*Available online at* www.scholarsresearchlibrary.com

Scholars Research Library

**Der Pharmacia Lettre, 2016, 8 (12):251-259**
*(http://scholarsresearchlibrary.com/archive.html)*

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

# Spectrophotometric determination of Zn(II) in food and water samples using 2-hydroxy-N'-(1-(pyridin-2-yl)ethylidene) benzohydrazide as a sensitive and selective analytical reagent

**P. Nityananda Kumar Reddy[1], G. Trivikram Reddy[2], Sangita D. Kumar[3], A. V. R. Reddy[3], S. Nazaneen Parveen[1] and N. C. Gangi Reddy*[2]**

[1]*Department of Environmental Science, Yogi Vemana University, Kadapa, A.P., India*
[2]*Department of Chemistry, Yogi Vemana University, Kadapa, A.P., India*
[3]*Analytical Chemistry Division, Bhabha Atomic Research Centre (BARC), Trombay, Mumbai, India*

---

**ABSTRACT**

*2-hydroxy-N'-(1-(pyridin-2-yl) ethylidene) benzohydrazide (HPEBH) is found to be a selective and sensitive reagent for the determination of Zn(II) in food, water and synthetic samples. HPEBH forms colour less complex (Zn-HPEBH) with Zn(II) in aqueous DMF at pH 5.0.* Ligand shows maximum absorbance at 312 nm, whereas the complex shows maximum absorption at 351 nm. *Beer's law is obeyed in the range of 0.653-6.53 µg $mL^{-1}$ of Zn(II), with a correlation coefficient 0.9996. The molar absorptivity (ε) and Sandell's sensitivity of the complex are $6.27\times10^4$ $L.mol^{-1}.cm^{-1}$and 0.026 µg $mL^{-1}$ respectively. The metal to reagent ratio 1:1 is confirmed by the Job's and mole ratio methods. The optimized method has been successfully applied for the determination of Zn(II) in food, water and synthetic samples in the presence of diverse ions. It is also found that the obtained results are in good agreement with AAS method. Hence, the proposed method is fairly sensitive and reproducible.*

**Keywords**: Zn(II), food, water samples, spectrophotometry and 2-hydroxyN'(1(pyridine-2-yl) ethylidene) benzo hydrazide.

---

## INTRODUCTION

Zinc is the second most abundant transition metal ion in the human body. It is an important element for all animals including human beings. Zinc is found in several foods such as rice, cereals, meat, liver, oysters, cheeses nuts, in several enzymes and DNA-binding proteins. It plays a significant physiological role in human beings like mammalian reproduction, gene transcription, immune function, brain function and pathology [1]. Zinc is a contributory factor in neurological disorders like epilepsy and Alzheimer's disease and also it can directly control several enzymatic and cellular functions [2]. The suggested daily dosage of zinc for women is 12 mg and for men 15 mg. Zinc is also used in galvanizing ferrous metals, brass alloys and in dermatology as an antiseptic, mineral-vitamin preparations, eye drops, mouth washes, creams, ointments and against to different types of infection.

The symptoms of zinc deficiency lead to loss of hair, decrease of fertility, slow healing of wounds, reduction in the senses of taste and smell, retardation of children's growth, skin problems, loss of appetite, mental lethargy, diabetes, sickle cell disease, chronic liver disease, chronic renal disease and increased susceptibility to infections. Very high

levels of zinc cause Wilson's disease, damage the pancreas, increase the chance of developing prostate cancer in the the human system, disturb the protein metabolism and in plants, it reduces the chlorophyll content. Widespread exposure to zinc chloride can cause respiratory disorders [3]. Hence, there is a great need to determine Zn(II) in environmental matrices using simple reagents.

Determination of zinc at micro levels by several analytical techniques such as spectrophotometry, AAS, polarography, ICP-AES, voltametry, X-Ray fluorescence spectroscopy, and other techniques[4-12]. Among them, spectrophotometric methods are preferred because they are cheaper and comparable sensitivity. Some of the previously reported methods [13-17] are given in the table. 1. However, the methods are having some disadvantages like less selectivity and sensitivity, time-consuming, etc. So there is a great need to develop a simple, efficient and eco-friendly method for the determination of Zn(II) in environmental matrices. Herein we demonstrated a rapid spectrophotometric method for the determination of Zn(II) in food, water and synthetic samples using 2-hydroxy-N'-(1-(pyridin-2-yl) ethylidene) benzo hydrazide (HPEBH) as a selective and sensitive analytical reagent.

