Spectrophotometric determination of zinc in blood serum of diabetic patients using bis-[2,6-(2´-hydroxy-4´-sulpho-1´-napthylazo)]pyridine disodium salt

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Abstract

Bis-[2,6-(2´-hydroxy-4´-sulpho-1´-napthylazo)]pyridine disodium salt (HSNP), a new heterocyclic azo dye is proposed as a sensitive spectrophotometric reagent for zinc. A selective spectrophotometric method is presented for the trace determination of zinc in blood serum using HSNP as spectrophotometric reagent (λ_{max} = 565 nm) in basic aqueous solution (pH range = 7.5 to 9.1). The HSNP forms a 1:1 purple coloured complex. The Sandell's Sensitivity is 1.37×10^{-3} dm^3 mol^{-1} cm^{-2} with molar absorptivity 4.6×10^4 mol L^{-1} cm^{-1}. The proposed method has been successfully applied to the determination of zinc in blood samples of diabetic patients. The precision and the accuracy obtained were satisfactory.

Key words: Bis-[2, 6-(2´-hydroxy-4´-sulpho-1´-napthylazo)] pyridine di sodium salt, zinc, blood serum, trichloro acetic acid

Introduction

Zinc is an essential element for all living animals including the human being and different microorganisms and it plays an important physiological role. In normal human blood, zinc is distributed 75-85% in erythrocytes and carbonic anhydrase, 12-22 % in plasma and almost 3% in leukocytes. One third of zinc in plasma is loosely bound to serum albumins and the remaining part being more firmly attached to α-globulins with minor fractions complexed in histidine and cysteine [1,2].

Zinc is associated with many enzyme system, both as metalo enzyme and enzyme activator. Zinc deficiency leads to impaired DNA synthesis , delayed wound healing and decrease in collagen synthesis. Deficiency of zinc is also leads to retarded growth, lower feed efficiency, inhibits the general well being, causes ulcers, scaling of the skin and affect the bones and joints. . Zinc deficiency during pregnancy may produce serious defects and foetal loss.

The excess amount of zinc can cause nausea and vomiting. Toxic effects, which may also include abdominal pain, fever and severe anemia can result from eating acidic foods or drinking liquids that have stored in galvanized containers [3,4].

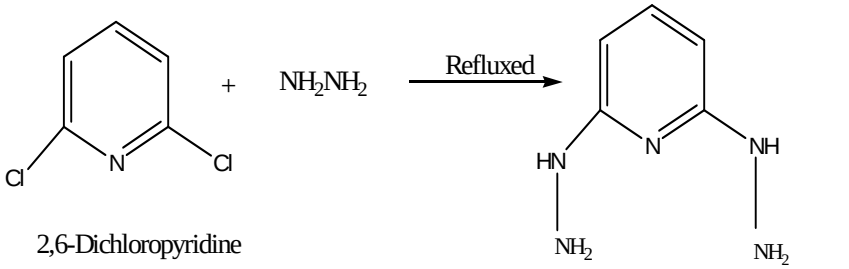
Zinc (II) has been determined spectrophotometrically by using different heterocyclic azo dyes.A new heterocyclic azo dye, Bis-[2,6-(2´-hydroxy-4´-sulpho-1´-napthylazo)] pyridine disodium salt, HSNP has been synthesized by *barman et al*. [5] and has been successfully used for spectrophotometric determination of nickel[5] and zinc in pharmaceutical samples [6].
The review of literatures indicates that not much attention has been paid for the spectrophotometric determination of zinc (II) in biological samples . This has promoted the researchers to make a systematic investigation for utilizing bis-[2,6-(2′-hydroxy-4′-sulpho-1′-napthylazo)]pyridine disodium salt for the first time for spectrophotometric determination of zinc (II) in ppm level. The established method is successfully applied for the spectrophotometric determination of zinc (II) in human blood serum of diabetic patients. The proposed method when compared with other spectrophotometric methods was found to be quite sensitive and selective. It also offers advantages like realiability and reproducibility in addition to its simplicity, instant colour development and less interference.

