

Synthesis, Characterization and Antibacterial Activity of Mixed Ligand Derived for Vanillin with some Transition Metals

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ABSTRACT

A new series of solid metal complexes of (Co^{+2} , Ni^{+2} , Cu^{+2} , Zn^{+2} , Cd^{+2} , and Hg^{+2}) were prepared from the complexation reaction between the azo-schiff base ligand and azo compound. Azo-azomethine ligand was synthesized by reaction of vaniline with (3-bromo aniline) to produce Schiff base in one step then reacted Schiff base with (2-amino acetophenone) to form azo-schiff base ligand in two steps. This ligand and its complexes were characterized by (C,H,N) elemental analysis, FT-IR, UV-vis, ^1H -NMR, mass spectrum, molar conductance, Atomic absorption, magnetic moment measurements. From the obtained results the octahedral geometrical shape was proposed for all prepared complexes. All complexes show that non-electrolyte properties. The biological activity of the ligand and the mixture of ligand and their metal complexes prepared on two types of bacteria, one of which is positive for the Gram stain (G^+), is (Staphylococcus) and another Gram negative (G^-) represented by bacteria (Escherichia coli).

KEYWORDS

Metal Complexes, Azo-azomethine, Antibacterial, Mixed Ligand, Spectral Studies.

Introduction

The name Schiff bases is given to organic compounds after their scientist discoverer Hugo Schiff, and its composition is on the azomethine group ($-\text{CH}=\text{N}-$). This type of compound contributed to the preparation of many complexes in harmonic chemistry and because of their spread and entry into various scientific fields, where they became the object of interest of researchers [1]. Schiff bases are characterized by relatively high stability and the reason for this stability is due to the aromatic characteristic relatively, to aliphatic Schiff bases [2]. Whereas, the compounds that contain aryl compensators are of high stability, are often solid, have relative thermal stability, and are colored, and their solubility depends in most cases on the nature of the associated groups in organic solvents, due to the high stability by resonance [3]. These compounds have importance and many uses in the scientific, industrial and biological fields due to the interest of research and studies in the preparation of lipids, as they were used in the preparation of nanoparticles of gold ion [4]. It also works to stop the division of cancer cells [5,6]. Azo compounds are organic compounds that contain the bridging azo group ($-\text{N}=\text{N}-$) in their chemical composition. They are linked on either side by two similar or different groups, which may be fatty or aromatic (homogeneous or heterogeneous) [7]. The aromatic azo compounds are more stable than their aliphatic counterparts [8]. It has played a major role in the formation of stable chelate complexes in the field of harmonic chemistry [9,10]. It has been used as an anti-bacterial and anti-fungal agent in terms of its biological effect, and it has also been used as an anti-cancer agent because it is used in the manufacture of medicines and medicinal drugs [11,12]. Azo-azomethine compounds This name is given to the organic compounds, which contain in their chemical composition the two active groups: (bridging azo) ($-\text{N}=\text{N}-$) and azomethine ($>\text{C}=\text{N}-$), and this type of compound is relatively recent if compared to the azo compounds and the chemical bases and that both groups contain in their composition a nitrogen atom that has a non-ionic double electron and this gives it many physical and chemical properties [13]. This type of compounds is used in various fields, including industrial, biological, and analytical, and the reason for this is due to the containment of these compounds on the two groups of azo and azomethine, which is highly effective towards the metal ions because they have non-electron pairs on the nitrogen atom of the azo and azomethine groups, and these two groups helped to be in harmony with different metal ions [14]. Some of the azomethine compounds were used in the industrial field as antioxidants [15].

Experimental

Materials and Measurements

All the chemicals used in the preparation of the azo-azomethine and its complexes had at least analytical

grade, except of 4,5-diphenyl imidazole was prepared as described earlier [16]. Melting point was determined by open capillary tube method and are uncorrected by using a Stuart melting point SMP10. The solid-state FTIR spectra of the compounds were recorded on Shimadzu FT-IR model 8400 S Spectrophotometer using KBr pellets in the range $4000 - 400 \text{ cm}^{-1}$. The UV-visible spectra of ligand solutions and their metal complexes were recorded in a dimethyl sulfoxide solvent by using the device Shimadzu UV-1700 spectrophotometer. The spectra of $^1\text{H-NMR}$ were done in a Bruker spectrophotometer (500 MHz) and using DMSO- d_6 as solvent and TMS as an internal standard. Elemental analysis (C, H, N) were recorded by C.H.Nmth E A 99. Mass spectrum of organic ligand was obtained using GC-MS QP- 2010 (Shimadzu Instruments). Magnetic susceptibilities were determined by Faraday method at room temperature using Sherwood scientific balance apparatus, and diamagnetic correction for the ligand were calculated using Pascal's constant. Molar conductivity of the metal chelate complexes were determined in DMSO using conductivity meter (Digital Conductivity Series Ino. Lab. 720) at 25°C .

Preparation of Ligand HL₁ = (BMHPE)

A- Preparation of SchiffBase

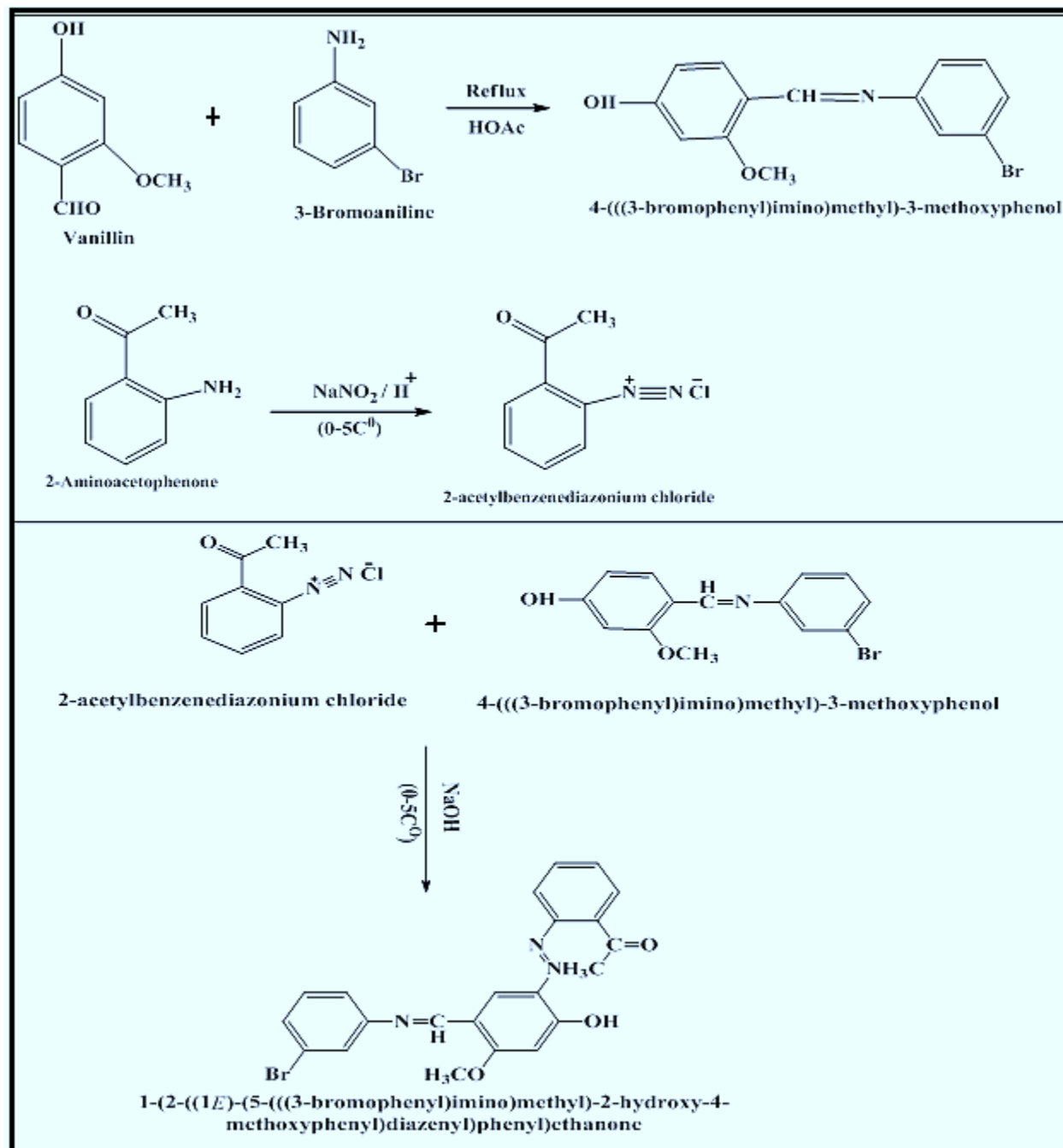
This ligand was prepared in two steps, the first of which was the preparation of a Schiff base of condensation [17,18]. Vanillin and (3-bromoaniline), and the reaction was done by mixing (1.52g, 0.01mol) of vanillin in a round flask of capacity (250ml) and (1.08ml, 0.01mol) of (3- Bromoaniline), followed by the addition of 3 drops glacial acetic acid, and the mixture was heated up for a period of (40 hours), after which the reaction was left to cool down and left aside to complete the sedimentation process and obtain an abundant product in the form of a dark brown precipitate, after which it was recrystallized with hot ethanol and its melting point was measured ($73- 76^\circ\text{C}$).

