Research Article

Spectrophotometric Determination of Zn (II) in Milk, Blood Serum and in Natural Water Samples

D. Sree vani, S. Sivaramaiah and K. Ashok Rao*

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

ABSTRACT

Zinc is ubiquitous in the environment, flora and fauna. Nutritional zinc supplementation has been increasing, due to the recognization that zinc deficiency may play a role in number of disorders such as sickle cell disease acrodermatitas- entreopathica and some forms of mental illness. Being a type of trace element both in toxic and essential nature of zinc, there has considerable interest in the determination of its contents in different types of samples involved in vital activities of flora and fauna. Among the several analytical methods of determination of zinc, direct and derivative spectrophotometric method is most versatile. Hence in the present paper a simple rapid and sensitive spectrophotometric method was developed for the determination of zinc (II) in blood serum, milk and in natural water samples. Di amino di hydroxy pyrimidine [DADHP] as a selective complexing agent, forms the ripen –mango colour complex at P^H - 6 in acetic acid and sodium acetate buffer in the presence of pyridinium chloride as salting out agent. The maximum absorbance is observed at 480nm. The Beer's law is obeyed in the range 1-6 µg. The molar absorptivity and sandell's sensitivity of the complex is $0.1484x10^4$ lit $mol^{-1}Cm^{-1}$ and 0.04545 µg. cm^{-1} respectively.

Keywords: Blood serum, milk, natural water, UV &Visible spectrophotometer, DADHP.

INTRODUCTION

Understanding the effects of trace metals on human beings is very tedious, complex and it is fascinating. The high concentrations may prove toxic and depletion may cause various metabolic instabilities. In recent years awareness about the trace elements role, either beneficial or harmful in human health has been increased. The alteration of concentration of trace elements in some body fluids especially blood serum and plasma brings so many changes in human health.[1] Interest in trace elemental research in biology, environmental studies, toxicology, clinical medicine and nutrition has become an exciting frontier. Among the trace elements zinc is an essential element for human beings and different microorganisms. Most of the zinc (75-80%) is in erythrocytes the remaining is in plasma and leucosites.[2-3] Zinc is associated as activator in vital activities of flora and fauna by distributing in body fluids such as blood serum, milk and also a micro nutrient in several enzymes. Several methods are found in the literature for the determination of zinc in body fluids[4-12] and water samples.[13-14]

In the present investigation zinc was determined in blood serum, milk and natural water using Di amino di hydroxy pyrimidine as a selective complexing agent in the conditions already established in our earlier communication.[15]

Experimental part

Preparation of solutions

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

Zn (II)-solution:

Stock standard Zn (II) solution was prepared by dissolving 0.4397gm of Zn (II) sulphate hepta hydrate in double distilled water containing 1000µg/ml. The solution was standardized by complexometric titration using EDTA.

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, 0.1M sodium acetate in the pH range 2-8.

Solutions of diverse ions:

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

Ligand solution

The reagent stock solution (0.1M) was prepared by dissolving 1.421gms of DADHP in Ethylene glycol. This was diluted to the required concentration using Ethylene glycol.

INSTRUMENTS

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

Construction of calibration plot

To examine the applicability of Beer's law for the present system, the following procedure is adopted.

An aliquots of standard solutions containing microgram quantities of Zn(II) are taken in a series of ten 20 ml comparison tubes followed by the addition of 2 ml of pyridine and 1 ml of 2M HCl, the pH was adjusted to 6.0 using acetic acid and sodium acetate buffer. To this 5 ml of Di Amino Dihydroxy Pyrimidine ($3x10^{-3}$M) solution are added and the contents are finally made upto the mark with double distilled water. The absorbance of the solutions is measured at 480nm against the reagent blank prepared under identical conditions .It was found that Beer's law is obeyed in the range 0.5-6.0 µg /ml.

Further for the above solutions the derivative spectras were also recorded with group size 9 and degree of freedom 5 in the wave length range 400-600nm. The derivative peaks were measured by the peak zero method at respective wave length. The peak heights were plotted against the amount of zinc gives a linear plot indicating the applicability of Beer's law in the range 0.5-6.0 µg /ml. The performance of the calibration plot has been verified for ten replicate determinations.(Table.1.Fig.1)

Applications

The proposed method was applied for the quantification of Zinc in different samples. The sample solutions were obtained by following the recommended procedure.

