Article

Preparation, Diagnosis and Study of the Effect of the Biological Activity of Multi Dentate Azo- Schiff Ligand and Cu(II), Zn(II) Complexes

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ABSTRACT

The new azo-Schiff ligand was prepared through two paths. The first path is the reaction of the primary aromatic amine(4-ethoxyaniline) with benzaldehyde to form the azo compound. The second path included condensation of the resulting compound with the aliphatic amine ethylenediamine and the addition of two drops of glacial acetic acid to prepare the azo-Schiff ligand. The ligand was characterized by using FTIR spectroscopy, ultraviolet-visible spectroscopy, mass spectrometry, proton nuclear resonance spectroscopy, and elemental analysis (C.H.N.). Solid metal complexes were prepared for the ligand prepared with the metal ions Cu(II), Zn(II), and the complexes were characterized by the above techniques, except for mass spectrometry and ^{1}HMNR measurements for the ligand only. As for the study of the molar conductivity and magnetic susceptibility of the metal complexes prepared and dissolved in ethanol at room temperature and at a concentration of (1×10^{-3})M proposed the geometric shape of the prepared complexes, all of which were octahedral, The last path includes studying the extent to which the biological activity of the azo-Schiff ligand and its complexes affects for two types of bacteria, for both *Escherichia coli* and *staphylococcus aureus* , at two concentrations(250ppm) and (500 ppm).

Keywords: 4-ethoxy aniline, ethylene diamine, benzaldehyde, biological activity, measurements of spectra.

INTRODUCTION

The organic chemistry of aromatic compounds has witnessed rapid development in recent times,and this development has led to diversity in the preparation of their various derivatives and the fields of their use,This diversity is attributed to the development of the necessary techniques in preparation and diagnosis[1,2].

In addition to the important roles that many of these compounds and their derivatives play in various medical fields, such as treating various types of cancer, such as lung, skin, eye, colon, and other cancers [3-5]. Azo-Schiff compounds are considered aromatic compounds, their impact and importance have grown in many fields of chemistry and their entry into various fields, including industrial, agricultural ones and in addition to their biological effect [6-8].

Azo compounds are distinguished by their bright colors[9],They are used in analytical chemistry as indicators[10] and have an important role in the field of green chemistry [11]. Because of the synthetic flexibilities of these Schiff-base ligands, their selectivity and sensitivity towards the transition metal ions, and the significant biological activities of their complexes, azo Schiff-base metal complexes have been studied extensively for years, in addition to their intriguing coordination properties [12]. The stability of Schiff bases is attributed to their aromatic character when compared to aliphatic Schiff bases[13].

EXPERIMENTAL SECTION

Synthesis of the ligand

The ligand is prepared in two paths:

The first path the azo compound is prepared from 4-Ethoxy aniline (0.01mol,1.30ml) dissolved in a solution of (3ml HCl+20ml D.W.)while maintaining the temperature (0-5)^{0}C. Then a solution consisting (0.01mol ,0.7g) $NaNO_2$ dissolved in (10ml) of distilled water is added to it slowly and dropwise. Solution 20min is left to complete the process and to obtain the diazonium salt [14] , after that the resulting mixture is added slowly, with stirring, to another solution prepared from(0.01mol,1.01ml) benzaldehyde dissolved in(50 ml) of absolute ethanol to which a solution consisting of (2g$NaHCO_3$ +5ml D.W. +0.3g NaOH) was added. The final solution was measured using litmus paper and the solution was neutral. Then the solution was left aside for 45 minutes until the color of the solution became dark. It was filtered and washed with ethanol, then filtered, dried and weighed and the result was brown colour, formula:$C_{15}H_{14}N_2O_2$,M.wt:254g/mol , yield (81%)as shows in diagram 1.

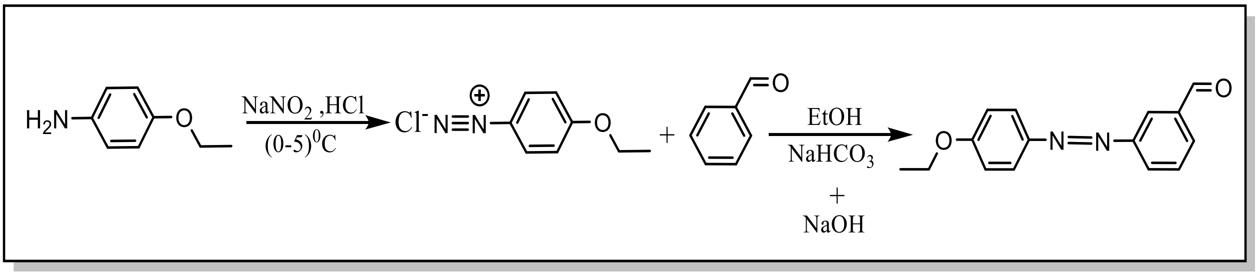


Diagram 1.Prepare of azo compound

The second path it was taken from the azo derivative (0.01mol ,1.95g)prepared in the previous step and dissolved in(50ml) ethanol with the addition of two drops of glacial acetic acid, then aliphatic amine was added to it, and the mixture was heated in a water bath at a temperature $(65\text{-}70)^0$c of 8 hours, while stirring the mixture, the precipitate was filtered and then crystallized with hot ethanol [15], so dark brown-coloured product was obtained, Melting point (dec. $365)^0$C,Yield (80%), formula:$C_{32}H_{32}N_6O_2$ and M.wt:532.7g/mol. As shows in diagram 2.