*Table: 1. Review on determination of zinc(II) using hydrazones by spectrophotometry*

| S. No. | Reagent | $\lambda_{max}$ (nm) | pH | Beer's range (ppm) | ε L.mol$^{-1}$ cm$^{-1}$ | Ref |
|---|---|---|---|---|---|---|
| 1 | 2,4-dimethoxy benzaldehyde-4-hydraoxybenzoyl hydrazone (DMBHBH) | 466 | | 0.16- 1.96 | 4.27 ×10$^4$ | 13 |
| 2 | Biacetyl mono (2-pyridyl) hydrazone | 440 | 10.0 | 0.04-1.20 | 5.20 ×10$^4$ | 14 |
| 3 | 2-hydroxy-1-naphthaldehyde-p-hydroxybenzoic hydrozone | 430 | 2.0-6.0 | 0.317-3.175 | 1.58 ×10$^4$ | 15 |
| 4 | 2,4-Dihydroxybenzaldehyde Isonicotinoyl hydrazone | 390 | 4.0-10.0 | 0.10-1.50 | 3.55 ×10$^4$ | 16 |
| 5 | Salicylaldehydecarbo hydrazone | 445 | 5.4 | | | 17 |
| 6 | 2-hydroxy-N'-(1-(pyridin-2-yl)ethylidene)benzohydrazide (HPEBH) | 351 | 5.0 | 0.653-6.53 | 6.27×10$^4$ | PM |

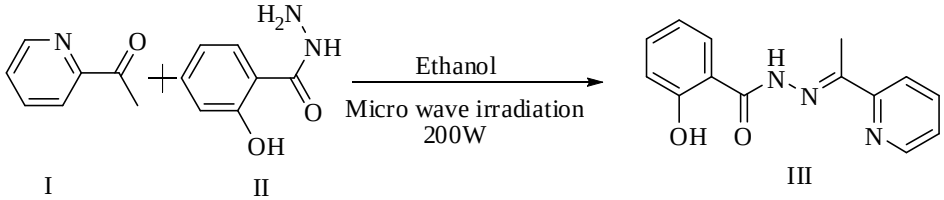
## MATERIALS AND METHODS

**Apparatus:** For absorbance studies, a Double beam UV-Visible spectrophotometer (Systronics model UV-2203) with a 1.0 cm quartz cell is used and for measurement of pH (Systronics model 3305) pH meter is used respectively. Melting point is determined and is uncorrected. For comparative analysis Flame atomic absorption spectrophotometer (Shimadzu model No: AA-6300) is used. $^1$H NMR spectrum of the ligand is recorded on Jeol 400 MHz NMR spectrometer (JNM-400) and mass spectrum of the ligand is recorded on Shimadzu-LCMS with ESI probe (LC-2010EV). All glassware are washed with a mixture of concentrated sulfuric acid and nitric acid (1:1) before use.

**Reagents and solutions:** All the chemicals used are of analytical reagent grade or the highest purity available (Across or Merck). DMF and double distilled water are used throughout the experiment.

**Preparation and characterization of 2-hydroxy-N'-(1-(pyridin-2-yl) ethylidene) benzohydrazide (HPEBH):** Synthesis of 2-hydroxy-N'-(1-(pyridin-2-yl) ethylidene) benzohydrazide (HPEBH) is reported by conventional method [18]. In the present study, we developed a green synthetic route for the synthesis of HPEBH by using microwave irradiation.

**Microwave method:** In a 100-mL beaker, 2-acetyl pyridine (I) [1 gm, 7.3 mmol] and salicyl hydrazide (II) [1.1 gm, 7.3 mmol] are dissolved in 10 mL of ethanol. The beaker is placed in a domestic microwave oven at 200 watts for 30-45 sec. The progress of the reaction is monitored by TLC. After completion of the reaction, the reaction mixture is cooled to RT and then washed twice with cold ethanol. Finally, the obtained crude product (III) is recrystallized from hot ethanol. The structure of the synthesized compound is confirmed by $^1$H NMR and mass spectral data [19].




I II III

**Scheme 1. Preparation of 2-hydroxy-N'-(1-(pyridin-2-yl) ethylidene) benzohydrazide (HPEBH)**

**Characterization data of HPEBH**
Colorless solid; Yield: 1.73 gm (92%); mp 238-240°C; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 11.80 (s, 1H, -NH), 11.47, (s, 1H, -OH), 8.63 (d, 1H, *J*=4.4 Hz, arom H), 8.14 (d, 1H, *J*=7.6 Hz, arom H), 8.02 (d, 1H, *J*=7.2 Hz, arom H) 7.89(t, 1H, *J*=7.6 Hz, arom H), 7.44 (t, 1H, *J*=7.6 Hz, arom H), 7.06-6.99 (m, 2H, arom H), 2.50 (s, 3H, -$CH_3$); MS (ESI): $(M+H)^+$ 256.10.

