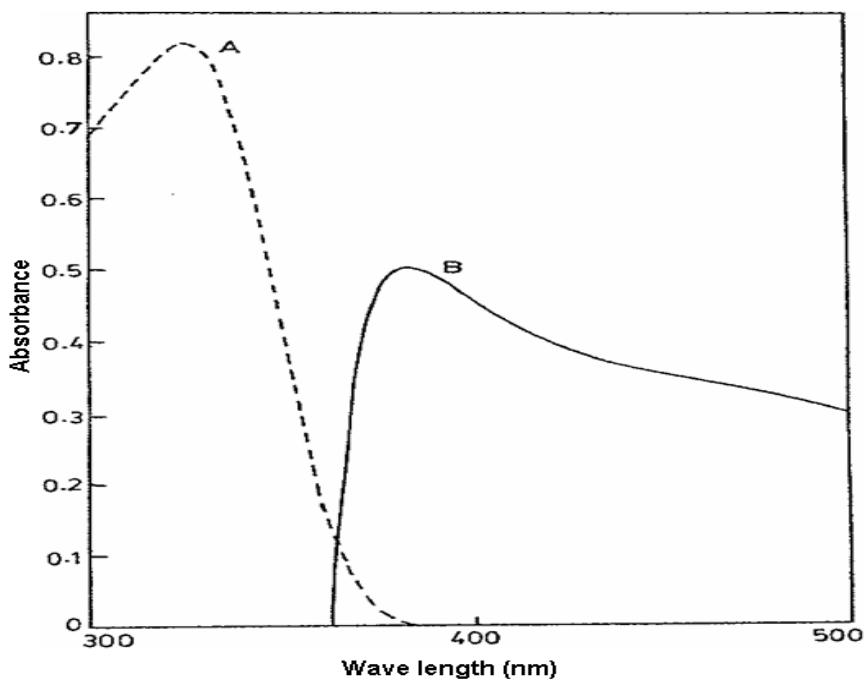


Fig.1. Absorption spectra of reagent and Cu(II) - THAPPH complex:
(A) Absorbance of the reagent;
(B) Absorbance of Cu(II)-THAPPH complex.

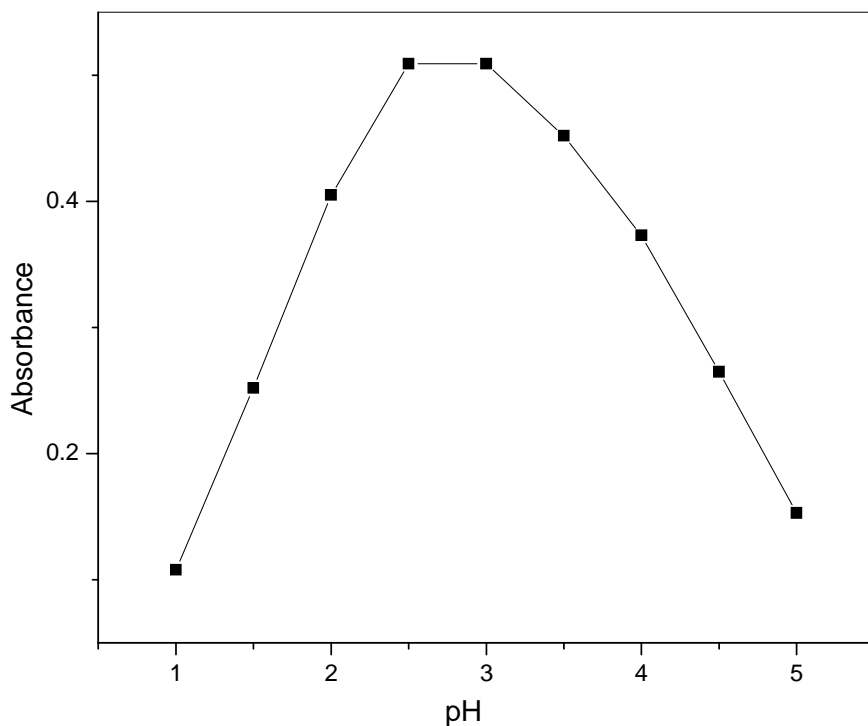


Fig.2. Effect of pH on Cu(II)-THAPPH complex.

