Spectrophotometric Determination of Copper Complex by Bismarck Brown Y in Environmental and Pharmaceutical Samples

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Abstract: *A simple and Sensitive method for the determination of trace amounts of copper by spectrophotometry is described, which is based on the formation of the copper- bismarck brown Y orange colored complex, in acetate buffer medium (pH = 4), which increases the sensitivity and the complex was extracted by chloroform. Under optimum conditions, the maximum absorption of the chloroform extract was measured at 420 nm. The Beer law was obeyed in the range of 0.40 to 5.2 µg ml $^{-1}$ of copper. The molar absorptivity and the Sandell's sensitivity of the complex were 1.8 x10^4 l mol $^{-1}$ cm $^{-1}$ and 0.2563 µg cm^{-2}, respectively. The detection limit was found to be 0.0120 µg ml^{-1}. A detailed study of various interfering ions made the method more sensitive. The method was successfully applied for the determination of Cu(II) in water and **pharmaceutical samples**. The performance of the present method was evaluated in terms of Student 't' test and Variance ratio 'f ' test, which indicate the significance of the present method over reported methods. It also provides a relative inexpensive alternative to more expensive instrumental method like AAS.*

Keywords: Spectrophotometry; Determination; Copper (II); Bismarck brown Y; Complex; Pharmaceutical samples

1. Introduction

Copper is available in nature in the free state in the form of sulphides, chlorides and carbonates. The major portion of the word's production of copper is utilized by the electrical industries. Copper is widely distributed and has diverse functions in plants and animals. It is an essential trace metal for animals and man because it is required for the formation of erythrocytes and hemoglobin as well as oxidative enzymes[1]. It is widely used in the form of wire, rods, cooking utensils. It is also used in anti fouling paints, in insecticides, as rot-proofing agent for fabrics and forms alloys [2]. Inhalation of dusts, fumes and mists of copper salts results in congestion of nasal septum. Excess of copper concentration can causes adverse effect on the human body. The diseases that arise due to the deficiency of copper are anemia and hair kinky, while excess of copper can result into a disease like jaundice. Copper metal fumes or salts cause salivation, nausea, vomiting, gastric pain etc. Contact with skin causes itching eczema. In chronic exposure, liver, kidney and spleen may be injured and may develop anemia[3]. Toxicity of dissolved copper(II) is considered less than mercury but greater than cadmium, silver, lead and zinc[4].

A limit of 0.1 mg m^{-3} for copper fume has been tentatively suggested. The Public Health Service for drinking water standard is 1 mg dm^{-3} or 1 ppm[3]. As a pollutant, copper is of particular concern, because of its high degree of toxicity to aquatic organism. In view of this, simple and sensitive methods are required for the trace determination of copper.

Several complexing agents have been reported for the extraction and spectrophometric determination of copper. Several methods like AAS[5], flow injection catalytic photometric method [6, 7], adsorptive cathodic stripping voltametry[8], derivative potentiometeric stripping analysis[9] and spectrophotometric methods [10-13] are reported in literature for the determination of copper(II). Spectrophotometric methods for the determination of copper are far more convenient and economical to other methods. However, the reported spectrophotometric methods suffer from certain drawbacks, such as few are extractive, less sensitive, time consuming while few others suffer from narrow range of determination.

The aim of the present work was to provide a facile, sensitive and rapid extractive spectrophotometric method for the determination of trace amounts of copper(II) in water and **pharmaceutical** samples and other natural samples of significant importance. In this study, new analytical reagent Bismarck brown Y for the determination of copper(II) in water and **pharmaceutical** samples was successfully sythesized. The experimental variables affecting color formation and the possible interference of foreign ions were studied.

2. Experimental

Instruments
A snap action thermostat and micro syringes were used for temperature control and Systronics UV-Vis spectrophotometric model 104 with matched silica cells was used for all spectral measurements. A Systronic pH meter model 335 was used for pH measurements. A Remi C-854/4 clinical centrifuge force of 1850 g with fixed swing out rotors was used for centrifugation. All reagents used were of Anal R grade. Double distilled demineralized water was used throughout.