**Preparation of 0.01M reagent (HPEBH) solution:** 0.01 M HPEBH solution is prepared by dissolving 256 mg of HPEBH in DMF and made up to the mark in a 100-mL volumetric flask with DMF. Dilute solutions are prepared from this stock solution.

**Preparation of Zinc(II) stock solution**
0.01M Zn(II) solution is prepared by dissolving 136.28 mg of zinc chloride (Merck) in double distilled water containing few drops of concentrated HCl and made up to the mark in a 100-mL volumetric flask. Aliquots of this solution are standardized with EDTA using xylenol orange as an indicator [20]. Dilute solutions are prepared from this stock solution.

**Sodium thiosulphate solution**: 100 ml stock solution of sodium thiosulphate ion (0.1% w/v) was prepared by dissolving 100 mg of sodium thiosulphate (Merck) in (100 mL) de-ionized water.

**Preparation of buffer solutions:**
Hydrochloric acid (1.0M) and sodium acetate (1.0M) are mixed to get the required pH (1.0-3.5), 0.2M sodium acetate and 0.2M acetic acid are mixed to get the required pH (4.0-6.0) and 0.01M Potassium dihydrogen phosphate and 0.01M disodium hydrogen phosphate are mixed to get the required pH (pH 7.0). The pH of the above buffer solutions are measured by a pH meter and finally adjusted suitably.

**2. General analytical procedure for the determination of Zinc (II)**
An aliquot (1.0 mL) of the solution containing known amount of zinc(II), 4.0 mL of sodium acetate-acetic acid buffer solution (pH 5.0), 1.0 mL of 0.01% thiosulphate solution and 1.0 mL of reagent (HPEBH) of required concentration are mixed in a 10-mL volumetric flask and the resulting solution is diluted up to the mark with double distilled water. The absorbance of this solution is measured at 351 nm, against the reagent blank.

## RESULTS AND DISCUSSION

In aqueous DMF, in the presence of 0.01% thiosulphate solution at pH 5.0 Zn(II) reacts with HPEBH and forms a white colored complex, which shows maximum absorbance at 351 nm, against the reagent blank. Hence a detailed study has been undertaken for the determination of Zn(II) using HPEBH by spectrophotometric method. The optimized method is successfully applied for the determination of zinc in water, food and synthetic samples alone or in the presence of diverse ions.

**3.1. Absorption spectra of HPEBH and Zn(II)-HPEBH complex**
Initially absorption spectrum of the HPEBH is recorded against the solvent blank. The absorption spectrum of Zn(II)-HPEBH complex is recorded against the ligand blank. The absorption spectrum of both complex and ligand are shown in the fig.1. From the absorption spectra, it is clear that the ligand shows maximum absorbance at 312 nm, whereas the complex shows maximum absorption at 351 nm. Therefore, all the spectral measurements are carried out at 351 nm.

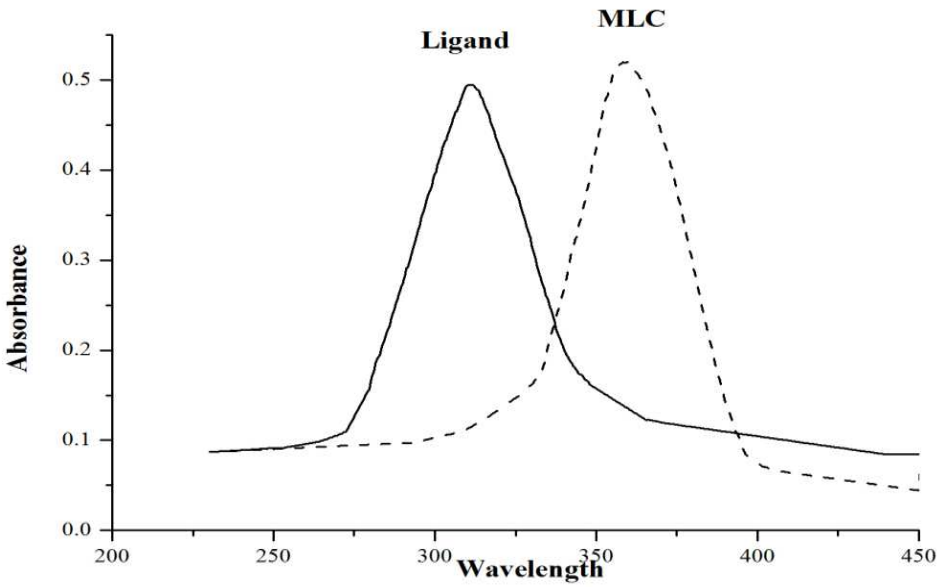



**Fig.1. (A) Absorption spectrum of ligand (HPEBH) Vs. solvent (DMF) blank (B) Absorption spectrum of Zn(II)-HPEBH complex (MLC) Vs. Ligand blank, Zn(II) =1.0 mL of $1.0\times10^{-4}$ M, HPEBH =1.0 mL of $1.0\times10^{-4}$ M and buffer solution of pH 5.0 = 4.0 mL**

