Spectrophotometric Determination of Zn(II) in Pharmaceutical Formulation Using a New Azo Reagent as Derivative of 2-Naphthol

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Abstract

For the fast investigation of Zn(II) using 2-hydroxy-5-(2-hydroxynaphthalen-1-yl) diazenyl benzoic acid, a specific novel spectrophotometric technique is suggested in this research. The interaction of the azo reagent with Zn(II) is instantaneous at pH 7, and the absorbance of the solution is stable for more than 24 h. The technique allows zinc levels of 1-18 ppm to be calculated, with a molar absorption of 1.516 $x10^4$ l mol^{-1} cm^{-1}. The suggested method for estimating zinc has been widely applied in many pharmaceutical formulations. The error of the determination does not exceed 4%.

Keywords: azo reagent; determination of zinc; spectrophotometry; pharmaceutical preparations
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1. Introduction

Zinc is commonly found in animal tissues at an average concentration of 20-30 mg/1 g of fresh tissue. Biochemically, zinc is a co-factor for several enzymes, for example carbonic anhydrase and alcohol dehydrogenase. Additionally, zinc is also attached to RNA. It is an essential element for healthy growth, and helps to maintain the plasma concentration of vitamin A, which has resulted in it being used in a large number of multi minerals preparations. In recent years, pharmaceutical preparations have incorporated a low concentration of zinc ion [1-3]. Many techniques for evaluating zinc (II) ions, particularly fluorometric techniques [4, 5], atomic absorption spectroscopy [6, 7] as well as spectrophotometric investigation [8, 9], have therefore been developed. In pharmaceuticals formulations, the zinc (II) ion has often been calculated through the use of azo dyes [10, 11]. The colored solutions of azo compounds have been widely studied in the field of inorganic and analytical chemistry and have generally been used during the spectrophotometric determination by UV-Vis spectra of metal ions in low concentration [12]. For example, the compound 2-carboxy-2'-hydroxy-5'-sulfoformazylbenzene, used as an analytical reagent, was observed to give a zinc complex that was stable over the pH range 8.5 to 9.5 and within the concentration range 0.1 to 2.4 ppm obeyed Beer's law [13]. Also, 4-(2-pyridylazo)

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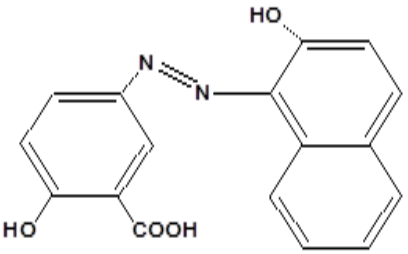
resorcinol (PAR) was used in the determination of zinc (II) and produced a colored complex that obeyed Beer's law in the range 0.025-13 ppm, and the elaborated method was applied successfully in the determination of zinc ions in pharmaceutical preparations [14]. In this research, 2-hydroxy-5-(2-hydroxynaphthalen-1-yl) diazenyl benzoic acid (HNABA) is used as the reagent in a basic yet efficient spectrophotometric approach for evaluating the low concentrations of Zn(II) found in pharmaceutical formulations. The reagent undergoes a sensitive color producing reaction with Zn(II) in the presence of different pH values, and the prevailing color characteristic of the resulting zinc-complex solution and its stability are ideal for the determination of zinc ions. A solid zinc chelate with ligand was prepared and characterized to confirm the formation of zinc-azo complex in solution and to identify the structure of the formed complex by FT-IR and UV-Vis spectra.

2. Materials and Methods

A Shimdzu 1800 double beam spectrophotometer was used for all absorbances, whereas FT-IR spectra were obtained on Shimadzu 8400s double beam spectrophotometer in KBr disks, including spectra in ethanol with apparatus-digital. The pH measurements were made by using HI 9321 HANNA pH meter.

Reagents: The HNABA as azo reagent (Figure 1) was prepared by dissolving the amine (5-amino salicylic acid) in a mixture of hydrochloric acid and absolute ethanol and stirring for 15 min an ice bath and then an ice-cold solution of $NaNO_2$ (10 %) was added dropwise into the solution over a period of 30 min. The solution was brown, and an ice-cooled 2-naphthol solution in alkaline ethanol was added with continuous stirring at 0-5 ºC, and left overnight. The mixture was made neutral at pH=7 with dilute hydrochloric acid or ammonia solution. The solid product was filtered, washed with cold distilled water, and left to dry. The synthesis was performed following previous research [15].