Preparation of water samples

The water samples were collected from Pinakini river basin located at Nellore near by the holy place of Hindu's named as Ranganayakula temple one and half kilometers from our laboratories. Similarly the water samples were also collected from well's, municipal taps in and around the Nellore town and the water samples from Bay of Bengal near Krishna patnam shipyard. 150 ml of the sample were stored in metal free polyethylene bottles then filtered through What Mann filter paper No 41. 15 ml of this solution was further diluted to 100 ml to obtain the working solution.(Table.2.)

Preparation of Milk samples

Ten mothers who intended to feed for at least two years of post-partum with breast milk the interest and goals of the study are informed in detailed and their consult was obtained. The milk samples were also collected from the buffalo's, cows and from dairy forms in and around the Nellore town.

100 ml of milk was added drop wise to a heated crucible to evaporate with frothing then heated strongly for one hour to remove the moisture. The dark ash obtained was dissolved in the minimum of 1:1 Nitric acid and evaporated. The process was repeated for thrice finally by adding dilute Hydrochloric acid to dried mass and filtered. The filtrate was diluted to 100 ml, 2 ml of aliquots of the solution is used for the determination at the pH 6.0. (Table.3.)

Analysis of blood samples

A total of 5 ml of blood was collected in a sterilic plastic test tube with screw cap from people of different age groups of normal and diabetic patients in Nellore town with the help of Jaya Bharat hospital clinical laboratories. Approximately 5 ml of drawn blood taken into centrifuged tubes and allowed to stand for 30 minutes then centrifuged at 3000 RPM for about 10 minutes. The serum separated is decanted. The total serum was treated with 1 ml of 20% Tri Chloro Acetic Acid (TCA) for the deprotinisation, then the sample is allowed for quantification of zinc.

RESULTS AND DISCUSSION

The optimal conditions for the complexation of zinc(II) i.e., pH 6 of acetic acid and sodium acetate buffer at 480 nm wave length with Di Amino Dihydroxy Pyrimidine was already established in detailed in our earlier communication[15] are now utilized for the quantification of zinc.

In the present study the content of the zinc in Pinakini water, tap water and well water (ground water) was found to be 1-2 µg/ml i.e., 1-2 mg/lit which is exceeding the pollution threshold value and ecological threshold value (0.2-1 µg/ml).[16-17] However in the areas of Nellore town that is in the extended periphery (extension areas) where the ground water is used as drinking water are with the permissible limiting value (1 µg/ml). So in the central town area to which the Nellore municipality is supplying the Pinakini water for drinking purpose is advisable for compulsory reduce the zinc to pollution threshold value.

In the human breast milk the zinc was quantified as 2.4-2.5 µg/ml which is in good agreement with the reported values.[5,18]

Similarly in buffalo's, cow's and pasteurized milk was found 3.5-5.1 µg/ml. Milk represents the most suitable pattern of nutrients to need the physiological requirements in young infants. Hence an accurate and complete knowledge of the composition of the human milk and other sources of milk is essential and adequate for the growth of infants and also to develop adequately defined formulas to be used as a substitute for human milk.[20-21] So our work is alarming the usage of breast milk and synthesis of milk products for the growth of neonates and infants.

The zinc content in the blood sample of different aged people in normal health conditions and suffering with diabetic was found to be 2.8-4 µg/ml and 1.15-1.5 µg/ml respectively. These values clearly indicating the important role of zinc deficiency in diabetic patients.

The determination of the circulating levels of zinc in serum has been most widely used approach for the assessment of zinc nutrition, metabolic states, stress, infection, food intake shorter fasting and hormonal state all appear to influence.[22] The decreased zinc concentration in serum decreases its excretion from the breast milk by several mediators because mammary glands gets zinc from the blood[23]. Further the decrease zinc concentration causes the leukemia.[24]

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.

Table 1: Performance data for the calibration proposed method

Concentration Range (µg/ ml)	Least square equation Y = A + BX A = Intercept B = Slope	Correlation Coefficient (r)	Standard Deviation	RSD %	REP %	Amount Determined in Ten replicate measurements (µg / ml)
0.5 – 6.0	Y = -0.0114 + 0.0309x	1.1281	0.1400	4.4217	0.6378	3.3404,3.1262,3.0254,2.9630 3.2412,3.1502,3.2502,3.3215 2.9764,3.2676.

