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RESEARCH ARTICLE

Determination of Copper in Water, Vegetables, Foodstuffs and Pharmaceuticals by Direct and Derivative Spectrophotometry

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Abstract: The quantification of copper in water, vegetables, foodstuffs, human hair and pharmaceutical samples was determined by a simple more sensitive and selective spectrophotometric method. Cu(II) forms an orange – red color complex with 5-{ά –methyl-3-hydroxy benzylidene} rhodanine [5M, 3H-BR], at pH 5.5 in sodium acetate and acetic acid buffer. The maximum absorbance was measured at 430 nm. The Beer's law is obeyed in the range of (0.05 µg -13 µg/mL). The molar absorptivity (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. First, second and third derivative spectrophotometry were also proposed and employed successfully for the determination of copper in the supra. The performance of the present method was also evaluated in terms of RMSEP, REP and RSD, students *t*- test. This indicates the greater importance of the method than other methods reported in the literature.

Keywords: Copper determination, Direct and derivative spectrophotometry, 5-{ά –Methyl-3-hydroxy benzylidene} rhodanine, RMSEP, REP, RSD

Introduction

Metals at trace levels are components of natural biosphere. Hence they are required for body structure, fluid balance, protein and to produce hormones. Some of them are considered essential, but at high concentration they are toxic. The range between essentiality and toxicity is often very small. Copper occurs in nature as mineral compounds, 75% copper that is mined is used in the electrical industries, house hold-utensil, metallic blends and pigments[1]. From these sources, it will enter as pollutant and pollutes the water, soil, foodstuffs, flora and fauna.

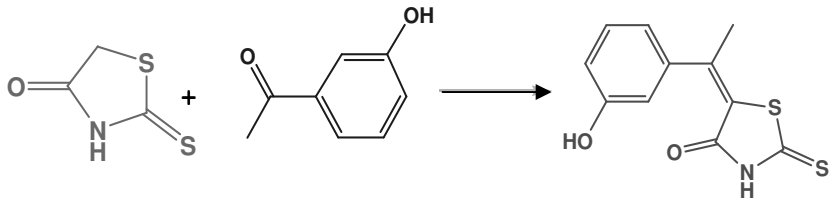
It is an essential to activate enzymes involved in[2] cellular respiration- (cytochrome-C oxidase), iron oxidation- (ceruloplasmin), connective tissue formation-(lysyl oxidase) neuro transmitter biosynthesis-(Mono Amine oxidase) and pigment formation-(Tyrosanase). On over healthy limit accumulates in the liver causing dizziness, vomiting, diarrhoea, transpiration and depending on its concentration it leads to death[3-7]. In chronic exposure, liver, kidney and spleen may be injured and may develop anemia. The deficiency of copper causes the coronary,

artery; heart diseases and can promote connection between sugar molecules and protein molecules which results in tissue damification in diabetic people[9-10]. Therefore, from this point of view, it is necessary to establish a rapid simple, sensitive and accurate procedure for the determination of copper concentration. Several techniques have been used for the determination of copper in different samples[11-15]. However these methods have the disadvantages that the operation of the instrumentation used, is complex and the price of the instrumentation is expensive compared with UV-visible spectrophotometry.

Hitherto several complexing agents[16-25] are reported for the spectrophotometric determination of copper. Spectrophotometry still represents an attractive technique for the determination of metal ions in aqueous media, because of its simplicity, being inexpensive and is readily availabble[26]. Therefore in the present investigation a selective reagent 5-{ά – methyl-3-hydroxy benzylidene}rhodanine [5M,3H-BR] was chosen for the UV- visible spectrophotometric determination of copper(II) in the samples selected.

Experimental

The ligand 5-{α-methyl-3hydoxy benzylidene}rhodanine, prepared according to the procedure reported previously[27]. 120 mg of ammonium acetate was added to a mixture of 360 mL glacial acetic acid and 13 mL benzene then 2 g of rhodanine was added. 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. Later it was allowed to cool at room temperature which gives a yellow precipitate. It is separated by filtration, washed with water and purified by recrystallization from methanol/ water (1:1) mixture melting point is 201 ^{0}C – 202 ^{0}C. The structure was confirmed from Mass IR, NMR spectra**.**



Scheme 1. Formation of 5-{ά –methyl-3-hydroxy benzylidene}rhodanine

Preparation of solutions

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

Cu(II) - solution

Stock standard Cu(II) solution was prepared by dissolving 0.3929 g of Cu(II) sulphate pentahydrte in double distilled water containing 1000 µg/mL. The solution was standardized by idometry[28]. The working standard solutions were prepared by suitable dilution of the stock solution.