(*E*)-2-hydroxy-5-((2-hydroxynaphthalen-1-yl)diazenyl)benzoic acid

Figure 1. Structural formula of azo reagent

Zn(II) standard stock solution: Zn(II) standard stock solution (100 ppm) was obtained by dissolving Zn(II) in 100 ml volumetric flask for a precisely calculated amount of zinc sulphate. The zinc complex was then obtained by dispersing (2 mmol) ligand in 25 ml ethanol as well as mixing dropwise to a stoichiometric quantity of 1:2 (metal: ligand) molar ratio of zinc sulphate salt, and dissolved in 20 ml of hot distilled water. The pH of the reaction mixture for the complex was adjusted at optimum pH. The resulting solution was stirred for 2 h under reflux. The

complex's solid product was filtered, washed with distilled water, recrystallized using absolute ethanol, and dried overnight at 50ºC.

Buffer solution: Buffet solution was prepared by dissolution of 0.7708 g ammonium acetate into 95 ml of water followed by the addition of a few drops of acetic acid as well as ammonia to modify the pH.

Pharmaceutical samples: The contents of ten tablets and capsules containing the ion were weighed, processed into a fine powder, and the equivalent amounts of one capsule or tablet (0.015 g -0.025 g) of drug were dispersed in 70 ml buffer solution, filtered and then brought up to 100 ml. For syrup formulation, the content of 16.6 ml of the drug equivalent to 0.01g of zinc of the product tested, the volume was introduced to the buffer solution, and checked.

3. Results and Discussion

Zinc (II) ion reacts with 2-hydroxy-5-((2-hydroxynaphthalen-1-yl) diazenyl) benzoic acid (HNABA) and produces a red colored complex in neutral medium at pH=7. HNABA was selected as the molecule because it has more than one functional group that has the ability to form a chelating complex with the metal ion. It has been found that the reagent solutions are characterized by their rapid reaction with the metal ion solution in addition to their high stability of the resulting complex.

3.1 Absorption spectra of the reagent and Zn(II)- complex

Under optimal conditions, the absorbance spectra of the zinc (II) complex against blank as well as the reagent solution were measured over the range 200-800 nm. The reagent 's electronic spectra clearly show two main bands of absorption attributing to π-π * transitions at 198-216 nm and another band at 466 nm related to n-π* transitions [15]. It is noted that this solution showed a spectral color change from yellow to red and showed a peak absorption at 500 nm of the zinc-complex solution that is distinguished by its appearance in a location that differs from the location of the reagent spectra and this is clear evidence that there is coordination between Zn(II) ion and the reagent (Figures 2 and 3).

