INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

DIRECT AND DERIVATIVESPECTROPHOTOMETRIC DETERMINATION OF COPPER (II) IN MILK, BLOOD AND WATER SAMPLES

K. Sarasubudhi, S. Sivaramaiah and K. Ashok rao*

Research Laboratories, Department of Chemistry, V.R. College, Nellore, Andhra Pradesh, India.

ABSTRACT

Copper is an essential micronutrient for human bodies. The accurate data in the distribution and the concentrations of Cu (II) in milk, blood and different organs and tissues is not thoroughly explained yet. Further issues which need clarification are the exact composition and constitution throughout the lactation is important. Breast milk contains the entire essential nutrients needed by neonates for growth development and maintaining of healthy condition. The physiological impacts of copper and copper enzymes are biomarkers in maintaining the sickness and health. Researches continue in an attempt to develop analytical method for the determination of copper in milk, blood and in drinking water, these discussions justify the purpose of our research related to studying the trace element Copper. In the paper a selective reagent {5M, 3H-B R} 5-{α Methyl -3 Hydroxy Benzylidene} rodanine was suggested for the development of simple and sensitive direct and derivative spectrophotometric method to the quantification of copper. In sodium acetate and acetic acid buffer at P^H 5.5. The maximum absorbance was measured at 430nm. The Beer's law is obeyed in the range of (0.05µg - 13µg/ml). The molar absorptive (ε) and the Sandal's sensitivity of the complex were $0.6027X10^4$ mole/cm and $0.01054\mu gcm^{-2}$ respectively. The breast milk obtained from ten lacting mothers not using any hormonal contraceptive devises aged 19to26 years in Nellore town collected and analyzed similarly cow milk,buffeloe's milk, blood samples, water samples were analyzed and an analytical concentration was made by comparing with WHO parameters.

Keywords: Milk, Breast milk, Blood, 5M-3H-BR, UV&Visible Spectrophotometry.

INTRODUCTION

The maintainens of optimal health requires an adequate supply of carbohydrates, proteins, lipids micronutrients and trace elements. Many trace elements play an essential role in the number of biological process through their action as activators or inhibitors of enzymatic reactions[1-2]. Copper is both vital and toxic for many biological systems by influencing the physiological functions and the interactions with other micronutrients [3-13]In this respect the exact composition structure of copper compounds generated in the organism is not fully established[14-22]. Several studies[23-24]shows the increasing copper levels of blood serum in malignant patients, remission is usually associated with the return of copper levels to normal ranges. Serum copper is suggested as a useful index for the extent of leukemia and malignant lymphoma, and may predict response to chemotherapy further the blood serum copper, zinc ratio is useful parameter for estimating the presence of and prognosis of malignant tumors[25-27]. So the determination of trace amounts of copper (II) in different samples is important for life and pollution. Hence the trace elements assay in biological fluids can be used as diagnostic or prognostic aid in patients with different hormonal disturbances alongside with other biochemical parameters.

Hitherto there are several methods depending on the colour reaction with spectrophotometric reagents, for the quantification of copper (II) in blood serum[28-31], water samples[32-42], and milk samples.[43-44] However in the present investigation highly selective chromogenic agent 5M3HBR was proposed for the determination of copper in blood serum, water samples and milk

samples. Availing the conditions developed in our earlier communication.[45]

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 0.3929gms of Cu(II)sulphate pentahydrte in double distilled water containing 1000µg/ml. The solution was standardized titrimetrically by Idometry. The working standard solutions were prepared by suitable dilution of the stock solution.

Buffer solutions: Buffer solutions were prepared by employing 0.1M acetic acid and 0.1M sodium acetate in the p^Hrange 3-10. Borate buffers are also prepared in the p^H range 3-12 from 1M Boric acid adjusting with 1Msodium hydroxid

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

Instruments used

Elico's Microprocessor based double Beam UV – Visible spectrophotometer SL.210.Equipped with 1cm Quartz cells were used for spectrophotometric measurements. The pH measurements are made with Elico digital pH meter L.I 127 model.

