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Original Article

Preparation and Characterization of New Hetrocyclic Azo Thiozal Dye Ligand and Its Use as a Reagent for Determination of Zn^{+2} Ion in Drug by New Analytical Method

Thekrayat Joodi Jassim[1,*], Haider M. Hessoon[2], Faiq F. Karam[2]

[1]*Department of Pathological Analysis, College of Science, University of Sumer, Thi-Qar, 64005, Iraq*
[2]*Department of Chemistry, College of Science, University of Al-Qadisiyah, Al-Diwaniyah, Iraq*

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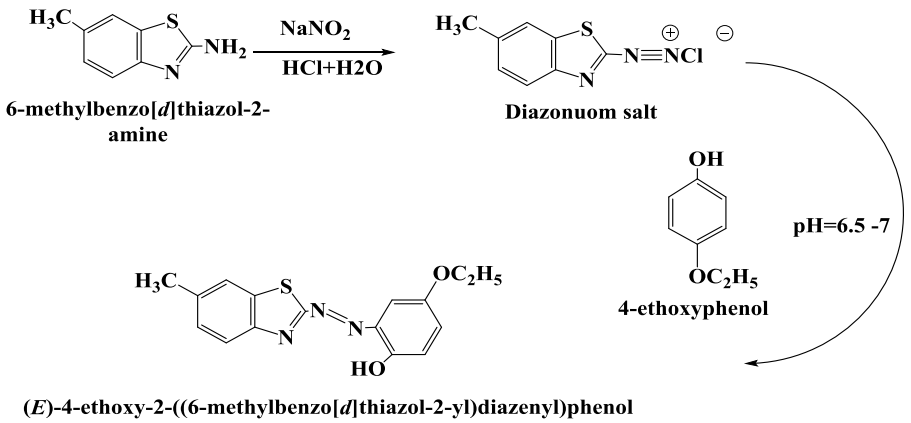
ABSTRACT

A novel heterocyclic organic ligand was synthesized by using a novel azo reagent and characterized by the organic diagnostic methods such as FT-IR, ^{1}H-NMR, and UV-Vis. The novel azo reagent was employed in the analytical approach to find trace levels of zinc(II) ion in several drug models. The complex was formed after studying the optimal conditions for its synthesis in a reagent: zn^{+2} ratio of 1:1. The largest absorption peak was seen at 620 nm in wavelength. In contrast, the reagent wavelength was 475 nm. The linear range was between concentration and absorption at longer wavelength ranges 0.3 - 20 mg/L. The approach has an accuracy of percent RSD 0.9259 and SD =0.0005. Sandal sensitivity, LOD, and LOQ are each 0.2632, 0.8772 g/mL, and 0.1754 $g.cm^{-2}$, respectively. The impact of various positive and negative ions on complex absorption was examined to demonstrate the method's selectivity. Under the optimal pH 6.5 to 7 conditions and an absorption temperature of 25 to 40 °C, the percentage recovery of the drug by the suggested method varied from 95 to 104 %.

GRAPHICAL ABSTRACT



* Corresponding author: Thekrayat Joodi Jassim
✉ E-mail: Email: asaljood3@gmail.com

Introduction

Azo compounds contain an azo group (-N=N-) bridge and have recently been used in many fields [1, 2]. These compounds have many advantages, including the ease of preparation, high purity, and abundant product. These facts motivated scientists to concentrate on researching this class of chemicals and the potential for using them in various applications. In addition, it has been utilized as an anti-cancer chemical and has successfully treated a variety of cancers [3]. Azo compounds are coordination complexes containing transition elements that may attach to the DNA bases via their coordination with nitrogen atoms to create a chelating ring, making them useful in medicine [4, 5]. Furthermore, Azo compounds are anti-bacterial and anti-fungal materials [6, 7].

Zinc was one of the earliest discovered metals. It was referred to as brass when alloyed with copper (40% Zinc and 60% copper) [8]. Zinc is resistant to attack in dry air at normal temperatures, but loses its luster in the moist air, creating basal carbonate, which functions as a protective cover, preventing further corrosion [9]. It is an essential component of human, animal, and plant life, as well as a common biological component of nature [10]. This ion is found in a few types of medicines [11]. Zinc has a boiling point of 904 °C and shines white at 424 °C. However, it loses its shine after a short time because of the exospheric condition [12]. Zn^{+2} has been characterized in several methods, including Flow Injection and Sequential Injection Techniques [13], Atomic Absorption Spectrophotometry [14], Cloud Point Extraction, Spectrophotometric Detection [15], Electrochemically [16], and development of HPLC method [17].