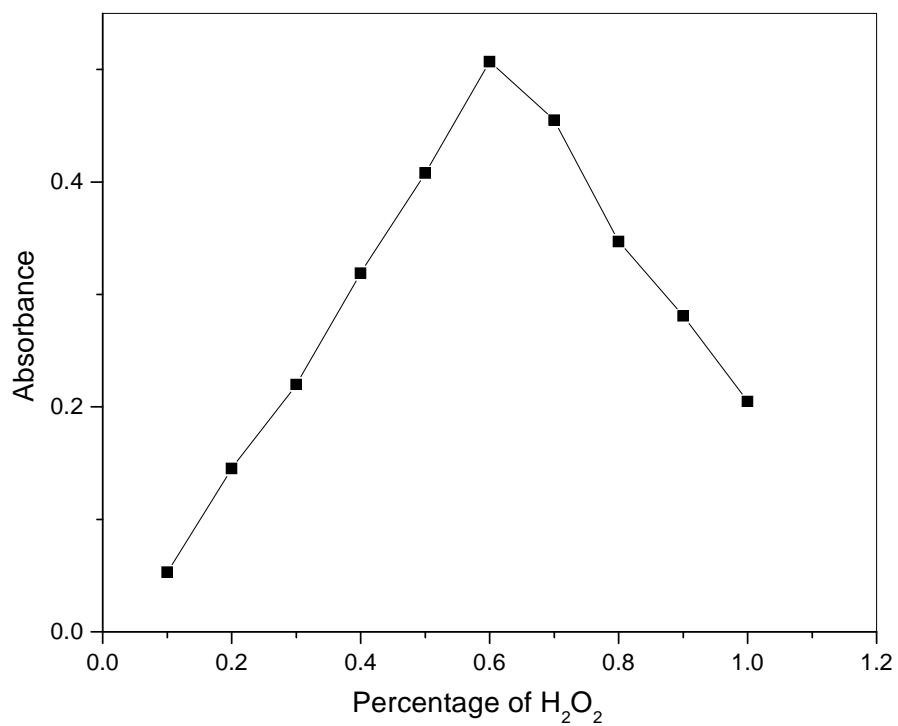


Fig.3. Effect of H₂O₂ on Cu(II)-THAPPH complex.

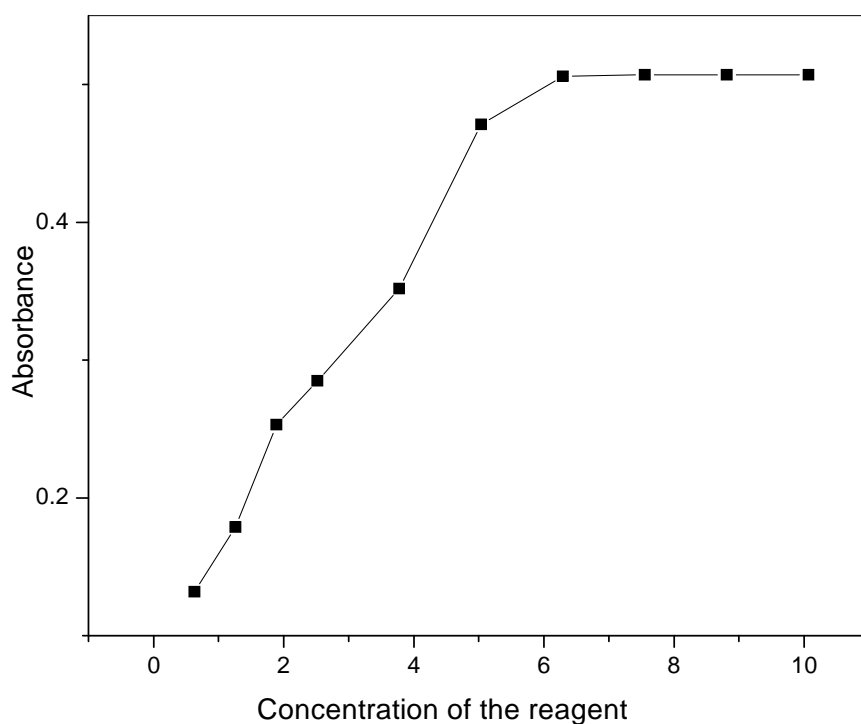


Fig.4. Effect of reagent concentration on Cu(II)-THAPPH complex.