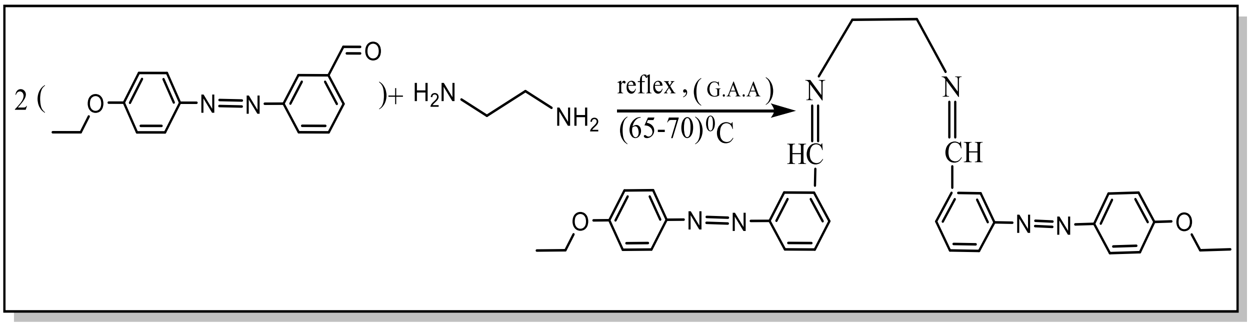


Diagram 2. Prepare of azo-Schiff ligand

Formation of ligand Complexes:

Formation of metal complexes by adding (0.01mol) of metal chloride to a solution consisting of (0.01mol , 4.02) of the ligand dissolved in (25ml) of hot absolute ethanol.Then the mixture is sublimated for 3 hours at a temperature (65-70)^{0}C. The complex is then washed with absolute ethanol and dried in the open air. As shown in the diagram 3 and in Table 3.

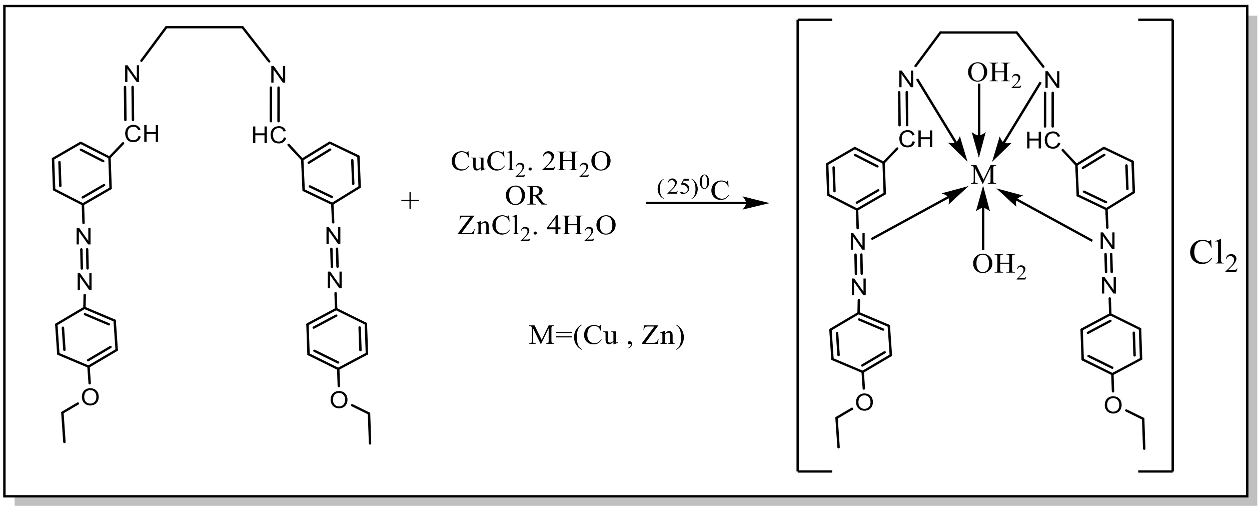


Diagram 3. Formation of azo-Schiff ligand complexes

Table 3. Analytical results and physical properties of azo-schiff ligand and its metal complexes

						Calculations (found)%			
No	Compound	Wt formula	color	yield	M.P.C^0	C	H	N	M
1	$L_1 = C_{32}H_{32}N_6O_2$	532.7	Drak Brown	88	des 365	72.16 (71.60)	6.06 (5.99)	15.78 (15.52)	
2	$C_{32}H_{36}N_6O_4CL_2Cu$	702.55	Olive	78.7	187-190	54.66 53.29	5.12 5.00	11.96 11.68	9.05 9.11
3	$C_{32}H_{36}N_6O_4Cl_2Zn$	704.38	Bronze	78.7	87-90.5	54.52 53.34	5.11 5.02	11.93 11.71	9.28 9.06

RESULTS AND DISCUSSION

Infrared spectra of the azo-Schiff ligand and its metal complexes

To interpret the spectrum of the new ligand and its metal complexes, the infrared spectra must be followed. An important band appeared in the ligand spectrum indicating the formation of the ligand, which is the (C=N) azomethine group, which appeared at 1593.25, and other bands appeared at (3117.07 cm^{-1}) for the aromatic ʋ(C-H) groups, the aliphatic ʋ(CH_2) at(2970.48 cm^{-1}), and ʋ(CH_3) at (2870.17 cm^{-1})Figure 1, [16]. The spectra of the metal complexes were traced and compared with the spectrum of the ligand. It was observed that new bands appeared and other shifts. The infrared spectrum of metal complexes shows vibrations of the aromatic bond ʋ(C-H) between (3155.65, 3267.52)cm^{-1},Aliphatic ʋ(-CH_2)between (2958.90, 2950.42)cm^{-}1 and ʋ(-C=N) between (1616.40, 1620.26)cm^{-1}[17,18]. This is evidence of the consistency between the ligand and the metal ion, as a new band of weak intensity appeared at the range (540.09, 590.24) cm^{-1} for the ʋ(M-O) group and at (420.50 , 470.65) for the ʋ(M-N) group in the spectra of the prepared metal complexes [19], which did not appear in the spectrum of the ligand [20] and new bands will appear like ʋ(O-H)of water in (3352.39, 3556.85) cm^{-1} for Cu^{+2} and Zn^{+2} complexes respectively Figure (2,3) and as shown in Table 2.