Materials and Methods

Synthesis and characterization of the reagent

(a) Synthesis of Hydrazide of 2,6-dichloropyridine

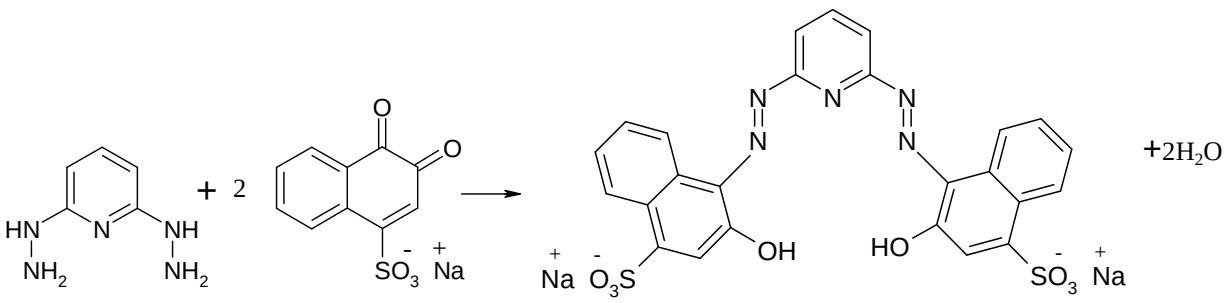
2,6-Dichloropyridine (Fluka) (0.05 mol) was refluxed with excess of 99% Hydrazine Hydrate (Qualigens) for two hours to get the corresponding hydrazide.



2,6-Dichloropyridine

Hydrazide of 2,6-Dichloropyridine

(b) The hydrazide of 2,6-Dichloropyridine (0.025 mol) was dissolved in minimum volume of dilute hydrochloric acid and coupling was done with aqueous solution of 1,2-Napthaquinone-4-sulphonic acid sodium salt (0.05 mol). A red precipitate appears, filtered and dried.



bis-[2,6-(2′-hydroxy-4′-sulpho 1′-naphthylazo)]pyridine disodium salt

The structure of HSNP was confirmed by means of IR spectrometry. The reagent was a dark red powder and highly soluble in water. The IR spectra of HSNP in KBr, recorded in FTIR-8400S Fourier Transform Infrared Spectrophotometer (Shimadzu). The IR-spectra of the ligand showed the following results:

ν_{-OH} bonded = 3510 cm^{-1} ; $\nu_{-N=N-}$ stretching = 1600 cm^{-1}; ν_{C-O} stretching = 1130 cm^{-1}

The above facts suggest that in solid state HSNP exist in the enol form.

Apparatus

The absorbance was measured by UV-1700 Pharmaspec UV-Visible Spectrophotometer (Shimadzu) and UV-Visible Spetrophotometer 108 (Systronics) with 10 mm quartz cells. The pH measurements were carried out with an Elico LI 120 digital pH meter.

Reagents

All reagents used were of analytical grade unless otherwise stated.

Buffer Solutions:

Borax buffer solution (pH=8.5) was prepared by the standard method for the pH adjustments.

Stock Solution of Zn(II)

A stock solution of zinc (II) was prepared by dissolving appropriate amount of zinc sulphate, $ZnSO_4.7H_2O$ (Analar, B.D.H.) in deionised doubly distilled water and was standardized complexometrically. Subsequent dilutions were made as and when required.

Recommended procedure

HSNP forms complex with Zn (II) in different pH ranges. A series of solutions containing 1.0 mL of 1×10^{-4} M Zn (II) and excess of the reagent (1.0 mL of 1.0×10^{-3} M) were made and pHs were adjusted at different levels in a total volume of 10 mL. It was found that only one complex is formed (λ_{max} = 565 nm) at all pH values (Fig.1). Plots of pH versus absorbance at λ_{max} value show that constant absorbance is exhibited in the pH range 7.5 – 9.1(Fig. 2).

