Determination of copper in natural Water, Vegetables by Direct and Derivative Spectrophotometry

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:Abstract

A selective and sensitive reagent with 5-{ά – methyl-3-hydroxy benzylidene} rhodanine [5M, 3H-BR], was used for the spectrophotometric determination of Cu(II) in vegetables and natural water samples. The trace metal copper forms an orange-red color complex with reagent at pH 5.5 in sodium acetate and acetic acid buffer. Beer's law was obeyed for Cu (II) – 5-{ ά –methyl-3-hydroxy benzylidene} rhodanine [5M, 3H-BR],   in the concentration range of 0.05   -13 μg /mL respectively. The molar absorptiviy (ε) and the Sandell's sensitivity of the complex were 0.6027×10^4 mol^{-1} cm^{-1} and 0.01054 $μg.cm^{-2}$ respectively. The method was validated with spiked in ,vegetables and water samples. The performance of the present method was also evaluated in terms of RMSEP, REP and RSD, students t-test. The validated method was successfully applied to selected real samples.

Key words: *Copper determination, 5-{ά –Methyl-3-hydroxy benzylidene} rhodanine, RMSEP, REP, RSD, spectrometer*.

1. Introduction:

Copper can be considered either essential or hazardous to life and plays a substantial role in the environment (1). As a micronutrient, copper is responsible for the proper functioning of several metalloenzymes and its deficiency reduces the activity of not only copper-containing enzymes but also some enzymes that do not contain copper (2). The deficiency of copper results in different health problems like anaemia, hair kinky, Wilson disease and jaundice (3). Contamination by heavy metal ions poses a serious threat to human health and the environment. Heavy metals are toxic and accumulate in living organisms throughout their lifetime. Certain trace elements, such as copper, iron, and cobalt are essential to organisms, which have a daily requirement of only a few milligrams. However, if ingested in high levels, such elements can be

harmful to humans, animals and plants. Excessive intake of copper can cause accumulation especially in liver cells and cause hemolytic crisis and neurological disturbances (4). Apart from the biological activity of copper, major portion of world's production of it is used in electrical equipments, roof sheeting, bronze paints and also finds its applications in agriculture as micronutrient fertilizers, fungicides, and insecticides (2). Thus the determination of trace amounts of copper in various types of environmental samples is becoming increasingly important.

Several analytical techniques have been used for determination of copper, including atomic absorption spectrometry, atomic emission spectrometry, electro analytical techniques, spectrophotometry, inductive coupled plasma-emission spectrometry, inductive coupled plasma-mass spectrometry, flow injection diode array spectrophotometry and X-ray fluorescence spectrometry (5,6). However, spectrophotometry methods are often preferred, as they involve inexpensive instrument, less labour-intensive, and provide comparable sensitivity when appropriate chromogenic reagents are available (7,8).

The aim of this work was to develop a highly sensitive, efficient and direct spectrophotometric method for Cu(II) in water and vegetables by using 5-{ ά –methyl-3-hydroxy benzylidene}rhodanine [5M, 3H-BR]. Various factors influencing the sensitivity of the proposed method such as the pH and ranges of applicability of Beer's law on the determination of Cu(II) were also studied. The

method was successfully applied natural water and vegetable samples as well and compared to standard method.

2. Experimental:

The ligand 5-{ά -methyl-3hydoxy benzylidene} rhodanine synthesis was followed by reported method previously (9). Firstly 13 mL benzene was added to 360 mL glacial acetic acid which gave mixture. 2 g of rhodanine was added to above said mixture. The reaction mixture was stirred and .

boiled for 5 minutes. 2 g of 3-hydroxy acetophenone was then added to the reaction mixture then refluxed to overnight. The yellow precipitate was obtained at room temperature. It is separated by filtration, washed with water and purified by recrystallization from methanol/ water (1:1) mixture melting point is 201^0C – 202^0 C. The synthesized compound was characterized and confirmed by Mass, IR and NMR spectra
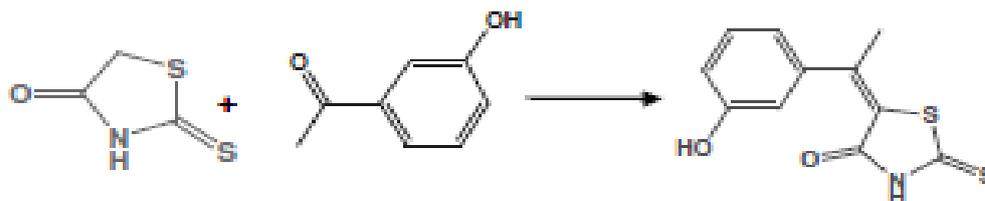
Scheme1.Formation of5-(ά -methyl-3hydoxy benzylidene} rhodanine.

