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Direct and Derivative Spectrophotometric Determination of Zinc (II) using 3,5 - Dimethoxy-4-hydroxybenzaldehyde isonicotinoyl hydrazone(DMHBIH).

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ABSTRACT

A simple and sensitive spectrophotometric method has been developed for the determination of Zinc (II) using newly synthesized reagent 3,5-Dimethoxy-4-hydroxy benzaldehyde isonicotinoyl hydrazone (DMHBIH). Zinc (II) forms an Yellow coloured water soluble complex with the reagent in acidic medium pH 3.5. The molar absorptivity and Sandell's sensitivity of coloured species are 2.42×10^4 L.mol^{-1} cm^{-1} and 0.0083 μg/cm^2 respectively. Beer's law is obeyed in the range 0.1635 to 1.635 μg/ml of Zn(II) at λ_{max} 473 nm. The developed derivative spectrophotometric method was employed for the determination of Zinc (II). This method has been satisfactorily applied for the determination of Zinc (II) in water samples, biological samples and pharmaceutical samples.

Keywords: Zinc(II), 3,5-dimethoxy-4-hydroxybenzaldehyde isonicotinoylhydrazone (DMHBIH), derivative spectrophotometry.

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INTRODUCTION

The potential analytical applications of hydrazone derivatives have been reviewed by Singh et al [1]. Hydrazones are important class of known analytical reagents [2-7]. They react with many metal ions forming colour complexes and act as chelating agents. The present paper describes a new, very simple, rapid, sensitive and derivative spectrophotometric determination of Zinc (II) is proposed. The method consists of the formation of Yellow coloured metal complex using 3,5-dimethoxy-4-hydroxybenzaldehyde isonicotinoylhydrazone (DMHBIH) with Zinc (II) and the stability of the complex is presented. In view of the importance of Zinc in industry and pharmaceutical preparations and the paucity of the literature using isonicotinoyl hydrazones for spectrophotometric determination of Zinc (II), this present method is proposed with DMHBIH.

Derivative spectrophotometry is a very useful approach for determining the concentration of components in mixtures with overlapping spectra, as it eliminates much of the interference. In recent years, the derivative method has been used to eliminate interference during spectrophotometric analysis [8-11]. It has been widely used in pharmaceutical analysis, amino acid and protein analysis, clinical industry and environmental analysis [12], etc., but less often in inorganic analysis [13,14].

In this paper, direct and first order derivative spectrophotometric methods are described for the determination of Zinc (II) in which the interference zero-order method is eliminated in the first order derivative method. The methods are applied for the determination of Zinc (II) in water, biological and pharmaceutical samples.

MATERIALS AND METHODS

A Shimadzu 160A, microcomputer based UV-VIS spectrophotometer equipped with 1.0cm quartz cells was used for all spectral measurements. The instrumental parameters are optimized and the best results were obtained with scan speed (2400) nm/min., slit width of 1nm and $\Delta\lambda$=2nm for first order derivative mode in the wavelength range 350-650nm. ELICO L1-120 digital pH meter was used for the pH adjustments.

All chemicals used were of A.R grade unless stated. All solutions were prepared with doubly distilled water. The standard Zinc (II) solution (0.01M) was prepared by dissolving accurately weighed 0.2875 g of $ZnSO_4 .7 H_2O$ in a few ml. of doubly distilled water containing a few drops of conc. Sulphuric acid and made up to the mark in a 100-ml of volumetric flask. The stock solution was standardised by using standard methods [15]. The working solutions were prepared by diluting the stock solution to an appropriate volume.

Buffer solutions were prepared by using 0.1M HCl, 0.1M NaOH, 0.1M disodium hydrogen phosphate and 0.1M potassium dihydrogen phosphate. Solutions of various ions of suitable concentrations were prepared using AR grade chemicals.