Reagents:
All reagent used were of analytical reagent grade. Double distilled water was used throughout the experiments. A stock solution of Cu(II) (0.01 M) was prepared by dissolving 2.505 g of $CuSO_4.5H_2O$ (Merck, Germany) in double distilled water in a 1000 ml volumetric flask. Copper(II) working standard stock solutions were prepared freshly by appropriate dilution of the standard stock solution with double distilled water.

Bismarck brown Y (Eastmann Kodak Co.) : A reagent solution (0.1 M) was prepared by dissolving 0.236 g of reagent in 100 ml of ethyl alcohol.

Acetate buffer was prepared by dissolving 8.2 g sodium acetate in 800 ml of water. It was adjusted to pH 4.0 with high purity glacial acetic acid and stored in a clean 1:l metal-free polyethylene bottle.

3. Procedure

To an aliquot of working standard solution containing 1–100 µl in a 50 ml separating funnel 5.0 ml of acetate buffer solution (pH 4.0), 2.0 ml of reagent solution and salting out agent (0.1 M Bismarck brown Y) were added. The mixture was shaken with 5.0 ml portions of chloroform for 20 s and allowed to stand for few minutes. The organic phases are collected and made up to the mark in a 25 ml volumetric flask with chloroform and the absorbance was measured at 420 nm against the corresponding reagent blank. A calibration graph was constructed.

Procedure for the determination of Cu(II) in natural water samples:
Different water samples (lake water, river water, spring water) were collected from various places in and around Bhilai – Durg Sector (Bhilai Steel Plant, largest Industries in Central india), Chhattisgarh, India. The sample 150 ml were stored at 0–5 °C in metal-free polyethylene bottles. The water samples were filtered through a whatman filter paper No. 41 and the clean solution was collected into a 250 ml beaker. The contents were diluted up to the mark with double distilled water. 15 ml of this solution was further diluted to obtain a working solution for the determination of Cu(II) as described in the above procedure and compared with the reported method [32] with statistical validation. The results are summarized in Table II.

Determination of copper in pharmaceutical samples:
 A multivitamin tablet (Supradyn tablet, Nicholas Piramal Ltd., India) containing copper was dissolved in aqua regia and was heated on a sand bath and evaporated to dryness. The residue was dissolved in water and again evaporated to dryness. The residue was dissolved in 5 ml of dilute hydrochloric acid and the volume made up to the mark with distilled water in a 100 ml standard flask. A suitable aliquot of diluted solution was taken and analyzed for copper according to the proposed method. Iron(III) can be effectively removed from solution by precipitation with 1-2 ml of saturated solution of sodium fluoride. The results are summarized in Table III.

Determination of copper in plant materials:
The proposed method was applied for the determination of copper in plant material like potato and papaya. The samples were dried in a muffle furnace at 500 °C for 8 h in porcelain crucible. Dried sample (0.5 g) was weighed and brought into solution by dry ash method[14]. The results show that the proposed method can be applied for the determination of copper in potato and papaya. The results (Table IV) are compared with reported method[15].

4. Results and Discussion

Copper (II) reacts with Bismarck brown Y to form a 1:1 orange colored complex in buffer of pH 4.0, and the complex was extracted into chloroform. The organic extract showed a maximum absorption at 420 nm. The formation of the complex and its extraction into chloroform are instantaneous. The complex is stable for more than 24 h.

Under the experimental condition, the absorption spectra of Bismarck brown Y and the (Cu(II)– Bismarck brown Y) complex were scanned. Whereas Bismarck brown Y exhibited an absorption maximum at 310 nm, the [Cu– Bismarck brown Y]$^{2+}$ complex gave an absorption peak at 420 nm. The difference between the two peaks is 110 nm and could obviously be distinguished. Thus, the absorption peak at 420 nm was chosen as the maximum wavelength for Cu(II)– Bismarck brown Y, as shown in Fig. I.

The Beer law was obeyed in the concentration range 0.40 to 5.2 µg ml^{-1} in Fig II. The molar absorptivity and the Sandell's sensitivity of the complex were 1.8 x10^4 l mol^{-1} cm^{-1} and 0.2563 µg cm^{-2}, respectively. The detection limit was found to be 0.0120 µg ml^{-1}. The correlation coefficient (r) for the experimental data was 0.9888.