Preparation of calibration plot

The quantification of copper in different samples is ensured evaluating the calibration plots. In different ranges obeying the Beer's law (0.05-13µg) using the standard solution of Cu(II)SO_4, consisting 1 µg/ml, 10 µg/ml, 100 µg/ml.

In each case different volumes ranging from 1-10ml are taken in comparison tubes, along with the 5ml buffer of p^H 5.5 followed by the 4ml reagent ($1.6x10^{-3}$M) solution, and finally diluted to 20ml. The absorbance of an aliquots sample (3.5ml) was measured. For the same solution the amplitude values are noted by examining the derivative spectras.

The molar absorptivity of the complex is $0.6027x10^4$ $mol^{-1}cm^{-1}$. The sandell's sensitivity of the method was found to be 0.01054 µg cm^{-2}, the standard deviation, correlation coefficient and other statistic parameters of the method are evaluated to ten replicate determinations.(Table.1&2)Fig.1.

Analysis of water samples

Water samples (Pinakini River and well water) collected in and around Nellore, A.P (.India.)150ml of the sample were kept at 0^0c-5^0c in metal free polyethylene bottles then filtered through what man No.42 filter paper and diluted to 250ml with double distilled water. Determinations are made in ten replicates at p^H5.5.(Table.3)

Analysis of milk samples

The Milk samples of Cow, Buffalo and mother's breast are collected.100ml of the sample is ignited by adding drop wise to a heated crucible without frothing, then heated strongly to 450-500^0c for 1hr after the moisture has been removed. We took utmost care to avoid loss by sputtering. The ash was dissolved in the minimum of dilute 1:1 Nitric acid and evaporated, the process was repeated for thrice finely by adding dilute hydrochloric acid to dried mass filtered and the filtrate was diluted to 100ml.2ml aliquots of the solution is used for determination at p^H5.5. (Table.4.)

Analysis of blood samples

10 samples of blood (5ml) were drawn from the people of Nellore of different age groups with the help of **J. B. Hospitals clinical laboratory**. The samples are centrifuged for 10 minutes with 3000 RPM, the blood serum separated is deprotanised by adding a drop of 20% TCA (Tri Chloro Acetic acid) then 3ml of the serum was taken for the quantification of Cu(II) as described in the procedure. The results are summarized in the table (5).

RESULTS AND DISCUSSION

The proposed direct and derivative spectrophotometric method were employed for the determination of Cu(11) in different samples such as natural water, biological samples, . The results are summarized in the table 4, 5&6 The WHO provisional guideline value of 2000µg/lit **(2µg/ml)** of copper in drinking water could produce an adverse reaction.[46]This is computable with the United states drinking water action level of **1300µg/ml (1.3µg/ml)**[47].In the present method it was found the copper content in the drinking water is less than **0.1µg/ml** (**0.1mg/lit**.) which is in good agreement with the reported value[48]So it is suggestive at least 8lit.of water to intake per day by ostensibly healthy individuals, which do not produce clinical symptoms of copper toxicity. It is in good agreement with the probable values suggested from the clinical and biochemical data **0.6-0.7mg** per day for either sex.[49]

In the present method, the content of copper in human breast milk was found to be 428-567µg/lit. It is in good coincidence with WHO/IAEA collaborative studies report on miner and trace elements[50].This content provides 50µg/kg of infant body weight per Day.Futher no clinical problems were associated in infants receiving breast milk alone[51].So it was suggested to mother's in India to feed the infants only with breast milk up to 3years age.

In the present method, the content of copper in buffalo's milk and Cow's milk were found to be 1065-1216µg/lit, 1230-1318µg/lit respectively. 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.[52]

The content of copper determined in blood serum of healthy people of different age groups are found to be 1.13-1.5 µg/ml which are in good coincidence with the values reported in WHO measurements.[53-54]

Table 1: Performance data for the calibration of proposed method

Conc. range (ug/ml)	Least square equation	Correlation coefficient (r)	Slope	Intercept	Standard deviation	RSD %	REP %	Amount determined in ten samples
0.05-0.5	A=-0.0020+2.3980C	1.000	2.3980	-0.0020	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	A=-0.0364+0.2466C	1.000	0.2466	-0.0364	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
5-13	A=0.6475+0.1053C	0.9869	0.1053	0.6475	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,