Buffer solutions

Buffer solutions were prepared by employing 0.1 M acetic acid and 0.1 M sodium acetate[29-30] 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.

Solutions 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

Reagent 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.

Instruments

Elico micro processor based double beam UV - visible spectrophotometer SL 210 equipped with 1 cm quartz cells were used for spectrophotometric measurements. The pH measurements are made with Elico digital pH meter L.I 127 model.

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[31-38] prepared, were computed from the standard calibration carves in the range 0.05 to 13 µg both by inspecting the direct and derivative spectra.

Results 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). It was observed that complex showed the maximum absorbance at 420 nm in borate buffers and at 430 nm in acetate buffers, whereas in acetate buffers the complex absorbance was found to be maximum. Hence, the 430 nm in acetate buffers were chosen for the proposed studies

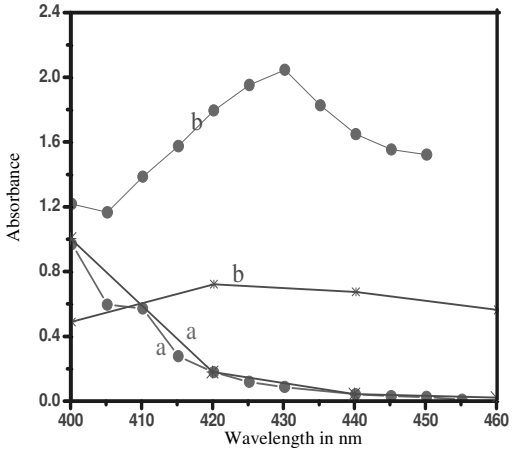


Figure 1. Absorption spectra of; a) 5M 3H BR *vs.* buffer blank; b) Cu(II)-5M 3H BR complex *vs.* reagent blank acetate buffer *Borate buffer Cu(II)=1.6x10^{-3} M (100 µg), 5M 3H BR =3x10^{-3} M

Effect of the pH

The pH of the aqueous solution is an important parameter 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). 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