Materials and Methods

High-purity analytical materials were employed to make the solvents and compounds in this investigation. Zinc chloride was supplied by ROMIL and had a purity of 98 percent. Osteocare, Ferglobin, Zainc, Vitadex, and Calcipare were the utilized drugs. The majority of these medications are manufactured by German firms Fluka, BDH, Merck, and Sigma Aldrich. On a Bruker MHZ spectrophotometer, H-NMR spectra were captured by using DMSO-d_6 as the solvent and TMS as the internal reference. The infrared spectra of Azo ligand were obtained as KBr discs by using a Shimadzu 8400 S FT-IR spectrophotometer in the 400 to 4000 cm^{-1} range.

Synthesis of thiazolyl azo dye ligand

The azo dye ligand was synthesized to use it in the same method [18, 19] with some modifications. 6-methylbenzo[d]thiazol-2-amine (1.64 g, 0.01 mol) was mixed with 3 mL of HCl, and 20 mL distilled water and diazotized below 4 °C with $NaNO_2$ (0.75 g, 0.01 mol), dissolved in 35 mL. The diazonium salt was combined with (1.388 g, 0.01 mol) of 4-ethoxyphenol in alkaline media within 4 °C. The solution had a reddish-orange tint. Therefore, it was stirred for 60 minutes at 0 to 5 °C. Later, the solution was filtered, washed with distilled water several times, crystallized with 100% ethanol, and dried in an oven at 45 °C for a few hours. Scheme 1 displays the process of diazotase coupling.

Preparation of standard stock solution

Preparation of zinc ion for complexity

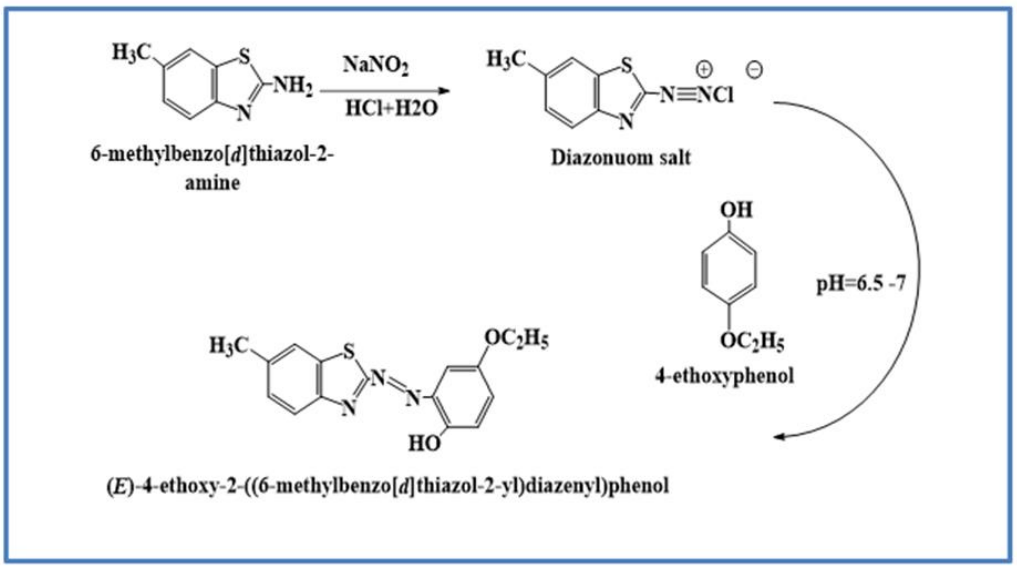
A 100 mg/L stock solution was prepared by dissolving 0.0209 g of water in 25 mL capacity Baker. The solution was then transferred to a 100 mL bottle, completed the size to the mark limit with pH=6.5 to 7, and adjusted by using the regulator solution to attend various concentrations by using the mitigation law.

Preparation of complex solution

A 1.5 mL of 5 mg/L and pH of 6.5 to 7 ion stock solution were mixed with 1.5 mL of the new organic reagent at 1×10^{-4} M concentration. Afterwards, the absorption was measured at 620 nm wavelength. The experiment was repeated under the same conditions, and average values were considered.

A.3. Positive ions interferences solutions

A 100 mg/L stock solution containing positive ions was prepared by dissolving certain weights



Scheme1: A heterogeneous synthesis of the new Azo ligand (*E*)-4-ethoxy-2-((6-methylbenzo[d]thiazol-2-yl)diazenyl)phenol

of each substance in 100 mL distilled water and attended concentrations of positive ions for successive dilution.

