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Research Article

Spectrophotometeric Determination of Zinc (II) in Food-Stuffs and Biological Samples with Tris-[2, 4, 6-(2-Hydroxy-4-Sulpho-1-Naphthylazo)] -S-Triazine, Trisodium Salt

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ABSTRACT

Tris-[2,4,6-(2-hydroxy-4-sulpho-1-naphthylazo)]-s-triazine, tri sodium salt (THT) is proposed as a sensitive and selective reagent for the spectrophotometric determination of zinc(II). An addition of dilute solution of zinc ions to the aqueous solution of THT resulted to form water soluble dark brown complex in the pH range 5.5-7.4, absorbing maximum at 510 nm. The reaction between THT and zinc(II) is instantaneous and the absorbance remains stable for over 24 h. Beer's law is valid over concentration range 0.0-1.45 ppm with molar absorptivity and Sandell's sensitivity of $4.75X10^4$ l. mol^{-1} cm^{-1} and 0.00145 µg cm^{-2}, respectively. The molar composition of the complex is 1:1 (M:L) as determined by Job's method of continuous variation. The tolerance limits for interfering ions have been investigated. All variable have been studied in order to optimize the reaction conditions. The efficiency of the proposed method is shown by the successful determination of traces of zinc(II) in food stuffs and biological samples.

Keywords: Tris-[2,4,6-(2-hydroxy-4-sulpho-1-naphthylazo)]-s-triazine, tri sodium salt (THT), zinc(II), spectrophotometery

INTRODUCTION

Zinc, an essential element for all animals including human beings plays an important physiological role in human blood and is distributed 75-85 % in erythrocytes (mostly as carbonic anhydrase), 12 to 22% in plasma and 3% in leukocytes. One third of zinc in plasma is loosely bound to serum albumins, the reminder being more firmly attached to α-globulins, with minor fractions complexed in histidine and cysteine[1-3]. Zinc is associated with many enzyme systems, both as metallo-enzyme and enzyme activator, as well as filling a structural role. In addition, it plays a number of important biological roles such as with the synthesis of deoxyribonucleic acid (DNA) and ribosomal ribonucleic acid (RRNA). Zinc deficiency leads to impaired DNA synthesis, delayed wound healing and decrease in collagen synthesis. Deficiency of zinc leads to retarded growth, lower feed efficiency, inhibits the general well- being, causes ulcers, scaling of the skin, besides affecting the bones and joints. Less severe zinc deficiency has been linked to a low sperm count and infertility. Zinc deficiency during pregnancy may produce serious defects and foetal loss[4].

Although a little zinc is vital to health, too much is harmful; a single 220 mg zinc sulphate capsule can cause nausea and vomiting. Toxic effects may include abdominal pain, fever and also severe anemia resulting from eating acidic foods or drinking liquids that have been stored in galvanized containers. It is clear that zinc is an essential element and has significant importance, both biological and industrially. For the quantative determination of Zn(II) in trace amount, there are several frequently adopted methods such as atomic absorption spectrophotometery, X-ray fluorescence spectroscopy, spectroflurimetry, spectrophotometry etc. Among these, spectrophotometric methods are preferred as they are economical, easy to handle, with a comparable sensitivity and accuracy and good precision. It is one of the most commonly used techniques for routine analysis of metals.

This paper reports, a water soluble tris-[2,4,6-(2-hydroxyl-4-sulpho-1-naphthylazo)]-s-triazine trisodium salt (THT) as an analytical reagent for the microdetermination of zinc(II), whereas, a very limited number of heterocyclic azo dyes find their uses for the determination of zinc. Comparatively this reagent has been found to have fair sensitivity and high selectivity for zinc (II). Thus the reagent was utilized to determine zinc in biological samples and food stuffs.

EXPERIMENTAL

Apparatus: A Bausch and Lomb spectronic 2000 spectrophotometer with 10 mm matched glass cells was used for recording spectra and a Beckman pH meter was used for pH measurements.