Effect of the reagent concentration
To a series of 25 ml separating funnels, each containing 1.0 ml of Cu solution (0.01 M) and 5.0 ml of buffer solution (pH 4.0) were added different volumes of the reagent solution and extracted with chloroform to obtain the maximum color formation. The absorbance of each solution was measured at 420 nm against the reagent blank. From the experimental observation, it was found that beyond a ten-fold excess of the reagent, the absorbance remained almost constant. Hence, for further studies, 2.0 ml of the reagent was recommended for complete color development.

Stoichiometry of the complex
On the basis of the Job's method of continuous variation as modified by Vosburg and Cooper for two phase systems, the composition of the extracted species was found to be Cu^{2+} : Bismarck brown Y = 1:1. The mole ratio of the method further con firmed the stoichiometry (Scheme I) of the complex as shown in Figs. III and IV.

Ringbom plot for the (Cu(II)− Bismarck brown Y) complex:

The Ringbom plot is the established standard adopted to determine the optimum range of concentration for a system that obeys the Beer's law. The plot is drawn between log c of Cu(II) and (1–T) (where T is the transmittance). The plot has a sigmoid shape with a linear segment intermediate

absorbance values (0.2 to 1.2 than constant Value) and concentration values (0.2 to 1.0 µg ml^{-1}). The slope of the Ringbom plot from Fig.V is 0.3562. Hence, the ratio between the relative error in the concentration and photometric error 0.40 for concentration of 0.60 for 0.45 % photometric error.

Precision and accuracy:
The precision and accuracy of the proposed method was evaluated by the good results which were obtained by a reported method. The results are shown in Tables II and III. The good correlation between the two methods indicate that the present method is more sensitive than the methods reported in the literature.

Detection limit:
Under the optimum conditions, the detection limits for determination of Cu(II) in various environmental samples was found 0.0120 µg ml^{-1} for a 250 ml volume of Cu(II) solution in Fig. III.

Effect of salting out agents:
Various salting out agents, such as lithium nitrate, thorium sulphate, magnesium sulphate, lithium chloride, ammonium chloride and ammonium sulphate were used for the enhancement of the extraction of the metal complex into the organic phase in a single step. It was observed that the presence of 1.0 ml of 0.1 M magnesium sulphate solution ensures the complete extraction. The aqueous phases was tested for the complete extraction of no copper(II) using chloroform, and no copper(II) was found in the aqueous phase. It was established that 1.0 ml of 0.1 M magnesium sulphate as salting out agent and a ten-fold molar excess of the reagent quantitatively extracts copper(II) into to the organic phase.

Effect of interfering species:
Several anions and cations were studied in detail. Table I summarizes the tolerance limits of interfering ions in the determination of 30 µg l^{-1} Cu(II). The tolerance limit was taken as the amount causing an error of ± 2 % at the peak height.

Applications of the extractive spectrophotometric method for the determination of copper(II) by the present method:
The results of the proposed extractive spectrophotometric method for the determination of Cu(II) in water and alloy samples are presented in Tables II and III. The obtained results were compared with reported methods [16-20] in terms of Student's '*t*' test and Variance ratio '*f*'-test. The analytical data summarized in Tables II and III suggest that the percentage recovery of Cu(II) from water and alloy samples range from 92.5 to 97.2 %, which is more reliable and sensitive than the reported methods[16-17].

5. Conclusions

The proposed extractive spectrophotometric method is simple, highly sensitive and selective for the determination of Cu(II) in water and pharmaceutical samples. The limit of detection of the proposed method is superior when compared to reported method[16-20]. The method has additional advantages over the reported methods due to easy preparation of complexation agent, high sensitivity of the very stable complex, low consumption of the reagent and minimal interferences.