Results and Discussion

Spectroscopic study for characterization of new hetrocyclic azo Ligand

^{1}H-NMR

As displayed in Figure 1, the spectra of azo ligand were measured by using (DMSO-d_6) as a solvent in (TMS). The azo ligand was used as an internal reference (500 MHz). The parameters recorded for the ligand are illustrated in Table 1 [10, 20].

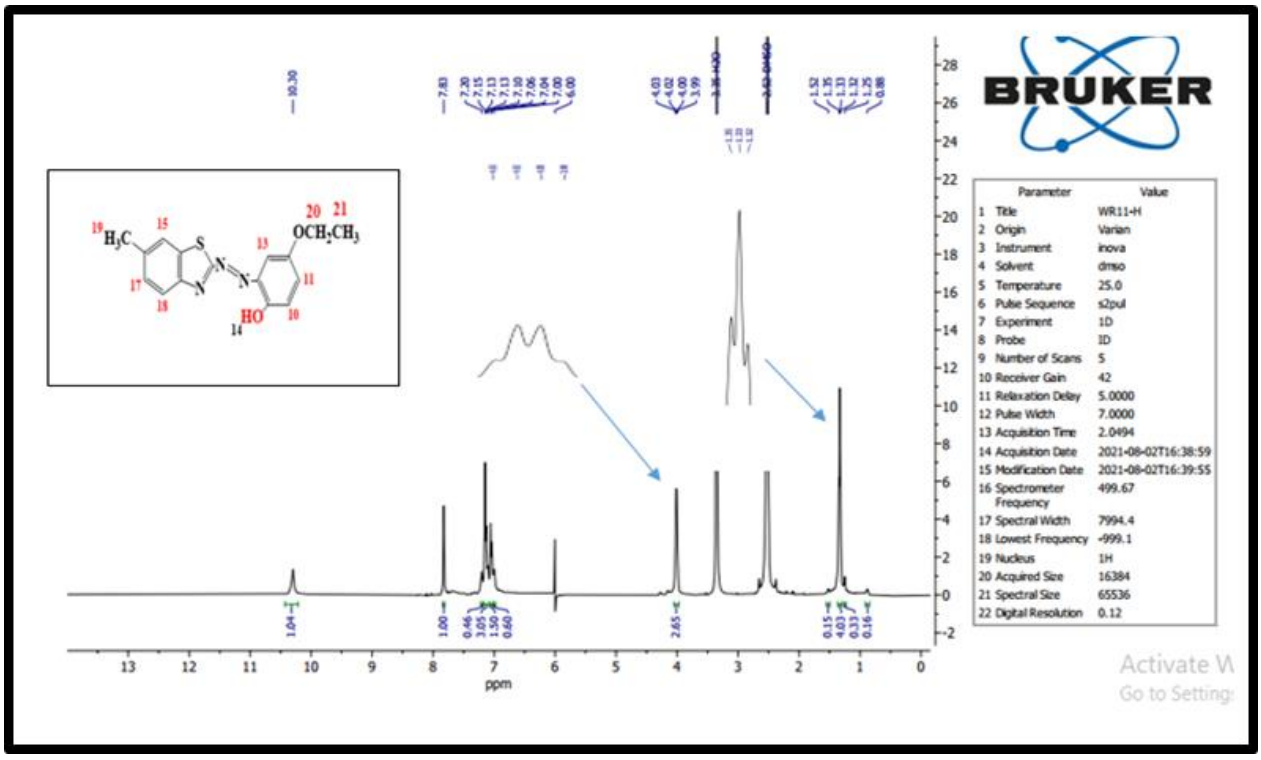


Figure 1: Spectrum ^{1}H-NMR for azo ligand

Table 1 : ^{1}H-NMR spectra of azo ligand

ligand (H atoms-peak-assignment)
1.32-1.35 (5H, t, 21)
2.4 (3H, S, 19)
2.52 solvent proton
3.99-4.03 (5H, q, 20)
6.00-7.10 (3H, S, 10, 11, 13)
7.13-7.20 (3H, S, 15, 17, 18)
10.30 (1H, S, 14)

Infrared spectra (FT-IR) for azo ligand

Table 2 presents the vibration bands stretching for functional groups in ligand and [Zn(L)Cl] in the 400-4000 cm^{-1} range of measurement [21, 22].