B-Preparation of the SchiffBase Using the Microwave Method ^[19]

The ligand was also prepared by microwave method where (1.52g, 0.01mol) of vanillin was dissolved in (8ml) of absolute ethanol, then adding (1.08ml, 0.01mol) of (3-bromoaniline) with the addition of some drops glacial acetic acid. Then, the mixture was exposed to microwave radiation for a period of (8) minutes, after which the product was cooled down, forming a dark brown crystalline precipitate with the product (90%) and recrystallized with hot absolute ethanol.

C- As for the Second Step of Preparing Azo

Azomethine ligand, it was obtained from preparing the diazonium salt of the compound (2-aminoacetophenone) by the traditional method [20]. From dissolving (1.21ml, 0.01mol) of amine in a solution (3ml) of HCl acid dissolved in (50ml) distilled water with cooling the solution at a temperature ($0-5^\circ\text{C}$) and adding (0.7g, 0.01mol) of sodium nitrite dissolved in (10ml) distilled water with shaking of the salt, taking into account that the temperature does not rise above (5°C). Then leave the nitrogenation solution aside for a period of (20 minutes) to complete the entry of the nitrogen molecule and add this solution gradually with cooling to a solution of (3.059 g, 0.01mol) from the previously prepared Schiff base dissolved in (50ml) of absolute alcohol and (0.6 g, 0.015mol). From NaOH, it was observed that the color of the solution changed from the moment of contact with the nitrogenous solution to reddish-brown color with the presence of a precipitate of the same mentioned color. Hydrogen ion, down to ($\text{pH} = 7$) The precipitate was filtered and washed with distilled water to get rid of the NaCl resulting from the reaction. It was dried with air and recrystallized in hot ethanol and its melting point was measured. ($121-124^\circ\text{C}$) scheme (1) reactions to obtain ligand.



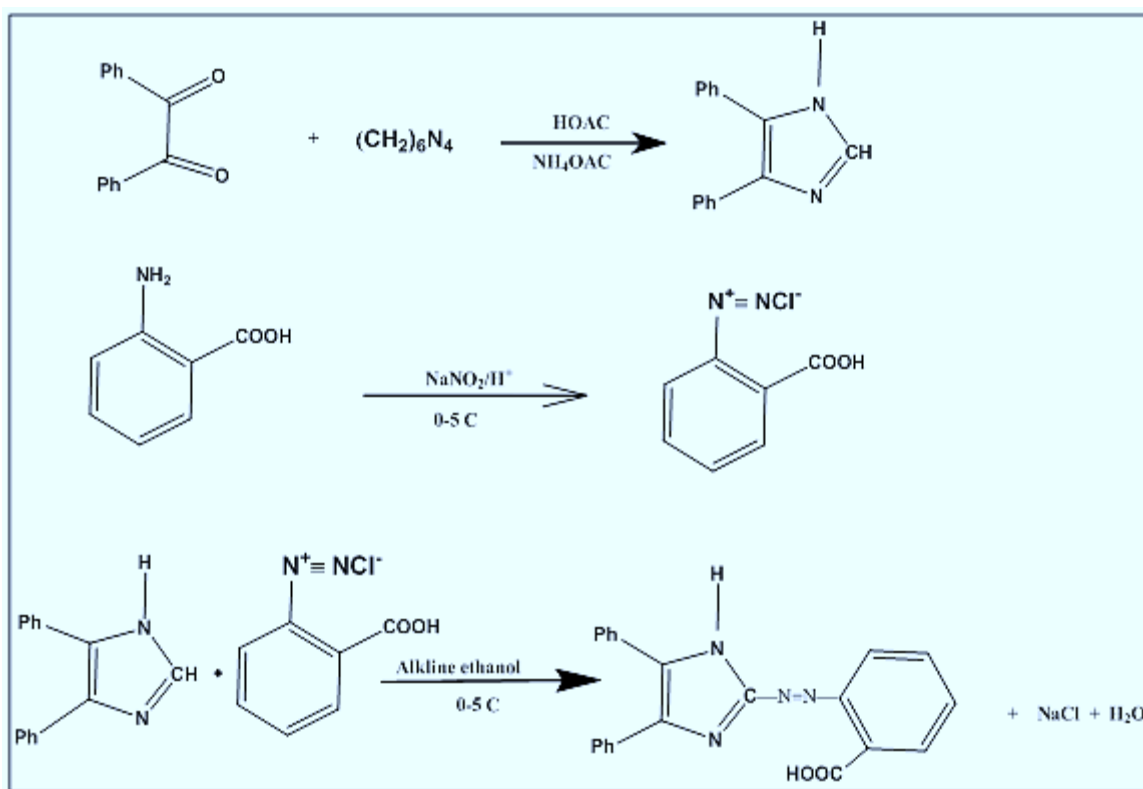
Scheme 1. The preparation of azo-azomethine ligand

Preparation of Ligand Complexes

All the complexes $[\text{M} (\text{C}_{22}\text{H}_{18}\text{N}_3\text{O}_3\text{Br})_2]$ were prepared in a molar ratio (1: 2) (metal: ligand) by adding (0.9016g, 0.002mol) of the dissolved ligand in (30ml) of gradually released ethyl alcohol with continuous stirring to (0.2379 g, 0.001mol) of the metals chloride (II) dissolved in (20ml) of distilled water. The color change was observed at the moment of mixing, after which heat the reaction mixtures for (30) minutes at a temperature of (75°C). Let the mixture cool and dry. With air, it was noticed that a precipitate was formed, then it was recrystallized with hot absolute ethanol and the solid product were dried and its melting point was recorded as shown in Table (1).

Preparation of Ligand HL₂ = (DPIB)^[20]

The ligand (E) -2- (4,5-diphenyl-1H-imidazole-2-yl) diazenyl) benzoic acid was conventionally prepared from 2-aminobenzoic acid by dissolving (0.01mol, 1.371g) of the mentioned amine in a mixture of (3ml) hydrochloric acid and (30 ml) distilled water and the solution was cooled by adding pieces of ice to reach a degree of (0-5C °), followed by adding a solution of sodium nitrite (0.01mol, 0.7g) dissolved in (10ml) of distilled water drop by drop with constant stirring and leave the solution for (15) minutes to complete the nitration process, after which the prepared nitrobenzene product was added gradually with continuous stirring to a base solution of a mixture consisting of (0.01mol 2.2 g) from (4,5 - diphenyl imidazole) dissolved in (50ml) of alcohol and (10ml) of sodium hydroxide solution (10%). The coloration of the solution was observed in orange, after which the mixture was left for the next day. The sedimentation was not complete. The acidic function by adding dilute hydrochloric acid, an orange precipitate was observed, filtered, and washed with distilled water the precipitate was dried and recrystallized from hot ethanol, and its melting point was measured (163 -166C °) and the proportion of the product was (80%) scheme (2).



