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A Rapid Spectrophotometric Method for Trace Determination of Zinc

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Abstract A simple, sensitive, and highly selective method is proposed for the determination of zinc(II) using a bis-azo dye, 2,6-bis(1-hydroxy-2-naphthylazo)pyridine as spectrophotometric reagent. At pH7.8, in 50% (*v*/*v*) ethanol–water medium, the complex is found to obey Beer's law up to 1.3 mg/L with an optimum concentration range between 0.19 and 1.0 mg/L. Sandell's sensitivity of the color reaction was calculated to be 0.0011 μg cm^{-2} with molar absorptivity of 6.0×10^4 L mol^{-1} cm^{-1} at 560 nm. The optimum conditions for the determination of Zn(II) with the reagent were ascertained. The complexation at different pH was studied in water–ethanol medium. The composition of the complex is 1:2. The action of some interfering ions was verified, and the developed method applied successfully for the estimation of zinc levels in food and milk samples, and the results were then compared with those obtained by using AAS.

Keywords Chromogenic · Zinc determination · Spectrophotometry · 2,6-bis(1-hydroxy-2-naphthylazo) pyridine (PBN)

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Introduction

Zinc is the most ubiquitous of all trace elements involved in human metabolism. More than 100 specific enzymes require zinc for their catalytic function. Zinc is released from food as free ions during digestion. These liberated ions may then bind to endogenously secreted ligands before their transport into the enterocytes in the duodenum and jejunum (Cousins 1996). The International Zinc Nutrition Consultative Group (IZiNCG) concurs with the upper limit of 40 mg zinc/day for adults by Food and Nutrition Board, but the upper limits proposed for young children are considered to be low. However, there is lack of adequate data to better define the upper limits for children. It is apparent that a large proportion of US children have usual zinc intakes greater than the proposed upper limit (Briefel et al. 2000). IZiNCG reviewed the data available from two recent supplementation studies in children (Bhandari et al. 2002; Lind et al. 2003). In the study conducted in India, children between 6 and 12 months of age received 10 mg zinc/day and those between 1 and 2 years of age received 20 mg/day Bhandari et al. 2002. Zinc occurs in a wide variety of foods but is found in highest concentrations in animal sources, particularly beef, pork, poultry, and fish and in lesser amounts in eggs and dairy products. Zinc content is relatively high in nuts, legumes, and whole grain cereals and is lower in fruits and vegetables. Individuals may be exposed to high intakes of zinc, either through supplemental zinc or by contact with environmental zinc. Overt toxicity symptoms, such as nausea, vomiting, epigastric pain, diarrhea, and lethargy may occur with acute high intakes (Fosmire 1990). Zinc compounds are known to have biocidal activity because they precipitate and denature the bacterial proteins. For this reason, it has been used in dermatology as an antiseptic and disinfectant,

in ophthalmic solutions, mouthwashes, and mineral-vitamin preparations (Matíes et al. 1997).

Heterocyclic azo dyes have been used as chromogens in spectrophotometric determination of metal ions. In our laboratory, the chromogens such as 2-(4,6-dimethyl-2-pyrimidylazo)-1-naphthol-4-sulfonic acid, 2-(4,6-diamino-4-pyrimidylazo)-phenol, 2,6-bis(7-hydroxyacenaphthyl-8-azo) pyridine, and 2,6-bis(1-hydroxy-2-naphthylazo)pyridine have been synthesized and used (Sharma and Singh 2008; Singh and Sushma 2000; Singh et al. 2000, 2003, 2006). In this paper, the application of the already reported bis-azo dye, 2,6-bis(1-hydroxy-2-naphthylazo)pyridine (PBN; Sharma and Singh 2008; Singh et al. 2006) using the Anderson and Nickless method (Anderson and Nickless 1967, 1968) and its complexation reaction with zinc(II) in ethanol–water medium was spectrophotometrically studied as an alternative method for the zinc determination. The method that showed a good sensitivity and high selectivity for zinc(II) ions as most of the common metal ions generally found associated with zinc do not interfere. The repeatability of the method was checked by finding relative standard deviation. Keeping in view the biological importance of zinc, the proposed method was evaluated for the determination of zinc in cereals, legume grains, milk, and tea samples commonly consumed by Indian vegetarians. This developed method is proven to be sensitive when compared to some other recently reported methods (Table 1).

Experimental

Reagents

PBN PBN having enol form structure was prepared by the method already reported (Sharma and Singh 2008; Singh et al. 2006).

A 5×10^{-4} mol/L reagent solution was prepared by dissolving 0.2092 g of PBN in 1-L ethanol.