Table 2: Direct spectrophotometric determination

Sample	Amount of Zinc Spiked µg/ml	*Amount of Zinc found µg/ml	Recovery %	RMSEP	REP %	RSD %	t-test
Pinakini water	-	1.866	-	0.0520	6.706	2.787	0.1216
	4	5.7890	98.7	0.0916	0.0014	0.022	0.2387
Tap water	-	2.0200	-	0.0267	0.4964	1.3168	0.6538
	4	5.989	99.5	0.0266	0.09	0.3950	0.1071
Well water	-	1.0400	-	0.0271	0.9780	2.5702	0.8524
	4	4.9800	98.8	0.0286	0.0120	0.5741	0.0331
Sea water	-	1.028	-	0.0216	0.4116	0.0021	0.6712
	4	4.987	99.2	0.0015	0.0120	0.0300	0.6409
Derivative spectrophotometric determination							
Pinakini water	-	1.866	-	0.0520	6.706	2.7867	0.1216
1st Derivative	2	3.789	98.0	0.1083	0.7342	2.8578	0.0992
	4	5.723	97.6	0.0868	0.5076	1.5166	0.7286
2nd Derivative	2	3.799	98.3	0.773	0.6582	2.0347	0.0409
	4	5.856	99.8	0.0346	0.3594	0.5905	1.1881
3rd Derivative	2	3.801	98.3	0.0608	0.2106	1.5995	0.1560
	4	5.864	99.96	0.0453	0.6325	0.7725	1.0470
Tap water	-	2.02	-	0.0266	0.4964	1.3168	0.6538
1st Derivative	2	3.866	96.2	0.1329	0.4907	0.1101	0.3521
	4	5.954	98.9	0.1159	0.4383	1.9465	0.6002
2nd Derivative	2	3.984	99.1	0.0975	5.4604	2.4472	0.3243
	4	5.978	99.3	0.0935	0.2176	1.5640	0.1352
3rd Derivative	2	4.001	99.5	0.0425	0.6000	1.0622	0.0744
	4	5.997	99.6	0.0317	0.3675	0.5285	0.9975

Well water	-	1.04	-	0.0270	0.4116	2.5702	0.8524
1st Derivative	2	2.946	96.9	0.0497	0.9873	1.6963	0.7635
	4	4.896	97.1	0.0336	0.3311	0.6862	0.9411
2nd Derivative	2	2.998	98.6	0.0300	2.1235	1.0006	0.0695
	4	4.90	97.2	0.0523	0.6578	1.0739	1.8111
3rd Derivative	2	3.01	99.0	0.0760	0.9006	2.539	0.5825
	4	4.986	98.2	0.0683	0.6070	1.370	0.5539
Sea water	-	1.028	-	0.0216	0.4116	0.0021	0.6712
1st Derivative	2	2.846	93.9	0.0348	0.9873	1.2227	0.9086
	4	4.722	94.0	0.0505	0.3311	1.0705	0.7131
2nd Derivative	2	2.964	97.9	0.0510	2.1235	1.736	1.2874
	4	4.886	97.1	0.1149	0.6578	2.353	1.4861
3rd Derivative	2	3.001	99.1	0.0159	0.9006	0.5332	0.1783
	4	4.969	98.8	0.1806	0.6070	0.363	0.4722

Average of ten replicate determinations

Table 3: Direct spectrophotometric Determination

Sample	*Amount of Zinc found µg/ml	RMSEP	REP %	RSD %	t-test
Milk(Buffalo)	4.01	0.0495	2.4206	1.2344	5.4555
Milk(Cow)	4.29	0.07951	0.5010	1.8534	0.7556
Breast Milk	2.49	0.0555	0.06	2.2311	0.0161
Pasturised Milk	5.01	0.0488	1.5287	0.9738	0.0129
Derivative spectrophotometric determination					
Milk(Buffalo)	4.01	0.0495	2.4206	1.2344	5.4555
1st derivative	3.98	0.0886	0.0559	2.2261	0.6638
2nd derivative	3.76	0.0577	0.0224	1.5345	0.9974
3rd derivative	3.64	0.1106	1.166	0.9279	1.1665
Milk(Cow)	4.29	0.0751	0.5010	1.8534	0.7556
1st derivative	4.09	0.0102	0.5237	0.2493	1.364
2nd derivative	4.08	0.0168	0.9806	0.4117	4.0468
3rd derivative	4.12	0.0362	0.3257	0.8786	0.1933
Breast Milk	2.49	0.0555	0.06	2.2311	0.0161
1st derivative	2.32	0.0518	0.2287	2.2327	0.1953
2nd derivative	2.29	0.0488	0.2671	2.1310	0.3499
3rd derivative	2.48	0.0855	0.5703	3.4470	0.2958
Pasturised Milk	5.01	0.0488	1.5287	0.9738	0.0129
1st derivative	4.99	0.0670	0.1041	1.3417	0.0188
2nd derivative	5.12	0.0814	1.936	1.5892	0.7264
3rd derivative	5.14	0.0105	0.3638	0.2041	0.7227