Reagents: ***Tris-[2,4,6-(2-hydroxy-4-sulpho-1-naphthylazo)]-s-triazine, trisodium salt (THT) solution***: THT as synthesized earlier[5, 6] was used as a $1X10^{-3}$M solution prepared by dissolving 0.90 g in 1 L of double distilled water. Solutions more than a week old were discarded.

Zinc (II) solution: A stock solution of zinc(II) was prepared by dissolving an appropriate amount of zinc sulphate heptahydrate (AnalaR) in acidulated double distilled water. The solution was standardized complexometrically with EDTA[7].

Phosphate buffer, pH 6.4: A phosphate buffer of pH 6.4, was prepared by diluting 250 ml of 0.2 M potassium dihydrogen phosphate and 63 ml of 0.02 M sodium hydroxide to 1l with distilled water[7]. All other chemicals used were of analytical reagent grade.

General procedure: To a suitable aliquot containing 5-32.5 µg of zinc add 1 ml of $2.5X10^{-3}$ M THT solution followed by 2.0 ml of phosphate buffer and make up the volume to 25 ml with double distilled water. Measure the absorbance at 510 nm against a reagent blank prepared under similar conditions.

RESULTS AND DISCUSSION

THT is multi-dentate water soluble heterocyclic azo dyes (**Fig. 1**) and is very sensitive towards zinc(II) ions. The metal ion forms a dark brown colored complex with maximum absorbance at 510 nm (**Fig. 2).** The color development is maximum and constant at pH 5.5-7.4 (phosphate buffer). The complex formation is instantaneous and stable for 24 h. The complex had maximum color development when 2-molar excess of THT was used. However, in further studies, at least 5-fold molar excess of THT was maintained. The composition of the complex as determined by Job's method of continuous variation was 1:1 (M:L). Optimum conditions and other optical constant determined for Zn-THT complex are shown in **Table-1**. A comparative study of the sensitivities of various spectrophotometric reagents known for zinc is given in **Table-2**, shows that the present reagent has a good sensitivity for the microdetermination of zinc.

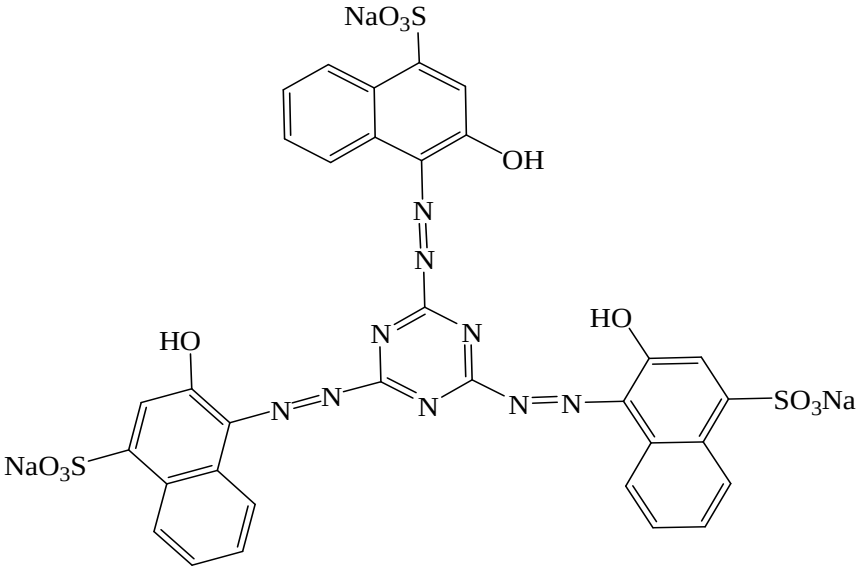

Fig.1: Chemical Structure of the Tris[2,4,6-(2-hydroxy-4-sulpho-1-naphthylazo)]-s–triazine, trisodium salt