Zinc(II) solution 0.01 mol/L stock solution of zinc(II) was prepared by dissolving calculated amount of zinc sulfate heptahydrate in deionized water, standardized complexometrically with EDTA. Working solutions of zinc were prepared by dilution of the stock solution with water.

Borate buffer solution (pH7.8) (Thomas and Chamberlin 1980) The buffer was prepared by diluting the solution containing 250 mL of 12.369 g of boric acid and 14.911 g of potassium chloride per liter and 13.25 mL of 0.2 mol/L sodium hydroxide to 1 L with distilled water.

Unless otherwise stated all reagents were of analytical-reagent grade.

Instruments

A Beckman spectrophotometer (PC based) with 10-mm matched glass cells was used for recording the spectra. An Elico pH-meter (model L1 614) was used for making pH adjustments. Atomic absorption spectrophotometer (AAS) ECIL model 4129 (PC-based) was used to analyze the samples for zinc.

Processing of Samples (Sandell and Onishi 1978; Thomas and Chamberlin 1980)

Milk samples Fresh samples of cow, buffalo, goat, and other samples were collected from milk booths and milk men of the university area. The zinc content of milk has been determined by the conventional dry ashing procedure; 100 mL of milk is pipetted into a heated crucible to evaporate without frothing. After the moisture has been

Table 1 Comparison of the proposed method with the other spectrophotometric methods for the determination of zinc(II)

Reagent	pH	λmax nm	Molar absorptivity L mol^{-1} cm^{-1}	Reference
2,6 bis(1-hydroxy-2-naphthylazo) pyridine (PBN)	7.8	560	6.0×10^4	Present method
di-2-pyridyl ketone salicyloyl hydrazone (DPKSH)	4.5	376	4.80×10^4	(Gaubeur et al. 2002)
7-(4-nitrophenylazo)-8-hydroxy quinoline-5-sulfonic acid (p-NIAZOXS)	9.2	430	3.75×10^4	(Korn et al. 1999)
biacetyl mono(2-pyridyl)hydrazone (BPH)	10	440	5.20×10^4	(Daniel et al. 1985)
4-(2-pyridylazo)resorcinol	8.5–10.3	410	9.40×10^4	(Chakravarty and Mishra 1993)
di-2-pyridyl ketone benzoylhydrazone (DPKBH)	5.5	–	3.60×10^4	(Vieira et al. 2008)
N-ethyl-3-carbazolecarboxaldehyde-3-thiosemicarbazone (ECCT)	3.0–5.5	420	1.50×10^4	(Reddy et al. 2007)
Benzildithiosemicarbazone (BDTSC)	–	405	0.42×10^4	(Reddy et al. 2002)
2,4-dihydroxybenzaldehyde iso nicotinoyl hydrazone (2,4-DHBINH)	6–8	390	3.50×10^4	(Sivaramaiah and Reddy 2005)

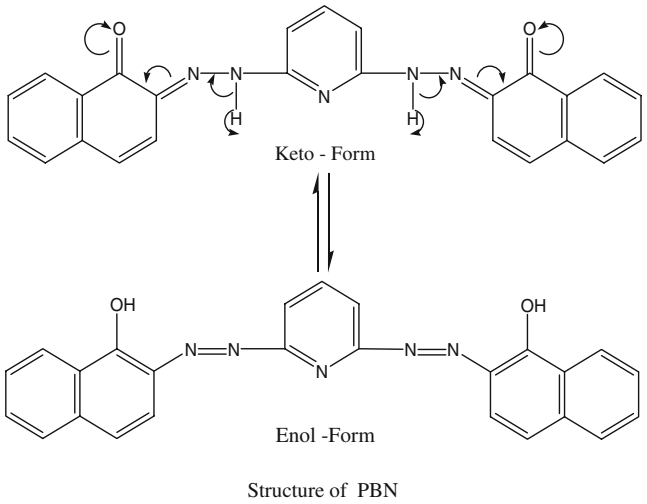

Fig. 1 Structure 2,6 bis(1-hydroxy-2-naphthylazo)pyridine (PBN)

removed, the residue is heated strongly to 450–500 °C for ~1 h. Utmost care was taken to avoid the loss by sputtering. The white ash obtained was dissolved in minimum volume of diluted nitric acid and volume was made up to 25 mL in a volumetric flask; 1–2 mL of the solution is pipetted out, the excess nitric acid is neutralized with dilute sodium hydroxide solution and the zinc content is determined following the recommended procedure.