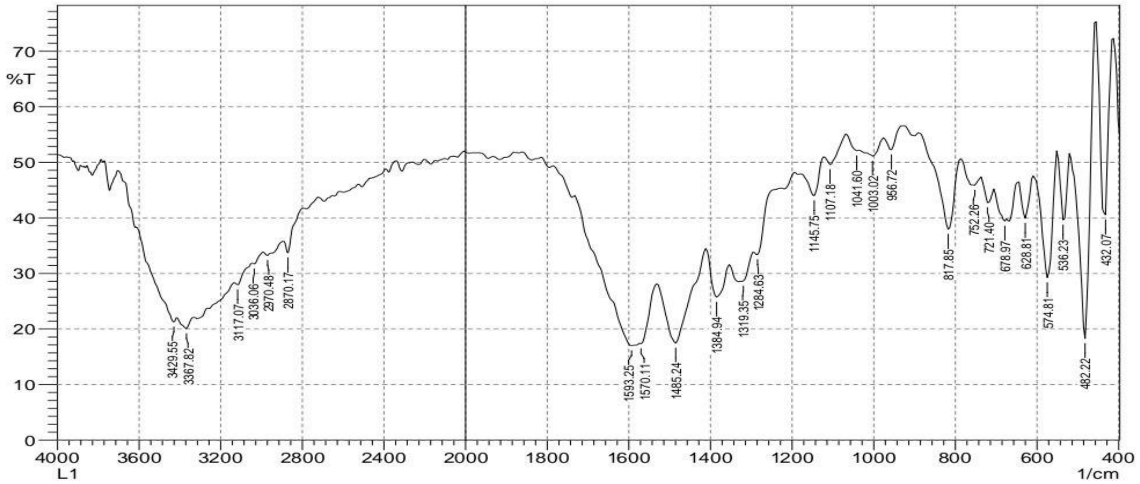


Figure 1. FT-IR for azo-Schiff ligand L_1

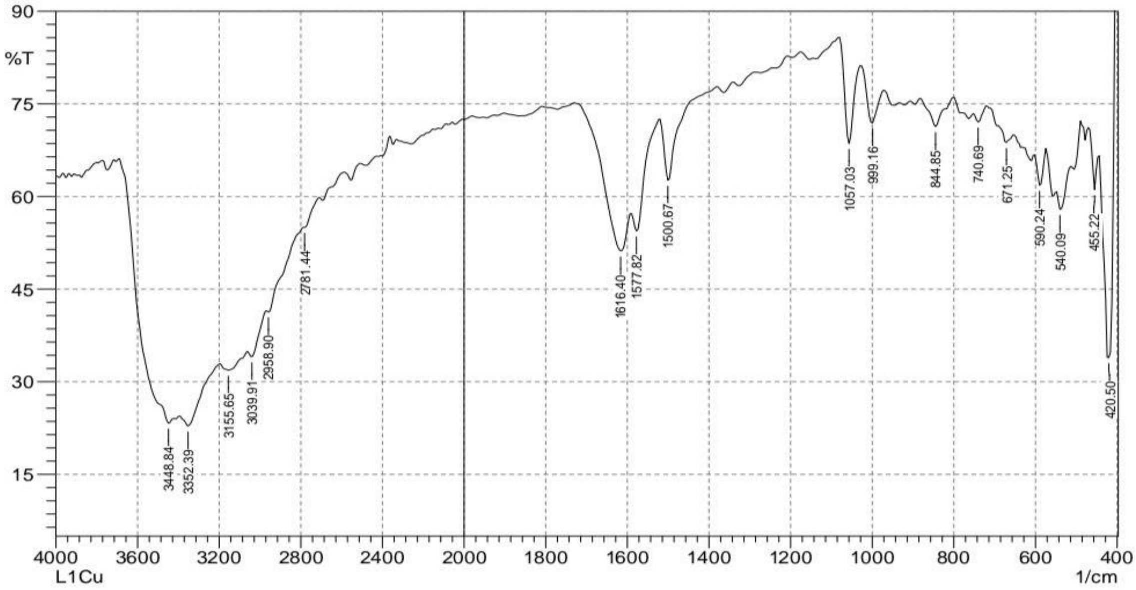


Figure 2. FT-IR for Cu complex

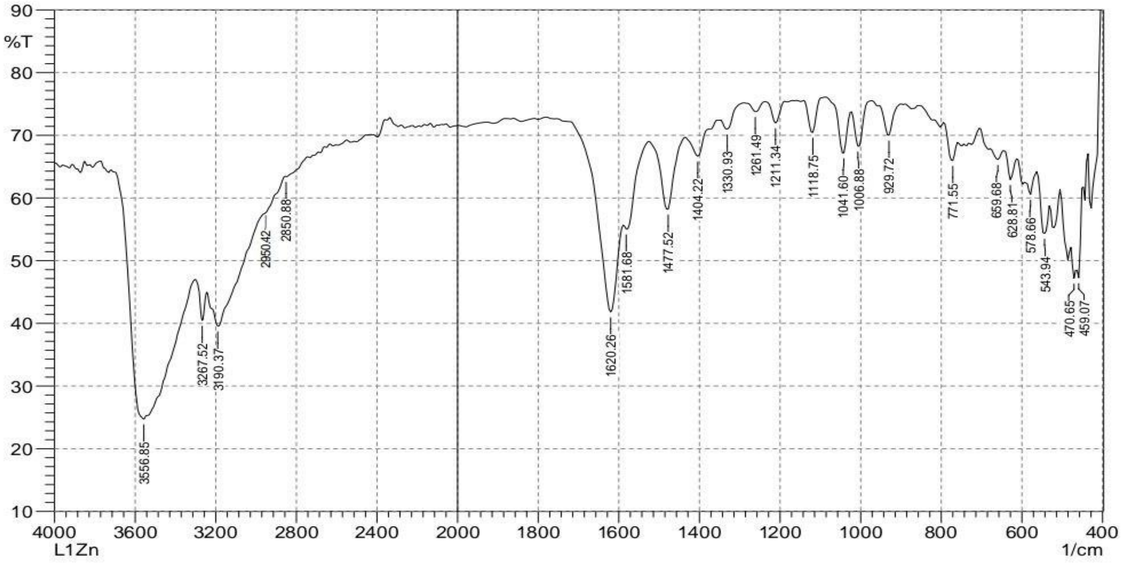


Figure 3. FT-IR for Zn complex

Table 2. shows the stretching bands of the active groups of the new ligand and its metal complexes.

Compound	V (O-H) water	V (CH) aromatic	V (CH_2) aliphatic	V (CH_3) aliphatic	V $(C = N)$	V $(N = N)$	V (C-O-C)	V $(M - N)$	V $(M - O)$
$L_1 = C_{32}H_{32}N_6O_2$		3117.07	2970.48	2870.17	1593.25	1485.24	1003.02		
$C_{32}H_{36}N_6O_4Cl_2Cu$	3352.39	3155.65	2958.90	2781.44	1616.40	1500.67	1057.03	455.22-420.50	590.24-540.09
$C_{32}H_{36}N_6O_4Cl_2Zn$	3556.85	3267.52	2950.42	2850.88	1620.26	1477.52	1041.60	470.65-459.07	578.66-543.94

Spectra of the ligand and its complexes in the visible and ultraviolet regions

The electronic spectra of solutions of the new ligand and its complexes prepared in absolute ethanol solvent were recorded, and the d-d electronic transitions were identified in addition to the (C.T.)charge transitions. The ultraviolet-visible spectra of the solutions of the coordination complexes showed absorption peaks at wavelengths different from the spectra of their free ligands. The spectrum of the new free ligand in absolute ethanol solvent showed two peaks at

(ʎ= 204nm) (Abs.= 3.347 cm^{-1}) , It goes back to the transition($\pi \rightarrow \pi^*$)and another at (ʎ = 246nm) (Abs.= 0.839 cm^{-1}) goes back to the transition ($n \rightarrow \pi^*$) [21]. As shown in the Figure (4).

As for the solution spectrum of the copper complex, it showed an absorption band at (ʎ= 286 nm, 3.835) dating back to the electronic transition (C.T.) and another at (ʎ= 860 nm , 0.223) dating back to the d-d type ($^2Eg \rightarrow {}^2T_2g$) transition, This conclusion is consistent with what was reported in the literature for the copper ion in its octahedral complexes[22]. The zinc ion spectra are interpreted as charge transfer spectra [23, 24], and the complex's spectra recorded absorption peaks at (ʎ= 282 nm, 1.618) and (ʎ= 200 nm, 2.801), The zinc complex is characterized by the absence of electronic transitions of the d-d type because it is of the system (d^{10}).Through the results of some spectroscopic and analytical methods, the stereoscopic form of the zinc complex is proposed, where the electronic spectra of the octahedral seat solution appear. As shown in the Figure (5,6).