2.1. Preparation of solutions

All the chemicals were of Anala R grades from Fisher Scientific Qualigens, India.

2.2Standard solution Preparation

0.3929 g of Cu(II) sulphate pentahydrte was dissolving in 1000 mL of double distilled water for preparation of standard solution. The solution was standardized by idometry (10). The working standard solutions were prepared by suitable dilution of the stock solution.

2.3Buffer solution

Buffer solutions were prepared by employing 0.1 M acetic acid and 0.1 M sodium acetate (11,12) in the pH range 3-10. Borate buffers were also prepared in the pH range 3-12 from 1 M boric acid adjusting with 1 M sodium hydroxide.

2.4Solutions of diverse ions

Solutions of diverse ions containing 1000 μg/mL were prepared by dissolving required amounts of salts of the corresponding ions in double distilled water

2.5Reagent solution

The reagent stock solution (0.1 M) was prepared by dissolving 1.255 g of [5M, 3H, BR] in DMF or methanol. This was diluted to the required concentration using 40% DMF.

2.6 Instruments

Lab India 3200 model double beam UV - visible spectrophotometer equipped with 1 cm quartz cells were used for spectrophotometric measurements. The pH measurements are made with systronics digital pH meter, 335 model.

2.7 General procedure for studies of different parameters

To ensure the complexation ratio between the Cu(II) and [5M, 3H-BR], and to quantification of the Cu(II) in the sample solutions, the following procedure was performed. To an aliquots of sample solution containing μg quantities of Cu(II) was added to a series of comparison tubes followed by 5 mL of acetic acid and sodium acetate buffer to adjust the pH5.5,then equilibrated with 5 mL of [5M, 3H- BR] solution (in 40% DMF) for 10 min and diluted to 20 mL with double distilled water. The absorbance of orange-red color complex formed was measured against a similarly prepared reagent blank at 430 nm. The composition of the complex was computed by Job's continuous variation, mole ratio and slope ratio methods. The amount of Cu(II) present in the sample solutions(13-20) prepared, were computed from the standard calibration carves in the range 0.05 to 13 μg both by inspecting the direct and derivative spectra.

3. Result and discussion

The absorption spectra of an orange–red color complex of [Cu(II) - 5M, 3H, BR] were recorded in the wave length region 400-600 nm against the reagent blank (Figure 1). The borate buffers showed maximum absorbance (430 nm) and acetate buffer showed lower absorbance (420nm). Therefore borate buffer was preferred for the proposed studies.
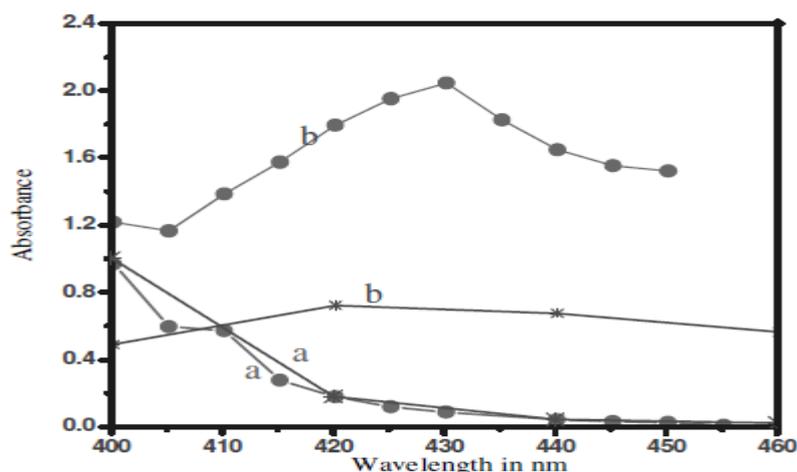
Figure.1: Absorption spectra of; a) 5M 3H BR vs buffer blank. b) Cu(II)-5M 3H BR Complex *vs.* reagent blank acetate buffer *Borate buffer Cu(II)=1.6x10-3 M (100 μg), 5M 3H BR =3x10^{-3} M.