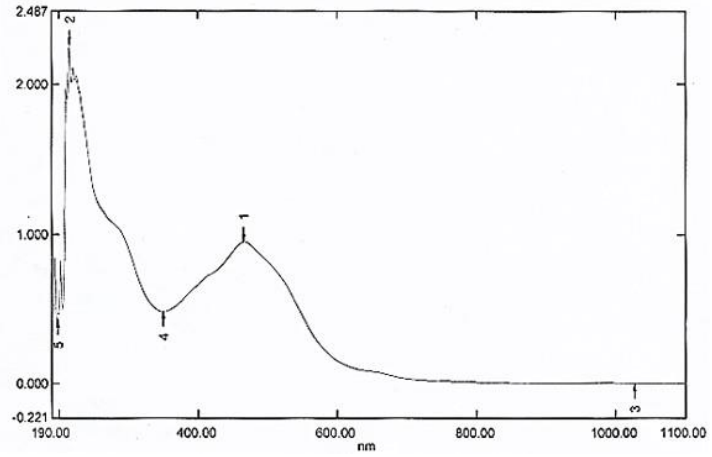


Figure 2. The absorption spectra of $1x10^{-4}$ M azo reagent

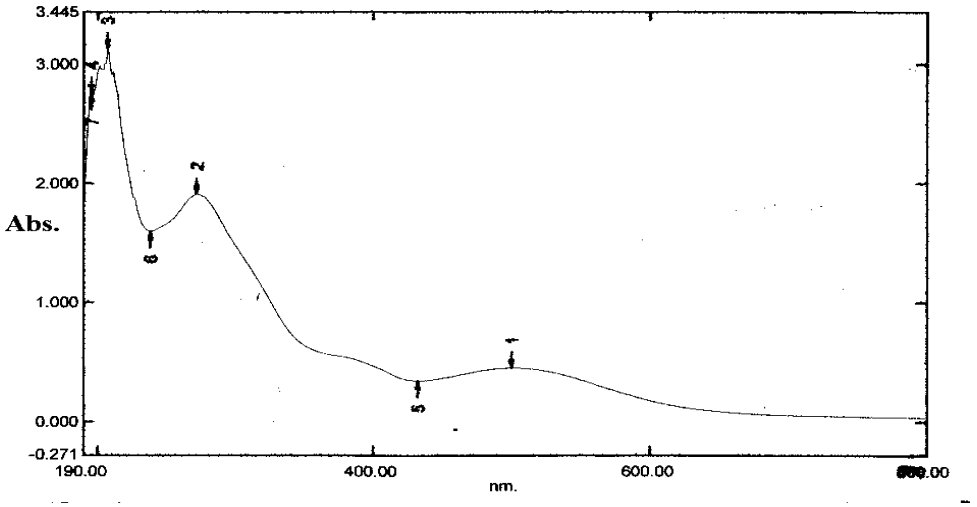


Figure 3. The absorption spectra of zinc-complex at pH 7 and 18 ppm (recorded against reagent blank)

3.2 Calibration curve

A wide range of concentrations (0.1-30 ppm) of mineral ions solutions were studied using the ligand, and it was observed during the preparation of mixing solutions with high concentrations (19-30 ppm) that precipitation occurred immediately after mixing the reagent and zinc ion solutions, which limited the spectral measurement process for these concentrations. As for the solutions that are less concentrated than the solutions mentioned, it has been shown that they obeyed the Lambert Bear law, but this did not apply right through the concentration range studied because some were almost colorless. The absorption values were measured at λ_{max} (500 nm), and the linear calibration curve followed the Lambert-Beer Law in a concentration range from 1 to 18 ppm. The coefficient (r^2) of 0.9988 and also the value of regression equation ($y = 0.0212x$) were noted and are shown in Figure 4. The analytical variables are described in Table 1.

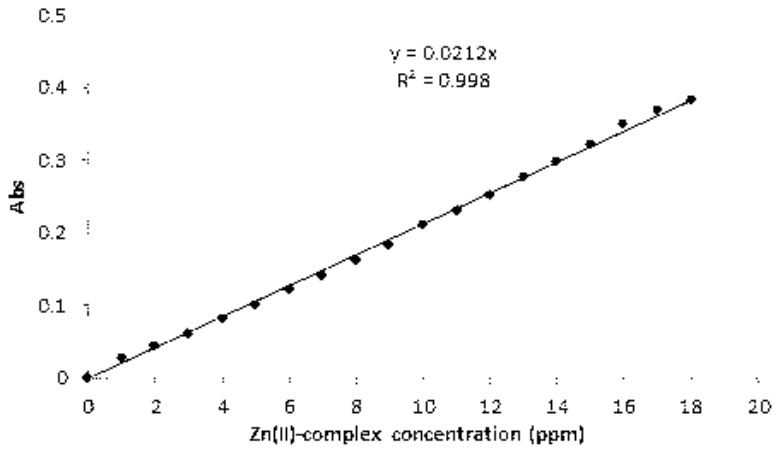


Figure 4. Linear relation between absorbance and Zn(II)-complex concentration (ppm)

Table 1. Analytical Parameters for Zn(II) - complex determination by azo reagent

Analytical Parameters	Value	Analytical Parameters	Value
λ_{max}(nm)	500	Sensitivity (µg cm^{-2})	0.003
Beers range (µg ml^{-1})	1-18	Coefficient (R^2)	0.998
Molar absorptivity (l mol^{-1} cm^{-1})	1.516 $x10^4$	(Metal: Ligand)	1:1
pH	7	Stability constant (β) (l mol^{-1})	6.5 $x10^5$
RSD%	0.433		

3.3 Optimum pH for formation of Zn(II)- complex

Appropriate pH levels were identified in the range of 5-10 for metal complex solutions. To assess the optimum pH value of metal-complex solutions, an ammonium acetate buffer solution was used. The pH against absorbance graph suggested that the optimal pH level for reagent-based Zn(II) complex forming was pH=7, as shown in Figure 5. and Table1. When the pH was increased to greater than 7, the probability of the formation of zinc hydroxide instead of zinc complex increased. The pH being lower than 7 led to the protonation of azo ligand and made the formation of complex difficult.