Scheme 2.Preparation of the (DPIB) ligand

Preparation of MixLigand Complexes

The chelate complexes of the ligand mixture prepared by the same steps were followed to dissolve known weights of the metal salts whose solid complexes were desired, which are all of the complexes of cobalt (II), nickel (II), copper (II), zinc (II), cadmium (II), and mercury (II). Its alcoholic solutions were combined with a solution of (0.4508 g, 0.001mol) of organic ligand (BMHPE) and (0.3683 g, 0.001 mol) of ligand (DPIB) in a molar ratio (1: 1: 1) (M: L: L) and the solutions of the complexes were heated. For one hour, then complex deposits were obtained after getting rid of the alcohol. Its crystals were returned from hot ethanol, the precipitate were collected and the melting degrees of the solid recrystallization were recorded. The melting points were fixed with the colors of these complexes in Table.1.

Results and Discussion

The ligands and its complexes were isolated in their pure solid form in preparation for proving their chemical formula by many spectral and analytical means, including the infrared, ultraviolet and visible spectra. The mass spectra and the nuclear magnetic resonance spectra of the proton were recorded, as well as the ratio of carbon, hydrogen, and nitrogen elements in their complexes, molar conductivity, and magnetic moments. The solid complexes are stable at room temperature and soluble in acetone, DMF and DMSO but insoluble in water. The elemental analyses and metal contents data were surmised in Table.1, for ligands and complexes are in a good agreement with the suggested formula.

Elemental Analysis

The ratio of carbon, hydrogen, and nitrogen elements was calculated, and in this study, the ratio of the aforementioned elements was calculated for the prepared organic ligands (BMHPE) and (DPIB) and the ligand complexes (BMHPE) and complexes of the ligand mixture, and the ratio of metallic elements in their complexes was calculated using the flame atomic absorption technique, the elemental analysis of the metal complexes indicate the (M:L) ratios were (1:2) and the chemical formula were $[M(C_{22}H_{18}N_3O_3Br)_2].H_2O$ and $[M(C_{22}H_{16}N_4O_2)].H_2O$, when $M = Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II)$. All prepared compound were quiet air stable

Table 1. Physical Data and Analysis of Ligands and its Complexes

NO	Formula	M:L	Color	m.p. C°	Yield%	Found,(calc.)%			
						C%	H%	N%	M%
1	$HL_1 = C_{22}H_{18}N_3O_3Br$	—	Reddish brown	121-124	80	58.26 (58.40)	3.82 (3.98)	9.07 (9.29)	—
2	$HL_2 = C_{22}H_{16}N_4O_2$	—	Orange	163-166	80	71.43 (71.73)	4.14 (4.34)	15.11 (15.21)	—
3	$[Co(C_{22}H_{18}N_3O_3Br)_2].H_2O$	1:2	Green	310 Dec.	79	53.82 (53.93)	3.24 (3.47)	8.33 (8.58)	5.92 (6.01)
4	$[Ni(C_{22}H_{18}N_3O_3Br)_2].H_2O$	1:2	Red	310 Dec.	70	53.21 (53.94)	3.22 (3.47)	8.41 (8.58)	5.66 (5.99)
5	$[Cu(C_{22}H_{18}N_3O_3Br)_2].H_2O$	1:2	Darkbrown	310 Dec.	82	53.42 (53.68)	3.25 (3.45)	8.38 (8.54)	6.24 (6.46)
6	$[Zn(C_{22}H_{18}N_3O_3Br)_2].H_2O$	1:2	Brightred	144-146	80	53.34 (53.58)	3.15 (3.45)	8.28 (8.52)	6.29 (6.63)
7	$[Cd(C_{22}H_{18}N_3O_3Br)_2].H_2O$	1:2	Orange	148-150	75	51.01 (51.14)	3.08 (3.29)	8.06 (8.13)	10.48 (10.88)
8	$[Hg(C_{22}H_{18}N_3O_3Br)_2].H_2O$	1:2	Darkred	138-140	83	47.02 (47.11)	2.91 (3.03)	4.20 (7.49)	17.71 (17.90)
9	$[Co(C_{44}H_{34}N_7O_5Br)].H_2O$	1:1:1	Red	310 Dec.	82	58.73 (58.99)	3.39 (3.57)	10.74 (10.95)	6.38 (6.58)
10	$[Ni(C_{44}H_{34}N_7O_5Br)].H_2O$	1:1:1	Brown	310 Dec.	74	58.89 (59.01)	3.37 (3.57)	10.81 (10.95)	6.29 (6.55)
11	$[Cu(C_{44}H_{34}N_7O_5Br)].H_2O$	1:1:1	Purple	310 Dec.	70	58.42 (58.69)	3.18 (3.55)	10.51 (10.89)	6.72 (7.06)
12	$[Zn(C_{44}H_{34}N_7O_5Br)].H_2O$	1:1:1	Purple	310 Dec.	88	58.27 (58.57)	3.21 (3.55)	10.47 (10.87)	7.19 (7.25)
13	$[Cd(C_{44}H_{34}N_7O_5Br)].H_2O$	1:1:1	Orange	310 Dec.	77	55.46 (55.67)	3.18 (3.37)	10.07 (10.33)	11.48 (11.85)
14	$[Hg(C_{44}H_{34}N_7O_5Br)].H_2O$	1:1:1	Red	310 Dec.	80	50.56 (50.93)	2.89 (3.08)	9.31 (9.45)	19.15 (19.35)

Infrared Spectra

The infrared spectrum of the ligand(**BMHPE**) showed a stretch band at the site (3360 cm^{-1}) assigned to the phenolic OH group.[21,22]The spectrum recorded a medium-intensity stretched band at (1662 cm^{-1}) related to ($\text{C}=\text{O}$) ketone,The spectrum showed a strong-intensity stretch band at the frequency (1593 cm^{-1}) belonging to ($\text{C}=\text{N}$) of the azomethine group.[23]The spectrum showed a weakly intense stretch band at (1427 cm^{-1}) belonging to the ($\text{N}=\text{N}$) group[24].The spectrum also showed a band at a frequency of (1066 cm^{-1}) belonging to ($\text{C}-\text{Br}$).When comparing the spectrum of the free ligand with the spectra of chelate complexes, we notice the occurrence of changes due to the occurrence of the symmetry process, where the spectra of the chelate complexes derived from this ligand showed the disappearance of the (OH) phenolic group and the emergence of a strong elastic band at the frequency (3406 cm^{-1}). This band was attributed to the presence of a crystallization water molecule that is included in the complexes to replace the phenolic (OH) group that participated in the coordination process after losing its proton.Also, in the spectrum of complexes, the frequency shift of the two groups ($\text{C}=\text{O}$) and ($\text{N}=\text{N}$) towards fewer wavenumbers with a clear change in the shape and intensity of these bands in most of the spectrums of these complexes due to their participation in the coordination process through the oxygen and nitrogen atoms.The spectra of the metal complexes showed the emergence of new bands of varying shape and intensity, but are generally weak at the range ($600-400\text{ cm}^{-1}$).These bands are due to the (M-N) and (M-O) bundle vibrations, respectively. Fig.1.

The ligand(**DPIB**) infrared spectrum showed a weak-intensity vibration band at the frequency (3412 cm^{-1}) belonging to the carboxylic (OH) stretch, and the spectrum showed a weak-intensity absorption band at the frequency (3147 cm^{-1}) attributed to the vibration of the (N-H) group for the imidazole ring [25,26].The spectrum also showed a strong band at the frequency (1695 cm^{-1}) that belongs to the ($\text{C}=\text{O}$) carboxylate group and showed a medium-intensity stretch at the frequency (1600 cm^{-1}), which belongs to the group ($\text{C}=\text{N}$) of the imidazole ring.The spectrum also showed a weak-intensity stretch band at the frequency (1456 cm^{-1}), which belongs to the bridge azo group ($\text{N}=\text{N}$) [27-29].As for the spectra of the complexes of the ligand mixture, they showed a clear difference from the spectrums of the ligands that are included in their composition, which indicates the occurrence of the coordination process and the formation of complexes, as it was observed that the phenolic (OH) group disappeared due to its participation in the coordination process after losing the proton.We also note the shifting of the frequencies of the azo-bridging group and the azomethine group of the imidazole ring towards a lower frequency, which confirms that the two groups entered the process of coordination and formation of chelate complexes.Table.2shows the values of the infrared spectra frequencies in units (cm^{-1}) for the HL_1 and HL_2 ligands and their chelate complexes. Fig.2.