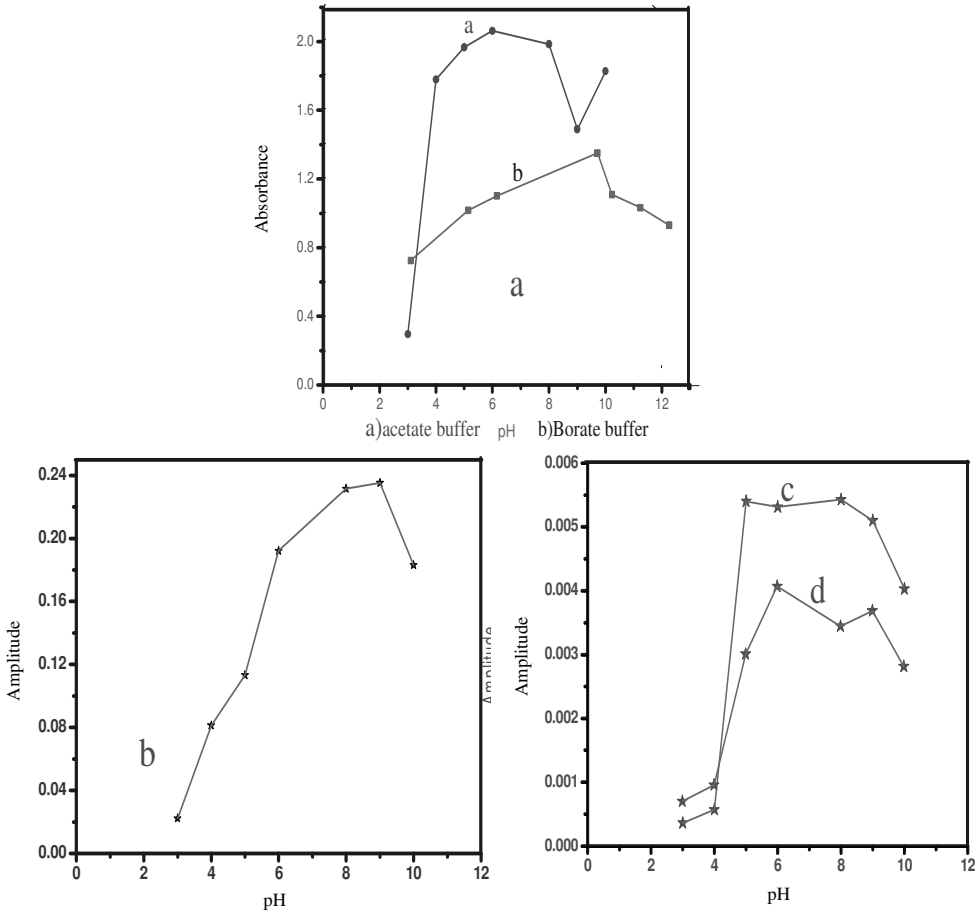


Figure 2. Effect of pH on the absorbance of [Cu(II)-5M 3H BR] system (a) Direct spectrophotometry (b) 1st derivative (c) 2nd derivative (d) 3rd derivative Cu(II)=[5M 3H BR] $=3x10^{-4}$ M

Effect of solvent and reagent concentration

A tenfold molar excess of the reagent was necessary for the maximum color development. 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**.**

Effect of salting out agent

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 enhancement of the color of the metal complex in the analysis of the different samples. It was observed that, the presence of 0.01 M sodium corbonate effectively increases the color (Figure 3).

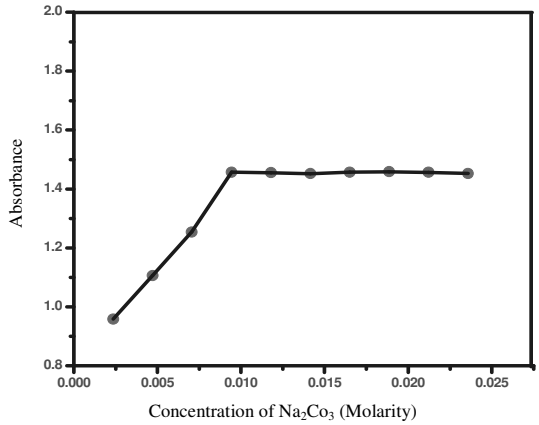


Figure 3. Effect of Na_2CO_3 on the complexation

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 bidental ligand (Figure 4-6).

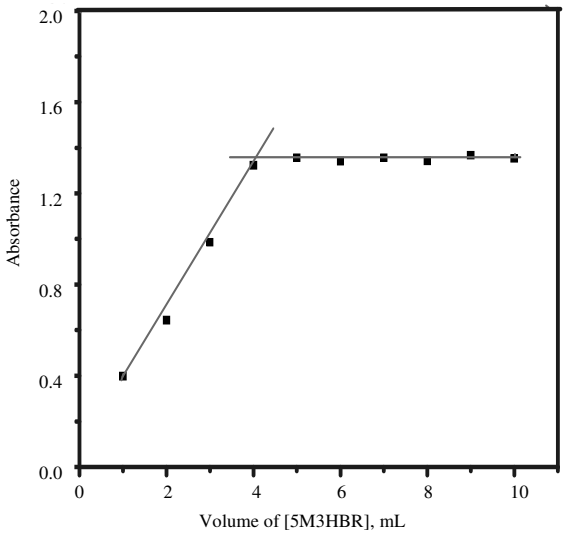


Figure 4. Mole ratio plot, pH:5.5 Cu(II)=[5M 3H BR]=$1.6x10^{-4}$ M, volume of Cu(II)=l mL (10 µg)