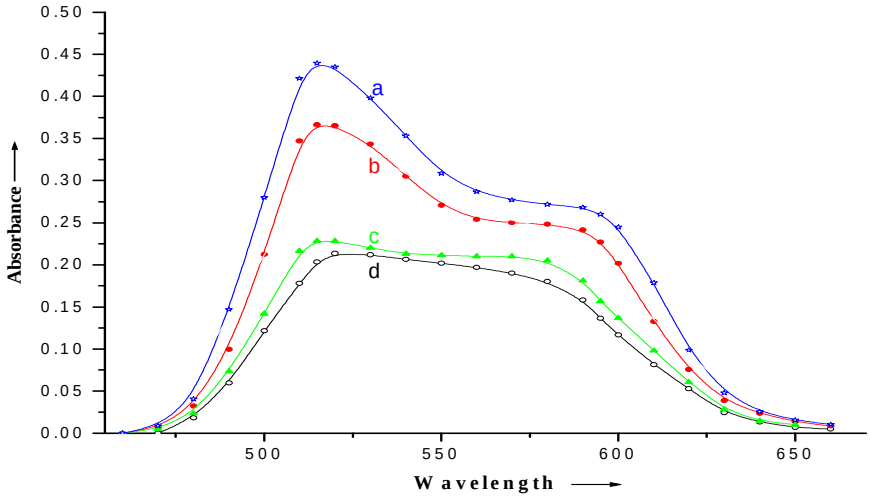

Fig.2: Absorption spectra of Zn(II)-THT complex at pH (a) 5.5-7.4 (b) 8.0 (c) 8.8 (d) 4.8. [Zn(II) = 8 X 10^{-6} M; THT = 4 X 10^{-5} M]

Table-1: Physico-chemical characteristics of zinc(II)-THT complex

Characteristics	Zn(II)-THT complex
λmax. (nm)	510
pH range	5.5 – 7.4
Reagent required for full complexation (mol.)	2
Beer's law range (ppm)	0.0 – 1.45
Optimum concentration range (ppm)	0.2 – 1.3
Sandell's sensitivity (µg cm^{-2})	0.00145
Molar absorptivity (ϵ) (l. $mol.^{-1}$ $cm.^{-1}$)	4.75 X 10^4
Composition (M : L) by Job's method	1 : 1

Table-2: Comparison of sensitivities of various spectrophotometric reagents for zinc (II).

Reagent	λmax (nm)	Molar absorptivity (l. $mol.^{-1}$ $cm.^{-1}$)	References
N-Ethyl-3-carbazole carboxaldehyde -3-thiosemicarbazone	420	1.55 X 10^4	14
Bis-[(2,6-(2'-hydroxyl-4'-sulpho-1'-naphthylazo)]pyridine disodium salt	565	4.6 X 10^4	15
2, 4 -Dihydroxybenzaldehyde isonicotinoyl hydrazone	390	3.55 X 10^4	16
Benzildithiosemicarbazone	395	0.42 X 10^4	17
7- (4-Nitrophenylazo)-8-hydroxyquinoline-5-sulfonic acid	520	3.75 X 10^4	18
5- Methylfuran-2-carboxaldehyde thiose-micarbazone	430	2.5 X 10^4	19
Xylenol orange and cetylpyridium chloride	580	1.1 X 10^4	20
3, 5-Dimethoxy -4-hydroxybenzaldehyde Isonicotinoyl hydrazone	473	2.42 X 10^4	21
2-Benzoylpyridine thiosemicarbazone	430	1.8 X 10^4	22
2, 6-Pyridinedicarboxyldehyde, phenylene-diamine	460	1.8 X 10^4	23
1, 3 -Cyclohexanedionedithiosemicarbazone	570	1.42 X 10^4	24
1, 2- Cyclohexanedionedithiosemicarbazone	415	0.73 X 10^4	25
Pyridoxal -4- phenyl-3-thiosemcarbazone	430	1.6 X 10^4	26
Tris[2,4,6-(2-hydroxy-4-sulpho-1-naphthylazo)] –s–triazine, trisodium salt	510	4.75 X 10^4	Present method