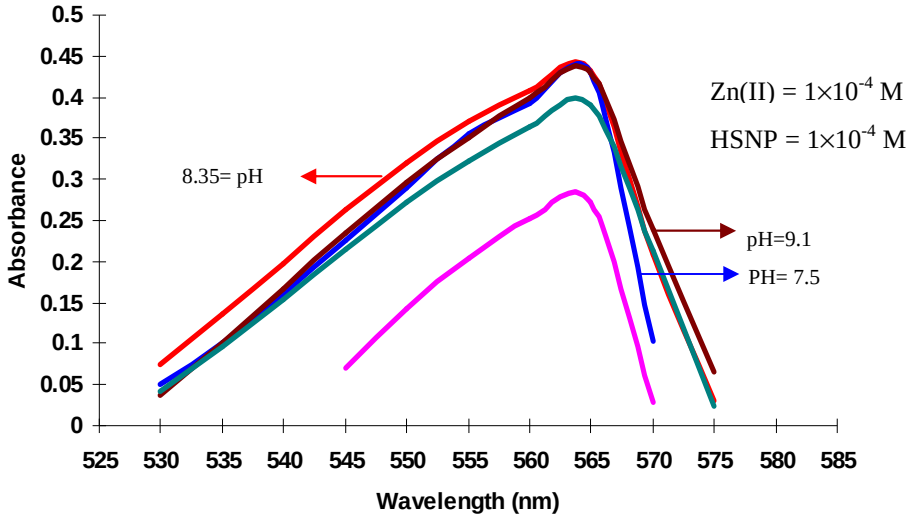


Fig. 1. Absorption Spectra of Zn(II)-HSNP Complex at different

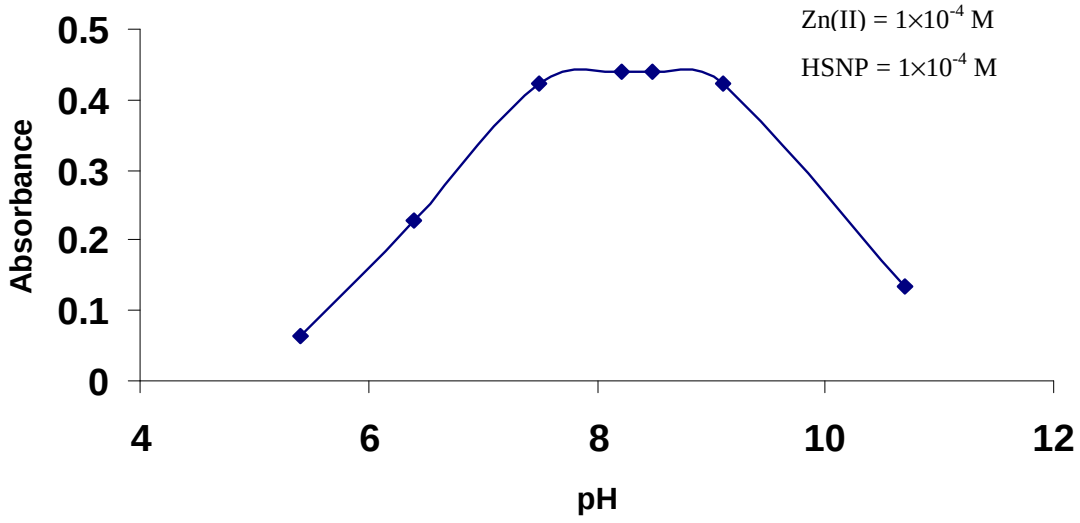


Fig. 2. Effect of pH on Zn (II)-HSNP Complex

Results and Discussion

Effect of reagent concentration and stability of the complex:
A series of solutions containing a fixed amount of the metal ion (of 1×10^{-4} M) and varying amounts of the reagent at appropriate pH value (8.3) was prepared. After making up the volume to 10 mL absorbance was measured at 565 nm. From the result (Fig.3), it is seen that at least five (05) times molar excess of HSNP is required for complete complexation. In subsequent studies, 10 times molar excess of the reagent was used.