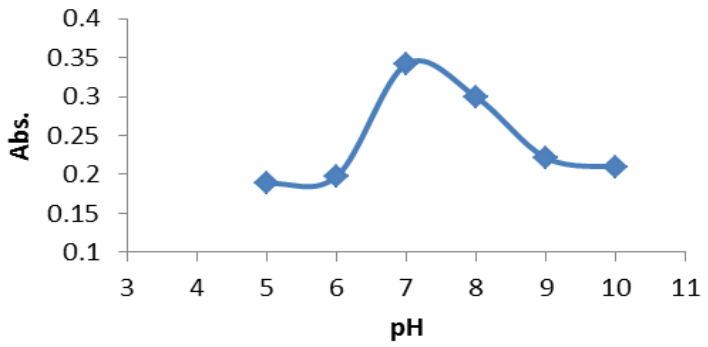


Figure 5. Effect of pH on the absorbance of Zn(II)-complex

3.4 Composition and stability constant for the complex

The formation of the complex was assessed by the molar ratio method [16] and under optimum conditions, the metal: ligand stoichiometry was determined to be 1:1. A stabilization constant was calculated from the absorbance data of ligand and metal ion mixture solutions at λ_{max} = 500 nm and pH=7, with the relationship $\beta=(1-\alpha)/(\alpha^2 c)$ for 1:1 metal complexes, where α=Am-As/Am. Am and As, in optimal level circumstance, are the absorbance values of the complete additionally partially formed-complex, respectively. Table 1 and Figure 6 display the measured (logβ) quantities for $[ZnL(H_2O)_2]$ complex. The reaction was performed at room temperature and its products stay stable for 24 h. This is due to the high stability of Zn(II)-complex as shown in Figure 7.

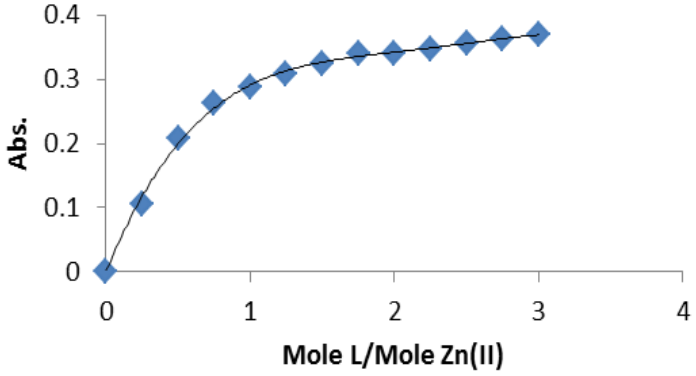


Figure 6. Mole ratio method for Zn(II)-complex

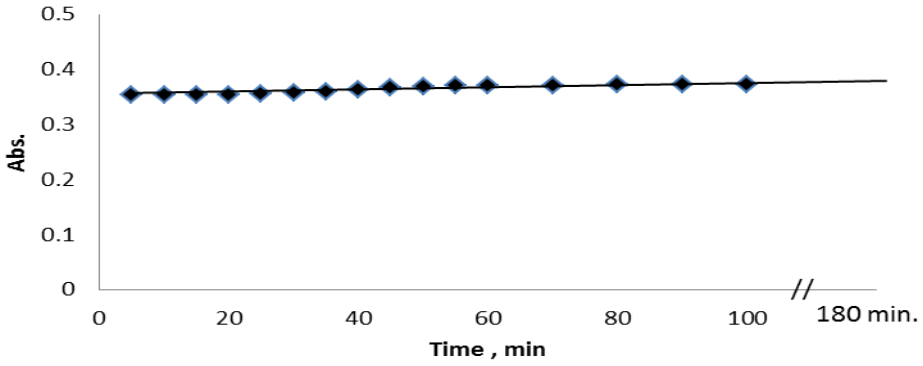


Figure 7. The Relation between absorbance and reaction time of Zn(II)-complex

3.5 Conductivity measurements

Table 2 describes the molar conductivity measurement data of 10^{-3} M Zn-complex in methyl alcohol and in DMF solvent, both at 25° C. The amount level for the molar conductance appears that the complex is non-electrolyte [17].