Average of ten replicate determinations

Table 4: Direct & Derivative spectrophotometric Determination :(Normal)

Blood Sample	Amount of Zinc found(normal) µg/ml	RMSEP	REP %	RSD %	t-test
Sample[a]	3	0.0501	0.6339	1.6703	0.1893
1st derivative	3.12	0.0770	0.5466	2.4624	0.0128
2nd derivative	3.14	0.0325	0.9584	0.0103	0.3076
3rd derivative	3.15	0.0336	0.1249	1.0687	0.0522
Sample[b]	3.12	0.0544	0.9508	1.7441	0.0509
1st derivative	3.102	0.0591	0.00038	1.9071	0.0276
2nd derivative	3.15	0.0247	0.0635	0.7847	0.1408
3rd derivative	3.09	0.0958	0.3625	2.2291	1.9323
Sample[c]	4.01	0.0722	1.1908	0.1802	2.0392
1st derivative	3.98	0.0844	0.2543	2.122	0.3184
2nd derivative	3.02	0.0141	0.9138	0.4682	1.5275
3rd derivative	3.86	0.0536	0.7724	1.4005	1.6636
Sample[d]	3.98	1.1017	1.117	2.5813	1.1939
1st derivative	2.98	0.1393	0.6482	0.4675	0.0123
2nd derivative	2.87	0.0714	0.4613	2.4910	0.3804
3rd derivative	2.84	0.0700	1.4814	3.9753	1.0811

Average of ten replicate determinations

Table 5: Direct & Derivative spectrophotometric Determination : (Diabetic)

Blood Sample	Amount of Zinc found(Diabetic) µg/ml	RMSEP	REP %	RSD %	t-test
Sample[a]	1.49	0.0278	0.0061	0.0187	0.8758
1st derivative	1.46	0.0205	0.9208	1.4074	0.7543
2nd derivative	1.42	0.056	0.5879	3.948	1.0489
3rd derivative	1.39	0.0253	0.4465	0.0182	0.1999
Sample[b]	1.31	0.0448	0.6961	3.4198	0.4471
1st derivative	1.289	0.0312	0.2112	2.4258	1.0722
2nd derivative	1.26	0.026	1.5117	2.0662	0.7177
3rd derivative	1.204	0.0099	0.0052	0.8259	0.3426
Sample[c]	1.301	0.0202	0.3234	1.5584	0.1747
1st derivative	1.296	0.0120	1.1436	0.9273	1.2014
2nd derivative	1.286	0.0432	0.7627	3.3596	0.0878
3rd derivative	1.249	0.0309	0.0139	0.0247	0.1806
Sample[d]	1.19	0.0186	0.6590	1.5630	1.0880
1st derivative	1.107	0.1460	0.4074	1.3194	1.4251
2nd derivative	1.18	0.1545	0.3821	0.1309	0.0491
3rd derivative	1.15	0.0038	0.0870	0.3304	0.6657

Average of ten replicate determinations
a = 29-31 y , b = 40-42 y, c = 51-55 y, d = 62-65 y.