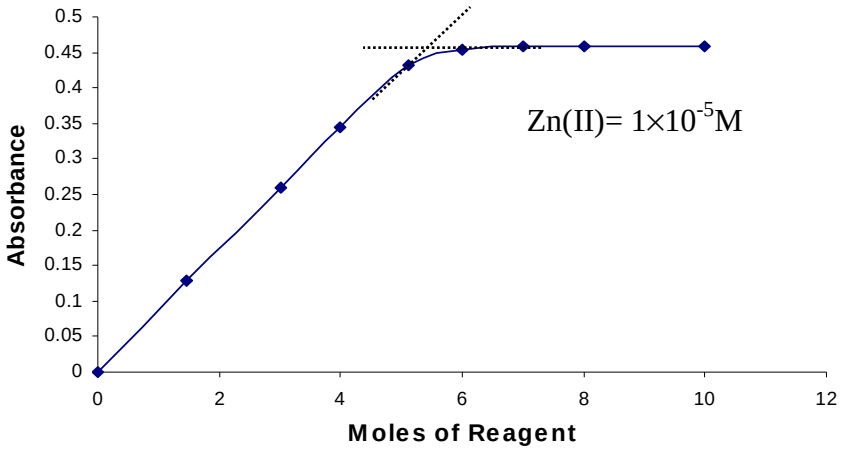


Fig. 3. Effect of Reagent Concentration on Zn (II)-HSNP

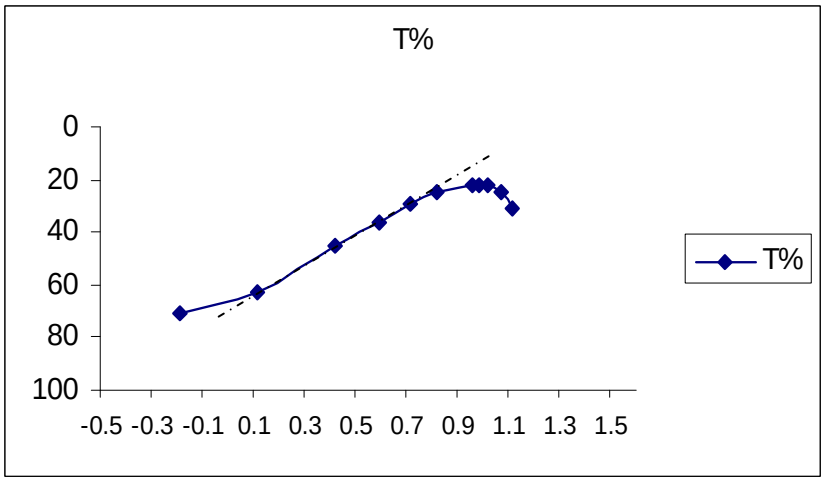


Fig. 4. Ringbom Plot for Zn(II) – HSNP Complex

Physico-chemical characteristics of the Zn(II)-HSNP complex

Linearity between the absorbance of the complex and Zn (II) concentration was examined by varying the concentration of Zn (II) in the solutions containing a fixed amount of the ligand solution at pH 8.3 and measuring the absorbance at 565 nm against the corresponding reagent blank. The result obtained for the validity of Beer's law, optimum range of concentration for accurate determination as calculated from Ringbom's plot (Fig.4), sensitivity and molar extinction coefficient are summarized in the following Table1.

Table-1: Physico-chemical characteristics of the Zn (II)-HSNP complex

01	λ_{max}	565(nm)
02	Beer's law validity	0.00- 1.12 (ppm)
03	Optimum concentration range	0.1 – 0.8 (ppm)
04	Sandell's sensitivity	0.00137 (μg Zn(II) cm^{-2})
05	Molar extinction coefficient (Є)	4.3×10^4 (1 mol^{-1} cm^{-1})

Molar composition of the complex

The stoichiometry of the complex was ascertained by making use of Job's method of continous variations. The curve obtained by plotting absorbance versus mole fraction of the metal ions at 565 nm and pH 8.3 shows that the metal to ligand ratio in the complex is 1:1. (Fig.5)