6. Acknowledgement

Determination of Cu^{2+} With Bismarck brown Y to form Complex
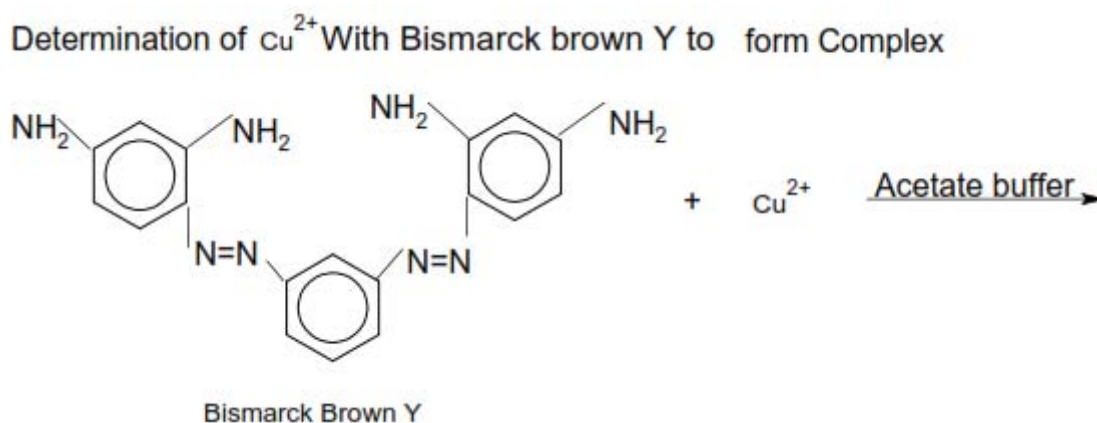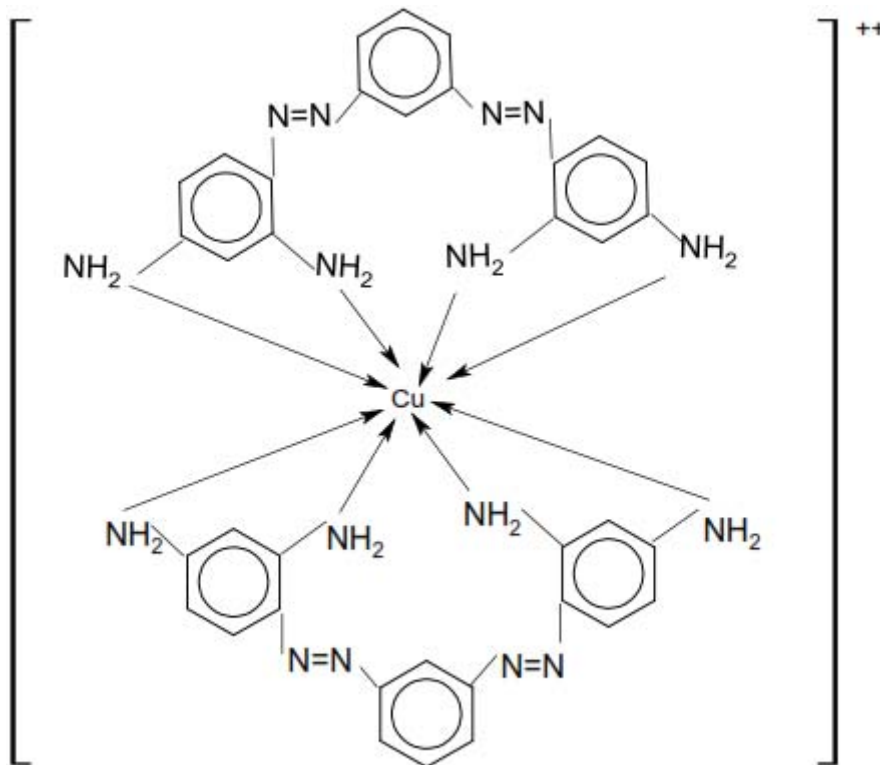
Bismarck Brown Y

Scheme 1: Colour Development of Complex Compund
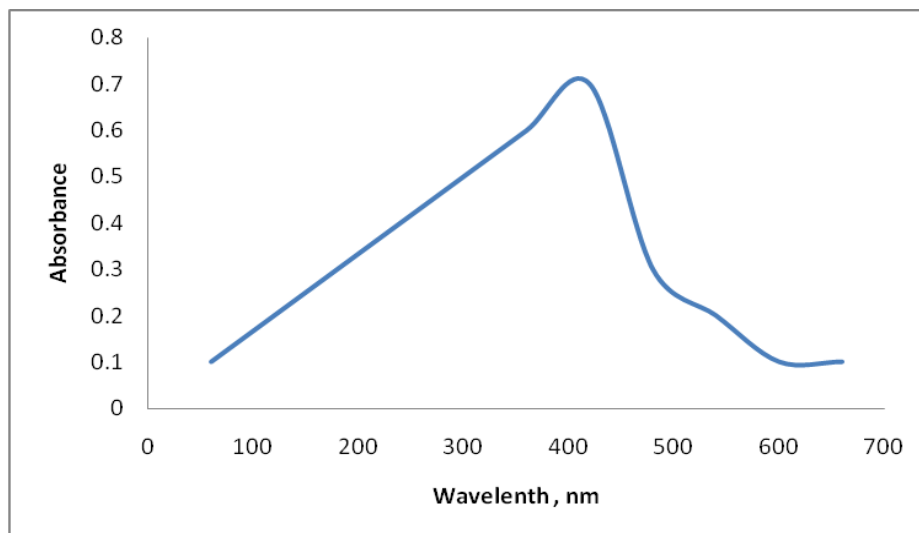