The reagent 3,5-dimethoxy-4-hydroxybenzaldehydeisonicotinoylhydrazone(DMHBIH) was synthesized by refluxing equimolar amounts of 3,5-dimethoxy-4-hydroxybenzaldehyde and isonicotinoylhydrazone. In a 250 ml round bottomed flask hot ethanolic solution of 3,5-dimethoxy-4-hydroxybenzaldehyde(1.8212g, 0.01 mole) and hot ethanolic solution of isonicotinoylhydrazone(1.3714g, 0.01mole) were mixed and refluxed using water condenser for 3 hours. On cooling the reaction mixture, a greenish yellow coloured product separated out, which was collected by filtration and washed with double distilled water. The resulting hydrazone was recrystalized using 50% ethanol (yield, 76%, mp 221^{0}C).

I R Spectral bands(cm^{-1}) of DMHBIH Reagent

Bands(cm^{-1})	**Assignments**
3429.03 (s, br)	ν NH stretching
2924.16 (m)	ν OH stretching
1658.62(s)	ν > C=O stretching
1578.92(s)	ν C=N stretching
1514.12 (m)	ν C=N stretching(pyridine ring)
1372.71 (w)	ν N-H stretching(primary amide)
1123.66 (m)	
836.12 (m)	ν C-H bending
749.239 (m)	
689.53 (s)	ν C-C bending

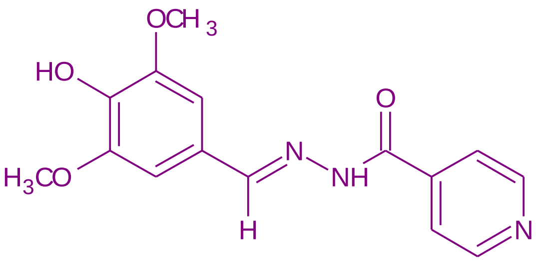
The 1H NMR (200 MHz) spectrum of the reagent was recorded in DMSO-d6 solvent.

δ value	**Assignments**
11.9 (S, 1H)	**NH**
8.97 (S, 1H)	OH (phenolic)
8.78 (d, 2H)	ArH(pyridine ring)
8.34 (S, 1H)	N=C-H
7.8 (d, 2H)	ArH (pyridine ring)
7.01 (S, 2H)	ArH (aldehydic)
3.8 (S, 6H)	$-OCH_3$ (2 Methoxy)
3.3 (S, 2H)	Water in solvent
2.5 (S, 6H)	DMSO(solvent)

The mass spectrum showed that molecular ion peak at m/z 302.2 (M+1) corresponding to molecular weight peak. The structure of DMHBIH was confirmed based on the IR, NMR and Mass spectral data and is given in Figure-1.

The reagent solution (0.01 M) was prepared by dissolving 0.3022 g of DMHBIH in 100 ml of Dimethylformamide (DMF). The reagent solution is stable for 48 hours.

Fig.1: Structure of 3,5-Dimethoxy-4-hydroxybenzaldehyde isonicotinoylhydrazone(DMHBIH)



EXPERIMENTAL

The reaction of some important metal ions were tested at different pH values. The samples were prepared in 10ml volumetric flasks by adding buffer solution 3.0 ml (pH 1-11), metal ion 0.5 ml of 5x10^{-4}M and DMHBIH 0.5 ml of 5x10^{-3}M solution. The mixture was diluted up to the mark with distilled water. The absorbance was measured in 300-700nm range against reagent blank. The results are summarized in Table-1.

For the spectrophotometric determination of Zinc (II), an aliquot of the solution containing 0.1635 to 1.635 µg/ml of Zinc (II), 3.0ml of buffer solution (pH 3.5), and 0.5ml of 5x10^{-3}M DMHBIH reagent solution were taken in 10 ml volumetric flask and the solution was diluted up to the mark with doubly distilled water. The absorbance was read at 473 nm in a 1.0cm cell against reagent blank prepared in the same way. The measured absorbance was used to compute the amount of Zinc (II) from the predetermined calibration curve.