Table2. The values of the infrared spectra frequencies in units (cm^{-1}) for the HL_1 and HL_2 ligands and its complexes

Compound	$\nu(\text{O}-\text{H})$ H_2O	$\nu(\text{C}=\text{O})$	$\nu(\text{C}=\text{N})$ Schiff	$\nu(\text{C}=\text{N})$ Imidazole	$\nu(\text{N}=\text{N})$	$\nu(\text{M}-\text{N})$	$\nu(\text{M}-\text{O})$
$\text{HL}_1=\text{BMHPE}$	3360(phenol)	1662	1593	_____	1427	_____	_____
$\text{HL}_2=\text{DPIB}$	3412(COOH)	1695	_____	1600	1456	_____	_____
$[\text{Co}(\text{L}_1)_2].\text{H}_2\text{O}$	3406	1637	1593	_____	1423	522	443
$[\text{Ni}(\text{L}_1)_2].\text{H}_2\text{O}$	3394	1635	1593	_____	1409	522	447
$[\text{Cu}(\text{L}_1)_2].\text{H}_2\text{O}$	3381	1643	1593	_____	1417	516	447
$[\text{Zn}(\text{L}_1)_2].\text{H}_2\text{O}$	3402	1641	1593	_____	1425	594	443
$[\text{Cd}(\text{L}_1)_2].\text{H}_2\text{O}$	3402	1633	1593	_____	1411	549	478
$[\text{Hg}(\text{L}_1)_2].\text{H}_2\text{O}$	3396	1637	1593	_____	1409	516	472
$[\text{Co}(\text{L}_1\text{L}_2)].\text{H}_2\text{O}$	3410	1710	1695	1598	1408	543	484
$[\text{Ni}(\text{L}_1\text{L}_2)].\text{H}_2\text{O}$	3415	1708	1662	1597	1409	542	470
$[\text{Cu}(\text{L}_1\text{L}_2)].\text{H}_2\text{O}$	3433	1706	1633	1598	1413	549	441
$[\text{Zn}(\text{L}_1\text{L}_2)].\text{H}_2\text{O}$	3404	1708	1641	1597	1446	538	439
$[\text{Cd}(\text{L}_1\text{L}_2)].\text{H}_2\text{O}$	3398	1707	1695	1593	1442	536	451
$[\text{Hg}(\text{L}_1\text{L}_2)].\text{H}_2\text{O}$	3396	1708	1641	1595	1423	542	468

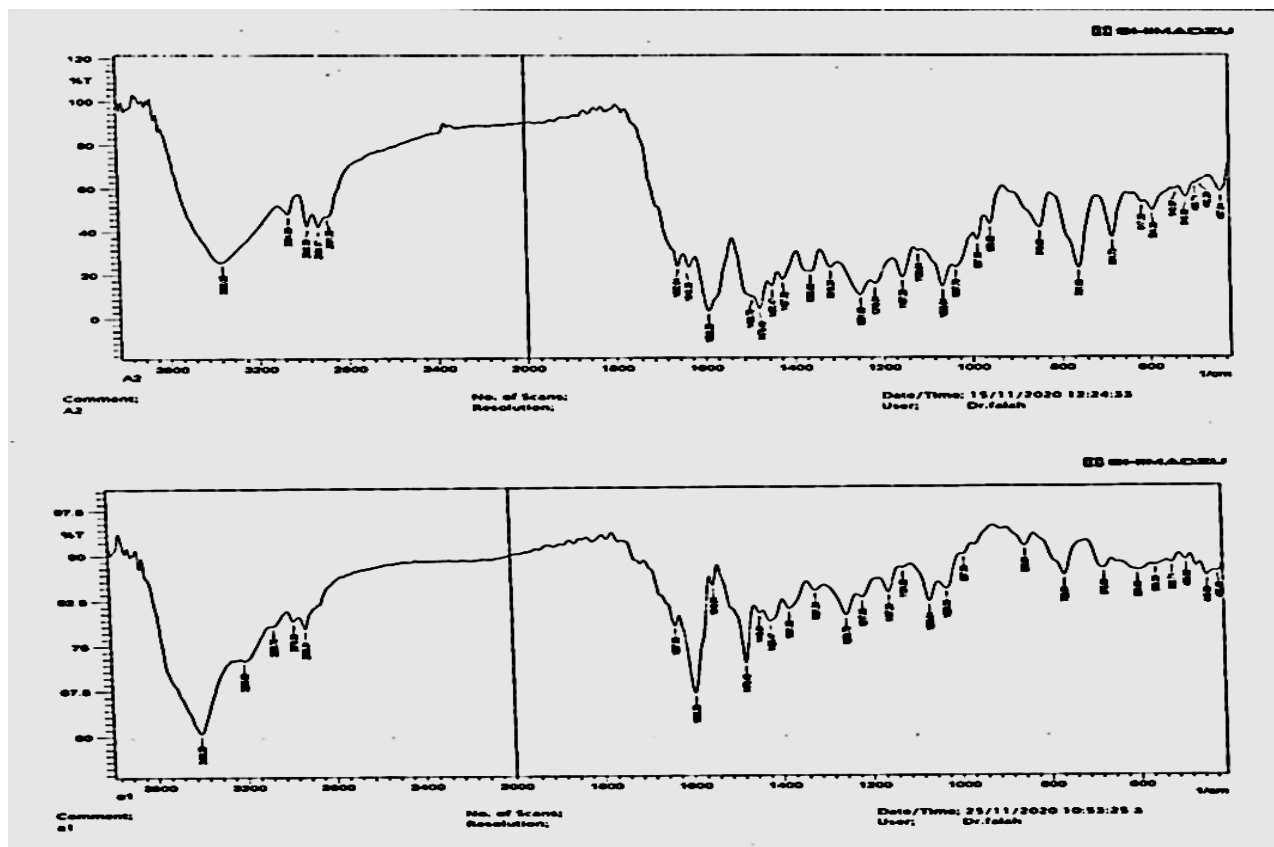


Fig.1. IR spectra of:(a)the azo-Schiff base&(b) $[Co (L_1)_2].H_2O$ complex

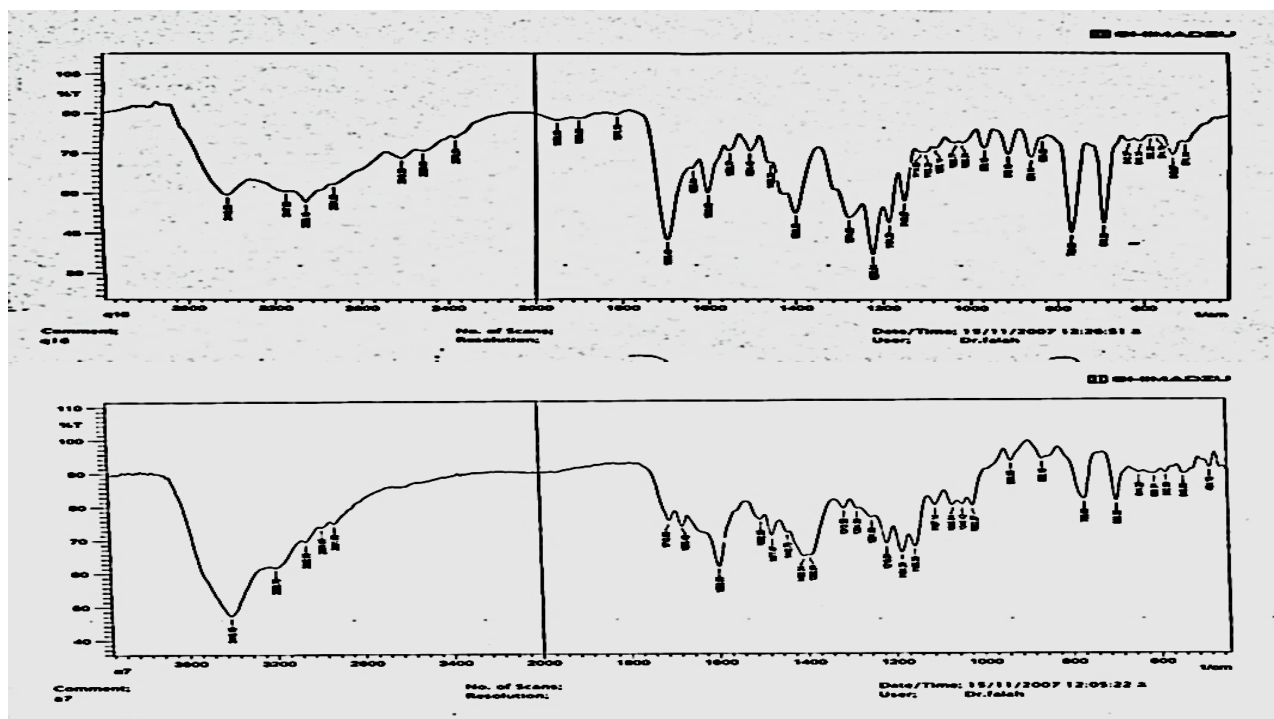


Fig. 2.IR spectra of (a) theAzo-amidazole ligand&(b) $[Co(L_1L_2)].H_2O$ mix complex