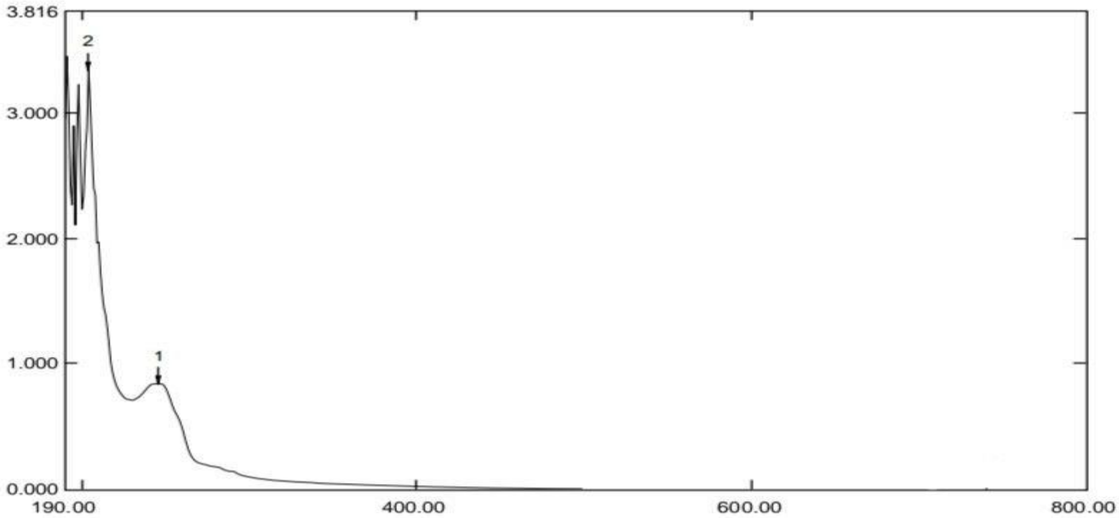


Figure 4. UV-Vis for azo- Schiff ligand

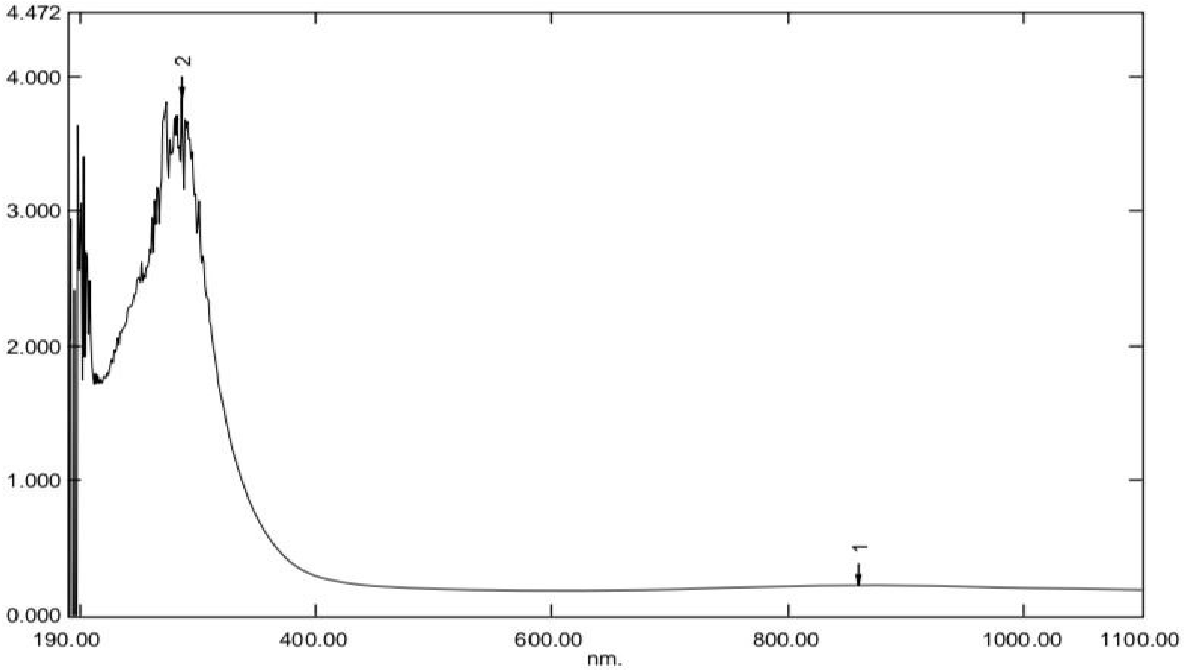


Figure 5. UV-Vis for Cu complex

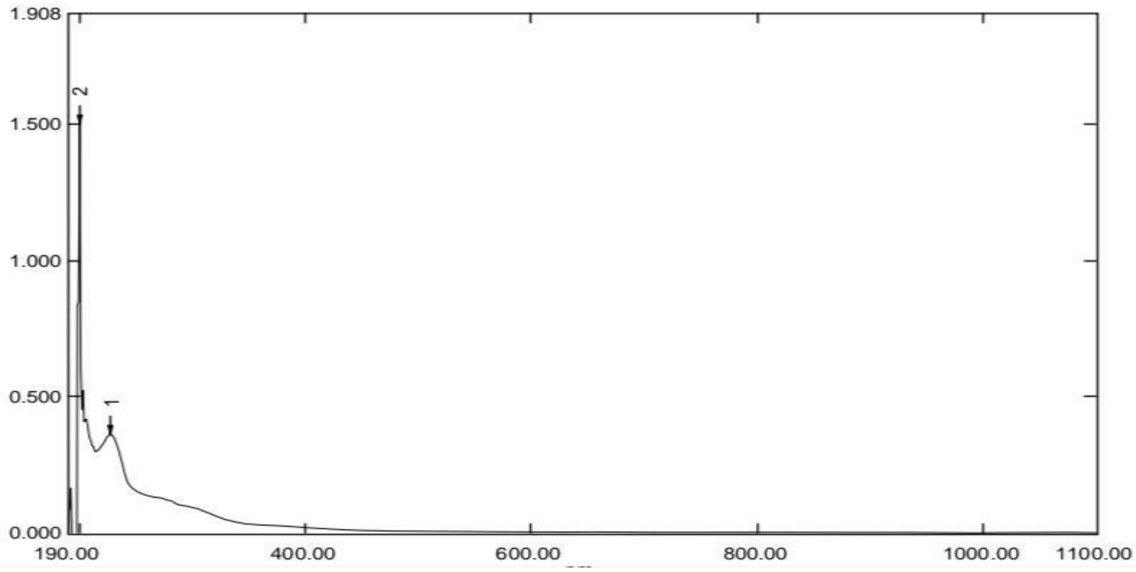


Figure 6. UV-Vis for Zn complex

Mass spectrum of the Azo-Scheff new ligand

The mass spectrum of the free ligand showed the charge to mass ratio (M/+Z),which is proportional to the molecular weight of the compound (M.wt =532.7 gm /mole) [25] Figure(7) and diagram(4), The demonstrates how to divide the compound's mass spectrum. Since the ligand's molecular weight and formula are known, this method can be utilized to ascertain the ligand partitioning pathways [26,27].