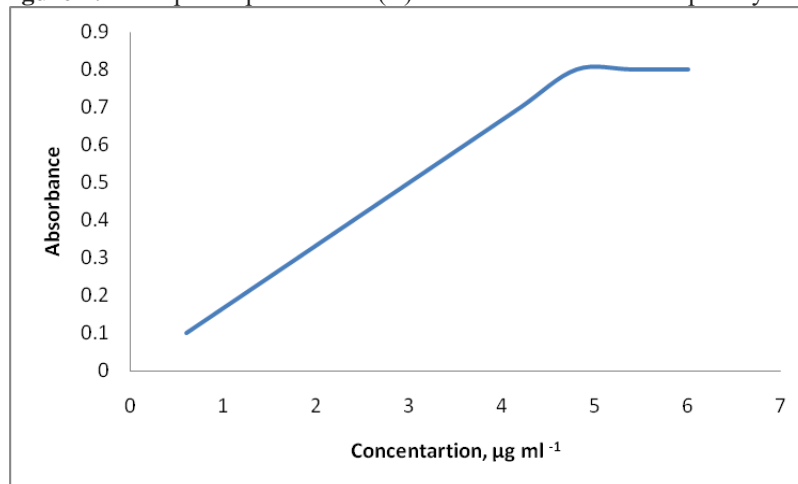**Figure 1:** Absorption spectra of Cu(II)– Bismarck brown Y complex system.
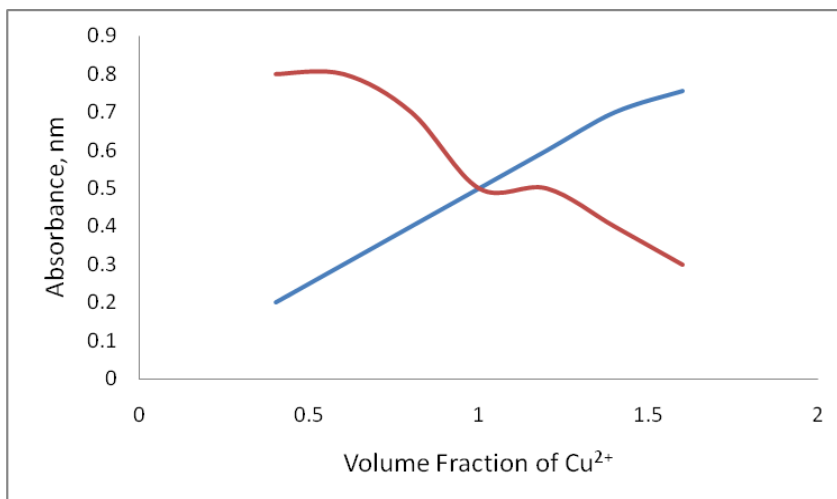