Electronic Spectra and Magnetic

The electronic spectral data of the ligands and metal complexes in DMSO field around the metal ion has been deduced from the electronic spectra. The magnetic moments and electronic spectral of the complexes are summarized in Table.3. The magnetic of the Co(II) has been found to be (4.77 and 4.81 B.M) which are with the range of the octahedral complexes [30]. For the Ni(II) complexes its magnetic moment (2.8 and 2.9 B.M) which are suggesting with octahedral configuration [31]. The magnetic moments value of the Cu (II) complexes (1.74 and 1.75 B.M) which may suggest an octahedral structure. Zn (II), Cd(II), Hg(II) complexes are diamagnetic moments for d^{10} ion and the electronic spectra of there complexes do not show any d-d bands [32].

Table 3. Electronic spectra, Conductivity and Magnetic Moment of Ligands and its Complexes

Compound	Absorption bands (nm)	Transition	Conductivity S. cm ² .mol ⁻¹ in (DMSO)	μ_{eff} (B.M)
HL ₁ = BMHPE	260 289 371	$\pi \rightarrow \pi^*$ C.T	—	—
HL ₂ =DPIB	293 445	$\pi \rightarrow \pi^*$ C.T	—	—
[Co(L ₁) ₂].H ₂ O	293 365 485 648	$\pi \rightarrow \pi^*$ $\pi \rightarrow \pi^*$ IL CT ML CT	9.1	4.77 B.M
[Ni(L ₁) ₂].H ₂ O	293 373 535	$\pi \rightarrow \pi^*$ IL CT ML CT	12.4	2.8 B.M
[Cu(L ₁) ₂].H ₂ O	283 522	$\pi \rightarrow \pi^*$ ML CT	8.2	1.74 B.M
[Zn(L ₁) ₂].H ₂ O	287 359	$\pi \rightarrow \pi^*$ ML CT	2.7	Di
[Cd(L ₁) ₂].H ₂ O	292 365	$\pi \rightarrow \pi^*$ ML CT	4.2	Di
[Hg(L ₁) ₂].H ₂ O	289 347	$\pi \rightarrow \pi^*$ ML CT	11.2	Di
[Co(L ₁ L ₂)].H ₂ O	341 521	IL CT ML CT	15.3	4.81 B.M
[Ni(L ₁ L ₂)].H ₂ O	259 310 516	$\pi \rightarrow \pi^*$ IL CT ML CT	14.8	2.9 B.M
[Cu(L ₁ L ₂)].H ₂ O	262 313 534	$\pi \rightarrow \pi^*$ IL CT ML CT	13.8	1.75 B.M
[Zn(L ₁ L ₂)].H ₂ O	304 417 517	IL CT IL CT ML CT	0.8	Di
[Cd(L ₁ L ₂)].H ₂ O	249 436	IL CT ML CT	5.3	Di
[Hg(L ₁ L ₂)].H ₂ O	245 515	IL CT ML CT	6.01	Di

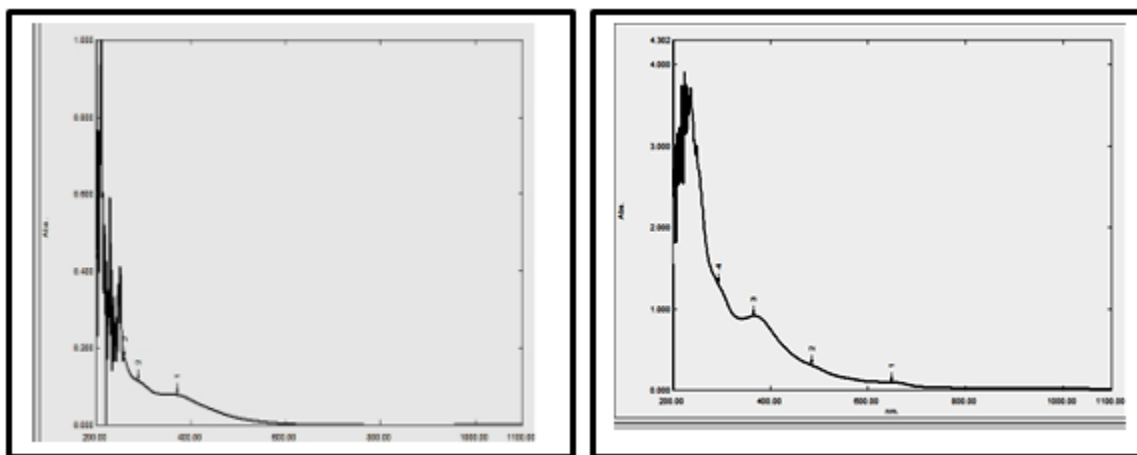


Fig.3. Uv-vis of (a)(BMHPE)ligand&(b) $[Co(L_1)_2] \cdot H_2O$ complex

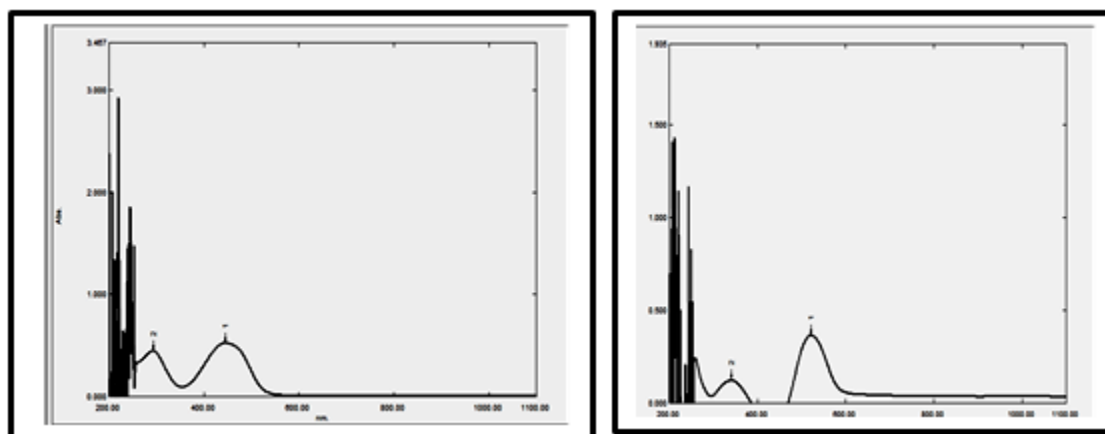


Fig.4. Uv-vis of (a)(DPIB) ligand&(b) $[Co(L_1)(L_2)] \cdot H_2O$ complex

1H -NMR Spectra of Ligand (BMHPE) [33,34]

The proton nuclear magnetic resonance spectrum of (BMHPE) ligand solution in the solvent (DMSO- d_6), using tetramethylsilane (TMS) as a reference in the measurement. (1H -NMR) spectrum of the ligand (BMHPE). Shows the following singnals,phenyl multiples at (6.53 -802 ppm) range,(CH=N) at(9.03 ppm),OH phenolic at (11.23 ppm),-OCH₃ at (3.87 ppm), -CH₃ at (2.64 ppm) and DMSO at (2.51 ppm) as shown in Fig. 5.