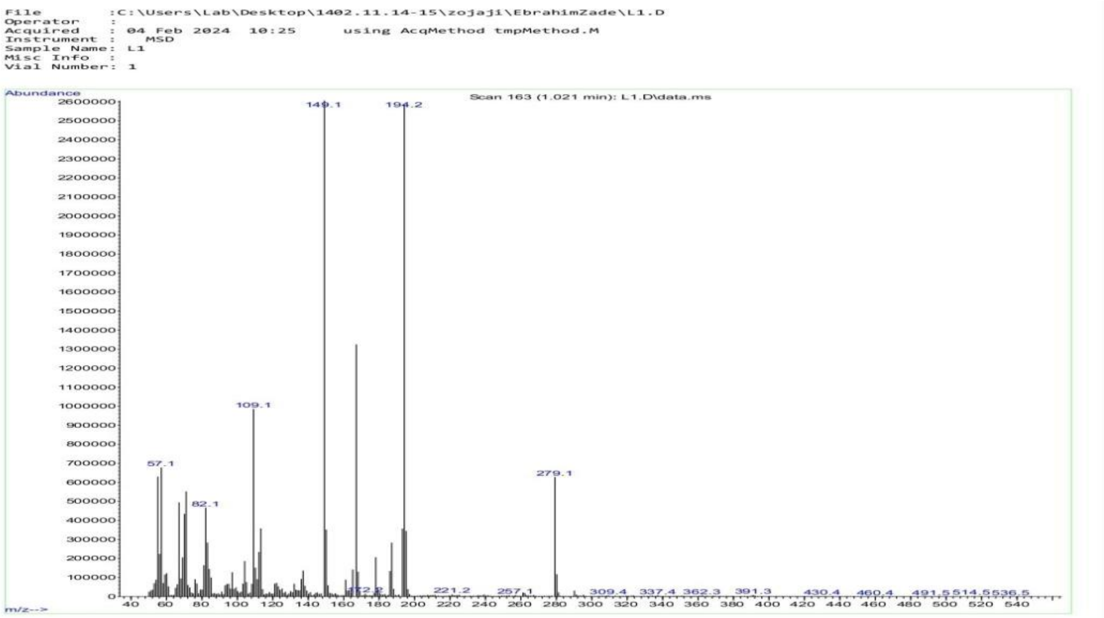


Figure 7. Mass spectrum of the new ligand

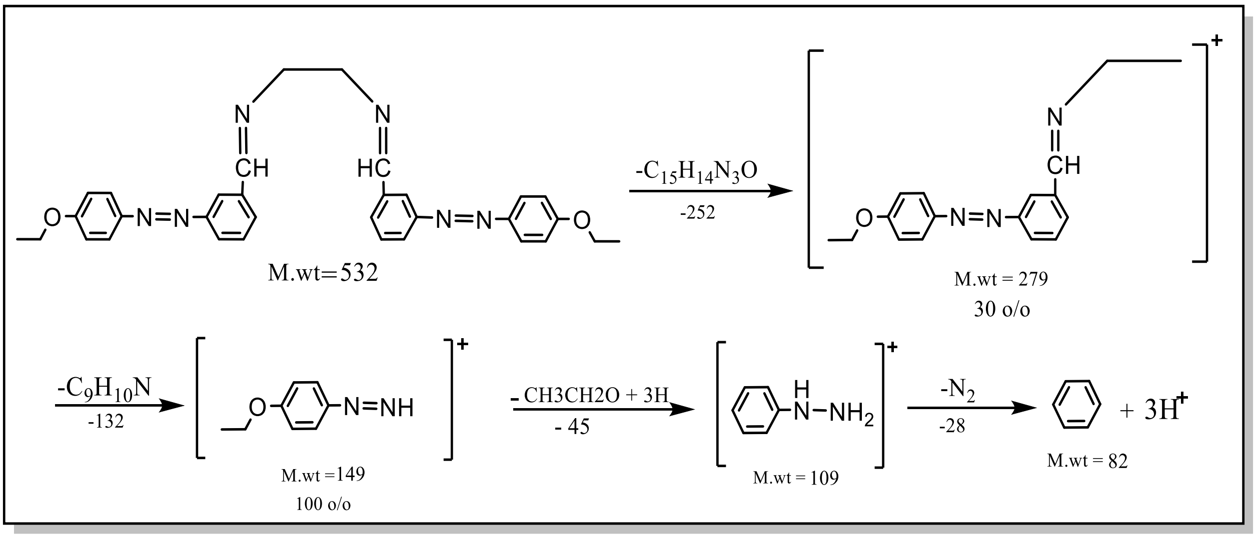
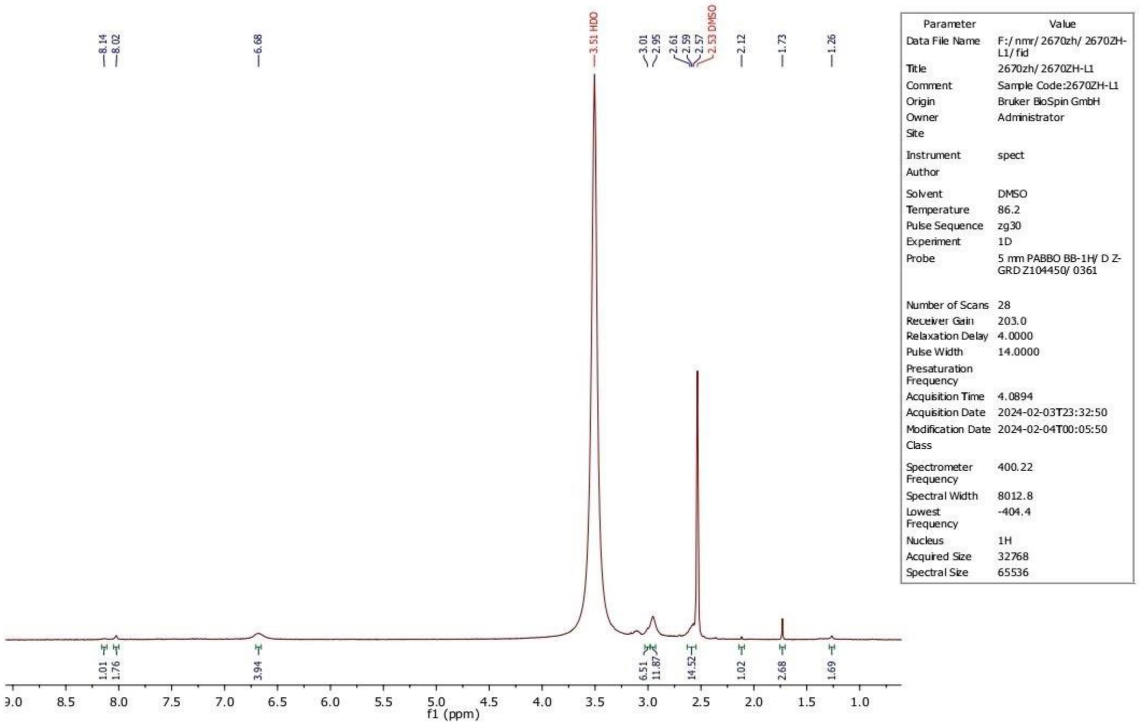