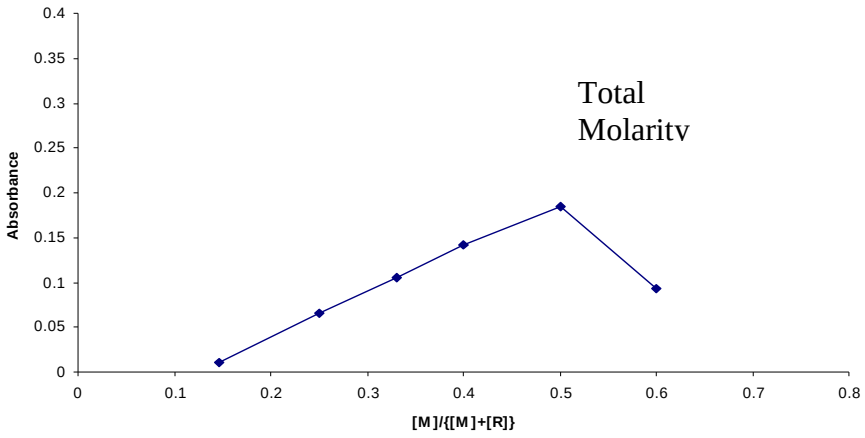


Fig.5. Composition of Zn (II)-HSNP complex by Job's Method

Effect of diverse ions

Effect of diverse ions in the determination of Zinc has been studied by preparing synthetic solutions containing 0.653 μg / mL of Zinc (II) and different amounts of diverse ions. The zinc

content in these mixtures was determined by following the recommended procedure. The amounts in ppm of various ions, given in parentheses, did not cause deviations of more than ± 2% in absorbance. It has been found that fluoride, chloride, bromide, iodide, nitrate, borate, sulphate phosphate (50ppm), thiocyanate (50 ppm), citrate (50 ppm), acetate (80 ppm); silver (I) (20ppm), magnesium (II), calcium (II), barium (II), strontium (II)(20ppm each), aluminium(III)(50 ppm), cobalt(II)(20 ppm), mercury(II)(50 ppm), copper(II)(50 ppm) did not interfere. However, sulphide, thiosulphate, thiourea, cyanide and EDTA interfere seriously.

Sensitivities of various reagents for the spectrophotometric determination of zinc (II)
The proposed method when compared with other reported spectrophotometric methods [7-15] was found to be more sensitive and selective (Table 2).

Determination of Zinc in Blood Serum of Diabetic Patients

Blood Samples
Fresh blood samples of Diabetic patients were collected from D'LARC (Danish Laboratory and Research Center), a pathological clinic, Guwahati, Assam, India. Prior to the collection of the blood samples the patients did not take any vitamin supplements containing zinc sulphate, $ZnSO_4$ as a component.

Separation of blood serum from blood
Place approximately 5.0 mL of freshly drawn blood in a 13 ×100 mm centrifuge tube and allow it to stand for about 30 minutes at room temperature. Centrifuge at 2500 rpm for about 10 minute and decant off the clear serum.

Recommended procedure for determination of zinc
Take 1.0 mL of serum in a 13 ×100 mm centrifuge tube, add 1.0 mL of 20% TCA(Trichloro Acetic Acid). Allow it to stand for ≈ 15 minutes and then centrifuge. To 1.0 mL of the supernatant liquid add 1.0 mL of 1×10^{-3} M HSNP solution and dilute to 10.0 mL adjusting the pH to 8.3. Record the absorbance at 565 nm against a reagent blank. Calculate the amount of zinc from a standard calibration curve.
The results are summarized in Table.3 & 4.

Table2. Comparison of present method with other reported spectrophotometric methods

Reagent	λ_{max}	pH	M: L	Molar Absorptivity ($Lmol^{-1}cm^{-1}$)$\times 10^4$	Linear Range (mg L^{-1})	Remarks	Reference
Pyridoxal-4-phenyl -3-thio-semicarbazone	430	5.5	1:1	1.60	1.0-18.0	Highly sensitive	3
7-(4-nitrophenylazo)-8-hydroxyquinoline-5-sulphonic acid	520	9.2	1:2	3.75	0.05-1.0	Cu(II), Ni(II), Co(II), Cd(II), Fe(III) & Fe(II)	4
Benzildithiosemicarbazone	395	9.5	1:1	0.42	1.0-18.0	Cu(II), Ni(II), Co(II), Pb(II), Mn(II), Ag(I)	5
1,3-Cyclohexandionedithiosemicarbazone	570	6.3	N.R.	1.42	N.R.	Less sensitive	6
1,2-Cyclohexandionedithiosemicarbazone	415	1.0-6.6	1:2	0.73	N.R.	Hg(II), Cu(II), Cd(II), Fe(II), Ni(II), Co(II)	7
Glyoxaldithiosemicarbazone	433	9.0-11.0	1:1	1.3	N.R.	Less sensitive	8
Methylglyoxal bis (4-phenyl-3-thiosemicarbazone)	445	6.0-8.5	1:1	0.21	0.2-0.4	Less sensitive	9
Bis-[2,6-(2'-hydroxy-4'-sulpho-1'-napthylazo)] pyridine	565	7.5-9.1	1:1	4.3	0.1-0.8	Highly selective and sensitive	P.M.