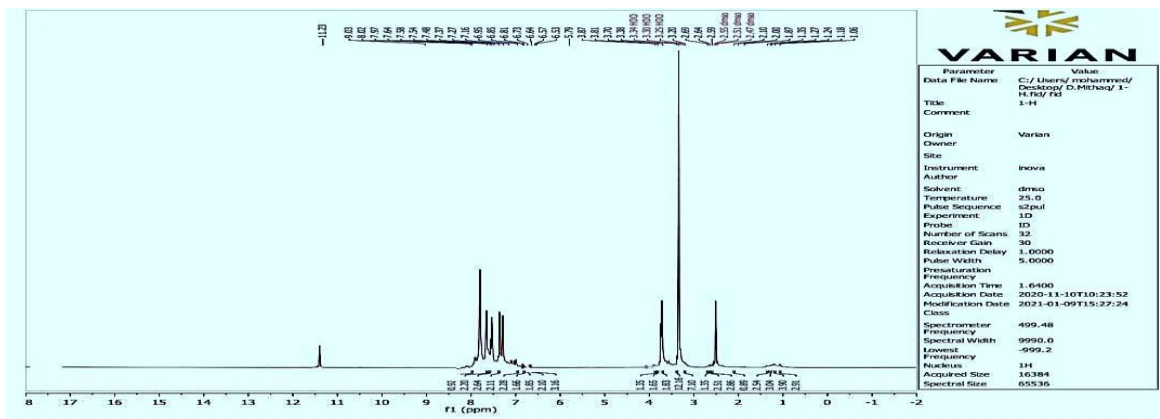


Fig.5. Spectrum of the 1H -NMR proton of the ligand (BMHPE)

Mass Spectrum of Azo-azometheneLigand(BMHPE)^[35]

The mass spectrum of the azo-azomethene (BMHPE) was recorded and it showed many fragments of mass fractionation with relative abundance, which differed according to the stability of that part of the compound against the surrounding conditions, including the peak of fragmentation (452) (M / z^+) attributed to a mother molecule (azo - Azomethene) ligand was prepared for the study of this research and is a statement of the correctness of its molecular structure. The fragment at ($m/z=396$ and 280) corresponding to $(C_{21}H_{11}O_3Br)^+$ and $(C_{13}H_{12}O_2Br)^+$ respectively. Another fragment at ($m/z^+=120$) which due to $(C_8H_8O)^+$. Fig 6 and scheme 3, the mass spectrum and fragmentation pattern of azo-Schiff ligand.

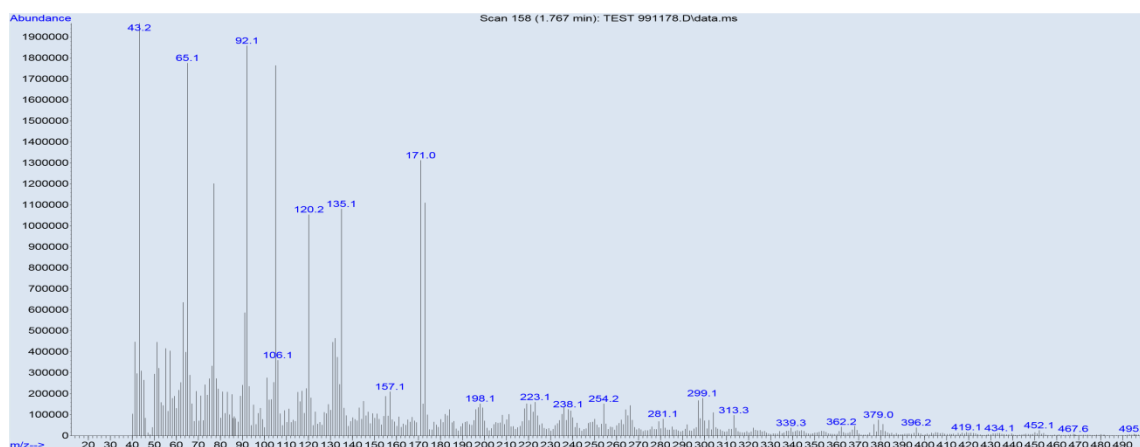
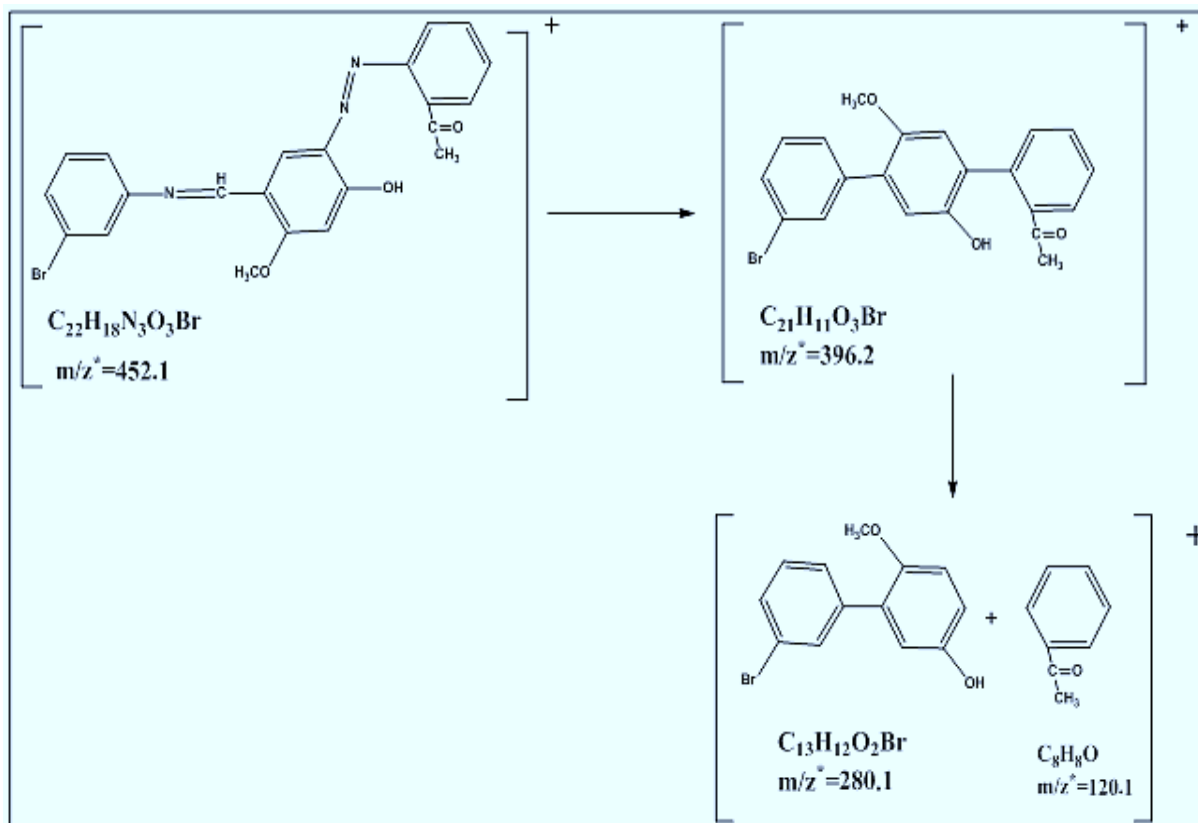


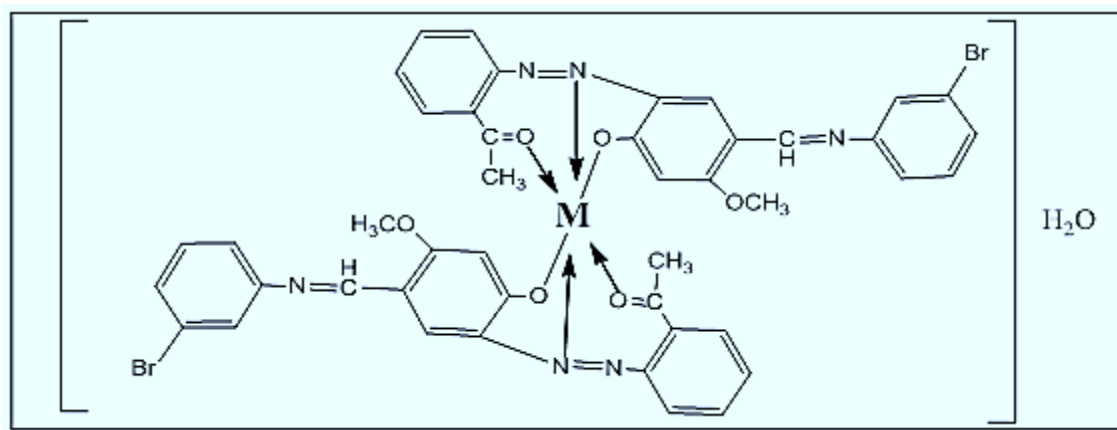
Fig.6. Mass Spectrum of the Ligand(BMHPE)