Figure 2: Beer law range

Figure 3: Job's Continuous variation method for the Cu(II)– Bismarck brown Y complex.
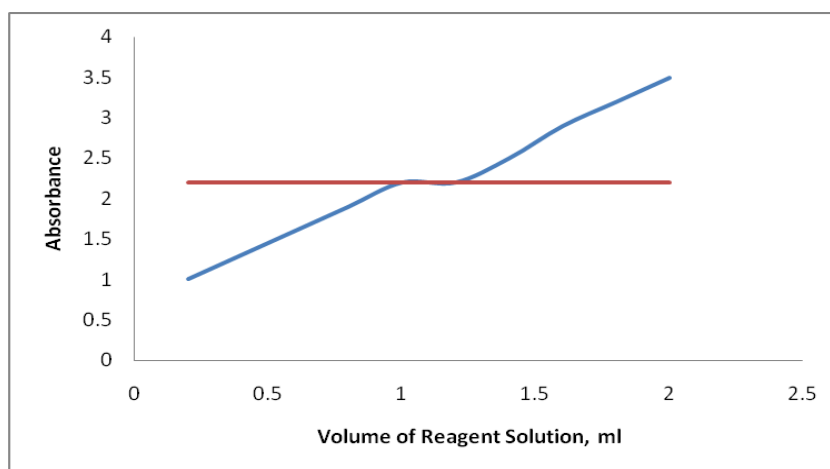

Figure 4: Mole ratio method for the Cu(II)– Bismarck brown Y complex
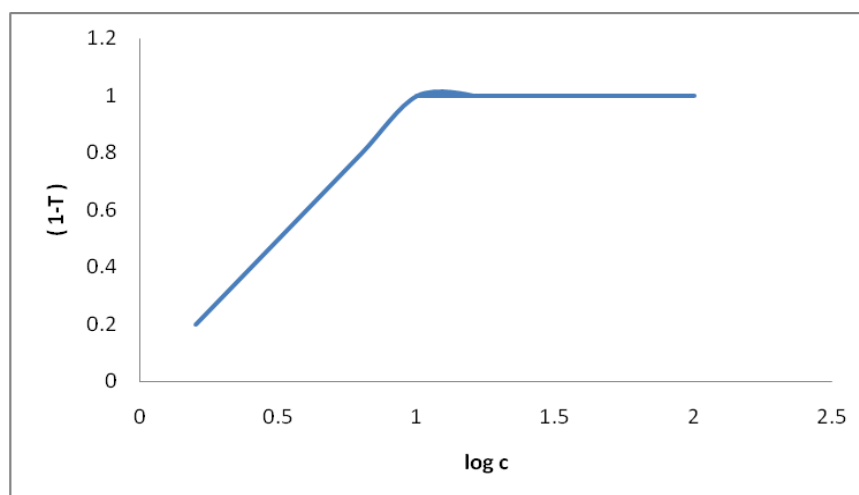
Figure 5: Ringbom plot of Cu(II)– Bismarck brown Y complex.

Table 1: Tolerance limits of interfering ions on the determination of 30 µg l^{-1} Cu(II) ion.

Foreign species	Tolerance limit (µg ml^{-1})[a]
K(I), Na(II), Ca(II), Mg(II), Cl$^-$, Br$^-$, PO$_3^{3-}$, CO$_3^{2-}$, SO$_4^{2-}$, Al(III), Cr(III)	40,000 – 50,000
NO$_3^-$, Zn(II), Hg(II), PO$_4^{3-}$, SO$_4^{2-}$	8000 - 10000
Sn^{4+}, Co^{3+}, As^{5+}, H$_2$O$_2$, NO$_2^-$	5000 - 6000
VO$_4^{3-}$, Cr$_2$O$_7^{2-}$, Se^{2+}, Fe^{3+}, Mn(II), Co(II), Ni(II)	2000 - 3000

a = Tolerance limit may vary the absorbance values by ± 2%.

Table 2: Determination of copper (II) in various water samples.

Sample	Copper added µg ml^{-1}	Proposed method		t-Test	f-test	Reported Method [32]	
		Found[a] µg ml^{-1}	Recovery %			Found µg ml^{-1}	Recovery %
Synthetic mixture	0.4	0.39	97.50	3.80	0.24	0.39	97.5
	1.0	0.96	96.00	2.46	0.62	0.90	95.0
	1.6	1.58	98.75	1.22	0.16	1.55	96.8
Lake water[b]	-	0.39	-	-	–	0.38	-
	0.8	1.19	99.16	2.36	0.02	1.18	98.3
	1.2	1.59	99.37	6.96	0.06	1.58	97.5
River water[b]	-	0.52	-	-	-	0.39	-
	0.8	1.18	98.33	3.46	0.08	1.19	95.0
	1.2	1.58	98.75	2.22	0.04	1.59	91.1
Springer water[b]	-	0.26	-	-	-	0.19	-
	0.4	0.76	95.00	4.80	0.46	0.59	97.5
	0.6	0.96	96.00	6.26	0.12	0.79	98.3
Lower lever	-	0.76	-	-	–	0.59	-
	0.9	1.28	98.46	2.82	0.24	1.49	95.5
	1.2	1.57	98.12	1.26	0.68	1.79	95.8
Polluted water	-	0.39	-	-	-	0.38	-
	0.4	0.79	98.75	2.30	0.03	0.98	96.6
	0.8	1.19	99.16	4.26	0.04	1.18	97.5