### 3.2. Effect of pH

Into a series of 10-mL volumetric flasks, 1.0 mL of Zn(II) solution ($1.0\times10^{-4}$M), 1.0 mL of ligand solution ($1.0\times10^{-4}$M), 1.0 mL of 0.01% thiosulphate solution and 4.0 mL of buffer of varying pH (1.0 - 7.0) are added and made up to the mark with double distilled water and the absorbance is measured against reagent blank at 351 nm. The absorbance increases from pH 1.0-5.0 then decrease. From this study, it is optimized that the pH 5.0 is the suitable pH for further studies (fig.2).

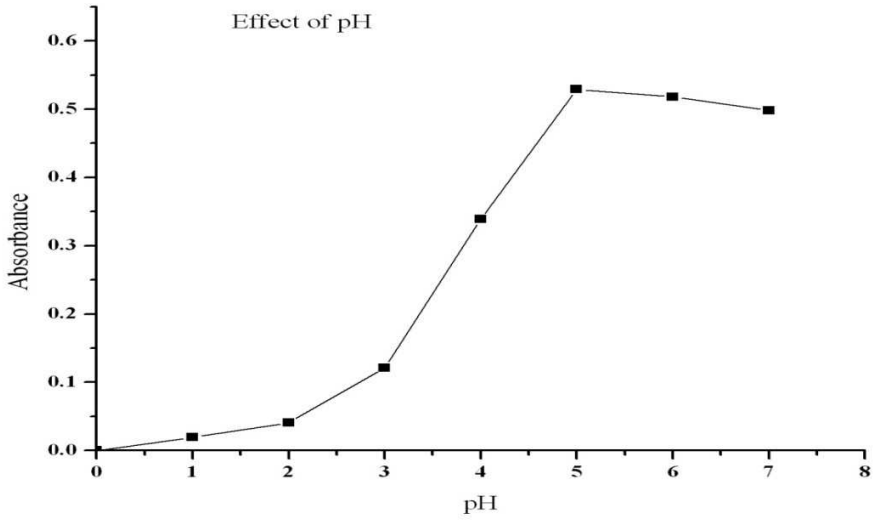



**Fig.2. Effect of pH on the absorbance of Zn(II)-HPEBH complex, Zn(II) = 1.0 mL of $1.0\times10^{-4}$M, HPEBH =1.0 mL of $1.0\times10^{-4}$ M, $\lambda_{max}$ = 351 nm**

### 3.3. Applicability of Beer's law

The known aliquots of 10.0 mL solutions, each containing constant volumes of 4.0 mL of buffer (pH 5.0), 1.0 mL of 0.01% of thiosulphate solution, 1.0 mL of $1.0\times10^{-4}$M of reagent and 1.0 mL of Zn(II) solutions of concentrations in the range from $0.10\times10^{-4}$ to $1.0\times10^{-4}$M (0.653-6.53 µg $mL^{-1}$) are prepared. The absorbance of these solutions is measured at 351 nm. A graph plotted between the amount of Zn(II) and its absorbance is as shown in fig.3. From the graph, it is observed that a linear plot passing through the origin obeys Beer's law in the range from 0.653-6.53µg $mL^{-1}$ of Zn(II). The correlation coefficient is 0.9996 which indicates the linearity between the two variables. The molar absorptivity coefficient and sandal's sensitivity of the complex are found to be $6.27\times10^{4}$ L $mol^{-1}$ $cm^{-1}$ and 0.026 µg $cm^{-2}$ respectively.