Table-3: Amount of Zn (II) in blood serum of Male Diabetic Patients

Sl. No.	Age (in years)	Gender	Amount of Zn (µg / mL)
01	70	Male	0.001555
02	70	Male	0.001385
03	58	Male	0.001052
04	56	Male	0.001396
05	50	Male	0.000966
06	50	Male	0.0009477
07	49	Male	0.001380
07	48	Male	0.0009566
08	46	Male	0.0015431
09	46	Male	0.0009781
10	43	Male	0.0009872

Table-4: Amount of Zn (II) in blood serum of Female Diabetic Patients

Sl. No.	Age	Gender	Amount of Zn (µg / mL)
01	77	Female	0.001545
02	70	Female	0.001170
03	67	Female	0.001035
04	64	Female	0.001165
05	62	Female	0.0009871
06	62	Female	0.000982
07	58	Female	0.001090
08	50	Female	0.001038
09	43	Female	0.001433
10	35	Female	0.001418

Conclusion

The p[resent method spectrophotometric determination of zinc by using bis-[2,6-(2´-hydroxy-4´-sulpho-1´-napthylazo)]pyridine disodium salt (HSNP) has been found to be

quite sensitive and can be made selective. The reagent is highly water soluble and its aqueous solutions are also highly stable. The dye compared well with other heterocyclic azo dyes in its sensitivity. The determination of zinc in blood samples by the proposed method is quite simple and is not time consuming. It has been observed that the average value of zinc is same in blood samples of both male and female diabetic patients. The maximum and minimum zinc found in the blood serum of diabetic patients are 0.001555µg /mL and 0.0009662 µg /mL respectively and also found that the amount of zinc (II) in the blood serum of diabetic patients are almost same as found in blood serum of normal human being. Thus HSNP can be used as a sensitive spectrophotometric reagents in the determination of metal ions in various biological samples.

References

1. William, D. R.; *Computer Models of Metal Bio-chemistry and Metabolism in Chemical Toxicology and Clinical Chemistry of Metals,* Academic Press: NY, 1983.
2. Fisher, G.L.; *Sci. Total Environ*. **1975**, *4*, 373.
3. Sarma, L. Subramanyam; Kumar, J. Rajesh; Reddy, K. Janardhan, Triveni, T.; Reddy, A. Varada; *J. Braz. Chem. Soc.* **2006**,17, 463
4. Korn, M.G.A.; Ferreira, A.C.; Teixera, L.S.G.; Costa, A.C.S.; *J. Braz. Chem. Soc.***1999**,10, 46
5. Barman, Banjit and Barua, Sudarsan; *Asian Journal of Chemistry*, **2009**, 21(7), 5469-5474.
6. Barman, Banjit and Barua, Sudarsan; *Proceedings, 53rd Annual Technical Session*, Assam Science Society, *2008***,** 9.
7. Reddy, B.K.; Kumar, J.R.; Sarma, L.S.; Reddy, A.V.; *Anal. Lett*. **2002**, *35*, 1415.
8. Nevado, J.J.B.; Leyva, J.A.M.; Ceba, M.R.;*Talanta* **1976**, *23*, 257.
9. Leyva, J.A.M.; Pavon, J.M.C.; Pino, F.; *Inform. Quim. Anal*. **1972**, *26*, 226.
10. Hoshi, S.; Yotsuyanagi, T.; Aomura, K; *Bunseki Kogaku* **1977**,*26*, 592.
11. Herrador, M.A.; Jimenez, A. M.; Asuero, A.G.; *Analyst* **1987**, *112*, 1237.
12. Reddy, K.J.; Kumar, J.R.; Thriveni, R.T.; Reddy, A.V.; *Food Chemistry* **2007**, *101*, 585.
13. Vogel, A. I.; *A Text Book of Quantitative Inorganic Analysis*, Longman Green: London, **1961**.
14 .Sandell, E. B.; *Colorimetric Determination of Traces of Metals*, Interscience, New York, **1959**.
15. Marczenko, Z.; *Spectrophotometric Determination of Elements*, John Wiley, New York, **1976**.