The first-order derivative spectrum was recorded with scan speed fast having a degree of freedom 9, in the wavelength range from 350-650nm. The derivative peak height was measured by peak-zero method at 540 nm. The peak height was plotted against the amount of Zinc (II) to obtain the calibration curve. The second order derivative spectrum of [Zn(II)-DMHBIH] system was recorded with reference to the reagent blank in a wavelength range 350-650nm. The derivative peak height was measured by peak-zero method at 590 nm. The peak height was plotted against the amount of Zinc (II) to obtain the calibration curve.

RESULTS AND DISCUSSION

The reagent 3,5-dimethoxy-4-hydroxybenzaldehyde isonicotinoylhydrazone(DMHBIH) was easily synthesized as any other Schiff base reagent. The new chromogenic reagent DMHBIH was not used for the spectrophotometric determination of Zinc (II). The colour reactions of some important metal ions with DMHBIH are summarized in Table-1. In acidic medium, the ligand presumably co-ordinates the metal ion to give neutral complexes.

The absorption spectrum of DMHBIH and its Zinc (II) complex under the optimum conditions are shown in Figure-2. The [Zn(II)-DMHBIH] complex shows the maximum absorbance at 473 nm, where the reagent blank does not absorb appreciably.

Zinc (II) reacts with DMHBIH in acidic buffer to give Yellow coloured water soluble species. The colour reaction between Zinc (II) and DMHBIH was instantaneous even at room temperature in pH range 3.0-5.0, the maximum colour intensity was observed at pH 3.5.

When varied the volume of reagent DMHBIH (5×10^{-3}M) from 0.5ml to 4.0ml, the constant absorbance was obtained from 0.5 ml. Therefore a 10 fold molar excess of reagent is adequate for full colour development.

The order of addition of buffer solution, metal ion and reagent has no adverse effect on the absorbance of [Zn(II) –DMHBIH] complex. Beer's law obeyed in the range 0.1635 to 1.635 μg/ml. The molar absorptivity and Sandell's sensitivity of [Zn(II)-DMHBIH] complex was obtained from the Beer's law. The linear regression analysis of absorbance at λ_{max} of the complex against metal ion (μg/ml) shows a good linear fit. The various important analytical characteristics of Zinc (II) and DMHBIH complex are summarized in Table-2.

The first order derivative spectral graph was shown in Figure-3. This shows that the derivative amplitudes measured at 540 nm for first order were found to be proportional to the amount of Zinc (II).

The stoichiometry of the complex was found to be 1:1 (M : L) investigated by Job's continuous variation method and molar ratio method, with a stability constant 3.97×10^{7}.

The effect of various diverse ions in the determination of 0.8175 μg/ml Zinc (II) and tolerance limit of foreign ions was studied in the present method. The tolerance limit of a foreign ion was taken as the amount of foreign ion required to cause an error of ±2% in the absorbance or amplitude. The results are given in Table-3. The data obtained in the derivative method is also incorporated. The data suggest that several associated anions and cations do not interfere when they are present in large excess, such as iodide, nitrate, thiosulphate, thiocyanide, bromide, sodium(I), calcium(II), bismuth(III), tungsten(VI). The tolerance limit values for many anions and cations are more in derivative method. The interference of associated metal ion such as Iron (III) is decreased by adding masking agent fluoride.

Applications

The proposed method was applied for the determination of Zinc (II) in water samples, Biological samples and Pharmaceutical samples. The sample solutions were obtained by following the recommended procedure.

Preparation of Water samples: The water samples were collected from Chitrvathi river basin, located at Puttaparthi which is located 85 Km from our laboratories. The water samples contain other elements well within the tolerance limits of the proposed method. Hence, the samples are directly taken for analysis.

Preparation of Blood samples: Fresh blood samples of diabetic patients were collected from a diagnostic centre, Anantapur, Andhra Pradesh, India. It was assured that the patients had not taken any zinc supplement prior to the collection of the sample.