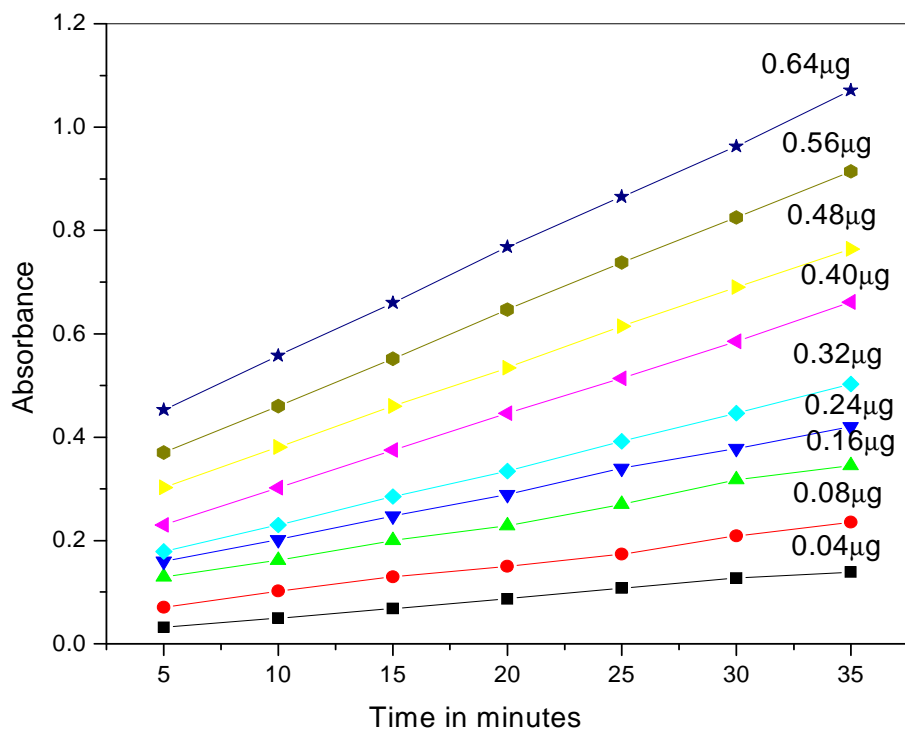


Fig.5. Variation of absorbance with time for different concentration of Copper(II).

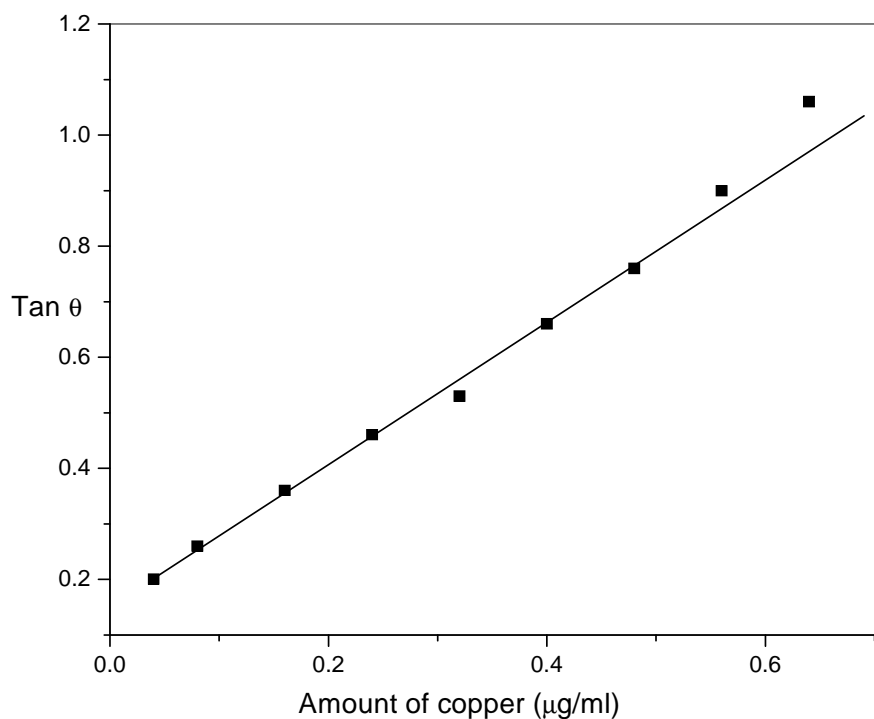


Fig.6. Calibration curve for the determination of copper(II) by tangents method.