Table 2: Calibration Data For Derivative Spectrophotometry

Linear range(ug/ml)	Calibration Equation	Wave length(nm)	Correlation coefficient
0.05-0.5	First-DerivativeSpectrophotometry $\partial A/\partial\lambda$= -0.0292+0.5640C Second-derivativeSpectrophotometry $\partial^2A/\partial\lambda^2$= 0.0089+0.0440c Third-derivative Spectrophotometry $\partial^3A/\partial\lambda^3$ =-0.000124+0.00446c	405 430 435-460	0.9985 1.0033 0.9989
0.5-5	First-DerivativeSpectrophotometry $\partial A/\partial\lambda$ =-0.0431+0.0588c Second-derivativeSpectrophotometry $\partial^2A/\partial\lambda^2$ =- 0.000526+0.001207c Third-derivativeSpectrophotometry $\partial^3A/\partial\lambda^3$ =- 0.000116+0.0002656c	405 430 435-460	0.9973 0.9980 1.000
5-13	First-DerivativeSpectrophotometry $\partial A/\partial\lambda$ =-0.0641+0.0534c Second-derivative Spectrophotometry $\partial^2A/\partial\lambda^2$ =- 0.008788+0.002201c Third-derivative Spectrophotometry $\partial^3A/\partial\lambda^3$ =- 0.00223+0.00087c	405 430 435-460	1.0032 0.9887 0.9907

Table 3: 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	4.186	4.562	0.8648
	1.3351	1.333±0.01	99.26	0.0101	1.78	0.757	0.7827
Pinakiniwater	-	0.2408	-	0.0182	3.872	7.558	1.2336
	0.76	0.982±0.03	97.9	0.0109	2.046	0.111	1.3054
	0.824	1.051±0.05	98.67	0.0283	5.783	0.2963	1.4637
Derivative spectrophotometric determination							
Tap water	-	0.0086	-	0.00033	9.696	3.820	3.3681
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
Pinakiniwater 1st Derivative	-	0.2408	-	0.0182	3.872	7.558	1.2336
	0.781	1.022±0.03	99.9	0.0412	3.359	4.040	2.1183
2nd 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
3rd 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
	1.7478	1.96±0.003	98.5	0.1914	3.826	9.770	1.2291

Table 4: Direct spectrophotometric Determination

Sample	Amount of Copper Spiked µg/ml	*Amount of copper found µg/ml	Recovery %	RMSEP	REP %	RSD %	t-test
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
Derivative spectrophotometric determination							
Milk(Buffalo)	-	1.216	-	0.1460	1.3990	3.4312	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	4.9157	3.5917	0.1469
1st derivative	-	1.2300	-	0.5835	6.559	13.55	0.0325
2nd derivative	-	1.2281	-	0.9981	3.014	23.21	1.355
3rd derivative	-	1.2582	-	0.5265	6.229	11.95	0.5321
Mother Milk	-	0.4841	-	0.0686	4.7901	2.0240	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

Table 5: Direct & Derivative spectrophotometric Determination

Blood Sample	*Amount of copper found µg/ml	RMSEP	REP %	RSD %	t-test
Sample[a]	1.323	0.0044	1.6042	0.3684	2.8747
1st derivative	1.348	0.0034	0.9852	0.2493	0.5176
2nd derivative	1.339	0.0013	0.2092	0.1014	1.8628
3rd derivative	1.342	0.0019	0.2982	0.1426	1.6521
Sample[b]	1.482	0.0013	0.2704	0.0905	2.1238
1st derivative	1.491	0.0010	0.2014	0.0670	1.8973
2nd derivative	1.501	0.0033	0.6666	0.2240	0.9402
3rd derivative	1.493	0.0024	0.4019	0.1626	0.1302
Sample[c]	1.329	0.0063	0.1129	0.4758	0.2501
1st derivative	1.298	0.0015	0.4633	0.1218	1.6001
2nd derivative	1.288	0.0060	1.0562	0.4703	0.2087
3rd derivative	1.299	0.0058	1.1011	0.4510	0.1619
Sample[d]	1.84	0.0027	2.5425	0.2312	1.8478
1st derivative	1.168	0.0012	1.1995	0.0976	1.1095
2nd derivative	1.159	0.0009	0.1812	0.0761	0.3583
3rd derivative	1.1601	0.0042	0.6875	0.2600	0.7595