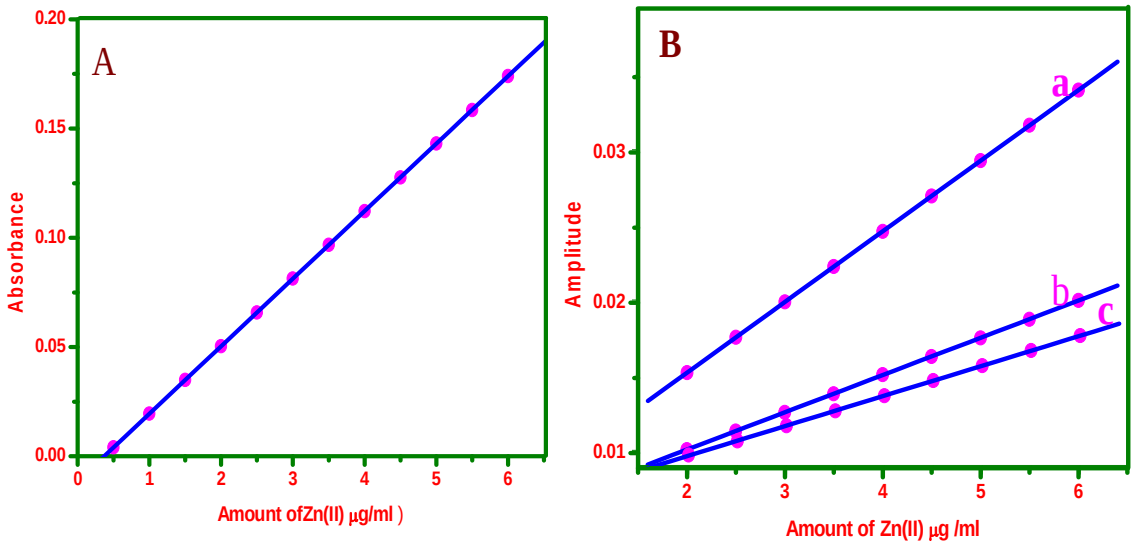

FIG.1. Calibration curve of Zn(II) obeying the Beer's law (0.5 -6.0 µg /ml)

(A)Directspectrophotomtry (B) (a)First (b) second (c) third Derivative spectrophotometry

Sample selected with help of Jaya Bharath Hospital clinical Laboratories

REFERENCES

1. Mc call JT, Gold stein NP and Smith LH. Fed Thoc. 1971;30:1011.
2. William DR. Computer Models of metal –Biochemistry and Metabolism in Chemical Toxicology and clinical chemistry of metals, Academic press: NY, 1983.
3. Fischer GL. Sci Total Environ. 1975;4:373.
4. Nagarjuna Reddy D, Vasudeva Reddy K and Hussain Reddy K. Der pharma chemical. 2011;3(2):496-504.
5. Ruchi Dubey Sharma and Sulbha Amlathe. Journal of chemical and Pharmaceutical Research. 2012;4(2):1097-1105.
6. Adesiyan AA,.Akiibinu MO, Olisekodiaka MJ, Onuegbu AJ and Adeyeye AD. Pakistan Journal of Nutrition. 2011;10(3):249-253.
7. Semaghiul Birghila, Simona Dobrinas, Gabriela stanciu and Alina soceanu. Environmental Engineering and Management Journal. 2008;7(6):805-808.

8. Shahnaz khaghani, Hamid Ezzatpanah, Najmeh Mazhari, Mohammad-Hadi Givianrad, Hossein Mirmiranpour and Fatemeh shahi sadrabadi, Iran J pediatr. 2010;20(1):53-57.
9. M. Sikiric, N. Brajenovic, I. Pavlovic, J.L.Havranek, N.Plavljanic. Czech J. Anim.Sci., 48, (11), 481-486.(2003).
10. Sapana Garg, Devender singh, Sonia verma and pratap singh kadyan. Journal of chemical, biological and physical sciences. 2012;2(4):1746-1752.
11. Adriana PR Silva, Marcia Regina vitolo, Luis Fabricio zara and Carlos Frederico S Castro. J pediatr (Rio J). 2006;82(3):227-331.
12. Rekha Dasari and Yugandhar Neelam. Eurasian J Anal chem. 2010;5(2):152-160.
13. KL MA. Analytical Sciences. 2001;17(4):561.
14. Valunth GVS, Chandra Sekhar KB and Deranna.N. Resarch J Pharmaceutical Biological and Chemical Sciences. 2010;1(3):739.
15. Sreevani D, Sarasubudhi K, Sivaramaiah S and Ashok Rao. K Chem Sci Trans. 2013;2(2): 513-523.
16. Georgiera N, Dosptalier L and Yaneva Z. Trakia Journal of Sciences. 2010;8(2):511-516.
17. Prasad PMN and Reddy YVR. Enree. 2010;7(1):9-17.
18. Krebs NF and Hambidge KM. Zinc requirements and zinc intakes of breast-fed infants. American Journal of Clinical Nutrition WHO (Geneva). 1986;43:288-292.
19. Ruth MF, Ronald R and Eitenmillar H. Am J Clin Nutr. 1983;37(3):443-48.
20. Al-Awadi FM and Sri Kumar TS. Nutrition. 2000;16(11-12):1069-73.
21. Casey CE and Neville MC, Hambidge KM. Am J Clin Nutr. 1989;49(5):773-785.
22. Hargovic M, Tessner CF and Thomas FB. Significance of Serum, Copper Levels in Adult Patients with Hogkin's disease. Cancer. 1973;31:1337-45.
23. Kelleher SL and Lonnerdal B. Mol Aspects Med. 2005;26(4-5):328-9.
24. Delves HT, Alexander FE and Lay H. Br J Haematol. 1973;24:525-33.