3.1 Effect of pH

The pH plays important role and creates favourable conditions for complex formation. The influence of pH of the aqueous solutions on the formation of [Cu(II) - 5M, 3H, BR] complex were investigated at 430 nm using various buffer solutions of different pH values (Figure 2). Two buffers were selected for present study those are namely acetate buffer and borate buffer. The complex with maximum absorbance was observed at pH values 5 to 8 in acetate buffers and 8 to 10 in borate buffers. However, the maximum absorbance was found in acetate buffers. In the light of these findings, all subsequent studies were carried out at pH 5.5 for direct and derivative spectrophotometry.
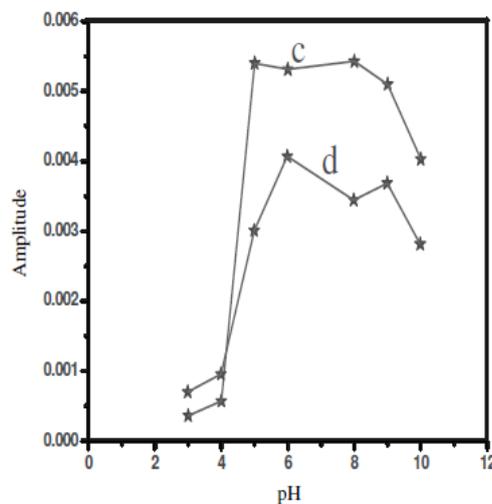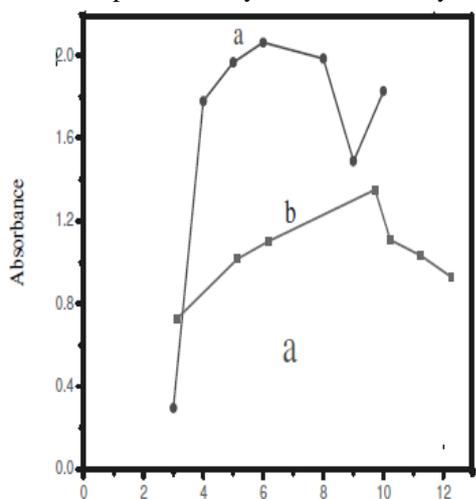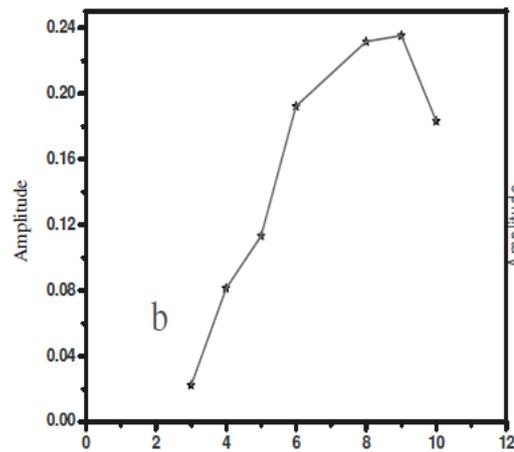
Figure 2. Effect of pH on the absorbance of [Cu(II)-5M 3H BR] system (a) Direct spectrophotometry (b) 1st derivative (c) 2nd derivative (d) 3rd derivative Cu(II)=[5M 3H BR] =3×10^{-4} M.

3.2 Effect of solvent and reagent concentration

The maximum color development was developing with reagent. An orange-red color formation between Cu(II) and reagent was instantaneous and the color was stable for more than 36 hours. The complex was found to soluble in 40% of DMF. So the reagent solutions were prepared in 40% (v/v) DMF.