Effect of diverse ions: In the determination of zinc(II) at the 0.65 µg/ ml level, chloride, bromide, nitrate, fluoride, sulphate, sulphite, nitrite, iodide, thiosulphate, tartrate, borate, oxalate, thiourea, thiosemicarbazide, cyanide, phosphate, alkaline earths, lanthanides, aluminium(III), chromium(III), vanadium(v), molybdenum (VI), tungsten(VI), gold(III) and platinum metals did not interfere at all. However, EDTA was found to interfere seriously. Studies also show that fair amounts of cyanide, iodide,

thiosulphate, phosphate, arsenate and sulphide are tolerated and these can be used to mask a number of transition metals which interfere in the determination of zinc(II). **Table-3** represents the tolerance limits in ppm of various ions in solution that caused a deviation smaller than ± 2% in absorbance for the determination of zinc(II).

Table-3: Tolerance limits of diverse ions on the determination of 0.65 µg/ml of zinc(II).

Foreign ions	Tolerance limits(ppm)	Masking agents
S^{2-}	400	----------
CNS^-	1600	----------
Citrate	1600	----------
Cd(II)	10	masked by I^-
Hg(II)	25	" " " I^-, $S_2O_3^{2-}$ or T.U.
Mn(II)	8	" " " AsO_4^{3-}
Fe (II)	15	" " " CN^- or PO_4^{3-}
Co (II)	15	" " " CN^-
Ni (II)	15	" " " CN^-
Cu (II)	40	" " " I^- or $S_2O_3^{2-}$
Ag(I)	25	" " " I^- or CN^-
Pb(II)	40	" " " PO_4^{3-}, $S_2O_3^{2-}$ or I^-
In(III)	50	" " " S^{2-}
Bi(III)	50	" " " S^{2-}
Sb(III)	50	" " " S^{2-}
Th(IV)	25	" " " PO_4^{3-} or F^-
UO_2 (II)	25	" " " $(NH_4)_3AsO_4$

APPLICATIONS

Determination of zinc in food stuffs[8, 9]: Wet ash 5gm food sample (dried for one day in oven) with nitric and perchloric acids. Evaporate the ash twice with 5 ml portions of 10% hydrochloric acid. Dissolve the dry residue in water. Filter the solution into a flask; add 1 or 2 drops of concentrated HCl. Make up the volume to 25 mL. Analyse 1 mL aliquots as described above.

Determination of zinc in biological samples[10-13]:

(a). Body- tissues and eye-lenses: Wet ash 5gm sample with 1:1 nitric–perchloric acids. Evaporate slowly the acid solution to dryness and ash the residue at 300 ^{0}C. Dissolve the ash in 2 ml of 1M sulphuric acid, dilute to a suitable volume and determine zinc in this solution as already described.

***(b). Blood sera*:** Take 3 ml of serum, add 1.5 ml of N-HCl, mix and heat in a boiling water bath for 5 minutes. Cool, add 1.5 ml of 10% trichloroacetic acid, mix and centrifuge. Remove the supernatant liquid to a 10 ml volumetric flask, add 2 ml of N-HCl to the residue in the centrifuge tube, mix and re-centrifuge. Take out the supernatant liquid again in the volumetric flask and make up to 10 ml with N HCl and determine zinc contents as already described.

Results for these analyses are given in **Table 4**.

Table-4: Contents of Zinc in various Foodstuffs and Biological samples

Sample	Number of Analysis	Zinc found in whole Sample (µg)	Mean	Standard Deviation (σ)
Food Samples				
1. Cicer arietinum (Gram)	5	35.5, 36.5, 36.0, 37.0, 35.0	36.0	0.7071
2. Zea mays (Maize)	5	28.5, 29.0, 28.0, 29.5, 30.0	29.0	0.7071
3. Triticum aestivum (Wheat flour)	5	34.5, 33.5, 34.0, 35.0, 34.5	34.3	0.5099
4. Oryza sativa (Bran-rice)	4	90.25, 91.5, 89.0, 92.0	90.69	1.1642
5. Pennisetum typhoideum	4	54.0, 55.0, 54.5, 55.5	54.75	0.5590
Biological Samples				
1. Eye lens (Pooled) normal, wet.	4	3.5, 4.0, 4.5, 4.0	4.0	0.3535
2. Eye lens (Pooled) mature cataract wet.	4	8.5, 7.5, 8.0, 8.25	8.06	0.3697
3. Human blood sera (Normal)	5	3.0, 3.6, 3.2, 2.5, 3.5	3.16	0.3929