Scheme3. The proposed fragmentation pattern for azo-azomethine ligand

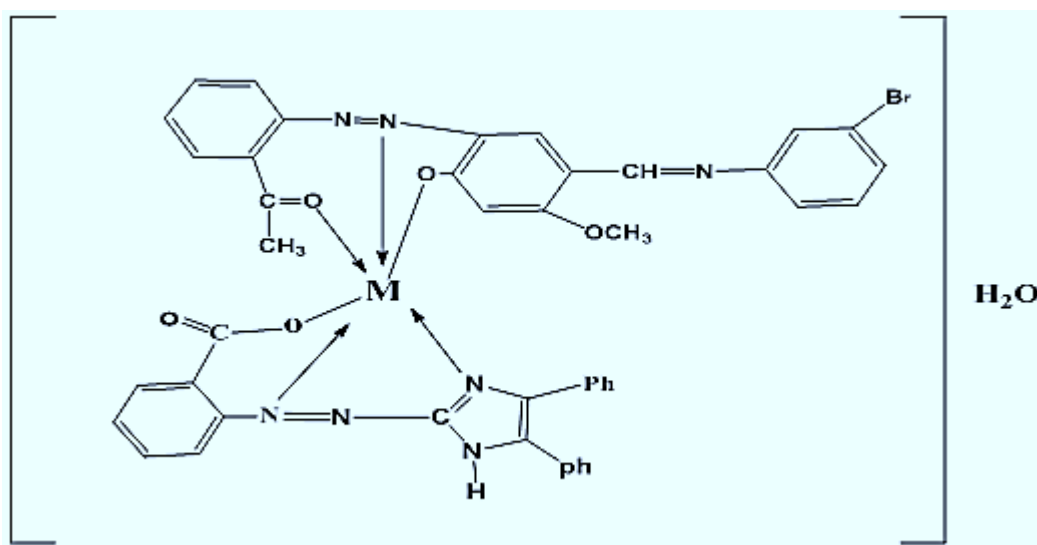
Conductivity Measurement

All prepared complexes low conductivity values ranging between (0.8-15.3) $\text{s.cm}^2.\text{mol}^{-1}$. As a result of these results, it was found that these complexes do not possess the ionic character, as shown in the proposed Fig 7,8.



M= Co(II), Ni(II), Zn(II), Cu(II), Cd(II), Hg(II)

Fig.7. The proposed structural formula of the metal chelate complexes with ligand (BMHPE)



M= Co(II), Ni(II), Zn(II), Cu(II), Cd(II), Hg(II)

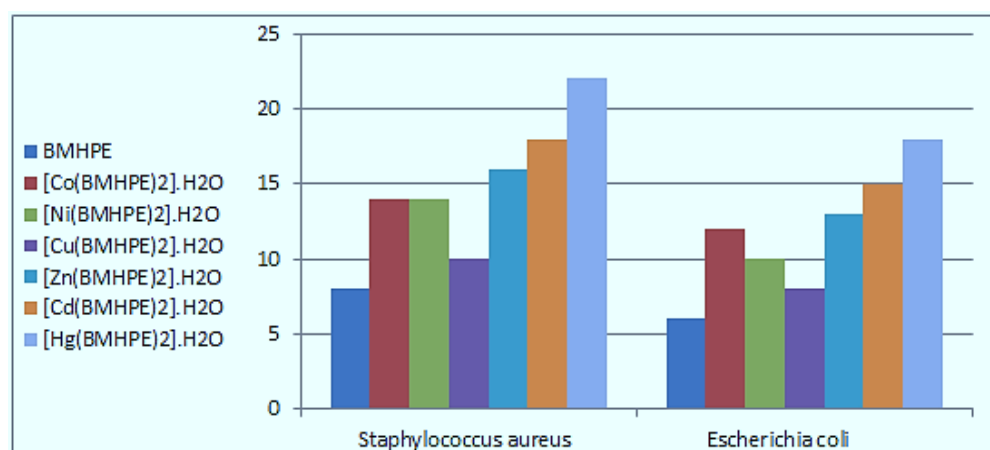
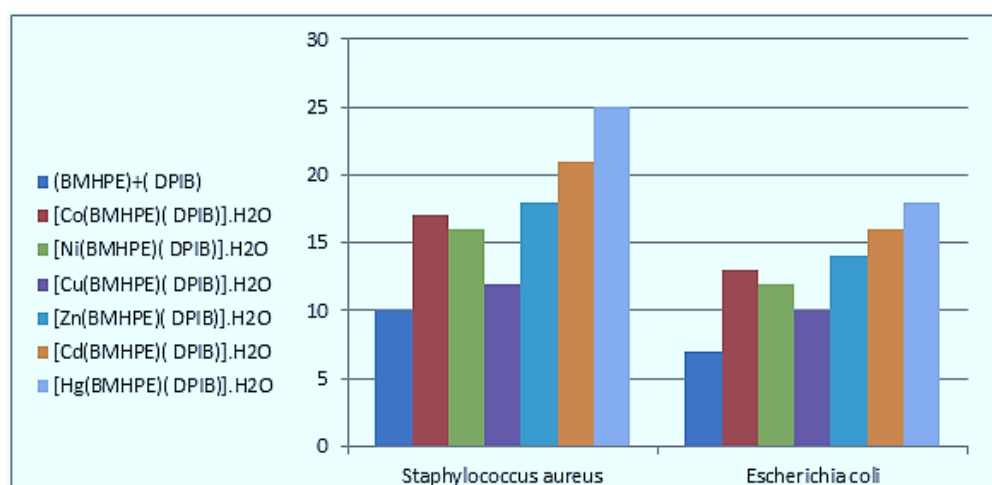
Fig.8. The proposed structural formula of the metal chelate complexes with mix ligand

The Study of Bioactivity^[36]

The organic and inorganic compounds have a clear effect on the micro-organisms, which cause many common diseases, so many researchers pushed him to research, investigate and study the inhibitory activity, to obtain an antibiotic that works to inhibit these types of bacteria and other microorganisms that cause these ailments. In this study, two types of bacteria were used, one positive for (Gram-positive), which is (*Staphylococcus aureus*) and the other negative for (Gram-negative), which is (*Escherichia coli*). The effect of the biological activity of the prepared ligand (BMHPE) and the mixture of ligand (BMHPE + DPIB) and their prepared metal complexes Co (II), Ni (II), Zn (II), Cu (II), Cd (II), Hg (II), on the bacteria mentioned above, using (DMSO) as a solvent, and the certifications of the spreading drilling method (Wells). Concentrations of (1000 ppm) were used, where all the compounds prepared for the ligand complexes (BMHPE) and the mixture of ligand (BMHPE + DPIB) showed positive results, clearly high, medium to weak, towards inhibiting the growth of the selected bacteria in the study as shown in the table (4).