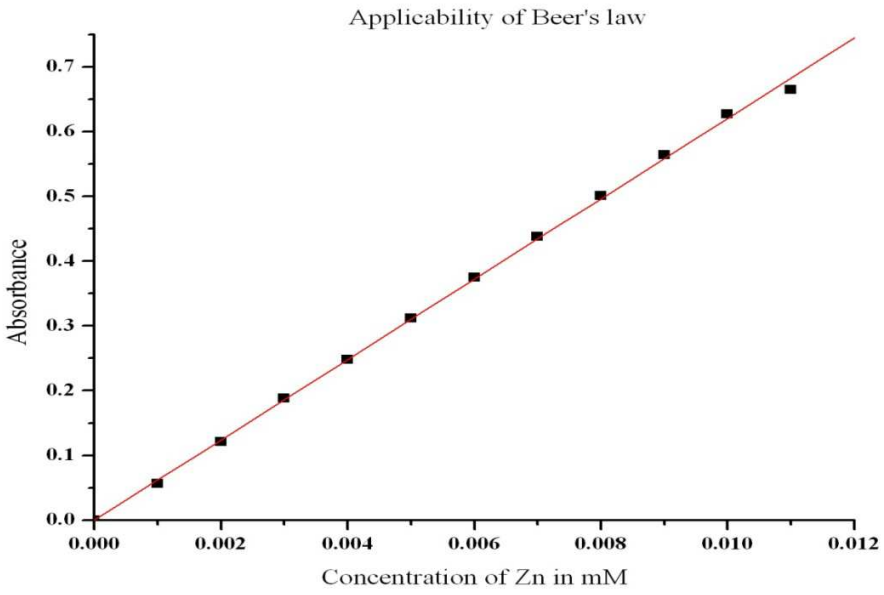



**Fig.3. Applicability of Beer's Law: HPEBH = 1.0 mL of $1.0\times10^{-4}$ M, Zn(II) =1.0 mL of $0.1\times10^{-4}$ M - $1.0\times10^{-4}$ M, buffer solution of pH 5.0 = 4mL and $\lambda_{max}$ = 351 nm**

### 3.4. Job's method of continuous variation

Equimolar solutions of Zn(II) ion and reagent HPEBH($1.0\times10^{-4}$M concentration each) is prepared. The metal and reagent solutions are mixed in different proportions, keeping the total volume of metal and ligand is constant at 5.0 mL. In each case, 4.0 mL of sodium acetate-acetic acid buffer (pH 5.0), 1.0 mL of 0.01% of thiosulphate solution are added to the mixture and the total volume of the solution is made up to 10.0 mL with double distilled water. The absorbances of all the solutions are measured at 351 nm against their reagent blanks. The corresponding graph (fig. 4) is drawn between absorbance and $V_M/V_L+V_M$ (where $V_L$ and $V_M$ are the volumes of the reagent and the metal, respectively). From the fig.4, it is identified that the composition of metal to ligand complex is 1:1 ratio. The composition of the M-L complex is further confirmed by molar ratio method (fig. 5 and fig. 6).

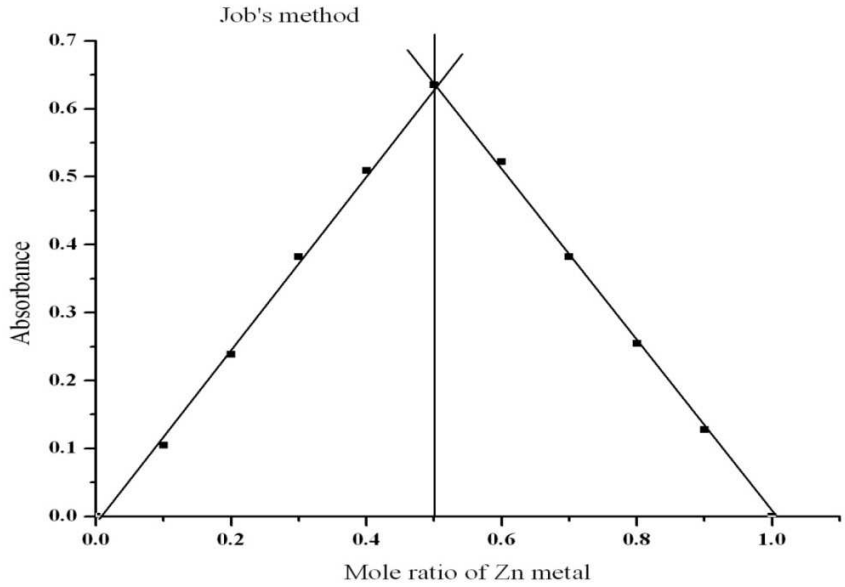



**Fig.4. Job's method of continuous variations for stoichiometric ratio between Zn(II) and HPEBH ($1.0\times10^{-4}$ M concentration each), buffer solution of pH 5.0 = 4mL and $\lambda_{max}$ = 351 nm**

### 3.5. Mole ratio method

**Effect of ligand concentration**

Into a 10-mL volumetric flask 1.0 mL of $1.0\times10^{-4}$M Zn(II) solution, 4.0 mL of buffer pH 5.0, 1.0 mL of 0.01% of thiosulphate solution and 1.0 mL of varying amounts of ($1.0\times10^{-5}$ to $2.0\times10^{-4}$M) reagent (HPEBH) are added and made up to the mark with double distilled water and measured the absorbance at 351 nm against the corresponding reagent blanks. From the results, it is clear that 1.0 mole of ligand is necessary for the maximum recovery of 1.0 mole of Zn(II). The results are plotted in the fig. 5.