Table 2. Conductance measurements data of Zn-complex

Complex	**Λm(S.mol^{-1}.Cm^{2})**	
	In Methanol	In DMF
[ZnL $(H_2O)_2$]	10.54	12.16

3.6 Infrared spectra

The reagent FT-IR spectrum showed a wide band at 3419 cm^{-1} υ(O-H), and two more bands at 1666 υ(C=O), and 1489 cm^{-1} υ(N=N). For the zinc-complex spectra, it was found that the band of υ(C=O) at 1666 cm^{-1} had shifted to 1618 cm^{-1} and a new weak band at 623 cm^{-1} related to υ(M-O), suggesting that the carboxylic group and hydroxyl group had coordinated with Zn(II) ion [18]. On the other hand, the band of the azo group in the zinc-complex spectra appeared at the same frequencies that it had in the free reagent, suggesting that the N=N group had not coordinate with zinc ion (Figures 8-9 and Table 3).

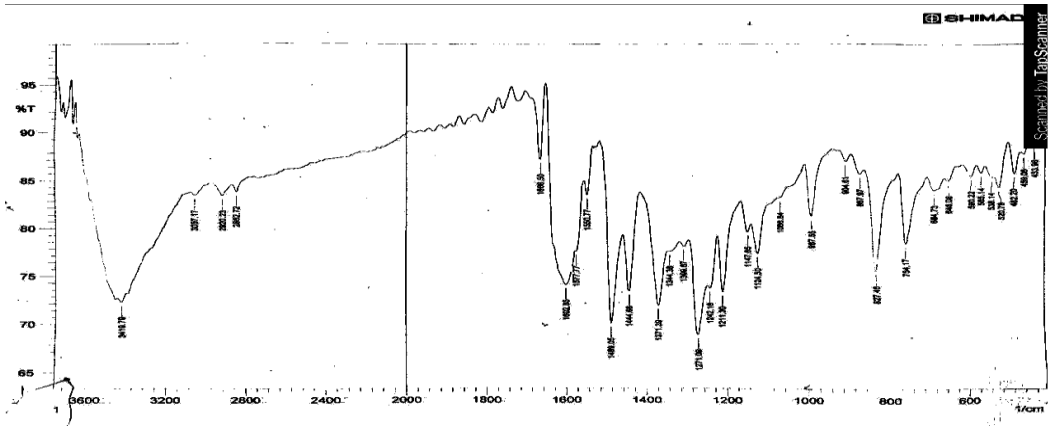


Figure 8. FT-IR spectra of the HNABA reagent

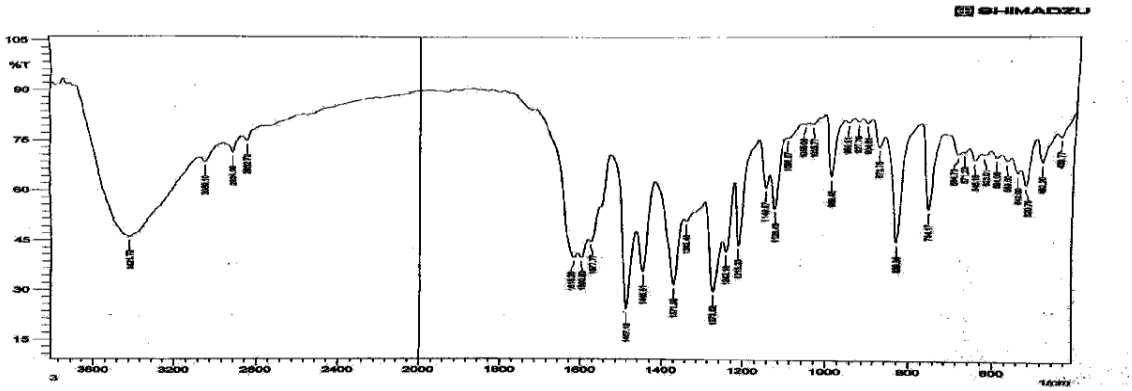


Figure 9. FT-IR spectra of Zn(II) complex

Table 3. FT-IR data of azo reagent and its complex with Zn(II) in cm^{-1} unit

Compound	υ(OH)	υ(C-H) arm.	υ(C=O)	υ(N=N)	υ(C=C)	υ(M-O)
Azo reagent	3419	3057	1666	1489	1602	----
$[ZnL(H_2O)_2]$	3421	3059	1618	1487	1602	623

3.7 Suggested structural formula of Zn- complex

Based on measurements, spectroscopic studies, and the molar conductivity for the reagent HNABA and its zinc-complex, we suggest that the reagent is a bidentate chelating moiety joined to Zn(II) metal ion through the O atoms of salicylic acid, with water molecules presenting as coordinating ions. These generate a tetrahedral structure (Figure 10).