Foodstuffs Five grams of food grains (dried for ~24 h at 70 °C in an oven) was wet-ashed with nitric and perchloric acids; 5 ml of hydrochloric acid (1+9) was added to the ash and evaporated to dryness. This step was repeated. The dry residue was dissolved in water and filtered into a 25-mL standard flask; one or two drops of conc. HCl were added and made up to 25 mL.

Tea leaves Two grams of the material was dry ashed at 450 °C and then digested with 2 mL of 3:1 mixture of nitric and perchloric acids. The sample was heated gently almost to dryness repeated again with 2 mL of acid mixture and diluted finally to 25 mL with water. The sample was set aside for overnight and filtered to remove impurities.

Recommended Procedure

Zinc(II) in a solution To an aliquot containing zinc(II) ions between 5.0–25 μg, add 2 mL of 1.0×10^{-3} mol/L PBN solution, 1 mL of borate buffer solution (pH 7.8), 1 mL of thiosemicarbazide (TSC) and make up the volume to 25 mL, maintaining 50%(*v*/*v*) ethanol concentration in the final solution. Record the absorbance at 560 nm against a reagent blank prepared under similar conditions.

Zinc(II) in processed samples Take 1 mL of the processed sample and analyze it for zinc(II) ions following the recommended procedure. Dilute the processed sample, if necessary.

Results and Discussion

The reagent and its color reaction As is evident from the literature, an azo dye of α-naphthol is obtained if 1,2-naphthoquinone is reacted with an aromatic hydrazine (Anderson and Nickless 1967, 1968; Kamel and Amin 1964). Thus, a bis-azo dye was obtained by reacting 2 mol of 1,2-naphthoquinone with 1 mol of 2,6-dihydrazinopyridine. Its infrared spectrum confirmed that the compound obtained had an enol form (Sharma and Singh 2008; Fig. 1). The dye showed a light orange color up to pH 9.0. It was observed that only one complex was formed absorbing maximum at 572 nm at all pH levels.

The ethanolic solution of PBN gave very deep color reactions with a number of metal ions at different pH levels: deep blue to violet color with zinc(II), cadmium(II), mercury(II), copper(II), silver(I), cobalt(II), nickel(II), manganese(II) in neutral to alkaline media; violet color with iron(II), vanadium(V) and thalium(I) in alkaline media; green color with palladium(II) at neutral to alkaline media and a pink color with chromium(III) in alkaline media. In all these color reactions, the ethanol content was kept above 50% otherwise precipitates appeared in most of the cases.

Studies on color reactions of PBN with metal ions also showed that in a borate buffered medium, the colors produced by all the transition metals except cadmium, manganese, and iron are masked by thiosemicarbazide while the color developed by cadmium, manganese, and iron are masked by citrate, thus, making the present method highly selective for zinc. With this view, detailed spectrophotometric studies were made on PBN as a reagent for zinc(II).

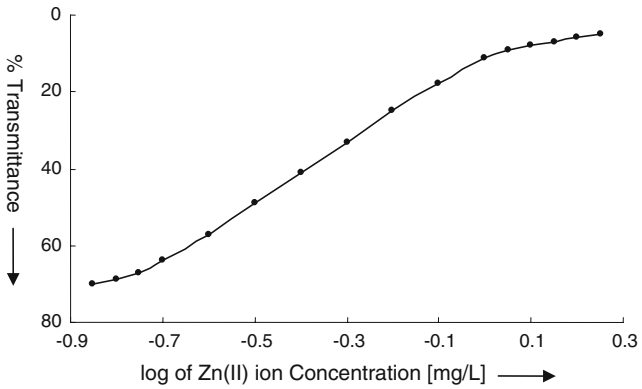

Fig. 2 Ringbom plot for Zn(II)-PBN complex

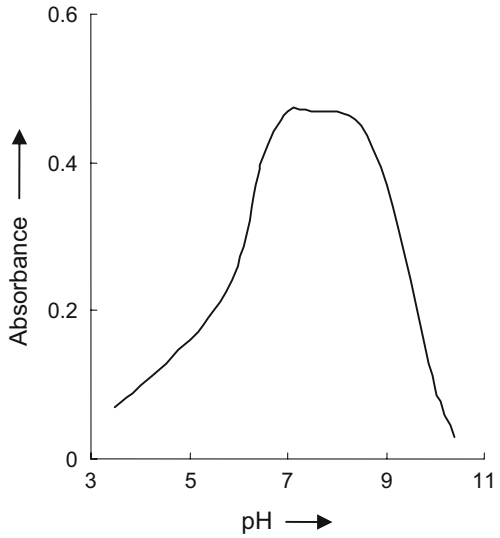

Fig. 3 Effect of pH on the formation of Zn-PBN Complex. (Zn=8 × 10^{-6}mol/L; PBN=4×10^{-5}mol/L)