Average of ten replicate determinations
a = 14 -18y, b = 24-30y, c = 42 -48y, d = 60 – 65y
Sample selected with help of Jaya Bharath Hospital clinical Laboratories

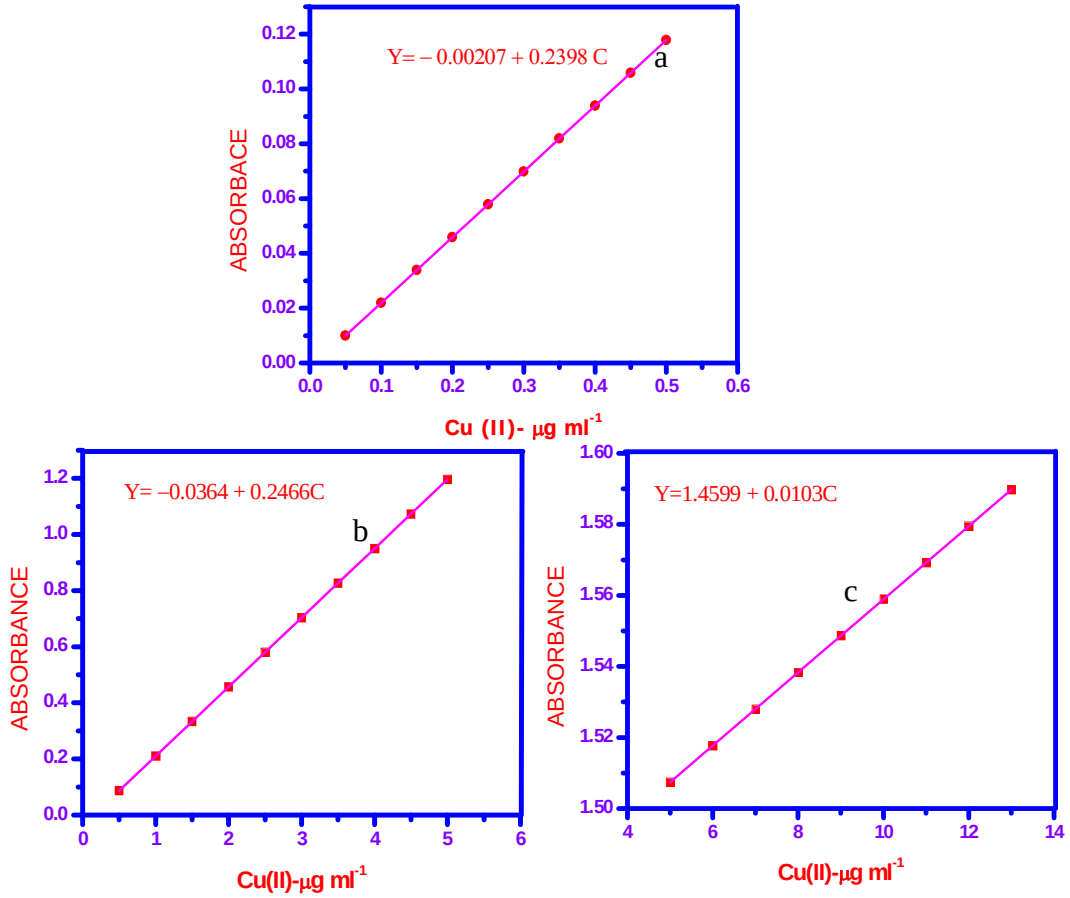

Fig:1 Calibration plot's for diffrent range

(a) 0.05-0.5µg⁄ml (b) 0.5-5.0µg⁄ml (c) 5-13µg⁄ml

ACKNOWLEDGEMENTS

The authors are thankful to Sri "Anam Vivekananda Reddy". M.L.A. Secretary and correspondent V.R. Colleges and High school committee Sri "Anam Ramnarayana Reddy"Finance Minister (Govt. of A.P.) for the continuous encouragement to carry out the proposed work.