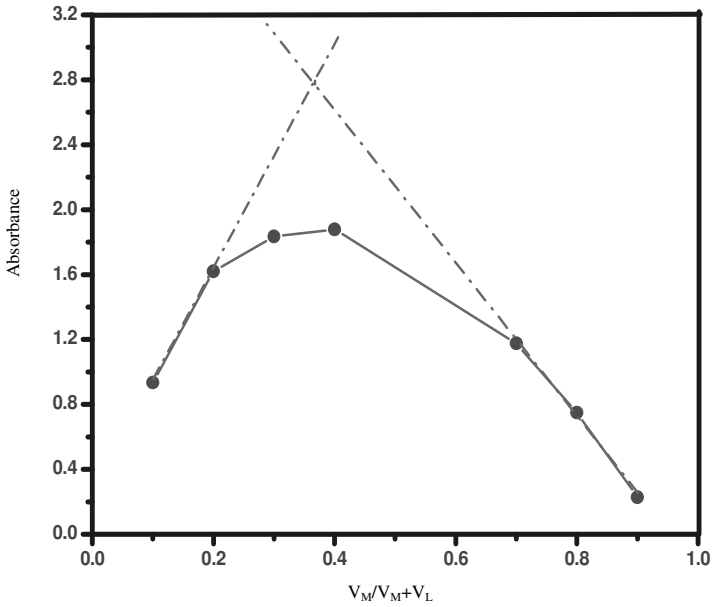


Figure 5. Job's continuous variation method for the Cu(II)-[5M 3H BR] complex, Cu(II)= [5M 3H BR]= 1.6x10-4 M, pH:5.5, λ_{max}: 430 nm

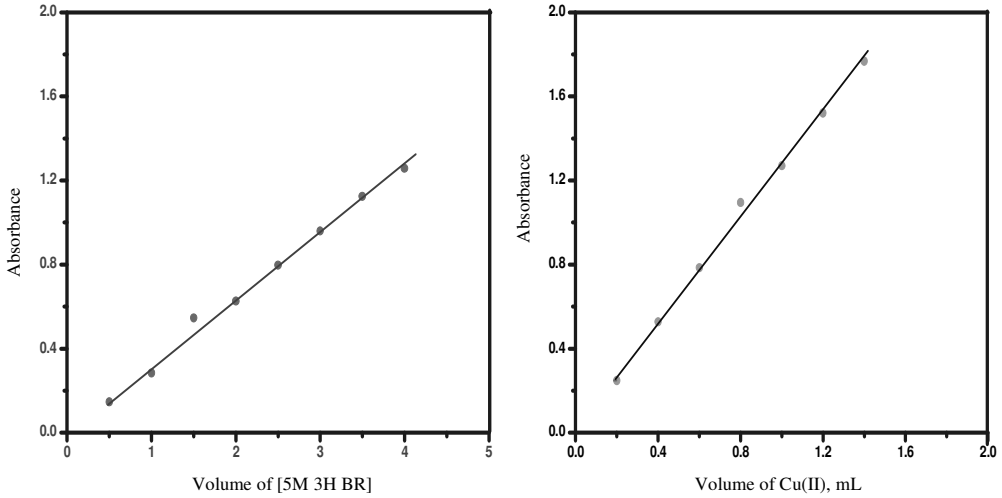


Figure 6. Slope ratio method, Cu(II)=[5M 3H BR]=1.6x10^{-4} M pH:5.5 λ_{max}=430

Performance for the calibration of proposed method

Beer's law was obeyed in the concentration range 0.05-13 µg/mL of Cu(II) in different sample solutions. The molar absorptivity of the complex was 0.6027×10^4 $moles^{-1}$ cm^{-1}. The Sandell's sensitivity of the method was found to be 0.01054 µgcm^{-2}. The standard deviation, correlation coefficient and other statistical parameters of the method are evaluated to ten replicate determinations Table 1**.**

Table 1. Performance data for the calibration of proposed method

Concentration Range, µg	Least square equation Y = A + B X A =Intercept B = Slope	Correlation Coefficient (ŗ)	Standard Deviation	RSD %	REP %	Amount determined In ten replicate samples, µg
0.05 -0.5	Y= - 0.00207 + 0.2398 X	1.0000	0.000769	0.1917	0.2991	0.4010,0.3997,0.4022,0.4012 0.4020,0.4012,0.4018, 0.4014, 0.4012,0.4024
0.5 -5.0	Y = - 0.0364 + 0.2466 X	1.000	0.0505	1.2241	2.6839	4.1625,4.1091,4.0275,4.1525 4.1592,4.0761,4.1475,4.1855 4.1572,4.0761.
5.0 – 13	Y = 1.4559 + 0.0103 X	0.9869	0.005957	0.0799	0.2014	7.4550,7.4469,7.4470,7.4481 7.4500,7.4470,7.4620, 7.4475, 7.4451,7.4598.