Separation of serum from blood: approximately 5.0 ml of freshly drawn blood was taken in a centrifuge tube and alloed to settle for 30 minutes at room temperature. Centrifuged it for 10 minutes and separated the clear serum.

Recommended procedure for the determination of Zinc(II): 1.0 ml of the serum was taken in a centrifuge tube and 1.0 ml of 20% Trichloro Acetic acid (TCA) was added to this. It was allowed to stand for 10 minutes and then centrifuged. 0.5 ml of the supernatant liquid, 3.0 ml of buffer solution(pH3.5) and 0.5 ml of DMHBIH were taken in a 10 ml standard volumetric flask and made up to the mark with doubly distilled water. Absorbance was Measured against reagent blank and the amount of Zinc(II) was calculated from a standard calibration curve. The results were summarized in Table-5.

Preparation of pharmaceutical samples: The pharmaceutical samples were prepared by destroying organic matter by an established procedure [16] based on the use of perchloric acid. The insulin was treated with 10% m/v chloroacetic acid for the precipitation of proteins. The other samples are previously diluted with doubly distilled water. The results were summarized in Table-6.

The Zinc (II) in these solutions was determined by the recommended derivative procedure from a predetermined calibration plot. Results are presented in Table-4, Table-5 and Table-6 respectively.

CONCLUSION

The present method using 3,5-dimethoxy-4-hydroxybenzaldehyde isonicotinoyl hydrazone(DMHBIH) as spectrophotometric reagent for the determination of Zinc (II) in aqueous medium is sensitive and simple. Many of the methods involve either heating at a specific temperature [17,18] or extraction [19,20] of the reaction mixture. However heating at a specific temperature for a long time is laborious and time consuming. The determination of Zinc (II) using DMHBIH is not laborious and there is no need of heating or extraction of the components. Further the reagent is easy to synthesize using available chemicals. More over the present method is simple, rapid, selective and more precise for the determination of Zinc (II) [21-23].

Table-1: Analytical Characteristics of 3,5-Dimethoxy-4-hydroxybenzaldehyde isonicotinoylhydrazone

Metal ion	pH	Colour	λ $_{max}$ (nm)	Molar absorptivity (ε) (L.mol^{-1}cm^{-1})
Fe(III)	4.0	Yellow	386	1.875×10^{4}
Hg(II)	4.0	Orange	428	2.16×10^{4}
Cu(II)	9.0	Yellow	440	3.37×10^{4}
Zn(II)	3.5	Yellow	473	$2.42 \times 10^{4*}$

*Present work

Table-2: Physico-Chemical and Analytical Characteristics of [Zn (II)-DMHBIH]

Characteristics	Results
Colour	Yellow
λ $_{max}$(nm)	473
p^{H} range (optimum)	3.0-5.05
Mole of reagent required per mole of metal ion for full colour development	10 folds
Molar absorptivity(L.mol $^{-1}$cm^{-1}) (ε)	2.42×10^{4}
Sandell's sensitivity(μg/cm^{2})	0.0083
Beer's law validity range(μg/ml)	0.1635-1.635
Optimum concentration range(μg/ml)	0.491-1.308
Composition of complex(M:L) obtained in Job's and mole ratio methods	1:1
Stability constant of the complex	3.97×10^{7}
Standard deviation	0.00008
Relative standard deviation (%)	0.13

Table-3: Tolerance limit of foreign ions in the determination of 0.8175 μg/ml Zinc(II)