Study of precise analysis of reagent elements CHNS

The accurate analysis technique was used to characterize the new reagent by calculating the proportions of carbon, hydrogen, sulfur, and nitrogen elements, as summarized in Table 3. When comparing the values listed in the above table that were obtained in practice with the theoretically calculated values, the great convergence between them is clearly evident, that confirms the correctness of the formation of the solid reagent.

The electronic spectra measurement

The spectra of azo ligand were measured at room temperature at 1×10^{-4} M absolute ethanol. The results are listed in Table 4 and Figure 2.

Table 2: Stretching vibration for function groups to the ligand

Compounds	*ν(OH)*	*ν (CH_3)*	*ν (C=N)*	*ν (N=N)*	*ν (C=C)ph*	*ν (C-S)thia*	*ν (C-N)thia*	*ν (M-N)*	*ν (M-O)*
Azo ligand	3376.13 m.br	3055.42 w	1599.22 w	1482.44 s	1412.59 w 659.99 w	1286.83 s	1177.86 s	-	-
[Zn(L) Cl]	-	3067.43 w	1576.44 w	1477.43 m	1433.65 w 633.43 w	1247.76 s	1171.44 s	566.87 w	*434.32 w*

Where, br = broad, w= weak, m= medium, and s=strong

Table 3: Accurate element analysis (C.H.N.S) results of the prepared solid reagent

Compound	C %		H %		N %		S%	
New Reagent	Theoretical	Practical	Theoretical	Practical	Theoretical	Practical	Theoretical	Practical
	61.32	61.20	4.83	4.79	13.41	13.37	10.23	10.20

Table 4: The electronic spectra cm^{-1} and nm

Compounds	λ_{max} nm	Absorption bands (cm^{-1})	Transition
Azo ligand	475	21052	$n \rightarrow \pi^*$
	340	29411	$\pi \rightarrow \pi^*$
Zn-complex	620	16129	M→L,CT
	430	23255	M→L,CT
	310	32258	Ligand centered

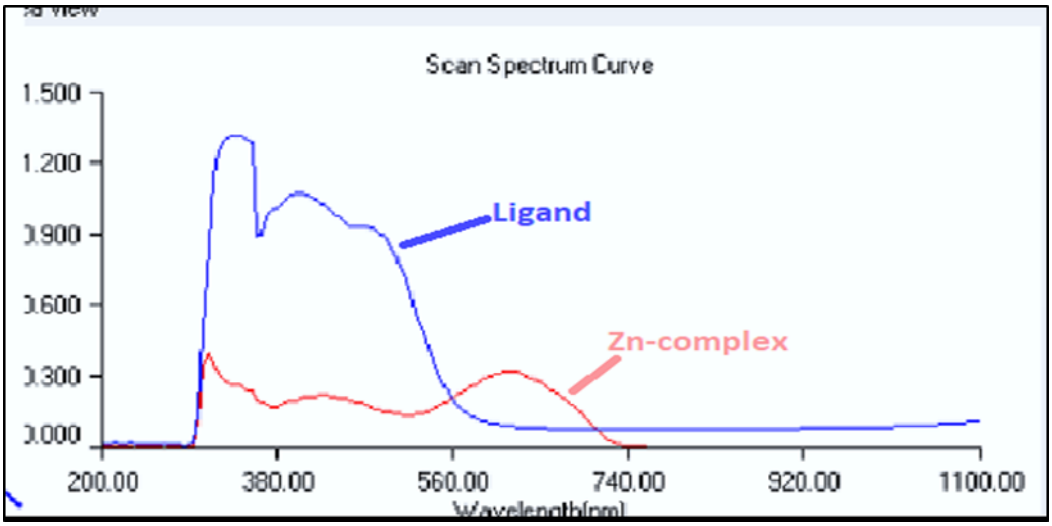


Figure 2: UV-Visible spectra of azo dye ligand and complex zinc ion

Study the optimal conditions of complexity

Effect of reagent concentration

This study was conducted at a pH of 6.5 to 7- and 1-mL zinc solution. The 5 mg/L concentration was blended with a 1 mL reagent to form a series of 1×10^{-3} to 1×10^{-5} M concentrations. The absorption increases rapidly with increasing reagent concentration in the reagent concentration of 1×10^{-5} to 1×10^{-4} M where the best (optimum) reagent's concentration is achieved at wavelength 620 nm, as depicted in Figure 3. After the optimum concentration, the absorption declines, reaching about 0. 005 M. The increase of the reagent after concentration 0.0005 M does not affect the absorption.