3.3 Effect of salting-out agent

It has been reported that the addition of salt to the samples is beneficial enhancement of the color of the metal complex in the analysis of the different samples. The complexation of Cu(II) with the (5M, 3H, BR) is certain and effective at pH 5.5. However various salting out agents such as sodium sulphate, sodium chloride, ammonium chloride, ammonium sulphate and sodium carbonate are used for the It was observed that the presence of 0.01 M sodium carbonate effectively increases the color.(Fig.3)
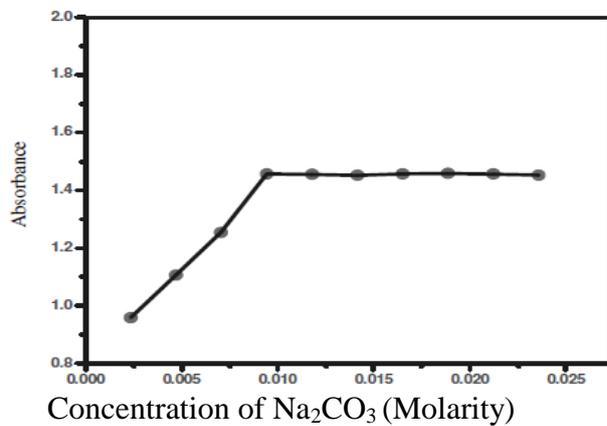

Concentration of Na_2CO_3 (Molarity)

Fig3, Effect of Na_2CO_3 on the complexation

3.4 Nature of the complex

The composition of the complex and stoichiometric ratio between the metal to ligand was determined by mole ratio, slope ratio and jobs continuous variation methods elating of these experimental .

results indicates the Cu(II) forming the 1:4 complex with the reagent and the stoichiometric ratio is 1:2. So the reagent was found to be a bio-dental ligand (Figure 4-6)
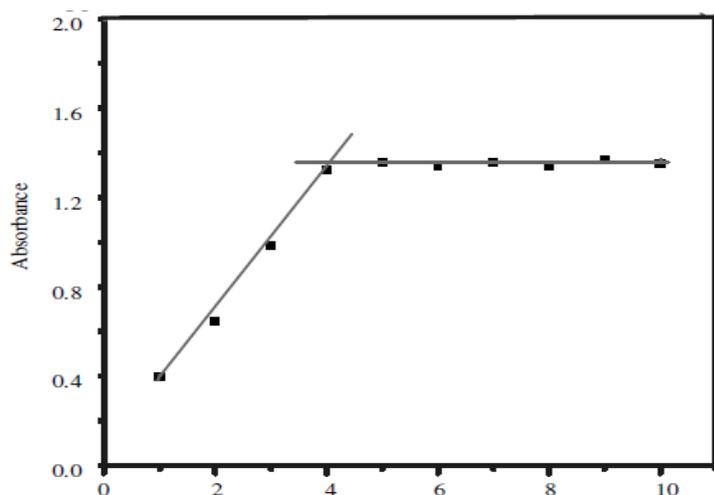
Figure 4. Mole ratio plot, pH:5.5 Cu(II)=[5M 3H BR]=1.6×10^{-4} M, volume of Cu(II)=l mL (10 μg).
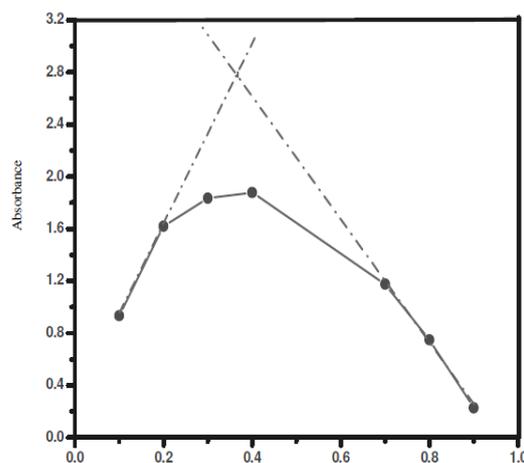
Figure 5. Job's continuous variation method for the Cu(II)-[5M 3H BR] complex, Cu(II)= [5M 3H BR]= 1.6×10^{-4} M, pH:5.5, λ max: 430 nm.
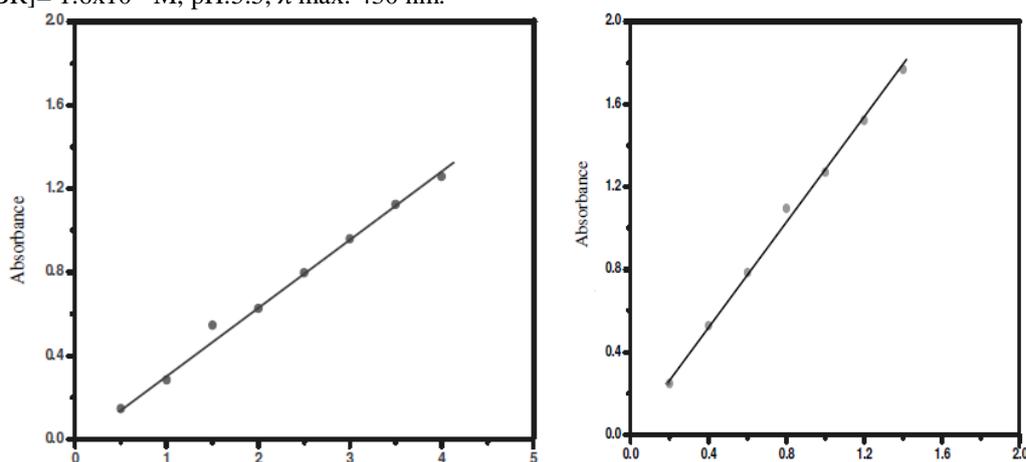
Figure 6. Slope ratio method, Cu(II)=[5M 3H BR]=1.6×10-4 M pH:5.5 λ max=430