REFERENCES

1. D. R. William, Computer Models of Metal Biochemistry and Metabolism in Chemical Toxicology and Clinical Chemistry of Metals, Academic Press, New York, 1983.
2. G. L. Fisher, *Sci. Total Environ.*, 1975, **4**, 373.
3. E.L. Giroux, M. Durieux and P. J. Schechter, *Bioinorg. Chem.*, 1976, **5**, 211.
4. M. Hernick and C.A. Fierke, *Arch. Biochem. Biophys.*, 2005, **71**, 443.
5. I. Singh and P.S. Kadyan, *Analyst*, 1985, **110**, 809.
6. Ibid, Idem, 1987, **112**, 891.
7. A. I. Vogel, A Text Book of Quantitative Inorganic Analysis, 3rd Edn, Longman, London, 1975.
8. M.M. Barling and C.V. Banks, *Anal. Chem.*, 1964, **36**, 2359.
9. J.F. Goodwin and B. Murphy, *Clin. Chem.*, 1966, **12**, 58.
10. Z. Marczenko, Spectrophotometric Determination of Elements, Ellis Hordwood Limited, Chichester, 1976.
11. B. A. Gringas, R. L. Somozjai and C. H. Bayley, *Can. J. Chem.*, 1961, **39**, 973.

12. E. B. Sandell and H. Onishi, Photometric Determination of Traces of Metals, Part 1, 4th Edn, Wiley, New York, 1978.
13. R. Bock, A Handbook of Decomposition Methods in Analytical Chemistry, International Text Book Company, Blackie group, Glassgow, 1979.
14. K. J. Reddy, J. R. Kumar, C. Ramachandraiah, T. Thriveni and A.V. Reddy, *Food Chemistry*, 2007, **101(2)**, 585.
15. B. Barman and S. Barua, *Arch. Appl. Sci. Res.*, 2009, **1(1)**, 74.
16. S. Sivaramaiah and P.R. Reddy, *J. Anal. Chem.*, 2005, **60(9)**, 933.
17. B. K. Reddy, J. R. Kumar, L. S. Sarma and A.V. Reddy, *Anal. Lett.*, 2002, **35(8)**, 1415.
18. M.G.A. Korn, A.C. Ferreira, L.S.G. Teixera and A.C.S. Costa, *J. Braz. Chem. Soc.*, 1999, **10**, 46.
19. D. N. Reddy, K.V. Reddy and K. H. Reddy, *Der Pharma Chemica*, 2011, **3(2)**, 496.
20. M. Benamor, K. Belhamel, and M. T. Draa, *J. Pharm. Biomed. Anal.*, 2000, **23**, 257.
21. G.V.S. Vallinath, K. B. Chandra Sekhar and N. Devanna, *Res. J. Pharm. Bio. and Chem. Sci.*, 2010, **1(3)**, 739.
22. D. Reddy K.V. Reddy and K.H. Reddy, *J. Chem. Pharm. Res.*, 2011, **3(3)**, 205.
23. M. D. Fakruddin Ali Ahmed and Y. Lingappa, *International Journal of Current Res.*, 2011, **3(3)**, 24.
24. B. Barman and S. Barua, *Asian J. Chem.*, 2009, **21(7)**, 5469.
25. B. K. Reddy, J.R. Kumar, L.S. Sarma and A.V. Reddy, *Anal. Lett.*, 2002, **35**, 1415.
26. L. Sarma, J. R. Kumar, K. J. Reddy, T. Triveni and A.V. Reddy, *J. Braz. Chem. Soc.*, 2006, **17**, 463.

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