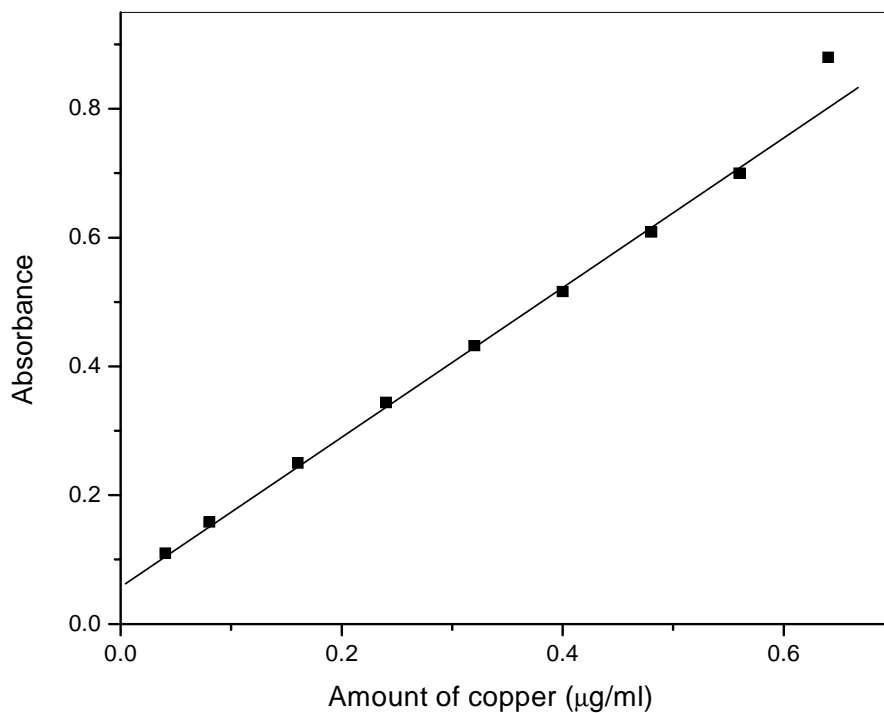


Fig.7. Calibration curve for the determination of copper(II) by fixed time method.

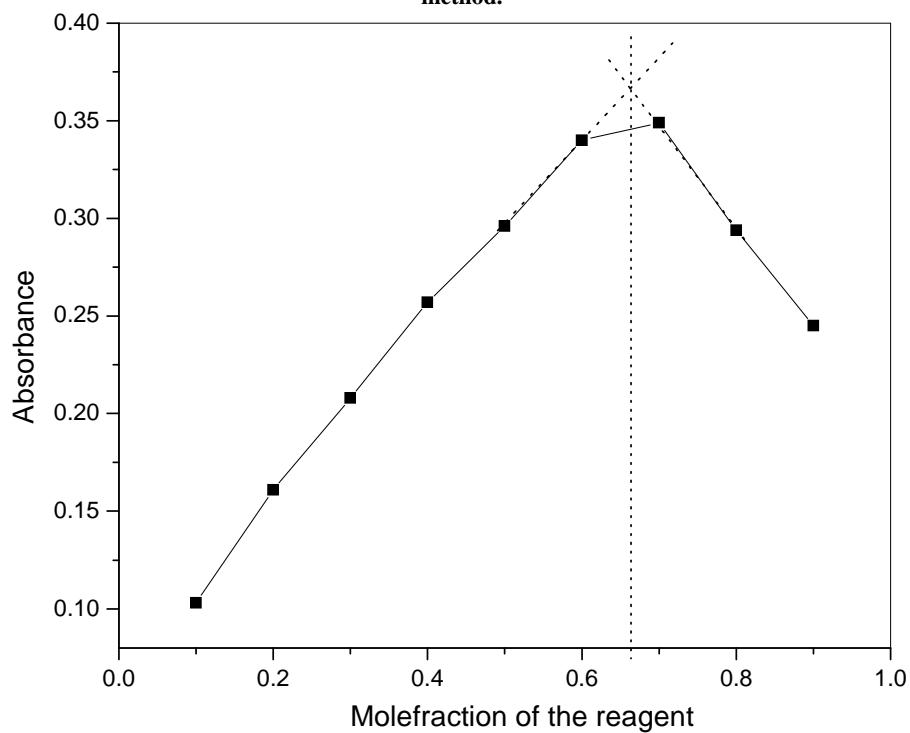


Fig.8. Job's method of continuous variation for the determination of composition of Cu(II)-THAPPH complex .