3.5 Performance for the calibration of proposed method

Under the optimum conditions, the linearity range, correlation coefficient, standard deviation and repeatability of the method were investigated. The calibration curve was constructed concentration vs. absorbance and it was obeyed Beer's law was obeyed in the concentration range of Cu(II) in different sample solutions. The molar absorptivity of the complex was 0.6027×104 moles^{-1} cm^{-1}.The

Sandell's sensitivity of the method was found to be 0.01054 $\mu g.cm^{-2}$. The linearity range for the optimized procedure was between 0.05-13 $\mu g/mL$ with three categories those are tabulated in table 1.The calibrations equations were y = -0.00207+0.2398X ,y=-0.0364 + 0.2466X ,y=1.4559+0.0103 X respectively. Good correlation coefficient (R^2) with excellent linearity for target analytes were 1.000,10000, 0.9869 respectively. The standard deviation, correlation coefficient and other statistical parameters of the method are evaluated to ten replicate determinations.

Table 1. Performance data for the calibration of proposed method

Concentration Rang(μg)	Least square equation Y = A + B X A =Intercept B = Slope	Correlation Coefficient (r^2)	Standard Deviation	RSD %	REP %	Amount determined in ten replicate samples, μg
0.05-0.5	Y=-0.00207 +0.2398X	1.0000	0.00069	0.19197	0.2991	0.4010,0.3997,0.4022,0.40120.4020,0.4012,0.4018,0.4014, .4012,0.4024 4.1625,4.1091,4.0275,4.1525
0.5-5.0	Y= - 0.0364+0.24466X	1.000	0.0505	1.2241	2.6839	4.1625,4.1091,4.0275,4.15254.1592,4.0761,4.1475,4.1855.4.1572,4.0761.7.4550,7.4469,7.4470,7.4481
5.0-13	Y=1.4559+0.0103X	0.9869	0.005957	0.0799	0.2014	7.4500,7.4470,7.4620,7.4475, 7.4451,7.4598

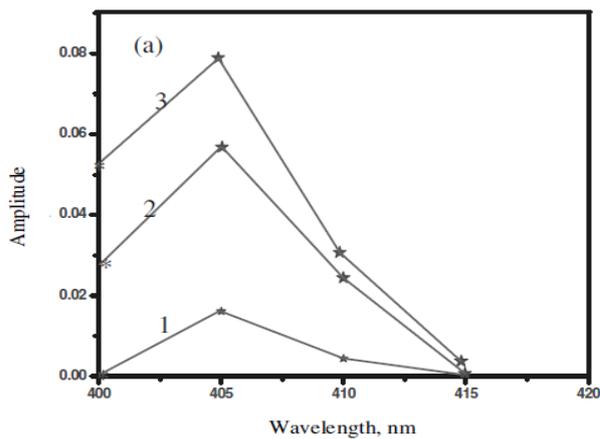## 6.5 Copper determination by Derivative spectrophotometry