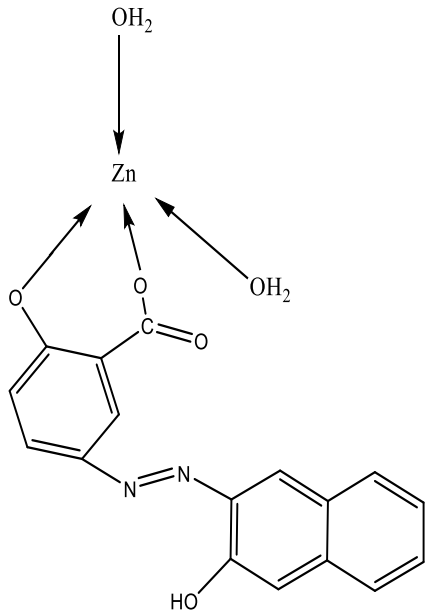


Figure 10. The suggested Zn(II)-complex fundamental formula

3.8 Effect of interference ions

The influence of different interference ions on the absorption of the solution comprising Zn(II) was calculated under optimal conditions. The different ions researched including Cu(II) as well as Fe(II), and the findings are described in Table 4. Foreign ions that do not interfere when introduce as masking agents (or not as masking agents) produce less than 4% error in an analytical recovery.

3.9 Identification of Zn(II) in pharmaceutical formulations

The described approach has indeed been extended to Zn(II) identification of Zn(II) in various pharmaceutical specimens (syrup, tablet and capsule) by spectrophotometric technique utilizing 2-hydroxy-5-(2-hydroxynaphthalen-1-yl) diazenyl benzoic acid as the azo reagent. The information collected in the evaluation is provided in Table 5, and the findings are described in comparison with other documented spectrophotometric technique (Table 6).

Table 4. Influence of interfering cations and anions as well as masking agent on Zn(II)'s relative error (E percent)

Foreign Ions	(E%)	Rec.%	R.S.D%
Fe(II)	2.09	102.09	0.70
Cu(II)	4	104	1.20
EDTA	1.16	101.16	0.90
Oxalate	3.5	96.5	1.30
Tartrate	0.24	99.76	0.88

Table 5. Evaluation of Zn(II) in Pharmaceutical formulations

Pharmaceutical formulations	(E%)	Rec.%	R.S.D%
Zinc tablet	-0.7	99.3	0.771
Zinc capsule	-1.2	98.8	0.562
Zinc plus with multivitamin syrup	1.6	101.6	0.812

Table 6. Comparison of chosen reagents utilized for Zn(II) spectrophotometric evaluation

Reagent	λ_{max} (nm)	pH	Ɛ(L mol^{-1} cm^{-1})	Linear rang (µg ml^{-1})	M:L	Reference
7-(4-nitrophenylazo)-8-hydroxyquinoline-5-sulphonic acid	520	9.2	3.75×10^4	0.05-1	1:2	[19]
3-(2,4-dihydroxy-1-phenylazo)-1,2,4-triazole)	490	10	4.86×10^4	2.6- 9	1:2	[20]
4-(2-arsonophenylazo) salicylic acid	525	6	1.36×10^4	0.5-7	1:1	[21]
2-(2,4-dihydroxyphenylazo) benzimidazole	540	9.23	----	0.06-1.44	1:2	[22]
3-(5-mercapto-1,2,4-triazolo-3-azo)-2,6-dihydroxybenzoas acid	480	7.35	----	0.05-2.25	1:2	[23]
HNABA (azo reagent)	500	7	1.516×10^4	1-18	1:1	P.M

4. Conclusions

The coloring-developing between HNABA azo reagent and Zn(II) was systematically investigated, as was the procedure for the assay of Zn(II) by using Zn(II)-complex coloring reaction. The proposed method showed that maximum absorbance was attained at 500 nm when using UV-Vis. Spectrophotometer, and optimum conditions at pH 7 and 1-18 ppm concentration. This new analytical method was relatively simple, rapid and sensitive in comparison with other spectrophotometric methods that involve the direct interaction between the zinc ion solution and the reagent solution used for estimation. In addition to the low limit of zinc that can be detected, the analysis of a wide range of concentrations follows the Lambert-Beer's Law, particularly in the concentration range from 1 to 18 ppm.

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