Diagram 4. Ligand fragmentation

^{1}H-NMR spectrum for ligand

The magnetic resonance spectrum of the free ligand, as shown in Figure (8), showed a clear band belonging to the DMSO d6 solvent at (δ= 2.53 ppm). A shift at range (δ = 1.26 – 1.73 ppm , 6H) appeared to belong to the two methyl groups (-CH_3) and another shift appeared at range (δ = 2.12 – 3.01 ppm , 8H) due to the methylene groups (-CH_2). Multiple peaks also appeared to belong to the phenyl groups present in different pikas at Range (δ = 6.68 – 8.02 ppm , 16H), and finally a band appeared at (δ= 8.14 ppm, 2H) belonging to the protons of the two azomethine groups (H-C=N) [28].



Parameter	Value
Data File Name	F:/ nmr/ 2670zh/ 2670ZH-L1/ fid
Title	2670zh/ 2670ZH-L1
Comment	Sample Code:2670ZH-L1
Origin	Bruker BioSpin GmbH
Owner	Administrator
Site	
Instrument	spect
Author	
Solvent	DMSO
Temperature	86.2
Pulse Sequence	zg30
Experiment	1D
Probe	5 mm PABBO BB-1H/ D Z-GRD Z104450/ 0361
Number of Scans	28
Receiver Gain	203.0
Relaxation Delay	4.0000
Pulse Width	14.0000
Presaturation Frequency	
Acquisition Time	4.0894
Acquisition Date	2024-02-03T23:32:50
Modification Date	2024-02-04T00:05:50
Class	
Spectrometer Frequency	400.22
Spectral Width	8012.8
Lowest Frequency	-404.4
Nucleus	1H
Acquired Size	32768
Spectral Size	65536

Figure 8. ^{1}HNMR spectrum for ligand L1

Magnetic Susceptibility and Molar Conductivity

The magnetic properties were measured using the Faraday method for ligand complexes Cu(II), Zn(II) and it was found that they had magnetic properties (1.81)B.M. and dia respectively, Equation (1,2,3),They are called Pascal's constants [29] was used to calculate magnetic susceptibility as shown in Table 3.

The molar conductivity of the complexes in DMSO solvent was recorded [30, 31] and it was shwon that all electrolytic complexes supported an octahedral geometry for all complexes.

$Xm = M.wt \times Xg$ Eqn.

$XA = Xm - (-D)$ Eqn.2

$\mu_{ef} = 2.828\sqrt{X_A T}$ B.M Eqn.3

Where Xm =Molar susceptibility, M.wt = Molecular weight , Xg = Gram sensitivity(weight), XA = Atoms susceptibility, D = Diamagnetic correction, μ_{ef} = Effective Magnetic momentum, T = Absolute temperature.

Table 3.Magneticsusceptibility and molar cnductivity for complexe

Complexes	Magnetic Susceptibility (B.M.)	molar conductivity	Hybeidization	Proposed structure
$C_{32}H_{36}N_6O_4Cl_2Cu$	1.81	79.99	Sp^3d^2	octahedral
$C_{32}H_{36}N_6O_4Cl_2Zn$	dia	75.10	Sp^3d^2	octahedral

Biological activity of the ligand and its complexes against bacteria

The research was conducted on Mueller Hinton agar plates, where a bacterial inoculum of two types, *Staphylococcus aureus* and *Escherichia coli*, was made. A number of bacterial colonies from (0.65-0.5)McFarland were dissolved and spread on Mueller Hinton agar plate and left to dry. Then a scuff was made on the surface of the plate, after which the scuff was filled with ligand and its complexes were placed in the incubator at 37 degrees for a period of 24 hours after which the results are read.

The ligand($C_{32}H_{32}N_6O_2$) showed through the results that its highest inhibitory effect on *Eseherichia coli* bacteria at a concentration of (500)ppm reached a diameter of (30)mm, and the lowest inhibitory effect on the same type of bacteria at a concentration of (250)ppm reached (8)mm. As for its inhibitory effect on *Staphylococcus aureus* bacteria at a concentration of (500)ppm, the diameter of inhibition reached (10)mm and no effect was shown,the trend of the same bacteria at a concentration of (250)ppm.