An aliquot of the solutions containing copper (II) in Beer's law validity range. A sensitive three derivative spectrophotometric methods are developing for copper determination. The 1st and 2nd derivative spectra show the maximum amplitude at 405 nm and 430 nm. The 3rd derivative curve amplitude becomes zero at 428 nm and maximum amplitude was shifted to 35-460 nm. The derivative amplitudes were measured for different concentration of copper (II) .The plots were linear, the plots drawn between the amplitude and the concentration of Cu(II) in the range of 0.05-13 $\mu g/mL$ respectively. The results are summarized in table 2and figure 4-6

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Table 2 Calibration data for the derivative spectrophotometric determination

Linear Range, μg/mL	Calibration Equation Y = A + BX, A = Intercept, B = Slope		Wave length (nm)	Correlation Coefficient (r^2)
0.05 – 0.5	First – Derivative Spectrophotometry		405	0.9985
	$\partial A/\partial \lambda =$ - 0.0292 + 0.564X			
	Second – Derivative Spectrophotometry		430	1.003
	$\partial^2 A/\partial \lambda^2$ = - 0.0089 + 0.0440 X			

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	Third – Derivative Spectrophotometry	435-460	0.9989
	$\partial^3 A/\partial\lambda^3 = -0.000124 + 0.0046\ X$		
	First – Derivative Spectrophotometry	405	0.9937
	$\partial A/\partial\lambda = -0.0431 + 0.0588\ X$		
0.5 – 5.0	Second – Derivative Spectrophotometry	430	0.9980
	$\partial^2 A/\partial\lambda^2 = -0.000526 + 0.001207\ X$		
	Third – Derivative Spectrophotometry		1.000
	$\partial^3 A/\partial\lambda^3 = -0.000116 + 0.0002656\ X$		
	First – Derivative Spectrophotometry	405	1.0032
	$\partial A/\partial\lambda = -0.0614 + 0.0534\ X$		
5.0 - 13	Second – Derivative Spectrophotometry	430	0.9887
	$\partial^2 A/\partial\lambda^2 = -0.008788 + 0.002201\ X$		
	Third – Derivative Spectrophotometry	435-460	0.9907
	$\partial^3 A/\partial\lambda^3 = -0.00223 + 0.00087\ X$		
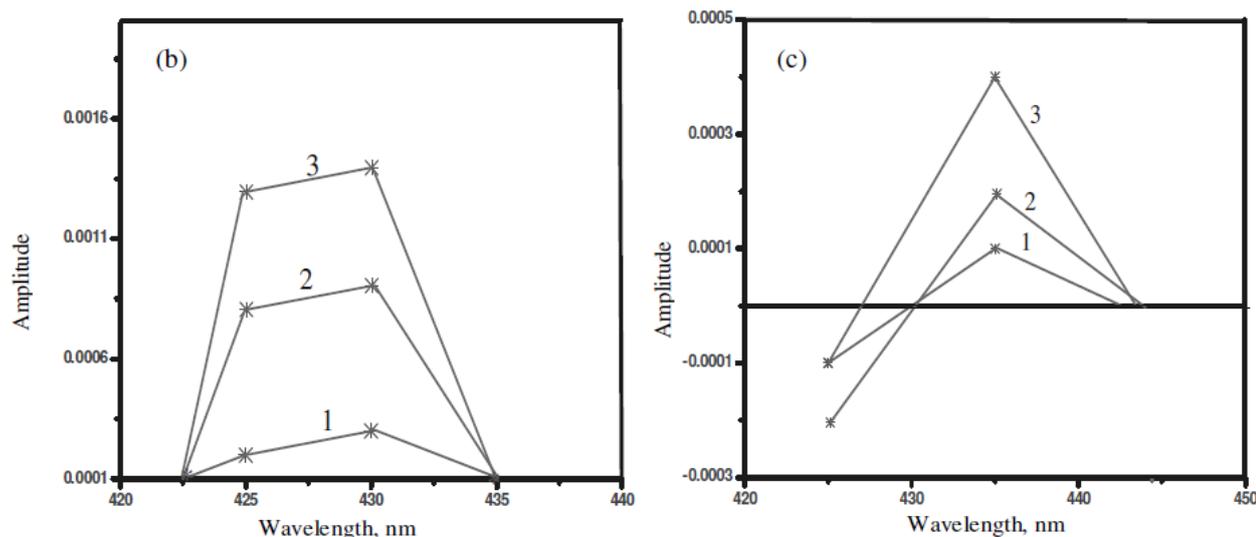
Figure 7. Derivative spectra of [Cu(II)-5M 3H BR] system (a) first order (b) second order (c) third order Cu(II) - μg/mL (1) 1.5 (2) 3 (3) 4.5.