Optimal reagent volume

The study was carried out through complex conditions, consisting of 1 mL ion size, 5 mg/L concentration, a series of 0.5 to 3 mL reagent at 1×10^{-4} M concentration, 620 nm wavelength, room temperature, and pH 6.5 to 7, as indicated in Figure 4. The maximum absorption is reached when the reagent volume is 1.5 mL. The absorption rises with increasing reagent volume. After reaching this concentration, the absorption starts to decline and eventually approaches 0.04 in the vicinity of reagent volume 3 mL.

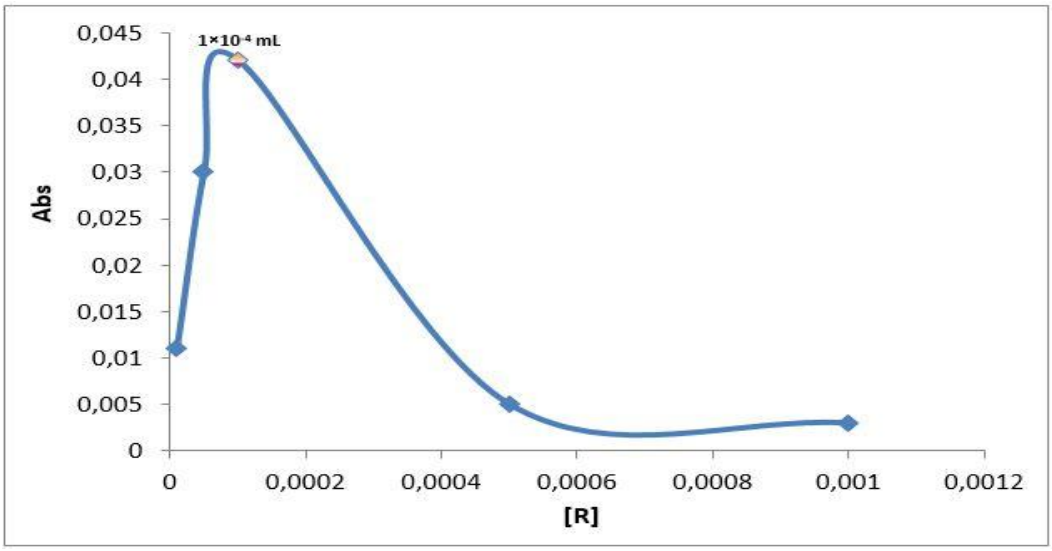


Figure 3: Effect of reagent concentration

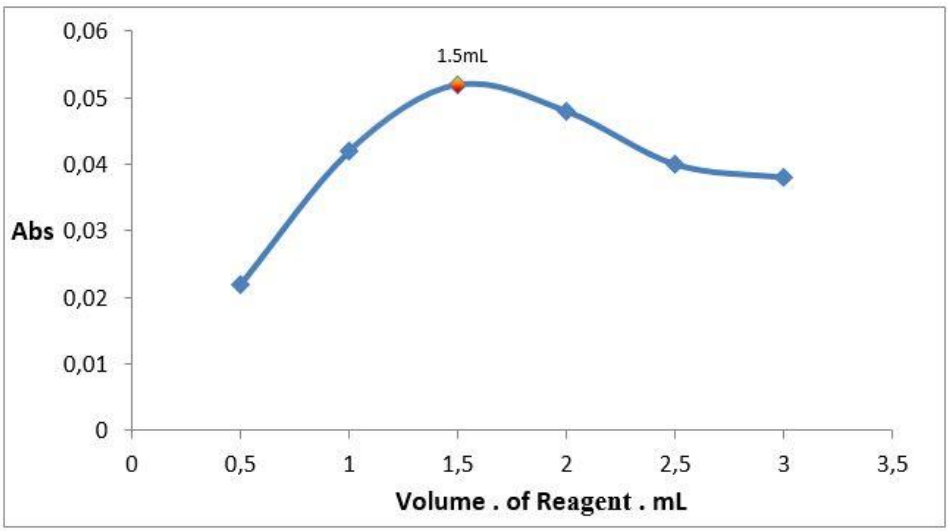


Figure 4: The optimal reagent volume

Optimal zinc ion volume

The optimal ion volume was studied by taking a series of ion volume from 0.5 to 2.5 mL at 5 mg concentration. Increasing the volume of zinc ions increases the absorption ability. The result shows that increasing volume zinc ions from 0.5 to 1 increased the absorption 10 times. The maximum absorption was achieved at 1.5 mL zinc ions volume at 1 $\times 10^{-4}$ M concentration of reagent and when increasing zinc ions volume more of 1.5 ml decreases the absorption ability, as demonstrated in Figure 5.