Derivative spectrophotometry

For the determination of copper derivative spectrophotometric methods are also developed. 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 maxis mum amplitude was shifted to 435-460 nm. Calibration plots drawn between the amplitude and the concentration of Cu(II) was found to be linear in the range (0.05-13 µg/mL) The derivative amplitudes were found to be proportional to the concentration of Cu(II). The results are summarized in Table 2, Figure 7.

Table 2. Calibration data for the derivative spectrophotometric determination

Linear Range, µg/mL	Calibration Equation Y = A + BX, A = Intercept, B = Slope	Wavelength, nm	Correlation Coefficient (ŗ)
0.05 – 0.5	First – Derivative Spectrophotometry $\partial A/\partial\lambda$ = - 0.0292 + 0.564 X	405	0.9985
	Second – DerivativeSpectrophotometry $\partial^2A/\partial\lambda^2$ = - 0.0089 + 0.0440 X	430	1.003
	Third – Derivative Spectrophotometry $\partial^3A/\partial\lambda^3$= - 0.000124 + 0.0046 X	435 - 460	0.9989
0.5 – 5.0	First – Derivative Spectrophotometry $\partial A/\partial\lambda$ = -0.0431 + 0.0588 X	405	0.9937
	Second – DerivativeSpectrophotometry $\partial^2A/\partial\lambda^2$ = - 0.000526 + 0.001207 X	430	0.9980
	Third – Derivative Spectrophotometry $\partial^3A/\partial\lambda^3$ = - 0.000116 + 0.0002656 X	435 - 460	1.000
5.0 - 13	First – Derivative Spectrophotometry $\partial A/\partial\lambda$= - 0.0614 + 0.0534 X	405	1.0032
	Second – DerivativeSpectrophotometry $\partial^2A/\partial\lambda^2$ = - 0.008788 + 0.002201 X	430	0.9887
	Third – Derivative Spectrophotometry $\partial^3A/\partial\lambda^3$ = - 0.00223 + 0.00087 X	435 – 460	0.9907