REFERENCES

1. Los Almos National Laboratory- copper, http:// en.wikipedia. Org/wiki/copper, 2005.
2. Saker B, Haussac J P, Hau S,Dakar,D. (Ed); Revan press, New York, p 159.(1983)
3. Haris ED. Copper. In: O'Dell BL, Sunde RA, eds. New York: Marcel Dekker, Inc; 231-273, (1997).
4. Food and Nutrition Board, Institute of Medicine. Washington, D.C., National Academy Press, 224-257, (2001).
5. Murthy A. S., Keutmann H. T., Eipper B.A., Molecular Endocrinol,1(4),290-9 (1987)
6. Johnson M.A., Fischer J.G., Kays S.E., Crit Rev Food Sci Nutr, 32(1), 1-31, (1992.)
7. Bowater L., Fairhurst S. A., Just V.J., Bornemann S., Febs Letters, 557, 45-48,(2004).
8. Iain S. Mac Pherson, Federico I. Rossell, Melanie Scofield, A. Grant Mauk and Michael E.P. Murphy, protein Eng Design and Selection, 23(3), 137-145,(2010).
9. Malte Rolff, Felix Tuczek. Angew Chem, 47(13), 2344-2347, (2008).
10. Turgut S., Polat A., Inan M., Turgut G., Emmungil G., Bican M., Karakus Ty., Genc O. Indian Journal of Pediatrics, 74(9),827-30,(2007).
11. Gurgoze M.k., Olcucu A., Augun A.D., Taskin E., Kilic M., Biol Trace Elem Res, 111(1-3), 23-29, (2006).
12. Finely E.B., Cerklewski F.L. J Clin Nutr, 37(4): 553-556, (1983).
13. Jacob R.A., Skala J.H., Omaye S.T., Turnlund J.R. J Nutr, 117(12), 2109-2115, (1987).
14. Rosalind S. Gibson, Oxford University, New York, pp697-711,(2005).