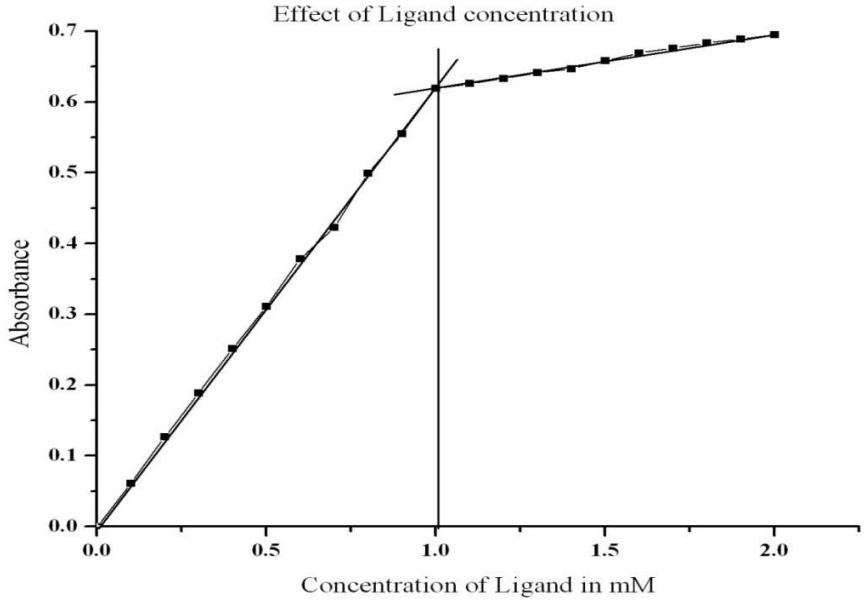



**Fig.5. Effect of Ligand concentration: HPEBH = 1.0 mL of $1.0\times10^{-5}$ -$2.0\times10^{-4}$M, Zn(II) =1.0 mL of $1.0\times10^{-4}$ M, buffer solution of pH 5.0= 4mL and $\lambda_{max}$ = 351 nm**

**Effect of metal ion concentration**

Different molar excesses of Zn(II) are added to the fixed amount of HPEBH and absorbance is measured according to the standard procedure. It is observed that the reagent (HPEBH) and the metal molar ratio is 1:1. Based on the above two methods the composition of the Zn(II)-HPEBH complex is confirmed as 1:1 ratio (fig.6).

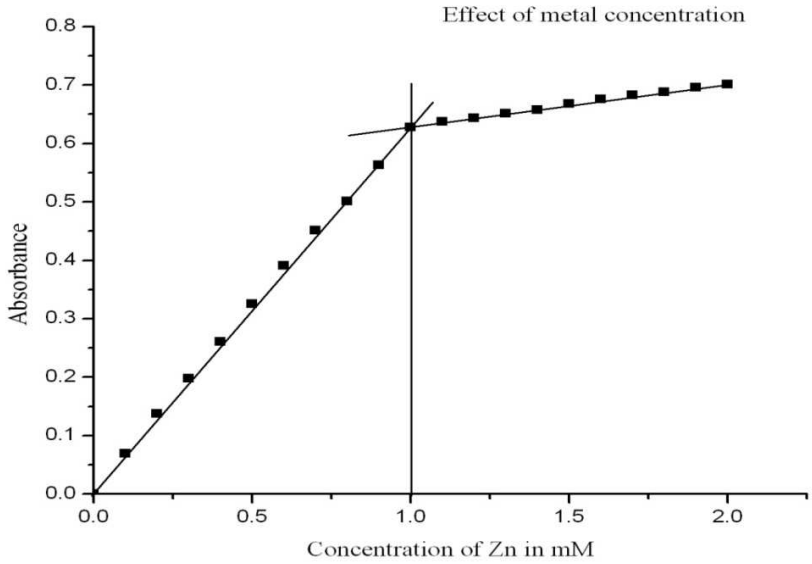



**Fig. 6. Effect of metal concentration: HPEBH =1.0 mL of 1.0× $10^{-4}$ M, Zn(II) =1.0 mL of 0.1×$10^{-4}$M-2.0× $10^{-4}$ M, buffer solution of pH 5.0 = 4mL and $\lambda_{max}$ = 351 nm**

### 3.6. Effect of foreign Ions

The effect of various cations which are generally associated with the metal ion on the determination of the Zn(II) is studied by measuring the absorbance of the zinc complex containing 1 µg $mL^{-1}$ of Zn(II) in solution. The effect of foreign ions on complexation is studied by taking 1.0 mL of Zn(II) solution, 1.0 mL of required concentration of the foreign ion solution, 4.0 mL of sodium acetate-acetic acid buffer (pH 5.0) and 1.0 mL of HPEBH solution in a 10-mL standard flask. The total volume of the solution is brought to 10.0 mL with double distilled water. The experiment is repeated by changing the concentration of the diverse ion. The absorbance is measured at 351 nm. A change of ± 0.02 is taken as the tolerance limit for interference. 1% potassium iodide is used for removal of interference of Co(II), Pb(II), Ni(II), and Cd(II) in the pH range studied.