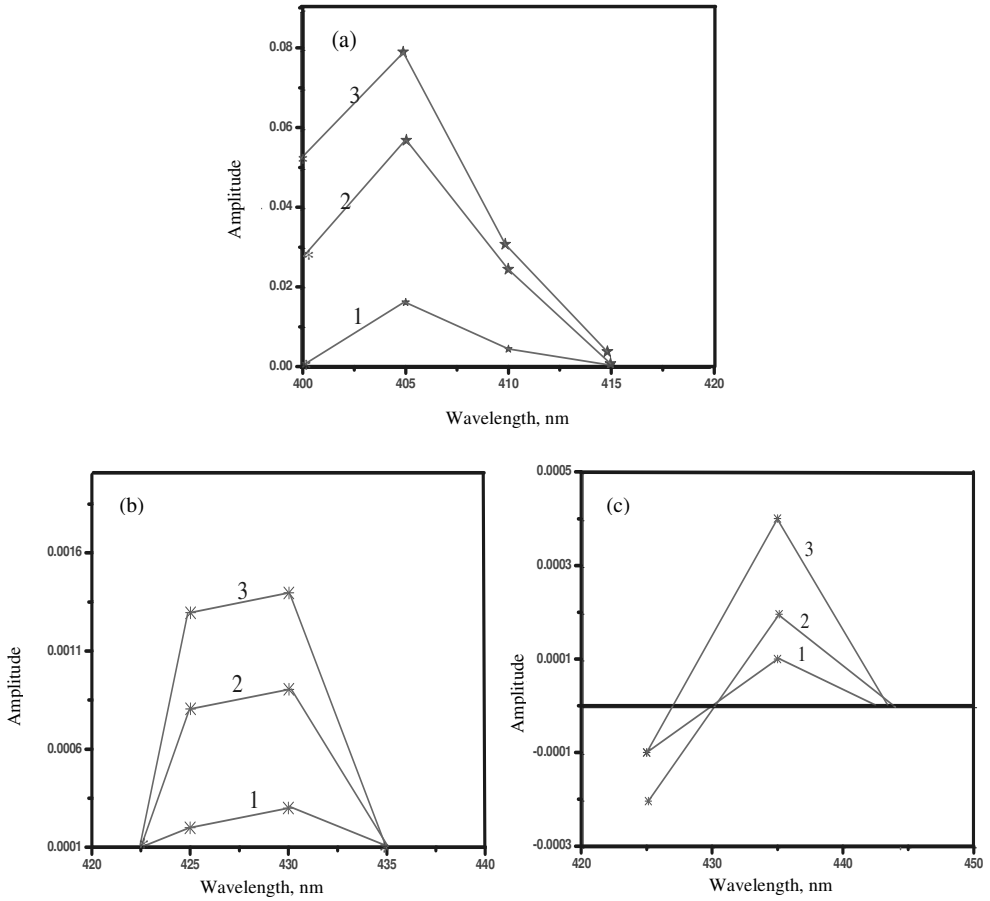


Figure 7. Derivative spectra of [Cu(II)-5M 3H BR] system (a) first order (b) second order (c) third order Cu(II) - µg/mL (1) 1.5 (2) 3 (3) 4.5

Effect of diverse ions

To examine the effect of the diverse ions, 10 µg of Cu(II) and diverse ion in question were transferred in to comparison tubes of 20 mL capacity followed by an excess of reagent solution at pH 5.5. However, in the case of interference were masked using citrate, tartarate, phosphate as masking agents. The tolerance limit was stated as the highest amount for an ion that produces an error not exceeding ±3% in the determination. The results are summarized in the Table 3.

Analytic conclusion

The proposed direct and derivative spectrophotometric method were employed for the determination of Cu(II) in different samples such as natural water, biological samples, foodstuffs and pharmaceutical samples. The results are summarized in the Table 4, 5 & 6. The WHO provisional guideline value of 2000 µg/L (2 µg/mL) of copper in drinking water could produce an adverse reaction[39]. This is computable with the United states drinking water action level of 1300 µg/mL (1.3 µg/mL)[40]. In the present method, the content of copper in buffalo's milk and Cow's milk were found to be 1065-1216 µg/L, 1230-1318 µg/L respectively.

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
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
Tap 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
Milk (Buffalo)	-	1.216	-	0.1460	1.3990	3.4312	0.3530
Milk(Cow)	-	1.318	-	0.1657	4.9157	3.5917	0.1469
Mother Milk	-	0.4841	-	0.0686	4.7901	2.0240	0.8205
		µg/g					
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	-	14.76	-	0.0642	0.8438	2.0742	1.6413
Banana	8.0	21.98±0.006	96.57	0.0563	0.4423	1.2197	2.2859
Amruthapani	-	16.4	-	0.1254	6.4778	3.633	0.2370
Banana	4.0	19.55±0.004	95.8	0.1718	1.6557	0.3871	1.0878
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
Rice(1010)	-	14.522	-	0.2017	0.7729	3.9680	2.2105
	5.0	17.7482±0.03	90.91	0.7770	4.7914	12.508	1.8041
Rice	-	21.68	-	0.1278	3.2275	2.8070	0.1855
(Masuria)	3.0	23.93±0.0001	96.96	0.1357	3.8526	2.6993	1.0136
Human hair	-	41.2	-	0.0329	0.4409	0.38506	1.6724

Average of ten replicate determinations

Table 5. Derivative spectrophotometric determination

Sample	Amount of copper Spiked, µg/mL	Amount of copper found 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	1.5676
	0.792	0.781±0.07	97.5	0.00712	2.929	0.913	0.9763
Pinakini	-	0.2408	-	0.0182	3.872	7.558	1.2336
water	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
Milk(Buffalo)	-	1.216	-	0.1460	1.399	3.431	0.3530
1st derivative	-	1.0668	-	0.4075	5.090	10.91	0.8147
2nd derivative	-	1.0071	-	0.5240	5.724	14.86	0.6577
3rd derivative	-	1.0654	-	0.3088	2.602	8.281	0.6492
Milk(Cow)	-	1.318	-	0.1657	4.915	3.591	0.1469
1st derivative	-	1.2300	-	0.5835	6.559	13.55	0.0325
2nd derivative	-	1.2281	-	0.9981	3.014	23.21	1.3550
3rd derivative	-	1.2582	-	0.5265	6.229	11.95	0.5321
Milk(Mother)	-	0.4841	-	0.0686	4.790	2.024	0.8205
1st derivative	-	0.4297	-	0.0535	4.259	1.779	2.1004
2nd derivative	-	0.4288	-	0.2672	2.942	8.901	1.9454
3rd derivative	-	0.5674	-	0.4747	6.256	11.95	0.3111
		µg/g					
Cabbage	-	17.2	-	0.0779	1.267	2.158	1.1731
1stderivative	-	17.91	-	0.2465	4.524	6.552	1.4855
	2.21	19.27±0.005	99.27	0.2175	2.949	4.502	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	3.697	0.3617
3rd derivative	-	12	-	0.0735	2.793	2.915	0.6582
	7.94	22.06±0.0003	97.2	0.2782	4.126	3.780	1.0900