15. Lockitch G, Reston L, Jacobson B., Clin Chem, 34(8), 1625-8,(1988).
16. Ghayour-Mobarhan, M., Taylora A., New S. A, Lamb D. J., Ferns G. A. A.,Cli Biochem, 42 (5),364-75, (2005).
17. Senofonate, O., Violante, N., Caroli, S., J Trace Elem Med Biol, 14(1), 6-13, (2000).
18. Carlos Diaz Romero, Patricia Henriquez Sanchez, Felix Lopez Blanco Elena Rodriguez and Lluis Serra Majem, J Trace Elem Med Biol, 16(2), 75-81, (2002).
19. Voskaki Irene, Arvanitidou Vasiliki,Athanasopoulou Helen, Tzagkaraki Angeliki, Tripsianis Gregory, Giannoulia-karantana Aglaia, Biol Trace Elem Res, 134(2), 136-45,(2010).
20. Kouremenou-Dona Eleni, Artemis Dona, John Papoutsis and Chara Spiliopoulou,Sci Total Environ, 359(1-3), 76-81, (2006).
21. Brunetto M. R., Alarcon O. M., Davila E., Contreras Y., Gallignani M., Rondon C., Burguera J.L., Burguera M., Angarita C., J Trace Elem Med Biol, 13(1-2), 40-50,(1999).
22. M. Ghayour-Mobarhan, A. Shapouri-Moghaddam, M. Azimi-Nezhad, H. S. M.R. Parizadeh, M.Safarian, S. M. R. Kazemi-Bajestani,G. H. Khodaei, S. J. Hosseini, S. M. J. Parizadeh and G. A. Fern, J Trace Elem Med Biol, 23(3),167-175, (2009).
23. Shah I, Lewkow LM, Khila-Nnana U.51,851-4.(1983)
24. Tessner CF, Hrgovic M,Brown BW, et al. 29,173-9.(1972)
25. Oyama T, Mastuno K, Kawamoto T, et al.Biol Trace Ele Res, 42, 115-27.(1994)
26. Gupta SK, Shukla V, et al. J Tropical pediatrics ,40.185-7.(1994)
27. Guptha SK,Shukla V, Vaidya MP, et al. J Surgical Oncology, 46.178-81.(1991)
28. J. Homolka, Biochemia Kliniczna, PZWL, Warsaw,394p, (1971).
29. J.Krawczynski,T.Osinski,Laboratoryjne Metody Diagnostyczne,PZWL, Warsaw,(1967).
30. L.Tomaszewski, Mikrometody Biochemiczne W Laboratorium Klinicznym, PZWL, Warsaw, (1970).
31. W. Ostrowski, Wybrane Metody z Chemii Klinicznej, PZWL, Warsaw, (1970).
32. S.T. Liu, Lihua Jianyan Xuaxue Fence, 30. 108.(1994)
33. K.wrobel, K. Wrobel, G. Cruz-Jimenez, F. Angulo-Romero, Anal. Chim. Acta 387,217.(1999)
34. M.S. Epstein, B. Buehler, M.F. Bullard, Talanta 47, 95.(1998)
35. D.Dong, Y. Shi, X.Z. Chi, J.S. Wang, Fenxi Shiyanshi 15, 45.(1996)
36. Wanda Winkler, Agata Arenhovel- Pacula, Talanta 53, 277-283.(2000)
37. I. Baranowska, Chem. Anal. (Warsaw) 31,851.(1986)
38. Orhan Turkoglu and Mustafa Soylak, Journal of the Chinese Chemical Society, 52, 575-579.(2005)
39. D. Rekha, K. Suvardhan, K. Suresh kumar, P. Reddyprasad, B. Jayaraj and P. Chiranjeevi, J. Serb. Chem. Soc. 72(3) 299-310(2007).
40. H. zavvar Mousavi and H. Shirkhanloo, The Malaysian Journal of Analytical Sciences, Vol 12, No 2, 486-490.(2008)
41. S. E. Ghazy, R.M. E1-Shazly, M.S.E1-Shahawi, G.A.A. Al-Hazmi and A.A. E1-Aamy, Journal of the Iranian Chemical Society, Vol. 3, No. 2, pp.140-150.(2006)
42. Ogbonna O, Jimoh W.L, Awagu E.F. and Bamishaiye E.I. Advances in Applied Science Research, 2(2): 62-68.(2011)
43. Ruchi Dubey Sharma, Sulabha Amlathe j.chem. pharm.res.,4(2),1097-1105(2012)
44. Chan-il Park, Hyun –Soo Kim, and Ki-Won Cha. Bull.Korean Chem. Soc.20 (3),352-354(1999)
45. K.Ashokrao,S.Sivaramaiah,K.Sarasubudhi,D. Srivani,Y.Umamhaswari,G.V.Haritha, Chem Sci Trans., 1(3), 590-603.(2012)
46. Guidelines for drinking water quality, 2nded.v1.WHO Geneva (1993)
47. Washington, D.C, National Academy of science, 117.(1997).
48. PMN Prasad andYVR Reddy, Electronic news. Letter on renuwal energy and environment, 7(1), 9-16. (2010)
49. J.R.Turnlund, W.R.Keyes.H.Anderson.and L.L Accord, Am.J.Clin.Nutrition. 49, 870-878. (1989).
50. Minor and Trace elements in breast milk, WHO 9, Geneva.(1989).
51. Salmenpera.L. Perheentupa J and Simes M A, Am.J.of Clinical nutrition 43,251-257. (1986).
52. Under wood.e.J Trace elements in human and animal nutrition 4thedition Academicpress Newyork, 56-108. (1977).
53. Candian Soil Quality Guidelines for Copper Environmental and Human Health."Candian council of Ministers of the Environment. March 1997.
54. V.M.Shorrocks and B.J.Alloway, TN 35, 1988. Internet: www.Copper.info.co.uk