Effect of time on Zn(II) complex formation

This study was carried out in the best above mentioned conditions and at 25 to 40 °C. In these conditions, the complex has a good stability in the time frame between 0.5 to 120 min, as demonstrated in Figure 6.

Calibration curve to complex Zn(II)

After obtaining the best conditions, a series of ion metal concentrations were performed 0.01 to 30 mg/L. A linear relation was obtained in the range of (0.3 to 20 mg/L). The results outcomes were 0.1754 $\mu g.cm^{-2}$. Sandal sensitivity was 0.2632 mg/L LOD, 0.8772 mg/L LOQ, respectively, as illustrated in Figure 7. The R^2 value was 0.9997, indicating that the experimental data on absorption and Zinc(II) concentration were well fit by the linear equation.

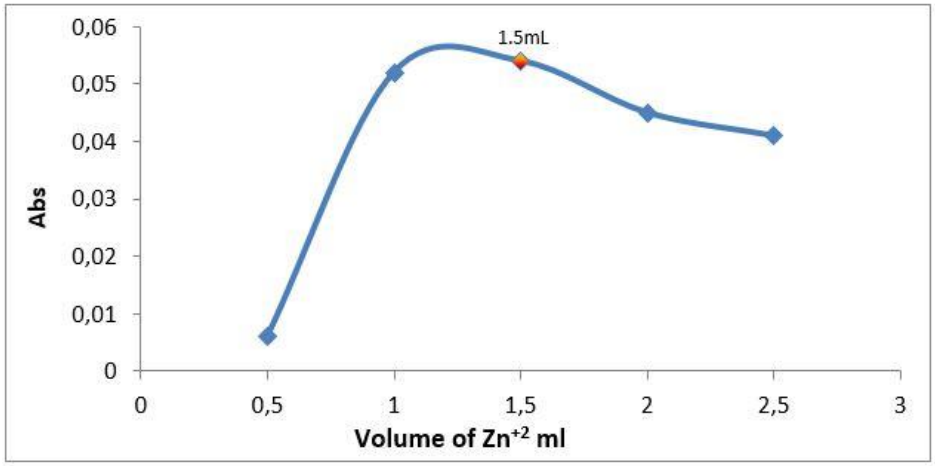


Figure 5: Optimal zinc ion volume

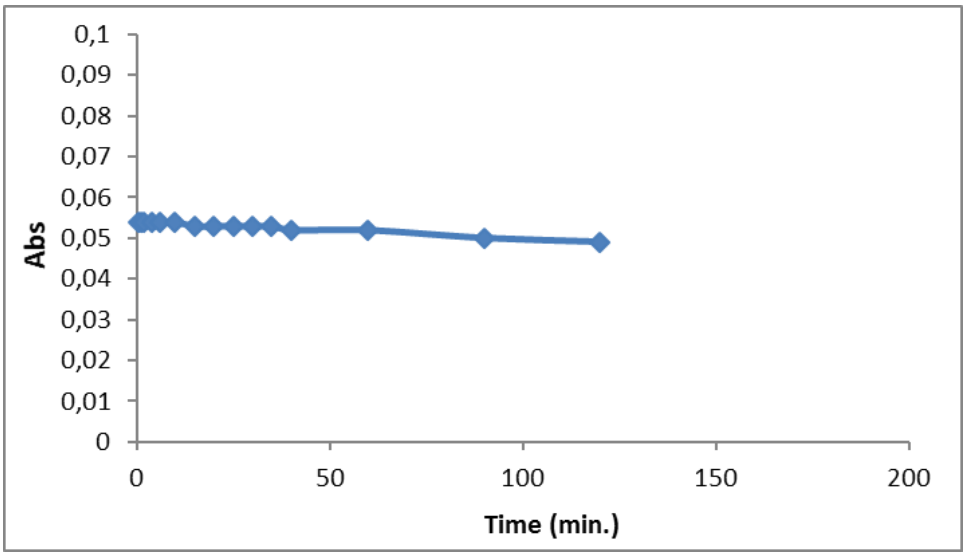


Figure 6: Effect of time on the Zn(II) complex formation