[a] n =5, [b] Collected around Bhilai - Durg area, Chhattisgarh

Table 3: Determination of copper in plant material and pharmaceutical samples

Sample[a]	Copper found[b]	
	Present method	Reported method[31]
Potato	0.52 ± 0.40	0.45 ± 0.03
Papaya	3.6 ± 0.03	3.5 ± 0.40
Multivitamin tablet[c] (Supradyn)	3.56±0.40	3.36 ± 0.02

[a]Amount of sample = 0.500 g. [b]Copper found in potato (mg), papaya (µg) in 0.5 g sample and tablet (mg per tablet). [c]Certified value of copper in tablet (Supradyn, Nicholas Piramal Ltd., India): 3.39 mg per tablet.

Table 4: Comparison of analytical parameters of the present method with other spectrophotometric method for the determination of copper

Reagent	^max /nm	Optimum pH range	Beer's law validity range/ppm	Molar Absorptivity cm^{-1} mol^{-1}	M:L	Remarks
8-Methoxy-2-chloroquinolin-3 -carbaldehyde Thiosemicarbazone [21]	410	5.0	3.0	0.0026768	1:1	Interference no. of metal ions and poor senstivity and selectivity
7-Methoxyl-2-chloroquinoline-3-carbaldehyde Thiosemicarbazone [22]	400	4.0	5.0	343.4	-	Mo(V) interfere, very poor sensitivity
2,5-Dihydroxy acetophenone ben Zoichydrazone [23]	–	5.00	0.3–3.00	0.011	-	High reagent consuming and poor selectivity
Pyruvaldehyde (*N,N*-dibutyl)bis-thiosemicarbazone [24]	488	5.12	–	10900	1:1	Less sensitivity
2-Carboxy benzaldehyde thiosemicarbazone [25]	346	-	0.5–5.0	12000	1:1	Less sensitivity
2,4-Dihydroxy-5-bromoacetophe none thiosemicarbazone [26]	400	6.00	12.7	1450	1:1	Very poor sensitivity and selectivity
2,2-Dipyridyl-2-pyridye hydrozone [27]	-	11.9–12.6	Up to 1.0	0.038	-	Interference of mutual ions and expensive reagent
Benzaldehyde-4-(2-hydroxy-5-sulfo phenyl)-3-thiosemicarbazone [28]	325	4.5	7.62	7.44	1:2	very poor sensitivity
4-Chlorosemimmonsoacetophenone Thiosemicarbazone [29]	400	7.5–8.5	0.2–2.0	2518	1:2	Ag(I),Ni(II),Co(II),Pb(II),Cd(II) cyanide, tartarate and edta interfere, less sensitive
2,4-Dihydroxy benzophenone benzoic hydrazone [30]	-	4.0	0.31–2.20	0.0155	-	Less sensitive and sable
2,4-Dihydroxy benzophenone isonicotinoyl hydrazone [16]	-	2.0	0.063–2.550	0.0165	-	Less sensitive more reagent consumption
Benzildithiosemicarbazone [17]	380	4.0	0.5–4.0	16347	1:1	Low detection limit and detection unit
2,5-dimercapto-1,3,4 thiadiazole [18]	390	0.02–0.014	–	0.000565	1:2	High reagent consumption and highly commercial
3-Methoxy, 4-hydroxy benzaldehyde 4-bromophenyl hydrazone[19]	462	2.0–4.0	–	-	1:1	Low reagent consumption, economical

						color forming reagent, less interference, highly stable.
leucocrystal violet [31]	590	-	0.004 - 0.04	1.47×10^6	-	Highly sensitivity but it is colour development method
Present method	420	4.0	0.40 to 5.2	1.8×10^4	1:1	Highly sensitivity and more useful for determination of copper(II) compound

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