Contd…

Amruthapani banana	-	16.4	-	0.1254	6.477	3.633	0.2370
1st derivative	-	13.09	-	0.3098	2.175	11.26	1.037
	3.79	19.68±0.0005	97.5	0.1978	4.408	0.416	1.2293
2nd derivative	-	14.99	-	0.3502	5.014	11.11	1.358
	4.22	20.31±0.01	99.5	0.3309	1.745	0.260	1.120
3rd derivative	-	21.59	-	0.1388	3.193	3.0600.7	1.155
	3.86	19.22±0.002	94.9	0.5060	2.534	12	0.5681
Tomato	-	18.35	-	0.2239	6.918	5.782	1.7173
1st derivative	-	14.13	-	0.2068	4.220	6.967	0.6712
	3.98	21.59±0.004	96.7	0.3850	4.962	6.879	0.8615
2nd derivative	-	23.75	-	0.3709	4.837	7.433	2.2669
	4.22	22.23±0.001	98.5	0.1383	2.157	1.313	0.8894
3rd derivative	-	21.55	-	0.2805	1.951	6.197	0.1352
	4.25	21.44±0.0002	94.9	0.2302	3.398	3.250	0.3461
Wheat flour	-	15.7	-	0.0506	3.143	1.532	1.681
1st derivative	-	14.85	-	0.0835	2.792	2.677	1.0528
	5.25	20.27±0.002	96.8	0.0956	1.018	1.616	1.3032
2nd derivative	-	16.65	-	0.6531	2.398	18.68	1.7672
	4.89	19.58±0.04	94.7	0.0711	0.603	0.557	1.5477
3rd derivative	-	16.83	-	0.1115	2.947	3.154	1.3074
	4.87	20.34±0.0005	98.9	0.333	2.576	3.462	1.3218
Cucumber	-	24.28	-	0.0268	1.495	0.526	2.2065
1st derivative	-	17.93	-	0.2942	5.742	7.809	0.8276
	3.92	27.26±0.0019	96.7	0.4363	2.619	13.83	0.6667
2nd derivative	-	23.21	-	0.17086	3.024	3.504	1.984
	4.24	27.15±0.05	95.2	0.0865	1.598	1.295	0.2449
3rd derivative	-	14.02	-	0.1527	5.010	5.138	0.9028
	4.09	26.72±0.0006	94.2	0.3989	1.488	3.416	0.3052
Rice(1010)	-	14.5222	-	0.2017	0.772	3.968	2.2105
1st derivative	-	8.4805	-	0.0595	4.471	2.004	1.445
	5.00	13.280±0.001	98.5	0.4499	3.666	7.695	0.6403
2nd derivative	-	13.7077	-	0.1770	1.810	3.689	0.8146
	5.00	17.997±0.0003	96.19	0.1118	1.962	2.098	0.5798
3rd derivative	-	11.808	-	0.2502	4.406	6.053	1.0907
	5.00	16.00±0.02	95.19	0.3112	0.141	6.183	0.9409
Rice(Masuria)	-	21.68	-	0.1278	3.227	2.807	0.1855
1st derivative	-	17.4059	-	0.1078	1.161	2.948	1.3288
	3.00	20.222±0.0001	99.0	0.1547	1.859	3.282	0.8421
2nd derivative	-	19.589	-	0.2576	3.141	6.261	1.080
	3.00	21.669±0.02	95.9	03870	6.875	8.503	0.2279
3rd derivative	-	18.244	-	0.1483	2.601	3.870	1.0746
	3.00	20.62±0.001	97.0	0.2166	3.412	4.999	1.2175
Human Hair	45.02[33]	41.2	91.5	0.0329	0.440	0.380	1.6724
1st derivative		45.73	101.5	0.0776	1.672	1.034	0.175
2nd derivative		44.59	99.0	0.0550	2.849	0.949	1.880
3rd derivative		45.59	101.2	0.0622	1.899	1.004	0.9556