**Table: 2. Tolerance limit of foreign ions**

| Foreign ions | Tolerance limit | remarks |
|---|---|---|
| Mg(II), Ca(II), Hg(II) and Mn(II) | 5000 µg | Less interference |
| Cr(III) and Al(III) | 2000 µg | Less interference |
| W(V) and Mo(VI) | 1000 µg | Moderate interference |
| Cu(II), [a]Co(II), [a]Ni(II), [a]Fe(II), [a]Cd(II) and [a]Pb(II) | 200 µg | More interference |

*amasked with 1.0 % Potassium iodide*

### 3.7. Scope of the method

The optimized method is successfully applied for the determination of Zn(II) in environmental matrices like food and water samples.

#### 3.7.1. Determination of Zn(II) in food and water samples

Food and water samples are collected from various places in and around Kadapa, A.P., India. The collected samples are digested as per the procedure reported in the literature [21, 22]. The prepared solutions are analyzed according to the optimized procedure. The obtained results are shown in the table: 3 and table: 4.

**Table: 3. Concentration levels of Zn(II) in food samples**

| S. No. | Name of the Sample | Scientific name | Concentration of Zn(II) by AAS Method[a](ppm) | Concentration of Zn(II) in the present method(ppm)* | Standard Deviation |
|---|---|---|---|---|---|
| 1 | Wheat grain | *Triticum aesivum* | 0.010 | 0.009 | 0.0006 |
| 2 | Cabbage | *Brassica oleracea* | 0.61 | 0.59 | 0.0040 |
| 3 | Carrot | *Daucas carota* | 0.037 | 0.035 | 0.0004 |
| 4 | Potato | *Solanum tuberosum* | 0.000 | 0.000 | 0.0000 |
| 5 | Tomato | *Lycopersicon esculentum* | 0.02 | 0.01 | 0.0020 |
| 6 | Vepaku | *Azadirachta indica* | 0.309 | 0.307 | 0.0006 |
| 7 | Thotakura | *Amaranthus Gangeticus* | 0.110 | 0.107 | 0.0010 |
| 8 | Chukkaku | *Rumex Vesicarius* | 0.093 | 0.091 | 0.0006 |
| 9 | Cauliflower green | *BrassicaDeraceavar batuties* | 0.160 | 0.157 | 0.0010 |

**Average of five readings* *[a]Atomic absorption spectroscopy*

**Table: 4. Determination of Zn(II) in water samples**

| Sample | Zn(II) (µg/L)-(Spiked) | Concentration of Zn(II) by AAS Method | concentration of Zn(II) by the present method | Recovery (%) |
|---|---|---|---|---|
| Waste water[1] | 100 | 102.10 | 101.98 | 101.98 |
| | 500 | 510.10 | 510.00 | 102.00 |
| Sea water[2] | 100 | 101.80 | 101.50 | 101.50 |
| | 500 | 507.6 | 507.55 | 101.51 |
| Tap water[3] | 100 | 101.00 | 100.98 | 100.98 |
| | 500 | 505.03 | 504,50 | 100.90 |

*[1]Collected from industrial area, Kadapa.* *[2]Bay of Bengal, Nellore.* *[3]Yogi Vemana University, Kadapa.*

### 3.7.2. Preparation of synthetic mixtures

Metal ion solutions of $Cu^{2+}$, $Fe^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Mn^{2+}$, $Hg^{2+}$, $Pd^{2+}$, $Pb^{2+}$ $Cd^{2}$ and $Zn^{2+}$are prepared from Merck-analytical grade stock standards of concentration 1000 mg/L. The synthetic water solutions are then prepared by mixing the different metal ions as prescribed in the table: 5. The aliquot is analyzed for Zn(II), using HPEBH adopting the recommended procedure.

**Table: 5. Determination of Zn(II) in synthetic mixtures**

| Samples | Metal 1 (2µg/mL) | Metal 2 (2µg/mL) | Metal 3 (2µg/mL) | Metal 4 (4µg/mL) | by AAS method (µg/mL) | by Present Method (µg/mL) | % of recovery |
|---|---|---|---|---|---|---|---|
| 1 | Fe | Co | Ni | Zn | 3.97 | 3.96 | 99.00 |
| 2 | Co | Ni | Mn | Zn | 3.99 | 3.98 | 99.50 |
| 3 | Fe | Cd | Ni | Zn | 3.97 | 3.95 | 98.75 |
| 4 | Pb | Co | Ni | Zn | 3.96 | 3.96 | 99.00 |
| 5 | Cu | Pd | Mn | Zn | 3.98 | 3.96 | 99.00 |