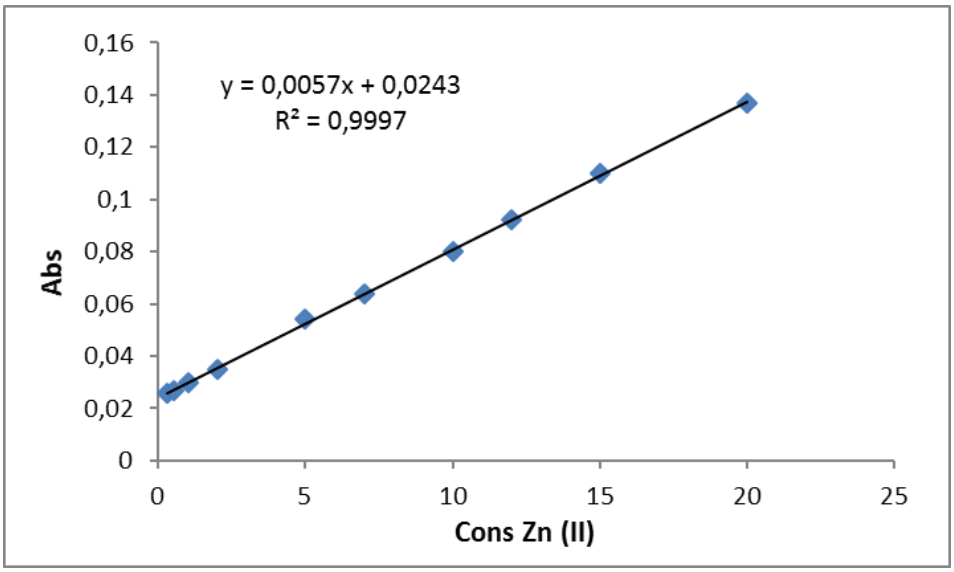


Figure 7: Calibration curve to complex Zn(II)

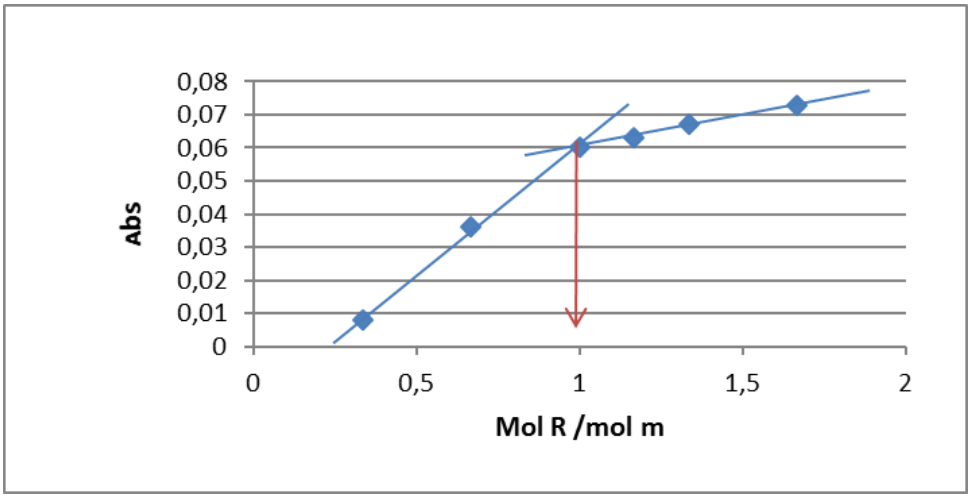


Figure 8: Mole ration method

$$M + L \rightarrow ML$$

$$\alpha C \quad \alpha C \quad (1-\alpha)\,C$$

$$K = \frac{[ML]}{[M][L]}$$

$$K = \frac{(1-\alpha)C}{\alpha C \cdot \alpha C}$$

$$K = \frac{(1-\alpha)C}{\alpha^2 C^2}$$

$$K = \frac{(1-\alpha)}{\alpha^2 c}$$

$$\alpha = \frac{A_m - A_S}{A_m}$$

$$\alpha = \frac{0.073 - 0.060}{0.073} = 0.178$$

$$K = \frac{(1-0.178)}{(0.178)^2 \times 1\times10^{-4}}$$

$$K = \frac{0.822}{0.001\times31.68\times1\times10^{-4}}$$

$$K = \frac{0.822}{31.68\times10^{-7}}$$

$$K = 0.0259\times10^{7}$$

$$K = 2.59\times10^{5}$$

Determination of possible composition

Mole ration method

The molar ratio of the reagent to the metal ion was studied extensively and illustrated in Figure 8. The results show that the crossing of the two fitting lines was at reagent/ion of 1:1 ratio.