Ion added	Tolerance limit μg/mL	Ion added	Tolerance limit μg/mL
Bromide	3196.16	Pb^{2+}	103.6
Iodide	2538	Co^{2+}	88.4
Urea	1500	Ca^{2+}	60.15
Chloride	1064	Sn^{2+}	59.35
Tetraborate	970	Cd^{2+}	56.21
Sulphate	940	Zr^{4+}	45.61
Oxalate	880	Ni^{2+}	29.34
Nitrate	620	U^{6+}	12.0
Acetate	590	Ag^{+}	11.0
Thiocyanide	581	Mo^{6+}	9.59
Phosphate	475	As^{3+}	7.5
Ascorbic acid	176.13	Sb^{3+}	6.08
Tartarate	148	Pd^{2+}	5.32
Thiourea	115	Ru^{3+}	5.05
Fluoride	95	Al^{3+}	2.69
Ba^{2+}	206	Cr^{3+}	2.59
W^{6+}	184	V^{5+}	2.54
Sr^{2+}	175.2	Fe^{3+}	0.63 1.27*
Mn^{2+}	110		
Bi^{3+}	104.5		

*masked by fluoride 70 μg/ml.

Table -4: Determination of Zinc (II) in water samples.

Sample	Amount of Zinc (II)* found(µg/ml)		Error(%)
	AAS method	Present method	
01	2.47	2.44	+1.21
02	2.05	2.07	-0.98
03	1.68	1.69	-0.59
04	1.61	1.61	0.00
05	0.58	0.57	+1.72

*average of best three determinations among five determinations

Table -5: Determination of Zinc (II) in Blood serum of Diabetic patients.

Sl.No.	Age (in years)	Gender	Amount of Zinc (II) found (**µg/ml**)
1	70	Male	0.001554
2	70	Male	0.001383
3	70	Female	0.001169
4	67	Female	0.001032
5	58	Male	0.001054
6	58	Female	0.001087
7	50	Male	0.009473
8	50	Female	0.001040
9	35	Female	0.001421

*average of best three determinations among five determinations

Table -6: Determination of Zinc (II) in Pharmaceutical samples.

Sample	Amount of Zinc (II)* found(µg/ml)		Error(%)
	AAS method	Present method	
Zingisol (JCPA Health Products Pvt. Ltd.)	3.99	4.03	-1.00
Insulin Zinc Suspension (Knoll Pharma Ltd.)	1.01	1.03	-1.98
Biocosules Z (Omni Protech Drugs Ltd.)	3.01	3.04	-0.99

*****average of best three determinations among five determinations

Table -7: Determination of Zinc (II)

S.No	Reagent	pH	λ_{max} (nm)	Molar absorptivity	Reference
1	Furfural-2-benzthiozolyl hydrazone (FBTH)	9.4	418	0.8 x10^4	21-90
2	o-hydroxybenzaldehyde isonicotinoyl hydrazone	5.0	380	1.5 x10^4	22-93
3	o-hydroxybenzaldehyde benzoyl hydrazone(BBH)	--	380	1.35 x10^4	23-92
4	3,5-dimethoxy-4-hydroxy-benzaldehyde isonicotinoyl hydrazone (DMHBIH)	4.0	428	2.16x10^{4*}	Present work

* present work

Fig.2: Zero order Absorption spectra: (a): [Zn(II)-DMHBIH] complex peak. (b): Reagent (DMHBIH) Vs DMF blank