Average of ten replicate Determinations

Table 6. Determination of copper in pharmaceutical preparations

Pharmaceuticals	Form	Certified value mg / tablet	Found mg /tablet	Recovery %	RMSEP	REP %	RSD	*t* – test
Supradyne	$CuSO_4$.	3.39	3.41	100.6	0.0459	3.2319	1.346	3.8511
1st derivative	$5H_2O$		3.57	105.3	0.0800	3.2740	2.2405	2.2925
2nd derivative			3.78	111	0.4535	3.9085	10.910	0.6177
3rd derivative			3.10	91.3	0.2079	2.7644	6.7150	2.1522
MULTIRICH	Copper	50	53	106	0.4780	2.8202	9.684	2.4166
1st derivative			48.86	97.72	0.3005	4.4118	7.873	1.1470
2nd derivative			49.47	98.94	0.2348	2.3565	5.634	0.4754
3rd derivative			52.07	104.14	0.3959	4.2369	10.032	1.5487
MULTIVITE	$CuSO_4$.	0.1	0.108	108	0.1420	0.0358	3.749	2.5074
1st derivative	$5H_2O$		0.092	92	0.1459	2.1845	4.531	1.3329
2nd derivative			0.101	101	0.1642	3.0030	3.541	0.3624
3rd derivative			0.105	105	0.3002	4.9610	7.4187	1.0059
GBION	Copper	2.0	1.36	68.0	0.5284	0.0679	11.074	0.4895
1st derivative			0.8623	43.1	0.1170	2.2943	3.8440	0.8891
2nd derivative			0.8922	44.6	0.2831	4.1110	9.0652	0.1854
3rd derivative			0.954	47.7	0.0872	2.4457	2.6300	1.5883
NEXBLEND	CuO	0.5	0.40	80	0.3149	4.5997	8.0009	1.8416
1st derivative		mg/15 mL	0.3047	60.94	0.074	3.4494	2.4279	0.1666
2nd derivative			0.3542	70.84	0.1780	2.1879	1.5519	4.6484
3rd derivative			0.3148	62.96	0.0872	2.4457	2.6306	1.5883

The infants not given breast milk, fed with buffalo's milk and cow's milk may have to been increased the bioavailability of copper and is associated with the acute phase reactions of number of diseased states, is always almost accompanied by hypercaeruloplasminaemia[45]. The content of copper determined in the human hair is 41.2-45.4 µg/g. It is good coincidence with the values reported in the literature[33].

The quantity of copper(II) in the common man dietaries like cabbage (13.5-18 µg/g), vegetable banana (12-14.7 µg/g), Amruthapani banana (13.1-21.5 µg/g), tomato (14.1-23.7 µg/g), wheat flour (14.9-16.9 µg/g), cucumber (14-24.2 µg/g), rice 1010 (8.5-14.5 µg/g), rice masuria (17.4-19.6 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[46]. So the above diets are suggestive as good dietary for healthy individuals to supplement the require copper.

The estimation of copper in the pharmaceutical samples shows the efficiency of the method and sensitivity of the reagent than the methods reported in the literature[33-38]. However, in the case of GBion tablet determined value of Cu(II) is very low than the certified value, in all other cases the certified and the determined values are in good agreement hence, it was concluded the GBion tablets [Cotec health care pvt.ltd Uttaranchal. India.] Maintain substantial values than the certified.

The proposed spectrophotometric method is more selective. The standard addition method was used to determine Cu(II) in real samples, because of the incomplete release due to the interfering effects. The relative standard deviations representing the reproducibility

and low detection limits in the determinations. The reagent used is highly specific. Hitherto no information in the literature used for the trace metal analysis. The proposed method shows the possibility of determination of ultra trace levels without the use of sophisticated instrumentation.

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