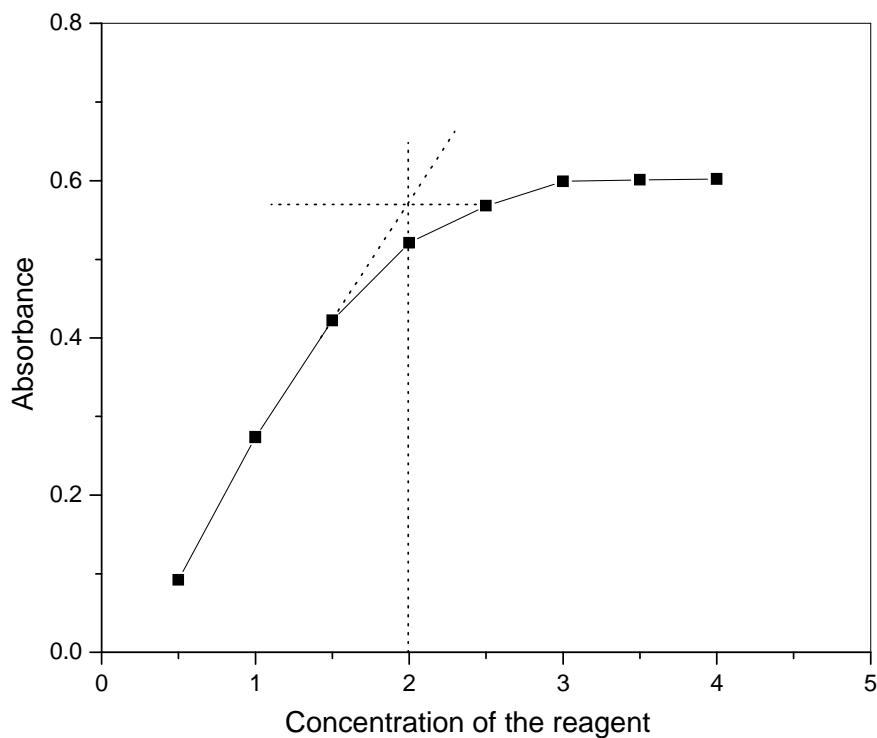


Fig.9. Molar ratio method for determination of composition of Cu(II)-THAPPH complex.

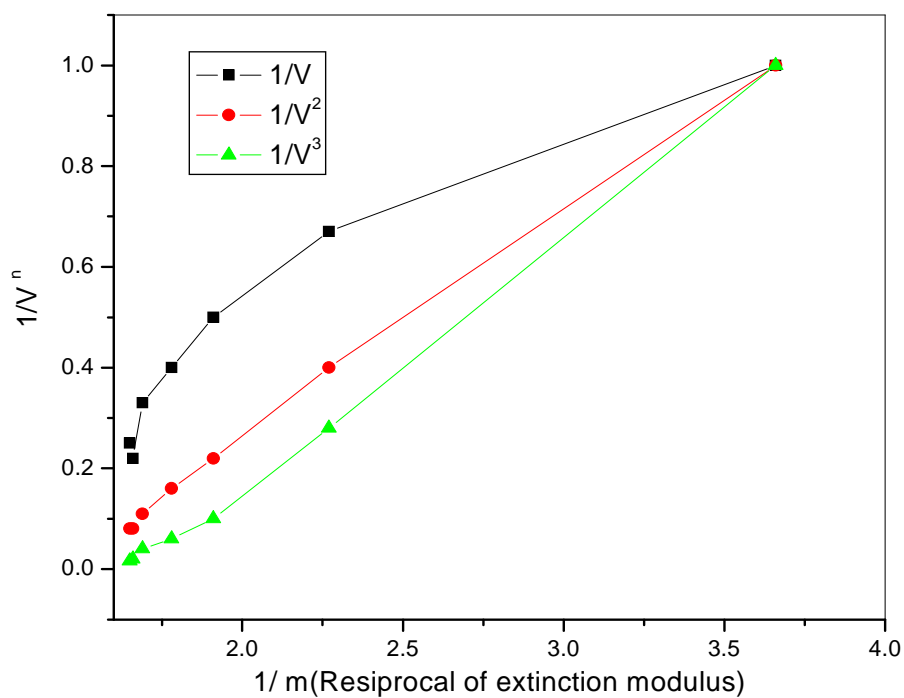


Fig.10: Asmus' method for the determination of composition of Cu(II)-THAPPH complex.

CONCLUSION

The present investigation has proved that 2,3,4-trihydroxy acetophenonephenylhydrazone (THAPPH) was a selective and sensitive chelating reagent for kinetic spectrophotometric determination of Cu(II). The present method was not laborious and there was no need of heating the components. The molar absorptivity ($1.0053 \times 10^5 \text{ L. mole}^{-1} \text{ cm}^{-1}$) value of the complex reveals that the reagent was more sensitive for Cu(II) than earlier reagents. A number of associated elements do not interfere with its determinations. The selectivity of the reagent was also improved by judicious use of masking agent to suppress the interference of metal ions like Al(III), Bi(III), Th(IV), Ti(IV), V(V), W(VI), Ag(I) and Cr(IV). This method has successfully applied for the determination of Cu(II) in standard alloy samples, food and medicinal leafy samples.

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