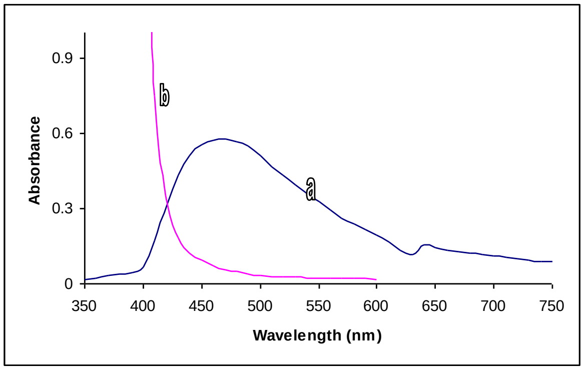


Fig.3: First order derivative spectra of [Zn(II)-DMHBIH] complex

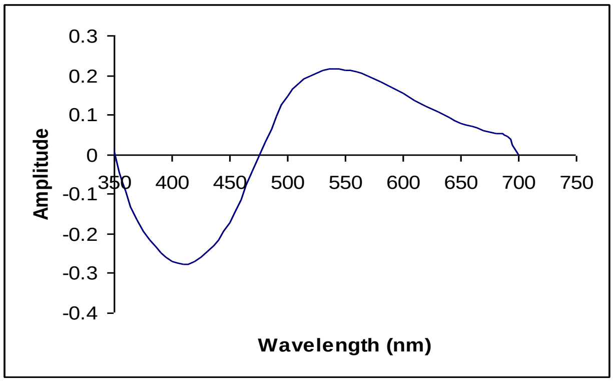
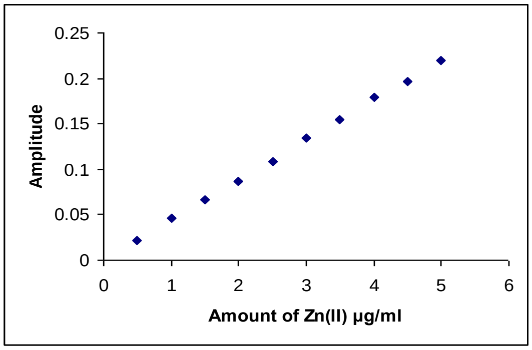


Fig.4: Beer's law First order derivative spectra of [Zn (II)-DMHBIH] complex



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REFERENCES

[1] Singh RB, Jain P, Singh RP. Talanta 1982; 29: 77.
[2] Chandrasekhar KB, Hussain Reddy K. Indian J Chem 2002; 41:1643.
[3] Hussain Reddy K, Chandrasekhar KB. Indian J Chem 2001; 40: 727.
[4] Ramesh M, Chandrasekhar KB, Hussain Reddy K. Indian J Chem 2000; 39:1337.
[5] Rameswara rao M, Hari K, Devanna N, Chandrasekhar KB. Asian J Chem 2008; 2 :1402.
[6] Narender Reddy G, Chandrasekhar KB, Devanna N, Jayaveera KN. Asian J Chem 2008;3:2257.
[7] Kiran Kumar V, Rameswara rao M, Chandrasekhar KB, Devanna N. Asian J Chem 008; 3: 2197.
[8] Such V, Travast J, Ganzaly R, Gelpi E. Anal Chem 1980; 52: 412.
[9] Davidson AC, Elsheik H. Analyst 1982; 107: 879.
[10] Kitamura K, Mazima R. Anal Chem 1983; 55: 54.
[11] Hassan SM, Davidson AG. J Pharm Pharmacol 1984; 36: 7.
[12] Sanchez RF, Bosch-Ojeda C, Cano PJM. Talanta 1988; 35: 753.
[13] Bermejo-Berrera A, Bermejo-Berrera P, Bermejo Martinez. Analyst 1985; 110:1313.
[14] Kurodo R, Kurosaki M, Hayashibe V. Talanta 1990; 77: 619.
[15] Vogel AI. " A Text book of Qualitative Inorganic Analysis" 4th edn., London: ELBS and Longman 1985; 489.
[16] Official methods of analysis. William S Ed., Arlington, VA: Association of official Analytical Chemists 1984; 444.
[17] Naik BR, Desai KK. Asian J Chem 2006; 18(4): 3176.

[18] Yoshiaki Sasaki, Shoji Tagashira, Yoshilo Murakami, Musafumi Ichikawa. Anal sciences 1998; 14:1603.

[19] Rameshwar Dass, Anil Kumar. Indian J Chem 2004; 43A: 576.

[20] Mallik AK, Kaul KN, Lark BS, Faubel W, J Rao AL. Turk J Chem 2001;25: 99.

[21] Odashima T, Ishii H. Anal Chim Acta 1976; 83: 431.

[22] Vasilikiotis GS. Dissertation, University of Thessaloniki 1968.

[23] Vasilikiotis GS, Kouimitzis THA, Vasiliades VC. Microchem J 1975; 20: 173.