6.6 Effect of diverse ions

The influence of coexisting ions on the determination of 10 μg of Cu(II) was investigated the diverse ion in question were transferred in to comparison tubes of 20 mL capacity followed by an excess of reagent solution at pH 5. The tolerance limit of various ions is defined as the largest amount making a variation of less than ±5% in the determination of Cu(II). As shown in Table 3, most of divalent ions whose complexes with color complex of [Cu(II) - 5M, 3H, BR] generally have maximum absorption around 405–460 nm, do not produce remarkable interfere at the measured wavelength. The results are summarized in the Table 3.

6.7 Application

The proposed approach was applied to the determination of total copper in natural water and
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selected vegetables. A 5.0 mL prepared water or food samples were performed the procedure described in previous sections. The results are listed in Table 4,5&6. To examine the accuracy, the recovery experiments were carried out and all samples were spiked with copper standard solutions in two levels. The obtained recoveries were reasonable for copper in samples in a range of 94.9–99.9%. The accuracy of the proposed method was also validated by determination of Certified Reference Materials and the results are given in Table 3. The obtained results were performed by statistical analysis using student's t-test. As can be seen in Table 3, the calculated t values were less than tabulated value at 95% confidence level indicating no significant difference between the results obtained and the certified values

Table 3. Effect of diverse ions

Diverse ion	Added as	Tolerance limit μg/ 20 mL
Mg^{+2}	$MgSO_4$	1000
Ba^{+2}	$BaCl_2$	984
Co^{+2}	$Co(NO_3)_2$	675
Ag^+	$AgNO_3$	750
Pb^{+2}	$Pb(NO_3)_2$	688
Se^{+2}	Na_2SeO_3	943
Ca^{+2}	$CaCl_2$	920
Sn^{+2}	$Sn(NO_3)_2$	1000
Te^{+2}	Na_2TeO_3	1000
Li^{+2}	$LiNO_3$	785

Al^{+3}	$Al(NO_3)_3$	1034
Cr^{+3}	$K_2Cr_2O_7$	1000
Zn^{+2}	$ZnSO_4$	902
Cd^{+2}	$CdCl_2$	1220
Hg^{+2}	$HgCl_2$	980
Mn^{+2}	$MnCl_2$	650

Table.3(Continued)

Ni^{+2}	$NiSO_4$	730
Fe^{+3}	$FeSO_4$	945

Table 4. Direct spectrophotometric determination

Sample	Amount of Copper Spiked µg/mL	Amount of copper found µg/mL	Recovery %	RMSEP	REP %	RSD %	t-test
Tab water	-	0.0086	-	0.00033	9.696	3.820	3.3681
	1.066	1.05±0.02	97.7	0.0479	4.1862	4.562	0.8648
	1.3351	1.333±0.01	99.26	0.0101	1.7802	0.0757	0.7827
Pinakini	-	0.2408	-	0.0182	3.8725	7.558	1.2336
Water	0.76	0.982±0.03	97.9	0.0109	2.0460	0.111	1.3054
	0.824	1.051±0.05	98.67	0.0283	5.7831	0.2693	1.4637
Cabbage	-	17.2	-	0.0779	1.2671	2.158	1.1731
	2.0	18.6±0.001	96.8	0.2319	4.6434	5.927	2.5153
Vegetable Banana	-	14.76	-	0.0642	0.8438	2.0742	1.6413
	8.0	21.98±0.006	96.57	0.0563	0.4423	1.2197	2.2859
Tomato	-	18.35	-	0.2239	6.9181	5.782	17173
	4.0	21.53±0.01	96.33	0.4036	5.8121	5.500	1.6715
Wheat flour	-	15.7	-	0.0506	3.1433	1.532	1.6810
	5.0	20.4±0.03	98.55	0.4040	4.1543	7.4252	0.0821
Cucumber	-	24.28	-	0.0268	1.4955	0.5268	2.2065
	4.0	27.11±0.001	95.86	0.1526	4.8579	1.6495	2.4825