Table 4. Inhibition regions resulting from the biological effect of a ligand (BMHPE) and the mixture of ligand (BMHPE + DPIB) and their metal complexes at a concentration of (1000ppm) with the bacteria under study measured in units (mm)

Compound	Inhibition zone (mm).	
	G(+)	G(-)
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
BMHPE	8mm	6mm
(BMHPE)+(DPIB)	10mm	7mm
[Co(BMHPE) ₂].H ₂ O	14mm	12mm
[Ni(BMHPE) ₂].H ₂ O	14mm	10mm
[Cu(BMHPE) ₂].H ₂ O	10mm	8mm
[Zn(BMHPE) ₂].H ₂ O	16mm	13mm
[Cd(BMHPE) ₂].H ₂ O	18mm	15mm
[Hg(BMHPE) ₂].H ₂ O	22mm	18mm
[Co(BMHPE)(DPIB)].H ₂ O	17mm	13mm
[Ni(BMHPE)(DPIB)].H ₂ O	16mm	12mm
[Cu(BMHPE)(DPIB)].H ₂ O	12mm	10mm
[Zn(BMHPE)(DPIB)].H ₂ O	18mm	14mm
[Cd(BMHPE)(DPIB)].H ₂ O	21mm	16mm
[Hg(BMHPE)(DPIB)].H ₂ O	24mm	18mm

**Fig.9.** The biological activity of BMHPE and its complexes at a concentration of (1000 ppm)**Fig.10.** The biological activity of a mixture of (BMHPE + DPIB) ligand and its metal complexes at a concentration of

(1000 ppm)

Conclusion

The research included the preparation of ligand azo- Schiff and ligand Azo, followed by the preparation of a set of complexes with ligand azo-azomithene alone with the ions Co(II), Ni (II), Zn (II) Cu (II), Cd (II), Hg (II), as well as the preparation of a set from chelate complexes with aforementioned ligands with a mixture of ligand. The isolation compound were characterized by available techniques. All complexes are stable and nonionic nature and the geometry is proposed for all chelate complexes show octahedral stereochemistry. The biological effect of the prepared complexes and their potential use in the medical field has been shown through their remarkable effect on inhibiting the growth of many types of bacteria, such as (*Staphylococcus aureus*), a type of (Gram positive bacteria), and (*Escherichia coli*), a type of (Gram negative bacteria).

References

- [1] Davagi. S, Degani. Y; *"The Chemistry of Carbon Nitrogen Double Bond"* Ed. S. Patai John Wiley and Sons, Interscience, New York. (1970).
- [2] El-Sherif, A. A, & Aljahdali, M. S; *J. Coord. Chem*, 66, 3423–3468. (2013).
- [3] Martin, K; *Molecules*, 22 (15- 73), 1-15, (2017).
- [4] Ahlam. J. A, & Sajakhahl, M; *Der pharma. Chemical*, 9 (8), 109 – 116, (2017).
- [5] Kumar. J, Rai. A, Raj. V; *Organic & Medicinal Chem II*, 1(3), 1-15, (2017).
- [6] Momle. G. R, Vedpathak. S. G, & Kakade. G. K; *EJPMR*, 5(3), 225-235, (2018).
- [7] Hunger. K ; *" Industrial Dyes"*; WILEY –VCH Verlag GmbH & Co. KGa A., p. 19, (2003).
- [8] Armino. D. M, Hand book of colorant. in, Hand book of US colorants. food, Drugs, cosmetics and medical devices. 3rd edn. Wiley New York, USA. 23- 26. (1999).
- [9] Widad. I. Y, Raheem T.M, & Hussein. K.A; *Journal of Physics: Conf. Series* 1294. (2019).
- [10] Radha. V. P, Mahalakshmi. D, Jonekirubavathy. S & Chitra. S; *J. Serb. Chem. Soc.*, 85 (2), 215–225, (2020).
- [11] Surbhi. V. U, Raksha V. Z, & Keyur D. B; *An Scientific journal*, 145, 1-15, (2020).
- [12] Jasmine. M. C, Tim. R. B, Alexander. D. H. K, Robert. P, David. M. P, & James. W. W; *molecules*, 25, 2383. (2020).
- [13] M. Kand, F. Galil, L. Abdelwahab, and A. Psman; *J. Fur Park Tische Chemie.*, 313, 1011, (1971).
- [14] Ispir. E, Ikiz. M, Inan. A, Sünbül. A. B, Tayhan. S. E, Bilgin. S, & Elmastaş. M; *Journal of Molecular Structure*, 1182, 63-71, (2019).
- [15] Danish. S, Aamer. S, Fayaz. A. L, Pervaiz. A. C, Qamar. A, Mohamed. F. A, Ifzan Arshad. M, Mauricio. F. E, Mubashir. H, Hussain. R, Sung-Yum. S, & El-Seedi. H. R; *Molecules*, 24, 1511. (2019)
- [16] Bourgeois I, Pestel-Caron M, Lemeland JF, Pons JL y Caron F, Tolerance to glycopeptides, vancomycin and teicoplanin in coagulase negative staphylococci, *Antimicrob Agents Chemother*, 2007; 51 (2): 740–743.
- [17] Amanullah. M, Sadozai. S. K, Rehman. W, Hassan. Z, Rauf. A, & Iqbal. M; *Afr. J. Biotech*, 10(2), 209, (2011).
- [18] Singh. U. K, Pandeya. S. N, Sethia. S. K, Pandey. M, Singh. A, Garg. A, & Kumar. P; *Int. J. Pharma. Sci. Drug Res*, 2(3), 216, (2010).
- [19] Mitthu. L. G, Chetna. A, Rakshit. A, Kiran. M, & Pinki. B. P; *Eur. Chem. Bull*, 7(2), 52-58, (2018).
- [20] Rabab Mahdi Ubaid Mahmood., Synthesis, Spectral Investigation and Microbial Studying of Pyridine-Heterocyclic Compounds., *European Journal of Molecular & Clinical Medicine*, 2020, Volume 7, Issue 11, Pages 4444-4453

- [21] T. EL-Sayed and A.Aboelnaga, *Egypt.J.Chem.* Vol. 63, No. 1. pp. 101,115 (2020).
- [22] L. Ali. Mohammed, N. Izet Mahdi, R. Abdul BaqiAldujaili, *Egypt.J.Chem.* Vol. 63, No.1. pp. 289- 300 (2020).
- [23] F.A.Saad and A.M.Khedr,*Bulgarian Chemical Communications*, 47, 2, p.654-663, (2015).
- [24] Al-Adilee, K. J., and Dakheel, H. K.(2017). Synthesis, Spectral Characterization and Biological Studies of new Heterocyclic Azo-Schiff Base Compound (E)-5-(E)-(1H-benzo [d] imidazol-2-yl) diazenyl)-N-(4-chlorobenzylidene)-2-amine-4, 6-dimethylpyridin with some Metal Ions.
- [25] B.Nagakumar, Kavith, B. and Kewn, W.L., *Bull. Korean Chem. Soc*, 27, 266,(2006).
- [26] T.D. Bradshaw, Stevens, M.F.G. and Westwell, A.D., *Curr. Med. Chem*, 8,203, (2001).
- [27] Habiban, A. M., Mahmoud, W. A., and Kareem, T. A. (2015). Preparation and Characterization of Some Metal Complexes with Heterocyclic Azo Ligand (4-SuBAI). *Baghdad Science Journal*, 12(3), 503-515.
- [28] M.H.Muzael; *World Journal of Pharmacy and Pharma Ceutical Sciences*, 4(10), p.264-273 (2015).
- [29] Singh. D, Kumar. K, Kumar. R, & Singh; J. J. *Serb. Chem. Soc*, 75(2), 217-228, (2010).
- [30] Sahar. S, Hoda. P, Naser. F, Mehran. D, & Fereshteh. M; *Appl.Sci.*, 8 (85), 1-12, (2018).
- [31] K. J. Al-Adilee, H. A. H. Al-shamis, & M. N. Dawood; *Research Journal of Pharmaceutical, Biological and Chemical Science*. 7(4), 2882-2905, (2016).
- [32] Rabab Mahdi Ubaid Mahmood., Synthesis, Characterization of Diazepine-Bicycles System and Study of their Bio-Behavior.,*International Journal of Pharmaceutical Research*, 2021, Volume 13, Issue 1, Pages 4225-4233.
- [33] Ommenya F. K, Nyawade. E. A, Andala. D. M, & Kinyua. J;*Hindawi Journal of Chemistry*, 8 pages, (2020).
- [34] Sadeek. S. A, El-Hamid. S.M. Abd, Rashid. N.G; *Egypt. J. Chem.* 63, 3, 939-951, (2020).
- [35] Afonso. C, Lesage. D, Fournier. F, Mancel. V,&Tabet. J. C; *Int. J. Mass Spectrom*, 312, 185–194, (2012).
- [36] L.Armand-Lefevre, Angebault, C., Barbier, F., Hamelet, E., Defrance, G. and Ruppe, E. Emergence of imipenem-resistant Gram-negative bacilli in intestinal flora of intensive care patients. *Antimicrob. Agents*, (2013).