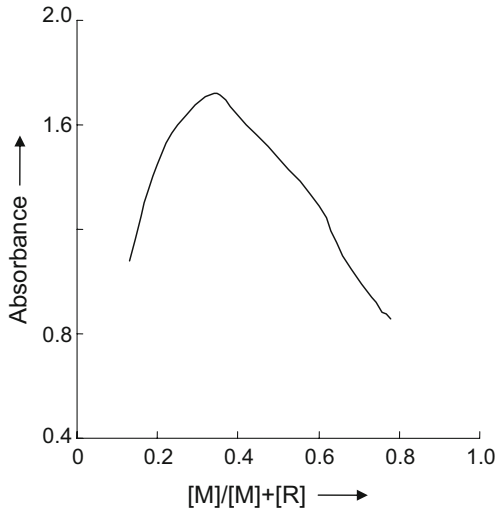

Fig. 5 The continuous variation plot for Zn(II)-PBN complex by Job's Method. [M] represents [Zn(II)] ions; [R] represents [PBN]; Total Molarity=1.2×10^{-4}mol/L

Spectrophotometric studies on zinc(II)-PBN complex For Beer's law validity, the linearity between the absorbance of the complex was examined by varying the concentration of zinc(II) in the solutions containing a fixed amount of ligand (five molar times). Ethanolic solution of PBN gave a violet colored complex with zinc(II), containing not less than 50% ethanol, otherwise precipitated. The optimum range, for highest precision, has been determined from the linear portion of the ~ shape curve obtained by plotting percent transmission against the log of Zn(II) ion concentration expressed in milligram per liter (Ringbom plot; Ringbom 1939; Fig. 2).

Plot of pH against absorbance at λmax of the complex show that constant and maximum absorbance was exhibited in pH range of 7.0 to 8.5 (Fig. 3). In subsequent studies, pH of the reaction mixture solutions was maintained in this range and 1 mL of borate buffer solution of pH7.8 was suitable for this purpose.

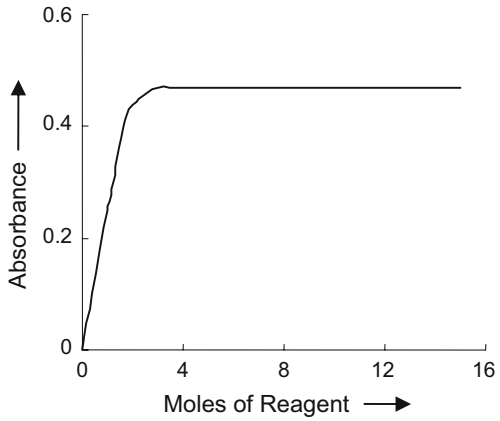

Fig. 4 Effect of reagent concentration on the formation of Zn-PBN Complex (Zn=8×10^{-6}mol/L)

Effect of the reagent concentration For this study a series of solutions containing a 8×10^{-6}mol/L of zinc(II) ion and varying amounts of the reagent were prepared. One milliliter of borate buffer (pH7.8) to each solution was added to maintain pH. The solution was diluted to 25 mL keeping 50% (*v*/*v*) ethanolic concentration. It was observed that at least three times molar excess of PBN was required for complete complexation (Fig. 4). In subsequent studies, however five times molar excess of the reagent was used.

Various physicochemical constants of the complex formed are; λmax 560 nm, Beer's law validity 0.0–1.3 mg/L, optimum concentration range 0.19 to 1.0 mg/L, molar extinction coefficient (ε) of the color developed 6.0×10^4L mol^{-1} cm^{-1}. Job's method of continuous variation was employed to elucidate the composition of the complex (M/L) which was found to be 1:2 (Fig. 5; Job 1928).

Absorbance deviations and accuracy of the method The mean absorbance of a series of solutions containing 1 mL

Table 2 Precision and accuracy of the method

Sr. No.	Zn(II) Added (mg/L)	Zn(II) Found (mg/L)	% Recovery	SD	RSD %
1	6.50	6.40	98.50	0.0791	1.23
2	9.70	9.65	100.0	0.0396	0.41
3	13.0	13.15	99.50	0.1581	1.21
4	16.2	16.35	100.9	0.0791	0.49
5	19.5	19.60	100.5	0.0791	0.40
6	22.7	23.0	101.3	0.0791	0.35