Table5. Derivative spectrophotometric determination

Sample	Amount of copper spiked (µg/mL)	Amount of copper founded (µg/mL)	Recovery	RMSEP	REP%	RSD %	t-test
Tap Water	-	0.0086	-	0.00033	9.696	3.820	3.3700
1st derivative	1.037	1.04±0.01	99.4	0.0778	9.556	7.487	0.8454
	1.60	1.59±0.04	98.8	0.0283	5.720	1.780	5.7992
2nd derivative	0.896	0.87±0.06	96.5	0.0424	2.607	4.857	1.3424
	1.635	1.63±0.03	99.4	0.0264	3.400	0.162	3.6413
3rd derivative	1.102	1.086±0.001	97.8	0.0470	9.410	4.328	1.5686
	0.792	0.781±0.07	97.5	0.00712	2.929	0.913	0.9763
Pinakini water	-	0.2408	-	0.0182	3.872	7.558	1.2336
	0.781	1.022±0.03	99.9	0.04127	3.359	4.040	2.1183

1st derivative	1.16	1.384±0.05	99.1	0.0800	5.618	5.780	0.5217
	0.744	0.96±0.02	97.3	0.0149	0.524	1.555	1.2203
2nd derivative	1.18	1.39±0.003	97.9	0.0409	3.629	2.940	1.2370
	0.93	1.16±0.01	98.9	0	0	0	0
3rd derivative	1.7478	1.96±0.003	98.5	0.1914	3.826	9.770	1.2291
Cabbage	-	17.2	-	0.0779	1.267	2.158	1.1731
1st derivative	-	17.91	-	0.2465	4.524	6.552	1.4855
	2.21	19.27±0.005	99.27	0.2175	2.949	0.6806	1.3681
2nd derivative	-	18.01	-	0.3781	2.196	9.994	0.6806
	1.98	18.35±0.02	95.7	0.3612	3.621	2.941	0.3992
3rd derivative	-	13.52	-	0.3644	0.891	12.83	1.3693
	2.14	18.70±0.04	96.7	0.3871	2.358	4.510	0.8414
Vegetable banana	-	14.76	-	0.0642	0.843	2.074	1.6413
1st derivative	-	12.32	-	0.3348	2.565	11.59	0.3848
	7.84	21.67±0.001	95.9	0.3295	3.707	6.281	1.4971
2nd derivative	-	12.07	-	0.2805	6.287	3.664	0.0183
	8.12	22.55±0.04	98.6	0.4677	1.807	3.664	0.3617
3rd derivative		12	-	0.0735	2.793	2.915	0.6582
	7.94	22.06±0.003	97.2	0.2782	4.126	3.780	1.0900

The quantity of copper(II) in the common man dietaries like cabbage (13.5-18 µg/g), vegetable banana (12-14.7 µg/g), tomato (14.1-23.7 µg/g), wheat flour (14.9-16.9 µg/g), cucumber (14-24.2 µg/g), were determined by this method, it was widely believed that most ostensibly healthy individuals consumed diets are to provide 2000 µg of copper/day(21).So the above diets are suggestive as good dietary for healthy individuals to supplement the require copper. The results are summarized in the Table 4and 5 . The WHO provisional guideline value of 2000 µg/L (2 µg/mL) of copper in drinking water could produce an adverse reaction (22). This is computable with the United states drinking water action level of 1300 µg/mL (1.3 µg/mL)(23)

4. Conclusion

A simple and high-selective method was employed for rapid determination of total copper in water and vegetable in this paper. Total copper can be efficiently determined based on the colored complex formation of Cu(II)- 5-{ ά –methyl-3-hydroxy benzylidene}rhodanine [5M, 3H-BR], followed by dispersive and derivative complex and subsequent UV–vis spectrophotometry detection. The absorption spectra of an orange–red color complex of [Cu (II) - 5M, 3H, BR] were recorded in the wave length region 400-600 nm. The method can be successfully proposed for the total copper assay in routine water and vegetable sample analyses.

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