The ($C_{32}H_{36}N_6O_4Cl_2Cu$) complex showed the highest inhibitory effect on both *Staphylococcus aureus* bacteria and *Eseherichia coli* bacteria at a concentration of (500)ppm, where it reached (10)mm. It did not show any inhibitory effect on the two types of bacteria at a concentration of (250)ppm. As for the ($C_{32}H_{36}N_6O_4Cl_2$ Zn) complex, it showed the highest inhibitory effect on *Staphylococcus aureus* bacteria at both concentrations of (250, 500)ppm, where the diameter of inhibition reached (20)mm. The complex showed the lowest inhibition against *Escherichia coli* bacteria at a concentration of (500)ppm, reaching (8)mm, and did not show any inhibitory effect at a concentration of (250)ppm. As shown in the Figure (9,10) and in Table 4.

Table 4. It shows the inhibitory effect of the ligand and its complexes against two types of bacteria

Bacteria Compound	G^+ve 500ppm	G^+ve 250ppm	G^-ve 500ppm	G^-ve 250ppm
$L_1 = C_{32}H_{32}N_6O_2$	10mm	R	30mm	8mm
$C_{32}H_{36}N_6O_4Cl_2Cu$	10mm	R	10mm	R
$C_{32}H_{36}N_6O_4Cl_2Zn$	20mm	20mm	8mm	R

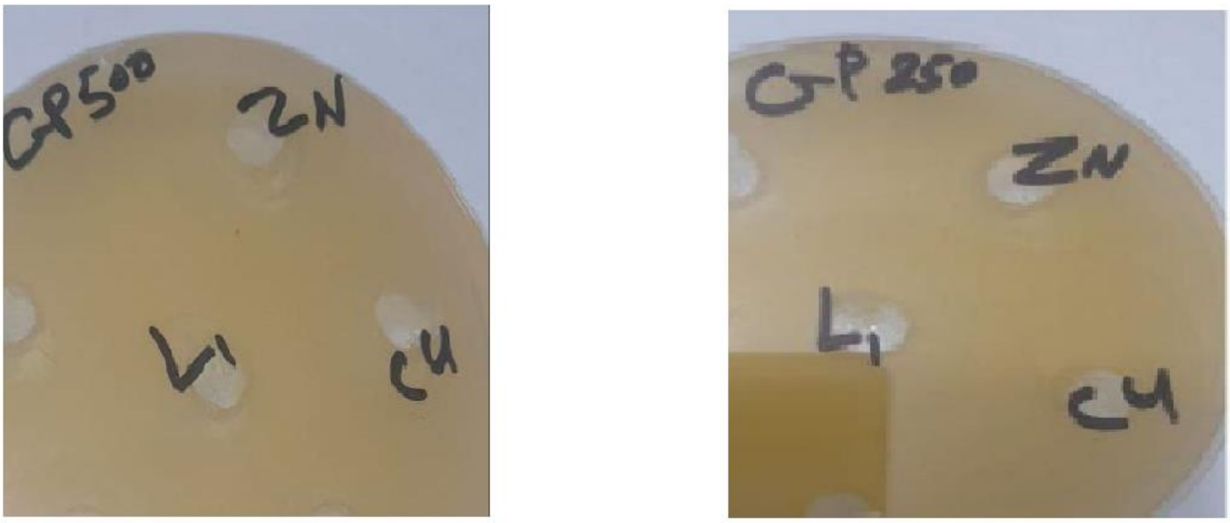


Figure 9.The extent of the inhibitory effect on bacteria G^+ve and of ligand and its complexes at concentrations of (250,500) ppm.

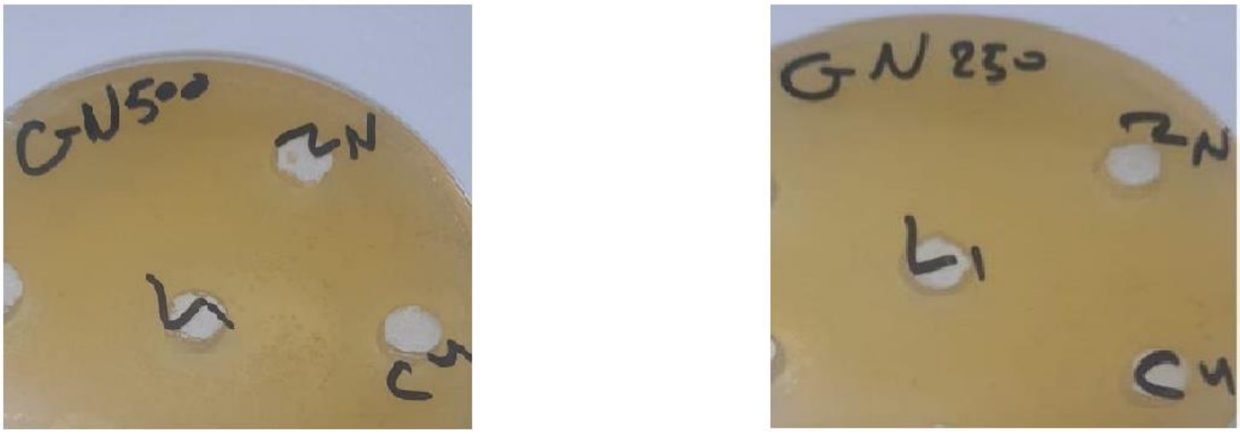


Figure 10.The extent of the inhibitory effect on bacteria and $G^{-}ve$ of ligand and its complexes at concentrations of (250,500) ppm.

Conclusion

The reation of the new azo-Schiff ligand involved two paths: the first was the diazotization reaction, which produced the azo compound; the second was the condensation reaction between the benzaldehyde and the azo compound, which produced the ligand. The compounds that were produced were colored, Stable in air and temperature; the success stability was confirmed by spectroscopic data, FT-IR, UV-Vis, ^{1}H-NMR and magnetic susceptibility, The proposed shape octahedral geometry surrounding the Cu(II) and Zn(II) ions. Molar conductivity show that complexes were electrolytic.

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