Stability Constant (β) for Complexes Zn^{+2} ion

To find out the stability of the formed zinc compound (II) and the possibility of its study by spectroscopic methods, the dissociation degree and its stability constant were calculated according to the following equations:

Study of interference

The positive and negative ions that may interfere with the zinc ion during its complexity were studied with the new reagent (Cu, Ni, Co, Fe, Mg, Mn, Cd, F, So_4, I, CH_3Coo, and Br). At the end of the experiment, it was found that all ions did not interfere except copper and iron and the interference was removed by using a blocking agent S_2O_3 [23].

Applications

The analytical method used to determination the Zinc(II) ion was applied to different drug samples at a concentration of 5 mg/L and comparing its results with the results of the FAAS technique for the same drug samples. The results outcomes are listed in Table 5. Based on Table 5, we deduced the recovery rate for Spec. technique (95-104) %, while for FAAS technique (94 -106%).

Statistical values for applications at technical Spec and FAAS

Table 6 shows the statistical values of different drug samples in the two techniques Spec. and FAAS.

According to the above mathematical equations, we deduce that since $t_{calculated}$ value is less than $t_{scheduled}$ value, the zero theorem is accepted.

Table 5: Determination of Zinc(II) ion by Spec. and FAAS techniques in different drug samples

Drug samples	Taken value (mg/L)	Found value (mg/L) Spec:FAAS	$Er_{spec:FAAS}$	$Rec_{spec:FAAS}$
Osteocar	5	5.079: 4.700	1.58:-6	101.58:94
Ferglobin	5	4.850: 4.900	-3:-2	97:98
Zinc	5	4.800: 5.300	-4 :6	96:106
Vitadex	5	4.750: 4.670	-5:-6.6	95:93.4
Calcicar	5	5.200: 5.01	4:0.2	104:100.2

Table 6: Statistical Values for Applications with Spec and FAAS technique

Spec.	5.079	4.850	4.800	4.750	5.200	4.936	0.176	3.566
FAAS	4.700	4.900	5.300	4.670	5.010	4.916	0.210	4.272

$$S^2 = \frac{(n_1-1)\ S_1^2 + (n_2-1)\ S_2^2}{n_1 + n_2 - 2}$$

$$S^2 = \frac{(5-1)(0.176)^2 + (5-1)\ (0.210)^2}{5 + 5 - 2}$$

$$S^2 = \frac{(4)\times 0.031 + (4)\ \times 0.044}{10-2}$$

$$S^2 = \frac{0.124+0.176}{8}$$

$$SP = \sqrt{S^2} \qquad S^2 = 0.038$$

$$SP = \sqrt{0.038}$$

$$SP = 0.195$$

$$t = \frac{\bar{X}_1 - \bar{X}_2}{SP}\sqrt{\frac{n_1\ n_2}{n_1 + n_1}}\ ,\ t = \frac{4.936 - 4.916}{0.195}\sqrt{\frac{25}{10}}$$

$$t = \frac{0.02}{0.195}\sqrt{2.5}$$

$$t = 0.103 \times 1.581 = 0.163$$

$$t\ scheduled = 2.306$$

Table 7: Analytical characteristics of Zn (II) complex with EMBTYDP

Characteristics	Zn(II) Complex with EMBTYDP
Absorption maximum	620nm
pH range	6.5-7
Law ranges‘ Beer	(0.3-20) mg/L
LOD	0.2632mg/L
LOQ	0.8772 mg/L
R^2	0.9997
Sandal sensitivity K	0.1754 µg.cm^{-2} 2.59×10^5

Conclusion

The analytical method is straightforward and achieves high results, as it is easy to prepare and diagnose the new organic reagent, the ease of spectral estimation of the zinc binary complex, and its complexity with a group of other metal ions such as Ni and Cu, after fixing the optimal conditions of concentration and size of the reagent, acidity function, time, temperature, and molar ratio. After estimating the ion under study, we obtained high stability, control, efficiency, and accuracy at the greatest absorption wavelength of the zinc ion complex 620 nm under the optimal conditions. Except for the copper and iron ions that interfere with the metal ion and are blocked by S_2O_3, the recovery rate of the drug for the proposed method ranged from 95% to 104% when compared with the flame atomic absorption spectrum in which the recovery rate ranged between 94-106%. The results of the technique under study showed that it has a higher sensitivity compared with FAAS. Moreover, the complex stability constant is $K=2.59\times10^5$, and when studying the statistical values for both techniques, it turns out. The t calculated value is less than the t scheduled value. Thus, the zero theorem is accepted.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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