Table 3 Effect of diverse ions

Foreign ions	Tolerance limits	Remarks
CH_3COO^-	250-fold	–
Thiourea	50-fold	–
Tartrate	40-fold	–
Cyanide	250-fold	–
Chromium(III)	100-fold	Masked by TSC
Manganese(II)	5-fold	Masked by citrate
Iron(II)	6-fold	Masked by citrate
Cobalt(II)	10-fold	Masked by TSC
Nickel(II)	3-fold	Masked by TSC
Copper(II)	6-fold	Masked by TSC
Cadmium(II)	5-fold	Masked by citrate
Mercury(II)	10-fold	Masked by TSC
Silver(I)	10-fold	Masked by TSC
Lead(II)	20-fold	Masked by citrate
Thallium(I)	25-fold	Masked by TSC

TSC thiosemicarbazide

of 2×10^{-4} mol/L zinc(II) and excess of PBN in a total volume of 25 mL at pH 7.8 was calculated. The accuracy of the method was checked by preparing a series of solutions containing different amounts of zinc(II) (5.0–25.0 μg) and following the recommended procedure. The recoveries of known additions to different samples lay within the range 98.5–100.9%. The standard deviation and relative standard deviation values clearly indicate that the precision and accuracy of the method are within permissible limits (Table 2).

Effect of diverse ions In the determination of zinc(II) at a 0.52 μg mL^{-1} level, fluoride, chloride, bromide, iodide, nitrate, nitrite, thiosulfate, thiocyanate, TSC, oxalate, citrate, borate (upto 1,000-fold), alkaline earths, lanthanides, platinum metals except palladium(II) (up to 100-fold) did not interfere at all. However, EDTA and phosphate interfered seriously even at equivalent molar concentrations. The results of the tolerance limits of various ions in solution that caused deviation smaller than 2% in the absorbance are given in the Table 3.

Zinc(II) in milk, foodstuffs, and tea samples The procured samples were processed using standard methods (Anderson and Nickless 1967, 1968) and were analyzed for zinc following the recommended procedure as stated above. The samples were also analyzed for zinc using an AAS ECIL model 4129(PC-based). The amounts of zinc(II) found in various samples using the present method and by AAS are summarized in Table 4.

Conclusion

The PBN reagent, for the determination of trace amounts of zinc in different samples, is proposed. Ethanolic solution of PBN formed a violet colored complex with dilute solution of zinc ion at pH 7.8. The colored complex has high molar absorptivity and is made basis of the spectrophotometric determination of the metal ion. The potentiality of the reagent was further explored by analyzing zinc in milk and

Table 4 Contents of zinc in various food stuffs and Milk samples

Sample	No. of Analyses	Sample ashed mL/g	Zn found in whole sample using PBN(μg)	Zn found in whole sample using AAS(μg)	Range of Zn levels (mg/100g) or (mg/100mL)
Milk samples					
Cow	4	100	279, 271, 276, 277	280, 273, 276, 274	0.271–0.279
Buffalo	4	100	225, 220, 223, 228	220, 221, 223, 226	0.220–0.228
Goat	4	100	201, 206, 204, 201	202, 205, 204, 201	0.201–0.206
Food samples					
Cicer arietinum (Gram)	3	5	34.28, 34.81, 33.74	34.30, 34.86, 33.76	0.674–0.696
Oryza sativ (Bran- Rice)	4	2	89.27, 92.02, 92.70, 91.32	89.30, 92.0, 92.75, 91.28	4.463–4.635
Pennisetum typhoidem (Bajra)	4	5	53.29, 53.83, 53.56, 54.10	53.31, 53.80, 53.50, 54.0	1.065–1.082
Zea mays (Maize)	4	4	27.32, 27.85, 27.58, 28.38	27.36, 27.89, 27.60, 28.32	0.683–0.709
Lens culinaris(Masur)	3	4	94.08, 96.14, 96.82, 95.45	94.12, 96.06, 96.80, 95.54	2.352–2.420
Triticum aestivum (Wheat flour)	3	5	32.27, 32.96, 34.33	32.30, 32.92, 34.31	0.645–0.686
Milk Powder	4	5	111.9, 112.6, 111.2, 113.9	111.7, 112.8, 111.3, 113.6	2.225–2.278
Tea samples					
Lipton	3	2	144.9, 140.4, 143.4	144.6, 140.8, 143.9	7.020–7.245
Brooke-Bond	3	2	128.5, 132.9, 135.9	128.8, 133.0, 136.0	6.425–6.795
Taj	3	2	80.60, 77.68, 82.17	80.70, 77.75, 82.28	3.884–4.108

foodstuffs consumed by the predominantly vegetarian gentry of North India. The results were compared with amounts of zinc(II) found in experimental samples using AAS. The studies reveal that PBN can successfully be used determine zinc(II) ions in diverse samples.

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