## CONCLUSION

A rapid spectrophotometric method for the determination of Zn(II) in food materials, water and synthetic mixture has been developed by using HPEBH as a fairly sensitive and selective analytical reagent. The proposed method offers advantages like good sensitivity, selectivity, reliability, reproducibility, less interference and immediate color development. The developed method is found to be quantitative comparable to other standard methods. The molar absorptivity of the complex ($6.27\times 10^4$ L $mol^{-1}cm^{-1}$) reveals that the ligand is highly sensitive for Zn(II) when compared with other hydrazones. Hence, HPEBH is an alternative ligand for the spectrophotometric determination of trace amount of Zn(II) in various environmental matrices.

**Acknowledgement**

We gratefully acknowledge the Department of Atomic Energy-Board of Research in Nuclear Sciences (Bhabha Atomic Research Centre), Mumbai, India for providing the financial support of this work through a major research project (No. 2011/37C/52/BRNS/2264).

**REFERENCES**

[1] C.J. Frederickson, J.Y. Koh, A. I. Bush, *Nat. Rev. Neurosci*., **2005**, 6, 449-453.
[2] J. H. Weiss, S. L. Sensi, J. Y. Koh, *Trends Pharmacol. Sci*., **2000**, 21, 395–401.
[3] B. Krishna Reddy, J. Rajesh Kumar, L. Subramanyam Sharma, A. Varada Reddy, *Anal. Lett*., **2002**, 35(8), 1415-1427.
[4] D. Nagarjuna Reddy, K. Nagabushan Reddy, *Int. J. Anal. Bioanal. Chem*., **2011**, 1(3), 77-81.
[5] D. Nagarjuna Reddy, K. Vasudevareedy, K. Hussain Reddy, *Der Pharma Chemica*., **2011**, 3(2), 496-504.
[6] K. Janardhan Reddy, J. Rajesh Kumar, C. Ramachandraiah, T. Thrivani, A. Varada Reddy, *Food. Chem*., **2007**, 101, 585-591.
[7] A. Fakruddin, Y. Lingappa, *Int. J. Curr .Pharm. Res*., **2011**, 3, 24-26.
[8] M. Tazul Islam, M. Jamaluddin Ahmed, *Pak. J. Anal. Environ. Chem*., **2013**, 14(1), 1-15.
[9] Lengoc, Le van Tan, Nguyen Xuan Chien, *Eur. Chem. Bull*., **2013**, 2(6), 311-314.
[10] S. Sivaramaiah, P. Raveendra Reddy, *J. Anal. Chem*., **2005**, 60(9), 933-937.
[11] Sapana Garg, Devender Sing, Sonia Verma, Pratap Sing, Kadyan, *J.Chem.Bio. Phy. Sci*., **2012**, 2(3), 1746-1752.
[12] D. Nagarjunareddy, K. Vasudevareddy, K. Hussain Reddy, *J. Chem. Pharm. Res*., **2011**,3(3), 205-213.
[13] C. Viswanatha Radhakrishna, N. Devanna, *Research Journal of Pharmaceutical, Biological and Chemical Sciences*., **2015**, 6(1), 1106-1111.
[14] S. Lakshmi Narayana, S. Young Kyo, B. Sung Ok, A. Varada Reddy, e-*Journal of Chemistry*, **2012**, 9(3), 1288-1304.
[15] G. Sumathi, T. Sreenivasulu Reddy, *J. Chem. Bio. Phy. Sci*. Sec. A., **2016**, 6(1), 001-006.
[16] S. Sivaramaiah, P. Raveendra Reddy, *Journal of Analytical Chemistry*, **2005**, 60, 828-832.
[17] M. E. U. Pozo, A. G. D Torres, J. M. C. Pavon, *Analyst*., **1988**, 113, 547-549.
[18] S. Shit, J. Chakrabarty, B. Samanta, A. M.Z. Slawin, V. Gramlich, S. Mitra, *Struct Chem* **2009**, 20(4), 633-642.
[19] P. Nityananda Kumar Reddy, G. Trivikram Reddy, Sangita D. Kumar, A. V. R. Reddy,S. Nazneen Parveen, N. C. Gangi Reddy , *J. Chem. Pharm. Res*., **2015**, 7(11), 121-130.
[20] A.I. Vogel. A Textbook of Quantitative Inorganic Analysis, 3rd edition, Longman Green publishers, London, U.K, **1961**, 444.
[21] S. Satyaveni, Ph.D thesis, Sri Venkateswara University, (Tirupati, AP, **2007**).
[22] S. Adinarayana Reddy, K. Janardhan Reddy, A. Varada Reddy, *J. Chin. Chem. Soc*., **2010**, 57, 236-243.